FACTORS AFFECTING THE INFECTIVITY AND EARLY SPOROGONIC DEVELOPMENT OF *PLASMODIUM FALCIPARUM* IN THE MALARIA VECTOR, *ANOPHELES GAMBIAE S.S.*

By

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DECLARATION.

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

Bernard Achero Okech — Date: 23rd March 2004

We confirm that the candidate under our supervision carried out the work reported in this thesis.

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DEDICATION

I dedicate this thesis to my sons Craig Malinke Achero and Neville Fabu Achero, my loving wife Eva Livere Achero and my parents Samson Okech and Joyce Okech.

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ABSTRACT.

This study investigated the effects of abiotic factors particularly temperature and biotic factors (mosquito related) on early malaria parasite development and infectivity. Volunteer gametocyte carriers were recruited following malaria infection surveys in the schools and community and requested to donate blood (~3ml) for artificial infection of *An. gambiae* mosquitoes via the immediate membrane feeding assay using glass feeders and parafilm membrane. In studies related to the impact of temperature on malaria development, 2 experiments were carried out, (1) to determine if *P. falciparum* development can continue beyond 32^0C temperature threshold and, (2) to determine the influence of indoor microhabitat temperature prevailing in the village houses on malaria development. Mosquitoes were exposed to these microclimatic conditions and later dissected to determine parasite densities in the mosquito midguts. Mosquito factors such as age and the previous dietary history was also studied for their influence on malaria infectivity to mosquitoes. Similarly, the impact of microclimatic and dietary factors on mosquito survival was studied both in the semi-natural and in the natural environment.

The study showed that malaria parasites were able to develop at high temperatures above 30^0C. Mean ookinete densities were decreased with increase in temperature and were significantly different (ANOVA: $F = 3.705$, d.f. = 2,49, $P = 0.032$). However, oocsyt densities were not statistically different (ANOVA: F=0.301, d.f.=2, P=0.741) between 27^0C, 30^0C and 32^0C temperature levels. Similarly, the indoor microclimates prevailing in the villages were suitable for malaria development as parasite densities in the mosquito midguts were not different (ANOVA: F=0.546,

df=2, P=0.580) for experiments done in the semi-natural environments and also experiments done in the field (ANOVA: F=0.671, df=2, P = 0.523) conditions. The study also revealed that the infectivity of *P. falciparum* to mosquitoes was not influenced by the age of the mosquito (Chi-square, χ^2 =3.604, df = 2, P = 0.165) rather by the dietary factors the mosquito had previously fed on. Under similar microclimatic conditions, the survival potential of mosquitoes could enable malaria parasite transmission. In addition, the presence of sugar in the diet improved survival but increased feeding on blood in the absence of sugar and the presence of parasites in the blood meal lowered the survival probability.

Although temperatures affects the rates of parasite and vector survival, this study has shown that parasites can resist high temperatures and as such cannot stop transmission in hot areas. However, mosquito related biotic factors are more important determinants of malaria infectivity and development in the *An. gambiae* and influences malaria transmission in nature. This information is very useful in predicting how temperature changes can affect the distribution of malaria transmission in nature and also the role of dietary factors in transmission of malaria by mosquitoes.

ABBREVIATIONS

AMA – Apical Membrane Antigen

ANOVA – Analysis of Varaince

ARPPIS – African Regional Postgraduate Programme in Insect Science

BOG – Board of Governors

CI – Confidence Interval

CS - Circumsporozoite

DF – Degrees of Freedom

EBA – Erthrocyte Binding Antigen

EIR – Entomological Inoculation rates

FITC – Fluorescein isothiocyanate

GAF – Gametocyte Activation factor

ICIPE – International Centre of Insect Physiology and Ecology

ITN – Insecticide Treated Net

KEMRI – Kenya Medical Research Institute

MOH – Ministry of Health

MRTC – Malaria Research and Training Centre

MSP – Merozoite Surface Protein

NIH – National Institutes of Health

NLM – National Library of Medicine.

PBS. – Phosphate Buffered Saline

PM –Peritrophic matrix

PROB. Probability

RESA – Erythrocyte Surface Antigen

TBV – Transmission Blocking Vaccine

TRAP – Thrombospondin Related Adhesive Proteins

W/V – Weight to volume

WHO – World Health Organization

XA – Xanthurenic Acid

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW.

1.1 GENERAL INTRODUCTION.

Malaria is an infectious parasitic disease of man transmitted from an infected to a non-infected individual by anopheline mosquitoes. The disease occurs in tropical areas of the world, mainly in Africa, Asia and Latin America. In these areas of the world, malaria continues to present an immense public health challenge with the World Health Organization (WHO) estimating that 300 to 500 million people are at risk of infections and close to 1.5 to 2.7 million people dying annually (WHO, 1996; RBM/WHO, 2001). More than 80% of these cases occur in sub-Saharan Africa with an estimated 66 million infants exposed to the disease and 0.43 to 0.68 million deaths in children below 5 years old (Snow *et al.,* 1999a, b). The identification of malaria infections in people involves the microscopic examination of an appropriately stained thick blood smear spread on a glass slide (Trape, 1985). This enables the identification of the parasite species involved and the life cycle stage of the parasite so that suitable treatment can be given to the infected persons. In addition to the treatment of individuals with anti-malarial drugs to kill the parasites, vector control to kill the malaria vectors has been employed in an effort to control the disease. However, the emergence of pyrethroid insecticide resistance in the vector (Quinones *et al.,* 1998; Hargreaves *et al.,* 2000) and anti-malarial drug resistance in *Plasmodium falciparum* (Yeung, 2002) has significantly reduced the viability of many control programs. Many anti-malarial drugs have

little or no effect on the gametocytes that are responsible for initiating infections in mosquitoes (Strickland *et al*, 1986). As a result malaria transmission in the community continues with individuals who receive treatment becoming re-infected quickly. An increase of malaria in Africa has also been enhanced by the changes in the climatic conditions and population movement amongst others (Nchinda, 1998). Climate is an important determinant of malaria transmission because pools of water forming as a result of rain provide ideal breeding grounds for mosquitoes whereas temperature affects both the development and survival of mosquito and parasite. Therefore there is a complex temperature related interaction between mosquito survival that affects vector distributions and parasite growth, which affects the probability of malaria transmission in nature.

1.2 LITERATURE REVIEW.

1.2.1 The development of malaria parasites in mosquitoes.

The development of malaria parasites in mosquitoes (sporogony) involves a sequential process of developmental steps after an appropriate anopheline mosquito vector ingests gametocytes from a human that will develop into zygotes, ookinetes, oocysts and eventually sporozoites in the salivary glands (Beier and Vanderberg, 1998). All of these developmental steps within the mosquito are easily quantifiable by microscopy. Failure of the parasite to complete these essential steps would prevent transmission from the mosquito to the vertebrate host; and this has become the basis of some of vaccine-based strategies for blocking transmission in nature (Miller *et al*, 2002). The process of *P. falciparum* sporogony in mosquitoes is temperature dependent; for example, extending from about 9 days at 30^0C to 10 days at 25^0C and to 11 days at 23^0C (Beier and Vanderberg, 1998). While an increase in temperature will result in shorter extrinsic development period for malaria, it has been observed that development may continue between 32^0C as the upper temperature threshold while 18^0C is the lower in classic laboratory models (Boyd, 1949; MacDonald, 1957). Therefore the geographical distribution of malaria is confined within climates favoring its extrinsic cycle of development (Martens, 1994; Craig *et al.,* 1999; Snow *et al.,* 1999a) provided conditions do not limit mosquito survival (Martens, 1994; Craig *et al.,* 1999). Some of the *P. falciparum* malaria stages during the process of sporogonic development are particularly sensitive to environmental factors. Temperatures above 30^0C even for short time periods after blood feeding disrupts the development of NF-54 strain of *P. falciparum* in

Anopheles gambiae (Noden *et al.*, 1995). However this observation contradicts observations in nature where temperatures above 30^0C occur in hot seasons where malaria transmission is intense, such as in Mali and in Burkina Faso or in dry season periods in western Kenya. In addition, several environmental factors other than temperature also affect malaria infectivity and development in the mosquito. Studies have shown that nutritionally deprived smaller sized mosquitoes were less competent vectors than the larger sized ones (Lyimo and Koella, 1992). In addition the number of previous blood meals (Vaughan *et al.,* 1994) or even previous sugar feeding may influence the susceptibility of *An. gambiae* infected with *P. falciparum* (Kelly and Edman, 1997).

1.2.2 Malaria vectors in Kenya.

Several species of *Anopheles* mosquitoes are found in the Afro-tropical region with some also in Kenya, but the most important of these in terms of malaria transmission in Kenya are the *An. gambiae* Giles, *An. arabiensis* Patton and *An. funestus* Giles even though the other minor vectors such as *Anopheles merus*, and *Anopheles pharoensis* (Mukiama and Mwangi, 1989) may also contribute. In Kenya, all these species are involved in malaria transmission especially in western Kenya (White, 1972), in central Kenya (Mwangi and Mukiama, 1989) and in the coastal area (Mbogo *et al.,* 1993; Mbogo *et al.,* 1995). *An. gambiae* Giles and *An. arabiensis* Patton are closely related and they cannot be distinguished on morphological basis (White, 1974; Coluzzi, 1984). They constitute members of the six sibling species of the *Anopheles gambiae* complex group generally referred to as *Anopheles gambiae sensu lato (s.l).* In Kenya, they include *Anopheles gambiae* ss, *An. arabiensis,* and *An. merus.*

1.2.3 Malaria transmission in Kenya.

In endemic areas of Africa, exposure rates as high as 1000 infective bites per year have been reported (Trape and Rogier, 1996). In Kisumu District, Kenya, the average bites in some areas is as high as 300 infective bites per person per year (Githeko *et al.,* 1992) whereas recent studies in Suba District have estimated the Entomological Inoculation rates (EIR) at between 0 – 5 infectious bites per person per month (Shililu *et al.,* 2000). Any increase in malaria transmission has been characterized by upsurges in the densities of the mosquito vectors and such increases in transmission can be identified by high malaria case admissions in health facilities (Githeko and Ndegwa, 2001). The transmission in highland areas, where environmental factors could not permit, has been reported in the western part of the country (Shililu *et al.,* 1998; Githeko and Ndegwa, 2001) and even in the hotter semi arid regions in the North Eastern province of Kenya. However these may have been due to changes in the climatic or environmental factors to favor transmission (Lindsay and Birley, 1996). In tropical African countries, the climatic and ecological conditions are quite ideal for the prolific breeding of vectors of malaria and favor malaria parasite sporogony, hence increasing transmission (Snow *et al.,* 1999a). This calls for a greater and in-depth study on how these environmental and climatic factors interact to favor transmission.

1.2.4 Vector competence studies.

An elucidation of malaria transmission dynamics would require a greater understanding of the interaction between vector and parasite, which is a key component in vector competence studies (Beier, 1998). The deeper understanding of epidemiology of insect borne diseases requires the study of the vector competence, because vectors not biologically capable of transmitting disease agents would be of minimal health importance. In this field, studies on the developmental steps including the interaction between *Anopheles* mosquitoes and malaria parasite have been done with the aim of understanding better factors that make the *Anopheles* mosquitoes capable of maintaining and transmitting the malaria parasites. Most of the studies looking into the development of the *P. falciparum* parasites in anopheline mosquitoes have used culture-derived parasites and laboratory bred *Anopheles* mosquitoes (Vaughan *et al.,* 1992, 1994a, b). Infection of mosquitoes is achieved by letting the mosquitoes feed directly on gametocyte infected human volunteer or experimentally feeding infected blood through artificial membranes using glass feeders (Ponnudurai *et al.,* 1989; Githeko *et al.,* 1993; Robert *et al.,* 1995; Robert *et al.,* 1998). However, very few studies have used parasites strains obtained from human volunteers. The interaction between malaria parasite and mosquito vector may sometimes affect the survival of mosquitoes (MacDonald, 1957) and experimental data from laboratory studies supports this observation (Chege and Beier, 1990; Robert *et al.,* 1990). On the other hand, data from other studies does not show any detrimental effect of parasite on vector survival (Klein *et al.,* 1986). However such studies examined only direct mortality as a result of the physical presence of parasites in mosquito tissues. Lyimo and Koella (1992) studied a natural population of mosquitoes caugh t in the wild and they presented data to show that high oocyst burdens as

found in larger mosquitoes induced mortality. Other causes of mortality in natural populations of mosquitoes are related to humidity, temperature and even differences in body energy reserves (Clements, 1963; 1992). Such factors that might alter vectorial capacity are important determinants of malaria transmission in an area (Beier and Vanderberg, 1998).

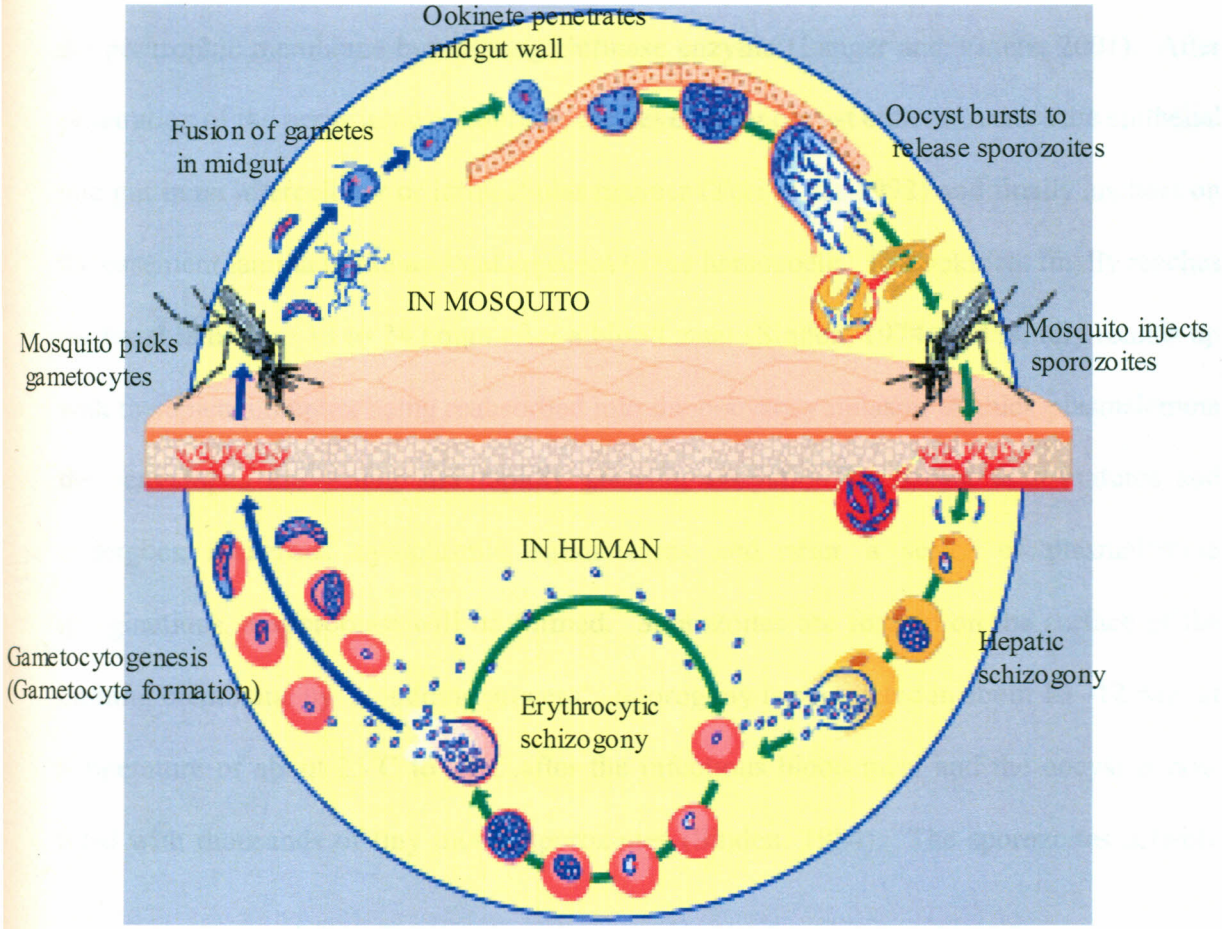
1.2.5 Life cycle of malaria parasites.

1.2.5.1 Development in the human host.

Malaria infections occur when an infective female *Anopheles* mosquito bites a human during the blood feeding process. Infective sporozoites from the salivary glands of the *Anopheles* mosquito are injected into a human host when the mosquito takes a blood meal. The injected sporozoites vary in number with the innoculum size ranging from 10 – 10,000 sporozoites (Beier *et al.,* 1991; Kabiru *et al.,* 1997) and the sporozoite dose may or may not necessarily determine the clinical episode and disease pattern in the human population (Greenwood *et al.,* 1991; Kabiru *et al.,* 1997). The sporozoites enter the host passively from the saliva into the bite site and in less than 30 minutes, they invade the parenchymal hepatocytes where they reside and begin the exo-erythrocytic schizogony (Garnharm, 1966). The time for this cycle until the first appearance of liver merozoites in the circulation is the pre-patent period and it is variable within individual *Plasmodium* species (Bruce-Chwatt, 1985). It is 11 – 13 days, 15 –16 days, 9 – 10 days and 10-14 days for *P. vivax*, *P. malariae*, *P. falciparum* and *P. ovale*, respectively (Bruce-Chwatt, 1985). These liver merozoites then enter the blood circulation and invade the erythrocytes where a vacoule forms and the parasite assumes a "ring form" or early trophozoite (Bruce-Chwatt, 1985). Growth follows in the next 12 – 24 hours until the now more solid parasite occupies a large space in the red

blood cells and is called a mature trophozoite. After this stage, nuclear division will commence and continue until maturity when second intra-erythrocytic schizogony occurs with formation of merozoites, a schizogonic process that occurs regularly with merozoites flooding the blood circulation resulting in the febrile chills, paroxysms and fever usually in *P. falciparum* infections (Bruce-Chwatt, 1985). These merozoites stay in the blood circulation for a period of up to eight days until of some of them differentiate into gametocytes, the sexual stages (Garnham, 1966). It is still unclear what triggers this differentiation. These gametocytes can persist in circulation for up to four weeks (Carter and Graves, 1988) before an appropriate anopheline vector picks them up for the sexual cycle to begin in the mosquito (Figure 1).

Figure 1. The life cycle of malaria parasite in human and mosquito hosts.



1.2.5.2 Development in the mosquito host.

When an appropriate mosquito vector ingests blood along with gametoctytes, the extrinsic cycle begins. The male gametocyte (microgametocyte) goes through exflagellation, to become motile gametes (microgametes) while the female gametocyte (macrogametocyte) transforms into a macrogamete (Gosh *et al.,* 2000). After the formation of the gametes that usually occurs in the first hour, there is fusion and a zygote is formed. The zygote or "round form" can persist in the midgut about 12 hours post feeding when it starts to transform itself into a motile ookinete (Gouagna *et al.,* 1998). The early stages of the ookinete (also called retort form) usually starts forming at 10 hours post infection and mature into an ookinete at between 20 –30 hours post infection (Vaughan *et al.,* 1992). The motile ookinete penetrates the peritrophic membrane by utilizing chitinase enzyme (Langer and Vinetz, 2001). After penetration of the peritrophic membrane, the developing oocyst either traverses the epithelial mid gut in an intercellular or intracellular manner (Tori *et al.,* 1992) and finally anchors on the basement lamina of the mid gut adjacent to the hemocoele. The ookinete finally reaches the basal lamina in 18 to 24 hours after a blood meal (Sinden, 1974) and rapidly rounds up with the apical complex being reabsorbed into the oocyst cytoplasm. A thick plasmalemma then envelopes the young and rapidly growing oocyst. The oocyst then matures and undergoes numerous cytoplasmic subdivisions and after a series of plasmalemma invaginations, a sporoblast will be formed. Sporozoites are formed on the surface of the limiting membrane by 'a budding process'. Sporogony is completed in about 10 –12 days at temperature of about 25^0C to 27^0C after the infectious blood meal and the oocyst is now filled with thousands of tiny motile sporozoites (Sinden, 1984). The sporozoites actively

escape the oocyst through tiny holes on the weakened oocyst wall and flood the hemocoele of the insect. After about two days, the sporozoites invade the salivary glands where they accumulate and are infective (Vaughan *et al.,* 1992) to man and the cycle continues.

1.3 Factors influencing malaria parasite development in mosquitoes.

1.3.1 Mosquito factors.

1.3.1.1 Melanization.

Melanotic encapsulation of the developing oocyst which usually occurs extracellularly between the basolateral epithelial cell membrane and the basement membrane (Collins *et al.,* 1986; Paskewitz *et al.,* 1988; Zheng, 1997) is a factor preventing infection by the ookinete. Ronald Ross first observed this phenomenon in 1898 and called them "Ross black spores". Melanization, in the salivary glands and within the oocyst in the midgut, involves the production of melanizing enzymes by the mid gut cells or it could be the result of a humoral response of the prophenoloxidase cascade (Paskewitz and Christensen, 1996). Incubation of infected mosquitoes with dihydroxyphenylalanine (DOPA) or dopamine results in heavy deposition of melanin around the encapsulated parasite in vesicular structures that seem to be confined to a single cell proximity to the parasites. Thus melanization is a standard defense mechanism used by insects against invading microbials and parasites.

1.3.1.2 Refractoriness.

Apparent physiological incompatibility between the mosquito and the parasite is commonly seen in mosquito/ parasite interaction and is termed as a refractory condition and this has also been observed as a major barrier to parasite infection of mosquitoes (Al-Mashhadani *et al.,* 1980; Feldmann and Ponnudurai, 1989; Curtis, 1994; Feldmann *et al.,* 1998b). Mosquito and parasite species are quite specific and this might have been the result of co-evolution over several years so that there is genetic and physiological compatibility between them (Koella, 1999).

1.3.1.3 Gametocyte Activating Factor (GAF).

Extensive studies done with *P. elongatum* show a strong dependence on a vector specific insect factor that induces microgametogenesis (exflagellation) where the absence of this factor has been shown to inhibit further development of this parasite in *Culex nigripalpus* vector (Carter and Graves, 1988). Several attempts to identify this insect factor have recently indicated a molecule thought to be GAF as Xanthurenic acid (XA) (Bilker *et al.,* 1998). It is the mosquito-derived factor connected to the mutation in eye pigmentation seen in *Anopheles* (Beard *et al.,* 1995). Anophelines have eye color variations that are predominantly controlled by the only significant eye color pigment omochrome seen in these mosquitoes (Beard *et al.,* 1995). During the biosynthesis of omochrome, a by-product xanthurenic acid is produced. Xanthurenic acid (XA) that is mosquito derived is hypothesized to be a trigger for gametogenesis even though Bilker *et al.,* (1998) indicated the presence of a yet another unknown factor. Xanthurenic acid is naturally also found in

mammalian blood at low concentrations and the probability of successful gametogenesis and XA levels in the mosquito blood meal could affect eventual sporogony.

1.3.1.4 The pH of the mosquito midgut.

A rise in pH of the mosquito midgut may also influence gametogenesis. Nijhout and Carter (1978) found that gamete development was dependent on the stimulation by the presence of bicarbonates (that is regulated by CO_2 tensions in the atmosphere) that increase the midgut pH to 7.8 upto 8.1, higher than the blood plasma pH (range 7.1 to 7.2). Other factors that could also interact to control mosquito gut pH include blood meals, consisting of proteins that increase the activity of acid activated protein digestion enzymes like trypsin and pepsin (Briegel and Lea, 1975; Billingsley and Hecker, 1991). However, several workers have been able to demonstrate that the pH rise is not the absolute trigger of gametogenesis. Bishop and McConnacchie, (1960) demonstrated that exflagellation took place before the pH reached 7.4 in *P. gallinaeceum*. This suggests the involvement of other factors for the initiation of gametogenesis.

1.3.1.5 Other mosquito derived factors.

It has also been demonstrated that exflagellation or syngamy by the malaria parasite is controlled by another mosquito-derived factor which when absent prevents parasite development (Nijhout, 1979). This heat stable molecule was shown to be present in the mosquito head and thorax tissues and may contribute to the initiation of early sporogony in mosquitoes.

1.3.1.6 Peritrophic membrane.

This membrane may prevent the ookinete from penetrating and anchoring on the basal lamina to mature into an oocyst (Billingsley and Rudin, 1992). The mosquito peritrophic membrane is a type I membrane secreted immediately after a blood meal (Billingsley, 1990). It is a membranous sac primarily composed of chitin, proteins and proteoglycans (Spence, 1991) separating the contents from the mid gut epithelium, protecting midgut epithelium and aiding digestion. It is usually formed in varying times from 24 hours – 36 hours after a blood meal (Miller and Lehane, 1993). Ookinetes are formed 10 – 24 hours after a blood meal and it should be expected that most of the ookinetes would have traversed the membrane before it is formed. However there are reports of ookinete penetration failures (Shahabuddin *et al.,* 1993). However, evidence indicates the presence of a penetrating chitinase enzyme produced by the developing oocyst (Huber *et al.,* 1991; Shahabuddin, 1995) that enables the parasite to reach the basal lamina for anchorage.

1.3.1.7 Midgut microbes.

Bacterial associations in the midguts of haematophagous insects are well documented. These microbes live as symbionts and may not be of benefit to the invertebrate insect host (Maudlin, 1991). *Anopheles gambiae* mosquitoes have been shown to have over 20 genera resident bacteria in their midguts (Straif *et al.,* 1996). Mosquitoes may acquire bacteria transtadially or through sugar feeding, but whichever the case the bacterial flora multiplies exponentially after blood-feeding and can adversely affect the development of the malaria parasites (Beier *et al.,* 1994; DeMaio *et al.,* 1996). However the bacterial population usually subsides to previous numbers after 2 – 3 days. The number of *P. falciparum* oocysts on the

mosquito midgut was shown to significantly reduce with increased density of bacteria (Pumpuni *et al.,* 1996). *Pseudomonas*, *Enterobacter* and *Flavobacterium* were present in *An. gambiae*, *An. stephensi* and *An. albimanus* midguts, respectively (Pumpuni *et al.,* 1996).

1.3.2 Parasite related factors.

1.3.2.1 Sex ratio and gametocyte number.

The ratio of the sexual gametes has been found to affect the outcome of a mosquito infection. Robert *et al.,* (1996) showed that there were significant differences in the sex ratios of gametocytes in gametocyte carriers and that the mean ratio in gametocytes was 3.6 females to 1 male. The number of gametocytes ingested has been shown to influence the oocyst rates (Tchuinkam *et al.,* 1993) so that there was a direct linear relationship in the oocyst infection rates and the number of gametocytes ingested. Some studies have also found that there is an increase in the midgut oocyst numbers in mosquitoes fed on a 9-fold dilution in gametocyte numbers due to less intense intra-specific competition for nutrients by the gametocytes (Ponnudurai *et al.,* 1989). Therefore the sex ratio and gametocyte number can influence infectivity and success of sporogony.

1.3.2.2 Gametocyte age.

The maturation of gametocytes before they become infective to mosquitoes can determine the mosquito infectivity with malaria parasites (Jeffrey and Eyles, 1955). Morphological maturity may take 8- 10 days (Smalley, 1976). However, Hogh, (1998) discovered a reduction in infectivity in gametocytes that were present in circulation for over

one week. Therefore it seems likely that there is an optimum age of gametocytes required to achieve maximum infectivity.

1.3.2.3 Parasite chitinase.

To traverse the peritrophic membrane, *P. gallinaceum* ookinetes secrete a chitinase enzyme (Huber *et al.,* 1991). Experimental feeding of allosamidin, an inhibitor of chitinase, blocks both *P. falciparum* and *P. gallinaceum* development in the mosquito suggesting that the enzyme is necessary to cross the peritrophic membrane (PM) barrier (Shahabuddin *et al.,* 1993; 1995). Secreted *P. gallinaceum* chitinase is a pro-enzyme that is activated with *Aedes aegypti* midgut trypsin. This implies that the parasite chitinase and the mosquito trypsin act together to allow the parasite to cross the mosquito PM.

1.3.3 Human host factors.

1.3.3.1 Immunity.

The immune status of the vertebrate human host for instance has been reported to influence the levels of infection in mosquitoes due to the presence of antibodies against the malaria parasite (Lensen *et al.,* 1996). It was shown that mosquitoes that were infected using whole blood from residents of malaria endemic areas had a low oocyst count as compared to those fed using non-immune blood from people with no previous history of malaria (Mulder *et al.,* 1994; Lensen *et al.,* 1996). This clearly suggests that humans in endemic areas develop factors which block the transmission of the parasite to mosquitoes.

1.3.3.2 Sickle cell traits.

Some studies have suggested that host sickle cell traits may affect the infectivity of mosquitoes (Robert *et al.,* 1996). Gametocyte carriers with AS hemoglobin were twice as likely to infect mosquitoes as AA carriers indicating that heterozygous sicklers increased malaria transmission (Robert *et al.,* 1996).

1.3.4 Environmental factors.

1.3.4.1 Temperature.

The development of malaria parasite inside the mosquito is influenced by temperature. Any drop in the temperature below 37.5^{0}C outside the human host triggers the transformation of gametocyes into gametes (Sinden and Croll, 1975), before malaria sporogonic development. Inside the mosquito host, malaria parasites will not develop beyond certain temperature thresholds. For instance, studies have shown that beyond 30^0 C, malaria sporogony does not continue (Noden *et al.,* 1995; Eling *et al.,* 2001). Reports based on laboratory models indicate that the development of malaria can occur between 18^{0}C and 32^{0}C (Boyd, 1949; MacDonald, 1957). This implies that in nature, malaria transmission occurs where the lower and upper temperature thresholds are 18^{0}C and 32^{0}C, provided that conditions do not limit mosquito survival (Martens, 1994; Craig *et al.,* 1999). This however is quite in contrast to the situation in many countries particularly in the Sahel where temperatures reach 40^{0}C. Many studies directed at establishing the ideal temperature conditions for malaria parasite development have largely focused on fixed laboratory or incubator temperatures (Vanderberg and Yeoli, 1966; Noden *et al.,* 1995; Eling *et al.,* 2001).

This has led to questions as to whether natural parasites, found in the human hosts, can develop in the hot climates experienced during the hot seasons or in hot areas.

1.3.4.2 Mosquito diet.

The vectors of malaria especially the *An. gambiae* complex mosquitoes exhibit unique feeding behavior that often influences greatly their lifespan and fecundity (Foster, 1995). Sugar feeding in the form of nectaries from plants provides the essential energy needed for survival (Clements, 1992; Foster, 1995). Blood feeding, critically needed for completion of vitellogenesis and subsequent oviposition, usually provides much needed substitute energy source for survival and longevity (Nayar and Sauerman, 1975) especially during the dry season when nectar sources are often scarce. However, the influence of sugar feeding or previous blood feeding on malaria transmission in nature is still unknown and remains a difficult area of study. Some studies have shown that the previous feeding on sugar meals enhances the infection rates in mosquitoes (Kelly and Edman, 1997). The shortcoming in many of such studies is the use of culture parasites that may not be representative of the parasites that exist in nature. Studies have shown that the number of previous blood meals reduces the malaria infectivity in *Anopheles* mosquitoes due to increased enzyme activity that is detrimental to the early ookinetes and thickened peritrophic membrane (Vaughan *et al.,* 1994b). However, the association between mosquito age and oocyst infection rates has received very little attention. This study investigated the effect of age and nutrition of *An. gambiae* mosquitoes on the development of *P. falciparum* using natural gametocytes obtained from human volunteers.

1.4 STRATEGIES FOR THE CONTROL OF MALARIA IN AFRICA.

1.4.1 Personal protection.

Encouraging people to personally protect themselves against biting mosquitoes can go along way in stopping the spread of malaria. Several approaches can be used to curb mosquito bites. These include use of insect repellents in the form of repellent creams and soaps that can be bought from the retail shops and pharmacies. Physical barriers like insecticide treated nets (ITNs) and curtains in houses, wearing long sleeve shirts and long trousers to cover most parts of the body will also help shield against biting mosquitoes.

1.4.2 Chemotherapy.

Malaria control in Africa is based almost exclusively on chemotherapy. However increasing evidence shows that there is a heavy price to pay in terms of the number of deaths owing to drug resistance (Trape, 2001). In Kenya, the mainstay for the control of malaria is treatment of infected individuals with anti-malarial drugs. The first line dug of choice is a Sulphadoxine–Pyrimethamine (SP) drug after there was widespread resistance to chloroquine®. However there is increasing parasite resistance to the currently available SP drugs (Sibley *et al*, 2001) such as Metakelfin®, Orodor®, and Fansidar® and this is hampering the control of the disease. The administration of chemotherapy to the whole population is not encouraged. However there could be selective treatment of cases especially those who are non-immune such as travelers, pregnant women and children under 5 years old. This may not be easy to implement because of the enormous cost involved (Menon *et al.,* 1990).

Pregnant women are particularly vulnerable to malaria infection due to the lowered immunity (Weinberg, 1984). As a result serious harm to mother and unborn child could occur such as retarded fetal` growth that leads to low birth weights in the unborn child due to placental malaria (McGregor, 1984, Menendez, 1995). Low birth weight children have a higher chance of dying. In high transmission areas such as in the tropical Africa, the effects of malaria in first time pregnant women (primigravidae) are particularly marked (Menendez, 1995). Chemotherapy to primigravidae has been shown to increase the birth weight of unborn babies and to reduce the risk of malaria infection in pregnant mothers (WHO, 1994).

1.4.3 Vector control.

Anopheline mosquitoes sustain the transmission of malaria in nature. Hence by limiting the mosquito population density, malaria transmission can be reduced. A variety of methods have been used to control the *Anopheles* mosquitoes.

1.4.3.1 Residual house spraying.

This involves the spraying of interior walls and ceilings of a house with insecticides such as dieldrin, fenitrothion (OMS-43) and malathion. However since most of these pesticides are no longer used due to the toxic effects seen on the environments and also prohibitive costs, alternative methods to synthetic insecticides are being encouraged. This has seen the revival in interest in the use of repellents products from plants (Seyoum *et al.*, 2003). However, synthetic insecticides have been particularly effective against insects that prefer to rest indoors, particularly for endophilic anophelines. This was the major method used to in the 1950's in trying to eradicate malaria problem in Brazil with great success

(Gladwell, 2001). This method could be revived and used together with other methods in an integrated fashion.

1.4.3.2 Use of insecticide treated nets, ITNs.

This method involves the treatment of bed nets with a pyrethroid insecticide (Deltamethrin), which repels and kills mosquitoes that come into contact with it. By this action, the net prevents the mosquito from biting. A bed net may last for very many years but the insecticide has to be replenished every six months as the activity of the pyrethroid gradually decreases with time. In addition the effect of the insecticide has been rendered inactive due to insecticidal resistance that has emerged and the excito-repellency behavior exhibited by the mosquitoes has made insecticide treated nets (ITN) of limited value for killing the mosquitoes (Quinones *et al.,* 1998; Hargreaves *et al.,* 2000). Therefore new methods need to be sought in the fight against malaria.

1.4.3.3 Bio-control methods.

The use of larvivorous fish for the control of mosquito larvae has been tried especially in areas where agricultural practices have led to an increase in malaria cases, especially in rice irrigation schemes. The species of fish are introduced into flooded areas as a source of food but importantly to feed on mosquito larvae. There are certain spiders and other insect species, especially emisine bugs that have been identified to play a role in the controlling mosquito numbers in the house (White *et al.,* 1972; Service, 1973). In addition, there are certain strains of bacteria that have been identified to kill insect vectors of disease (Fillinger *et al.,* 2003). *Bacillus thuringiensis* and *B. sphaericus* work by releasing a toxin that kills

insects. *Metarrhizium anisophilae* is also an entomopathogenic bacterium that has been used extensively in controlling some disease vectors (Kaaya *et al.,* 1989; Samish *et al.,* 2001 and is being tried against *An. gambiae* mosquitoes. Although these biological agents have been shown to be effective in the few studies, they are yet to be tested on a wider scale, and still doubts are cast as to whether they can work unilaterally without the incorporation of other tools.

1.4.3.4 Genetic manipulation of vectors.

Genetic manipulation of the mosquitoes is another strategy being evaluated for the control of the malaria parasite transmission by mosquitoes (Curtis, 1994). The arrested development of malaria oocysts, generally known as melanization (Paskewitz and Christensen, 1996) or the inability of mosquitoes to be infected also known as refractoriness (Feldmann *et al.,* 1998a), are regulated by certain genes within the genetic code of the mosquito. In addition, closely related species to the *An. gambiae* is known to be zoophagic and there are attempts to identify the genes that control this zoophagic behavior and transfer it to *An. gambiae* mosquitoes. In that way, the vectors would instead bite animals in the homestead. Because animals cannot develop malaria, this would be a dead end for the malaria parasite development. This is yet to materialize and it could take sometime before it becomes a reality, but before then malaria continues to take its toll.

1.4.4 Vaccines.

This is a new strategy, which is being developed due to failures of the traditional control methods of vectors using insecticides and malaria parasites using antimalarial drugs. A malaria vaccine can target the antigen of the blood stages parasite to prevent other red blood cells from becoming infected (Etlinger *et al,* 1991; Perkins, 1992) and thus reducing the pathology associated with malaria. This vaccine would react to erythrocyte surface antigen (RESA), merozoite surface antigen 1 and 2 (MSP1 and MSP 2), erythrocye bindng antigen 175 (EBA-175) (Sim *et al.,* 1990) or apical membrane antigen (AMA-1). These are known as the asexual blood stage vaccines. The other vaccine would be a transmission blocking vaccine (TBV) that would interfere with the developing parasite in the mosquito mid gut so that the mosquito does not become infected (Kaslow *et al.*, 1991). Many antigens have been identified as targets for TBV including the pfs 25, pfs 28, pfs 45/48 antigens on the surface of gametes, zygotes and ookinetes (Lensen, 1998). Vaccine that would block the sporozoites from entering the liver and inhibit the liver stage development are also being developed and so far several candidate antigens have been identified including circumsporozoite protein (CS), the Spf66 that is a mixture of synthetic peptides (Pattaroyo *et al.,* 1997) and the trombospondin related adhesive proteins (TRAP) (Muller *et al.*, 1993).

The strategy has been to design a cocktail vaccine that would attack the parasite at all the stages in its life cycle. A lot of progress has been made in some of these in designing such a vaccine; unfortunately they are unable to reduce the transmission when tested in the field.

1.4.5 Environmental management.

Management of the environment to minimize potential breeding grounds and hiding places of the malaria vectors can also help to reduce malaria transmission. Bushes and tall grass provide resting places of malaria vectors soon after emergence form larval habitats and also shields them from being blown away by strong winds. Thus clearing of vegetation around homesteads will reduce the number of malaria vectors around the homesteads. Clogged and blocked drainages, that leads to the accumulation of water to form pools, should not be allowed as these waters provide good breeding grounds for mosquito (Service, 1989). Although Anopheles gambiae has not been identified to breed in waste cans and old tires, as has been culicine mosquitoes, they should nevertheless be disposed off in appropriate places as rainwater may collect in them to encourage stagnant waters. However, mosquitoes being biologically functional organisms, may resort to unusual places to lay their eggs particularly when their preferred sites are scarce as can happen during the dry season.

1.4.6 Community participation.

Any malaria control effort cannot be effective without the active involvement of the community. The community should be involved in all aspects of the malaria control activities. Although this has been very difficult to achieve, community mobilization through education and awareness can be achieved. The community's efforts can be put into promotion of ITN usage, the correct and responsible usage of antimalarial medication, environmental management, and in the training of mosquito control personnel in an area (WHO, 1992).

Rationale and justification of the study.

Malaria parasite transmission depends on the vector competence of the *Anopheles* mosquitoes. *Anopheles gambiae*, an efficient malaria vector in Africa exhibits substantial natural variation among individual populations in their susceptibility to the parasite (Beier, 1996). Understanding the main environmental and genetic factors underlying the observed phenotypic variation could contribute significantly to malaria control. Most studies of the vector-malaria parasite interactions over the past 100 years have been restricted to the laboratory environment, a good understanding is lacking of how the efficiency of natural strains of *P. falciparum* sporogonic development is affected by exposure of infected mosquitoes to the natural environmental conditions.

Studies with the NF-54 strain of *P. falciparum* in the laboratory have shown that exposure of infected mosquitoes to temperatures above 30^0C effectively prevents the pre oocyst stages of the parasite from developing (Noden *et al.,* 1995). This is seemingly inconsistent with the situation in many African settings where indoor temperatures exceed 35^0C in periods of intense transmission suggesting that there could be parasites that are adapted to high temperature survival in these areas. Results from this study would give a better understanding of how interrelationships among mosquitoes, malaria parasites and natural climate-driven environmental factors affect the transmission of malaria parasites. The information will ultimately be useful for predicting how environmental factors affect the distributions of malaria transmission in nature.

1.5 Objectives of the study.

To evaluate the effects of abiotic and biotic environmental factors on the infectivity and early sporogonic development of *P. falciparum* in *An. gambiae* malaria vectors.

1.5.1 Specific objectives.

1.5.1.1 To determine the effects of high temperature on the early stages of sporogonic development of *P. falciparum* in malaria vectors.

1.5.1.2 To determine the effects of temperature variation on the early stages of sporogonic development of *P. falciparum* in malaria vectors.

1.5.1.3 To determine the effect of sugar feeding on the infectivity of malaria vectors with *P. falciparum* malaria.

1.5.1.4 To determine the effect of aging and previous blood feeding on the infectivity of malaria vectors with *P. falciparum* malaria.

CHAPTER TWO

MATERIALS AND METHODS.

2.1 Study area.

The study was carried out in Mbita Point, a rural area of western Kenya, Suba District (between longitudes 34° E 34° 20 " E and latitudes 0° 20 " S 0° 52 " S (Figure 2) on the shores of Lake Victoria. Mbita Location is constituted of several small housing groups adjacent to the Lake Victoria where approximately 8000 people, most of whom are fishermen while others are traditional farmers. It serves as a central trading hub for other parts of Suba District, especially Rusinga and Mfangano Islands where approximately 15,000 and 20,000 people, respectivey, also subsist on fish and farm produce. Mud walled houses are predominant in this area, 60% of which are grass-thatched and 25% are corrugated iron sheet roof. Other types of houses in this area include wooden, corrugated iron sheet, stone walled with corrugated iron sheet roofs and tiled roofs. The EIR in this study area has been estimated at 1.55 infectious bites per person per month (Shililu *et al.*, 2000). The closest health facility is Mbita Health Centre that serves a majority of the people in Mbita. The area typically has two rainy seasons, the main rains extending from March to May and lesser rains in September to December. Annual rainfall ranges from 700 mm to 1200 mm. The area experiences high temperature throughout the year ranging from 17°C to 34°C (ICIPE-Mbita

2.2 *Plasmodium falciparum* gametocytes.

2.2.1 Surveys of *P. falciparum* gametocytes in outpatients attending Mbita Health Centre.

Malaria parasite stages in human blood, either the asexual stages (trophozoites and merozoites cause pathogenicity) or the sexual stages (gametocytes are transmissible), are routinely detected by microscopic examination of blood smear samples at many health facilities. The study of malaria parasite transmission and its regulating factors requires availability of human blood donors carrying infectious gametocytes (Tchuinkam *et al.,* 1993, Toure *et al.,* 1998b). Health facilities could therefore represent a sampling site through which symptomatic gametocyte carriers may be selected for studies. The potential of these volunteer patients with gametocytes to infect *An. gambiae* mosquitoes via experimental membrane feedings was also examined. The local health center, Mbita Health Centre, Suba District, western Kenya was chosen because the area is representative of the basin region of Lake Victoria with high malaria transmission intensity (Beier *et al.,* 1990). The information on malaria parasite infectiousness is critical for understanding the mechanisms that regulate parasite development and transmission under natural conditions and for developing malaria control strategies that block parasite transmission (Tchuinkam *et al.,* 1993; Miller and Hoffman, 1998). In collaboration with the Ministry of Health, Suba District, the ICIPE malaria research project conducted daily malaria surveillance for all admissions in the pediatric ward having children within the age group of 3-15 years as this age group yields the highest gametocyte infection in mosquitoes (Githeko *et al.,* 1992). However, the age group was revised upwards up to and including 45 years by the National Institute of Health because

of the insufficient numbers of gametocyte found. The surveillance system included a clinical and parasitological diagnosis of malaria. During consultation and examination by the Clinician, the goals and objectives of the study were explained to the patients in the local language and informed consents were obtained from volunteers or parents/guardians. The volunteers were screened by examining thick blood films that were collected on blood slides stained with 10% giemsa solution under a light microscopic (100 x immersion lens) to identify and quantify *P. falciparum* trophozoites and/or gametocytes.

2.2.2 Surveys of *P. falciparum* gametocytes in schools and communities in Suba area.

The screening in schools and village communities was carried out under the medical supervision of the Medical Officer of Health of Mbita Health Centre. Consent to screen in schools was obtained from the area education office in Suba while consent to screen in villages was obtained from the area chiefs and village elders. All children attending the primary school of age within the age limit and apparently healthy looking as approved by the Kenya Medical Research Institute (KEMRI) and Tulane University,USA, ethical review boards were accepted to participate in the study. In the village children <1 year and adults living in the local area of Suba District were selected to participate in the study. Upon signing informed consent form, a thick blood smear was taken and microscopical techniques were employed to identify malaria parasites. Those found harboring *P. falciparum* gametocytes were requested to donate blood for infection experiments.

2.2.3 Ethical considerations.

Pregnant women, children weighing <5 kg, and patients with signs of severity of concomitant disease requiring hospital admission were excluded from the study according to the inclusion/exclusion criteria approved by the ethical review boards in Kenya Medical Research Institute, Kenya and in Tulane University, USA. Uncomplicated malaria cases and asymptomatic subjects with slide-confirmed malaria with densities exceeding 1000 parasites per microliter of blood were treated with Sulfadoxine-pyrimethamine (Fansidar®) according to the Ministry of Health policy. *Plasmodium falciparum* gametocyte carriers with gametocyte density of 16 gametocytes per microlitre and above were recruited to donate blood for the experimental infections.. Only persons who had signed the consent agreement form and with pure *P. falciparum* infections were recruited in the study (see appendix 1). Persons who were slide positive with asexual parasites of 5000 parasites per microlitre or lower parasitaemias with malaria-like symptoms were referred and accompanied to the Mbita Health Centre for first line anti-malarial treatment with pyrimethamine-sulphadoxine (Fansidar®) according to the National Malaria Treatment Guidelines in Kenya.

2.3 Mosquitoes used in the study.

2.3.1 Rearing of 'Ifakara' and 'Mbita' strain of *An. gambiae* s.s mosquitoes.

Anopheles gambiae mosquitoes, originally colonized from specimens collected at Njage, 70km from Ifakara, south west Tanzania in April 1996 and a local "Mbita" strain of *An. gambiae s.s* mosquito collected from Lwanda village, 10 km from ICIPE Mbita Point

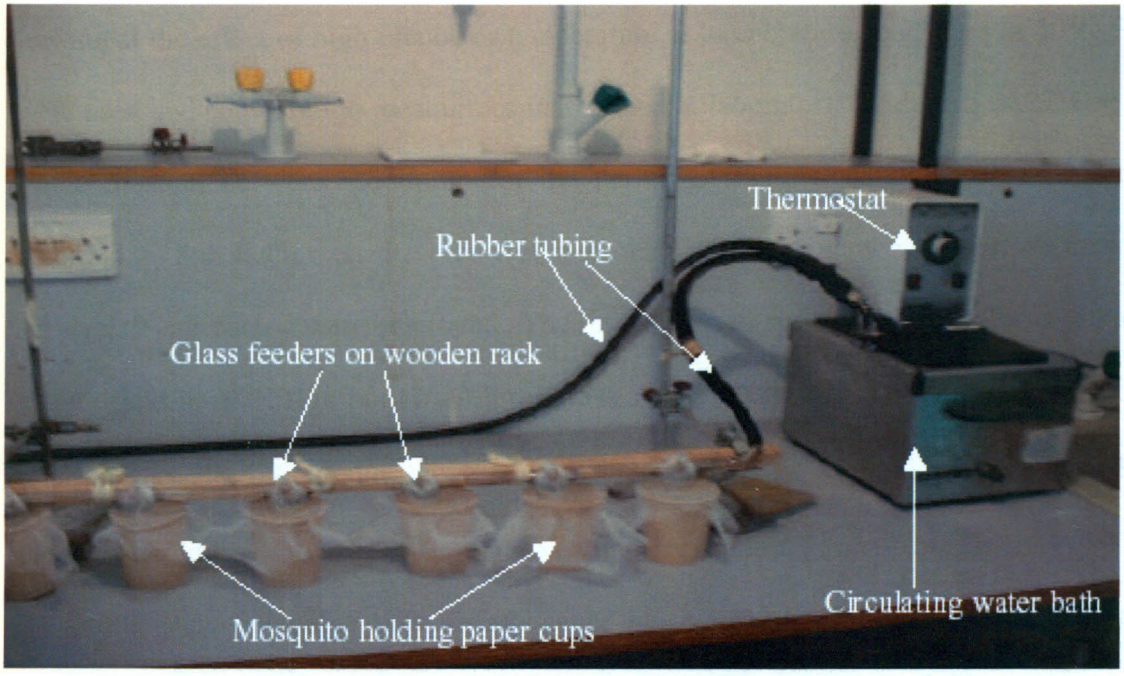
Field Station in August 2000 were established in the insectary and reared as previously described (Benedict, 1997). The two stocks of mosquitoes (i.e. 'Ifakara' and 'Mbita' strain) were kept separately and utilized for different sets of experiments, because the two populations were sympatric. Briefly, about 200 larvae per 20 × 15 cm tray were reared by feeding on 10% slurry of parts 2:1 of Tetramin Baby E® fish food and yeast. Pupae were collected and kept in 30 × 30 × 30 meshed cages where emerging adults had access to 6% glucose and water from where they could be used for experiments. The Ifakara strain of mosquitoes was used in survival studies while the Mbita strain was used in the experimental infection studies using local parasites by artificial membrane feeding. This was because the ethical review boards of the National Institutes of Health (NIH) and the KEMRI did not allow for direct skin feed on human volunteers. Therefore, for successful experimental infection via artificial parafilm membrane with *P. falciparum* from human volunteers, the Mbita strain of mosquitoes had to be adapted to feed via the parafilm membrane. Membrane feeding as an infection method has been shown to yield equally good infection results when compared to mosquitoes that feed directly on human skin (Awono-Ambene *et al.,* 2001).

2.3.2 Adaptation of mosquitoes to artificial membrane feeding.

To adapt mosquitoes to feed through artificial membrane involves selection of mosquitoes that quickly respond to a heat source and those that manage to engorge from the parafilm membranes. Briefly, a human forearm may be placed on top of the cage to attract mosquitoes. Those that make an active attempt to reach the forearm to feed are aspirated into a cage. Also a polythene bag with warm water at a temperature of approximately 37 -

40°C can be used so that the mosquitoes could be attracted to the heat source. These mosquitoes were fed on human blood via the artificial parafilm membrane feeding system (Figure 3) using mini glass feeders. Those that engorged represented the first generation parental stock of a membrane competent. They were kept in a cage where they were allowed access to oviposition dishes after 3 days. The subsequent generations were also selected until a high membrane feeding success rate was achieved. Membrane feeding ability in mosquitoes is difficult to acquire but can be lost easily and hence the mosquitoes must be continuously maintained on a non-human source such as rabbit blood.

Figure 3. The experimental feeding system via artificial membrane.

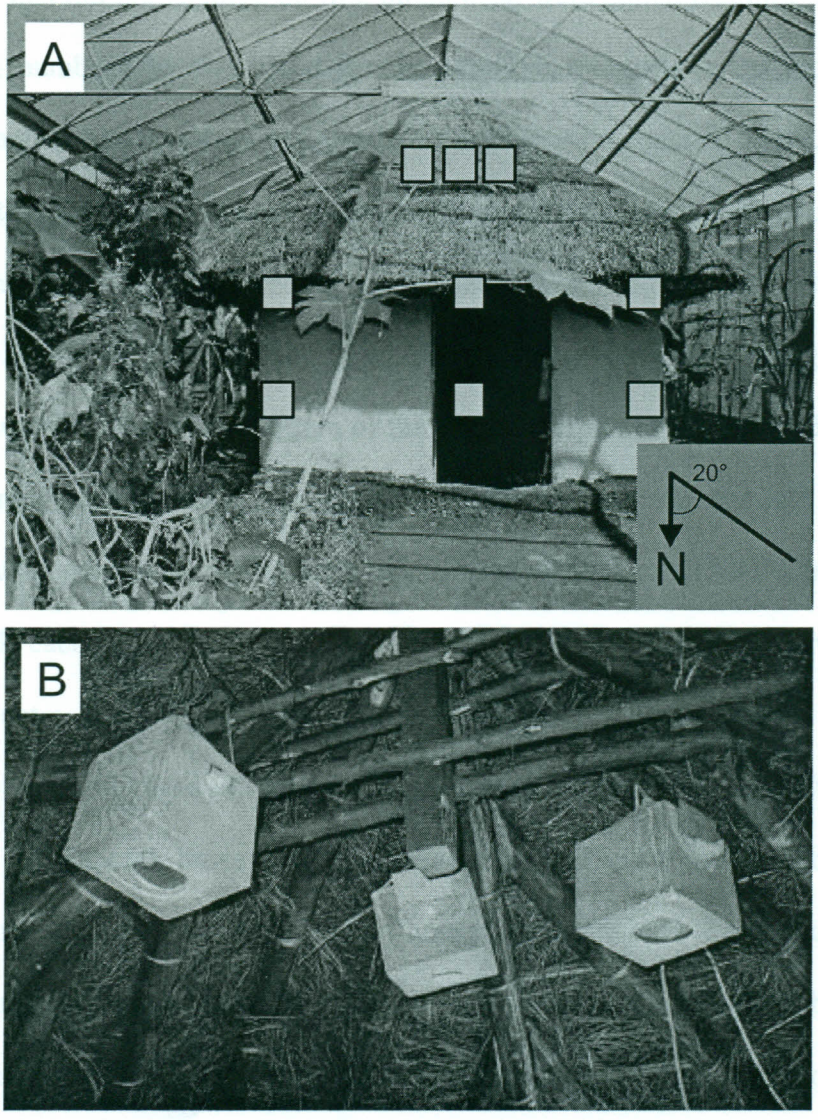


2.3.3 Preliminary studies on the survival of *An. gambiae* mosquitoes under semi natural and natural microhabitats.

In order to assess the influence of high temperatures in incubators and variable temperature of the environment on malaria parasite development, it would require the holding of mosquitoes in the preset high temperature conditions inside incubators or under variable temperature conditions in simulated microhabitats in modified screen houses (semi-natural microhabitats) and in natural microhabitats inside village houses. Of major concern was the impact of microclimatic conditions on mosquito survival that may have led to mosquito mortality. Therefore experiments were designed to assess the effect of high incubator temperatures and effect of microhabitat temperature variation on mosquito survival. Batches of mosquitoes (30 – 50) each were separately fed on sugar meal and blood meal and each maintained under experimental conditions being investigated. For studies looking at the effect of high incubator temperature, 2 temperature conditions of 30°C, 32°C were used and compared to mosquitoes maintained at laboratory conditions of 27°C $\pm$ 2°C. These mosquitoes were monitored for the period of interest of 10 days and mortality recorded. For studies on the effect of microhabitat temperature variation on mosquito survival, 2 approaches were employed. The first approach; mosquito survival was studied in a semi field local village hut (wall dimensions 3.2m $\times$ 2.8m $\times$ 1.7m) constructed within a large mesh-walled screen-house (dimensions 11.4m $\times$ 7.1m $\times$ 4.2m) exposed to ambient climatic conditions and used to simulate a natural mosquito microhabitat (Figure 4). Within the house, each of the nine experimental cages was assigned to one combination of vertical heights (0.5, 1.5 and 2.5 m) and one of three sides of the house (walls facing approximately

east, south and west). Each cage was suspended from a pole or directly from the ceiling using steel wire, the first centimeter of which was coated with Tanglefoot®, a castor oil-based odourless formulation for protecting against crawling insects, ants in particular.

Figure 4. The experimental system and design used in the mosquito survival studies under semi field environment. Cages shown as white boxes inside the hut at the 3 heights (0.5, 1.5 and 2.5 m) and aligned in the middle of each of the 3 sides of the hut (A) on the east, south and west-facing walls and grass thatch roof (B).



The three cages at each level were randomly assigned to either blood, sugar or a combination of the two and in the subsequent two experiments, this arrangement was rotated so that cages at each position (height and side combination) received each of the three diets once over the course of the experiment. The sugar-fed cages were allowed *ad libitum* access to 6 - 10 % (w/v) glucose solution in a cotton pad whereas blood fed cages, after covering them with black cloth, were offered a human forearm from the same individual every second day. Mosquitoes that did not engorge on first blood meal were culled resulting in a reduction in the starting numbers for the blood group. For the mosquitoes fed blood, oviposition substrates in this experiment were not provided because as mentioned above, earlier attempts in the field to incorporate oviposition along with survival data proved difficult as the identity of the mosquitoes that laid could not be established from the cage. Similar observations were recorded as (Straif and Beier, 1996) in which the mosquitoes deposited eggs on dry paper towels that were placed on the bottom of the cage to help in collection of dead mosquitoes and to maintain cleanliness. So oviposition data were not collected and the experiment proceeded with no provision for extra oviposition substrates. HOBO® data loggers were placed inside each cage to record temperature and humidity at hourly intervals throughout the experiment.

The second approach, mosquito survival was assessed inside 6 local village houses that were selected for experiments, however, 2 houses were vacated and demolished by owners in the middle of the experiments and thus only 4 houses remained with 2 of corrugated iron roof and 2 of grass thatched. The head of the households were consulted to obtain consent to conduct the mosquito survival experiments in their houses. A questionnaire was also

distributed to find out the major household features and activities that could influence the microclimates inside the houses. The microclimatic conditions were also monitored with mini-climate recorders (HOBO® Data logger, Onset computer corporation, Bourne, MA, USA) of humidity and temperature over the entire period of experiments. Within these houses, 2 experimental cages were placed on one side of the house wall facing the rising sun. Each cage was suspended directly from the ceiling using a steel wire, the first centimeter of which was coated with Tanglefoot®. The cages were placed at a level of 1.5 metres above the ground. The mosquitoes were either provided blood alone or 10% glucose solution. The sugar-fed cages were allowed *ad libitum* access to 6 - 10 % (w/v) glucose solution in a cotton pad whereas blood fed cages, after covering them with black cloth, were offered a human forearm from the same individual every day. In addition the blood fed cages were provided oviposition dishes in an attempt to integrate oviposition data into the studies. However, this data did not prove useful because mosquitoes that had oviposited and those that had not could not be distinguished. In both experimental set ups, the experiments started in the evening of each day. Dead mosquitoes were counted and removed from the cages and blood meals provided at this time. The experiment proceeded until all the mosquitoes died.

2.4 Experimental infection of mosquitoes with *Plasmodium falciparum* gametocytes from human volunteers.

Two strains of *An. gambiae s.s* mosquito described above that were adapted for the ability to feed on artificial parafilm membrane as described above were used in experimental infections. Batches of approximately 50 to 100, 3 - 5 day old female mosquitoes were aspirated into paper cups and used in the experimental infections. These mosquitoes were starved for 6-8 hours prior to feeding on infectious blood. For experiments looking at the effect of aging on mosquito infectivity, 3 batches of emerging mosquitoes were collected shortly after emergence and were provided with a daily access to 10% glucose solution in cotton wicks that were changed every day, until they reached the desired age for experiments. Three groups of 100 mosquitoes were aspirated into a holding cup for experiments. The first group was 1 to 3 days old mosquitoes, the second 4 to 7 day old mosquitoes and the third 8 to 11 day old mosquitoes. In the experiments studying the effect of previous diet on infectivity, 3 cages of emerging mosquitoes were also set up in this study. All the mosquitoes used for infection experiment were in the same age group of 8 – 11 days old. The first cage was provided 1 blood meal every 4 days for 8 days so that overall it received only 2 blood meals, second group was provided 1 blood meal in 8 days, and another group was provided no blood meals but sugar meals for 8 days. This meant that the first cage received 2 previous blood meals, second cage 1 previous blood meal and third cage no previous blood meals. In the first set of experiments, 3 groups of 100 mosquitoes from each of the 3 cages were each fed on infectious blood meal containing *P. falciparum* gametocytes from infected volunteers. In the second set of experiments, 2 cages of emerging mosquitoes

were set aside with one cage receiving 10% glucose solution immediately on emergence and the other cage receiving distilled water. These mosquitoes were aspirated into 2 experimental cups of 100 each. The 0-2 day old mosquitoes where one group received 10% sugar meals and the other received none were also fed on infectious blood meal containing *P. falciparum* gametocytes. During experimental infections, the clinician withdrew 2 ml of venous blood into heparinized tubes from each gametocyte carrier which was dispensed into 6 glass mini membrane feeders prewarmed to 37°C as previously described (Tchuinkam *et al.,* 1993) and immediately fed to mosquitoes. Mosquitoes were allowed to feed for 15 minutes after which fed mosquitoes were sorted form unfed mosquitoes. The cups holding the mosquitoes were divided so as to accommodate the different experimental study conditions. Mosquitoes were maintained on with 10% glucose solution on cotton wicks that were changed daily. For experiments on parasite effect on mosquito survival, mosquitoes were experimentally infected with blood containing gametocytes and another group with naïve blood with no history of malaria infection. After the feeding the mosquitoes were offered 10% sugar solution on wetted cotton wool for the entire duration of holding. The mosquitoes were monitored daily for mortality up to 7 days and the dead mosquitoes were counted. The wings of the dead mosquitoes were mounted on glass slides and the size measured using ocular micrometer. On day 7, the mosquitoes in the experimental group were dissected to observe for oocyst infection. Oocyst densities and prevalence was recorded.

2.5 Parasite detection in mosquito midguts.

Mosquito dissections to detect and quantify ookinetes followed the method previously described by Gouagna *et al.,* (1999). Briefly, 5 mosquitoes were dissected in phosphate-buffered saline (PBS, pH 7.2) between 18 - 24hr post feeding according to Noden *et al.,* (1995) and Robert *et al.,* (1995). Their entire midguts were disrupted and suspended in a vial containing 50µL of specific FITC-labeled monoclonal antibodies against Pfs 25 gamete stage antigen in 0.05% Evans blue (Robert *et al.,* 1995). After 30 minutes incubation in darkness at room temperature, the suspension was washed by centrifugation in 1000µL PBS at 5000 rpm for 2 minutes. The pellet was then resuspended in 50µL PBS and homogenized. The preparation was then placed between slide and cover slip and the entire slide screened for ookinetes with the aid of a fluorescent light microscope at the × 50 oil immersion objective lens.

The detection of oocysts on the mosquito mid gut was done on day 7-post infection. Mosquitoes were dissected and oocysts detected by staining in 2% mercurochrome and examining under light microscope at 10x objective lens. The intensity expressed as number of oocyst per midgut and infection rate as number of infected mosquitoes were estimated for each experimental group of mosquitoes.

2.6 DATA MANAGEMENT AND STATISTICAL ANALYSIS.

2.6.1 Surveys of gametocytaemia in symptomatic and asymptomatic volunteers.

All statistical analyses were carried out using SPSS version 10.0.1 and Microsoft Excel 2000 for Windows, as described in the results section in relation to the outcomes examined. Parasitological indexes using age as grouping factor and for comparison between groups were compared, Chi-square test was used to test hypothesis of difference between variables and to examine possible source of variation. The independent contrast between clinical symptoms, asexual positivity, previous treatment and gametocyte carriage was regressed using forward conditional selection procedures to obtain parameter values, which were tested by Chi-square to determine possible predictors and to select the most probable underlying predictor when faced with two or more covariates.

2.6.2 Mosquito survival studies.

The differences in survival times and mean survival times for mosquito groups on different dietary regimes, pooled over all variables, were calculated using the Kaplan-Meier survival analysis. The predictors of mosquito survival were estimated by a Cox proportional hazards regression model as a function of diet, temperature, humidity, height and side of hut or the hut in which the mosquitoes were held. The proportion surviving was calculated as the proportion of mosquitoes surviving longer than any time, t. The effects of continuous variables such as temperature and humidity were tested in the Cox regression model directly and as transformations thereof because their relationship to mosquito survival is known to be

non-linear (Martens, 1994; Craig *et al.,* 1999). The model computed the proportions of surviving mosquitoes, baseline survival function for any given day, survival function at mean of covariates, hazard function and estimated regression coefficients for each variable in the standard Cox model. The survival functions at any time, $S_1(t)$, were calculated by raising the baseline survival function, $S_0(t)$, to the power of C, where C is the exponential of Z, the summation of the regression coefficients of the covariates, (β), multiplied by means of covariate factors that affect survival (x) as described by Everitt, (1994) as follows:

$S_1(t) = S_0(t)^{C_1}$ where $C_1 = \exp^Z$ and $Z = (\beta_0 + \beta_1 x_1 + ..)$

All models were fitted and the most appropriate covariates selected by exploratory fitting, using forward conditional stepwise selection procedures. The SPSS Cox regression procedure allows calculation of regression coefficients and means for categorical covariates using a reference group that is specified or coded during contrasting of categorical covariates.

2.6.3 Malaria parasite infection parameters in mosquitoes.

The parameters or indicators of infection that were considered included ookinete density, oocyst prevalence, oocyst density and the killing power or K representing the successive loss in parasite numbers (Vaughan *et al.,* 1992). To account for individual differences in infectiousness between gametocyte carriers (Muirhead-Thomson, 1954; Githeko *et al.,* 1992) the analysis was done per individual carrier. Comparisons of the mean densities of ookinete and oocysts and the mean prevalence were done using the analysis of

variance. The loss in parasite numbers is estimated by a population mortality coefficient, *k*, representing the difference between densities at 2 consecutive stages calculated for the transitions in early parasite development from macro-gametocytes to ookinetes (*k-1*) and ookinetes to oocysts (*k-2*) as previously described (Vaughan *et al.,* 1992) where *k*-1 represents $\log_{10}$ [macro-gametocytes] minus $\log_{10}$ [ookinetes], *k*-2 represents $\log_{10}$ [ookinetes] – $\log_{10}$ [oocysts] while overall mortality, *K,* is the sum of *k*-1 and *k*-2. The antilog of *K* gives a quantitative measure of the magnitude of that loss (Vaughan *et al.,* 1992, 1994a). However, this may sometimes be represented by parasite yield, *Y*, which is the inverse of the antilog *K*, expressed as a percentage.

CHAPTER THREE

RESULTS.

3.1 Parasitological surveys for *Plasmodium falciparum* gametocytes from symptomatic and asymptomatic carriers.

3.1.1 Clinic attendance profile and malaria burden in Mbita Health Centre.

Over the course of the year long survey at the clinic, 3987 patients were screened for malaria parasites, 55.5% of whom were females. The dominant age groups attending the clinic were young children, particularly infants, and young adults aged 15-30 years (Table 2). The vast majority of the patients attending Mbita clinic, came either from Mbita itself (54.2%) or from nearby Rusinga Island (35.5%). *P. falciparum* was highly prevalent and parasites were identified in thick smears from almost half of the patients (Table 1).

Table 1. Malaria parasite infection (trophozoites and gametocytes) distribution within age groups of patients attending Mbita Health Centre, Suba District.

Age Group:	<5	5-15	16-30	>30	Overall
Number of patients (%):	1417 (37.7)	635 (16.9)	1163 (31.0)	539 (13.5)	3754 (100)
Proportion infected (%)	54.8	57.3	36.6	33.4	46.5
With >2000 parasites per µl (%)	12.8	8.2	2.8	3.5	7.6
Proportion with gametocytes (%)	6.6	3.2	0.9	2.8	3.7

The only other parasite species identified was *P. malariae* with a prevalence of 0.9% (19 cases of which 15 were mixed infections with both species). High infection prevalence was distributed across all age categories and over half of all children up to 14 years of age harbored patent infections, including very young infants. Based on this distribution, all screened patients as young children (<5 years), older children (5-15 years), young adults (16-30) and older adults (>30) were categorized for subsequent analyses. The prevalence of malaria parasites was highest in children and high-density infections (>2000 per µl) were particularly found in the youngest age groups (Table 2). Only 2.9% of all patients reported having taken medication within two weeks prior to presentation, the most common treatments being pyremthamine-sulphadoxine combinations such as Fansidar® and Metakelfin® (56 patients), followed by chloroquine (Malaraquine®, Homaquine®), amodiaquine (Malaratab®) and quinine (34, 22 and 3 patients for each drug, respectively). Logistic regression using a forward conditional stepwise selection procedure revealed that age group but not previous treatment, or location of residence significantly influence the probability of patients having parasitaemia at low threshold ($\chi^2 = 149.0$, d.f. = 3, $P<0.001$) or high densities ($\chi^2 = 91.5$, d.f. = 3, $P<0.001$). Consistent with Table 1, children were more than twice as likely to exhibit patent infections than adults (Table 2). Children aged >5 and 5-15 years were four- and two-fold more likely to harbor high-density infections than adults, respectively (Table 2).

Table 2. The influence of age group upon patients' probability of having malaria infection at presentation.

Age Group	Odds Ratio [95% confidence interval]	
	Detectable parasitemia [b]	Heavy parasitemia
≤5	2.42 [1.97-2.98]	4.03[2.49-6.54]
6-15	2.67 [2.10-3.39]	2.44 [1.43-4.18]
16-30	1.15 [0.93-1.43]	0.80 [0.45-1.49]
>30	1.00[c]	1.00[c]

[b]Limit of sensitivity for thick smear screening procedure, >2000 per µl., [c]Reference group; confidence intervals not applicable.

3.1.2 Clinical symptoms associated with the presence of gametocytes in symptomatic volunteers.

Only 3.7% of the 3987 patients were found to carry gametocytes, of whom the majority (63%) were young children (Table 1). The logistic regression (forward conditional stepwise selection) determined that age group ($\chi^2 = 35.0$, d.f. = 3, $P<0.001$) and the presence of asexual parasites ($\chi^2 = 63.9$, d.f. = 1, $P<0.001$) but not previous treatment or parasite density significantly influenced the probability of patients being found to carry gametocytes at presentation because of both the obvious association between asexual infection and gametocytaemia and also the fact that infected individuals were screened more rigorously (Table 3).

Table 3. The influence of age group and the presence of asexual malaria parasites upon patients' probability of carrying gametocytes at presentation[a].

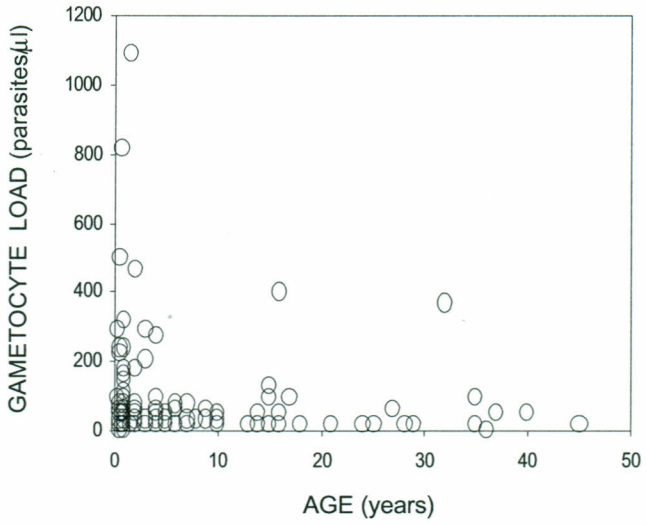
Factor	Odds Ratio [95% confidence interval]
Age group	
<5	1.67 [0.95-2.94]
5-15	0.72 [0.36-1.44]
16-30	0.30 [0.14-0.67]
>30	1.00[b]
Presence of asexual parasites	13.1 [7.2-23.8]

[a] Identified and quantified as significant risk factors by logistic regression using forward conditional selection. [b] Reference group; confidence intervals not applicable.

Possible associations between the presence of gametocytes and various clinical symptoms were therefore tested using partial correlation analysis, controlling for age group and the presence of asexual parasites. Out of 32 symptoms that were recorded, the presence of gametocytes was significantly related to joint pains and was found to be negatively but loosely correlated with this common symptom of clinical malaria ($P = 0.026$, correlation coefficient = -0.036). Gametocyte carriage was not significantly related to any of the other symptoms recorded or to the total number of symptoms. This observation was confirmed by including joint pains as a potential predictor in the logistic regression analysis: reporting joint pains was selected as a significant predictor of gametocytaemia ($P = 0.023$) and included in the model without making any significant changes to the odds ratios described in Table 4. Patients reporting joint pains (20.2%), were less than half as likely to carry gametocytes,

compared with those that did not (OR=0.46, 95% confidence interval [CI] = 0.24-0.90). Gametocyte densities were quite low overall with a geometric mean density of 39 (95% CI = 33 - 46) parasites/μl, that decreased with age (Figure 5).

Figure 5. Gametocyte load, among carriers, as a function of age.

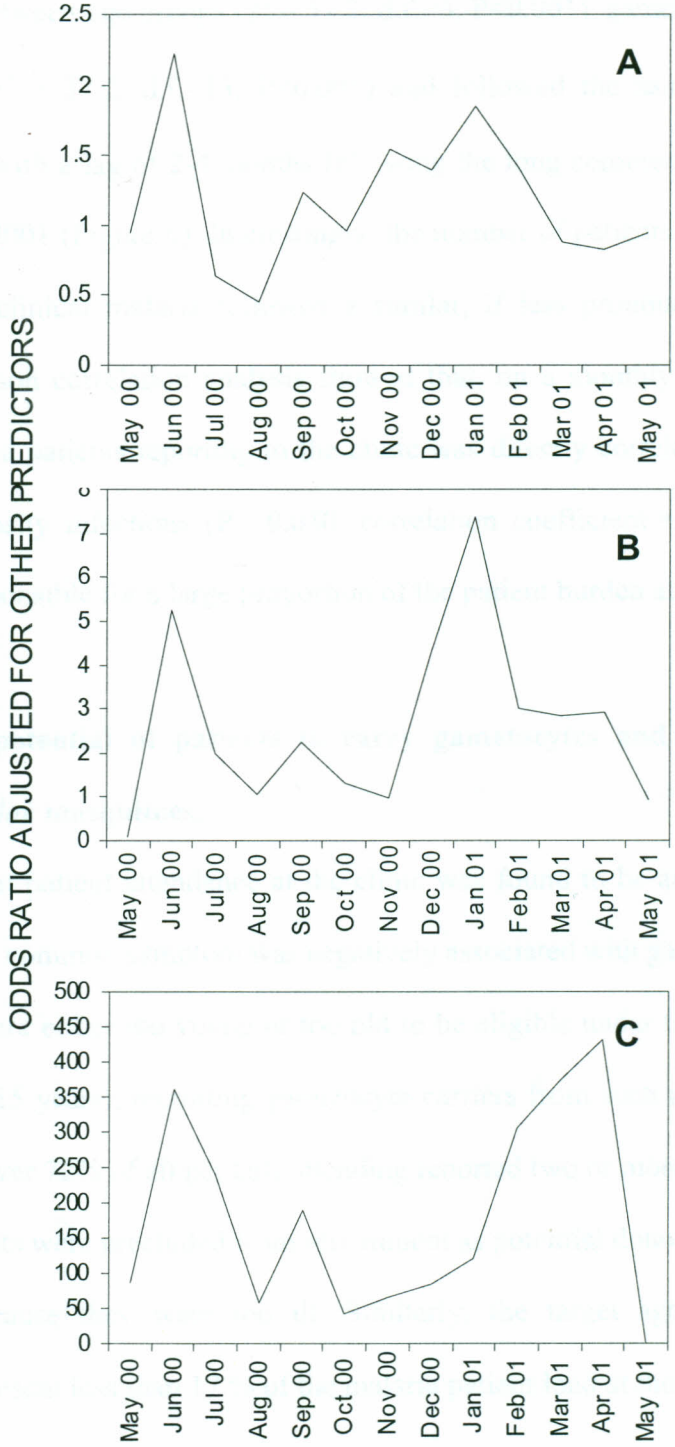


3.1.3 Seasonal prevalence of malaria infection in Mbita Health Centre.

Parasite prevalence in those reporting to the clinic was found to fluctuate seasonally (Figure 6). Inclusion of month as a predictor in the logistic regression model described in Table 2, demonstrated that, allowing for age group (χ^2 = 148.3, d.f.=3, P<0.001) there was significant variation from month to month (χ^2 = 166.4, d.f.=13, P<0.001). Prevalence peaked briefly in June 2000, following the heavy long rains and then more steadily between September 2000 and February, 2001, during which time the usual short rains extended, with essentially no continuous dry period, into the long rains of 2001 (Figure 6). Allowing for age

group (χ^2 = 82.6, d.f.=3, P<0.001), the prevalence of high-density parasitemias fluctuated even more (χ^2 = 87.8, d.f.=13, P<0.001), with two severe peaks in June and December to January (Figure 6).

Figure 6. Seasonality of malaria infection, patients attendance, probability of carrying infection (**A**) estimated by odds ratio, high density infection (**B**) and gametocytes (**C**) at Mbita Health Centre.



Allowing for fluctuations in asexual infection prevalence ($\chi^2 = 62.7$, d.f.=1, $P<0.001$) as well as differences between age groups ($\chi^2 = 31.2$, d.f.=3, $P<0.001$), gametocyte prevalence was also seasonal ($\chi^2 = 37.2$, d.f.=13, $P<0.001$) and followed the same pattern as asexual parasitemia but with a lag of 2-4 months following the long combined rainy season of late 2000 and early 2001 (Figure 6). Interestingly, the number of patients reporting to the clinic with suspected clinical malaria followed a similar, if less pronounced seasonal pattern (Figure 6). Pearson correlation analysis showed that, on a monthly basis, the number of suspected malaria patients reporting to the clinic was directly correlated with the adjusted odd of high-density infections ($P = 0.030$, correlation coefficient = 0.60), indicating that malaria was responsible for a large proportion of the patient burden at Mbita Health Centre.

3.1.4 The potential of patients to carry gametocytes and to infect *Anopheles gambiae* mosquitoes.

Given that patient attendance at the clinic was found to be associated with clinical malaria, that one common symptom was negatively associated with gametocytaemia and that most patients were either too young or too old to be eligible under the approved protocols (target group 5-15 years), recruiting gametocyte carriers from such a health centre proved very difficult. Over 72% of all patients attending reported two or more clinical symptoms so that many patients were precluded from recruitment as potential donors for studies of vector competence because they were too ill. Similarly, the target age groups selected for recruitment represent less than 17 % of the malaria patient load at the clinic and only 14.6%

of all gametocyte carriers. This combination of factors resulted in only 66 potential donors that fulfilled both the inclusion and exclusion criteria of the protocol, of whom only 22 consented and were recruited. Blood from each of these donors was successfully fed to batches of 150-200 mosquitoes. Out of over 4,000 mosquitoes fed on blood from the 22 volunteers only 5 of the volunteers infected any mosquitoes. In total only 12 mosquitoes were infected with only one or two oocysts each. With this trend, there was an urgent need to revise the human use protocol to allow for the screening and recruitment of gametocyte carriers from schools and the communities in Suba district for mosquito infection studies.

3.1.5 Malaria infection and gametocytaemia in asymptomatic subjects in schools and community.

A total of 2228 school children in 11 schools scattered in Suba district were screened for malaria parasite infection and gametocyte carriage. About 51% of the children were males and 49% were girls within the age groups ranging from 3 – 18 years. The prevalence of *P. falciparum* asexual parasitaemia was 31.8% (709/2228) whereas there was a prevalence of 1.7% (37/2228) of *P. malariae* parasites. The overall positivity for gametocytes was very low, a total of 81 children were positive for gametocytes (3.6%) from which some were selected and recruited into the study. Table 4 shows the schools and the number of children screened in each school.

Table 4. The numbers and prevalence of malaria infection and gametocytaemia in asymptomatic school children in Suba district.

Primary school	No.screened	Age (yrs)	Positive cases (%)	Gam positive (%)
Kirindo	325	10.79	29.5	3.4
Kirambo	233	10.78	28.7	3.0
Nyamasare	131	10.14	22.9	3.8
Nyasumbi	154	12.52	29.2	3.2
Kisui	277	10.2	30.7	5.1
Usare	453	10.2	21.2	4.6
Wanyama	404	6.80	25.1	3.2
Mbita Point International	82	5.15	15.8	2.4
Chamakoa	169	10.69	43.2	2.4
Gingo	328	9.50	34.4	2.1
Mother Teresa Academy	75	7.2	69.3	12

No. – number ; Gam. – gametocyte

In the community, a total of 1228 assymptomatic subjects were screened in about 8 villagers namely Kaugege beach (101), Kisui beach (198), Lwanda market (316), Nyagina beach (205), Nyachebe beach (161), Tom Mboya community (100), Lower Kachola (483), Nyatemba (102), Ang'iya (65), Wanga (153) for a period of 6 months. A total of 52 % were males and 48% females. The mean age of participants in this subpopulation was 15.7 years (range 0-94 years). A total of 34.7% of the individuals had *P. falciparum* asexual parasitaemia with relatively low density since only 1% of them had 1000 asexual parasite per μL. Other species other than *P. falciparum* was *P. malariae* that had a prevalence of

3.3%. The gametocyte prevalence was 4.6% (57/1228) from where volunteers with gametocytes in their blood were recruited for experiments.

3.2 STUDIES ON THE SURVIVAL OF *ANOPHELES GAMBIAE* MOSQUITOES.

3.2.1 Survival of mosquitoes at 30^{0}C and 32^{0}C.

The overall mosquito mortality in each experimental incubator was not different for mosquitoes fed blood and maintained on sugar meals. There were no significant differences observed between the mean survival times at the 3 incubator temperatures, namely 27^{0}C, 30^{0}C and 32^{0}C for mosquitoes fed on either sugar or blood.

Table 5. The mean survival times and confidence intervals in parentheses of sugar fed and blood fed mosquitoes held inside incubators preset at 3 temperatures.

	Mean survival times in days	
Experimental temperature	Sugar fed (C.I.)	Blood fed (C.I.)
27^{0}C	9 (7 - 13)	11 (9 - 13)
30^{0}C	12 (11 - 14)	11 (9 - 15)
32^{0}C	11 (10 - 13)	11 (9 - 14)

C.I – confidence intervals

The mean survival times for the mosquitoes was on average about 12 days, a time period which is sufficient for malaria parasite sporogony. Because the experimental mosquitoes

were going to be kept in these incubators, the results show that the mosquitoes can survive long enough until the day of dissection, which in the experimental infection study was 7 days post infection. Regardless of whether the mosquitoes were fed sugar or blood there were no significant differences in their survival between all the dietary and temperature groups (Table 6).

Table 6. A log rank pairwise comparison of the differences in survival of sugar fed and blood fed mosquitoes held inside incubators set at 3 preset temperatures.

Experimental groups	Sugar fed	Blood fed
27^0C vs. 30^0C	0.56 (P =0.453)	0.16 (P =0.687)
27^0C vs. 32^0C	0.25 (P =0.618)	0.49 (P =0.484)
30^0C vs. 32^0C	0.4 (P =0.842)	1.67 (P =0.197)

It was concluded that the incubator temperatures were ideal for holding of infected mosquitoes and was suitable for the experiments.

3.2.2 Influence of indoor microclimate on survival of blood fed and sugar fed *An. gambiae* under semi field environment.

Initially, the effects of diet, height and side of hut were tested by Cox regression to see if any of these factors influenced survival within the hut. The diet ($P<0.0001$) offered to mosquitoes as well as the side of hut ($P=0.003$) at which they were placed significantly affected their survival probability. Height ($P=0.707$) of the cage within house did not

influence mosquito survival. The effect of diet on survival was clear with mean survival times of 6.25 ± 0.30 days for mosquitoes fed blood alone, 14.67 ± 0.48 days for sugar alone and 15.86 ± 0.65 days for blood plus sugar. The availability of sugar to the mosquitoes decreased the odds of mortality (calculated from exponential of the Beta covariate coefficient) by more about 70% compared to the mosquitoes on blood alone (Table 7).

Table 7. Cox regression analysis of the combined influence of environmental and dietary variables upon survival of mosquitoes.

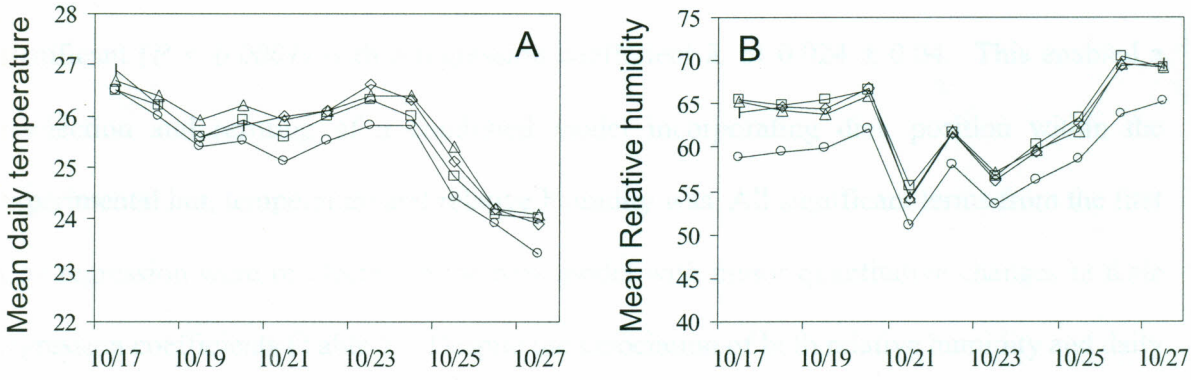
Variable	â	SE	DF	Prob.	Exp(â)
Diet[b]			2	0.0001	NA
Blood alone	0[a]	0[a]	NA[a]	NA[a]	NA[a]
Sugar	-1.086	0.092	1	0.0001[c]	0.338
Sugar +Blood	-1.376	0.099	1	0.0001[c]	0.253
Side of Hut[b]			2	0.002	NA
East side of hut	0.085	0.087	1	0.328[c]	1.089
South side of hut	0[a]	0[a]	NA[a]	NA[a]	NA[a]
West side of hut	0.298	0.088	1	0.001[c]	1.347
Height of habitat	NS	NA	2	0.827	NA
Temperature, (T')[d]	0.021	0.004	1	0.0001	1.021
Relative humidity	NS	NA	1	0.412	NA

[a]Reference group for categorical variables[b]. [c]Significance in relation to the reference group as specified (coded) during contrast selection of the Cox regression model fitting. [d]The square of mean temperature minus the optimum, $(T-20)^2$; NA - Not applicable; NS - not significant at P = 0.05 level.. Beta (β) - estimatable regression coefficient, SE- standard error. A negative beta (β) value indicates increased survival compared to reference group. Exponential of Beta, (Exp â) is a value by which odds of mortality changes per unit increase in covariate factors: Exp(â) > 1, odds of mortality increase, < 1, odds of mortality decrease.

Although the height of cages within the hut had no influence upon survival, the modest but clear effect of the side at which mosquitoes were placed demonstrated that there is considerable heterogeneity in the suitability of different sites within a single house for mosquito survival.

In a related study to compare the climatic conditions of the semi field hut versus 3 field houses, done over a 1-week period, there were significant differences between mean temperature and mean relative humidity's between all the houses (ANOVA: $F = 27.94$; df = 6, 4816; $P < 0.001$ and $F = 18.87$; df = 6, 4816; $P < 0.001$ respectively). The indoor climatic conditions for the experimental hut and field houses was greatly influenced by the prevailing weather conditions as seen in Figure 7.

Figure 7. Temperature (A) and relative humidity (B) profiles from experimental hut and 3 grass thatched huts from Mbita point area, Suba District. Experimental hut (o), Field Hut 1 ( ) Field hut 2 ( ) and Field hut 3 ( ).



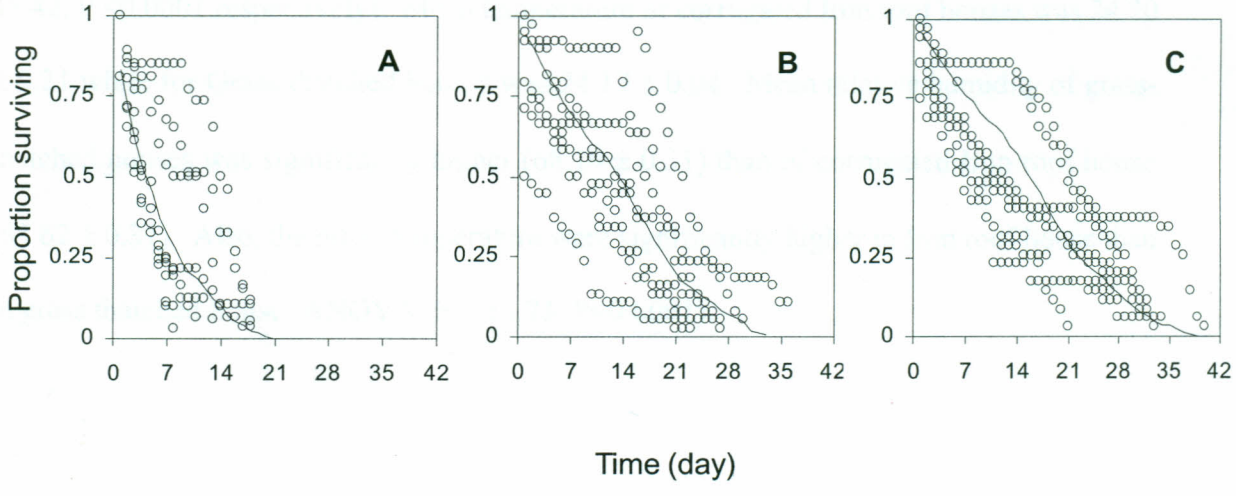
In the experimental hut, temperature ranged between 14.1 and 28.1 °C over all experiments and mean daily temperature varied with height but not side of house (ANOVA: $F = 11.06$; df = 2, 643; $P < 0.0001$ and $F = 0.08$; df = 2, 643; $P = 0.924$, respectively by ANOVA). It increased slightly but consistently with height (23.50 ± 0.07, 23.98 ± 0.08 and 24.04 ± 0.09 °C for 0.5, 1.5 and 2.5 m, respectively). Mean daily relative humidity, which ranged between 23.3 and 90.6 %, varied with height and side of house (ANOVA: $F = 28.75$; df = 2, 643; $P < 0.001$ and $F = 40.14$; df = 2, 643; $P < 0.001$, respectively). Mean daily relative humidity decreased with height (69.2 ± 0.6; 64.3 ± 0.9, 58.1 ± 1.4 % for 0.5, 1.5 and 2.5m, respectively) and was 57.15 ± 1.1, 70.28 ± 0.6 and 63.02 ± 1.2 % for east, south and west facing sides, respectively. Upon examination of these trends, it was hypothesised that such micro heterogeneities of climate, humidity in particular, might be responsible for the differences in survival of mosquitoes in different sides of the house.

In order to test whether microheterogeneities of temperature was responsible for the different survival probabilities on different sides of the hut, a model incorporating temperature and humidity both directly and as non-linear functions was tested to see if they were potential determinants of mosquito survival. The transformed temperature function T′ was selected as significant ($P < 0.0001$) with a regression coefficient â, of 0.024 ± 0.04. This enabled a reselection and refitting of a combined model incorporating diet, position within the experimental hut, temperature and relative humidity was. All significant terms from the first Cox regression were reselected in the new model with minor quantitative changes in their regression coefficients (Table 7). Despite the association of both relative humidity and daily survival with the side of the house at which mosquitoes were placed, neither relative

humidity nor any tested non-linear function thereof proved to be a significant determinant of survival probability over the range observed here (Table 8). However, the non-linear function of temperature (T′), did influence survival, with an optimum of approximately 20 °C that was identified as optimal under these semi-field conditions. By including both temperature and side of house in the same regression model (Table 7), these factors failed to negate the significance of either one, resulting in parameter estimates of both terms that are essentially the same as when separately fitted. Therefore the differential survival of mosquitoes at each side of the hut was independent of temperature and humidity and was not caused by microheterogeneities in these microclimatic factors.

Using the final model the influence of diet on survival over the entire lifespan of female mosquitoes was examined. It was clear that sugar, either alone or in combination with blood confers much greater longevity than blood alone (Figure 8).

Figure 8. Survival probability of female *Anopheles gambiae* fed on blood alone (A), sugar alone (B) or a combination of sugar and blood (C). Observations are represented by points whereas the predictions indicated by the lines.



The minimum sporogonic incubation period of *P. falciparum* was estimated to be just over 14 days under the climatic conditions recorded in the experimental hut with an overall mean temperature of 23.8 °C using the model of Craig *et al.,* (1999). Thus using equation 3, the post-sporogonic survival potentials of mosquitoes fed blood alone, sugar alone, and a combination of the two was estimated as 0.29, 3.63 and 5.37 days respectively. Thus the availability of sugar under these experimental conditions served to increase the survival of female vectors to ages greater than 14 days, during which time they can transmit malaria, by more than 18-fold.

3.2.3 Influence of microclimate of village households on the survival of *Anopheles gambiae* mosquitoes.

Microclimate recorders (Hobo® data loggers) recorded indoor temperature and humidity and the summarized results are shown in Table 8. The temperature and humidity for each house type are summarized in Table 8. The overall temperature and humidity conditions significantly varied between house types (ANOVA: $F = 15.65$, $P < 0.0001$; $F = 18.42$, $P < 0.0001$ respectively). Mean temperature of corrugated Iron roof houses was 24.20 ± 0.21 while for Grass thatched houses was 24.17 ± 0.04. Mean relative humidity of grass-thatched houses was significantly higher (68.19 ± 0.31) than of corrugated Iron roof house (65.62 ± 0.31). Also, the mean temperature were significantly higher in Iron roof house than in grass thatched house (ANOVA: $F = 33.73$, $P < 0.0001$).

Table 8. House types climatic conditions (Mean temperature and Mean relative humidity ± standard error of mean) shown with activity and ventilation levels.

Hut No.	Roof	Activity	Ventilation	Temp. ± SEM	RH ± SEM	Condition
1	Grass	Low	Poor	23.80 ± 0.06	68.91 ± 0.45	Cooler/ Humid
2	Grass	High	Poor	24.31 ± 0.04	66.10 ± 0.47	Cool/ Dry
3	Grass	Low	Good	24.72 ± 0.05	66.17 ± 0.51	Warm /Dry
4	Iron	Low	Poor	22.12 ± 0.57	68.31 ± 0.37	Cool/Humid
5	Iron	High	Good	24.87 ± 0.08	64.10 ± 0.57	Warm / Drier
6	Iron	Low	Good	24.30 ± 0.04	69.50 ± 0.50	Warm /Humid

The median survival times for mosquitoes maintained on sugar and on blood alone for studies under different house conditions were determined. Under the grass-thatched house conditions, the mean survival times were 8 and 10 days for mosquitoes fed blood and sugar meals, respectively. In these conditions, mosquitoes on sugar lived 2 - 3 days longer than those on blood meal alone. The log rank analysis showed significant differences between the survival times for mosquitoes fed on blood and those fed sugar (Table 9). The mean survival times under the corrugated Iron roof houses was 7 days and 10 days for mosquitoes on blood and sugar, respectively. Pair wise log-rank analysis showed significant differences in the mean survival times ($P < 0.0001$) for mosquitoes held under the Iron roof houses.

Table 9. The median survival times of mosquitoes fed on two diets and held in two house types. Log rank statistics shows differences in survival times between the mosquito groups on blood and sugar.

	Mean survival time (d)			
House (No.)	Blood	Sugar	Log rank statistic	P-value
Grass 1	7 (5 – 9)	10 (9 – 11)	11.31	0.0008
Grass 2	8 (7 – 8)	10 (9 – 11)	14.88	0.0001
Iron 1	7 (6 – 8)	9 (8 – 9)	23.03	<0.0001
Iron 2	7 (7 – 8)	10 (9 – 11)	15.32	0.0001

However no significant differences in survival of mosquitoes were found between the grass houses (P=0.124) and the Corrugated Iron roof houses (P = 0.095) for either group of mosquitoes fed blood or sugar meals. However, significant differences were observed between Grass house number 1 and Iron roof house number 2 for the two groups of mosquitoes fed on blood (P = 0.019) and on sugar (P = 0.0321). There were no differences in the survival of mosquitoes between all the other remaining houses (Table 9).

All the dietary and microclimatic factors interacting in the indoor environments, were all included in a Cox regression model in order to predict the factors that influenced most the survival of mosquitoes in the village houses. The diet offered to the mosquitoes, temperature and relative humidity were selected as significant factors affecting the indoor survival of mosquitoes. Diet offered to mosquitoes greatly improved the odds of survival of mosquitoes

by 38%, temperature by 9.5% where as humidity reduced the chances of survival by 4.6 % (Table 10).

Table 10. Cox regression analysis of the combined influence of indoor environmental conditions and diets upon survival of mosquitoes.

Variable	â	SE	DF	Prob.	Exp (â)
Diet[b] -Blood[a]	0[a]	0[a]	NA	NA	NA
-Sugar	-0.470	0.087	1	<0.0001[c]	0.625
Temperature	-0.100	0.020	1	<0.0001	0.905
Relative Humidity	0.045	0.008	1	<0.0001	1.045

[a]Reference group for categorical variables[b]. [c]Significance in relation to the reference group as specified (coded) during contrast selection of the Cox regression model fitting. NA; Not applicable, Beta (β); estimatable regression coefficient, SE; standard error. A negative beta (β<0) value indicates increased survival compared to reference group.

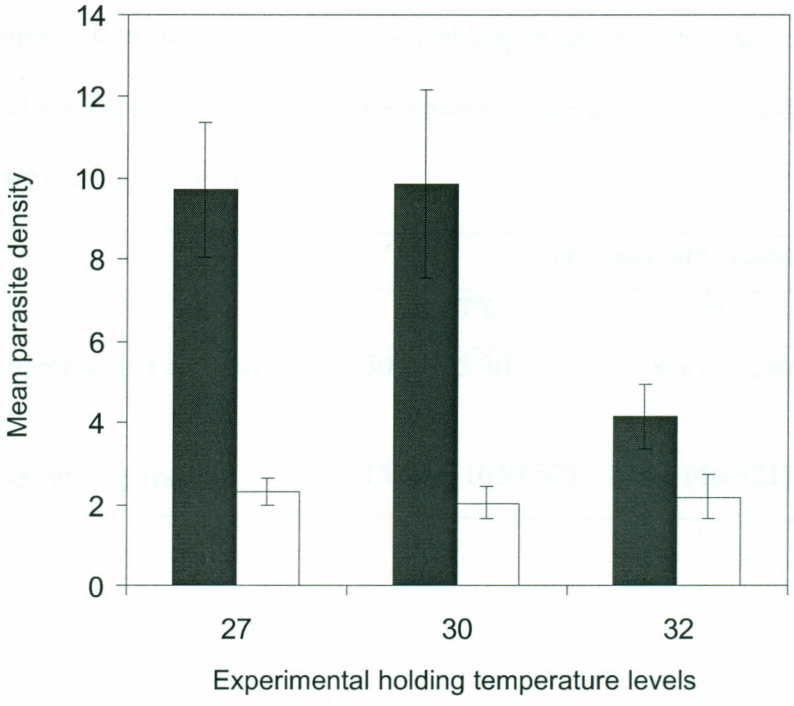
3.3 Effect of high temperatures on early *Plasmodium falciparum* development in *Anopheles gambiae* mosquitoes.

3.3.1 Ookinete densities in midguts of mosquitoes exposed to high temperatures.

Thirty gametocyte carriers [mean age, 5.5 years and mean gametocyte density, 264.1 per μL of blood (range 16 – 1536 per μL)] out of 50 were successfully used for experimental infections where 63% (19/30) resulted in ookinete infections while 50% (15/30) yielded

oocyst infections in mosquitoes. For the comparisons of parasite density and prevalence between experimental temperature groups, only infections (= gametocyte carriers) yielding positive ookinete/or oocysts were used in the analysis. The mean ookinetes density per mosquito mid-gut was significantly different between the experimental temperature groups (ANOVA: $F = 3.19$, d.f. $= 2$, $P = 0.05$) and decreased as the temperature increased (Figure 9)

Figure 9. Mean parasite density ± SEM of ookinete (dark bars) and oocyst (clear bars) in the midguts of artificially infected *Anopheles gambiae* mosquitoes held at 27°C, 30°C and 32°C.



Ookinete densities at 27°C and 30°C were not significantly different (Least significant difference, LSD, test: $P = 0.954$) after performing a multiple comparison analysis for the differences in ookinete density between groups. The only significant difference was found

between 27^0C vs. 32^0C (LSD test: $P = 0.031$) and 30^0C vs. 32^0C (LSD test: $P = 0.031$) indicating a significant effect of high temperature on early parasite development.

3.3.2 Oocyst prevalence and densities in mosquitoes exposed to high temperatures.

A total of 1, 507 mosquitoes were dissected for detection of oocysts with an average batch size of 18.83 mosquitoes dissected per replicate and experimental condition. Examining the data by carriers that yielded one or more oocyst infections, the number of carriers infecting at least a single mosquito decreased with an increase in temperature (Table 11).

Table 11. Proportion of gametocyte carriers yielding positive infections and of infected mosquitoes held at the 3 temperature levels. (Numbers in parentheses are the division of the number positive by total number examined)

	Temperature conditions		
	27°C	30°C	32°C
Proportion of carriers infecting ≥ 1 mosquito	50% (15/30)	44.8% (13/29)	32.1% (9/28)
Proportion of mosquitoes with ≥ 1oocyst	15.8% (103/652)	8.7% (46/531)	6.25% (32/512)

suggesting that high temperatures reduce the infection outcome. The oocyst prevalence was influenced by the experimental temperature conditions (χ^2 = 29.98, d.f. = 2, P < 0.0001) and decreased from 15.8%, 8.7% and 6.25% for 27°C, 30°C and 32°C respectively (Table 12). In contrast to ookinete stage infection densities, there was no significant difference in the mean oocyst density across the temperature conditions (ANOVA: F = 0.301, d.f.. = 2, P = 0.741).

Table 12: Oocyst prevalence and oocyst density listed as per individual gametocyte densities used in membrane feeding experiments for groups of mosquitoes held at the 3 experimental temperatures of 27°C, 30°C and 32°C. Thirty infections were carried out but the table only shows experiments that resulted in oocyst infections.

		27°C		30°C		32°C	
Carrier	Gametocytes/mm^3	‡Prevalence (%)	†Density	‡Prevalence (%)	†Density	‡Prevalence (%)	†Density
1	800	2/10 (20)	4.00	1/10 (10)	1.00	0/11 (0)	0
2	64	7/27 (26)	2.00	5/25 (20)	1.80	1/20 (5)	1.00
3	112	12/24 (50)	1.75	2/18 (11.1)	2.00	6/26 (23.1)	1.17
4	320	22/28 (78.7)	6.32	16/23 (69.6)	6.56	13/25 (52)	5.69
5	32	4/35 (11.4)	1.25	4/30 (13.3)	1.75	4/23 (17.4)	2.00
6	144	8/21 (38.1)	1.88	0/22 (0)	0	ND	ND
7	64	3/24 (12.5)	2.33	2/21 (9.52)	1.50	0/15 (0)	0
8	48	3/21 (14.3)	1.67	4/26 (15.4)	2.25	1/17 (5.9)	20
9	48	9/47 (19.2)	2.00	2/39 (5.13)	1.50	0/21 (0)	0
10	128	2/23 (8.7)	1.50	1/20 (5)	1.00	0/19 (0)	0
11	1536	9/32 (28.1)	1.89	2/29 (6.9)	2.50	2/22 (9.1)	1.50
12	32	2/21 (9.5)	1.50	0/19 (0)	0	0/23 (0)	0
13	32	6/19 (31.6)	1.83	2/8 (25)	2.00	0/11 (0)	0
14	96	14/39 (35.9)	2.57	4/10 (40)	1.75	3/31 (9.68)	1.33
15	16	1/30 (3.3)	2	ND	ND	2/12 (16.67)	1
Mean ± SE	194.13	104/651 (15.9)	2.3 ± 0.33	46/539 (8.5)	2.04 ± 0.4	33/498 (6.6)	2.18 ± 0.54

‡Prevalence is calculated by division of the number of positive mosquitoes by total number of dissected mosquitoes.
†Density is calculated as the number oocysts on all positive midguts divided by total number of dissected mosquitoes
ND - Not done

In order to determine where the greatest impact of temperature occurred during parasite development, the interstage parasite loss was estimated using a mortality index based on a *k*-value representing the differences of densities between two consecutive stages (Vaughan *et al.,* 1992). *K*-values between macrogametocyte-ookinete and ookinete-oocyst were calculated for all the carriers at each of the temperature levels. There was a 10.82 fold loss in parasite numbers during the interstage transition from macro gametocyte to ookinete, *k*-1, at 27^0C, a fold loss which increased with temperature and differed significantly between the 3 experimental temperature groups (ANOVA: F = 3.693, df = 2, P = 0.032) and indicating that parasite mortality increased with temperature (Table 13).

Table 13. Stage specific mortalities of *P. falciparum* parasites developing in *An. gambiae* held at 3 temperature levels. Figures in parentheses represent fold decrease in parasite numbers.

Temperature	*k*-1	*k*-2	K
27	1.034 (*11*)	0.437 (2.9)	1.471 (*31*)
30	1.118 (*12.6*)	0.558 (3.8)	1.676 (*47*)
32	1.415 (*26.1*)	0.504 (2.7)	1.933 (*86*)

k-1 = $\{\log_{10}$ [macrogametocytes] – $\log_{10}$ [ookinetes]$\}$/N; *k*-2 = $\{\log_{10}$ [ookinetes] – $\log_{10}$ [oocyts]$\}$/N; *K* = {*k*-1 + *k*-2}/N N represent the number of gametocyte carriers that yielded positive ookinete infections.

3.3.3 Relationship between parasite mortality and infection parameters.

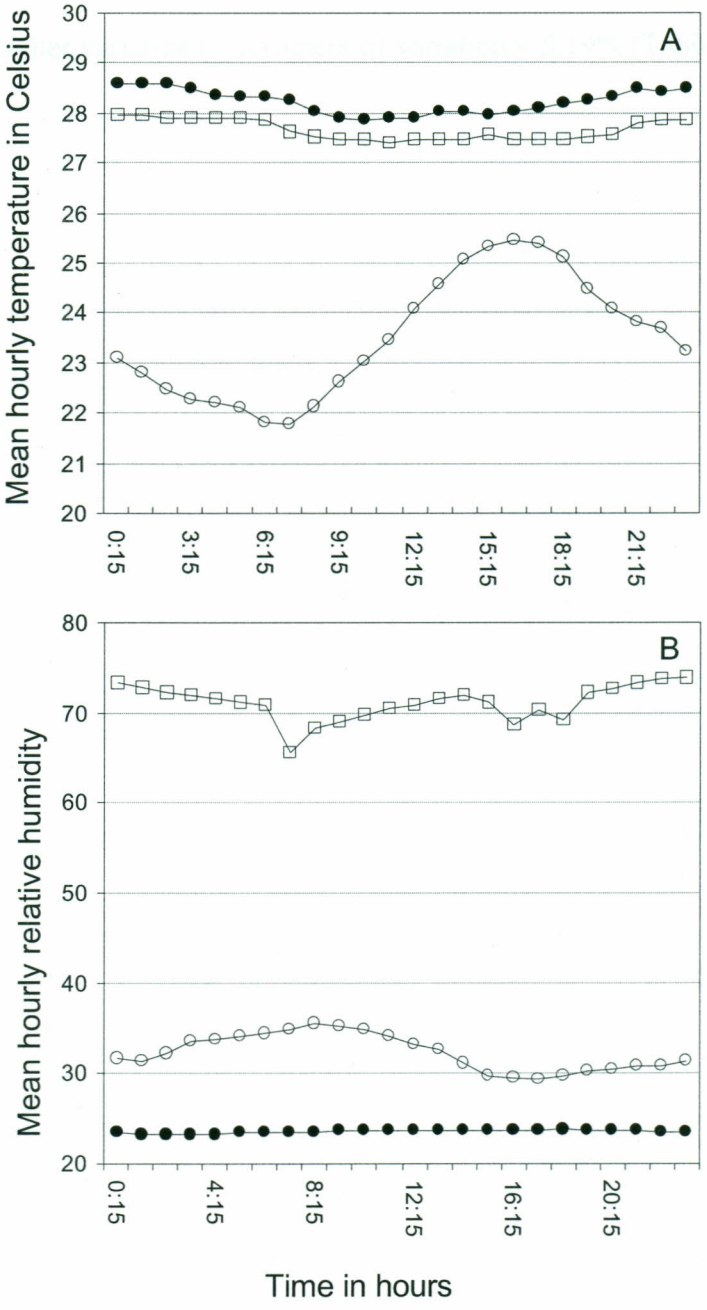
There was no clear relationship between parasite mortality at the transition between ookinete-oocyst stage (*k*-2) and temperature levels. However, the parasite loss between ookinete and oocyst was not significantly different between the 3 temperature levels (ANOVA: F= 0.549, df = 2, P = 0.581). Overall, at the transition from macrogametocyte to oocyst development, there was a 1.6 times and 2.9 times higher loss at the 30^0C and 32^0C compared to 27^0C, respectively. However, the total mortality (*K*) overall increased with temperature (Table 13). This result suggests that high temperatures affect parasite prior to oocyst formation.

3.4 Effect of temperature variation inside semi natural and natural microhabitats on the early development of malaria parasites in *Anopheles gambiae*.

3.4.1 Climates under semi-natural and natural microhabitats.

The mean temperatures were higher in the laboratory (28.22^0C) than in the grass hut within the green house (23.60^0C). Climatic conditions in the laboratory showed less fluctuation than the grass hut in the green house. Overall, temperature in the laboratory was significantly higher with lower relative humidity than the green house hut (Figure 10).

Figure 10. The mean hourly temperature and humidity over a one-month period of the semi natural environments used for the experimental holding of mosquitoes. Blocked circles ( ) represents laboratory environment, open circles ( ) represents semi field hut and open squares ( ) represents incubator as control.



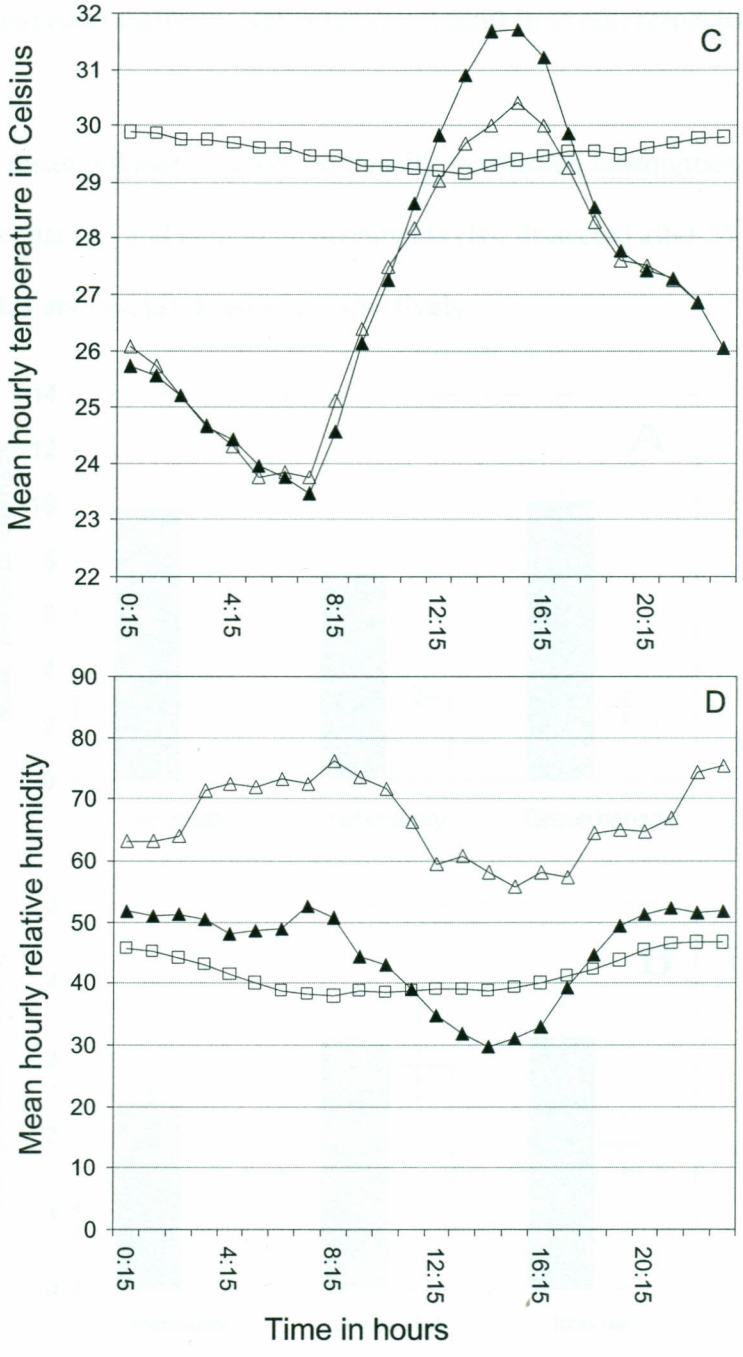
The temperature variation (expressed as the range between the maximum and minimum temperatures) for the control, laboratory and the grass hut in the green house, were significantly different (ANOVA: df = 2, F = 1154, P < 0.001) the grass hut in the green house having higher variation (Coefficient of variation = 5.19% (Table 14).

Table 14. The climatic data readings from the semi natural and natural microhabitats.

	Semi natural microhabitats				Natural microhabitats			
Climatic parameter	Control	Screen house hut	Laboratory	P-value.	Control	Grass hut	Iron hut	Prob.
Temperature								
[a]Max	28.8	26.6	29.9	<0.0001	30.6	31.3	33.0	<0.0001
Mean	27.6	23.7	28.2	<0.0001	29.1	26.9	27.2	<0.0001
[b]Min	26.4	21.3	27.0	<0.0001	27.5	22.6	21.5	<0.0001
Range	2.4	5.3	2.9	<0.0001	3.17	8.9	11.5	<0.0001
[c]C.V	0.8%	5.2%	0.9%	ND	0.8%	7.9%	9.6%	ND
Relative humidity								
[a]Max	78.2	38.4	24.6	<0.0001	85.7	55.7	76.0	<0.0001
Mean	71.2	32.1	23.6	<0.0001	73.7	41.7	50.8	<0.0001
[b]Min	52.9	26.1	23.0	<0.0001	61.8	27.7	25.6	<0.0001
Range	25.3	12.3	1.5	<0.0001	0.29	28.1	50.5	<0.0001
[c]C.V	2.8%	6.2%	0.6%	ND	7.5%	9.6%	17.4%	ND

[c]Max – maximum, [b]Min – Minimum, [a]CV – coefficent of variation, ND – not done. All comparisons were tested by ANOVA.

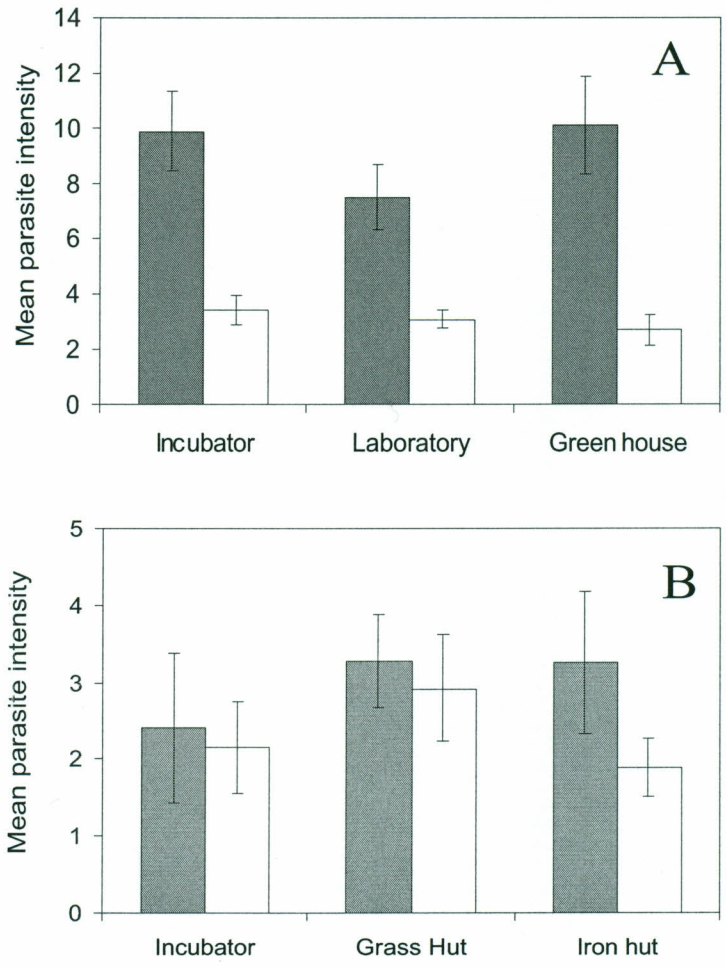
Figure 11. The mean hourly temperature and humidity over a one-month period of the natural environments used for the experimental holding of mosquitoes. Blocked triangles ( ) represents Iron roof house, open triangles ( ) represents grass thatch hut and open squares ( ) represents incubator as control



3.4.2 Mosquito infections in semi natural and natural microhabitats.

Eighty seven percent (27/31) of gametocyte carriers resulted in ookinete infections for mosquitoes held in incubator and semi field hut compared to 84% positive ookinete infections under laboratory conditions. The mean ookinete density was 9.81, 7.50 and 10.11 per mosquito exposed to incubator, laboratory and semi field hut, respectively (Figure 12).

Figure 12. The mean ookinete and oocyst density in infected mosquitoes exposed to semi natural environments (A) and natural environments (B), dissected after 24 hours and 7 days to detect ookinetes and oocyst densities, respectively.



However, the ookinete densities were not significantly different between the 3 holding conditions (ANOVA: $F = 0.915$, $df = 2$, $P = 0.405$). On day 7 post-feeding, 43% (13/30) of the infection experiments resulted in oocyst stage infections in mosquitoes held in the incubator, while 65.3% (17/26) and 64% (16/25) experiments yield oocyst in mosquitoes that were held in semi field hut and laboratory environment, respectively. Whereas the prevalence of infected mosquitoes was influenced by the microhabitat conditions where they were exposed ($\chi^2 = 6.668$, $df = 2$, $P = 0.035$), the mean oocyst density did not vary significantly between groups of mosquitoes exposed to various conditions (ANOVA: $F = 0.546$, $df = 2$, $P = 0.580$) (Table 15).

Table 15. Proportion of *Plasmodium falciparum* gametocyte carriers yielding positive infections, prevalence of oocyst infections and the mean oocyst density in infected mosquitoes exposed to semi natural and natural environments.

Mosquito infection	Semi natural environments				Natural environments			
	Incubator	Semi-field hut	Laboratory	Prob.	Incubator	Grass hut	Iron hut	Prob.
Carriers with infections	61.9 (13/21)	68.2 (15/22)	57.1 (12/21)	ND	53.8 (7/13)	38.5 (5/13)	58.3 (7/12)	ND
Average macros ingested								
Oocyst prevalence (%)	10.2 (68/669)	8.4 (64/762)	12.5 (91/730)	0.04	6.9 (13/189)	9.0 (25/278)	9.4 (26/278)	0.62
Mean oocyst density ± SE	3.43 ± 0.52	2.70 ± 0.57	3.08 ± 0.34	0.58	2.15 ± 0.6	2.92 ± 0.69	1.88 ± 0.38	0.37

An analysis of variance for the differences in mean macrogametocyte mortality (k-1) between the mosquito groups exposed under semi natural conditions was not significant (ANOVA: df = 2, F = 0.367, P = 0.694). Similarly there was no significant difference in ookinete mortality (k-2) even though quantitatively there was more oocyst development in mosquitoes held in the laboratory and screen house hut than in the mosquitoes held in the incubator. There was a 5.5-fold decrease in oocyst numbers in mosquitoes held in the incubator higher than in any other experimental group (Table 16).

Table 16. The comparison of the interstage parasite mortality during the early *P. falciparum* development in experimentally infected *An. gambiae* held in the semi natural and natural microhabitats (Values in parenthesis are the parasite yield in percentage).

Experimental group	*k-1*	*k-2*	*K*
Semi field experiments			
Incubator	0.742 (18)	0.734 (18)	1.48 (3.3)
Laboratory	0.858 (14)	0.551 (28)	1.43 (3.7)
Semi field hut	0.766 (17)	0.699 (20)	1.47 (3.4)
Field experiments			
Incubator	1.26 (5.5)	0.10 (2 79)	1.362 (4.3)
Grass thatch house	1.285 (5.2)	0.143 (72)	1.428 (3.7)
Iron roof house	1.287 (5.2)	0.075 (84)	1.361 (4.4)

Yield % = [1/antilog (mortality coefficient, k)] × 100 where

Ookinete mortality coefficient, k-1 = $\log_{10}$ [macrogametocytes +1] – $\log_{10}$ [ookinetes + 1]

Oocyst mortality coefficient, k-2 = $\log_{10}$ [ookinetes +1] – $\log_{10}$ [oocyts + 1]

Total mortality, K = {k-1} + {k-2}

For the natural microhabitat conditions, 54% (7/13) and 31% (4/13) of infection experiments yielded ookinete infections in mosquitoes held inside the grass thatch house and in the corrugated iron roof house, respectively while 39% (5/13) for the control (inside the incubator) group. The mean ookinete densities were 0.34 ± 0.16, 0.66 ± 0.22 and 0.37 ± 0.24, respectively per mosquito exposed to the 3 conditions. There were no significant differences between groups (ANOVA: df = 2, F = 0.671, P = 0.523) (Figure 12). Higher infection prevalence of 9.4% (26/278) was recorded in the iron roof house as compared to 8.99% (25/278) and 6.87% (13/189) in the grass thatch house and incubator. The mean oocyst count in the mosquitoes held in the grass thatch house was 2 fold (0.50 ± 0.45 per mosquito mid-gut) higher than those held in Iron house (0.254 ± 0.18 oocyst per mid-gut) but these differences in oocyst densities were not statistically significant (ANOVA, df = 2, F = 0.166, P = 0.848).

To estimate the parasite mortality between 2 consecutive stages in the field experiments, only ookinete positive infections were used. Regardless of the developmental transition (ookinete or oocyst), parasite mortality rate was not significantly different between mosquito groups held under different roof types (ANOVA; df = 2, F = 0.148, P = 0.864, ANOVA; df = 2, F = 0.024, P = 976, respectively) though there was higher parasite mortality rate on the transition between ookinete and oocyst when mosquitoes were held in grass thatched house environment (Table 17). Although ambient temperatures were higher in corrugated Iron roof house than in the grass thatched house, overall, there were no significant differences in

parasite mortality rate observed in mosquitoes exposed in 2 environments as compared to the control (Table 17).

Table 17. The interstage parasite mortality during the developmental transitions from *P. falciparum* macro-gametocytes to oocysts in *An. gambiae* exposed to 3 different natural microhabitats.

	Holding environment			
Parameter	Control	Grass	Iron	P - value
k-1	1.373	1.286	1.379	0.909
k-2	0.306	0.297	0.053	0.054
K	1.679	1.583	1.432	0.549

k-1 = $\log_{10}$ [macrogametocytes +1] – $\log_{10}$ [ookinetes +1]

k-2 = $\log_{10}$ [ookinetes +1] – $\log_{10}$ [oocyts +1]

$K = \{k\text{-}1\} + \{k\text{-}2\}$

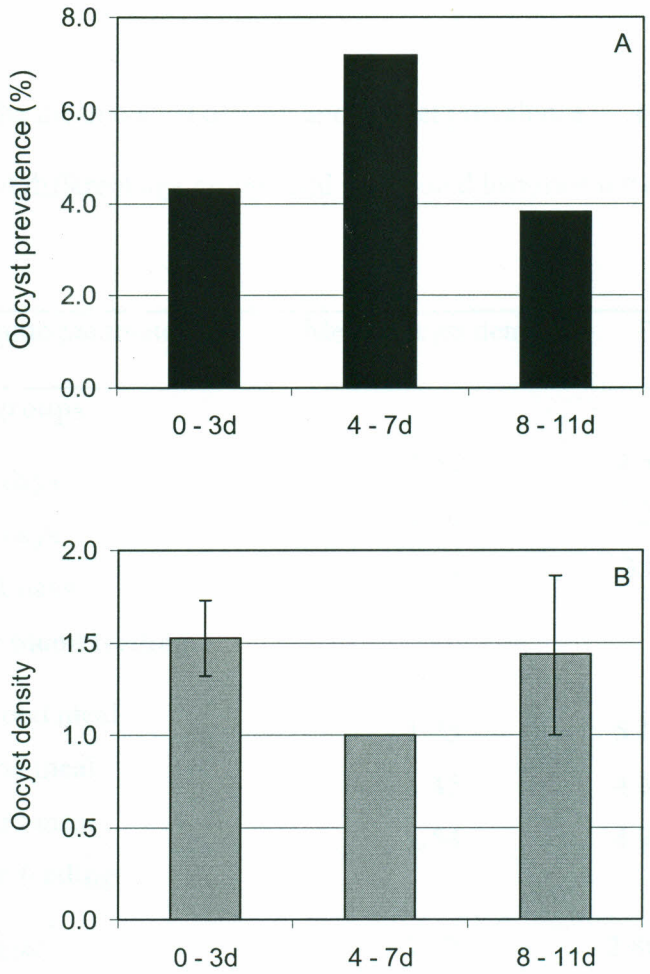
3.5 Effect of age and previous diet on the infectivity of *Anopheles gambiae* with *Plasmodium falciparum* parasites from human volunteers.

3.5.1 Effect of mosquito age on malaria parasite infectivity to *Anopheles gambiae* mosquitoes.

The number of mosquitoes surviving in the experimental groups reduced with increasing age of mosquitoes across the groups from 488 to 265 to 160 mosquitoes for the mosquito age groups of 0-3d, 4-7 d and 8-11 days, respectively. However, the oocyst

prevalence rate was 4.3% (21/487) for 0-3 days, 7.2% (19/265) for 4-7 days and 3.8% (6/160) for 8-11 days. There were no significant differences in mean oocyst count between the mosquito age groups (ANOVA, F = 0.044, df = 2, P = 0.957) even though minor quantitative differences were observed (Figure 13). Mosquitoes in the older age categories of 8 - 11 d were found to be larger in size (mean wing size 5.05) than the 2 other age categories of 0 - 3d, and 4 - 7d with wing sizes of 4.94 and 4.94, respectively. However the sizes of the mosquitoes were not significantly different (ANOVA, df = 2, F = 4.444, P = 0.12). Further analysis showed that there was no significant association between the age of the mosquito and oocyst prevalence (Chi square, $\div^2 = 3.604$, df = 2, P = 0.165).

Figure 13. The mean oocyst prevalence and oocyst density in experimentally infected *An. gambiae* mosquitoes of different age groups.



3.5.2 Effect of previous diet on malaria parasite infectivity to *Anopheles gambiae* mosquitoes.

The prevalence of oocyst infections was not influenced by the type of feed that the mosquitoes had been subjected in the past 8 –11 days (Chi square, χ^2 = 0.188, df = 2, P =

0.910), but could have been influenced by the category of age class. However, there were no significant differences in the mean oocyst density between mosquitoes fed on 1 blood meal, 2 blood meals or none (ANOVA, F = 0.476, df = 2, P = 0.625) (Table 18).

Table 18. The mean oocyst density and oocyst prevalence in *Anopheles gambiae* mosquitoes of different age groups and nutritional histories fed on gametocytes from human volunteers.

Mosquito parameter	Mean oocyst density	Prevalence
Age groups		
0 – 3 days	1.52	4.3% (21/487)
4 – 7 days	1.00	7.2% (19/265)
8 – 11 days	1.43	3.8% (6/160)
Prior blood feeding		
No blood meal	1.25	5.1% (16/315)
1 blood meal	1.45	4.3% (11/254)
2 blood meal	1.54	4.9% (13/263)
Sugar feeding		
No sugar	1.17	2.89% (17/588)
10% glucose	3.03	2.2% (13/592)

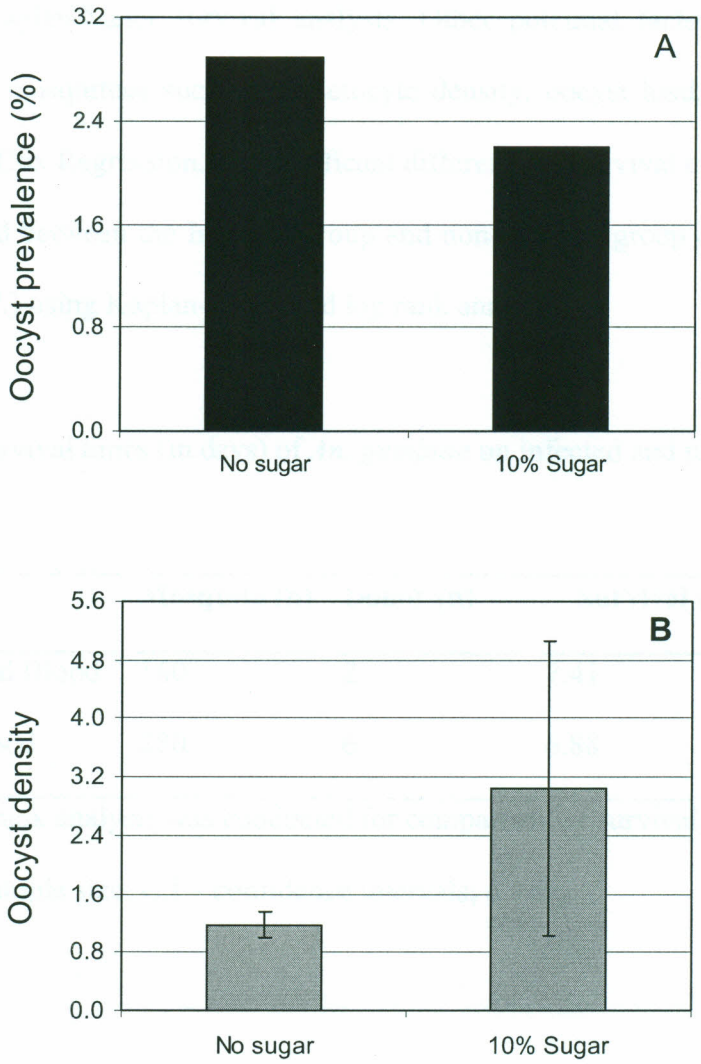
The prevalence of oocyst infection was higher in no blood meal group, followed by the single blood meal group then the double blood meal group. However when the mosquitoes were categorized into 4 size classes, a significant association between size and oocyst

infection prevalence was observed for the group of mosquitoes that were not given blood (Chi square, $\chi^2 = 7.943$, df = 3, P = 0.047) whereas the mosquitoes that were fed 1 or 2 blood meals did not differ significantly (Chi square: $\chi^2 = 2697$, df = 3, P = 0.441 and $\chi^2 = 3.609$, df = 3, P = 0.307, respectively). Similarly, when grouped by the diet given (0, 1 or 2), a significant difference (T-test, P = 0.003) was found in oocyst numbers between mosquitoes given sugar alone and those given 1 or 2 blood meals. In addition the infection rates were higher for the blood fed (8.77%) than the sugar fed mosquitoes (5.35%) even though the sizes of sugar fed mosquitoes (4.91 ± 0.30) were significantly larger (T-test, P < 0.0001) than blood fed mosquitoes (4.79 ± 0.32). When the prevalence and oocyst numbers in single and double blood fed mosquitoes were compared, a higher oocyst prevalence was found in mosquitoes provided 2 blood meals (11.66%) than in mosquitoes provided single blood meal (5.66%) even though the sizes of mosquitoes for these groups did not differ significantly (T-test, P = 0.066).

3.5.3 Effect of sugar feed on malaria parasite infectivity to *Anopheles gambiae* mosquitoes.

The prevalence of oocyst infection was higher in mosquitoes that had not fed on sugar 2.89% (17/588) compared to the group fed sugar 2.2% (13/592) even though the group of mosquitoes fed on sugar (3.03 ± 2.02) had a higher mean oocyst density than that of the no sugar group (1.17 ± 0.17) (Figure 14). However, there were no significant differences between the mean oocyst density between the 2 groups with or without to 10% sugar (T – test, df = 1, P = 0.298).

Figure 14. The prevalence of oocyst infection (A) and the mean oocyst density (B) in 2 groups of experimentally infected mosquitoes, one fed 10 % glucose and the other none.



3.5 Effect of *Plasmodium falciparum* parasite infection on survival of *Anopheles gambiae* under laboratory conditions.

The survival between the infected mosquitoes and uninfected mosquitoes was tested using the Kaplan-Meier survival analysis. Other potential factors that could influence mortality of mosquitoes such as gametocyte density, oocyst load, and donor blood were tested using Cox Regression. A significant difference in survival curves and survival times was observed between the Infected group and non-infected group (*P=0.03*) (see Figure 16 and Table 19.) using Kaplan-Meier and log rank analysis.

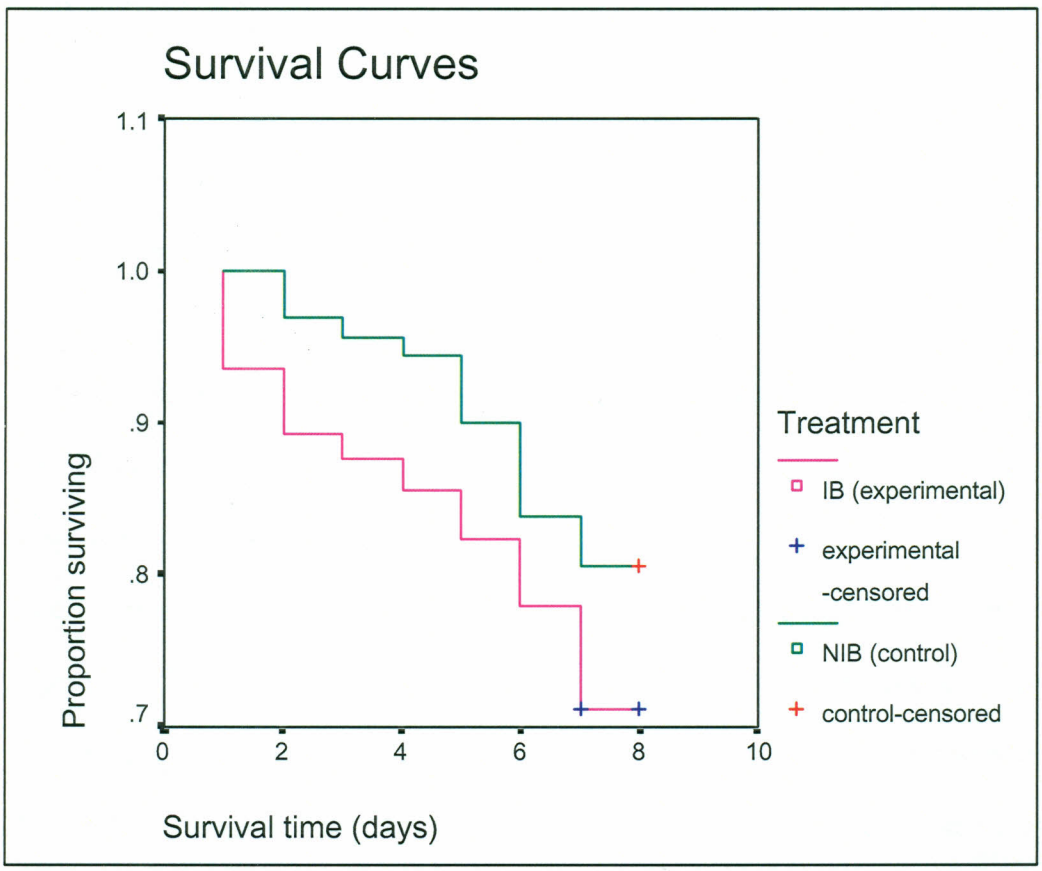
Table 19. Survival times (in days) of *An. gambiae* on infected and non-infected blood.

Treatment	Mosquito (n)	Donor (n)	Survival (d)	95%-CI	P-value[§]
Non-infected Blood	160	2	7.41	7.20-7.63	0.03
Infected Blood	250	6	6.88	6.61-7.14	

[§]Log rank analysis was conducted for comparison of survival curves.

n – sample size; C.I – confidence intervals; d - day

Figure 15. Survival curves of *An. gambiae* fed on *P. falciparum* infected blood (IB) and non-infected blood (NIB).



The infected group suffered a greater mortality than the non-infected group. Mean survival times for Infected and non-infected groups were 6.88 and 7.41 days respectively (Table 19) Treatment was seen as a significant factor (i.e. whether mosquitoes received infectious blood or did not receive infectious blood) using Cox Regression (P = 0.03). However,

gametocytaemia and the blood donor were not seen as significant factors that affected survival.

CHAPTER FOUR

DISCUSSION.

4.1 Parasitological surveys for *Plasmodium falciparum* gametocytes from symptomatic and asymptomatic carriers.

4.1.1 Malaria parasite infection and gametocyte carriage in symptomatic volunteers.

The level and clear seasonality of parasite prevalence indicate that malaria transmission intensity is relatively low and seasonal in the Mbita-Rusinga area where most of the patients come from. The observation that reporting antimalarial drug use prior to presentation had no influence on infection probability is somewhat alarming and suggests that many of the people reporting to the clinic may be presenting with recrudescing, resistant infections. Although this may be partially explained by reluctance of patients to divulge such information, preliminary school surveys in the area suggest that over 10% of infected children may harbor parasites that are resistant to pyremethamine-sulphadoxine (Bousema *et al.,* 2003) and widespread self-treatment and under-dosing was noted in the community. Although location of origin did not influence infection probability, despite the clear differences in vector abundance between marshy areas like Luanda and relatively dry areas around Mbita itself, this may be accounted for by the fact that the vast majority of patients came from the latter.

The clear seasonality of malaria infection, gametocyte prevalence and patient attendance, illustrates that these indicators may indeed be quite responsive to natural fluctuations and artificial perturbations of transmission intensity in the area. The clinical impacts of changes in vector population dynamics, ecology and control in the surrounding area may therefore be evaluated through continued surveillance at this central health facility. Although the reason is not clear, gametocyte prevalence and density do appear consistently low, compared with reports of active community surveillance (Githeko *et al.,* 1992; Toure *et al.,* 1998a; Drakeley *et al.,* 2000) or other surveys of clinical cases reporting to health facilities in Africa (Akim *et al.,* 2000; Robert *et al.,* 2000). Furthermore, of those that did carry gametocytes, the vast majority could not be recruited because of their age, clinical symptoms, low gametocyte loads or unwillingness to participate.

The negative association between gametocyte carriage and body aches, after controlling for age and asexual prevalence, is an interesting example of how clinical malaria and gametocytogenesis occur at different phases of infection. Gametocytes are typically associated with well-established infections and typically appear after the initial peak of asexual parasitemia, in response to a variety of stimuli such as drug pressure, erythrocyte lysis and host reactions (Carter and Miller, 1979; Smally *et al.,* 1981; Schneweis *et al.,* 1991). Clearly in a clinic where patients attend specifically because they are ill, the prevalence of gametocytes reported here are probably misrepresentative of prevalence in the area and preliminary school surveys indicate considerably higher levels among asymptomatic community members (Bousema *et al.,* 2003).

Given the low levels of gametocytemia observed, and the necessity to exclude most potential donors from recruitment, even by expanding the target age group for recruitment, very few

patients attending Mbita Health Centre are suitable gametocyte donors for studies of human infectiousness or vector competence at initial presentation. Although Mbita Health Centre is indeed an excellent surveillance point for monitoring local infection dynamics, regular access to gametocyte-infected blood will require alternative approaches such as active community surveys surveillance (Price *et al.,* 1999; Akim *et al.,* 2000; Robert *et al.,* 2000) or follow up of patients following treatment. (Graves *et al.,* 1988; Githeko *et al.,* 1992; Drakeley *et al.,* 1999).

4.1.2 Malaria parasite infection and gametocyte carriage in asymptomatic volunteers.

This study investigated the prevalence of *P. falciparum* trophozoites and gametocytes in apparently healthy children naturally exposed to endemic malaria. On average about 3% of the children presented with gametocytes at enrolment but this prevalence was higher in children below 5 years of age. The age dependency of gametocyte prevalence is consistent with findings from other studies (Hogh *et al.,* 1988; Akim *et al.,* 2000) as is the age dependency of asexual parasite prevalence and density (Bloland *et al.,* 1999). The gametocyte prevalence of 3.7% in asymptomatic children below 5 years in this area was about 3 times lower than the prevalence of 10% in symptomatic children of the same age from Tanzania (Akim *et al.,* 2000) exposed to an EIR of 30 infectious bites per month (Charlwood *et al.,* 1998; Smith *et al.,* 1998) five times greater than the EIR in this study area. Whether there is a direct relationship between transmission intensity and risk of gametocyte carriage remains to be clarified. Both gametocyte prevalence and gametocyte density of carriers was lower in this study with only sporadic cases of high density gametocyte carriers.

Gametocyte prevalence was consistently highest in the youngest age group and decreased with age as has been described previously (Hogh *et al.,* 1998; Akim *et al.,* 2000; Drakeley *et al.,* 2000). This does not mean that this age group contributes most to the infectious reservoir since a lower prevalence in older people, representing a greater proportion of the population, can be equally important (Hogh *et al.,* 1998; Akim *et al.,* 2000; Drakeley *et al.,* 2000). The correlation between gametocyte carriage and age probably reflects the influence of host immunity, supporting the hypothesis that gametocyte prevalence varies with level of endemicity.

4.2 Survival of *Anopheles gambiae* mosquitoes under semi natural and natural microhabitats.

4.2.1 Influence of indoor microclimate on survival of blood fed and sugar fed *An. gambiae* under semi field environment.

It is demonstrated here that the availability of sugar and, to a lesser extent, the microhabitat within houses in which mosquitoes rest, can substantially influence their longevity and thus the potential to transmit malaria. An important role for sugar feeding in vector survival and possibly malaria transmission was suggested by these studies, indicating that sugar feeding behaviour and the roles of natural carbohydrate sources warrant much more extensive investigation. This represents the first demonstration that resting in different parts of a house can influence a mosquito's chance of survival and that factors other than temperature and humidity may be involved.

Although the availability of sugar clearly increases the longevity of female *An. gambiae* and their potential to transmit malaria under these conditions, the striking impact of sugar observed here may be somewhat exaggerated. Here access to blood only on every second day was allowed whereas vectors may feed more often upon blood if given the opportunity to do so (Briegel and Horler, 1993; Beier, 1996; Straif and Beier, 1996; Gary and Foster, 2001). Although this helped to demonstrate clearly the role of sugar as a supplement to blood and is consistent with other studies in which daily access to blood was allowed (Gary and Foster, 2001), others have shown that *An. gambiae* can survive almost as long on blood alone (Straif and Beier, 1996), because they can increase the number of blood meals they take to make up for this energetic shortfall (Briegel and Horler, 1993).

In terms of malaria vectorial capacity, it has been suggested that the shorter lifespan of *An. gambiae* feeding on blood alone is more than made up for by increased feeding frequency (Gary and Foster, 2001) and actually results in increased transmission potential. Although blood-feeding frequency was not measured, an increased activity in blood feeding such as doubling and trebling of blood feeding in the absence of sugar in the diet would still result in a substantially reduced transmission potential based on the post sporogonic potentials estimated. Also, wild mosquitoes taking extra blood meals are not provided with them within the safety of a cage so the cumulative hazards associated with host seeking and blood feeding may result in even shorter life spans in natural populations of vectors feeding predominantly on blood alone compared with those reported here. Overall, the results suggest that malaria transmission may be substantially enhanced by the availability of sugar sources to wild vector populations and that, in addition to host and larval habitats, this may be a crucial and, as yet understudied resource for malaria vector populations.

We have demonstrated that very small differences of temperature but not humidity within a hut can influence mosquito survival in an area like Mbita Point, western Kenya. This does not mean that relative humidity cannot limit vector survival and malaria transmission, as reported elsewhere (Ijumba *et al.,* 1990; Craig *et al.,* 1999), but rather that the wide but high range of humidity observed on the shores of Lake Victoria (Figure 6) are all equally hospitable to mosquitoes. The extreme temperature sensitivity of *An. gambiae* females under these semi-field conditions contrasts sharply with laboratory based studies. Although the temperature optimum of 20 °C identified is consistent with other reports (Martens, 1994; Craig *et al.,* 1999), in the semi-field experimental hut, mosquitoes are clearly much more sensitive to small temperature increases than those maintained in laboratory incubators (Martens, 1994; Craig *et al.,* 1999). An interesting observation is that some factor or factors other than temperature and humidity, influences the chances of mosquito survival, depending upon which side of the hut they were placed in. Although some obvious possibilities such as airflow or light intensity heterogeneities can be considered, it remains to be seen what precisely makes such a clear difference between sites within such a small, enclosed space.

The results of this study suggest some important questions that may form the basis of further investigations into somewhat overlooked determinants of mosquito behavior, life histories and transmission potential. Although the importance of larval habitat and blood meal host availability is well established, if somewhat poorly understood (Killeen *et al.,* 2000a; Killeen *et al.,* 2000b), the role of sugar sources remains essentially unexplored. Some studies have established a clear relation between nectar feeding and the availability of nectar sources for *Aedes aegypti* (Martinez-Ibarra *et al.,* 1997). Increased efforts should be focused on establishing which sugar sources are most commonly used by wild malaria vectors and how

their availabilities influence mosquito population dynamics and malaria transmission potential. Additionally, the potential influence of seemingly minor heterogeneities of indoor microclimate may merit further investigation. Furthermore, uncaged vectors may select particular locations within houses that favor or inhibit parasite development and may even constitute good targets for mosquito control measures such as indoor spraying, or traps.

4.2.2 Influence of microclimate of village households on the survival of *Anopheles gambiae* mosquitoes.

This study has shown that there are differences in survival of mosquitoes when these are held in different houses constructed with different materials. It has also shown that the heterogeneous microclimates observed between houses have an effect on the survival of mosquitoes and by and large are determined by the human activities that go on inside these houses. In addition, indoor microclimates are governed to a greater extent by the type of material used for construction and the factors such as household items and furniture that may prevent the flow of air indoors.

Mosquito densities and infection status is heterogeneous between and among house types (Copeland, 1994) and may be determined by the topography. This study only used 4 houses found in an approximate area of 10 square kilometers with uneven terrain and as such could have obvious differences in microclimates. However, host seeking mosquitoes have been caught in these houses (Mathenge *et al.,* unpublished data) and the fact that mosquitoes seek wherever there is a potential blood meals (Mutero *et al.,* 1999), the study focused on these 4 houses and then by an extension, study a relatively larger area for longer time in order to establish the stability in the relationship between house hold microclimates and factors that

influence it (Ventilation and activity levels). Therefore broad generalizations about heterogeneities of micro-climate and even of mosquito survival in these houses cannot be made because it is impractical to monitor survival and microclimates in as many houses in an area. Only 2 house types were used here because these are the hoses that predominate the rural area where the bulk of mosquitoes have been found.

The indoor microclimates are to a large extent determined by the prevailing climatic conditions, however, the study found that indoor activities and the house design may also influence the microclimate. Many of the houses that were used in the study had some level of habitation with families and their children. However these houses had to be similar in as many attribute as was possible. Houses that had low activity and poor ventilation were found to be cooler and more humid regardless of the roof type. Warmer and drier houses had high activity and good ventilation even though this trend was variable and heterogeneous for the different house types. Microclimatic factors and obviously the diet type were selected as determinants of mosquito survival and could play an important role in elucidating some aspects of poorly understood mosquito behaviors and malaria transmission dynamics indoors (Lindsay and Snow, 1988; Ribeiro *et al.,* 1996).

The temporal and spatial distribution of mosquitoes may be influenced by climatic factors (Ijumba *et al.,* 1990; Snow *et al.,* 1999a) even within indoor environments (Okech *et al.,* 2003) and the availability of potential hosts and food sources (Martinez-Ibarra *et al.,* 1997; Mutero *et al.,* 1999). The results have demonstrated that the survivorship of mosquitoes indoors may be largely influenced by the availability of food resources and microclimatic factors that are governed by house type, activity levels and ventilation indoors. This in turn may account for the heterogeneous distribution of mosquito densities observed inside houses.

4.3 Effect of high temperatures on early malaria parasite development in *Anopheles gambiae* mosquitoes.

This study examined the effect of high temperature on the early sporogony of natural *P. falciparum* parasites. This study differs from other similar studies in that parasites obtained from natural gametocyte carriers were used to infect a local strain of *Anopheles gambiae* ss mosquito. Using natural parasites, it was found that sporogony would still go on even at temperatures greater than 30 even though parasite intensity is reduced in contrast to other previous studies (Noden *et al.,* 1995; Eling *et al.,* 2001). Whereas many studies have instead used culture parasites for their infections (Vaughan *et al.,* 1992; Noden *et al.,* 1995), for the first time the question of how the extrinsic development of natural wild *P. falciparum* malaria parasites is affected by high temperatures that prevail in natural situations has been effectively addressed. Previous studies using cultured parasites, particularly NF-54 have demonstrated that sporogony is impeded at temperatures $\geq 30^0$C (Noden *et al.,* 1995). Parasite development was quite efficient at 27^0C judging from the high parasite prevalence and intensity observed in this study even though it was reduced at higher temperatures. Mechanisms that underlie this adaptation to survival at such temperature conditions are unclear and may need to be investigated further. In addition, the wild genotype pool of *P. falciparum* malaria parasites used could provide vital clues to specific gene systems encoding this phenotypic adaptation. It is known that blood stage malaria parasites secrete heat shock proteins (Biswas and Sharma, 1994) that may also be expressed during early preoocyst stages as the gene encoding for this heat shock protein is present in both asexual and sexual stage of

the *P. falciparum* malaria parasite (Bonnefoy *et al.,* 1994). Heat shock proteins may help counter the damaging effects that high temperature would have on the developing ookinetes. Our study has also shown that the ookinete is the most vulnerable stages of the developing parasite on which high temperatures above 30°C show a significant effect. However the fact that ookinete densities are similar at 27°C and 30°C may indicate that natural *P. falciparum* malaria may also suitably develop at 30°C. It may also raise important questions into overlooked aspects of temperature dependent kinetics of sporogony or aggregation of parasite strains in mosquitoes, a question yet to be investigated at different temperatures. Parasite efficiency was higher at 27°C, which suggest that in the laboratory experiments, 27°C temperature was closer to the optimum temperature for parasite development in mosquitoes in nature. An interesting observation was the reduction of infection rates in mosquitoes with an increase in temperature even though this trend did not hold for infection intensity. This relationship may be superficial because the low density under natural conditions obscure the effect of temperatures when infection prevalence is considered more relevant as previously indicated (Medley *et al.,* 1993).

The loss in parasite numbers is estimated by a population mortality coefficient, *K,* the difference between densities at 2 consecutive stages. An antilog *K* gives a quantitative measure of the magnitude of that loss (Vaughan *et al.,* 1992). At the ookinete stage, the highest impact was seen at the highest temperature of 32°C where there was an overall 25-fold reduction in parasite numbers compared to 10-fold and 13-fold reduction at 27°C and 30°C, respectively. This dramatic loss occurring at temperatures beyond 30°C, may suggest that under natural conditions, temperatures greater than 30°Cmay bear a strong impact on malaria transmission by reducing the prevalence of infected mosquitoes.

It has been demonstrated the sporogonic process of NF-54 parasites is hampered at temperatures above 30°C in studies conducted under laboratory settings (Noden *et al.,* 1995; Eling *et al.,* 2001). This study did not confirm these previous findings but demonstrated that wild parasites develop and produce oocyst at temperatures above 30°C. This observation is in agreement with natural situations in many countries with tropical climates where malaria transmission continues despite the high temperatures, particularly during hot season. Although exposure of parasites at high temperatures greater than 30°C may reduce parasite load of early pre-oocysts stages, the overall outcome is particularly saddening because a single oocyst can still make a mosquito infective.

In conclusion, the experiments have demonstrated that local parasites strains are not completely impeded or knocked out by temperatures just exceeding 30°C, temperatures that have been shown not to affect mosquito survival adversely (Clements, 1963, 1992) but to affect sporogonic stages of cultured parasite strains (Noden *et al.,* 1995; Eling *et al.,* 2001). Similarly, the strongest impact of high temperature was shown to be on the early pre-oocyst stages, which is the weakest link for successful sporogony and often a major target for transmission blocking vaccines. The low parasite intensities in infected mosquitoes under natural conditions (Beier *et al.,* 1992) may reflect the environmental conditions that the mosquitoes experienced during parasite development. To elucidate how environmental factors affect transmission dynamics, the mechanisms underlying the developmental relationship of the vector and parasite, which directly influences the disease transmission, should be addressed because environmental parameters may interact with other factors to influence or regulate parasite survival and transmission.

4.4 Effect of temperature variation inside semi natural and natural microhabitats on the early development of malaria parasite in *Anopheles gambiae* mosquitoes.

This study has shown that *Plasmodium falciparum* ookinete development is efficient resulting in higher densities under conditions of cool temperatures as observed in the grass-thatched houses in both the semi-field and field environments. As observed in the Iron roofed houses, higher temperatures of 34^0C, beyond previously identified temperature thresholds for malaria development (Boyd, 1949; MacDonald, 1957; Vanderberg and Yeoli, 1966; Noden *et al.,* 1995; Eling *et al.,* 2001) did not prevent malaria parasite development to the oocyst stage. However, the relatively low numbers of ookinetes and oocysts found in mosquitoes exposed to semi field and field conditions suggest an inefficient malaria parasite development under these conditions (Beier *et al.,* 1992). Although available data shows low parasite numbers in infected mosquitoes, microclimatic factors are often not considered, and when examined as in this study it is clear that cycling temperature impacts negatively and substantially lowers the parasite densities in mosquitoes in nature.

Although laboratory studies have shown that the sporogony of *Plasmodium falciparum* in *Anopheles gambiae* mosquitoes is quite inefficient resulting in a 2000-fold loss in parasite numbers (Vaughan *et al.,* 1992), environmental factors, particularly variable and cycling environmental temperature, that might have a major influence have not been addressed. In these studies conducted using field parasite populations, experimental mosquitoes exposed to both the semi natural and natural microhabitats experienced a loss in parasite numbers in the range of 20 to 30-fold; sharply contradicting results of earlier laboratory studies (Vaughan *et*

al., 1992). This study with natural parasites from infected human volunteers, suggest that using culture strains of *P. falciparum* parasites, likely yielded bias results on parasite development efficiency in nature.

On separate and closer examination of the interstage parasite development, only a 3-5 fold loss and a 1-2 fold loss in total parasite ingested by mosquitoes was observed due to temperature fluctuation in semi field and field conditions suggesting that cycling climatic conditions experienced in the field does not impact on the efficiently of parasite development. This contrast sharply with earlier studies (Vanderberg and Yeoli, 1966; Noden *et al.,* 1995; Eling *et al.,* 2001) where high parasite loss before ookinete formation was reported. One explanation for this difference may be differential responses of diverse and different parasite genotypes to temperature fluctuation. In high temperature conditions the bulk of early parasites ingested would die soon after exposure of mosquitoes to the microhabitat during the peak temperatures in the field houses (Figure 3). This effect would be most detrimental to ookinetes resulting in fewer oocysts in mosquitoes resting in the hotter shelters. However the fact that mosquitoes are still infected strongly indicates that parasites were able to withstand these temperature conditions and survive through the ookinete stages. Moreover, the prevalence of infected mosquitoes increased as the temperatures of the microhabitats became hotter. Yet the results show that high temperatures in the Iron roof house may not be enough to knockdown the parasites during early mid-gut stages of development. It is also possible that parasite genotypes have adapted to survival at high temperatures in the tropics.

The mortality rate of the macrogamete and ookinetes in mosquitoes held in the semi field environment were consistently low and were not associated with temperature fluctuation.

However, in field experiments where mosquitoes were held in local huts, higher ookinete mortality and lower oocyst mortality was recorded. This result may be explained by differences in the magnitude of temperature fluctuation. More variation was seen in the field microhabitats (coefficient of variation: 9.58% and 17.35% for the field and 6.19% and 0.63% for semi field) than in the semi field microhabitats. Of particular importance is the negligible impact of peaking temperatures in the Grass thatch and Iron roof village houses that was expected to suppress parasite within mosquitoes held under the drastic field microhabitats. Other studies have shown that the bulk of field-collected mosquitoes originate from grass thatched houses or in cooler places outside such as in grass thatched sheds and have a higher prevalence of infection with parasite stages (Service *et al.,* 1978). Such variable climatic conditions may be influenced greatly by house design parameters. In this study, mosquitoes experimentally held in the cooler grass thatched house developed similar densities of ookinetes, oocysts and infection prevalence. This suggests that increase in the construction of Iron roof houses in the rural replacing grass thatched houses with Iron roof may not affect malaria parasite development efficiency. Whether construction of Iron roof houses which becomes increasingly popular in the rural community of western Kenya may affect mosquito host seeking behaviour, survival distributions and malaria transmission risks remains to be studied.

4.5 Effect of age and previous diet on the infectivity of *Anopheles gambiae* with *Plasmodium falciparum* parasites from human volunteers.

This study has shown that the previous diet history particularly of *An. gambiae* mosquitoes that have fed multiple blood meals may influence the infectivity with *Plasmodium falciparum* parasites. In addition sugar diets increase the malaria infectivity in mosquitoes. The study also demonstrates that age does not influence malaria infectivity in mosquitoes. The results show that there was high oocyst prevalence in mosquitoes that did not feed on blood than those that fed on either 1 blood meal or 2 blood meals. The low infection prevalence in the group of mosquitoes that fed on many blood meals is expected because blood feeding increases the proteinase enzyme secretions that would readily digest the developing parasite in the mid gut along with the blood and this has been demonstrated in other studies ((Vaughan *et al.,* 1994b). The mid gut secretions of these proteinases have been shown to increase with the number of blood meals ingested (Gass, 1977; Billingsley and Hecker, 1991; Chadee and Beier, 1995).

For the group of mosquitoes fed blood only (either 1 or 2 previous blood meals) this study found the oocyst prevalence higher in mosquitoes that had previously fed on 2 blood meals and not 1 blood meal. This is in contrast to expectation as several blood meals will thicken the peritrophic membrane (Billingsley, 1990; Miller and Lehane, 1993) and make difficult parasite penetration thus interfering with infectivity. A possible reason for the observations here might be that several blood meals increase the nutrient resource in the mosquito heamocoel that the oocysts may use to grow and develop. This is similar for sugar fed mosquitoes that developed more oocysts than mosquitoes that were not fed anything.

However the wing sizes of mosquitoes that received more dietary resources were compared to the other group, they were not significantly different suggesting that there was no influence of size on oocyst infection rates or prevalence.

In conclusion, the age of the mosquito does not seem to have any particular impact on malaria parasite infectivity to *Anopheles gambiae*. However the fact that older mosquitoes are liable to die sooner may result in younger mosquitoes transmitting malaria. The previous diet may have an effect on infectivity particularly if the nutritional resource interferes with the mid gut by either reducing the amount of blood meal ingestible or by triggering the laying of more peritrophic matrix on the mid gut wall that may interfere with ookinete passage through the mid gut. The fact that *Anopheles gambiae* mosquitoes are anthropophillic, a behavior that leads them to acquire more nutrients from human blood may result mosquitoes developing more parasites and increasing malaria transmission in the community.

4.6 Effect of *Plasmodium falciparum* parasite infection on survival of *Anopheles gambiae* under laboratory conditions.

This study has shown that the human malaria parasite, *P. falciparum* causes some mortality on its vector, *An. gambiae* mosquito, during the seven days period after being fed with *P. falciparum* gametocyte infected blood. Many studies have tried to assess whether the malaria parasite may affect the survival of the vector. However, the conditions under which some of these studies were done were not very well controlled (Anderson *et al.*, 2000). This study

has now attempted to control conditions and to monitor the mortality of *An. gambiae* mosquitoes that have been given parasite infected blood meal.

The results show that there is more mortality in infected *An. gambiae* as compared to uninfected ones. However, this mortality is not a significant one considering that during the 7 days not even half of the mosquitoes died. Non-infected mosquitoes (81%) and infected mosquitoes (71%) still remained alive after initial infection. It must be pointed out that the sample size used was small thus the results of the study should be interpreted with caution. Longer survival in non-infected mosquitoes suggests that there may be slight pathogenicity associated with the *P. falciparum* parasite. However the extent of this pathogenicity needs further investigation. The work conducted in this experiment seems to be in line with other studies (Klein *et al.,* 1982; Klein *et al.,* 1986). On the other hand, other stuides still contradict this claim (MacDonald, 1957; Chege and Beier, 1990; Robert *et al.,* 1990) on the ground that the parasite needs the mosquito to survive in order to continue its transmission. Potentially, there may be different strains in *P. falciparum,* which have a virulent effect on the survival of the mosquito. Since the wing sizes of living mosquitoes were not measured it is difficult to say whether size plays a role in mosquitoes surviving on infectious blood meals. However it can be assumed that size may play some role in survival of mosquitoes. Since bigger mosquitoes take up more blood they also take up more parasite (Lyimo and Koella, 1992). The largest mosquitoes should therefore suffer most from mortality induced by parasites. Thus the possibility of the mosquito taking up a virulent strain of parasite is increased. On the other hand bigger mosquitoes may have better immune capabilities, and high- energy reserves thus being able to protect themselves from such virulent parasites. Gametocyte density can also affect the survival of mosquito during its initial stage of

exflagellation and invasion of the mid gut wall as enzymes could be produced during this process that may induce mortality. The result for this experiment has shown that with the natural parasite in human volunteers, there was a detrimental effect on vector survival.

CHAPTER FIVE.

CONCLUSIONS AND FUTURE WORK.

5.1 Major conclusions.

1. Anti-malarial drug resistance is prevalent in the Mbita area. In addition there is a low gametocyte prevalence suggesting that the area is highly endemic for malaria.

2. The survival of mosquitoes is enhanced by sugar availability and may be influenced by minor microheterogeneities of temperature even within an indoor environment.

3. Survival of mosquitoes in village houses is largely influenced by the diet and the prevailing inter-house climatic differences.

4. Natural parasite strains of *P. falciparum* obtained from humans are able to survive high temperatures during the early development in the mid guts of *An. gambiae* mosquitoes.

5. The microclimates prevailing in indoor microhabitats in Mbita area of western Kenya do not negatively impact on early malaria parasite development in *Anopheles gambiae* mosquitoes.

6. The infectivity of malaria to *An. gambiae* mosquitoes is influenced by the previous diet that increases energy reserves within the mosquito. However the age of the mosquito does not have any particular influence on malarial infectivity.

7. The survival of *An. gambiae* mosquitoes is negatively affected by *Plasmodium falciparum* malaria parasites infection.

5.2 Suggestions for future work.

1. Studies to determine the existence of high temperature adapted genotypes of malaria parasites and threshold temperature for their development in *An. gambiae* mosquitoes need to be carried out.

2. Studies to look at the factors affecting the spatial survival distributions of *An. gambiae* mosquitoes and how it affects the vectorial potential of mosquitoes in nature should be conducted.

3. Field studies to look at the availability of potential host and vector resources on mosquito population density and malaria parasite transmission should also be done.

4. Field studies on the mosquito energetics and parasite development needs to be done.

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APPENDICES.

Appendix 1.

INFORMED CONSENT FORM FOR MINORS

I, ...(name of parent/guardian) being the lawful parent/guardian for my child has been explained the purpose of this study in a language to which I am fluent and as such do hereby consent for the child named.....to participate in the research project titled: **'Vector competence of the African malaria vector'**. My participation in this study is totally voluntary and I understand that I may revoke this consent at any time without penalty or loss of benefits, if any.

Parent/Guardian signature and date.....

ID no.....

Village address .....

Witness name, signature and date.....

Investigator's name, signature and date.....

Appendix 2.

INFORMED CONSENT FORM FOR ADULTS

I, ...(name of volunteer) do hereby consent to participate in the research project titled: 'Vector Competence of African Malaria Vectors'.

I have been given the opportunity to ask questions concerning this project. Any such questions have been answered to my full satisfaction. Should any further questions arise concerning the right of this child, I may contact Dr. John Githure, the Major Foreign Collaborator, ICIPE, P.O. Box 30772, Telephone: 861680, Nairobi.

I also understand that I may revoke this consent at any time without penalty or loss of benefits, if any.

Volunteer's signature and date.....

ID No.....

Village address .....

Witness name, signature and date.....

Investigator's name, signature and date.....