SPECIES ABUNDANCE, COMPOSITION AND COLONIZATION BEHAVIOUR OF MALARIA VECTORS IN A SEMI-ARID ECOSYSTEM OF BARINGO DISTRICT, KENYA.

By

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DECLARATION

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

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DEDICATION

I dedicate this research work to my wife Beryl Katiba, and my brother, Moses Odhiambo.

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LIST OF ABREVIATIONS AND ACRONYMS

An.	*Anopheles*
ANOVA	Analysis of variance
CDC	Center for Disease Control
DVBD	Division of Vector Borne Diseases
GPS	Global Positioning System
PSC	Pyrethrum Spray Collection
SAS	Statistical Analysis System
SNK	Student Newman Keul’s test
WHO	World Health Organization

DEFINITION OF TERMS

Anthropophilic	Mosquitoes which feed almost entirely on human’s blood
Crepuscular	Mosquitoes which suck blood in the late hours of the night
Endophilic	Mosquitoes which rest indoors after blood meal
Endophagic	Mosquitoes which prefer feeding indoors
Exophagic	Mosquitoes which prefer feeding outdoors
Exophilic	Mosquitoes which prefer to rest outdoor after blood meal
Nocturnal	Mosquitoes which generally suck blood at night
Zoophilic	Mosquitoes which feed on other animals’ blood

ABSTRACT

Malaria is one of the public health problems facing people in many parts of Kenya including semi-arid areas. It is caused by an infectious bite of female *Anopheles* mosquitoes. To effectively implement malaria control program, the knowledge of colonization, resting behavior of the vectors and effect of distance between houses and breeding habitats on mosquito abundance is required. This research set out to determine seasonal dynamics, outdoor resting habits and colonization of larval habitats by *Anopheles* vectors of malaria. The study was conducted in Kamarimar village in Baringo District adjacent to Loboi swamp. Larval survey was conducted once weekly by making 10-20 dips per habitat using standard dipper (350 ml). Ten houses were sampled by use of Pyrethrum Spray Catches (PSC) once a week for adult mosquitoes and aspiration method once a fortnight. Data was analyzed using SAS version 9.2. Mosquito breeding habitats comprised pan dams, ditches, marshes and canals. Marshes were relatively productive larval habitats and produced 35 out of the total 74 larvae collected in the dry season. However, no larvae were collected from canals. Pan dams, ditches and culverts produced 28, 9 and 2 *Anopheles* larvae respectively. Out of 281 *Anopheles* larvae collected from all the breeding habitats, 207 were collected during the rainy season. *An. gambiae* was found to breed in pan dams and ditches in both seasons and were constantly available during the rainy season in which 91 larvae were collected while the dry season realized only 22 larvae. *An. funestus* larvae were few but during the dry season, 21 larvae were collected from the swamp marshes and pan dams while in the rainy season, only 9 larvae were collected. The larvae of *An. gambiae* and *An. funestus* were rarely detected in the canals. Altogether, 2488 adult *Anopheles* mosquitoes were collected indoors of which 1166 were *An. gambiae*, 63 *An. funestus*, 11 *An. coustani* and 1248 *An. pharoensis*. 115 *Anopheles* mosquitoes were collected outdoors including most potential vectors of malaria. 2019 (81%) *Anopheles* mosquitoes were collected during the rainy season while 463 (19%) were collected in the dry season. The effect of distance between a house and a breeding habitat on natural logarithm transformed mosquito numbers was significant, $r = 5.48$, $df = 1$, $p = 0.026$. There also was a difference in mosquito abundance between outdoor habitats, $\chi^2 = 29.87$, $df = 2$, $p < 0.0001$. A fitted Poisson regression revealed that interaction between seasons and habitats was significant $\chi^2 = 12.6$, $df = 1$, $p = 0.027$. The findings of this study may be useful in a vector control program targeting resting and breeding habits of malaria vectors as a way of controlling their population.

CHAPTER ONE: INTRODUCTION

1.1 Background

Malaria which is caused by protozoan parasites of the genus *Plasmodium* is transmitted to humans by an infectious bite of the blood seeking female mosquitoes of the genus *Anopheles* (Diptera; Culicidae). According to Kiszewski and Teklehaimanot (2004), the genus *Anopheles* consists of approximately 484 species. The main malaria vectors in sub-Saharan Africa include *Anopheles gambiae, An. arabiensis* and *An. funestus*. These species are confronted with highly variable and challenging climatic conditions especially in the semi-arid regions and especially during the dry seasons (Mattingly, 1971). In semi- arid ecosystems with seasonal dynamics of *Anopheles* populations, larval habitats of *An. gambiae* complex are considerably reduced during the dry season (Taylor *et al.,* 1993; Charlwood *et al.*, 2000), and adult vector densities are thus very low in dry seasons (Mbogo *et al.*, 1995; Lindsay *et al.*, 1991). Permanent breeding sites during the dry season may serve to seed the additional larval habitats formed during the rainy season (Charlwood *et al.*, 2000). It has been observed that the abundance of malaria vector species declines dramatically with the onset of the dry season and this may depress the incidence of severe malaria (Wilkinson, 1978; Snow and Marsh, 1993). The onset of rains, however, brings a rapid explosion in mosquito numbers and concomitant increase in malaria infections (Omer and Cloudsley-Thomson, 1970; Mbogo *et al.*, 1995 and Snow and Marsh, 1993).

There have been no studies conducted to show how colonization, abundance and species composition vary between the seasons in semi-arid areas in Kenya. Anopheline mosquito eggs are susceptible to prolonged desiccation which likely prevents the egg stage from making significant contribution to the long term survival during the dry season (Deane and Causey, 1943). Studies in the laboratory however, have demonstrated that eggs may survive and hatch after 12 days of storage in moist conditions (Beier *et al.*, 1990). These observations suggest that the egg stage could be important for a short-term survival, as larvae typically cannot survive for long periods without water (Muirhead – Thomson, 1945). Adult stages could also make an important contribution to dry season and semi-arid population dynamics.

Previous studies conducted in Burkina Faso and the Sudan in Africa revealed that *An. gambiae* adults enter a state of dormancy at the onset of dry season (Omer *et al.*, 1970). In semi-arid environments and periods of extended drought, there is normally scarcity of water sources for mosquito breeding. Nonetheless, transmission of malaria is observed throughout the year at low level (Omer *et al.*, 1970). Most semi-arid complexes are currently affected by malaria epidemics and these ecosystems present peculiar physical conditions which must be investigated. A rapid change in environmental temperature is an important factor governing the behavior of many insects. Study of the breeding and resting behavior of mosquitoes, particularly vectors of malaria is important for understanding vector bionomics. Most of the species which are in close contact with human and domestic animals are found resting inside houses or in animal shelters, thus giving the impression that these are the only resting places (Bhatt *et al.,* 1989). However, it is a well known fact that many mosquito species including some of the major vectors of malaria prefer to rest in natural shelters in vegetation, in hollow trees, under culverts, animal burrows among others. The degree of outdoor resting varies considerably between different species and in the same species in different areas and seasons (Sharma *et al.,* 1986). A search for outdoor resting mosquitoes usually proves time consuming and unrewarding. However, a collection of outdoor resting mosquitoes occupies an important place in the studies of mosquito ecology and behavior (Sharma *et al.,* 1986). Evaluating seasonal dynamics therefore aimed at determining resting and colonization habits of malaria vectors which contribute to their survival over periods of extended dry season and in semi-arid regions. This study investigated colonization, composition and abundance of the vectors resting indoors and outdoors during rainy and dry seasons with the view of finding out how these habits could be utilized in managing dynamics of *Anopheles* population.

1.2 Statement of the problem

Malaria exists in tropical regions which share problems of poverty, lack of adequate health care and poor infrastructure. However, the intensity of malaria transmission is not uniform in these regions. There are approximately 430 species of *Anopheles* mosquitoes about 70 of which are known malaria vectors (White, 1974). Malaria in Africa especially Sub-Saharan Africa is mainly transmitted by three mosquito species *An. gambiae*, *An. arabiensis* and *An. funestu*s (Coetzee, 2004 and Mattingly, 1971).

The global distribution ranges of these three species intersect in Africa, which carries approximately 90% of the global malaria burden (Nora and Catherine, 2004). This threatens health of people as most semi-arid regions in Kenya such as Kamarimar village in Baringo District; there is no access to good health facilities and poor and unreliable transport network puts lives at risk.

1.2.1 Justification of the study

All age groups experience malaria, but the highest mortality occurs in children under the age of five years and pregnant women (Philips, 2001) purportedly due to their lower level of immunity (WHO, 2006). More than 90% of deaths caused by malaria occur in Sub-Saharaan Africa and the disease is responsible for 50% of outpatient cases and 20% of hospital admissions (Boutin *et al.*, 2005). Previous studies by Aniedu, (1993) revealed that there are dynamics of malaria vectors in semi-arid ecosystems between the rainy and dry seasons. The populations get much reduced during the dry seasons due to scarcity of water sources for breeding. However, the most intriguing observation is that malaria transmission persists throughout the year (Omer *et al.,* 1970). This suggests that malaria vectors are capable of utilizing limited habitats during the dry seasons for successful breeding and subsequent transmission of the disease. Behavioral habits such as colonization of breeding habitats by malaria vectors in semi-arid habitats have not been investigated in Kenya. This poses a major challenge in handling malaria vectors in these areas which lack accessibility to health facilities due to poor infrastructural development.

The effect of distance between a house and a breeding habitat on resting behavior of malaria vectors is an important factor in determining the success or failure of control programmes using indoor residual spraying with insecticides. Most anophelines rest outdoors to some extent, but if a species is wholly or largely exophilic, a large proportion of the mosquito population will escape contact with the insecticide and transmission will not be interrupted. The aim of this study was to find out mosquito breeding patterns in semi-arid habitat and the population dynamics of larvae, pupae and adults as affected by seasonal changes to ascertain the abundance and dynamics of malaria vectors. A detailed investigation of vector behavior was undertaken in Kamarimar village of Baringo District. Resting behavior and colonization patterns were established.

1.3 Research questions

a) How does distance between a homestead and a mosquito breeding habitat affect abundance of malaria vectors resting indoors in Kamarimar village?

b) How does colonization of breeding habitats by malaria vectors vary in Kamarimar village?

c) What are the possible outdoor resting habitats for malaria vectors in Kamarimar village?

1.4 Hypotheses

a) The distance between a homestead and a breeding habitat has no effect on the abundance of malaria vectors resting indoors in Kamarimar village.

b) Colonization of breeding habitats by malaria vectors in Kamarimar village is not variable.

c) There are no outdoor resting habitats preferred by malaria vectors in Kamarimar village.

1.5 Objectives of the study

1.5.1 General objective

To determine the species abundance, resting and colonization behavior of malaria vectors in a semi-arid ecosystem of Baringo district.

1.5.2 Specific objectives

a) To determine the effect of distance between homestead and a breeding habitat on the abundance of malaria vectors resting indoors in a semi-arid ecosystem.

b) To establish the colonization of breeding habitats by malaria vectors in a semi-arid ecosystem.

c) To identify the outdoor resting sites of mosquito vectors in Kamarimar village of Baringo district.

CHAPTER TWO: LITERATURE REVIEW

2.1 Biology of *Anopheles* mosquitoes

The lifespan of adult mosquito is generally 3-4 weeks, although it may be reduced in nature by various mortality factors including natural enemies and environmental factors such as humidity and temperature (Cheesbrough, 1987).

2.1.1 Life cycle of *Anopheles* mosquitoes

Anopheles mosquitoes develop through four stages of life cycle: egg, larva, pupa, and adult. The first three stages are mainly aquatic and the cycle from egg to adult stage may last for 5-14 days but this is dependent on species, humidity and ambient temperature (Clement, 1992). In tropical climates, development is rapid and therefore the egg-adult cycle may be completed in 6 days (Gillies and De Meillon, 1968).

After emergence, the adult mosquito takes at least one day to reach sexual maturity. The adult stage is when the female *Anopheles* mosquito acts as a vector and is capable of transmitting malaria parasites. They are highly anthropophilic and females take more than 90% of their blood meal from human hosts. Blood is needed for egg development which takes about two days (Clement, 1992). After mating and blood feeding, a gravid female mosquito lays about 50-500 eggs the second day after blood feeding (Clement, 1992).

Eggs hatch into larvae approximately two days after oviposition and this is largely dependent on ambient temperature and humidity (Clement, 1999). The aquatic stages (egg, larva and pupae) of *Anopheles* species occur in a variety of habitats. The most common are shallow open sun-lit pools of water such as burrow pits and drains (Jensen *et al.*, 1994). During periods of dry weather, breeding occurs in temporary pools left by receding rivers and streams or water collections associated with human activities (Coetzee, 2004).

Larvae spend most of their time feeding on algae, bacteria and other micro-organisms in the surface micro layer of water (Gillies and Coetzee, 1987). It takes less than seven days to develop through four larval instars. Duration of the larval stage is however influenced by environmental temperature and availability of food (Gillies and De Meillon, 1968). The pupal stage duration varies from 1-3 days to develop into adult and this also depends on the environmental conditions such as ambient temperature and humidity which may affect duration in this developmental stage. The pupae frequently come to the surface of water to breathe which they do through a pair of trumpets on the cephalothorax. When pupae emerge into female it attains sexual maturity and assumes host seeking for a blood meal.

2.1.2 Species of *Anopheles* mosquitoes

Anopheles mosquitoes in Africa consist of two groups which have been incriminated in malaria transmission. These two broad groups include *An. gambiae* complex group and *An. funestus* group (Coetzee, 2004).

The *An. gambiae* complex consists of morphologically indistinguishable sibling species namely *An. gambiae* s.s, *An. arabiensis*, *An. bwambae, An. merus, An. melas, An. quadriannulatus* species A and B (Coetzee *et al.*, 2000). Two species within the complex namely *An. gambiae* and *An. arabiensis* are responsible for malaria transmission in Africa (Gillies and Coetzee 1987). *An. funestus* group on the other hand consists of *An. funestus* Giles, *An. rivulorum, An. parensis* Gillies, *An. brucei* Service and *An. leesoni* Evans (Gillies and Coetzee 1987). *An. funestus* which is endophilic and anthropophilic, and *An. rivulorum* are the only members of *An. funestus* group involved in malaria transmission (Cohuet *et al.,* 2004).

2.2 Mosquito behaviour

Behaviour pattern in mosquitoes vary from one species to another but they are all aimed at increasing their chances of survival (Mathews and Mathews, 1978). Consequently, vector behaviour determines its vectorial capacity. Therefore, to control vector populations, it is important to study the behavior patterns which directly or indirectly influence malaria vectors (Klowden, 1996).

2.2.1 Flight behaviour of vector species

Flight is influenced by temperature, humidity, wind velocity and physiological stage of the female (Bidlingmayer, 1964). Species with a tendency for extensive flight activities usually show two different non-oriented dispersal behaviours (Provost, 1953); adrift with the wind or passive migration and an active dispersal using appetitive flight. Dispersal serves mostly to bring the blood-sucking insect into contact with suitable signal from their potential host. It is likely therefore that species which breed in areas where few hosts are available develop stronger tendency for migration than those which breed in the vicinity of their host (Schafer, 1997).

2.2.2 Host-seeking behaviour

In mosquitoes, oogenesis can only be completed when females take blood meal. Therefore, they have developed complex host-seeking behavior to locate potential hosts (Takken, 2004). Foraging and feeding follow circadian rhythm and are maintained by physiological clock within the organism (Takken and Knols, 1999; Clements, 1999; Laarman, 1955). This must first be “set” by an external stimulus, such as change from light to dark, but thereafter maintains the rhythm without further time cues (Takken, 1991; Harker, 1958, 1961). Primarily, location of the host is based on olfactory, visual and thermal stimuli (Takken, 2004). Host-seeking behaviours differ within species depending on season and availability of host (Senior White *et al.*, 1945).

2.2.3 Feeding behaviour of vector species

Feeding behavior of malaria vectors may vary considerably geographically, seasonally, and even locally due to availability of hosts. Females of many mosquito species take a sugar meal obtained from plants before blood feeding. The sugar meal is a source of energy to sustain the host-seeking flight (Takken, 2004). A considerable proportion of *Anopheles* females take blood meal prior to mating (Takken and Knols 1999). There are different feeding patterns among mosquitoes.

Some species such as *Anopheles gambiae* and *An. funestus* which mainly prefer to rest indoors feed almost entirely on humans due to their resting habit and are referred to as anthropophilic (Cohuet, 2004). Other mosquito species especially those with exophagic feeding behavior feed mostly on animals such as cattle and goats found outdoors and are considered to be zoophilic (Takken, 2004) and others readily on both (Kettle, 1990). According to feeding behaviour, mosquitoes are classified either as anthropophilic or zoophilic.

Mosquitoes bite for a restricted period during each 24 hour and those species which will bite throughout the 24 hour bite most readily during one or two limited periods (Cohuet, 2004). Most malaria vectors with anthropophilic habits prefer seeking for a blood meal early in the night and not later than midnight (Mbogo *et al.*, 1995). The biting drive is independent of nectar feeding and is not known to be caused by hunger (Laarman, 1955, 1958). The feeding stages involve activation, orientation, landing and probing. Blood feeding follows a circadian rhythm with most species being nocturnal or crepuscular and smaller number diurnal. Many mosquito species including *An. gambiae* bite in the early hours of the night (Mbogo *et al.*, 1995) while other species bite in the late hours of the night (Muirhead-Thomson, 1951).

2.2.4 Resting behaviour of *Anopheles* mosquitoes

Although only comparatively few mosquito species regularly rest in human and animal habitations, those that do are important vectors of malaria and other diseases (Service, 1976).

Most mosquito species rest exclusively outdoors in natural resting places such as vegetation and only relatively few species rest in man-made shelters (Aniedu, 1993). However, it is usually difficult to find mosquitoes that rest outdoors than those that rest in buildings such as houses and animal quarters (Service, 1976). This is because outdoor populations are widely distributed over large areas. Some mosquitoes prefer resting indoors after feeding indoors (endophilic) while others prefer outdoors (exophilic) (Takken, 2004). Preference of resting site by mosquitoes is determined by feeding behavior of mosquito species and anthropogenic activities in the mosquito resting sites (Takken and Knols 1999). *Anopheles gambiae* and *An. funestus*, the bulk of daytime indoor resting females consist of fed individuals, characteristic associated with indoor resting species (Holstein, 1954; Subra, 1980). Many mosquito species rest amongst grassy and shrubby vegetation and on the foliage of bushes and shrubs, even species such as *An. gambiae* which is regarded as highly endophilic; a certain proportion of the population may be found resting outdoors (Gillies, 1954). These can be collected by slowly walking through vegetation and capturing them in small hand nets as they are disturbed and fly out (McClelland, 1957; McClelland and Weitz, 1963).

2.3 Colonization of breeding habitats by malaria vectors

In mosquito survey, it is important to determine whether permanent ponds or small scattered temporary pools are colonized by the vectors which may eventually contribute to adult population.

Colonization of breeding habitats greatly varies depending on the prevailing environmental condition of a given area (Mutero *et al.,* 2000; Clements, 1999; Sumba *et al.,* 2004). Various ecological traits and behavior of adult anophelines are rooted in differences of their larval habitats (Charlwood, 1985). Temporary pools and other newly formed habitats have been observed to favour certain anopheline species in regions of Guinea and peak populations occur during rainy season when there is maximum colonization of temporary pools and puddles (Haramis, 1985).

2.3.1 Oviposition behaviour of mosquitoes

Mosquitoes select oviposition sites by using visual and chemical cues. Chemical cues originate from water bodies as breakdown of bacterial origin or from mosquitoes as oviposition pheromones (Bentle and Day, 1989). The stimuli are responsible for the aggregation of eggs in sites suitable for egg development (McCall and Cameron, 1995). *Anopheles gambiae* and *An. funestus* mosquitoes are nocturnal in their oviposition activities and the time of oviposition is determined by factors including ambient temperature, light conditions and time the mosquito obtain the blood meal (Clements, 1999). Studies have also shown that *Anopheles gambiae* oviposition time is regulated by light dark cycle and oviposition habitat characteristics (Sumba *et al.*, 2004).

2.3.2 Mosquito breeding habitats

Mosquitoes breed in permanent or any temporary body of water that is present for more than a week. The larvae generally live where the water is shallow, one foot or less (Aditiya *et al.,* 2006). The positive breeding habitats, and their quantitative characters (water depth) and qualitative characters (natural/artificial, permanent/temporary, shady/lighted, water movement, vegetation condition and turbidity), determines presence or absence of different mosquitoes species (Rueda *et al.,* 2006). Relatively few mosquito species actually breed in permanent bodies of water such as marshes or swamps and most of the mosquito species associated with marshes or swamps actually breed in temporary pools along the margins of these habitats (Pemola and Jauhari, 2005). Both quantitative and qualitative characters of the mosquito breeding habitats have contributed to understanding requirements of different larval species of mosquitoes. Different types of habitats, both natural and artificial, nature of vegetation, water movement and water depth are the main characters that explain the observed population among mosquito larvae in a breeding habitat (Almiron and Brewer, 1996).

2.3.2.1 Effect of biotic and abiotic factors on larval mosquito population

The limiting factors in mosquito breeding are the longevity of the aquatic habitat and the duration of the mosquito species' lifecycle (Edillo *et al.,* 2002). In a breeding habitat biotic factors such as predation and availability of food resources also determine population of mosquito larvae Mahesh and Jauhari, (2002b). However, the authors also pointed out that weeds, debris, emergent grasses or some sort of aquatic vegetation shelters the mosquito larvae from fish and other predators thus largely contributing to larval population in a breeding habitat. Abiotic factors such as ambient temperature, humidity and chemical properties of water are ideal in a breeding environment (Edillo *et al.,* 2002). *An. gambiae* s.l. larval densities for instance increase with increasing humidity (Gimning *et al.*, 2001) and prevailing environmental temperature in the area (Minakawa *et al.*, 1999).

2.4 Outdoor resting habitats of malaria vectors

Most malaria vectors rest in natural shelters, such as vegetation, hollow trees, animal burrows, and crevices in ground and only few mosquito species commonly rest in man-made shelters (Takken, 2004). Some mosquitoes which are known to be endophilic such as *Anopheles gambiae* have been collected from some outdoor habitats (Boutin *et al.,* 2005). The change in resting habits of mosquitoes from indoor to outdoor has been attributed to the pressure of human activities such as use of ITN's, mosquito coils, indoor residual spraying (WHO, 2003) and other traditional practices such as use of smoke in the houses (Nahlen *et al.*, 2003). Searching for outdoor resting mosquitoes has usually proved time consuming and unrewarding due to nature of the habitats however, worthwhile numbers of mosquitoes have been caught (Breeland, 1972a, b; Service, 1973; Senior White, 1951). The distribution of resting mosquitoes amongst vegetation may also change during the day so that they avoid sunlight (Senior White *et al.,* 1945; Service, 1971a).

2.5 Exophily of *Anopheles* mosquitoes

The possibility that certain anopheline species prefer moving outdoors after feeding was first suggested by (Muirhead –Thomson, 1951) after he had observed an exodus of females from houses in Dar-es-Salaam on the night they had fed. There were few significant findings of outdoor resting until Iyengar, (1962) discovered exophilic populations in Pemba, a coastal village. Outdoor resting sites were found close to houses and cattle shelters, and most of the females collected were blood-fed, indicating facultative outdoor resting habits. In coastal Tanzania, Bushrod, (1978) could not find any female *An. gambiae s.l*, resting indoors although they were abundant in the human bait catches. It was therefore speculated that in the area, there was a population which preferred resting outdoors and anthropophagic, a phenomenon currently referred to as exo-anthropophily (Coetzee, 2004). Control measures directed towards all the locally potential outdoor resting sites might help reduce the adult population significantly.

2.6 Mosquito control measures

Vector control is one of the methods employed to reduce transmission of many vector borne diseases and is ranked as one of the best methods of protecting a community against malaria (WHO, 1997). Vector control is important if adequate treatment is not available and diagnosis of the disease is difficult. Besides, treatment of malaria is complicated by the spread of strains of *P. falciparum* to commonly used anti-malarial drugs. Thus, the main objective of vector control is the reduction of malaria morbidity and mortality by reducing the levels of transmission. The particular vector control method to be applied in a community depends on the local situation and the preferences of the population (WHO, 1997).

Control strategies adopted towards malaria include use of insecticides (Palchick, 1996), biological control agents (Lacey, 1994) and environmental management (Mitchell, 1996). Of all the vector control approaches employed, chemical control methods have been widely used due to their effectiveness and rapid action.

2.6.1 Larval mosquito control

Control of mosquito larvae involves the use of chemicals and living agents to kill mosquito larvae in aquatic habitats. Larval control such as larviciding is feasible and effective when breeding sites are relatively few in number and easily identified and treated (Bashir *et al.*, 2008). Larval control is a practice which has taken different dimensions with environmental management and larviciding of breeding habitats being practiced in Mwea Kenya (Minakawa, 2002).

2.6.1.1 Environmental management

Since the discovery of the role of *Anopheles* mosquitoes in malaria transmission, malaria control programmes have targeted potential mosquito breeding habitats which have helped to reduce malaria transmission in many parts of the world (Utzinger *et al.,* 2001). Environmental management is a re-emerging vector control strategy in the world (Utzinger *et al.,* 2001). It involves the performance of activities that lead to modification or elimination of aquatic habitats so as to reduce mosquito breeding (WHO, 2003). Two forms of environmental management are recognized.

Environmental modification is long term and may be achieved through alteration of the breeding sites of the vectors by filling the habitats on a permanent basis (Lindsay *et al.,* 2004). Environmental manipulation which is short-term refers to activities that reduce larval breeding sites through temporary changes in the aquatic environment in which larvae develop. Techniques include repeated removal of vegetation from pools and canals (WHO, 1997) and flooding or temporarily draining man-made or natural wetlands and changing water salinity (WHO, 2003).

2.6.1.2 Chemical larvicides

The main aim of larviciding is to eliminate or reduce vector population by killing the immature stages of mosquitoes. A range of chemicals has been successfully used as larvicides against malaria vectors (Grats and Pal, 1998). Heavy petroleum oils have been replaced with lighter products such as monolayer films which may show good efficacy against anophelines under certain conditions (Bashir *et al.*, 2008). Temephos powder exhibited very low mammalian toxicity (Grats and Pal, 1998) and has been used in larval control in several countries including Mauritius (Gopaul, 1995).

2.6.1.3 Biological control of larval mosquitoes

Many organisms help to regulate *Anopheles* mosquito population naturally through predation, parasitism and competition. Biological control is the introduction or manipulation of these organisms to suppress vector population. At present, fish and bacterial pathogens (*Bacillus thuringiensis* var *israelensis* and *Bacillus sphaericus*) that attack larval mosquito stages are employed (Das and Amalraj, 1997).

Several pathogens in the fungal genera *Matarhizium* and *Beauveria* show promise as larvicides (Scholte *et al.,* 2003). Another biological control agent includes the nematode *Romanomermis culicivorax* and aquatic plant *Azolla* (Lacey, 1994). Predatory fish (particularly in the family Cyprinodontidae) that eat mosquito larvae have been used for mosquito control for a long period (Meisch, 1985). The most common fish species used was mosquito fish *Gambusia affinis affinis* (Cyprinodontiformes: Peociliidae). The practice has since been discouraged as the efficacy is highly variable and negative impacts of the fish on native fauna have been quite significant (Scholte *et al.,* 2003).

2.6.2 Control of adult mosquitoes

Current malaria control strategies emphasize domestic protection against adult mosquitoes with insecticides, and improved access to treatment. However, malaria prevention by killing adult mosquitoes is generally favored because moderately reducing mosquito longevity can suppress community level transmission (Killeen and Knols, 2002).

2.6.2.1 Indoor residual spraying

This is the application of long-acting chemical insecticides on walls and eves of houses in order to kill mosquitoes that land and rest on these surfaces. It is expected to 1) reduce the lifespan of mosquitoes so that they cannot transmit the parasite and 2) reduce the number of mosquitoes that enter the house (WHO, 2006).

However, insecticide resistance by mosquitoes hampers the effectiveness of the chemicals used (Nauen, 2007)

2.6.2.2 Personal protection

Since some malaria vectors enter houses to bite and rest, the use of insecticide treated nets (ITN's) for personal protection against *Anopheles* mosquitoes has been shown to significantly reduce morbidity and mortality due to malaria (Nahlen *et al.,* 2003; Zaim and Nakashima, 2000). Some of the personal protection measures include, screening of windows and doors and electrically heated vaporizing mats and have become important in protection against malaria (WHO, 2006).

Insecticide treated bed nets (ITN's) have become an important defence against malaria transmission (WHO, 2006). The initial pyrethroid-treated bed nets had the main drawback that they had to be re-treated every 6 to 12 months. Long lasting insecticidal nets (LLITN's) which could last for 3 to 5 years have since been introduced. The use of ITN's alone has been shown to significantly reduce morbidity and mortality due to malaria (Nahlen *et al.*, 2003). However, the existing widespread use of bed nets and indoor residual spraying is expected to enhance insecticide resistance and these calls for more approaches in protection against the vectors. (Nauen, 2007; WHO, 2006).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study site

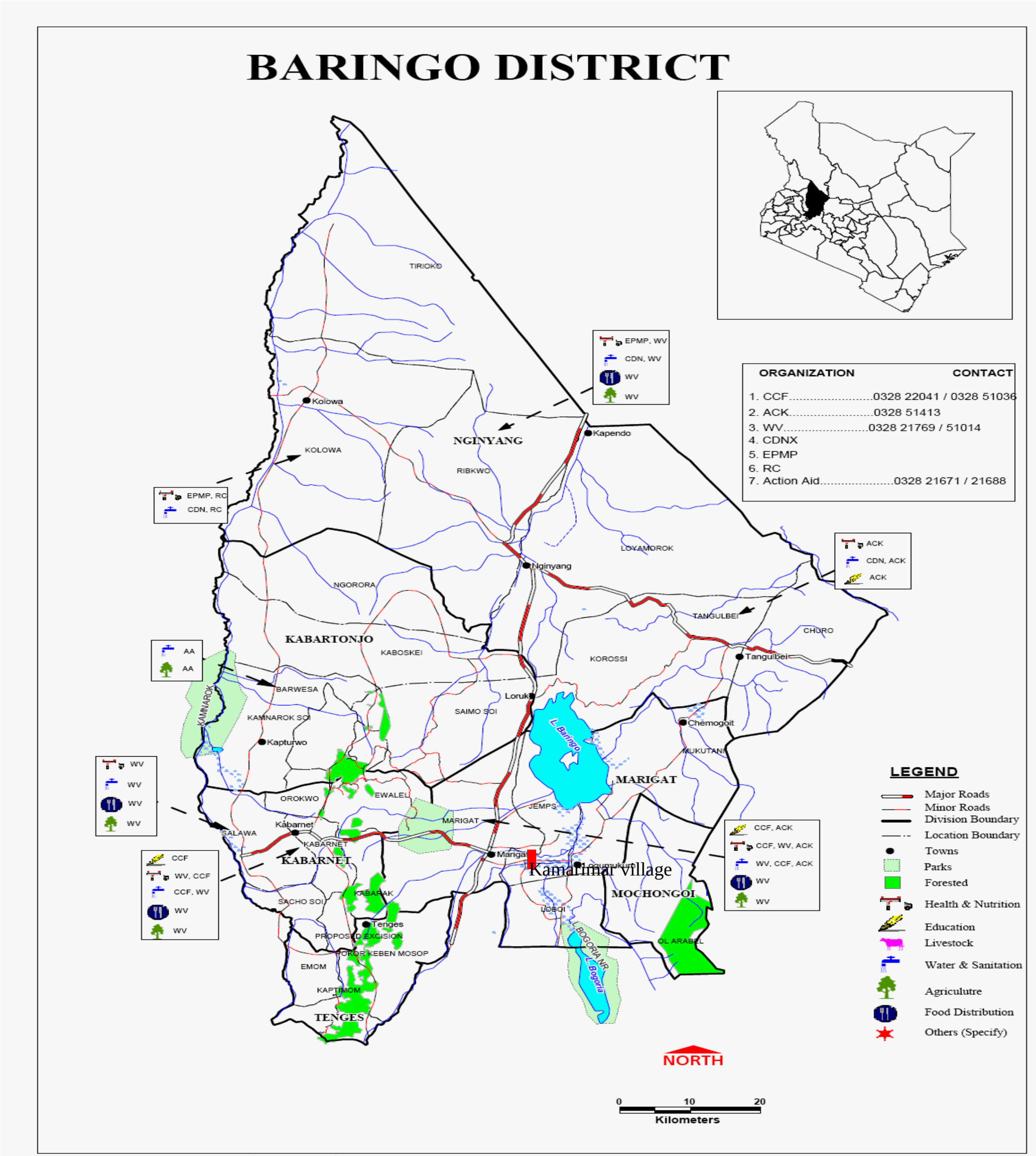
This study was conducted in the Kamarimar village in a semi-arid region of Baringo District situated in the Rift Valley Province of Kenya (Fig. 1). The District is approximately 250 km North West of Nairobi (045ºN, 36ºE). It is divided into three agro-ecological zones namely; the highlands, midlands and lowlands.

The highlands have an altitude of 1,815 meters above sea level and located on the eastern edge of the Kerio Valley. Views include east over the Rift Valley towards Lake Baringo and Lake Bogoria, and west to the Elgeyo escarpment and the Kerio Valley. The rainfall is about 50% reliable. It varies from 1000 to 1500mm in the highlands. The region has different agro-ecological zones necessitating different agricultural activities. Major cropping activities are concentrated in the highland areas, which have adequate rainfall. The midlands have an altitude of 1,030 meters above sea level with an average annual rainfall of 960mm. Minimal cropping activities take place in this area and most families engage in livestock rearing as a way of generating income through sale of milk and other livestock commodities.

The lowlands in the district have an average altitude of about 700 meters above sea-level and most of it is rangeland. The region experiences short rains between May and October followed by long hot dry season between December and March. The annual rainfall varies from 400mm to 600mm with an average of 550mm per year.

Temperatures in this zone are above 32°C. The village has a population of a bout 200 inhabitants. The people live in compounds comprising 2-3 houses. Most of the inhabitants of this village live in traditional houses with mud walls and roofs of corrugated iron while others dwell in mud walled and grass thatched houses. The main activity of the inhabitants is livestock rearing; goats, cows, sheep, and chicken are bred in the village. Crop production is not feasible; however, the land around the swamp is used for growing crops such as onions, tomatoes and cabbages in small plots fed by drainage canals from the swamp.

The study was conducted in the drier lowland regions of the district where malaria is the leading cause of morbidity. This is due to frequent flooding during the rainy season creating favourable breeding ground for malaria vectors and presence of some permanent mosquito breeding habitats in the area. The region also has unreliable communication and transport network making accessibility to the nearest health facility difficult.



The boundaries and names shown on this map do not imply official endorsement or acceptance by the United Nations

Prepared by OCHA June 2001

Figure 1: Map of Kenya showing location of the study site in Baringo District

3.2 Monitoring adult mosquito population

The collection of mosquitoes in the rainy and dry seasons was done once a week indoors by Pyrethrum Spray Collection (PSC) and once a fortnight outdoors by use of aspirators, sweep and drop nets. Mosquito collection was conducted in 10 houses in the village. The typical house types in the study area included grass thatched roof and mud walled house (Plate 1) and iron sheet roofed and wooden wall house (Plate 2). The two house types dominated in the community which could be attributed to the economic status of the inhabitants of this village. The houses were randomly selected based on their location as either in the center or periphery of the village. Outdoor collection was conducted from vegetation, hideouts around stores and culverts.

Plate 1: A typical grass thatched roof and mud walled house

Plate 2: A photograph showing iron sheet roofed house and wooden wall house

3.2.1 Pyrethrum spray collection (PSC)

Sampling for mosquitoes resting indoors was carried out using Pyrethrum Spray Collection (PSC) procedure as described by WHO, (1975 and 1997). Sampling from the selected households started at 0600hrs to 0900hrs. This was done to estimate the density and composition of daytime indoor resting malaria vectors in relation to proximity of the house to a breeding site. Ten houses in the village were randomly selected for mosquito collection. During the sampling activity, all occupants, animals and easily removable objects were first removed from the house to be sprayed. White calico sheets were laid over entire floor, over beds, and other furniture not removed. All doors and windows were closed and hut space was sprayed with 3% pyrethrum diluted in one litre of water (Plate 3). The insecticide used had a weak persistence, had no human toxicity under normal condition of use as an indoor spray. The treated houses were ready to be used approximately 20 minutes after spraying. The spray was first directed at all potential escape roots such as eaves, closed doors and windows, thereafter aimed at the roof or ceiling.

To reduce the number of mosquitoes escaping through eaves that existed in some huts between top of walls and roofs, spraying of these eaves was conducted.

Plate 3: Pyrethrum spray collection of mosquitoes

After spraying, the house was closed for 15 minutes before collection of the knocked down mosquitoes. During the collection, the contents of the sheet were carefully handled and transferred onto a single sheet from all the sheets spread during spraying; the mosquitoes were collected using forceps and placed into mosquito vials. The sheets were routinely cleaned once in a fortnight. The total number of *An. gambiae, An. pharoensis* and *An. funestus* collected identification numbers, method of collection, type of the house and the number of people sleeping in the houses were recorded on data sheets. The abundance of each species between the seasons was recorded during the study.

3.2.2 Sampling of outdoor resting vectors

Outdoor resting habitats utilized by vectors were identified and sampled by active search once a fortnight. Adult mosquitoes were collected from traditional stores using battery powered aspirator and torch for 15 min per site selected at random. Natural outdoor resting places such as vegetation were sampled on a timed basis. Shelters not used as houses, vegetations around human habitation, culverts and other natural resting surfaces were sampled by aspirators and hand nets using method described by Arunachalam, (2004). The sites were given specific identifiers, their characteristic described and distance from the nearest house recorded in meters using a ribbon plastic tape measure. The collected mosquitoes were used to assess the abundance of vectors in outdoor natural resting sites and preferred outdoor resting sites.

3.2.2.1 Searching of mosquitoes from vegetation

Outdoor day resting mosquitoes were collected during dusk between 1700 and 1800 hrs by a drop net (total three attempts) from the ground level vegetation and small bushes surrounding human dwellings and cattle sheds in the village. The drop-net measured 2m x 2m x 2 m high (Mutero *et al.*, 1982). Using loops of cloth attached to all corners, two people suspended the net on four 2-m poles fixed at selected sampling sites. The enclosed shrub was disturbed and the escaping mosquitoes were collected within the net using battery-powered aspirators for 15 minutes.

3.3 Mosquito handling and processing

All the *Anopheles* species captured by PSC and aspiration were first grouped by sex and the male *Anopheles* mosquitoes counted, the numbers recorded and thereafter disposed. The female *Anopheles* were classified into species by sorting them according to the morphological identification keys using specules on wings and legs as well as body colour (Gillies and De Meillon, 1968; Gillies and Coetzee, 1978). They were further analyzed and categorized by their abdominal condition as unfed, fed, semi-gravid or gravid and specimens stored in vials containing desiccant dry rite crystals and labels of the date of collection, house number and village from which they were collected stuck on the vial. Samples were then transported to the Division of Vector Borne Diseases (DVBD) laboratory in Marigat Division for further analysis.

3.4 Identification and characterization of the potential *Anopheles* breeding habitats

Identification and geo-location of larval habitats and houses for indoor spray collection was done in the village from May to July 2008. The study area covered a radius of 2 km inclusive of the entire village and the swamp region. Mapping of existing and potential larval habitats in the study village was done using geographical positioning system (GPS) Garmin 12/12XL Model. Location of the house and mapping of the larval habitats was carried out with the assistance of local guides and inhabitants who had knowledge of the area. Stagnant waters were identified by traversing the study area on foot. This technique especially relied on information from inhabitants when locating the more remote habitats, which may have been under-represented in the survey. Once identified, habitats were evaluated for presence or absence of mosquito larvae using standard aquatic dippers. Villagers were questioned about their awareness of open water bodies around the village including those which persisted into the dry season. Identification was assigned to all breeding habitats according to location, and types as pools, marsh, pan dam, ditch and canals. The geographic coordinates for all identified larval habitats were recorded using a global positioning system to ascertain the distance recorded between the habitats and houses. All identified water habitats were assessed for the presence of larvae. During weekly survey, information was recorded on the characteristics of water bodies including type of larval habitat, presence or absence of vegetation, water turbidity, presence of arthropods and productivity of the habitats rated by presence or absence of mosquito larvae. The larvae were morphologically identified as described by Gillies and Coetzee, (1987). Mesopleural basal hook was used for identification. Presence of large curved and sharply pointed basal spine of pleural hairs and poorly developed inner shoulder hairs were used to distinguish the larvae of *An. gambiae* complex from other anophelines.

3.4.1 Colonization of breeding habitats by *Anopheles* mosquitoes

During rainy season temporary and permanent larval habitats colonized by mosquitoes were identified and sampled once weekly for ten months. These larval habitats included temporary pools of water, canals, ditches, marshes, hoof prints and pan dams within the village. The types, distance of the habitats from the house and habitat characteristics were recorded. Interaction between seasons, larval counts and habitats were examined. Seasonal abundance of species in these habitats was determined and preferred habitats by anopheline vectors in dry and wet season identified.

3.4.2 Collection of mosquito larvae from breeding habitats

Larval collection was carried out to establish colonization of breeding habitats in this semi-arid ecosystem. Standard aquatic dippers were used for the collection of mosquito larvae that occurred in flooded pan dams (Plate 4), canals (Plate 5), and culverts (Plate 6) within the study area. During larval collection a dipper was used to make five to ten dips depending on the size of the larval habitat. Dipping was performed around the perimeter of the habitat, at approximately one meter intervals. It was however, not done more than one meter into the breeding habitats, which may have led to an under representation of less abundant species. It also considered the fact that the larval stages prefer the edges of the habitats to deep sites. The dipper was lowered at 45° until one side was just below water (WHO, 2003). Larvae collected from ten dips were transferred in a sieve which released water of collection and left behind the larvae and predators. The collection was thereafter cleaned by passing clean water through the sieve and the contents transferred to a white tray. Larvae were then searched for and identified in the tray by eye observation. The larvae below or on the surface of water in the tray were picked by a pipette and transferred to collecting vials labeled for habitat, date and location for identification and quantification.

The dippers and tray were white in colour for efficient detection of larvae and predators present in the samples collected. The predators were however, not identified.

For consistency, habitats of the same category were subjected to same treatment in reference to the number of dips performed. The larvae collected were examined and categorized according to their developmental stage, counted and numbers recorded on appropriate data forms while in the field. The third and fourth instar larvae were preserved in vials containing 70% ethanol. The vials were put in a cool box cushioned with cotton wool and transported to the Division of Vector Borne Diseases laboratory in Marigat for microscopic examination and identification. The pupae were kept in whirl packs with sufficient clean water placed in cool box and taken to the laboratory where they were transferred into emergence cages then reared till adult emerges for confirmation or identification of species. The main types of the breeding habitats in the study area included pan dams and culverts which were shallow and temporary water bodies and lasted for approximately three to four weeks after the rains. Drainage canals were permanent and turbid water bodies without vegetation cover.

Plate 4: A flooded pan dam during the rainy season

Plates 5: A drainage canal; sampled mosquito breeding habitat

Plate 6: A culvert; sampled mosquito breeding habitat

3.5 Data processing and management

Statistical analysis of the collected data was carried out using Statistical Analysis System (SAS – Version 9.2 statistical package) (SAS Institute Inc, 1997). Regression analysis was used to evaluate the effect of distance between a house and a breeding habitat on transformed mosquito populations in ten houses of the Kamarimar village. Fitted Poisson regression was used to evaluate the effect of seasons, habitats and their interaction on the larval count on seven different larval habitats sampled. The key factors included in the model included were type of habitats and season. The chi-square test was used to compare seasonal differences in larval density. Poisson was used to evaluate the outdoor habitats in terms of mosquito population numbers using PROC GENMOD.

3.6 Ethical consideration and clearance

The study involved the use of pyrethrum spray for collection of mosquitoes in households. Ethical clearance was sought from the Ministry of Health and Kenyatta University. Signed consent was also sought from community participants.

CHAPTER FOUR: RESULTS

4.1 Proportions of *Anopheles* mosquitoes in Kamarimar village in Baringo District

Four *Anopheles* species were captured resting indoors namely, *Anopheles gambiae s.l*, *An. funestus*, *An. pharoensis* and *An. coustani* over a period of twelve months. Of the four species, only *An. gambiae* and *An. funestus* were malaria vectors collected and had been incriminated in the intermittent transmission of malaria parasite in the same area by Aniedu (1993). *An. gambiae* was the most abundant vector compared to *An. funestus* and they constituted 46.9% (1166) and 2.5% (63) of the total *Anopheles* collected respectively. Other anopheline species were composed of *An. pharoensis*, 50.2% (1248) whereas *An. coustani* only constituted 0.4% (11) (Table1).

Table 1: The proportions of *Anopheles* species in Kamarimar village of Baringo District

Anopheles sp.	Total (n)	Adults (%)
An. gambiae	1166	46.9
An. funestus	63	2.5
An. pharoensis	1248	50.2
An. coustani	11	0.4
Total	**2488**	**100**

n is the total number of *Anopheles* mosquitoes collected indoors during the study.

4.2 Effect of distance between a house and a breeding habitat on mosquito abundance

The effect of distance between a house and a breeding habitat on mosquitoes resting indoors revealed a significant variation between the distance of a house and abundance of the mosquitoes collected indoors (Table 2). The monthly mean of *Anopheles* mosquitoes in relation to distance was significantly higher (r =5.48, p= 0.026). The regression results indicated that houses closer to a breeding habitat had higher numbers of mosquitoes resting indoors and over 60% of mosquitoes collected were from these houses. The regression coefficient for distance was negative implying that increase in the distance between a house and a breeding site reduced the abundance of mosquitoes inside the houses.

Table 2: Regression coefficient and standard error of the relationship between distance of a house from a breeding habitat and mosquito population

Parameter	Estimate	Standard error	T – value	P > | t |
Intercept	3.639	0.682	5.34	<.0001
Distance	-0.003	0.0013	-2.34	0.0259

Other factors that may have a bearing on mosquito population resting indoors such as, cooking, number of insecticide treated nets in the house and house type may have contributed to the reduced number of the vectors resting indoors since the R square for the model in relation to effect of distance between a house and a breeding habitat on mosquito abundance was 15% as revealed in this study.

4.3 Seasonal variation in monthly numbers of *Anopheles* mosquitoes

A study of seasonal population dynamics of the malaria vectors revealed abundance of mosquitoes in the rainy months between August 2008 and July 2009. Altogether 2019 *Anopheles* mosquitoes were collected in the rainy season. However, the population of *An. funestus, An. gambiae* and *An. pharoensis* decreased to 463 between December and April coinciding with the dry season. After the dry period, there were short rains in the months of May and June in which 229 mosquitoes were collected. Mosquitoes were most abundant in the rainy season during the months of August, September and October after which the population declined at the onset of the dry season in December (Figure 2). In spite of weekly rounds of residual spraying, densities of *Anopheles* mosquitoes were observed to be higher in August, September and October compared to November - February, which could be attributed to seasonal variations.

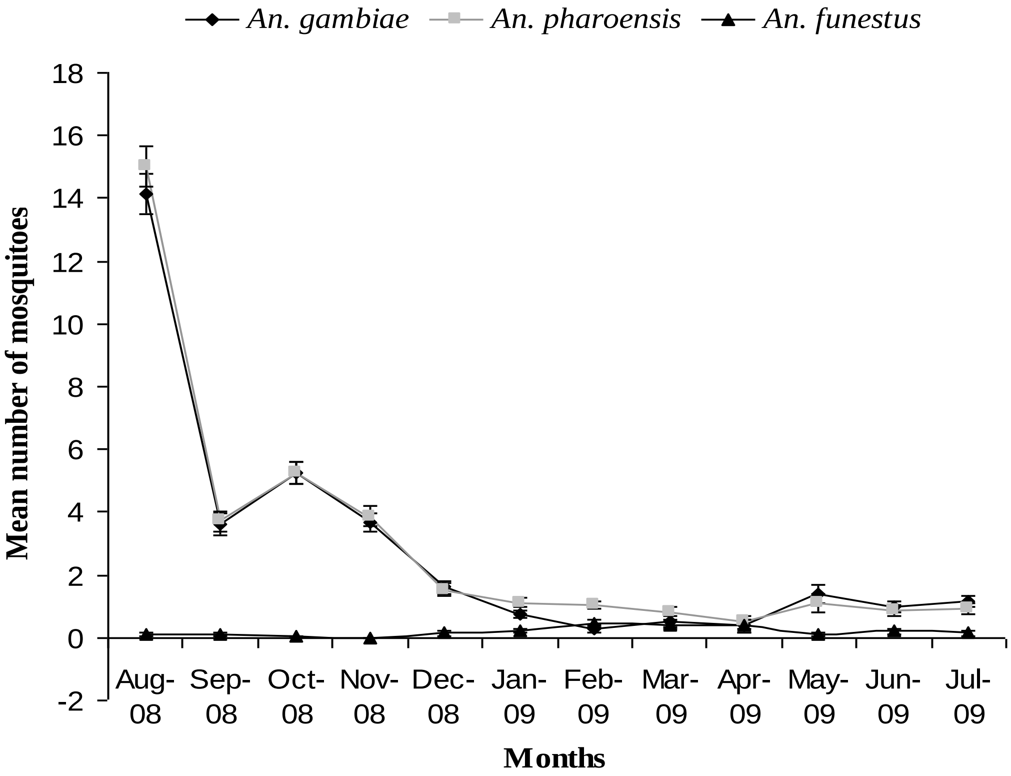


Figure 2: Monthly abundance of *An. gambiae, An. funestus* and *An. pharoensis*

On the contrary, the abundance of *An. funestus* was observed between December and April which corresponded with the dry season. *An. funestus* population was rarely encountered in the rainy season and constituted less than < 5%. February which was peak of the dry season recorded the highest population mean of 0.5 mosquitoes. The proportion of *An. funestus* was high above 80% in the dry season (Figure 3). However, the abundance of this vector gradually declined during early rainy season in May when some sporadic rains were experienced.

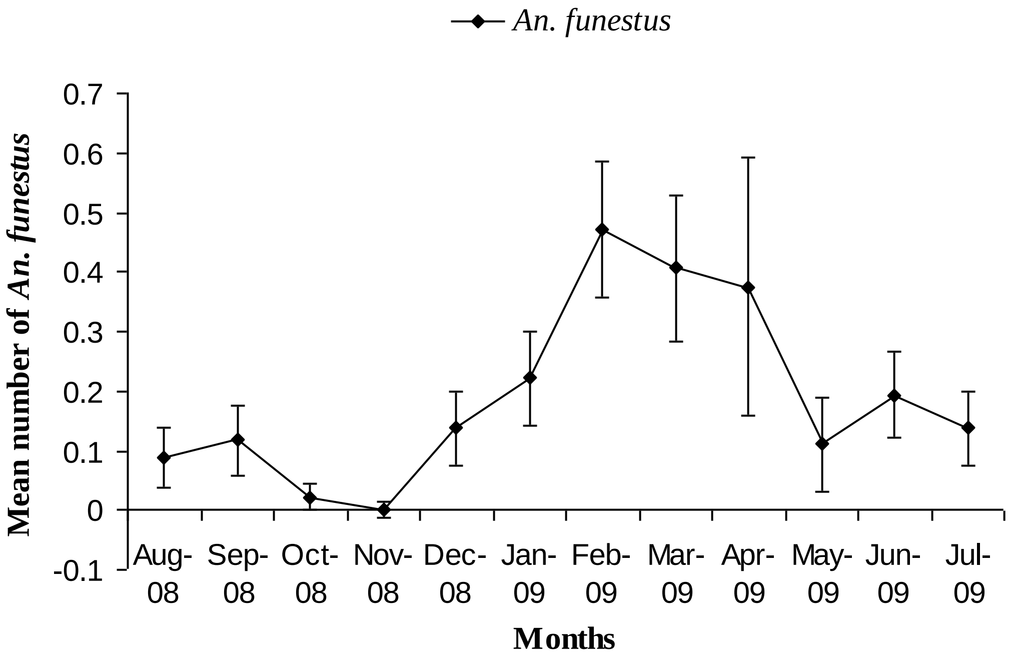


Figure 3: Mean monthly numbers of *An. funestus* during the rainy and dry seasons

During the rainy season, 1790 of *An. gambiae* and *An. pharoensis* mosquitoes were collected in the months of August, September, October and November of the year 2008. About 20 *An. funestus* were collected during early November and they were more abundant during the months of January, February and March respectively which coincided with the dry season. *An. coustani* was captured in late November at the onset of the dry season but recorded very low counts of about 0.4% and were therefore ignored in the study.

4.4 Colonization of the breeding habitats of *Anopheles i*n Kamarimar village

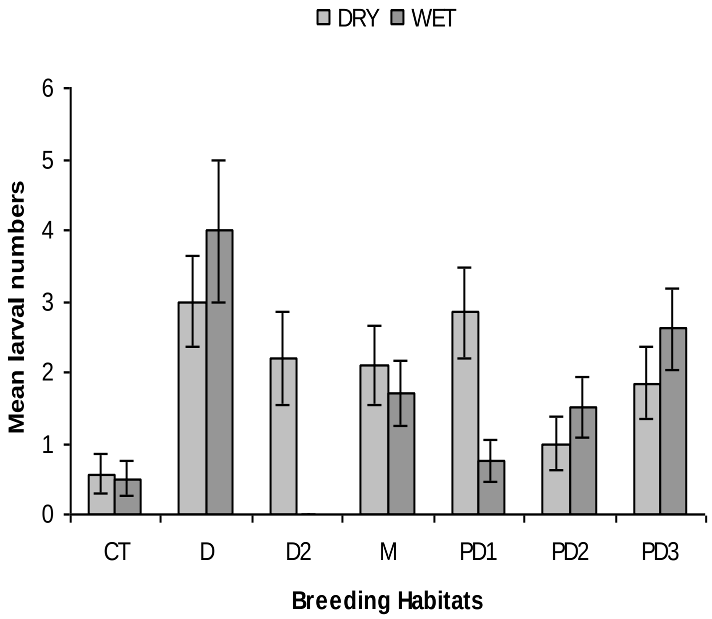
The larval habitats were identified and characterized in two ecological settings of the study area namely Tirion and Kamarimar village adjacent to the permanent Loboi swamp. The habitats belonged to five major types comprising marshes, pan dams, canals, ditch, and culverts. Colonization of the habitats by mosquito larvae was highly variable between the seasons. Similarly the abundance of anopheline larvae greatly varied in the habitats between the rainy and dry seasons. Comparing the larval abundance, the composition of the larval catches was dominated by *Anopheles pharoensis* followed by *An. gambiae* in rainy season. The same trend of abundance was observed in dry season but with reduced numbers.

Ditches were readily colonized than other habitats and had the highest monthly mean of 4.0 and 3.0 larvae during the rainy and dry seasons respectively. Culverts and marshes were mostly colonized in the dry season. The colonization of pan dams took place in both the dry and rainy seasons. Quantity, transparency of water and vegetation cover determined colonization at any time of the season.

Density of the third and fourth instar larval stages of malaria vectors revealed seasonal variation in habitat preference in the study area (Figure 4). Culvert was the least preferred breeding habitat and had lowest mean of 0.57 and 0.50 larvae in the dry and wet seasons respectively. Ditches had the highest mean of 4.0 larvae during the wet and 3.0 larvae in the dry seasons. Ditches were temporary, lasted for a month and were the most readily colonized mosquito habitats. Clear water, high vegetation cover

(80%), non-interference from grazing animals justified its high productivity. However, similar habitat D2 formed along a riverbed was only productive during the early dry season and had mean of 2.20 mosquito larvae. The habitat had no mosquito larvae during the wet season which may have been due to persistent overflows from River Chepkornis which was highly seasonal and presumably made it unsuitable for colonization during the wet season.

Marshes recorded high mean larval numbers of 2.10 larvae during the dry season compared to the rainy season (1.70), an indication that it was a potential breeding site in the absence of rain dependent breeding habitats. Marshes received constant water supply from the Loboi swamp which is a permanent water source in the study area. Pan dams had variable mean densities between the seasons. PD2 and PD3 had higher mean larval numbers in the wet season compared to the dry season (1.50 and 2.62 larvae) respectively. The means were low in rainy season at (1.00 and 1.85 larvae) implying that the two habitats were readily colonized by the vectors during the rainy season. Mean larval numbers during the dry season in PD1 was 2.85 larvae which was higher than 0.75 larvae in the wet season. The duration taken by a temporary habitat, its location, plant cover >50% and minimal animal interference contributed to larvae collected from the habitats in both seasons.



Key:
CT- Culvert
D- Ditch
M- Marsh
PD-Pandam

Figure 4: Seasonal mean numbers of mosquito larvae in breeding habitats

4.4.1 Effects of seasons and habitats on mosquito larvae population

During the dry season, the only potential breeding sites were marshes around the swamp and pan dams which recorded a low number of *Anopheles* larvae. At the onset of the rainy season additional habitats were mainly ditches and pools. Ditches and pools were found to be highly colonized by anopheline larvae but lasted for a period of four weeks after the rains which attested their dependence on rainfall.

Fitted Poisson was used to evaluate the effect of seasons, habitats and their interaction on larval numbers in different larval habitats sampled. Interaction between season and habitat evidently created variation in larval numbers chi- square value $\chi^2 = 12.6$, p=0.027 (Table 3).

This demonstrated that both season and habitat types significantly determined the extent to which larval mosquitoes colonize particular breeding grounds at any given time of either the rainy or dry season.

Table 3: Effect of interaction between season and habitats on population of mosquito larvae

Change	df	Deviance	Mean deviance	Deviance ratio	Approx Chi pr
Season	1	0.971	0.971	0.97	0.325
Habitat	6	34.498	5.750	5.75	<.001
Season. Habitat	5	12.598	2.520	2.52	0.027
Residual	78	241.080	3.091		
Total	**90**	**289.147**	**3.213**		

During late the dry season, *An. pharoensis* was the most common *Anopheles* species in most habitats totaling 48% of larval catches followed by *An. gambiae* which was relatively abundant and constituted up to 40% of the collections. *An. funestus* was common mainly in the swamp marshes; they were mainly detected during the dry seasons and constituted only 12 % of the total larval collection. Only 80 *Anopheles* larvae were collected before the habitats dried up in the peak dry season.

Nearly all larval habitats were human-made and rain-dependent except the unproductive canal draining from the swamp to small scale farms in the village. The dry season therefore experienced low number of adult mosquitoes collected in the houses due to the reduced breeding sites. The seasonal patterns in the area revealed that the dry season typically lasted from late November through January to late March. However, there were sporadic rainfalls not lasting two days in March.

4.5 Outdoor resting habitats of *Anopheles* mosquitoes in Kamarimar village

Outdoor resting habits of mosquitoes were detected in the Kamarimar village. Outdoor resting population of mosquitoes was low in January through February which coincided with the dry season and again in November and December. *An. gambiae* was most numerous around traditional stores compared to *An. pharoensis* which predominated in vegetation. *An. funestus* was rarely captured outdoors especially in vegetation. In the dry season however, some were caught resting in store hideouts with the high population in February after which it declined later with the onset of the rainy season.

Altogether 115 mosquitoes were collected from vegetation, culverts, and hideouts round traditional stores. The collected mosquitoes comprised *An. gambiae* complex 42 (36.5%) *An. pharoensis* 49 (42.6%) and *An. funestus* 24 (20.9%).Vegetation and hideouts around traditional stores were highly productive with up to 100 anophelines of the total collection being obtained in these habitats. There were differences in mosquito population between the habitats studied, (χ^2 =29.87, df =2, $p < 0.0001$).

Vegetation had the highest mean of 1.270 mosquitoes followed by hideouts around stores at 0.812 mosquitoes and culverts least mean of 0.312 mosquitoes showing that these mosquitoes had higher preference for vegetation as outdoor resting habitats than culverts and hideouts around stores (Table 4).

Table 4: Monthly mean and standard error of mosquito numbers in the outdoor habitats

Habitat type	Numbers of mosquitoes	Mean Numbers	Standard error
Culvert	13	0.3125	0.0803
Stores	42	0.8125	0.1300
Vegetation	58	1.2708	0.1626

The deviation reflected on the mean number of mosquitoes in the habitats by the standard error significantly demonstrates the relative preference of outdoor habitats such as vegetation based on the number and mean of mosquitoes collected. Low standard error is associated with low mean which is a reflection of the low number of mosquitoes collected from a given habitat. Shrubs around human dwellings and cattle sheds were collectively categorized as vegetation. They were the most favoured resting sites and many *An. gambiae* and *An. pharoensis* were caught from such outdoor sites followed by the hideouts around traditional stores while culverts had the least abundance of mosquitoes collected outdoors during the study period.

4.5.1 Abundance of *Anopheles* species resting outdoors in Kamarimar village

Evaluation of the outdoor resting behaviour by three anopheline mosquitoes collected revealed that *An. pharoensis* was the most abundant outdoors compared to *An. gambiae* and *An. funestus* (Figure 6). *Anopheles gambiae* complex constituted 36% (n= 42) of the mosquitoes collected and identified from outdoor shelters, *An. funestus* constituted 20% (n= 24) while *An. pharoensis* represented 42% (n= 49). It should however, be noted that the indoor and outdoor collections represented two separate populations collected using different methods. The indoor populations of *Anopheles gambiae* complex were significantly higher (n=1166) compared to outdoor population (n=42). *An. gambiae* caught outdoors were from drop-net collections compared to aspiration from hideouts around storage structures. More than 50% of *An. pharoensis* collected outdoors using drop-net were mainly from vegetation and culverts while 20% of *Anopheles funestus* were collected from hideouts around stores and they were rarely encountered in vegetation. Abundance of the mosquitoes outdoors coincided with the rainy season in which 69.5% (n= 80) was recorded compared to 29.5% (n= 35) collected outdoors during dry season.

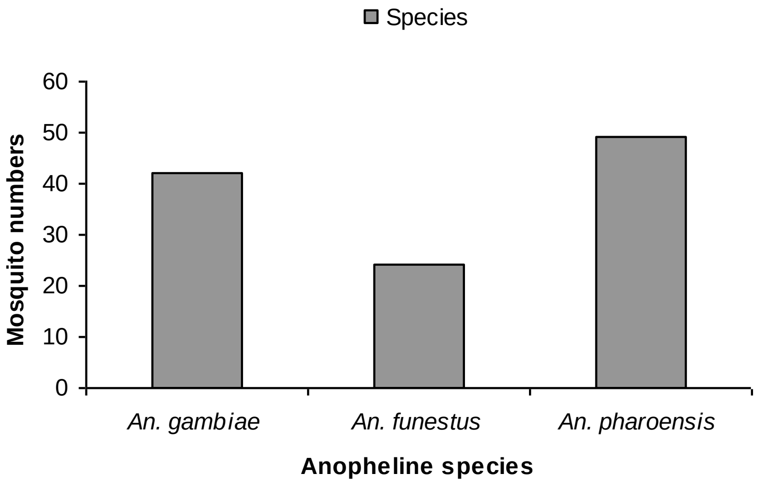


Figure 5: Abundance of *Anopheles* species resting outdoors in Kamarimar village

4.5.2 Comparison between outdoor resting habitats of mosquitoes

Significant differences in mosquito resting preference were observed between all the habitats. The significance of the contrasts estimates are presented in (Table 5). A comparison between culverts and vegetation was highly significant with p-value = 0.0001 demonstrating that most anophelines preferred vegetation as resting habitats Mean = 1.27 to culverts Mean = 0.31. Comparison between culverts and stores p = 0.0017 followed suggesting that stores Mean = 0.8, were the most preferred to culverts. Contrast between vegetation and stores was the least significant at p =0.0291 suggesting that these habitats shared considerably equal preference.

Table 5: Contrast estimate results between the outdoor resting sites

Habitats	Estimate of Mosquitoes	Error	Chi-Alpha	Confidence Limits		Chi-Square	P- value
Culverts vs Stores	0.9555	0.3038	0.05	0.3600	1.5510	9.89	0.0017
Culverts vs Vegetation	1.4028	0.2882	0.05	0.8380	1.9677	23.69	<.0001
Vegetation vs Stores	0.4473	0.2050	0.05	0.0455	0.8492	4.76	0.0291

The results above shows significant difference between the paired mean numbers and p-values of mosquitoes collected from different habitats. This demonstrates how mosquitoes may prefer one habitat over the other when the two are compared. Outdoor resting vectors were mostly abundant in vegetation around human dwellings which contributed 51% of the outdoor collection. This was followed by stores at 37% and culverts 11% as scored from the number of mosquitoes collected from each habitat. Percentage composition of *An. gambiae*, and *An. pharoensis* revealed that their distribution among outdoor and indoor shelters was nearly equal.

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

This study presents significant differences in the proportions of *An. gambiae* and *An. funestus* mosquitoes between rainy and dry seasons suggesting that seasonal variation influenced abundance and distribution of malaria vectors in the semi-arid ecosystem of Baringo District. This observation is most likely associated with the preference of breeding habitat by the vectors which in most cases depend on seasons. Studies conducted by Aniedu in Kerio Valley Kenya, (1993) described abundance of mosquitoes but with little emphasis on breeding habitats and their significance on the abundance of adult mosquitoes. The study also revealed that some anopheline species never showed significant abundance for comparative studies. *An. coustani* for instance has been considered incidental vector in other parts of Africa (Gillies and De Meillon, 1968). However, the low numbers of these species observed in the study area shows that they may be unimportant in malaria transmission.

Distance between a house and larval habitat was significantly associated with the abundance of malaria vectors resting in the houses (p =0.026). This finding was consistent with that reported in studies conducted by Utzinger *et al.* (2001) on factors influencing abundance of mosquitoes resting indoors in the republic of Eritrea. There was presence and abundance of *Anopheles* mosquitoes in houses less than 500 meters from larval habitats which contributed more than 80% of mosquitoes collected from the village suggesting that most anopheline mosquitoes probably fed and rested in houses closer to breeding habitats. This is in agreement with work of Manga *et al.* (1993) on survey of mosquito resting preference in the republic of Ghana. *Anopheles gambiae, An. funestus* and *An. pharoensis* contributed a significant proportion of mosquitoes collected indoors. However, the abundance of some of these vectors such as *An. funestus* was found restricted to dry season while others were present in both seasons. During the study, it was observed that *An. gambiae* rested in the thatch, dark corners and cracks of the houses, but also in relatively exposed situations on damp as in river banks and culverts along the highly seasonal River Chepkornis which flows through the village during rainy season. Bulk of day time indoor resting females of *An. gambiae s. l.* and *An. funestus* in Kamarimar consisted of fed individuals and according to Killeen and Knolls, (2002) they mainly feed on human blood due to their anthropophagic behaviour which is characteristic commonly associated with indoor resting species, this is in agreement with findings obtained by Holstein, (1954) and Subra, (1980).

The two species were the main vectors of malaria detected in the area and made up 49% of the indoor resting mosquitoes. Other vectors were dominated by *An. pharoensis* 50% of the total *Anopheles* mosquitoes while composition of the rest of *Anopheles* species was just about 1%. The mosquitoes caught indoors indicated unexpectedly low number of gravid females relative to freshly fed ones.

This suggested that some indoor resting females left the house during the period between feeding and becoming gravid for either outdoor resting or breeding habitats. This subsequently influenced the abundance of mosquitoes resting indoors. Similar observation was also made by Subra in Kumar India, (1980) while studying resting behaviour of mosquitoes in relation to their physiology. Distribution and abundance of indoor resting mosquitoes could also be due to the pressure human activities in the house which may have expelled the gravid female mosquitoes to other habitats. Generally, male *Anopheles* mosquitoes were rarely caught indoors. Unfed females constituted above 45% of indoor resting mosquitoes. However, percentage of fed females found outdoors was <5%. Preference of indoors for resting by fed mosquitoes over outdoors could be attributed to anthropogenic activities outdoors, humidity and environmental temperature which could lead to dehydration of fed mosquitoes and unfavourable conditions for the development of eggs. This is supported by the findings of Killeen and Knolls (2002) in Brazil during their study of ecology of malaria vectors. The numbers of fully fed females were more than semi gravid and gravid females resting indoors.

During the rainy and dry season, significant differences were observed in the population dynamics of *An. gambiae*, *An. pharoensis* and *An. funestus*. These variations in proportions between seasons suggested that there was seasonal preference by the vector species which subsequently affected their distribution in the region.

These differences could be ascribed to breeding habitat preference by anopheline mosquitoes, their availability, quality and nature of the habitats which are determined by the seasons as was reported by Ijumba *et al.* 1990 in Mwea Tabere, Kenya. The present findings documented the highest numbers of *An. pharoensis* in most habitats sampled for larvae and adults collected indoors and outdoors revealing its dominance in either season. This result supports the findings by Gillies and De Meillon, (1968) in a research conducted in the Ethiopian region. These authors documented that, in the absence of the availability of breeding habitats during the dry season, *An. pharoensis* would be expected to have a competitive advantage on habitat colonization over *An. gambiae* as former species would utilize a broad range of breeding habitats.

Few adult mosquitoes were collected during the dry season and it was not possible to get samples from all the sampled houses in the village. This was due to the distance of the house to the nearest available breeding habitats which determined the indoor abundance of mosquitoes in either season. This saw the houses far from a breeding habitat consistently recording no mosquitoes during the survey. Comparison of the proportions of *An. gambiae* and *An. pharoensis* in the dry and rainy season revealed that both species were equally abundant in the rainy season compared to the dry season which could be attributed to increased breeding habitats as opposed to dry season when most habitats dried out. *An. funestus* remained closely associated with houses in the dry season suggesting that it was the most important dry season malaria vector in this semi-arid ecosystem.

The vector survived at low density probably as hidden refugia populations before the dry seasons when their population increased considerably. Presence of *An. gambiae* was evident in both seasons but greatly reduced in the dry season. *An. pharoensis* though not a vector of malaria at present, was studied and its population dynamics monitored due to its abundance. Both *An. pharoensis* and *An. gambiae* recorded high population in the wet season and this may be mainly attributed to the diversity of the breeding habitats in the rainy season. The diversity of breeding habitats in the rainy season and persistence in the dry season is an important aspect in managing the population of malaria vectors by focusing on their sources in either season in the semi-arid regions (Fillinger, *et al.*, 2004). Utilization of the habitat as a control measure in either rainy or dry season was also demonstrated by Kitron and Spielman, (1989) as a source reduction approach for handling malaria vectors. The range of species recorded in the current study reflected variety and complexity of larval habitats that may exist between the seasons. The results revealed differences in seasonal dominance by different species p =0.027 and this could be associated with the effects of environmental and meteorological conditions, principally temperature ranges and rainfall, in creating or diminishing larval habitats. This supports the findings by Girardin *et al.* (2004) in the republic of Tanzania.

Canals were not positive for larvae throughout the dry and rainy season in spite of their persistence. This may have been due to their turbidity, water movement characteristics and continuous interruption by human activities. Three months after the rainy season a considerable number of the major larval habitats in the area dried up and sampling continued only in the canals from the swamp which remained uncolonized by anopheline larvae. Observation of high proportion of mosquito larval habitats in August and September and the low proportion in January and February defined the rainy and dry seasons.

During the dry season all the larval habitats were almost dry in the village indicating that pan dam was one of the key potential manmade sources of mosquitoes in the dry season as it continued to produce mosquito larvae. Most habitats which were drying up had none or sometimes less than five mosquito larvae, surveillance of these habitats revealed that there was low vegetation cover ranked at approximately 20% in pan dams which may have had impact on mosquito larvae considerably reducing their numbers. This is consistent with the results of the studies conducted by Fillinger, *et al.* (2004) and Service (1977) in the studies conducted in Western Kenya.

Larval habitats which mainly composed of ditches and pan dams in the village were replenished with water during the short rains and there was evidence of *Anopheles* larvae presence in these habitats. This demonstrated the rain dependency of breeding habitats in the area suggesting that mosquitoes probably laid their eggs immediately at the onset of the rainy season, (Haramis, 1985; Beier *et al.,* 1990). The re-colonization of breeding habitats by mosquitoes at the onset of the rainy season demonstrated the survival of refugia adult population hibernating in the swampy plains during the dry season. This supports the findings by Jansen *et al.* (1994) in Eritrea while studying survival mechanisms of *Anopheles* mosquitoes. However, this refilling of most larval habitats did not translate into high anopheline larvae productivity in the subsequent month of April as continued sampling indicated. This was presumably because of higher temperature (> 40C) and low relative humidity which occurred in the early days of this month, Charlwood, (1985). However, during the dry season in areas adjacent to the receding marshes of the Loboi swamp, there were numerous small natural habitats that were productive for *Anopheles* larvae. As a result, the mosquito abundance was relatively higher in the houses adjacent to the swamp compared to those far away during the dry season.

The quick re-colonization of the larval habitats shortly after a rainfall in the area suggested that mosquitoes that emerged from the swampy plains were also important seed of the rain-fed water bodies such as the pan dams and ditches which were mainly rain dependent. These results are consistent with that observed by Ribiero *et al.* (1996) on re-colonization of temporary breeding habitats by mosquitoes in Ethiopia. Three major anopheline species were recorded and some seasonal similarities were found between habitats. In the early dry season *An. pharoensis and An. gambiae* were generally dominant in most of the breeding habitats, constituting between 40% - 48% of the total collections. *An. pharoensis* were relatively abundant in the pan dams and pools while *An. gambiae* dominated mostly in ditches and fewer in marsh.

However, few numbers occasionally colonized pan dams especially during the peak dry season. Some larvae were occasionally encountered in pools formed by canals during the dry season especially from stagnant parts. This ascertained vector dependence on the man-made larval habitats which are known to play an important role in *Anopheles* proliferation (Holstein, 1954; Minakawa, 2002; Girardin *et al.*, 2004), especially in the dry season. *Anopheles* mosquito larvae co-exist in most breeding habitats (Mutero *et al.*, 2000). In the current study it appeared that there was differential habitat use and this was dependent on seasons and species. *An. gambiae* was collected from different habitats at different times of the season but *An. funestus* was mainly collected from swamp or marsh habitats and was not found in pan dams and canal in either season. The existence of macro habitat variation in the area could subsequently play an important role in the seasonal distribution of the larval stages of malaria vectors.

Other genera observed included *Culex* which dominated in all habitats sampled for mosquito larvae. Their numbers may have had effect on *Anopheles* larvae numbers in the breeding habitats since some of the *Culex* species such as *Culex tigripes* observed in some habitats are known for their predatory habits (Minakawa *et al.*, 2002). In this area, most productive larval habitats are human-made and rain-dependent, drying out within two months after the rainy season. Numerous anopheline larvae may be found in favorable ecological conditions (e.g., along the swamps or marshes), which may sustain malaria transmission at a low level during the dry season and may serve as inoculums in the surrounding areas at the onset of rainy season.

This scenario is similar to those in other areas of seasonal malaria transmission and provides an opportunity for a mosquito control strategy targeting the dry season larval control and environmental management.

The present study revealed that the abundance of larval anopheline species in pan dams was associated with the late dry and early wet seasons. This suggested that these habitats could be important breeding grounds for propagating mosquito population in dry season when there is scarcity of breeding habitats. Colonization of habitats at different times could be explained from studies conducted by Rejmankova *et al.* (1991) in Chiapas Mexico which revealed that local environmental features of larval habitats such as high temperatures being responsible for some of these disparities. The nature of some parts of Kamarimar village has changed with time since the introduction of small scale irrigation farming in the area. Water is drained from Loboi swamp via canals into small reservoirs adjacent to some homes for farming activities. These measures have initiated the formation of potential mosquito larval habitats which may influence the vector population dynamics during rainy and dry season in the area.

The study revealed that vegetation, hideouts around stores and culvert shelters harboured *Anopheles* mosquitoes in the semi-arid areas. Although only comparatively few mosquito species regularly rest in human and animal habitations, those that do are important vectors of malaria and other diseases. However, considerable numbers also rest in the outdoor habitats especially the day time (Service, 1976). During the outdoor survey, some mosquitoes which were collected from outdoor habitats rested in the natural resting places such as vegetation. However, large proportion of the species also rested in the man-made shelters as was also reported by Aniedu (1993) in Kerio Valley, Kenya. Service (1976) reported that it is usually difficult to find mosquitoes that rest outdoors than those that rest in buildings such as houses and animal quarters due to the fact that outdoor populations are widely distributed over large areas. The present study found that mosquitoes preferred to rest outdoors in small vegetation near cattle sheds or around human dwellings and in hideouts around storage structures. Even species such as *An. gambiae* which is regarded as highly endophilic, about 5% of the population were found resting outdoors. This is consistent with the results reported by Gillies (1954) in the republic of Ghana.

The population of *An. funestus* which were collected outdoors was however low about 3% compared to the indoor catches. This suggested that *An. funestus* was more endophillic compared to other *Anopheles* mosquitoes in the area. These results are consistent with the one obtained by Aniedu, (1993) on seasonal transmission of malaria by anopheline mosquitoes in a semi-arid ecosystem of Kenya. Certain degree of exophily was exhibited by *An. gambiae* which were collected in the village. It is not clear whether, there is any change in vector behaviour due to the pressure of the use of insecticide treated nets or cooking and other routine practices in the house since the previous surveys did not study the exophilly of *Anopheles* mosquitoes. Exophillic behavior of the vectors may contribute to inefficiency of the indoor residual spraying since mosquitoes may not come into contact with the chemicals used and as a result non-interruption of malaria transmission as was pointed out in the studies conducted by Sahu (1990) in the coastal region of Madagascar. *An. pharoensis* was found to be equally exo - and endophilic. However, it was much more abundant outdoors than other species in both seasons. The collection of some potential vectors of malaria outdoors indicated that after biting, some did not take rest in the houses and preferred to rest in small vegetation outdoors. These findings are similar to that found by Hati, (1986) in India and concluded that the control of malaria vectors should not only target the indoor resting vectors. Both outdoor and indoor resting habits require equal attention in any activity geared towards the control of mosquitoes considering that the composition of both outdoor and indoor resting mosquitoes included malaria vectors.

5.2 Conclusions

Distance between a house and a breeding habitat as a variable showed a significant correlation with adult densities. More than 60% percent of the collected adult mosquitoes were found in the houses within 500 meters from the larval habitat.

There was a large seasonal variation in the abundance of anophelines studied. *An. gambiae* and *An. pharoensis* were almost three times higher in the wet season than in the dry season. In contrast, *An. funestus* was more common in the dry season.

The study revealed a seasonal variation in colonization of the breeding habitats by the vectors. There was colonization preference for pan dams, ditches and marshes by *Anopheles* mosquitoes depending on the availability of these breeding habitats.

Exophilic behaviour was observed in *Anopheles gambiae*, *An. pharoensis* and *An. funestus* in Kamarimar village. Significant preference for outdoor habitats was revealed and the vectors caught outdoors were found resting in vegetation, under stores and culverts.

5.3 Recommendations

1. Based on the high density of malaria vectors observed in the houses closer to the breeding habitats, indoor residual spraying as a control strategy should go hand in hand with treatment or larviciding of breeding habitats in proximity to reduce larval density.

2. Surveillance on seasonal population dynamics of malaria vectors in the semi-arid ecosystem to ascertain mosquito abundance is recommended.

3. Larval mosquito control should be emphasized and environmental management of breeding habitats encouraged in controlling larval stages of the vectors.

4. There is need to explore methods of managing and controlling outdoor resting vectors. Survey of other natural outdoor harborages to find out which type of shelter is preferred by different mosquito species should be emphasized.

REFERENCES

Aniedu, I. (1993). Biting activity and resting behavior of malaria vectors. *Insect Sci-applic. Vol. 13 No. 2 pp. 151-161.*

Aditya, G.; Pramanik, M. K. and Saha, G. K. (2006). Larval habitats and species composition of mosquitoes in Darjeeling Himalayas, India. *Journal of Vector Borne Diseases 43: 7-15.*

Almiron, W. R. and Brewer M. E. (1996). Classification of immature stage habitats of Culicidae (Diptera) collected in Cordoba, Argentina. *Memorias do Instituto Oswaldo Cruz, Rio de Janeiro 91: 1-9.*

Arunachalam, N. (2004). Japanese encephalitis in Kerala, South India: Can *Mansonia* (Diptera: Culicidae) play a supplemental role in transmission? *Journal of Medical Entomology, 6: 105-108.*

Bashir A.; Sharma, R.C. and Rahman, R.S. (2008). Efficcacy of monolayer film asainst immature stages of An. arabiensia in Khatoum, sudan. *Southeast Asian journal of tropical medicine and public health, 39 222-228.*

Bentle, M. D and Day, J. L. (1989). Chemical and behavioural aspects of mosquito oviposition. *Annual Review of Entomology, 34: 87-94.*

Beier, J.C.; Copeland, R.; Oyaro, C.; Masinya, A.; Odago, W.O.; Odour, W.; Koech, D.K. and Roberts, C.R. (1990). *Anopheles gambiae* complex egg stage survival in dry soil from larval development sites in Western Kenya. *Journal of American Mosquito Control Association, 6: 105-108.*

Bhatt, R.M.; Sharma, R.C. and Yadav, R.S. (1989). Resting of mosquitoes in pit shelters in Kheda District, Gujarat. *Indian Journal of Malariology, 26 (2) 75-81.*

Bidlingmayer, W. L. (1964). The effect of moonlight on the flight activity of mosquitoes. *Ecology, 45: 87-94.*

Boutin, J. P.; Pages, F.; Legros, F. and Rogier, C. (2005). Epidemiology of malaria. *La Revue du Praticien, 38:223-230.*

Breeland, S.G. (1972a). Studies on the diurnal resting habits of *Anopheles albimanusin* El Salvador. *Mosquito news, 32: 99-106.*

Breeland, S.G. (1972b). Methods of measuring anopheline densities in El Salvador. *Mosquito news, 32: 62-72.*

Bushrod, F. M. (1978). Field and laboratory studies the vectors of Bancroftian filariasis in Tanzania with special reference to their control. Ph.D. Thesis, University of Liverpool.

Charlwood, J. D.; Vij, R. and Billingsley P. F. (2000). Dry season refugia of malaria-transmitting mosquitoes in dry savannah zone of east Africa. *American Journal of Tropical Medicine and Hygiene, 62: 726-732.*

Charlwood, J. D. (1985). Influence of larval habitats on ecology and behaviour of anopheline species from pupae. *New Guinea pp. 399-406.*

Cheeseborough, M. (I987). Medical Laboratory Manual for Tropical Countries. Volume 1. Butterworth-Heinemam Ltd. Halley Court, Jordan hill, *Oxford OX2 8EJ. pp 398*

Clement, A.N. (1999). The biology of mosquitoes volume 2. Chapman and Hall, London.

Clement, A.N. (1992). The Biology of Mosquitoes. *Chapman and Hall***.** *New York.*

Coetzee, M.; Craig M.; and Lesuer, D. (2000). Distribution of African malaria mosquitoes belonging to *Anopheles gambiae* complex. *Parasitologia Today, 16:73-77.*

Coetzee, M. (2004). Distribution of African malaria vectors of *Anopheles gambiae* complex. *Am. J. Trop. Med. Hyg.* 70*:103-104.*

Cohuet, A; Simard, F.; Wondji, C. S. and Fontenille, D. (2004). High malaria transmission intensity due to African malaria vectors of *Anopheles gambiae* complex. *Journal of Medical Entomology,* 41*:901-904.*

Das, B. J. and Amalraj, K. (1997). Adaptation to intermittently flooded swamps by *Anopheles quadrimaculatus* (Diptera: Culicidae). *Environ. Entomol., 20: 1050-1056.*

Deane, M.P. and Causey, O.R. (1943). Viability of *Anopheles gambiae* eggs and morphology of unusual types found in Brazil. *American Journal of Tropical Medicine and Hygiene, 23: 95-102***.**

Edillo, F. E.; Toure, Y. T.; Lanzaro, G. C.; Dolo, G. and Taylor, C. E. (2002). Spatial and habitat distribution of *Anopheles gambiae* and *Anopheles arabiensis* (Diptera: Culicidae) in Banambani Village, Mali. *Journal of Medical Entomology 39: 70-77.*

Fillinger, U.; Sonye, G.; Killeen, G.F.; Knols, B.G. and Becker, N. (2004). The practical importance of permanent and semi permanent habitats for controlling aquatic stages of *Anopheles gambiae sensu lato* mosquitoes: operational observations from a rural town in western Kenya. *Trop. Med. Int. Health, 9: 1274-1289.*

Gillies, M.T. (1954). Role of secondary vectors of malaria in north east Tanganyika. *Trans. Soc. Trop. Med. Hyg. 58 (2) 154-158.*

Gillies, M.T. and De Mellion, B. (1968). The anopheline of Africa, south of Sahara (Ethiopian zoogeographical region) *South African Institute for Medical research, Johannesburg, South Africa.*

Gillies, M.T. and Coetzee, M. (1987). A supplement of the anophlinae of Africa south of the Sahara (Afrotropical Region). Johannesburg: *South Africa Institute of Medical Research, No. 55.*

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

Girardin, O. and Dao, D. (2004). Opportunities and limiting factors of intensive Vegetable farming in malaria endemic area. *Acta Tropica., 89: 109 -123.*

Gopaul, B. (1995). The control of anophelines of Sub-Saharan Africa, *South African Institute for Medical research, Johannesburg, South Africa.*

Grats, N. and Pal, R. (1998). Malaria vector control: larviciding. In Malaria-Principles and practice of malariology, Edinbugh;Churchi Livingstone, *2:1213-1236.*

Haramis, **L.D. (1985).** Larval nutrition, adult body size and biology of *Aedes triseriatus.* Pp. 431-437 in ecology of mosquitoes. *Florida Med. Entomology.*

Harker, J. E. (1958). Diurnal rhythms in animal kingdom. *Biol. Rev. 3: 1-52.*

Harker, J. E. (1961). Diurnal rhythms. *Ann. Rev. Ent. 6: 131-144.*

Hati, A. K. (1986). Studies on Culex and other related vectors in rural west Bengal, Department of Entomology, *Calcuta School of Tropical Medicine, Calcuta. 281-493.*

Holstein, M. H. (1954). Biology of *Anopheles gambiae*. Research in French West Africa. *World Health Organization Monogr. Sept. 9, pp. 173.*

Ijumba, J. N.; Mwangi, R.W. and Beier, J.C. (1990). Malaria transmission potential of *Anopheles* mosquitoes in the Mwea-Tabere irrigation scheme, Kenya. *Medical and Veterinary Entomology, 4: 425-32.*

Iyengar, R. (1962). The bionomics of saltwater *Anopheles gambiae* in East Africa. *Bulletin of the World Health Organization, 27: 223-229.*

Jensen, T.; Kaiser, P.E. and Barnard, D.R. (1994). Adaptation to intermittently flooded swamps by *Anopheles quadrimaculatus* species C1 (Diptera: Culicidae). *Environ. Entomol., 23: 1150-4.*

Kettle, D. S. (1990). Medical and Veterinary Entomology. *Oxford University Press, U.S.A.*

Killeen, G. F. and Knols, B.G.J. (2002). Eradication of *An. gambiae* from Brazil: lessons for malaria control in Africa. *The Lancet: infectious diseases, 2: 618-627.*

Kiszewski, A. E. and Teklehaimanot, A. (2004). A review of the clinical and epidemiologic burdens of epidemic malaria. *American Journal of Tropical Medicine and Hygiene, 71 (Suppl. 2): 128-135.*

Kitron, U. and Spielman, A. (1989). Suppression of transmission of malaria through source reduction: anti-anopheline measures applied in Israel, the United States, and Italy. *Rev. Infect. Dis. 11: 391-406.*

Klowden, M.J. (1996). Vector behavior. In: the biology of Disease Vectors. *University Press of Colorado, U.S.A. pp. 73-83.*

Laarman, J. J. (1955). The host seeking behaviour of malaria mosquito *Anopheles maculipennis atroparrus. Acta laidensia, 25: 1-144.*

Laarman, J. J. (1958). The host-seeking behavior of malaria mosquitoes. *Trop. Med. 10: 293-305.*

Lacey, L.A. (1994). The role of biological control of mosquitoes in integrated vector control. *American Journal of Tropical Medicine and Hygiene 50(6): 97-115.*

Lindsay, S.; Mathew. K. and Robert, B. (2004). Environmental management for malaria control in East Asia region. Warshington, DC, World Health Organization.

Lindsay, S.W.; Wilkins, H.A.; Zieler, H.A.; Daly, R.J.; Petrarca, V.; and Byass, P. (1991). Ability of *Anopheles gambiae* mosquitoes to transmit malaria during the dry and wet seasons in an area of irrigated rice cultivation in The Gambia. *American Journal of Tropical Medicine and Hygiene 94: 313-24.*

Mahesh, R. K. and Jauhari, R. K. (2000b). Interspecific association and index of association among different aquatic forms of Anophelines collected from Saharanpur block of Doon Valley. *Journal of Parasitic Diseases 24: 147-150.*

Manga, L.; Fondjo, E.; Carnevale, P. and Robert, V. (1993). Importance of low dispersion of Anopheles gambiae (Diptera: Culicidae) on malaria transmission in hilly towns in south Cameroon. *Journal of Medical Entomology, 30: 936-938.*

Mathews, S.R. and Mathews, J. R. (1978). Insect behaviour. *John Wiley & Sons, New York.*

Mattingly, P.F. (1971). Ecological aspects of mosquito evolution. *Parasitologia, 13: 31-65*

Mbogo, C.N.; Snow, R.W.; Khamala, C.P.; Kabiru, E.W.; Ouma, J.H.; Githure, J.I.; Marsh, K. and Beier, J.C. (1995). Relationships between *Plasmodium falciparum* transmission by vector populations and the incidence of severe disease at nine sites on the Kenyan coast. *American Journal of Tropical Medicine and Hygiene 52: 201-206.*

McCall, P. and Cameron, M. (1995). Oviposition pheromones in insect vectors. *Parasitologia Today, 45: 87-94.*

McClelland, G.A. (1957). Methods of collection of blood-fed females in the field. E. Afr. Virus Res. Inst. Rep., 1956-1957. *Government printer, Nairobi pp. 47-55.*

McClelland, G.A and Weitz, B. (1963). Serological identification of natural hosts of *Aedes egypti* and some other mosquitoes caught resting in vegetation in Kenya and Uganda. *Ann. Trop. Med. Parasitol., 57: 214-224.*

Meisch, M. (1985). *Gambusia Affinis Affinis.* Biological mosquito control. *Ann. Trop. Med. Parasitol., 40: 110-116.*

Minakawa, L.; Mutero, C. M.; Githure, J. I.; Beier, J. C. and Yan, G. (1999). Spatial distribution and habitat characterization of Anopheline mosquito larvae in Western Kenya. *American Journal of Tropical Medicine and Hygiene 61: 1010-1016*

Minakawa, L. (2002). Influence of host and larval habitat distribution on the abundance of African malaria vectors in western Kenya. *American Journal of Tropical Medicine and Hygiene, 67: 32-38.*

Mitchell, C.J. (1996). Environmental management for vector control. The biology of disease vectors, Eds Barry J.B. and Williams C.M. *University Press of Collorado, U.S.A., 492-495.*

Muirhead-Thompson, R.C. (1945). Studies on the breeding places and control of *Anopheles gambiae* Var. melas in coastal district of Sierra Leon. *Bull. Entomol. Res. 36: 185-252.*

Muirhead-Thompson, R.C. (1951). Studies on salt-water and fresh-water *Anopheles gambiae* on the east African coast. *Bull. Entomol. Res. 41: 487-502.*

Mutero, C.M.; Blank, H.; Konradsen, F.; and van der Hoek, W. (1982). Water management for controlling the breeding of *Anopheles* mosquitoes in rice irrigation schemes in Kenya. *J. Am. Mosq. Control Assoc. 10: 507-510.*

Nahlen, B.L.; Clarke, J.A. and Alnwick, D. (2003). Insecticide-treated bed nets. *Amrican Journal of Tropical Medicine and Hygiene, 68:1-2.*

Nauen, R. (2007). Insecticide resistance in disease vectors of public health importance. *Pest Management Science* 63:628-*633.*

Nora, J.A. and Catherine, J. (2004). The burden of malaria. Geneva. *World Health Organization. WHO/CTD/MAL/96.10.*

Omer, S.M and Cloudsley-Thomson, (1970). Survival of female *Anopheles gambiae* Giles through a 9-month Dry-season in Sudan. *Bulletin of World Health Organization 42: 319-330.*

Omer, S.M.; Philips, D.; Kennedy, L. and Cloudsley-Thomson, (1970). Seasonal diversity of malaria vectors *Anopheles gambiae* Giles in a depressed breeding condition. *Bulletin of World Health Organization 22: 205-210.*

Palchick, S. (1996). Chemical control of vectors. The biology of disease vectors, Eds Barry J.B. and Williams C.M. *University Press of Collorado, USA: 502-508.*

Pemola, N. and Jauhari R. K. (2005). Species diversity patterns among mosquitoes (Diptera: Culicidae) from certain parts in Garhwal Himalayas, India. Journal of Applied Bioscience 31: 105-113.

Philips, R.S. (2001). Current status of malaria and potential for control. *Clinical Microbiology Review,* 14*:208-226.*

Provost, M. W. (1953). Motives behind mosquito flight. *Mosquito news 13: 106-109.*

Rejmankova, E.; Savage, H.M.; Rejmanek, M.; Arredondo, J.I. and Roberts. D.R. (1991). Multivariate analysis of relationships between habitats, environmental factors and occurrence of anopheline mosquito larvae *Anopheles albimanus* and *An. pseudopunctipennis* in southern chiapas, mexico. *Journal of Applied Ecology, 28: 827-841*

Ribiero, J.M.; Seulu, F. and Kidane, G. (1996). Temporal and spatial distribution of anopheline mosquitoes in an Ethiopian village: Implication for malaria control strategies. *Bulletin World Health Organization, 74: 299-305*

Rueda, L. M.; Kim, H. C.; Klein, T. A.; Pecor, J. E.; Sithiprasasna. R.; Debboun, M. and Wilkerson, R. C. (2006). Distribution and larval habitat characteristics of *Anopheles hyrcanus* Group and related mosquito species (Diptera: Culicidae) in South Korea. *Journal of Vector Ecology 31: 199-206.*

Sahu, H. W. (1990). Host behaviour; its influence on the feeding success of mosquitoes. *Ann. Entomol. Soc. 64**:** 513-516.*

Schafer, W.W. (1997). Larval and adult biology of mosquitoes. *Ann. Trop. Med. Parasitol., 50: 399-414.*

Scholte, D.E.; Takken, W. and Knolls B.W. (2003). Infection of malaria and filariasis vectors with fungus *Metarhizium anisoplia* . *Malaria journal 2: 1-8.*

Senior White, R. A. David, B. and Gosh, R. (1945). On the adult bionomics of some Indian anophelines: with special reference to malaria control by pyrethrum spraying. *J. Malaria Inst. India 6: 129-245.*

Senior White, R. A. (1951). Studies on the bionomics of *Anopheles aquasalis* Curry, 1932 (contd.) part 2. *Indian Journal of Malariology, 5: 465-512.*

Service, M. M. (1971a). The daytime distribution of mosquitoes resting in vegetation. *Journal of Medical Entomol*ogy 8*: 271-278.*

Service, M. W. (1973). Flight activities of mosquitoes with emphasis on host-seeking behaviour. In: Hudson A. biting fly b control and environmental quality. Proc. Symp. *University of Alberta, Canada, pp. 125-132.*

Service, M. W. (1976). Mosquito ecology. Field sampling methods. *Applied Sciences Publishers, pp. 255-350.*

Service, M.W. (1977). Mortalities of the immature stages of species B of the *Anopheles gambiae* complex in Kenya: comparison between rice fields and temporary pools, identification of predators, and effects of insecticidal spraying. *Journal of Medical Entomol. 1: 535-45.*

Sharma, V.P.; Shanna, R.C. and Gautam, A.S. (1986). Bio-environmental control of malaria in Nadiad, Kheda district, Gujarat. *Indian Journal of Malariology, 23 (2): 95-117.*

Snow, R.W. and Marsh, K. (1993). Malaria transmission and morbidity. In: Coluzzi, M. & Bradley, D. editors. *The malaria challenge after one hundred years of malariology. Parasitologia, 41(1-3), 241-246.*

Statistical Analysis System Institute Inc. (1997). SAS/STAT Software: *SAS Institute Inc., Cary, North Carolina*

Subra, R. (1980). Biology and control of *Culex* mosquitoes (Diptera: Culicidae) with special reference to Africa. *WHO document, WHO/UBC/80,781.*

Sumba, L. A.; Kenneth, O. and Githure, J. (2004). Daily oviposition patterns of African malaria mosquito *Anopheles* gambiae on different substrates. *Journal of circadian rhythms 2:1-7.*

Takken, W. (1991). The role of olfaction in host seeking of mosquitoes: a review. *Insect Science and its Application, 12: 287-295.*

Takken, W. and Knols, B.G. (1999). Odor mediated behaviour of Afrotropical malaria mosquitoes. *Annual Review of Entomlogy, 44: 131-157.*

Takken, W. (2004). Behavioural response of *Anopheles* mosquitoes between emergence and first feeding. *Bulletin of Entomological Research, 52: 155-166.*

Taylor, C. E.; Toure, Y.T.; Coluzzi, M. and Petrarca, V. (1993). Effective population size and persistence of *Anopheles arabiensis* during the dry season in West Africa. *Medical and Veterinary Entomology, 7: 351-357.*

Utzinger, J.; Tozan, Y. and Singer, B.H. (2001). Efficacy and cost effectiveness of environmental management for malaria control. *Trop. Med. Int. Health, 6: 677-687.*

White G. B, (1974). *Anopheles gambiae* complex and disease transmission in Africa. .

Am. J. Trop. Med. Hyg. 38:223-230.

Wilkinson, L. M. (1978). Malaria studies and control in vector prone areas of Brazil. *Am. J. Trop. Med. Hyg. 38:223-230*.

World Health Organization (1975). Manual on practical entomology in Malaria**.** Part II. Methods and techniques. World Health Organization Offset Publication, Geneva, *No***.** *13*

World Health Organization (1997). Vector control methods for use by individuals and communities. *World Health Organization. Geneva, 10-12.*

World Health Organization (2003). The Africa Malaria Report and Strategic Plan. WHO, Geneva**.** Switzerland.

World Health Organization (2006). The Africa Malaria Report. WHO, Geneva**.** Switzerland.

Zaim, M. and Nakashima, N. (2000). Safety of pyrethroid-treated nets. *Medical Veterinary Entomology, 4: 1-5.*