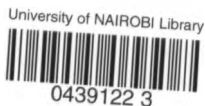


**ECOLOGICAL AND PHYSIOLOGICAL ADAPTATIONS OF MALARIA VECTORS
IN A SEMI-ARID AREA IN BARINGO DISTRICT, KENYA**

By

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AWARD OF DOCTOR OF PHILOSOPHY (APPLIED PARASITOLOGY) DEGREE,
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2011

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ABSTRACT

Longitudinal entomological sampling was executed to study physiological and ecological adaptations of malaria vectors at two semi-arid villages (Kamarimar and Tirion) in Kenya. Adult mosquitoes were sampled using CDC light traps and pyrethrum spray catch collections. Mosquito larvae were sampled using standard dipping methods. Parity rates, length of gonotrophic cycle duration and insemination rates of female mosquitoes were assessed. Host choice and sporozoite rates were determined by ELISAs. Sub-species of the *Anopheles gambiae* complex were identified by PCR. Out of 16,683 mosquitoes collected in both villages *An. gambiae sl* was most predominant followed by *An. pharoensis*, *An. funestus* and *An. coustani*. *An. arabiensis* was the most predominant member of the *An. gambiae* complex. Only 13.77% of all adults were blood-fed and 0.02% were *Plasmodium falciparum*-infected. Some 30.44% of the mosquitoes had fed on humans; 28.46% and 42% had fed on bovines and goats, respectively. Larval productivity was low all year. During the wet season, larvae occupied all habitats but more so in pan dams. All hoof prints, stream beds, tyre tracks, concrete tanks and ditches were rain-fed and produced larvae for short periods only. Habitat stability was positively associated with larval density. Duration of gonotrophic cycles varied significantly between wet and dry seasons. Christophers stage I incidence differed significantly between dry and wet seasons. Prevalence of stage II, III, IV and V did not vary with season. There was no significant difference in insemination frequency between seasons. Breeding of malaria vectors was predominantly driven by human factors. *An. arabiensis* and *An. funestus* bred at low levels in permanent breeding sites throughout the year. Permanent water sources available during the dry season served as inocula that provided "larval seed" to fresh habitats formed during the rainy season. The vectors fed mainly on humans and to a lesser but substantial extent on goats and bovines. Malaria transmission was undetectable through most of the year but occurred at low-level in a few wet months. The duration of gonotrophic cycles was longer during the wet than the dry season indicating that these vectors adapt to hot dry weather by ovipositing more frequently. Shortened gonotrophic cycles during peak dry seasons apparently compensated for the low vector densities.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background and rationale

The main malaria vectors in Sub-Saharan Africa (*Anopheles gambiae* Giles, *An. arabiensis* and *An. funestus*) are confronted with highly variable and challenging climatic conditions especially in the semiarid regions and during the dry season (Mattingly, 1971). The abundance of malaria vectors drops dramatically with the onset of the dry season, depressing the incidence of severe malaria in the process (Wilkinson et al., 1978; Snow *et al.*, 1993). The onset of the rains, however, brings a rapid explosion in mosquito numbers and a concomitant increase in malaria transmission (Omer and Cloudsley-Thomson, 1970, Mbogo *et al.*, 1993, Snow *et al.*, 1993). Data on the Anophelinae is not conclusive with respect to how each life stage contributes to long-term survival in semi-arid conditions and the rapid population rise that occurs following the onset of the rains. Contributions to long-term survival during the dry season and in semiarid zones could be made during the egg, larval, and/or adult (including pupal) stages. Anopheline mosquito eggs are susceptible to prolonged desiccation, which likely prevents the egg stage from making a significant contribution to long-term survival during the dry season (Deane and Causey, 1943). Studies in the laboratory have demonstrated that egg stages may survive and hatch after 12 days of storage in moist conditions (Beier *et al.*, 1990). These observations suggested that the egg stage could be important for 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 survival during the dry season. Studies conducted in Burkina Faso and the Sudan in Africa indicates that *An. gambiae* adults enter a state of dormancy at the onset of the dry season (Omer, 1970). The mechanisms

underlying this physiological adaptation are however, poorly understood (Mala *et al.*, 2011a, Mala *et al.*, 2011b).

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 a low level. If the focal sites where mosquitoes breed in semi arid environments and during the dry season can be identified and managed, then the reservoir of vector species that form “seed” at the onset of the rains would be eliminated. Most semi arid areas are currently hit by malaria epidemics (Grover-Kopec *et al.*, 2005, Kiszewski and Teklehaimanot, 2004, Thomson *et al.* 2006) and present peculiar physical conditions that have to be understood for sustainable vector management. This will also provide site-specific knowledge about *Anopheles* ecology in such areas as a basis for targeted malaria control. It is envisioned that countries lying within the semi-arid regions of Africa would have more sustained approach to the control of malaria vectors if the ecology of the vector species is adequately understood. The results of this study shed light on spatial and temporal heterogeneities experienced in malaria transmission in these regions.

1.2 Socio-economic impact of malaria

The burden of malaria has a potentially large economic impact, limiting the productivity of a country's two major assets, its people and its land. The cost of malaria prevention and treatment consumes the scarce household resources and in turn, the burden on one public health sector impacts on the allocation of already scarce resources. The annual economic burden of malaria in Africa is estimated at about \$11.7 billion, or 1% of the Gross domestic product; the cost of treating a single case from a society's viewpoint is estimated at \$9.84 or 12 days equivalent of active productivity, and the cost of treatment per household ranges

from \$ 0.2 -\$15 per month (WHO, 1999). Highly malarious countries are among the very poorest in the world and typically have very low rates of economic growth (Gallup and Sachs, 2001). The disease, which affects mainly children and pregnant women has lifelong effects on cognitive development and education levels through the impact of chronic malaria, induced anaemia and time lost or wasted away from the classroom due to malarial illness (Holding and Snow, 2001). Pregnant women are reportedly four times more likely to suffer from malaria attacks leading to the delivery of low weight babies and still births, endangering the health of the women and the prospects for the newborn (Brabin, 1983, Diagne *et al.*, 1997). Much of the mortality in endemic areas is concentrated among children under the age of five. In areas of stable endemic transmission about 25% of all-cause mortality in children aged 0 to 4 years has been attributed directly to malaria (Snow *et al.*, 1999). Malaria may have adverse demographic consequences as well. Malaria substantially raises the chances of infant and child mortality, and households respond to this increased risk by having more children, thereby increasing the overall rate of population growth. In addition, investments which parents of many children can afford to make in the well being of each child is limited— so that average levels of health care and education per child tend to be reduced. As a result, mothers of large numbers of children are less able to participate in the formal labor force, thereby also reducing the household income. Malaria imposes a heavy cost not only on a country's current income, but also on its rate of economic growth, and therefore on the level of economic development in the long run. There is need for more research to document the household and institutional impacts of epidemic malaria in sub Saharan Africa where malaria epidemics are on the rise.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Malaria presentation and disease symptoms

Malaria is caused by protozoan parasite genus *plasmodium*. Five species of the plasmodium parasite can infect humans; the most serious forms of the disease are caused by *plasmodium falciparum*. Malaria caused by *plasmodium vivax*, *plasmodium ovale* and *plasmodium malariae* cause milder disease in humans that is not generally fatal. A fifth specie *plasmodium knowelsi*, cause malaria in macaques but can also infect humans. This group of human – pathogenic *plasmodium* species is usually referred to as malaria parasites. The species that causes the greatest illness and death in Africa is *P. falciparum* (WHO, 2000). *Plasmodium falciparum* occurs in most malaria affected areas of the world. In tropical Africa and Asia, 85-90% of malaria cases are due to this parasite species. *Plasmodium vivax* is common in South Asia and Central America, and is predominant in South America. *Plasmodium ovale* is found mainly in tropical Africa, in West and South Africa, with sporadic reports from other continents, e.g the South Pacific islands. *Plasmodium malariae* is the least common species of malaria to infect humans, and is infrequent all over the world (Hombhange, 1998).

The general symptoms of malaria include headache, nausea, fever, vomiting and flu-like symptoms, although these symptoms may differ depending on the type of *Plasmodium* species that caused the infection (Schmitz and Gelfand, 1976; Wernsdorfer and Mc Gregor, 1986). *Plasmodium falciparum* normally takes 7 to 14 days for symptoms to show while *P. vivax* and *P. ovale* takes 8 to 14 days (but in some cases can survive for months in the human host) while *P. malariae* takes 7 to 30 days (Vinetz *et al.*, 1998; D'Avanzo *et al.*, 2002). It is however important to mention that these figures are indications only because the onset of

symptoms varies tremendously depending on the *Plasmodium* species infecting and the level of host immunity. Symptoms may appear and disappear in phases and may come and go at various time frames. These cyclic symptoms of malaria are caused by the life cycle of the parasites as they develop, mature, reproduce and are once again released into the blood stream to infect more blood and liver cells. Fever is the main symptom of malaria (Schmitz and Gelfand, 1976). A high swinging fever can develop when this happens, with marked shivering and intense perspiration. Further serious complication involving the brain and kidneys can then develop leading to delirium and coma. The most severe manifestations are cerebral malaria, anaemia, kidney and other organ dysfunctions such as respiratory distress (WHO, 1986). Individuals that have been repeatedly exposed to malaria infections acquire considerable degrees of clinical immunity, which provides them with protection against future infections (Baird, 1995).

2.2 Vectors of Malaria and genetic transformation of mosquitoes

Over 80 species of *Anopheles* mosquitoes have been known to transmit malaria. The importance of each species in malaria transmission however varies by region and geographical distribution. For example, the *An. minimus* complex, *An. aquasalis*, *An. albimanus* complex are important vectors in South America (Laubach *et al.*, 2001, Marquetti *et al.*, 1991). In sub-saharan Africa, the *An. gambiae* complex and the *An. funestus* complex are the drivers of malaria transmission

2.2.1 The *Anopheles gambiae* complex

The *An. gambiae* Giles complex comprises seven sibling species, namely *An. gambiae* s.s, *An. arabiensis*, *An. melas*, *An. merus*, *An. bwambae* and the non-malaria transmitting *An. quadriannulatus* species A and B, and several incipient species all differing in various ways

(White, 1975, Coluzzi *et al.*, 1985; Gillies and Coetzee 1987; 1995; Hunt *et al.*, 1998). At least three species within *An. gambiae* s.s have been reported in West Africa (Fanello *et al.*, 2003). Members of the *An. gambiae* complex are morphologically indistinguishable, but exhibit distinct genetic and eco-ethological differences, as reflected in their ability to transmit malaria.

An. gambiae sensu stricto is the most important vector of *P. falciparum* malaria in sub-Saharan Africa. This species is the most adapted to humans and has the highest malaria parasite inoculation rates with entomological inoculation rates ranging from between <1 to >1000 infective bites per person per year (Trape and Rogier, 1996). It is also remarkably stable and versatile in a wide range of bio-ecological and seasonal conditions, both in exploiting new man-made environments and in their response to malaria control activities (Coluzzi, 1984).

Members of the *An. gambiae* complex have a wide geographical distribution and have been reported in most African countries (Coetzee *et al.*, 2000; Coluzzi, *et al.*, 1979; Chinery, 1984). *Anopheles melas* is confined to the West Coast of Africa (Coluzzi and Sabatini, 1968) whereas *An. merus* is confined to the coast of East Africa and islands off its coast, and has also been recorded in Somalia in the North to Natal in the South (Paterson *et al.*, 1964). Two species of the *An. gambiae* complex namely *An. gambiae* s.s and *An. arabiensis* are not only the most broadly distributed but also the most efficient vectors of malaria (Coetzee *et al.*, 2000).

The distribution range and relative abundance of *An. gambiae* and *An. arabiensis* is strongly influenced by climatic factors, especially total annual precipitation (Lindsay *et al.*, 1998).

An. gambiae is dominant in humid zones (Lindsay 1998, Coetzee *et al.*, 2000) while *An. arabiensis* tends to predominate in arid savannas. In situations where these two species occur in sympatry, changes in species composition usually occur, with *An. arabiensis* mainly predominant during the dry season and *An. gambiae* more abundant during the rainy season. This pattern may vary depending on either local ecology and/or species adaptation. *Anopheles quadriannulatus* has been reportedly found in Ethiopia and Eritrea (Hunt *et al.*, 1998, Shililu *et al.*, 2003) and *An. bwambae* has been reported breeding in mineral water springs in Uganda (Davidson and Hunt, 1973).

In Kenya *An. gambiae* s.s, *An. arabiensis* and *An. merus* are the most common members of the *An. gambiae* complex, with the range of distribution and relative abundance of *An. gambiae* and *An. arabiensis* defined by climatic factors, mainly annual precipitation and annual and wet season temperatures (Minakawa *et al.*, 2002). *Anopheles merus* prefers to breed in salty water habitats and is mainly found along the coastal strip, and is a minor vector in Kenya. Studies in West Africa have provided evidence for further subdivision of the *An. gambiae* species. The evidence for this division is shown by the micro-morphological differences of the second chromosome in different populations, due to inversions. These chromosomal forms, which also carry different strain specific combinations have been well studied in Mali, West Africa where three chromosomal forms, 'Bamako', 'Mopti' and 'Savanna' appear to be reproductively isolated in nature (Toure *et al.*, 1998; Favia *et al.*, 1997; Lanzaro *et al.*, 1998). None of these forms have been reported in Kenya, and in the East African region.

2.2.2 The *Anopheles funestus* complex

Anopheles funestus Giles is one of the three major vectors of malaria in Africa, together with *An. gambiae* Giles and *An. arabiensis* Patton of the *An. gambiae* complex. *Anopheles funestus* is emerging as an important vector of malaria, especially during the dry season when *An. gambiae* are less active (Fontenille, 1997). Taxonomically, *An. funestus* belongs to a group of nine morphologically similar species that were classified by Gillies and De Meillon (1968). *An. funestus* s.s., *An. aruni* Sobti, *An. parensis* Gillies, and *An. vaneedeni* Gillies and Coetzee are the first four species that belong to the *funestus* sub-group and have been described on the basis of adult characters, whereas *An. confusus* Evans and Leeson, *An. fuscivenosus* Leeson, *An. lesoni* Evans and *An. rivulorum* can be distinguished from each other and from the *funestus* subgroup based on larval characters.

Anopheles funestus is a versatile species as evidenced by its wide distribution and ability to occupy regions ranging from lowland to high altitudes. The species is highly endophilic and anthropophilic and is found in almost all bioclimatic areas near swamps or rivers (Faye *et al.*, 1985). The other species are limited in density and distribution, mainly zoophilic, but can avidly feed on humans outdoors in the absence of other hosts (Gillies and De Meillon, 1968). *Anopheles rivulorum* is the second most abundant and widespread species in the group. It is occasionally collected indoors along with *An. funestus* s.s., and has been shown to be a vector of malaria in Africa (Wilkes *et al.*, 1996). Other studies have shown that *An. vaneedeni* can be experimentally infected with *P. falciparum* in the laboratory (De Meillon *et al.*, 1977), but no evidence has been availed on its role in malaria transmission so far. This species has been recorded only from South Africa. Studies in Kenya have indicated the presence of *An. funestus* s.s., *An. parensis*, *An. lesoni*, and *An. rivulorum* (Kamau *et al.*, 2002; 2003). *Anopheles funestus* s.s. has been found almost exclusively inside human dwellings while *An.*

rivulorum was found exclusively outdoors, *An. parensis* both indoors and outdoors and *An. lesoni* was found indoors (Kamau *et al.*, 2003). Only *An. funestus* s.s. has been implicated as an important vector of *P. falciparum* in Kenya, even though its importance in malaria transmission varies depending on locality. The paucity of studies on this species group indicates the need for proper identification of members of the *An. funestus* species complex to make available data on biological and behavioral characteristics that will elucidate their role in malaria transmission, and that are relevant to vector control.

2.3 Malaria Transmission

Malaria transmission is complex, and its intensity is a function of the interaction between the parasite, the female *Anopheles* mosquito vector, the human host and the environment. Vector abundance, the duration of the extrinsic incubation period and the survival rate of the vector, when combined with the probability of the vector feeding on a human host; determine the risk of malaria infection, the stability of disease transmission and seasonal patterns. These biological parameters influenced by meteorological variables such as rainfall, temperature, and relative humidity, which determine mosquito survival and development. Presence of mosquito breeding sites, vegetation, land use, house construction and use of preventive measures determines the degree vector-host contact.

2.3.1 Factors influencing malaria transmission

Malaria transmission is influenced by both intrinsic and extrinsic factors. Extrinsic factors are mainly climatic and environmental factors such as rainfall, temperature, and elevation whereas intrinsic factors include host, parasite and vector factors.

2.3.1.1 Climatic factors

Malaria is particularly influenced by mainly rainfall, temperature and relative humidity (WHO, 1990).

I. Rainfall

Rainfall affects the availability of breeding sites. Oviposition of mosquito eggs and their maturation to larvae and adults occurs aquatic breeding habitats and is therefore dependent on rainfall (Le Sueur and Sharp, 1991; Molyneux, 1988).

Several studies have demonstrated the association between *An. gambiae* abundance and rainfall (Charlwood *et al.*, 1995) but no direct predictable relationship has so far been established. *Anopheles gambiae* prefers to breed in temporary turbid waters, whereas *An. funestus* prefers more permanent water bodies with emergent aquatic vegetation. However, the availability of both types of habitats depends on availability of adequate rainfall, which is also related to the saturation deficit and affects mosquito survival (Molyneux, 1988). This provides a good basis for using rainfall as a predictor of the presence of vectors, their survival and possible malaria transmission. The natural boundaries of the geographical distribution of malaria are determined by temperature and rainfall (McMichael *et al.*, 1996). Rainfall not only provides the medium for the aquatic stages of the mosquito life cycle but also increases the relative humidity and then the longevity of the adult mosquito.

The impact of rainfall on malaria transmission is however complicated, and varies with the circumstances of particular geographic regions and depending on local habits of mosquitoes. Rain may prove beneficial to mosquito breeding when it is moderate but it may destroy larval habitats and flush out the mosquito larvae when it is excessive. Considerable evidence exist

to show that heavy rainfall and flooding can increase mosquito breeding (Ramasamy *et al.*, 1992) and increase mosquito densities leading to a higher probability for mosquito bites, and disease transmission. It is not only the total amount of rainfall that is important but also its weekly and monthly distribution. Rainfall patterns in known malarious and non-malarious regions indicate the requirement for stable malaria transmission is around 80mm for 5 months and that neither 60mm for 5 months nor 80mm for less than 5 months is sufficient to sustain endemic malaria transmission (Lindsay *et al.*, 1996).

II. Temperature

Temperature plays an important role in driving both malaria vector and parasite development rates within mosquito vectors. The rate of egg development through the larval and pupal stages to the adult mosquito largely depends on temperature. As temperature increases, the time required for mosquito development shortens (Rueda *et al.*, 1990). For example at 16⁰C, larval development may last more than 45 days, compared to only 10 days at 30⁰C. Tekleheimanot *et al.*, (2004) reports that at lower temperatures, the larval and pupal stages of mosquitoes take longer time to complete 47 days at 16⁰C and in Ethiopia and a small but drops to 37 days at 17⁰C with a just small rise in temperature. Long development cycle due to low temperatures result in a reduction in the number of mosquito generations, puts larvae at the risk of predators, and eventually reduces adult population size. It is clear that by affecting the duration of the aquatic stage in the mosquito life cycle, temperature determines the timing and abundance of mosquitoes following adequate rainfall.

Female adult mosquitoes seek a blood meal after emergence and may ingest malaria parasites (gametocytes) with the blood of infected hosts. Martens (1995) reports that the blood feeding frequency of mosquitoes increases with temperature, leading to more host vector contact and

resulting in increased proportions of infective mosquitoes. Very low temperatures limit vector development by lengthening the gonotrophic cycle and reducing survival rate. Temperature also affects vector survival and the sporogonic cycle of the parasite. *Anopheles gambiae* for example, only exists in frost-free regions (De Meillon, 1934) or where absolute minimum temperatures in winter remain above 5⁰C in Africa (Leeson, 1931). Empirical studies have demonstrated the duration of the sporogonic cycle varies inversely with environmental temperature.

The duration of the extrinsic phase of the malaria parasite, which is the development of the ookinete in the midgut of the anopheline mosquito, also depends on temperature. The development of malaria parasites and the biting activities of mosquitoes decreases with temperature. In addition, the incubation time for *P. falciparum* is 26 days at 20°C but when the temperature is increased to 25°C, the incubation period is shortened to 13 days (Buynavanich and Landrigan, 2003). On average, the sporogonic cycle lasts about 10 days, but shortens as temperature increases becoming as short as five days when the temperature exceeds 30°C (Macdonald, 1957). These biological characteristics explain why effective malaria transmission can occur only in areas with a temperature higher than 20°C (Lindsay and Martens, 1998) and indicate the potential role that increasing temperature can play in the transmission of malaria and other vector-borne diseases.

Several studies have investigated the effects of climatic variables/weather on transmission of vector borne diseases. Mbogo *et al.*, (2003) observed variation in the relationship between the mosquito population and rainfall in different districts of Kenya and attributed the variation to environmental heterogeneity. Similarly, Zhou *et al.*, (2004) showed that there was high

spatial variation in the sensitivity of malaria outpatient numbers to climate fluctuations in the East African highlands.

One of the most striking uncertainties in the literature on weather and malaria is the variability in the reported relationship between rainfall and malaria, with several studies showing the importance of rainfall as a precipitating factor for malaria transmission (Loevinsohn, 1999; Lindblade *et al.*, 1999, Kilian *et al.*, 1999; Bouma *et al.*, 1996) while other studies show negative or neutral effects (Lindsay *et al.*, 2000; Woube, 1997). Occurrence of a positive association between rainfall and malaria cases requires that temperatures be warm enough to support mosquito and parasite development; enabling the impact of rainfall on malaria cases to become immediate in warmer temperatures. This is consistent with laboratory findings that established mosquito populations peak early at higher temperatures, while low temperatures are characterized by slow, steady growth with a delayed peak (Alto and Juliano, 2001). On the other hand, data from studies on the timing of the mosquito life cycle suggest that malaria cases should peak following periods of increased temperature and rainfall. Because temperature accelerates several steps in the process of mosquito and parasite development, the time lag between the appearance of suitable weather conditions and the appearance of new malaria cases should shorten as temperature rises. For example, at an average temperature of 20°C, the aquatic phase of the mosquito is usually completed in about 28 days (5 days for the eggs to hatch and 23 days for the larva to develop to the adult stage); and sporogony is completed in about 28 days. At this temperature, malaria cases should appear 9-10 weeks following rainfall, assuming an average incubation period of about 10-16 days. The number of malaria cases should be positively related to increases in temperature i.e. when the mean temperature is higher, e.g 30°C, the aquatic stages of the mosquito and the sporogony cycles are completed in about 12 and 8 days respectively, and

malaria cases should appear 4-5 weeks following rainfall. It is expected that the time lag in the effect of temperature should also be shorter. An integration of the climatic and environmental factors governing the distribution of insect vectors and the pathogens they transmit provides a powerful system for assessing disease vector dynamics in relation to disease patterns and impact of control measures.

III. Humidity

Humidity is an important environmental parameter with respect to the survival of mosquitoes. Relative humidity is defined as the ratio of water vapor content of the air to its total capacity at a given temperature (Woodward and Hales, 1998). Relative humidity affects dispersal, mating, feeding behavior, and oviposition of vector species. Humidity also affects the rate of evaporation of water at breeding sites. High relative humidity favours metabolic processes in the vector, prolongs survival of the mosquito vector and allows the parasite to complete the necessary life cycle so that it can transmit the malaria parasite to several hosts (Lindsay and Mackenzie, 1997). Low humidity levels may force vectors to feed more frequently to compensate for dehydration. Under conditions of optimal humidity, mosquitoes tend to survive for longer periods, which allow them to disperse farther and to have greater opportunity to participate in malaria transmission cycles (Lindsay and Mackenzie, 1997; Liehne, 1998).

Anopheles mosquitoes transmit malaria parasites when the environmental parameters, such as water availability, temperature and humidity permit. In many parts of the world where temperature is not a limiting factor, malaria transmission is highly seasonal, with its peak following the period of peak rainfall. Understanding how malaria varies in the community as a result of seasonal or year-to-year changes in environmental factors is important for the

planning of national malaria control programs since it may allow interventions to be adapted to specific sites or times of the year. This is essential for effective disease control.

2.3.1.2 Vector factors and refractory mosquitoes

Out of over 80 *Anopheles* species implicated in malaria transmission, only 40 are of major public health importance (Bruce Chwatt, 1980), with *An.gambiae*, *An. funestus* and *An. arabiensis* being the most efficient vectors for *P. falciparum* transmission in sub-Saharan Africa. Vector efficiency in malaria transmission is determined by factors such as the degree of preference for human blood feeding (Mbogo *et al.*, 2003), vector abundance (Minakawa *et al.*, 2002) and the survival probability (Charlwood *et al.*, 1997) of female mosquitoes.

Malaria transmission is directly proportional to vector abundance, the number of infective bites per day per mosquito and the probability of the mosquito surviving long enough to become infective (Trips and Hausermann, 1986). Mosquito survival through the sporogonic stages is most important because the successful completion of this stage ensures parasite development from gametocyte ingestion to inoculation, a process which can last as long as 8 to 30 days depending on ambient temperature. In a highly anthropophilic vector population, with high adult mosquito population densities, the successful completion of sporogony is likely to result in high entomologic inoculation rate (EIR). EIR is a measure of the intensity of malaria transmission, expressed as the number of sporozoite positive mosquito bites per year. The EIR is likely to be high if vector density and man biting rate is high. In Africa, EIRs are highly variable, ranging from <1 to >1000 infective bites per person per year (Beier *et al.*, 1999). Generally, higher EIRs are associated with stable and intense malaria transmission (Mbogo *et al.*, 1995). Beier and others (1999) found that the relationship between EIR and malaria transmission in Africa is such that any detectable EIR is associated

with prevalence rates of *P. falciparum* malaria, large enough to have an impact on public health. Mbogo *et al.*, (1995) however recorded *P. falciparum* prevalence rates of 44.7% and 49.3% in two respective communities on the Kenyan coast; where EIRs were 0.001 and another where no infected mosquitoes were found. This indicates high malaria prevalence rates can be observed with extremely low or non detectable EIRs. The implication of this for malaria vector control is that the extent of control efforts employed in any epidemiological setting will have to be specific to the level of parasite transmission. Substantial reductions in transmission intensity are necessary to reduce the prevalence of malaria infection in human populations.

Genetic alteration of mosquitoes offers a possible solution to the complex malaria problem. Though genetic manipulation as a vector control method has been discussed since the 1960s (Curtis 1968), improvements in the last 15-20 years associated with genetic technologies have revived the concept (Atkinson *et al.* 2001). Genetic transformation is the introduction of heritable genetic material from one individual to another. There has been previous success with genetic control programs, such as screwworm fly (*Cochliomygia hominivorax*) eradication through release of sterile males (Krafsur *et al.* 1986). The concept behind genetically modified mosquitoes is the idea that potential vectors can be modified through the introduction of novel genes that prevent parasite transmission.

Three main challenges impede the creation of transgenic mosquitoes (O'Brochta 2002). The first challenge is delivering the desired DNA (via fine glass needle) into the germ cells of a mosquito egg without physical damage. The second challenge is that, once inserted, this introduced DNA plasmid must rapidly recombine with the mosquito's chromosomal DNA.

Significant research has been dedicated to discovering new gene insertion methods. In the past two decades, techniques involving transposable elements, viruses and in vivo recombination systems have all been studied as potential gene vectors (Atkinson *et al.* 2001). The third challenge is integration of the inserted DNA. A transposable element introduced with the desired gene solves this problem.

An. gambiae was the first mosquito species for which genetic transformation was reported (Miller *et al.* 1987). Miller *et al.* attempted transformation with the *Drosophila* transposable P-element. After thousands of attempts, one embryo was transformed with a gene that confers resistance to a neomycin analog, G-418. Despite this success, the results indicated that this transformation was not a result of the P-element transposition. Clearly, further gene vector development was needed (Moreira *et al.* 2002). The advent of mosquito gene vector and recombination technology reignited genetic modification efforts for *An. gambiae*. With the publication of the *An. gambiae* genome revealed new potential targets for genetically- based control. Furthermore, the mosquito genes and pathways involved in vectoring malaria can be pinpointed; this discovery will elucidate previously unknown details about malaria transmission. In 2000, Grossman *et al.* (2001) reported stable transformation of *An. gambiae* to express the EGFP marker using the newly developed *piggyBac* transposable element. Recently, this success was extended when *Plasmodium* refractory genes were inserted into a transformed *An. gambiae* germ-line (Kim *et al.* 2004).

In addition, other Anopheline species have been genetically modified in the effort to eliminate malaria. *An. stephensi*, the major malaria vector on the Indian subcontinent, has also been a subject of genetic control research. This species has been transformed with the *Minos* transposon to express an EGFP marker (Catteruccia *et al.* 2000) and with the *piggyBac*

transposon to express the red fluorescent protein, dsRED (Nolan *et al.* 2002). *An. albimanus* is a significant South American malaria vector, also present in the southwestern United States. Germ-line transformation of this species was also achieved with a *piggyBac* transposon containing the exogenous EGFP marker (Perera *et al.* 2002).

Genetic modification of mosquito vectors from other disease systems has also been accomplished. *Aedes aegypti*, a vector of yellow fever and dengue virus, has been a focus of genetic modification efforts. In 1998, stable integration of the *Drosophila melanogaster* cinnabar gene with the transposon *Mariner* (*Mos1-cn+*) into an *Aedes aegypti* germ-line was one of the first successful mosquito transformation experiments (Coates *et al.* 1998). *Ae. aegypti* has also been transformed to express markers *Drosophila melanogaster* cinnabar (*cn*) (Jansinskiene *et al.* 1998), *luciferase* (*luc*) (Coates *et al.* 1999) and EGFP (Pinkerton *et al.* 2000) with the *Hermes* transposon. The *piggyBac* transposable element has also been a gene vector for EGFP transformation (Kokoza *et al.* 2001) and for *cn*, the *Drosophila* eye color gene. Finally, *Culex quinquefasciatus*, a vector for West Nile Virus, has been transformed to express EGFP with the *Hermes* transposable element (Allen *et al.* 2001).

That many species that have been successfully transformed in the past few years demonstrates the feasibility of genetic transformation (O'Brochta, 2002). The ultimate goal in the development of transgenic *An. gambiae* is their release into a natural system. Instead of attempting to eradicate all malaria mosquitoes, this approach proposes to eliminate mosquitoes' ability to vector the disease. Ideally, genetically modified mosquitoes would displace or out-compete endemic mosquito populations in hyper-endemic areas of Africa.

For this strategy to succeed, the released transgenic mosquitoes must have fitness equal or greater to their wild counterparts (Scott *et al.* 2002).

2.3.1.3 Parasite factors

Four *Plasmodium* parasites, namely *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae* are responsible for malaria infection in humans. Of the four, *P. falciparum* is the most virulent (Gupta *et al.*, 1994). The other *Plasmodium* species have also been reported to cause serious illness, for example *P. vivax* is widespread in Asia and Central and South America where it reportedly causes fewer severe complications but relapses months after infecting the host because of secondary exo-erythrocytic cycles. *Plasmodium malariae* can persist in humans as an asymptomatic erythrocytic disease for many years following an untreated or incompletely treated primary infection. This parasite has been associated with renal complications (Kulwichit *et al.*, 2000). *Plasmodium ovale* is the rarest of the four species and is apparently more restricted in distribution. However, it is common in the West African countries of Ghana, Liberia, and Nigeria and in neighboring areas (Chin and Contacos, 1966; Richter *et al.*, 2004).

The resistance of *Plasmodium* parasites to antimalarial drugs has complicated the treatment of malaria in sub-Saharan Africa and is one of the most serious threats to the control of malaria. Drug resistance has led to withdrawal of cheap drugs such as chloroquine from the malaria drug regimen in almost all parts of Africa, necessitating the use of drugs that are more expensive and may have serious side effects.

Plasmodium parasites have extremely complex genomes and the ease with which they can switch between the microenvironments in different hosts, and the metabolic changes required illustrates the difficulty in studying the exact modes of action of the anti-malarial drugs on parasite metabolism (WHO, 1987). Resistance develops more quickly where large populations of parasites are exposed to drug pressure. The increasingly rapid spread of resistant malaria may be due to an increasingly efficient mosquito vector. This phenomenon may be explained by the increased oocyst formation efficiency that has been observed in resistant species (Bruce Chwatt, 1980) and reduction in gametocyte formation rates, and transmission after treatment with co-artemether (Sutherland *et al.*, 2005). The resistant forms undoubtedly have a biological advantage in influencing malaria transmission.

2.4 Evaluation of Malaria Parasite Transmission

The evaluation of malaria parasite transmission rates in nature is performed by the identification of the source of blood meals using direct Enzyme-Linked Immunosorbent Assay (ELISA) [Beier *et al.*, 1988] and determination of the sporozoite loads in the salivary glands

2.4.1 Detection of mosquito blood meal sources

The identification of blood meals taken by malaria vectors is an important procedure in malaria epidemiology because the degree of human feeding influences the probability that mosquitoes will come into contact with gametocyte carriers and thus acquire and transmit *Plasmodium* infections. Garret-Jones *et al.* (1980) demonstrated that the most successful vectors of malaria fed most commonly on humans and secondarily on cattle and other domestic animals, depending on host availability. In tropical Africa, it has been estimated that 80% of *Anopheles* species feed on any large mammal that is available. In areas where active

malaria transmission is being reintroduced after a period of eradication or low transmission, the identification of blood meals from mosquitoes can provide important epidemiological information on host preference by different species, and a useful guide on which species to target for control. Along the Kenyan coast, a high degree of preference to human feeding by *Anopheles* vectors of malaria has been reported as a major factor contributing to their efficiency of *P. falciparum* transmission. Mbogo *et al.* (1993b) reported a human blood feeding rate of 94.4% in *An. gambiae* s.l and 90.8% in *An. funestus* Giles along the Kenyan coast. The high preference for human feeding, facilitating frequent vector contact with gametocyte carriers provides evidence for the high malaria transmission observed along the Kenyan coast. Several methods have been developed for the detection of the different sources of blood meals in disease vectors. These include the use of enzyme linked immunosorbent assay (ELISA), precipitin tests, haemagglutination assays, counter current immunoelectrophoresis and immunofluorescence. The ELISA method developed by Beier *et al.* (1988) is the most commonly used test. The ELISA assay meets the criteria for a field operational kit for the detection of blood meals in mosquitoes and has been the most commonly used technique. A brief description of this method is given in the section on methods of this thesis.

2.4.2 Determination of sporozoite infection rates in wild mosquitoes

The rate of mosquito infectivity in the field has always been measured by determining the proportion found to be carrying *Plasmodium* sporozoites. Previously, the determination of sporozoites rates was done by the dissection of the salivary glands, and this was obviously a tedious, and labour intensive process, especially in areas where mosquito density was high. In addition, this process requires fresh female mosquito samples. This method was not easily applicable in the field. Wirtz *et al.* (1987) developed an ELISA method for the detection of

the *P. falciparum* circumsporozoite protein in field-collected mosquitoes. ELISA combines the specificity of antibodies with the sensitivity of simple enzyme assays, by using antibodies or antigens coupled to an easily-assayed enzyme. There are two main variations of this method: The ELISA can be used to detect the presence of antigens that are recognized by an antibody or it can be used to test for antibodies that recognize an antigen. In the sporozoite ELISA technique, monoclonal antibodies are used to detect circumsporozoite proteins of *P. falciparum* (Wirtz *et al.*, 1987) and the results are read visually by using an ELISA reader (Wirtz *et al.*, 1987; Beier *et al.*, 1988). The sporozoite ELISA technique is more rapid, and is very useful in detecting infectious mosquito species from either fresh or dry stored mosquito specimen. Sporozoite rates are then inferred from the proportion of human biting anophelines that test positive for *P. falciparum* circumsporozoite protein by ELISA.

2.4.3 Entomological inoculation rates (EIR)

The intensity of malaria parasite transmission in the field is estimated by determination of the entomological inoculation rate (EIR). EIR is the product of the mosquito biting rate and the proportion of mosquitoes carrying sporozoites in their salivary glands (Wirtz *et al.*, 1987; Beier *et al.*, 1999). EIR can be used to estimate the level of transmission to individuals living in a particular location at a time and as a measure to differentiate transmission intensity between geographic areas (different villages or parts of villages) over a period of time. In Africa, EIRs in malaria endemic areas have been reported to range from between <1 to >1000 infective bites per person per year (Trape and Rogier, 1996). This reflects the extent to which the malaria transmission situation is variable in different parts of Africa, hence the need for more area specific control measures. Any control measures aimed at reducing malaria

prevalence may need to substantially reduce EIR's in order to reduce the prevalence of malaria infection.

2.5 Dry season survival mechanisms

2.5.1 Hidden refugia populations

Lewis (1944) found *An. gambiae* in the Gezira area of the Sudan hiding in earth cracks during the dry season. Species survival and perpetuation could be maintained through the establishment of refugia populations normally hidden with respect to conventional sampling i.e., at high densities in restricted areas. These populations can only be found by active search (Charlwood and Billingsley, 2000). Meillon (1934) found adult females, which took frequent blood meals, resting under stones during the dry season. Holstein (1954) found female *An. gambiae* passing the dry season in dwelling huts, ruined or uninhabited huts, holes in rocks and cracks in soil, covered pigsties, rabbit hutches, hencoops, cattle pens and dry wells, at Bobo-Dioulasso, Upper Volta.

2.5.2 Exploitation of container and continuous breeding

The availability of alternative container breeding sites might make physiological diapause in females unnecessary due to presence of oviposition sites (Omer *et al.* 1970). Numerous records of breeding in underground or container water collections during periods when suitable surface water is absent, or rare, are summarized by Gillies &, de Meillon (1968). Evidence of continuous breeding by *An. arabiensis* during extended dry periods in parts of Africa where surface waters are scarce (De Meillon, 1938). This is achievable through breeding in household water storage containers. *An. arabiensis* can adapt to maintain itself in the drier parts of Africa by breeding in non-obvious aquatic sites with the following order of

preference: surface water, water in pits or shallow wells and container (tanks) water (Omer *et al.* 1970).

2.5.3 Diapause

The adaptive incidence and intensity of diapause varies from one environment to another (Andrewartha, 1952. Holstein (1954) found female *An. gambiae* passing the dry season in hidden refugia with completely developed ovaries and were able to feed whenever the opportunity arose. There was no observable evidence of fat reserves, leading him to conclude what he had observed was a state half way between gonotrophic dissociation and gonotrophic concordance. Omer and Mustafa (1986) observed significantly more females during the cool season than during the rest of the hotter parts of the year. It has been mentioned by other workers this may be related to the retardation of ovarian development due to low prevailing ambient temperatures. Several other workers previously pointed out the possibility of the gonotrophic cycle of *A. gambiae s.l.* being extended to more than five days under temperature conditions below 70F (21⁰C) (Muirhead-Thompson, 1945; Omer, 1968). Holstein (1954) was able to demonstrate the ability of females to survive for many months with curtailed gonotrophic activity under low humidity weather conditions in an arid area of West Africa. Omer & Cloudsley-Thompson (1968, 1970) gave a report of female *An. gambiae* being able to survive, in a state of retarded ovarian development, through the nine months long dry season west of the Nile. However, there was concurrent low level breeding throughout the dry season in the Nile Valley.

2.5.4 Reduced feeding activity

It is hypothesized that it is possible to ascertain the size of a blood meal within the gut of anopheline females to investigate feeding behavior. Omer and Cloudsey (1970) observed blood-feeding as being basically for nutrition and survival during the dry season and not necessarily for egg development among diapausing females with gonotrophic dissociation.

2.6 Statement of the problem

Most semi arid areas are currently hit by malaria epidemics. These areas present peculiar physical conditions that have to be understood for sustainable vector management and providing site-specific knowledge about *Anopheles* ecology in semi arid ecosystems as a basis for targeted malaria control.

2.7 Justification

Malaria is a major cause of mortality and morbidity in drylands. Data on the Anophelinae are not conclusive with respect to how malaria vectors survive in semi-arid conditions and extended periods of dry weather.

2.8 Research Hypotheses

- 1) Abundance, species composition, diversity and resting behaviour of malaria vectors in semiarid areas in Kenya vary with season.
- 2) Aggregation sites of immature stages of malaria vectors vary with season.
- 3) Malaria vectors are physiologically and reproductively adapted to survive semi-arid conditions and extended periods of dry weather

2.9 Objectives

2.9.1 General Objectives

To provide critical information on the ecology of malaria vectors in semi-arid ecosystems as a model for understanding the dynamics of *Anopheles* mosquito populations and malaria transmission for targeted control interventions in Kenya.

2.9.2 Specific Objectives

1. To evaluate the abundance, species composition, and diversity of malaria vectors in semiarid sites in Baringo District
2. Identify and characterize mosquito aggregation sites in semi arid sites as a basis for targeted vector control;
3. Study the physiological adaptations and reproductive behaviour of vector species during extended periods of dry weather.

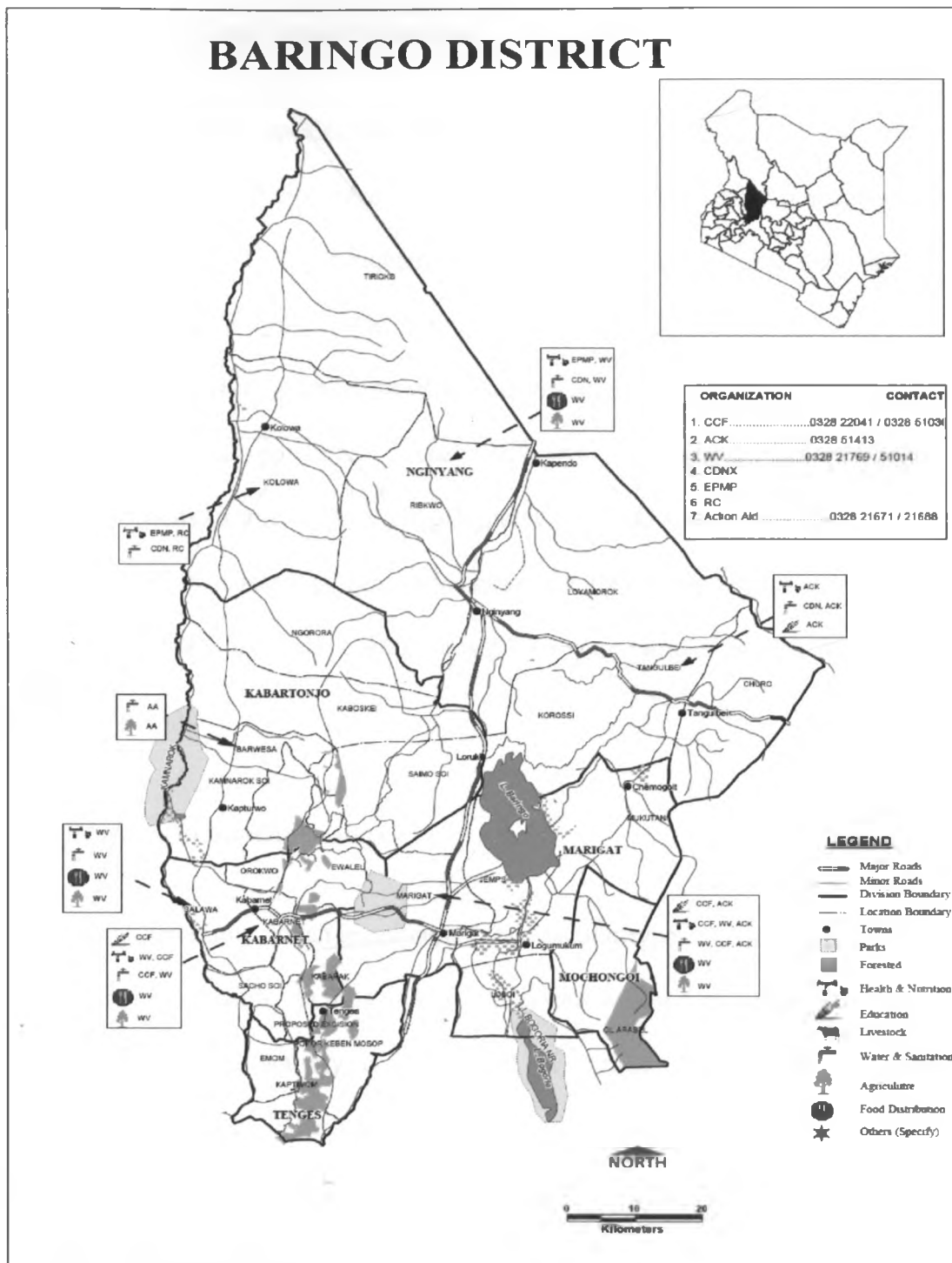
CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study site

The study carried out was in Kamarimar and Tirion villages in Marigat division of Baringo district in Kenya. The two villages are located approximately 20 km and 17 km respectively, away from Marigat town (Plate 1). The town is about 250 km north-west of Nairobi and is situated 0.45N and 36E activity. Accessibility and availability of known breeding sites are the factors that influenced site choice. The division is semi-arid with an average but unreliable annual rainfall of between 500 and 600mm, coupled with high average temperature of above 32°C that results in elimination of temporary standing water in a matter of days. The average altitude of the study area is about 700 meters above the sea level and most of it is rangelands with pastoralism being the main activity. The main rainy season occurs between the months of March and June. The short rains come between October and December but in some years these are scanty or totally absent. There is usually a long dry period from October to February whenever the short rains fail, characterized by high temperatures and strong dusty winds, especially from January; with little rainfall. These harsh ecological conditions ensure only permanent water sources remain the foci of *Anopheles gambiae* s.l and *Anopheles funestus* breeding, which occurs in low numbers through out the year (Aniedu *et. al.*, 1989, Aniedu, 1993, Aniedu 1997)

BARINGO DISTRICT



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

Prepared by OCHA June 2001

Plate 1: Map of Baringo District showing the study area

3.2 Study design

3.2.1 Species composition and resting behaviour

Changes in vector population, species composition and diversity between the wet and dry season in the selected semiarid site were assessed under this task. Vector populations are adapted to surviving in limited refugia (aquatic habitats) during the dry season and may have variable potential for malaria transmission in the course of time. It is likely that changes in the risk of malaria transmission is linked to variations in vector composition and abundance, which determines vectorial potential. These variations are dictated by changes in meteorological conditions in a given area. It is hypothesised that different species and sub species of malaria vectors coexist in semi arid areas and that these vectors vary in their feeding, resting and reproductive habits. Adult mosquitoes were sampled and processed using different methods.

3.2.2 Aggregation sites of immature stages

This activity aims to determine which anopheline larval habitat types are most productive in terms of larval and pupal densities in the dry and wet seasons within semi-arid environments, and how vector species productivity is partitioned over time. It is hypothesised that permanent and semi-permanent water sources such as rivers, lakes and swamps are the main sites where female malaria vectors oviposit their eggs and that the temporary breeding sites dry out at the onset of the dry season.

3.2.3 Physiological adaptations

It is hypothesized that during dry season mosquitoes go through reduced reproductive activities. It is therefore expected that the duration between two consecutive gonotrophic

cycles will be much wider in the dry season as compared to normal humid conditions. Parity, gonotrophy, measurement of reproductive activity and fecundity are the key parameters that were investigated under this exercise.

3.3 Sample collection and processing

3.3.1 Adult mosquito sampling and identification

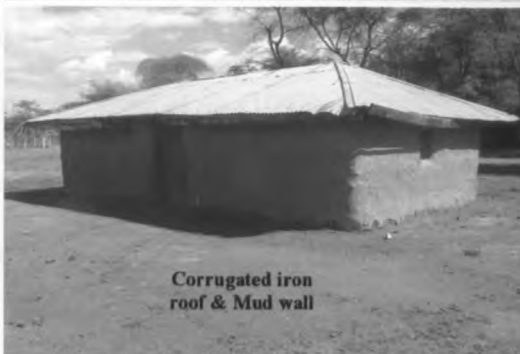
In each study site, mosquitoes were collected in 10 randomly selected houses by Pyrethrum Spray collections (PSC). The collections were carried out once every week between 0700 and 1100 hours for a period of 22 months (July 2008 – April 2010). Houses were categorized depending on materials used to construct the walls and the roofs (Plate 2). Other household characteristics such as size of eaves, distance to the nearest animal shed, use of insecticide treated bed nets and distance to the nearest habitat were recorded. Every week, a Centres for Disease Control (CDC) and prevention light trap (J.W. Hock Ltd, Gainesville, FL, U.S.A.) was operated in the main bedroom of each of the houses between 1800 and 0600 hours to estimate the human biting rates. Ten CDC light traps were operated outdoor during the night preceding PSC collection once weekly, to sample outdoor mosquitoes.



Grass thatched roof & Mud wall



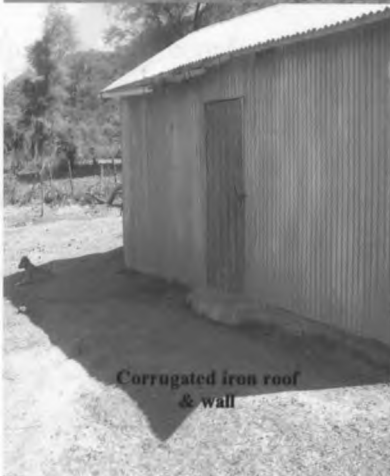
Grass thatched roof & stick wall



Corrugated iron roof & Mud wall



Corrugated iron roof & Wooden



Corrugated iron roof & wall



Traditional Kalenjin hut ((Porriet)



Corrugated iron roof & mud wall

Plate 2: Photographs of house types in Baringo

3.3.2 Mosquito identification

The samples were scored as unfed, blood-fed, semi-gravid or gravid by examining their abdomen under a dissecting microscope, and preserved in labelled vials containing anhydrous calcium sulphate. Collected specimens were identified to species morphologically (Gillies and De meillon 1968). A sub set of *Anopheles gambiae* s.l. mosquitoes were identified to sibling species of the *An. gambiae* complex using species-specific polymerase chain reaction technique of Scott *et al.* (1993).

Larvae were transferred from the dipper by pipetting into a white collecting tray with clear water for categorization into different instar stages, followed by counting, morphological identification and recording (WHO, 1975). The 3rd and 4th instar anophelines were identified morphologically using taxonomic keys of Gillies and De Meillon (1968) and Gillies and Coetzee (1987). Larvae were reared to adult stage and 500 randomly selected emerged *Anopheles gambiae* s.l adults identified to sub-species by polymerase chain reaction (PCR) method (Scott *et al.* 1993).

3.3.3 Determination of sporozoite infection rates in malaria vectors

The female mosquitoes were assayed for sporozoites using the ELISA technique. The head and thorax of individual female *Anopheles* mosquitoes were removed and put in 1.5 ml micro-centrifuge tubes with perforated cups and kept dry with desiccant in zip-lock bags until used. The head and thorax of individual female mosquitoes were homogenized in 250 µl of grinding buffer (PBS, pH 7.4 containing 0.5% NP-40 and 0.5% casein) using a glass pestle.

CS protein micro-plate ELISA using 50 µl /well of the homogenate was done in 96-well microtitre plates coated with anti-*P. falciparum* monoclonal antibodies at 22–25°C for 30 min (Wirtz *et al.* 1987). Captured CS antigen were revealed by monoclonal antibody (MoAb) horseradish peroxidase conjugate incubated for 1 h. Addition of ABTS [2,2,6,6-tetramethyl-3,4-dihydro-2H-pyridine-3,5-dithione] substrate was used to give colour changes for positive results which were read by visual assessment of the colour reactions, and OD measured within 30 min using spectrophotometer (Multiskan Ascent, Model 354; ThermoLabsystems, Finland) at 414 nm.

Sample positivity was determined by titration of PfCSP-positive control antigen using cut-off OD values equivalent to 12pg of PfCSP or 50 sporozoites (Collins *et al.* 1988). The PfCSP concentrations of test samples was determined by extrapolation from a graph of PfCSP OD versus protein concentration in the controls, and the corresponding sporozoite loads estimated. Slides containing asexual stages and gametocytes were dehemoglobinized with 0.3 N HCl and slides with sporozoites fixed with methanol. The procedure, which was the same for all 3 antigens, involved first adding to each spot a 5 µl aliquot of a 1:50 dilution of mosquito blood meal in phosphate buffered saline (pH 7.4-). After, incubating for 30 min at room temperature in a moist chamber, each spot was washed twice with PBS, and then incubated for another 30 min with 5 µl of a 1:10 dilution of FITC anti-human IgG (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD). After washing 3 times with PBS, a cover slip mounted with glycerol buffer was added. Slides were examined at 400x using fluorescent microscopy. The degree of fluorescence observed for each sample, for all parasite antigens, was evaluated against reactions for 1:20 to 1:80 dilutions of control and immune sera. Circumsporozoite protein rates were estimated.

3.3.4 Determination of host preference of malaria vectors

The abdomen from each blood-fed mosquito was homogenized in 50 μ l of phosphate buffered saline (PBS) and 950 μ l of grinding buffer. Blood meals were identified by direct enzyme-linked immunosorbent assay (ELISA) anti-host IgG conjugate against human and bovine in a single-step assay. Any non-reacting samples were subsequently tested using goat IgG. Fifty microlitres of the mosquito triturate was added to U-shaped bottom 96-well microtitre plates and incubated overnight at room temperature. Each well was then washed twice with PBS containing 0.5% Tween 20, followed by addition of 50 μ l of host specific conjugate in 0.5% boiled casein containing 0.025% Tween 20. The ELISA results were read visually according to the protocol of Beier *et al.* (1988). The human blood index (HBI) and bovine blood index (BBI) were calculated as the proportion of blood-fed mosquito samples that had fed on either human or bovine out of the total tested. The sporozoite rate (SR) was computed as the proportion of mosquitoes positive for *P. falciparum* CSPs out of the total tested. EIR was derived as the product of human biting rate (HBR) estimated as the geometric mean number of vectors caught in a light trap/ per house and the sporozoite rate.

Using forceps, PSC and light trap collected mosquitoes were placed in wide mouthed vacuum flasks or in commercial cold boxes to keep them cool. On return to the laboratory, mosquitoes from each house were identified, and stored dry at room temperature in screw-capped tubes filled to the quarter level with drierite with a layer of cotton wool at the top. This layer of cotton wool served the purpose of separating mosquitoes from drierite. The tubes were tightly corked to reduce influx of free flowing air. The tubes were labeled according to species, site, house number and collection date. Tests were performed on individual, blood fed *Anopheles gambiae* s.l. The selection process involved choosing, at random, up to 5 tubes (i.e., representing up to 5 houses) from Kapkuikui and 5 tubes from

Tirion before the rainy season and the same for after the rainy season. For each of the tubes, up to 3 blood-fed *An. gambiae* s.l. per vial were selected at random for testing. Individual *An. gambiae* s.l. were triturated in 50 µl PBS, samples were centrifuged at 16,000 g for 5 min, and supernatants stored at -2 °C. Samples; diluted to 1:50 with PBS were screened first for the presence of human ,bovine, small ruminant,hippopotamus, suidae, canidae, wild ruminant, buffalos, felidae avian or bovine IgG using a direct ELISA for blood meal identification (Beier et al. 1988). Samples testing positive for human IgG were then tested by IFA against the 3 *P. falciparum* antigens as described below. Anthropophilic rates were estimated.

3.3.5 Habitat census

All water bodies were located and mapped with geopositioning equipment (GPS) in July 2008. A total of 25 discrete habitats (14 and 11 in Kamarimar and Tirion respectively) were mapped and assigned numbers. Each habitat was sampled by visual inspection, dipper, and hand-picking with a pipette for preliminary classification by presence or absence of anopheline and/or culicine larvae. Distance of each water body to the nearest house was estimated from Geographic Information System (GIS) maps of the study area.

3.3.6 Water chemistry analysis

Physical characteristics of the larval habitats, including water depth, turbidity, presence of floating and/or emergent vegetation were recorded. Water depth was measured using a metal ruler. Turbidity was measured through visual examination of water against a white background and categorized as either clear or turbid. A record of whether the habitat was wet or dry at the time of the visit was taken. Water pH, conductivity, and temperature were

measured using hand-held YSI 650 Multiparameter Display System (YSI Environmental, YSI Incorporated, Yellow Springs, OH). Salinity and TDS were measured in the field using the hand-held YSI EC 300 (YSI Environmental).

3.3.7 Larval sampling

All potential breeding sites were sampled longitudinally using a standard mosquito dipper (350 mL) once weekly for a period of 22 months from July 2008 to April 2010. Ten dips were taken from each habitat. In small habitats where this was not practical, larvae were collected individually using plastic pipettes on a daily basis.

3.3.8 Distinguishing nulliparous from parous Mosquitoes

The ovary tracheation method of Detinova (1962) was used to distinguish nulliparous from parous females. Adults were dissected in a drop of phosphate-buffered saline (PBS) on a glass microscope slide under a dissecting microscope at 40x magnification. One ovary from each female was placed in a drop of distilled water, and allowed to air dry at room temperature on the slide to reveal ovary tracheation (Detinova, 1962). The second ovary was dissected and further observations of ovarioles made under a compound microscope fitted with differential interference contrast (DIC) optics at 200x magnification (Olympus Optical, Tokyo, Japan). Ovary development was classified according to Christophers (1911) stages, as modified by Clements and Boocock (1984). Females were classified by one investigator (AOM) as nulliparous or parous based on the condition of ovary tracheation. A second technologist familiar with the Detinova (1962) method observed the same ovaries and provided an independent estimate of the parity status.

3.3.10 Assessment of mating frequency

At the time of capture, the *Anopheles* females were kept in cool-boxes for the trip to the laboratory where they were placed in test tubes and immobilized by chilling just prior to being placed on the stage of the dissecting microscope. The terminal portion of the abdomen was placed in a drop of physiological saline on a glass microscope slide. A minuten probe was introduced between the cerci, and with a slight twisting and withdrawal motion, the spermatheca was removed, and observed for the presence of sperm. By grasping the 7th abdominal" segment with a pair of fine tweezers and gently pulling the end of the abdomen, the reproductive organs and most of the alimentary canal were exposed. By changing the magnification and altering the incidence of light reflected from the sub stage mirror, the stomach and ovary tracheation could be observed.

3.3.11 Experimental design for estimation of gonotrophic cycle duration

Two hundred 4-d-old virgin F1 female *Anopheles arabiensis* mosquitoes were placed into a 30 by 30 by 30-cm³ cage and bloodfed on a rabbit for 30 min. Mosquitoes that failed to feed were aspirated out of the cage. An equal number of virgin *Anopheles arabiensis* male mosquitoes were introduced into the cages to allow females to mate for 24 h. Cages were suspended from the laboratory roof at a distance of 2 m above the ground in the laboratory using greased suspension twines to block ants from reaching the cages. Each of the 100 female blood-fed mosquitoes was transferred into an individual oviposition cup after 24 hours. The oviposition cups, 10 cm in width and 12 cm in height, each contained a piece of filter paper on a wet cotton wool pad to provide an oviposition substrate, and the cups were placed on a table inside the laboratory. Grease was applied on table legs to prevent ants from accessing the oviposition cups. The number of eggs oviposited per female mosquito during the first gonotrophic cycle was counted under a dissection microscope and recorded. Females that oviposited were given a second blood meal to determine the duration of the second gonotrophic cycle but no egg counts were done. The studies were carried out in during the dry season in September 2009 and repeated during the rainy season in the month of February 2010.

HOBO data loggers (Onset Computer Corporation, Bourne, MA) were placed inside the laboratory where the gonotrophic cycle length was measured, to record temperature and relative humidity from 1st July 2008 to April 31 2010. The data loggers were suspended from the roof, 2 m above the ground. Outdoor temperature was recorded by placing three HOBO data loggers in standard meteorological boxes, 2 m above the ground for the same time period described above. These data were offloaded from the data loggers using a Hobo Shuttle Data

Transporter (Shuttle, Onset Computer Corporation) and then downloaded to the computer using BoxCar Pro 4.0 (Onset Computer Corporation).

3.3.12 Determination of parity states

Females at any stage of blood digestion and ovarian development were used for dissection exercises. The ovaries were examined under a binocular microscope. During dissection the ovaries were always kept in a small drop of physiological saline. The ovary was moved to the edge of the drop and the ovarioles examined in a small quantity of liquid. One of the ovaries lying in a drop of physiological saline was immobilized on the slide by means of a needle held in the left hand a needle held in the right hand was used to pierce the outer ovarian membrane in several places and then to remove it bit by bit. Where the membrane had been removed, the ovarioles appeared separated one from another. This made further examination much easier. The internal oviduct and the ovary were then pierced with the left-hand needle to immobilize them. The ovariole under examination was carefully moved aside from the internal oviduct with the right-hand needle. At the same time the complete terminal portion of the ovariole, which up until then had been pressed up against the internal oviduct by the developing follicle, were straightened out and its dilatations became visible. It is possible to count the dilatations only when the ovariole has been preserved in its entirety and when the site of its connection with the internal oviduct is visible. The numbers of dilatations in the ovarioles were counted using a manual counter followed by recording in data sheets. Parity rates were estimated. Comparisons of these proportions between dry and wet seasons were performed by Chi-square test.

3.3.13 Determination of fecundity rates

To examine fecundity, blood fed females of the *Anopheles* species were kept individually in plastic vials containing 30 ml tap water, and allowed to oviposit. The incidence of oviposition (percentage) by the blood fed females and percentage of eggs with at least an embryo among the total number of eggs laid was examined. Fecundity was recorded as the sum of oviposited and retained eggs.

3.3.14 Dissection of spermathecae to estimate insemination rates

Females were dissected in a drop of 0.8% saline under a stereoscopic microscope and examined for sperm in spermathecae as a measure of reproductive activity. At the time of capture, *Anopheles* females were placed in half-pint containers, which were in turn placed in a portable ice chest for the trip to the laboratory. In the laboratory, the mosquitoes were placed in test tubes, and immobilized by chilling just prior to being placed on the stage of the dissecting microscope. The terminal portion of the abdomen was placed in a drop of physiological saline on a glass microscope slide.

A minuten probe was introduced between the cerci. With a slight twisting and withdrawal motion, the spermatheca was removed and observed for the presence of sperm. By grasping the 7th abdominal" segment with a pair of fine tweezers and gently pulling the end of the abdomen, the reproductive organs and most of the alimentary canal' were exposed. By changing the magnification and' altering the incidence of light reflected from the sub stage mirror, the stomach and ovary tracheation could be observed.

3.3.15 Collection of meteorological data

A rain gauge (Tru-Chek®, Rain Gauge Division, Edwards Manufacturing Co. Albert Lea, MN, U.S.A.) was placed in each of the study villages and rainfall data recorded daily over a period of 22 months (July 2008 to April 2011). Temperature and relative humidity were measured using temperature and relative humidity data loggers (Onset Computer Corporation, Bourne, MA, U.S.A.).

3.4 Statistical Analysis

Data was analyzed using Statistical Analysis Software (SAS) Version 9.2 (SAS Institute, 2010). Data was checked for normality and homogeneity of variances and analysis of variance (ANOVA) was used to compare the mean differences in adult mosquito densities between villages and months. Chi-square and Fishers exact tests were done (as appropriate) to compare the differences in the human blood index (HBI), SR and HBR of *An. arabiensis* between seasons and villages. Pearson Correlation analysis was used to assess the relationship between rainfall and adult mosquito densities while multivariate logistic regression analysis done to assess the relationship between the measured micro-epidemiological characteristics and the occurrence of *An. arabiensis*.

Physical habitat characteristics such as habitat size, stability, and distance to the nearest house were categorized as dichotomous variables for analysis. The cut-offs for each variable was selected to maximize the number of habitats within each category using the methods of Mutuku *et. al.* (2006). Habitats were classified as large if their areas were greater than 5 m². For stability, habitats were classified as stable if they were flooded for at least 18 days. For distance to the nearest house, habitats were classified as near if they were within 50 m of a

human dwelling and far if they were greater than 50 m from a human dwelling. Variation in larval counts between villages and seasons was compared by Student *t*-test, and differences in larval counts among habitat types and months analyzed using one-way analysis of variance (ANOVA). Where significant differences were observed in ANOVA, the Tukey test was used to separate the means. Variation in diversity of habitat types between villages was compared using the Chi-square test. Pearson correlation analysis was used to assess the relationship between water chemistry covariates and larval counts in different habitat types and villages. Variation in larval densities and categories of habitat characteristics were analyzed using one-way analysis of variance (ANOVA). Larval counts were expressed as the number of larvae per 20 dips because the number of larvae sampled was low. Absolute larval counts were used for small habitats where dipping was not possible. Statistical analyses was done using log-transformed ($\log_{10} n + 1$) larval counts to normalize the data. Results were considered significant at $P < 0.05$.

Monthly average minimum or maximum temperatures were calculated from the daily record of minimum or maximum temperature. The daily mean temperature is the arithmetic mean of the 24 hourly temperature records of a day, and mean monthly temperature is the average of daily mean temperatures. Mean gonotrophic cycle duration is defined as the average number of days that female mosquitoes took to lay eggs after taking a blood meal. We compared the number of eggs laid in the dry season on one hand and wet season on the other to check for any interseasonal variation using the chi-square test. *t*-tests were conducted to evaluate the effect of ambient temperature and humidity on fecundity for each season. Fecundity data were square root-transformed. Comparisons of fecundity, parous states, insemination frequency and gonotrophic cycle duration between seasons by species were analyzed using the Chi-square test.

CHAPTER FOUR

4.0 RESULTS

4.1 Vector species abundance and resting behaviour

4.1.1 Species composition

Four *Anopheles* species were collected in the two study sites during the 22-month period (Table 1). These included *An. gambiae* s.l. (66.8%), *An. funestus* (17.9%), *An. pharoensis* (14.5%) and *An. coustani* (0.8%). *An. gambiae* s.l. and *An. coustani* were the most and the least abundant species in both study sites. *An. pharoensis* was second most abundant species in Kamarimar while *An. funestus* was the second most abundant species in Tirion. For all species, light trap collections were more productive than PSC. rDNA PCR analysis of 500 *An. gambiae* s.l. samples revealed *An. arabiensis* as the only sibling species present in the study area.

Table 1: Relative abundance of Anopheles mosquito species

| Mosquito species | Kamarimar | | Tirion | |
|-----------------------------|----------------|-------------|----------------|-------------|
| | Light trap (%) | PSC (%) | Light trap (%) | PSC (%) |
| <i>Anopheles funestus</i> | 800 (11.9) | 280(22) | 1840(24.7) | 72(5.8) |
| <i>Anopheles gambiae</i> | 4186 (62.2) | 799(62.9) | 5246(70.4) | 907(73.7) |
| <i>Anopheles pharoensis</i> | 1700 (25.3) | 180(14.2) | 300(4.0) | 240(19.5) |
| <i>Anopheles coustani</i> | 42(0.62) | 12(0.9) | 67(0.9) | 12(1.0)) |
| Overall Total | 6728 | 1271 | 7453 | 1231 |

4.1.2 Spatial and temporal distribution of mosquitoes

The densities of *An. arabiensis* were significantly higher in Tirion than in Kamarimar ($F = 10.76$, $df = 1$, $P = 0.001$) and were mostly collected in CDC light traps (outdoors) than in pyrethrum spray collections ($F = 7.58$, $df = 1$, $P = 0.001$). There were significant differences in adult densities across months in both Kamarimar ($F=6.66$, $df=18$, $P=0.001$), and Tirion ($F=6.77$, $df=18$, $P=0.001$). Adult densities were generally low with peaks in the months of July and November 2008, February, April and October 2009. However there was no clear-cut difference in monthly adult densities in the year 2010 (Figure 1). These populations were virtually undetectable in October 2008 and July 2009. The year 2010 experienced a lot of rainfall in the months of March and April, with *Anopheles arabiensis* densities remaining generally high but with low densities being observed in the dry months of January and February. When mosquito densities between wet the months and dry months were compared, no significant differences were observed. Similarly, there was no significant correlation between rainfall and adult mosquito density ($r = 0.01$, $df = 20$, $P>0.05$).

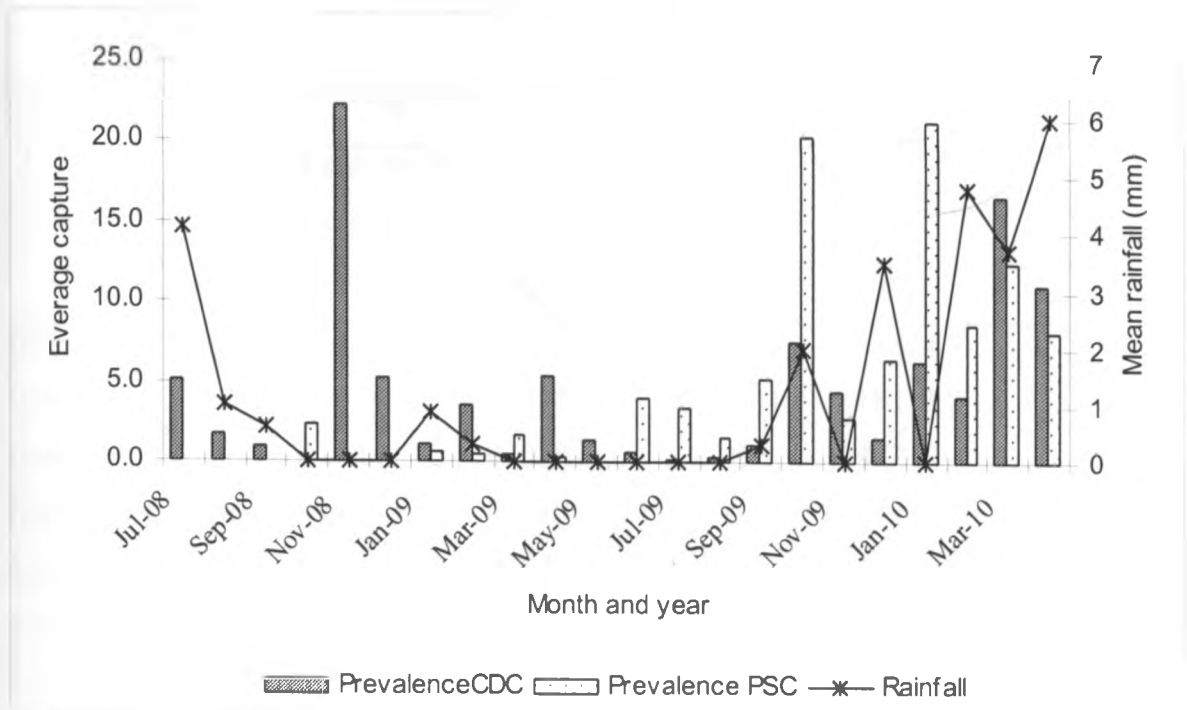


Figure 1: Mean number of indoor resting mosquitoes relative to rainfall

4.1.3 Blood meal sources

ELISA (Table 2) successfully identified blood meal hosts of 417 (95%) samples out of the 421 tested. In Kamarimar, the human blood index (HBI) including those with mixed blood meals for outdoor collected (CDC light traps) samples was 0.63 and significantly higher than 0.11 for indoor collected (PSC) samples ($\chi^2 = 30.938$, $P < 0.0001$). Similar values for Tirion were 0.48 and 0.43, for outdoor and indoor collected samples respectively and did not differ significantly from each other ($\chi^2 = 0.522$, $P = 0.478$). The HBI for outdoor collected samples was significantly higher in Kamarimar (0.65) than in Tirion (0.48, $\chi^2 = 4.899$, $P = 0.027$). In contrast, the HBI for indoor collected samples was significantly higher in Tirion (0.43) than in Kamarimar (0.11, $\chi^2 = 14.847$, $P < 0.0001$). Mixed blood feeding was also observed, the most common being bovine and goat blood meals. Some samples contained blood meals from all the three hosts tested.

Table 2: Blood meal sources of *Anopheles arabiensis* mosquitoes

| | Kamarimar | | Tirion | |
|------------------|-----------|-----------|-----------|-----------|
| | CDC | PSC | CDC | PSC |
| | n (%) | n (%) | n (%) | n (%) |
| Human | 45 (51.1) | 0 (0) | 56 (37.6) | 33 (24.3) |
| Bovine | 0 (0.0) | 21 (47.7) | 30 (20.1) | 18 (13.2) |
| Goat | 0 (0.0) | 0 (0.0) | 8 (5.4) | 4 (2.9) |
| Human and Bovine | 9 (10.2) | 0 (0.0) | 5 (3.4) | 18 (13.2) |
| Human and Goat | 0 (0.0) | 0 (0.0) | 2 (1.3) | 3 (2.2) |
| Bovine and Goat | 33 (37.5) | 18 (40.9) | 40 (26.8) | 55 (40.4) |
| All | 1 (1.1) | 5 (11.4) | 8 (5.4) | 5 (3.7) |
| Total | 88 | 44 | 149 | 136 |

4.1.4 Human biting density, sporozoite rates and EIR

The HBR of 9.51 bites per person per month in Kamarimar was not significantly different from 8.93 bites per person per month in Tirion ($F= 0.79$, $df=1$, $P= 0.3812$). For each of the three years, there was a marked monthly variation in HBR. HBR was undetectable in some months, and quite high in other months e.g. 96 bites/person for the month of November 2008. Sporozoite rates were very low (16 out of 7,322, 0.02%) and were only detected in two months in Kamarimar (October 2009 and March 2010) and four months in Tirion (October and November 2009 and January and March 2010 (Table 3). Similarly, EIR was undetectable throughout much of the study period except in October 2009 in Kamarimar and October 2009, November 2009 and January 2010 in Tirion when low levels were detected (Table 3).

Table 3: Sporozoite rates, Human biting rates and entomological inoculation rates

| Month | Year | Kamarimar | | | Tirion | | |
|-----------|------|-----------|---------|--------|--------|---------|--------|
| | | SR | HBR | EIR | SR | HBR | EIR |
| July | 2008 | 0.0000 | 46.6226 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| August | 2008 | 0.0000 | 12.0532 | 0.0000 | 0.0000 | 3.8843 | 0.0000 |
| September | 2008 | 0.0000 | 2.9356 | 0.0000 | 0.0000 | 3.1748 | 0.0000 |
| October | 2008 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| November | 2008 | 0.0000 | 13.8905 | 0.0000 | 0.0000 | 95.7889 | 0.0000 |
| December | 2008 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| January | 2009 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| February | 2009 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| March | 2009 | 0.0000 | 3.1910 | 0.0000 | 0.0000 | 3.5652 | 0.0000 |
| April | 2009 | 0.0000 | 4.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| May | 2009 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| June | 2009 | 0.0000 | 15.6254 | 0.0000 | 0.0000 | 7.1680 | 0.0000 |
| July | 2009 | 0.0000 | 6.8903 | 0.0000 | 0.0000 | 7.0405 | 0.0000 |
| August | 2009 | 0.0000 | 3.1072 | 0.0000 | 0.0000 | 3.5569 | 0.0000 |
| September | 2009 | 0.0000 | 9.7898 | 0.0000 | 0.0000 | 12.4831 | 0.0000 |
| October | 2009 | 0.0019 | 62.1170 | 0.1176 | 0.0058 | 22.8941 | 0.1339 |
| November | 2009 | 0.0000 | 31.0782 | 0.0000 | 0.6667 | 38.4435 | 0.0000 |
| December | 2009 | 0.0000 | 11.2909 | 0.0000 | 0.0000 | 11.0009 | 0.0000 |
| January | 2010 | 0.0000 | 32.5275 | 0.0000 | 0.0085 | 33.3302 | 0.0000 |
| February | 2010 | 0.0000 | 39.7993 | 0.0000 | 0.0000 | 34.5888 | 0.0000 |
| March | 2010 | 0.0027 | 0.0000 | 0.0000 | 0.0066 | 0.0000 | 0.0000 |
| July | 2010 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

* HBR =human biting rate; SR =sporozoite rate; EIR =entomologic inoculation rate. The sporozoite rate was derived from indoor resting collection. EIR =infective bites/person/month.

4.1.5 Household characteristics and vector occurrence

Multivariate logistic regression analysis was done to determine the relationship between the measured household characteristics and occurrence of *An. arabiensis* (Table 4). The odds of *An. arabiensis* occurrence increased with decreasing distance to the animal shelter and the nearest larval habitat and increasing number of houses, sleepers and size of eaves. *An. arabiensis* was also more likely to be encountered in grass-thatched than in metal-roofed houses and in the absence than in presence of animals.

Table 4: House characteristics and indoor vector resting densities

| Characteristic | Coefficient | S.E. | df | Sig. | Odds ratio |
|--|-------------|------|----|----------|------------|
| No. of houses | 0.16 | 0.04 | 1 | < 0.0001 | 1.17 |
| No. of sleepers | 0.28 | 0.07 | 1 | < 0.0001 | 1.76 |
| Presence of animals | -0.91 | 0.42 | 1 | 0.031 | 0.40 |
| Distance to animal shelter | -0.13 | 0.02 | 1 | < 0.0001 | 0.88 |
| Roofing material | -0.76 | 0.39 | 1 | 0.048 | 0.47 |
| size of eaves | 0.06 | 0.01 | 1 | < 0.0001 | 1.06 |
| Distance to the nearest larval habitat | -0.04 | 0.00 | 1 | 0.003 | 0.81 |

4.2 Aggregation sites of immature stages

4.2.1 Habitat survey and census

A total of 25 discrete habitats were mapped and their mode of formation recorded (Plate 3). In Kamarimar, a majority of breeding sites (78.57%) were man-made in origin, 7.14 % were livestock-associated, and the remainder were naturally occurring. In Tirion Village, 90.9% of all habitats were man-made and the remainder naturally occurring. Chances of sampling anopheline mosquito larvae were higher in marshes and canals in Kamarimar but highly heterogeneous in Tirion where a majority of habitat types were supportive to anopheline larval development (Table 5).

Table 5: Habitat types and mosquito larval prevalence

| Village | Habitat type | No. of times with Anopheline larvae only | No. of times with Culicine larvae only | No. of times with both Anopheline and Culicine larvae | No. of times without larvae | Total (%) |
|-----------|-------------------|--|--|---|-----------------------------|------------|
| Kamarimar | Concrete tank | 2 | 19 | 8 | 11 | 40 (11.1) |
| | Hoof print | 0 | 0 | 0 | 4 | 4 (1.1) |
| | Marsh | 7 | 3 | 7 | 10 | 27 (7.5) |
| | Canal | 17 | 121 | 85 | 52 | 275 (75.6) |
| | Tyre tracks | 0 | 0 | 0 | 3 | 3 (0.8) |
| | Stream beds | 0 | 0 | 0 | 13 | 13 (3.6) |
| | Total/mean | 26 | 143 | 100 | 93 | |
| Tirion | Concrete tank | 2 | 0 | 2 | 3 | 7 (1.8) |
| | Culvert | 4 | 11 | 10 | 7 | 32 (8.2) |
| | Ditch | 13 | 6 | 19 | 15 | 53 (13.6) |
| | Marsh | 4 | 2 | 10 | 11 | 27 (6.9) |
| | Canal | 1 | 3 | 2 | 145 | 151 (38.8) |
| | Pandam | 27 | 7 | 28 | 57 | 119 (30.5) |
| | Total/mean | 51 | 29 | 72 | 238 | |



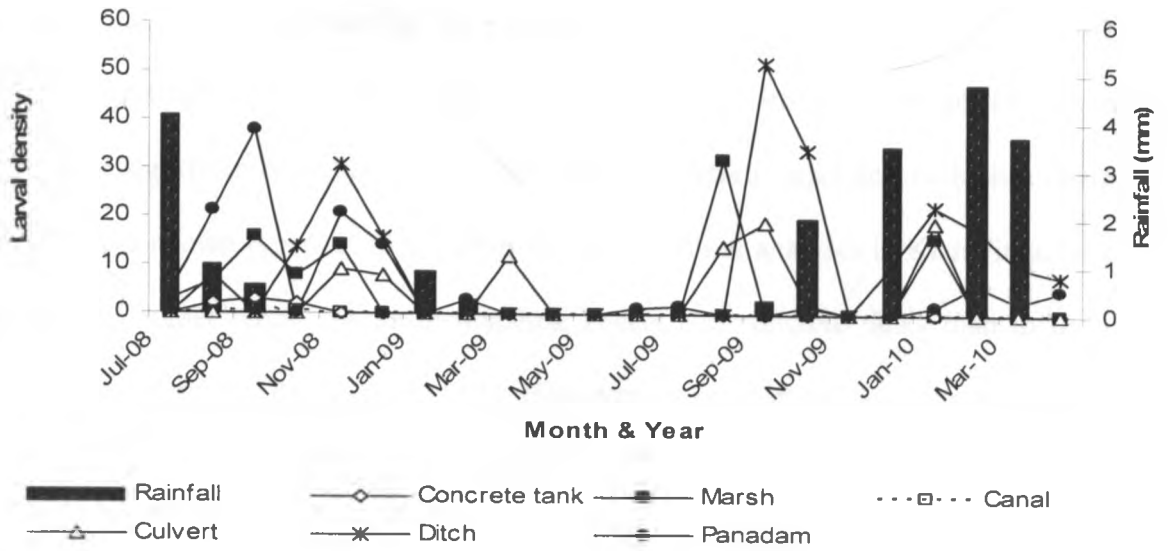
Plate 3: Types of breeding sites in Baringo

4.2.2 Larval abundance and habitat diversity

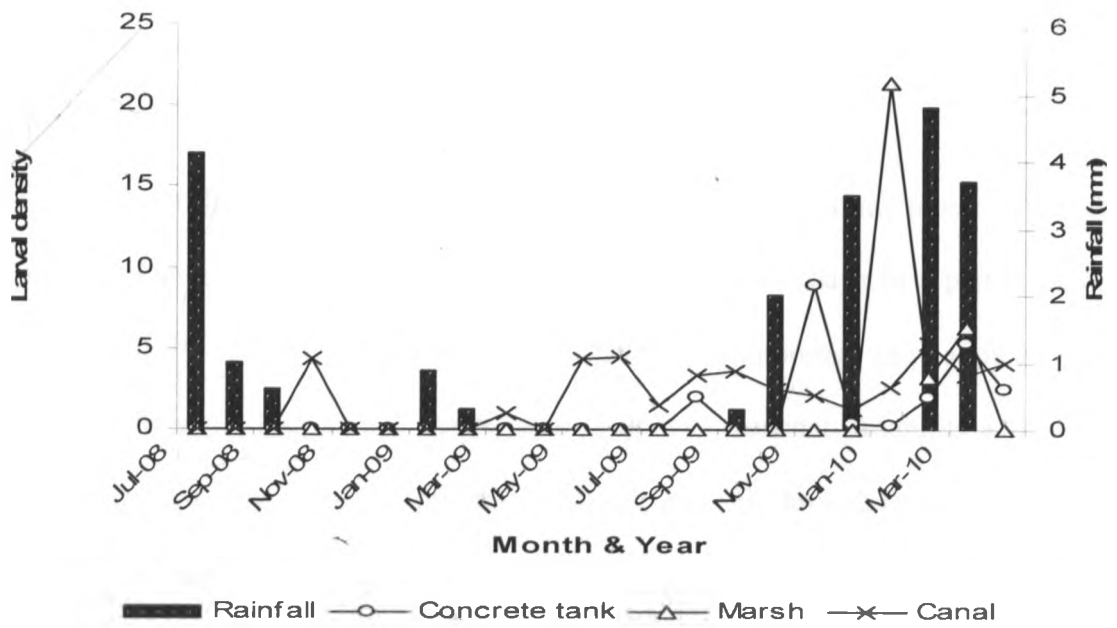
A total of 590 larvae (371 early instars, 219 late instars) were collected in Kamarimar and 1249 (1000 early instars, 294 late instars) in Tirion. (Table 6). Habitat support for larval development varied in the two villages. In Kamarimar, 26 habitats had anopheles larvae only and were visited 363 times compared to 51 in Tirion which were visited 389 times resulting in an overall tally of 752 longitudinal samples in 22 months (Table 6). The relative abundance of early ($t=3.87$, $df=1$, $P < 0.0001$) and late instars ($t=5.91$, $df=1$, $P < 0.0001$) were significantly higher in Tirion than Kamarimar respectively. Larval densities for early and late instars were two-fold and five-fold respectively, higher in Tirion than Kamarimar. The temporal dynamics of different habitat types with regard to larval presence and productivity is shown in figure 2.

Table 6: Relative abundance of anopheline larvae

| Village | Habitat type | No. of habitats | No. of samples taken | Percentage positive Anopheline larvae | Counts of early instars/10 dips | Counts of late instars/10 dips | |
|-------------------|-------------------|-----------------|----------------------|---------------------------------------|---------------------------------|--------------------------------|------------|
| Kamarimar | Concrete Tank | 2 | 36 | 25 | 24 | 27 | |
| | Hoof print | 1 | 4 | 0 | 0 | 0 | |
| | Marsh | 3 | 27 | 51.85 | 100 | 26 | |
| | Canal | 6 | 280 | 37.09 | 247 | 166 | |
| | Tyre tracks | 1 | 3 | 0 | 0 | 0 | |
| | Stream beds | 1 | 13 | 0 | 0 | 0 | |
| | Total/mean | 14 | 363 | | | 371 | 219 |
| | Tirion | Concrete tank | 1 | 7 | 57.14 | 7 | 0 |
| Culvert | | 1 | 33 | 43.75 | 80 | 36 | |
| Ditch | | 2 | 53 | 60.38 | 310 | 126 | |
| Marsh | | 1 | 27 | 51.85 | 106 | 13 | |
| Canal | | 3 | 150 | 1.99 | 7 | 0 | |
| Pandam | | 3 | 119 | 40.34 | 490 | 119 | |
| Total/mean | | 11 | 389 | | | 1000 | 294 |



(b) Tirion



(a) Kamarimar

Figure 2: Contribution of habitat types to larval production

Six distinct habitat types were identified in each village (table 6). Canal, marsh, and concrete tank habitats constituted most of the samples in Kamarimar, while pan dam, ditch, marsh, and culvert habitats constituted most of the samples in Tirion. Results of ANOVA and Turkey's honestly significant differences test showed counts of late instars of anopheline larvae in Tirion were significantly higher in pan dams, canals, concrete tanks and in ditches compared with the other habitat types ($F = 5.82$, $df = p < 0.001$). Similar analyses in Kamarimar revealed significantly higher larval counts in marshes, canals and concrete tanks than in the other habitat types ($F = 5.82$, $df = 2$, $p < 0.001$). However, in relation to long-term contribution to larval productivity, canals were more important because they had water available for anopheline larval development long after most of the other habitats had dried up. They were therefore sampled more times for mosquito larvae compared with other habitat types (Table 6).

4.2.3 Seasonal larval habitat productivity

The importance of habitats in larval production was dependent upon the month of collection and village (Figure 2). Some habitats were important in one village in a particular month ($F = 3.80$, $df = 20$, $P < 0.0001$), but were either absent or less important in relation to habitats in the other village. In Kamarimar, concrete tanks showed seasonal variability and supported larval development only in the wet months of August 2009 all through to April 2010 (Figure 2a). Marshes supported larval development for only three wet season months (January to March) out of 22 months. Canals were the most stable breeding sites and supported year-round larval production regardless of season. They supported larval survival through the long dry season that extended from March to August in the year 2009. In Tirion, canals supported minimal larval development in September, October and November in the year 2008 with no evident seasonal variation (Figure 2b). Larval production was largely varied with season in pan dams, culverts, concrete tanks, ditches and marshes. These habitat types

supported larval development through most of the wet months of September, December and November in the year 2008, in January, February, July, November and December in the year 2009 and in April in the year 2010. Overall, there were no significant differences in larval densities among different months in both villages. Mean larval densities were higher in the dry season (0.61 ± 0.97) than the wet season (0.51 ± 0.88) but the differences in density were not statistically significant ($F=0.04$, $df=1$, $p=0.8501$).

4.2.4 Larval Species composition and abundance

Some 41.33% ($n=212$) of late stage anopheline larvae were positively identified. *Anopheles gambiae* s.l. constituted 55.04% and *Anopheles pharoensis* 46.7% in Kamarimar. In Tirion, 44.30% of anophelines were *Anopheles gambiae* s.l. while 50.63% were *Anopheles pharoensis* (Table 7). *Anopheles coustani* and *Anopheles funestus* were available in Tirion Village only.. PCR results showed all 500 *Anopheles gambiae* complex mosquitoes were *Anopheles arabiensis*, making this sub-species by far the most abundant Anopheline mosquito in both villages. Overall, *Anopheles gambiae* and *Anopheles pharoensis* jointly accounted for 96.7% of all mosquitoes and were represented in all habitat types except in hoof prints, tyre tracks and stream beds in Kamarimar. In Tirion, these species were absent in canals and concrete tanks but present in marshes ditches, pan dams and culverts.

Table 7: Distribution of anopheline larval species in different larval habitat types

| Village | Anopheles Species | Marsh | Canal | Concrete tank | Hoof print | Tyre tracks | Stream beds | Ditch | Pandam | Culvert | Total (%) |
|-----------|-----------------------------|-------|-------|---------------|------------|-------------|-------------|-------|--------|---------|-----------|
| Kamarimar | <i>Anopheles gambiae</i> | 18 | 46 | 7 | 0 | 0 | 0 | * | * | * | 71(55.04) |
| | <i>Anopheles coustani</i> | 0 | 0 | 0 | 0 | 0 | 0 | * | * | * | 0(0.0) |
| | <i>Anopheles funestus</i> | 0 | 0 | 0 | 0 | 0 | 0 | * | * | * | 0(0.0) |
| | <i>Anopheles pharoensis</i> | 31 | 26 | 1 | 0 | 0 | 0 | * | * | * | 58(44.96) |
| | Total | 49 | 72 | 8 | 0 | 0 | 0 | * | * | * | 129 |
| | <i>Anopheles gambiae</i> | 5 | 0 | 0 | * | * | * | 21 | 9 | 0 | 35(44.30) |
| Tirion | <i>Anopheles coustani</i> | 3 | 0 | 0 | * | * | * | 2 | 0 | 0 | 5(6.32) |
| | <i>Anopheles funestus</i> | 0 | 0 | 0 | * | * | * | 0 | 2 | 0 | 2(2.53) |
| | <i>Anopheles pharoensis</i> | 5 | 0 | 0 | * | * | * | 18 | 17 | 1 | 40(50.63) |
| | Total | 13 | 0 | 0 | * | * | * | 39 | 26 | 1 | 83 |

Key: *... indicates that habitat type was absent.

4.2.5 Factors associated with larval development

Significantly higher densities of larvae were collected in shaded habitats holding turbid, temporary and still water (Table 8). Presence of emergent or floating vegetation, depth, size and distance to the nearest house did not significantly affect larval density among the two villages. There was a positive relationship between larval density and salinity ($r=0.19$, $p<0.05$), conductivity ($r=0.05$, $p=0.45$) and total dissolved solids ($r=0.17$, $p<0.05$). However, the relationship between water temperature and larval density was negative ($r=0.15$, $p=0.35$).

Table 8 : Larval habitat characteristics and mean densities of Anopheles gambiae larvae

| Habitat characteristics | | Mean±SD | F | Sig. |
|---------------------------|--------------------|-------------|------|--------|
| Shade intensity | Light | 1.54 ± 3.74 | 4.70 | 0.036 |
| | Shade | 4.08 ± 9.12 | | |
| Turbidity | Clear | 1.42 ± 3.53 | 3.79 | 0.029 |
| | Turbid | 5.32 ± 9.72 | | |
| Water depth | Deep | 2.09 ± 5.95 | 0.59 | 0.447 |
| | Shallow | 3.42 ± 7.27 | | |
| Vegetation cover | Floating | 4.03 ± 8.88 | 2.53 | 0.089 |
| | Emergent | 1.73 ± 4.28 | | |
| | Floating+ Emergent | 3.48 ± 9.70 | | |
| Permanence | Permanent | 1.54 ± 3.74 | 4.70 | 0.036 |
| | Temporary | 4.08 ± 9.12 | | |
| Size | Large | 2.21 ± 6.01 | 0.62 | 0.43 |
| | Small | 3.42 ± 7.52 | | |
| Water current | Moving | 1.30 ± 3.14 | 6.70 | 0.0132 |
| | Still | 3.97 ± 8.74 | | |
| Distance to nearest house | Near | 2.44 ± 6.37 | 0.14 | 0.714 |
| | Far | 3.19 ± 6.27 | | |

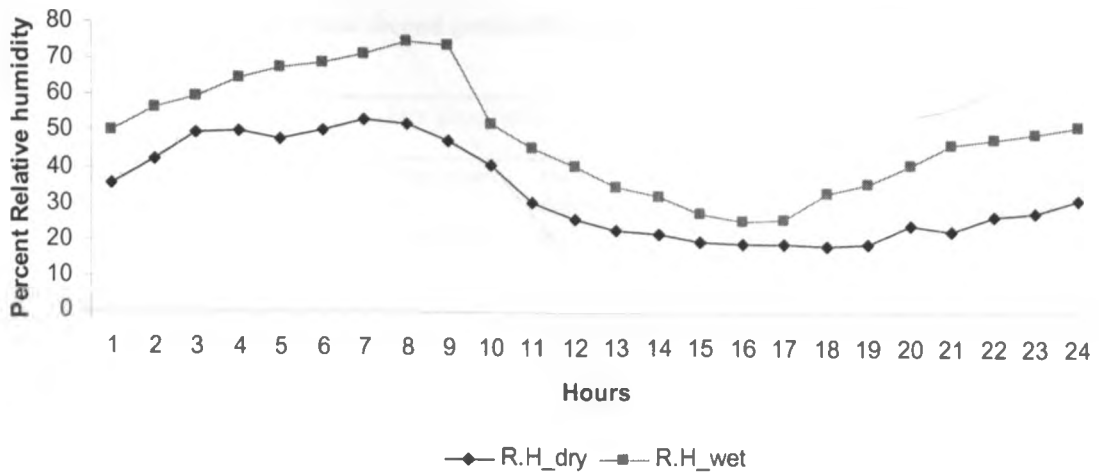
4.3 Physiological adaptations and reproductive behaviour

4.3.1 Meteorology

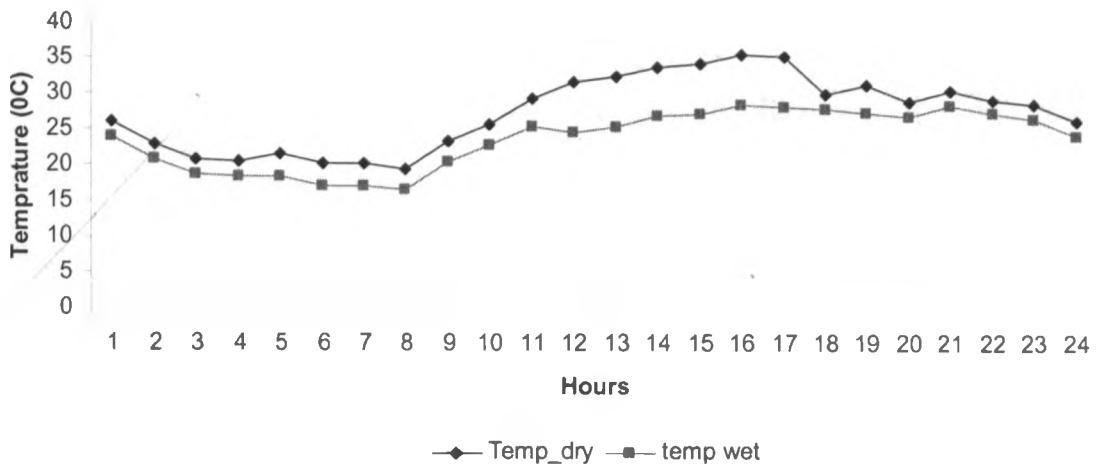
The average monthly maximum outdoor temperature during the study period was 28.40 and 23.65 °C in the dry and wet seasons respectively. However, the average minimum temperature (12.5 °C) in the dry season was 4.91 °C lower than that in the wet season (7.59 °C) ($t=5.98$, $df=16$, $p=0.001$). On the other hand mean monthly outdoor temperature was 26.32 °C during the dry season and 24.82 °C during the wet season. The average monthly temperature during the dry season was 1.5 °C higher than that of the dry season ($t= 2.59$, $df=16$, $P=0.0075$), and the average monthly maximum temperature was 4.75 °C higher ($t = 8.85$, $df=16$, $p<0.001$).

Figure 3 shows the 24-h indoor temperature and relative humidity profile during the dry and wet seasons. The 24-h cycle profile was calculated as the average of the hourly readings of temperature and relative humidity over the dry season or rainy season. During the dry season, the average indoor temperatures in the study site were 1.1 °C higher than that of the wet season ($t= 2.39$, $df=16$, $P<0.001$). Between 1200 and 1400 hours, indoor temperatures in the dry season were consistently 6.6-7.0 °C higher than in the wet season (Fig. 3b). Mean outdoor relative humidity during the dry season was significantly lower than that in the wet season by an average of 3.3% ($t=3.84$, $df =16$, $P =0.004$). Mean indoor relative humidity in the dry season was not significantly different from that in the wet season ($t=3.84$, $df =16$, $P >0.05$). Overall mean hourly readings of relative humidity in both seasons were lowest between 1200 and 1400 hours when indoor temperature was also higher (Fig. 3a).

A. Dry and wet season, relative humidity



Dry and wet season temperature



24-h daily cycle in Insectary, (a) Average hourly relative humidity (b) Average hourly temperature during dry season (September 2009) and wet seasons (February 2010)

Figure 3: Mean indoor temperature and relative humidity

4.3.2 Seasonal variation in gonotrophic cycle duration

Table 9: Duration of the first and second gonotrophic cycles of *A. gambiae* mosquitoes

| Season | First gonotrophic cycle | | | | Second gonotrophic cycle | | | |
|--------|-------------------------|-------------------|---|-------------------|--------------------------|---|-------------------|----------------|
| | Mean indoor Temp | Mean outdoor Temp | Proportion of mosquitoes that laid eggs (%) | Mean duration (d) | Mean fecundity | Proportion of mosquitoes that laid eggs (%) | Mean duration (d) | Mean fecundity |
| Dry | 28.22± 1.1 | 26.32 ± 0.33 | 43 | 4.52± 0.12 | 70.65± 0.74 | 8.7 | 3.0± 0.2 | 69.02± 0.1 |
| Wet | 27.12± 1.2 | 24.82 ± 0.33 | 64 | 6.14± 0.27 | 54.24± 1.02 | 6.2 | 4.1 ± 0.3 | 50.36± 0.2 |

Field collected F1 female mosquitoes that had blood fed and mated were used. Standard deviation for mean indoor and mean outdoor temperatures, gonotrophic cycle duration, and fecundity is shown.

During the dry season, significantly fewer mosquitoes (38.2%) laid eggs than those in the wet season (61.8%) ($t=8.85$, $df=1$, $p<0.05$). The average duration of the first gonotrophic cycle in the wet season was 4.1 days after blood feeding, 1.1 days (36%) longer than those in the dry season (3.0 days) ($z=11.1$, $P <0.001$; Table 9).

The average duration of the second gonotrophic cycle in the wet season was 2.9 days after second blood meal, 0.7 days (31.8%) longer than those in the dry season (2.2 days) ($Z=7.1$, $P<0.001$; Table 1). Chi-square tests showed that the duration of the gonotrophic cycle of *Anopheles arabiensis* females was significantly different between the wet and dry seasons ($X^2 =96.68$, $df=2$, $p<0.001$). During the first gonotrophic cycle, mosquitoes collected during the wet season exhibited significantly lower fecundity than those collected in the dry season (54.2 versus 70.7 eggs; $t=3$, $df=1$, $p<0.05$). However we found no significant difference in mean fecundity between the first and the second gonotrophic cycles ($t=0.21$, $df=1$, $p=0.85$).

4.3.3 Seasonal trends in parity

Parity varied over time, ranging between 88% and 100%. Four peaks in parity were recognized in November 2008, June 2009 and in March and April 2010 (Figure 4).

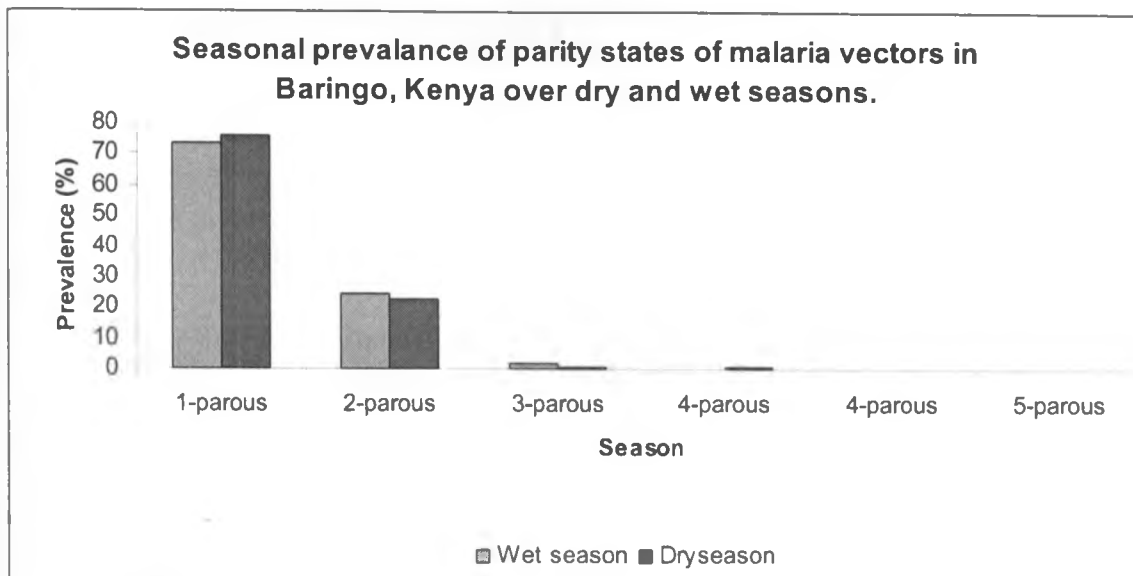


Figure 4: Prevalence of multiparous groups of *Anopheles arabiensis*

Parous females were categorized into different multiparous groups to determine the number of gonotrophic cycles the females had undergone and the results are shown in table 10. A majority of the mosquitoes were parous-1, suggesting a largely young vector population. During the dry season, a majority of females were parous-1 at 75.5 % (n=3916) followed by parous-2 at 22 % (n=1107). No parous-5 mosquitoes were encountered during the dry seasons sample collections for dissection, but three parous-4 females were collected. Overall no mosquitoes were found that had undergone more than five gonotrophic cycles in either season throughout the study period, raising questions on vector longevity in this area, a subject that requires further investigation. Chi-square tests showed significant interseasonal variation in parity ($X^2=4.46$, $df=1$, $P<0.0001$). Populations of parous-2 ($X^2=07.0$, $df=1$, $P=0.008$) and parous-3 ($X^2=4.35$ $df=1$, $P=0.04$) significantly varied between dry and wet

seasons. Populations of parous-1 ($X^2=3.46$, $df=1$, $P=0.06$) parous-4 ($X^2=1.43$, $df=1$, $P=0.23$) and parous-1 females ($X^2=1.21$, $df=1$, $P=0.27$) did not significantly vary between the dry and wet seasons.

Table 10: Parity status of *Anopheles arabiensis*

| Month/year | Dilatation number and total in each category | | | | |
|-------------------|--|------------|-----------|----------|----------|
| | 1 | 2 | 3 | 4 | 5 |
| July 2008 | 345 (3.24) | 210 (7.39) | 0(0.00) | 0(0.00) | 0(0.00) |
| August2008 | 120 (1.13) | 80 (2.82) | 4(2.03) | 4(13.79) | 0(0.00) |
| September 2008 | 76 (0.71) | 30(1.06) | 0(0.00) | 0(0.00) | 0(0.00) |
| October 2008 | 0 (0.00) | 0(0.00) | 0(0.00) | 0(0.00) | 0(0.00) |
| November 2008 | 2100 (19.74) | 520(18.31) | 0(0.00) | 0(0.00) | 0(0.00) |
| December 2008 | 520 (4.89) | 140(4.93) | 0(0.00) | 0(0.00) | 0(0.00) |
| January 2009 | 123 (1.16) | 27(0.95) | 0(0.00) | 5(17.24) | 0(0.00) |
| February2009 | 356 (3.35) | 80(2.82) | 0(0.00) | 7(24.14) | 0(0.00) |
| March2009 | 21 (0.20) | 30(1.06) | 2(1.02) | 1(3.45) | 1(9.09) |
| April2009 | 400 (3.76) | 271(9.54) | 0(0.00) | 2(6.90) | 0(0.00) |
| May2009 | 121 (1.14) | 50(1.76) | 0(0.00) | 1(3.45) | 0(0.00) |
| June2009 | 152 (1.43) | 5(0.18) | 0(0.00) | 5(17.24) | 6(54.55) |
| July2009 | 68 (0.64) | 13(0.46) | 6(3.05) | 1(3.45) | 2(18.18) |
| August2009 | 43 (0.40) | 21(0.74) | 10(5.08) | 0(0.00) | 1(9.09) |
| September2009 | 62 (0.58) | 50(1.76) | 0(0.00) | 0(0.00) | 1(9.09) |
| October 2009 | 1170 (11.00) | 200(7.04) | 0(0.00) | 0(0.00) | 0(0.00) |
| November 2009 | 450 (4.23) | 57(2.01) | 43(21.83) | 0(0.00) | 0(0.00) |
| December 2009 | 230 (2.16) | 76(2.68) | 4(2.03) | 0(0.00) | 0(0.00) |
| January 2010 | 940 (8.84) | 250(8.80) | 19(9.64) | 0(0.00) | 0(0.00) |
| February 2010 | 301 (2.83) | 348(12.25) | 45(22.84) | 3(10.34) | 0(0.00) |
| March 2010 | 1800 (16.92) | 379(13.35) | 64(32.49) | 0(0.00) | 0(0.00) |
| April 2010 | 1240 (11.66) | 300(10.56) | 0(0.00) | 0(0.00) | 0(0.00) |
| Total/mean | 10638 | 2840 | 197 | 29 | 11 |

4.3.4 Seasonal patterns of follicular development

A majority of follicles were in Christopher Stage I during the wet season at 57.08% (n=3051) as shown in table 11. Some 24.43% were in Stage II (n=1306%), 13.4 % (n=680) in stage III, 2.81% (n=250) in stage IV while stage V had the least number of females at 2.28% (n=255). During the dry season, however, Christopher stage II dominated at 56.68% (n=2742). Stage V mosquitoes were more prevalent during the dry than wet season collections by more than double at 5.27% (n=255). There was a reduction in the number of females with follicles in stage I to 18.83% (n=911). Stage III and IV follicle prevalence doubled during the dry season at 5.17% (n=250) and 5.27% (n=255) respectively.

Stage I prevalence was significantly different between dry and wet seasons ($X^2=6.35$, $df=1$, $P=0.01$). However prevalence of stage II ($X^2=0.20$, $df=1$, $P=0.65$), stage III ($X^2=1.76$, $df=1$, $P=0.18$), stage IV ($X^2=0.51$, $df=1$, $P=0.47$) and stage V ($X^2=0.08$, $df=1$, $P=0.78$) did not significantly vary with seasons. There was no significant difference in insemination frequency between dry and wet seasons ($X^2=3.36$, $df=1$, $P=0.06$).

Table 11: Follicular stages of *Anopheles arabiensis*

| Month/year | Christopher stages of follicular development | | | | |
|-------------------|--|------------|------------|-----------|------------|
| | I | II | III | IV | V |
| July 2008 | 200 (3.46) | 310(5.55) | 3(0.18) | 40(8.51) | 2(0.37) |
| August2008 | 56(0.97) | 132(2.36) | 2(0.12) | 2(0.43) | 16(2.99) |
| September 2008 | 6(0.10) | 60(1.07) | 20(1.22) | 5(1.06) | 15(2.80) |
| October 2008 | 0(0.00) | 0(0.00) | 0(0.00) | 0(0.00) | 0(0.00) |
| November 2008 | 1500(25.95) | 820(14.67) | 150(9.15) | 30(6.38) | 120(22.43) |
| December 2008 | 280(4.84) | 300(5.37) | 57(3.48) | 3(0.64) | 20(3.74) |
| January 2009 | 30(0.52) | 101(1.81) | 10(0.61) | 3(0.64) | 11(2.06) |
| February2009 | 56(0.97) | 272(4.87) | 43(2.62) | 32(6.81) | 40(7.48) |
| March2009 | 8(0.14) | 29(0.52) | 4(0.24) | 3(0.64) | 11(2.06) |
| April2009 | 187(3.23) | 348(6.23) | 58(3.54) | 4(0.85) | 76(14.21) |
| May2009 | 18(0.31) | 143(2.56) | 4(0.24) | 2(0.43) | 5(0.93) |
| June2009 | 38(0.66) | 84(1.50) | 23(1.40) | 18(3.83) | 5(0.93) |
| July2009 | 27(0.47) | 62(1.11) | 1(0.06) | 0(0.00) | 0(0.00) |
| August2009 | 12(0.21) | 48(0.86) | 3(0.18) | 6(1.28) | 6(1.12) |
| September2009 | 28(0.48) | 65(1.16) | 3(0.18) | 8(1.70) | 10(1.87) |
| October 2009 | 148(2.56) | 833(14.91) | 315(19.22) | 70(14.89) | 4(0.75) |
| November 2009 | 39(0.67) | 420(7.52) | 36(2.20) | 37(7.87) | 18(3.36) |
| December 2009 | 132(2.28) | 115(2.06) | 10(0.61) | 50(10.64) | 3(0.56) |
| January 2010 | 297(5.14) | 565(10.11) | 194(11.84) | 97(20.64) | 56(10.47) |
| February 2010 | 320 (5.54) | 246(4.40) | 7(0.43) | 7(1.49) | 117(21.87) |
| March 2010 | 1700(29.41) | 214(3.83) | 276(16.84) | 53(11.28) | 0(0.00) |
| April 2010 | 699(12.09) | 421(7.53) | 420(25.63) | 0(0.00) | 0(0.00) |
| Total/mean | 5781 | 5588 | 1639 | 470 | 535 |

4.3.5 Meteorology

The total precipitation for the period September 2008 to March 2010 was 100mm. The rainy season for the year 2008 was concentrated in July-August, bimodal in 2009 concentrated in January-February and September-December. The months of February-March were wet in the year 2010. The average daily temperature was 26.24(range=21.60-30.68) and the average relative humidity was 20.94% (range=2.74-94.8%)

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Abundance, species composition and resting behaviour

Anopheles arabiensis was the main vector of malaria in the study area feeding readily on humans and domestic animals. Malaria transmission by this species was largely undetectable throughout much the study period except in a few wet months when low levels of transmission were detected. This suggests that malaria transmission in arid areas of Africa is seasonal occurring mainly during the rainy season when larval habitats for *An. arabiensis* become abundant. The finding that *Anopheles arabiensis* is the only sibling species of *Anopheles gambiae* s.l. present in the study area is consistent with previous cytogenetic studies (Mnzava *et al.*, 1994, Aniedu *et al.*, 1989, Aniedu, 1993, Aniedu 1997). Some *Anopheles funestus* were also collected during the study period. This species is known to thrive in hot and humid environments as opposed to the hot and dry conditions that prevailed in the study area (Charlwood *et al.*, 2000, Ramsdale and Fontaine 1970b, Ramsdale and Fontaine 1970, 1970 Taylor *et al.* 1993). Thus the lack of suitable long-lasting larval habitats preferred by this species may partly account for its low densities in the current study (Munga *et al.*, 2006). Previous studies in the study area reported that production of *Anopheles arabiensis* in Kamarimar is sustained mainly by permanent or semi-permanent larval habitats including pan dams, marshes and adjoining drainage canals used for irrigation that are less dependent on rainfall (Ijumba *et al.*, 2000, Wirtz and Burkot, 1991)

A significant fraction of anthropophilic mosquitoes were sampled outdoors from the two villages, a clear evidence of either house-exiting by vectors after feeding on humans or

feeding on humans engaged in outdoor activities. Lightly dressed residents in the villages stay out late in the evening and rise up as early as 3 am to irrigate their farms before temperatures rise to uncomfortably hot levels during day time. These activities may expose them to mosquito bites. Another possible channel for human exposure to these vectors can be found in herding practices. The largest communal grazing field in Marigat division that is used by hundreds of pastoralists from different villages is located in Tirion, exposing herders to cattle-seeking *An. arabiensis* mosquitoes. Conversely, large fractions of zoophilic mosquitoes were collected indoors illustrating the potential for zoophylaxis as a strategy to control malaria in semi-arid areas of Africa. This strategy has successfully been used to control malaria in some parts of Africa and may have played a role in earlier efforts to eradicate malaria in Europe and USA (Muriu *et. al.*, 2008). However, this strategy may be counterproductive under certain circumstances where high livestock densities lead to an increase in vector densities and thus the human biting rates (Shililu *et. al.*, 2003].

The intensity of malaria transmission by *An. arabiensis* as measured by EIR was extremely low and seasonal. This could be due to low infectiousness of the human population in the area (Lindblade *et. al.*, 2000) or because a majority of mosquitoes caught by light traps were mostly newly emerged and had not had an opportunity to acquire an infectious blood meal (Mala *et. al.* 2011). Further, it is also likely that adult lifespan of this species was shorter than the extrinsic incubation period for malaria parasites but this possibility was not evaluated in the current study. These findings are consistent with those from studies conducted by Shililu and others (2003) in a semi-arid area in Eritrea that showed the risk of exposure to sporozoite-laden *An. arabiensis* was highly heterogeneous and seasonal, with high biting and EIRs during the rainy season, and with little or no transmission during the dry season. Year-long studies conducted in the Sudan by Dukeen and Omer (1986) in the 1970s showed *An.*

arabiensis adult densities peaked seasonally during certain months when Nile water levels were low but persisted at low level throughout the year. Both of these studies were conducted in semi-arid ecosystems similar to those in Baringo.

Entry and/or exit of mosquitoes was largely determined by house type. The average number of indoor resting mosquitoes collected in the two villages varied significantly depending on house type highlighting the importance of this micro-epidemiological factor in malaria epidemiology. There was a strong preference for grass-thatched houses. House type modification to limit mosquito flight into houses could be an important control strategy but would depend on the ability of residents to put up such houses.

A low EIR value was realized in only one out of the 22 months in which sampling was done. Intensifying control measures could lower the prevailing EIR levels below one infective bite per person per month; potentially eliminating malaria in these areas by reducing parasite rates to levels that can interrupt malaria transmission. We believe that these findings have important implications and will inform policy on vector control in epidemiological zones with low malaria transmission patterns.

5.1.2 Aggregation sites of immature stages

The results of this study show a highly restricted structure in terms of habitat type and larval species abundance and diversity; with permanent and localized water sources being the drivers of year-round low-level larval production. Lack of diversity in habitat types had a marked effect on *Anopheles* species diversity. Tirion, which had significantly more diverse habitat types, had a richer *Anopheles* mosquito fauna than Kamarimar, which had fewer habitat types. Previous findings have reported a close association between larval habitat

diversity and mosquito fauna (Beier *et al.*, 1990, Shililu *et al.*, 2003). An alternative explanation for lack of larval species diversity could be found in the fact that a permanent swamp was the sole water source in the two villages. The villages are joined by a major irrigation canal that drains from this swamp and provides residents with water for domestic use. This created a scenario in which sampling was done in two different habitat types (marshes and drainage canals) but was essentially done from the same water body, especially during the dry season when only these permanent water sources supplied larvae.

Human activities and climatic changes tremendously influenced larval breeding. Over 90% of all habitats in both villages were by-products of human activities, attesting to the human-dependent ecology of Afrotropical *Anopheles* (Coluzzi, 1999). By 4 weeks after the end of the rainy season, most water bodies had dried up and few mosquito larvae could be found. As a result, the number of adult mosquitoes collected in surrounding houses dropped drastically (Mala *et al.* 2011). Season affected mosquito counts in breeding sites, confirming previous reports from studies conducted in Mali where malaria transmission during the dry season was found to be undetectable (Toure' *et al.*, 1998). Rainfall played a minor role in habitat hydrology in Kamarimar but, interestingly, a major one in Tirion. It was noted that larval production significantly peaked in the latter during the wet season when semi-permanent pan dams got filled with rain water. This was accompanied with increased adult catch sizes in adjacent houses (Mala *et al.* 2011). Pan dams were unique to Tirion only, had the highest larval counts but were mainly important during the wet season. Marshes and canals had low larval counts but continued to churn out larvae in dry and wet seasons in both villages. These findings corroborate past findings that showed the most productive habitats per surface area might not necessarily be the most important for spatial and temporal proliferation of vector numbers (Gillies and deMeillon, 1968).

Anopheles gambiae s.s were not encountered in adult collections, perhaps because the subspecies prefers temporary, sunlit pools (Gillies and deMeillon, 1968, Gillies and Coetzee; 1987) unlike marshes and drainage canals that were the dominant breeding sites in Baringo. In the rare occasions when temporary habitats were observed after sporadic rain showers, they hardly lasted beyond five days to sustain a complete cycle of larval cohorts to adult stage. These findings are consistent with those of Toure' *et al.* (1998) in Mali which showed *Anopheles gambiae* predominated in humid areas; with larval production occurring almost exclusively during rainy periods.

The high catch sizes of *Anopheles arabiensis* recorded in the present study were expected as these species are known to be more versatile under dry weather conditions than the other sibling species of the *Anopheles gambiae* complex (Toure' *et al.*, 1998). The nature and seasonal design of habitats also suited their ecology, as they are known to exploit permanent, artificial habitats such as rice fields and marshes (White *et al.*, 1972), White and Rosen, 1973). Toure' *et al.* (1998) noted *Anopheles arabiensis* prevailed in arid areas and likely reproduced throughout the year. Past studies have noted incidences of vectorial complex variation in which certain sibling species dominate during certain times of the year, depending on season. In Tanzania and Nigeria, *Anopheles arabiensis* predominated during the dry season and *Anopheles gambiae* , just after the long rains [19, 20]. It would be sensible and logical to conclude from these findings that malaria vectors in semi-arid settings adapt to dry season survival by allowing more hardy sibling species to take up ecological space during the dry season and vice versa.. The availability of permanent water sources complement vector survival by ensuring species that are best adapted to these kinds of habitats such as members of the *Anopheles funestus* group are able to breed and sustain

malaria transmission. Large permanent habitats with emergent vegetation are known to favor proliferation of *Anopheles funestus* (Gillies and deMeillon, 1968). These were the main types of breeding sites in Baringo and where *Anopheles funestus* mosquitoes were collected, a pointer to alternative adaptive behavior among vectors based on habitat suitability.

The alternate utilization and quick recolonization of habitats shortly after rainfall in Tirion was interesting. Semi-permanent pan dams in this village were the main drivers of larval production during the wet season while permanent irrigation drainage canals in Kamarimar supported larval breeding during both dry and wet seasons. It is possible that adult mosquitoes carried over from the dry season permanent water sources provided larval “seed” to newly formed water bodies during the wet season. Further seeding effects could have been provided by other permanent water sources located outside the study villages but from where no sampling was done. A good example is found in River Lobo located less than 3 km away from the furthest breeding site in either of the two villages; well within the flight range of gravid *Anopheles gambiae* females (Kaufman and Briegel, 2004). We did not observe any potential obstacles that could have hindered free flight of mosquitoes between the villages and this river, a situation that was favored by an open shrub land that allowed free wind flow.

In various areas with seasonal-malaria transmission in Africa, it has been possible to identify local reservoirs of transmission during the dry season (Charlwood *et. al.*, 2000, Omer and Cloudsey-Thompson, 1970, Sattler *et. al.*, 2005). Identifying sources of mosquito larvae during the dry season may provide a basis for selective larval control, which may impact on subsequent malaria transmission in the rainy season. The findings of this study provide solid data that can make this dream a reality in Baringo and other semi-arid complexes with similar ecological conditions.

4.3 Physiological adaptations and reproductive behaviour

The same challenges encountered by previous workers who have attempted to determine the physiological age of afrotropical malaria vectors were met during this study. The small body and ovary sizes of *Anopheles arabiensis* mosquitoes made dissections difficult. The greatest difficulties occurred in determining the age of females that had just oviposited or in those with fully developed ovaries. Such females possess ovarioles from which the mature eggs have been passed and are still in the form of sacs, and it is impossible to tell the age of the females from them. When dilatation formation is just beginning, the stalks of the ovarioles rupture easily when stretched and part of the sac that has begun to contract comes away with the next follicle; making false diagnosis highly probable.

The high proportions of parous females in the population observed in this study points not only to continuous small-scale breeding of malaria vector species but also to a low mortality among those that have emerged. Female age composition plays a key role in malaria transmission dynamics. Older parous females that have previously blood fed, as indicated by previous gonotrophic cycles, have a higher probability of being infected with malaria parasites. Since a majority of females encountered quite frequently among those dissected were young (parous-1 and parous-2), it can be assumed that these age-groups are epidemiologically the most important in relation to malaria transmission in Baringo. This can help explain the extremely low sporozoite rates among females realized in this study (Mala *et al.* 2011) since such young females have most likely just emerged and are unlikely to be infected by sporozoites.

Results on follicular stages show high proportions of post teneral mosquitoes. Such wild caught females have ovaries that are not yet developed and are yet to take their first blood meal. Instead, at least one carbohydrate meal is required by females with stage I follicles.

Examination of spermatheca of these females revealed no evidence of sperm presence, a confirmation that such females are largely uninseminated. However, almost all females with follicles in stage II showed evidence of insemination but were all nullipars. Most of the mosquito biting populations during the dry season were in stage II. This is an indication that gonotrophic concordance prevails for *Anopheles arabiensis* in Baringo. Usually, ovaries of nulliparous anopheline females do not develop beyond Christophers and Mer's stage II without a blood meal (Detinova 1962). As initially suggested by Mer (1936), gonotrophic concordance applies only to females whose ovaries are in stage II at the time of blood feeding

This study has demonstrated that the duration of their first and second gonotrophic cycle by 1.1 days and 0.7 days shorter respectively during the dry than the wet season. Fecundity rates were lower during the dry than the wet season but neither mating frequency varied with season.

Fecundity rates were higher during the dry than the wet season with a larger proportion of females laying eggs in the dry season. The reason of not laying eggs by a few mosquitoes may be improper feeding or some fertilization factors. These observations suggest malaria incidence in semi-arid areas may increase based on seasonal temperature increase alone and not necessarily due to increased vector density alone (Bown *et al.*, 1991, PAHO 1996, Rua *et al.*, 2003)

Reductions in gonotrophic cycle duration is likely to increase malaria incidence due to increased egg laying and biting frequency especially among old sporozoite-laden females (Macdonald 1957, Detinova 1962, Bruce-Chwatt 1980, Molyneaux 1988). These cycles are especially sensitive to changes in environmental temperature (Lindsay & Birley 1996, Martens 1997, Martens *et al.* 1999), being reduced with increments in temperature. The

kinetics of mosquito oocyte development, maturation of the ovaries, and duration of the gonotrophic cycle are temperature driven, and all of these variables influence frequency of human-vector contact. A reduction in the duration of the gonotrophic cycle would make the vectors bite more often, and increase the probability of malaria transmission, taking account the reduction in longevity with increments in temperature. It is expected that a balance between all these variables would explain the effect of climate on malaria transmission.

This study showed that the average duration of the first gonotrophic cycle was longer than that of second cycle one in both seasons. Wild caught fed females used in the study consisted of both nulliparous and parous individuals as is usually found in natural populations. The possible explanation for shorter duration observed in the second gonotrophic cycle may be that most of the wild-caught mosquitoes were parous. Parous females have been known to take a shorter time in oviposition than the nulliparous females because in parous females the ovaries are already in the middle or late Christopher's stage II of ovarian development (WHO 1975). In Tanzania, Gillies and Wilkes (1965) established that the gonotrophic cycle in parous *Anopheles gambiae* was 3 days. This study covered both wet and dry seasons. Low temperature during the wet seasons most likely slowed down blood digestion and ovary development (Gillies 1954). Muirhead-Thomson (1951) also reported that over wide areas of the tropics, the gonotrophic cycle in several species of *Anopheles* was two days but in the wet season but extended to three, four or even more days in the same species. Rodriguez et al. (1992) found the gonotrophic cycle of *Anopheles albimanus* Wiedemann to be 4 days for nulliparous mosquitoes and 2 days for parous mosquitoes in southern Mexico. Charlwood and Graves (1986) reported a gonotrophic cycle duration of 2.4-3.2 days for *Anopheles koliensis* Owen, 2.7-3.7 days for *Anopheles punctulatus* Donitz, and 2.1-3.0 days for *Anopheles farauti* Laveran in Papua New Guinea. Hii et al. (1990) observed a 2-3 days

gonotrophic cycle duration in *Anopheles balabacensis* Baisas in Malaysia. Few such studies have been conducted in the Afrotropical anopheline mosquitoes, but our results on the gonotrophic cycle duration of *A. gambiae* by using confined adult mosquito rearing cages are consistent with the studies on other mosquitoes.

Negative correlation between gonotrophic cycle duration and temperature has been reported in other mosquito species. For example, Mahmood and Crans (1997) reported that the amount of time required for bloodmeal digestion and oviposition decreased significantly with each increment in temperature from 10 to 28°C in *Culiseta melanura* Coquillett. Neto and Navarro-Silva (2004) exposed *Aedes albopictus* (Skuse) to different cyclic temperatures of 25/18°C and 27/20°C and found that the gonotrophic cycle was shorter for the higher temperatures.

5.2 Conclusions

This study arrived at three conclusions

- a) Malaria transmission in the study area was seasonal and vectored by *An. arabiensis*. This species was found to feed readily on humans and domestic animals suggesting that zooprophylaxis may be a potential malaria control strategy in the study area and other semi-arid regions of Africa. However, further studies are needed to assess the negative impacts of this strategy in target sites.

- b) Breeding of malaria vector mosquitoes in Baringo is driven by predominantly shaded, human-made and permanent breeding sites in which *Anopheles arabiensis* and *Anopheles funestus* breed at low level throughout the year. During the dry season, permanent water sources serve as “inocula” by providing “larval seed” to freshly formed rain-fed habitats during the rainy season. The highly localized and focal nature of breeding sites in these semi-desert environments provides a good opportunity for targeted larval control since habitats are few, well-defined and easily traceable

- c) These findings suggest the duration of gonotrophic cycle among *Anopheles arabiensis* in drylands with scarce breeding sites is longer during the wet than the dry season. These findings suggest the duration of gonotrophic cycle among *Anopheles arabiensis* in drylands with scarce breeding sites is longer during the wet than the dry season. Shortened gonotrophy during peak dry seasons ensure vectors are able to compensate for low densities known to prevail in semi-arid areas by rapid breeding through increased biting intensities and frequent egg-laying. Low fecundity rates during the

dry season could be a sign of reduced reproductive activity. However lack of variation in seasonal mating frequency is a clear indicator that oviposition and mating kinetics are influenced differently even under the same environmental conditions. Consequently higher mating frequency may not necessarily translate into high oviposition rates, or high vector densities for that matter.

5.3 Recommendations

- It is recommended that mass larviciding campaigns be carried out to take advantage of the few and well defined breeding sites to reduce the populations of immature stages of malaria vectors
- The role of flight range in perpetuating adult dry season survival warrants investigation, preferably through MRR studies. Flight ranges that are longer than normal are likely to enable the vectors access human hosts that would have otherwise been out of their reach.
- The role of egg stages in supporting dry season survival should also be investigated further

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LIST OF APPENDICES

Appendix I: Pyrethrum spray collection protocol

Knock down space spraying with pyrethrum is a standard, quick and easy method of catching mosquitoes in huts and animal shelters. All occupants, animals; easily removable objects such as small tables and chairs, exposed food and drinking water were removed from the house to be sprayed. Two people carefully laid white cotton sheets over the entire floor; over the bed; and over furniture and miscellaneous objects that had not been removed. Sheets were regularly washed to avoid overlooking mosquitoes in dirty sheets. All windows and doors were closed and the hut space sprayed with 10% synergised pyrethrum in distilled water. Strong spray guns of medium capacity and along plunger stroke were used. A pin or other suitable implement was used to clear the nozzle whenever the nozzle was blocked. The spray was first directed to all potential escape routes, such as closed doors, windows and eaves; it was then aimed towards the roof and ceiling. Sprayers left the house after spraying and then re-entered the house after 15 minutes to retrieve mosquitoes.

Mosquitoes were collected by carefully picking up each cloth sheet by the corners and shaking the contents onto a single sheet. Weather permitting, two collectors took this sheet outside. If it was either windy or raining, mosquitoes were collected from the sheet inside the house; using a torch for illumination. Using forceps, mosquitoes were placed in wide mouthed vacuum flasks or in commercial cold boxes to keep them cool and transported to the laboratory for further processing. If not dissected immediately mosquitoes were stored in deep freeze. Mosquitoes were counted, sexed and the gonotrophic stages and species recorded by individual house.

Appendix II: CDC light trap usage

CDC light-trap collections were used to carry out this exercise. The light-traps were suspended about 1.5m from the ground next to animal enclosures to trap outdoor mosquitoes. The traps were tied to roof trusses and suspended over occupied mosquito net-secured beds. House occupants and Field staff were instructed in the proper operation of the light-trap: to turn the trap sunset, to close the neck of the trap collection bag sunrise, to prevent the mosquitoes from escaping, and to turn off the motor. The traps were collected in the morning by project staff, inquiries made as to whether the trap functioned properly all night. Proper light-trap operation during the night was ensured by periodic inspection by project staff.

Appendix III. Sporozoite ELISA protocol

Protocols for Enzyme-linked immunosorbent assays (ELISAs) were developed to detect *Plasmodium falciparum*, *P. vivax*-210, and *P. vivax*-247 circumsporozoite (CS) proteins in malaria-infected mosquitoes. The sensitivity and specificity of these molecular methods are based on the monoclonal antibodies (Mabs) used. The ELISAs detect CS proteins, which can be present in the developing oocysts, dissolved in haemolymph, and on sporozoites present in the haemocoel or in the salivary glands.

Procedure

- vials were labeled with the corresponding numbers as marked in the ELISA working sheet
- 50 microlitres of BB-np-40 was added into each vial
- Using sharp clean surgical blade the mosquito was cut between the thorax and the abdomen
- the head /thorax was picked with forceps and transferred to the sporozoite marked vial and the abdomen to the corresponding vial marked blood meal if the mosquito is blood fed. If not blood fed or no blood meal analysis was required and the mosquito was discarded.
- the head /thorax was left to soak in NP-40 for 20 minutes

Using a non-absorbent glass or plastic rod the mosquito was ground in the vial

The volume was adjusted by adding 200 microlitres of the blocking buffer.

-the pestle cleaned and wiped dry with gauze before grinding the next sample to avoid contamination. This was repeated until all samples were prepared.

ii. Plate coating

- a) PVC plates were labeled with appropriate number
- b) Into each well of clean PVC plate 50 microlitres of diluted capture MAb was added
- c) The plates were covered and incubated for 30 minutes at room temperature in subdued light

iii. Blocking plates

- a) Using an eight-channeled manifold attached to a vacuum pump, capture MAb was aspirated from the microtitre plate
- b) The plate was banged hard on absorbent tissue paper to ensure complete dryness of the plate
- c) Each well was filled with blocking buffer (Ph 7.4) using the manifold attached to a 60 ml syringe followed by incubate for one hour at room temperature in subdued light.

IV. Loading the plates

- a) The blocking buffer was aspirated from the wells using the manifold attached to a vacuum pump and banged to complete the dryness
- b) Using the labeled ELISA processing sheets, as a guide, 50ml of 100,50,25,12,6,3,1.5, and 0 pgs positive controls was put on the first column wells. Into the next column wells 50ml of the negative controls was added.
- c) The first mosquito sample (50 microlitres) was loaded into the third column well (A3) and the horizontal order was continued up to the last well in the plate
- d) The plate was covered and incubated for 2 hours at room temperature in subdued light.

v. Addition of conjugate (peroxidase enzyme)

- a) After two hours the triturate was aspirated from the wells
- b) the plates were washed 2 times with PBS-TWEEN 20
- c) 50 microlitres of the peroxidase labeled enzyme was added and incubated for one hour at room temperature

vi. Addition of substrate

- a) the enzyme was aspirated from the wells and washed three times with PBS-Tween 20 and banged to dryness
- b) Using an octapate multichannel pipette 100 microlitres of the substrate mixture and was added and incubated for 30 minutes. Results were read visually or at 414 nm using an ELISA plate reader

Appendix IV. Blood meal ELISA protocol

a). Sample preparation

1. Negative controls- male mosquitoes were ground in 500ml of PBS per mosquito
2. Positive controls- For each host serum: to 500ml PBS 500mls of host serum control was added
3. Blood- meal samples: - each mosquito abdomen sample was diluted in 1000 microlitres of PBS. To ensure proper grinding, 100 microlitres of PBS is first put in mortar and ground with a grinder, followed by addition of 900 ml PBS to raise to the required volume.

b).Technique

1. To a PVC flex plate , the following additions were made:
 - column1 50 microlitres added per well of eight negative controls
 - column 2 50 microlitres added per well of eight positive controls(Chicken, human, pig, cat, horse, cow, goat and dog)

It is critical that one well be designated as a blank control and receive 50 ml of PBS alone. The remaining wells should receive 50 mls/well of mosquito blood meal sample

2. Incubation was allowed overnight at room temperature
3. The plates were washed with PBS-Tween 2 times.

Enzyme conjugate preparation

1. To 5mls of BC-Tw (enzyme diluent) , the following additions were made:
 - HRP phosphate anti-human..... 2.5 microlitres (1:2000 dilution)
 - Bovine phosphatase conjugate.....20.0 microlitres (1:250 dilution), and Sera of each host except the one being tested for.....10.0 microlitres (1:500)
 - serum for all hosts was added except those for which enzyme-conjugate was added.

2. All HRP conjugates were diluted 1:2000. Cow which is a phosphatase conjugate was diluted 1:250.
3. 50ml/well of the prepared enzyme conjugate solution was added
4. Incubation was allowed for one hour at room temperature
5. the plate were washed with PBS-TW 3 times

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

7. 100 microlitres of substrate was added to each well
8. Incubation was allowed for 30 minutes
9. Absorbance was read visually or at 414nm with ELISA reader.
10. The plate was washed with PBS-TW 3 times

Phosphatase substrate preparation

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

11. 100ml of the phosphatase substrate was added to each well
12. Incubation was allowed for five hours and absorbance read at 414 nm (read from 2 hrs onwards)

Preparations for PBS (7.4), B.C 0.5% & PBS-Tween are as for sporozoite ELISA. For goat blood meal ELISA the same directions were followed except for the use of the conjugate HRP goat was used. Dilution is similar to human i.e. 1:2000(2.5ml).

Appendix V: Standard Solutions for PCR

The following standard solutions were prepared using sterile double distilled water (sddw). Where appropriate, the solutions were autoclaved at 121lb/sq in. for 15 minutes in an Eylea Autoclave (Rikikakki Tokyo).

Bender buffer

-0.1M NaCl, 0.2M sucrose. 0.1M Tris-HCl pH 7.5, 0.05M EDTA pH 9.1, 0.5% SDS. Stored at 40 C

0.5 M EDTA (pH8.0)

-186.1 g/l in water, ph adjusted with NaOH pellets and stored at room temperature.

EtBr (10mg/ml)

-1g of EtBr was completely dissolved in 100ml sddw and stored in the dark at room temperature.

KAc (5M K 8M Acetate)

-60ml of 5 M KAc and 11.5 ml glacial acetic acid in 28.5 ml distilled water

TE (pH 8.0)

-10Mm Tris-HCl (pH), 1mM EDTA (pH8.0). Stored at room temperature.