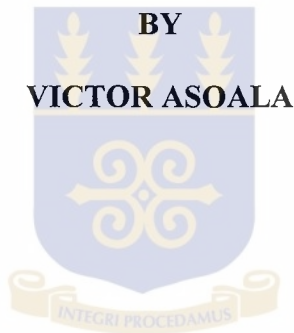




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**A COMPARATIVE STUDY OF HUMAN LANDING
CATCHES AND PYRETHRUM SPRAY CATCHES
USED IN MALARIA VECTOR STUDIES IN THE
KASENA NANKANA DISTRICT (KND) OF GHANA**



**THIS DISSERTATION IS SUBMITTED TO THE SCHOOL OF
PUBLIC HEALTH, UNIVERSITY OF GHANA, LEGON IN
PARTIAL FULFILMENT OF THE REQUIREMENT FOR
THE AWARD OF MASTER OF PUBLIC HEALTH DEGREE.**

SEPTEMBER 2003

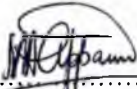
DECLARATION

I hereby declare that this dissertation is produced by me from a study undertaken in Kassena Nankana district under supervision in partial fulfilment of the program for award of Master of Public Health (MPH) Degree.



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DR. BAWA AWUMBILA

DEDICATION

To Miss Hawa Selma Ali, for all the reasons she knows so well



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TABLE OF CONTENTS

Declaration	i
Acknowledgement	iii
Table of Contents	iv
List of Tables	vi
List of Figures.	vii
List of Abbreviations	viii
Abstract	ix
CHAPTER 1.	1.1. INTRODUCTION								1
	1.1.1 Primary Objectives				5
	1.1.2 Specific Objectives				5
									1
CHAPTER 2.	LITERATURE REVIEW				6
	2.0. THE BURDEN OF MALARIA								
	2.0.1 Morbidity and Mortality of Malaria				6
	2.0.2. Economic and Social Burden of Malaria				7
	2.0.3 Distribution of Malaria				8
	2.1. LIFE CYCLE OF MALARIA PARASITE				9
	2.1.1 Life Cycle of Malaria Parasite in the Mosquito				9
	2.1.2 Life Cycle of Malaria Parasite in the Mosquito				10
	2.2.0. MALARIA VECTORS				13
	2.2.1. Life Cycle of Malaria Vectors				13
	2.2.2 Malaria Vector Behaviour				17
	2.2.2.1 Feeding Behaviour				17
	2.2.2.2 Biting Cycle				18
	2.2.2.3 Resting Behaviour				19
	2.2.3 Factors Affecting Distribution of Malaria Vectors				20
	2.2.4 Factors Affecting Transmission of Malaria by Vector				21
	2.3. ESTIMATION OF MALARIA TRANSMISSION								
PARAMETERS					23
	2.3.1. Entomological Inoculation Rate				23
	2.3.2. Man Biting Rate				24
	2.3.3 Sporozoite Rate				25

	2.4.0. MOSQUITO SAMPLING METHODS	25
	2.4.1. Light Trap Catches (LTC)	26
	2.4.2. Human Landing Catches (HLC)	28
	2.4.3. Pyrethrum Spray Catches (PSC)	30
CHAPTER 3.	MATERIALS AND METHODS	33
	3.1 Study Site	33
	3.2 Mosquito Collection	35
	3.2.1. Human Landing Collections	35
	3.2.2. Pyrethrum Spray Collections	36
	3.3.0. Processing of Mosquitoes	36
	3.3.1. Mosquito Identification	36
	3.3.2. Determination of Sporozoite Infectivity	37
	3.4. Analysis	38
	3.5. Ethical Considerations	38
CHAPTER 4	RESULTS	40
	4.0. Species of Anopheles captured by Human Landing catches and Pyrethrum Spray catches	40
45 ...	4.1. Man biting rates of anophelines captured using HLC and PSC	
	4.2. Sporozoite infectivity	50
	4.3 Entomological Inoculation Rates (EIR) estimated from HLC and PSC	50
CHAPTER 5.	DISCUSSION	54
CHAPTER 6	CONCLUSIONS AND RECOMMENDATIONS	56
	6.0 CONCLUSIONS	
	6.1 Recommendations	56
REFERENCES		57

LIST OF TABLES

- Table 1. Number of Anopheles mosquitoes captured by HLC and PSC in the irrigated areas of KND
- Table 2. Number of Anopheles mosquitoes captured by HLC during the wet and dry seasons.
- Table 3. Number of Anopheles mosquitoes captured by PSC during the wet and dry seasons.
- Table 4. Transmission parameters estimated from HLC and PSC in the irrigated area of KND during the wet and dry seasons.
- Table 5. Seasonal changes in entomological inoculation rates (EIR) of *Anopheles gambiae* and *Anopheles funestus* using HLC and

LIST OF FIGURES

- Figure 1. Diagram depicting the life cycle of human
- Figure 2. Life cycle of the Anopheles Mosquito
- Figure 3. Map of the Kassena Nankana District
- Figure 4. Man biting rates estimated from PSC during the west season
- Figure 5. Man-biting rates estimated from HLC during the wet season
- Figure 6. Man-biting rates estimated from HLC during the dry season
- Figure 7. Man-biting rates estimated from PSC during the dry season

LIST OF ABBREVIATIONS

EIR	Entomological Inoculation Rate
ELISA	Enzyme-linked Immunosorbent Assay
HLC	Human Landing Catches
KND	Kassena Nankana District
LTC	Light Trap Catches
MBR	Man-Biting Rate
PSC	Pyrethrum Spray Catches
SR	Sporozoite Rate
WHO	World Health Organisation

ABSTRACT

The most direct and reliable method for estimating the man-biting rate is the human landing catches (HLC). It is considered the most representative and usually considered as the “Gold standard”, for determining human biting activity of mosquitoes. However, of recent there has been concerns about the ethics of using humans as baits to collect mosquitoes and therefore there is the need for an alternative method. This study compared Human Landing and Pyrethrum Spray methods in estimating malaria transmission intensity in an irrigated area in Kassena Nankana District of Northern Ghana. Both methods were used to collect mosquitoes during the wet and dry seasons of 2001-2002. Man biting rates, sporozoite rates and Entomological inoculation rates (EIR) were estimated for both methods. Using Human landing catches with a total of 192 man-nights of yielded 59.8% (6270) of *Anopheles gambiae s.l.*, whereas Pyrethrum spray catches (PSC) contributed 40.2% (4220). Biting rates estimated from HLC during the wet season were significantly higher ($P<0.05$) than that of the dry season (60.9 versus 26.6), whereas, rates from PSC during the wet and dry seasons were not different ($P>0.05$). There were significant differences ($P<0.05$) between the biting rates obtained for *Anopheles funestus* for the two seasons using both methods. The overall sporozoite rate estimated using PSC, 7.1% (29/407) was significantly higher ($P<0.05$) than 4.9% (204/4353) for HLC. EIR per year using HLC was 123.9 and 1323.0 infective bites/ man/ year for the dry and wet seasons respectively while, it was 34.3 and 52.3 respectively using PSC. The annual EIR estimated for the two vectors, *Anopheles gambiae* and *Anopheles funestus* from HLC were similar (731 vrs 517.9) whilst that estimated from PSC was higher for *Anopheles gambiae* than *Anopheles funestus* (36.0 vrs 13.0).

Although the estimated values using the two methods varied considerably, the transmission patterns were similar. In areas where mosquito numbers may be very low, for example, in the dry season, PSC may be useful in the assessment of malaria transmission intensity. The study provides information on the comparative use of HLC and PSC in vector transmission studies, which is necessary for the planning and implementation of future vector control strategies.

CHAPTER ONE

1.0 INTRODUCTION

Globally, malaria is a major public health problem. It is by far the most important insect transmitted disease (Gilles and Warrel, 1993). The WHO estimates that there are at least 270 million infected, 110 million new cases and 1.2 million deaths due to malaria annually (WHO, 1999). Over one million of these deaths occur in children aged under five years (Trigg and Kondrachiane, 1998). About 90% of all malaria deaths in the world occur in Africa. This is because the most dangerous malaria parasite, *Plasmodium falciparum* and the most effective vector, *Anopheles gambiae* occur in Africa (WHO, 2002).

Malaria is not just a public health problem; it is also an important obstacle to economic development in most developing countries particularly in Africa. It inhibits economic growth and places an economic burden on these countries (Liese, 1998).

In Ghana, malaria is by far the most important parasitic disease. It accounts for 7.8% of all certified deaths. For children under five years, about 25% of all deaths is due to malaria and also accounts for about 40% of all out patients seen in health facilities (Ahmed, 1989; Binka *et al.*, 1996).

Malaria is hyper endemic in the Upper East Region of Ghana. The region is characterized by harsh environmental conditions typical of much of the Guinea Savannah belt of West Africa. It is the leading cause of both morbidity and mortality and accounts for about 60% of hospital admissions and 35% of deaths in the Kassena Nankana District located in the region (Owusu-Agyei, 2001). Significant variations in malaria morbidity and mortality in the Kassena Nankana district have been observed, with higher rates occurring during the

rainy season (Binka et al, 1996). The major vectors in the district have been reported to be *Anopheles gambiae s.l.* Greater numbers of these vectors are found in areas around the irrigated dam found in the district (Appawu et al 2001b). While transmission is estimated to be between 100 –1000 bites per person per annum (Binka et al., 1996).

The intensity of malaria parasite transmission is best expressed in terms of the entomological inoculation rate (EIR), which is defined as the number of infective mosquito bites received by a person during a certain period of time. (Bier et al., 1999; Hay et al., 2000). The magnitude of the EIR is influenced by the rate at which vectors feed on humans, which is largely dependent on the mosquito density and to some extent, the feeding habits of the vector species (Shilulu et al., 1998). The EIR can be determined by multiplying mosquito-biting rates by the proportion of mosquitoes carrying sporozoites in their salivary glands referred to as, the sporozoite rate (Hee-II Lee et al., 2001).

The EIR has been shown to be influenced by several factors such as vector bionomics including the vector species, feeding preferences, resting behaviour and vector efficiency, among others and therefore, field based research on malaria vectors and malaria parasite transmission is vital to the success of any coordinated malaria control intervention (Bier, 2000).

Entomological studies of malaria can seriously be hampered by the choice of collection methods for assessing mosquito vector populations (Hii et al., 2000). Different methods can be used for the collection of mosquitoes and each of these methods has its own limitation and because adequate sampling and processing is essential for obtaining significant information, these methods are chosen based on the objectives of a study.

Human landing catches (HLC) is a method that involves the collections of malaria mosquitoes, which land on human ‘collectors when they attempt to feed on them. It is considered the most representative and usually considered as the “Gold standard”, for determining human biting activity (WHO, 1975). The principle behind the use of this method is that, humans act as baits to attract blood-seeking mosquitoes.

Human landing catches gives information on the different *Anopheles* species biting people and the relative changes in abundance. The biting activity throughout the night can be obtained and hence the times of maximum exposure to malaria transmission. The method can also be used to estimate the man biting rate per night and adults caught can be dissected to determine parity and for the calculation of sporozoite rate and entomological inoculation rates (Le Goff *et al.*, 1993; Service, 1976 ; WHO,1975).

Certain biases may be introduced when HLC is used to estimate malaria transmission parameters. For example, the rate at which a mosquito approaches and attacks an exposed person performing HLC may be higher than under normal conditions. This is because, the baits in HLC are relatively more available to host seeking mosquitoes than if they were under normal circumstances, that is, either asleep (covered with bedding) or more active instead of posing as baits (Mathenge *et al.*, 2000). Biases introduced by human factors such as the differential attractiveness of collectors acting as baits and also the method being labour intensive, also affect estimates using this method (Knols *et al.*, 1995). The use of human baits also raises the ethical problem of the deliberate exposure of collectors to mosquito bites, increasing the risk of contracting malaria (Magbity *et al.*, 2001).

Pyrethrum spray sheet collection (P.S.C) is another sampling method used to collect indoor resting mosquitoes after being knockdown by space spraying with a pyrethrum

solution. The main principle of the method is that, mosquitoes tend to rest after feeding and therefore any mosquitoes captured with this method may have either fed on people in the room or are coming to feed (Molineaux *et al.*, 1988). This method has been used as a standard quick and easy method of catching mosquitoes resting in huts and animal shelters (Service, 1993). It gives valuable information on relative changes in seasonal abundance of endophilic vectors, provides adults for determining sporozoite and inoculation rates and information on host preferences and degree of exophily. PSC method also provides unfed adult mosquitoes for determination of parity and half gravids for chromosomal identifications. The blood fed mosquitoes are useful in determining the host preferences of mosquitoes (Appawu *et al.*, 2001; Service, 1976 ; WHO, 1975). It is the best method to use when large numbers of mosquitoes are required. This is because, this method collects mosquitoes of different physiological stages such as, resting blood-fed gravid females and unfed females in search of blood meal (Mwandawiro *et al.*, 1997; Appawu *et al.*, 2001). However, It results in the sampling of different species of only endophilic vectors found in an area. In addition, PSC is faster and does not require many workers and skill is not very important.

Unlike HLC, estimation of man biting rate from PSC is done indirectly. The man-biting rate is estimated by dividing the number of blood-engorged females by the number of bedroom occupiers. What is obtained is based on the assumption that exophily, zoophagy and exophagy have been excluded (Service, 1976). Not all the mosquitoes may have fed on humans and therefore the value obtained is usually multiplied by a factor known as the human blood index. Also mosquitoes captured using the method may not represent all the

mosquitoes in the room since some may escape through eaves and others may fall in places where they cannot be collected.

PSC has the potential to provide indirect but reliable estimates of man biting rates and other malaria transmission parameters and therefore present an alternative to HLC and avoid the possible ethical problem that arises when mosquito collectors deliberately expose themselves to disease vectors. This study is therefore aimed at comparing HLC and PSC as methods for sampling mosquitoes for studies of the transmission dynamics of malaria in the Kassena Nankana District.

1.1. OBJECTIVES

1.1. 1 Primary Objective

To compare Pyrethrum Spray Catch (PSC) and Human Landing Catches (HLC) as sampling methods for the determination of transmission dynamics of malaria.

1.1.2. Specific Objectives: are to

1. To determine the population of mosquitoes captured by each method
2. To determine biting and sporozoite rates of mosquitoes captured by each method
3. Determine the entomological inoculation rates (Intensity of transmission) by each method
4. To determine the seasonal variation in the above parameters for the two methods

CHAPTER TWO

LITERATURE REVIEW

2.0 THE BURDEN OF MALARIA

2.0.1 Morbidity and mortality Of Malaria

Human malaria is by far the most important insect transmitted disease (Gilles and Warrel, 1993). Malaria is caused by protozoan parasites of the genus *Plasmodium*, which comprise four species namely, *Plasmodium falciparum*, *P. vivax*, and *P. malariae* and *P. ovale*. They occur throughout the tropics but *P. falciparum* causes the most deadly form of malaria (Gilles, 1985). The World Health organization (WHO, 1999) estimates that globally, there is at least, 276 million infected, 110 million new cases and 1-2 million deaths due to malaria annually (WHO, 1999). Over a million of these deaths occur in children aged less than five years but also includes women in their first and second pregnancy, older children, young adults and non-immune travellers (Trigg and Kondrachine, 1998). Studies have shown that 58% of malaria related deaths occur among 20% of the world's population, a percentage higher than any other disease of major public health importance (Hamudi and Sachs, 1999). While these numbers may be shocking, they may probably be under-estimates of the world's malaria burden given that only a fraction of malaria cases are reported each year and deaths among children with chronic malaria are often attributed to other illnesses (WHO, 1999; Hamudi and Sachs, 1999). Recent studies indicate that, malaria causes at least 20% of all deaths in children less than five years of age in Africa. Although respiratory diseases caused by a variety of infectious agents result in similar proportion of deaths, *plasmodium falciparum* is the most

important single infectious agent causing deaths among young children in Africa (Africa malaria report, 2003). Children who get malaria may suffer long-term consequences of the infection. For instance, an estimated 2% of children who recover from cerebral malaria infections suffer learning impairments and disabilities due to brain damage including Epilepsy and Spasticity (Murphy and Breman, 2001). Repeated malaria infections make young children more susceptible to other common childhood illnesses such as diarrhea and respiratory infections and therefore contribute indirectly to mortality (Molineaux, 1997). Children who survive malaria may suffer long-term consequences of infections including repeated fever and illness, reduced appetite, play and social interaction, which contribute to poor development (Murphy *et al.*, 2001). In highly endemic areas, malaria is responsible for about 30-50% of fever cases, 30% out patient consultations and 10-15% hospital admissions (Trigg and Kondrachine, 1998). The widespread and increasing resistance of *P. falciparum* to chloroquine, the first line drug for the treatment of acute uncomplicated malaria in most African countries coupled with constrained resources has led to a rising burden of the malaria disease (Trape *et al.*, 1998).

2.0.2 Economic and Social Burden of Malaria

Malaria is not just a public health problem; it is also an important obstacle to economic development in most developing countries particularly Africa. It is the only vector-borne disease to be placed on the WHO's Disability Adjusted Life Years (DALYs) (Arrese, 2001). Malaria causes 10.6% of lost DALYs, second to only HIV/AIDS (WHO, 1999). Estimates suggest that a single bout of malaria cost the equivalent of ten working days (Liese, 1998). It has been shown that although poverty does not appear to determine the

risk of malaria, the prevalence of malaria has an enormous impact on a country's economy. The disease dramatically inhibits economic growth and places an economic burden on countries in Africa (Gallus and Sachs, 1998). Studies in Northern Ghana have shown that both direct and indirect cost associated with a malaria episode represents a substantial burden on poor households. While the cost of malaria was just 1 % of the income of the rich; it was 34% of that of poor households (Akazili, 2002). It has been estimated that the gross domestic product (GDP) of African countries is reduced by an average of 1.3% annually because of malaria. Malaria also undermines the effectiveness of investment in education (Liese, 1998). In endemic areas, learning capacity of between 35% and 60% of all school children may be impaired. In cases where adults become seriously ill with malaria, the need for extra additional labor results in the interruption of a child's education or sometimes, total withdrawal (Liese, 1998). In Ghana, malaria is also the most important parasitic disease. It accounts for 7.8% of all certified deaths. For children under five years, about 25% of all deaths is due to malaria. And also accounts for about 40% of all out patients seen in health facilities (Ahmed, 1989; Binka *et al.*, 1994).

2.0.3. Distribution Of Malaria

Malaria is not a homogeneous disease but varies from region to region, from country to country and from place to place. The disease occurs mostly in tropical and subtropical countries, particularly Africa south of the Sahara, South East Asia and the forest fringe zones in South America. Malaria is endemic in 42 of the 46 African countries covered by WHO and very few of these countries have any malaria free zones (WHO, 2000).

About 90% of all malaria deaths in the world today occur in Africa south of the Sahara. This is because the majority of infections are caused by *P. falciparum*, the most

dangerous of the four human malaria parasites. It is also because the most effective vector, *Anopheles gambiae* mosquito is most widespread in Africa and most difficult to control (WHO, 2002).

2.1 LIFE CYCLE OF MALARIA PARASITE

The life cycle of all malaria parasites is essentially the same. It comprises an exogenous sexual phase (sporogony) with multiplication in certain species of *Anopheles* and an endogenous asexual phase (schizogony) with the multiplication in the human host. The life cycle is illustrated in Figure 1. The endogenous asexual phase includes a development cycle in the red blood cells (erythrocytic schizogony) and a phase taking place in the cells of the liver, pre-erythrocytic schizogony (Gilles, 1986).

2.1.1 Life Cycle of the Malaria Parasite in the Mosquito

Mosquitoes have been conventionally called the definitive host of malaria because the sexual cycle of the malaria parasite occurs in the *Anopheles* vector and man is the intermediate host (Service, 1980). When a female *Anopheles* mosquito ingests blood from a human host with malaria parasites in circulation, the asexual parasites are digested together with the red blood cells. The mature sexual cells known as gametocytes undergo further development. The nuclei of the male gametocytes usually divide into between 4-8 daughter nuclei, each of which develops a flagellum, which breaks off after some time. These are the male gametes (micro gametes). The female gametocytes undergo maturation to form the female gametes (macro gametes). In the gut of the mosquito, the male gametes fertilize the female ones to form zygotes. The zygote within 24 hours elongates and develops a cytoskeleton and becomes mobile and is now known as an

ookinete. This ookinete then settles on the outer surface of the gut wall, becomes rounded into a small sphere with an elastic membrane and now called an oocyst. The oocyst gradually increases in size and as it enlarges, the nucleus divides repeatedly to form sporozoites. These sporozoites are motile and burst out of the oocyst to invade the body cavity of the mosquito (Service, 1980 ; Bradley, 1996) When the sporozoites enter the salivary glands of the mosquito, it becomes infective. It has been estimated that, on average, one single oocyst of *P. falciparum* may produce and release 1000 sporozoites (Gilles, 1986).. Oocysts can be seen in the stomach walls of vectors about four days following a blood meal and after eight days, they become fully grown.. Sporozoites are usually found in the salivary glands after 9-12 days but the time required for this cyclical development depends on temperature and species of the parasite. (Service, 1980).

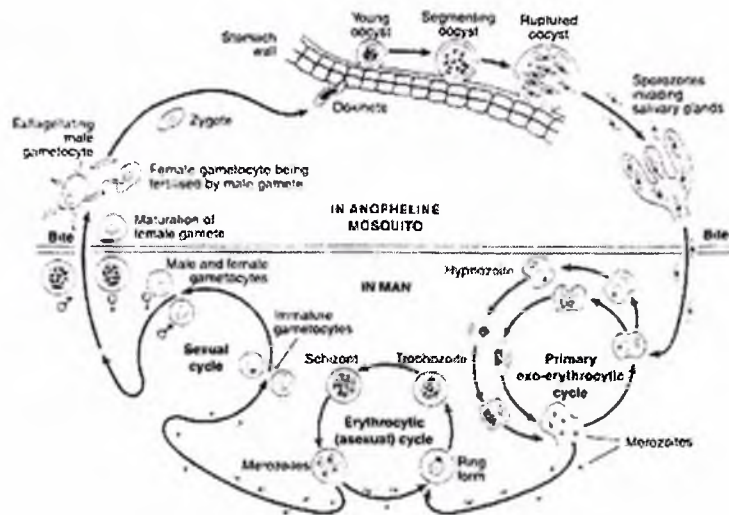
2.1.2 Life Cycle of Malaria Parasite in Man

The sporozoites from the mosquito salivary glands are injected into the human during a blood meal and the host becomes infected. Phagocytes destroy many of the sporozoites but some enter the liver cells (hepatocytes) and undergo a process of development and multiplication, known as pre-erythrocytic schizogony. This process results in the production of schizonts, which divide asexually to produce thousands of merozoites. At the end of this phase, the merozoites are released into the bloodstream and most of them invade the red blood cells. The merozoites develop in the red blood cells forming trophozoites. As they grow, these trophozoites undergo asexual division (erythrocytic schizogony) to produce merozoites and the red blood cells burst to release them into the bloodstream. These merozoites then invade fresh red blood cells and the same process

produces another generation of parasites. The erythrocytic schizogony is repeated over and over in the course of the infection leading to the progressive increase in parasitaemia (Service, 1980; Bradley, 1996 ; Gilles, 1986) while merozoites from pre-erythrocytic schizogony may also give rise to sexually differentiated gametocytes, it is usually after several rounds of erythrocytic schizogony that these forms are produced in greater numbers. After invading fresh red blood cells, these gametocytes grow and remain in the blood until mosquitoes take them up during feeding (Gilles, 1985).

Figure 1: Diagram depicting the life cycle of human malaria

(Asexual phase in human body and sexual phase in the mosquito Life Cycle of the Malaria Parasite)



2.2 0 MALARIA VECTORS

It is known that only mosquitoes of the genus *Anopheles* can transmit *plasmodium* species, which causes malaria in man (Curtis, 1996). The geographical distribution of malaria corresponds with the geographical spread of *Anopheles*. It has been found that no transmission of malaria takes place in continents without *Anopheles* (Cutter, 1998). There are about 360 described species of *Anopheles* and of this number, 45 are considered to be involved in malaria transmission (White, 1977).

The knowledge of the major vectors of malaria in Africa, whether less efficient or locally important remains incomplete (Fontenilli and Lochonarn, 1999). The vector population in Sub-Saharan Africa is effective, with six species of *Anopheles gambiae* Giles complex being the most efficient vectors of human malaria and often considered the most important in the world. *Anopheles funestus* Giles has also been found to be capable of producing very high inoculation rates in a wide range of geographic, seasonal and ecological conditions (Coluzzi, 1984).

2.2.1 Life Cycle Of Malaria Vectors

The mosquito goes through four separate and distinct stages of its life cycle: Egg, larva, Pupa and Adult. *Anopheles* and many other mosquitoes lay their eggs singly on water surfaces. The adult female *Anopheles* takes a blood meal approximately every two to three days. This is necessary for the development of a batch of eggs, which are normally

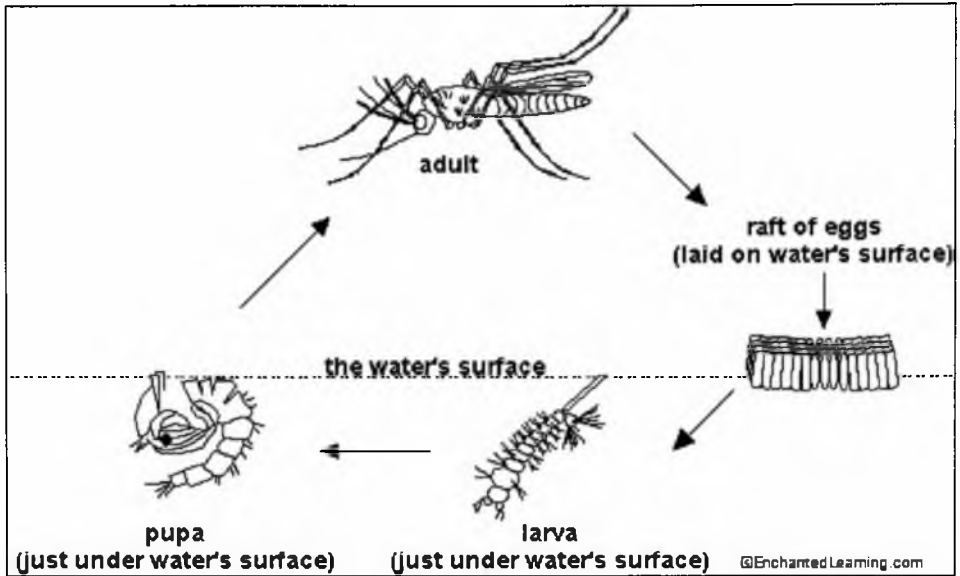
laid before another blood meal is taken. Temperature and humidity influence the time taken for a blood meal to be digested and the eggs to develop. Eggs are laid on water in batches of about 100-150. The sites for egg laying varies from small amounts of residual water in places like hoof-prints and coconut husks to larger water bodies such as streams, canals, rivers, ponds and lakes depending on the species. Female mosquitoes will usually continue to lay their eggs throughout their lives. Under favorable conditions the average life of female *Anopheles* mosquitoes is about three weeks and most lay between one and three batches of eggs (Service, 1993).

The eggs usually hatch after two to three days and larvae emerge. These generally live just below the surface of water to enable them to breathe. There are four larval stages known as instars and the time required for each stage depends on the water temperature.

The pupa stage lasts about two to three days at normal tropical temperatures and it is this stage that major transformation to the adult stage occurs. The pupa, which is comma shaped stays on the surface of water but does not feed and eventually it's skin splits releasing the adult mosquito.

Most mosquitoes mate shortly after emergence from the pupa. With a few exceptions, the female mosquitoes must bite a host and take a blood meal to obtain the necessary nutrients for the development and maturation of the eggs in the ovaries. A few species can sometimes develop at least the first batch and possibly, subsequent batches without a blood meal (Service, 1980). The speed of digestion of the blood meal depends on temperature and in most species, takes between two to three days. As the blood is digested, the white eggs in the ovaries enlarge making the abdomen to become whitish posteriorly and dark reddish anteriorly. This marks the midpoint of blood digestion. and

the mosquito is said to be half-gravid. When all the blood is digested and the abdomen becomes dilated and whitish carrying fully developed eggs, the mosquito is said to be gravid and searches for a suitable habitat to oviposit. The mosquito then takes another blood meal again to develop another batch of eggs. This process is repeated several times and is referred to as the gonotrophic cycle (Service, 1980).

Figure 2 Life cycle of *Anopheles* mosquito

2.2.2. Malaria Vector Behaviour

Most mosquitoes are nocturnal in their activities and thus, their emergence from pupae, mating, blood feeding and oviposition will normally occur either in the evenings, at night or early morning around sunrise. Some species bite man only outdoors (exophagic), whereas other species bite mainly indoors (endophagic). Also while some *Anopheles* species rest in houses after feeding (endophillic), others rest outdoors in natural shelters like animal pens, tree holes and artificial sheds (exophillic). It has been found that most *Anopheles* species are not exclusively exophagic, endophagic, exophillic, endophillic but rather exhibit a mixture of the extremes of behaviour. A few *Anopheles* feed exclusively on either man or animals, depending on the species. (Service, 1980)

2.2.2.1 Feeding Behaviour

Mosquitoes can either bite man indoors or outdoors. Two factors have been found to affect the relative number of species biting indoors and outdoors: the reaction of the vector to the house itself and the timing of the biting activity. A species with both late biting and outdoor feeding habits for instance will clearly make little contact with man (Elliot, 1972). Many *Anopheles* that feed readily on any large mammals outdoors, including man, fail to orient to the same species once he is confined within a house (Gillies, 1988). Certain features of a house could be involved in blocking or offsetting the mosquito's response to attractant stimuli emerging through apertures on the walls. These could be visceral, tactile, micro climatic changes in the air coming from occupied houses or the reluctance of low flying mosquitoes to enter through elevated apertures such as eaves (Coluzzi et al., 1979; Gillies, 1988). Certain genetic variants in *Anopheles*

arabiensis have been found to occur at different frequencies in indoor and outdoor biting populations and this has been interpreted to mean that carriers of certain chromosomal types sense the difference in saturation deficit between air issuing from houses and that in open air and for this reason, fail to respond to the attractant plume issuing from houses (Coluzzi *et al.*, 1979).

Biting behaviour could also have seasonal variations. There have also been findings in tropical America where outdoor biting by *Anopheles albimanus* is greater during the dry season than during the wet season (Muirhead-Thompson and Mercier, 1952 ; Elliot, 1968). In Thailand also, outdoor biting by *Anopheles minimus* was much higher in the dry season than during the rains (Ismail *et al.*, 1974). On the other hand, Hanney (1960) showed that in the rather harsher climate of northern Nigeria, the reverse was true for *Anopheles funestus* which were caught in fewer numbers biting outdoors in the dry season than during the rains. It is possible that seasonal changes in wind speed could play a part (Gilles, 1988).

2.2.2.2 Biting Cycle

The change from day to night and back again to daylight conditions has a dominating influence on the behaviour of *Anopheles*. Laboratory studies have shown that many cyclical changes in the behavior of *Anopheles* are true circadian rhythms, controlled by an internal clock set by the transition from light to darkness (Jones *et al.*, 1972; Chalwood and Jones, 1979). A synchronized departure of unfed and gravid mosquitoes from houses has been observed at twilight (Gilles, 1988). Late feeding of the *Anopheles gambiae* complex and *Anopheles funestus* has been seen to contrast with the pattern of activity of other non-vector species caught biting man. The association of late feeding with

efficiency can be explained by the activity of human beings at night. Usually human activities in the early part of the night tends to hinder successful attacks by mosquitoes while a sleeping host is usually less easily disturbed by bites and thus, making feeding success to be higher (Elliott, 1972). Appawu *et al.*, (2001) reported that the majority of infective bites by mosquitoes in coastal forest and savanna area in Ghana, occurred during the second half of the night coinciding with hours biting vector densities are high.

2.2.2.3 Resting Behaviour

After feeding, malaria vectors may either leave the house during the course of the night on which it fed, it may also leave the following evening at dusk or it may complete the rest of the blood digesting phase of the gonotrophic cycle indoors (Gillies, 1988).

In some species, the number of fed females found indoors during daytime does not necessarily represent all those that had fed the previous night. A high proportion of certain vectors like, *Anophele gambiae*, *Anophele funestus* and *Anopheles culicifacie* are found indoors during the daytime (Molineaux and Gramiccia, 1988). Studies of *Anopheles gambiae* complex mosquitoes with exit traps have generally shown that less than 15% of females of fresh water species of the complex leave houses after feeding to seek shelter outside or in other houses nearby. The bulk of the population therefore remains indoors from the time of feeding till at least the following evening (Muirhead-Thompson, 1948; Gelfaud, 1955). A study in Ghana showed a small proportion of indoor resting mosquitoes leaving the room after feeding while others moved indoors after feeding on cattle outdoors (Appawu *et al.*, 2001). Females that have fed outdoors or those that have recently emerged after feeding indoors are usually presented with the

problem of selecting a resting site that will provide them with seclusion and a suitable microclimate (Gilles, 1988).

2.2.3 Factors Affecting Distribution of Malaria Vectors

Different vector species breed in widely different types of surface water. The availability of the appropriate types of surface water determines the distribution and may be affected by many factors. For example, local rainfall may produce rain pools, which favour the breeding of species like *Anopheles gambiae* and *Anopheles arabiensis* (Molineaux, 1988). Rainfall provides the available surface water for breeding of mosquitoes for the aquatic stages of the *Anopheles* and therefore has an important role to play in mosquito breeding and malaria epidemiology (Kruiyf *et al.*, 1973). It has the secondary effect of increasing atmospheric humidity and hence increases the longevity of adult mosquitoes. Several attempts have been made in the past, to predict mosquito production and the likelihood of malaria epidemics using rainfall data (Kruijf *et al.*, 1973; Scorza *et al.*, 1981). However, with the advent of large irrigation schemes, the significance of local rainfall has been reduced to some extent, as there are usually small pools of water on the farmlands and in partially dry irrigated canals, suitable for mosquito breeding. (Molineaux, 1988; White, 1974 ; Ijumba and Lindsay, 2001).

The range of temperatures allowing the development of the aquatic and gonotrophic stages of the mosquito varies between vector species. Temperatures of between 20⁰-30⁰C represent an optimal range for most malaria vectors (Molineaux, 1988). Within this range, a rising temperature tends to increase the growth rate of vector populations by shortening the interval between successive ovipositions and the interval between oviposition to emergence (Gillies and Demeillon, 1968). Generally, within limits, the longevity of adult vectors increases with the relative humidity of air over 60% (Pampana, 1963).

Radiation as an ecological factor has a very important influence on *Anopheles* populations, both directly and indirectly. For example, in influencing reproduction and feeding behaviour and through its effect on temperature, in controlling the rate of development of larval stages and the rate of digestion of blood meals (Molineaux, 1988).

2.2.4 Factors Affecting Transmission of Malaria by the Vector

Several factors influence the transmission of malaria. One such factor is the vector density. To be of importance, as a vector of human disease, an insect must be relatively abundant and its habitat, adjacent to human settlement. The population density of a species is largely dependent on its larval ecology (Gillies, 1988). Human activities may play a role in the increased number of vectors in an area. For example, Irrigated fields are favourable breeding grounds to many malaria vectors.

The susceptibility of the vector to infection also affects malaria transmission. This refers to the effectiveness of the vector in acquiring the infection after feeding on an infected person. There is also the need for the parasite to undergo development within the vector. And this period of incubation is necessary before a vector can be infective and is determined by the species of parasite and temperature. At a given temperature, the duration of sporogony increases in the following order: *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium malariae*. (Coatney *et al.*, 1971 ; Shute and Maryon, 1952). Generally, an infective vector remains so for the rest of its life span and lightly infected mosquitoes may discharge all their sporozoites in one or two feeds. (Shute and Maryon, 1952).

There is a wide variation in the ability of different species and geographical strains of vectors to transmit different species and strains of malaria parasites . This ability is

genetically determined (Molineaux, 1988). There is usually co-adaptation between the vectors and parasites from the same geographical areas and this may offer protection from imported parasites. However, vectors like *Anopheles gambiae* s. l, have been found to be effective in transmitting malaria parasites from different geographical areas (Soper and Wilson, 1943; Coatney *et al.*, 1971).

The man-vector contact which is measured as the frequency of biting and is expressed as the man biting rate also plays an important role in transmission of malaria by the vector. The frequency of biting also affects the rate of transmission of the disease. Usually, this must be high enough to sustain transmission. The rate of anthropophily is an important attribute of human disease vectors. The proportion of vectors that feeds on humans is important for transmission to occur in an area. Studies in Southern Ghana showed a higher transmission in an area where 93% of the mosquitoes fed on human compared to another area where about 45% of mosquitoes fed on cattle (Appawu *et al.*, 2001). The fraction of blood meals that is taken on man depends on the availability and accessibility of man and alternative sources of blood. Some vectors may split their blood meals between two or more hosts, and therefore, the probability that a vector acquires a malaria infection is likely to vary with the volume of blood ingested, and also on the amount of saliva (and sporozoites) injected before a second and subsequent bites (Boreham *et al.*, 1979).

Another factor that affects transmission is the longevity of a vector. This can be defined as, the fraction of infected vectors that survive the incubation period and the number of blood meals that they take after that (Molineaux, 1988). The longevity is determined by genetic and environmental factors, in particular the temperature and humidity. A rising

temperature tends to increase transmission by increasing the feeding frequency and shortening the incubation period, but tends to decrease transmission by reducing longevity (Detinova, 1962).

Sympatric vectors may differ significantly with respect to one or more of the factors mentioned above. For example, in an area of sympatry of *Anopheles gambiae* and *Anopheles melas* in The Gambia, both reached similar densities but *Anopheles gambiae* had much higher sporozoite rates and this was attributed to the greater longevity and anthropophily of *Anopheles gambiae* while susceptibility to infection was the same (Bryan, 1983). The combination of several of these factors determines the suitability of a mosquito as a vector of malaria.

2.3 ESTIMATION OF MALARIA TRANSMISSION PARAMETERS

2.3.1 Entomological Inoculation Rate

The intensity of malaria parasite transmission is best expressed as the entomological inoculation rate (EIR), which is defined as the number of infective mosquito bites received by a person during a certain period of time. (Bier *et al.*, 1999; Hay *et al.*, 2000).

The magnitude of the EIR is influenced by the rate at which infective vectors feed on humans, which is largely dependent on the mosquito density and to some extent, the feeding habits of the vector species (Shilulu *et al.*, 1998). The EIR is determined by multiplying mosquito-biting rates by the proportion of mosquitoes carrying sporozoites in their salivary glands (Hee-II Lee *et al.*, 2001). It is expressed in terms of the average number of infective bites per person, per unit time (Beier *et al.*, 1999; Hay *et al.*, 2000 ; Hee-I Lee *et al.*, 2001). The precise and specific measurement of EIR is important in

quantifying the potential risk of malaria infections and also in numerically quantifying the dynamics of transmission. Such information is essential in the epidemiological assessment and control of malaria. (Molineaux *et al*, 1988 ; Onon and Grab , 1980).

Mathematical models are currently being developed to help gain a better understanding of the dynamics of malaria transmission (Koella, 1991) and for predicting the potential impact of malaria control methods on EIR (Killeen *et al.*, 2000). Even though these models may be accurate and simple in their understanding and application, it is necessary that they are based on meaningful values of critical parameters obtained from the field (Service, 1977). Therefore, the accurate assessment of the man biting rate as well as the distribution of age, infection and other physiological properties within the biting population is important, as these are critical determinants of EIR (Molineaux *et al*, 1988). EIR for *Plasmodium falciparum* has not been estimated in most malaria endemic countries in Africa (Hay *et al.*, 2000). The identification of entomological variables in different malaria areas such as prevalent species, density, seasonal variation, larval habitats and EIRs may assist in the development of effective malaria control programmes (Adenildo *et al.*, 2002).

2.3.2 Man Biting Rate

The man-biting rate of mosquitoes is an essential component of the EIR, an important concept for describing and comparing transmission intensities in entomological terms (Burkot and Graves, 1995). Measuring biting rates therefore constitutes a very important aspect of entomological monitoring of vector control interventions, such as insecticide treated nets (Service, 1993). The most direct and reliable method for estimating the man

biting rate is the human landing catches (HLC), whereby a team of people act as baits and collect mosquitoes coming to bite them (Service, 1993; Mathenge, 2002). The HLC method of mosquito collection which would be discussed in more detail shortly has problems associated with it and objections to its use and this has led to a search for surrogate methods such as Pyrethrum spray catches, Light trap catches and collections in man baited traps which can have the potential of providing reliable estimates of man biting rates. (Lines *et al.*, 1991).

2.3.3 Sporozoite Rate

The sporozoite rate is an important epidemiological parameter for the assessment of malaria transmission. This is usually measured as the proportion of female mosquitoes with their salivary glands infected with malaria parasites in a given sample (Mboera and Magesa, 2001). The understanding of the entomological aspects of the epidemiology of malaria depends in part, on the accurate assessment of this rate, which is generally estimated by salivary gland dissection for the presence of sporozoites or by the presence of circumsporozoite protein by Enzyme-Linked immunosorbent Assay (ELISA) (Beier *et al.*, 1999; Molineaux *et al.*, 1988).

2.4.0. MOSQUITO SAMPLING METHODS

Insect distributions are generally aggregated, that is, individuals are more clustered than would be expected if a random distribution applied (Southwood, 1978). This clustering occurs as a result of biological and environmental factors (Magbity and Lines, 2002).

Several sampling methods have been used to estimate the population density of mosquitoes searching for a blood meal. The methods commonly used include the Human Landing catches, Pyrethrum spray catches, and Light trap catches (Service, 1993).

2.4.1. Light Trap Catches (LTC)

Light traps are mechanical devices that attract mosquitoes to light, a phenomenon that may not be directly associated with the act of feeding on humans (Mathenge *et al.*, 2002). The main principle behind the use of light traps as a collection method is that, mosquitoes that have come into the room in search of a blood meal get attracted to the light in the trap and are sucked in by a fan and therefore any mosquitoes captured by the trap can be taken as those that would have bitten the occupants of the room. The use of light traps for sampling mosquitoes has the advantage of trapping mosquitoes in search of a blood meal and therefore can be used to estimate man-biting rates. It also enables comparisons to be made from different traps in different geographical locations. In addition to this, light traps can be used to sample mosquitoes throughout the night.

The New Jersey mosquito light trap evolved in 1932 and has been until recently the most widely used light trap for mosquito sampling. Several modifications have been made including the ultra violet light trap and the CDC miniature light traps. Light traps have been widely used for the routine sampling of Culicine mosquito populations and for the study of Culicine vectors of viral diseases. They have also been used especially in Africa for collecting species of mosquitoes such as *Anopheles gambiae* but have not however been adopted in malaria eradication studies (WHO, 1975).

The Light trap method of sampling mosquitoes gives information on seasonal incidence of endophilic *Anopheles*. The adults can also be processed for sporozoite rates and the unfed mosquitoes, used for parity dissections.

Light traps have however been used in the sampling of man-biting mosquitoes with varying degrees of success (Odetoyinbo, 1969; Chandler *et al.*, 1975). One of the disadvantages of using light traps to sample mosquitoes is that, they collect a wide variety of insects as well as mosquitoes and therefore, much labour is required to sort for the mosquitoes. There is also the tendency for the mosquitoes collected to die and dry up, causing them to lose some of their parts. This makes identification and their dissection difficult.

Light traps underestimate the relative abundance of some species of *Anopheles* (Zaim *et al.*, 1986; Le Goff *et al.*, 1993 ; Hii *et al.*, 2000) and they have been found to have a bias in sampling older or gravid mosquitoes (Carnevale and Pont, 1973 ; Mbogo *et al.*, 1993 ; Hii *et al.*, 2000).

Modifications have been made in recent years by hanging light traps beside untreated bed nets occupied by human subjects and this increases the efficiency of sampling host seeking mosquitoes because, the attraction of the host seeking to the person inside the bed net increases the chance of being captured by the light trap (Lines *et al.*, 1991; Mbogo *et al.*, 1993 ; Mboera *et al.*, 1998). This method has however been shown to have a bias towards sampling specific cohorts of mosquitoes (Mbogo *et al.*, 1993). Also, the catch size and parity rates may differ according to the trap position relative to the host occupying the bed net (Mboera *et al.*, 1998).

2.4.2 Human Landing Catches (HLC)

This method involves the collections of *Anopheles* mosquitoes landing on human ‘in an attempt to feed.. It is considered the most representative and usually considered as the “Gold standard”, for determining human biting activity (WHO, 1975). The principle behind the use of this method is that, humans act as baits to attract blood-seeking mosquitoes. Human landing catches gives information on the different *Anopheles* species biting people either indoors or outdoors.. The biting activity throughout the night can be obtained and hence the times of maximum exposure to malaria transmission. The method can also be used to estimate the man biting rate per night and season and adults caught can be dissected to determine parity and for the calculation of sporozoite and inoculation rates (Le Goff *et al.*, 1993; Service, 1976 ; WHO,1975).

The HLC has two variations where either the collector himself can act as the bait; by sitting on a small stool with bare legs on which he catches the mosquitoes as they come to bite or, the collector catches the mosquitoes on a local inhabitant or a member of the collecting team who acts as a bait. In this case, the one acting as bait may lie down or even sleep. Normally all night collections start at sunset and stop at sunrise. These collections are made both indoors and outdoors depending on the behaviour of the local inhabitants. In some tropical areas, the period between 18:00hours and 06:00 hours represent the main period of biting activity and movement of majority of local vectors. The environmental conditions at the time of collection such as wind-speed, rainfall, moonlight, temperature and humidity all have influence on the movement of mosquitoes and therefore their biting activity (WHO, 1975; Service, 1976).

The unique advantage of the method is that it directly samples the mosquitoes as they land to bite humans and therefore the samples obtained can be projected to represent mosquitoes for the transmission of malaria (Mathenga *et al.*, 2002).

One of the disadvantages of using HLC is that, the rate at which a mosquito approaches and attacks an exposed person performing HLC may be higher than under normal conditions. This is because, the baits in HLC are relatively more available to host seeking mosquitoes than if they were under normal circumstances, that is, either asleep (covered with bedding) or more active instead of posing as baits (Mathenge *et al.*, 2000). This method therefore over estimates the biting rate although it is considered as the most representative measure of man biting rates of mosquitoes (Lines *et al.*, 1991). There is also the problem of a variation in the attractiveness of the individual collectors to vectors and their ability to catch mosquitoes (Knols *et al.*, 1995; Lindsay *et al.*, 1993). To reduce the effect of this variation, the collectors are rotated at regular intervals to include indoor and outdoor catching (Shadrawi *et al.*, 1974). Another disadvantage in the use of this method is that, it is labour intensive and requires working throughout the night and difficult to supervise. Usually, the collectors break for some minutes to stretch up and change stations for assembled cups for the next hours catch. An alternative is that, a group collects from 18:00 hours to midnight and is replaced by another group till 06:00 hours (Service, 1977; Trape, 2001).

The use of human baits also raises an ethical problem of the deliberate exposure of collectors to mosquito bites, thereby increasing the risk of contracting malaria (Magbity *et al.*, 2001). To reduce the risk of getting malaria, local inhabitants are used as collectors and those who show any symptoms of malaria are treated.

2.4.3 Pyrethrum Spray Catches (PSC)

Pyrethrum spray sheet collection (*PSC*) is used to collect indoor resting mosquitoes after knockdown by space spraying of a pyrethrum solution. The main principle of the method is that, mosquitoes tend to rest after feeding and therefore any mosquitoes captured with this method might have fed on people in the room or were not able to feed in the night and were resting (Molineaux, 1988). This method has been used as a standard quick and easy method of collecting mosquitoes resting in huts and animal shelters. (Service, 1993). It collects mosquitoes of varying physiological conditions, gives valuable information on relative changes in seasonal abundance of endophilic vectors, provides adults for determining sporozoite and inoculation rates and information on host preferences and degree of exophily. The PSC also provides unfed adult mosquitoes for dissections to determine parity and half gravids for chromosomal identifications (Appawu *et al.*, 2001; Service, 1976; WHO, 1975).

PSC is not repeated in the same hut or room at short intervals as some repellent effect of the pyrethrum extract might persist for some days (Service, 1993; WHO, 1975).

This method has several advantages, such as collection of large numbers of mosquitoes. In addition, PSC is faster and does not require many workers and skill is not very important.

Unlike HLC, estimation of man biting rate from PSC is done indirectly since the mosquitoes are not caught directly biting. The man-biting rate is estimated by dividing the number of blood-engorged females by the number of bedroom occupiers. What is obtained is based on the assumption that exophily, zoophagy and exophagy have been excluded (Service, 1976). Not all the mosquitoes might have fed on humans and therefore

the value obtained is usually multiplied by a factor known as the human blood index. The human blood index is defined as the proportion of *Anopheles* that had fed on human blood. Also mosquitoes captured using the method may not represent all the mosquitoes in the room since some may escape through eaves and others may fall in places where they cannot be collected.

Many studies on malaria vectors have been undertaken using HLC and PSC as the methods for mosquito sampling. It has been reported that that *Anopheles gambiae* s.l was the most predominant species in both coastal forest and coastal savanna areas followed by *Anopheles funestus* in the coastal forest area and *Anopheles pharoensis* in the coastal savanna area using both methods (Appawu *et al.*, 2001). They also reported that PSC sampled more mosquitoes than HLC from both the coastal forest and the coastal savanna; it sampled 77% and 58% of the mosquitoes collected from these areas respectively.

A comparative study of HLC and PSC in a Sudanese Savanna area from April 1996 to March 1997 found the mosquitoes collected using PSC to be 1.9 times higher than HLC. (Dia *et al.*, 2002). Awolola *et al.* (2002) conducted studies in South Western Nigeria using both HLC and PSC. They observed that, of the female *Anopheles* collected, 37.3% were from HLC and 62.7% from PSC. They also observed an overall *Anopheles* collection in the wet season to be significantly higher than the number collected in the dry season. In that study, there was no statistical significance in the sporozoite rates of mosquitoes caught by both methods. And that, the percentage of mosquitoes positive for the *Plasmodium falciparum* circumsporozoite fell from 2.9% in the wet season, to 1.8% in the dry season.

Studies on the bionomics of some species of *Anopheles* in Ngari, Senegal, using HLC and PSC revealed that *Anopheles gambiae* was the most captured species, accounting for 48.81% and *Anopheles funestus* accounted for 17.16%. From PSC, *Anopheles gambiae* was also predominant (92.5%) and the proportion of species by the two sampling methods was statistically different with PSC recording higher numbers. *Anopheles gambiae*, *Anopheles arabiensis*, *Anopheles funestus* and *Anopheles nili* collected were infected with *Plasmodium falciparum* (Dia *et al.*, 2003).

Using the two methods in another study in Senegal, Robert *et al.*, (1998) reported sporozoite rates of 1.6% and 1.8% from PSC and HLC respectively with no statistical difference. Estimations of sporozoite rates and EIRs, in an irrigated area in Western Kenya by Githeko *et al.*, (1993) showed the EIRs using PSC to be a more realistic index of EIR.

CHAPTER THREE

MATERIALS AND METHODS

3.1. STUDY SITE

Mosquito collections were carried out in the Kassena Nankana district located in the Upper East Region of Ghana. It covers about 1,674 kilometers in the Sahelian Savannah. The average annual temperature ranges from 18C to 45C with an annual rainfall of 850mm, all of which almost occurs in the rainy season from May-September with the rest of the year being relatively dry. The Navrongo demographic surveillance system (NDSS) is used by the Navrongo Health Research Center to monitor the population dynamics of the study area. Another important feature of this system is that, it has a geographical information system where all the compounds in the study area has been mapped and numbered systematically making the location of compounds easy.

The district has a population of about 140,000 living in roughly 14,000 compounds (Binka et al, 1999). The population is mainly rural with the exception of about 20,000 people who live in the Navrongo town, the administrative capital. The main occupation of the people is subsistence farming of millet, sorghum and livestock. Most of the people live in multi-family compounds, which form the basis of the address system used by the Navrongo Demographic Surveillance System. The houses are built with mud and roofed with thatch. There is a large water reservoir (Tono dam) situated in the district. This study was carried out in the irrigated areas of the district, which includes areas around the Tono dam. Figure 3 shows the map of the Kassena Nankana District where the study was conducted.

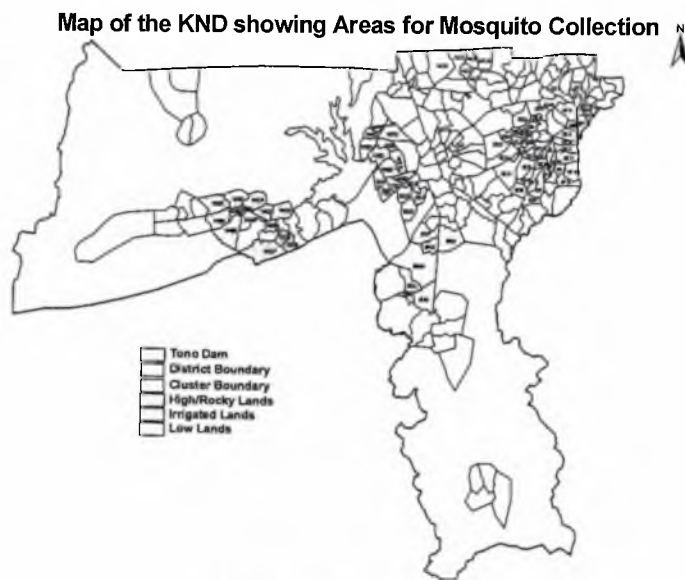


Figure 3.

3.2. Mosquito Collection

Compounds visited for mosquito collection were selected randomly using the Navrongo Demographic Surveillance System. Mosquitoes were collected once weekly from two different compounds for the months of July to September 2003 during the wet season and dry season samples were collected from January to March 2002 from the irrigated clusters in the district using HLC and PSC. Mosquito samples were collected from the irrigated area shaded green in Figure 3.

3.2.1. Human landing collections (HLC)

Eight trained collectors with a supervisor collect mosquitoes once a week from two randomly selected compounds, in the irrigated zone of the district. At the selected compound, two collectors sat indoors and two others sat outdoors for 50 minutes of each of the hours between 1800hrs and 0600hrs to aspirate mosquitoes, which will land on the exposed legs with the help of flashlights.

In order to compensate for differences in individual attractiveness or repulsiveness to mosquitoes and as a precaution against dozing and inappropriate techniques, the two teams of collectors rotated between indoors and outdoors hourly after taking ten minutes break. In addition, the supervisor made surprised visits throughout the night for quality assurance and to gather the mosquitoes collected.

The captured mosquitoes were placed into paper cups covered with nets to prevent the mosquitoes from escaping and labeled according to the location, date of collection, site of collection (indoors or outdoors), collector's name, hour of collection. The collected mosquitoes were placed upright in specially made wooden boxes and transported to the

laboratory. Pieces of wet cotton wool were placed over the tops of the cups to maintain the mosquitoes at an appropriate humidity. To prevent ants from reaching the captured mosquitoes, the supervisor ensured that the box containing the cups was kept far from areas where they could be found.

3.2.2. Pyrethrum Spray Collections

On the days that the human landing collections were performed, rooms from the same compound were selected for pyrethrum spray collection. The spraying is done early in the morning between 06:00hours and 07:00hours. The inhabitants of the selected rooms were made to cover any food and stored water. The floors of rooms were then covered completely with white cotton sheets and sprayed with Raid which contains 0.15%Tetramethrin, 0.25% Allethrin, and 0.015% Delmethrin, produced by Johnson Wax European BV Holland. One of the collectors sprays the eaves, if any from outside to prevent mosquitoes from escaping out of the rooms. After ten minutes the door is opened for the mosquitoes to be collected. The white sheets were removed gently, lifting them and moved gently out of the rooms for the mosquitoes to be picked into petri dishes lined with wet cotton wool for laboratory examination and analysis. The number of people who slept in the sprayed room during the night was recorded.

3.3.00. Processing of Mosquitoes

3.3.1. Mosquito Identification

Mosquitoes captured from the human landing activities were killed with chloroform and the female *Anopheles* were sorted out from other genera and identified morphologically to species (Gilles and De Meillon, 1968). Mosquitoes were put into tubes(5 mosquitoes of

the same species per tube) and labeled according to species, physiological states of abdomen, area and hour of collection. They were kept dry with a desiccant until needed.

In the same way, mosquitoes obtained from the pyrethrum spray method were sorted and placed into zip lock bags and kept in the laboratory

3.3.2. Determination of Sporozoite Infectivity

The head and thorax of individual mosquitoes were separated from the abdomen by means of a forceps and blade and the separated parts were put into different tubes. These tubes were labelled, according to species, and collection method. For the mosquitoes collected using PSC, only the blood feds were processed for the ELISA assays.

Circumsporozoite Enzyme Linked Immunosorbent Assay (ELISA)

The head and thorax of individual female *Anopheles* mosquitoes were homogenized in a total of 250µl of grinding buffer (PBS, pH7.4 containing 0.5% NP-40 and 0.5% casein) using a glass pestle. *Plasmodium falciparum* circumsporozoite protein (PfCSP) micro-plate ELISA utilizing 50µl/well of the homogenate was carried out in 96-well micro-titre plates coated with anti-*P. falciparum* monoclonal antibodies at 22-25°C for 30 minutes (Wirtz *et al.*, 1987). Captured PfCS antigen was revealed by monoclonal antibody (MoAb) horseradish peroxidase conjugate incubated for 1 hour. Addition of ABTS (2,2'-azino-di-(3-ethylbenzthiazoline-6-sulfonate) substrate gave a green colour reaction for positive results which were read by visual assessment of the colour reactions, and optical densities (OD) measured within 30min using spectrophotometer (Multiskan Ascent, Model 354, ThermoLabsystems, Finland) at 414nm. Sample positivity was determined by titration of PfCSP positive control antigen using cut off OD values equivalent to 12pg of PfCSP or 50 sporozoites (Collins *et al* 1988).

3.4. ANALYSIS

Data obtained was analyzed with Microsoft Excel. The parameters for estimating malaria transmission were determined as follows:

The man-biting rate (MBR) from HLC was calculated as the number of mosquitoes caught biting /Number of collectors x Number of captures

The MBR for PSC as the Number of blood fed mosquitoes collected x human blood index/Number of sleepers X Number of captures). The human blood index was assumed to be = 1 (that is, mosquitoes had fed on humans).

The Sporozoite rate (SR) was calculated as the number of mosquitoes that were positive for *P falciparum* circumsporozoite protein (PfCSP)/Total number of mosquitoes tested

(3.)The Entomological Inoculation Rate (EIR) was calculated as the product of the man-biting rate and sporozoite rate.

3.5. ETHICAL CONSIDERAIIONS

Informed consent was obtained from heads of compounds and owners of rooms of selected compounds after carefully explaining the study to them to allow the use of their rooms. Although the field workers were trained to efficiently catch mosquitoes that landed on them before they could bite, any field worker who complained of sickness was sent to the district hospital for proper diagnoses and treatment. The study area is also endemic for filariasis and is a beneficiary of the yearly mass Albendazole and Ivermectin

treatment program of the Ministry of Health. Field workers who had not taken the drugs were made to do so before the commencement of work.

CHAPTER FOUR

RESULTS

4.0 Species of *Anopheles* captured by Human landing catches (HLC) and Pyrethrum Spray (PSC)

A total of 17019 *Anopheles* mosquitoes were captured using both collection methods. Out of this, 56.4% (9594) was captured by HLC and 43.6% (7425) by PSC (Table 1). Using Human landing catches with a total of 192 man-nights; HLC contributed 59.8% (6270) of *Anopheles gambiae s.l.* of the catches, whilst Pyrethrum spray catches made up 40.2% (4220). On the other hand, *Anopheles funestus* constituted 51.4% (3078) of the PSC, while HLC made up the remaining 48.6% (2914) (Table 1). Significant numbers (98.5%) of *An. pharoensis* were collected by HLC compared to the PSC whereas the reverse was true for *An. rufipes*.

Table 1 Number of *Anopheles* mosquitoes captured by Human Landing Catches and Pyrethrum spray catches in the irrigated areas of the KND

Species	Human Landing Catches		Pyrethrum Spray Catches		Total	
	NO	%	NO	%	NO	%
<i>An. gambiae</i>	6270	59.8	4220	40.2	10490	100
<i>An. funestus</i>	2914	48.6	3078	51.4	5992	100
<i>An. pharoensis</i>	394	98.5	6	1.5	400	100
<i>An. rufipes</i>	16	11.7	121	88.3	137	100
Total	9594	56.4	7425	43.6	17019	100

More than 70% of both *An. gambiae* and *An. funestus* were collected by HLC during the wet season (Table 2). Similarly, PSC produced over 66% of both species during the wet season compared to the dry season (Table 3). *An. pharoensis* and *An. rufipes* were collected by both methods during the seasons. PSC captured more *An. rufipes* than HLC whereas HLC captured more *An. pharoensis* than PSC and most of them were collected during the dry season.

Table 2 Number of *Anopheles* mosquitoes captured by Human Landing Catches during the wet and dry seasons

Species	WET		DRY		Total
	NO	%	NO	%	NO
<i>An. gambiae</i>	4577	73.0	1693	27.0	6270
<i>An. funestus</i>	2119	72.7	795	27.3	2944
<i>An. pharoensis</i>	120	30.5	274	69.5	394
<i>An. rufipes</i>	7	43.8	9	56.3	16
Total	6823	71.1	2771	28.9	9594

Table 3 Number of *Anopheles* mosquitoes captured by Pyrethrum Spray Catches during the wet and dry seasons

Species	WET		DRY		Total
	NO	%	NO	%	NO
<i>An. gambiae</i>	2824	66.9	1396	33.1	4220
<i>An. funestus</i>	2305	74.9	773	25.2	3078
<i>An. pharoensis</i>	5	83.3	1	16.7	6
<i>An. rufipes</i>	1	66.9	120	99.2	121
Total	5135	69.2	2290	30.8	7425

4.1 Man biting rates of anophelines captured using HLC and PSC

Man biting rates estimated for *Anopheles* captured by HLC was 44.4 bites/man/night whilst that for PSC was 1.8 bites/man/night. Biting rates estimated from HLC during the wet season were higher and statistically significant ($P < 0.05$) from that of the dry season (60.9 versus 26.6), however, biting rates from PSC during the wet and dry seasons were not statistically significant ($P > 0.05$). Both collection methods showed that *Anopheles gambiae* was the most dominant biting mosquito during the wet and dry seasons with rates significantly higher ($P < 0.05$) than that of *Anopheles funestus*. The man biting rates for *Anopheles gambiae* were 40.87 and 16.28 for the wet and dry seasons respectively using HLC. While using PSC, they were estimated as 1.35 and 1.88 for the wet and dry seasons respectively. Both methods showed statistical differences ($P < 0.05$) in the biting rates for the two seasons. Estimated man biting rates for the wet and dry seasons for *Anopheles funestus* were 18.92 and 7.64 respectively, using HLC and 0.87 and 0.74 for wet and dry seasons respectively for PSC. . There were significant differences ($P < 0.05$) between the values obtained for *Anopheles funestus* for the two seasons using both methods.

Monthly biting rates of *Anopheles* estimated by HLC and PSC during the wet season increased progressively from July and peaked in September as shown in Figures 4 and 5. However, biting rates estimated by HLC during the dry season, showed almost similar biting rates for the months of February and March whilst biting rates estimated from PSC indicated high biting in the month of March (Figure 4 & 5).

Figure 4 Man biting rates estimated from PSC during the wet season in KND

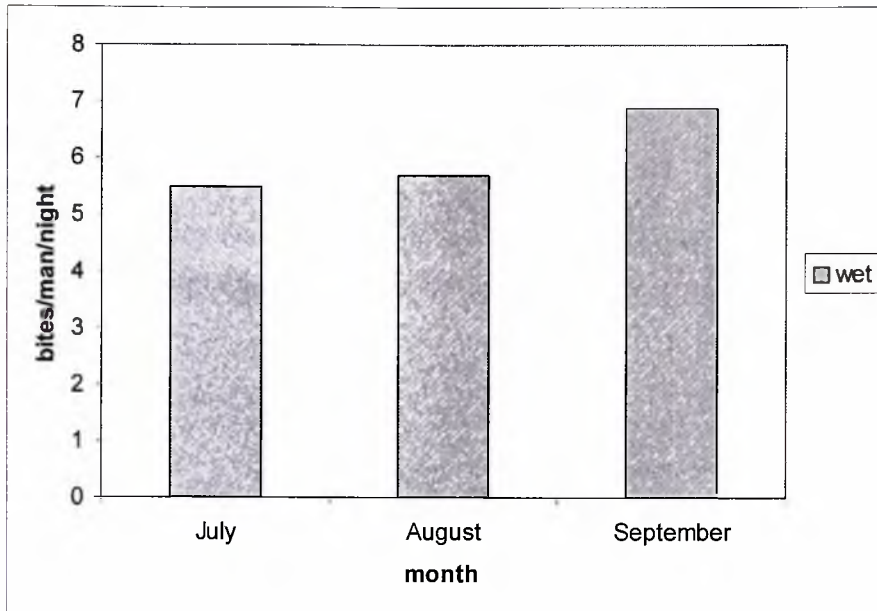


Figure 5 Man biting rates estimated from HLC during the wet season in KND

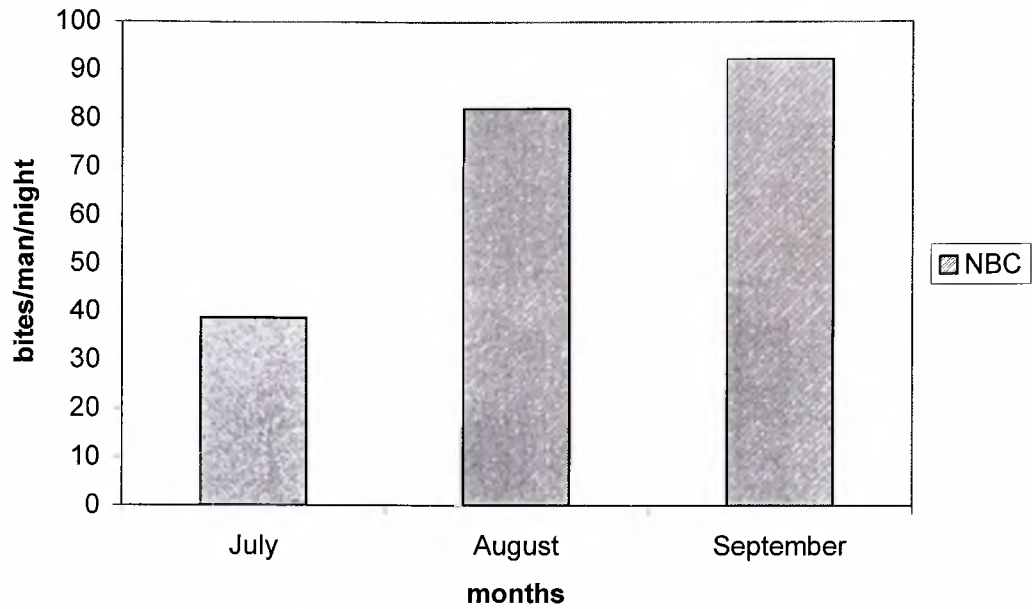


Figure 6 Man biting rates estimated from PSC during the dry season in KND

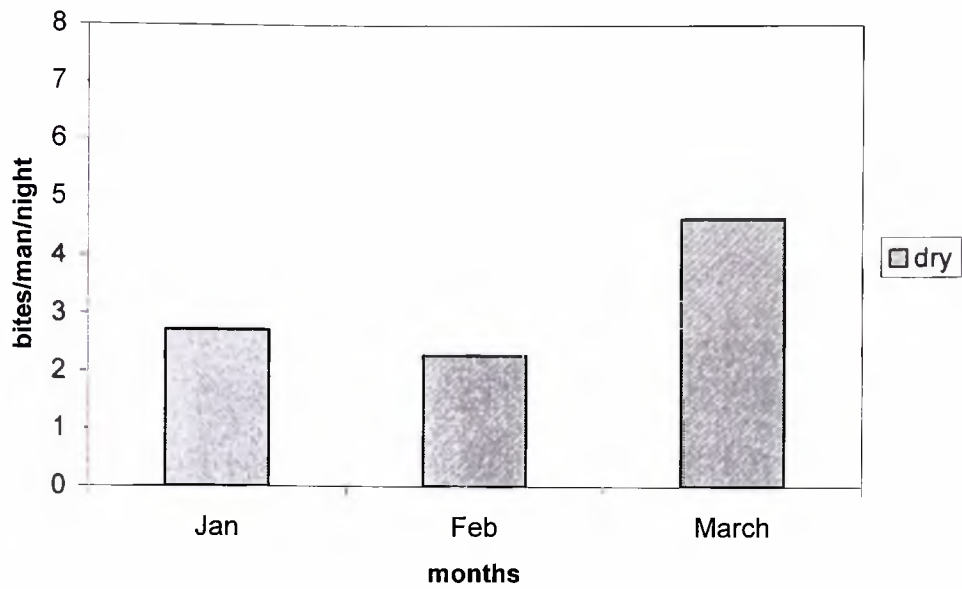
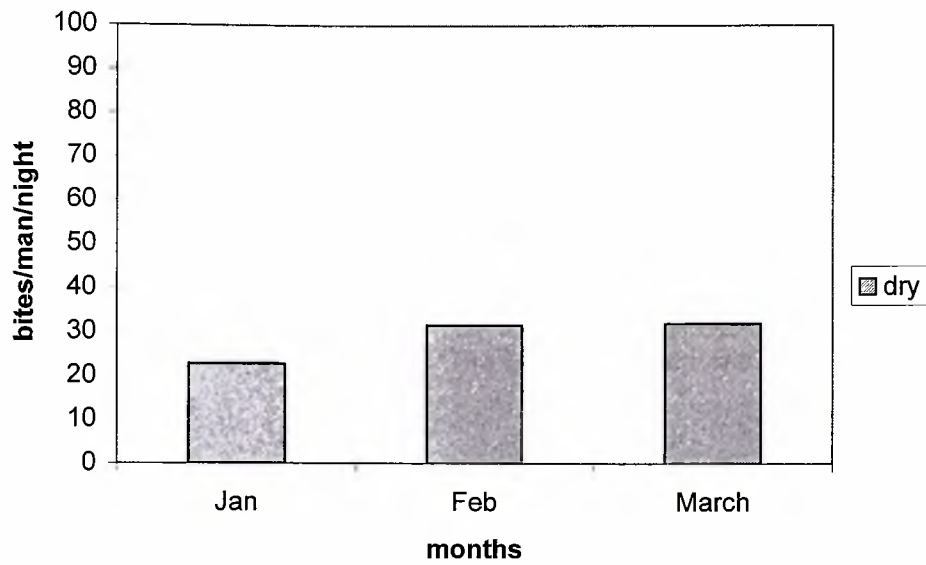


Figure 7 Man biting rates estimated from HLC during the dry season in KND



4.2 Sporozoite infectivity

Plasmodium falciparum sporozoite rates of vectors captured by the different methods (HLC and PSC) from the study area are shown in Table 3. The overall sporozoite rate estimated using HLC was 4.9% (204/4353) while that for PSC was 7.1% (29/407). The rate for PSC was significantly higher ($P < 0.05$) than that of HLC. During the wet season the sporozoite rate estimated using HLC, 6.0% (189/3176) was significantly higher ($P < 0.05$) than the dry season, 1.3% (15/1177). However, the sporozoite rates estimated during the dry and wet seasons using PSC samples gave similar rates, for 7.0% (11/143) and 6.0% (18/264) respectively (Table 4). The rates of *Anopheles gambiae*, 4.7% and *Anopheles funestus* estimated during the dry and wet seasons were statistically significant when for both methods.

No positive mosquitoes were detected from samples collected in March using HLC, but some positive mosquitoes were detected using PSC method in the same month.

4.3 Entomological Inoculation Rates (EIR) estimated from HLC and PSC

The entomological inoculation rate, which indicates intensity of transmission, was obtained from the product of the man-biting rate (MBR) and sporozoite rate (SR). EIR per year using HLC was 123.9 and 1323.0 infective bites/ man/ year for the dry and wet seasons respectively while, it was 34.3 and 52.3 respectively using PSC.

There were also seasonal differences in the EIRs estimated for *Anopheles gambiae* and *Anopheles funestus* using the two methods as shown in Table 5. *Anopheles gambiae* had an EIR that was almost 10 times higher than that of *Anopheles funestus* (926 versus 92.3 infective bites per year) in the dry season when the estimation was done using HLC.

However, with PSC, EIR for *Anopheles funestus* was 1.4 times higher (44.8 versus 30.88) than that of *An. gambiae* as shown in Table 5. During the wet season, EIR estimated using HLC for *Anopheles gambiae* and *Anopheles funestus* were similar (731 vrs 517.9) whereas that estimated using PSC was higher for *Anopheles gambiae* than *Anopheles funestus* (36.0 vrs 13.0).

Table 4 Transmission parameters estimated for Human Landing catches and Pyrethrum Spray catches in the irrigated area of KND during the wet and dry seasons

Parameters	Human Landing Catches		Pyrethrum Spray Catches	
	WET	DRY	WET	DRY
Numbers captured	6696	2488	5129	2169
Man biting rates ^a	60.9	26.6	2.2	1.3
Sporozoite rates	0.059	0.013	0.065	0.074
EIR/year ^b	1323	123.94	52.25	34.39

a –bites /man/night

b –infective bites/man/year

Table 5. Seasonal changes in the entomological inoculation rates (EIR) of *Anopheles gambiae* and *Anopheles funestus* sampled using HLC and PSC.

Species	WET		DRY	
	HLC	PSC	HLC	PSC
<i>An. gambiae</i>	731.0	36.0	926.0	30.9
<i>An. funestus</i>	517.9	13.0	92.3	44.8

CHAPTER FIVE

5.0 DISCUSSION

The most direct and reliable method for estimating the man-biting rate is the human landing catches (HLC) (Service, 1993; Mathenge, 2002). It is considered the most representative and usually considered as the “Gold standard”, for determining human biting activity (WHO 1975). However, of recent there have been concerns about the ethics of using humans as baits to collect mosquitoes and therefore the need for an alternative method is urgent. This study was designed to compare Human Landing and Pyrethrum Spray methods in estimating malaria transmission intensity in an irrigated area in Northern Ghana.

The study identified *Anopheles gambiae* and *Anopheles funestus* to be the vector species in the study area, using both HLC and PSC methods. However, *Anopheles gambiae* was the most dominant. This is similar to observations made in studies conducted in two ecological zones in the coastal parts of Southern Ghana using the same sampling methods (Appawu *et al.*, 2001).

HLC captured a higher number of mosquitoes than PSC. This is different from what was reported by Appawu *et al.*, 2001 but similar to findings from a study in coastal Lagos (Awolola *et al.*, 2002). In real terms, the total numbers captured by the HLC will depend on the total man-nights put in to capture mosquitoes and the numbers from PSC will also depend largely on the number of rooms sprayed per period and the number of times sprayed. Thus it is really difficult to compare the numbers collected by the two methods since they will be influenced by the factors mentioned. Whereas PSC sampled more

Anopheles rufipes (94.2%) compared to HLC, most of the *Anopheles pharoensis* were collected by HLC. This suggests that *Anopheles rufipes* were not readily biting the human collectors but could be more endophilic while *Anopheles pharoensis* exhibits higher level of anthropophily. This finding was consistent with Appawu *et al.*, 2001 in studies in southern Ghana.

There was a progressive increase in both the numbers and biting rates of *Anopheles gambiae* and *Anopheles funestus* in the wet season between July and September using both methods. As the rainfall increased from July onwards, more breeding sites were created thus causing the increase in mosquito numbers. Similar observations have been made in Sierra Leone (Buckarie *et al.*, 1994), Nigeria (Awolola *et al.*, 2002) and Senegal (Diatta *et al.*, 1998). The irrigation scheme in this study area also played a role in the variations in numbers and biting rates of *Anopheles* that were collected from the area during the dry season, by making surface water readily available when there were no rains.

The EIR estimated from HLC was higher than that from PSC but the pattern of variation between the wet and dry seasons were similar for the two sampling methods. In areas without irrigation systems, man biting and sporozoite rates may be difficult to estimate using HLC during the dry season because of relatively lower numbers of mosquitoes biting.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.0 CONCLUSIONS

In conclusion, the study provides information on the comparative use of HLC and PSC in vector transmission studies, which is necessary for the planning and implementation of future vector control strategies. The study also recommends that PSC can be used as a surrogate method to replace HLC in estimating transmission intensity. Although the estimated values using the two methods may vary considerably, the pattern will be similar. In areas where mosquito numbers may be very low, for example, in the dry season, PSC may be useful in the assessment of transmission intensity. However, further studies are needed on the suitability of PSC in areas not influenced by irrigation schemes.

6.1 Recommendations

1. This study should be extended to the non-irrigated areas of the district so that a more accurate comparison can be made
2. Pyrethrum Spray Catches can be used in the study area to assess malaria transmission parameters in the study area.
3. The high inoculation rates measured in the study confirms that malaria is endemic in the area and therefore the introduction of vector control methods such as insecticide treated materials and Biological control is recommended.

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PLASMODIUM CIRCUMSPOROZOITE ELISA

- (1) Coat MAb [50µl/well (96-well plate)] 30min, RT or 4° overnight .
- (2) Flip plate empty and bang.
- (3) Fill wells with blocking buffer (BB); 200µl/well, 1hr, RT.
- (4) Flip plate empty, bang and incubate with test mosquito extracts and controls (50µl/well; RT, 2hr).
- (5) Wash plate twice with washing buffer (PBS/Tw-20), flip empty and bang.
- (6) Incubate MAb-peroxidase conjugate (0.05µg/50µl/well) in BB; 1hr; darkness.
- (7) Flip plate empty, ^{wash 5x}rinse ~~2x~~ and bang
- (8) Incubate substrate [100µl/well; (30-60min)*], RT
(30-60min)*: Start reading plate after 30min and finish by 60th min.

MOSQUITO/PLASMODIUM ANTIGEN (MPA) PREPARATION

(a) *Grinding Solution (GS)*

- (1) Mix blocking buffer (BB) and Nonidet P-40 (NP-40) in the ratio of 5 μ l NP-40: 1ml BB (store at 4°C)

(b) *MPA Preparation*

- (1) Put test mosquito sample in a pre-labelled 1.5ml micro-centrifuge (Eppendorf) tube containing 50 μ l GS.
 - (2) Grind with a pestle to obtain a homogenous suspension.
 - (3) Rinse pestle with more GS onto the suspension to obtain a total of 200 μ l of MPA suspension.
- * MPA extract can be tested immediately or stored frozen.

PHOSPHATE BUFFERED SALINE (PBS)

(a) *Calibration of flask*

- (1) Pour 1,2 or 3 litres of tap water into the flask.
- (2) Gently slide a stirring rod **to be used** along the side into the flask.
- (3) Mark the final volume of water in the flask.

(b) *Preparation of buffer*

- (i) Dulbecco's PBS (DPBS), Sigma No. D5773 pH 7.4
- (1) Discard the water in (a) above and rinse flask with distilled water.
- (2) Pour about 750ml of distilled water into the calibrated flask.
- (3) Rinse stirring rod with distilled water and slide it into the flask.
- (4) Place flask on an electronic magnetic stirrer and stir.
- (5) Empty one bottle of DPBS powder into flask while content is stirring.
- (6) Rinse bottle thoroughly and add to content of the flask.
- (7) Adjust pH according to the manufacturer's specification if necessary.
- (8) Add more distilled water to the content of the flask to the level of the calibrated mark. Store at 4°C; Shelf life-2weeks

Usually 1 bottle of DPBS prepares 1 litre of solution. Refer to the manufacturer's specification.

(ii) Ordinary Laboratory PBS pH 7.4

Reagent	Amount in			
	1.0 litre	5.0 litre	10.0 litre	20.0 litre
NaCl	8.0g	40.0g	80.0g	160.0g
Na ₂ HPO ₄	1.4g	7.0g	14.0g	28.0g
KH ₂ PO ₄	0.2g	1.0g	2.0g	4.0g
KCl	0.2g	1.0g	2.0g	4.0g
Na Benzoate	0.1g	0.5g	1.0g	2.0g

- (1) Pour about 600ml of distilled water into the calibrated flask and slide the stirring rod into it.
- (2) Place flask on an electronic magnetic stirrer and stir.
- (3) Add amount of salts for preparation of 1.0 litre solution (check table above).
- (4) Adjust pH to 7.4 by adding HCl dropwise.
- (5) Add more distilled water to the level of the calibrated mark.

(iii) Washing buffer [PBST(tween)]

PBST = PBS plus 0.05% Tween 20

Preparation

- (1) Follow the usual procedure for preparation of PBS above to the stage of pH adjustment.
 - (2) Add 0.5ml Tween 20 (to 1 litre PBS) while stirring.
- NB: For prompt dissolution, Tween should be released slowly (a little at a time, but continuously) into the content of the flask.
- (3) Add more distilled water to the level of the calibrated mark.

(iv) Blocking Buffer (BB)

BB can be mixed in two ways (A or B)

(A) *Boiled casein BB*

Reagent	Amount in	
	0.50 liter	1.00 litre
Casein (0.5%)	2.50g	5.00g
0.1N NaOH	50.00ml	100.00ml
PBS, pH 7.4	450.00ml	900.00ml

- (1) Suspend casein in 0.1N NaOH and bring to a boil.
- (2) After casein is dissolved, slowly add the PBS, allow to cool and adjust the pH to 7.4 with HCl.

(B) *Unboiled casein BB*

Reagent	Amount in	
	0.50 liter	1.00 litre
BSA (0.1%)	0.50g 5.00g	1.00g 10.00g
Cascin (0.5%)	2.50g	5.00g
PBS, pH 7.4	500.00ml	1000.00ml

- (1) Suspend bovine serum albumen (BSA) and casein in PBS and mix for 2hr or until dissolved. Some casein may not dissolved.

SUBSTRATE SOLUTION

- (1) Mix KP&L ABTS (Solution A) and hydrogen peroxide (Solution B) 1 : 1 immediately before use.

(Incubate at 100 μ l/well).

COATING OF MAb to PLATES

Preparation of solution for coating one micro-titre plate

- (1). Stock capture MAb = $0.5\mu\text{g}/\mu\text{l}$.
- (2). Coating rate is $0.1\mu\text{g}/50\mu\text{l}$ /well.
- (3). One micro-titre plate contains $96 + *4$ wells = (100 wells).
*Provision against spillage.
- (4). $10 (100 \times 0.1)\mu\text{g}$ MAb is needed to coat one plate
- (5). From (1) above, $10\mu\text{g}$ MAb is contained in
$$\frac{10 \times 1}{0.5} = 20\mu\text{l}$$
 solution
- (6). From (2), $5000 (50 \times 100)\mu\text{l}$ of MAb Solution is needed to coat one micro-titre plate.
- (7). Add $4, 980\mu\text{l}$ PBS to $20\mu\text{l}$ ($10\mu\text{g}$ MAb) solution.
- (8). Coat immediately, 4°C overnight or store frozen until use.
Coat at $50\mu\text{l}$ ($0.1\mu\text{g}$ MAb)/well.