THE IMPACT OF AN IRRIGATION SCHEME ON MALARIA TRANSMISSION BY *ANOPHELES* (DIPTERA: CULICIDAE) IN A COASTAL SAVANNA AREA OF GHANA

BY

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DECLARATION

I hereby declare that with the exception of references to other peoples work which I have duly acknowledged all the experimental work described in this thesis was carried out by me and this thesis, either in whole or in part, has not been presented elsewhere for another degree.

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DEDICATION

To my parents, Mr and Mrs M. O. Okoye and to my siblings,

Mrs Chidi Opurum and Master Chinaecherem Okoye

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LIST OF ABBREVIATIONS

bp	base pair
dATP	deoxyadenosine triphosphate
ddw	double distilled water
dCTP	deoxycytidine triphosphate
dGTP	deoxyguanosine triphosphate
DNA	deoxyribonucleic acid
dTTP	deoxythymidine triphosphate
EDTA	Disodium ethylene diamine tetraacetate. $2H_2O$
EtBr	Ethidium bromide
H_2O	Water
M	Molar
NaOH	Sodium hydroxide
PCR	Polymerase chain reaction
pH	Hydrogen-ion exponent
RNase	ribonuclease
rDNA	ribosomal DNA
rpm	revolutions per minute
s.l	Sensu lato
s.s	Sensu stricto
TAE	Tris - acetate EDTA
Tm	Melting temperature
Tris	2 –amino-2-(hydroxymethyl)-1,3 propanediol
µl	microlitre
µM	micromolar
HLC	Human landing catches
PSC	Pyrethrum spray catch
B/m/n	Bites/man/night
i/b/m/n	infective bites/man/night
EIR	Entomological inoculation rates
ABTS	2,2'-azino-di-3[ethyl-benzthiazoline sulfonate]
RT	Room temperature
Tween 20	Polyoxyethylenesorbitan, monoalurate
HBI	Human blood index

ABSTRACT

The impact of irrigation schemes on malaria risk has been a perennial subject of debate in that transmission in irrigated areas can appear higher, less than or similar in neighboring non-irrigated villages. To study its impact on malaria transmission in the coastal savanna area of Ghana, adult *Anopheles* mosquito populations were studied during the dry season of 2002 – 2003 in two villages, Okyereko which is situated within a rice-growing irrigation scheme and Bewadze located 9.5km away. Indoor and outdoor human landing catches of adult mosquitoes were carried out simultaneously over 2 days each month at the villages followed by pyrethum spray catches. The mosquitoes were morphologically identified and sibling species of *Anopheles gambiae* s.l. identified using polymerase chain reaction based (PCR) method. Molecular forms of *Anopheles gambiae* s.s were further identified using *HhaI* restriction of the PCR products. *Anopheles* mosquitoes were dissected for parity and *Plasmodium falciparum* sporozoite infections and blood meal source were determined by ELISA. A total of 8893 mosquitoes; 8233 from Okyereko and 660 from Bewadze were caught using both HLC and PSC during the study period. *Anopheles gambiae* s.l. was the dominant species at Okyereko comprising 73.9% of the total collection followed by *Culex* species, *An. pharoensis, An. funestus* and *Mansonia* species, *Aedes* species and *An. coustani* respectively. At Bewadze, *An. funestus* was the dominant species accounting for 33.5% of the total collection followed by *An. gambiae* s.l., *Culex* species, *Mansonia* species, *An. phaoensis* and *Aedes* species repectively. A total of 798 *Anopheles gambiae* s.l. from both sites (602 from Okyereko and 196 from Bewadze) were all identified as *Anopheles gambiae* s.s by PCR. The M and S molecular forms constituted 91.7% (66/72) and 8.3% (6/72) at Okyereko and 66.7% (52/78) and 33.3% (26/78) at Bewadze respectively. The mean biting rate of anopheline mosquitoes was approximately 17 times higher at Okyereko than at Bewadze (126.3 vs 7.5 bites/man/night). Parous rates however were similar being, 65% (874/1345 dissected) at

Okyereko and 63.8% (157/ 246 dissected) at Bewadze. At Okyereko, the sporozoite rates of *An. gambiae* s.s. and *An. funestus* were 0.2% (4/2,411) and 0.5% (1/213) respectively. However, it was higher at Bewadze, where it was 2.1% (4/188) and 5% (9/179) respectively for the two species. The overall infection rate (due to both *An. gambiae* and *An. funestus*) was about 17.5 times lower at Okyereko compared to Bewadze (0.2% vs 3.5%). None of the *An. pharoensis* and the *An. coustani* examined was sporozoite-positive. The estimated yearly EIR for *An. gambiae* s.s. and *An. funestus* were 66.7 and 12.9 at Okyereko, and 28 and 69.7 infective bites/man/night respectively at Bewadze. The yearly EIR (due to both species) was 1.2 times lower at Okyereko than at Bewadze (81.9 vs 95.6 infective bites/man/night). The HBI of *An. gambiae* s.s. and *An. funestus* was 85.3% and 82.8% at Okyereko, and 92% and 62.2% respectively at Bewadze. The results obtained indicate that despite the higher number of *Anopheles* vector species at the irrigated area, the malaria challenge was lower there than in the neighbouring non-irrigated community.

CHAPTER ONE

INTRODUCTION

Africa has a very high human population growth rate which is not matched by the rate of increase in agricultural production per capita (Robert *et al.*, 1992). The human population of Africa increases by nearly 20 million people annually (USBC, 2000) and it is projected to reach 1.58 billion by the year 2005. The population has already outstripped food production in all but three of the 41 sub-Saharan countries the exceptions being Cameroon, Central African Republic and Cote d'ivoire (Ijumba *et al.*, 2002). In addition to this, almost half of the potential arable land in Africa is too dry for rain-fed agriculture. Moreover precipitation fluctuates greatly from year to year and is generally insufficient for reliable cultivation (WRI/IIED, 1986). Irrigation therefore is considered to be the most effective way to increase crop production through increased yield, acreage, number of cropping cycle per year and by reducing the risk of crop failure (Oomen *et al.*, 1994).

At present, only 4% of Africa's arable land is irrigated and 70% of this is in four countries: Egypt, Madagascar, Nigeria and Sudan (FAO, 1987, 1996) but there are plans to increase this in several countries. In Ghana, only 2 per cent or 10 000 ha out of a potential area of 500 000 ha has been developed so far for irrigation (Owusu *et al.*, 2003). However, there are concerns that irrigation may aggravate the health risks of local communities, by enhancing conditions favouring transmission of vector borne diseases, including malaria, schistosomiasis, lymphatic filariasis, etc (Bradley, 1988; Gratz, 1988; Hunter *et al.*, 1993). Also, new rice varieties and management systems have been introduced into the traditional culture system in order to achieve high and stable yields. Unfortunately, these high yield rice varieties require flooded lands for optimum growth

and this inevitably results in changes in the agro-ecosystems which determine the abundance of mosquitoes (Surtees, 1970).

Irrigation schemes in general increase mosquito breeding. Studies carried out in Africa have shown that irrigation can generate prodigious numbers of mosquitoes (Ijumba and Lindsay, 2001). Rice fields provide a wide range of mosquito breeding habitats, particularly suitable for pioneer species such as members of the *Anopheles gambiae* complex, the main vectors of malaria in Africa (Grainger, 1947; Bruce-Chwatt, 1954; Chandler & Highton, 1976; Snow, 1983; Coosemans *et al.*, 1984; Robert *et al.*, 1985; Service, 1989; Lacey & Lacey 1990; Ijumba & Lindsay 2001).

Malaria is one of the major tropical diseases associated with irrigation schemes, but the changes in transmission pattern of this disease following irrigation have been a perennial source of debate. It has often been assumed that high numbers of malaria vector mosquitoes resulting from irrigation schemes lead inevitably to increased malaria in local communities. However, recent studies carried out have revealed a more complex picture. Increased numbers of vectors following irrigation can lead to increased malaria in areas of unstable transmission such as the African highlands and desert fringes where people have little or no immunity to malaria parasites (Ijumba & Lindsay, 2001). The increase in malaria incidence in large-scale irrigation developments (Anonymous, 1989) has been attributed partly to demographic aspects related to the influx of susceptible human populations with no immunity against the parasite, and partly to vector-related aspects such as increased abundance, human contact and field survival. In some instances, increased transmission has been due primarily to increased populations of established vectors (Ault, 1989).

According to Ijumba and Lindsay (2001), for most of sub-Saharan Africa, where malaria transmission is stable, the establishment of irrigation schemes has had little impact on malaria transmission. However other studies reveal that malaria transmission intensity in irrigated settlements can be higher, similar or less than in neighbouring villages outside the irrigation scheme. For example, in the rice-growing area of the Rusizi valley, Burundi, the vectorial capacity of *An. gambiae* s. l. was found to be 150 times higher in the irrigation scheme than in an adjacent area (Coosemans *et al.*, 1985). Alternatively, in the rice-growing areas of Bobo Dioulasso, Burkina Faso, the number of infective bites received in the local community was similar to that in the control area (Robert *et al.*, 1985). Whilst in the lower-Moshi rice irrigation scheme in Tanzania, the number of infective bites was 2.6 times lower in the control village (Ijumba, 1997).

1.1 Rationale of the study

Malaria is considered the greatest challenge of all health problems in tropical countries (Ghosh *et al.*, 2000). It is a major public health problem in 90 countries inhabited by a total of 2,400 million people, almost one third of the world's population (WHO, 2000). An estimated number of 2 – 3 million people, are at risk of infection with the malaria parasite (WHO, 1999). World wide the prevalence of the disease is estimated to be in the order of 300 – 500 million clinical cases each year of whom about one million are children under the age of five years in Africa, south of the Sahara (WHO, 1993). In absolute numbers, malaria kills 3,000 children per day under the 5 years of age. Its main impact is in sub-Saharan Africa where at least 90% of deaths occur (Greenwood & Mutabingwa, 2002).

In Ghana, malaria is the most important parasitic disease, accounting for 7-8% of all certified deaths and ranks fifth as the commonest cause of death in the 0 – 4 year age groups (Ahmed, 1989). About 40-42% of all outpatient attendance in Ghana hospitals is attributed to malaria (Ahmed, 1989).

The main vectors of malaria that occur in Ghana are *Anopheles gambiae* s.l. and *An. funestus* (Dzodzomenyo *et al.*, 1999; Appawu *et al.*, 1994; 2001). Of the six member species species of the *An. gambiae* complex, *An. gambiae* s.s., *An. arabiensis* and *An. melas* are known to occur in the country (Dzodzomenyo *et al.*, 1999; Appawu *et al.*, 2001).

There are approximately 22 main irrigation schemes in Ghana and these include Okyereko, Tono, Vea, Dawhenya, Kpong, Ashiaman, Afife, Aveyime, Kpando-Torkor, Mankessim, Amate, Dedeso, Akumadam, Nobewam, Sata, Subinja, Tanoso, Bontanga,

Golinga, Libga, Kikam and Weija irrigation projects and other forms of informal urban irrigation (Kranjac-Berisavljevic, 2001). Most of these irrigation projects are for rice or/and vegetables production.

Malaria and mosquito populations in irrigated areas of Ghana have been poorly studied. Also there is no baseline information of species populations and malaria transmission during pre-irrigation period, so it is not known which species have replaced what. This not withstanding, a comparative study involving an irrigated and a nearby non-irrigated site can provide some information since this assumes that apart from the irrigation scheme present in one village, the bio-ecological factors acting on them are the same.

Gomoa Okyereko the site within the irrigation scheme is a village located about 50 km west of Accra. The Okyereko Irrigation Project (OIP) was constructed in 1974 for sustainable rice agriculture all year round. Since no pre-irrigation base-line data was collected, the malaria transmission in this village was compared to that of a neighbouring village, Bewadze which is located about nine and half kilometres away.

Good indicators of the level of malaria transmission in a given area include man – vector contact, sporozoite rate and vector survival rate (Macdonald, 1957). When properly estimated, they can also be useful parameters for evaluating control measures and for planning intervention trials (Young *et al.*, 1985). The entomological inoculation rate (EIR) for example is an important parameter for assessing malaria situations and predicting epidemics (Onori and Grab, 1980).

1.2 Overall Objectives

1.2.1 General objective

The main objective of this study is to assess the effect of irrigation on malaria transmission in a coastal area of Ghana. This will be achieved by comparing the relevant data; sporozoite rates, biting rates, entomological inoculation rate (EIR), vectorial role, vector population density, vector species, parity, etc. of an irrigated site with that of a neighbouring site within the same ecological zone.

1.2.2 Specific objectives

1. To conduct field entomological survey for malaria. This will involve human landing catches (HLC) (indoor and outdoor) and pyrethrum spray catches (PSC).
2. To identify *An. gambiae* s. l complex and *An. funestus* morphologically.
3. To identify by PCR the sibling species of the *An. gambiae* s.l and to further identify and determine the distribution of the M and S forms of *An. gambiae* s.s. mosquitoes by restriction enzyme analysis of the PCR products
4. To dissect mosquitoes caught by HLC for parity
5. To use ELISA techniques to determine *falciparum* sporozoite rates and also host blood meal source in mosquitoes obtained by PSC.
6. To determine entomological parameters; vector abundance, indoor resting densities, biting rates, entomological inoculation rate (EIR) and vectorial role of the vectors.

CHAPTER TWO

LITERATURE REVIEW

2.1 Malaria: The Disease and Symptoms

Malaria is a febrile illness caused by protozoa of the class sporozoa, genus *Plasmodium* (Farr, 1988) and transmitted by infected female *Anopheles* mosquitoes. In endemic regions where transmission is high, people are continuously infected and some may develop immunity to the disease (Allison, 1984). Malaria is often described as a great imitator of other diseases, sharing various characteristics with other childhood illnesses like influenza, typhoid, brucellosis and urinary tract infections. Even more confusing, malaria may coexist with other diseases. Most of the morbidity due to malaria is caused by cerebral malaria and anaemia (Marsh, 1992). Malaria is diagnosed by the clinical symptoms and microscopic examination of blood films.

The symptoms first appear some 10 to 16 days after the infectious mosquito bite and coincide with the bursting of infected red blood cells. When many red blood cells are infected and break at the same time because of the parasites growth, malaria attacks can recur at regular time periods – every two days for *P. vivax* malaria and *P. ovale* and every three days for *P. malariae* (Berendt *et al.*, 1994). In *P vivax* malaria, the patient may feel entirely well between attacks. Even without treatment, the paroxysms subside in a few weeks. However, a person with *P. falciparum* malaria is apt to feel very ill between attacks and without treatment, is in danger of dying. One reason why *P. falciparum* malaria is so virulent is that the parasite can infect red blood cells in all stages of development, and the number of parasites in the blood can reach extremely high levels (Bruce–Chwatt, 1985). In contrast, *P. vivax* parasites infect only young red blood cells

which means the number of parasites in the blood does not reach the same high levels as seen in *P. falciparum* infection.

Malaria typically produces a string of recurrent attacks or paroxysms, each of which has three stages: chills, followed by fever, pain in the joints and then sweating (Cheesbrough, 1991). Along with chills, the patient is likely to have headache, nausea and repeated vomiting (Commey, 1989) and within an hour or two, the patient's temperature may rise. One of the commonest causes of death in children and adults with *falciparum* malaria is cerebral malaria, which is due to parasitized red cells and fibrins blocking the capillaries and venules in the brain (Marsh, 1992). Cerebral oedema and peri-capillary haemorrhaging from these small blood vessels may occur, causing irreversible injury to the brain (Warrell *et al.,* 1982). In adults, cerebral malaria often develops after several days of fever and other non-specific symptoms (Warrell, 1983; Molyneux *et al.,* 1989), but in children, the history is usually less than two days. Cerebral malaria often starts with a generalized convulsion followed by persisting unconsciousness. It is frequently associated with major metabolic and physiologic changes, particularly hypoglycaemia, decreased cerebral perfusion/hypoxia and cytokine induction, resulting in tissue damage and generalized seizures, paralysis, speech and behaviour disorders and hearing impairment (Commey, 1989). Severe anaemia is often the attributable cause of death in areas of intense malaria transmission (Commey, 1989).

2.2 The Global Burden of Malaria

Around the world, the malaria situation is seriously getting worse. It threatens the lives of 40% of the world's population – over 2,200 million people. Malaria is responsible for up to 500 million clinical episodes and 2.7 million deaths a year, predominantly among young children in sub-Saharan Africa (WHO, 1996). In tropical Africa the total cost in terms of health care, treatment and lost productivity is estimated to be over US$1800 million a year (WHO, 1992).

The estimated costs of malaria, in terms of strains on the health systems and economic activity lost are enormous. In affected countries, as many as 3 in 10 hospital beds are occupied by victims of malaria. In Africa, where malaria reaches a peak at harvest time and hits young adults especially hard, a single bout of the disease costs an estimated equivalent of 10 working days. In all malaria-endemic countries in Africa, 25-40% (average 30%) of all outpatient clinic visits are for malaria (with most diagnoses made clinically). In these same countries between 20% and 50% of all hospital admissions are a consequence of malaria. With high case fatality rates due to late presentation, inadequate management, and unavailability or stock-outs of effective drugs, malaria is a major contributor to deaths among hospital inpatients (WHO, 2003).

Malaria affects all age groups, but the most affected are children under 5 years of age (Breman and Campbell, 1988) and pregnant women (Lindsay *et al.*, 2000). It is also one of the leading causes of death among young children. Together with pneumonia, diarrhoea, measles and malnutrition, malaria is responsible for over 70% of deaths in young children especially in developing countries. The disease during pregnancy causes severe maternal illness and anaemia, and is also associated with a low birth weight among newborn infants, a leading risk factor for infant mortality (UNICEF, 2000).

Malaria's cost to human and social well being is enormous. The disease typically strikes its victims not once but repeatedly. As a result, workers output is diminished, and children miss school, often for periods of a week or more at a time. Taken together, the effects of malaria on lives and livelihoods are devastating for economic progress in hard-hit countries. A stricken family spends an average of over one-quarter of its income on treatment and prevention. It is estimated that such a family can only harvest 40% of crops harvested by healthy families (WHO, 1990).

Malaria is a major cause of poverty, and poverty exacerbates the malaria situation. Poor people are at increased risk of becoming infected with malaria and of becoming infected more frequently. Child mortality rates are known to be higher in poorer households and malaria is responsible for a substantial proportion of these deaths. In a demographic surveillance survey in rural areas of the United Republic of Tanzania, under-5 mortality following acute fever (much of which would be due to malaria) was 39% higher in the poorest socio-economic group than in the richest (Mwageni *et al.*, 2002).

In Ghana, malaria is the most important parasitic disease, accounting for 7 8% of all certified deaths and it ranks fifth as the commonest cause of death in the 0 – 4 year age groups (Ahmed, 1989). About 40-42% of all outpatient attendance in Ghana hospitals is attributed to malaria. Studies on malaria can be traced back to 1910 in Accra where 50% of children below 3 years of age were infected with malaria parasite (Ahmed, 1989).

Both direct and indirect costs are associated with a malaria episode and this represents a substantial burden on the poorer households. A study in northern Ghana found that, while the cost of malaria care was just 1% of the income of the rich, it was 34% of the income of poor households (Akazili, 2002). Also, estmates of direct and indirect costs of malaria

in the country are pegged at an average of US$8.67 for every disease episode (Assenso-Okyere and Dzator, 1997). Knowledge about malaria is markedly low among affected populations. In one recent survey in Ghana, for example, half the respondents did not know that mosquitoes transmit malaria (WHO, 1999).

Outside Africa malaria is also a major public health problem in parts of Asia, Latin America, the Middle East, Eastern Europe and the Pacific. In India, epidemics are frequently reported from areas that previously were not associated with malaria. In Bangladesh, the malaria situation has been steadily deteriorating since the late 1980's; the number of cases increased fivefold between 1988 and 1994. In Latin America, Brazil is worst affected with over 50% of all malaria cases in Americas (UNICEF, 2000).

2.3 Global Distribution of Malaria

Malaria is a public health problem in more than 90 countries inhabited by 40% of the world's population. (Yeboah-Antwi *et al.*, 2001). Malaria is believed to have had a major impact on humans only since the introduction of agriculture within the past 10,000 years (Pennisi, 2001; Tishkoff *et. al.* 2001).

Indigenous malaria has been recorded as far north as latitude 64°N in Archangel in the former USSR and as far south as latitude 32°S in Cordoba in Argentina (Figure 2.1). It has occurred in the Dead Sea area at 400m below sea level and at Londiani (Kenya) at 26000m above sea level and at 2800m in Cochamba (Bolivia). Within these limits of latitude and altitude there are large areas free of malaria, which is essentially a focal disease, since the transmission of malaria depends greatly on local environmental and other conditions. The prevalence and severity of malaria as well as the magnitude of associated social and economic effects vary widely in different geographical areas where the disease occurs. The duration of the transmission period of malaria, as well as its intensity is closely related to the amount of rainfall and its distribution throughout the year (Mouchet *et al.*, 1998).

Plasmodium vivax has the widest geographical range; it is prevalent in many temperate zones and also in the tropics and subtropics. *Plasmodium falciparum* is the commonest species throughout the tropics and subtropics, although it may occur in areas with a temperate climate. *Plasmodium. malariae* is patchily present over the same range as *P. falciparum* but much less common. *Plasmodium ovale* is found chiefly in tropical Africa, but also occasionally in West Pacific (Ombhanje, 1998).

The geographical area affected by malaria has reduced over the past 50 years, with successful eradication and cessation of transmission in large areas of North America, Southern Europe, and the former Soviet Union and some territories of Asia and South America (WHO 1998). The disease is now confined to poorer tropical areas of Africa, Asia and Latin America because of the tropical climate, which is suitable for vector development and survival.

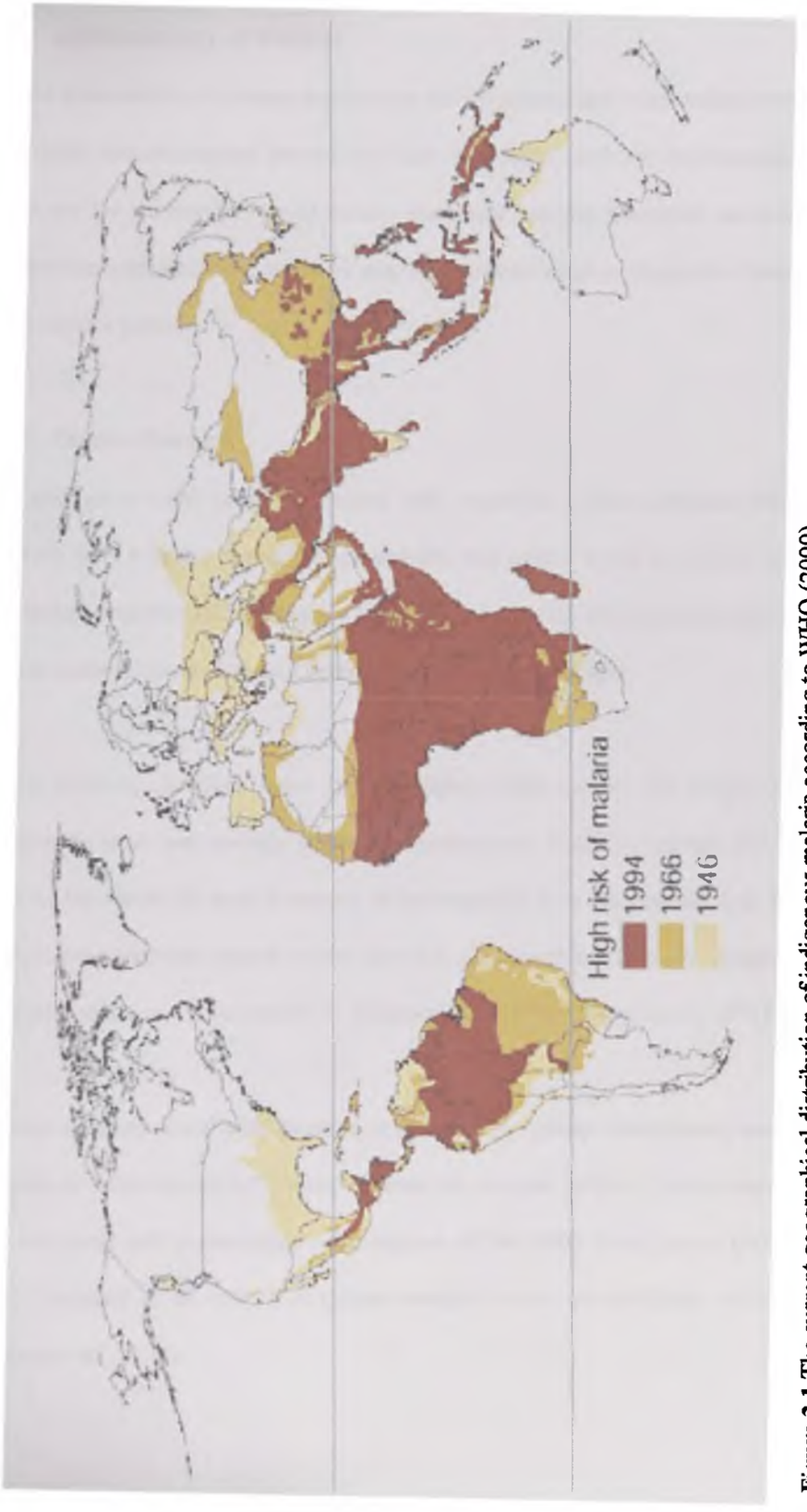

Figure 2.1 The current geographical distribution of indigenous malaria according to WHO (2000)

2.4 Epidemiology of Malaria

Natural transmission of malaria depends on the occurrence and relationships between the three basic epidemiological factors; the host, the vector, and the environment. Human beings are the vertebrate host of human plasmodia and the *Anopheles* mosquito is the invertebrate host. However, the latter may also be considered as the agent of transmission of the malaria parasite.

2.4.1 Human Factors

Sex and age are not important factors with regard to malaria infection but children generally have a higher degree of susceptibility than adults. There are factors involved in the immune response to the parasites for example people of African origin seem to have a greater innate immunity to some types of malaria than other races.

Innate immunity involves many intrinsic factors that govern the ability of malaria parasites to enter and multiply within the erythrocytes. There is evidence that in certain parts of the world the high frequency of haemoglobin S in the population is due to the evolutionary selection related to the fact that the presence of this haemoglobin has a mitigating effect on the severity of *P. falciparum* infections (Pasvol *et al.*, 1978)

Persons with red blood cells negative to Duffy blood group determinants are relatively immune to infections with *P. vivax,* because the receptor of the *P vivax* merozoites on the red blood cell is associated with antigens of the Duffy blood group (Miller *et al.*, 1973; Barnwell *et al.*, 1989). A typical example is seen in individuals of West Africa (Mason *et al.*, 1977).

Sickle cell trait provides a survival advantage over people with normal hemoglobin in regions where malaria is endemic. Sickle cell trait provides neither absolute protection nor invulnerability to the disease. Rather, people (and particularly children) infected with *P. falciparum* are more likely to survive the acute illness if they have sickle cell trait. When these people with sickle cell trait procreate, both the gene for normal hemoglobin and that for sickle hemoglobin are transmitted into the next generation. Researchers found that the sickle cell gene is especially prevalent in areas of Africa hard-hit by malaria. In some regions, as much as 40 percent of the population carries at least one HbS gene (Nagel, 2001).

The precise mechanism by which sickle cell trait imparts resistance to malaria is unknown. A number of factors likely are involved and contribute in varying degrees to the defense against malaria. It turns out that, in these areas, HbS carriers have been naturally selected because the trait confers some resistance to malaria. Their red blood cells, containing some abnormal hemoglobin, tend to sickle when they are infected by the malaria parasite. Those infected cells flow through the spleen, which culls them out because of their sickle shape and the parasite is eliminated along with them.

Other investigations suggest that oxygen radical formation in sickle trait erythrocytes retards growth and even kills the *P. falciparum* parasite (Anastasi, 1984). Sickle trait red cells produce higher levels of the superoxide anion (O_2-) and hydrogen peroxide (H_2O_2) than do normal erythrocytes. Each compound is toxic to a number of pathogens, including malarial parasites. Homozygous hemoglobin S red cells produce membrane associated hemin secondary to repeated formation of sickle hemoglobin polymers. This membrane-associated hemin can oxidize membrane lipids and proteins (Rank *et al.*, 1985). Sickle trait red cells normally produce little in the way of such products. If the

infected sickle trait red cells form sickle polymer due to the low oxygen tension produced by parasite metabolism, the cells might generate enough heme to damage the parasites (Orjih *et al.*, 1985).

Protection against malaria, acquired by individuals living in endemic areas is largely governed by transmission pattern. MacDonald (1957) identified two epidemiological extremes within which the disease exists. In one case, instability of transmission is the dominant feature where the disease is characterized by the incidence of substantial seasonal fluctuations, producing cyclical epidemics. In the other case, transmission and morbidity remain stable over the seasons and years. In those parts of the world where malaria occurs in unstable extreme, the entire population regardless of the age is vulnerable and infections tend to be followed by clinical disease. Populations exposed continually to intense malaria in highly endemic areas develop a degree of immunity to infection. Mosquitoes derive the infection from an individual with parasites in the blood, but not necessarily from an individual with fever and other manifest signs of diseases. When malaria is endemic a proportion of the population is usually a carrier of gametocytes and this may be particularly the case amongst young children. In endemic malarious areas the prevalence of gametocytaemia decreases with rising age, until it reaches low levels in adults (Gilles, 1999).

2.4.2 The Vector factors

The transmission of malaria depends significantly on the characteristics of the vectors, such as their abundance, susceptibility to infection and their longevity and their contact with humans.

Anopheles species the main vectors of malaria have a worldwide distribution, occurring not only in tropical areas but also in temperate regions. They are however absent from Polynesia and Micronesia (Service, 1980). The most efficient vectors of human *Plasmodium* the members of the *An. gambiae* complex and *An. funestus* occur in sub – Saharan Africa. *Anopheles funestus* also remains the main vector in southern Africa (Coetzee *et al.*, 2000).

The *An. gambiae* species complex comprises of 6 sibling species; *An. gambiae* sensu stricto, *An. arabiensis, An. merus, An. melas, An. quadriannulatus* and *An. bwambae.* Three of these species (*An. gambiae* s.s., *An. arabiensis,* and *An. quadriannulatus*) are adapted to fresh water breeding sites. *An. gambiae* s.s. predominates in humid areas and is highly anthropophilic. *An. arabiensis* extends more into savanna areas, and in many areas is more zoophilic and exophilic. *An. quadriannulatus* is more zoophilic and consequently does not transmit malaria in humans. The last fresh water species, *An. bwambae* has a very restricted distribution in a small area of the rift valley, west of Ruwenzori, where it breeds in geothermal waters (Coetzee *et al.*, 2000). The two brackish water species, *An. merus* of East Africa and *An. melas* of West Africa are generally more exophagic and zoophilic and thus less efficient as vectors than *An. gambiae.* s.s (Gilles, 1999).

In tropical Africa the main *Anopheles* species i.e. *An. gambiae* s.l. and *An. funestus* bite predominantly late at night between the hours of 22.00HR and 05.00HR (Curtis, 1997). Some species such as *An. albimanus,* a malaria vector in South America, bite humans mainly outdoors (exophagic) from about sunset at 21.00HR whereas *An. gambiae* complex bite indoors (endophagic) (Bryan *et al.*, 1987).

Sporozoite rates, that is, the percentages of infective female vectors vary considerably not only from species to species of mosquitoes, but also according to locality and season. Sporozoites are often about 1-5% in species such as *An. albimanus* and *An. culicifacies* (Mendis *et al.*, 2001). In the Upper East Region of Ghana the sporozoite rate has been reported to be as high as 100-1000 infective bites per human per year (Binka *et al.*, 1996) and 75-400 in South Burkina Faso (Robert *et al.*, 1988).

Both before and after blood-feeding some species will rest in houses (endophilic) whereas others will rest outdoors (exophilic) in a variety of natural shelters such as amongst vegetation, in rodent burrows, in cracks, crevices in trees, under bridges, in culverts, and cracks in the ground. Most *Anopheles* species are not exclusively exophagic or endophagic, exophilic or endophilic but exhibit a mixture of these extremes of behaviour. Similarly, few *Anopheles* species feed exclusively on both humans and animals but the degree of anthropophilism and zoophilism varies according to species. For example, *An. culifacies* an important malaria vector in India frequently feeds on cattle and still maintains a strong vector – human contact. This is one of the reasons why *An. gambiae* is considered a more efficient vector than *An. culifacies,* for example. In tropical countries adult mosquitoes probably live on average only about two to three weeks, but in temperate climates the average life may be four to six weeks or longer. It seems that males have a shorter life span (Service, 1980).

Anopheles pharaoensis is exophilic, females enter houses at night mainly for feeding purposes, only to retreat before day break (Haddow, 1942; Lewis, 1948). This nocturnal activity is widely recognised (Kerr, 1933; Haddow, 1942; Hanney, 1960; Service, 1963; Haddow & Ssenkubuge, 1974). Daytime activity has been observed to be mainly limited

to resting outdoors among vegetation for the majority, with only small proportion staying indoors (Gillet, 1971).

Anopheles funestus complex are sympatric with *An. gambiae* s.s. and *An. arabiensis*, *An. funestus* mosquitoes succeed the members of the *An. gambiae* complex in the colonization of the breeding sites. For reasons which are not totally clear (but may possibly be due to competitive exclusion), towards the end of the rainy season, when the densities of the *An. gambiae* complex mosquitoes start decreasing, the grassy edges of the water pools become the favorite breeding sites for *An. funestus* mosquitoes. They are anthropophilic and endophilic and they are vectors which may promote the extension of the malaria transmission season to the first months of the dry season.

2.4.3 Environmental factors

Variation in climatic conditions has a profound effect on the life of a mosquito and on the development of malaria parasites, hence its influence on the transmission of the disease and on its seasonal incidence. The most important factors are temperature and humidity. Malaria parasites cease to develop in the mosquito when the ambient temperature is below 16°C. The best conditions for the development of *plasmodia* in the *Anopheles* and the transmission of the infection are when the mean temperature is within 20-30°C, and the mean relative humidity is at least 60% (MacDonald, 1957). A high relative humidity lengthens the life of the mosquito and enables it to live long enough to transmit the infection to several persons.

Temperature affects the sporogonic cycle of the parasite and vector survival. Very low temperatures limit the vector by lengthening the gonotropic cycles and reducing the survival rate. *Anopheles gambiae* for example only exists in frost-free regions (de

Meillon, 1934) or where absolute temperatures in winter remain above 5°C (Leeson, 1931). Strong winds affect the flight of *Anopheles* and may prevent their egg laying; however, in some cases they may extend their flight range well beyond the normal limits. Rainfall provides mosquito-breeding sites and increases the humidity, thus enhancing mosquito survival. However, the relationship between abundance and rainfall is not direct, as a specific amount of rain does not lead to a specific mosquito density. By examining rainfall patterns in known malaria and non-malarious areas, Craig *et al.*, (1999), estimated that an average of 800mm per month for at least three to five months is a reasonable requirement for the availability of mosquito breeding sites to sustain malaria transmission. Rainfall normally increases the amount of surface water and may have a negative effect on the amount of transmission of malaria. Excessive rainfall may transform small streams into rapid torrents and thus strand many larvae and pupae on the edges of the water channel. Conversely, deficient rains in other parts of the world change many rivers into string of pools in which certain *Anopheles* would breed in profusion. The latter is often observed in West Sri Lanka where during years of relative drought increased breeding of *An. culifacies* takes place and is followed by severe epidemics of malaria (Tren *et al.*, 2003).

The presence of water bearing plants (which favour the breeding of *Anopheles*) or the presence of cattle (on which some *Anopheles* feed) constitute factors within the environment that impacts on the epidemiology of malaria.

Social and economic factors, such as sanitation, housing, occupation, poverty, etc have important effects since malaria is more prevalent in underdeveloped countries. Also, development projects in pursuits of economic development such as water schemes, construction of dams and bridges, all drilling and mining activities, urban planning and

development, logging activities, urban planning and development, logging activities inadvertently lead to an increase in mosquito breeding sites, thereby increasing their numbers, human-mosquito contact and therefore transmission (Okenu, 1999). Building of dams and man made lakes raises the ground water table and often causes seepages and flooding, thus contributing to the creation of new larval habitats.

Wars and large-scale population movement whether of pilgrims or labour forces from endemic areas, have often been a factor in the spread of malaria. The movement of infected people from areas where malaria is still endemic to areas where the disease had been eradicated has led to the resurgence of the disease (Gubler, 1998). As people move, they increase their risk for acquiring the disease through the technology they introduce, for example through deforestation for resettlement and irrigation systems (Service, 1991). Such environments create more favourable mosquito breeding habitats and at the same time, workers may have increased exposure to the vector. Population movement is also implicated in the spread of drug resistance in malaria (Rajagopalan *et al.*, 1986), with people carrying resistant parasites from endemic to non-endemic areas and accelerating transmission of resistant strains.

2.5 The Life Cycle of Human *Plasmodium* parasites

Anopheles is the only mosquito genus that can transmit human *Plasmodium* species. These include *P. falciparum, P. malariae, P. vivax* and *P. ovale.*

The life cycle of malaria parasites comprises of an exogenous sexual phase (sporogony) with multiplication in certain *Anopheles* mosquitoes and an endogenous asexual phase (schizogony) with multiplication in the vertebrate host (Figure 2.2). The latter phase includes the development cycle in the red blood cells corpuscles (erythrocytic schizogony), and the phase taking place in the liver parenchyma cells (exo-erythrocytic schizogony).

The female *Anopheles* mosquito ingest male and female gametocytes during blood feeding, and the asexual parasites which are also present in the blood meal are digested. Within a few minutes in the stomach of the mosquito the gametocytes pass though a rapid maturation process resulting in the formation of male and female gametes. In the case of the female, this starts by the parasite breaking out of its host red blood cell so that it becomes a free body within the stomach contents. It then throws off several polar bodies from its nucleus; the female gamete then remains dormant awaiting fertilization. In the case of the male, the male gametocyte escapes from the red blood cell and then extrudes a number of threadlike processes (flagella), which are formed by breaking up of the nucleus. These are the microgametes and 4-8 of these are produced by each male gametocyte. The male gametes lash about vigorously in the stomach fluid, in search of a female gamete. Probably by chemotaxis the female attracts the male and fertilization takes place

Following fertilization the female gamete becomes the zygote which remains dormant for several hours. It gradually becomes elongated (ookinete) and starts moving towards the stomach wall. The ookinete penetrates the wall of the midgut and becomes an oocyst and through sporogony the oocyst develops into enlarged motile sporozoites, which burst into the abdominal cavity of the mosquito. The whole developmental process takes about 10-24 days (depending on the species of *Plasmodium* involved). The sporozoites, which are now infective then migrate to the salivary glands of the mosquito and are injected into the wound when the mosquito takes the next blood meal.

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

Release of the merozoites from the erythrocytes brings on an attack of malaria, and the interval between the attacks is the length of the schizogonic cycle. This may last for several hours. The merozoites repeat the cycle and invade other erythrocytes. After a number of cycles of schizogony, the trophozoites do not divide but become gametocytes,

which develop no further in man but circulate in the bloodstream until a mosquito takes them up during a blood meal (Kettle, 1992).

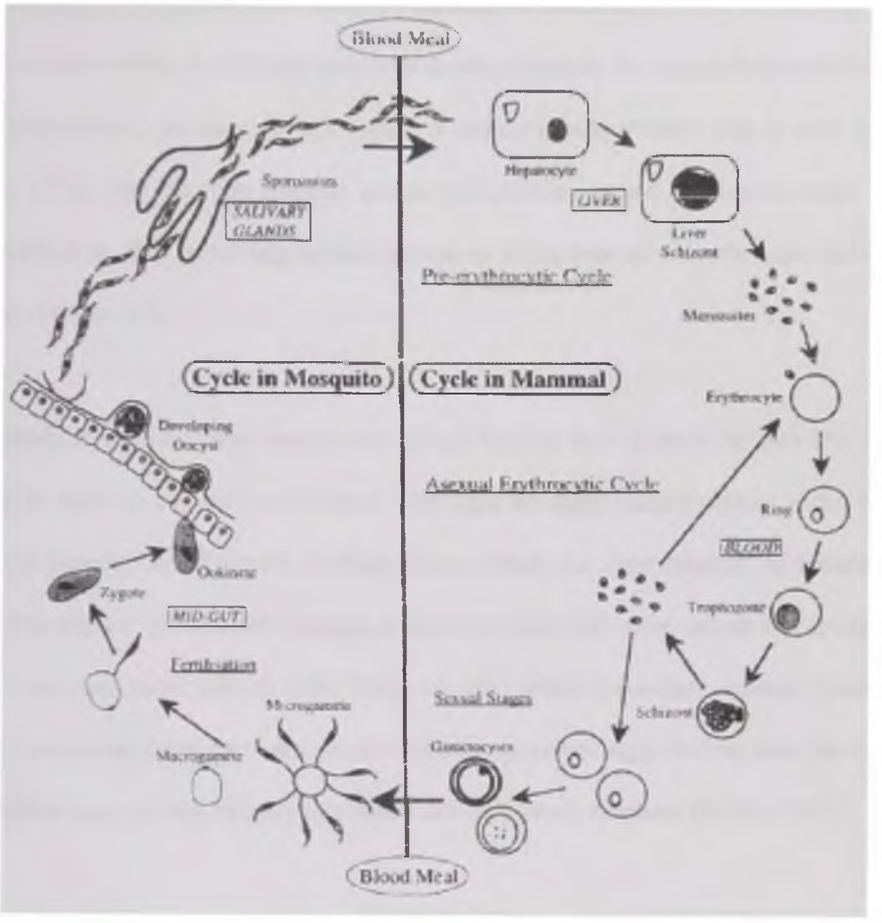

Figure 2.2 Life cycle of *Plasmodium spp.* showing the various developmental stages of the parasite in the mosquito and the vertebrate host (Philips, 2000).

2.6 Life cycle of *Anopheles* mosquitoes

The life cycle from egg to adult takes a minimum of about two weeks or slightly less. The time required for the different stages to develop depends on various factors including water temperature; the development period is shorter in warm water than in cool water (WHO, 1992). The life cycle involves aquatic pre-adult stages and a terrestrial stage. It is holometabolous, that is, the egg hatches into larva which then develops to pupa and then to adult (Figure. 2.3).

The female *Anopheles* after mating and blood feeding lays a batch of 100-150 eggs usually at night on the surface of water. The eggs are cigar shaped objects about 1mm long and bearing paired lateral air-filled floats, which are characteristic of anopheline eggs. The surface structure of the eggs is such that they will either attach end-to-end or side to side but never end to side. They can also attach by surface tension forces to objects projecting from the water surface and this prevents eggs drifting into the open. Anopheline eggs develop directly into larvae and only rarely diapause (Kettle, 1992).

Viable eggs hatch into larvae within 2-3 days in the tropics, but in cooler temperature regions they may not hatch till after 4-7 days or longer (Service, 1980). The larva emerges from the egg by using a small tooth, located postero-dorsally on its head. It grows steadily and under optimal conditions will pupate in 7-10 days but the duration of the larval stage is also temperature dependent, and in the cool season in subtropical areas the larval stage may last several months, e.g. *An. pharoensis* in Egypt (Kettle, 1992). The larva undergoes four moults to reach the pupal stage.

The head and thorax of the pupa are combined into a single division, the cephalothorax, which is joined posteriorly to a segmented abdomen. The pupa does not feed but it

breathes through a pair of broad trumpets dorsally placed on the cephalothorax, i.e. it is propneustic. The most distinctive characteristic of *Anopheles* pupae is the presence of short peg like spines laterally near the distal margins of abdominal segments. The pupal period lasts two to three days in warm climates.

The pupal skin splits dorsally and the adult emerges. The newly emerged adult inflates its wings and grooms its head appendages before flying away. When the progeny of any one egg batch of the mosquito emerge as adults the male emerges first. This is because the male terminalia have to rotate about 180^0 before the males are ready for mating. This process takes about 24 hours so that by the time the females emerge the males are ready for mating. Mating is often preceded or accompanied by swarming in which the males associate over a marker and fly in a particular manner. Most male mosquitoes usually die after mating. The females require a blood meal for ovarian development and egg maturation (Gillies, 1955).

Blood feeding follows a circadian rhythm and most species follow a circadian rhythm. Most species of *Anopheles* are nocturnal and many, including major disease vectors such as *An. gambiae, An. minustus* and *An. farauti* have their peak biting rate in the early hours of the morning after midnight (Muirhead-Thomson, 1951). Most mosquitoes exhibit gonotrophic concordance in which there is strict alternation of blood feeding and oviposition, with each blood meal being followed by the maturation and oviposition of a batch of eggs. *An. gambiae* and *An. funestus* require two blood meals to mature the first batch of eggs but only 2 per cent and 4 per cent, respectively, feed twice in subsequent ovarian cycles (Gillies, 1955). Under favorable environmental conditions, an *Anopheles* mosquito can live for more than 3 - 4 weeks, but the mean survival in nature is much

shorter (6 - 9 days). Thus, only a relatively small fraction of the population lives long enough for the sporogonic cycle to be accomplished

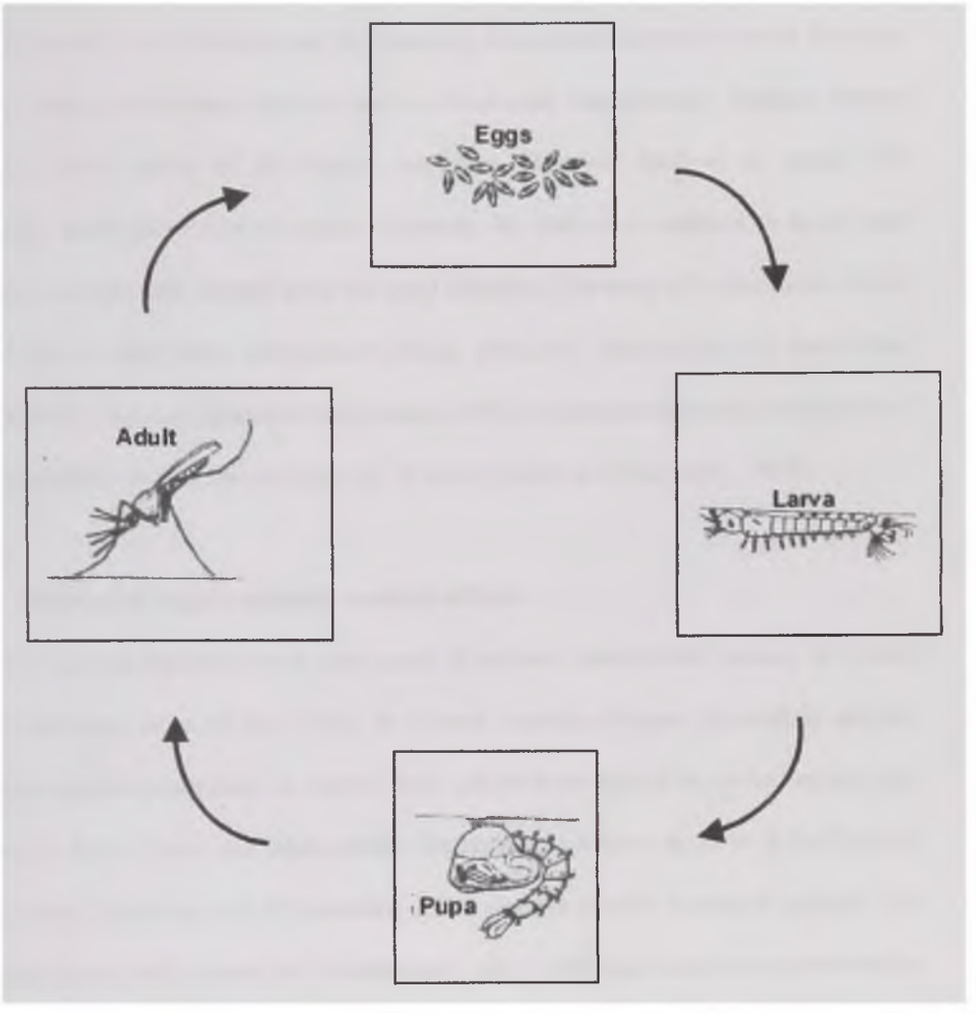

Figure 2.3: Schematic diagram of the life cycle of *Anopheles* mosquitoes.

2.7 Malaria Control

The strategy of malaria control is based on breaking the chain of transmission of the parasites between humans and mosquitoes. There are in principle two approaches to malaria control: chemotherapy and by breaking the contact between human host and vectors using insecticides, bednets and environmental management. Malaria vectors exhibit a wide variety of life history strategies, therefore there is no simple and universally accepted method of control. Generally the method or methods to be adopted in malaria control will depend upon the local situation. The most effective public health tools however have been anti-parasitic drugs, especially chloroquine and insecticides most notably dichlorodiphenyltrichloroethane (DDT), organochlorines and pyrethroids of which many have been in use for the past 50 years (Collins and Paskewitz, 1995).

2.7.1 History of major malaria control efforts

Relatively simple materials have been used to achieve considerable success in vector control in many parts of the world. In Central America Gorgas successfully applied measures against mosquitoes to control both yellow fever spread by *Aedes aegypti* and malaria, at first in Cuba and subsequently eliminate yellow fever by 1906 in the Panama Canal Zone (Spielman and D'Antonnio, 2001). Larval control measures included the draining swamps and marshes or flooding them, when breeding would be concentrated at the edge of the resultant lake and accessible to chemical control (Collins and Paskewitz, 1995).

The early control efforts using chemicals involved the use of oil and Paris green, a compound containing both copper and arsenic, against *Anopheles* larvae and pyrethrum against adults. Paris green applied as a dust at 1kg/ha selectively poisoned surface feeding *Anopheles* larvae, leaving the habitat apparently otherwise undisturbed. Oil was

messy and changed the habitat, but was administratively convenient because its application could be easily checked. House spraying with pyrethrums in the evening and early morning was directed at reducing longevity of the vector. When this was combined with other measures, communities in endemic areas where maintained relatively malaria free and expatriate personnel operating in tropical countries were protected (Kettle, 1992).

Malaria in northern Europe and most of the US began to decline during the mid 1800's, in part because of drainage of swamps for agricultural use. Subsequently better housing and screening, increased availability of quinine, education and improved economic status contributed to the eradication of malaria in these areas. Large scale spraying with DDT was implemented in the 1950s by the US Public Health Service and this program is believed to have contributed to the elimination of endemic malaria from the US although the vectors still remain (Spielman *et al.*, 1993).

The successful programs resulting in the eradication of *An. gambiae* in Brazil during 1934 - 1949 (Soper and Wilson, 1943) and in Egypt in 1948, and interruption of transmission on Sardinia and Cyprus during 1946 - 1950, made worldwide eradication of malaria through vector control seem a feasible goal. In 1955, the World Health Assembly launched the malaria eradication initiative with the use of DDT as the primary tool. The goal was to sufficiently reduce infected vector populations feeding on humans sufficiently to interrupt transmission of parasites, rather than to eradicate all vectors. Eradication was most successful in countries where the transmission was unstable (e.g. Tunisia, Morocco) and on islands (Molineaux, 1988). However, success was sometimes short-lived. For example, Sri Lanka began the attack phase of the Malaria Eradication Program in 1958 and reduced its malaria incidence from a high of 2.8 million cases

recorded in 1946 to 17 per year by 1963 (Gramiccia & Beales, 1988; Wijensundera, 1988). In 1964, the consolidation phase was initiated and spraying was halted but by 1967, an epidemic occurred and spraying was reintroduced. Unfortunately, the vectors were then resistant to DDT. By the mid 1960s the general agreement was that eradication would not work in many areas, and by 1969, the goal was revised from eradication to control.

The situation in sub-Saharan Africa however told a different story. For example the WHO initiated a large-scale control programme in the Garki district of northern Nigeria. The study collected substantial baseline data to assess the usefulness of residual spraying alone or combined with mass drug administration. The findings of this work which, lasted from 1969 to 1976, constitute one of the best illustrations of the problem of malaria control in much of tropical Africa (Molineaux & Gramiccia, 1980). The baseline studies revealed very high transmission intensity in all villages far in excess of the critical value required for the maintenance of malaria. During peak transmission period, intensity could reach almost 2000 times the critical value. The number of infectious bites per person was also very high, reaching 132 per person during the 6 months of the wet season in one village. The insecticide Propoxur was applied as a residual spray to houses and careful management resulted in very complete coverage, and tests showed that the insecticide was very effective against the vectors (Curtis & Mnzava, 2000). Nonetheless, the study revealed that even a 90% reduction of vectorial capacity reduced malaria prevalence by only 25%. Immigration of vectors or humans from sprayed neighbouring sites was not significant. Instead, the principal reasons for the apparent failure of the program seemed to be a tendency for the vectors to rest outdoors after blood feeding (exophily) and a very high cumulative human-biting rate by the vectors *An. gambiae*, *An. arabiensis* and *An. funestus*. Presumably the same factors also explained why the

addition of mass drug administration, even at frequent intervals and with very good community participation (80%), transmission was not interrupted, although it did reduce disease prevalence to very low levels. Finally it was concluded that even though combinations of drugs and insecticides could reduce the disease prevalence in the short term, over the long term the control could not be sustained and therefore would fail (Collins and Paskewitz, 1995).

DDT was removed from the marketplace in the US because of environmental concerns (Gilles, 1999) during the late 60's and alternative pesticides were more expensive and less effective; some were even toxic to humans (Gramiccia & Beales, 1988; Oaks *et al.*, 1991). At this time also major malaria programs were abandoned, because of widespread resistance to DDT and other classes of insecticides. By 1985, at least 117 mosquitoes species had been reported as resistant to either one or more insecticides with 67 of these in the genus *Anopheles* (Pant, 1988, Shidrawi, 1990).

2.7.2 Chemotherapy

For several hundred years, the back of the *Cinchona* tree was used for treatment of malaria. Quinine which is the active ingredient in the bark was identified in 1820 but limitations of its accessibility and undesirous side effects stimulated the search for new antimalarials. Thus, the advent of new and effective therapeutic drugs particularly the 4 – aminoquinolines in the 1940's provided for effective treatment and for chemoprophylaxis (Jeffery *et al.*, 1985). Chloroquine especially became the antimalarial drug of choice because it was safe, inexpensive and highly effective against malaria parasites (WHO, 1967). Chloroquine works by interfering with the ability of the trophozoites (red blood cell stage) to polymerise the haem molecules that result from digestion of haemoglobin (Slater and Cerami, 1992). The first documented cases of *P. falciparum* resistance to

chloroquine were in South America in the 1950s (Peters, 1970), and confirmed in Thailand in 1959 (Harinasuta *et al.*, 1962). In Africa, *P. falciparum* resistant parasites first appeared in Kenya and Sudan in 1978 (WHO, 1978) and over a period of a decade have spread over virtually all of Africa (Collins and Paskewitz, 1995). Chemotherapy has also been complicated further by the development and spread of resistance to other antimalarials such as Fansidar (WHO, 1998). Currently, drug combination therapy is recommended for malaria control in areas where resistance to one drug is highly prevalent.

2.7.3 Vector control

Insecticides were first used on a large-scale mosquito control in 1935. Pyrethrum was the first, and good results were achieved with it in southern Africa and in parts of India. During the 1940's, the organochlorine insecticide DDT became available during the Second World War, malaria control by house spraying was initiated on a small scale in certain countries and on a large scale in Madagascar, Mauritius, South Africa (Natal and Transvaal), Swaziland, and Zimbabwe, formerly southern Rhodesia (Bruce-Chwatt, 1963).

The increasing problem of resistant mosquitoes to most insecticides together with the growing concern for the environment impact of their repeated applications has been largely responsible for the absence of organised large scale vector control programs.

Significant progress has been made since the 1980's in research and development of new tools for use in malaria control activities. The use of insecticide treated bed nets (ITNs) and curtains with pyrethroids seems to be the most promising available method of controlling malaria in endemic tropical countries. The present ''Roll Back Malaria'' by

WHO promotes the use of insecticide treated mosquito bed nets (ITN) to reduce vector-human contact. Roll Back Malaria programme aims at reducing overall mortality due to malaria by 50% by the year 2010. Studies carried out in Senegal (Alonso *et al.*, 1991) and China (Cheng *et al.*, 1995) demonstrated the efficiency of ITNs for reducing infant mortality. These findings have been confirmed by subsequent large-scale multicenter studies in 6 countries across Africa (Nevill *et al.*, 1996; Binka *et al.*, 1996; Lengeler *et al.*, 1996).

However, there have been reports of the emergence of resistance to pyrethroid (the choice insecticide for impregnating nets) in the *Anopheles* vectors (Curtis *et al.*, 1998). Some cases of such resistance are known, notably in *An. gambiae* in West Africa where the *kdr* type of resistance has been selected probably due to the use of pyrethroids in cotton production (Zahar, 1984). Moreover, Doannio (1999) reports that despite the known repulsion effect of permethrin treated bednets on mosquitoes, their use had no signficant impact on transmission. On the other hand, other studies have shown that the use of ITNS is effective in reducing morbidity and mortality due to malaria (Lengeler, 2000).

Environmental management has also been used in the control of mosquitoes, usually in the form of a long-lasting or permanent changes of land, water or vegetation to reduce the vectors' habitats. Environmental manipulation which could be a temporary or recurrent activity have also been attempted as a means of controlling the mosquito vectors. For e.g. in deltaic regions of lower Bengal, the land is flooded annually by the rise of water level in the great rivers to reduce breeding of vectors and also expose mosquito larvae to larvivous fish and other predators (Bruce-Chwatt, 1999).

Several viruses (cytoplasmic polyhedrosis, baculovirus, etc) have shown to have little practical applicability for mosquito control. On the other hand, considerable attention has been given to *B. thuringiensis* serotype $H\text{-}_{14}$ and its products. *B. thuringiensis* serotype $H\text{-}_{14}$ form spores which produces endotoxins that are harmful to dipteran larvae. The endotoxins are potent gut poison when ingested by mosquitoes and other aquatic insects, but harmless to plants, animals and humans. The crystallized toxin of *B. thuringiensis* serotype $H\text{-}_{14}$ is now produced commercially for large-scale larviciding operations against blackflies and mosquitoes (Lacey and Undeen, 1986). *Bacillus sphaericus* spores also produce a toxin harmless to animals and humans and is also highly potent against various mosquitoes. *Bacillus sphaericus* was used against mosquito populations in Thailand and its effectiveness increased with dosage (Mulla *et al.*, 2001).

The potential of fungi belonging to the genera *Coelomomyces*, *Culicinomyces*, *Lagenidium*, etc for mosquito control have also been studied. *C. ilensis* is a parasite of mosquito larvae and adult and it has a high infectivity for *Anopheles* and decreases their ability to transmit malaria. However, field trials have not proved the economic practicability of microsporidia (Collins and Paskewitz, 1995).

Nematodes of the family Mermithidae are now being studied as potential biological control agents and the most interesting is *Romomanomermis culicivorax*, which in laboratory settings can greatly reduce larval populations and survives in a variety of habitats (Chapman, 1974; Collins and Paskewitz, 1995).

The use of larvivorous fish as a biological control agent has been most successful in many parts of the world. This has been used in malaria control in Spain, Italy, Greece and other countries in Southern Europe and northern Africa and also Georgia, India, Papua,

New Guinea, Malaysia, Madagascar, etc (Bruce-Chwatt, 1999). Naturally, the use of larvivorous fish is limited to some special situations where the water and other conditions are suitable. The introduction of fish such as carp, into rice field in China has helped to reduce mosquito breeding and the incidence of malaria in certain areas (Service, 1989).

Alternate wet/dry irrigation method (AWDI) is an effective means for vector control in areas where the main breeding sites of the principal vectors of malaria or Japanese encephalitis are the rice fields that contribute significantly to the overall abundance of adult mosquitoes. Alternate wet/dry irrigation method implies that rice fields are not kept continuously submerged but are allowed to dry intermittently during the rice growing stage. It seems most suitable for areas with unstable malaria transmission and extensive areas of irrigated rice cultivation, as is the case in many parts of Asia and parts of East Africa. In hyperendemic areas such as West Africa, rice fields are often just one of many possible breeding sites and the control of mosquito breeding in rice fields may not have any effect on the level of malaria transmission and the overall incidence of the disease. Larval control in general might not be an option in areas where breeding places of the vector are diffuse and various such as for *An. dirus* in South East Asia and *An. gambiae* in Africa. In East Africa there are larger schemes (> 10,000 hectares) with a more uniform environment than in West Africa and there it could be feasible to implement AWDI. However, this is not certain, for example, in Kenya it was effective against *An. funestus* and *An. coustani*, but not against *An. gambiae* and *An. pharoensis* (Grainger, 1947). In Africa multiple interventions are always needed at the same time in order to achieve effective malaria control (Van der Hoek *et al.*, 2001).

2.8 Systematics of *Anopheles gambiae* complex and *An. funestus*

Before 1962, *An. gambiae* was considered to be a single, biologically variable species. However, there were many reports of variations in the larval habitats and adult female resting behavior and feeding preferences (Gillies and De Meillon, 1968). The first real evidence for the specific distinctiveness of the saltwater breeding members of the group was demonstrated in hybridization experiments by Ribbands (1944) and Muirhead-Thomson (1945) in West Africa and in East Africa by Muirhead-Thomson (1951). Hybrid male sterility between two freshwater breeding populations were obtained, but it was not considered then to be an evidence of interspecific differences between the two (Muirhead-Thomson, 1951).

However by 1964, five species were recognized, with the evidence that the three fresh water forms did not mate in nature (Paterson, 1964). Holstein (1952) had earlier suggested that the species consisted of 2 races. However on the basis of studies carried out by Davidson & Jackson (1962), Paterson (1964) concluded that these authors were dealing with sibling species. Paterson *et al.* (1964) later provided direct evidence for the specific distinctiveness of three freshwater forms which he designated A, B and C that did not mate in nature. Later, another species breeding in heavily mineralized water in Uganda, originally documented by Haddow *et al.* (1947) was designated species D (Davidson & Hunt, 1973). This brought the *An. gambiae* complex to a total of six member species.

Mattingly (1977) proposed the formal names *gambiae* Giles, *arabiensis* Patton and *quadriannulatus* Theobald for species A, B and C respectively, and species D was named *bwambae* by White (1985). The two recognized salt-water breeding species are *melas* Theobald in West Africa and *merus* Donitz in East Africa (Paterson *et al.*, 1964; Mahon

et al., 1976). Recently, Hunt *et al.* (1998) reported a new member of the complex designated *quadriannulatus* species B from Ethiopia.

While the existence of the six formally named species of the *An. gambiae* complex is well established, there is the indication that further subdivision within species of the complex may exist. The evidence for cryptic taxa within *An. gambiae* s.s is the observation that the micromorphology of the second chromosome for different populations differ by inversions. These chromosomal forms, which are far from Hardy-Weinberg equilibrium at certain times of the year in West Africa also carry different strain specific combinations of inversions and differ in vectorial capacity. This has been well studied in Mali where three forms "Bamako", "Mopti" and "Savanna" have been described (Toure *et al.*, 1983). These forms appear to be fully or partially isolated reproductively, in nature. In sympatric areas, hybrids between the "Savanna" and the two other forms have been observed at frequencies lower than expected. Also, no individuals carrying heterozygous complements of the "Mopti" and "Bamako" inversions are seen in nature, even though the two produce viable progeny under laboratory conditions (Coluzzi *et al.*, 1985). Two other forms of '*gambiae*' namely "Forest" and "Bissau" have also been proposed (Bryan *et al.*, 1982).

Anopheles funestus is widespread and it is also very heterogenous. *Anopheles funestus* group comprises of at least nine members (Gillies & De Meillon, 1968; Gillies & Coetzee 1987). Members of this group include *An funestus* s.s., *An. vaneedeni*, *An. parensis*, *An. aruni*, *An. confusus*, *An. leesoni*, *An. rivulorum*, *An. evans* and *An. brucei*. *Anopheles funestus*, *An confusus*, *An leesoni*, *An rivulorum* and *An. brucei*, can be distinguished at the larval stage, while the species of the subgroup *funestus*, *An funestus*, *An. parensis*, *An. aruni*, *and An. vaneedeni* (Gillies & De Meillon, 1968) can be

identified by minor morphological differences between the adults (Gillies & Coetzee, 1987). Their biology and their vectorial capacity are very different. With the exception of *An. funestus*, these species are essentially zoophilic. Human *Plasmodium* have only been found in *An. funestus,* which is an excellent vector having a high vectorial capacity and rarely in *An. rivulorum* in Tanzania (Wikes *et al.*, 1996).

2.8.1 Distribution of *Anopheles gambiae* complex and *An. funestus*

In Africa, members of the *An. gambiae* complex and the *An. funestus* group are the two most competent and efficient vectors of *Plasmodium* species causing malaria (Service, 1980; Coluzzi, 1984). *Anopheles gambiae* s.s. and *An. arabiensis* have the widest distributions. The two species show dissimilar distributional limits and asynchronous seasonal prevalence probably due to divergent responses to climatic factors.

Members of the *An. gambiae* complex have been reported from most countries of Africa and its adjacent islands, including Madagascar, as well as Saudi Arabia and Yemen (Coetzee *et al.*, 2000). The *An. gambiae* s.l. shows high adaptability to changing environments and is mostly found living in sympatry with *An. funestus* in many parts of Africa (Coluzzi, 1984).

Anopheles gambiae predominates in forest and humid savanna zones are more predominant in the rainy season (Faye *et al.*, 1995). It colonizes clean and well-lit temporary pools of rainwater, shallow slow moving streams, bore holes and burrow pits, roadside puddles formed by tire tracks, irrigation ditches and other artificial bodies of water (Gillies and DeMeillon, 1968; White *et al.*, 1972; Woodbridge, 1980; Charlwood & Edoh, 1996; Gimnig *et al.* 2001). Their larval and pupal developmental periods are short, 3-4 days depending on their population density, surface water temperatures and

algal growth (Gillies and DeMeillon, 1968; Gimnig *et al.*, 2001) and are suitably adapted for the short span the water lasts before drying out.

Anopheles arabiensis is more successful in arid savannas and steppes, including those of the south-western part of the Arabian Peninsula (Coetzee *et al.*, 2000). It occurs in most areas of tropical Africa and is absent only from the equatorial region (Fontenille & Lochouaran, 1999).

Shifts in seasonal prevalence are observed showing an increase in the relative frequency of *An. arabiensis* during the dry season (Coluzzi *et al.*, 1979). In areas where rainfall is less than 1000mm, *An. arabiensis is* recorded more often than An. *gambiae* and the reverse is true where rainfall is greater than 1000mm. Where *An. arabiensis* occurs in equatorial rainforest regions, it is usually associated with a history of extensive land clearance e.g. in Benin City, Nigeria (Coluzzi *et al.*, 1979), although this is not always the case. Diallo *et al.* (2000) has reported that *An. arabiensis* was found in large numbers only during the rainy season in Dakar (Senegal), especially in September, when they accounted for 2.25 bites per man per night, and were generally more abundant in the dry season in sahelian and sudan savanna areas of Senegal.

Anopheles quadriannulatus occurs sympatrically with *An. arabiensis* and less frequently with *An gambiae* s.s. It appears to have a relict (narrow) distribution occurring in southeast Africa, Ethiopia and Zanzibar (Hunt *et al.*, 1998). In Ethiopia, it occurs at localities with mean annual rainfall of less than 1000mm while in southern Africa it occurs in localities with a mean of around 700mm (Coetzee *et al.*, 2000).

An. melas and *An. merus* are mainly salt and brackish water breeders on the West and East African coast respectively, although *An. melas* has been found breeding in fresh water streams in the Gambia (Chinery, 1984). Evidence points to a short dispersal range from preferred breeding sites by *An. melas* and the adults are usually not found at distances more than 3km from the saline environment (Bryan *et al.,* 1987). *Anopheles. bwambae* is restricted to the Semliki forest in the Rift Valley near the Zaire border where it breeds in geothermal mineral springs (White, 1985).

An. funestus s.s. has the widest distribution of all the members of the *funestus* group, and is also highly anthropophilic (Gillies & De Meillon, 1968). They breed preferentially in semi-permanent water pools and are often associated with rice fields (Gillies & De Meillon, 1968; Takken, *et al.,* 1998). A comparison of the vectorial capacities of both the *An. gambiae s.s.* and *An. funestus* reveals noticeable differences in their salivary gland sporozoite loads that incriminates the *An. gambiae* s.s. as the more competent and efficient *Plasmodium* species vector (De Meillon, 1947; Pringle, 1966). The two species, *An. gambiae* s.s and *An. funestus* have narrow host ranges and exhibit preferentially anthropophagic and endophilic characters that make humans their main source of blood meal (Burkot, 1988).

Previous vector studies in Ghana have shown the occurrence and importance of *An. gambiae* s.l. in malaria transmission in Ghana (Appawu *et al.,* 1994). Chinery (1984) speculated that *An. arabiensis* might have replaced *An. gambiae* s.s in Accra, the capital of Ghana, because of the changing breeding habitats and the relatively low sporozoites rate recorded for *An gambiae* s.l. during 1964-1965. Dzodzomenyo *et al.* (1999) found the most abundant *Anopheles* to be *An. gambiae* s.s. followed by *An. funestus* at

Okyereko, Central region of Ghana which is a site for this study. Appawu *et al.* (2001) reported ***An. gambiae*** s.s. to be the predominant vector followed by ***An. funestus*** in the Upper East region of Ghana.

2.8.2 Identification of *Anopheles gambiae* complex and *An. funestus*

A number of methods have been developed which can be employed in identifying the members of *An gambiae* s.l. with varying degrees of specificity. The methods are largely based on universal characters that cut across geographical zones for particular sibling species members and are applicable in field situations. Established identification methods of the anopheline mosquitoes include the use of morphological characters described by Gillies & De Meillon (1968) and later improved upon by Gillies & Coetzee, (1987). *Anopheles gambiae* adult females are identified by their smooth palps with 3 pale bands on the 3rd 4th and 5th segments; the wing field is pale with yellowish or creamy markings and has pale fairly long costal spots. The number of pale spots (5) on the costa of the wings can be used to differentiate between *An. gambiae* and other anophelines (Figures 2.4a-d). The femora, tibia and 1st tarsal segment speckled to a variable degree. The abdomen is light brown and hairy with scales on the 8th tergite and scales on the cerci.

Anopheles funestus adult females are distinguished by the three pale bands on the 2nd, 3rd and 5th segments of the palps, it also has pale scales on its characteristic dark wings, with the costa having four pale spots usually shorter than the intervening dark areas. The legs are usually dark with a small apical white spot on the tibia. The abdomen is dark brown and devoid of scales including the cerci.

Cuticular hydrocarbon analysis has also been applied in the identification of the *An. gambiae* s.l. (Carlson and Service, 1979; 1980) and in distinguishing the variant strains of the larvae of *An. gambiae* s.s. (Anyanwu *et al*, 2000). The technique involves chemical analysis of cuticular waxes that vary in composition of carbon content between different species. Using discriminant analysis Anyanwu *et al.* (2000) observed high segregation of hydrocarbon content among four strains of *An. gambiae* s.s. larvae. Similar techniques have been successfully applied in the identification of other insect sibling species like the *Simulium damnosum* species complex (Carlson & Walsh, 1981). Despite the successes achieved with cuticular hydrocarbon analysis, and its advantages of distinguishing geographic variants, it needs highly skilled workers and sophisticated equipment, therefore not practical for routine fieldwork. It is however best suited for analysing museum collections (Crampton, 1994).

Cytotaxonomy is another identification method that classifies organisms based on the detection of differential banding patterns of the polytene chromosomes. Coluzzi & Sabatini (1967) used the banding patterns of chromosomes from the ovarian nurse cells of the semi-gravid adult females to distinguish between species A and B of the *An. gambiae* complex. The chromosomes can also be obtained from the fourth larval instar of the same species. All six members of the *An. gambiae* s.l. have been identified using this method (Coluzzi *et al.*, 1979). The method has also been used to detect species genetic polymorphism due to paracentric inversions (Coluzzi, 1993) Skill and experience limits its wide application and routine use in the field.

Recent molecular techniques developed for the identification of the malaria vectors include PCR assays that are usually targeted at specific regions of repeat gene families, such as the ribosomal RNA (rRNA) gene family. These gene families differ among

cryptic species of rapidly evolving regions like the internal transcribed spacers (ITS1 and ITS 2) or intergenic spacer regions (IGS) of the rRNA DNA sequences. A diagnostic assay for the identification of the *An. gambiae* complex based on IGS and ITS sequence differences has been developed and applied routinely (Scott *et al,* 1993; Favia & Louis, 1999). Similar techniques has also been developed for the identification of *An. funestus* s.l. (Koekemoer *et al*., 1999)

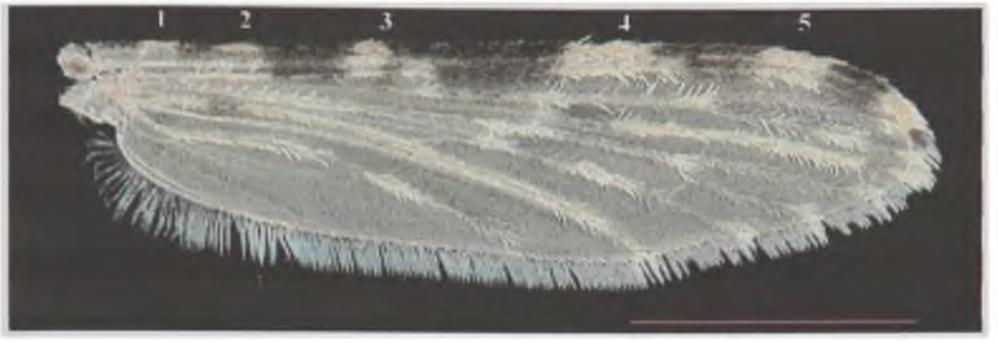

Figure 2.4a Wing markings of *An. gambiae* species showing the characteristic 5 pale spots (numbered) on the costa (Hervy *et al.*, 1998).

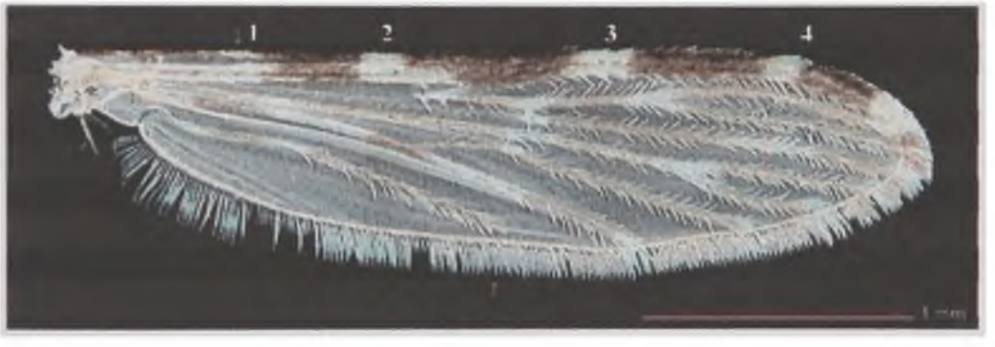

Figure 2.4b Wing markings of *An. funestus* species showing the characteristic 4 pale spots (numbered) on the costa (Hervy *et al.*, 1998).

Figure 2.4c Wing markings of *An. pharoensis* species showing the characteristic 6 pale spots (numbered) on the costa (Hervy *et al.*, 1998).

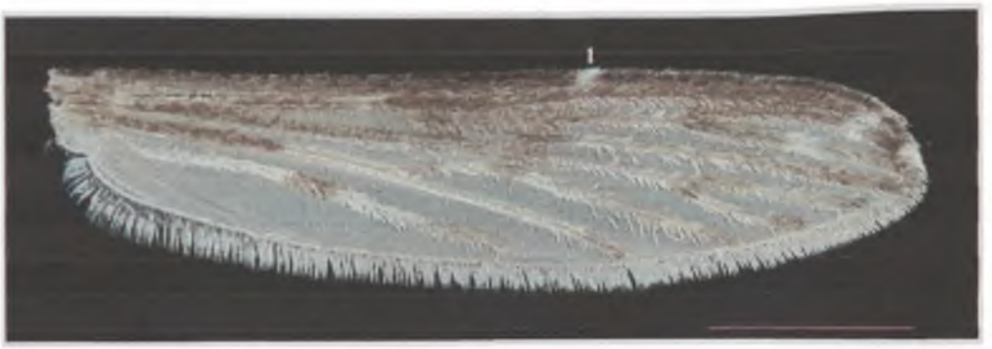

Figure 2.4d Wing markings of *An. coustani* species showing the characteristic 1 pale spot (numbered) on the costa (Hervy *et al.*, 1998).

2.9 Malaria and Irrigation

For nearly half of the world's population, rice is the staple food providing 35-60 percent of the calories consumed (Guerra *et al.* 1998). Rice provides more than a third of cereal calorie intake in West Africa and up to 85% in traditional rice producing countries like Gambia, Guinea-Bissau, Guinea, Sierra Leone, Liberia and Côte d'Ivoire. Rice production in sub-Saharan Africa has more than doubled in the last 3 decades increasing from 2,081 tonnes in 1984 to 4,567 in 2000 (WARDA, 2000).

Several studies have demonstrated that the transformation of arable land into rice irrigation might create suitable habitats for large populations of disease vectors (Van der Hoek *et al.*, 2001). The improper elevation of canal beds, erosion from unlined canals and seepage from poorly designed and constructed canals can all lead to the creation of stagnant and sunlit pools which are often ideal mosquito breeding sites (Goonasekere & Amerasinghe, 1988). Moreover, in dry regions/seasons, irrigation will elevate relative humidity that aids survival of these vectors and also provide suitable breeding sites for anopheline mosquitoes (Lindsay *et al.*, 1991). Irrigated-rice cultivation, depending on the number of cropping cycles, may also extend their breeding season and hence increase the annual duration of transmission.

Rice fields have led to the proliferation of malaria vectors such as *An. gambiae, An. funestus, An. pharoensis* and *An. arabiensis* in Africa, and *An. culifacies, An. philippinensis* and *An. albimanus* in South America (Guthmann *et al.*, 2002). In addition to rice fields providing the principal breeding habitats, irrigation canals and isolated ponds adjacent to fields could also produce vectors such as was observed for *An. culicifacies* in India (Service, 1989).

Although many species of mosquito thrive in rice fields, flooded paddy fields provide ideal breeding sites for the vectors of malaria in Africa: members of the *Anopheles gambiae* complex (Bruce-Chwatt, 1954; Chandler & Highton, 1976; Coosemans *et* al., 1984; Robert *et al.*, 1985; Carnevale *et al.*, 1999), especially *An. Arabiensis* in particular prefer to breed in open sunlit pools created by irrigation schemes (Giles & De Meillon, 1968; Surtees, 1970; White, 1974; Snow, 1983; Coluzzi, 1984). These vectors are pioneer species which rapidly colonize recently flooded fields although they decline in abundance as the rice grows and begins to cover the water surface (Snow, 1983; Lindsay *et al.*, 1991; Ijumba, 1997).

Although *An. gambiae* s.l is generally associated with irrigated rice, in certain situations *An. funestus* also thrives in paddy fields, especially in parts of Madagascar. *Anopheles funestus* species typically favours breeding sites shaded by vegetation (Gillies & De Meillon, 1968) and its presence tends to be indicative of more persistent wetland habitats. In rice fields *An. funestus* occurs later in the growth of rice and when the land is fallow (Marrama *et al.*, 1995). Under some circumstances, however, *An. funestus* becomes excluded by well-managed irrigation schemes such as those that the West Africa Rice Development Authority, (WARDA) maintain under guidance (Henry *et al.*, 1998). In general, the predominant vector in irrigated rice systems is that found in surrounding areas, although there is at least one exception to the rule. The "Mopti" (or M) form of *An. gambiae sensu stricto* found in West Africa thrives in rice fields located in the northern fringes of the Sahel. Thus, in Burkina Faso this cytotype was common in the center of the rice fields but at the edge of the irrigated area the "Savannah" or S form was more abundant (Robert *et al.*, 1989).

Malaria is one of the major tropical diseases associated with irrigation schemes, and changes in the transmission pattern of this disease following irrigation development have been a perennial subject of debate. It has often been assumed that high numbers of malaria vector *Anopheles* mosquitoes resulting from irrigation schemes lead inevitably to increased malaria in local communities. However, recent studies in Africa have revealed a more complex picture. Increased numbers of vectors following irrigation can lead to increased malaria in areas of unstable transmission, where people have little or no immunity to malaria parasites, such as the African highlands and desert fringes. But for most of sub-Saharan Africa, where malaria is stable, the introduction of crop irrigation has had little impact on malaria transmission (Ijumba & Lindsay, 2001).

The development of an irrigation scheme can change the pattern of malaria transmission in an area (Surtees, 1970; Najera, 1988; Service, 1989). However, there is no simple association between irrigated rice fields and the degree of exposure to malaria parasites as measured using classical entomological methods. For example, in the sub-Saharan region of Senegal, irrigation increased mosquito population densities and particularly that of *An. gambiae* s.l., but malaria transmission does not seem to be influenced by this increase. Thus, transmission intensity in irrigated settlements can appear higher, similar or less than in neighbouring villages outside the irrigation scheme.

The association of rice cultivation with ill-health has been known for centuries. In the 1930's for example, the cultivation of rice in Valencia, Spain was forbidden because of health reasons. The term 'rice malaria' was thus coined in the 1930s to describe the association of rice cultivation in Europe with malaria. It is also believed that malaria epidemics during the 1600s in California, USA (which made parts of the State the most malarious on the Pacific Seaboard), resulted from the introduction of rice agriculture

(Boyd, 1941). Rice irrigation schemes in particular have been associated with high incidences of malaria and arboviral diseases (Surtees, 1970; Service 1989; Lacey & Lacey 1990). For instance, forest clearing and rice field extension in the 17th century U.S. was accompanied by intense breeding of *An. quadrimaculatus* and endemic malaria, while in Europe, rice growing was associated with malaria in Portugal and Czechoslovakia (Surtees, 1970).

In the rice growing area of the Rusivi valley, Burundi, the vectorial capacity of *An. gambiae s. l.* was 150 times in the rice irrigation scheme than in an adjacent area growing cotton (Coosemans, 1985). There are a number of possible reasons for this finding. Perhaps most importantly it may have resulted from the large-scale migration of semi-immune or non-immunes moving into a malarious area. Following development of the irrigation scheme, more than 50,000 people, amounting to about 30% of the entire population, migrated from the mountains into the area (Hunter *et al.*,1993). Such a large influx of people presumably from non-endemic areas into an area of moderate transmission, where chloroquine and sulfadoxine and pyrimethamine resistance was widespread (Coosemans *et al.*, 1985; Coosemans *et al.*, 1987; Coosemans & Barutwanayo, 1989), could have resulted in the observed increased malaria. This situation may also have been exacerbated by the low income of migrant farmers (Coosemans & Barutwanayo, 1989), who were unable to afford bednets to protect themselves from malaria mosquitoes bites.

In the Mwea rice irrigation scheme in the Kenyan highlands, malaria prevalence was found to be 26% higher than in the surrounding areas (WHO/FAO/UNEP, 1987, 1990; Hunter *et al.*, 1993). Again, this is an area where many people may have little immunity against malaria. Although anti-malarials were regularly given to children under 10-years-

old, pregnant women and National Irrigation Board staff and their families (Thitai, 1991), medical facilities were not readily accessible to some villages in the scheme because of distance (Wang'ombe & Mwabu, 1993), and protection against mosquitoes was minimal (Mwabu, 1991).

The Madagascar highlands were once malaria free, but the disease became established when irrigated rice farming was introduced into the area (Carnevale *et al.*, 1999). Malaria had remained in check due to effective antimalaria control, although it has resurged in recent years after the cessation of indoor-spraying with DDT.

In Sri Lanka, the Mahaweli Development Project caused a sharp increase in malaria incidence (Goonasekere & Amerasinghe 1988). The introduction of irrigated rice production was also associated with the first ever-recorded outbreak of Japanese Encephalitis in Sri Lanka in 1985/86. It has been well established that the large-scale irrigation development following the Lagdo dam in the North Province of Cameroon, has resulted in increased human malaria incidence (Slootweg and Schooten, 1990), although in these rice-fields the anopheline density can be high, but its anthropophilic biting fraction has short-live span.

Generally, the increase of malaria incidence following irrigation developments has been attributed partly to demographic aspects related to influx of susceptible human populations, and partly to vector related aspects such as increased abundance, field survival, and human contact (Amerasinghe *et al.*, 1991). In some instances, increased transmission has been attributed primarily to increased populations of established vectors (e.g. *An. gambiae* s.s. and *An. arabiensis* in many irrigation schemes in Africa [Service, 1989], and *An. culifacies* in the Mahi-Kadan Project in India [Ault, 1989]. In others,

existing vectors have been supplanted by species previously regarded as non-vectors (e.g. *An. darlingi* replaced by *An. aquasilis* in Guyana, and *An. superpictus* by *An. pulcherrimus* and *An hyrcanus* in the Kunduz Valley of Afghanistan).

Similar levels of malaria transmission have also been reported in ricefield communities and control villages in the same district. For example, following rice irigation development in the Senegal River valley, the prevalence of malaria remained unchanged (Faye *et al.*, 1993b). Similar results were reported from a study involving rice-growing areas in the Senegal River delta. In this area, despite the high man-biting density of vectors, following the development of rice irrigation, malaria transmission incidence rates remained unchanged (Faye *et al.*, 1995). Also, in the rice-growing areas of Bobo Dioulasso, Burkina Faso, the number of infective bites received in the local community was similar to that in the control area (Robert *et al.*, 1985). Although there were more adult mosquitoes near the area of irrigation the sporozoites rate was less than in the surrounding savannah area, this was attributed to the use of bed nets by the population in the rice field area.

Alternatively, in the lower-Moshi rice irrigation scheme, Tanzania, the number of infective bites was 2.6 times lower in the irrigation scheme than in the control village (Ijumba, 1997). The irrigated village was more affluent with better nutritional status of children and greater use of bed nets. Also Boudin *et al.* (1992) recorded in Kou valley, near Bobo-Dioulasso (Burkina Faso) that the inoculation rate was 6 times lower in the irrigated area compared to the savanna, the sporozoite rate in this vector is one-twelfth of those in non-irrigated areas. Service (1989) has suggested that the low infectivity in the Kou valley may also be due to the fact that different vector karyotypes are found in the irrigated and non-irrigated areas. Robert *et al.* (1989) reported that this epidemiological

paradox was due to a permanent use of bed nets on account of mosquito nuisance; a lower survival rate of *An. gambiae* in rice fields, which did not allow the complete development of the sporogonic cycle of *Plasmodium*, and a zoophilic deviation of the parous females (*An. gambiae)*, that contrasts with the human blood preference of young *Anopheles*. In this area, malaria transmission is characteristically intense during the wet season and limited, or absent, at other times of the year when there are few potential breeding sites and vectors are markedly reduced in numbers (Greenwood *et al.*, 1987; Lindsay *et al.*, 1989).

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

The reasons given for less malaria risk are many and varied. One of such is that the introduction of irrigated-rice cultivation results in wealth creation in local communities (Robert *et al.*, 1985; Audibert *et al.*, 1990; Boudin *et al.*, 1992), and with this additional wealth, farmers make improvements to their homes, their standard of living rises and they have more disposable income with which to use to protect themselves from nuisance mosquitoes and malaria. In Kenya, it was found that at a certain threshold of income the situation becomes favourable for adoption of malaria control measures at the family level, and this goes hand in hand with improvement of the living standard of the family

or community (Mwabu, 1991). Therefore, a community with relatively higher economic development would be associated with greater use of anti-malarial measures and also, within a community, wealthy individuals would be more likely to use such measures. Support for this comes from studies that have shown that bednet ownership was related to affluence in Gambia (D'Alessandro *et al.*, 1994) and Tanzania (Ijumba, 1997).

There is enough recent and historical evidence to support the view that economic development in general has an overall positive impact on health (Carrin, 1984; Stewart, 1985). The disappearance of malaria in some parts of Europe was associated with economic development as a result of agricultural expansion rather than vector control or chemotherapy (Cambournac, 1994; Najera, 1994; Zulueta, 1994). At a certain threshold level of income, it becomes possible for the family to invest in antimalaria measures in order to save on direct costs associated with malaria treatment (Wang'ombe & Mwabu; 1993). Increased incomes lead to better nutrition, habitation and protection - factors that often influence the overall health status more than public health expenditure on health care. Therefore, if irrigation contributes to increased incomes, this will be reflected in better health - although this process may not be particularly rapid (Oomen *et al.*, 1988).

Large-scale irrigated rice cultivation can result in several thousand mosquitoes entering a local house during the night. For most people such high biting rates are unacceptable and they will protect themselves by sleeping under bednets. Studies in Gambia have shown that more people start using bednets when mosquito numbers begin to increase (Aikins *et al.*, 1993; Thomson *et al.*, 1994; Bøgh, 2000). Bednets in good condition reduce biting rates (Lindsay *et al.*, 1989) and can protect against malaria (Bøgh, 2000). Thus, it is likely that part of the association between rice and moderate malaria may be explained by the high bednet coverage in communities living near irrigated rice production. High net

use has been reported from rice villages in Burkina Faso (Robert *et al.*, 1985), Cameroon (Robert *et al.*, 1992) and Gambia (Lindsay *et al.*, 1991).

Moreover, at high mosquito densities, density-dependent effects become important. With high net use, individual mosquitoes find it difficult to locate and obtain a human bloodmeal. Reanalysis of data from Gambia (Lindsay *et al.*, 1993) shows that the proportion of human-blood fed mosquitoes collected from individual villages declined with increasing numbers of mosquitoes collected from light traps ($z = 3.059$, $P = 0.002$). Even with badly torn nets, blood feeding is density dependent (Lindsay *et al.*, 1992).

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

Widespread use of anti-malarials (Carnevale & Robert, 1987; Faye *et al.*, 1993b) in ricefield communities and the existence of a well established health infrastructure (Faye *et al.*, 1993a) may also contribute to the general lower level of malaria, although the growing problem of chloroquine resistance will make control more difficult in the future. It is of course also important that irrigation schemes are well designed and maintained to

reduce standing water to a minimum and thus limit the opportunities for mosquito breeding.

2.10 Molecular and Biochemical Methods for Malaria Studies

2.10.1 Molecular methods for identification of malaria vectors

Although it has long been known that only some members of anopheline species complexes are important vectors of malaria, these sibling species have proven difficult, if not impossible to distinguish by traditional taxonomic measures. The species specific differences DNA sequences of the genome have been exploited for distinguishing adults of *Anopheles gambiae* complex (Gale and Crampton, 1987; Collins *et al.*, 1987; Besansky, 1997). Most noptable is the PCR-based methods for the specific identification of members of the *An. gambiae* complex (Scott *et al.* 1993) and *An. funestus* group (Koekoemer *et al.* 1999).

2.10.1.1 Principles of Polymerase Chain Reaction (PCR)

The PCR is an *in vitro* method developed for the enzymatic synthesis of specific DNA sequences, using two oligonucleotide primers that hybridize to opposite strands and flank the region of interest in the target DNA (Saiki *et al.*, 1985). Figure 2.5 shows a schematic diagram of the PCR process. The reaction utilizes the DNA polymerase enzyme and occurs in a repetitive series of cycles resulting in the exponential accumulation of large amounts of the target DNA sequences. The first experiments with PCR used the Klenow fragment of *Escherichia coli* DNA polymerase (Saiki *et al.*, 1985; Mullis & Faloona, 1987). The high temperatures used to denature the template DNA diminished the activity of the klenow fragment, therefore, more enzyme had to be added at the start of each cycle. This was both labour intensive and expensive. The technique was revolutionized by the discovery of a heat stable polymerase isolated from the thermophilic bacteria *Thermus aquaticus* and therefore called *Taq* polymerase (Saiki *et al.*, 1985). The fact that *Taq* polymerase enzyme is added to the reaction once, at the beginning of the reaction has transformed the PCR into a simple and robust reaction, which is now automated by

programmable thermal cycling devices (Saiki *et al.*, 1985). The reaction constituents (DNA template, oligonucleotide primers, *Taq* polymerase, deoxynucleotides and reaction buffer which contains salts) are all included in a mix and the amplification reaction carried out by simply cycling the temperature within the reaction tube (Saiki *et al.*, 1988). The PCR reaction involves three steps of repeated cycles of heat denaturation of the DNA, primer annealing to their complementary sequence at a lower temperature and extension of the annealed oligonucleotide by the DNA polymerase.

The PCR involves complex kinetic interactions between the template or target DNA, product DNA, deoxynucleotide primers (polymers made up of 10-30 nucleotides), deoxynucleotide triphosphates (dNTPs), buffer and enzyme (DNA polymerase). These relationships change during the course of the reaction. Though PCR works well with most DNA targets, usually adjustments are needed in reaction parameters in order to improve specificity and yield. These include altering the reaction buffer (particularly the $MgCl_2$ concentration), relative concentrations of template DNA, primers, dNTPs and enzyme, and annealing time and temperature. No single protocol is appropriate for all situations and each new application will require optimization. For example, amplifying a 100 bp DNA fragment is not equivalent to amplifying a 10kb fragment.

The specificity of PCR depends on base pairing by the two oligonucleotide primers to the target DNA that serves as the template. The primers which are sequences that flank the sequences to be amplified anneal to the strands of the denatured template DNA. Repeated PCR cycles involve heat denaturation to separate the two DNA strands, annealing of primers to the complementary DNA sequence and extension of the new DNA strands from the 5' – 3' end by DNA polymerase, with the base sequence of the new strands determined by the template DNA. The primers are constructed so that the DNA synthesis

proceeds across the region between the annealed primers. The mixture is repeatedly heated and cooled until the desired amount of amplification of the template DNA is achieved.

2.10.1.2 PCR-based method for the identification of *An. gambiae* sibling species

A PCR method developed by Scott *et al.* (1993) is currently widely used in the identification of member species of the *An. gambiae* complex. The method, which is based on species-specific nucleotide sequences in the ribosomal DNA (rDNA) intergenic spacer regions may be used to identify both species and interspecies of hybrids regardless of life stage using either extracted DNA or fragments of a specimen. The non-coding rDNA spacer sequences can be highly variable in length and sequence between closely related species. Concerted evolution acting on rDNA assays maintains sequence homogeneity within species as it drives differentiation between species, a pattern that explains the utility of rDNA for species diagnostic assays (Collins & Paskewitz, 1995). In using this method, parts of a mosquito specimen as small as an egg or a homogenate of a leg may be placed directly into the PCR mixture for amplification and analysis (Scott *et al.*, 1993). The method uses a cocktail of five 20-base oligonucleotides to identify *An. gambiae, An. arabiensis, An. quadriannulatus,* and either *An. melas* in West Africa or *An. merus* in East and South Africa.

2.10.1.3 Principles of restriction fragment length polymorphism (RFLP)

Restriction fragment length polymorphism (RFLP) provides a means of discriminating between similar DNA samples at the species or strain level. Extracted DNA is digested using restriction endonucleases, which cleave at specific recognition sites. The resulting restriction fragments are separated according to size by electrophoresis on agarose gels

revealing diagnostic polymorphisms with distinct DNA fragments. However, in most cases, the digestion products appear as a smear with fragments ranging upto approximately 25kb in size. To gain useful information on these gels, the DNA must first be transferred to a nylon or nitrocellulose membrane by Southern blotting (Sambrook *et al.*, 1989) and selective fragments visualized by hybridization with a suitable labeled DNA probe.

Favia *et al.* (1997) developed a polymerase chain reaction (PCR-RFLP) assay, which unambiguously separates the *An. gambiae* s.s. "Mopti" from "Savanna" form. This involves a multiple of steps where individual mosquitoes are first identified by PCR (Scott *et al.*, 1993). In a second step, *An. gambiae* s.s. specimens are identified to the molecular form level by either of two methods, both based on the presence of two nucleotides substitutions (GC in the S-form vs. AT in the M-form) within the amplified rDNA region. The first method is based on a PCR-RFLP method, whereby the PCR amplification of a 1.3kb r DNA fragment is followed by digestion with restriction enzymes *Hha* I (or *Tru* I) and a specific pattern is obtained for each form (Favia *et al.*, 1997). The second method consists in a polymorphism allele-specific PCR based on form-specific primers that amplify fragments of different size within the above-mentioned 1.3kb rDNA region (Favia *et al.*, 2001). Therefore the identification of each *An. gambiae* s.s. specimen requires either two PCR reactions followed by an enzyme digestion, or only two PCR reactions.

Fanello *et al.* (2002) have recently proposed a new method involving combination of the protocols established by Scott *et al.* (1993) and Favia *et al.* (1997). This method enables the simultaneous identification of the species of the *An. gambiae* complex and S and M forms of *An gambiae* s.s. It is based on the observation that the 5'-GCG/C-3' restriction

site for *Hha* I enzyme lies within the *An. gambiae*-specific fragment amplified by the method of Scott *et al.* (1993) for the S form but not in the M form (Favia *et al.* 1997). Therefore, it is possible to digest this fragment to differentiate M and S molecular forms.

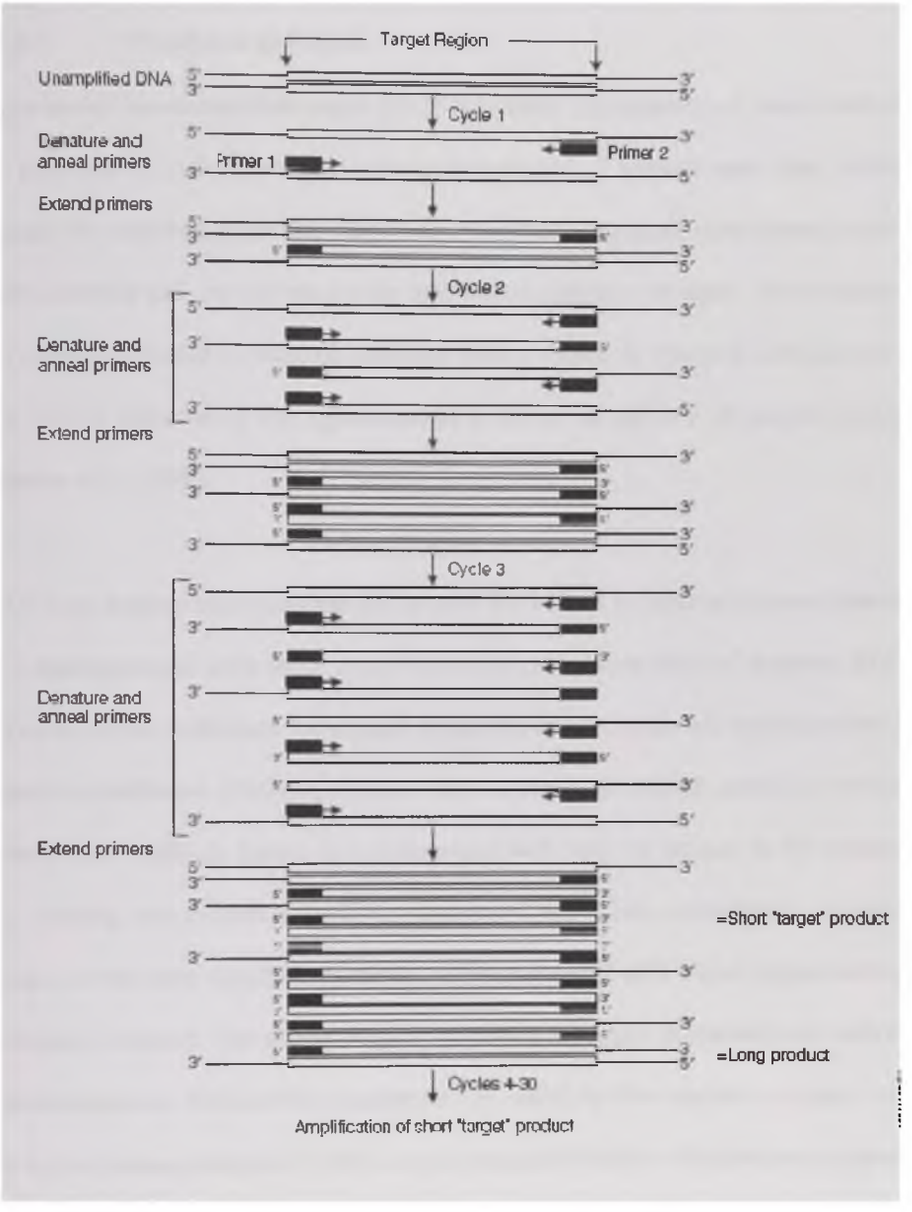

Figure 2.5: Schematic diagram of polymerase chain reaction (PCR)

2.10.2 Biochemical methods for the detection of sporozoite infections and Blood meal source

2.10.2.1 Principles of ELISA

Enzyme-linked immunosorbent assays (ELISA) employ the property of plastic materials (e.g. polyvinyl or polystyrene) to adsorb monolayers of protein onto their surface. Although the adsorbed molecules may lose some of their antigenic determinants, enough remain unaltered and can still react with their corresponding antibodies. The presence of these antibodies bound to antigens adsorbed onto a plastic, is detected with an enzyme which can be detected by the appearance of a colour on addition of proper substrate (Benjamin *et al.*, 1996).

ELISA is an enzyme immunoassays (EIA) used for antigen concentration measurements. It is a heterogeneous solid phase assay that requires the separation of reagents. ELISA has two available techniques for antigen measurement, the sandwich technique and the competitive technique (Monroe, 1984). The sandwich or double antibody technique begins with an antibody bound to a polystyrene well plus the antigen to be measured. After washing the microtiter well to remove all the other components an enzyme conjugate of the same specific antibody is added to the well with bound antigen-antibody (or immune complex). The excess enzyme – antibody conjugate is washed away and then a suitable substrate followed by a substrate are added to the enzyme conjugate which binds to the immune complex. If the enzyme conjugate binds to the immune complex, a positive test or color change will occur. The amount of product formed in a given time is directly proportional to the amount of antigen originally present (Holme & Peck, 1983). It should be emphasized that after coating the plastic surface with antigen, it is imperative to "block" any uncoated plastic surface to prevent it from absorbing the other reagents, most importantly the labelled reagent. Such "blocking" is achieved by coating the plastic

surface with a high concentration of an unrelated protein, such as gelatin, after the application of the antigen (Monroe, 1984).

The antigen competitive inhibition assay begins with an antibody bound to a polystyrene well plus a test sample containing an antigen mixture to which an antigen-enzyme conjugate is added. At this point competitive inhibition occurs between the antigen-enzyme conjugate and an unlabeled antigen, depending on which antigen type is in excess, two different outcomes can follow when binding to a specific antibody occurs. After the formation of an immune complex from an antigen-antibody binding, the reagents are separated by washing. Next a substrate is added to the immune complex, if the antigen-enzyme conjugate is the antigen in excess a color change will occur indicating that the substrate has chemically changed as a result of the enzyme conjugate being bound to the immune complex. If it is the unlabeled antigen that is in excess there will be little to no change in color because the test sample contains antibody-type-specific antigen (Monroe, 1984).

2.10.2.2 Determination of sporozoites in mosquitoes by ELISA

Sporozoite infection rate in mosquitoes, together with abundance, is a direct measure of the intensity of malaria transmission (Gu, 1995). Traditionally, sporozoite infection rates have been based on the microscopic detection of sporozoites in the salivary glands after dissection. However, this process is labour intensive and requires highly trained technicians (Coleman *et al,*. 2000). The development of monoclonal antibodies specific for the circumsporozoite (CS) protein, a major component of the malaria sporozoite (Zavala *et al.,* 1982; Burkot *et al.,* 1984) heralded a major advance in malaria epidemiology. The use of immunological assays, in particular the circumsporozoite enzyme-linked immunoassay (CS-ELISA), has widely replaced mosquito dissection for

determining mosquito sporozoite rates (Zavala *et al.*, 1982; Wirtz *et al.*, 1987a; Wirtz & Burkot 1991).

A monoclonal antibody-based radioimmunoassay which detects *P. falciparum* sporozoites in mosquitoes was reported in 1982 (Zavala *et al.*, 1982). The target of the assay is a region of the circumsporozoite (CS) protein on the surface of the malaria sporozoite which contains repeated amino acid sequences (Dame *et al.*, 1984; Enea *et al.*, 1984). A monoclonal antibodies (Mab) specific for an epitope in this region was used to capture the protein and an ^{125}I-labeled aliquot of the same Mab was used as the detector. Subsequent research has found out that the CS proteins of other *Plasmodium* species are also characterized by similar repeating epitope regions, but the actual repeat sequences differ between species (Zavala *et al.*, 1985). Mabs produced against the repeat of region one species therefore generally do not cross-react with the sporozoites of other species. A similar assay has been developed using a Mab specific for *P. vivax* sporozoites (Wirtz *et al.*, 1985), and both the *P. falciparum* and *P. vivax* assays have been modified to use enzyme-labelled rather than radionuclide-labeled Mabs (Wirtz *et al*, 1987b). These sporozoite detection assays have been field-tested on sporozoites and infected mosquitoes from Africa, South America and Asia (Collins *et al.*, 1984; De Arruda *et al.*, 1986).

The main advantages of CS-ELISA are that it is species-specific and can detect sporozoites in pooled samples. Processing samples is a highly efficient and economic method for determining sporozoite rates, particularly when vector infection rates are low (Wirtz *et al.*, 1987a; Gu, 1995). The CS-ELISA has been used in numerous epidemiological investigations on the vectorial capacity of a wide variety of Anopheline mosquitoes for *Plasmodium* parasites (Rosenberg *et al.*, 1989, 1990) and has increased our understanding of the transmission dynamics of malaria (Rosenberg *et al.*, 1989). In

addition, the assays are more sensitive because low sporozoites may be overlooked in salivary gland dissections; sensitivities of 25 or fewer sporozoites per assay have been reported for both the *P. falciparum* and *P. vivax* ELISAs. Futhermore, gland assays can occasionally result in the loss of one or more of the six gland lobes. In mosquitoes with low-level gland infections, sporozoites have been observed to concentrate in one gland or even one salivary gland lobe. The ELISAs detect CS protein, which can be present in the developing oocyst, dissolved in heamolymph, and on sporozoites present in the salivary glands. Moreover, the repeating epitope region of the sporozoite remains detectable even in air-dried mosquitoes (Collins *et al.*, 1985). The ELISAs can be carried out on fresh, frozen, or dried mosquitoes. The application of the ELISA method on dried adult mosquitoes to detect all the human species of malaria (although by separate ELISAs), the commercial availability of ELISA kits and the introduction of portable ELISA plate reader have made its use popular.

However, the disadvantages include the inability to distinguish between infective and infected mosquitoes and difficulty in determining the cut-off points when a result should be taken as negative. Also, a positive ELISA on a mosquito does not establish the mosquito species as vector, and ELISA results may not be synonymous with salivary gland sporozoite rates.

2.10.2.3 Determination of source of mosquito blood meal by ELISA

Identification of the blood meal taken by a haematophagous insect provides information on host preferences under natural conditions. The anthropophilic index (percentage feeding on humans) is a vital component of vectorial capacity, while knowledge of other hosts reveals the relative importance of reservoirs of vector borne zoonotic or enzootic infections (Boakye *et al.*, 1999).

Several methods can be used to identify blood meals, e.g. the haemoglobin crystallization test, passive haemaglutination inhibition tests, the latex agglutination test, fluorescent antibody techniques and the precipitin test. The precipitin test, although relatively simple and inexpensive lacks sensitivity and specificity, and can be somewhat time –consuming unless an automatic dispenser is used (Weitz, 1957). Passive haemagglutination tests offer greater sensitivity and specificity but are variable, time consuming and are difficult to use routinely. The latex agglutination test, although much easier to perform, cannot distinguish between closely related hosts and is less sensitive. Immunofluorescent techniques (Gentry *et al.*, 1967; McKinney *et al.*, 1972) require sophisticated laboratory equipment and technology and they are not suitable for field work. The complement fixation test requires highly skilled personnel and is subject to many technical problems (Service *et al.*, 1986).

Identification of blood meal by ELISA is a specific, relativeely sensitive, reproducible and practical test for the identification of *Anopheles* blood meal up to 24 hours after ingestion and holding in the conditions of an insectary (Edrissian & Hafizi, 1982). ELISA can identify blood in half-gravid females mosquitoes and even when they are about three-quarters gravid and only a trace of blood visible externally. ELISA is a well-suited objective and sensitive method, a simple filter paper eluate or abdomen homogenate can be analysed for a wide variety of hosts. It is quite simple to combine wide variety of species identification systems in any microtiter plate, and the combination can be varied for any particular application (Service *et al.*, 1986).

The disadvantages of blood meal identification by ELISA are that, firstly, in order to unambiguously identify a given blood meal it is necessary to produce polyclonal antisera

against the blood of the host in question. Secondly, it is also necessary to carry out preabsorption steps against the blood antigens of other species before the specificity of the antibody reagent can be ensured. Third, it is necessary to test the specimen with antisera produced against all potential hosts in order to unambiguously identify the blood meal. Finally, it may not be possible to identify a particular blood meal if a suitable antibody is not available (Boakye *et al.*, 1999).

2.11 Entomological Indices

The roles that the *Anopheles* vectors play in the malaria cycle, necessitates the understanding of the complexity of the mathematical relationships describing the rules which allow the intensity of malaria transmission to be "predicted" on the basis of measurements of several entomological parameters (Esposito & Habluetzel, 2003).

2.11.1 Vector density

The vector density is the average number of mosquito vectors per inhabitant. The reference method for measuring vector density is the human landing catch. With the help of a simple aspirator, mosquitoes are caught when they land on a human volunteer for a bloodmeal. The rationale for the choice of this method is straightforward and is based on few considerations. The feeding and resting habits of *Anopheles* not only vary both inter- and intra-specifically, but they may also vary under the selective pressure exerted by intervention measures. Coluzzi *et al.* (1979) have shown that certain karyotypes of *An. arabiensis* are found with different frequencies biting outdoors or indoors and *An. farauti* shifted from prevailing endophily to exophily after indoor spray operation were implemented in Solomon Islands (Taylor, 1975). It would be therefore risky to determine the *Anopheles* density using the results of catches performed at the presumed resting sites. In addition, the fraction of the mosquito population which actually "counts" in terms of malaria transmission is constituted by the females which bite human and thus it seems very appropriate to target the catch method to them.

Practical and ethical considerations however argue against the human bait catches. From the practical point of view, since most malaria vectors bite at night (sometimes late at night), landing catches are very labour demanding in terms of execution and supervision. From the ethical point of view, the deliberate exposure of human volunteers to mosquito-

borne diseases (not only malaria) is definitely questionable. Alternatives have been proposed, like the human baited double bednets: a first bednet is partly uprisen or purposedlly torn and works as a trap, while the second is properly located and protects the volunteer from being bitten.

Recently, a light trap designed at the Center for Diseases Control in Atlanta, USA, has become increasingly popular. Located near a volunteer sleeping under a bednet, at a height of about 0.5 m from the ground, the trap exploits the attractiveness of the human bait "from far" When the mosquito approaches to bite, the light of a small battery operated bulb may exert an attraction "from near" and bring the insect close to the fan which aspirate it into the trap bag. Practical and independent on the collector skill, the CDC light traps are definitely less efficient than the human bait catch. This may lead to an underestimation of the real amount of transmission in baseline epidemiological studies, but does not represent a major disadvantage in control programmes where usually either historical or parallel reference data for measuring the impact are available (Esposito & Habluetzel, 2003).

For special purposes, the human bait (in landing or in CDC trap catches) can be substituted by animal baits, for instance when sampling the least anthropophilic or the zoophilic fraction of the population is required. When the *Anopheles* populations thriving in a certain area are quite stable in their habits, and their resting places are well known and easily accessible, other methods can be used. Aspirators can be used to collect living mosquitoes at their resting places

For strictly endophilic vectors another practical and widespread method consists in spraying a pyrethrum suspension in a closed room/house. The knocked-down mosquitoes can then be collected on a white sheet previously laid on the floor.

2.11.2 Parous rates

Methods exist which directly assess morphophysiological characteristics directly related to the mosquito age (Muir, 1988). The unravelling of the tracheoles of the ovaries associated with follicle growth allows the nulliparous to be distinguished from the parous females which have gone through one or more gonotrophic cycles. The ratio parous/nulliparous is obviously related to the probability of vector survival, as parous specimens are certainly older than nulliparous, but requires repeated measures on large samples, being strongly influenced by short-term meteorological variables. The almost simultaneous emergence of young adults from the breeding sites, as frequently occurs during the rainy season, may suddenly, i.e. from one day to the next, increase the nulliparous fraction. In addition, the partition between the two age categories - parous and nulliparous - occurs at an age (4 - 5 days) which is not really crucial in relation to malaria transmission. More detailed and useful information can be gathered from the dissection and microscopical examination of the dilatations of the ovarioles. These dilations are thought to represent the "footprints" of previously laid eggs. Assuming a constant (and known) time interval between subsequent ovipositions, by multiplying this time by the number of dilatations the age of the mosquito could be estimated. Applying this method, Gillies (1988) found for a Tanzanian population of *An. gambiae* that 65% of the mosquitoes died within the first week and 92% within a fortnight. Doubts on the interpretation of the dilatations have been raised by Hoc & Charlwood (1990). In addition, the method is very elaborate and to process a limited number of specimens substantial amount of time and remarkable skills are required. Age grading is crucial in

malaria control programmes targeted at the vector (e.g. indoor spraying or impregnated bednet/curtains).

2.11.3 Sporozoite rates and entomological inoculation rates (EIR)

The EIR is the number of infective mosquito bites received per person per unit time. The sporozoite rate is often used to estimate the entomological inoculation rates (EIR). Although sporozoite rates alone are useful, a better understanding of the epidemiology of malaria is gained by comparing the daily EIR. This has been described as the most important single determinant of malaria prevalence (Burkot *et al.*, 1981). The EIR is a product of the man biting rate and sporozoite rate and because of this, it provides the most effective indicator of the intensity of malaria transmission. Muliplying the mean daily EIR for all sampling periods by 365 gives an approximate estimate of the number of infective bites a person receives over a year.

The estimation of inoculation rates can be very problematic. There are, for example, often difficulties in measuring accurately man-biting rates or the biting rates of different age groups within a community to determine their role in malaria transmission. When extremely low *Anopheles* densities are encountered as in a vector control project in Burkina Faso, reliable man-biting rates cannot be obtained thus precluding the calculation of EIR (Habluethzel *et al.*, 1988). Instead, transmission patterns were monitored by applying an ELISA technique to measure the prevalence of antibodies to sporozoites (spo-abs) in the human population (Habluethzel *et al.*, 1988).

The EIR is used to determine the entomological impact of an intervention and can establish a relationship between the entomological and parasiotological variables in areas with several vector species with different biting rates and human blood index (HBI). The

annual EIR is the preferred measure of assessing malaria endemicity, suitability of vector control and risk of epidemic development.

2.11.4 Human blood index (HBI)

The HBI of anopheline mosquitoes is an important factor in determining the role different *Anopheles* mosquitoes play in malaria transmission (Edrissian & Hafizi, 1982). The importance of this parameter resides in the fact that it is one of the parameters in the formula quantifying the "power" of a vector (vectorial capacity) for transmitting malaria (Macdonald, 1957).

The Human blood index parameter is largely influenced by host availability, however clear-cut differences for host-specific tendencies have been demonstrated. When humans and cattle were equally made available to *An. merus* and *An. gambiae,* the former took the majority of meals on animals and the latter on humans (Gillies, 1967), indicating that a primarily ecological adaptation had become genetically fixed. Bearing in mind that these habits can be partially or totally reverted by host availability, various *Anopheles* species can be ranked according to their degree of anthropophily, measured by the human blood index, i.e. the proportion of mosquitoes fed on humans.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Chemicals, Reagents and Equipment

The sources and/or manufacturers of reagents and equipments used for the study are listed in Appendix I.

The various buffers and solutions used were prepared as described in Appendix II

3.2 Study Areas

The studies were carried out at Gomoa Okyereko (irrigated) and Gomoa Bewadze (non-irrigated), both coastal areas in the Central Region of Ghana.

3.2.1 Gomoa Okyereko (irrigated)

Gomoa Okyereko (Figure. 3.1) is a village located about 50 km west of Accra and about 10 km northeast of Winneba, on the western extension of the coastal savannah zone in Ghana. It lies between latitudes 5° 24' - 35'N and longitudes 0° 25' - 36'W. The area has an average annual rainfall range between 760-1000 mm, a mean temperature range of 26-30°C. The main rainy season lasts from May to July with a minor season of light shower between September and October. The vegetation is a typical coastal savanna grassland interspersed with very few trees. On the western margin of the village is the River Ayensu which is the main source of water to the village. This river normally has a large volume of water in the rainy season leading to periodic flooding but reduces in volume in the dry season.

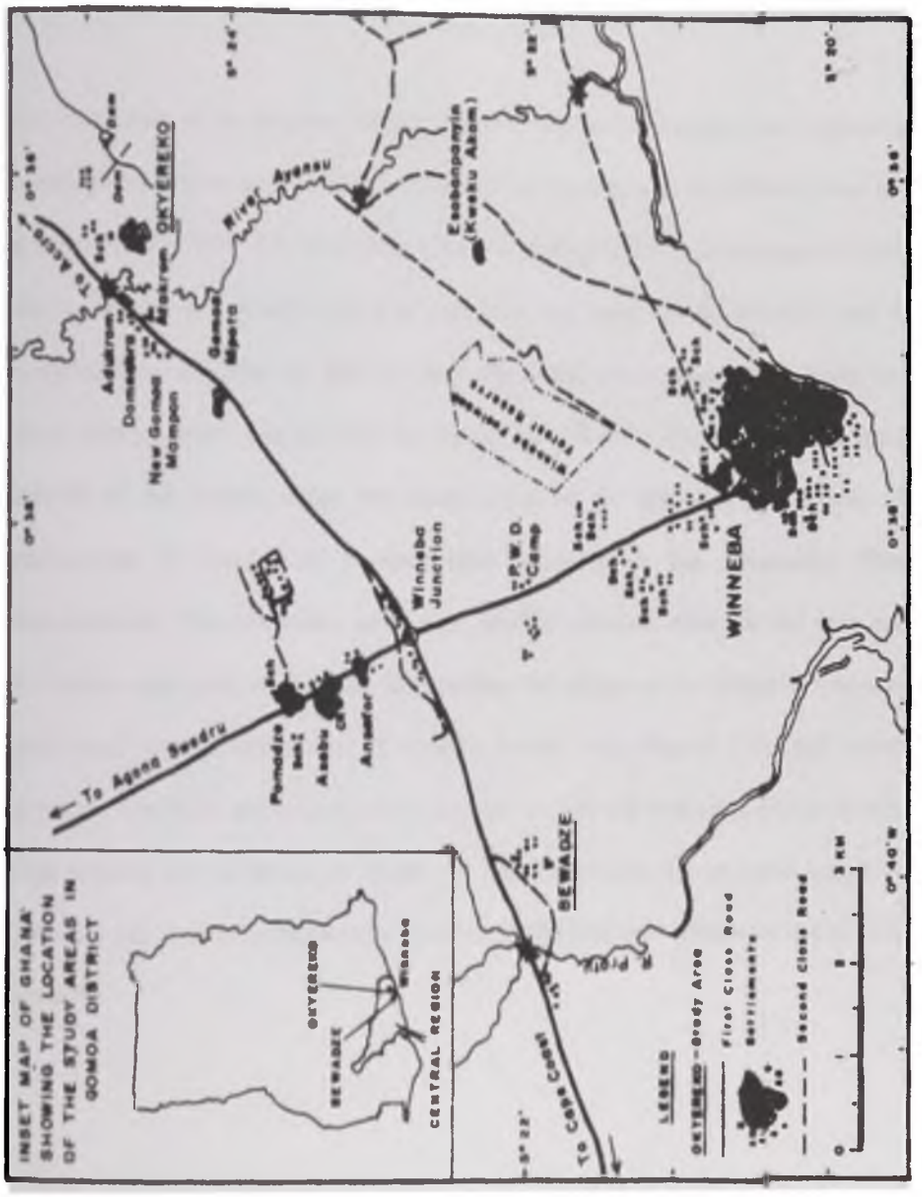

Figure 3.1 Map showing the two study sites Okyereko and Bewadze (underlined)

The inhabitants, mainly ethnic Gomoa, are generally peasant farmers and to permit sustained agricultural activities during the dry season, the installation of irrigation facilities was necessary. The estimated population size for year 2000 was 706 inhabitants.

The construction of an irrigation facility (Figure 3.2) which involved the construction of an earth dam across a small tributary of the River Ayensu was therefore started in 1973 and completed in 1974. The Okyereko Irrigation Project (OIP) thus envisaged an increase in the production of rice and vegetables during the dry season in the Winneba area. It was also intended to minimize the drift of the youth to the urban areas by allocating plots to farmers where it was expected that the income would go a long way to raise the living standards of the people. Since the construction of the dam and the canals, clinical manifestations of bancroftian filariasis have appeared in the community (Personal communication). The Okyereko settlement itself is situated between the dam and the river Ayensu and most of the land surrounding the village is the irrigable land which is mainly used for the cultivation of cereals, mainly rice (Figure 3.3) and maize and vegetables. The fields are irrigated from 8.00am to 6.00 pm everyday except Sundays. A typical housing unit is shown in Figure 3.4 and the houses are grouped together. The community has a clinic which caters for their health but most indigenes use it only as a last resort.

Figure 3.2: A typical canal at the Okyereko Irrigation Scheme

Figure 3.3: A typical rice farm at Okyereko

Figure 3.4: A typical housing unit at Okyereko

3.2.2 Gomoa Bewadze (non-irrigated)

Gomoa Bewadze (Figure 3.1) is a small village located about nine and half kilometers from Okyereko. Gomoa Bewadze lies between latitudes 5° 20' - 50'N and longitudes 0° 40' - 30'W. It is within the same ecological zone as Okyereko and the climatic conditions are similar to that described for Okyereko above. The estimated population size for the year 2000 was 155 inhabitants. The people are mainly peasant farmers and the community has a typical village setting. The houses at Gomoa Bewadze are clustered into households comprising of extended families with most of them headed by men. A typical housing unit is shown in Figure 3.5.

Figure 3.5: A typical housing unit at Bewadze

3.3 Field sampling of mosquitoes

Adult mosquitoes were collected over a period of six months (September, 2002 to January 2003 and March 2003) using both human landing catch (HLC) and pyrethrum spray catch (PSC) methods (WHO, 1992). Two day catches of mosquitoes were carried out simultaneously each month at the two villages. Each village was divided into two halves and on each collection day two structurally typical housing compounds were randomly selected from each sector for HLC and PSC. Both indoor and outdoor HLC were conducted.

3.3.1 Human landing catches (HLC)

The catches were on hourly basis and were carried out between 18.00 hours and 06.00 hours. At Okyereko, collections were made by two teams (two persons per team) of village collectors, one team collected indoors and the other team collected outdoors. This was because the collectors at Okyereko have had years of experience of mosquito survey and were therefore used to collecting for long hours. One team collected between 18.00 hours and 24.00 hours indoors while the other team collected outdoor within the same period. At midnight, they changed places and collected from 24.00 hours to 06.00 hours.

However, at Bewadze collections were made by four teams (two teams for indoor catches and two teams for the outdoor) because the collectors did not have any previous experience in mosquito collection. Thus, one team collected indoors while another team collected outdoors simultaneously between 18.00 hours and 24.00 hours. At the end of this period, the team left and the other two teams of collectors collected indoors (by one team) and outdoors (by the other team) between 24.00 hours and 06.00 hours. The teams were rotated during the period in order to compensate for differences in the attractiveness of the collectors to mosquitoes and their catching abilities.

Outdoor collections were carried out in an open place where the villagers normally gathered in the evening. The collectors had their clothing adjusted so that their legs as far as their knees were exposed. Mosquitoes were caught using glass tubes and aided by illumination from flash – lights when they landed before they could take blood meals. At the end of each hour's collection, the mosquitoes caught were transferred into labeled empty paper cups covered with netting. At the end of each night's collection, the paper cups were placed inside a labeled carton and transported to the laboratory for identification, dissection and molecular studies. The location, date, site of collection (indoors or outdoors) and hour of collection were noted.

3.3.2 Pyrethrum spray catches (PSC)

To assess indoor resting mosquito density and the monthly changes in resting densities, adult mosquitoes were sampled by the PSC method. Spray catches were carried out in the morning after the occupants of the rooms had woken up usually around 06.30 – 07.00 hours (WHO, 1995). To prepare the room for spraying, all foods items were removed or covered as well as all openings with cloth. White sheets were then spread so that they completely covered the floor and the furniture. The sheets on the furniture were placed in such a way that they did not hang down to the floor because this would prevent the spray from reaching mosquitoes under the furniture. Sheets were also spread under the bed, tables and all other potential resting places of mosquitoes. The doors and windows were then closed and the room sprayed with "Raid ™" insecticide containing pyramin forte (0.050%), neopynamin (0.050%), deltamethrin (0.015%) and, solvent and fragrance (99.885%). The insecticide was sprayed towards the ceiling in a clockwise direction until the room was filled with a fine mist.

After ten minutes, starting from the doorway the sheets were lifted up one at a time by their corners and carried outside. Knocked down mosquitoes were then collected from the sheets using forceps and placed into petri dishes that were lined with damp filter paper and taken to the laboratory for further studies. The location, date and time of collection, and the number of people who slept in the rooms the previous night were noted.

3.4 Specimen Preservation and Dissection

3.4.1 Preservation

The mosquitoes obtained were all identified and stored at $-20^{O}C$ prior to dissection for parity after which each carcass was given an identification number, placed in 1.5ml eppendorf tube with perforated caps, and kept dry over silica gel in ziplock bags until needed for PCR and ELISA.

Data on the number of specimens collected for each house, study area by species, collection times and dates and methods of collection were recorded and the data entered into Microsoft Excel ™ spreadsheet and later imported into an SPSS ™ for subsequent analysis.

3.4.2 Parity Determination

To determine the proportion of female *Anopheles* which had previously laid eggs and therefore likely to carry the malaria parasites from previous bites, the parity of the mosquitoes were determined. Parity status was determined using the method of Detinova (1962) and is summarised as follows; ovaries from adult *Anopheles* were dissected with fine point forceps and individual ovarioles were removed, place on slides to dry and observed for the condition of the tracheal skeins. Ovaries which had stretched tracheoles were scored as parous while those that were coiled were scored as nulliparous.

3.5 Identification of Mosquitoes

3.5.1 Morphological identification of *Anopheles* species

Mosquito specimens were identified morphologically using the keys of Gillies and De Meillon (1968) and a computer-base software of taxonomic keys developed by HERVY *et al.* (1998). *Anopheles* species were differentiated from other mosquitoes by their characteristic pale and black spots on the wings. Male and female *Anopheles* were differentiated by the antennae, which are plumose in the male and sparsely feathered in the female.

Anopheles species were distinguished, basically by the number of white spots on the costal margin, anal vein colouration, number of white rings on maxillary palps and tibia ornamentation. *Anopheles gambiae* sensu lato was identified using the following features: presence of three white rings on the maxillary palps; presence of five pale spots on the costal margin of the wings; two white spots and a white apical fringe on the anal vein; and the presence of white speckles or spots in the median part of the tibia.

Anopheles funestus was identified using the following features: entirely dark tibia; presence of four pale spots on the costal margin of the wings; and presence of two white spots and a dark apical fringe on the anal vein.

Anopheles pharoensis was identified by the presence of four white rings on the maxillary palps and six pale spots on the costal margin of the wings; three white spots and a white apical fringe on the anal vein; and the presence of white speckles or spots in the median part of the tibia.

Anopheles coustani was identified using the following features: entirely dark femur; presence of four white rings on the maxillary palps; presence of one pale spots on the sub-costal margin of the wings and presence of two white spots and a dark apical fringe on the anal vein.

3.5.2 PCR identification of member species of *Anopheles gambiae* complex

Mosquito DNA extractions were performed by homogenizing one individual mosquito leg in 50 ul of sterile double distilled water in 1.5 ml Eppendorf tubes with plastic pestles. The homogenate was then boiled on a hot plate (model B212, J.BIBBY Science Products Limited, UK) for 10 minutes, allowed to cool and then kept at 4°C until required for PCR.

Five sets of primers designed from the DNA sequences of the intergenic spacer region of *An. gambiae* complex ribosomal DNA (rDNA) were used in PCR for their identification (Scott *et al.*, 1993). The sequence details of these primers abbreviated as UN, GA, ME, AR and QD and the expected sizes of the PCR products are given in Table 3.1. The UN primer anneals to the same position on the rDNA sequences of all five species, but the reverse primers, GA anneals specifically to *An. gambiae* s.s. ME to both *An. merus* and *melas*, AR to *An. arabiensis* and QD to *An. quadriannulatus*.

Each reaction mix of 20 µl contained 1 x reaction buffer C (300 mM Tris – HCL; 75 mM $(NH_4)_2SO_4$; 2.5 mM $MgCl_2$), 200 µM of each of the four deoxyribonucleotide triphosphates (dNTPs), 0.25 µM of each of the primers and 0.5 U of DNA *Taq* polymerase enzyme. Five microliters of the isolated mosquito DNA was added as template for the amplification reaction. The reaction was thoroughly mixed, centrifuged

briefly at 10 krpm and overlaid with 20 µl of mineral oil to avoid evaporation and refluxing during thermocycling.

The cycling parameters for the reaction were as follows; 94°C for 3 minutes (initial melt) followed by 35 cycles of 94°C for 30 seconds (denaturation), 50°C for 30 seconds (annealing), 72°C for 1 minute (extension) and a final cycle of 72°C for 10 minutes. For each reaction a negative control that contained no DNA template was included. PCR amplifications were carried out using a PTC 100 thermal cycler (MJ Research Inc., USA).

The PCR products were then electrophoresed in a 2% agarose gel stained with 0.5ug/ml ethidium bromide. Eight micro litres of each sample were added to 1ul of orange G (5x) gel loading dyes. The gels were prepared with 1x TAE buffer, electrophoresed at 100V for one hour using either a midi or maxi gel system (BIORAD). The PCR products were visualized and photographed over a UV transilluminator (UPC, USA) at short wave length using a Polaroid camera fitted with an orange filter and Polaroid film (type 667). The sizes of the PCR products were estimated by comparison with the mobility of a 100bp molecular weight ladder.

Table 3.1: DNA sequence details of the synthetic oligonucleotide primers used for the PCR identification of *An. gambiae* s.l. species, their melting temperatures and diagnostic band sizes of amplified DNA products.

Primer	Sequence (5'-3')	Melting Temp (Tm°C)	Expected amplified DNA size (bp)
UN	GTG TGC CCC TTC CTC GAT GT	58.3	468
GA	CTG GTT TGG TCG GCA CGT TT	59.3	390
ME	TGA CCA ACC CAC TCC CTT GA	57.2	464
AR	AAG TGT CCT TCT CCA TCC TA	47.4	315
QD	CAG ACC AAG ATG GTT AGT AT	42.7	153

3.5.3 Identification of the molecular forms of *Anopheles gambiae* s.s.

The numbers and sizes of DNA fragments obtained after restriction of *An. gambiae* s.s. PCR products distinguish the M and S molecular forms. The *An. gambiae* S form digestion is characterised by two fragments of 257 and 110bp, which are as a result of the presence of *HhaI* restriction site within the DNA sequence. The *An. gambiae* M form DNA sequence does not have this restriction site and is characterised by a single 367bp fragment.

The *HhaI* enzyme digestions were carried out using the recommended protocol of the manufacturers (Sigma-Aldrich, USA). The final reaction volume of 20 µl contained 10 µl of amplified product, 1 U of *HhaI* in 1 x Buffer C (Promega, USA) and was digested at 37°C for three hours. Digested fragments were electrophoresed through an ethidium bromide stained 2% agarose gel and photographed under ultraviolet light illumination as described in Section 3.5.2.

3.6 ELISA detection of *P. falciparum* Sporozoites in *Anopheles* Mosquitoes

3.6.1 Mosquito/*Plasmodium* Antigen (MPA) preparation

The head and thorax of an individual *Anopheles* female mosquito were separated from the abdomen and placed in sterile 1.5ml Eppendorf tubes, and homogenised in 50 µl grinding buffer using plastic pestles. The pestle was rinsed with more grinding buffer onto the homogenate until a final volume of 250 µl was reached. The homogenates were stored frozen at –40°C until required for ELISA.

3.6.2 Detection of sporozoites

The ELISA method used was that described by Wirtz *et al.* (1987a) and was carried out in a 96–well round bottom microtiter plate. Each well was filled with 50 µl of capture Mab solution and the plates incubated overnight (12-18 hr) at 4°C. The capture Mab was removed and the wells filled with 200 µl of Blocking buffer (BB) for one hour at room temperature (RT). The plate was emptied and 50 µl of the mosquito homogenate was added to each well. Positive and negative controls were also added to specific wells at the same time. These were removed after a 2-hr incubation at RT, and the wells were washed 2 times with washing buffer (PBS/Tw-20). The plates were incubated in Mab- peroxidase conjugate mixed with BB at the concentration of 0.05µg/50µl/well for 1 hour in darkness. The wells were emptied and washed five times with washing buffer. Finally, 100 µl of freshly prepared ABTS (2,2'-azino-di-3[ethyl-benzthiazoline sulfonate) substrate were added to each well.

The results were read visually and scored and then also measured spectrophotometrically at 404nm using a plate reader (Model 354, Multiskan Ascent, Thermolabsystems,

Finland) 30 – 60 minutes after the addition of the substrate. A test was considered positive if there was a colour change to green and had a minimum optical density (OD) value equal to or greater than the mean of the positive control for the D row of that plate which contained 12pg of the sporozoite antigen.

3.7 ELISA identification of blood meal source in *Anopheles* mosquitoes

The abdomen of an individual blood fed *Anopheles* female mosquito was separated from the head and thorax and placed in a sterile 1.5ml Eppendorf tube and then homogenised in 150 µl PBS/Tw-20 using a plastic pestle. The homogenate that remained on the pestle was rinsed with more grinding buffer until a final volume of 200 µl was obtained. The homogenates were stored frozen at –40°C until required for ELISA.

The method used was a modification of the ELISA technique described by Burkot *et al.* (1981). Fifty microlitres of the homogenised mosquito abdomen was placed in the wells of a 96 – well round bottom microtiter plate. Positive and negative controls were also added to specific wells at same time. The plate was covered and incubated for 2 hours. The microtiter plate was then emptied, washed twice with PBS/Tw-20 and 100µl of the diluted appropriate conjugate (peroxidase - labelled antihuman IgG) was then added to each well of the microplate and incubated for one hour. The microtiter plate was emptied and washed 3 times with PBS/Tw-20. 100µl of freshly prepared ABTS substrate solution were then added to each well. The plate was placed in the dark for 30 minutes. The results were read visually and scored.

3.8 Data Analysis

SPSS® and Sigma Stat® softwares were used for statistical analysis. Data was entered into an Excel ™ spreadsheet and later imported into SPSS® The observed differences of *Anopheles* abundance (indoors and outdoors), parity, sporozoite rate, entomological inoculation rate and human blood index between the two villages were tested for statistical significance using Chi-squared tests.

3.8.1 Estimation of sample size for ELISA studies

Power calculations were made to arrive at sample sizes needed for this aspect of the work using EPI-INFO 6® (CDC Atlanta) software at 1% and 0.01% as the expected and worst acceptable frequencies of sporozoite rate. These limits of sporozoite rates were assumed from results of studies carried out in Ghana and as in other parts of Africa.

3.8.2 Estimation of entomological indices

Entomological parameters were calculated as follows:

i) Man biting rates: $\frac{\text{Number of mosquito collected}}{\text{Number of collectors x Number of captures}}$

ii) Sporozoite rate = number positive/ number processed

iii) Entomological inoculation rate (EIR) = man biting rate x sporozoite rate

iv) Parity = (number parous)/ (number parous + number nulliparous)

v) Human blood index (HBI) = number positive/ number processed

vi) Room resting density (PSC) = $\frac{\text{Number of mosquitoes}}{\text{Number of rooms sprayed}}$

CHAPTER FOUR

RESULTS

4.1 Mosquito Populations in the Study Area

A total of 8893 mosquitoes, 8233 at Okyereko and 660 at Bewadze, were caught using both HLC and PSC during the study period.

4.1.1 Species Composition

The details of species composition of the total mosquitoes collected at each site are given in Table 1. Briefly, *Anopheles gambiae* s.l. formed the dominant species at Okyereko comprising 73.9% of the 8233 collected. *Culex* species, *An. pharoensis, An. funestus* and *Mansonia* species accounted for 11.7%, 5.0%, 4.9%, and 4.5%, respectively, whilst, *Aedes* species and *An. coustani* each formed 0.02%.

At Bewadze, *An. funestus* was the dominant species accounting for 33.5% of the 660 collected. *An. gambiae* s.l., *Culex* species, *Mansonia* species, *An. phaoensis* and *Aedes* species accounted for 31.1%, 24.2%, 10%, 1.1% and 0.2% of the total collected, respectively.

Figures 4.1 and 4.2 also show the species composition of the monthly mosquito collections at Okyereko and Bewadze respectively. At Okyereko, *An. gambiae* s.l was the most abundant mosquito collected every month throughout the study period. The highest collection of *An. gambiae*, 81.5% (1592/1953), was recorded in September 2002 whilst the lowest, 53.1% (696/1371) was recorded in December 2002. In contrast, the populations of *Culex* species, *An. funestus* and *An. pharoensis* peaked in December accounting for 20.9%, 14.0% and 7.3% respectively of the total 1371 caught. *Anopheles*

funestus was the least anopheline in abundance except in December when it was higher than *An. pharoensis*. The lowest population of *An. funestus* was 0.8% (7/1014) and this was recorded in March. *Anopheles coustani* and *Aedes* species were only caught in March 2003 and accounted for 0.02% and 0.1% of the month's collection respectively. The population of *Culex* species was higher than that of *Mansonia* species in all the months except in September 2002.

At Bewadze, *An. funestus* was the dominant species from September 2002 to November 2002, but was replaced by *Culex* species and *An. gambiae* s.l in the months of December 2002, January 2003 and March 2003. The *An. funestus* populations peaked in October, when it formed 73.4% (58/79) of the total mosquitoes caught that month. It was lowest in March 2003 when it formed only 0.6% of that month's collection. *Anopheles gambiae* s.l. was highest in the same month forming 64.4% (103/160) of the mosquitoes collected, whilst it was lowest in September 2002 when it accounted for only 9.8% (23/235) of that month's collection. *Anopheles pharoensis* species was caught only in September 2002 and in March 2003 accounting for 2.1% (5/235) and 1.3% (2/160) respectively. *Aedes* species were caught only in March and they accounted for 0.6% (1/160) of the collection. The population of *Culex* species was higher than that of *Mansonia* species except in the month of September when they formed 19.6% (46/235) and 20.0% (47/235) of the collection respectively. No *Mansonia* species were recorded in the month of December.

Table 4.1: Species composition of the total mosquitoes obtained at Okyereko and Bewadze.

Species	Okyereko n (%)	Bewadze n (%)
An. gambiae s.l.	6080 (73.9)	205 (31.1)
An. funestus	405 (4.9)	221 (33.5)
An. pharoensis	415 (5.0)	7 (1.1)
An. cousiani	2 (0.02)	0 (0)
Culex species	959 (11.7)	160 (24.2)
Mansonia species	370 (4.5)	66 (10_
Aedes species	2 (0.2)	1 (0.2)
Total	8233 (100)	660 (100)

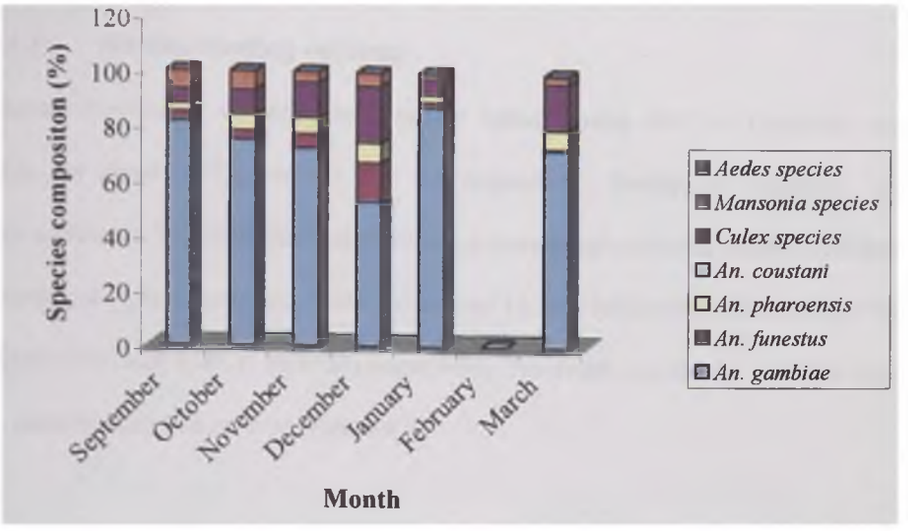

Figure 4.1: Monthly distribution of the mosquito species obtained at Okyereko

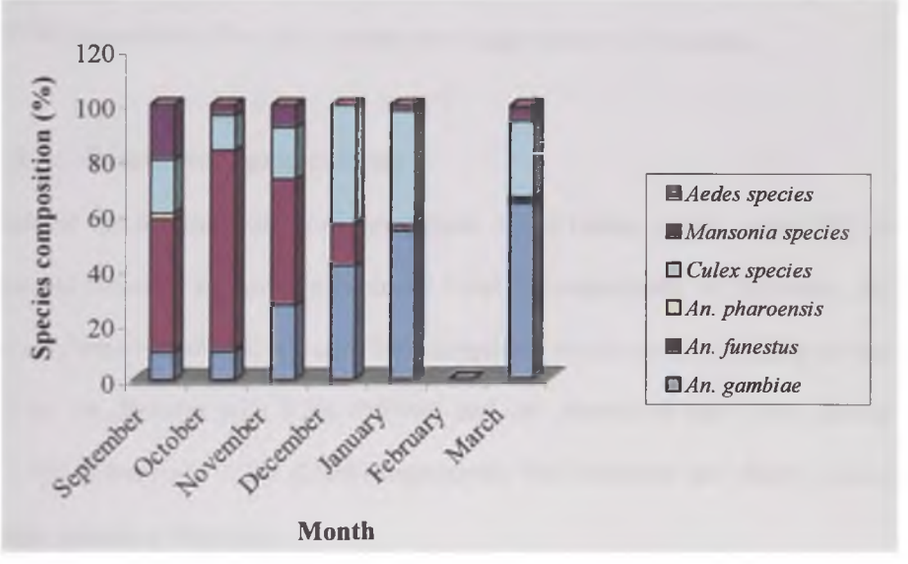

Figure 4.2: Monthly distribution of the mosquito species obtained at Bewadze

4.1.1.1 Indoor Populations

4.1.1.1.1 Human landing catches

The species distribution of mosquitoes caught indoors using HLC at Okyereko and Bewadze are given in Figures 4.3 and 4.4 respectively. Briefly, at Okyereko, *An. gambiae* s.l formed 76.3% of the total 3559 mosquitoes caught indoors, whilst *Culex* spp, *An. funestus*, *An. pharoensis* and *Mansonia* formed 10.3% (367/3559), 5.8% (207/3559), 4.5% (160/3559) and 3.0% (108/3559) respectively. No *Aedes* spp and *An. coustani* were caught indoors using this method (Figure 4.3).

At Bewadze, *An. funestus* was the most dominant indoor biting species forming 48.3% of the total 288 mosquitoes caught, followed by *An. gambiae* s.l. with 29.9% (86/288), with *Culex* spp, *Mansonia* spp and *An. pharoensis* being 12.2% (35/288), 8.7% (25/288) and 1.0% (3/288) respectively. No *Aedes* species was caught indoors at Bewadze.

4.1.1.1.2 Pyrethrum spray catches

The details of species composition of mosquitoes caught resting indoors using PSC at Okyereko and Bewadze are given in Figures 4.5 and 4.6 respectively. At Okyereko, *An. gambiae* s.l formed 93.6% of the total 846 mosquitoes caught resting indoors. It was followed by *An. funestus* with 5.2% (44/846) and *An. pharoensis* and *Culex* species formed 0.9% (8/846) and 0.2% (2/846) respectively. No *Mansonia* and *Aedes* species were caught indoors at Okyereko.

At Bewadze, *An. funestus* was the most abundant species and formed 39.6% of the total 96 caught resting indoors, followed by *Culex* species with 30.2% (29/96) and *An. gambiae* s.l and *Mansonia* species which formed 28.1% (27/96) and 2.1% (2/96)

respectively. At Bewadze, neither *An. pharoensis* nor *Aedes* species were caught resting indoors.

The estimated monthly room resting densities and abundance of *An. gambiae* s.l and *An. funestus* at Okyereko are shown in Figure 4.7 and 4.8 respectively. At Okyereko, *Anopheles gambiae* mosquitoes recorded the highest overall mean room density for the entire study period with 66/room with a peak of 139/room in January 2003 and the least was in December 2002 with 38/room. The overall mean room resting density of *An. funestus* for the entire study period was 3.7/room with a peak of 6/room in January 2003 and the least was in March 2003 with 1.0/room. *Anopheles pharoensis* was found resting in rooms only in September 2002 and October 2002 and the values were 2.0/room for each month.

The estimated monthly room resting densities and abundance of the *An. gambiae* and *An. funestus* at Bewadze are shown in Figure 4.9 and 4.10 respectively. At Bewadze, the overall mean density for *An. gambiae* was 2.3/room with its highest monthly room resting density being 6.5/room in March 2003. No *An. gambiae* was found resting in the rooms during October and November 2002. *An. funestus* recorded the highest overall mean room resting density for the entire study period with 3.2/room with a peak of 13.5/room in September 2002. No *An. pharoensis* was found resting in the rooms at Bewadze throughout the entire period.

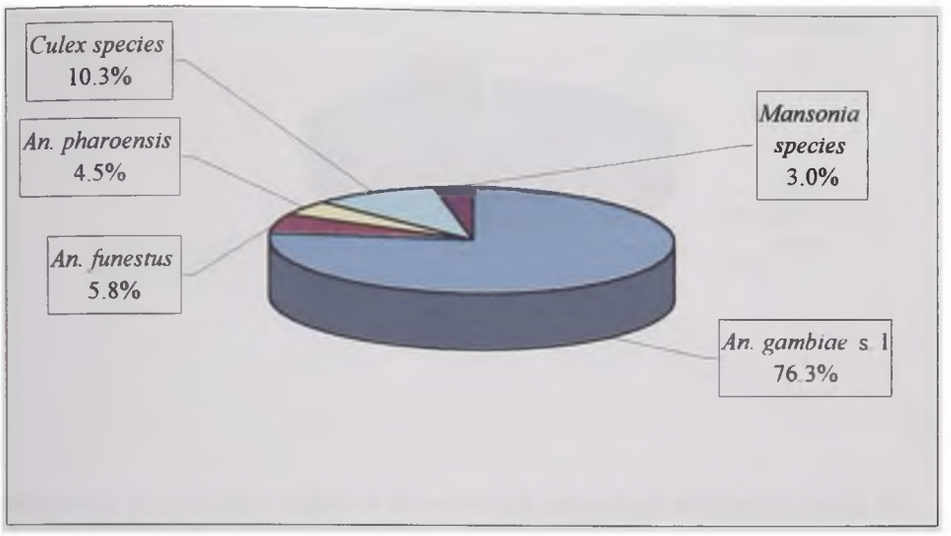

Figure 4.3: Species composition of mosquitoes caught indoors at Okyereko using HLC

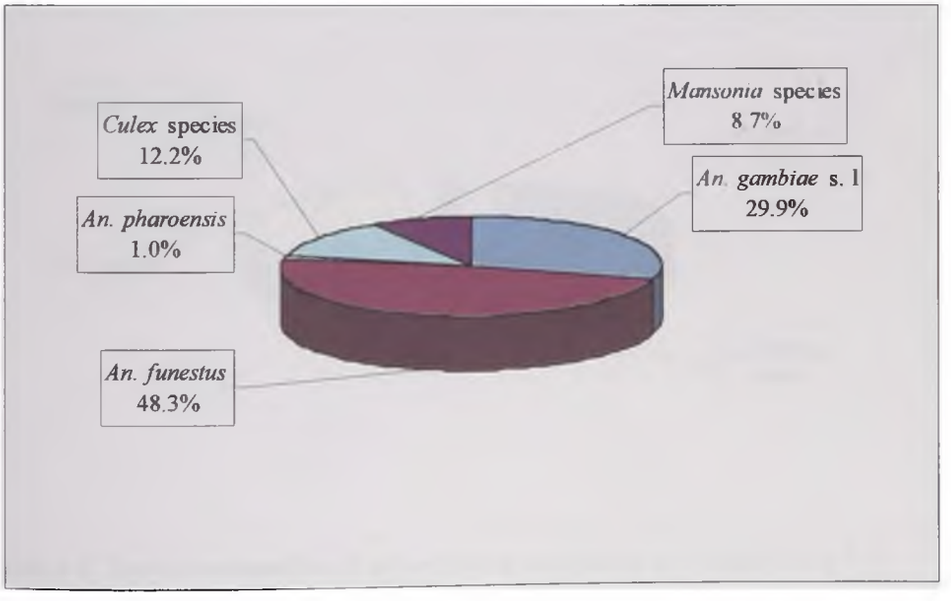

Figure 4.4: Species composition of mosquitoes caught indoors at Bewadze using HLC

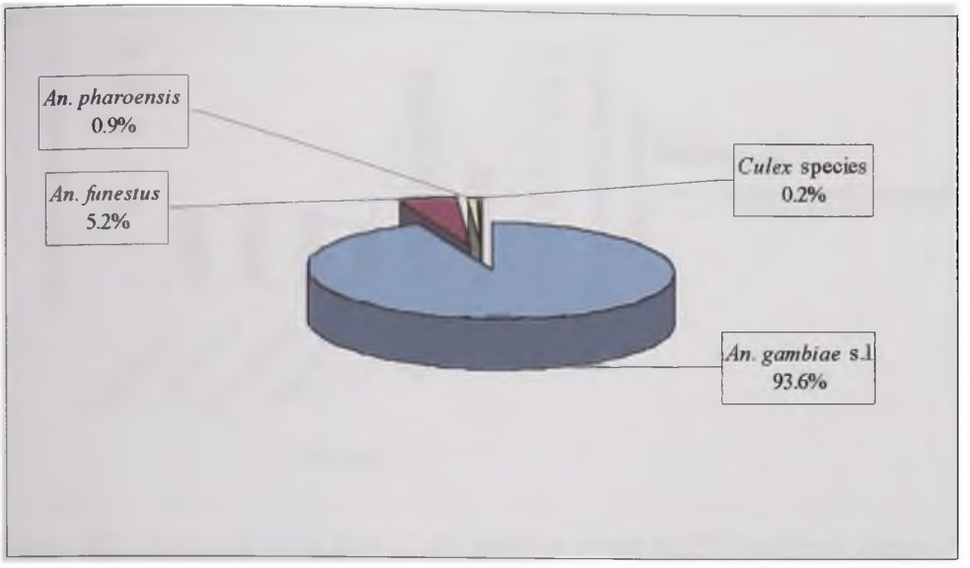

Figure 4.5: Species composition of indoor resting mosquitoes at Okyereko using PSC

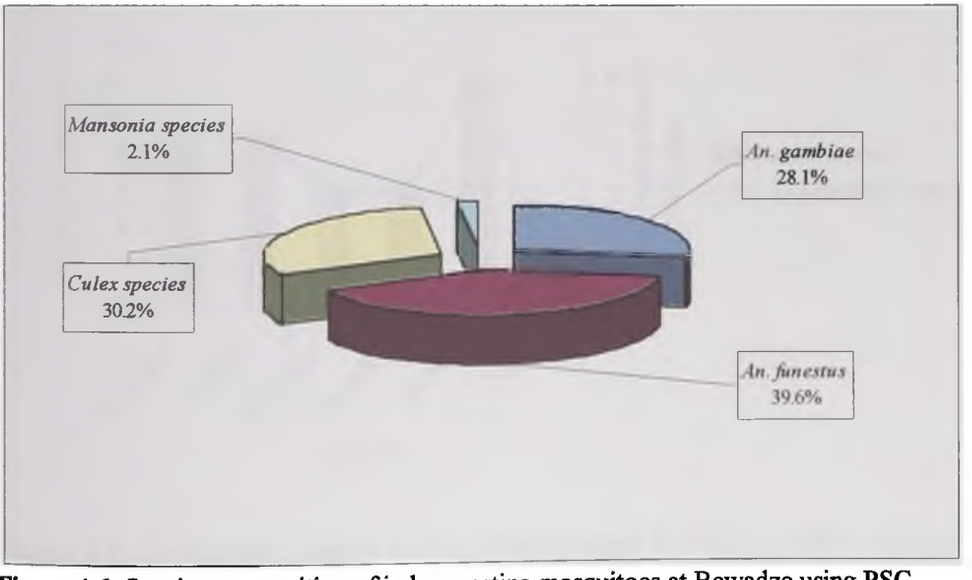

Figure 4.6: Species composition of indoor resting mosquitoes at Bewadze using PSC

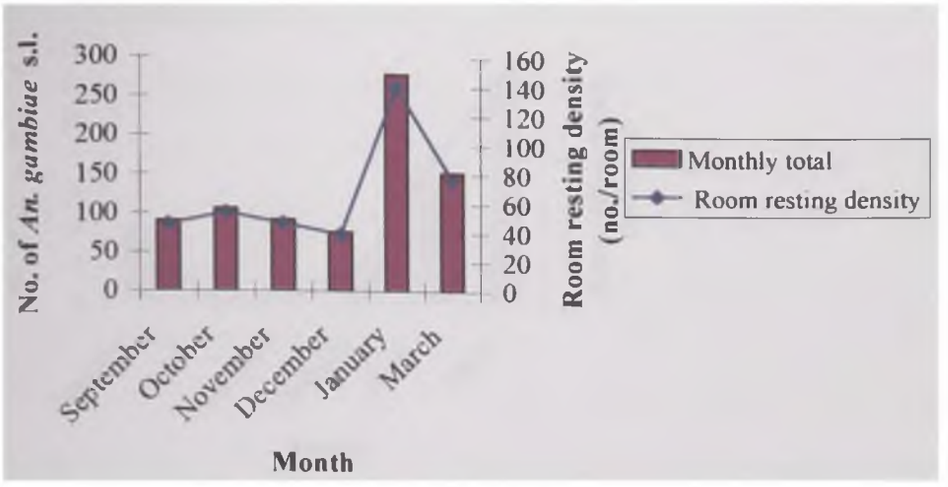

Figure 4.7: Monthly total number of *An. gambiae* caught by PSC and their respective room resting densities at Okyereko

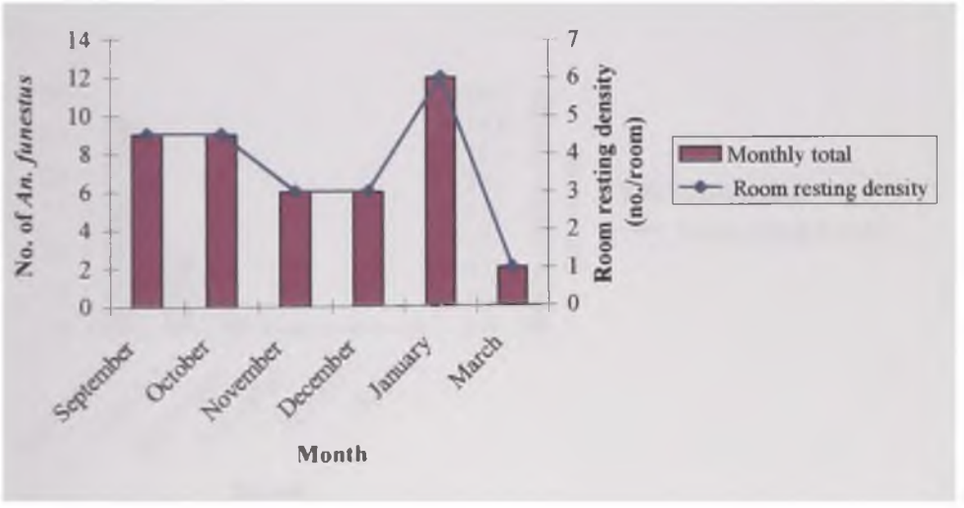

Figure 4.8: Monthy total number of *An. funestus* caught by PSC and their respective room resting densities at Okyereko

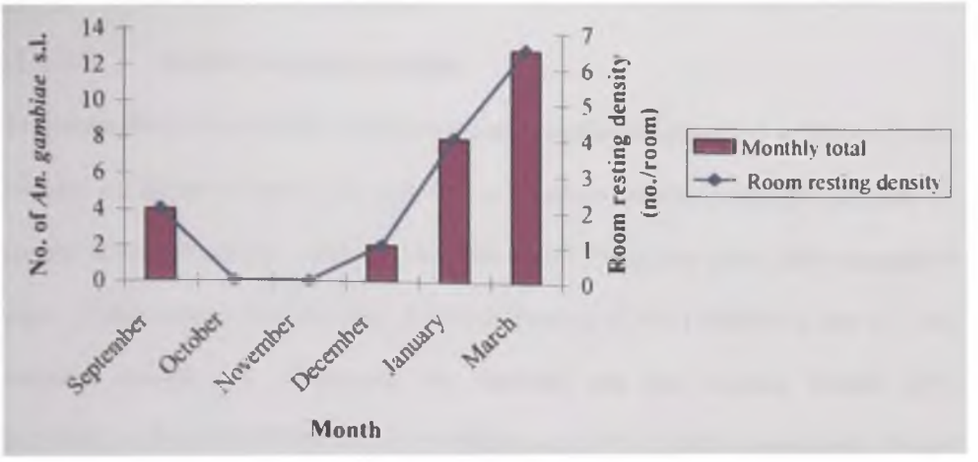

Figure 4.9: Monthly total number of *An. gambiae* caught by PSC and their respective room resting densities at Bewadze

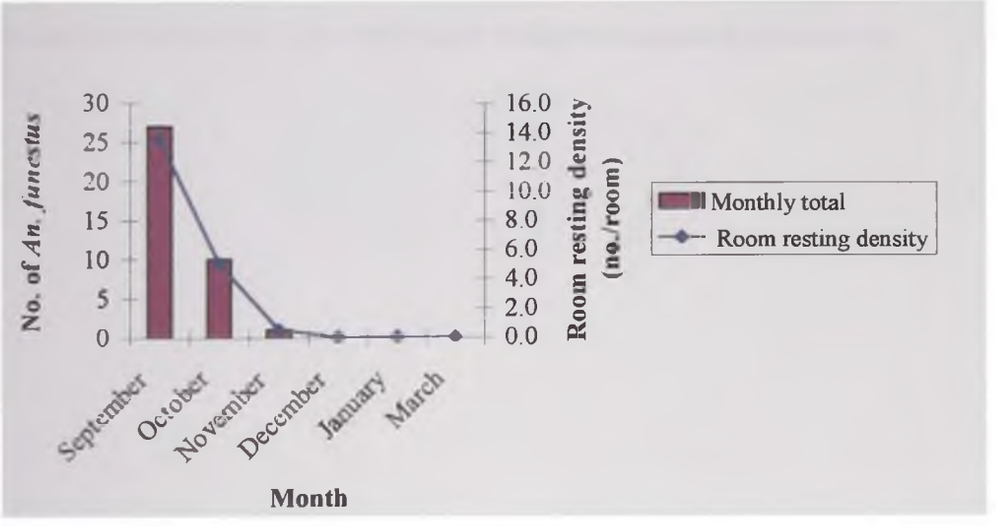

Figure 4.10: Monthly total number of *An. funestus* caught by PSC and their respective room resting densities at Bewadze

4.1.1.2 Outdoor Populations

4.1.1.2.1 Human landing catches

The species distribution of the mosquitoes caught outdoors using HLC at Okyereko and Bewadze are given in Figure 4.11 and 4.14 respectively. At Okyereko, *An. gambiae* s.l. was the dominant species outdoors and formed 67.2% of the total 3828 mosquitoes caught. *Culex* species was the next dominant forming 15.4% (590/3828), and the rest; *Mansonia* species, *An. pharoensis*, *An. funestus*, and *An. coustani* formed 6.8% (262/3828), 6.5% (247/3828), 4.0% (154/3828) and 0.1% (2/3828) respectively (Figure 4.11).

At Bewadze, *Culex* species was the most abundant mosquito outdoors and formed 34.8% of the total 276 caught by HLC. *Anopheles gambiae* s.l. was the next dominant with 33.3% (92/276), followed by *An. funestus*, *An. pharoensis* and *Aedes* species and they formed 15.9 % (44/276), 1.4% (4/276) and 0.4% (1/276) respectively (Figure 4.12).

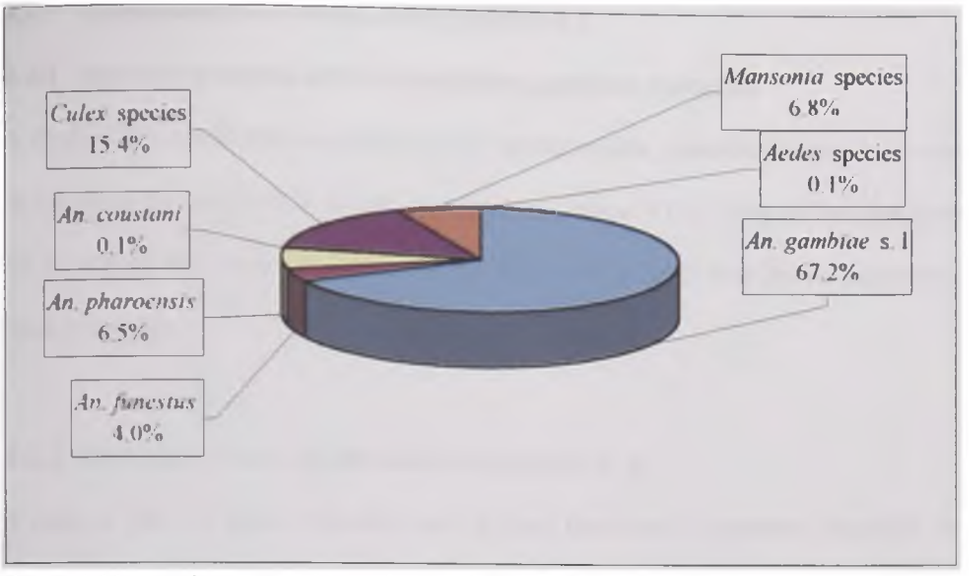

Figure 4.11: Species composition of mosquitoes collected outdoors by HLC at Okyereko

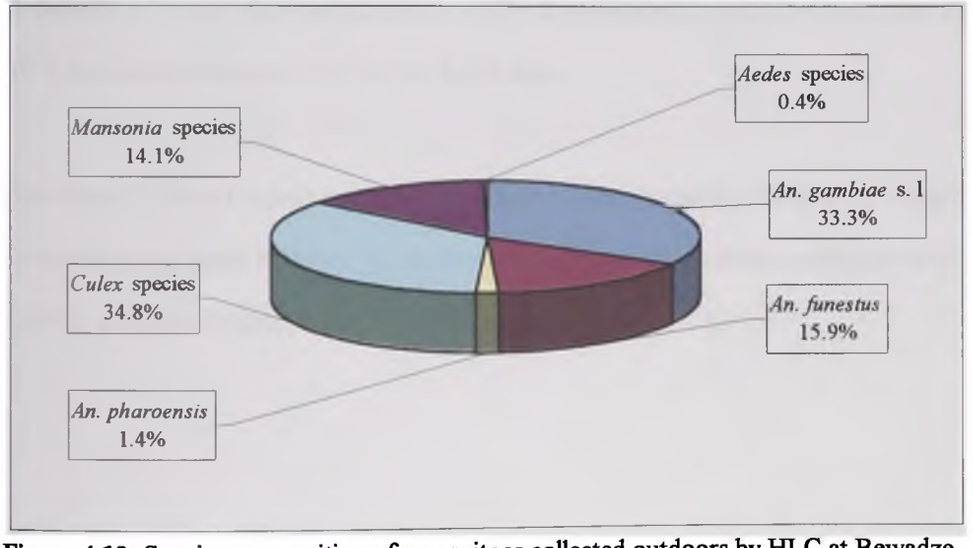

Figure 4.12: Species composition of mosquitoes collected outdoors by HLC at Bewadze

4.2 Identification of *Anopheles gambiae* s.l.

4.2.1 Member species of the *Anopheles gambiae* complex

A total of 798 out of 826 mophololologically determined *An. gambiae* s.l specimens were all identified further by PCR as *An. gambiae* s.s. (Figure 4.13). Twenty-four specimens out of 626 (3.8%) from Okyereko and six out of 202 (3.0%) from Bewadze however failed to amplify.

4.2.2 Molecular forms of *Anopheles gambiae* s. s.

A total of 150 (72 from Okyereko and 78 from Bewadze) molecularly identified *An. gambiae* s.s mosquitoes were further analysed by PCR-RFLP method to determine the strain(s). Figure 4.14 shows the DNA bands produced after amplification and digestion of the PCR products which resulted in two DNA fragments of about 257bp and 110bp for the S-form and a fragment of 367bp for the M form.

Out of the 72 from Okyereko analysed, the M form constituted 91.7% (66/72), while S form constituted about 8.3% (6/72). At Bewadze however, the M form constituted 66.7% (52/78), whilst the S form constituted the remaining 33.3% (26/78).

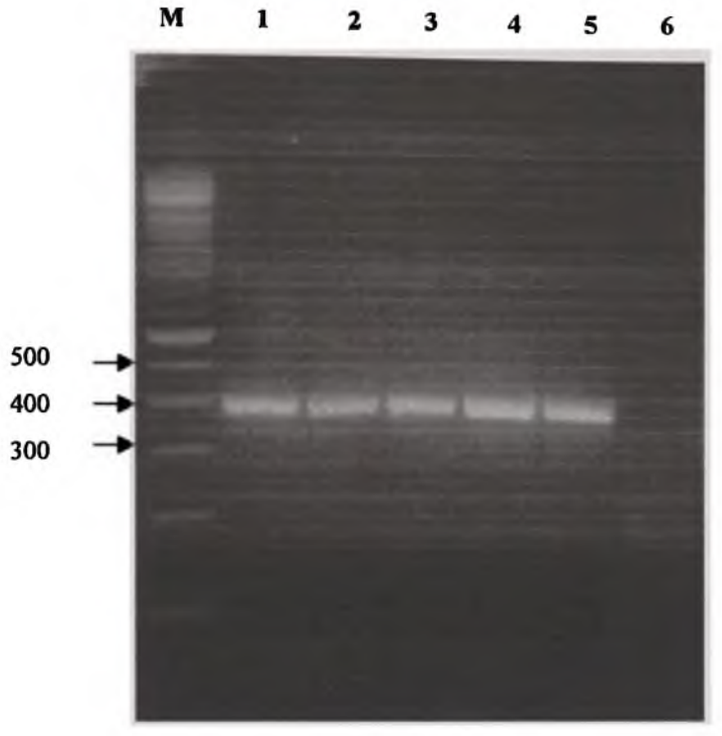

Figure 4.13: An example of 2% agarose gel electrophoresis of PCR amplified rDNA sequences of *An. gambiae* s.s mosquitoes. Lane M = 100bp DNA molecular weight marker, Lanes 1-4 = *An. gambiae* mosquitoes, Lane 5 = positive control, Lane 6 = negative control

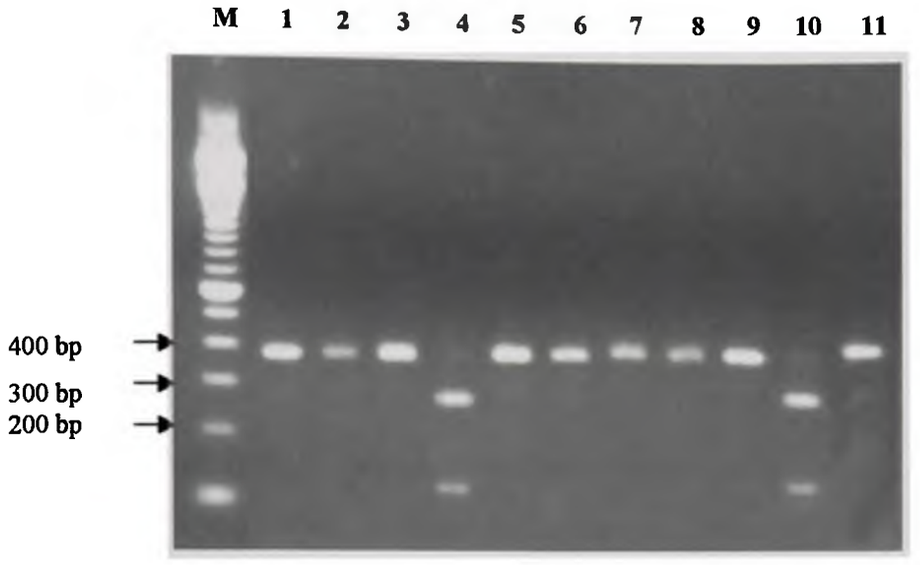

Figure 4.14: An example of 2% agarose gel electrophoresis of *HhaI* digests of PCR DNA products of *An. gambiae* s.s. specimens for the identification of M- and S-forms. Lane M = 100bp DNA molecular weight marker; Lanes 1-3 = M form; Lane 4 = S form; Lanes 5-9 = M form; Lane 10 = S form; Lane 11 = undigested *An. gambiae* s.s. PCR product.

4.3 Entomological Indices of Biting And Malaria Transmission

4.3.1 Biting rates

At Okyereko, the overall mean biting rates for *An. gambiae* s.s. and *An. funestus* for the entire period of study were 110.2 bites/man/night (b/m/n) and 7.5 b/m/n respectively. The overall biting rate due to both *An. gambiae* s.s. and *An. funestus* for the entire period was 117.7 b/m/n. The overall mean biting rates estimated for *An. pharoensis* and *An. coustani* were 8.8 b/m/n and 0.04 b/m/n respectively.

At Bewadze, the overall mean biting rate for *An. gambiae* s.s. and *An. funestus* estimated for the period of study were 3.6 b/m/n and 3.8 b/m/n respectively. Together, the overall mean biting rate due to both *An. gambiae* s.s. and *An. funestus* for the entire period was 7.4 b/m/n. The mean biting rate estimated for *An. pharoensis* was 0.1 b/m/n. Thus, the overall mean biting rate due to all anophelines for the entire study period was 126.5 b/m/n at Okyereko and 7.5 b/m/n at Bewadze.

Although *An. pharoensis* is the vector of malaria in Egypt, it has not been implicated in the disease transmission in Ghana, so it was excluded from the analyses. Also no sample was collected in February so it was excluded from the analyses.

4.3.1.1 Hourly biting rates

Figures 4.15 and 4.16 detail the indoors and outdoors hourly biting rates of *An. gambiae* and *An. funestus* at Okyereko. *Anopheles gambiae* s.s. recorded higher biting rates both indoors and outdoors than *An. funestus*. The indoor hourly biting rate of *An. gambiae* s.s. at Okyereko ranged between 1.4 –14.7 bites/man/hour (b/m/hr) and the average hourly rate was 9.4 b/m/hr. Biting was lowest from 7.00 – 8.00 pm then rose steadily to peak at 14.7 b/m/hr between 1.00-2.00 am and then fell but never to zero at 6.00 am when

sampling was stopped (Figure 4.15).The indoor hourly biting rate for *An. funestus* ranged between 0 –1.5 b/m/hr and the average hourly rate was 0.7 b/m/hr. Biting was lowest from 6.00 – 7.00 pm when no biting was recorded and then rose and fell intermittently but never to zero till 6.00 am. The peak of 1.5 b/m/hr was recorded between 12.00 – 1.00 am (Figure 4.15).

The outdoor hourly biting rates for *An. gambiae* s.s. at Okyereko ranged between 1.3 – 14.5 b/m/hr and the average hourly rate was 8.9 b/m/hr. Biting was lowest from 6.00 – 7.00 pm and rose steadily to peak at 14.5b/m/hr between 11.00 –12.00 midnight, and then fell but never to zero at 6.00 am when sampling was stopped (Figure 4.16). The outdoor hourly biting rates for *An. funestus* ranged between 0 – 1.3 b/m/hr and the average hourly rate was 0.5 b/m/hr. Biting was lowest from 6.00 – 7.00 pm when no biting was recorded and rose steadily to peak at 1.3b/m/hr between 11.00 – 12.00 midnight, and then fell but never to zero by 6.00 am when sampling was stopped (Figure 4.16). Thus the peak of biting outdoors for *An. gambiae* s.s. and *An. funestus* were the same at Okyereko).

At Bewadze, the indoor hourly biting rate of *An. gambiae* s.s. ranged between 0 – 1.0 b/m/hr and the average hourly rate was 0.3 b/m/hr. Biting was lowest from 6.00 – 7.00 pm and 8.00 – 10.00 pm when no biting was recorded and rose steadily and maintained a steady biting rate of 0.4 b/m/hr 1.00 – 4.00 am to peak at 1.0 b/m/hr between 4.00 – 5.00 am (Figure 4.17). The indoor hourly biting rate of *An. funestus* at Bewadze ranged between 0 – 1.4 b/m/hr and the average hourly rate was 0.5 b/m/hr. Biting was lowest from 6.00 – 9.00 pm when no biting was recorded and the peak of 1.4 b/m/hr was recorded between 5.00 – 6.00 am. At Bewadze, *An. funestus* had a higher biting rate than

An. gambiae s.s. from 11 pm to 6 am but both species had the same biting rate of 1.0 b/m/hr between 4.00 – 5.00 am (Figure 4.17).

At Bewadze, *An gambiae* s.l. had a higher outdoor biting rate than *An. funestus* except at 1 – 2 am and at 5 – 6 am when the biting rate of *An. funestus* was higher. The outdoor hourly biting rate of *An. gambiae* ranged from 0 – 0.8 b/m/hr and the average hourly biting rate was 0.3 b/m/hr. No biting was recorded for this species from 6.00 – 9.00 pm. The peak outdoor biting rate of *An. gambiae* was 0.8 b/m/hr recorded between 2 – 3 am (Figure 4 18). The outdoor hourly biting rate of *An. funestus* at Bewadze ranged from 0 – 0.4 b/m/hr and the average hourly biting rate was 0.2 b/m/hr. No biting was recorded outdoors for this species from 6.00 – 12.00 midnight. *An. funestus* showed two peaks of 0.4 b/m/hr between 2 and 3 am and between 4 – 5 am respectively (Figure 4.18).

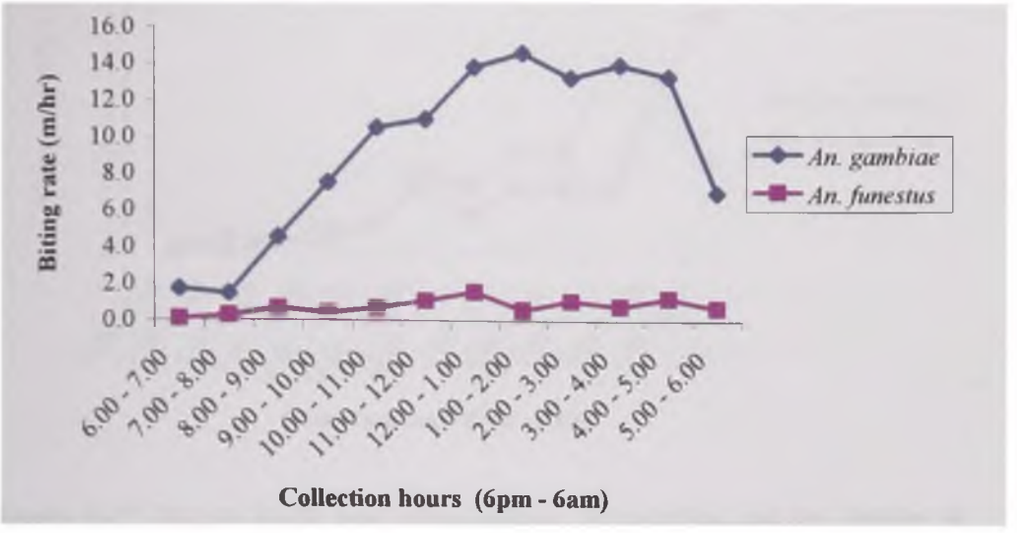

Figure 4.15: Indoors hourly man biting rates for *An. gambiae* and *An. funestus* at Okyereko

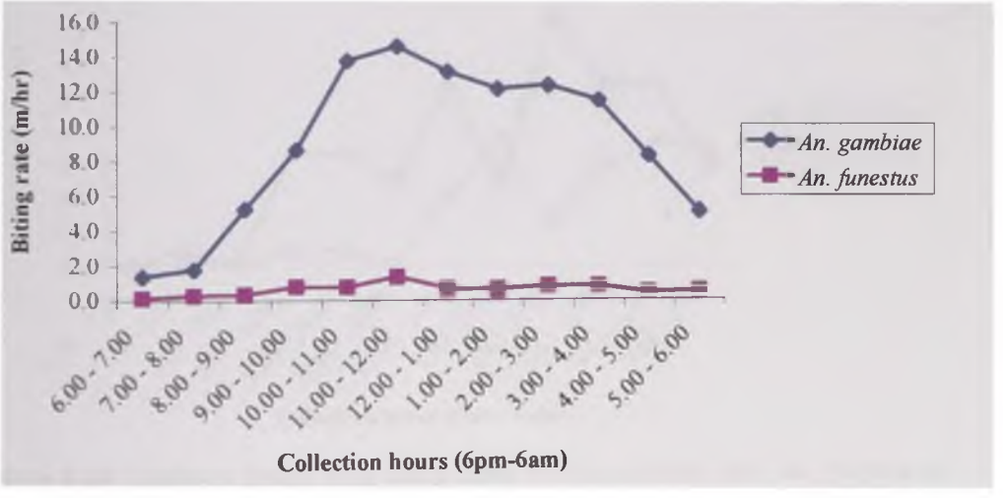

Figure 4.16: Outdoors hourly man biting rates for *An. gambiae* and *An. funestus* at Okyereko

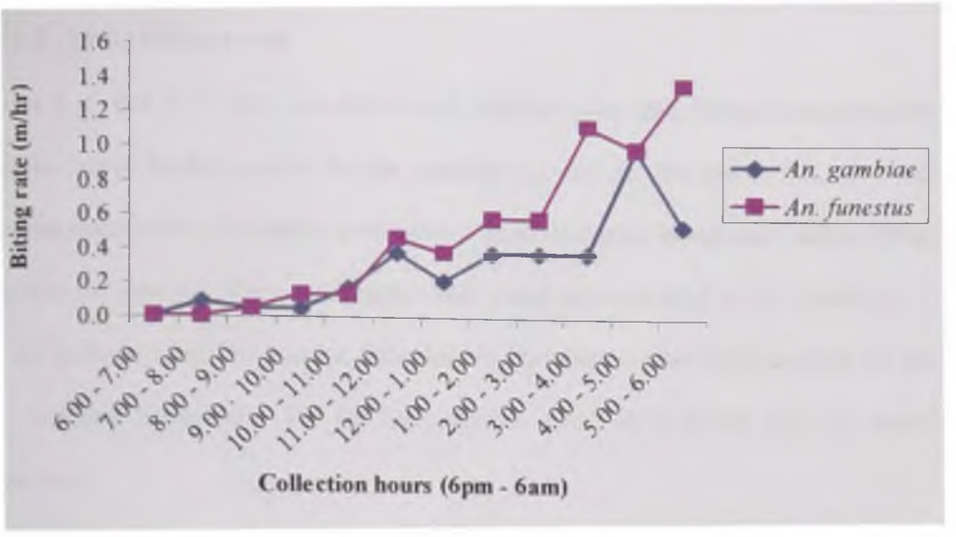

Figure 4.17: Indoors hourly man biting rates for *An. gambiae* and *An. funestus* at Bewadze

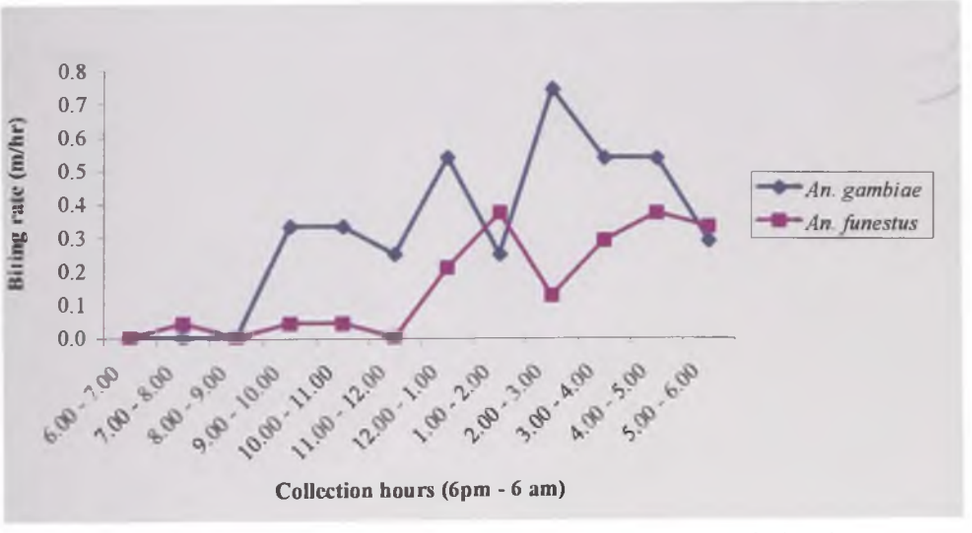

Figure 4.18: Outdoors hourly man biting rates for *An. gambiae* and *An. funestus* at Bewadze

4.3.1.2 Daily biting rates

Figures 4.19 and 4.20 show the indoor and outdoor mean daily biting rates computed from the human landing catches for *An. gambiae* s.s. and *An. funestus* at Okyereko and Bewadze respectively. *Anopheles gambiae* s.s. recorded higher indoor and outdoor biting rates than *An. funestus*. The overall indoor daily biting rates recorded for *An. gambiae* s.s. and *An. funestus* were 113.2 and 8.6 bites/man/night (b/m/n) respectively and that for the daily outdoor biting rates for the two species were 107.1 b/m/n and 6.4 b/m/n respectively.

At Bewadze, *An. funestus* recorded a higher indoor biting rate than *An. gambiae* s.s. while the reverse was the case outdoors. The overall indoor daily biting rates recorded for *An. gambiae* s.s. and *An. funestus* were 3.6 b/m/n and 5.8 b/m/n respectively. The overall daily outdoor biting rates for *An. gambiae* s.s. was 3.7 b/m/n while that of *An. funestus* was 1.7 b/m/n.

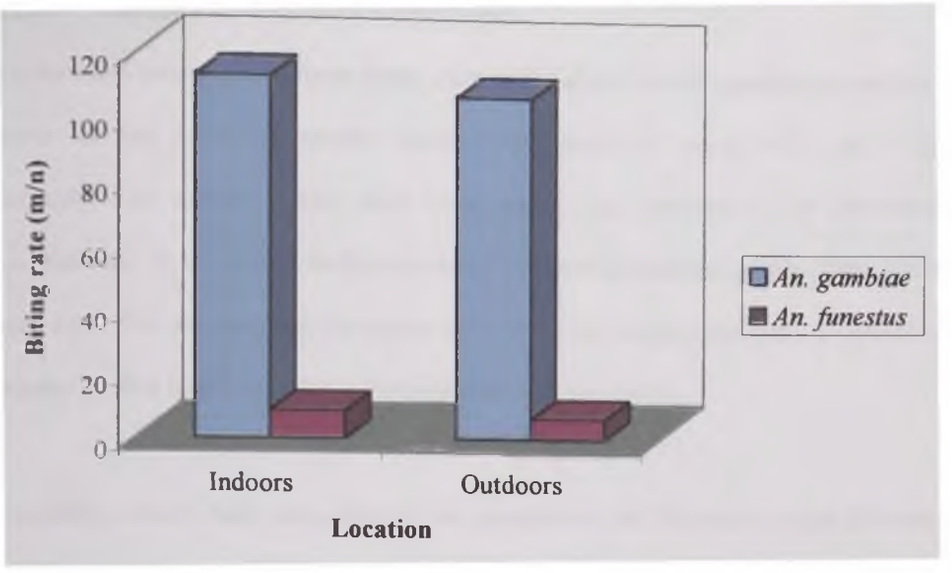

Figure 4.19: Indoor and outdoor daily biting rates of *An. gambiae* and *An. funestus* at Okyereko for the entire period of sampling

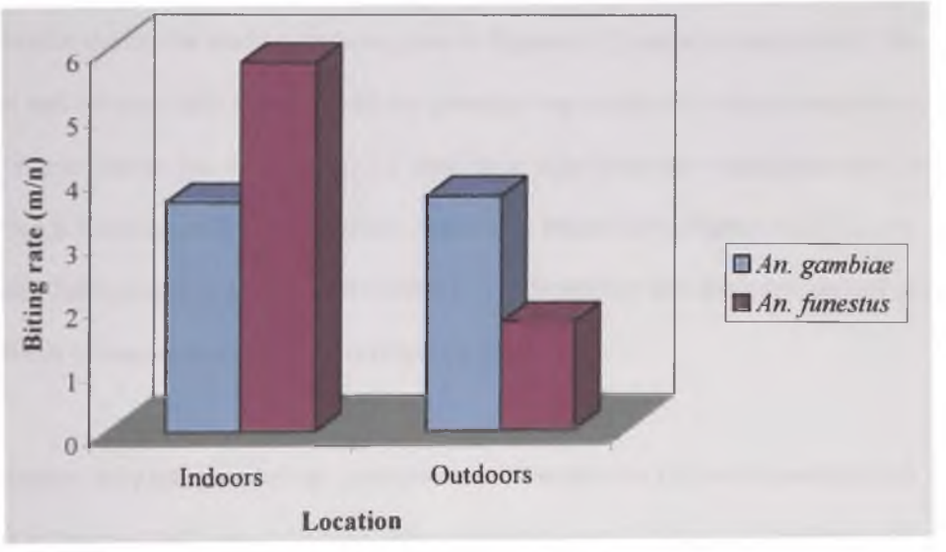

Figure 4.20: Indoor and outdoor daily biting rates of *An. gambiae* and *An. funestus* at Bewadze for the entire period of sampling.

4.3.1.3 Trends in mean daily biting rates

The indoor and outdoor daily mean biting rates at Okyereko for *An. gambiae* s.l and *An. funestus* for the individual months sampled are given in Figures 4.21 and 4.22 respectively. The monthly indoor daily biting rate of *An. gambiae* s.s. at Okyereko ranged between 73.5 recorded in October and 229.3 b/m/n recorded in September 2002 (Figure 4.21). For *An. funestus*, the indoor daily biting rate ranged between 3.0 recorded in October to 28.8 b/m/n recorded in December 2002 (Figure 4.22).

The monthly outdoor daily biting rates of *An. gambiae* s.s. at Okyereko ranged between 83.0 recorded in March 2003 and 168.8 b/m/n recorded in September 2002 (Figure 4.21). The outdoor daily biting rates of *An. funestus* at Okyereko ranged between 0.8 recorded in March 2003 and 20.5 b/m/n recorded in December 2002 (Figure 4.22).

The details of indoor and outdoor daily biting rates for *An. gambiae* s.s. and *An. funestus* at Bewadze during the study period are given in Figures 4.23 and 4.24 respectively. The indoor and outdoor daily biting rate of *An. gambiae* was steady until January and then a sharp rise in March. For *An. gambiae* s.s. the indoor daily biting rate ranged between 0.8 recorded in October 2002 and 10.3 b/m/n recorded in March 2003 (Figure 4.23). For *An. funestus*, the highest biting rate of 18.0 b/m/n was in September and decreased steadily to 0.25 b/m/n in January and March 2003 (Figure 4.24).

The outdoor daily biting rate of *An. gambiae* s.s. at Bewadze for the months sampled was lowest in January 2003 with 1.0 b/m/n, while the highest was 12.3 recorded in September 2002 (Figure 4.23). For *An. funestus*, the value was zero in January and March 2003, while the highest of 3.8 was recorded both in September and November 2002 (Figure 4.24).

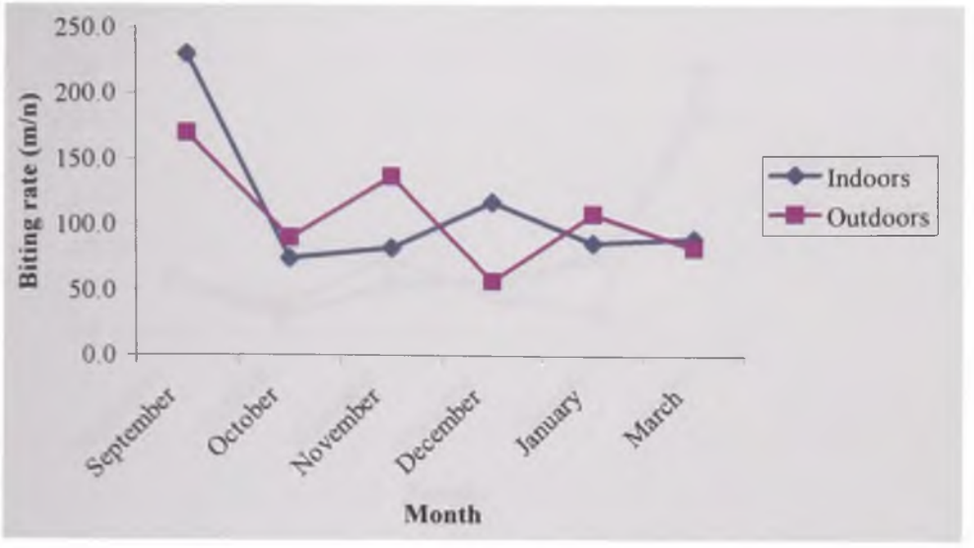

Figure 4.21: Indoor and outdoor daily biting rate of *An. gambiae* at Okyereko for the months sampled

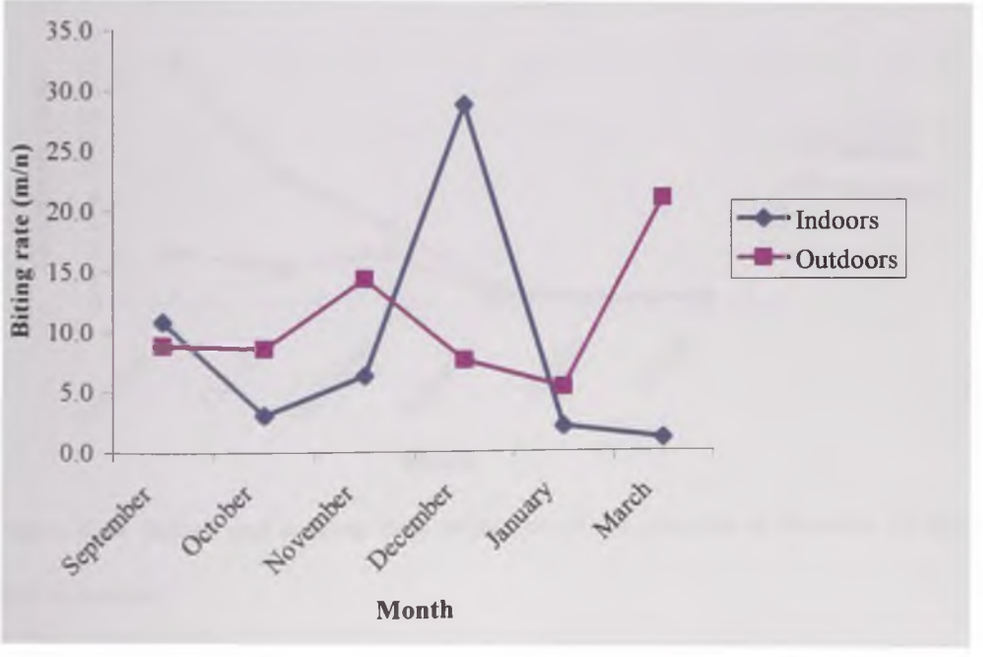

Figure 4.22: Indoor and outdoor daily biting rate of *An. funestus* at Okyereko for the months sampled

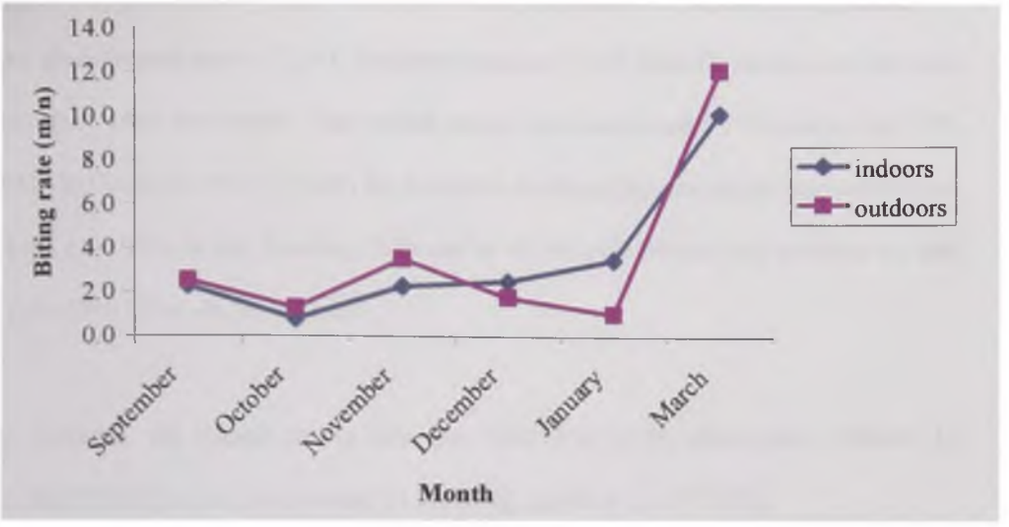

Figure 4.23: Indoor and outdoor daily biting rate of *An. gambiae* at Bewadze for the months sampled

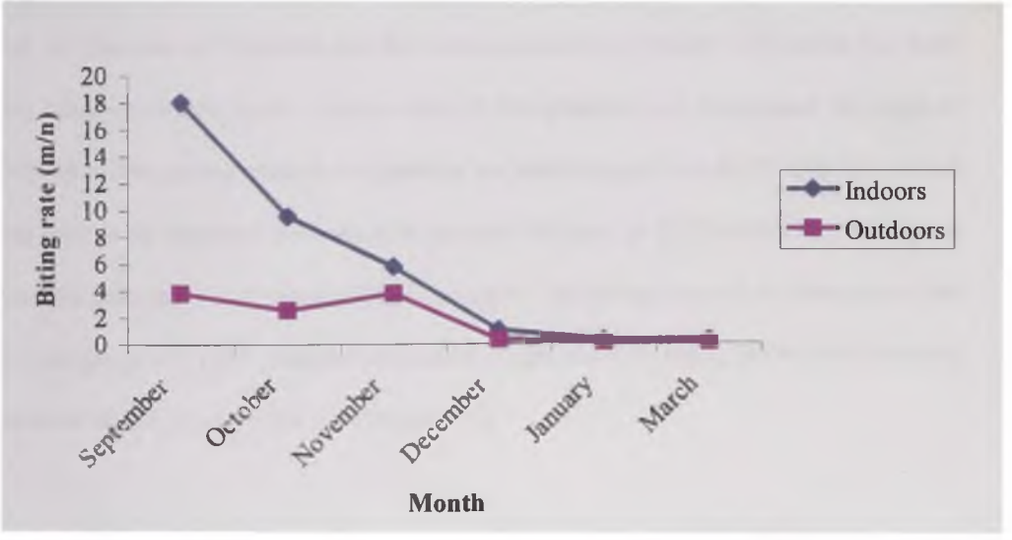

Figure 4.24: Indoor and outdoor daily biting rate of *An. funestus* at Bewadze for the months sampled

4.3.2 Physiological ages of *Anopheles* populations at the study sites

The physiological ages of 1,591 *Anopheles* species (1,345 from Okyereko and 246 from Bewadze) were determined. The overall parous rates calculated for Okyereko was 65% (874/1345) and 63.8% (157/ 246) for Bewadze. At Okyereko, the highest parous rate was 74.0% (134/181) in *An. funestus*, followed by 64.7% (641/991) in *An. gambiae* s.s. and 57.2% (99/173) in *An. pharoensis*.

At Bewadze, the highest parous rate was 75% (3/4) in *An. pharoensis*, followed by 65.8% (77/117) in *An. funestus* and 61.6% in *An. gambiae* s.s. (77/125).

4.3.2.1 Hourly distribution of parous rates

Figures 4.25 and 4.26 show the hourly distribution of the parous rates of *An. gambiae* s.s. and *An. funestus* at Okyereko and Bewadze respectively. Figure 4.25 shows that there was relatively stable hourly parous rates of *An. gambiae* s.s. throughout the night at Okyereko. The parous rates of *An. gambiae* s.s. there ranged from 51.9% (54/104), which was the lowest recorded between 3.00 am and 4.00 am, to 73.3% (85/116), the highest recorded between 11.00 pm and 12.00 midnight. The parous rate of *An. funestus* at this site ranged from 0 (0/2) recorded between 6.00 pm and 7.00 pm to 100% (9/9) recorded between 10.00 pm and 11.00 pm (Figure 4.25).

At Bewadze, the parous rate of *An. gambiae* s.s. ranged from 40.0% (2/3) which was the lowest between 9.00 pm and 10.00 pm to 81.3% (13/16) the highest recorded between 5.00 am and 6.00 am. No parous *An. funestus* was recorded between 8.00 pm – 9.00 pm (0/1) and between 10.00 pm – 11.00 pm (0/4). The highest, 88.9% (8/9) was recorded between 11.00 pm and 12.00 midnight (Figure 4.26).

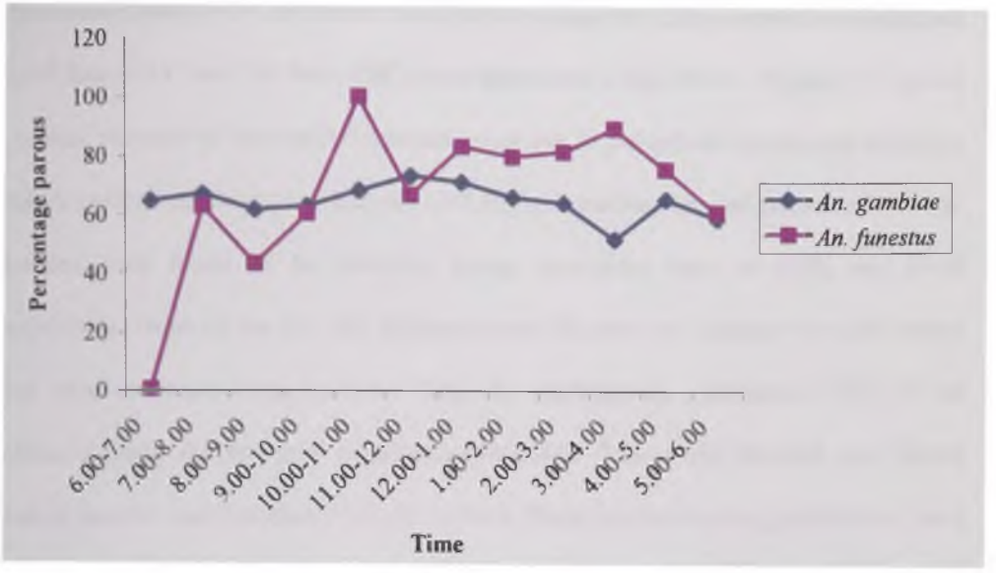

Figure 4.25: Hourly distribution of parous rates of *An. gambiae* s.s. and *An. funestus* at Okyereko

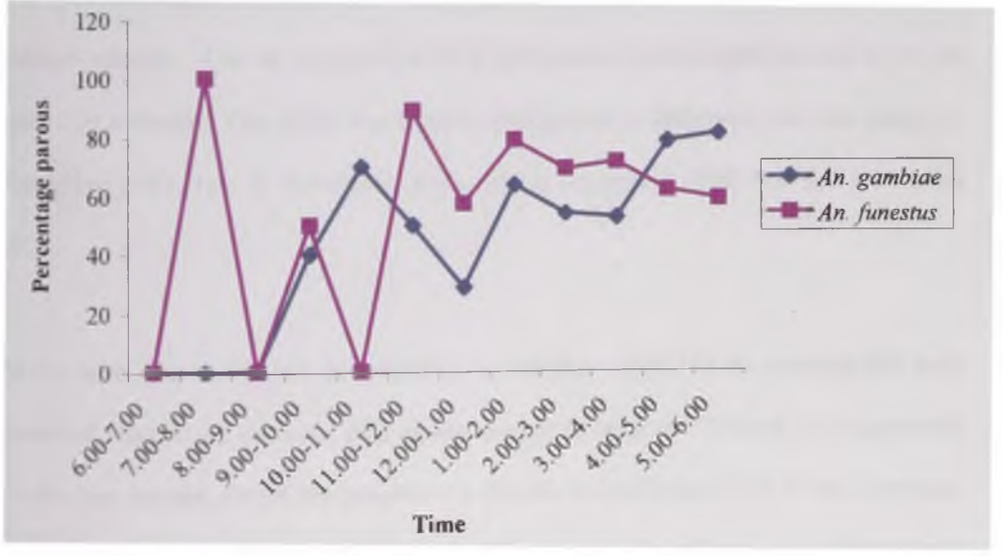

Figure 4.26: Hourly distribution of parous rates of *An. gambiae* s.s. and *An. funestus* at Bewadze

4.3.3 Vector infectivity rates

Plasmodium falciparum sporozoite infections in a total of 3,265 *Anopheles* mosquitoes (2,925 from HLC and 340 from PSC) were determined using ELISA. Figure 4.27 shows a typical example of the results obtained using the *P. falciparum* sporozoite detection ELISA method. At Okyereko, four out of 2,411 *An. gambiae* s.s. and one out of 213 *An. funestus* were found to be infective, giving sporozoite rates of 0.2% and 0.5% respectively. None of the 265 *An. pharoensis* and the two *An. coustani* from Okyereko that were examined were positive. Thus, *An. gambiae* s.s. contributed 80% of the infections while *An. funestus'* contribution was 20%. The overall infection rate due to malaria vectors was therefore 0.2% (5 / 2,624). The four infective *An. gambiae* s.s. were found to be the M form.

At Okyereko, three of the five infections occurred in the HLC samples (2,647), whilst the rest (2) were in the PSC samples (244). Of the three HLC infections, two occurred in the outdoor samples. Also all the infective HLC mosquitoes were caught between 10.00 pm and 12.00 midnight. Out of the five infective mosquitoes at Okyereko, one was caught in September 2002, two in November 2002, one in December 2002 and one in January 2003.

At Bewadze, four out of 188 *An. gambiae* s.s. and nine out of 179 *An. funestus* that were examined were found infective, thus giving sporozoite rates of 2.1% and 5% respectively for the two species. *Anopheles gambiae* s.s. therefore contributied 31% of the infections, whilst *An. funestus* contributed 69%. None of the seven *An. pharoensis* examined were found positive. The overall infection rate was therefore 3.5% (i.e. 13/367). Twelve of the 13 mosquitoes occurred in the HLC sample (n=305) and only one in the PSC sample (n=62). Of the 12 infective HLC mosquitoes, seven occurred in the indoor mosquitoes

and three out of the 12 were caught before midnight and the rest (9) were caught afterwards. Of the total thirteen infective mosquitoes at Bewadze, two were caught in September 2002, three in October, two in November 2002, two in December 2002, one in January 2002 and three in March 2003. Three out of the four infective *An. gambiae* s.s. were found to be the S form while one was the M form.

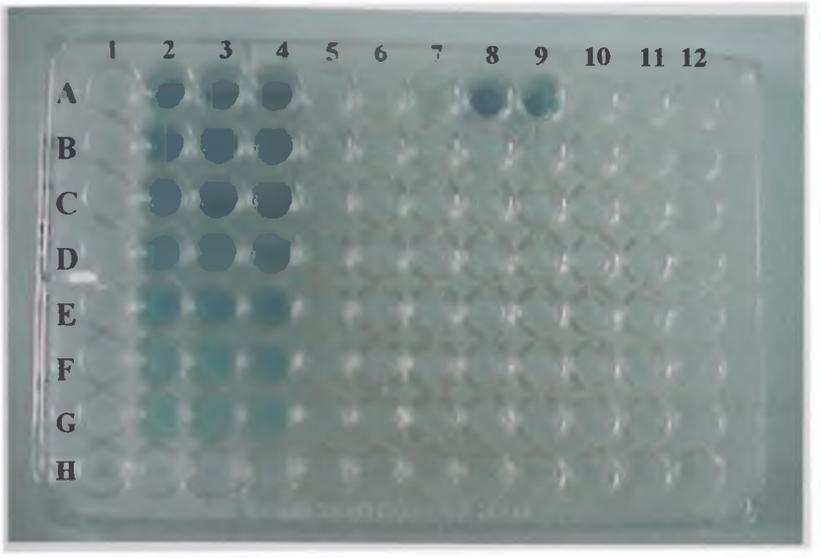

Figure 4.27: An example of *P. falciparum* sporozoite detection ELISA showing arrangement of samples and controls in a 96-well microtiter plate. 1A-1H= Negative controls; 2A - 4G = Positive controls; 2H-4H = Blanks; 5A – 12H = Mosquito test samples; 8A and 9A = *Plasmodium falciparum* - positive samples.

4.3.4 Entomological inoculation rates (EIR)

The estimated daily *P. falciparum* EIR due to *An. gambiae s.s.* and *An. funestus* at Okyereko were 0.182 and 0.035 infective bites/man/night (i/b/m/n) respectively. Thus, the estimated daily EIR due to both species was 0.224 i/b/m/n. The estimated yearly EIR for *An. gambiae* s.s. and *An. funestus* were 66.73 and 12.85 i/b/m/n respectively and the estimated yearly EIR due to both species at Okyereko was 81.85 i/b/m/n.

At Bewadze, the estimated daily *P. falciparum* EIR for *An. gambiae* s.s. was 0.077 i/b/m/n and 0.191 i/b/m/n for *An. funestus*. Thus, the estimated daily EIR due to both species was 0.262 i/b/m/n. The estimated yearly EIR for *An. gambiae* s.s. and *An. funestus* was 27.96 and 69.74 i/b/m/n respectively and the estimated yearly EIR due to both species at Bewadze was 95.62 i/b/m/n.

4.3.5 Human blood index (HBI)

Figure 4.28 shows an example of the results obtained for the human blood meal detection by ELISA. In all a total of 390 blood-fed indoor resting mosquitoes comprising of 361 *An. gambiae* s.s. and 29 *An. funestus* from Okyereko and 62 made up of 25 *An. gambiae s.s.* and 37 *An. funestus* from Bewadze were studied.

At Okyereko, the estimated HBI of *An. gambiae* s.s. was 85.3% (308/361) of the mosquitoes examined while for *An. funestus* it was 82.8% (24/29)..

At Bewadze, the HBI of *An. gambiae* was 92% (23/25) mosquitoes examined while for *An. funestus* it was 62.2% (23/37)

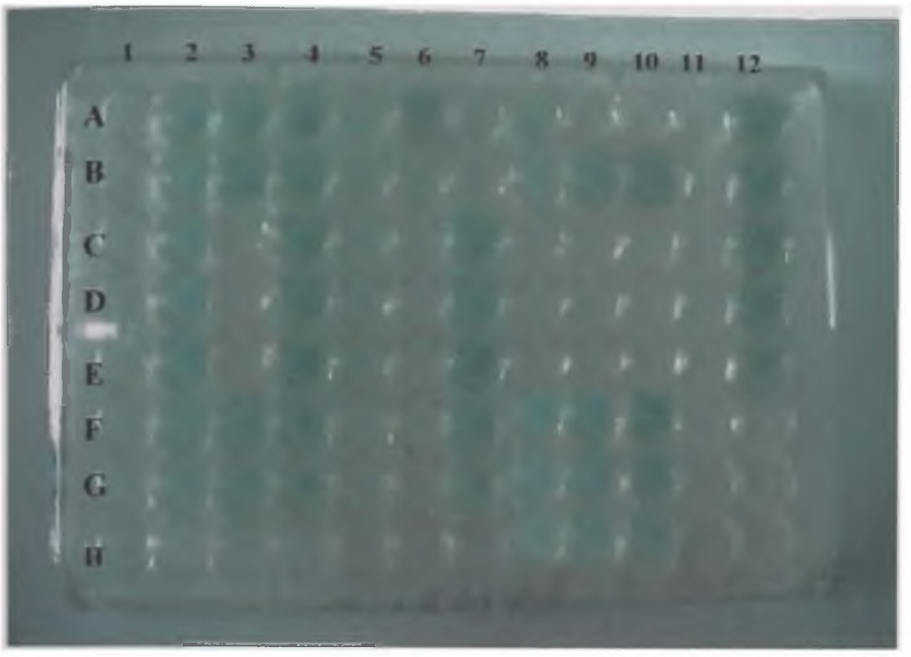

Figure 4.28: An example of human blood meal detection by ELISA showing arrangement of samples and controls in a 96-well microtiter plate. 1A-1H= Negative controls; 2A – 2D = Positive controls; 1E – 12H = Mosquito test samples (wells with green colouration indicate positive samples).

CHAPTER FIVE

DISCUSSION AND CONCLUSION

Africa has a very high human population growth rate which is not matched by the rate of increase in agricultural production per capita. One solution often sought by many African governments is to increase the area of irrigated land, permitting two crops per year. However, irrigation has often been blamed for aggravating diseases in local communities (Ijumba & Lindsay, 2001). Malaria is one of the major tropical diseases associated with irrigation schemes and changes in the transmission pattern of this disease following irrigation development have been a perennial subject of debate, since transmission in irrigated areas can appear higher, less than or similar in neighbouring non-irrigated villages.

This study was carried out to find the impact of irrigation on malaria transmission in a coastal savanna area of Ghana and it showed that approximately 12.5 fold more mosquitoes were caught at Okyereko irrigated area than that caught at the non-irrigated village, Bewadze. Furthermore, *An. gambiae* s.l. was also the most dominant vector caught at Okyereko. These findings are expected because the rice fields are flooded daily and these provide several mosquito breeding habitats, particularly suitable for pioneer species such as members of the *Anopheles gambiae* complex (Bruce-Chwatt 1954; Grainger 1947; Surtees, 1970; Chandler & Highton 1976; Snow 1983; Coosemans *et al.*, 1984; Robert *et al.*, 1985; Service 1989; Lacey & Lacey 1990; Ijumba & Lindsay 2001). The large numbers of *An. gambiae* s.l. collected as Okyereko also agrees with the findings of Dzodzomenyo *et al.* (1999) at the same site. *Anopheles funestus* on the other hand, was the dominant vector at Bewadze. This is also to be expected since *An. funestus* typically favours semi permanent water-bodies and breeding sites shaded by vegetation

(Gilles & de Meillon, 1968), and the presence of these at Bewadze would favour its production. No reason could be given for the disappearance of *An. funestus* at Okyereko from January to March 2003.

The high numbers of mosquitoes present throughout the period of study at Okyereko, with fewer numbers at Bewadze could be as a result of the daily irrigation of the rice fields at Okyereko creating abundant breeding sites for the mosquitoes. The high numbers of the different species caught indoors at Okereko was significantly higher than those caught indoors at Bewadze ($\lambda^2 = 645.702$, df = 4, P= 0.000). This was also the case outdoors ($\lambda^2 = 211.695$, df = 6, P= 0.000).

This study identified *An. gambiae* s.s as the only sibling species present in the two villages and this agrees with what has been found in other coastal savanna areas of Ghana (Appawu *et al.*, 1994, 2001 and Midega, 2001). Although *An. arabiensis* has been found in irrigated fields in some parts of Africa, this species was not observed at Okyereko during the study period. *Anopheles gambiae* s.s exhibited endophagic and endophilic tendencies as expected (Bryan *et al.*, 1987).

The "Mopti" and "Savanna" forms of *An. gambiae* s.s were also found present in the two villages. There was a significant difference between the "Mopti" and "Savanna" forms in the two sites ($\lambda^2 = 12.493$, df = 1, P< 0.001). The "Mopti" forms were more dominant at Okyereko (permanent waters). The higher proportion of "Mopti" form occurring at Okyereko is not surprising because this molecular form has a remarkable ecological flexibility and is known to prevail in inundated areas where dry season breeding opportunities exist (e.g. rice fields) [Coluzzi *et al.*, 1985; Petrarca *et al.*, 1986, 1987; Robert *et al.*, 1989; Appawu *et al.*, 1994].

The mean biting rate for the anophelines for the entire period of study at Okyereko was found to be about seventeen times higher than that of Bewadze. This finding agrees with what has been recorded in other parts of Africa where the biting rate has been found to be higher in irrigated areas than in neighbouring non-irrigated areas (Robert *et al.*, 1985; Ijumba & Lindsay, 2001). The mean indoor biting rate for the anophelines collected at Okyereko was about fourteen fold higher (indoors) and twenty two fold higher (outdoors) than at Bewadze. A plausible reason for this was the higher number of *Anopheles* caught at Okyereko compared to Bewadze.

At Okyereko, *An. gambiae* s.s. was biting more both indoors and outdoors, however, at Bewadze *An. funestus* was biting more indoors as compared to *An. gambiae* s.s. which was biting more outdoors. This could be as result of the absence of *An. funestus* outdoors between 6.00 pm and 12.00 midnight at Bewadze.

The biting of *An. gambiae* s.s. peaked in March 2003 at Bewadze and this corresponded with the month in which the highest number of *An. gambiae* s.s. was caught. This could have been as a result of the rains that occurred some days prior to the day of sampling. Contrary to the expectation that *An. funestus* will be higher in abundance in the dry months i.e. during January and March 2003, *An. funestus* rather was biting more from September to December and then was completely displaced by *An. gambiae* s.s. during these dry months. No reasons could be attributed to this.

The high parous rates for both *An. gambiae* and *An. funestus* observed at Okyereko and Bewadze indicates that the biting species are mainly older individuals with previous biting experience and therefore have high potentials to harbour the parasites picked from

the previous blood meal. This also implies relative high survival rates of the mosquitoes and the potential for repeated infections because sporozoites can remain viable in the vector for the remainder of its life span (Ghosh *et al.*, 2001). Also, high proportions of parous anophelines were biting later than the nulliparous ones, an observation that was also reported by Bockarie *et al.* (1996) for *An. gambiae* and *An. punctulatus* in Sierra Leone and Papua New Guinea respectively.

Among the indoor resting *An. gambiae* females, proportionately more had human blood meal (i.e. HBI was higher) at Okyereko compared to Bewadze though the differences in the HBI at the two sites were not statistically significant ($\lambda^2 = 2.358$, df = 1, P= 0.125). The HBI for *An. funestus*, however, was lower at Bewadze despite it having more sporozoites. This appears contradictory because a higher number of *An. funestus* were biting indoors than outdoors thus it will be expected that its HBI would be higher. It is possible that there are probably two sympatric *An. funestus* populations, one relatively more zoophilic and the other anthropophilic.

The sporozoite rate of *An. funestus* was about two and half fold more than that of *An. gambiae* at both Okyereko and Bewadze and this was significant ($\lambda^2 = 4.206$, df = 1, P= 0.040 at Okyereko; $\lambda^2 = 10.454$, df = 1, P= 0.001 at Bewadze). This finding indicates that *An. funestus* could be a more efficient vector than *An. gambiae* species in the study areas. This finding however, contradicts what has been reported for *An. funestus* elsewhere (De Meillon, 1947; Pringle, 1966). Thus, it is likely *An. funestus* might be a more efficient vector than *An. gambiae* in ceratin cases.

The majority of infective bites of the vectors in the study areas occurred after 10.00pm. which coincides with the hours when the biting densities of the vectors are high, and also

when most of the human inhabitants are in bed. This finding agrees with what was reported by Appawu *et al.*, (2001) for *An. gambiae* and *An. funestus* in Dodowa where most of the infective bites were between 01.00 – 02.00 hours. This suggests that if the inhabitants slept under impregnated bed-nets, human-vector contact and thus the risk of infective bites could be reduced.

The absence of spororozoite-positive *An. pharoensis* in the study area was as expected because *An. pharoensis* has not been incriminated as a malaria vector in Okyereko and in other parts of Ghana. This species is regarded as a vector in parts of Egypt but usually not in Afrotropical regions (Gillies & Coetzee, 1987, Gillies & de Meillon, 1968) except Senegal (Carrara *et al.*, 1990).

Exposure to malaria estimated in terms of EIR was lower in Okyereko compared to Bewadze. At Okyereko, the EIR of *An. funestus* was significantly lower than that of *An. gambiae* s.s. while the reverse was the case at Bewadze ($\lambda^2 = 51.692$, df = 1, $P < 0.001$). At Okyereko, the low man biting rate and the small population size of *An. funestus* made its EIR low despite the high infectivity rate recorded for this species. This finding agrees with what was observed by Appawu *et al.* (2001) in Prampam. The higher EIR recorded for *An. funestus* at Bewadze could be the result of the higher biting and sporozoite rates recorded for this species there.

The differences in the intensity of malaria transmission reported for these two neighbouring villages, is most likely the result of the irrigation scheme and practices at Okyereko. The explanation for the lower EIR recorded in Okyereko could be that the nuisance associated with the high mosquito biting rates are unacceptable to the inhabitants forcing most to protect themselves. It could also be that as a result of the

greater wealth acquired by the community through agriculture, most are able to have access to anti malarial drugs and screened houses and use of insecticide repellents. Existence of a well established health infrastructure in Okyereko might also contribute to the general lower level of malaria. This explanation is plausible because by casual observation, relatively few people lived in screened houses and also there was poor access to efficient healthcare at Bewadze as compared to Okyereko.

Another probable reason for the lower malaria transmission at Okyereko could be due to the prevalence of the "Mopti" form of *An. gambiae* s.s. It has been observed that where this form is prevalent, there is frequently a reduced vectorial ability for *P. falciparum* malaria (Appawu *et al.*, 1994). Robert *et al.* (1986, 1991) pointed out, that this phenomenom is probably due to differences in population dynamics between "Mopti" form and the other taxa, rather than to intrinsic factors.

The results of this study confirm other findings in Africa, where the cultivation of irrigated rice gives rise to large numbers of potential vectors but low sporozoite and inoculation rates. For example, in rice growing areas of Rusivi valley, Burundi, the biting density of *An. gambiae* s.l. was about 10 – fold higher than in adjacent cotton growing areas, but the sporozoite rate was three fold lower in the rice irrigation scheme (Coosemans *et al.*, 1985). Likewise, the sporozoite rate of *An. gambiae* s.l in the Ahero rice irrigation scheme in Kenya was five-fold less than in the nearby sugar belt (Ghitheko *et al.*, 1993). In the rice growing areas of Bobo Dioulasso, Burkina Faso, the man biting density of *An. gambiae* s.l was 10-fold higher than in the nearby savanna areas, but the sporozoite rate was 10 fold lower in the rice growing area (Robert *et al.*, 1985).

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APPENDIX I

CHEMICALS AND REAGENTS

The reagents for PCR, 10X PCR Buffer, magnesium chloride (50mM $MgCl_2$), *Taq* DNA polymerase, deoxyribonucleoside triphosphates: deoxyadenosine triphosphate (dATP), deoxycytidine triphosphate (dCTP), deoxyguanosine triphosphate (dGTP), deoxythymidine triphosphate (dTTP) and molecular weight marker (100 bp ladder), were purchased from Sigma Chemical Company, St Louis U.S.A, and GIBCO BRL (Life Technologies U.S.A). Sodium hydroxide, silica gel dessicant, mineral oil, agarose (molecular biology grade) and ficoll, were also purchased from Sigma Chemical Company, St Louis, U.S.A. Ethidium bromide was obtained from Fluka AG Granite Chemika, Switzerland. Orange G was obtained from Kanto Chemical Company Ltd., Japan.

The restriction enzyme *Hha*I was purchased from Promega (USA).

The reagents for ELISA: Polyoxyethylenesorbitant monoalurate (Tween 20), Dulbecco Phosphate Buffered Saline, Igepal, Casein, Anti-Human IgG (peroxidase labelled) and Microtiter plates were purchased from Sigma Chemical Company, St Louis, U.S.A. Lyophilised monoclonal antibodies (Pf capture Mab, Pf peroxidase Mab and Pf positive control protein) were obtained from the Center for Disease Control and Prevention, Atlanta, U.S.A. KP&L ABTS (2,2'-azino-di-3[ethyl-benzthiazoline sulfonate]) was purchased from Kirkegaard and Perry Laboratories, inc. Maryland, U.S.A.

APPENDIX II

PREPARATION OF REAGENTS

Standard Solutions

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

Solutions for Electrophoresis

Agarose Gel

10X TAE buffer

242g Tris Base, 57.1 ml glatial acetic acid, 100 ml 0.5 M EDTA, pH adjusted to 7.7 (with glatial cetic acid) and the volume made to one liter with sterile distilled water

Gel loading buffers

5X orange G

20% w/v Ficoll, 25 Mm EDTA, 2.5 mM EDTA. 2.5% (w/v) orange G. Stored at room temperature.

DNA molecular weight size markers

The 100bp DNA molecular weight size marker obtained from Sigma were diluted according to the manufactures specifications and used. For the 100 bp ladder, the first band size is 100 bp, the subsequent ones are 200, 300 to 1000 bp.

Ethidium bromide, EtBr (10mg/ml)

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

0.5 M EDTA (pH 8.0)

186g of EDTA, dissolved in 800ml ddw, pH adjusted with NaOH pellets, the volume made to 1000ml with sddw and stored at room temperature.

Solutions used in ELISA studies

Dulbecco's Phosphate Buffered Saline (PBS)

The flask was calibrated by pouring 1 litre of tap water into the flask, a stirring rod was gently slided alongside the flask and the final volume of the water in the flask was marked.

The water was discarded and the flask rinsed with distilled water. About 750ml of distilled water was poured into the flask, a stirring rod was rinsed and slided into the flask. The flask was placed on an electronic magnetic stirrer and stirred. 9.6g of Dulbecco's PBS was emptied into the flask while content was stirring. The pH was adjusted to 7.2 and more distilled water added to the content of the flask to the level of the calibrated mark and stored at room temperature.

Washing Buffer (PBST (tween 20)

0.5ml Tween 20 was added to 750ml of PBS (prepared as described above) while stirring. More distilled water was added to the level of the calibrated mark.

Blocking Buffer (BB)

5g of casein was suspended in 100ml of 0.1N NaOH and left to boil. 900ml of PBS was slowly added after casein is dissolved and allowed to cool. The pH was adjusted to 7.2 and stored at 4^0C.

Grinding solution (GS)

2.5ml of IGEPAL was added to 500ml of blocking buffer and stored at 4^0C.

Substrate solution

KP&L ABTS (solution A) and hydrogen peroxide were mixed 1 : 1 immediately before use.

Unlabelled monoclonal antibody (Mab)

To get a coating rate of 0.1µg / 50 µl / well, 20µl of unlabelled Mab was added to 4,980µl of PBS immediately before use.

Peroxidase - conjugate monoclonal antibody (Mab)

To get an incubation rate of 0.05 µg / 50 µl / well, 40 µl of peroxidase - conjugate Mab was added to 4,960 µl of Blocking buffer immediately before use.

Anti human IgG

40µl of anti human IgG was added to 2ml of blocking buffer immediately before use.

APPENDIX III

HUMAN LANDING CATCHES (INDOORS AND OUTDOORS)

APPENDIX IIIA OKYEREKO

OKYEREKO (INDOORS) SEPTEMBER, 2002							
Time	Total no. collected	*An gambiae* s.l.	*An. funestus*	*Culex.* Spp	*Aedes* Spp	*Mansonia* Spp	*An. pharoensis*
6.00 - 7.00	8	8	0	0	0	0	0
7.00 - 8.00	11	7	0	1	0	3	0
8.00 - 9.00	44	27	4	0	0	9	4
9.00 - 10.00	41	32	0	0	0	5	4
10.00 - 11.00	82	65	4	1	0	10	2
11.00 - 12.00	107	96	2	3	0	3	3
12.00 - 1.00	120	97	9	6	0	1	7
1.00 - 2.00	159	147	4	2	0	4	2
2.00 - 3.00	142	132	6	3	0	0	1
3.00 - 4.00	102	97	2	3	0	0	0
4.00 - 5.00	145	137	5	1	0	0	2
5.00 - 6.00	79	72	7	0	0	0	0
TOTAL	**1040**	**917**	**43**	**20**	**0**	**35**	**25**

OKYEREKO (OUTDOORS) SEPTEMBER, 2002							
Time	Total no. collected	*An gambiae* s.l	*An. funestus*	*Culex* Spp	*Aedes* Spp.	*Mansonia.* Spp	*An. pharoensis*
6.00 - 7.00	18	4	0	8	0	4	2
7.00 - 8.00	26	10	0	7	0	7	2
8.00 - 9.00	54	28	1	11	0	11	3
9.00 - 10.00	95	46	2	20	0	16	11
10.00 - 11.00	112	82	2	11	0	14	3
11.00 - 12.00	95	67	5	6	0	14	3
12.00 - 1.00	129	110	0	6	0	9	4
1.00 - 2.00	101	85	0	6	0	6	4
2.00 - 3.00	93	84	0	3	0	5	1
3.00 - 4.00	71	57	3	5	0	4	2
4.00 - 5.00	78	68	0	6	0	4	0
5.00 - 6.00	41	34	0	2	0	5	0
TOTAL	**913**	**675**	**13**	**91**	**0**	**99**	**35**

OKYEREKO (INDOORS)							
OCTOBER, 2002							
Time	Total no. collected	*An gambiae* s.l.	*An. funestus*	*Culex.* Spp	*Aedes* Spp	*Mansonia* Spp	*An. pharoensis*
6.00 - 7.00	0	0	0	0	0	0	0
7.00 - 8.00	4	3	1	0	0	0	0
8.00 - 9.00	9	5	0	0	0	0	4
9.00 - 10.00	41	34	1	1	0	4	1
10.00 - 11.00	51	38	3	3	0	2	5
11.00 - 12.00	36	26	3	3	0	1	3
12.00 - 1.00	53	44	1	3	0	0	5
1.00 - 2.00	31	29	0	1	0	1	0
2.00 - 3.00	18	18	0	0	0	0	0
3.00 - 4.00	44	37	1	4	0	2	0
4.00 - 5.00	37	33	1	2	0	1	0
5.00 - 6.00	29	27	1	0	0	1	0
TOTAL	**353**	**294**	**12**	**17**	**0**	**12**	**18**

OKYEREKO (OUT)							
OCTOBER, 2002							
Time	Total no. collected	*An gambiae* s.l.	*An. funestus*	*Culex.* Spp	*Aedes* Spp	*Mansonia* Spp	*An. pharoensis*
6.00 - 7.00	3	1	0	0	0	2	0
7.00 - 8.00	18	3	1	10	0	3	1
8.00 - 9.00	32	15	0	7	1	8	1
9.00 - 10.00	52	27	1	5	0	12	7
10.00 - 11.00	91	43	3	20	0	9	16
11.00 - 12.00	78	47	1	10	0	13	7
12.00 - 1.00	66	54	0	8	0	4	0
1.00 - 2.00	36	29	1	4	0	2	0
2.00 - 3.00	44	42	0	1	0	0	1
3.00 - 4.00	49	45	0	3	0	1	0
4.00 - 5.00	29	23	1	4	0	1	0
5.00 - 6.00	38	28	4	4	0	1	1
TOTAL	**536**	**357**	**12**	**76**	**1**	**56**	**34**

OKYEREKO (INDOORS)							
NOVEMBER, 2002							
Time	Total no. collected	*An gambiae* s.l.	*An. funestus*	*Culex.* Spp	*Aedes* Spp	*Mansonia* Spp	*An. pharoensis*
6.00 - 7.00	4	2	0	2	0	0	0
7.00 - 8.00	20	4	0	14	0	0	2
8.00 - 9.00	37	17	3	12	0	3	2
9.00 - 10.00	46	20	2	14	0	0	10
10.00 - 11.00	48	39	0	7	0	1	1
11.00 - 12.00	42	31	2	8	0	1	0
12.00 - 1.00	72	49	6	14	0	0	3
1.00 - 2.00	65	46	2	12	0	2	3
2.00 - 3.00	59	47	4	7	0	0	1
3.00 - 4.00	36	32	1	3	0	0	0
4.00 - 5.00	36	29	5	1	0	1	0
5.00 - 6.00	14	13	0	1	0	0	0
TOTAL	**479**	**329**	**25**	**95**	**0**	**8**	**22**

OKYEREKO (OUTDOORS)							
NOVEMBER, 2002							
Time	Total no. collected	*An gambiae* s.l.	*An. funestus*	*Culex.* Spp	*Aedes* Spp	*Mansonia* Spp	*An. pharoensis*
6.00 - 7.00	1	0	0	0	0	0	1
7.00 - 8.00	24	9	2	7	0	4	2
8.00 - 9.00	50	22	1	14	0	6	7
9.00 - 10.00	98	58	6	15	0	6	13
10.00 - 11.00	121	84	6	11	0	6	14
11.00 - 12.00	132	91	10	15	0	7	9
12.00 - 1.00	82	55	4	12	0	5	6
1.00 - 2.00	73	63	4	2	0	3	1
2.00 - 3.00	77	67	4	4	0	0	2
3.00 - 4.00	50	47	2	0	0	1	0
4.00 - 5.00	45	40	1	2	0	1	1
5.00 - 6.00	11	10	0	0	0	0	1
TOTAL	**764**	**546**	**40**	**82**	**0**	**39**	**57**

OKYEREKO (INDOORS)							
DECEMBER, 2002							
Time	Total no. collected	*An gambiae* s.l.	*An. funestus*	*Culex.* Spp	*Aedes* Spp	*Mansonia* Spp	*An. pharoensis*
6.00 - 7.00	6	2	1	2	0	0	1
7.00 - 8.00	29	7	2	13	0	2	5
8.00 - 9.00	59	20	6	28	0	3	2
9.00 - 10.00	76	35	5	22	0	11	3
10.00 - 11.00	72	28	7	21	0	2	14
11.00 - 12.00	103	34	15	30	0	5	19
12.00 - 1.00	136	74	20	27	0	3	12
1.00 - 2.00	92	57	6	17	0	4	8
2.00 - 3.00	111	78	15	11	0	2	5
3.00 - 4.00	112	75	14	12	0	7	4
4.00 - 5.00	74	45	18	7	0	1	3
5.00 - 6.00	31	15	6	8	0	2	0
TOTAL	**901**	**470**	**115**	**198**	**0**	**42**	**76**

OKYEREKO (OUTDOORS)							
DECEMBER, 2002							
Time	Total no. collected	*An gambiae* s.l.	*An. funestus*	*Culex.* Spp	*Aedes* Spp	*Mansonia* Spp	*An. pharoensis*
6.00 - 7.00	14	0	1	13	0	0	0
7.00 - 8.00	19	2	0	14	0	0	3
8.00 - 9.00	23	5	4	8	0	1	5
9.00 - 10.00	41	17	8	8	0	3	5
10.00 - 11.00	49	29	4	11	0	3	2
11.00 - 12.00	54	14	12	15	0	7	6
12.00 - 1.00	53	26	10	7	0	8	2
1.00 - 2.00	68	44	8	12	0	0	4
2.00 - 3.00	48	28	13	4	0	1	2
3.00 - 4.00	59	37	12	7	0	2	1
4.00 - 5.00	24	14	6	4	0	0	0
5.00 - 6.00	18	10	4	3	0	1	0
TOTAL	**470**	**226**	**82**	**106**	**0**	**26**	**30**

OKYEREKO (INDOORS)							
JANUARY, 2003							
Time	Total no. collected	*An gambiae* s.l.	*An. funestus*	*Culex.* Spp	*Aedes* Spp	*Mansonia* Spp	*An. pharoensis*
6.00 - 7.00	20	18	0	0	0	1	1
7.00 - 8.00	9	8	1	0	0	0	0
8.00 - 9.00	24	23	0	0	0	1	0
9.00 - 10.00	38	31	1	1	0	2	3
10.00 - 11.00	54	47	1	2	0	0	4
11.00 - 12.00	26	24	1	0	0	0	1
12.00 - 1.00	40	39	1	0	0	0	0
1.00 - 2.00	40	38	1	0	0	1	0
2.00 - 3.00	29	26	0	2	0	1	0
3.00 - 4.00	44	42	0	0	0	1	1
4.00 - 5.00	38	37	0	0	0	1	0
5.00 - 6.00	15	13	2	0	0	0	0
TOTAL	**377**	**346**	**8**	**5**	**0**	**8**	**10**

OKYEREKO (OUTDOORS)							
JANUARY, 2003							
Time	Total no. collected	*An gambiae* s.l.	*An. funestus*	*Culex.* Spp	*Aedes* Spp	*Mansonia* Spp	*An. pharoensis*
6.00 - 7.00	8	7	0	0	0	0	1
7.00 - 8.00	24	10	2	12	0	0	0
8.00 - 9.00	36	22	0	9	0	1	4
9.00 - 10.00	35	26	0	5	0	4	0
10.00 - 11.00	56	42	0	9	0	0	5
11.00 - 12.00	95	85	2	3	0	2	3
12.00 - 1.00	53	47	0	4	0	0	2
1.00 - 2.00	48	37	0	9	0	1	1
2.00 - 3.00	49	41	0	7	0	0	1
3.00 - 4.00	69	60	0	6	0	1	2
4.00 - 5.00	28	23	0	2	0	2	1
5.00 - 6.00	39	35	0	1	0	2	1
TOTAL	**540**	**435**	**4**	**67**	**0**	**13**	**21**

OKYEREKO (INDOORS)							
MARCH, 2003							
Time	Total no. collected	*An gambiae* s.l.	*An. funestus*	*Culex.* Spp	*Aedes* Spp	*Mansonia* Spp	*An. pharoensis*
6.00 - 7.00	12	10	0	2	0	0	0
7.00 - 8.00	7	5	1	0	0	1	0
8.00 - 9.00	20	16	1	2	0	0	1
9.00 - 10.00	35	28	0	7	0	0	0
10.00 - 11.00	39	36	0	0	0	0	3
11.00 - 12.00	62	53	2	6	0	0	1
12.00 - 1.00	34	31	0	3	0	0	0
1.00 - 2.00	42	36	0	5	0	1	0
2.00 - 3.00	20	20	0	0	0	0	0
3.00 - 4.00	60	55	0	3	0	1	1
4.00 - 5.00	45	42	0	3	0	0	0
5.00 - 6.00	33	29	0	1	0	0	3
TOTAL	**409**	**361**	**4**	**32**	**0**	**3**	**9**

OKYEREKO (OUTDOORS)								
MARCH, 2003								
Time	Total no. collected	*An gambiae* s.l.	*An. funestus*	*Culex.* Spp	*Aedes* Spp	*Mansonia* Spp	*An. pharoensis*	*An. coustani*
6.00 - 7.00	36	19	0	14	0	0	3	0
7.00 - 8.00	25	7	0	10	0	1	5	2
8.00 - 9.00	62	32	0	15	1	0	14	0
9.00 - 10.00	69	32	0	14	0	4	19	0
10.00 - 11.00	80	49	1	13	0	2	15	0
11.00 - 12.00	73	45	0	15	0	3	10	0
12.00 - 1.00	31	22	0	4	0	1	4	0
1.00 - 2.00	68	32	0	29	0	0	7	0
2.00 - 3.00	55	33	0	18	0	1	3	0
3.00 - 4.00	50	28	0	19	0	1	2	0
4.00 - 5.00	45	30	0	12	0	1	2	0
5.00 - 6.00	11	3	2	5	0	1	0	0
TOTAL	**605**	**332**	**3**	**168**	**1**	**15**	**84**	**2**

APPENDIX IIIB BEWADZE

BEWADZE (INDOORS)							
SEPTEMBER, 2002							
Time	Total no. collected	*An gambiae* s.l.	*An. funestus*	*Culex.* Spp	*Aedes* Spp	*Mansonia* Spp	*An. pharoensis*
6.00 - 7.00	2	0	0	2	0	0	0
7.00 - 8.00	3	0	0	2	0	1	0
8.00 - 9.00	1	0	0	1	0	0	0
9.00 - 10.00	4	0	1	3	0	0	0
10.00 - 11.00	3	1	1	0	0	1	0
11.00 - 12.00	5	1	4	0	0	0	0
12.00 - 1.00	10	1	5	0	0	2	2
1.00 - 2.00	12	0	7	1	0	4	0
2.00 - 3.00	13	1	8	0	0	4	0
3.00 - 4.00	23	2	15	2	0	4	0
4.00 - 5.00	16	1	10	0	0	5	0
5.00 - 6.00	23	2	21	0	0	0	0
TOTAL	**115**	**9**	**72**	**11**	**0**	**21**	**2**

BEWADZE (OUTDOORS)							
SEPTEMBER, 2002							
Time	Total no. collected	*An gambiae* s.l.	*An. funestus*	*Culex.* Spp	*Aedes* Spp	*Mansonia* Spp	*An. pharoensis*
6.00 - 7.00	0	0	0	0	0	0	0
7.00 - 8.00	0	0	0	0	0	0	0
8.00 - 9.00	3	0	0	2	0	1	0
9.00 - 10.00	12	0	0	5	0	7	0
10.00 - 11.00	5	1	0	2	0	2	0
11.00 - 12.00	10	1	0	2	0	7	0
12.00 - 1.00	7	2	2	0	0	3	0
1.00 - 2.00	4	1	2	0	0	0	1
2.00 - 3.00	9	2	2	5	0	0	0
3.00 - 4.00	8	2	1	1	0	4	0
4.00 - 5.00	8	1	2	4	0	1	0
5.00 - 6.00	8	0	6	0	0	0	2
TOTAL	**74**	**10**	**15**	**21**	**0**	**25**	**3**

BEWADZE (INDOORS)							
OCTOBER, 2002							
Time	Total no. collected	*An gambiae* s.l	*An. funestus*	*Culex.* Spp	*Aedes* Spp	*Mansonia* Spp	*An. pharoensis*
6.00 - 7.00	0	0	0	0	0	0	0
7.00 - 8.00	0	0	0	0	0	0	0
8.00 - 9.00	2	0	1	1	0	0	0
9.00 - 10.00	2	0	1	1	0	0	0
10.00 - 11.00	1	1	0	0	0	0	0
11.00 - 12.00	8	0	7	1	0	0	0
12.00 - 1.00	1	0	1	0	0	0	0
1.00 - 2.00	4	1	3	0	0	0	0
2.00 - 3.00	3	0	2	0	0	1	0
3.00 - 4.00	9	1	8	0	0	0	0
4.00 - 5.00	7	0	7	0	0	0	0
5.00 - 6.00	8	0	8	0	0	0	0
TOTAL	**45**	**3**	**38**	**3**	**0**	**1**	**0**

BEWADZE (OUTDOORS)							
OCTOBER, 2002							
Time	Total no. collected	*An gambiae* s.l.	*An. funestus*	*Culex.* Spp	*Aedes* Spp	*Mansonia* Spp	*An. pharoensis*
6.00 - 7.00	1	0	0	1	0	0	0
7.00 - 8.00	2	0	1	1	0	0	0
8.00 - 9.00	0	0	0	0	0	0	0
9.00 - 10.00	1	1	0	0	0	0	0
10.00 - 11.00	3	0	1	1	0	1	0
11.00 - 12.00	0	0	0	0	0	0	0
12.00 - 1.00	1	1	0	0	0	0	0
1.00 - 2.00	3	1	2	0	0	0	0
2.00 - 3.00	2	0	0	1	0	1	0
3.00 - 4.00	2	0	1	1	0	0	0
4.00 - 5.00	5	1	4	0	0	0	0
5.00 - 6.00	2	1	1	0	0	0	0
TOTAL	**22**	**5**	**10**	**5**	**0**	**2**	**0**

BEWADZE (INDOORS)							
NOVEMBER, 2002							
Time	Total no. collected	*An gambiae* s.l.	*An. funestus*	*Culex.* Spp	*Aedes* Spp	*Mansonia* Spp	*An. pharoensis*
6.00 - 7.00	0	0	0	0	0	0	0
7.00 - 8.00	3	2	0	1	0	0	0
8.00 - 9.00	1	0	0	1	0	0	0
9.00 - 10.00	1	0	1	0	0	0	0
10.00 - 11.00	2	0	2	0	0	0	0
11.00 - 12.00	3	2	0	0	0	1	0
12.00 - 1.00	3	0	3	0	0	0	0
1.00 - 2.00	5	1	4	0	0	0	0
2.00 - 3.00	6	2	4	0	0	0	0
3.00 - 4.00	1	0	1	0	0	0	0
4.00 - 5.00	9	2	6	1	0	0	0
5.00 - 6.00	2	0	2	0	0	0	0
TOTAL	**36**	**9**	**23**	**3**	**0**	**1**	**0**

BEWADZE (OUTDOORS)							
NOVEMBER, 2002							
Time	Total no. collected	*An gambiae* s.l.	*An. funestus*	*Culex.* Spp	*Aedes* Spp	*Mansonia* Spp	*An. pharoensis*
6.00 - 7.00	1	0	0	1	0	0	0
7.00 - 8.00	0	0	0	0	0	0	0
8.00 - 9.00	0	0	0	0	0	0	0
9.00 - 10.00	5	2	1	1	0	1	0
10.00 - 11.00	0	0	0	0	0	0	0
11.00 - 12.00	2	1	0	0	0	1	0
12.00 - 1.00	8	4	3	1	0	0	0
1.00 - 2.00	7	0	5	1	0	1	0
2.00 - 3.00	10	5	1	2	0	2	0
3.00 - 4.00	6	1	4	1	0	0	0
4.00 - 5.00	4	0	1	3	0	0	0
5.00 - 6.00	1	1	0	0	0	0	0
TOTAL	**44**	**14**	**15**	**10**	**0**	**5**	**0**

BEWADZE (INDOORS)							
DECEMBER, 2002							
Time	Total no. collected	*An gambiae* s.l.	*An. funestus*	*Culex.* Spp	*Aedes* Spp	*Mansonia* Spp	*An. pharoensis*
6.00 - 7.00	0	0	0	0	0	0	0
7.00 - 8.00	0	0	0	0	0	0	0
8.00 - 9.00	1	0	0	1	0	0	0
9.00 - 10.00	0	0	0	0	0	0	0
10.00 - 11.00	1	0	0	1	0	0	0
11.00 - 12.00	1	1	0	0	0	0	0
12.00 - 1.00	0	0	0	0	0	0	0
1.00 - 2.00	2	2	0	0	0	0	0
2.00 - 3.00	1	1	0	0	0	0	0
3.00 - 4.00	3	2	1	0	0	0	0
4.00 - 5.00	5	4	1	0	0	0	0
5.00 - 6.00	2	0	2	0	0	0	0
TOTAL	**16**	**10**	**4**	**2**	**0**	**0**	**0**

BEWADZE (OUTDOORS)							
DECEMBER, 2002							
Time	Total no. collected	*An gambiae* s.l.	*An. funestus*	*Culex.* Spp	*Aedes* Spp	*Mansonia* Spp	*An. pharoensis*
6.00 - 7.00	1	0	0	1	0	0	0
7.00 - 8.00	1	0	0	1	0	0	0
8.00 - 9.00	5	0	0	5	0	0	0
9.00 - 10.00	3	0	0	3	0	0	0
10.00 - 11.00	2	1	0	1	0	0	0
11.00 - 12.00	2	1	0	1	0	0	0
12.00 - 1.00	2	1	0	1	0	0	0
1 00 - 2.00	0	0	0	0	0	0	0
2.00 - 3.00	4	3	0	1	0	0	0
3.00 - 4.00	7	1	1	5	0	0	0
4.00 - 5.00	6	3	2	1	0	0	0
5.00 - 6.00	2	0	1	1	0	0	0
TOTAL	**35**	**10**	**4**	**21**	**0**	**0**	**0**

BEWADZE (INDOORS)							
JANUARY, 2003							
Time	Total no. collected	*An gambiae* s.l.	*An. funestus*	*Culex.* Spp	*Aedes* Spp	*Mansonia* Spp	*An. pharoensis*
6.00 - 7.00	0	0	0	0	0	0	0
7.00 - 8.00	0	0	0	0	0	0	0
8.00 - 9.00	0	0	0	0	0	0	0
9.00 - 10.00	2	1	0	1	0	0	0
10.00 - 11.00	1	0	0	1	0	0	0
11.00 - 12.00	1	0	0	1	0	0	0
12.00 - 1.00	0	0	0	0	0	0	0
1.00 - 2.00	3	3	0	0	0	0	0
2.00 - 3.00	2	2	0	0	0	0	0
3.00 - 4.00	3	2	1	0	0	0	0
4.00 - 5.00	6	5	0	1	0	0	0
5.00 - 6.00	1	1	0	0	0	0	0
TOTAL	**19**	**14**	**1**	**4**	**0**	**0**	**0**

BEWADZE (OUTDOORS)							
JANUARY							
Time	Total no. collected	*An gambiae* s.l.	*An. funestus*	*Culex.* Spp	*Aedes* Spp	*Mansonia* Spp	*An. pharoensis*
6.00 - 7.00	0	0	0	0	0	0	0
7.00 - 8.00	2	0	0	2	0	0	0
8.00 - 9.00	3	0	0	3	0	0	0
9.00 - 10.00	1	0	0	1	0	0	0
10.00 - 11.00	4	1	0	3	0	0	0
11.00 - 12.00	1	0	0	1	0	0	0
12.00 - 1.00	0	0	0	0	0	0	0
1.00 - 2.00	1	0	0	0	0	1	0
2.00 - 3.00	0	0	0	0	0	0	0
3.00 - 4.00	2	1	0	1	0	0	0
4.00 - 5.00	4	2	0	2	0	0	0
5.00 - 6.00	0	0	0	0	0	0	0
TOTAL	**18**	**4**	**0**	**13**	**0**	**1**	**0**

BEWADZE (INDOORS)							
MARCH, 2003							
Time	Total no. collected	*An gambiae* s.l.	*An. funestus*	*Culex.* Spp	*Aedes* Spp	*Mansonia* Spp	*An. pharoensis*
6.00 - 7.00	1	0	0	0	0	1	0
7.00 - 8.00	1	0	0	1	0	0	0
8.00 - 9.00	2	1	0	1	0	0	0
9.00 - 10.00	5	0	0	4	0	0	1
10.00 - 11.00	2	2	0	0	0	0	0
11.00 - 12.00	7	5	0	2	0	0	0
12.00 - 1.00	5	4	0	1	0	0	0
1.00 - 2.00	3	2	0	1	0	0	0
2.00 - 3.00	4	3	0	0	0	1	0
3.00 - 4.00	3	2	1	0	0	0	0
4.00 - 5.00	12	12	0	0	0	0	0
5.00 - 6.00	12	10	0	2	0	0	0
TOTAL	**57**	**41**	**1**	**12**	**0**	**2**	**1**

BEWADZE (OUTDOORS)							
MARCH, 2003							
Time	Total no. collected	*An gambiae* s.l.	*An. funestus*	*Culex.* Spp	*Aedes* Spp	*Mansonia* Spp	*An. pharoensis*
6.00 - 7.00	7	0	0	6	0	1	0
7.00 - 8.00	1	0	0	1	0	0	0
8.00 - 9.00	6	0	0	5	0	1	0
9.00 - 10.00	9	5	0	3	0	1	0
10.00 - 11.00	6	5	0	1	0	0	0
11.00 - 12.00	7	3	0	2	0	2	0
12.00 - 1.00	9	5	0	2	1	0	1
1.00 - 2.00	5	4	0	1	0	0	0
2.00 - 3.00	10	8	0	2	0	0	0
3.00 - 4.00	10	8	0	1	0	1	0
4.00 - 5.00	7	6	0	1	0	0	0
5.00 - 6.00	6	5	0	1	0	0	0
TOTAL	**83**	**49**	**0**	**26**	**1**	**6**	**1**

APPENDIX IV

PYRETHRUM SPRAY CATCHES (PSC)

OKYEREKO						
DATES	Total no. collected	*An. gambiae* s.l	*An. funestus*	*Culex* spp	*An. pharoensis*	No./room
9/9/2002	62	50	9	0	3	2
12/9/2002	41	40	0	0	1	3
8/10/2002	94	84	9	0	1	2
10/10/2002	24	21	0	0	3	2
8/11/2002	18	18	0	0	0	4
11/11/2002	79	73	6	0	0	4
4/12/2002	70	66	4	0	0	3
9/12/2002	12	10	2	0	0	2
29/01/2003	83	77	4	2	0	3
31/01/2003	209	201	8	0	0	3
26/03/2003	48	48	0	0	0	3
28/03/2003	106	104	2	0	0	2
TOTAL	**846**	**792**	**44**	**2**	**8**	**33**

BEWADZE						
DATES	Total no. collected	*An. gambiae* s.l	*An. funestus*	*Culex* spp	*Mansonia* spp	No./room
9/9/2002	16	3	11	0	1	1
12/9/2002	36	1	16	14	0	5
8/10/2002	8	0	6	1	0	1
10/10/2002	11	0	4	1	0	6
8/11/2002	4	0	1	2	0	1
11/11/2002	3	0	0	1	1	1
4/12/2002	1	0	0	0	0	1
9/12/2002	3	2	0	0	0	1
29/01/2003	10	6	0	1	0	3
31/01/2003	9	2	0	2	0	5
26/03/2003	8	4	0	3	0	1
28/03/2003	18	9	0	4	0	5
TOTAL	**127**	**27**	**38**	**29**	**2**	**31**