KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

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DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY

Malaria Transmission Dynamics and Pyrethroid Insecticide Resistance Status of *Anopheles Gambiae* Sensu Lato Gilles (Diptera: Culicidae) in two Districts of the Brong Ahafo Region, Ghana.

A Thesis Submitted to the School of Graduate Studies of the Kwame Nkrumah University of Science and Technology for Doctor of Philosophy (Ph.D.) Degree in Biological Sciences.

By:

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September, 2009.

DECLARATION

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institution of higher learning, except where due acknowledgment has been made in the text.

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DEDICATION

The work is dedicated to my parents, Auntie Esi Amammoah and Papa Kwesi Amadu Eduatsey (Senya Beraku) all of blessed memory. I am grateful to the Almighty God for lifting up a monument for them. The work is also dedicated to my siblings, Frank Onomah Hayford, Sandra Acquah-Hayford, Georgina Acquah-Hayford, Emma Arfoduba Hayford, Isaac Etsibah Hayford; my nieces and nephews, Aba Amammoah Afful, Cynthia Hayford, Mabel Hayford, Paa Kwesi Afful, Leonidas Papa Afful, Leticia Essel, Helena Essel and Christian Essel Jnr. as well as my dearest friend, Mary Ann Sorotow Paintsil to whom higher education is an obvious priority. It's my exceptionally fervent wish that they develop into the world's firstclass intellectuals for the sake of our mother Ghana and Africa at large.

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ABBREVIATIONS/ACRONYMS AND SYMBOLS

%	.....	Percentage
&	.....	And
()	.....	Parenthesis (bracket)
(-)	.....	Negative
(+)	.....	Positive
/	.....	Solidus
=	.....	Equal to
±	.....	Plus or minus sign
√	.....	Square root
˚C	.....	Degree Celsius
µ	.....	Micro
µg	.....	Microgram
µl	.....	Microlitre
µM	.....	Micromolar
BB	.....	Blocking Buffer
bp	.....	Base pair
CDC	.....	Centres for Disease Control
dATP	.....	Deoxyadenosine triphosphate
dCTP	.....	Deoxycytidine triphosphate
DDT	.....	Dichlorodiphenyltrichloroethane
dGTP	.....	Deoxyguanosine triphosphate
DNA	.....	Deoxyribonucleic Acid
dNTPs	.....	Deoxyribonucleotide triphosphates
DPBS	.....	Dulbecco's Phosphate Buffered Saline

dTTP	.....	Deoxythymidine triphosphate
E	.....	Exposure mortality
EDTA	.....	Ethylene diamine tetra acetic acid
EIR	.....	Entomological Inoculation Rate
estEIR	.....	Estimated Entomological Inoculation rate
ELISA	.....	Enzyme-linked immunosorbent assay
EtBr	.....	Ethidium Bromide
EtOH	.....	Ethanol
G	.....	Gonotrophic cycle
g	.....	Gram
GABA	.....	γ Aminobutyric Acid
GMA	.....	Ghana Meteorological Agency
GIS	.....	Geographic Information System
GPS	.....	Global Positioning System
GS	.....	Grinding solution
H_2O	.....	Water
H_2O_2	.....	Hydrogen peroxide
HBR	.....	Human-biting rate
HCL	.....	Hydrochloric acid
HLCs	.....	Human landing catches
KAc	.....	Potassium acetate
kb	.....	kilobase
kdr	.....	Knockdown resistance
KDT	.....	Knockdown Time
l	.....	Litre

log	.....	Logarithm
m	.....	Milli
M	.....	Molar
ma	.....	Man biting rate
MAb	.....	Monoclonal antibodies
MoH	.....	Ministry of Health
MPA	.....	Mosquito/*Plasmodium* antigen
mtDNA	.....	Mitochondria deoxyribonucleic acid
Mw	.....	Molecular weight
n	.....	Length of sporogonic cycle
N	.....	Sample size
NaCl	.....	Sodium chloride
NaOH	.....	Sodium hydroxide
nDNA	.....	Nuclear deoxyribonucleic acid
NMCP	.....	National Malaria Control Programme
NP-40	.....	Nonidet P-40
p	.....	Probability of daily survival
P	.....	Proportion parous
PBS	.....	phosphate buffered saline
PBST	.....	PBS-Tween
PCR	.....	Polymerase chain reaction
pH	.....	Hydrogen ion concentration
RAPDs	.....	Randomly amplified polymorphic DNA
RBM	.....	Roll Back Malaria
rDNA	.....	Ribosomal deoxyribonucleic acid

RFLP	.....	Restriction fragment length polymorphism
RNA	.....	Ribonucleic acid
RNAse	.....	Ribonuclease
rpm	.....	Revolution per minute
RR	.....	Resistance ratio
s	.....	Sporozoite rate
s.l.	.....	*sensu latu*
s.s.	.....	*sensu stricto*
$SddH_20$	.....	Sterile double distilled water
SDS	.....	Sodium dodecylsulphate
TAE	.....	Tris acetate EDTA
TE	.....	Tris-EDTA
Tm	.....	Melting temperature
Tris	.....	2-amino-1-hydroxymethyl-1,3-propanediol
w/v	.....	Weight per volume
WHO	.....	World Health Organisation
χ^2	.....	Chi square

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABSTRACT

Malaria results from infection with *Plasmodium* species transmitted mostly through the bite of female *Anopheles* species. The Brong Ahafo Region (BAR) of Ghana is endemic for malaria with endemicity greater than 75%. The Ghana National Malaria Control Programme (NMCP) has prioritised the use of pyrethroid-treated bednet and/or indoor residual spraying as a key strategy for combating malaria transmission. Control of malaria transmission using insecticide treated bed-nets and/or IRS impacts on both morbidity and mortality due to malaria. Evidence of insecticide resistance in malaria vectors in different areas necessitates surveillance studies to allow prompt detection of resistance should it arise and thus enable its management. Also, in preparation for the future IRS expansion programme planned by the NMCP, data on indices of malaria transmission would be required from several parts of Ghana, including the BAR. Hence, density, diversity, biting habits, feeding behaviour, parity rates, survival rates, sporozoite rates and entomological inoculation rates (EIRs) as well as pyrethroid insecticide resistance in the main malaria vector were investigated in some communities in two districts of the BAR. Mosquito larvae were collected using the dipping technique and reared to the adult stage and used for the insecticide susceptibility bioassays. Adult mosquitoes were also collected using human landing catches. The susceptibility bioassays were carried out using the standard WHO diagnostic bioassay kits. Bioassays were performed on non-blood fed female mosquitoes 2- to 4-day old. Knockdown rates were recorded after 5, 10, 15, 20, 30, 40, 50, 60, and 80 minutes. Mortality rates 24 hours post-exposure were also noted. *Anopheles gambiae* complex mosquitoes were analysed using the *An. gambiae* species specific PCR protocol. DNA from specimens identified as *An. gambiae* s.s. were subjected to PCR assays for the identification of the M and S molecular forms, and the

detection of the leucine to phenylalanine knockdown resistance (*kdr*) gene mutation. Ovaries of wild-captured adult mosquito samples were dissected to examine the ovarian tracheations in order to determine parity, survival rates and life expectancy. The heads and thoraces of the parous mosquito samples were tested for the presence of circumsporozoite antigens of *P. falciparum* using enzyme-linked immunosorbent assay. A total of 15,384 *Anopheles* was collected, 13,088 in the rainy season and 2,296 in the dry season. Three *Anopheles* species were identified in both seasons: *An. gambiae* s.l. Gilles (98.80%), *An. hancocki* Edwards (1.20%) and *An. coustani* Laveran (0.03%). *Anopheles gambiae* s.s. was the only member of the *An. gambiae* s.l. found, consisting of both the M and S molecular forms but the S form was predominant (72.30%). No M/S hybrids were found. The susceptibility tests showed resistance to deltamethrin and permethrin. That is, mortality rates below 80% were observed ranging from 45% to 59% for the 0.05% deltamethrin bioassays conducted at Ahafo Kenyasi and Sunyani respectively. Mortality rates for the 0.75% permethrin bioassays ranged from 27% to 35% for the Ahafo Kenyasi and Sunyani samples respectively. Lastly, 46% mortality was observed for the Ahafo Kenyasi samples tested with 1.5% permethrin. The leucine to phenylalanine *kdr* gene mutation was detected in the two molecular forms but it was more frequent in the S form. That is, approximately 80.1% and 31.0% of the S and M molecular forms of the *An. gambiae* s.s. respectively, had the mutation. *Anopheles gambiae* s.s had its peak biting activity in the third and fourth quarters of the night in the rainy season but activity in the dry season peaked in the third quarter. In the rainy season, human-biting rates did not differ significantly between Sunyani, Ahafo Kenyasi and Hwidiem but the rates in these communities were significantly higher than in Chiraa ($p = 0.034$). In the dry season, however, there were no significant differences in HBR between the study sites ($p = 0.898$). Overall

biting rates were higher in the rainy season ($p < 0.001$). Parity and survival rate did not differ significantly between sites but they were higher in the dry season. The sporozoite rate of both the *An. hancocki* and *An. coustani* was zero. There were no significant differences in *P. falciparum* sporozoite rates of *An. gambiae* between sites and seasons, however, EIRs were higher in the rainy season. The average inoculation rate was 2.864 infective bites per man per night (ib/m/n) in the rainy season, which gives an estimated 85.92 infective bites per man per month (ib/m/m). Comparative figures for the dry season were 0.468 ib/m/n and 14.07 ib/m/m. An overall average inoculation rate was 1.649 ib/m/n, which gives an estimated 49.48 ib/m/m. The results of this study suggest that the *An. gambiae* s.s. populations are resistant to the pyrethroid (deltamethrin and permethrin) insecticides tested. The implication of this is that malaria vector control measures in these communities using pyrethroid insecticides may be compromised due to the existence of insecticide resistance in the main malaria vector, *An. gambiae* s.s. The results of this study also suggest that the present study communities are areas of high malaria transmission intensity with transmission occurring all year round even though it is significantly higher in the rainy season.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Introduction

Malaria results from infection with intracellular protozoan parasites of the genus *Plasmodium* transmitted mostly through the bite of female *Anopheles* mosquito species, although blood transfusions and congenital transmission also contribute. Four major species of plasmodial parasites, namely, *Plasmodium ovale* Stephens, *P. vivax* Grassi and Feetti, *P. falciparum* Welch and *P. malariae* Laveran cause human malaria worldwide. Of these, *P. falciparum* is the most predominant and is responsible for most of the morbidity and mortality due to malaria transmission (Garnham, 1988; Cox, 1991; Hombhange, 1998; WHO, 2000, 2003; CDC, 2004a). In Ghana, *P. falciparum, P. malariae and P. ovale* cause malaria with *P. falciparum* being the main species (Asenso-Okyere & Dzatow, 1997; MoH, 2008). About 400 species of *Anopheles* mosquitoes have been identified and there is evidence of additional sibling species (White, 1982; WHO, 2003; CDC, 2004b), which are members of species complexes, such as the *Anopheles minimus* complex, *An. gambiae* complex, *An. funestus* complex. Of these, 60 to 80 species have been found to transmit malaria, although only about 40 are of major importance (White, 1982).

Members of the *An. gambiae* complex and the *An. funestus* complex are the most competent vectors of the *P. falciparum* malaria in Ghana (Appawu *et al.* 2001, 2004; Afrane *et al.* 2004; Appawu, 2005) and sub-saharan Africa on the whole (Coetzee *et al.* 2000). Among these complexes, the *An. gambiae* is more widespread in Ghana (Appawu *et al.* 2001, 2004; Kristan *et al.* 2003; Stile-Ocran, 2003; Afrane *et al.* 2004; Yawson *et al.* 2004; Sakyi, 2004; Appawu, 2005; Kabulah, 2007; Stiles-

Ocran *et al.* 2007) and throughout tropical Africa, and has higher vectorial capacity (Coetzee *et al.* 2000). The *An. gambiae* complex comprises six named species (Gillies & Coetzee, 1987) and one unnamed species (Hunt *et al.* 1998). The named species are *An. gambiae* s.s Gilles, *An. arabiensis* Patton, *An. quadriannulatus* Theobald, *An. merus* Donitz, *An. melas* Theobald and *An. bwambae* White. The unnamed species has been designated *An. quadriannulatus* species B due to its close similarity to this species (Hunt *et al.* 1998). Only *An. arabiensis*, *An. melas* and *An. gambiae* s.s have been reported in Ghana with *An. gambiae* s.s as the most predominant sibling species (Appawu *et al.* 2001, 2004; Appawu, 2005; Stiles-Ocran *et al.* 2003; 2007; Afrane *et al.* 2004). There is evidence for at least five chromosomal forms or species within *An. gambiae* s.s. in West Africa (Fanello *et al.* 2003) namely Forest, Savanna, Mopti, Bamako and Bissau form (Bryan *et al.* 1982; Coluzzi *et al.* 1985). Three of these forms have been reported in Ghana, namely the Forest, the Savanna and the Mopti form (Appawu *et al.* 1994). Among the *An. gambiae* complex, *An. gambiae* s.s. is reported to be the most predominant vector of *P. falciparum*, followed by *An. arabiensis* (Gillies & Coetzee, 1987; Coetzee *et al.* 2000).

Further analysis of the *An. gambiae* s.s using nucleotide sequences from the intergenic spacer region of rDNA of the X-chromosome has revealed two molecular forms of this sibling species referred to as S and M (Favia *et al.* 1997; della Torre *et al.* 2001). In Mali and Burkina Faso, the M molecular form always corresponded to chromosomal Mopti whereas the S molecular form corresponded to sympatric populations of Savannah and Bamako. Outside Burkina Faso and Mali this linkage has broken down, and both molecular genotypes are found in the Savanna and Forest chromosomal forms (della Torre *et al.* 2001). Studies done in Navrongo revealed the M molecular form in northern Ghana (Lehmann *et al.* 2003; Donnelly *et al.* 2004).

Another study reported both forms in sympatry in southern Ghana but not in the central and northern part, where only S and M forms, respectively, were found (Yawson *et al.* 2004) though preceding study in the Kassena Nankana District reported both forms in the north (Appawu *et al.* 2004).

Malaria is a worldwide public health problem. Between 350 and 500 million people fell sick and over one million people died from malaria worldwide in 2005 with 90% these deaths occurring among sub-Saharan African children below age five years (WHO, 2005a). There were an estimated 247 million malaria cases among 3.3 billion people at risk of malaria infection in 2006, causing almost a million deaths, mostly of children under five years (WHO, 2008). One hundred and nine countries were endemic for malaria in 2008, 45 within the WHO African region (WHO, 2008). In Ghana, malaria is endemic and everyone is at risk of contracting malaria with children under-five years, pregnant women and non-immune visitors being at the greatest risk. Presumptively diagnosed malaria cases form 37.5% of all outpatient illnesses, 36% of all admissions and 33.4% of all deaths in children under-five years. The disease accounts for approximately 13.8% of all outpatient attendances, 10.6% of admissions and 9.4% of mortalities among pregnant women. Most morbidities and mortalities due to malaria occur at home and are not reported (MOH, 2008).

The social and economic costs of malaria justifies the need for more progress in malaria control interventions (Asante & Asenso-Okyere, 2003), at least to decrease morbidity and mortality rates. Malaria control interventions are now concentrating on case definition, accurate diagnosis, prompt and effective disease treatment, use of insecticide treated nets (ITNs), indoor residual spraying and other vector control tactics (WHO, 2000, 2005a, 2006a,b,c,d). The use of insecticide treated bed nets for example is a key strategy for preventing malaria transmission in Africa (Nabarro &

Taylor, 2000; RBM, 2005) because they could reduce bites from infected vectors and thus save lives significantly (Binka *et al.* 1996; Jones *et al.* 2003; Lengeler, 2004). However, malaria control in general have been met with some technical hitches, such as lack of proper healthcare facilities and infrastructures (Foster, 1995; Kager, 2002; Moeman *et al.* 2003; Wiseman *et al.* 2008) as well as emergence of drug resistant *Plasmodium* parasites (Cotran *et al.* 1998; Abuaku *et al.* 2005). There has also been an increase in the existence of counterfeit and substandard drugs, including antimalarial drugs (WHO, 1993; Afu, 1999; Wondemagegnehu, 1999; Reithinger, 2001) in the developing countries. Counterfeit and substandard drugs may cause clinical aggravation leading to complications and even death from the malaria itself or probable poisonous ingredients in the drug, increased health costs to attain cure, and selection of drug-resistant *Plasmodium* parasites. Ineffective or partially effective drugs may also lead to biased data on anti-malarial drug efficacy and to discordant results between clinical efficacy and molecular markers (Basco, 2004).

Malaria transmission control or prevention strategies employing insecticide has also been hindered drastically due to insecticide resistance development in the vector. The global malaria eradication efforts emphasised eradication of all potential vectors. Beginning in the 1940s, the use of DDT was considered ideal for the eradication of malaria vectors and the disease. Global efforts of malaria vector control or prevention using chemical insecticides were initially successful at reducing morbidity and mortality due to malaria transmission (Sachs, 2002). However, by the 1960s, insecticide use was largely reduced due to widespread vector resistance to insecticides and the rates of transmission increased to the levels observed before DDT usage (Hemingway *et al.* 2002, Sidhu *et al.* 2002, Wellems, 2002).

Generally, efforts against malaria transmission are aimed at reducing malaria related morbidity and mortality through a combination of manifold interventions that disrupt the parasite-vector-human cycle at several points. This stratified strategy is based on the premise that the usefulness of different malaria control options may depend heavily on local conditions (WHO, 1992, 2003). For instance, the fact that malaria tends to be intense among the poorest nations of the world reduces the feasibility of a large-scale implementation of more effective malaria transmission control and/ or prevention methods requiring more money, careful maintenance or a high level of technical skills. It is for this reason that the large-scale control of malaria vector became feasible only after the introduction of residual insecticides and has remained largely dependent on diverse forms of chemical control (Najera & Zaim, 2003). Effort to combat malaria transmission through vector control in Africa depends mostly on interventions targeted at adult vectors using insecticide-treated nets or indoor residual spraying (Mittal *et al.* 1991). For example, the use of pyrethroid treated bed-nets for preventing and/ or controlling malaria transmission is a key strategy of the National Malaria Control Programme (NMCP) and the Roll Back Malaria Initiative in Ghana (RBM, 2005; MOH, 2008). However, high pyrethroid resistance, resulting from a single point mutation in the gene that encodes the sodium channel (Martinez-Torres *et al.* 1998) have been reported (Yawson *et al.*, 2004; Stiles-Ocran *et al.* 2007; Kabulah, 2007; Adasi & Hemingway, 2008) and this could hamper the use of treated bednets since pyrethroids are the only insecticides approved for treating bednets (Lines & Zaim, 2000; Zaim & Nakashima, 2000; WHO, 2000).

Malaria transmission control and/ or prevention is multi-dimentional in nature and adopting a more integrated malaria control strategy in the endemic areas of sub-saharan Africa may have a greater impact in reducing morbidity and mortality due to

malaria. Malaria vector control effort, which is one of the core aspects of integrated malaria control operations, has been the means of eradicating the disease from several parts of the world, and has greatly reduced its incidence in several other areas (Bruce-Chwatt & de Zulueta, 1981; WHO, 2003). Since the *Anopheles* mosquito is still the intermediate host in *Plasmodium* transmission, there is the need for research and control effort in regions where malaria transmission remains a health burden. It should be recalled that success in combating malaria transmission has been largely attributed to the vector control and that this has mostly failed in Africa due to the emergence of vector resistance to insecticides, limited capital input and gaps in the fundamental biological knowledge of the vector populations among several other important issues (Lounibos & Conn, 2000; Shiff, 2002).

In order to effectively control malaria transmission intensity in a particular locality, adequate information on the gravity of malaria burden, local malaria vector ecology, distribution, transmission patterns and factors affecting transmission are required to design interventions suited to the locality in question (Githeko *et al.* 1993). In Ghana as in many endemic countries of Africa, reported malaria cases represent only a small fraction of the actual number of malaria episodes in the population because the majority of people with symptomatic infections are treated at home and are not reported (RBM, 2005; MOH, 2008; WHO, 2008). Moreover, the reported cases do not give a true picture of the actual malaria transmission intensity since most communities in these countries do not have adequate healthcare systems (Foster, 1995; Kager, 2002; Moeman *et al.* 2003; Wiseman *et al.* 2008). Therefore, basic surveillance data is required, among other things, to determine the level of malaria endemicity, assess the seasonality of malaria transmission intensity, and identify the level of risk among different population groups. Studies to identify the

main vectors for selecting suitable vector control options (Banerjee & Nayak, 2001; WHO, 1987) and evaluation of the success of the goals for morbidity and mortality reduction (Adams *et al.* 2004) are also required in several communities in Africa including Ghana. An estimation of daily survival rate and life expectancy of the *Anopheles* vectors of malaria for example provides a means of assessing the efficacy of malaria control methods (Weidhaas *et al.* 1974; Garrett-Jones & Shidrawi, 1969; Garret-Jones & Grab, 1964; Dietz *et al.* 1974; WHO, 2003). Also, an estimation of the entomological inoculation rate (EIR) [Fontenille *et al.* 1997], provides a standard and relatively simple means of quantifying levels of exposure to infected mosquitoes. This EIR is considered a favoured parameter for measuring malaria endemicity, the suitability of malaria vector control methods and the risk of malaria epidemic development in a particular community (Burkot & Graves, 1995; Onori & Grab, 1980; reviews in Clements & Paterson, 1981).

Insecticides constituted 70% of an average of 814 tons of pesticides officially imported into Ghana between 1995 and 2000, and their use is believed to have increased over time (Gerken *et al.* 2001). These pesticides have been reported to be used improperly or in hazardous combinations in the country (Obeng-Ofori *et al.* 2002) for both domestic and agricultural pest control purposes. Also, ITNs and indoor residual spraying (IRS) have been prioritised as key strategies for malaria transmission prevention in Ghana (RBM, 2005; MoH, 2008). The role of domestic and agricultural insecticide usage in vector resistance development has been widely argued. For example, in the Kwazulu/Natal Province of South Africa (Coetzee *et al.* 2000), in Haiti (Brogdon *et al.* 1988a) and in Sudan (Lines, 1988), the impact of public health spraying on insecticide resistance development is obvious. Also, insecticide resistance appears rapidly in communities where insecticide-impregnated

bednets are used to control mosquitoes or sandflies (Vulule *et al.* 1994, 1999; Rivet *et al.* 1994). Likewise, insecticide use for agricultural purposes has been implicated in resistance development in for example the West African *An. gambiae* complex (Hamon *et al.* 1968; Yawson *et al.* 2004; Diabate *et al.* 2002, 2004) and the Central American *An. albimanus* (Brogdon *et al.* 1988b). These observations warrant a research into the status of insecticide resistance development among insect vector populations in areas where insecticides are used for insect pest control. This will ensure early detection of resistance should it arise and thus enable its management.

In Ghana, the published data on insecticide resistance in the main malaria vectors are mainly from the south and the north (Yawson *et al.* 2004; Adasi & Hemingway, 2008) with limited data from the middle belt (Yawson *et al.* 2004; Stiles-Ocran *et al.* 2003, 2007), such that no published data exists on insecticide resistance in the Brong Ahafo Region. Meanwhile, insecticide resistance monitoring is vital to sustainable malaria vector control programmes that involve insecticide application because data on vector susceptibility to insecticides and their operational implications are required to guide insecticide use. These data would offer a basis for choosing insecticide(s), for examining the continual susceptibility to, and efficacy of, insecticide(s) in use, and for insecticide resistance management (WHO, 1998a). Despite the fact that insecticide resistance distribution studies have been conducted in some communities in Ghana, the need to survey the others is justified due to the focal nature of insecticide resistance (Brogdon & MacAllister, 1998). Levels of insecticide resistance in the *Anopheles* vectors of malaria can differ even within comparatively small geographical scales and during different seasons of the year (Diabate *et al.* 2002, 2004). The dominant resistance mechanisms in these vectors may also vary as was reported in the Guatemalan populations of *An. albimanus*, where both the

resistance levels and mechanisms differed within short distances. Also, higher levels of insecticide resistance have been reported to occur in areas of ongoing vector control activities using insecticides (reviews in Brogdon & McAllister, 1998; Reimer *et al.* 2005). However, it should be noted that DDT resistance was not detected in South Africa from 1946 –1995 when DDT was employed for public health insect control purposes. This is possibly because stringent measures were taken to prevent its diversion to agriculture where *Anopheles* larvae could develop resistance if under selection pressure (Sadasivaiah *et al.* 2007). Development of vector resistance to insecticide may also be due to immigration of insecticide resistance genes. A typical example of this situation is the migration of esterase resistance to organophosphates in *Culex pipiens* into certain areas of France (Rivet *et al.* 1994). These observations imply that an extrapolation from one insecticide resistance situation to another may be misleading. Studies in Haitian populations of *An. albimanus* species reported that resistance frequencies to fenitrothion increased from 20 to 60% over a period of six months (Brogdon *et al.* 1988). This emphasises the need for insecticide resistance monitoring, even where there is no evidence of insecticide resistance in the *Anopheles* vectors as in several parts of Ghana, where insecticides are used for both public health and agricultural insect control (Gerken *et al.* 2001; Obeng-Ofori *et al*. 2002).

The Brong Ahafo Region (BAR) of Ghana is endemic for malaria with endemicity greater than 75% (www.mara.org.za/pdfmaps/GhaDistribution.PDF). The Ghana National Malaria Control Programme (NMCP) has prioritised the use of pyrethroid-treated bednet and/or indoor residual spraying (IRS) as a key strategy for combating malaria transmission in the country. The control of malaria transmission using chemical insecticide impregnated bed-nets and/or IRS is known to impact on both morbidity and mortality due to malaria. In preparation for the future IRS

expansion programme planned by the NMCP (MOH, 2008), data on vector population ecology for example and the factors regulating it would be required for various communities in Ghana, including those of the BAR. This research was therefore, designed to explore certain key indices of malaria transmission in some localities in two districts of the BAR and the association between these parameters and climatic factors. The study also explored the resistance status of the main malaria vector to pyrethroids. It is expected to describe the species composition, density, diversity, biting rhythm, feeding behaviour, longevity and the entomological inoculation rates in relation to the main malaria vector *An. gambiae* s.s in the rainy and dry seasons. The study is also expected to describe the resistance status of the *An. gambiae* s.s. population to deltamethrin and permethrin of the pyrethroid class and the distribution of the *kdr* gene mutation in this species. Finally, the study is expected to form part of the basis for a malaria vector control programme planning framework, which will employ the above information in planning future malaria vector control activities.

1.1.1 Aims and Objectives

The study aims at determining malaria transmission dynamics and pyrethroid insecticide resistance status of *An. gambiae* s.l. Gilles (Diptera: Culicidae) in the Sunyani Municipality and Asutifi District, both in the Brong Ahafo Region of Ghana.

1.1.1.1 Specific objectives

The specific objectives were to determine;

(1) diversity of *Anopheles* mosquito species, *An. gambiae* s.l. and the *An. gambiae* s.s. M and S molecular forms.

(2) spatial and temporal variation in respect of (a) the distribution patterns of *An. gambiae* s.s. population (density, biting habit and feeding behaviour) in relation to climatological factors: rainfall, minimum and maximum temperatures and relative humidity. (b) parity, survival rates and life expectancy of *An. gambiae* s.s. populations. (c) *plamodium falciparum* sporozoite rates, entomological inoculation rates and risk of malaria infection.

(3) status of pyrethroid resistance in the *An. gambiae* s.s populations using the standard WHO bioassay kits and procedures.

(4) distribution of leucine to phenylalanine knockdown resistance (kdr) mutation in *An. gambiae* s.s. using molecular methods.

CHAPTER TWO

REVIEW OF RELEVANT LITERATURE

2.1. Human malaria: the parasite, the disease and the symptoms

Human malaria results from an infection with parasites of the genus *Plasmodium,* family Plasmodiidae, suborder Haemospororina, order Eucoccidiorida, subclass Coccidiasina, class Sporozoasida, phylum Apicomplexa, subkingdom Protozoa and kingdom Protista (Cox, 1991; Peters & Gilles, 1995; Keas, 1999a). There are five human malaria parasite species: *Plasmodium falciparum, P. vivax, P. malariae, P. ovale* and *P. knowlesi* (WHO, 2000, 2003; Cox-Singh *et al.* 2008)**.** *Plasmodium falciparum* causes the greatest morbidity and mortality in Africa (WHO, 2000). In tropical Africa and Asia, 85-90% of malaria cases are due to this species. *Plasmodium ovale* is found mostly in the tropical Africa with sporadic reports from other parts of the world such as the South Pacific Islands. *Plasmodium vivax* is not widespread in sub-saharan Africa, but widespread in South Asia and Central America, and is the major species in South America. *Plasmodium malariae* is found in the tropical and subtropical areas of Central and South America, Africa, and Southeast Asia. Lastly, *P. knowlesi* is found in Southeast Asia in areas such as across Malaysian Borneo and Peninsular Malaysia (Hombhange, 1998; Keas, 1999b; Cox-Singh *et al.* 2008).

The general symptoms of malaria include fever, headache, nausea and flu-like symptoms, but the manifestation of these symptoms may differ depending on the type of *Plasmodium* species involved and the immunity of the person infected (Schmitz & Gelfand, 1976; Wernsdorfer & Mc Gregor, 1986; NIH, 2001; Stevenson & Riley, 2004; CDC, 2004a). After the infective bite from the *Anopheles*, the parasites undergo the incubation period before the first symptoms appear. *Plasmodium malariae*

normally takes 7 to 30 days for symptoms to show but *P. ovale and P. vivax* are usually incubated for about 8 to 14 days (or in some cases can survive for months in the host) and for *P. falciparum,* 7 to 14 days. Symptoms of malaria may be cyclic or periodic due to the nature of the life cycle of the parasite species involve – as they develop, mature, reproduce and be discharged from the erythrocytes into the blood stream to infect more erythrocytes and hepatocytes (D'Avanzo *et al.* 2002). Where *P. vivax* or *P. ovale* is involved in infections, the victims even after recovering from the initial occurrence of malaria may suffer several added attacks after months or even years without symptoms. These relapses occur since *P. vivax* and *P. ovale* have dormant liver stage parasites known as hypnozoites that may reactivate the illment (Garnham, 1988; CDC, 2004a).

Fever is the main symptom of malaria infection (Schmitz & Gelfand, 1976), which develops when the merozoite stages of the parasite life invade and destroy the erythrocytes. The destruction of these blood cells spill wastes, toxins, and other debris into the host blood stream. The host body's response to this spillage is the fever, an immune response that speeds up other immune defences to fight the foreign attackers in the blood (CDC, 2004a). The immune response also leads to spleen enlargement. The spleen rates in children aged two to nine years is used as an index for malaria endemicity determination (Kettle, 1995; Wanji *et al.* 2003). Other complications involving the brain and the kidneys can then develop leading to delirium and coma. The most severe manifestations are cerebral malaria, anaemia, kidney and other organ dysfunction such as respiratory distress (WHO, 1986). According to WHO, (1986) the cerebral malaria mostly occurs among children and people without previous immunity whereas the anaemia occurs among children and pregnant women. People who are frequently infected with malaria parasites will generally acquire a considerable level

of clinical immunity, which provides them with partial protection against future infections (Strickland, 1991; Baird, 1995; NIH, 2001; CDC, 2004a).

2.2 Human Malaria: The *Anopheles* Mosquito Vectors

Female mosquitoes require blood to produce their eggs and hence are capable of transmitting vector-borne diseases such as malaria. It is only the female *Anopheles* mosquitoes that transmit the human malaria parasites from an individual to another (White, 1982; WHO, 2003; CDC, 2004b). For any female *Anopheles* species to be able to transmit malaria, it must be able to ingest the parasites and promote their maturation until the infective stage. The mosquito must also be able to survive until the next blood meal (Failloux *et al.* 1995). Among the about 430 *Anopheles* species, 60-80 have been implicated in malaria transmission, only up to 40 of which are identified in most transmission studies. Of these, only 15 are most important vectors (White, 1982; WHO, 2003; CDC, 2004b). Although only few species of female *Anopheles* mosquitoes are able to meet the requirements for being human malaria vectors, the species and forms involved in malaria transmission are very diverse (Coetzee *et al.* 2000; Appawu, 2005). The significance of each species or form in malaria transmission differs by region, as does their geographical distribution. For example, the *An. albimanus* complex, *An. minimus* complex and *An. aquasalis* are the major human malaria vectors in South America (Laubach *et al.* 2001; Marquetti *et al.* 1991). *Anopheles gambiae* complex and *An. funestus* complex are the main malaria vectors in sub-saharan Africa, transmitting *P. falciparum*, which is the most important parasite of public health. The *An. gambiae* complex is predominant among the major sub-saharan *Anopheles* species complexes (Gillies & Coetzee, 1987; Service, 1985).

2.2.1 Anopheles gambiae Gilles sensu lato

Anopheles gambiae Gilles s.l. consists of six named sibling species: *An. gambiae* sensu stricto Gilles, *An. arabiensis* Patton, *An. bwambae* White, *An. quadriannulatus* Theobald, *An. merus* Donitz and *An. melas* Theobald (Gillies & Coetzee, 1987); one unnamed species designated *An. quadriannulatus* species B (Hunt *et al.* 1998) and several incipient species all differing in diverse ways (Coluzzi *et al.* 1979; Favia *et al.* 1997). At least five cytological forms of *An. gambiae* s.s. have been reported in West Africa (Fanello *et al.* 2003) namely Savanna, Forest, Bamako, Mopti and Bissau forms (Bryan *et al.* 1982; Coluzzi *et al.* 1985; Toure *et al.* 1998). Members of the *An. gambiae* s.l. are isomorphic or morphologically indistinguishable, but demonstrate distinct genetic and eco-ethological differences, as reflected in their ability to transmit malaria parasites. *Anopheles gambiae* s.s is the most important vector of *P. falciparum* malaria in sub-saharan Africa. This species is the most specifically adapted to human beings and has the highest entomological inoculation rates (EIRs) ranging from between less than one to greater than 1000 infective bites per person per year (Trape & Rogier, 1996; Beier *et al.* 1999). It is also stable in a wide range of bio-ecological and seasonal conditions, and therefore appears to be very flexible, both in exploring new man-made environments and in their response to malaria vector control efforts (Coluzzi, 1984; Pothikasikorn *et al.* 2007).

The sibling species of the *An. gambiae* s.l. have a wide geographical distribution and have been reported from various African countries (Coluzzi *et al.* 1979; Chinery, 1984; Coetzee *et al.* 2000). *Anopheles melas* is confined to the coast of West Africa (Coluzzi & Sabatini, 1968; Coluzzi, 1984; Akogbeto, 1995, 2000; Fonseca *et al.* 1996; Diop *et al.* 2002) whereas *An. merus* is confined to the coast of East Africa and islands off its coast. *Anopheles merus* has also been observed in

Somalia in the North to Natal in the South (Paterson *et al.* 1964). *Anopheles melas* has low anthropophily and a short life expectancy as it does not appear to live above 15 days in natural conditions (Bryan, 1983). According to the studies by Akogbeto & Romano, (1999) when *An. melas* is the only species present at a given area, the plasmodial indices observed in man are always very low. It is the low anthropophily and short lifespan of *An. melas* that make it less efficient at transmitting malaria in most places where it is found (Bryan, 1983). However, in areas where this species is very abundant, it may play appreciable role in malaria transmission (Diop *et al.* 2002).

Anopheles gambiae s.s and *An. arabiensis* are the most widespread and most efficient disease transmitting sibling species of the *An. gambiae* s.l. in Africa (Coetzee *et al.* 2000; Kelly-Hope *et al.* 2006). Their distribution and relative abundance appear to be strongly affected by climatic factors such as total annual precipitation (Lindsay & Martens, 1998; Kelly-Hope *et al.* 2006). *Anopheles gambiae* s.s is largely found to be dominant in humid areas (Lindsay & Martens, 1998; Coetzee *et al.* 2000) but *An. arabiensis* dominates arid savannas (Kelly-Hope *et al.* 2006). Where these species occur in sympatry, changes in species composition usually occur, with *An. arabiensis* mostly dominant in the dry season and *An. gambiae* s.s more abundant in the rainy season. Nevertheless, this pattern may differ considerably based on local ecology and species adaptation. *Anopheles quadriannulatus* is found in Ethiopia and Eritrea (Hunt *et al.* 1998; Shililu *et al.* 2003), but *An. bwanbae* has been reported breeding in mineral water springs in Uganda (Davidson & Hunt, 1973).

Three of the sibling species of *An. gambiae* s.l. have been reported in Ghana, namely *An. gambiae* s.s, *An. arabiensis* and *An. melas* with significant variations in their ability to transmit malaria. *Anopheles melas* has been reported in the coastal zone of southern Ghana (Appawu *et al.* 2001, 2004; Kristan *et al.* 2003;

Yawson *et al.* 2004; Appawu, 2005) but has not been implicated in malaria transmission (Appawu *et al.* 2001). *Anopheles arabiensis* has been found in the southern coastal zone and northern savannah zone of Ghana (Appawu *et al.* 2004; Appawu, 2005; Kelly-Hope *et al.* 2006). It transmits malaria in the northern savannah zone where the entomological inoculation rate (EIR) or the intensity of malaria transmission by *An. arabiensis* was estimated to be 0.02 infective bites per man per night (ib/m/n) or 5.70 infective bites per man per year (ib/m/y) [Appawu, 2005]. *Anopheles gambiae* s.s. on the other hand, has been observed in almost every ecological zone of Ghana. For example, studies by Adasi, (2000); Apawu *et al.* (2001); Adeniran, (2002); Yawson *et al*. (2004) and Kabulah, (2007) reported *An. gambiae* s.s. in the coastal savannah and coastal forest zone in the Greater Accra Region of Ghana. *Anopheles gambiae* s.s. has also been reported from the strand and mangrove zones of the Central Region as well as the rainforest zone of the south western region of Ghana (Kristan *et al.* 2003; Yawson *et al.* 2004; Sakyi, 2004). In the rainforest middle belt or central part of Ghana, this species has been reported mainly from the Kumasi Metropolis (Stiles-Ocran, 2003; Afrane *et al.* 2004; Yawson *et al.* 2004; Stiles-Ocran *et al.* 2007) and the Obuasi Municipality (Stiles-Ocran, 2008). Similarly, studies by Apawu *et al.* (2004) and Appawu, (2005) have reported *An. gambiae* s.s from three ecological zones in the Kassena Nankana District of the northern savannah zone of Ghana. It is *An. gambiae* s.s which has been found to be the strong force behind the hyper-endemicity of malaria in the country (Apawu *et al.* 2001, 2004; Afrane *et al.* 2004; Appawu, 2005). For example, in the Kassena-Nankana District in the northern savannah zone of Ghana, the EIR or the intensity of malaria transmission by *An. gambiae* s.s. was estimated to be 1.85 ib/m/n or 676.67 (95% CI, 575.1–779.3) ib/m/y (Appawu, 2005).

Genetic and behavioural variations within *An. gambiae* s.s. have been verified and the different cytological forms described (Coluzzi *et al.*, 1979, 1985). These forms include 'Bamako', 'Mopti', 'Savanna', 'Forest' and 'Bissau'. The 'Mopti', 'Savanna', and 'Forest' forms have been reported in Ghana (Appawu *et al.* 1994; Appawu, 2005). These chromosomal forms show restricted or no inter-breeding in the field (Bryan *et al.* 1982; Coluzzi *et al.* 1985; Toure *et al.* 1998) and their distribution depends on environmental factors (Toure *et al,* 1994). In sympatric areas, hybrids between Savanna and the other forms have been observed at low frequencies but no individual with heterozygous complements of the Mopti and Bamako inversions have been seen in nature. However, the two forms produce viable offspring in the laboratory (Coluzzi *et al.* 1985; Persiana *et al.* 1986). Genotyping x-linked rDNA of *An. gambiae* s.s. has led to the detection of molecular forms that differ in the transcribed and nontranscribed spacers in the rDNA repeat unit (Favia *et al.* 2001; della Torre *et al.* 2001; Gentile *et al.* 2002). These forms designated as M and S have also been reported in Ghana (Appawu *et al.* 2004; Appawu, 2005; Yawson *et al.* 2004; Kabulah, 2007). Interbreeding between M and S forms of the *An. gambiae* s.s. in the laboratory produces fertile progeny but M/S hybrids are rare in nature. Where these forms overlap in time and space, the rate of heterogamous insemination is 31% (Tripet *et al.* 2001) implying an existence of a pre-mating barrier. Such a pre-mating isolation mechanism may include the alternative swarming habits of the molecular forms observed in an area where they occurred in sympatry (Diabete *et al.* 2003).

2.2.1.1 Methods for distinguishing between sibling (cryptic) species of the An. gambiae s.l. and other Anopheles species complexes

Morphological keys are still the most widely used methods for taxonomic and systematic studies of anophelines (Harbach, 1994). In the field, these keys are used to identify unrelated species, and enables the initial sorting of samples prior to the use, if needed, of other techniques. This method allows appreciable saving of time and money and the benefits it offers have warranted it persistent use. Unfortunately, the evolution of species groups and complexes within the anopheline taxa is making morphological features less useful for the identification of vectors (Beebe & Cooper, 2000). However, it seems the morphological studies on cryptic species have not been thorough enough and that more exhaustive observations on all stages of the life cycle may yet yield diagnostic features (White, 1977).

The sibling species of anopheline mosquito complexes are reproductively distinct but cannot be distinguished reliably using morphological procedures alone and, therefore, require alternative more specific method for identification. Crossing experiments of the offspring from unknown specimens against laboratory colonies of known species (Davidson & Hunt, 1973) called mating incompatibility test (Collins & Paskewitz, 1996) is one technique that has been of immense value in exposing species within complexes. However, this method is laborious and time consuming for routine specimen identification and therefore led to the design of more simple reliable techniques such as the polytene chromosome analyses used to distinguish between species (within cryptic complexes) and chromosomal forms (Coluzzi & Sabatini, 1968; Gatti *et al.* 1977; Coluzzi *et al.* 1979; Toure *et al.* 1998), quantitative variations in cuticular hydrocarbon profiles (Carlson & Service, 1980; Hamilton & Service, 1983; Philips & Milligan, 1986; Philips *et al.* 1988), electrophoretic analysis of gene-

enzyme systems (Mahon *et al.* 1976; Miles, 1978; Narang *et al.* 1981, 1989a, b; Lanzaro *et al.* 1995) and hybridisation with DNA probes (Collins *et al.* 1987; Gale & Crampton, 1987; Cockburn & Seawright, 1988; Panyim *et al.* 1988).

The discovery of the polymerase chain reaction (PCR) has revolutionised the application of the DNA technology in the study of sibling (cryptic) species of the malaria vectors. The shift from the other biochemical techniques to the present DNA-based processes that make use of the PCR allows the identification of very old specimen because DNA is easily preserved by desiccation in alcohol. The DNA-based methods also have the advantage of being applicable to all developmental stages of insects and with the PCR, very minute amount of template DNA is needed for analysis (Sambrook *et al.* 1989). These DNA-based tools include the mtDNA methods (Caccone *et al.* 1996; Besansky *et al.* 1997; Lehmann *et al.* 1997; Thelwell *et al.* 2000), single nucleotide polymorphisms (De Merida *et al.* 1999) and species-specific restriction fragment length polymorphism analysis of rDNA (Collins *et al.* 1988; Yasothornsrikul *et al.* 1988; Torres *et al.* 2000; Favia *et al.* 2001). Other DNA-based techniques are the randomly amplified polymorphic DNAs (RAPDs) [Wilkerson *et al.* 1993; Favia *et al.* 1994a], microsatellite DNAs (Zheng *at al.* 1996, 1997; Lanzaro *et al.* 1998; Conn *et al.* 2001; Sharakov *et al.* 2001a), amplified fragment length polymorphisms (AFLPs) (Black & Lanzaro, 2001), sequence-tagged amplified RAPDs and single-copy markers (Mukabayire *et al.* 2001).

The DNA – based methods used in studying mosquito populations such as the randomly amplified polymorphic DNA (RAPD-PCR) requires no prior knowledge of the genome and tends to target repetitive and rapidly evolving genome regions (Wilkerson *et al.* 1995) or targeting known specific sequences such as rDNA. Currently, the identification of the main *Anopheles* vectors of malaria is based on

ribosomal DNA (rDNA) method. Some of the most widely applied PCR assays target nuclear rDNA, which is present as a single X-linked locus in *Anopheles* species (Rai & Black, 1999). The rDNA is organised as a tandem repeated array of conserved genes (18S, 5.8S and 28S) punctuated by rapidly evolving non-coding spacers such as the internal transcribed spacers ‘1 and ‘2 (ITS1 & ITS2) and the intergenic spacer (IGS). The most recently developed *Anopheles* mosquito species identification assays are based on differences within the ITS2 (Scott *et al.* 1993; Proft *et al.* 1999; Koekemoer *et al.* 2002a).

2.2.2 Anopheles funestus Gilles sensu lacto

Anopheles funestus Gilles s.l. is one of the three important malaria vectors in Africa, together with the *An. gambiae* s.s. and *An. arabiensis*. The significance of *An. funestus* s.l. in malaria transmission is mostly noticed in the dry season when *An. gambiae* s.l. population density has declined significantly (Fontenille *et al.,* 1997). *Anopheles funestus* belongs to a group of nine morphologically indistinguishable species (Gillies & De Meillon, 1968). These includes *An. funestus* s.s., *An. aruni* Sobti, *An. parensis* Gillies and *An. vaneedeni* Gillies and Coetzee which belong to the *funestus* sub-group. The members of this sub-group have been described on the basis of characters in the adult. Other members of the *An. funestus* s.l., namely *An. confusus* Evans and Leeson, *An. fuscivenosus* Leeson, *An. leesoni* Evans and *An. rivulorum* can be distinguished from each other and from the *funestus* subgroup based on larval features. *Anopheles funestus* s.s. has a wide distribution and the ability to occupy regions ranging from lowland to high altitudes. It is abundant, widespread, highly endophilic, anthropophilic and is found in almost all bio-climatic areas near swamps or rivers (Faye *et al.,* 1995). The other member species of the *An. funestus* s.l. are

more limited in density and distribution, and demonstrate high degree of zoophily although they can bite human especially outdoors and in the absence of other hosts (Gillies & De Meillon, 1968). *Anopheles rivulorum* is the next abundant and widespread species in the group. It is occasionally caught indoors together with the *An. funestus* s.s. and has been reported to transmit malaria in Africa (Wilkes *et al.*, 1996). It has also been demonstrated that *An. vaneedeni*, which has been recorded only from South Africa, can be experimentally infected with *P. falciparum* in the laboratory (De Meillon *et al.*, 1977), although there is no published data on its role in malaria transmission in nature.

Studies in Ghana have shown the presence of *An. funestus* especially in the southern and northern parts (Appawu *et al.*, 1994, 2001; Yawson *et al*., 2004). In the central Ghana, *An. funestus* has been reported in the Obuasi Municipality of the Ashanti Region (Stiles-Ocran *et al*., unpublished) but not in the Kumasi Metropolis of the same region (Yawson *et al*., 2004; Afrane *et al*., 2004; Stiles-Ocran 2003, Stiles-Ocran *et al*., 2007). There is also no published data on the presence of *An. funestus* group in the Brong Ahafo Region, also of the middle/central part of Ghana. Among the members of the *An. funestus* group, only the *An. funestus* s.s has been found to transmit malaria in Ghana and it is known to be the second major malaria vector (Appawu *et al*., 1994, 2001, 2004; Appawu, 2005; Stiles-Ocran *et al*., unpublished).

There is the need for proper identification of the *An. funestus* s.l. to make available data on biological and behavioural characteristics to help clarify their role as malaria vectors. Cytogenetic studies have indicated that *An. funestus, An. parensis, An. rivoulorum, An. leesoni,* and *An. confuses* each have matchless chromosomal inversion arrangements that can be used to sort them out (Green, 1982). *Anopheles vaneedeni* is homosequential with *An. funestus* differing from it only in having a

polymorphic inversion on arm 2 (Green & Hunt, 1980). However, a study by Green & Hunt, (1980) has shown that where the inversion is homozygous for the standard arrangement, these two species cannot be separated via cytogenetic methods.

The use of cytogenetics for routine species identification is disadvantageous in various ways including, the inability to identify immature stages or males and the fact that large sample sizes are required. The large sample size needed is further limited by the requirement of half gravid females, which are not easy to comeby and hence the need for development of DNA-based techniques similar to those used for the *An. gambiae* s.l. Molecular identification procedures for species complexes have primarily used the ribosomal DNA locus because it is represented in multiple copies in the mosquito genome, and it contains highly variable regions (Collins & Paskewitz, 1997). The first molecular studies of the *An. funestus* group were published by Koekemoer *et al.*, (1998) who demonstrated that restriction enzymes could be used to distinguish between the morphologically similar and chromosomally homosequential species namely, *An. funestus* and *An. vaneedeni*. Using PCR primers for the D3 region in the 28S ribosomal gene, amplified products digested with HpaII restriction endonuclease yielded dissimilar fragments on an agarose gel, enabling the differentiation of these two species (Koekemoer *et al*., 1998). Subsequently, single-strand conformation polymorphism analysis for the identification of four members of the *An. funestus* group namely, *An. funestus*, *An. rivulorum*, *An. leesoni* and *An. vaneedeni/parensis* using polyacrylamide gel electrophoresis was designed (Koekemoer *et al*., 1999). Unfortunately, this process has the disadvantage of overlap of banding patterns for *An. vaneedeni* and *An. parensis*, and is more expensive and difficult to perform than agarose gel techniques.

The rDNA internal transcribed spacer (ITS2) sequences have offered helpful methods for the identification of individual species (Koekemoer *et al*., 1999; 2002a). However, during *An. funestus* group vector control programmes, a multiplex system that can recognize all members of the group, such as that developed by Scott *et al.*, (1993) for the *An. gambiae* s.l. is what is really required. Therefore, Koekemoer *et al*., (2002b) designed a cocktail polymerase chain reaction assay to identify five members of the *An. funestus* group, which has been useful in several studies (see e.g. Appawu *et al*., 2004; Weeto *et al*., 2004; Apawu, 2005). It should, however, be noted that this multiplex PCR method for detecting the five *An. funestus* group members (Koekemoer *et al*., 2002b) did not yield species distribution records different from those observed by Gillies & De Meillon (1968), with the exception of the occurrence of *An. parensis* species in Ethiopia (Weeto *et al*., 2004).

Studies in West Africa on the *An. funestus* group using ITS2 sequence varriations demonstrated that *An. rivulorum* from West Africa differs from *An. rivulorum* from East or southern Africa (Hackett *et al*., 2000). Cohuet *et al*., 2003 have confirmed this observation in Cameroon, implying the taxon *An. rivulorum* is made up of more than one species. The role of the new species, designated ‘‘*An. rivulorum*-like’’, in malaria transmission remains to be explored.

2.2.3 The Anopheles nili group

Anopheles nili Theobald is widely distributed across tropical Africa (Hamon & Mouchet, 1961). Morphological, ecological and ethological variations among *An. nili* populations have been reported revealing that *An. nili* actually represents a group made up of at least four species: *An. nili* s.s., *An. somalicus*, *An. carnevalei* and the newly characterised malaria vector *An. ovengensis* (Gillies & de Meillon, 1968;

Carnevale *et al*., 1992b; Kengne *et al*., 2003; Awono-Ambene *et al*., 2004). Allele-specific PCR assay have been developed for the rapid identification of all members within the *An. nili* group (Kengne *et al*., 2003).

The role of *An. nili* in malaria transmission is usually restricted to localised forest area. For example, it seems to be the major malaria vector in some rural forest area of central Africa with an entomological inoculation rate (EIR) of 104 infective bites/person/year (ib/p/y) [Carnevale *et al*., 1992a] and in some parts of West Africa with EIR of up to 275 ib/p/y. *Anopheles ovangensis* and *An. carnevalei* have also been reported to be secondary vectors of malaria in Cameroon with EIR values of up to 70 and 8.6 ib/p/y respectively (Antonio-Nkonjio *et al*., 2006).

2.2.4 The Anopheles moucheti group

Anopheles moucheti is localised in distribution and is deemed a subsidiary malaria vector but its infectivity rates are usually high enough to be deemed a major vector in some communities (Mouchet *et al*., 2004). It is found in central and western Africa (Gillies & de Meillon, 1968; Antonio-Nkonjio *et al*., 2006). Morphological and behavioural variations imply at least three taxa may belong to this group namely, *An. moucheti moucheti*, *An. moucheti nigeriensis* and *An. bervoetsi*. However, comparison of DNA sequences of specimens from several populations shows that possibly, only two truly different species, both vectors, exist (Kengne *et al*., 2003).

2.2.5 The Anopheles pharoensis group

Anopheles pharaoensis group is made up of at least twin species and is abundant and widespread throughout the Afro-tropical region outside areas of close canopy forests (Gillies & de Meillon, 1968). They breed largely in large vegetated

swamps but can also be in irrigation ditches, rice fields, reservoirs, river edges, lake shores, small streams, ponds, pools and overgrown wells. The highest larval populations of this *Anopheles* species group are seen in large water surfaces loaded with aquatic floating vegetations such as *Pistia* and *Potamogeton*.

Anopheles pharoensis vector malaria in Egypt (Madwar, 1936; Barber & Rice, 1937) but its role in malaria transmission in other sub-saharan African countries is not clear in spite of several records of sporozoite-positive specimens (Gillies & de Meillon, 1968; Gillies & Coetzee, 1987). Furthermore, reports from Senegal (Carrara *et al*., 1990; Dia *et al*., 2008) and Cameroon (Antonio-Nkonjio *et al*., 2006), have shown that *An. pharoensis* transmit malaria in these areas. These contrasting views about the vector status of this species have been attributed to the possible existence of different sibling species within this group (Miles *et al*., 1983).

2.3 Human Malaria: The Transmission Cycle

The female *Anopheles* mosquito species bites the human host and introduces sporozoites from its salivary glands into the host's bloodstream. The sporozoites leave the circulating blood in 30 minutes and invade the parenchyma cells of the liver where a cycle of schizogony occurs to produce a large unpigmented schizont. This is called the exo-erythrocytic or pre-erythrocytic cycle (Shortt & Garnham, 1948). The schizont contains thousands of infectious merozoites, which when released into circulation invade the erythrocytes through a receptor-ligand-mediated mechanism. Their release marks the end of the prepatent period. In *Plasmodium vivax* and *P. ovale* some parasites may delay development and remain as hypnozoites for variable periods (Garnham, 1988). Depending on the intensity of the initial parasite infection,

there may be one or two erythrocytic schizogonic cycles before clinical symptoms are manifested and end the incubation period (Kettle, 1995).

The merozoite intimately attaches itself to the erythrocyte and by invagination of the erythrocyte membrane, it enters the cytoplasm and eventually live within the parasitophorous vacuole. In the erythrocyte, it grows from the ring form to become trophozoite and later schoint. The schizont produces 6-16 new merozoites. The released merozoites repeat the cycle and invade other erythrocytes. It is this erythrocytic cycle with its parasite-induced sequestration of infected red blood cells in capillaries deep within many organs which causes many of the pathological hallmarks of malaria infection. The interval between malaria attacks is the length of the schizogonic cycle (Garnham, 1966; Kettle, 1995).

Merozoites cannot be transmitted to another host by a mosquito bite, but a small proportion of merozoites follow an alternative developmental pathway that produces a transmissible form called gametocyte (Hemingway & Craig, 2004). According to Wernsdorfer, (1980) gametocytes mature in four days in *P. vivax* and in eight days in *P. falciparum.* The long-lived non-dividing gametocytes circulate in the bloodstream, awaiting uptake by the mosquito. The gametocytes are taken up by a female *Anopheles* in a blood meal. Within the female *Anopheles* mosquito midgut, the male gametocyte called microgametocyte undergoes a rapid nuclear division, producing eight flagellated microgametes in 10-15 minutes. This transformation is under the influence of environmental changes including temperature, bicarbonate and mosquito factors (Sinden & Smalley 1976; Nijhout & Carter 1978; Nijhout 1979). The microgamete fertilises the female macrogamete to produce first an immobile zygote and then the motile ookinete, which invades the midgut epithelium, thereby infecting the mosquito. The active ookinetes then differentiate into oocysts under the

basal laminar of the midgut epithelium during a period of apparent latency lasting about 10 days during which period about 10,000 sporozoites are produced (Pringle, 1965). The oocysts then rupture and discharge the sporozoites into the mosquito's body cavity, where they migrate to infect the salivary glands (Sinden, 1975).

2.4 Human Malaria: The Distribution

2.4.1 The Heterogeneity in the Global Distribution

There is huge heterogeneity in the distribution of malaria risk and burden between Africa and other regions of the world (WHO, 2008). This situation has been attributed to several factors. For example, variation in the parasite-vector-man transmission dynamics influences the risk of disease and death from malaria (WHO, 2008). Also, the *Anopheles* vector species differ in their vectorial capacity and global distribution due to the differences in climate. Studies have shown that the tropical areas of the world have the best combination of adequate rainfall, temperature and relative humidity which supports breeding and survival of mosquitoes (Favia *et al.* 1997; Lindsay *et al.* 1998; Lindsay & Martins, 1998; Coetzee *et al.* 2000; Kelly-Hope *et al.* 2006). Furthermore, different levels of socio-economic issues such as poverty, quality of housing, access to health care and education, and the existence of control efforts also influence malaria transmission and/or distribution. The poorest nations have the least resources for adequate malaria control interventions. Among these nations, exposure to malaria is enhanced by migrations enforced by poverty and/or conflict (Bryan *et al.* 1982; Petrarca *et al.* 1983; Koram *et al.* 1995; Gallop & Sachs, 2001; reviews in Heggenhougen *et al.* 2003; CDC, 2004a; RBM, 2005).

2.4.2 Distribution of Malaria in Ghana

Malaria is hyper-endemic in Ghana and studies show that the reported malaria cases represent only a small fraction of the actual number of malaria episodes since the majority of people with symptomatic infections are treated at home and are not reported to the health service (RBM, 2005; Abuaku *et al.* 2005; MoH, 2008). Even at the formal health service, only 15–20% of the suspected malaria cases are confirmed (WHO, 2008) and laboratory diagnosis are done microscopically, which may not give the true picture of the malaria situation in the country.

There is high heterogeneity in malaria transmission intensity in the various ecological zones in Ghana. For example, in the Kasena Nanakana District (KND) of the northern Ghana, entomological inoculation rates (EIRs) have been estimated to be between 100-1000 infective bites/man/annum, contributed mainly by *An. gambiae* s.l and *An. funestus* (Binka *et al*, 1994; Binka *et al.* 1996). Estimated EIR of 6-21 infective bites/man/night has also been reported from Dodowa in the coastal forest zone and Prampram in the coastal savannah zone in the southern part of Ghana with *An. gambiae* s.l and *An. funestus* group being the main vectors (Appawu *et al.* 2001). However, there is no published data on the distribution of infective bites in the major malaria vectors in the rainforest middle belt of Ghana, especially the Brong Ahafo Region although malaria endemicity is estimated to be greater than 75% in these areas (www. mara. org.za/ pdfmaps/GhaDistribution.PDF). Three of the four human malaria parasites; *P. falciparum*, *P. malariae* and *P. ovale* infect the population of Ghana (Appawu *et al.* 1994; Assenso-Okyere & Dzator, 1997). Infections detected via slide examination showed that *P. falciparum* accounts for approximately 90%, *P. malariae* for 9.9% and *P. ovale* for 0.1% (WHO, 2005b).

2.5 Human Malaria: The Socio-Economic Importance

The socio-economic importance of malaria is enormous. The economic burden of malaria in Africa is about 0.6-10% of the gross domestic product, although other reports indicate the disease burden on national income is likely to be higher. The cost of a malaria case from the society's view point is $9.84 or 12 days equivalent of productivity, and the cost of treatment per household ranges from $ 0.2- $15 each month in Africa (WHO, 1999). The prevalence of malaria also has a huge impact on a country's economy (Gallup & Sachs, 2001) since it could inhibit economic growth by restricting worker productivity, tourism, foreign investment and transportation. Poverty does not appear to determine malaria risk (Gallup & Sachs, 2001) but in The Gambia malaria has been associated with poor quality housing and crowding and with travel to rural areas where the level of malaria transmission is higher than in urban areas (Koram *et al.* 1995). The impact of malaria on the growth in real GDP in Ghana is negative and decreases (-0.41%) for every increase in the malaria morbidity rate (Asante & Asenso-Okyere, 2003). According to Asante & Asenso-Okyere, (2003) this significant negative association between malaria and economic growth in Ghana corroborates earlier studies undertaken elsewhere (Gallup & Sachs, 2001; McCarthy *et al.* 2000) but is less than the 1.3% established by Gallup & Sachs (2001) and the average of 0.55% estimated for sub-Saharan Africa (McCarthy *et al.* 2000).

2.6 Human Malaria: Control and/ or Prevention

One of the key malaria control strategies is effective case management which involves early recognition, diagnosis and prompt treatment of cases to reduce severity of the morbidity especially in children and to prevent mortality (WHO, 1993, 2003; 2008; Trigg & Kondrachine, 1998). In most malaria endemic areas, however, the

diagnosis and treatment of malaria are mostly done presumptively. Previous studies carried out in Ghana by Ahorlu and his colleagues (1997), for example, showed that antimalarials are usually given at home in wrong doses with the last resort, after the sickness has failed to respond to home treatment, being the formal health sector and thereby resulting in delay in obtaining effective treatment (Ahorlu *et al.* 1997). Lately, however, there has been a progress in improving access to prompt and effective treatment in Ghana and several other endemic part of Africa (RBM, 2005, WHO, 2008). Based on evidence from drug efficacy studies (e.g. Abuaku *et al.* 2005), Ghana has also changed from chloroquine to artesunate-amodiaquine and artemether-lumefantrine combination therapies for treatment of uncomplicated malaria (RBM, 2005; Adjei *et al.* 2008). Also, intermittent preventive treatment using sulfadoxine-pyrimethamine for pregnant women has begun in Ghana (RBM, 2005). A follow up study showed that artesunate-amodiaquine and artemether-lumefantrine combination therapies are effective for treatment of children with uncomplicated malaria and that drug-related adverse events are rare in treated subjects (Adjei *et al.*, 2008). Another study carried out in the Offinso District of the Ashanti Region, Ghana demonstrated that the use of sulfadoxine-pyrimethamine for intermittent preventive treatment of malaria in pregnant women is effective at reducing maternal morbidity and ensuring good neonatal health (Osei Tutu, 2009). In spite of these recent success stories, it has been noted that the widespread of potentially amodiaquine resistant parasites in some part of Africa seeks to question the effectiveness of amodiaquine as a partner drug for artemisinin combination therapy in Ghana and necessitates constant monitoring and evaluation of this combination therapy in the country (Adjei *et al.* 2008). Again, the reports of moderate to high resistance to sulfadoxine-pyrimethamine in some part of Africa call for an alteration of doses along with the evaluation of impact in malaria

control on ongoing basis, and the need for the strengthening of malaria vector control strategies (WHO/AFRO/MAL/MIP.IPT/05).

Malaria vector control augments the impact of early diagnosis and prompt treatment of malaria cases (WHO, 2003). The most advocated malaria control and/or prevention method appears to be the use of pyrethroid-impregnated bed nets (**Ne**vill *et al.* 1996; Binka *et al.* 1996; Lengeler *et al.* 1996). Evaluation of insecticide treated bed nets showed reduction of transmission, clinical disease, and childhood mortality in at least three large controlled trials in several areas, including some communities in Ghana (Alonso *et al.*, 1991, 1993; Binka *et al.* 1996; Navill *et al.* 1996). However, a subsequent study that evaluated a national malaria control programme that employed insecticide treated bed nets under routine use indicated that the intervention did not yield any significant reduction in child mortality (D'Alessandro *et al.* 1997). Also, the use of deltamethrin treated nets did not have major impact in reducing malaria incidence in a setting where the vectors rest outdoors (Banerjee & Nayak, 2002). In spite of such conflicting reports, the Roll Back Malaria campaign emphasises the use of insecticide-treated bed nets due to their effectiveness in reducing human contact with mosquito vectors (RBM, 2005). Other studies also imply that ITNs can reduce malaria episodes by 48–50% (Lengeler, 2004) and, if universally used, could prevent an estimated 7% of death in under-fives and save more children than any other single intervention except breastfeeding and oral rehydration therapy (Jones *et al.* 2003).

2.6.1 Human Malaria: Factors Involved in Transmission and Control

Factors that affect malaria transmission and control include biological and environmental and/or climatological factors. The biological factors include those those that are in relation to the host, the parasite and the mosquito vector. For

example, a mosquito's ability to transmit malaria parasite includes its innate susceptibility to the *Plasmodium* parasite, host choice and longevity. Other biological factors that affect malaria control efforts are the preferred feeding and resting place of the adult mosquito vectors, and the susceptibility of these vectors to insecticides. The environmental and/or climatological factors on the other hand include rainfall, temperature, relative humidity and elevation. The environmental and/or climatic factors may affect the biological factors such as the longevity of the mosquito vector (WHO, 1990; Linsay & Birley, 1996; Martins, 1995; Snow *et al.* 1993; 1994; Greenwood *et al.* 1987; Molineux *et al.* 1989; Wellem & Fairhurst, 2005).

2.6.1.1 Climatic factors

Malaria is influenced by climatic factors, mostly rainfall, relative humidity and temperature (WHO, 1990). Rainfall, for example, affects the availability of mosquito breeding habitats. The oviposition of mosquito eggs by gravid females and their maturation to larvae through pupae to adults requires the availability of aquatic breeding habitats (Le Sueur & Sharp, 1991; Molineux, 1988). Several studies have reported the association between *An. gambiae* abundance and rainfall (Ramasamey *et al.* 1992, Molineux, 1988; Appawu *et al.* 2001, 2004; Appawu, 2005). While *An. gambiae* prefers to breed in temporary clear waters, *An. funestus* prefers more permanent water bodies. Nevertheless, the availability of both types of breeding grounds depends on sufficient rainfall, which is also related to the saturation deficit and affects mosquito survival (Molineux, 1988). This offers a good basis for using rainfall as a predictor for the presence of vectors, their survival rates and malaria transmission potential. The natural boundaries of the geographical distribution of malaria transmission are primarily determined by rainfall and temperature patterns.

Temperature drives vector development. After oviposition, the rate of egg development through the larval and pupal stages to the adults mainly depends on temperature. As temperature increases, the time required for mosquito development shortens (Rueda *et al.* 1990). For example larval development may last for more than 45 days at 16°C but only 10 days at 30°C (Tekleheimanot *et al.* 2004). Tekleheimanot *et al.* (2004) also observed that it takes 47 days at 16°C and 37 days at 17°C for the larval and pupal stages to be completed. Thus, by affecting the duration of the development of the aquatic stage in the mosquito life cycle, temperature determines the timing and abundance of mosquitoes following adequate rainfall. The blood feeding frequencies of mosquitoes increase with temperature, leading to more vector host contact and therefore increased proportions of infective mosquitoes. Very low temperatures limit malaria vector development by prolonging the gonotrophic cycles and reducing the survival rate (Martens, 1995).

Temperature affects both vector survival and the sporogonic cycle of the parasite. *Anopheles gambiae* for example, exists in areas where absolute minimum temperatures in winter remain above 5°C in Africa (Leeson, 1931; De Meillon, 1934; MARA, 1998). Studies show that the duration of the sporogonic cycle varies inversely with environment temperature (Macdonald, 1957; Buynavanich & Landrigan, 2003). The duration of the development of the ookinete stage of the malaria parasite in the midgut of the *Anopheles* mosquito also depends on temperature. As temperature declines, the development of malaria parasites and biting activities of mosquitoes also decline. Also, the incubation time for *P. falciparum* is 26 days at 20°C but shortens to 13 days at 25°C (Buynavanich & Landrigan, 2003). On average, the sporogonic cycle lasts about 10 days, but shortens to about five days as temperature increases to over

30°C (Macdonald, 1957). These explain why effective malaria transmission can occur only in areas with temperatures higher than 20°C (Lindsay & Martens, 1998).

There are some controversies regarding the relationship between weather and malaria transmission. While some studies have shown the significance of rainfall as a precipitating factor for malaria transmission (Loevinsohn, 1999; Lindblade *et al.* 1999; Kilian *et al.* 1999; Bouma *et al.* 1996), other studies show negative or neutral effects (Lindsay *et al.* 2000; Woube, 1997). For rainfall to have a positive correlation with malaria cases the temperature must be warm enough to support mosquito and parasite development. Hence, the impact of rainfall on malaria cases becomes more immediate in warmer temperatures and this is consistent with laboratory findings that a mosquito population peaks early at high temperatures but experiences slow, steady growth with a delayed peak at low temperatures (Alto & Juliano, 2001). Other studies on the timing of the mosquito life cycle imply that comparatively higher malaria cases should follow periods of increased temperature and rainfall. Since temperature speeds up several steps in the process of mosquito and parasite development, the time lag between the appearance of suitable weather conditions and the manifestation of new malaria case should shorten as temperature increases. For example, at an average temperature of 20°C the aquatic phase of the mosquito will be completed in about 28 days (5 days for the eggs to hatch and 23 days for the larva to develop to the adult stage); and sporogony is completed in about 28 days. At this temperature, malaria cases should appear 9 – 10 weeks following rainfall, assuming an average incubation period of about 10 – 16 days. The number of malaria cases should be positively related to increases in temperature. That is, when the mean temperature is higher, say 30°C, the aquatic stages of the mosquito and the sporogony cycle are completed in about 12 and 18 days respectively, and malaria cases should appear 4 – 5 weeks

following rainfall and the time lag in the effect of temperature should also be shorter. An integration of the climatic and environmental factors that influence the distribution of insect and pathogens they transmit offers a powerful system for assessing disease vector dynamics in relation to disease patterns and the impact of control efforts.

Humidity is a key environmental parameter with respect to the survival of mosquitoes. Relative humidity influences dispersal, mating, feeding behaviour, oviposition of vectors and the rate of evaporation of water from breeding sites (Lindsay & Mackenzie, 1997). To survive, *Anopheles* mosquitoes need at least 50 or 60 % relative humidity (Martin & Lefebvre, 1995). Higher relative humidity favours metabolic processes in mosquitoes and lengthens their life-span. The survival of the mosquitoes allows the malaria parasites to complete their life cycle and be transmitted to several hosts (Martin & Lefebvre, 1995; Lindsay & Mackenzie, 1997). Low relative humidity also causes the mosquito vector species to feed more often to pay off for dehydration and hence increase man-vector contact. Optimal relative humidity causes mosquitoes to survive for a longer period. This enables them to disperse farther and to have a greater chance to take part in malaria transmission cycles (Lindsay & Mackenzie, 1997; Liehne, 1998).

Environmental and/ or climatic factors such as rainfall, temperature and humidity influence malaria transmission. At places where temperature is not a limiting factor, malaria transmission is highly seasonal, with its peak following the period of peak rainfall. Undertanding how malaria transmission varies in a given area as a result of seasonal or annual changes in environmental and/ or climatic factors is important for the planning of large-scale malaria control intervention as it may allow interventions to be tailored to particular communities or periods of the year.

2.6.1.2 Pattern of feeding, preferred sources for blood meals and resting place of Anopheles mosquitoes.

The patterns of feeding, resting and preferred sources for blood meals of the *Anopheles* mosquitoes influence their ability to transmit malaria (WHO, 1975; 2003). Time of biting varies among different malaria vector species (Pates & Curtis, 2005) and may be influenced by geographical location and climate. In Ghana, for example, significantly low biting rates were observed from malaria vectors in the early mornings mostly after 05:00 hour at Prampram in the coastal savannah and Dodowa in the coastal forest zones (Appawu *et al.* 2001). In the Kassena Nankana District of the northern savannah zone, however, significantly high biting rates were observed from the same vectors during the period in question (Appawu *et al.* 2004). These spatial variations in the biting time imply that the likelihood of malaria transmission in the early morning hours may be higher in northern Ghana than in the south.

The choice of human and/or animal as source of blood meal may also vary among *Anopheles* mosquitoes and even among sibling species of a complex. For example, *Anopheles melas* can be highly anthropophilic in the presence of man and very zoophilic where there is no human host (Akogbeto, 2000; Diop *et al.*, 2002; Awolola *et al.* 2002). However, *An. gambiae* s.s and *An. funestus* are mostly anthropophilic and therefore are two of the most efficient human malaria vectors worldwide (Coetzee *et al.* 2000; McEwen, 2000).

Malaria transmission is also influenced by the preferred choice of feeding place by the malaria vectors (WHO, 1975, 2003; Coetzee *et al.* 2000). Some *Anopheles* species: *An. gambiae* s.s and *An. funestus* in Africa, *An. culicifacies* in India, and *An. minimus* in East and Southest Asia are naturally endophilic (reviews in Pates & Curtis, 2005). However, the 2 brackish water species, *An. melas* distributed

along the coast of West Africa and *An. merus* of the East Africa are generally more exophagic and thus are less efficient in transmitting malaria (Coetzee *et al.* 2000).

2.6.1.3 Longevity of Anopheles mosquitoes as a factor affecting malaria transmission and the techniques involved in its determination in the field.

Adult *Anopheles* mosquito survival rates affect their potential to be vectors of diseases including human malaria. For example, female *Anopheles* mosquitoes infected during blood meal must survive the incubation period of the parasite, before they can transmit the disease (de Moor & Steffens, 1970; Miller *et al.* 1973; Onori & Grab, 1980; Kettle, 1995: WHO, 2003). The survival or longevity of adult *Anopheles* mosquitoes is, therefore, one of the variables used in determining their competence or vectorial capacity in malaria transmission (WHO, 1975, 2003).

It is difficult to assess mosquito survival and mortality rates directly in nature. The indirect method requires knowledge of the proportions of the population that are of a specific age and those that are older. Often the proportions of nulliparous and parous females are measured. With knowledge of this ratio and of the duration of the first gonotrophic cycle, the daily survival rates can be estimated (Davidson, 1954). However, the method is valid only when death rates are independent of age, and it is subject to severe sampling problems. Survival rates have been estimated in nature using mark-recapture method (Sheppard *et al.* 1969; Conway *et al.* 1974; MacDonard, 1977a). When the exact ages of recaptured samples are known, the mark-recapture data can offer a direct measure of survival rates but the data cannot be analysed using methods which assume a constant survival rate (Fisher & Ford, 1947).

The observation that irreversible changes occur progressively in the internal reproductive organs of females, provided a direct method for finding the physiological

age of mosquitoes. When females complete some gonotrophic cycles, the ovarioles bear dilatations, which results from the distension of ovarian membranes by the developing oocyte or from the residue of a resorbed follicle. There is a 1:1 ratio between the gonotrophic cycles completed and the largest number of dilatations in any ovariole (Polovodova, 1949; Samarawickkrema, 1962, 1967; Gilles & Wilkes, 1965). The duration of gonotrophic cycle is equal to the ovipositional interval, which is the mean number of days between ovipositions (Garrett-Jones & Grab, 1964).

Generally in nature, few organisms die of senescence, most are killed by predators, diseases, and hazards long before they reach old age (Krebs, 1972). This belief coupled with the accounts of mosquito survival in the laboratory and in the field led to the assertion that the full life span of female mosquitoes is measurable in months. It was also established that the said hazards of wildlife fall more or less equally on all adult age classes so that death rate is independent of age. Based on this and other works, an exponential model of mortality was assumed, which enables the calculation of the probability of a mosquito surviving a day (Macdonald, 1952, 1957). Also, estimates of the survival rate, *p*, have been used as constants in models of population dynamics (Miller *et al.* 1973; Weidhaas *et al.* 1974), in studying the vectorial capacity of a population of *An. gambiae* species (Garrett-Jones & Shidrawi, 1969) and for assessing efficacy of malaria vector control measures (Garret-Jones & Grab, 1964; Dietz *et al.* 1974; WHO, 1975, 2003).

The assertion that in wild populations of mosquitoes the adult death rates are independent of age has not been accepted by all researchers. Studies by Clements & Paterson, (1981) revealed that in most wild populations of 11 tropical mosquitoes, the adult female death rates increase with age. The patterns of death of most of the populations were well described by the Gompertz mortality function (Gompertz,

1825), according to which the rate of mortality increases with age in such a way that its logarithm is directly proportional to age. The epidemiological implications of these findings were examined through the calculation of the 'longevity factor', which is part of vectorial capacity. Here, huge differences were obtained in estimates of the longevity factor when the Gompertz model of death was substituted, in appropriate cases, for the widely used exponential model. Despite the shortfalls of the rates formulae, it has been established that they apply when the age-structure is steady, or for a seasonal 'insect' when a whole season's data is combined (Clements & Paterson, 1981) and it has been used in several recent malaria transmission studies (Appawu *et al.* 2001; Vythilingam *et al.* 2003; Wanji *et al.* 2003; Appawu, 2005).

2.6.1.4 Entomological inoculation rates (EIR)

Malaria parasite transmission intensity in the field is estimated through the determination of the entomological inoculation rate (EIR). The EIR is the product of the *Anopheles* mosquito biting rate and the proportion of mosquitoes carrying sporozoites in their salivary glands. It can be used to estimate the level of parasite transmission to people dwelling in a given community at a given time and as a parameter to differentiate transmission intensity between communities over a period (Macdonald, 1957; Burkot & Graves, 1995; Beier *et al.* 1999).

Studies on malaria transmission have been done in Africa where sporozoite rates usually range from 1 to 20% (Wirtz & Burkot, 1991) with the EIRs in endemic areas ranging from between less than one to greater than 1,000 infective bites per year (Trape & Rogier, 1996). The implication for such a wide range of values for the sporozoite rates and the EIR is that malaria transmission intensity varies significantly in different parts of Africa and therefore the need for more area specific control

interventions. Significant reductions in the transmission intensity are necessary to reduce the prevalence of malaria infection in human populations.

2.6.1.4.1 Methods for the determination of the parameters of the EIR

2.6.1.4.1.1 Methods for the determination of human biting rates or man biting rates

The number of *Anopheles* mosquito vectors biting humans is one of the major determinants of malaria transmission (Macdonald, 1957; Burkot & Graves, 1995; Beier *et al.* 1999). The most direct way to measure human-biting rate is the human landing catches (HLCs) technique. This technique is important since it determines which anophelines are anthropophagic; which of the anthropophagic ones transmit malaria; human-biting rates and seasonal variations; whether the vectors are endophagic or exophagic and their peak biting time (WHO, 1975, 2003). Although the method is expensive, technically difficult to replicate and unethical in areas of drug-resistant malaria, it is special as it directly samples man-biting species (Le **G**off *et al.* 1997). It involves a team waiting at a place, usually at night, collecting all the mosquitoes that attempt to feed on them (WHO, 1975, 2003).

Other ways of measuring human biting rates, namely, pyrethrum spray catches and light and exit traps rely on behaviours that are less directly linked with feeding on man (Garret-Jones, 1970; Service, 1993b). The sampling biases between HLCs and light-traps have been explored (Lines *et al.* 1991; Faye *et al.* 1992; Mbogo *et al.* 1993a; Davis *et al.* 1995; Smith, 1995; Magbity *et al.* 2002). The outcomes of these studies were rather controversial. For example, some studies have established that light-traps can be employed as a surrogate for HLCs in estimating human biting rates (Lines *et al*. 1991; Magbity *et al.* 2002) given that the differences in the mean numbers of mosquitoes caught from light-traps and paired bait collections were not

significant. However, Faye *et al.* (1992) found that while the mean numbers of mosquitoes captured by both methods may not differ significantly indoors, outdoor light-traps may capture significantly less mosquitoes depending on the season of the year. Furthermore, the studies by Hii *et al.* (2000) & Davis *et al.* (1995) suggest not only the fact that light-traps could under-sample some *Anopheles* species but also the fact that these light-traps have the tendency of selectively sampling older mosquitoes.

2.6.1.4.1.2 Methods for the determination of sporozoites infection rates in mosquitoes

Mosquito infectivity in the field has always been estimated by determining the proportion of field collected mosquitoes carrying *Plasmodium* sporozoites. This may be done by the dissection of the salivary glands (Ljungström, 2004; WHO; 1975, 2003) but this method has three major drawbacks. First, in areas where vectors have low sporozoite rates it is necessary to dissect many samples to establish sporozoite rates and this is laborious. Second, it cannot distinguish between the human plasmodia or these and bird or reptile plasmodia. Finally, it requires fresh female mosquito samples and so it is not easily applicable in the field (WHO, 1975; 2003). Wirtz *et al.* (1985) developed an enzyme-linked immunosorbent assay (ELISA) method for the detection of *Plasmodium*-specific circumsporozoite antigens (CS) from the mosquito head and thorax. The ELISA combines the specificity of antibodies with the sensitivity of enzyme assays, by using antibodies or antigens coupled to an easily-assayed enzyme. It can be used to test for antibodies that recognise an antigen or it can be used to detect the presence of antigens that are recognised by an antibody. The sporozoite ELISA, for example, involves the use of the monoclonal antibodies to detect circumsporozoite (CS) proteins of *P. falciparum* (Burkot *et al*. 1984; Wirtz *et al*. 1985; 1987a, b) and the results are read using an ELISA plate reader (Wirtz *et al.*

1985; Beier *et al*. 1988) or visually (Beier & Koros, 1991). This sporozoite ELISA is more rapid and is very valuable in detecting infectious mosquito species from either fresh or dry stored mosquito samples. It is also useful in quantifying sporozoite load and detecting precise parasite species invloved (Beier & Koros, 1991; Wirtz *et al*., 1992). Sporozoite rates are then derived from the proportion of man biting Anopheles species that test positive for P. falciparum circumsporozoite protein by ELISA.

2.6.1.5 Insecticide susceptibility or resistance in the Anopheles mosquitoes as a factor that affects malaria transmission and control.

Insecticide-based malaria vector control interventions are ideal for killing indoor feeding vectors so long as the vectors remain susceptible to the insecticides being employed in the control measures. However, after prolonged exposure of the malaria vectors to insecticide over several generations, insecticide resistance may develop. Insecticide resistance was a key problem to the Global Malaria Eradication Campaign launched in 1955 (CDC, 2004b; www.who.int/bulletin/volumes/86/2/07-050633/en/; http://en.wikipedia.org/wiki/*Anopheles*). When controlling malaria vectors via insecticides, the survivors of susceptibility tests should be studied to identify the sibling type and the chromosome type of sibling since non-uniform selection pressure could result from exposure of different cytotypes to insecticides. Such directional selection for insecticide resistance in the vector population could affect the progress of malaria control programmes (CDC, 2004b).

The ability of vectors to ingest parasites, to promote their maturation until the infective stage and the rate of vector survival until parasite maturation can vary according to geographic mosquito strain (Crans, 1973; McGreevy *et al.* 1982) possibly due to insecticide selection pressure (McCarroll & Hemingway, 2002).

Insecticide resistance is assumed to increase disease transmission but studies involving insecticide resistant *C. quinquefasciatus* (Hemingway, 2000; McCarroll *et al.* 2000) indicated that resistant species are unable to transmit parasites compared to susceptible ones. However, this issue remains unresolved as data on the success of ookinete to oocyst development in resistant and susceptible *An. stephensi* did not indicate any variation in vectorial capacity between resistant and susceptible ones (Vontas *et al.* 2004). Knowledge of the influence of resistance in the *Anopheles* vectors to the available chemical insecticides used in vector control on *Plasmodium* parasites development within the vectors may be important in designing more valuable malaria vector control techniques in future.

2.6.2 Chemical Insecticides used in Malaria Vector Control

Insecticides are chemical or biological agents that control insects by killing them or preventing them from engaging in destructive behaviours. The two types of chemical insecticides used in vector control are organic and inorganic insecticides. Modern vector control methods largely involve the use of organic insecticides. Although there are several organic insecticides used for insect pest control (Ware & Whitacre, 2004), organochlorines (OPs), carbamates and pyrethroids are the main organic insecticides used to control insects of public health importance (WHO, 1998b). Of these major insecticides of public health importance, only pyrethroids are used for net treatment (Lines & Zaim, 2000; Zaim & Nakashima, 2000; WHO, 2000). Pyrethroids are synthetic chemicals similar to the natural pyrethrin obtained from chrysanthemum flowers. They are usually degraded by sunlight and do not affect groundwater quality significantly. Some of the pyrethroid insecticides which are

widely used for agricultural and domestic pest control purposes are deltamethrin and permethrin (Stafford, 1999).

Pyrethroids in genral are axonic poisons and work by keeping open the sodium channels in neuronal membranes. There are two types of pyrethroids. Type I, among other physiological responses, has a negative temperature coefficient, resembling that of DDT. Type II, however, has a positive temperature coefficient, showing increased kill with increased ambient temperature. Pyrethroid insecticides affect the peripheral and central nervous system (CNS) of insect by stimulating nerve cells to produce repetitive discharges and thereby causing paralysis (Ware & Whitacre, 2004).

2.6.3 Chemical Insecticide Resistance in Malaria Vector Control

Synthetic insecticides have been useful in many ways in malaria vector control efforts but their very wide use has resulted in several problems. One of these problems is the appearance of resistant strains (WHO, 1980; Reimer *et al.* 2005). A population of insects is considered to be resistant if its response to an insecticide in detection tests drops much below its normal response. Insecticide resistance is a dynamic process, developing at widely different rates in different species and in the same species subjected to diverse conditions of insecticidal pressure (WHO, 1980). It shows uniform evolution in which the susceptible insects are killed during vector control efforts, leaving behind only those that are genetically resistant to the poison. Insecticide resistant species form an increasing large part of the population and pass their insecticide resistance mechanism to the next generations. Thus, insecticide resistance in insects simply represents the survivors of a stringent biological selection mechanism, the insecticide, over several generations. The greater the number of

generations exposed to insecticides, the greater the potential for developing insecticide resistance due to this intensive selection mechanism (Ware, 1978).

2.6.3.1 Tolerance and resistance to chemical insecticides in vector control

A population of insects considered resistant and a different species considered tolerant may both survive exposure to similar levels of insecticide. However, species-wide tolerance and population resistance are different, and distinguishing tolerance and resistance requires knowledge of the insecticide's toxicity level to some of the insects under study and some means to estimate changes in toxicity over time. Tolerance describes species-wide ability to survive a given level of insecticide exposure. Innate tolerance does not involve reduced susceptibility in selected populations or changes in susceptibility in return to exposure of the insecticide. Also, innate tolerance can involve non-selective shifts in susceptibility over time and no clear differences among separate populations of the same species. Resistance, however, refers to differences in susceptibility that arise among populations of the same species exposed to an insecticide. These variations are identified through observation of a statistical shift in the lethal dose either to kill 50% or 95% of the population. Individual differences in susceptibility exist within each species. Individuals much less susceptible may be present, usually at low frequencies, in at least some of the wild populations. Thus, a population must be labelled resistant or tolerant with care as many supposed cases may be due to poor application process or a bad batch, along with species-innate behavioural and/or ecological differences. A series of in-depth tests is needed to prove an evolution of resistance (Breaud, 1993).

2.6.4 Mechanisms of Chemical Insecticide Resistance in Insect Vectors

Insecticide resistance in insect vectors is generally mediated by behavioural, metabolic and/or physiological factors. It normally results from at least one of the three different mechanisms namely, reduction in insecticide penetration into the insect, increased metabolism of insecticide by metabolic enzymes secreted by the insect and modification of the insecticide target site in the insect (reviews in Brogdon & McAllister, 1998; Hemingway *et al.* 2004). Another mechanism of resistance based on thermal stress response has been proposed (Patil *et al.* 1996) but its impact on disease vector control interventions is not known (Brogdon & McAllister, 1998).

The increased metabolism of insecticide by metabolic enzymes also called the detoxification enzyme-based resistance mechanism occurs when enhanced levels or modified activities of certain enzymes prevent the insecticide from reaching its site of action. The target-site resistance mechanism, however, occurs when the insecticide no longer binds to its target site. Some studies have explored the distribution of the detoxification enzyme-based resistance mechanism in some West African Countries (Charo, 2005; Corbel *et al.* 2007; Awolola *et al.* 2008; Müller *et al.* 2007). Likewise, the target-site resistance mechanism especially the knockdown resistance (*kdr*) has been studied extensively in West Africa (see e.g. Martinez-Toress *et al.* 1998; 1998; Corbel *et al.* 2007; Awolola *et al.* 2008; N'Guessan *et al.* 2007; Kabulah, 2007).

2.6.4.1 Detoxification Mechanisms

Three major detoxifying enzymes implicated in insecticide resistance are esterases, cytochrome P450 monooxygenases and glutathione-S-transferases (WHO, 1998b; Oppenoorth, 1985). Increased activities of these enzymes have been linked to resistance development due to the fact that most insecticides can be detoxified by at

least one of these enzymes in insects (Vulule *et al*. 1999). Cross-resistance among different classes of insecticides, irrespective of their target sites, may also be due to the broad substrate spectrum of each of the metabolic enzymes and the multiplicity of individual enzymes (ffrench-Constant *et al*. 1999).

2.6.4.1.1 Esterase-mediated resistance

Esterase-mediated insecticide resistance is the key mechanism in mosquitoes for organophosphates (OPs) and carbamates (Herath *at al.* 1987; Karunaratne *et al.* 1993; Hemingway, 1981; Pasteur & Raymond, 1996; Hemingway & Ranson, 2000). In *Culex* species, esterase-based resistance to OPs results from sequestration (Hemingway & Karunaratne, 1998; Hemingway, 1999; Hemingway & Ranson, 2000). However, several *Anopheles* species have a non-elevated esterase mechanism that confers resistance specifically to malathion via increased rates of metabolism (Hemingway, 1982, 1983, 1985, 1999). Most pyrethroids also contain an ester linkage making them susceptible to hydrolysis by esterases (Oppenoorth, 1985). Esterase-based resistance has been found in some pyrethroid-resistant *Anopheles* species namely, *An. albimanus* from southern Mexico (Penilla *et al.* 1998), *An. gambiae* from Kenya, Mozambique and Ghana (Vulule *et al.* 1999; Charo, 2005; Casimiro *et al.* 2006) as well as *An. arabiensis* from Mozambique (Casimiro *et al.* 2006).

2.6.4.1.2 Monooxygenase-mediated resistance

Monooxygenases are a group of enzymes with the rate-limiting enzyme usually being cytochrome P450. They catalyze multiple oxidative reactions and are able to metabolise diverse endogenous and exogenous substrates (Guengerich, 1996). Earlier studies on the insect P450s have reported the operation of a distinctive

electron transport system involving multiple forms of cytochrome P450s. They metabolise insecticides via: O-, S-, and N-alkyl; hydroxylation, aliphatic hydroxylation and epoxidation; aromatic hydroxylation, ester oxidation, and nitrogen and thioether oxidation (Wilkinson, 1976). Modifications in P450s can regulate the levels of insect vector resistance to OPs, carbamates, pyrethroids and organochlorines (Hemingway & Ranson, 2000).

Pyrethroid resistance due to enhanced P450 monooxygenase activity has been observed in some mosquitoes (Vulule *et al.* 1994; Chandre *et al.* 1998; Hemingway & Ranson, 2000). For example, elevated monooxygenase activity have been associated with pyrethroid resistance in *An. funestus* populations from South Africa and Mozambique (Hargreaves *et al.* 2000; Brooke *et al.* 2001; Casimiro *et al.* 2006), *An. gambiae* and *An. arabiensis* from Mozambique (Casimiro *et al.* 2006), *An. gambiae* from Kenya and Cameroon (Vulule *et al.* 1999) and *An. gambiae* s.s. M molecular form from southern Ghana (Müller *et al.* 2007).

2.6.4.1.3 GST-mediated resistance mechanism

Detoxifying GSTs are a family of multifunctional dimeric enzymes that catalyse the nucleophilic attack of reduced glutathione (GSH) on the electrophilic centers of lipohpilic substrates including insecticides (Hemingway & Ranson, 2000). GSTs are involved in the detoxification of organophosphates through O-dealkylation or dearylation (Hayes & Wolf, 1988), and as a primary detoxifying enzyme of DDT to the non-toxic DDE (Pasteur & Raymond, 1996). GSTs implicated in DDT insecticide resistance exist as clusters of genes that have been further shuffled through the genome by recombination (Zhou & Syvanen, 1997). A number of resistance GST

genes, including multiple forms in the same insect, have been found in several insect vectors (Grant *et al.*, 1991; Prapanthadara, 1996).

Although earlier studies showed that pyrethroids do not serve as substrates for GSTs (Grant & Matsumura, 1989; Reidy *et al.* 1990), induction of GST activity has been reported after exposure to OPs and organochlorines and pyrethroids (reviews in Hemingway *et al.* 2004). GSTs-based resistance to DDT in *An. gambiae* (Prapanthadara *et al.* 1993, 1995, 1996; Ranson *et al.* 2001), *An. arabiensis* (Hargreaves *et al.* 2003) and *An. albimanus* (Penilla *et al.* 1998) have also been reported.

2.6.4.2 Target-site (kdr) insecticide resistance

Target-site insecticide resistance in general is due to functional modifications of insecticide target proteins in insects rendering the resistant ones less sensitive to the impacts of insecticides compared with the susceptible ones. The major insecticide target sites in insects include the acetylcholinesterases, the voltage-gated sodium channel, and gama-aminobutyric acid (GABA)-gated chloride channel. Reduced sensitivity of these insecticide target sites in insects has been reported by some studies (reviews in Brogdon & McAllister, 1998; Hemingway *et al.* 2004). Feyereisen, (1995) and ffrench-Constant *et al.* (2000) have indicated that the reported modifications have been exclusively point mutations of structural genes encoding the target site protein. For example, pyrethroid insecticides act on the nervous system through the modification of the gating kinetics of the voltage-sensitive sodium channels (Lund & Naharashi, 1983). One important resistance mechanism against pyrethroids results in a marked reduction in the intrinsic sensitivity of the insect nervous system to these compounds. This mechanism confers resistance to both pyrethroid insecticides and

DDT as they share similar mode of action. It was first identified in DDT-resistant *Musca domestica* and was called knockdown resistance (*kdr*) [Milani, 1954].

A single mutation (leucine to phenylalanine) in the S6 transmembrane segment of domain II in the sodium channel sequence is linked with *kdr* to pyrethroids and DDT in *Blattella germanica* (Miyazaki *et al.* 1996), *An. gambiae, Plutella xylostella* and *M. persicae* (Martinez-Toress *et al.* 1997; 1998). DDT-pyrethroid cross-resistance appears to cause a shift in the sodium current activation curve and cause low sensitivity to pyrethroids (Vais *et al.* 1997). Also, leucine to histidine mutation at this same site exists in pyrethroid-resistant tobacco budworm (Park & Taylor, 1997). In *super-kdr* houseflies, this mutation is linked with a second substitution further upstream in the domain which replaces a methionine with a threonine (Williamson *et al.* 1996a). Since the *kdr* gene mutation is due to a change of the insecticide affinity for its binding site on the sodium channel (Pauron *et al.* 1989), according to Martinez-Toress *et al.* (1998) it is possible that a limited number of changes in the insecticide target site can promote nerve insensitivity against insecticides and cause insecticide resistance in the insect vector.

The knockdown resistance (*kdr*) type of the target-site insecticide resistance mechanism is known to be widespread in some parts of Ghana including the Greater Accra Region (Adasi *et al.* 2000; Adeniran, 2002; Yawson *et al*. 2004; Achonduh, 2005; Kabulah, 2007) and the Ashanti Region (Stiles-Ocran *et al.* 2003; Yawson *et al*. 2004). For example, *kdr* frequencies of 88% and 91% were observed at Dodowa and Accra respectively in the Greater Accra Region (Adasi & Hemingway, 2008). Also, a *kdr* frequency of 100% was observed in the S form of *An. gambiae* s.s in Kumasi in the Ashanti Region (Yawson *et al.* 2004). The *kdr* is also known to be the most widespread insecticide resistance mechanism in some other countries in West Africa

(Martinez-Toress *et al.* 1997; 1998; Darriet *et al.* 1998; Chandre *et al.* 1998, 1999a, b, 2000; Diabete *et al.* 2002, 2003b, 2004; Elissa *et al.* 1993; Akogbeto, 2002; Corbel *et al.* 2007; Awolola *et al.* 2008; N'Guessan *et al.* 2007; Reimmer *et al.* 2005).

2.6.5 Cross-Resistance and Multiple Resistance to Insecticides in Insect Vectors

Since insecticides have in many cases a similar target site in the insect, cross-resistance and multiple-resistance can take place (Breaud, 1993). Cross-resistance is resistance of a strain of insect to compounds other than the selecting agent due to same biological mechanism (Kumar, 1984; Gullan & Cranston, 1994). Pyrethroid resistance has evolved despite early optimism that due to its rapid toxicologic action (Malcolm, 1988) and less persistence in the environment it would not produce resistance in disease vectors. It evolved via enhancement of existing mechanisms and cross-resistance. For example in Guatemala, pyrethroid resistance was first reported in *An. albimanus* population resistant to fenitrothion. When deltamethrin was used afterwards, the esterase conferring fenitrothion resistance was enhanced by selective pressure to yield deltamethrin cross-resistance (Brogdon & Barber, 1990). DDT-permethrin cross-resistance due to both oxidase cross-resistance in *An. albimanus* (Brogdon & McAllister, 1998) and *kdr* in other mosquitoes (Breaud, 1993; Soderlund & Knipple, 2003) has been found. However, no study has explored the phenomenon of DDT-pyrethroid cross-resistance mechanism in insect vectors in Ghana and so there is the need for surveillance studies to characterise the distribution of cross resistance mechanism in the country and in other unexplored areas of Africa.

Multiple resistance occurs when an insect's resistance to insecticides is conferred by dissimilar yet coexisting mechanisms (Gullan & Cranston, 1994). For example, in Sri Lanka, multiple insecticide resistance mechanisms involving

metabolic changes [elevated esterases, gluthathione S-transferases (GSTs) and monooxygenases] and insensitive target sites selected in the malaria vectors *An. culicifacies* and *An. subpictus* have been reported (Perera *et al.* 2008). Similarly, multiple insecticide resistance mechanisms involving the *kdr* and *ace-1*^{R} in *An. gambiae* and *Culex quinquefasciatus* have been found in Benin (Corbel *et al.* 2007). Evidence of multiple pyrethroid resistance involving the *kdr* gene and three detoxifying genes; *CYP325A3*, *GSTS1-2* and *TPX4*, and possibly a novel set of two cuticular candidate genes, *CPLC8* and *CPLC#* in *An. gambiae* s.s. from Nigeria has also been reported (Awolola *et al.* 2008). However, there is no published data on the existence of multiple pyrethroid resistance in Ghana and so there is the need for surveillance studies to characterise the distribution of multiple resistance mechanisms in the country and in unexplored areas of Africa at large.

The operational impact of pyrethroid resistance including cross-resistance and multiple resistance in malaria vectors on the efficacy of ITNs is not fully understood (Darriet *et al.* 1998; Chandre *et al.* 1998, 2000; Corbel *et al.* 2007; N'Guessan *et al.* 2007). However, the fact that an insecticide resistant population at a given area may contain a multiplicity of insecticide resistant mechanisms (Awolola *et al.* 2008) and/or cross-cross resistance mechanism can be disturbing. This is because although alternative insecticide application and rotational use of insecticides has been advocated for insecticide resistance management practice (Hemingway & Ranson, 2000; Coleman *et al.* 2006), there are few alternative insecticides available at present for malaria vector control programme operations. Therefore, even though the use of DDT for malaria vector control operations has been advocated (WHO, 2006), there is the need to find alternative insecticides to minimise the spread of cross-resistance between DDT and the pyrethroids (Awolola *et al.* 2008).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study sites

3.1.1 Sunyani Municipality

The Sunyani Municipality has a land mass of 2488 km^2. It is located in the Brong Ahafo Region, between Latitudes 07°35'N and 07°55'N and Longitudes 02°00'W and 02°30'W. It shares boundaries with the Asutifi District to the south, Tano South District to the east, Berekum and Dormaa Districts to the west, and Wenchi District to the north. The mean monthly temperature varies between 23°C and 33°C (the lowest in August and the highest in March/April). The municipality enjoys heavy to moderate rainfall, recording about 945.69 mm as annual mean. There are two rainfall regimes: a major rainy season from April to the end of July and a minor wet season from September to late October. The municipality occurs within a transitional zone with the southern part covered with tropical soft woods whilst the northern sector has mainly Guinea Savannah woodland. The forest cover is degenerating into the Savannah type through activities, such as agriculture and settlement expansion. Suburbs within the municipality are Odumasi, Sunyani, Nsoatre, Antwikrom, Fiapre, Abesim and Chiraa. The municipality is endemic for malaria with endemicity greater than 75% (www. mara. org.za/ pdfmaps/GhaDistribution.PDF). Sunyani (07°20N, 02°20W) and Chiraa (07°24N, 02°11W) with their respective altitudes of 308.8 m and 323.1 m were selected for the study (Figure 3.2).

3.1.2 Asutifi District

Asutifi District is located between latitudes 6°40' and 7°15' North and Longitudes 2°15' and 2°45' West. The district capital is Ahafo Kenyasi, which is about 50 km from Sunyani through Atronie and Ntotroso. It shares boundaries with Sunyani Municipality in the North, Tano South District to the North East, Dormaa to the North West, Asunafo North and South Districts in the South West and Ahafo Ano Districts (Ashanti) in the South East. It covers an area of 1500 km^2. The population was estimated to be 94, 486 in 2004 and 99, 928 in 2006 using 2.8% Growth Rate.

The forest cover is degenerating into the Savannah type through activities, such as agriculture and settlement expansion, although there are still large areas of forest reserves covering a total of about 475.63 km^2. There are dual rainfall maxima: June and October with a mean annual rainfall mostly between 125 and 200 cm. The major rainy season is from May to July and the minor from September to October. There is a sharp dry season between the two rains with the main one occurring between November and March. Relative humidity is usually between 75 - 80% in the rainy seasons and 70 - 80% during the rest of the year.

The district continues to exhibit rural characteristics but Kenyasi No. 2 and Hwediem are described as urban by the classification of the 2000 Population and Housing Census. The household sizes are large with mean lying between 6 and 7 persons per household. Ahafo Kenyasi (06°59N, 02°23W) and Hwediem (06°56N, 02°21W) were selected for the present study. Ahafo Kenyasi is 261.4 m above sea level and Hwidiem is 198.1 m above sea level. The entire Asutifi District is endemic for malaria with malaria endemicity greater than 75% (www.mara.org.za/ pdfmaps/GhaDistribution.PDF).

3.1.3 Reasons for the selection of the Present Study Sites

Newmont Ghana Gold Limited is carrying out mining operations in the Asutifi District (www.newmontghana.com). Ahafo Kenyasi was selected for the present study because it is located in the mining area. The mining operations may alter the breeding potential of the malaria vectors in this site as well as increase migration into this area and this could contribute to environmental factors that may result in increased malaria transmission, morbidity and mortality. Conversely, environmental alterations due to urbanisation and the mining operations may decrease malaria vector breeding sources and, hence, the health, social and economic burden of the disease.

Hwidiem is also located about 10 km from Ahafo Kenyasi and/or the mining operations. Its malaria transmission indices are not likely to be affected by the mining. Data from this site could be compared to that of Ahafo Kenyasi as part of the effort to measure the impact of mining activities on malaria transmission in the Asutifi area.

Sunyani and Chiraa were selected as a control area located about 50 km from Ahafo Kenyasi and/or the mining activities but under similar local political and health administration as Ahafo Kenyasi and Hwidiem respectively. Thus, data generated for Sunyani and Chiraa would also be useful for comparisons with Ahafo Kenyasi (mining area) and Hwidiem (which is relatively closer to the mining area).

On the whole, the present study would inform the implementation of large scale malaria vector control planned by the National Malaria Control Programme for these areas. The data will also be useful in deciding on vector control operations for other communities in the Brong Ahafo Region with similar ecological characteristics. Figure 3.2 shows that there was no published data on malaria entomology in these areas and the Brong Ahafo Region in general prior to this study. These current study sites were selected to help bridge the knowledge gap indicated on the map.

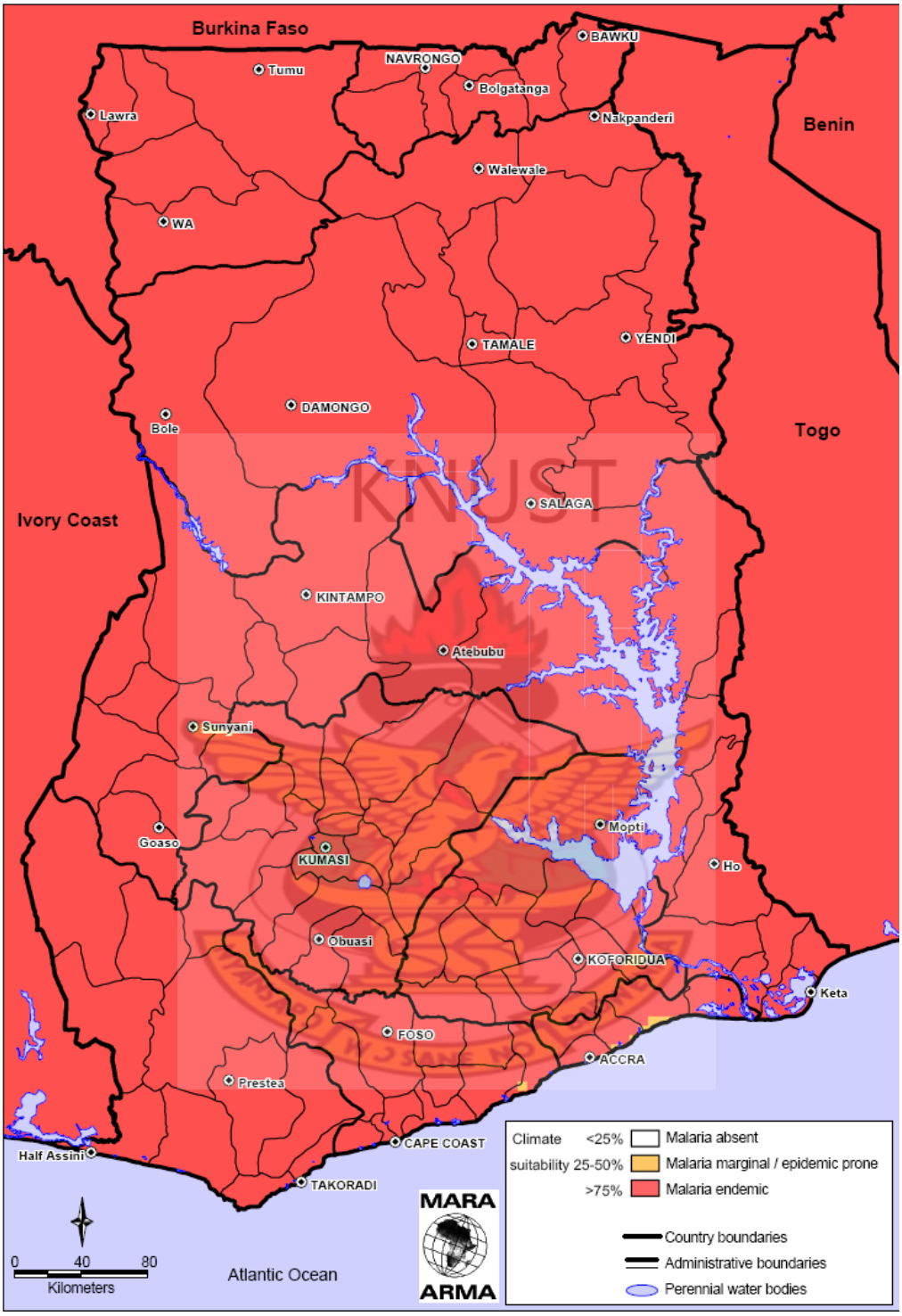


Figure 3.1 Distribution of malaria in Ghana. Retrieved on March 11, 2009 from the MARA/ARMA website: http://www.mara.org.za/.

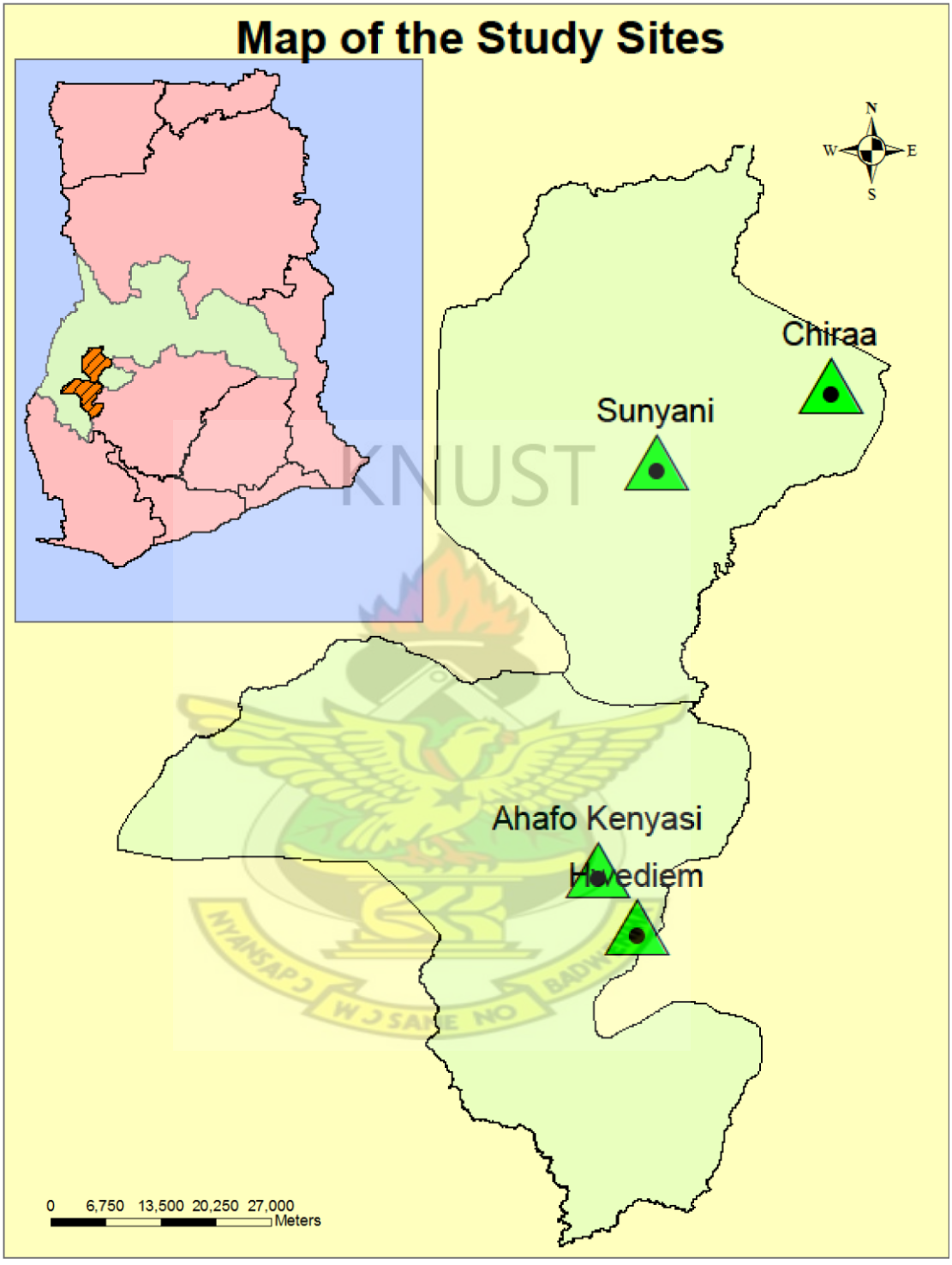


Figure 3.2: Regions in Ghana where there were published entomological data prior to the present study (coloured pink), the region where there were no published data (coloured cream), the two study districts (coloured orange with black lines) and the individual communities proposed for this study.

3.2 Meteorological Data Collection

Data on rainfall, relative humidity and temperature recorded from 2005 to 2007 were collected from the Ghana Meteorological Agency (GMA), East Legon-Accra.

3.3 Mosquito Collection

3.3.1 Mosquito larvae and pupae collection

Mosquito larvae and pupae were sampled from Sunyani and Ahafo Kenyasi in June and July during the main rainy season and in October during the minor rainy season. Breeding sites were identified by sampling of small pools of stagnant water and gutters, exposed to sunlight (Fig. 3.4). Most sites were small shallow pools of clear stagnant rainwater but few chocked gutters were found and surveyed in Ahafo Kenyasi. Like the breeding site in the figure 3.3, *An. gambiae* were also found and collected from muddy water bodies.

The *Anopheles* mosquito larvae were identified by their typical resting position, with the body parallel to the water surface and just below the surface film. Larvae and pupae were collected into plastic containers with a 350 ml ladle used as a dipper. Samples from each collection point were transferred into separate plastic bottles. The samples were then transported to the insectary for rearing.

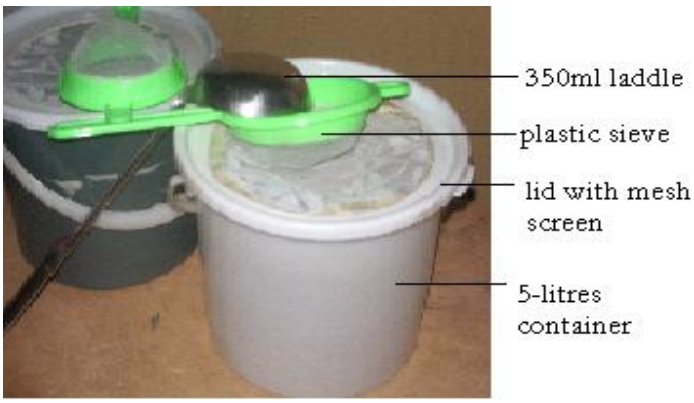


Figure 3.3 A labeled diagram of mosquito larval collection equipment.

Figure 3.4 Mosquito larval breeding site at Sunyani, created by articulated vehicle tyres. In the fore is accumulated stagnant water surrounded by green grasses and exposed to direct sunlight.

3.3.2 Insectary rearing of mosquitoes

Larvae collected from the breeding sites were transferred into mosquito-rearing plastic containers in the insectary. The batches were examined and non-mosquito species were discarded with the aid of a hand lens and hand-sucking pipette. In cases where larvae of culicines were present, they were identified by their angular position on the water surface and were also removed from the larval containers. The pupae were transferred into small plastic cups and placed in labelled wooden cages (Fig. 3.6) for adult emergence. The larvae were maintained in water from the collection site and were fed once a day on cerelac (Nestle Ghana Ltd.).

The larvae were reared to adults under conditions of 25 ± 2^{o}C. The insectary had a 12 hour photoperiod from 06:00 hour to 18:00 hour supplied by fluorescent tubes. Each morning, the pupae were collected and placed in labelled cages for adult emergence. The emerged adult females were transferred from the cages into small paper cups, using an aspirator, and used for the insecticide susceptibility bioassays.

Figure 3.5 Plastic bowls covered with mesh screen used for the rearing of mosquito larvae collected from the study sites.

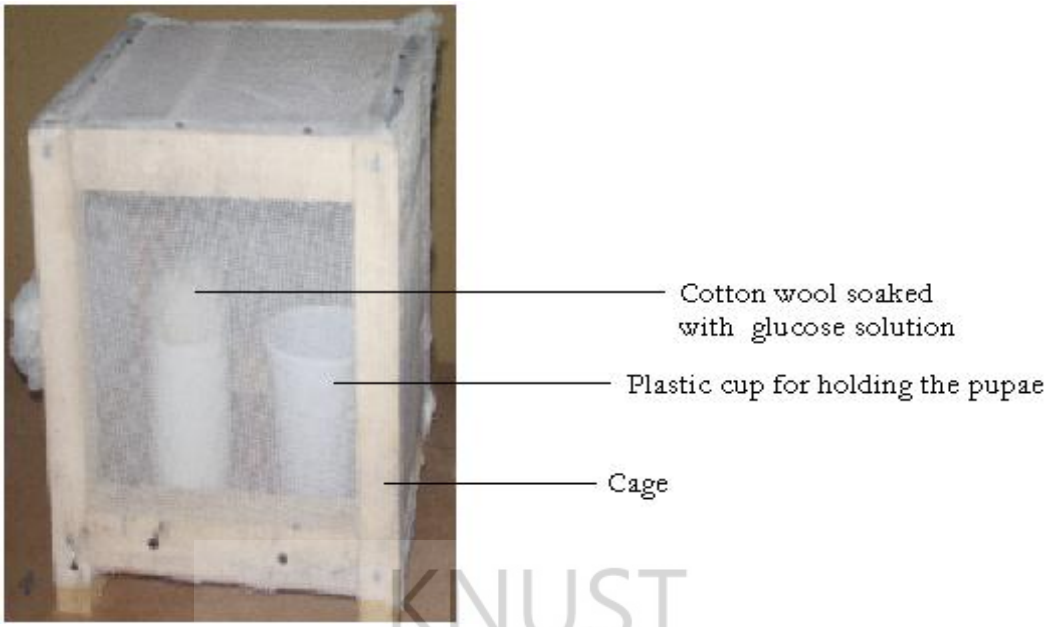


Figure 3.6 A labelled cage used for rearing pupae obtained from field collected larvae and maintaining emerged adult samples.

3.3.3 Adult mosquito collection and preservation

Mosquitoes were collected twice a month in randomly selected compounds within each study community in May, June and July 2006 corresponding to the beginning of the rainy season as well as in October 2006 corresponding to the end of the minor rainy season. Surveys were also done in August 2006 corresponding to the short cold dry period of the year as well as from November 2006 to January 2007 corresponding to the first three months of the main dry season.

Adult mosquitoes were collected using human landing catches (HLCs) method. The all night human landing catches (Fig 3.7) were made inside and outside human dwelling rooms. Mosquitoes attempting to bite the collectors were detected using flashlight, aspirated and placed in paper cups covered by mesh screen (WHO 2003). Two-man team of mosquito collectors and a supervisor were located at each collection point during the collection period. Collections were made for 50

minutes each hour from 18:00-06:00 hours. The collectors rotated between indoors and outdoors after each period of collection, to take care of differences in individual attraction or repulsion for mosquitoes. The collectors were provided access to malaria treatment in case of any infection.

The wild mosquitoes collected were morphologically identified, dissected for parity and preserved dry on silica gels in 1.5 mililitres eppendorf tubes until needed for molecular identification of sibling species complexes, molecular forms and the determination of knockdown resistance mutation as well as the *Plasmodium falciparum* parasite sporozoite rate studies.

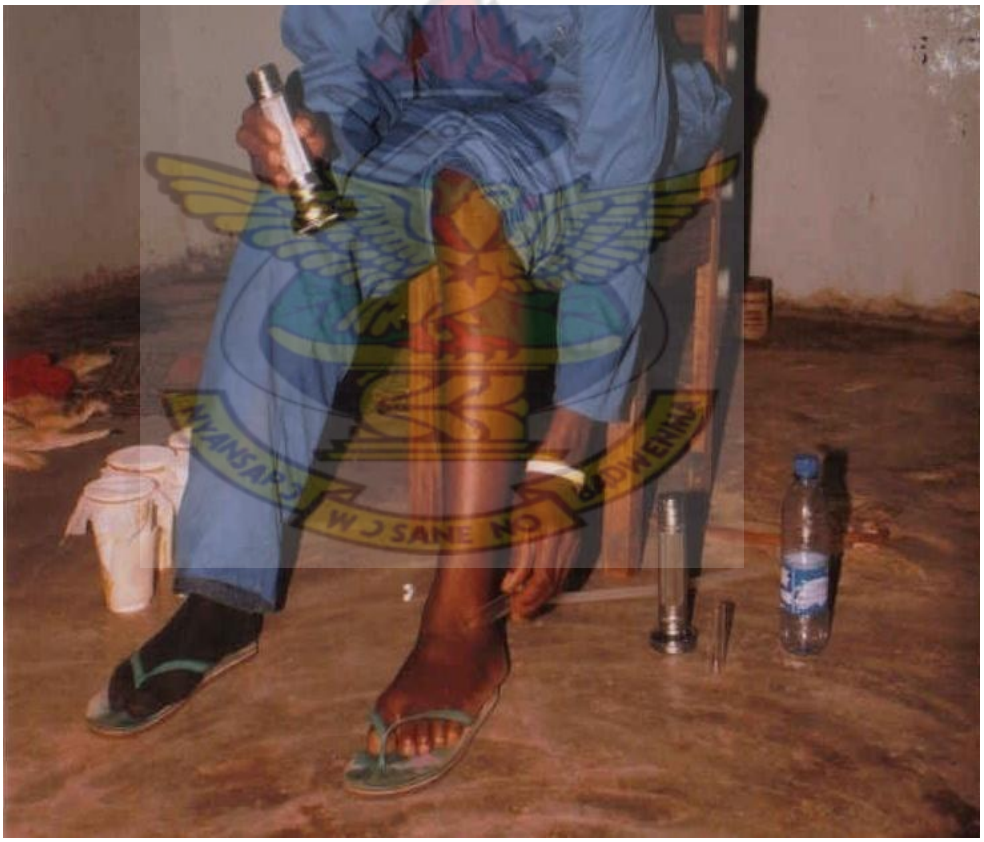


Figure 3.7 An illustration of the classical human landing catches (HLCs) technique used for collecting adult mosquito vectors in the field (WHO, 1975, 2003).

3.4 Georeferencing of the Mosquito Collection Sites

A handheld global positioning system (GPS) (Garmin: Geko, 301, US) was used to determine and record the precise grid co-ordinates of the study sites to enable these areas to be relocated in future.

3.5 Identification of *Anopheles* Species

3.5.1 Morphological identification of Anopheles species

Mosquito larvae were identified as *Anopheles* mosquito species based on the fact that they lie parallel to the water surface and do not possess siphon (WHO, 2003). Adult mosquitoes were identified as *Anopheles* species using the markings on the palps, the banding and speckling on the legs and the distinctive pattern of blocks of dark and pale scales on the vein of the wings, particularly along the costa. The *Anopheles gambiae* s.l. was distinguished from *Anopheles hancocki* species using the morphological keys of Foote & Cook, (1959); Gillies & de Meillon (1968). In brief, *An. gambiae* s.l. possessed five pale spots on the costal margin of the wings, anal vein coloration with three white spots, a dark apical fringe and white speckled (or spots in the median part) tibia ornamentation. *Anopheles hancocki* species also possessed five pale spots on the costal margin of the wings but there was a different pattern on hind tarsus (Ta-III). There was absence of a white speck on the joint between the first hind tarsus (Ta-III_1) and the second hind tarsus (Ta-III_2). The second segment of the hind tarsus (Ta-III_2) was partly white and partly dark in colour. The white part of the second segment of the hind tarsus adjoins the third segment of the hind tarsus (Ta-III_3). The last three segments of the hind tarsus, specifically segment three (Ta-III_3), four (Ta-III_4) and five (Ta-III_5) of *An. hancocki* species were completely white in colour. The *An. coustani* on the other hand, is darker than both the *An. gambiae* and

An. hancocki. The costal margin of the *An. coustani* wing had only two pale spot. Other external morphological features used to identify *An. coustani* included: the basolateral area of clypeus had a patch of dark laterally projecting scales, hindtarsomeres 4 and 5 (Ta-III4) were entirely pale. Hindtarsomere 1 (Ta-III) was broadly pale at base and apex, hindtarsomere 2 (Ta-1112) pale over approximately apical half, hindtarsomere 3 (Ta-IIIj) was dark at base only or entirely pale and lastly the abdominal sternum VII had a group of posteromedian dark scales. The morphologically identified wild-caught adult *An. hancocki, An. coustani* and *An. gambiae* s.l. were preserved dry on cotton in 1.5 mililitres eppendorf tubes containing silica gel. The eppendorf tubes were then labelled according to *Anopheles* mosquito species, date, time and site of collection. Based on these data, each *Anopheles* species was given a special ID numbers, which remained constant during the study period.

3.5.2 Molecular identification of the An. gambiae sensu lacto sibling species

3.4.2.1 Genomic deoxyribonucleic acid (DNA) extraction using the rapid method

Each mosquito specimen (i.e. legs and wings) was homogenized in 50 µl of sterile double distilled water in a 1.5 ml eppendorf tube using a plastic pestle. The homogenate was boiled for 10 minutes in a 100^{o}C water bath and was allowed to cool at room temperature and then kept at either 4^{o}C and used immediately or - 40^{o}C until required for polymerase chain reaction experiment.

3.5.2.2 Genomic DNA extraction using the Bender buffer method

Genomic DNAs from *Anopheles* vectors were extracted using the protocol described by Collins (1987). Whole female *Anopheles gambiae* s.l were homogenised in 1.5 ml eppendorf tube containing 100 µl Bender buffer: 0.1 M NaCl, 0.2 M

sucrose, 0.1 M Tris-HCl p^H 7.5, 0.05 M EDTA pH 9.1, 0.5% SDS (preheated at 65°C) using a sterile polypropylene rod followed by incubation at 65°C for 30 minutes. Then 125 µl of buffer saturated phenol were added to the homogenate and mixed by vortexing at 10000 rpm and spun at 14000 rpm for 10 minutes. The supernatant was transferred to a fresh tube and 250 µl of chloroform added; it was vortexed and spun at 14000 rpm for 10 minutes. The supernatant was again transferred into a fresh tube and 250 µl of pre-chilled absolute ethanol and 10 µl of 8 M potassium acetate added, followed by incubation at -40°C for an hour. The DNA was pelleted by centrifugation at 10000 rpm for 10 minutes and the supernatant was discarded. Two hundred micro litres of 70% ethanol were added to the pellet, the tube gently swirled and the DNA re-pelleted by centrifugation at 10000 rpm for 5 minutes. The supernatant was discarded and the tube opened and inverted over a dry paper towel and left overnight at 18°C to dry. The dried DNA pellet was redissolved in 25 µl of TE + RNase (5ug/ml). The resultant reaction mixture was then incubated on ice for about an hour and stored at -40°C until required for PCR process.

3.5.2.3 Methods of molecular identification of the Anopheles s.l.

The polymerase chain reaction (PCR) amplification protocol of Scott *et al.* (1993) designed for the species identification of single specimen of the *An. gambiae* s.l. was employed in the present study. The DNA extracted from the legs of female mosquito samples using the process described above were used for the polymerase chain reaction analysis. The amplification process used one universal primer and three species-specific primers each of 20 bases (Table 3.1). The universal primer designated UN anneals to the same position on the ribosomal DNA (rDNA) of each of the five species of *An. gambiae* s.l. namely, *An. gambiae* s.s. *An. arabiensis, An. melas, An.*

merus and *An. quadriannulatus*. The other three primers used in the amplification process were species specific and were the reverse primer GA, which anneals to the *An. gambiae* s.s. template; ME, which anneals to both the *An. merus* and *An. melas* templates and AR, which anneals to the *An. arabiensis* template. There was another primer in the protocol of Scott *et al.* (1993) designated QD, which anneals to the *An. quadriannulatus* template. The sizes of the amplified products were 153 base pairs (bp) for *An. quadriannulatus,* 315 bp for *An. arabiensis,* 390 bp for *An. gambiae* s.s, 464 bp for *An. merus,* and 466 bp for *An. malas* sibling species. In this work, UN, GA, ME and AR primers were used as *An. gambiae* s.s, *An. arabiensis* and *An. merus* are the only *An. gambiae* s.l. reported in Ghana (Appawu *et al.* 1994, 2004; Appawu, 2005; Kristan *et al.* 2003; Yawson *et al.* 2004; Afrane *et al.* 2004).

Table 3.1 *Anopheles gambiae* s.l. species-specific primer sequences with respective Tm temperatures (Scott *et al.* 1993).

Primer	Sequence (5' to 3')	Tm (^{o}C)
Universal primer UN	GTG TGC CCC TTC CTC GAT GT	58.3
Anopheles gambiae GA	CTG GTT TGG TCG GCA CGT TT	59.3
Anopheles melas ME	TGA CCA ACC CAC TCC CTT GA	57.2
Anopheles arabiensis AR	AAG TGA CCT TCT CCA TCC TA	47.4
Anopheles quadriannulatus QD	CAG ACC AAG ATG GTT AGT AT	42.7

3.5.2.4 Polymerase chain reaction (PCR) amplification

Each reaction mix of 2.0 µl contained 1x PCR buffer C (from the invitrogen PCR Optimiser Kit), 200 µM each of the four (4) deoxyribonucleotide triphosphates (dNTPs) [which are the nucleotides deoxyadenosine triphosphate (dATP),

deoxyguanosine triphosphate (dGTP), deoxycytidine triphosphate (dCTP), and deoxythymidine triphosphate (dTTP)], 0.25 µM each of oligonucleotide primers and 0.5 U of DNA *Tag* polymerase enzyme. One microlitre of extracted mosquito DNA was used as template in the PCR amplification. The reaction mix was thoroughly mixed, centrifuged briefly at 10 krpm and overlaid with 20 µl of mineral oil (nuclease free) to avoid evaporation and refluxing during thermocycling. The temperature profile for the PCR amplification was 94^{o}C for 3 minutes (initial melt) followed by 35 cycles of 94^{o}C for 30 seconds (denaturation), 50^{o}C for 30 seconds (annealing), 72^{o}C for 1 minute (extension), and a final cycle of 72^{o}C for 10 minutes. For each set of reactions, a negative control experiment, which had no DNA template, was conducted. The amplification reactions were conducted using a polymerase chain reaction Express Thermal Cycler (Hybaid Limited, United Kingdom).

3.5.2.5 The identification of the Anopheles gambiae sensu stricto molecular forms

The identification of M and S molecular forms of the *A. gambiae* s.s was done using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) protocol of Fanello *et al.* (2002). This protocol involves a combination of the protocols designed by Scott *et al.* (1993) and Favia *et al.* (1997). The method allows for concurrent identification of *An. gambiae* s.l. and the molecular forms of the *An. gambiae* s.s. It is based on the fact that GCG^C restriction site for *Hha* 1 enzyme (Favia *et al.* 1997) lies within the *An. gambiae* s.s. species specific fragment amplified by Scott *et al.* (1993) which makes it possible to digest this fragment directly in order to make a distinction between molecular M and S forms.

The procedure is based on the PCR reaction described in sections 3.5.2.3 to 3.5.2.4 based on the method of Scott *et al.* (1993). The RFLP analysis involved the

digestion of amplified PCR products from mosquito species, which has been identified as *An. gambiae* s.s with restriction enzymes *Tru* I and *Hha* I. Briefly, in the present study, the digestion was done in 20 µl volumes containing 0.2 µl of HhaI enzyme, 0.2 µl of BSA, 2.0 µl of digestion buffer, 10 µl of PCR product and 7.6 µl of sterile double distilled water to make up the 20 µl volume. The incubation was done at 37^{o}C for four (4) hours using a heat block (Thermomixer Compact, Eppendorf) or using a PCR Express Thermal Cycler (Hybaid Limited, United Kingdom).

The expected different band sizes between the PCR-amplified fragments and the fragments obtained after digestions were due to the presence of the restriction site for *Hha* 1 enzyme (Favia *et al.* 1997; Fanello *et al.* 2002) at position 469 in all taxa except *Anopheles merus,* and of a second restriction site at position 475 in *An. quadriannulatus, An. melas,* and *An. merus.* The *An. gambiae* molecular S form digestion profile is characterised by two fragments: 257 bp and 110 bp long, which results from the presence of the *Hha*1 restriction site. The *An. gambiae* molecular M form does not have this restriction site and thus has a single 367 bp fragment.

3.5.2.6 Analysis of polymerase chain reaction (PCR) products

Eight microlitres of each PCR product as well as each RFLP-PCR product were added to 1 ul of 10 x bromophenol blue gel-loading dye and electrophoresed in 2.0% agarose gels stained with 0.5 µg/ml ethidium bromide. The gels were prepared and run with 1x TAE buffer at 100v for an hour, visualised and photographed over an ultraviolet Polaroid (UVP) dual intensity transilluminator at short wavelength using a Polaroid direct screen instant camera fitted with an orange filter, a hood and a Polaroid Tupe 667 film. The film was processed as recommended by the manufacturer (Polaroid Inc. USA). Photographs were also taken using a Kodac direct

screen instant camera connected to a computer. The sizes of the PCR products and the RFLP-PCR products were estimated by comparison with the mobility of a standard 100 bp ladder (sigma, USA) for the identification of the sibling species of the *An. gambiae* s.l. as well as the molecular forms within the *An. gambiae* s.s.

3.6 Determination of the Indices of Malaria Transmission

3.6.1 Determination of parity in female Anopheles gambiae s.l.

Female *An. gambiae* s.l. samples were dissected following the protocol of the WHO, (2003) to obtain ovaries for parity determination. Briefly, a drop of distilled water was put on a cleaned labelled microscope slide. Under the stereoscope, each mosquito was gently grasped by the thorax with forceps, and placed ventral side up with her abdomen in the distilled water. The mosquito was then delegged and dewinged. While viewing the specimen under the stereoscope, a fine tip needle was used to gently remove the 7th and 8th abdominal segments by grasping them and pulling away slowly until the ovaries came out of the abdomen. The oviduct was cut and the ovaries separated from the rest of the specimen and transferred to a drop of distilled water on another slide and allowed to dry overnight at room temperature. The dried ovaries were examined under a compound microscope using x20 and x40 objectives. The females with coiled tracheolar skeins were identified as nulliparous and those with stretched-out tracheoles were identified as parous.

3.6.2 Estimation of daily survival and life expectancy of the An. gambiae s.l.

Daily survival rates were computed using the formula as described (Davidson, 1954). Briefly, $p = \sqrt[G]{P}$ (where p = probability of daily survival, G = gonotrophic cycle and P = proportion parous). Life expectancy was determined using the formula

$1/-\log_e p$ as described (Garrett-Jones & Grab, 1964). The gonotrophic cycle was taken to be three (3) days as stated in the publication of the WHO, (1975; 2003).

3.6.3 Detection of Plasmodium falciparum sporozoites infectivity of the Anopheles gambiae sensu lacto using enzyme-linked immunosorbent assay (ELISA)

The head and thorax of parous *An. gambiae* s.s. samples were tested for the presence of *P. falciparum* circumsporozoite protein (PfCSP) following the protocol of Beier & Koros, (1991) & Wirtz *et al.* (1987a, b). Flexible 96-well micro-titre plates (Becton Dickinson & Co. Oxnard, CA, USA) were coated with captured monoclonal antibodies (Kierkegaard & Perry Laboratories, Gaithersburg, Maryland, USA) overnight at 4°C. The coating was done such that each well contained 50 µl of the monoclonal antibodies. The plates were flipped empty, banged and then incubated at 200µl/well with blocking buffer (BB) at room temperature for 60 minutes. The plates were then emptied and incubated with the 50 µl aliquots of the homogenates at room temperature for 120 minutes. Plates were washed twice with excess washing buffer (PBS/Tween-20), flipped empty, banged and subsequently incubated with a monoclonal antibody peroxidase conjugate (0.5µg/50µl BB/well) for 60 minutes in darkness. The plates were emptied, rinsed twice with washing buffer, banged, washed again with excess washing buffer, banged and then incubated with the substrate solution (100µl/well) for 30 minutes at room temperature, following which the ELISA test results were determined visually based on colour change in the well. Wells which changed from colourless to green were deemed positive for PfCSP. Those which remained colourless were regarded to be negative for PfCSP.

The micro-titre plate was coated such that wells F11 and F12 contained positive controls, wells G11 and G12 contained the negative controls, wells H11 and

H12 were blank, and the rest of the wells contained the test samples. The positive control samples were obtained from the Parasitology/Entomology Department, Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Legon-Ghana. The negative controls on the other hand were made up of Phosphate Buffer Saline. Figure 3.8 shows a 96 well plate showing the arrangement for ELISA test to determine the presence of *P. falciparum* circumsporozoite antigens in *An. gambiae* s.s. mosquito samples.

	1	2	3	4	5	6	7	8	9	10	11	12
A	*	*	*	*	*	*	*	*	*	*	*	*
B	*	*	*	*	*	*	*	*	*	*	*	*
C	*	*	*	*	*	*	*	*	*	*	*	*
D	*	*	*	*	*	*	*	*	*	*	*	*
E	*	*	*	*	*	*	*	*	*	*	*	*
F	*	*	*	*	*	*	*	*	*	*	+	+
G	*	*	*	*	*	*	*	*	*	*	-	-
H	*	*	*	*	*	*	*	*	*	*	*B*	*b*

Figure 3.8 Illustration of a 96 well plate showing the arrangement for enzyme-linked immunosorbent assay (ELISA) technique to determine the presence of *P. falciparum* circumsporozoite antigens (PfCSP) in *An.gambiae* s.s. Legend: * represent the test samples, + represent the positive control samples and *b* represent the blanks.

3.6.4 Determination of the human biting rate (HBR), entomological inoculation rate (EIR) and risk of transmission of malaria of the Anopheles gambiae s.l.

The human biting rates (HBRs) were determined from mosquitoes obtained through human landing catches (HLCs), as the number of *Anopheles* biting per man per night or hour as described in the protocol of the WHO, (1975; 2003). The sporozoite rates were inferred from the proportion of man-biting *Anopheles* mosquito samples that tested positive for *Plasmodium falciparum* circumsporozoite protein (PfCSP) determined by the ELISA procedure. The EIRs were calculated as the product of the human-biting rates (HBRs) and the sporozoite rates at a given season and place. The risk of malaria transmission was calculated using the formula described by Krafsur, (1977). Briefly, the risk of being infected by *P. falciparum* through the bite of *An. gambiae* s.s. in each of the study sites in a given season is given by the formula: $1 - e^{-sn}$. The sn is the estimated entomological inoculation rate for the season under consideration.

3.7 Determination of the Status of Insecticide Susceptibility/Resistance

3.7.1 Insecticide susceptibility tests

Susceptibility tests were conducted to determine the proportion of the vector population that was physiologically resistant to 0.75% and 1.5% permethrin, and 0.05% deltamethrin insecticides. The susceptibility of *Anopheles* species obtained from the field-collected larvae were tested using the manual designed by the World Health Organisation (1998a; 2003) for testing insecticide resistance in the field.

Adult female *Anopheles* species (F1), 2 ± 4 days old were collected from mosquito cages using an aspirator and placed in paper cups covered with mesh screen. Twenty to twenty five mosquitoes were transferred to a special plastic holding tube

lined with insecticide free paper. A plastic tube lined with filter paper impregnated with mineral oil (used as control) was connected with the holding tube and 25 mosquitoes were transferred to the tube through a hole in the slide between the 2 tubes; also the same number of mosquitoes was transferred to plastic tube with 0.75% permethrin-impregnated filter paper. The slide was closed and the exposure and control tubes were allowed to stand upright for 60 to 80 minutes depending on the rate of knockdowns. The filter papers impregnated with mineral oil and permethrin were held in place by silver and copper rings respectively. Separate sucking tubes were used to transfer mosquitoes to the exposure and control tubes to avoid contamination. After the exposure period the mosquitoes were transferred back to the holding tube, which stood upright for 24 hours, with a piece of cotton wool soaked with 10% glucose on the gauze end. The experiment was conducted at a temperature of 25°C±2°C. Mosquitoes killed by contact with the permethrin insecticide impregnated paper and those killed in the control tube at the end of the study period were counted. Five replicates of the experiment were done to calculate percentage (%) mortality in the exposure and control tubes. Since there was no control mortality, percentage mortality was calculated using the equation E = number of dead mosquitoes divided by total number of mosquitoes in tube with insecticide multiplied by 100%. The same experimental procedure was repeated for 1.5% permethrin and 0.05% deltamethrin impregnated filter papers to determine the susceptibilities of the mosquitoes to these insecticide treated papers. Using insecticide treated papers of the same concentration and batch as the above; the experimental procedure was repeated for the standard susceptible *An. gambiae* s.s. from Kisumu maintained at the Parasitolgy/Entomology Department, Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Legon-Accran. The susceptibility/resistance levels of the wild *An. gambiae*

s.s. tested were then compared with those of the Kisumu *An. gambiae* s.s. to establish the extent to which resistance has been selected for in the wild mosquitoes.

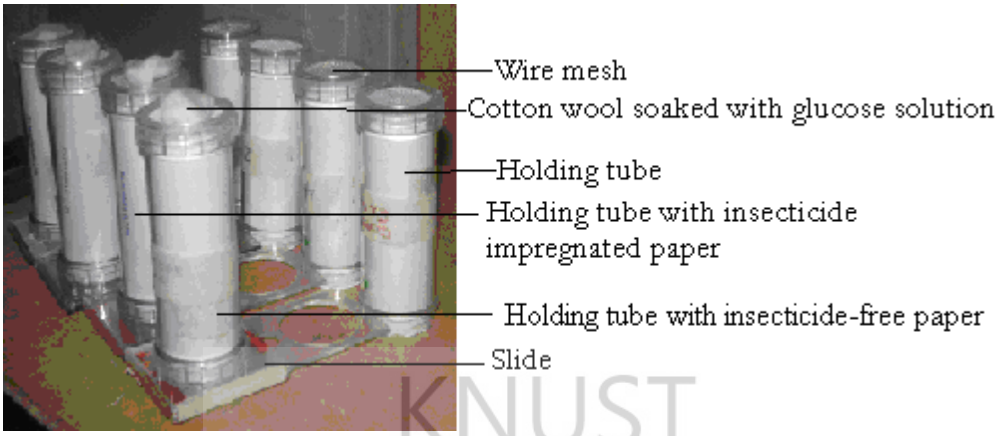


Figure 3.9 Illustration of adult mosquito insecticide susceptibility tests using the protocol of the World Health Organisation, (1998a, 2003).

3.7.2 Determination of knockdown resistance (kdr) mutation

3.7.2.1 The PCR amplification process

Molecular characterisation of the knockdown resistance (*kdr)* mutation was done on identified *An. gambiae* s.s. samples using the polymerase chain reaction (PCR) amplification method of Martinez-Torres *et al.* (1998). Deoxyribonucleic acid (DNA) used for the PCR amplification process was extracted using the rapid boiling method as well as the Bender buffer method described in section 3.5.2.1. Primers (Table 3.2) specific for *kdr* resistant strain of *An. gambiae* s.s designated as Agd1 and Agd3, and those specific for susceptible strain designated as Agd2 and Agd4 were used. The PCR reaction mixture of 25 µl contained 1x buffer C (300 mM of Tris-HCl, 75mM of $(NH4)_2$ SO4, 2.5 mM $MgCl_2$, pH 8.5), 200 µM of each of the four (4) oligonucleotide triphosphates (dNTPs) [which are the nucleotides deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP), deoxycytidine triphosphate (dCTP), and deoxythymidine triphosphate (dTTP)], 0.5 µM each of

Agd1 and Agd3 primers and 0.25 μM of Agd2 and Agd4, 50 ng of the extracted DNA and 0.625 U of Taq polymerase enzyme. The reaction mixture was overlaid with mineral oil (Sigma M-5904) to prevent it from evaporating. The knockdown resistance (*kdr)* mutation genotyping of susceptible and resistant individuals was possible after amplifying the DNA template from mosquitoes following the PCR conditions of 94^{o}C for 3 minutes (initial denaturation), followed by 45 cycles of 94 ^{o}C for 30 seconds, 50 ^{o}C for 30 seconds and 72 ^{o}C for one minute. There was a final extension cycle of 94^{o}C for 30 seconds, 50^{o}C for 30 seconds and 72^{o}C for 10 minutes followed by 4 ^{o}C for cooling.

3.7.2.2 Analysis of polymerase chain reaction (PCR) products

Eight microlitres of each PCR product were added to one microlitre of 10 x bromophenol blue gel-loading dye and electrophoresed in 2.0% agarose gels stained with 0.5 μg/ml ethidium bromide. The gels were prepared and run with 1x TAE buffer, using either a medium or maximum-gel system, at 100 v for an hour and were visualised and photographed over an ultraviolet Polaroid dual intensity transilluminator at short wavelength using a Polaroid direct screen instant camera fitted with an orange filter, a hood and a Polaroid Tupe 667 film. The film was processed as recommended by the manufacturer (Polaroid Inc. USA). The sizes of the PCR products were estimated by comparison with the mobility of a standard 100 bp ladder (sigma, USA) for the detection of the presence of the leucine to phenylalanine knockdown resistance (*kdr)* genes in the molecular forms of the *An. gambiae* s.s. The *kdr* genotypes of both the susceptible and resistant samples were then noted. Expected band sizes for susceptible, resistant and control samples were 137 bp, 195 bp and 293 bp respectively. The positive controls were susceptible laboratory reared Kisumu

strain of *An. gambiae* s.s. Table 3.2 shows the sequence details of oligonucleotide primers used for the characterisation of the *kdr* gene mutation in *An. gambiae* species in West Africa as described by Martinez-Torres *et al.* (1998).

Table 3.2 Sequence details of oligonucleotide primers used for the characterisation of insecticide knockdown resistance (*kdr)* mutation in *An. gambiae* species in West Africa (Martinez-Torres *et al*. 1998).

Primer Name	Sequence (5' to 3')	Tm (^{o}C)
Agd1 (forward)	ATA GAT TCC CCG ACC ATG	64.5
Agd2 (reverse)	AGA CAA GGA TGA TGA ACC	45.6
Agd3 (forward)	AAT TTG CAT TAC TTA CGA CA	45.2
Agd4 (reverse)	CTG TAG TGA TAG GAA ATT TA	60.0

3.8 Data Analysis

Indices of malaria transmission determined were: the sporozoite rate - the proportion of *Anopheles* mosquitoes with sporozoites in their salivary gland detected by PfCSP-ELISA; the biting rate - the number of *Anopheles* mosquito bites per collector per night (the nocturnal biting cycle is the hourly variation of biting rate); the entomological inoculation rate (EIR) or infective biting rate and the estimated entomological inoculation rate (estEIR) - the product of the biting rate and the sporozoite rate per unit time estimated from human-landing catches (HLCs); the risk of infection – the probability of acquiring one or more inoculations; the coefficient of endophagy - the ratio of the numbers of mosquitoes caught biting indoors to that caught biting outdoors in a given hour or study site; the parous rate - the proportion of *Anopheles* species samples found to have laid at least a batch of eggs (parous); daily

survival survival rate – the probality of *Anopheles* mosquito species surviving a day, life expectancy - the longevity of *Anopheles* mosquitoes measureable in days.

Chi-square analysis for homogeneity of proportions was performed to test for differences in endophagous rates between study sites or districts and between seasons. The same statistical method was used to examine the differences in parous rate within and between study sites as well as between seasons. The chi-square test of goodness of fit was used to test the significance of the differences in coefficient of endophagy, for example, per hour of night or study site. It was also used to test the differences in human-biting rates between quarters of night or different halves of night within each of the study sites. In brief, the night was divided into four quarters: the first (18:00-21:00 hours); second (21:00-00:00 hours), third (00:00-03:00 hours) and the fourth (03:00-06:00 hours). Again, the night was divided into two parts; the first part starting from dusk (18:00 hours) to midnight (00:00 hours) and the second from midnight (00:00 hours) to daybreak (06:00 hours). Statistical analyses were then performed on the nocturnal biting cycle data with reference to these night partitionings.

One-way ANOVA was used to test the similarity of meteorological data over a three year period, during the entomological surveys and between study sites. Spearman's correlation test was used to establish the linear relationship between the climatic factors: rainfall, minimum and maximum temperatures, and relative humidity at 0600 and 1500 hours as well as the linear relationship between these climatic factors and monthly biting and parous rates.

Deltamethrin and permethrin insecticide susceptibility levels were defined based on the WHO criteria for characterising susceptibility/resistance, where susceptibility is defined by mortality rates greater than 98% 24 hour post-exposure and resistance is defined by mortality rates less than 80% 24 hour post-exposure.

The results of the insecticide susceptibility tests were also analysed for dose/response relationship using regression probit analysis (Finney 1971). The linear log-time probit model was used to estimate knockdown times (KDTs), that is, time in minutes at which 50% and 95% of the mosquito populations assayed were knocked down. The KDT values were designated KDT_{50} and KDT_{95}, respectively. The KDTs of the wild mosquito populations were compared to those of the standard susceptible Kisumu colony using the calculated resistance ratios (RRs) for the KDT_{50} and KDT_{95} values designated RR_{KDT50} and $RRKDT_{95}$, respectively. Resistance ratio (RR) is the ratios of the KDTs estimated for the local mosquito populations to KDTs estimated for the Kisumu colony. The knockdown resistance (*kdr*) distribution refers to the frequency of the *kdr* gene in samples examined in each study site.

CHAPTER FOUR

RESULTS

4.1 Meteorological Data

4.1.1 Meteological data from 2005 to 2007

Rainfall, relative humidity and temperature data from 2005 to 2007 were available for Sunyani. Only rainfall data was available for Chiraa for the three years. There was rainfall data for Hwidiem for the year 2006. No meteorological data was available for Ahafo Kenyasi during the three year period. Mean rainfall recorded from 2005 to 2007 at the study sites are shown in Table 4.1. There were no significant differences in rainfall pattern in Sunyani ($F = 0.529$, $P = 0.594$) and Chiraa ($F = 0.133$, $P = 0.876$) over the three year period. There were also no significant differences in rainfall pattern between Sunyani and Chiraa for the years under consideration ($F = 0.155$, $P = 0.695$). Lastly, there were no significant differences in monthly rainfall between Sunyani, Chiraa and Hwidiem in the year 2006 when the entomological surveillance was undertaken ($F = 0.346$, $P = 0.710$).

Table 4.1 Mean monthly rainfall per year at the study sites.

Year	Sunyani	Chiraa	Hwidiem
2005	86.71	93.29	-
2006	96.36	85.94	119.7
2007	121.28	76.73	-

Rainfall ranges for Sunyani and Chiraa in 2005 were 1.10-233.60 and 8.00-184.20, respectively. Also, rainfall ranges for Sunyani, Chiraa and Hwidiem in 2006 were 6.30-267.60, 3.50-263.50 and 23.50-472.80, respectively. Similarly, rainfall ranges for Sunyani and Chiraa were 0.00-330.40 and 0.00-310.50.

The mean daily minimum and maximum temperatures recorded in Sunyani in the years 2005, 2006 and 2007 respectively are given in Table 4.2. There were no significant differences in minimum temperatures ($F = 0.046$, $P = 0.955$) and maximum temperatures ($F = 0.024$, $P = 0.977$) between these years. Nevertheless, there were significant differences between mean daily minimum and maximum temperatures over the three year period at Sunyani ($F = 16.263$, $P < 0.001$).

Table 4.2 Mean daily temperatures in degree Celsius (˚C) at Sunyani.

Year	Minimum temperature	Maximum temperature
2005	21.40	31.42
2006	21.54	31.58
2007	21.46	31.39

Daily minimum and maximum temperatures in 2005 were 17.90-23.00 and 27.40-35.10, respectively. Also, daily minimum and maximum temperatures in 2006 were 19.30-22.20 and 28.70-34.10, respectively. Lastly, daily minimum and maximum temperatures in 2007 were 18.40-22.60 and 27.70-35.40, respectively.

The mean daily relative humidity at 0600 and 1500 hours recorded in the years 2005, 2006 and 2007 at Sunyani are given in Table 4.3. There were no significant differences in the mean daily relative humidity at 0600 hours ($F = 0.534$, $P = 0.591$) and 1500 hours ($F = 0.110$, $P = 0.896$) during the three year period. However, there were significant differences between mean daily relative humidity at 0600 hours and 1500 hours during these years ($F = 24.237$, $P < 0.001$).

Table 4.3 Mean daily relative humidity (Rel. humid.) [%] at at Sunyani.

Year	Rel. humid. at 1500 hrs	Rel. humid. at 0600 hrs
2005	91.17	59.00
2006	92.25	56.58
2007	89.42	57.00

Daily relative humidity at 1500 and 0600 hours in 2005 were 72.00-95.00 and 28.00-73.00 respectively. In 2006, daily relative humidity at 0500 and 0600 hours were 89.00-95.00 and 41.00-67.00 respectively. Lastly, daily relative humidity at 0500 and 0600 hours in 2007 were 60.00-95.00 and 24.00-73.00.

There was a strong positive correlation between rainfall and relative humidity at 0600 hours (rho = 0.641, $p < 0.001$) and 1500 hours at Sunyani (rho = 0.557, $p < 0.001$). There was also a strong positive correlation between rainfall and minimum temperature (rho = 0.359, $p = 0.031$) and a weak negative correlation between maximum temperature and rainfall at Sunyani (rho = -0.301, $p = 0.075$). While there was a strong negative correlation between relative humidity at 0600 hours and maximum temperature (rho = -0.706, $p < 0.001$), there was a weak negative correlation between relative humidity at 0600 hours and minimum temperature (rho = -0.072, $p = 0.677$). There was also a strong negative correlation between relative humidity at 1500 hours and maximum temperature (rho = -0.891, $p < 0.001$) and a weak negative correlation between relative humidity at 1500 hours and minimum temperature (rho = -0.028, $p = 0.870$).

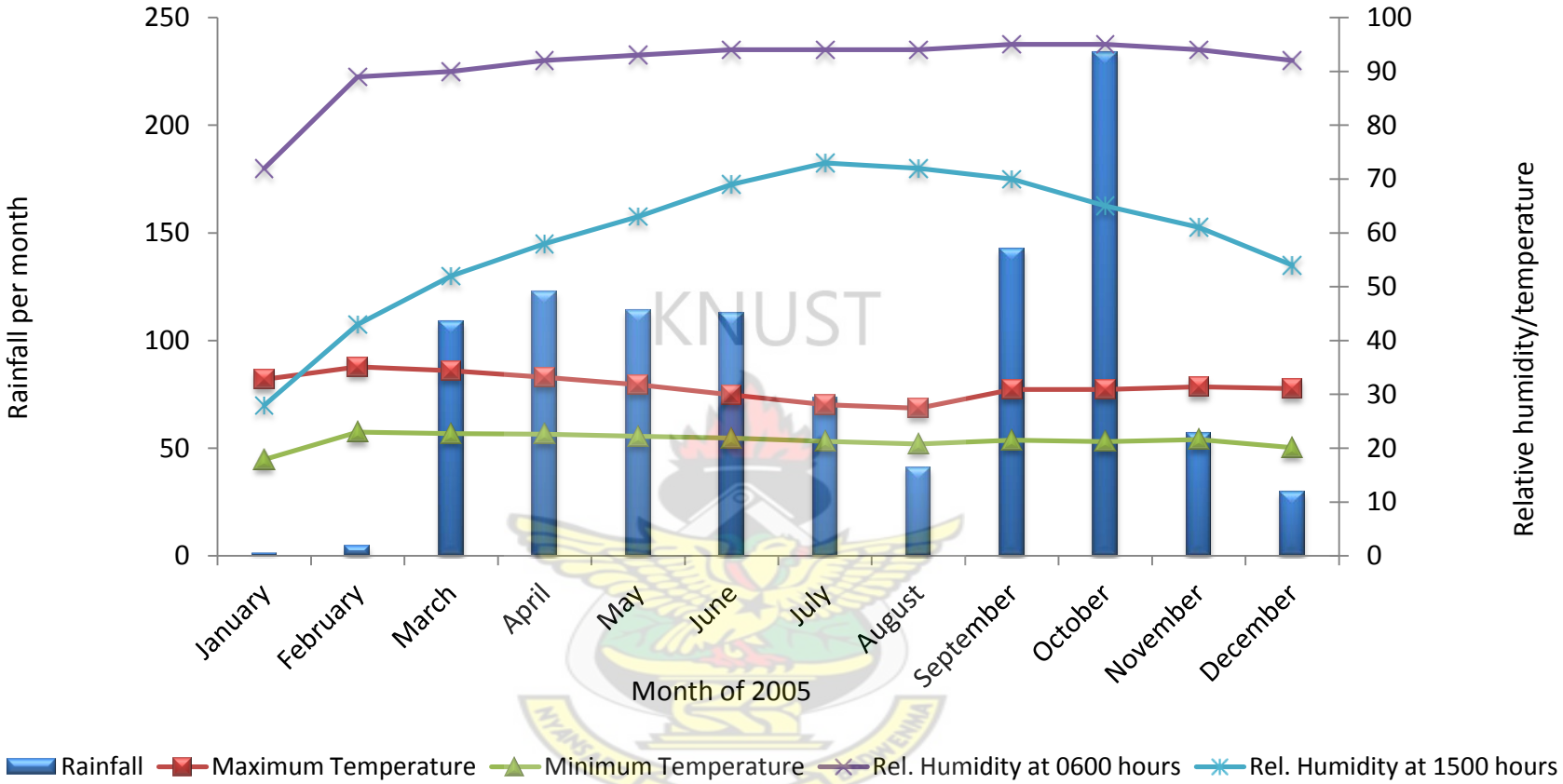


Figure 4.1 Monthly rainfalls (mm), mean daily minimum and maximum temperatures (˚C) per month, as well as mean daily relative humidity (%) at 0600 and 1500 hours per month at Sunyani in 2005.

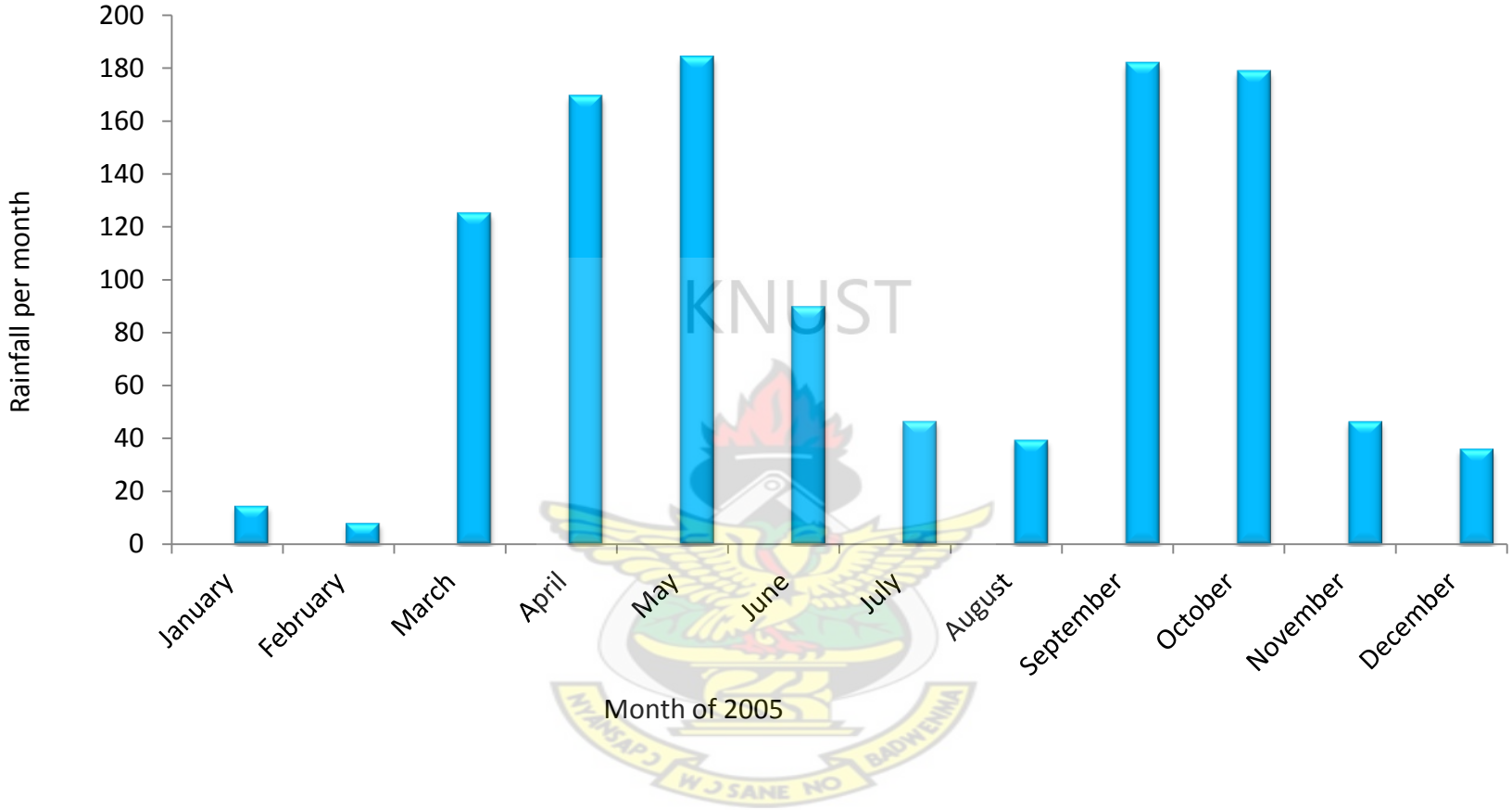


Figure 4.2 Monthly rainfalls (mm) recorded at Chiraa in the year 2005.

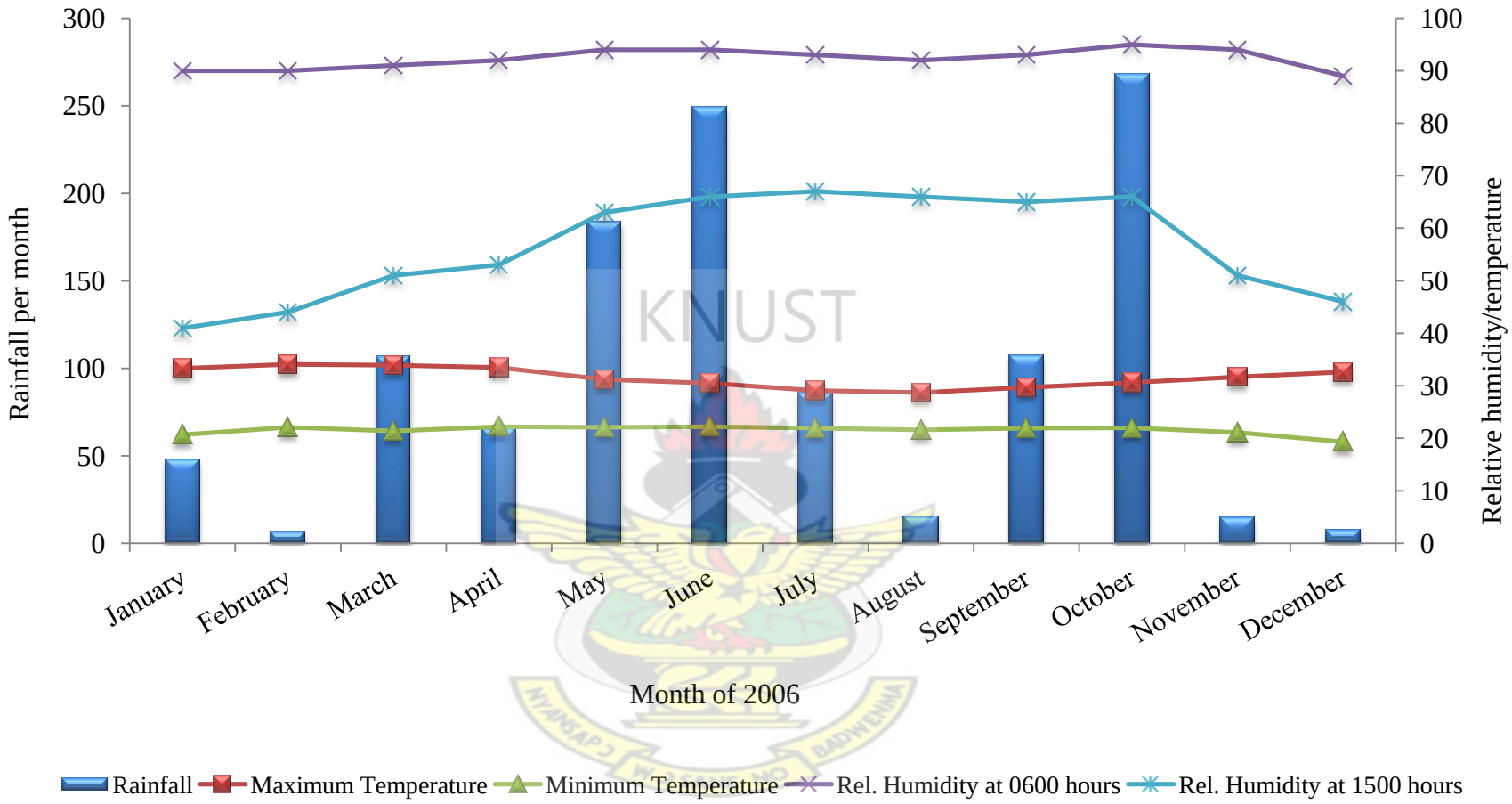


Figure 4.3 Monthly rainfalls (mm), mean daily minimum and maximum temperatures (˚C) per month, as well as mean daily relative humidity (%) at 0600 and 1500 hours per month at Sunyani in the year 2006.

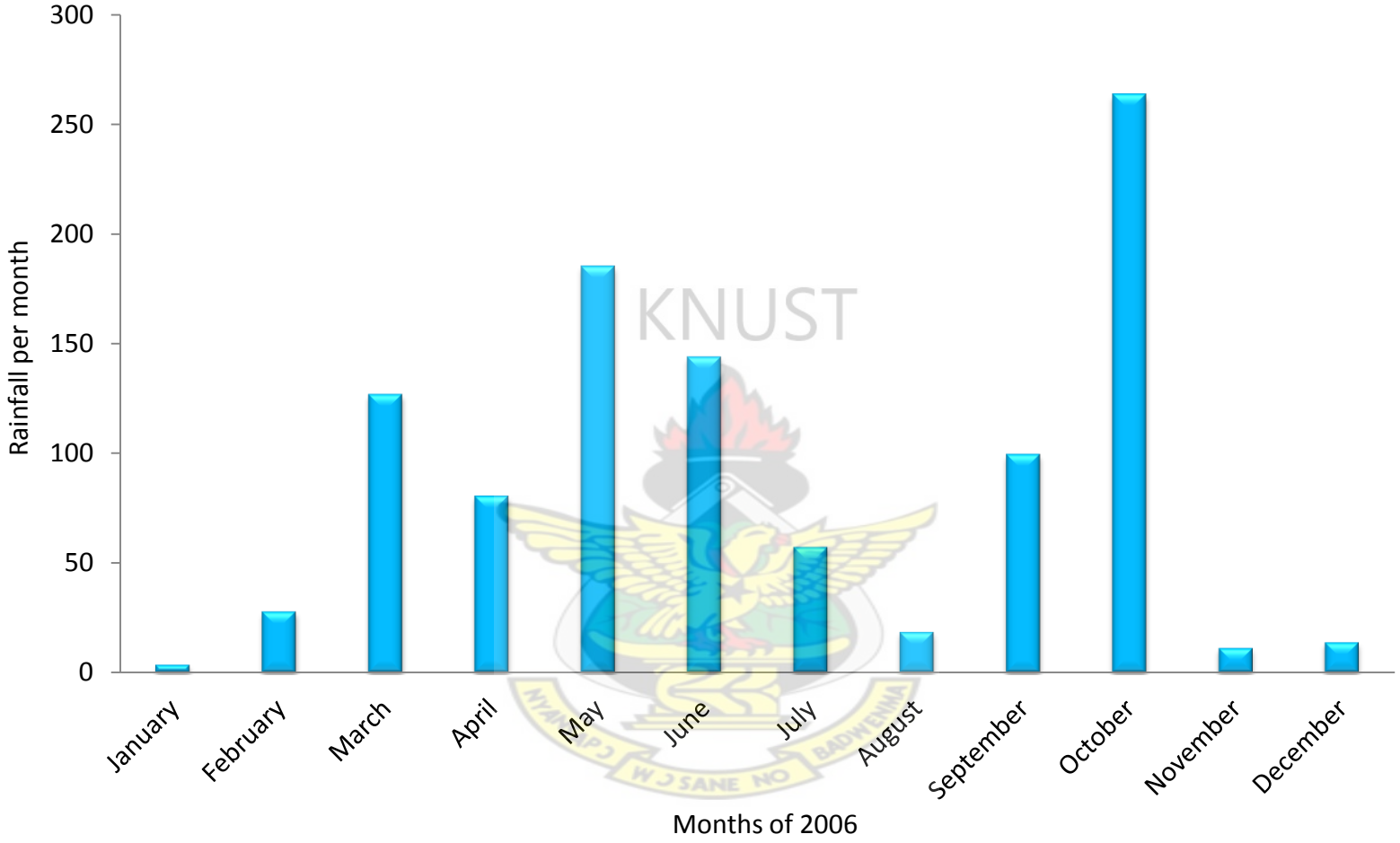


Figure 4.4 Monthly rainfalls (mm) at Chiraa in the year 2006.

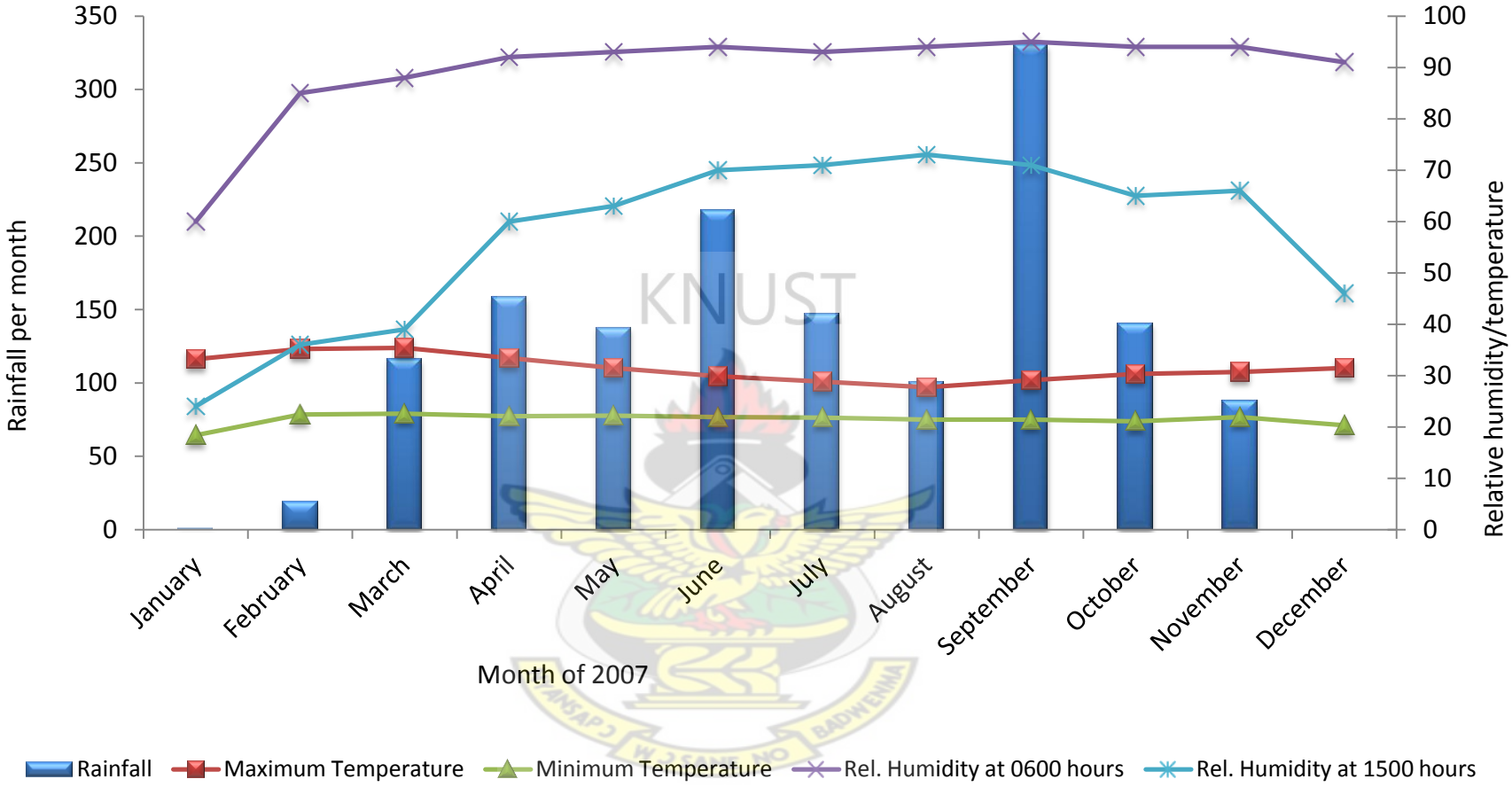


Figure 4.5 Monthly rainfalls (mm), mean daily minimum and maximum temperatures (˚C) per month, as well as mean daily relative humidity (%) at 0600 and 1500 hours per month at Sunyani in the year 2007.

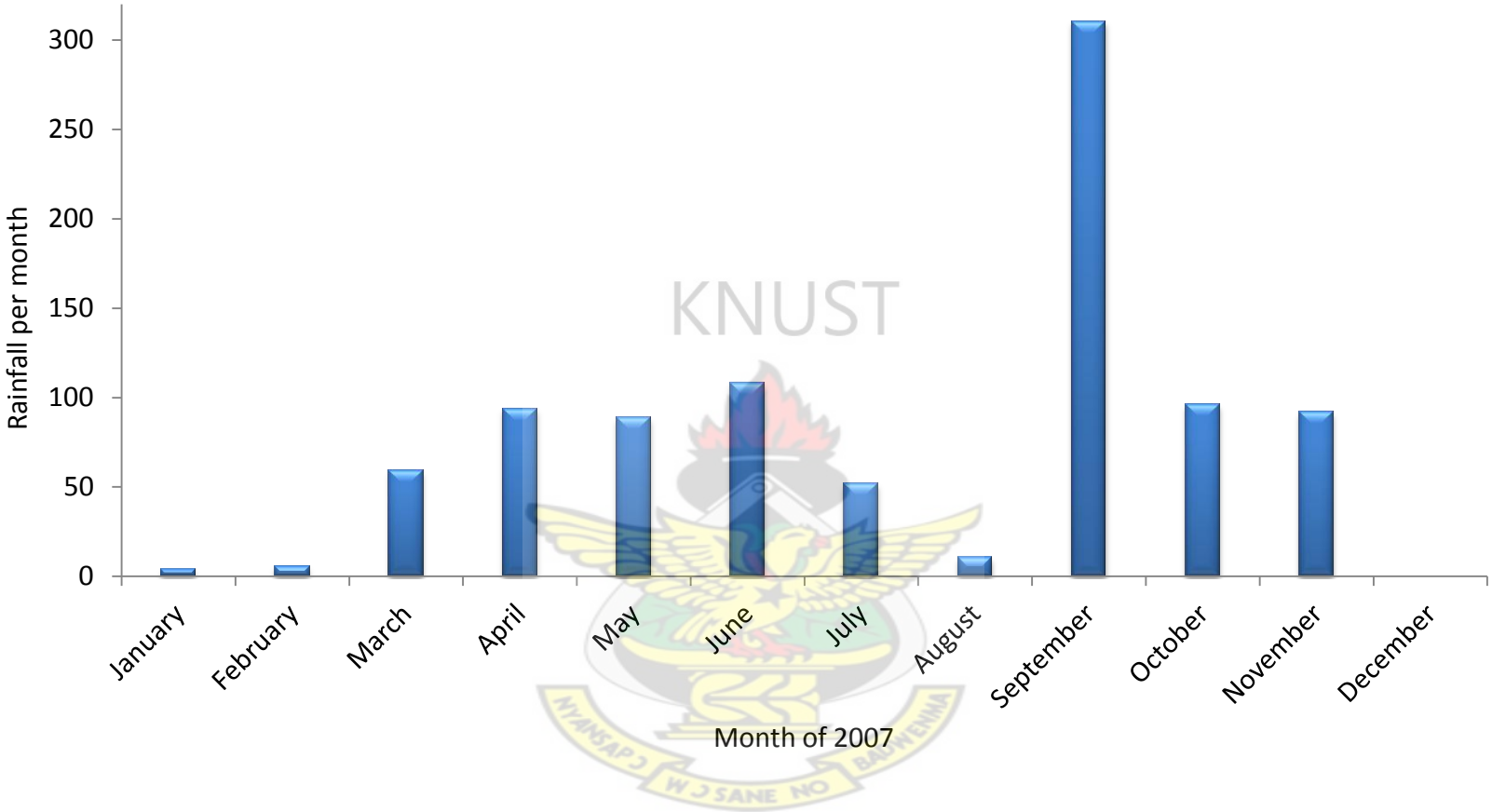


Figure 4.6 Monthly rainfalls (mm) at Chiraa in the year 2007.

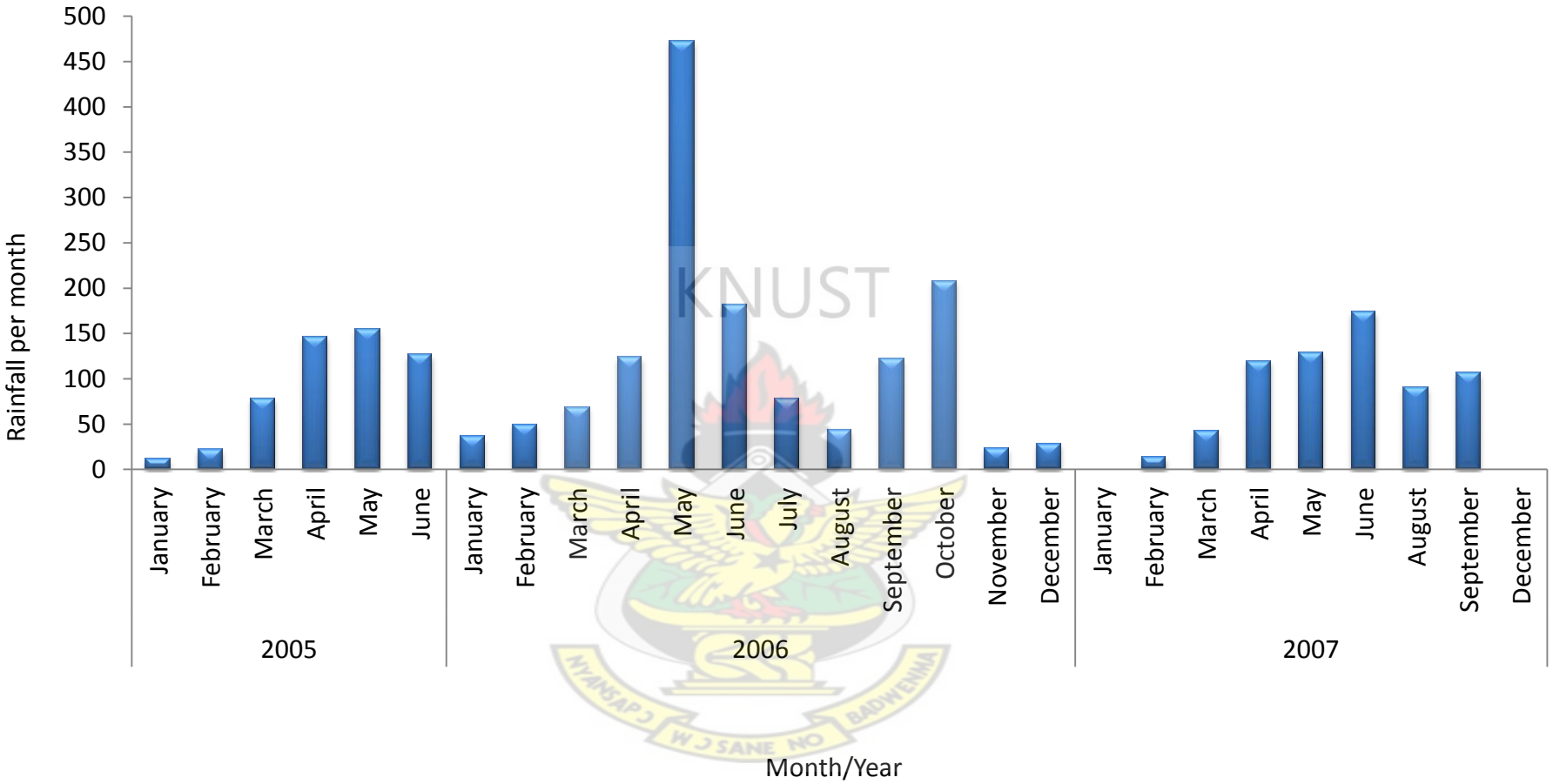


Figure 4.7 Monthly rainfalls (mm) at Hwidiem in the years 2005, 2006 and 2007. There was no rainfall data from July to December 2005 as well as in July, October and November 2007. According to the Ghana Meteorological Agency, the absence of rainfall data for the months in question was due to the fact that during these months, the equipment was out of use.

4.2 Indices of Malaria Transmission

4.2.1 *Anopheles species composition*

The *Anopheles* mosquito species caught in the study sites are given in Table 4.4. Three species of *Anopheles* mosquitoes; *Anopheles gambiae* s.l. Gilles, *An. hancocki* Edwards and *An. coustani* Laveran were identified from all the study sites. The predominant *Anopheles* species identified was *An. gambiae* s.l.

Table 4.4 *Anopheles* mosquito species composition in the study sites.

Species	No. caught (%)	95% Confidence interval
An. gambiae s.l.	15199 (98.80)	98.63 – 98.97
An. hancocki	181 (1.20)	1.01 – 1.35
An. coustani	4 (0.03)	0.00 – 0.06
Total	15384	

The distribution of the *An. hancocki* species are shown in Table 4.5. There was no significant differences in the number of *An. hancocki* in the study sites ($\chi^2 = 6.956$, d.f. = 3, $p = 0.073$). Although a greater proportion of *An. hancocki* mosquitoes was caught in the dry season (95, i.e. 52.49%) than in the rainy season (86, i.e. 47.51%), the difference was no significant ($\chi^2 = 0.45$, d.f. = 1, $p = 0.504$).

Table 4.5 Distribution of *An. hancocki* species in the study sites.

Site	No. caught (%)	95% Confidence interval
Sunyani	58 (32.04)	25.24 – 38.84
Chiraa	34 (18.78)	13.09 – 24.47
Ahafo Kenyasi	48 (26.52)	20.09 – 32.95
Hwidiem	41 (22.65)	16.55 – 28.75
Total	181	

The number of samples (n) from each of the study sites that were subjected to molecular analysis plus those that amplified (+s) and/or failed (-s) and their respective 95% confidence intervals (95% CI) are given in Table 4.6. In total, genomic DNAs were extracted from 388 adult *An. gambiae* s.l. for molecular analysis. These 388 *An. gambiae* mosquitoes comprised 322 adults collected through human landing catches (HLCs) and 66 adults raised in the insectary from field collected larvae. Out of the 388 *An. gambiae* s.l processed, PCR amplification for species identification was successful for 361 [93.04% (95% CI: 90.51- 95.57)]. Only a few DNA samples showed no amplification. All successful amplicons were *An. gambiae s.s*. (length of amplified sequence was 390 bp) (Fig. 4.8). No other member of the *An. gambiae* species complex was detected in the study sites.

Table 4.6 Results of the PCR analysis of *An. gambiae* s.l from the study sites.

Site	Number tested	Number positive	% (95% CI)
Sunyani	114	107	93.86 (89.45 – 98.27)
Chiraa	92	83	90.22 (84.15 – 96.29)
Kenyasi	100	94	94.00 (89.35 – 98.65)
Hwidiem	82	77	93.90 (88.72 – 99.08)

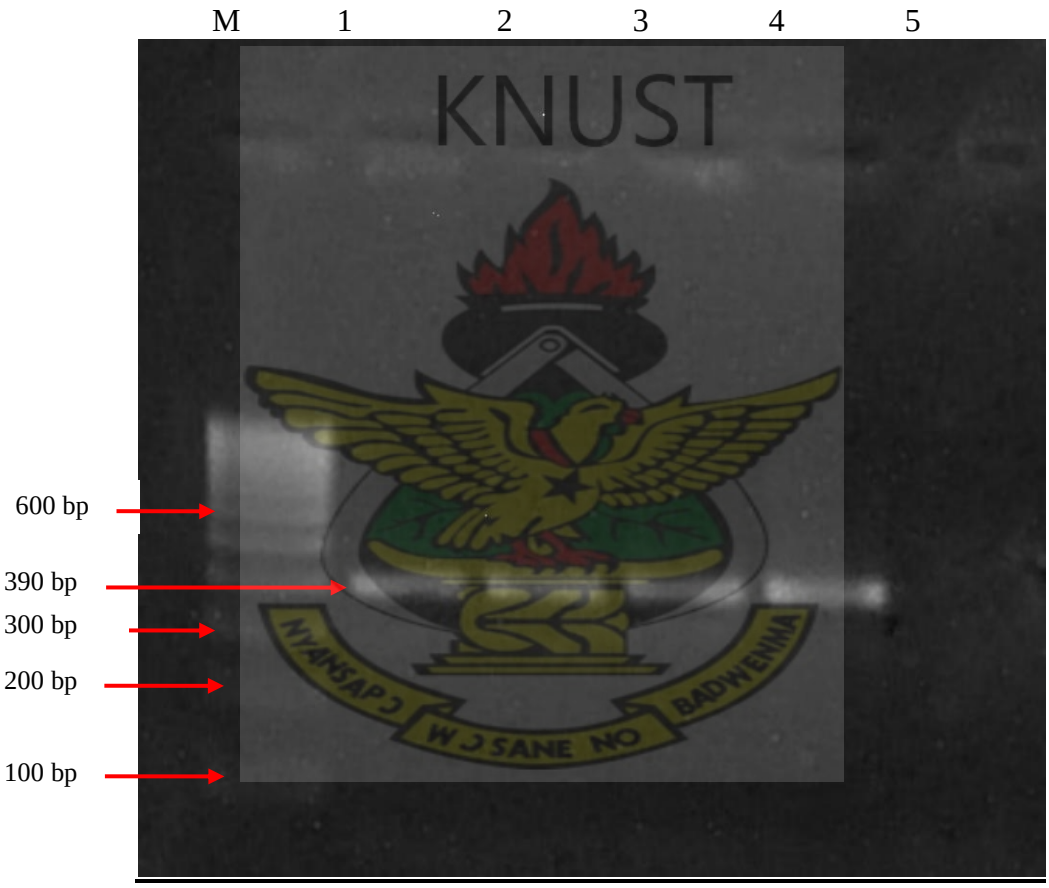


Figure 4.8 Ethidium bromide-stained 2.0% agarose gel electrophoregram of genomic DNA bands produced by the rDNA_PCR identification of *An. gambiae* s.l. Lane M is 100 bp ladder; lane 1 is previously identified *An. gambiae* s.s used as positive control; lanes 2, 3 and 4 are wild *An. gambiae* s.s and lane 5 was a negative control.

Table 4.7 shows the *An. gambiae* s.s. molecular forms identified in this study. Of the 361 *An. gambiae* s.s analysed, 261 (72.30%) were S forms and 100 (27.7%) were M forms. There were significant differences in relative frequencies of S and M molecular forms in the study sites (χ^2 = 8.44, p = 0.038). The frequency of S forms was significantly higher than that of the M forms in all site except for Chiraa where the frequency of S forms was not significantly higher (χ^2 = 3.48, p = 0.062). However, these forms were sympatric at all the study sites. The distribution of the M forms did not differ significantly between sites ($p > 0.05$). However, the frequencies of the M forms increased significantly from the months of the rainy season to the months of the dry season when rainfall was low or nil (χ^2 = 14.212, p = 0.048). No M/S molecular form hybrids were observed in any of the sites during the study period.

Table 4.7 *An. gambiae* s.s. molecular forms identified at the study site.

	Frequency of *A. gambiae* s.s molecular forms				
		S Form		M Form	
Study site	N	n_s	% (95% CI)	n_m	% (95% CI)
Sunyani	107	79	73.83 (65.50 – 82.16)	28	26.17 (17.84 – 34.50)
Chiraa	83	50	60.24 (49.71 – 70.77)	33	39.76 (29.23 – 50.29)
Kenyasi	94	74	78.72 (70.45 – 86.99)	20	21.28 (13.01 – 29.55)
Hwidiem	77	58	75.32 (65.69 – 84.95)	19	24.68 (15.05 – 34.31)

N = Total number of specimen analysed

n_s = Number of specimen found to be S forms

n_m = Number of specimen found to be M forms

CI = Confidence Intervals

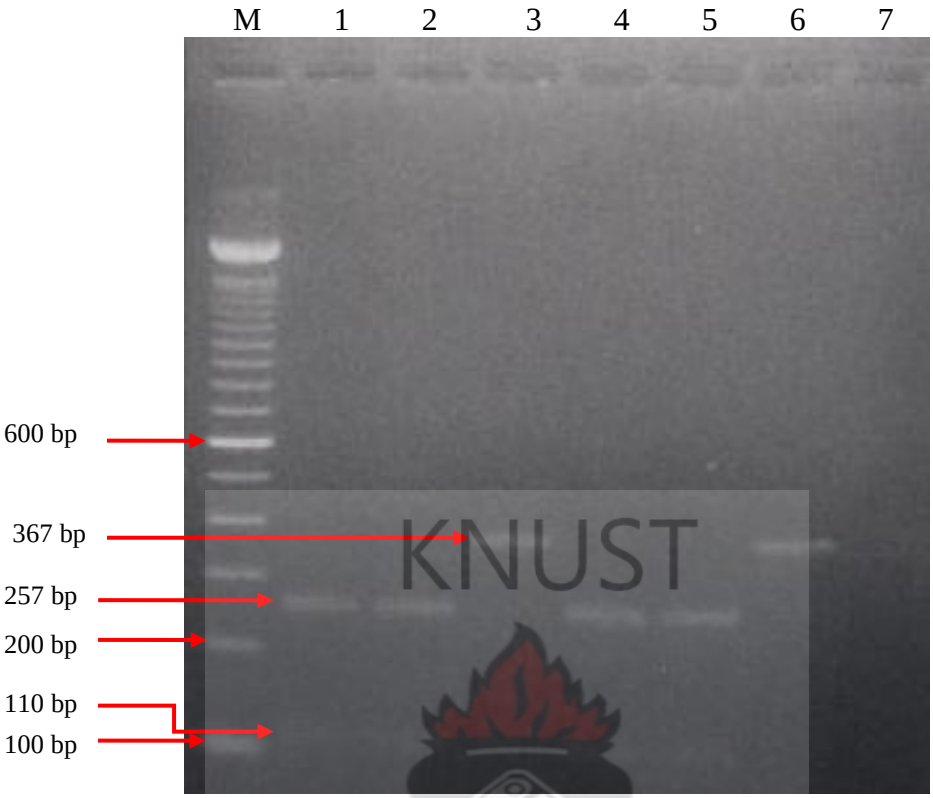


Figure 4.9 Ethidium bromide-stained 2.0% agarose gel electrophoregram of rDNA_PCR bands produced after the identification method for *Anopheles gambiae* s.s. Lane M = 100bp ladder (Sigma-Aldrich, USA); lanes 1, 2, 4 and 5 are molecular S forms of *An. gambiae* s.s; lanes 3 and 6 are molecular M forms of *An. gambiae* s.s, Lane 7 is a negative control.

4.2.2 Monthly human-biting rates (HBRs) of An. gambiae s.s. in relation to climatic factors in the study sites over the study period.

Monthly HBRs of *An. gambiae* s.s. in relation to three climatic factors in the study sites over the study period are shown in Figures 4.10 to 4.21. There were significant differences in monthly HBRs from May 2006 to January 2007 ($p < 0.001$) with the rates being higher in the months of high rainfall. However, whereas there were no significant differences in monthly HBRs between the months of high rainfall ($\chi^2 = 5.923$, $p = 0.748$), there were significant differences in HBRs between the months of low rainfall ($\chi^2 = 31.077$, $p = 0.0003$) at all study sites.

Monthly biting rates, monthly rainfall, relative humidity and temperature in Sunyani are given in Figure 4.10 to 4.12. Also, monthly biting rates and rainfall recorded at Hwidiem and Chiraa are given in Figures 4.13 and 4.14. There was a positive linear relationship between monthly bites per man per night (b/m/n) and rainfall at Sunyani (rho = 0.952, $p = 0.000$); Chiraa (rho = 0.714, $p = 0.047$) and Hwidiem (rho = 0.786, $p = 0.021$). Similarly, there was a positive linear relationship between b/m/n and relative humidity (%) measured at Sunyani during the 0600 hours [rho = 0.878, $p = 0.004$] and 1500 hours (rho = 0.756, $p = 0.030$). Lastly, there was a weak negative linear relationship between b/m/n and mean maximum daily temperature [rho = -0.548, $p = 0.160$] and a strong positive linear relationship between b/m/n and mean minimum daily temperature at Sunyani (rho = 0.857, $p = 0.007$) during the study period [Figs. 4.15 – 21].

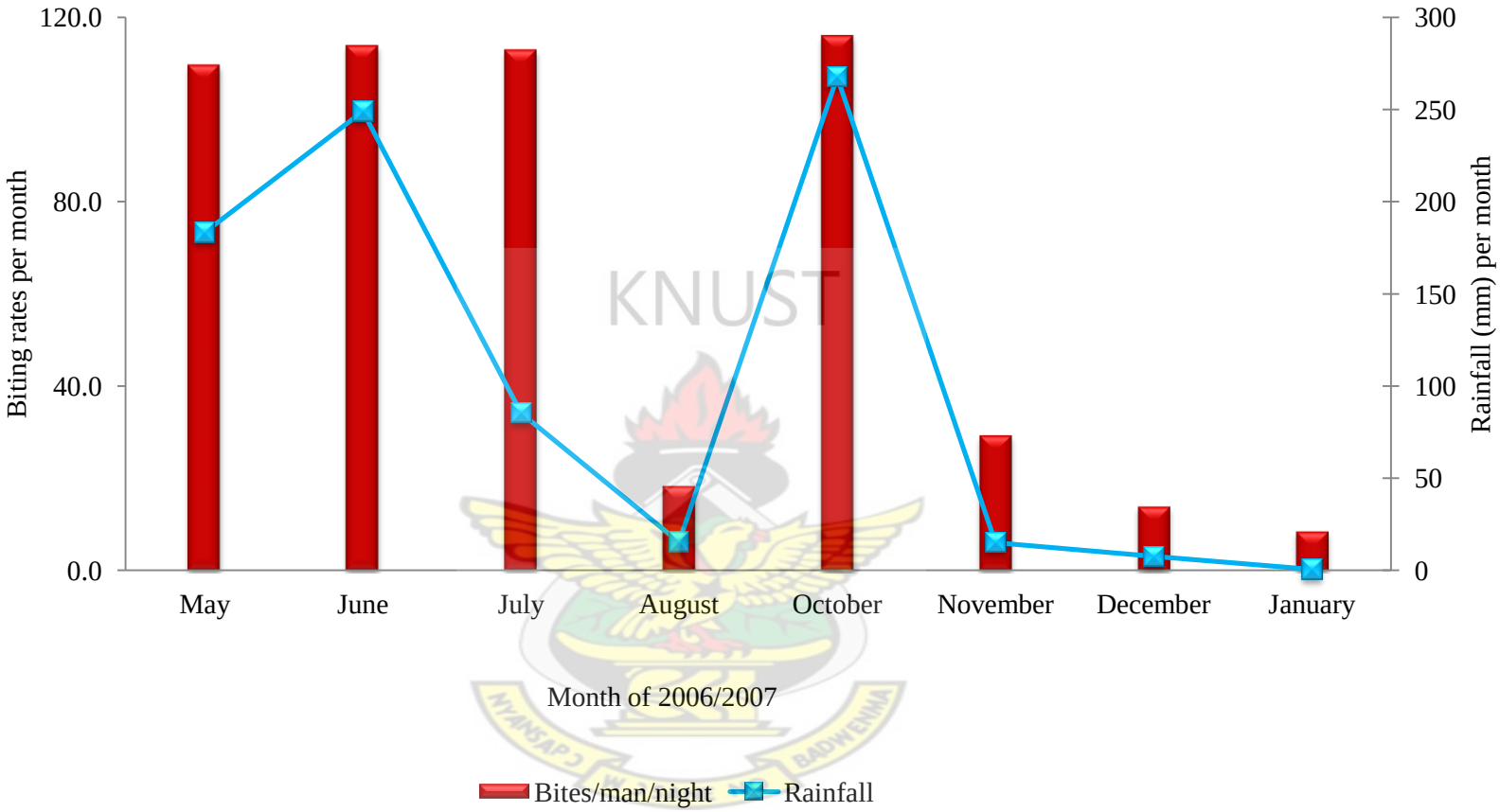


Figure 4.10 Monthly human biting rates of *An. gambiae* s.s. in relation to monthly rainfall at Sunyani.

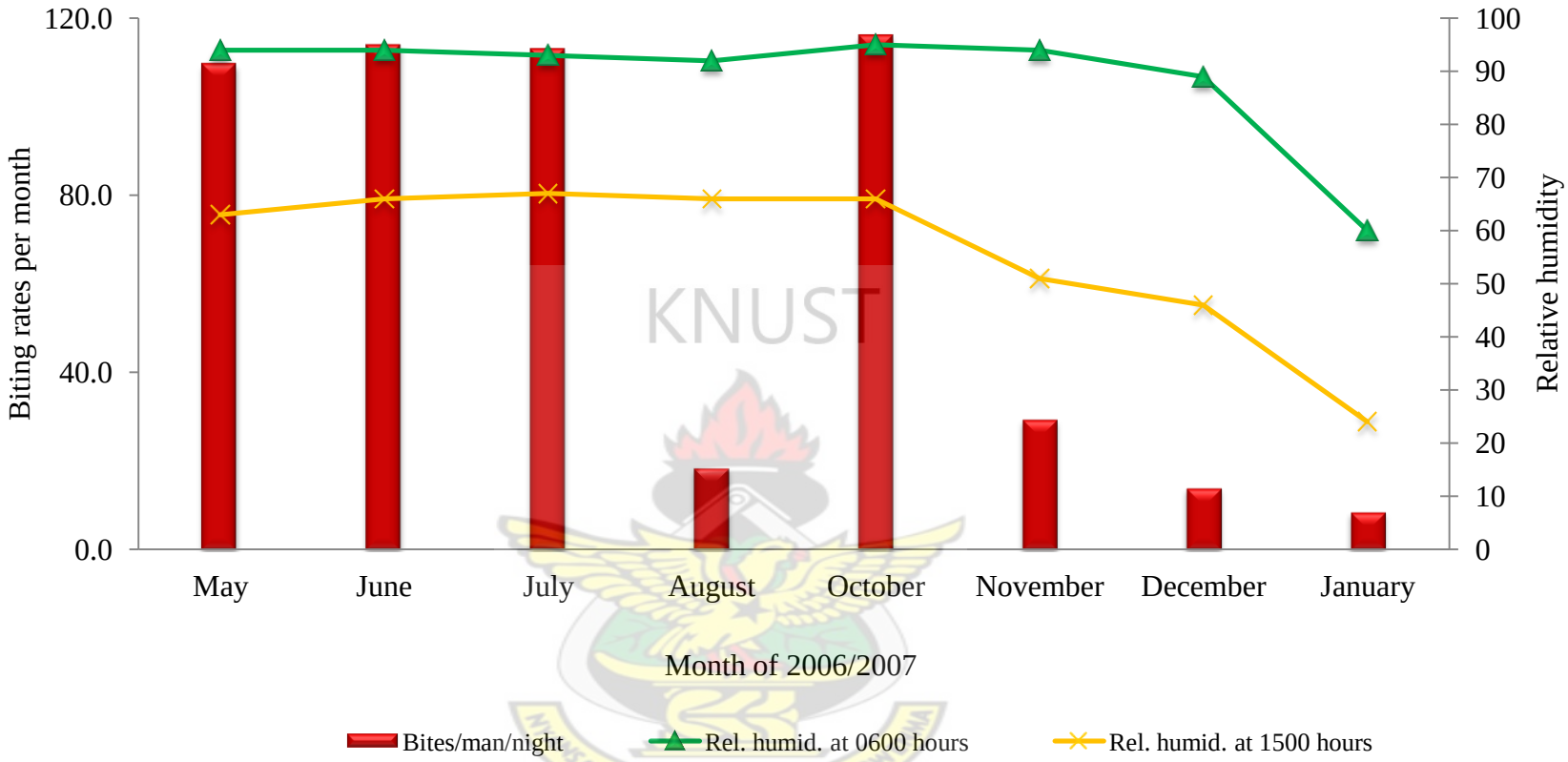


Figure 4.11 Monthly human landing rates of *An. gambiae* s.s. in relation to mean daily relative humidity (%) per month at 0600 and 1500 hours at Sunyani.

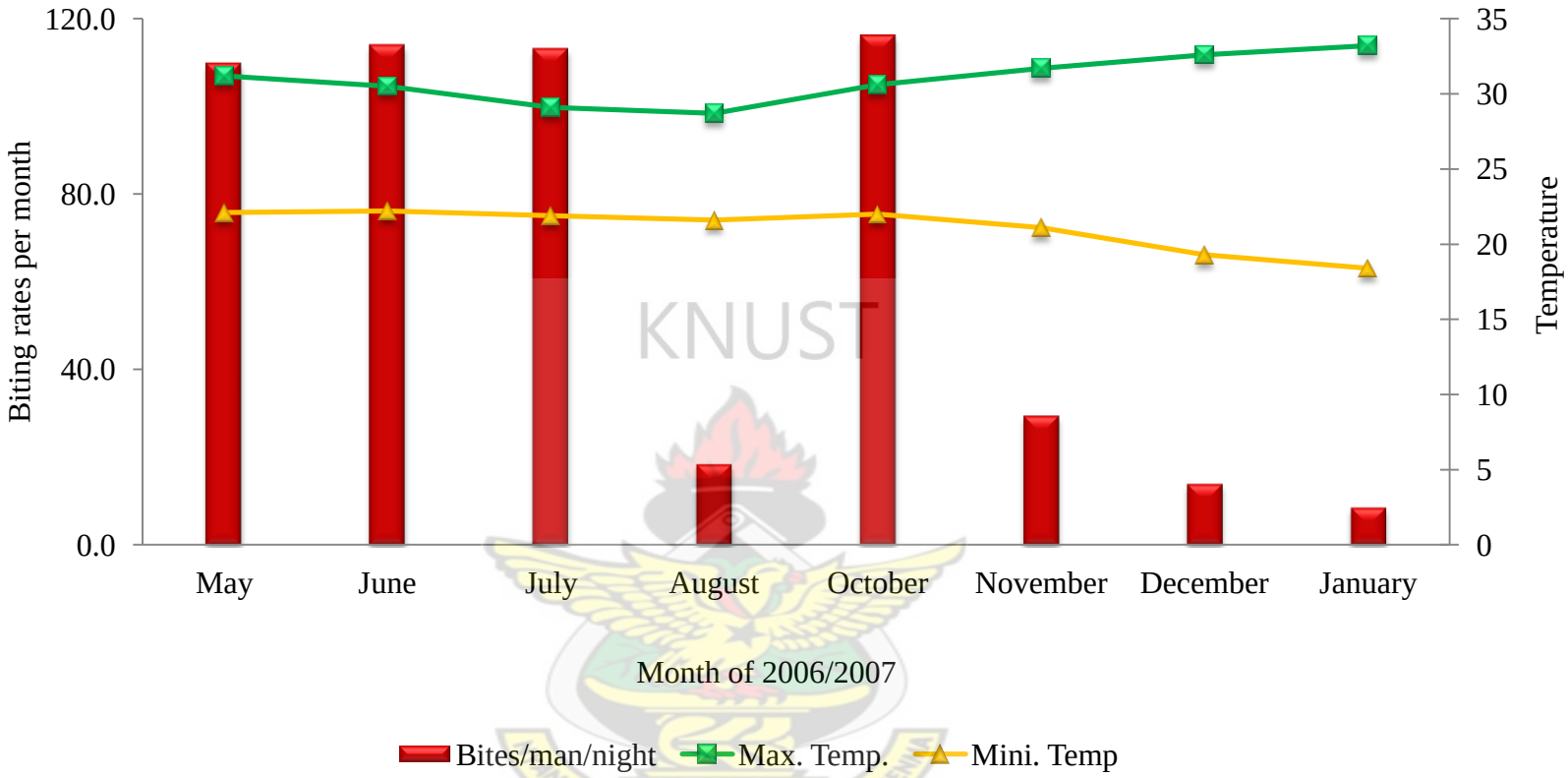


Figure 4.12 Monthly human biting rates of *An. gambiae* s.s. in relation to mean daily minimum and maximum temperatures (˚C) per month at Sunyani.

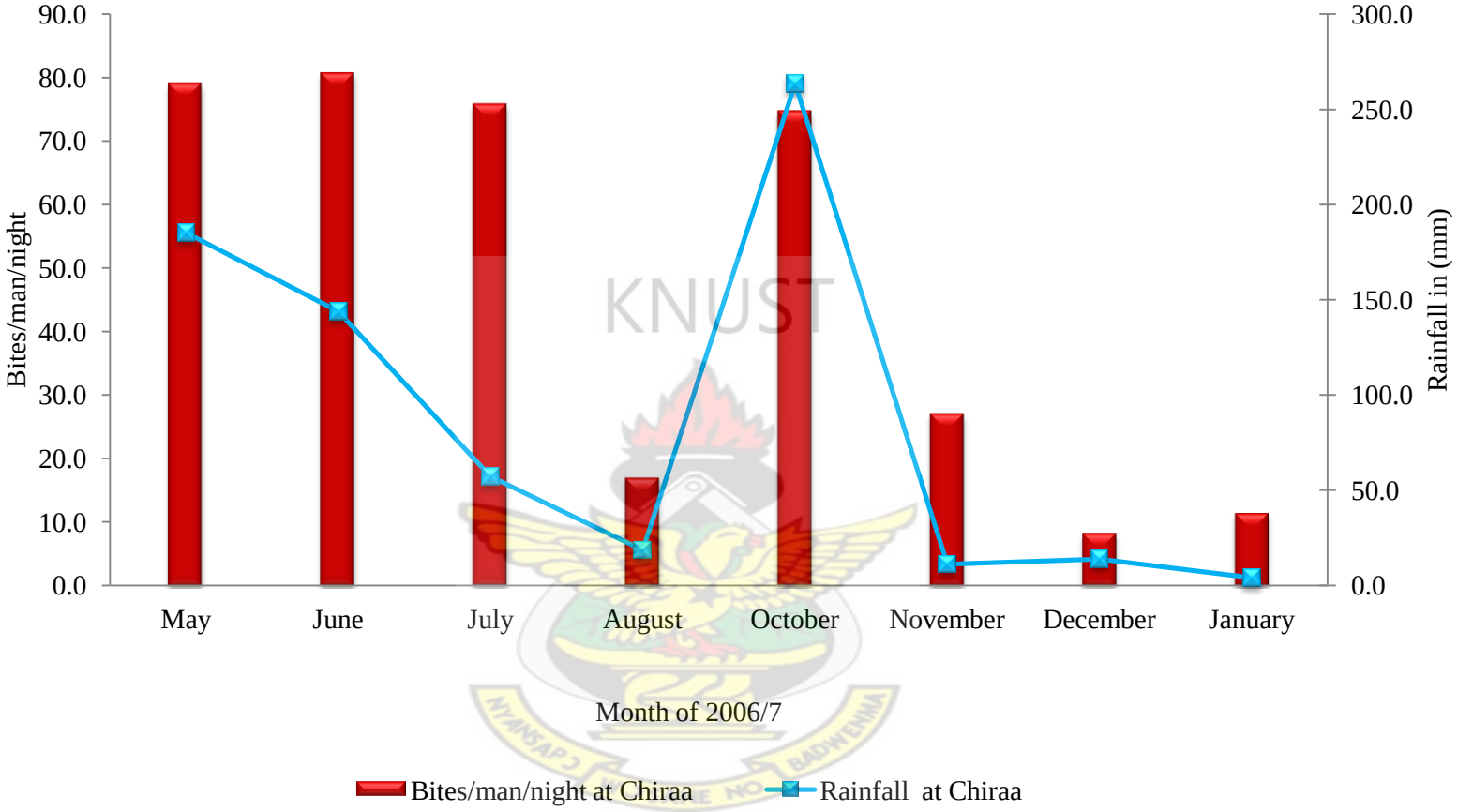


Figure 4.13 Monthly human landing rates of *An. gambiae* s.s. in relation to monthly rainfall at Chiraa.

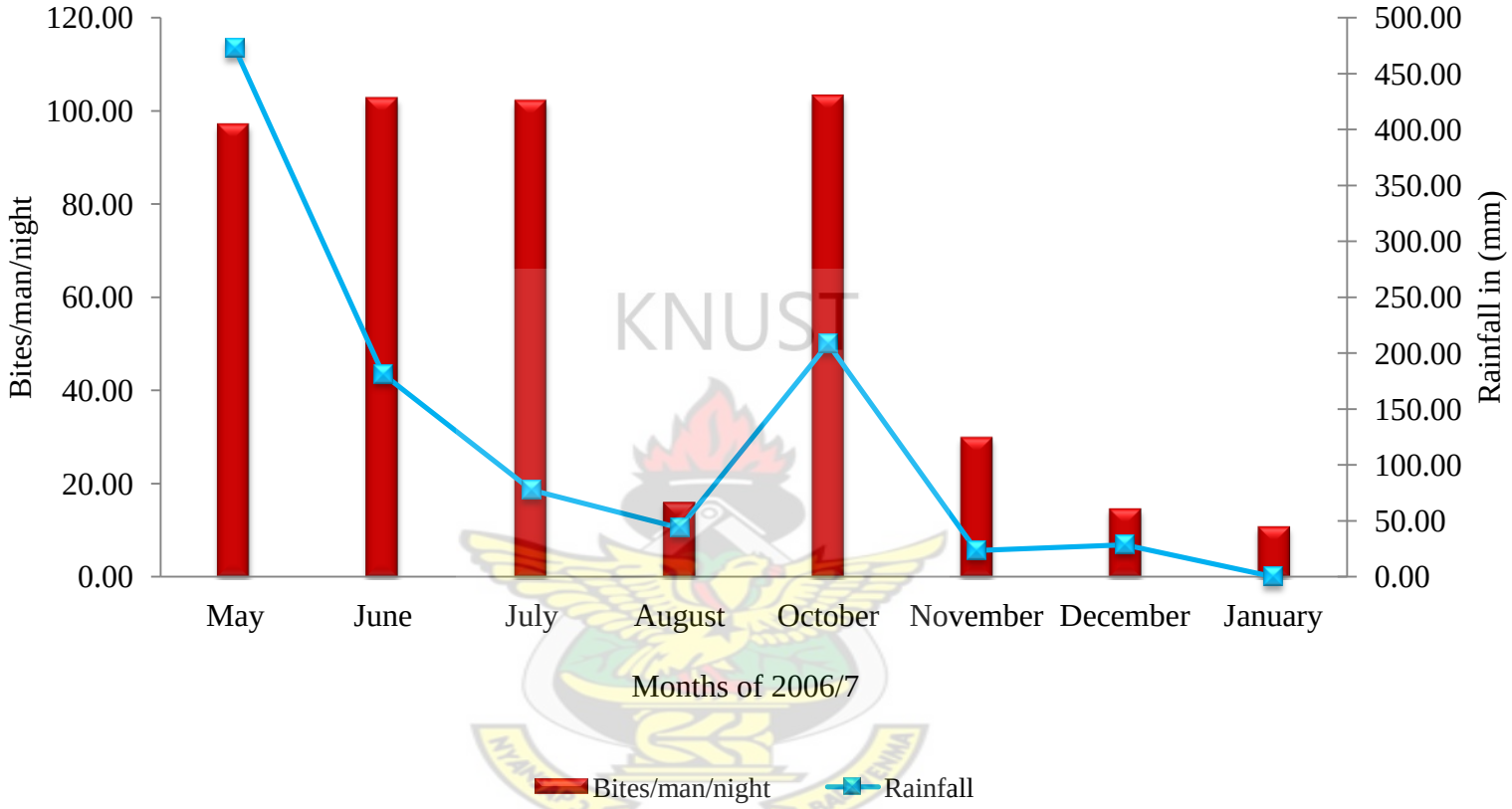


Figure 4.14 Monthly human biting rates of *An. gambiae* s.s. in relation to rainfall at Hwidiem.

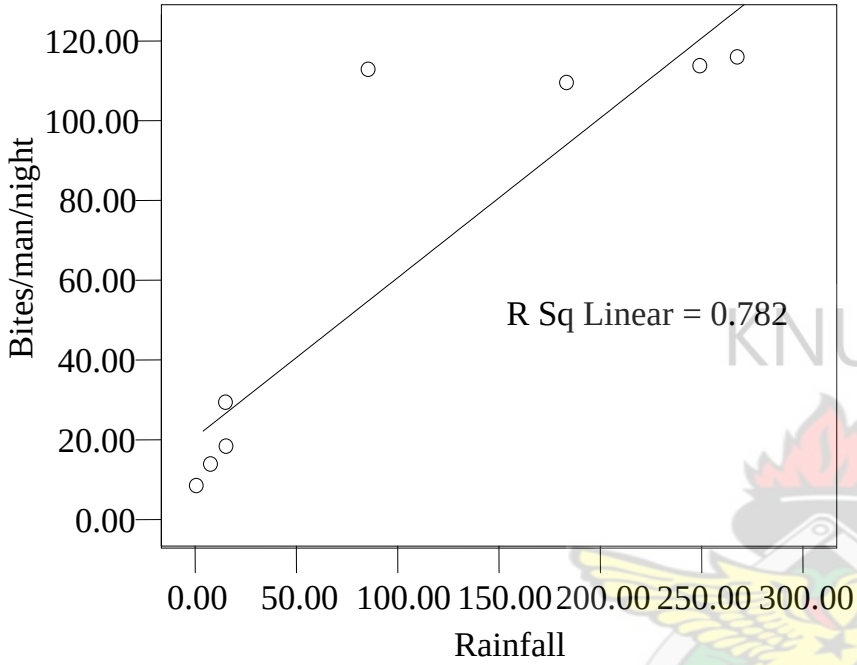


Figure 4.15 Correlation between monthly biting rates and monthly rainfall at Sunyani

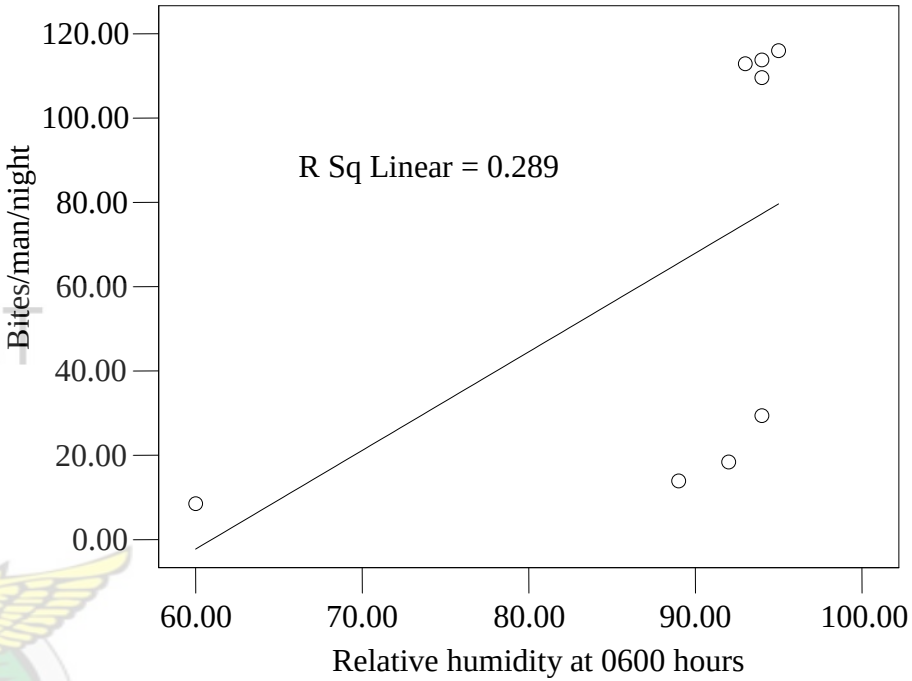


Figure 4.16 Correlation between monthly biting rates and mean daily relative humidity (%) at 0600 hours at Sunyani.

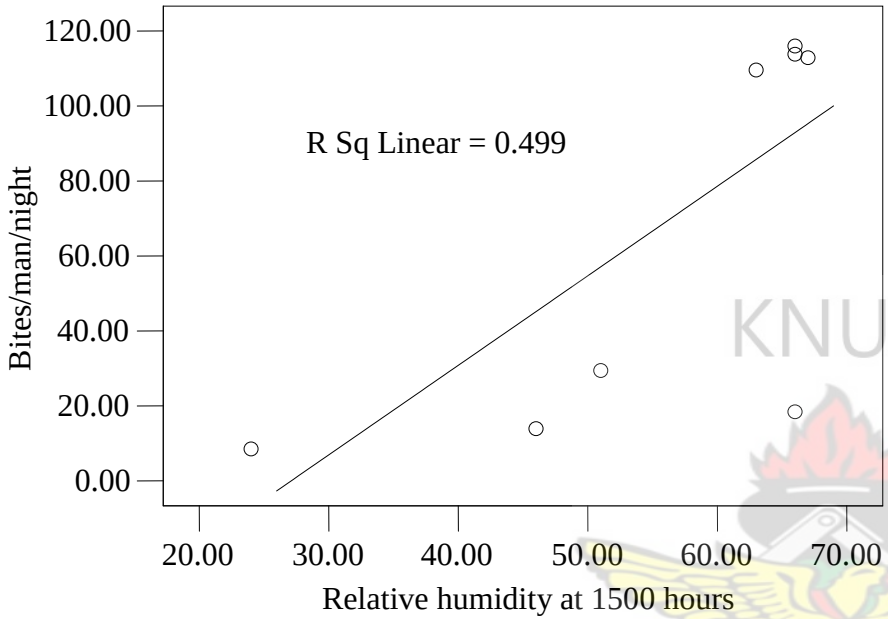


Figure 4.17 Correlation between monthly biting rates and mean daily relative humidity (%) at 1500 hours at Sunyani.

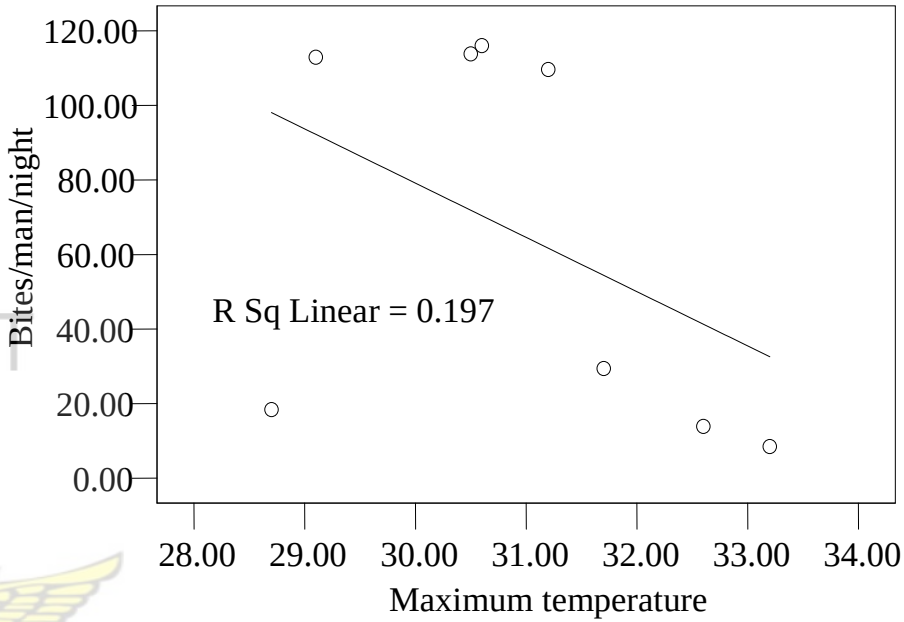


Figure 4.18 Correlation between monthly biting rates and mean daily maximum temperature (˚C) at Sunyani.

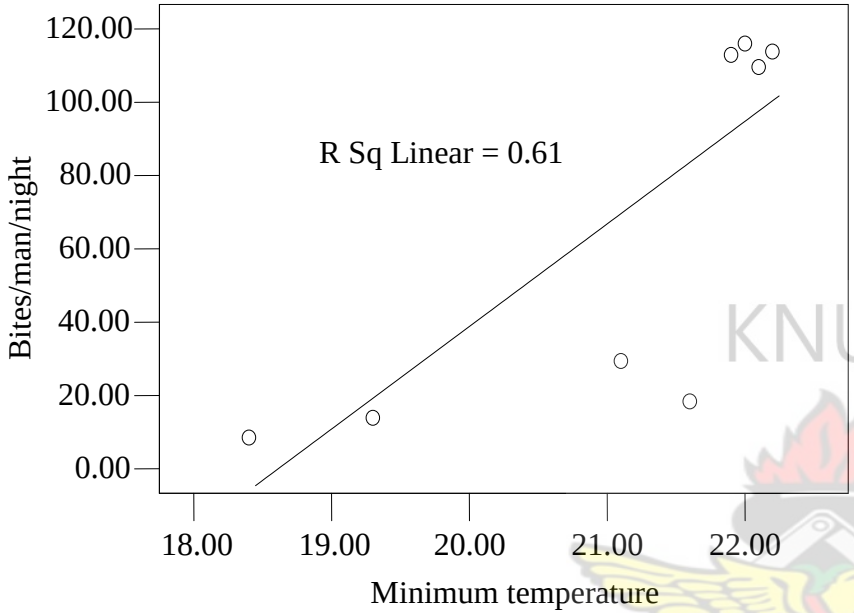


Figure 4.19 Correlation between monthly biting rates and mean daily minimum temperature (˚C) at Sunyani.

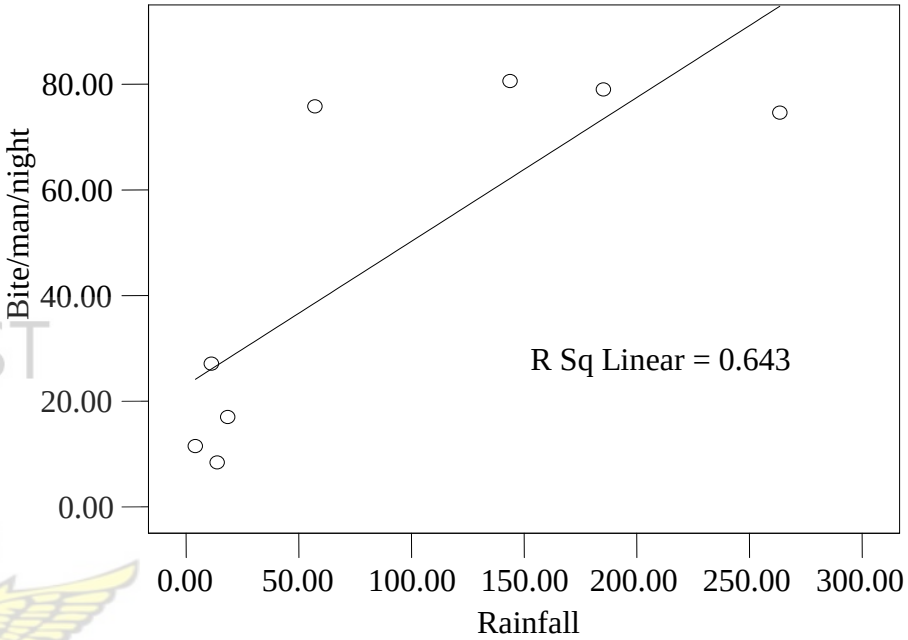


Figure 4.20 Correlation between monthly biting rates and monthly rainfall (mm) at Chiraa.

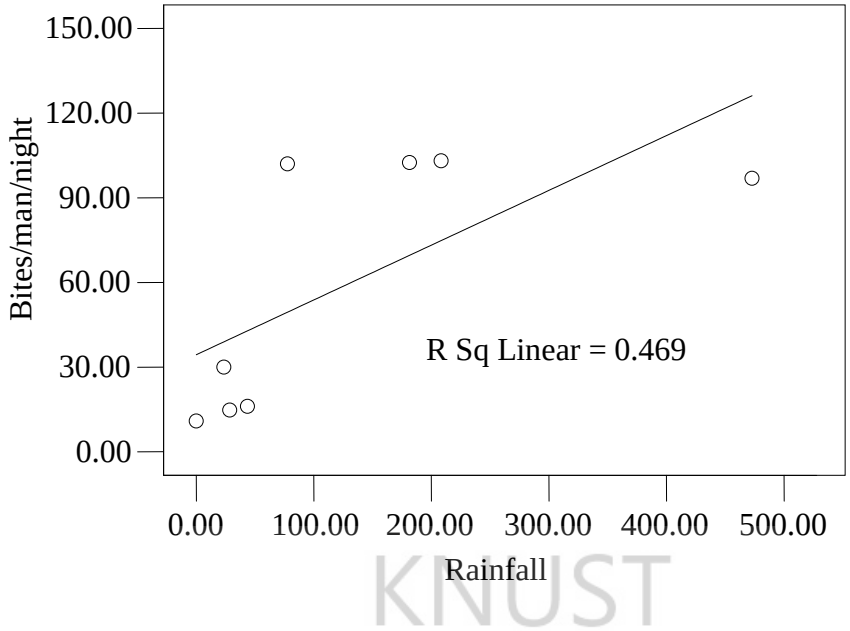


Figure 4.21 Correlation between monthly biting rates and monthly rainfall (mm) at Hwidiem.

4.2.3 Comparison of human-biting rates (HBRs) of An. gambiae s.s. between sites.

Table 4.8 shows the cumulative human-biting rates of *An. gambiae* s.s in the rainy and dry seasons at each of the study sites. During the rainy season, there were significant differences in HBR (b/m/n) between the study sites with the highest occurring at Ahafo Kenyasi and the lowest at Chiraa (χ^2 = 8.654, d.f. = 3, p = 0.034). Pairwise comparison showed that the HBR at Ahafo Kenyasi was not significantly higher than those of Sunyani (χ^2 = 0.009, d.f. = 1, p = 0.924) and Hwidiem (χ^2 = 0.830, d.f. = 1, p = 0.362) but it was significantly higher than that of Chiraa (χ^2 = 7.13, d.f. = 1, p = 0.008). During the dry season, however, there were no significant differences in HBRs between the study sites (χ^2 = 0.122, d.f. = 1, p = 0.898), although the highest occurred at Hwidiem and the lowest at Chiraa.

Table 4.8 The human biting rates (HBRs) of *An. gambiae* s.s. per season.

		Rainy season			Dry season		
Locality	Man-nights*	Total**	b/m/n	b/m/h	Total***	b/m/n	b/m/h
Sunyani	32	3618	113.1	9.4	561	17.5	1.5
Chiraa	32	2480	77.5	6.5	512	16.0	1.3
Kenyasi	32	3664	114.5	9.5	554	17.3	1.4
Hwediem	32	3236	101.1	8.4	574	17.9	1.5

*The number of man-nights of collections made per locality

**The number of *An. gambiae* s.s. caught per locality in rainy season

***The number of *An. gambiae* s.s caught per locality in dry season

4.2.4 Comparison of the human biting rates (HBRs) of Anopheles gambiae s.s. between the period of high and low rainfalls in the study sites.

Human biting rate was significantly higher in the rainy season (χ^2 = 65.323, d.f. = 1, $p < 0.001$) but followed similar trend in each season at all the study sites (χ^2 = 0.866, d.f. = 3, p = 0.834). In other words, HBRs at all the study sites, were high in the rainy season and low in the dry season when rainfall was much lower or nil and most of the temporary pools were dry. Of the 15,199 *An. gambiae* s.s. caught attempting to bite, 12998 (85.5%) and 2201 (14.5%) were caught in the rainy and the dry seasons respectively. On the whole, 128 man-nights gave rise to about 102 b/m/n (or 9 b/m/h) and 17 b/m/n (or 1 b/m/h) in the rainy and dry seasons respectively.

4.2.5 Endophagy and exophagy of the Anopheles gambiae s.s.

The degree of endophagy and exophagy exhibited by the *An. gambiae* s.s. refers to the proportions of this mosquito caught indoors and outdoors respectively. Estimates for the coefficient of endophagy of *An. gambiae* s.s. were obtained by

comparing the proportions of the mosquitoes attempting to bite indoors or outdoors in each study site (Tables 4.9 to 4.14). The coefficient of endophagy was estimated from the ratio of the numbers of mosquitoes caught biting indoors to that caught biting outdoors in a given hour or study site. The indoor and outdoor human-landing rates data were analysed both on hourly and night basis per study site.

4.2.5.1 Hourly endophagy and exophagy of An. gambiae s.s. in the study sites

Cumulative hourly *An. gambiae* s.s. human-landing rates (%) during the outdoor and indoor human landing catches (HLCs) over the study period are as shown in Tables 4.9 to 4.12. The hourly human-landing rates were generally higher indoor in most hours of the nights although outdoor rates were sometimes higher in some hours of early evening from 18:00 – 19:00 hours to the 20:00 – 21:00 hours and at times up to the 21:00 – 22:00 hours depending on the season and study site. It was noted that at most times, the differences between indoor and outdoor human-landing rates were not statistically significant ($p > 0.05$) during the early hours of the night.

4.2.5.1.1 Hourly endophagy and exophagy of *An. gambiae* s.s. at Sunyani

Hourly HBRs (%) in indoor and outdoor HLCs recorded at Sunyani in the rainy and the dry seasons are given in Table 4.9. During the rainy season, there were no significant differences in hourly indoor and outdoor HBRs until the 23:00 – 00:00 hours when the indoor HBRs significantly exceeded the outdoor HBRs. Also, hourly indoor HBRs were much higher during the 00:00 – 01:00 hours, 03:00 – 04:00 hours and 04:00 – 05:00 hours. However, outdoor HBRs exceeded the indoor rates during the 18:00 – 19:00 hours, 20:00 – 21:00 hours and 01:00 – 02:00 hours but the differences were not significant. During the dry season, there were no significant

differences in hourly indoor and outdoor HBRs until the 04:00 – 05:00 hours when the indoor rates significantly exceeded the outdoor rates (see Table 4.9).

Table 4.9 Hourly indoor and outdoor biting rates (%) of *An. gambiae* in the rainy and dry seasons at Sunyani

	Rainy season			Dry season		
Hour of night	In (%)	Out (%)	*p-value*	In (%)	Out (%)	*p-value*
18:00-19:00	41.67	58.33	0.197	69.23	30.77	0.166
19:00-20:00	55.91	44.09	0.254	66.67	33.33	0.157
20:00-21:00	44.19	55.81	0.127	59.38	40.63	0.289
21:00-22:00	52.99	47.01	0.360	47.92	52.08	0.773
22:00-23:00	51.70	48.30	0.580	56.52	43.48	0.289
23:00-00:00	59.32	40.68	0.0005	57.58	42.42	0.218
00:00-01:00	58.76	41.24	0.001	54.67	45.33	0.419
01:00-02:00	49.18	50.82	0.735	49.32	50.68	0.906
02:00-03:00	51.23	48.77	0.603	59.21	40.79	0.108
03:00-04:00	55.30	44.70	0.035	61.90	38.10	0.059
04:00-05:00	55.40	44.60	0.026	76.00	24.00	0.009
05:00-06:00	53.33	46.67	0.188	66.67	33.33	0.564

4.2.5.1.2 Hourly endophagy and exophagy of *An. gambiae* s.s at Chiraa

Cumulative hourly human landing rate (%) in indoor and outdoor HLCs at Chiraa in the rainy and dry seasons are given in Table 4.10. Indoor HBRs were generally higher but the differences were not always significant. In the rainy season, there were no significant differences in landing rates between indoor and outdoor until the 00:00 – 01:00 hours when indoor HBRs significantly exceeded the outdoor HBRs.

Similarly, indoor HBRs were much higher during the 01:00 – 02:00, 04:00 – 05:00 and 05:00 – 06:00 hours. Whereas outdoor HBRs exceeded the indoor HBRs during the 19:00 – 20:00 to 21:00 – 22:00 hours, the differences were not significant. In the dry season, there were no significant differences in indoor and outdoor HBRs until the 05:00 – 06:00 hours when the indoor HBRs significantly exceeded the outdoor HBRs. Again in the dry season, outdoor HBRs exceeded the indoor in the 18:00 – 19:00 hours to 20:00 – 21:00 hours but the differences were not significant (see Table 4.10).

Table 4.10 Hourly indoor and outdoor biting rates (%) of *An. gambiae* in the rainy and dry seasons at Chiraa

	Rainy season			Dry season		
Hour of night	In (%)	Out (%)	*p-value*	In (%)	Out (%)	*p-value*
18:00-19:00	57.14	42.86	0.317	42.86	57.14	0.593
19:00-20:00	54.29	45.71	0.473	43.75	56.25	0.617
20:00-21:00	52.94	47.06	0.552	34.78	65.22	0.144
21:00-22:00	46.47	53.53	0.357	51.16	48.84	0.879
22:00-23:00	45.86	54.14	0.265	54.39	45.61	0.508
23:00-00:00	52.70	47.30	0.420	54.55	45.45	0.500
00:00-01:00	57.48	42.52	0.017	57.14	42.86	0.232
01:00-02:00	57.59	42.41	0.010	57.38	42.62	0.249
02:00-03:00	53.64	46.36	0.205	58.57	41.43	0.152
03:00-04:00	55.19	44.81	0.068	51.61	48.39	0.799
04:00-05:00	56.20	43.80	0.040	51.52	48.48	0.862
05:00-06:00	58.91	41.09	0.004	87.50	12.50	0.034

4.2.5.1.3 Hourly endophagy and exophagy of *An. gambiae* s.s at Ahafo Kenyasi

Cumulative hourly human-landing rates (density) [%] in the HLCs recorded at Ahafo Kenyasi inside and outside human dwellings are indicated in Table 4.11. In the rainy season, about half of the *An. gambiae* s.s. was caught biting indoor and half outdoor during the 18:00 – 23:00 hours. However, during the 23:00 – 00.00 hours, there was a clear display of endophagy ($\chi^2 = 4.125$, $p = 0.042$). During the 00:00 – 02:00 hours, indoor human-landing rates still exceeded the outdoor rates although the differences were not significant ($p > 0.050$). After these hours of the night, indoor human landing rates considerably exceeded the outdoor human landing rates till daybreak, 05:00 – 06:00 hours. During dry season, there were no significant differences in the degree of endopagy and exophagy demonstrated until the 21:00 – 22:00 hours when outdoor human-landing rates significantly exceeded indoor rates. Indoor human landing rates were also significantly higher than the outdoor rates in the 03:00 – 04:00 hours, 04:00 – 05:00 hours and 05:00 – 06:00 hours (See Table 4.11).

Table 4.11 Hourly indoor and outdoor biting rates (%) of *An. gambiae* in the rainy and dry seasons at Ahafo Kenyasi

	Rainy season			Dry season		
Hour of night	In (%)	Out (%)	*p*	In (%)	Out (%)	*P*
18:00-19:00	51.02	48.98	0.888	48.9	51.1	0.527
19:00-20:00	49.56	50.44	0.924	38.5	61.5	0.109
20:00-21:00	48.52	51.48	0.700	43.0	57.0	0.239
21:00-22:00	51.52	48.48	0.645	44.6	55.4	0.022
22:00-23:00	52.96	47.04	0.315	51.7	48.3	0.052
23:00-00:00	55.89	44.11	0.042	52.3	47.7	0.225
00:00-01:00	53.17	46.83	0.217	50.3	49.7	0.083
01:00-02:00	53.24	46.76	0.178	51.9	48.1	0.569
02:00-03:00	55.53	44.47	0.023	52.9	47.1	0.079
03:00-04:00	55.71	44.29	0.017	52.9	47.1	0.024
04:00-05:00	55.48	44.52	0.022	54.3	45.7	0.003
05:00-06:00	56.02	43.98	0.015	52.1	47.9	0.0003

4.2.5.1.4 Hourly endophagy and exophagy of *An. gambiae* s.s at Hwidiem

Cumulative hourly human-landing rates (%) in the HLCs recorded at Hwidiem inside and outside human dwellings are indicated in Table 4.12. In the rainy season there were no significant differences in hourly indoor and outdoor human-landing rates except for the 18:00 – 19:00, 02:00 – 03:00 and 04:00 – 05:00 hours when the outdoor human-landing rates significantly exceeded the indoor rates. During the dry season, there were no significant differences in hourly outdoor and indoor human-landing rates except during the 03:00 – 04:00 hours, 04:00 – 05:00 hours and 05:00 – 06:00 hours when the indoor human-landing rates significantly exceeded the outdoor rates (See Table 4.12).

Table 4.12 Hourly indoor and outdoor biting rates (%) of *An. gambiae* in the rainy and dry seasons at Hwidiem.

	Rainy season			Dry season		
Hour of night	In (%)	Out (%)	*p*	In (%)	Out (%)	*P*
18:00-19:00	33.85	66.15	0.009	46.67	53.33	0.795
19:00-20:00	43.24	56.76	0.155	52.00	48.00	0.841
20:00-21:00	44.76	55.24	0.210	42.86	57.14	0.398
21:00-22:00	50.00	50.00	1.00	56.86	43.14	0.327
22:00-23:00	54.71	45.29	0.160	58.33	41.67	0.197
23:00-00:00	53.06	46.94	0.338	59.42	40.58	0.118
00:00-01:00	54.55	45.45	0.099	54.79	45.21	0.413
01:00-02:00	52.03	47.97	0.435	57.33	42.67	0.204
02:00-03:00	55.56	44.44	0.028	53.97	46.03	0.529
03:00-04:00	54.33	45.67	0.078	62.69	37.31	0.038
04:00-05:00	55.42	44.58	0.031	73.68	26.32	0.004
05:00-06:00	54.32	45.68	0.096	100.00	0.00	0.083*

* *z = 2.449, p = 0.014* (the data did not meet the assumptions of Chi-Square test).

4.2.5.2 Cumulative endophagy and exophagy of An. gambiae s.s per and between sites

An overall picture of nightly indoor and outdoor HBRs was obtained for each study site in view of the fact that the differences in the hourly figures fluctuated, giving significant or insignificant differences at certain hours and not at others. Tables 4.13 and 4.14 show the human-landing rates of *An. gambiae* s.s. and in/out ratio per site in the rainy and the dry seasons respectively. The coefficient of endophagy estimated in both the rainy and dry seasons deviated from the exact ratio of 1:1 at

each site. Analysis of the coefficient of endophagy within sites showed that indoor human-landing rates were significantly higher at all sites in both seasons ($p < 0.001$).

The pattern of *An. gambiae* s.s. human-landing rates inside and outside human dwellings in the rainy and the dry seasons was not the same at each site. While there were no significant differences in endophagous rates between sites in the rainy season ($\chi^2 = 1.807$, $p = 0.613$) [Table 4.13], endophagous rates varied significantly between study sites in the dry season ($\chi^2 = 8.162$, $p = 0.043$) [Table 4.14].

Table 4.13 Human-biting rate of *An. gambiae* and indoor (in)/ outdoor (out) ratio per study site during the rainy season.

		Number of *A. gambiae*		percentage of total		Ratio
Locality	Total	in	out	in	out	in/out
Sunyani	3618	1934	1684	53.5	46.5	1.15
Chiraa	2480	1350	1130	54.4	45.6	1.19
Kenyasi	3664	1982	1682	54.1	45.9	1.18
Hwidiem	3236	1710	1526	52.8	47.2	1.12

Table 4.14 Human-biting rate of *An. gambiae* and indoor (in)/ outdoor (out) ratio per study site during the dry season.

		Number of *A. gambiae* s.s.		percentage of total		Ratio
Locality	Total	in	out	in	Out	in/out
Sunyani	561	322	239	57.4	42.6	1.35
Chiraa	512	276	236	53.9	46.1	1.17
Kenyasi	554	346	208	62.5	37.5	1.66
Hwidiem	574	330	244	57.5	42.5	1.35

4.2.6 Nocturnal biting cycle of the Anopheles gambiae s.s.

4.2.6.1 Nocturnal biting cycle of An. gambiae s.s per site in rainy season.

The nature of nocturnal biting cycle of *An. gambiae* s.s. determined in the rainy season is illustrated in Figures 4.22 and 4.25. It was found in this study that biting activities essentially started from the 18:00 – 19:00 hours and were sustained until the 05:00 – 06:00 hours. Human-biting activities increased with night time, from the 18:00 – 19:00 hours to 01:00 – 02:00 hours but it was comparatively constant from 01:00-02:00 hours to 05:00-06:00 hours.

Of the 3618 *An. gambiae* s.s. from Sunyani, 325 (8.98%), 853 (23.58%), 1228 (33.94%) and 1212 (33.50%) were caught attempting to bite during the first, second, third and fourth quarters repectively. The lowest quarterly nocturnal biting activities occurred during the first quarter of night while the highest occurred during the third quarter (χ^2 = 594.451, p < 0.001). There were, however, no significant differences in biting activities between the third and fourth quarters (p > 0.050) although activities were higher in the third quarter. Overall, 1178 (32.56%) and 2440 (67.44%) of the 3618 biting activities occurred from dusk-midnight and from midnight-daybreak respectively (χ^2 = 440.20, p < 0.001).

At Chiraa, 221 (8.91%), 573 (23.10%), 846 (34.11%), 840 (33.87%) of the 2480 biting activities occurred during the first to the fourth quarters respectively. The lowest quarterly biting activities occurred in the first quarter of night whilst the highest occurred in the third quarter (χ^2 = 420.784, p < 0.001). Peak biting occurred during the 03:00 – 04:00 hours. Biting activities in the first quarter were lower than in the second (p < 0.001), those in the second were lower than in the third (p < 0.001) but there were no significant differences between the third and fourth quarters (p >

0.050). Overall, 794 (32.02%) and 1686 (67.98%) of the 2480 biting occurred in the first and second parts of the night respectively ($\chi^2 = 320.83$, $p < 0.001$).

At Ahafo Kenyasi, 331 (9.03%); 815 (22.24%), 1235 (33.71%) and 1283 (35.02%) of the 3664 samples were found biting during the first, second, third and fourth quarters respectively. The lowest activities occurred in the first quarter whilst the highest occurred in the fourth quarter ($\chi^2 = 642.878$, $p < 0.001$). Biting activities in the first quarter were lower than in the second ($p < 0.001$) and those of the second quarter were lower than in the third quarter ($p < 0.001$). However, there were no significant differences between the third and fourth quarters of the night ($p > 0.050$) although biting activities were more pronounced in the fourth quarter. Also, the 3664 biting activities recorded comprised 1146 (31.28%) from dusk to midnight and 2518 (68.72%) from midnight to daybreak ($\chi^2 = 513.751$, $p < 0.001$).

At Hwidiem, 319 (9.86%), 648 (20.02%), 1086 (33.56%) and 1183 (36.56%) of the 3236 bites occurred in the first, second, third and fourth quarters respectively. The lowest activities occurred in the first quarter of the night whereas the highest occurred in the fourth quarter ($\chi^2 = 596.571$, $p < 0.001$). Biting activities in the first quarter were signifiantly lower than in the second ($p < 0.001$) and those in the second were significantly lower than those in the third quarter of the night ($p < 0.001$). Also, biting activities in the third quarter were much lower than those in the fourth quarter of the night ($p < 0.012$). Lastly, of the 3236 human-biting activities observed, 967 (29.88%) occurred in the first part of night whereas the outstanding 2269 (70.12%) biting activities occured in the second part of the night ($\chi^2 = 523.858$, $p < 0.001$).

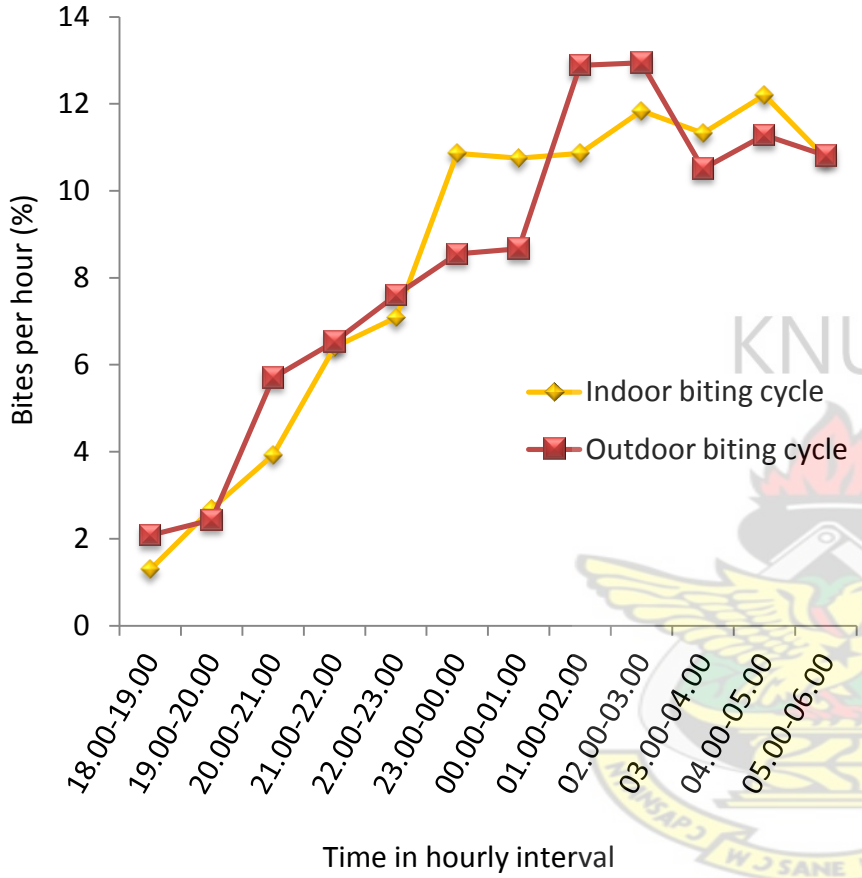


Figure 4.22 Nocturnal human-biting cycle of *An. gambiae* in the rainy season at Sunyani over the study period.

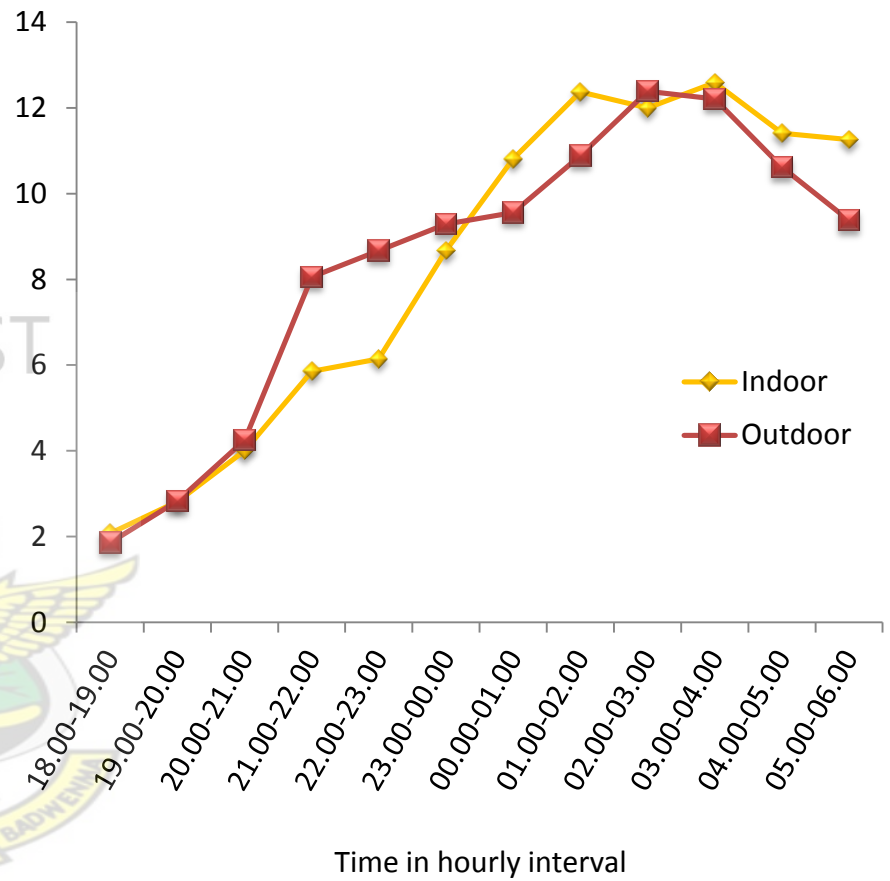


Figure 4.23 Nocturnal human-biting cycle of *An. gambiae* during the rainy season at Chiraa over the study period.

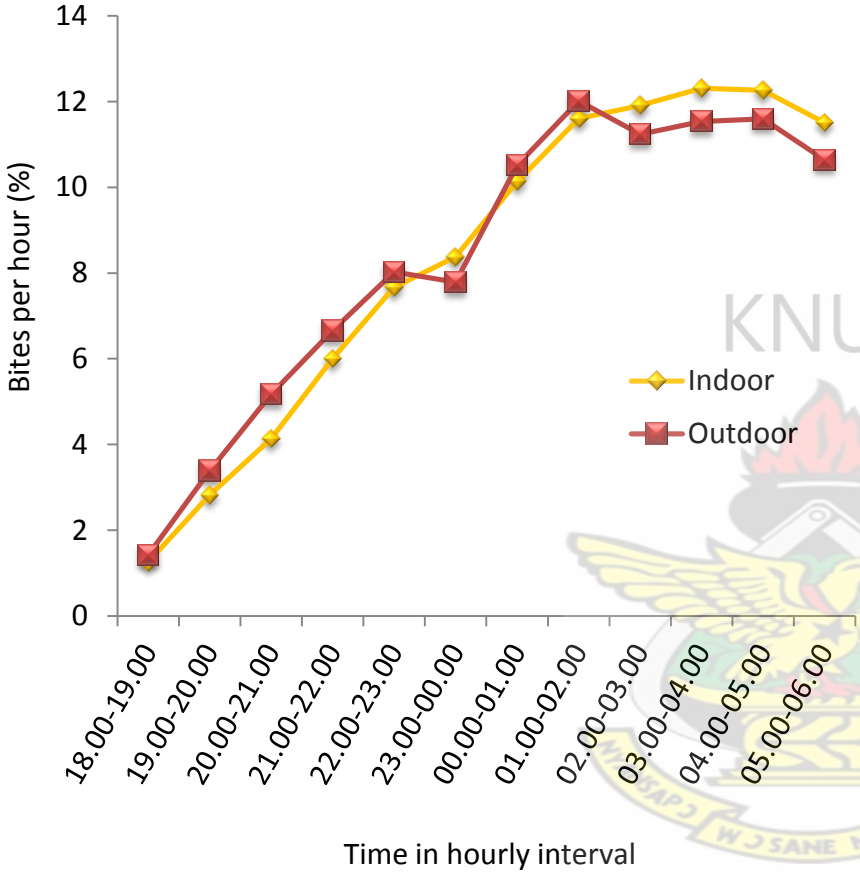


Figure 4.24 Nocturnal human-biting cycle of *An. gambiae* during the rainy season at Ahafo Kenyasi over the study period.

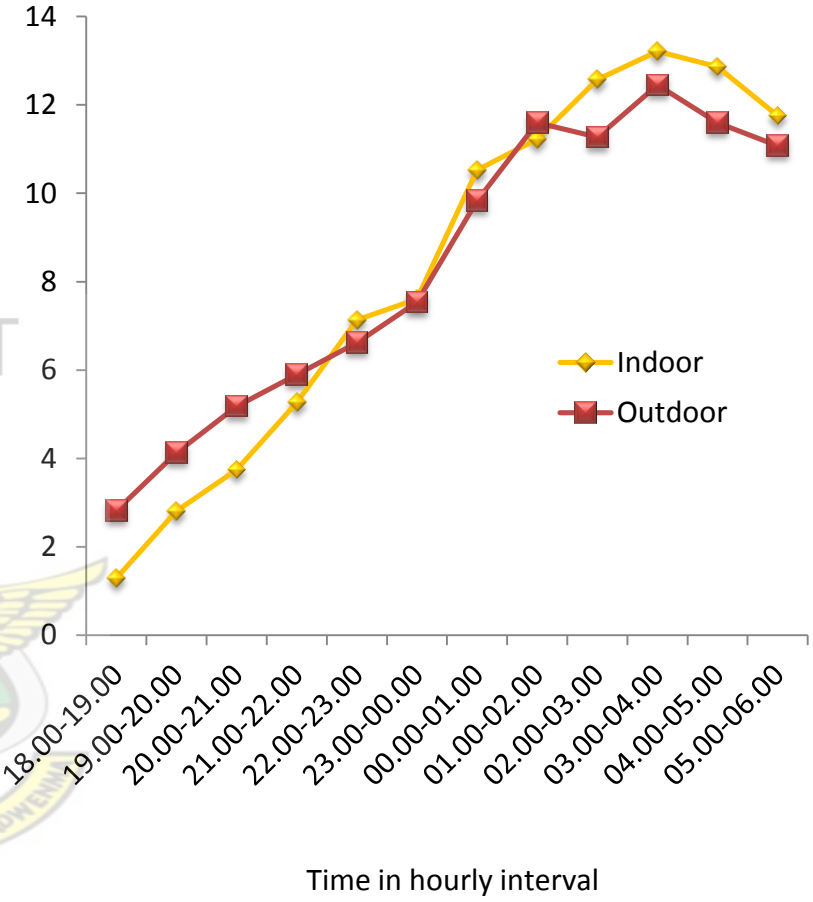


Figure 4.25 Nocturnal human-biting cycle of *An. gambiae* during the rainy season at Hwidiem over the study period.

4.2.6.2 Nocturnal biting cycle of An. gambiae s.s.*per site in the dry season*

The night biting cycle of *An. gambiae* s.s. observed in the dry season at the sites are given in Figures 4.26 – 4.29. Biting activities in the dry season were also analysed based on the night partitions as defined. Aggressiveness increased gradually from the first quarter of the night till the third quarter after which period it declined till daybreak. Aggressiveness was higher from the midnight-daybreak. Of the 561 bites from Sunyani, 63 (11.23%); 183 (32.62%), 224 (39.93%) and 91 (16.22%) were caught in the first, second, third and fourth quarters of the night respectively. Biting activities increased significantly until the third quarter ($p < 0.05$) and decreased significantly during the fourth quarter ($p < 0.001$). Of the 561 samples, 246 [43.85% (95% CI: 39.74 - 47.96)] and 315 [56.15% (95% CI: 52.04 - 60.26)] were caught during the first and second parts of the night respectively ($\chi^2 = 8.487$, $p = 0.004$).

Of the 512 samples from Chiraa, 53 (10.35%), 155 (30.27%), 201 (39.26%) and 103 (20.12%) were caught in the first, second, third and fourth quarters respectively. Biting activities increased from the first quarter until the third quarter ($p < 0.05$) after which it decreased significantly till daybreak ($p < 0.001$). Of the 512 samples from Chiraa, 208 (40.625%) were caught from dusk to midnight and 304 (59.375%) were from midnight to daybreak ($\chi^2 = 18.00$, $p = 0.00002$).

Of the 554 activities observed at Ahafo Kenyasi, 50 (9.03%), 175 (31.59%), 225 (40.61%) and 104 (18.77%) occurred in the first to the fourth quarters respectively. Biting activities increased significantly from the first till the third quarter of the night ($p = 0.002$) and decreased till the fourth quarter ($p < 0.001$). Similarly, the 554 samples from Ahafo Kenyasi comprised 225 [40.61% (95%) from dusk to midnight and 329 (59.39%) from midnight to daybreak ($\chi^2 = 19.523$, $p < 0.001$).

Lastly, of the 574 samples from Hwidiem, 75 (13.07%), 180 (31.36%), 211 (36.76%) and 108 (18.82%) were caught attempting to bite during the first to the fourth quarters respectively. Biting activities in the first quarter was significantly lower than any other quarter (χ^2 = 82.516, p < 0.001) but activities in the second quarter was not significantly lower than in the third quarter (p = 0.054). Lastly, biting activities in the third quarter was significantly higher than in the fourth quarter (p < 0.001). The 574 activities recorded at Hwidiem comprised 255 (44.43%) and 319 (55.57%) from first and second part of the night repectively (χ^2 = 7.136, p = 0.008).

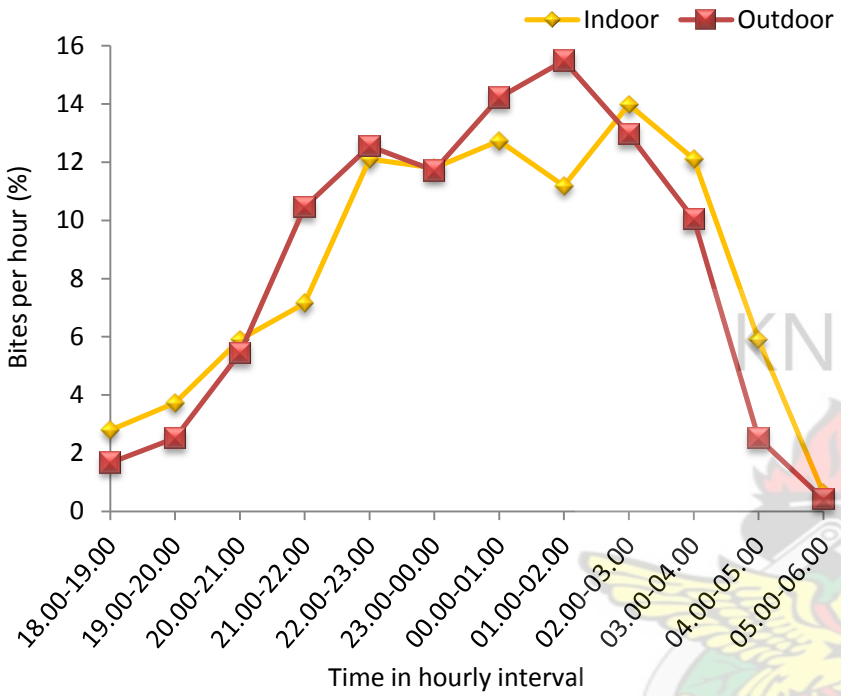


Figure 4.26 Nocturnal human-biting cycle of *Anopheles gambiae* during the dry season at Sunyani over the study period.

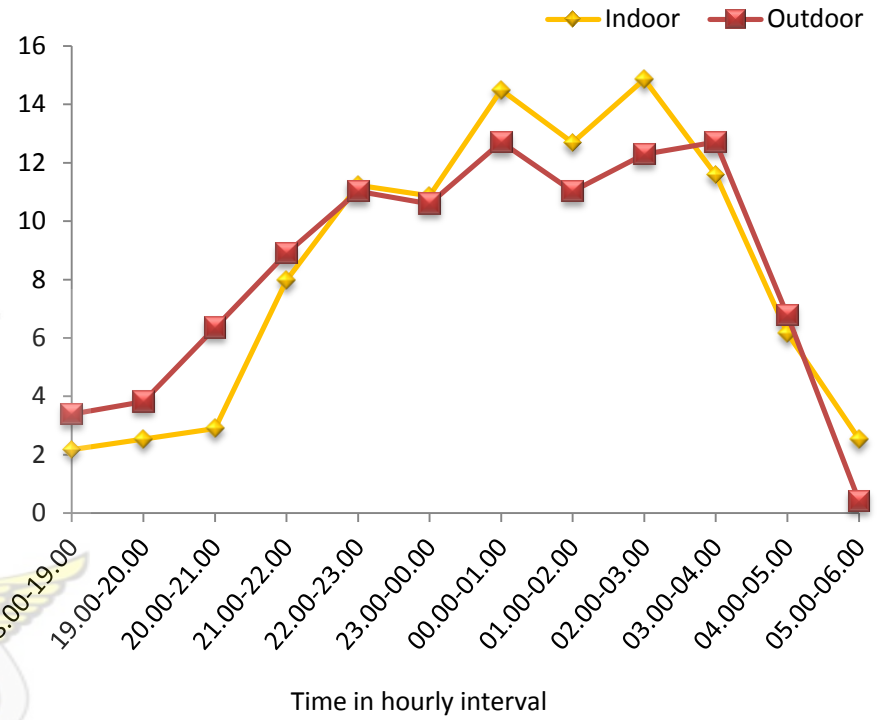


Figure 4.27 Nocturnal human-biting cycle of *Anopheles gambiae* during the dry season at Chiraa over the study period.

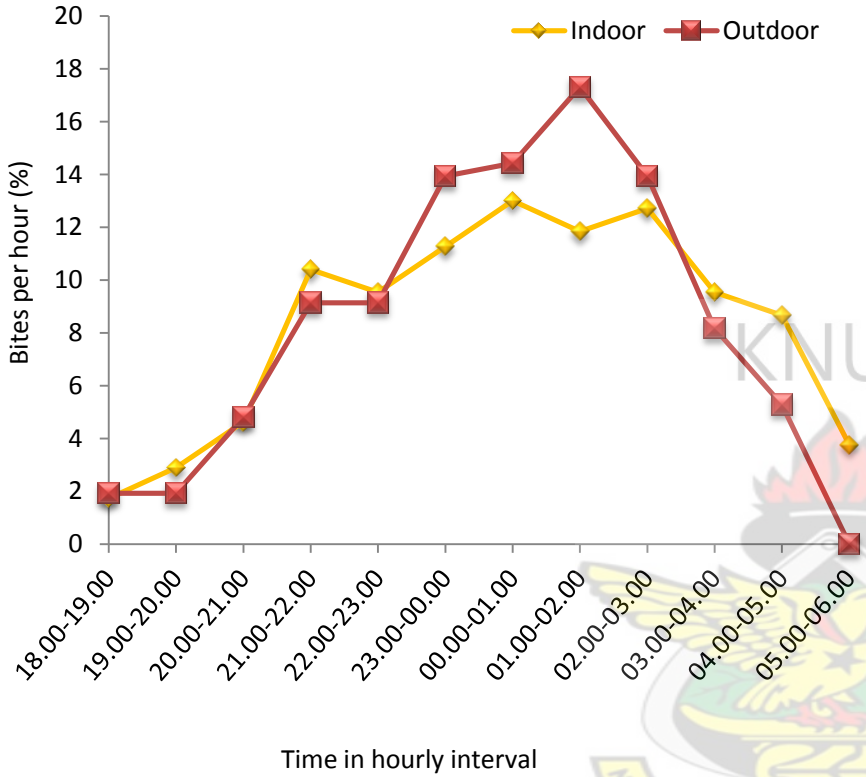


Figure 4.28 Nocturnal human-biting cycle of *An. gambiae* in the dry season at Ahafo Kenyasi over the study period.

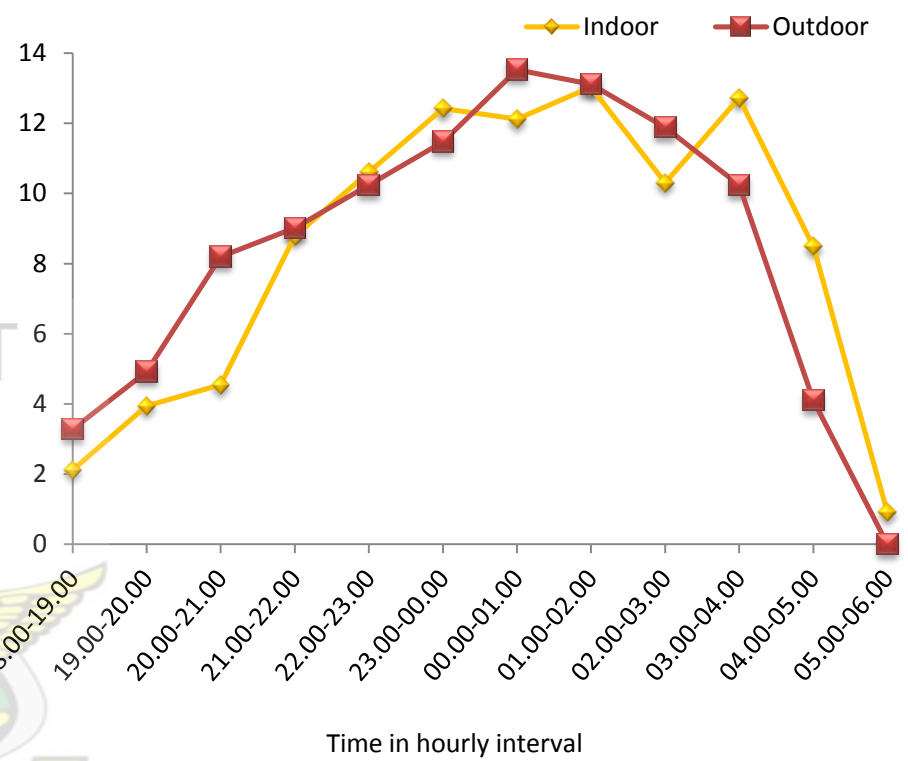


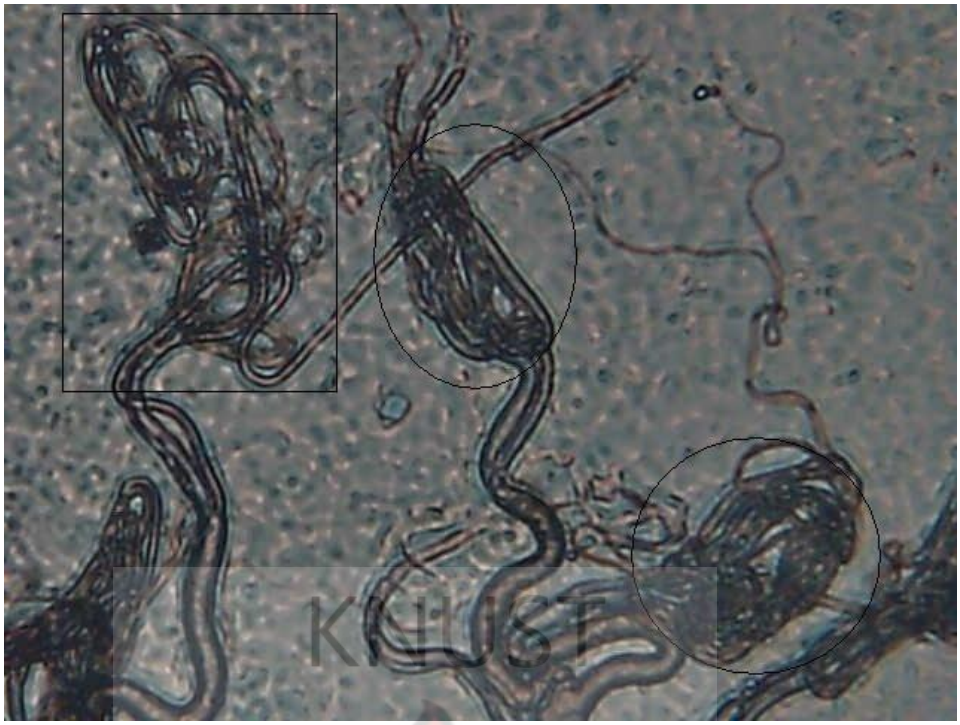
Figure 4.29 Nocturnal human-biting cycle of *Anopheles gambiae* during the dry season at Hwidiem over the study period.

4.2.7 Parous rates of Anopheles gambiae s.s. at the study sites

Examples of nulliparous and parous *An. gambiae* ovaries dissected in the present study are shown in Figures 4.30 to 4.32. *Anopheles gambiae* species were described as being nulliparous or parous based on the appearance of the tracheolar skeins in their dissected ovaries (WHO, 2003). Nulliparous mosquitoes had tightly coiled tracheolar skeins in their ovaries but parous mosquitoes had at least one distended tracheole in their ovaries, which resulted after oogenesis.

Figure 4.30 Tightly coiled tracheoles called “skeins” (shown inside the rectangles). This is a nulliparous female *Anopheles gambiae* s.s. This mosquito had not gone through the process of oogenesis.

A



B

Figure 4.31 Loose structures of tracheoles after oogenesis (shown inside the rectangles). This is a dissected ovary of a parous female *Anopheles gambiae* s.s.

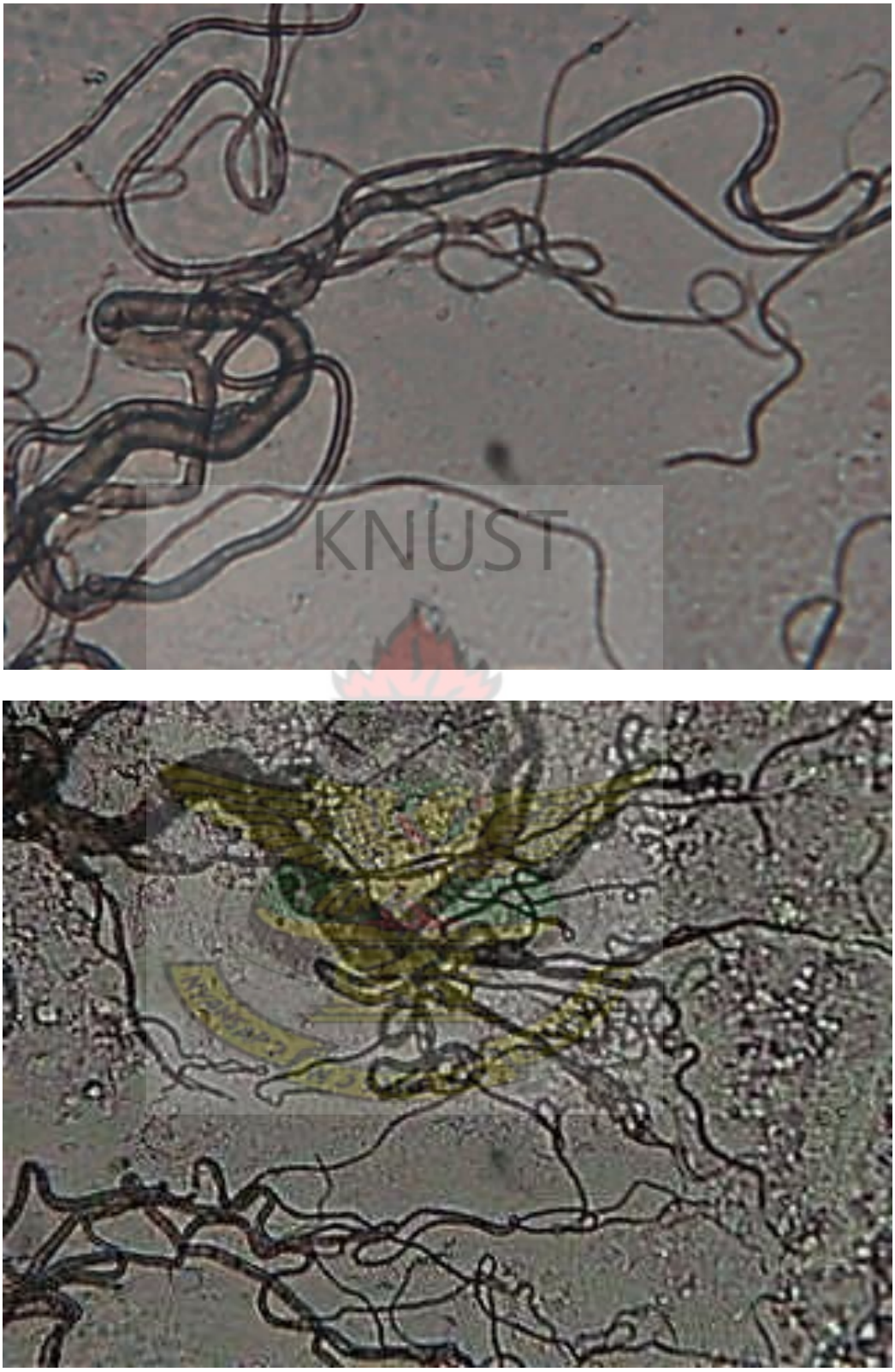


Figure 4.32 Fully distended tracheoles of *An. gambiae* species ovaries after several oogenesis. These female mosquitoes are also parous.

4.2.7.1 Monthly parous rates of Anopheles gambiae s.s. at the study sites

4.2.7.1.1 Monthly parous rates of *Anopheles gambiae* s.s. at Sunyani

Number of *Anopheles gambiae* dissected and parity rate (plus 95% confidence intervals) per month at Sunyani are given in Table 4.15. Figure 4.33 also shows the monthly parous rates in relation to monthly rainfall, mean daily relative humidity at 0600 hours and 1500 hours as well as the mean daily minimum and maximum temperatures recorded in this study at Sunyani. Figure 4.33 to 4.35 also illustrate the correlation between monthly parous rates and the meteorological parameters in question. Higher parous rates were observed during the months of dry weather than in the rainy season. The parous rates ranged from 65.2% in May in the major rainy season to 70.3% in October in the minor rainy season. Also, parous rates ranging from 73.8% in August (during the short dry period) to 91.9% in January in the main dry season of the year were recorded. Hence, there were significant differences in monthly parous rates with the lowest occurring in May and the highest in January (χ^2 = 21.121, d.f. = 7, p = 0.004). However, there were no significant diferences in parous rates between the months within seasons: rainy season (χ^2 = 1.895, d.f. = 3, p = 0.594) and dry season (χ^2 = 6.804, d.f. = 3, p = 0.078). There was a strong negative linear relationship between monthly parous rates and monthly rainfall (rho = -0.833, p = 0.010) and for that matter bites per man per night (rho = -0.762, p = 0.028). There was however, a weak negative relationship between monthly parous rates and mean daily relative humidity at 0600 hours (rho = -0.659, p = 0.076) and 1500 hours (rho = -0.683, p = 0.062). Lastly, there was a strong negative linear relationship between monthly parous rates and mean daily minimum temperature in each month (rho = -0.952, p = 0.000), and a weak positive correlation between monthly parous rates and mean daily maximum temperature (rho = -0.595, p = 0.120) [Figures 4.36 to 4.41].

Table 4.15 Number dissected, number parous and parous rate of *An. gambiae* per month in Sunyani, Sunyani Municipality, Ghana.

Month	Number dissected	Number parous	% Parous (95% CI)
May	287	187	65.16 (59.65 – 70.67)
June	297	198	66.67 (61.31 – 72.03)
July	295	198	67.12 (61.76 – 72.48)
August	80	59	73.75 (64.11 – 83.39)
October	303	213	70.30 (65.15 – 75.45)
November	127	95	74.80 (67.25 – 82.35)
December	60	50	83.33 (73.9 – 92.76)
January	37	34	91.89 (83.09 –100.69)

The months during which rainfall was low or nil and were therefore regarded dry were August, December and January.

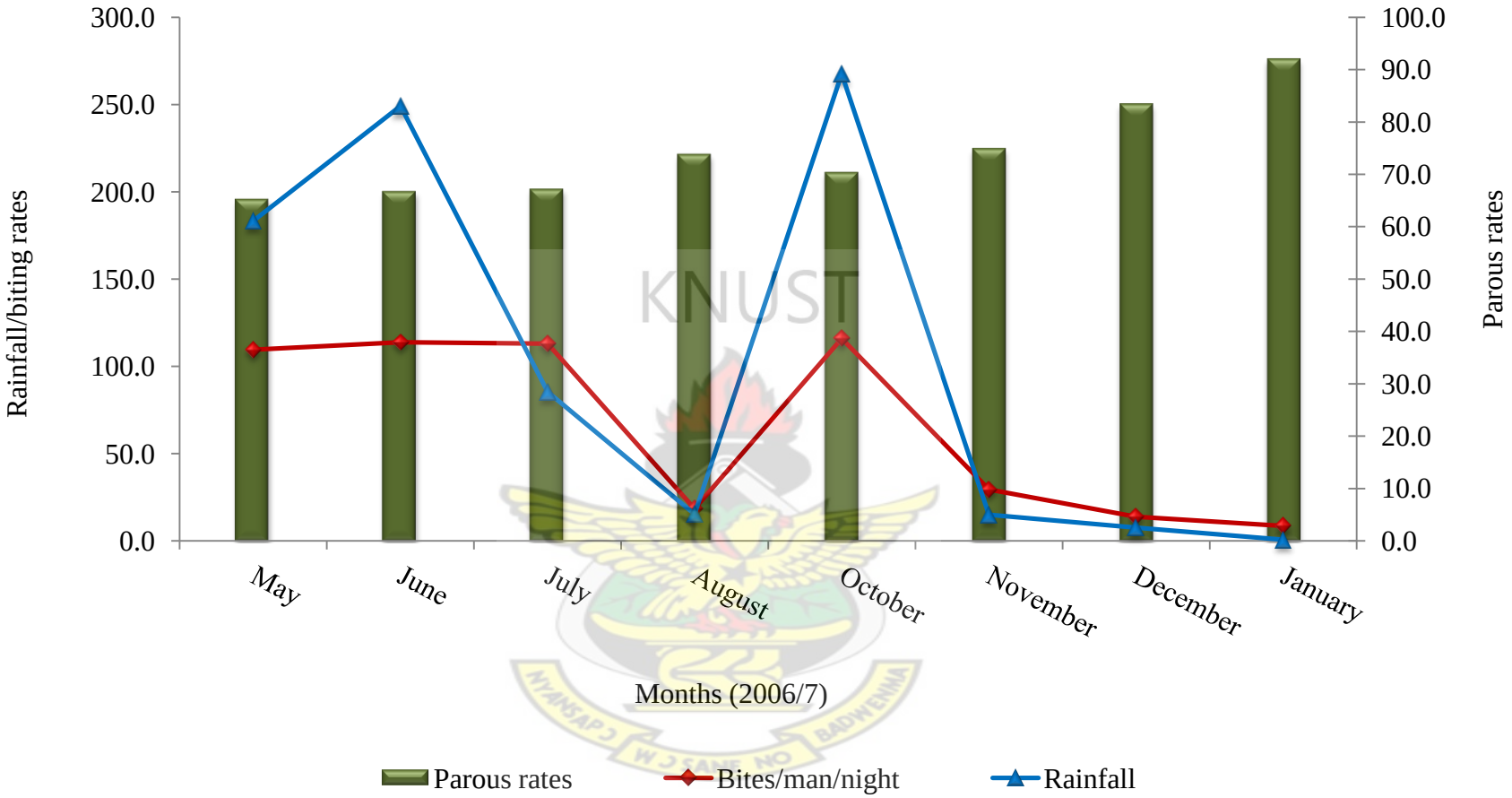


Figure 4.33 Monthly parous rates in relation to biting rates (b/m/n) and rainfall (mm) at Sunyani.

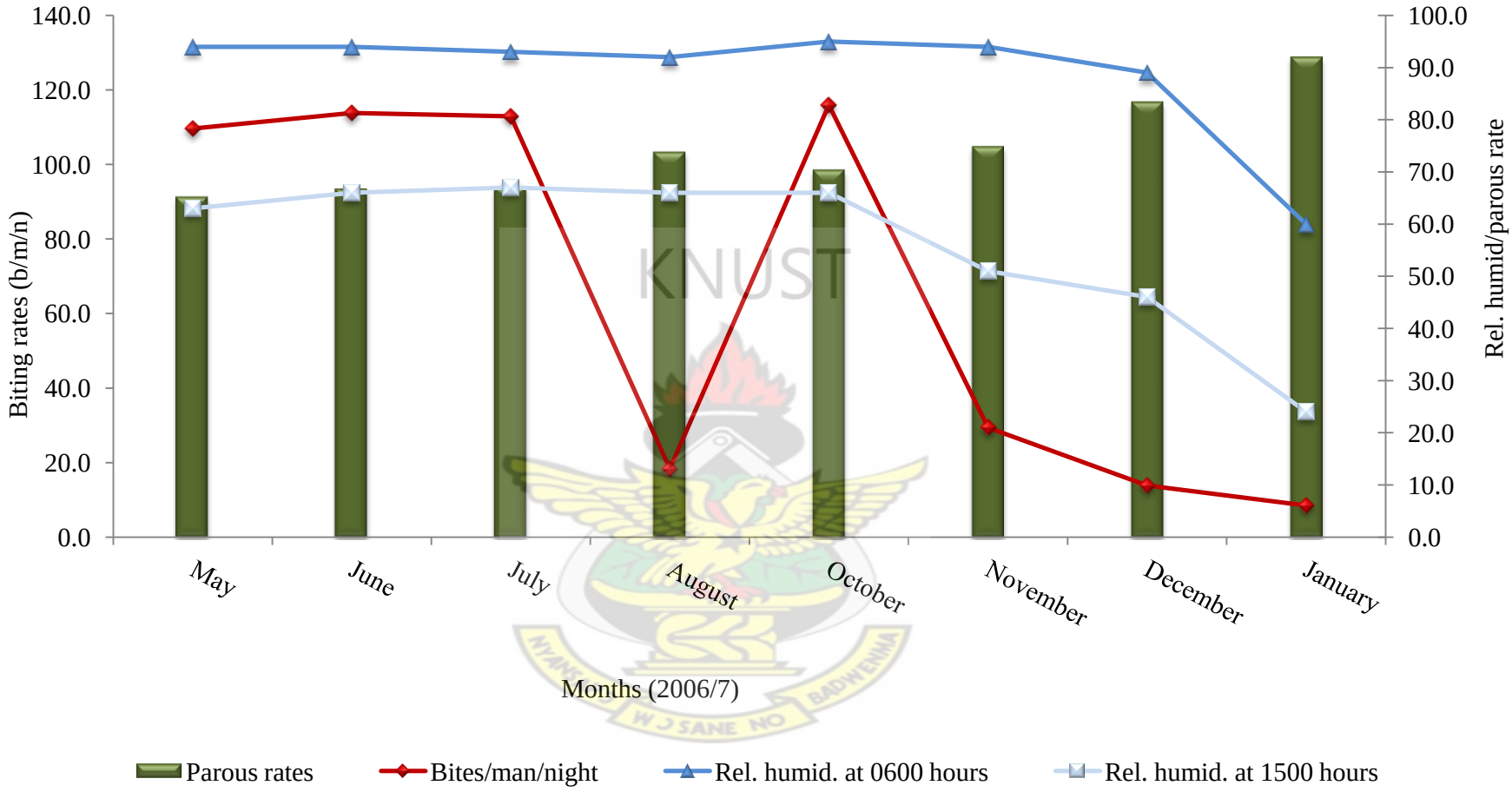


Figure 4.34 Monthly parous rates in relation to biting rates and mean daily relative humidity (%) at Sunyani.

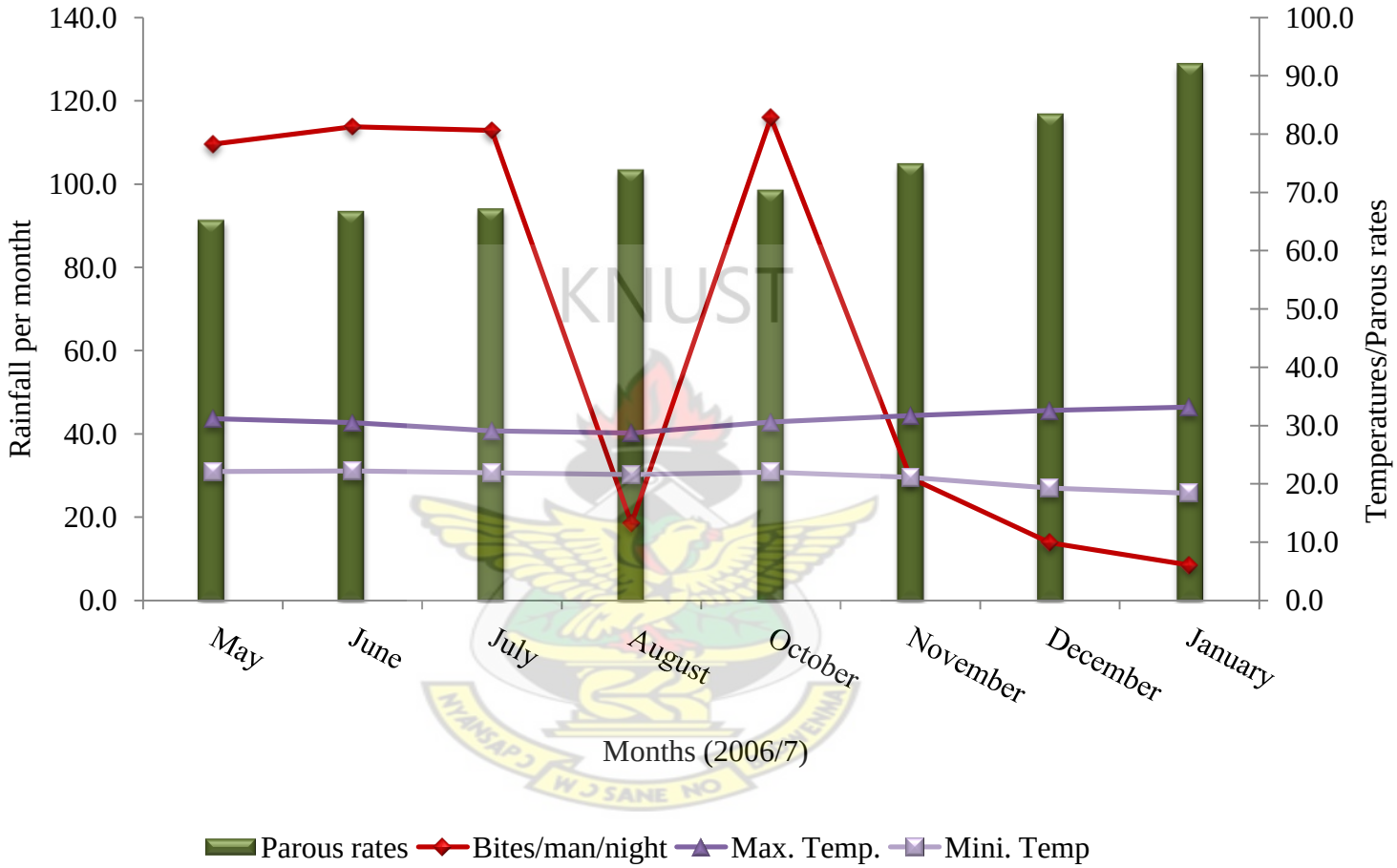


Figure 4.35 Monthly parous rates (%) in relation to biting rates, and daily minimum and maximum temperatures at Sunyani.

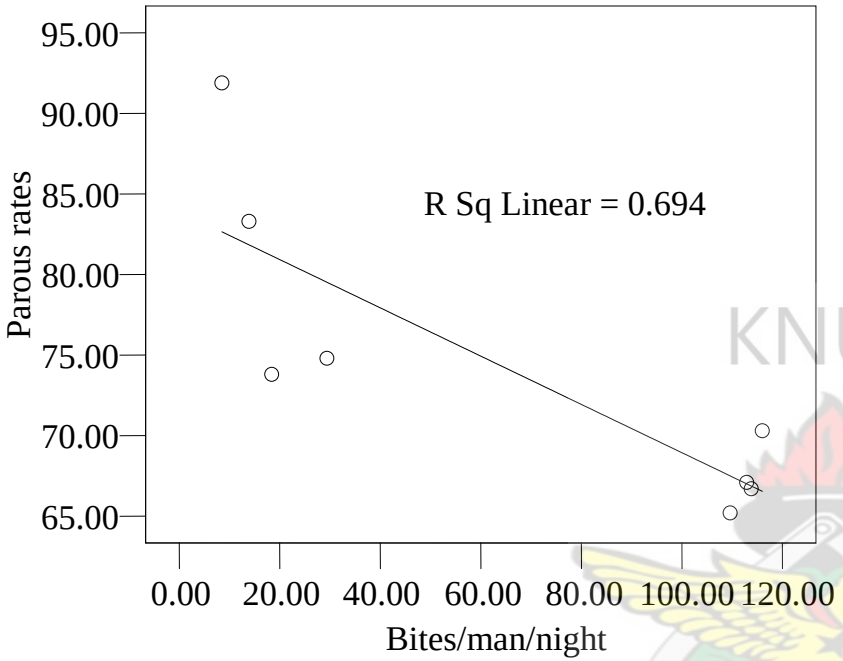


Figure 4.36 Correlation between monthly parous rates (%) and biting rates at Sunyani.

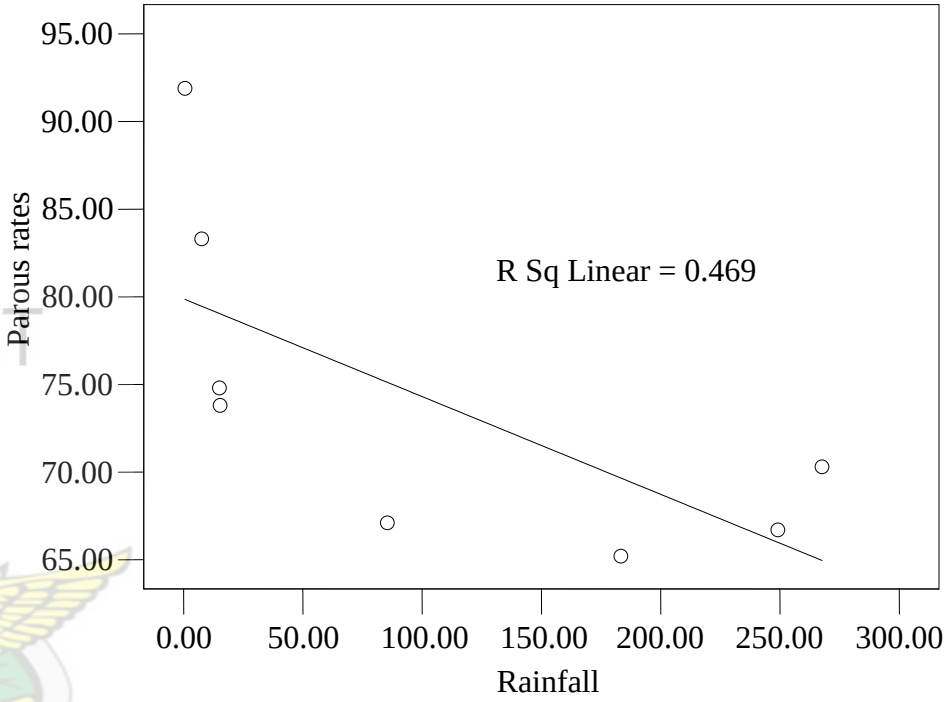


Figure 4.37 Correlation between monthly parous rates (%) and rainfall (mm) at Sunyani.

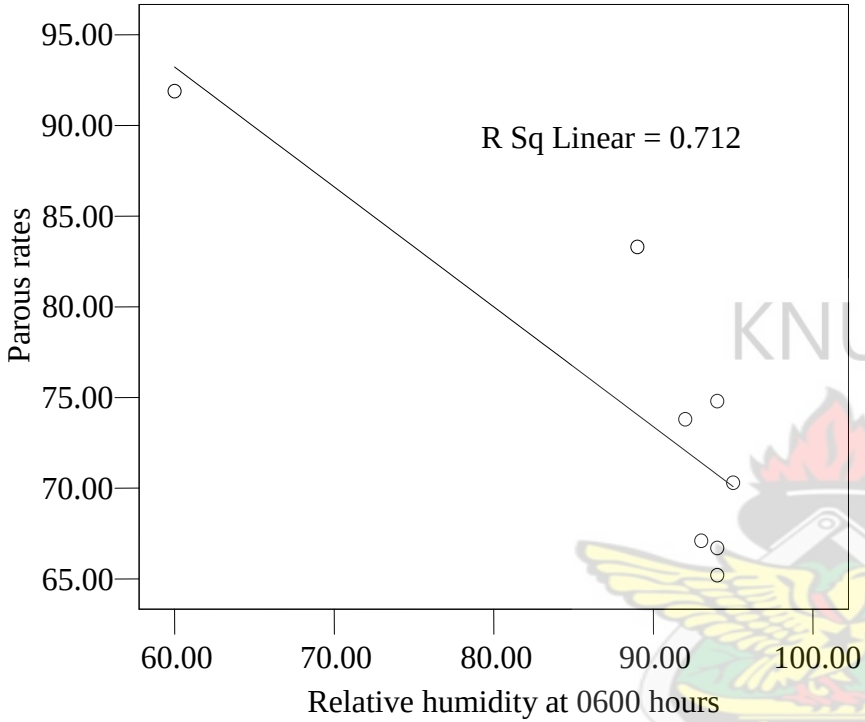


Figure 4.38 Correlation between monthly parous rates (%) and mean daily relative humidity (%) at 0600 hours at Sunyani.

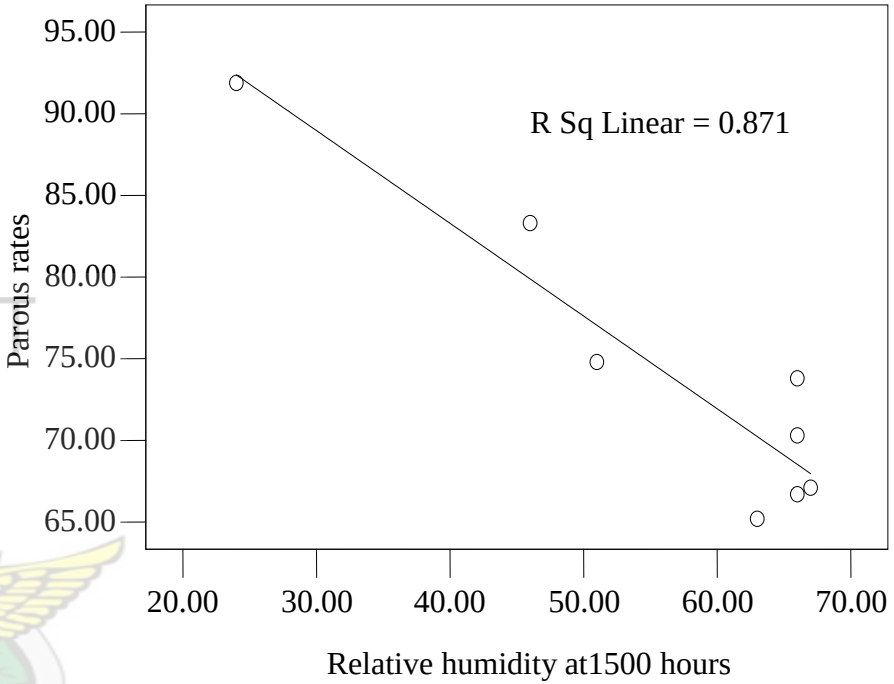


Figure 4.39 Correlation between monthly parous rates (%) and mean daily relative humidity (%) at 1500 hours at Sunyani.

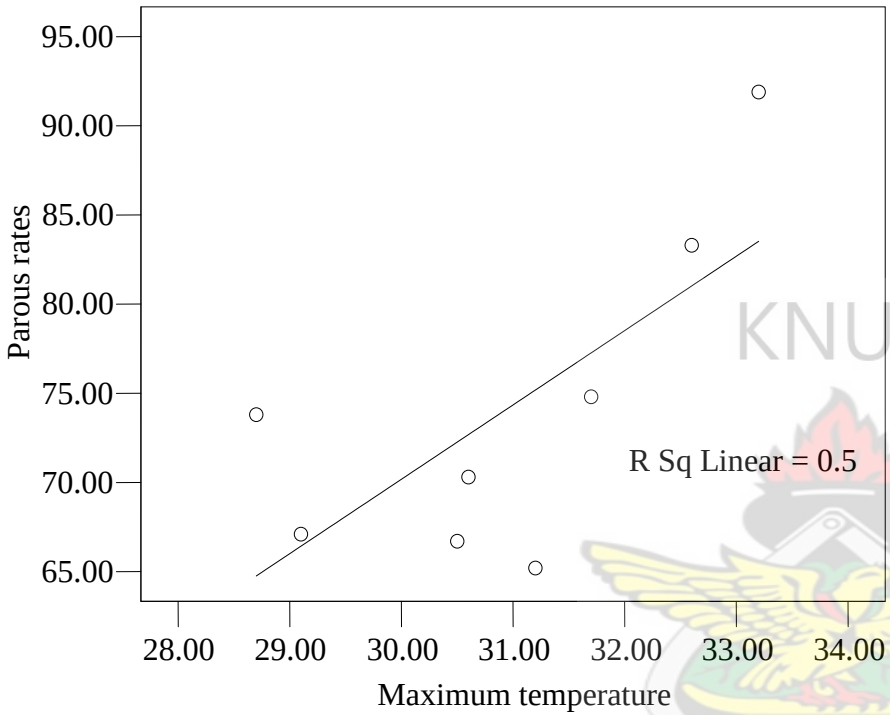


Figure 4.40 Correlation between monthly parous rates and mean daily maximum temperature (degree Celsius) at Sunyani.

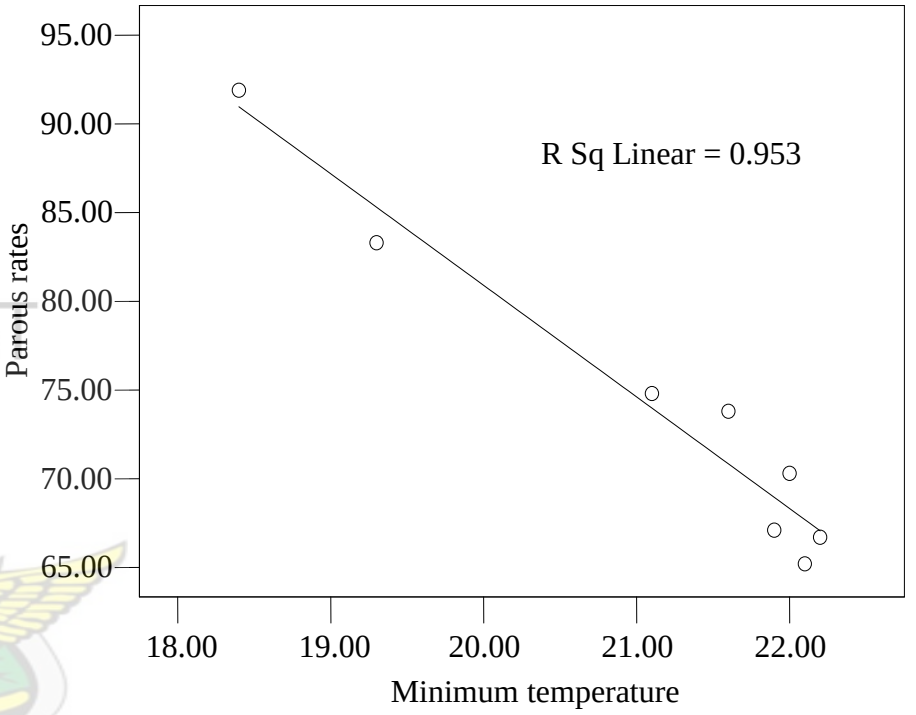


Figure 3.41 Correlation between monthly parous rates and mean daily minimum temperature (degree Celsius) at Sunyani.

4.2.7.1.2 Monthly parous rates of *Anopheles gambiae* s.s. at Chiraa

Number of *An. gambiae* dissected, number parous and parous rates (plus 95% CI) per month at Chiraa are given in Table 4.16. Correlation between parous rates and rainfall and biting rates are also given in Figures 4.42 to 4.44. In all sites there were high parous rates in the dry season than in the rainy season (χ^2 = 31.217, p = 0.0001). There was a weak negative correlation between the monthly parous rates and rainfall (rho = -0.524, p = 0.183) but a strong negative correlation between parous rates and biting rates (rho = -0.881, p = 0.004).

Table 4.16 Number dissected, number parous and parous rates of *Anopheles gambiae* s.l. at Chiraa.

Month	Number dissected	Number parous	% parous (95% CI)
May	186	119	63.98 (57.08 – 70.88)
June	190	137	72.11 (65.73 – 78.49)
July	178	113	63.48 (56.41 – 70.55)
August	74	60	81.08 (72.16 – 90.00)
October	176	135	76.70 (70.45 – 82.95)
November	118	89	75.42 (67.65 – 83.19)
December	36	32	88.89 (78.62 – 99.16)
January	50	44	88.00 (78.99 – 97.01)

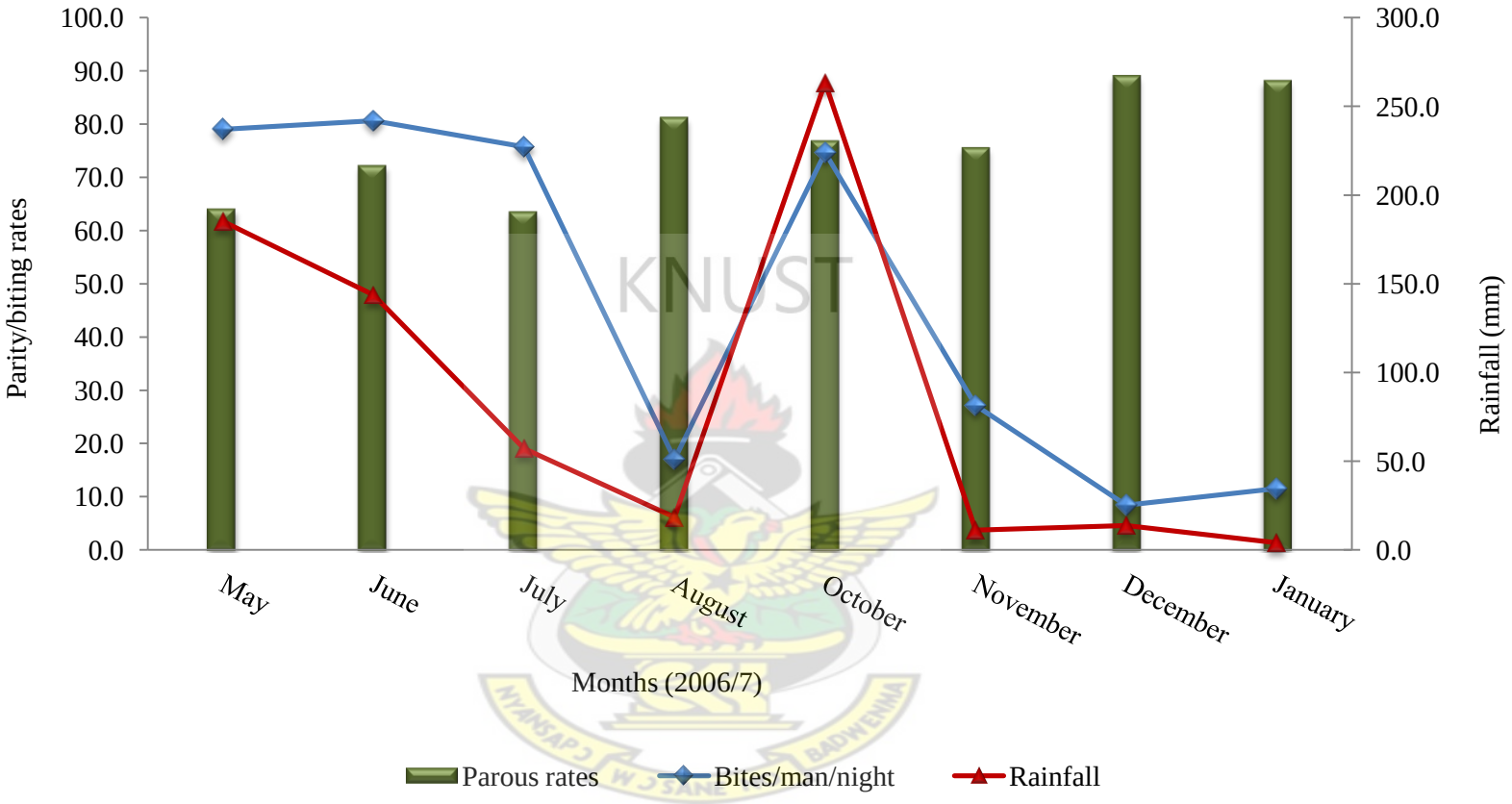


Figure 4.42 Monthly parous rates (%) of *An. gambiae* in relation to biting rates (b/m/n) and monthly rainfall at Chiraa.

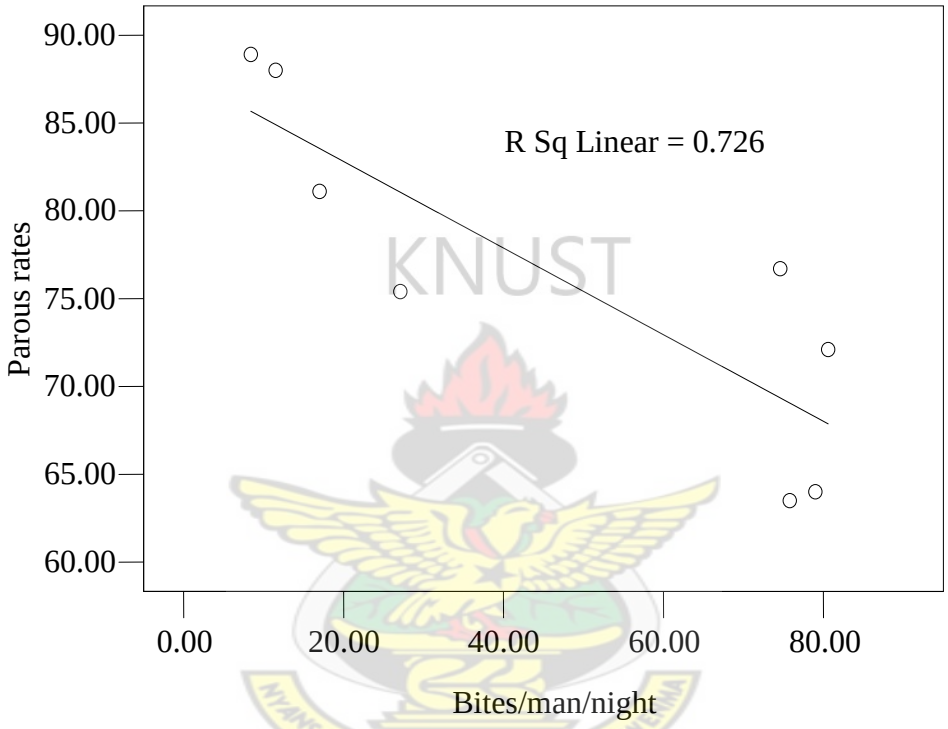


Figure 4.43 Correlation between monthly parous rates (%) and biting rate at Chiraa.

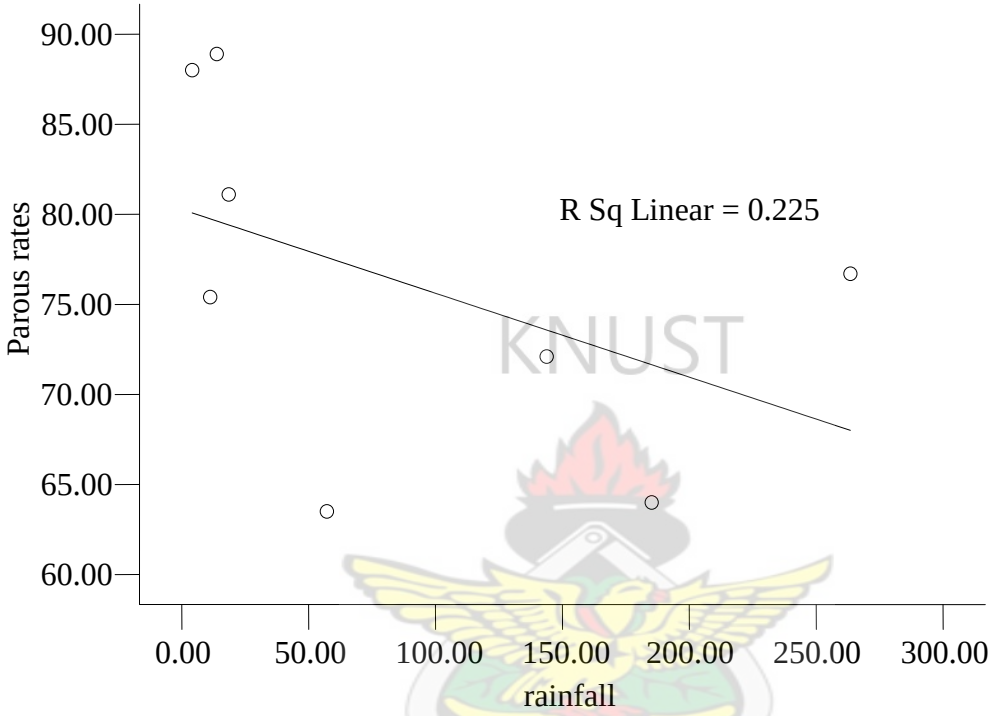


Figure 4.44 Correlation between monthly parous rates and monthly rainfall (mm) at Chiraa.

4.2.7.1.3 Monthly parous rates of *Anopheles gambiae* s.s. at Ahafo Kenyasi

Number dissected, number parous and parous rate (plus 95% CI) of *An. gambiae* per month at Ahafo Kenyasi are given in Table 4.17. Figure 4.45 shows the monthly parous rates in relation to biting rates while Figure 4.46 shows the correlation between parous rates and biting rates together with R^2 value. Higher parous rates were observed during the months of the dry season than in the rainy season. Specifically, there were significant differences in monthly parous rates with the lowest occurring in June and the highest in January ($\chi^2 = 21.208$, d.f. = 7, $p = 0.003$). However, there were no significant differences in parous rates between the months of the rainy season ($\chi^2 = 2.270$, d.f. = 3, $p = 0.518$) and dry season ($\chi^2 = 3.917$, d.f. = 3, $p = 0.271$). There was a strong negative linear relationship between monthly parous rates and bites per human per night (rho = -0.762, $p = 0.028$) over the study period (Fig. 4.46).

Table 4.17 Number dissected, number parous and parous rates of *Anopheles gambiae* s.l. at Ahafo Kenyasi.

Month	Number dissected	Number parous	% parous (95% CI)
May	279	188	67.38 (61.88 – 72.88)
June	274	175	63.87 (58.18 – 69.56)
July	284	192	67.61 (62.17 – 73.05)
August	86	69	80.23 (71.81 – 88.65)
October	291	203	69.76 (64.48 – 75.04)
November	147	110	74.83 (67.81 – 81.85)
December	53	43	81.13 (70.60 – 91.66)
January	30	27	89.31 (79.26 – 100.74)

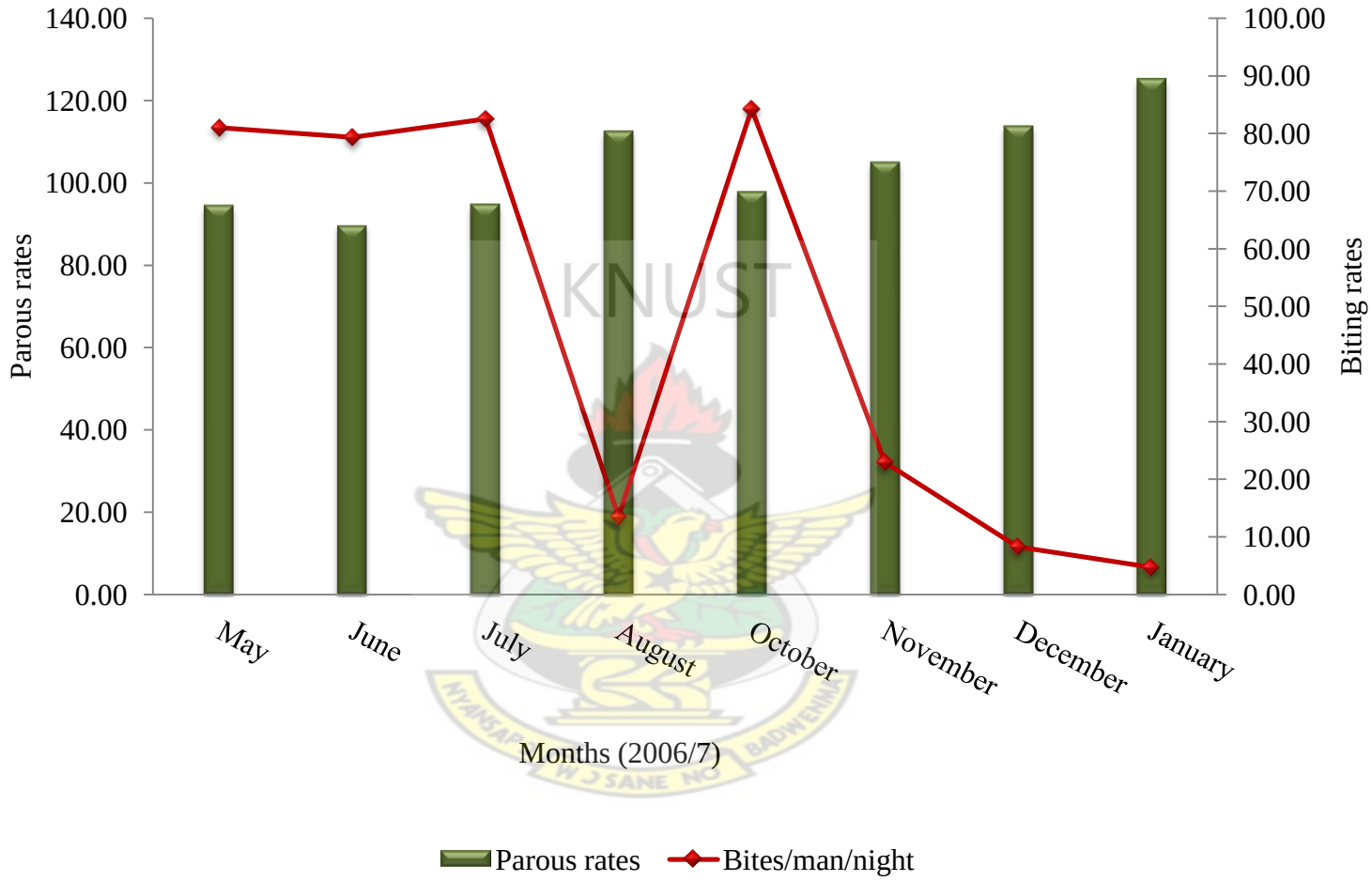


Figure 4.45 Monthly parous rates (%) of *An. gambiae* in relation to biting rates (b/m/n) at Ahafo Kenyasi

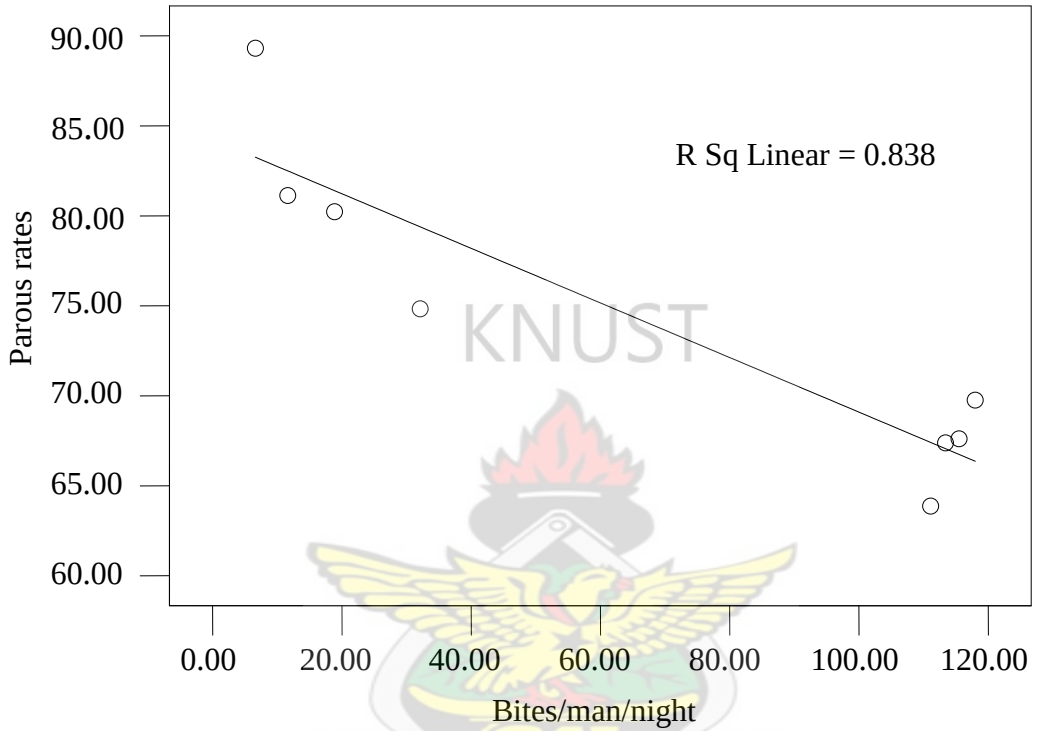


Figure 4.46 Correlation between monthly parous rates (%) and biting rates at Ahafo Kenyasi

4.2.7.1.4 Monthly parous rates of *Anopheles gambiae* s.s. at Hwidiem

Table 4.18 shows the number of *A. gambiae* dissected, the number parous and parous rate (plus 95% CI) per month at Hwidiem. Monthly parous rates in relation to rainfall and biting rates are given in Figure 4.47. Figures 4.48 and 4.49 also show the correlation between parous rates and biting rates as well as rainfall. Higher parous rates were observed during the months of the dry season than in the rainy season. Specifically, there were significant differences in monthly parous rates with the lowest occurring in May and the highest in January (χ^2 = 54.014, d.f. = 7, $p < 0.001$). However, there were no significant diferences in parous rates between the months of the rainy season (χ^2 = 4.276, d.f. = 3, p = 0.233) and the months of the dry season (χ^2 = 5.709, d.f. = 3, p = 0.127). There was a negative linear relationship between the monthly parous rates and mean monthly rainfall (rho = -0.857, p = 0.007) and between parous rates and biting rates (rho = -0.786, p = 0.021) over the study period.

Table 4.18 Numbers dissected, parous and parous rates of *An. gambiae* at Hwidiem.

Month	Number dissected	Number parous	% Parous (95% CI)
May	192	115	59.90 (52.97 – 66.83)
June	203	129	63.55 (56.93 – 70.17)
July	202	137	67.82 (61.38 – 74.26)
August	82	69	84.15 (76.25 – 92.05)
October	204	140	68.63 (62.26 – 75.00)
November	153	122	79.74 (73.37 – 86.11)
December	75	67	89.33 (82.34 – 96.32)
January	55	50	90.91 (83.31 – 98.51)

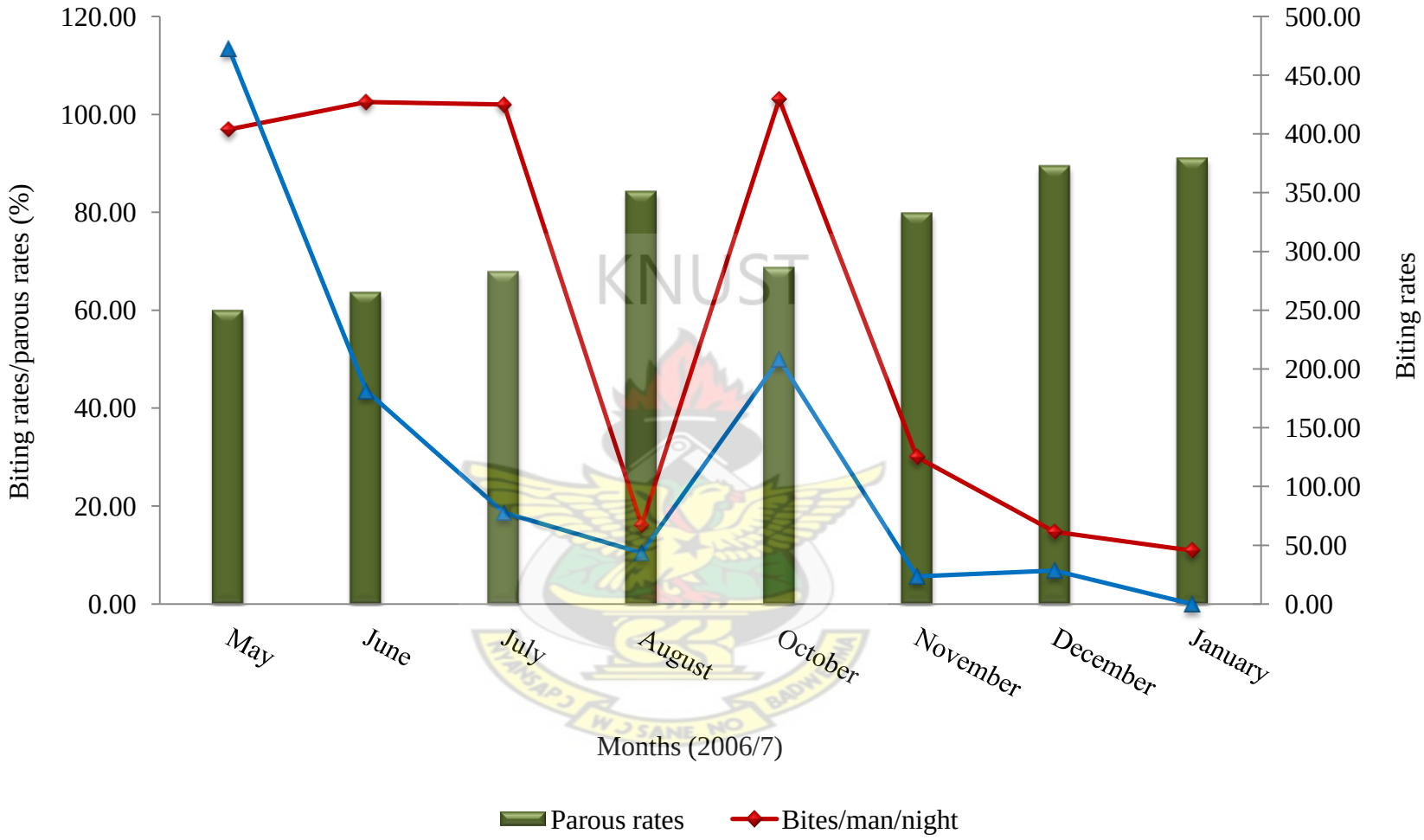


Figure 4.47 Monthly parous rates of *An. gambiae* in relation to biting rates and monthly rainfall (mm) at Hwidiem.

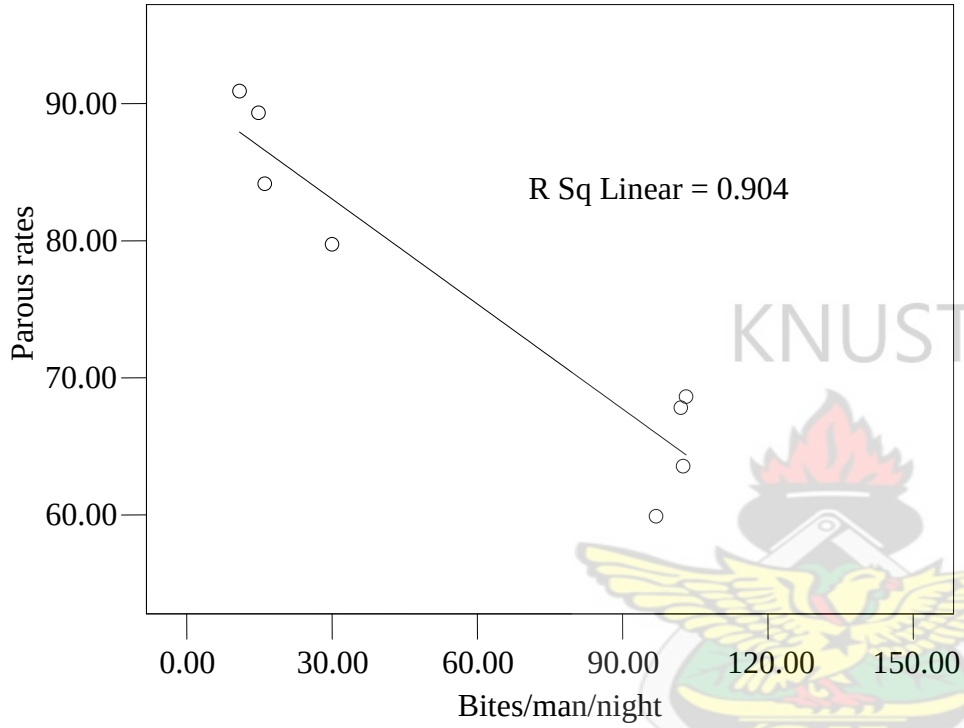


Figure 4.48 Correlation between monthly parous rates (%) and biting rates at Hwidiem.

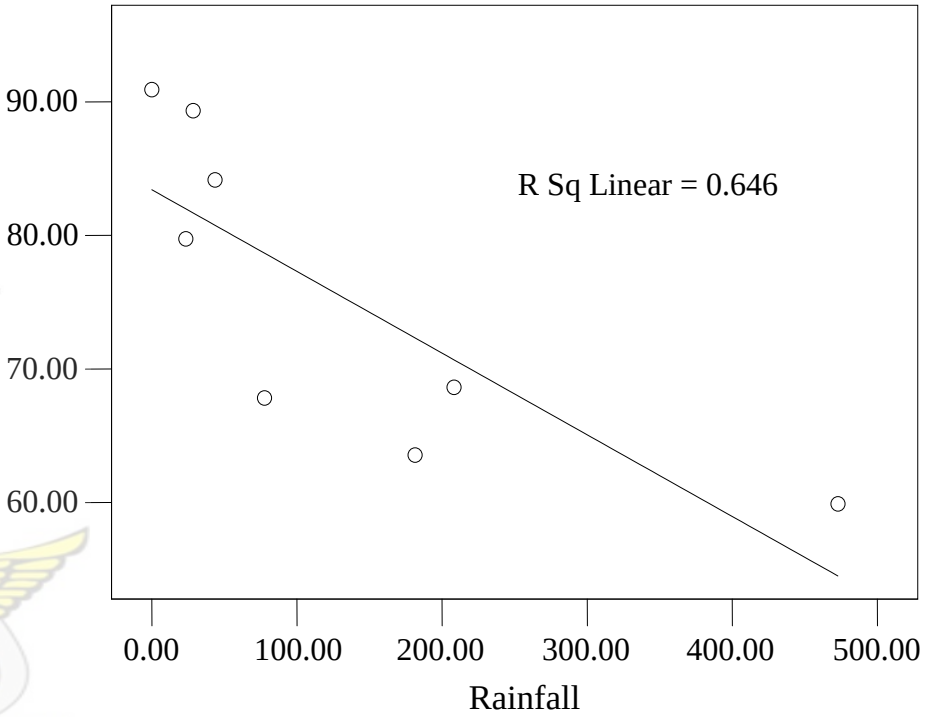


Figure 4.49 Correlation between monthly parous rates (%) and rainfall in mm at Hwidiem.

4.2.7.2 Parous rate, daily survival and life expectancy of An. gambiae s.s.

Parous rates (plus 95% CIs), daily survival rates and life expectancy during each season in each site during the study period are given in Tables 4.19 and 4.20. Approximately 66.67, 67.20, 65.25, 69.04% of female *An. gambiae* dissected during the rainy season at Sunyani, Ahafo Kenyasi, Hwidiem and Chiraa, respectively, were parous (Table 4.19). Parous rate was highest in Chiraa and lowest in Hwidiem but the differences between study sites were not significant (χ^2 = 2.562, d.f. = 3, p = 0.464). Probability of daily survival of females ranged from 0.87 at Sunyani and Hwidiem to 0.88 at Chiraa and Ahafo Kenyasi. Life expectancy in days also ranged from approximately 7 in Sunyani and Hwidiem to 8 in Ahafo Kenyasi and Chiraa.

The number dissected, parous, parous rates with 95% CI, daily survival rates and life expectancy of *An. gambiae* in the dry season are also shown in Table 4.20. About 78.29, 78.79, 84.15 and 80.94% of samples dissected from Sunyani, Ahafo Kenyasi, Hwidiem and Chiraa respectively were parous. Again, there were no significant differences in parous rates between sites (χ^2 = 4.680, d.f. = 3, p = 0.197). Probability of surviving a day ranged from was 0.92 at Sunyani and Ahafo Kenyasi, 0.93 at Chiraa and 0.94 at Hwidiem. Expected life expectancies (in days) were about 12, 13, 14 and 17 at Sunyani, Ahafo Kenyasi, Chiraa and Hwidiem respectively.

Table 4.19 Number dissected (N^d), number parous (N^p), parous rates, probability of daily survival (*p*) and life expectancy (*L*) of *An. gambiae* species during the rainy season in each study site.

Site	N^d	N^p	% Parous 95% CI	*p*	*L*
Sunyani	1182	788	66.67 (62.87-70.47)	0.87	7.40
Kenyasi	1128	758	67.20 (63.33-71.07)	0.88	7.55
Hwidiem	800	522	65.25 (60.58-69.92)	0.87	7.03
Chiraa	730	504	69.04 (64.30-73.78)	0.88	8.10

Table 4.20 Number dissected (N^d), number parous (N^p), parous rates, probability of daily survival (*p*) and life expectancy (*L*) of *An. gambiae* species during the dry season in each study site.

Site	N^d	N^p	% Parous 95% CI	*p*	*L*
Sunyani	304	238	78.29 (73.66-82.92)	0.92	12.3
Kenyasi	316	249	78.79 (74.29-83.31)	0.92	12.6
Hwidiem	366	308	84.15(80.41-87.89)	0.94	17.4
Chiraa	278	225	80.94 (76.32-85.56)	0.93	14.2

4.2.8 Plasmodium falciparum sporozoite rates of An. gambiae s.s.

On the whole, 720 *An. gambiae* mosquitoes were tested for the presence of *Plasmodium falciparum* circumsporozoite protein (PfCSP) using the qualitative ELISA technique. Of the total number of mosquitoes tested, 330 and 390 were collected in the rainy and the dry seasons respectively. An example of the PfCSP ELISA plate results obtained in the present research work is as shown in Figure 4.50.

Figure 4.50 ELISA plate results. Wells F11 and F12 are positive controls, G11 and G12 are negative controls, H11 and H12 are blank, B8 and G2 are wild positive samples and all other wells contained wild samples that were negative for PfCSP.

Number of *An. gambiae* tested, number of PfCSP positives and sporozoite rates at each site in the rainy and the dry seasons are given in Tables 4.21 to 4.22. During the rainy season, sporozoite rates ranged from 1.89% in Hwidiem to 4.05% in Chiraa but did not differ significantly between study sites (χ^2 = 0.771, d.f. = 3, p = 0.856). Also, during dry season, sporozoite rates ranged from 1.75 to 3.33% and did not differ significantly between study sites (χ^2 = 0.628, d.f. = 3, p = 0.890). Generally, there were no seasonal variations in sporozoite rates during the study period (p > 0.05). Of the 720 *An. gambiae* mosquitoes tested for PfCSP, 20 were found positive for PfCSP giving rise to an overall sporozoite rate of 2.78% (95% CI: 1.58 – 3.98).

Table 4.21 Number of *An.gambiae* tested, number of positives and sporozoite rates plus 95% CIs in each study site during the rainy season.

Locality	No. Tested	No. Positive	Sporozoite rate (%) (95% CI)
Sunyani	134	4	2.99 (0.11 – 5.87)
Kenyasi	76	2	2.63 (-0.97 – 6.23)
Hwidiem	106	2	1.89 (-0.70 – 4.48)
Chiraa	74	3	4.05 (-0.44 - 8.54)

Table 4.22 Number of *An. gambiae* tested, number of positives and sporozoite rates (%) plus 95% CIs in each study site in the dry season.

Locality	No. tested	No. Positive	Sporozoites rate
Sunyani	114	2	1.75 (-0.66 – 4.16)
Kenyasi	64	2	3.13 (-1.14 - 7.40)
Hwidiem	90	3	3.33 (-0.38 - 7.04)
Chiraa	62	2	3.23 (-1.17 - 7.63)

4.2.9 Entomological inoculation rates and the risk of malaria transmission.

Entomological inoculation rate (EIR) and the estimated EIR (estEIR) of *An. gambiae* at each site in the rainy season are shown in Table 4.23. The estEIR was obtained by multiplying the EIR by 30 (average number of days in a month). In the rainy season, infective bites per man per night (ib/m/n) ranged from 1.909 at Hwidiem to ib/m/n of 3.375 at Sunyani. There was an average of 2.864 ib/m/n during the rainy season. The estimated entomological inoculation rates (estEIRs) per month in the rainy season ranged from approximately 57.24 infective bites per man per month (ib/m/m) for Hwidiem to 101.25 ib/m/n for Sunyani. The average estEIR per month was 85.92 during the rainy season and the risk of an inhabitant receiving infection for *P. falciparum* of *An. gambiae* was 100% at each site in the rainy season.

Entomological inoculation rate and estEIR of *An. gambiae* at each study site in the dry season are given in Table 4.24. In the dry season, ib/m/n ranged from 0.306 at Sunyani to 0.596 at Hwidiem. On the whole, there was an average ib/m/n of 0.469 at all study sites in the dry season. The estimated infective bites per person per month ranged from 9.18 at Sunyani to 17.88 at Hwidiem. The overall average estEIR per man per month in the dry season at all the study sites was aproximately 14.10. The risk of an inhabitant receiving infection with *P. falciparum* from *An. gambiae* was approximately 100% at each study site in the dry season.

It is worth noting that although there were no significant differences in sporozoite rates between rainy and dry seasons, the EIR was generally higher during the rainy season than in the dry season. For instance, the highest ib/m/n, 3.375 and the lowest, 0.306 were both recorded at Sunyani during the rainy and the dry seasons respectively. As a result, an inhabitant of Sunyani may receive about three (3) infective bites every other night in the rainy season but about once every three nights

in the dry season. Generally, average infective bites per man per night of 1.649 and estimated infective bites per man per month of 49.47were recorded in this study.

Table 4.23 Human biting rates (ma), sporozoite rates (s), EIR, estEIR per month and the risk of being infected by *P. falciparum* through the bite of *An. gambiae* s.s. in each community during rainy season.

Site	ma	s	EIR	estEIR	Risk
Sunyani	113.1	0.0299	3.375	101.25	1.0000
Ahafo Kenyasi	114.5	0.0263	3.013	90.39	1.0000
Hwidiem	101.1	0.0189	1.908	57.24	1.0000
Chiraa	77.5	0.0405	3.142	94.26	1.0000

Table 4.24 Human biting rate (ma), sporozoite rates (s), EIR, estEIR per month and the risk of being infected by *P. falciparum* through the bite of *An. gambiae* s.s. in each community during dry season.

Site	ma	s	EIR	estEIR	Risk
Sunyani	17.5	0.0175	0.306	9.18	0.9999
Ahafo Kenyasi	17.3	0.0313	0.541	16.23	0.9999*
Hwidiem	17.9	0.0333	0.596	17.88	0.9999*
Chiraa	16.0	0.0323	0.517	15.51	0.9999*

4.3 Inscticide Susceptibility/Resistance Status of *Anophele gambiae* s.s species

A total of 4211 adult mosquitoes including 3223 anophelines and 988 culicines were raised in the insectary. The *Anopheles* species collected comprised 1819 females and 1404 males. About 946 of the females reared were collected from Sunyani while the remaning 873 were collected from Ahafo Kenyasi. Of the 946 females from Sunyani, 200 (21.1%) were selected at random and tested for their susceptibility/resistance to 0.05% deltamethrin and 0.75% permethrin. Of the 873 female samples raised from Ahafo Kenyasi, 300 (34.4%) were selected at random and tested for their susceptibility/resistance to 0.05% deltamethrin and 0.75% permethrin. Some mosquitoes were also exposed to 1.5% permethrin due to the low knockdowns and mortalities observed with the 0.75% permethrin. All the female *Anopheles* mosquitoes reared, including the ones used for the pyrethroid susceptibility/resistance tests were frozen at -5˚C and identified morphologically as *An. gambiae* s.l. The rates of knockdown (%) of the wild *Anopheles* mosquitoes from Sunyani and Ahafo Kenyasi and the standard susceptible controls from Kisumu (Kenya) recorded after 5, 10, 15, 20, 30, 40, 50 ,60 and 80th minutes of exposure time to the diagnostic doses of each insecticide used. The results are for mean knockdown (KD) across all batches of samples tested for each site. Knockdown rates (KDRs) of 49% (95% CI: 39.2 – 58.8) and 78% (95% CI: 69.88 – 86.12) were observed for samples from Sunyani and Ahafo Kenyasi respectively, exposed to 0.05% deltamethrin after 60 minutes (Fig. 4.52). About 24% (95% CI: 15.63 – 32.37) knockdown was observed for the mosquitoes from both sites exposed to 0.75% permethrin for 60 minutes (Fig. 4.53). Also, 60% (95%: 50.4 – 69.6) of the samples from Ahafo Kenyasi exposed to 1.5% permethrin were knocked down after 60 minutes (Fig. 4.54). Since knockdowns observed for the wild mosquitoes after 60 minutes of exposure were < 80%, the exposure time was

extended to 80 minutes. After the 80th minute, no significant differences in KDs were observed for all the insecticides tested at all sites ($p > 0.05$).

For the Kisumu samples, KDR after 60 minutes was 100% for 0.05% deltamethrin and 98% (95% CI: 95.26 – 100.74%) for both 0.75% permethrin and 1.5% permethrin. After 80 minutes, there was 100% KD for 1.5% permethrin but KDR for 0.75% permethrin increased to 99% (95% CI: 97.05 – 100.95).

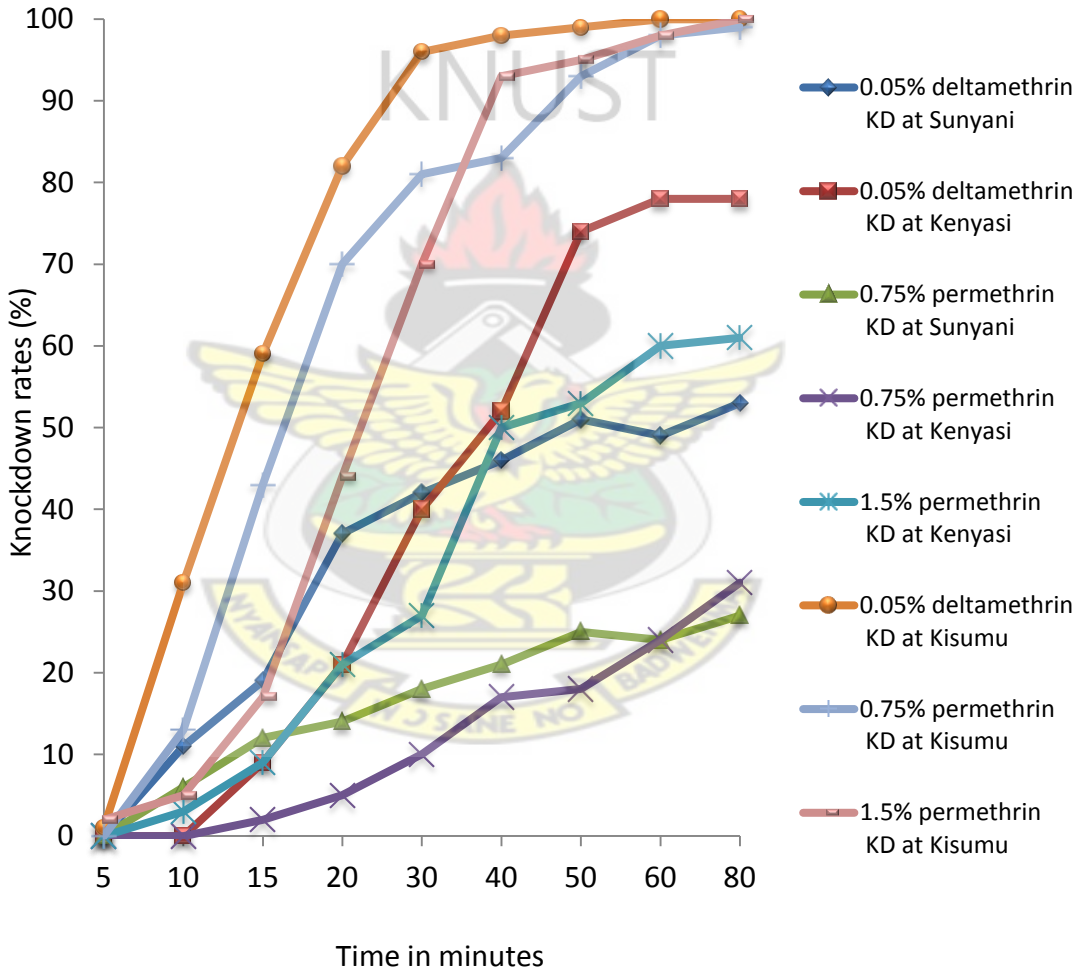


Figure 4.51 Pyrethroid knockdown (KD) rates (%) of wild *An. gambiae* from Sunyani and Ahafo Kenyasi and susceptible *An. gambiae* from Kisumu.

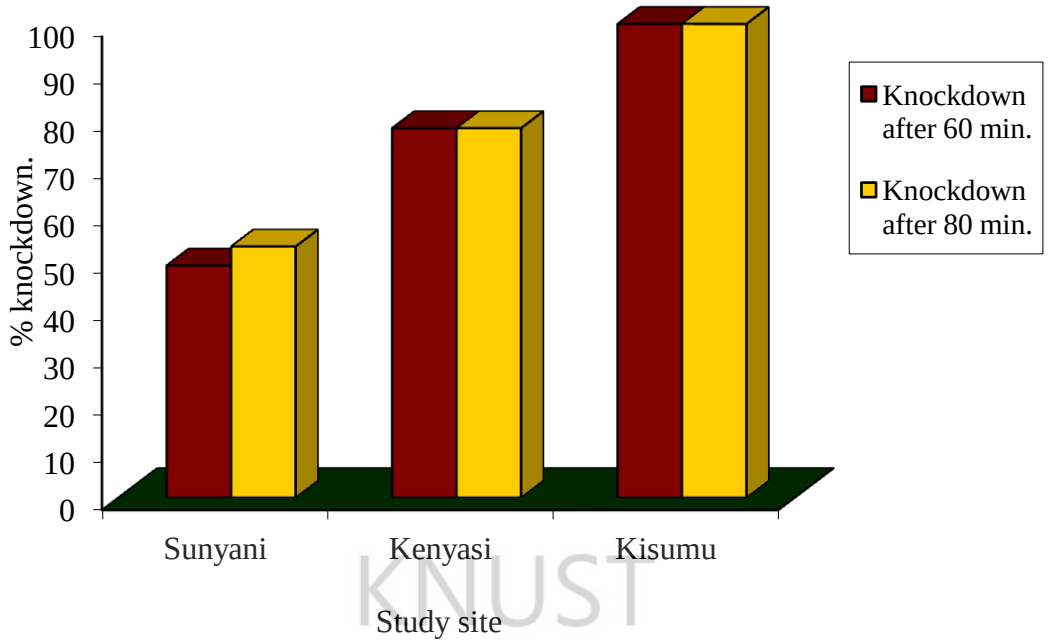


Figure 4.52 Percentage (%) knockdown of *An. gambiae* in each study site after the 60th and 80th minutes of exposure to 0.05% deltamethrin insecticide.

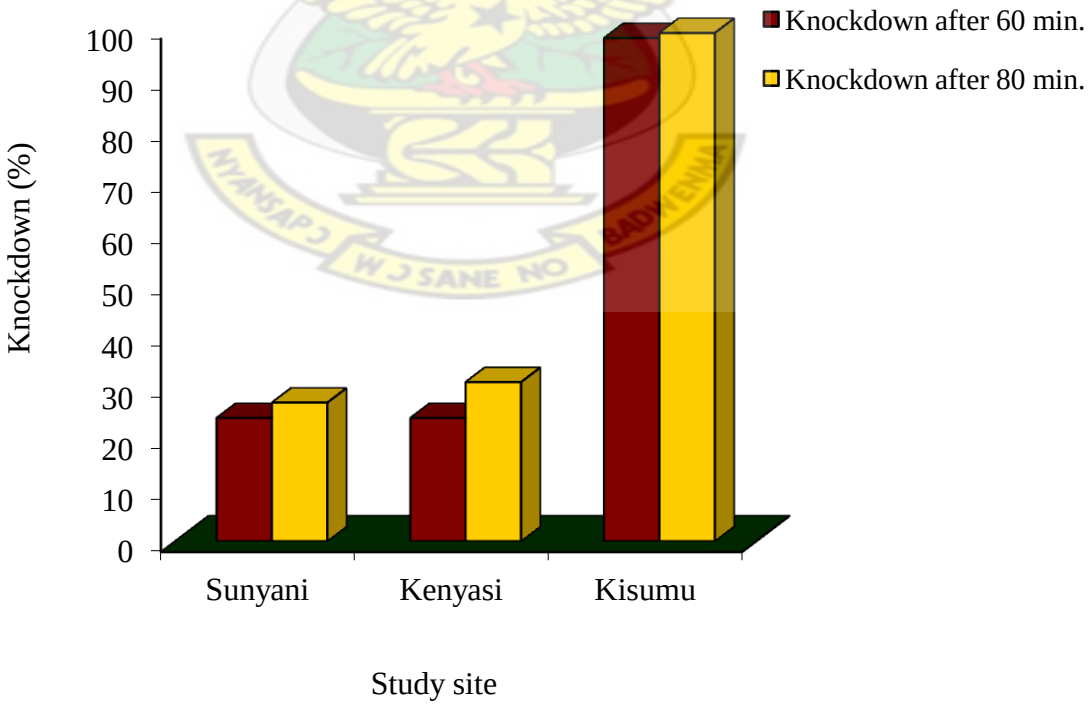


Figure 4.53 Percentage (%) knockdown of *An. gambiae* s.l in each study site after the 60th and 80th minutes of exposure to 0.75% permethrin insecticide.

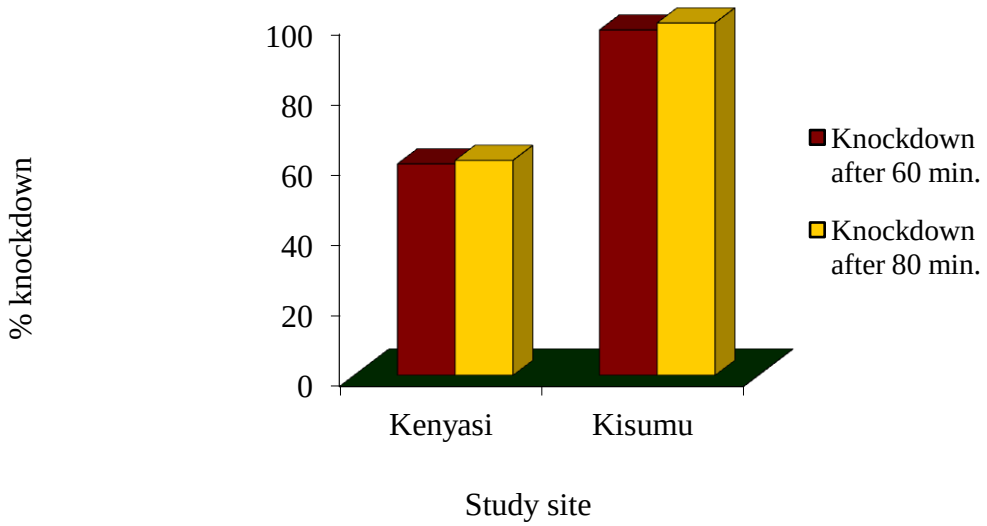


Figure 4.54 Percentage (%) knockdown of *An. gambiae* s.l in each study site after the 60th and 80th minutes of exposure to 1.5% permethrin insecticide.

The knockdown effects of the diagnostic concentration of permethrin and deltamethrin insecticides tested on *An. gambiae* after 60 minutes differed at each 'tests' sites (χ^2 = 87.997, p < 0.001). The 0.05% deltamethrin insecticide knocked down *An. gambiae* much more than the 0.75% permethrin at both Sunyani (χ^2 = 13.483, d.f. = 1, p < 0.001) and Ahafo Kenyasi (χ^2 = 58.343, d.f. = 1, p < 0.001). Also, the 0.75% permethrin insecticide had a much lower knock down ability on *An. gambiae* compared to the 1.50% permethrin insecticide at Ahafo Kenyasi (χ^2 = 26.601, d.f. = 1, p < 0.001). The 1.50% permethrin in turn had a much lower ability to knock down *An. gambiae* than the 0.05% deltamethrin at Ahafo Kenyasi (χ^2 = 7.574, d.f. = 1, p = 0.006). For the Kisumu control samples, the rates of knockdown after 60 minutes ranged from 98 – 100% and therefore did not differ significantly between the various diagnostic concentrations of the insecticides tested (p > 0.05).

The knockdown effects of the diagnostic concentration of insecticides tested on *An. gambiae* after 60 minutes of exposure generally differed between sites. The 0.05% deltamethrin knocked down *An. gambiae* at a much higher rate in Ahafo

Kenyasi than in Sunyani (χ^2 = 18.143, d.f. = 1, p = 0.00002). However, the 0.75% deltamethrin insecticide knocked down *An. gambiae* from Ahafo Kenyasi and Sunyani at equal rates (χ^2 = 0.000, d.f. = 1, p = 1.000). Also, the 0.05% deltamethrin insecticide had much higher knockdown effects on the standard susceptible *An. gambiae* from Kisumu than those from Sunyani and Ahafo Kenyasi ($p < 0.001$).

4.3.1 Knockdown Times of An. gambiae s.s. after 60 minutes of exposure

The estimated times at which 50% and 95% of the *An. gambiae* exposed to the various insecticide diagnostic concentrations were knocked down [with 95% CIs, and chi-square values (χ^2)] and resistance ratios (RRs) are given in Table 4.25. The linear log-time probit model used to estimate KDT_{50} and KDT_{95} did not fit the distribution of percentage KD with time for the 0.05% deltamethrin and 0.075% permethrin assays for Sunyani and Kisumu mosquitoes respectively (p - values for χ^2 tests of heterogeneity were < 0.05 in each of these cases). The KDT_{50} and KDT_{95} estimates in these cases were excluded in the comparisons as these would be unreliable although they were given in the Tables 4.25. The KDT_{50} and KDT_{95} estimated for the Ahafo Kenyasi 0.05% deltamethrin assay were 7.18 and 78.41 times respectively that of the Kisumu 0.05% deltamethrin assay. Also, the KDT_{50} and KDT_{95} for the Ahafo Kenyasi 1.5% permethrin assay were 8.86 and 20.91 times respectively that of the Kisumu 1.5% permethrin assay. It should also be noted that regardless of the KDT_{50} and KDT_{95} values, it was still obvious that deltamethrin and permethrin susceptibility tests of *An. gambiae* from Sunyani and Ahafo Kenyasi showed they were not knocked down as quickly as the standard laboratory reared Kisumu *An. gambiae* strain within the 60 minutes of exposure to the discriminating concentration of 0.05% deltamethrin, 0.75% permethrin or 1.5% permethrin, signifying *kdr*-type resistance.

Table 4.25 Knockdown times in minutes [with 95% confidence intervals and chi-square values (χ^2)] and knockdown resistance ratios of the KDT (RR_{KDT}) values of *An. gambiae* exposed to 0.05% deltamethrin 0.05%, permethrin 0.75% and permethrin 1.5% insecticides.

	Locality	N	KDT_{50}	95% C.I.	RR_{KDT50}	KDT_{95}	95% C.I.	RR_{KDT95}	χ^2	*P*
Deltamethrin 0.05%	Sunyani	100	45.35	(35.27 – 67.32)	1.90	364.66	(179.74 – 1568.04)	8.46	14.335	0.026
	Kenyasi	100	171.52	(109.50 – 385.20)	7.18	3378.48	(1101.03 – 27272.60)	78.41	5.192	0.519
	Kisumu	100	23.89	(22.70 – 25.12)	-	43.09	(39.96 – 47.23)	-	6.542	0.374
Permethrin 0.75%	Sunyani	100	35.81	(33.46 – 38.44)	1.63	98.97	(85.42 – 119.58)	1.98	5.078	0.534
	Kenyasi	100	45.49	(41.26 – 51.07	2.08	183.13	(141.92 – 259.26)	3.66	4.728	0.579
	Kisumu	100	21.91	(18.45 – 25.64)	-	50.00	(40.16 – 71.67)	-	25.635	0.000
Permethrin 1.5%	Kenyasi	100	117.00	(88.72 – 187.96)	8.86	619.06	(330.13 – 1991.64)	20.91	2.128	0.908
	Kisumu	100	13.21	(12.29 – 14.12)	-	29.61	(26.85 – 33.39)	-	3.518	0.742

χ^2 values are for the test of fit of the log-time probit model used to estimate the KDT_{50} and KDT_{95} values: deviation was not significant for all data, $p > 0.05$ except for data for deltamethrin 0.05% against Sunyani mosquitoes and 0.75% against Kisumu Strain, $p < 0.05^*$. n is the number of mosquitoes tested.

4.3.2 Mortality rates of An. gambiae s.s. after 24 hours of recovery period

Figures 4.54 to 4.56 show mortality rates 24 h post-exposure to the various diagnostic concentrations of insecticides. High resistance rates to 0.05% deltamethrin, 0.75% permethrin and 1.5% permethrin were detected in the samples from Ahafo Kenyasi and Sunyani. Mortality rates of 59% (95% CI 49.36 – 68.64) and 45% (35.25 – 54.75) were induced by 0.05% deltamethrin at Sunyani and Ahafo Kenyasi respectively. For 0.75% permethrin, 35% (95% CI: 25.65 – 44.35) and 27% (95% CI: 18.3 – 35.7) mortalities were recorded at Sunyani and Ahafo Kenyasi respectively. Lastly, 46% (95% CI: 36.23 – 55.77) mortality was observed for the Ahafo Kenyasi samples tested with 1.5% permethrin. Mortality for the controls was 100% for both 0.05% deltametrin and 1.5% permethrin but that of 0.75% permethrin was 99% (95% CI: 97.05 – 100.95).

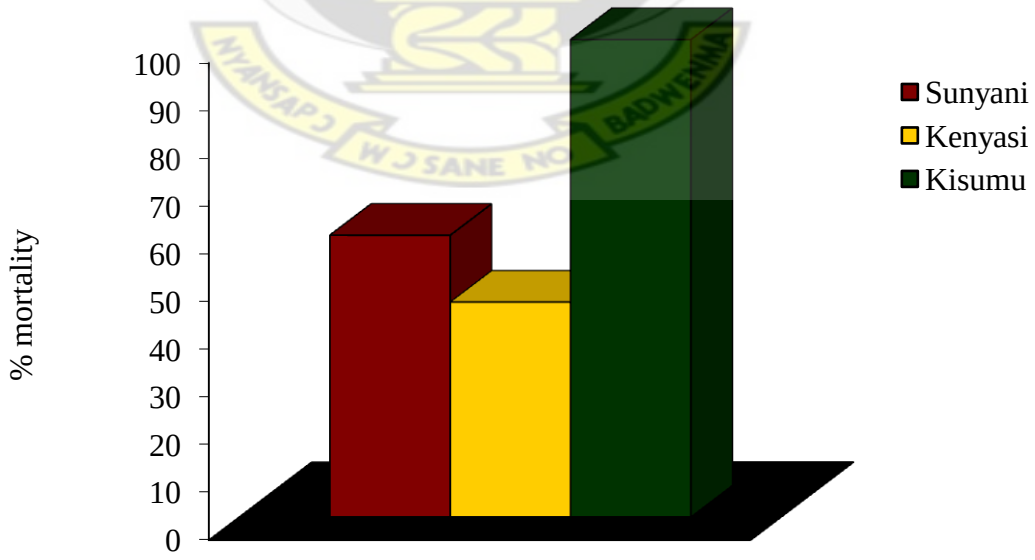


Figure 4.55 Percentage (%) mortality of *An. gambiae* s.l in each study site 24 hours after exposure to 0.05% deltamethrin insecticide.

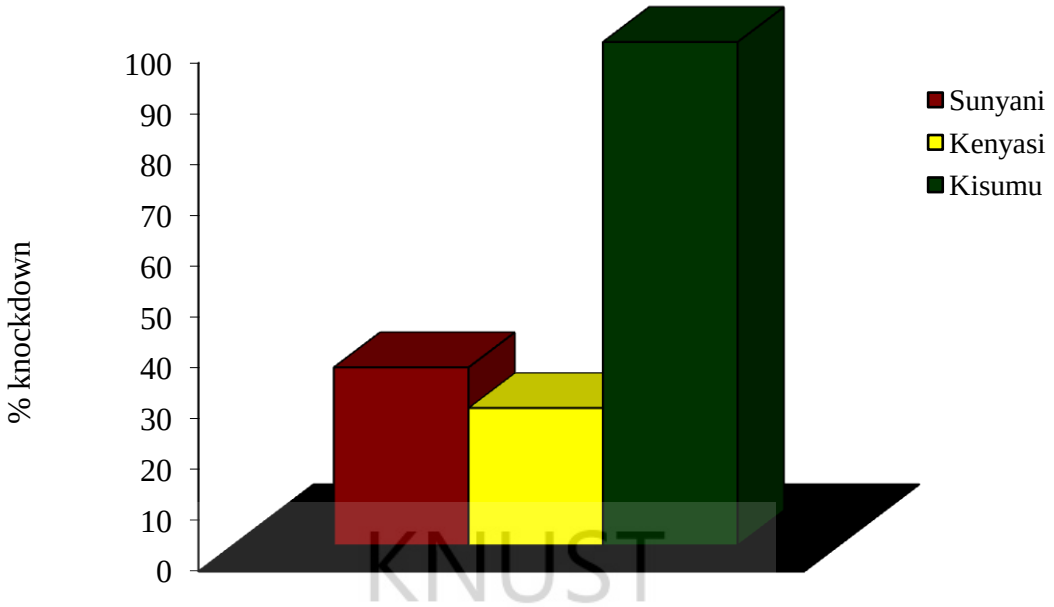


Figure 4.56 Percentage (%) mortality of *An. gambiae* s.l in each study site 24 hours after exposure to 0.75% permethrin insecticide.

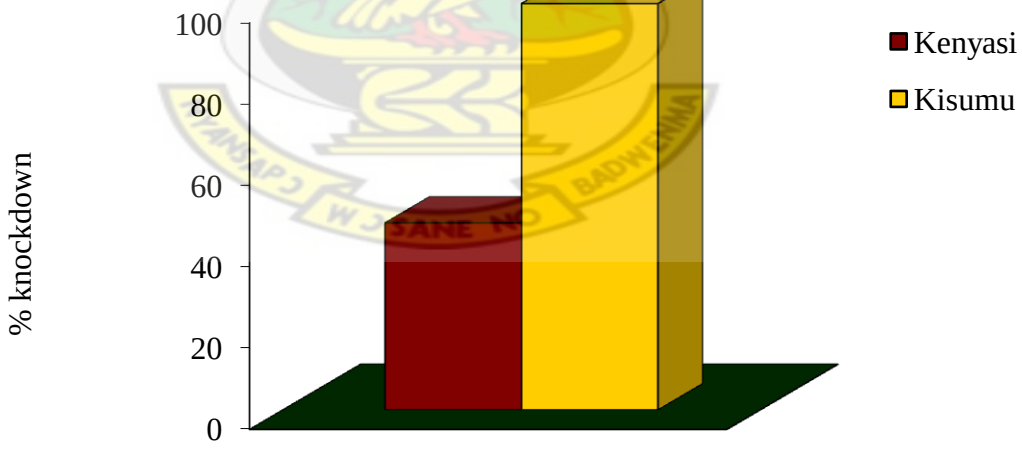


Figure 4.57 Percentage (%) mortality of *An. gambiae* s.l at Ahafo Kenyasi and the Kisumu colony 24 hours after exposure to 1.5% permethrin insecticide.

The mortalities observed with the various diagnostic concentrations of insecticides tested on *An. gambiae* after 24 hours post-exposure primarily differed significantly (χ^2 = 24.044, d.f. = 4, p = 0.0001). Specifically, the 0.75% permethrin was less lethal to the *An. gambiae* than the 0.05% deltamethrin at both Sunyani (χ^2 = 11.562, d.f. = 1, p = 0.0007) and Ahafo Kenyasi (χ^2 = 7.031, d.f. = 1, p = 0.008). Also, the 0.75% permethrin had a less lethal effect on *An. gambiae* compared to the 1.50% permethrin at Ahafo Kenyasi (χ^2 = 7.788, d.f. = 1, p = 0.005). However, the lethality of 1.50% permethrin on *An. gambiae* was not much higher than that of the 0.05% deltamethrin at Ahafo Kenyasi (χ^2 = 0.02, d.f. = 1, p = 0.888). For the Kisumu control strains, on the other hand, mortality rates after 24 hours of exposure did not differ between the different diagnostic concentrations of insecticides tested ($p > 0.05$).

Mortality observed with various diagnostic concentrations of insecticides on *An. gambiae* after 24 hours post-exposure was apparently homogenous between test sites ($p > 0.05$). The 0.05% deltamethrin insecticide appeared to be more lethal to *An. gambiae* from Sunyani than Ahafo Kenyasi (χ^2 = 3.926, d.f. = 1, p = 0.047). However, chi-square incorporating *Yates' correction for continuity* indicated no significant differences between these two mosquito populations (χ^2 = 3.385, d.f. = 1, p = 0.066). Also, the lethal effect of 0.75% permethrin on *An. gambiae* from Ahafo Kenyasi and Sunyani did not differ significantly (χ^2 = 1.496, d.f. = 1, p = 0.221). However, 0.05% deltamethrin and 0.75% permethrin were more lethal to *An. gambiae* from Kisumu than the test sites ($p < 0.001$).

4.4 Distribution of Leucine to Phenylalanine Pyrethroid Insecticide Knockdown Resistance (*kdr*) Mutation in *Anopheles gambiae* s.s. at the Study Sites.

The distribution of the *kdr* mutation in the M and S forms of *An. gambiae* s.s at the study sites are given in Table 4.26. The frequency of the *kdr* in the M forms ranged from 25% (95% CI: 6.02 – 43.98) at Ahafo Kenyasi to 33.33% (95% CI: 17.25 – 49.41) at Chiraa but the frequency in the S forms was much higher, ranging from 78.00% (95% CI: 66.52- 89.48) at Chiraa to 82.43% (95% CI: 73.76 – 91.1) at Ahafo Kenyasi. On the whole, 31% (95% CI: 21.02 – 38.98) of the M forms and 80.08% (95% CI: 75.23 - 84.93) of the S forms had the mutation. There were no significant variations in the frequency of the *kdr* mutation in both the M forms ($\chi^2 = 0.419$, $p = 0.936$) and S forms ($\chi^2 = 0.462$, $p = 0.927$) between study sites.

Table 4.26 Frequency distribution of the knockdown resistance (*kdr)* gene mutation in *An. gambiae* s.s M and S molecular forms from the study sites.

			kdr frequency in molecular forms							
			M Form				S Form			
Site	N	Total (%)*	n_m	+	-	%	n_s	+	-	%
Sunyani	107	66.36	28	8	20	28.57	79	63	16	79.75
Chiraa	83	60.24	33	11	22	33.33	50	39	11	78.00
Kenyasi	94	70.21	20	5	15	25.00	74	61	13	82.43
Hwidiem	77	67.53	19	6	13	31.58	58	46	12	79.31

N = Total number of M and S forms examined
Total (%)* = Percentage of *kdr* mutation observed in both forms
n_m = Number of M forms examined for the presence of *kdr* mutation
ns = Number of S forms examined for the presence of *kdr* mutation
+ = Number of M or S form samples found to possess *kdr* gene mutation
- = Number of M or S form samples which did not possess *kdr* mutation
% = percentage of *kdr* mutation in M or S form samples examined

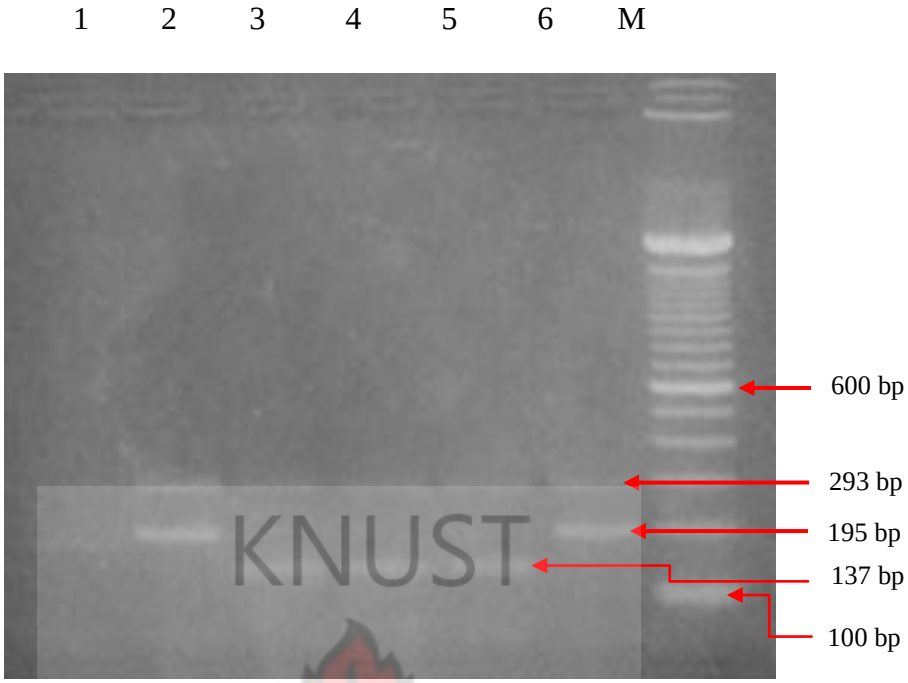


Figure 4.58 Ethidium bromide-stained 2.0% agarose gel electrophoregram of knockdown resistance (*kdr*) mutation obtained from the analysis of *An. gambiae* s.s rDNA_PCR product. Lane 1 is negative control; lane 2 is resistant mosquito from Korlebu, Accra used as positive control; lane 3 is a laboratory reared susceptible control from Kisumu, Kenya; lanes 4 and 5 are susceptible mosquitoes from Ahafo Kenyasi and Sunyani respectively; lane 6 = resistant mosquito from Ahafo Kenyasi and lane M = 100bp ladder (Sigma-Aldrich, USA).

CHAPTER FIVE

DISCUSSION

5.1 Introduction or overview of the present study

Human malaria is transmitted only by females of the genus *Anopheles.* Among the about 430 *Anopheles* species, only about 40 transmit malaria in nature. Of this number, only 15 are major vectors (White, 1982). This is because some *Anopheles* species prefer to bite other animals and transmit malaria to humans rarely. Others do not have the minimum lifespan required for the sporogonic cycle and/or are not susceptible to the malaria parasite. The ability of an *Anopheles* species to transmit malaria is also influenced by its abundance and the proximity of its habitat to human dwelling. An understanding of the relationship between the vector, its ecology and behaviour, the parasite and the host is required in order to develop and implement effective vector control strategies. Accordingly, the present study was undertaken to determine the distribution and role of *Anopheles* mosquito species in human malaria transmission and the susceptibility/resistance status of these mosquitoes to pyrethroid insecticides. The study was undertaken in some communities in two districts of the Brong Ahafo Region, Ghana where endemicity of malaria is reported to be greater than 75% (www. mara. org.za/ pdfmaps/GhaDistribution.PDF).

5.2 The diversity of *Anopheles* mosquitoes

Anopheles gambiae s.l. Gilles, *An. hancocki* Edwards and *An. coustani* Laveran were the only human-biting anophelines found in the present study areas: Sunyani and Chiraa in the Sunyani Municipality, and Ahafo Kenyasi and Hwidiem in the Asutifi District. Both districts are in the continually degrading semi-decidous rainforest

middle belt in the Brong Ahafo Region of Ghana. *Anopheles gambiae* s.l and *An. funestus* have been reported as the most abundant and widespread vectors in the coastal savannah zones of Ghana (Appawu *et al*., 1994; Yawson *et al*., 2004), and are the major human-biting species in Dodowa in the coastal savannah zone (Appawu *et al*., 2001; Yawson *et al*., 2004), in Okyereko and Mampong in the mangrove and strand zone, and in Osurogba, Odumase, Ayenya and Ayikuma in the coastal savannah zone of southern Ghana (Yawson *et al*., 2004). *Anopheles gambiae* s.l and *An. pharaoensis* have been reported as the most common biting anophelines in Prampam in the strand and mangrove zone (Appawu *et al*., 2001). *Anopheles gambiae* s.l., *An. funestus* and *An. rufipes* are the most common anophelines in the northern savannah zone of Ghana. On the whole, the most widespread malaria vectors in Ghana are *An. gambiae* s.l. and *An. funestus* (Appawu *et al*., 2001, 2004; Afrane *et al*., 2004; Yawson *et al*., 2004). The presence of the *An. gambiae* s.l. and the absence of the *An. funestus* in the present study communities are similar to the situation in some other areas in the middle belt of Ghana. For example, studies undertaken in the Kumasi Metropolis (Yawson *et al*., 2004; Afrane *et al*., 2004), Akomadan, Afrancho, Ejura and several other areas in the middle rainforest belt of Ghana (Stiles-Ocran, 2003; Stiles-Ocran *et al*., 2007) reported the *An. gambiae* s.l as the major malaria vector. None of these studies reported the presence of the *An. funestus* group in any of the communities surveyed. *Anopheles gambiae* s.l. may, therefore, be the most widespread *Anopheles* species in the middle belt of Ghana.

In most areas where *An. gambiae* s.l. was found to be a vector of malaria alongside the *An. funestus* s.l., the *An. gambiae* s.l. was found to be the leading vector especially in the rainy season (Appawu *et al.,* 2001, 2004; Appawu, 2005; Stiles-Ocran *et al.,*

unpublished). Studies conducted in other areas of Africa also indicate that the *An. gambiae* s.l. is the major malaria vector in most parts of Africa (Manga *et al.* 1997; Shililu *et al*. 1998; Lindblade *et al*. 1999; Coetzee *et al.* 2000; Jambou *et al.* 2001; Wanji *et al*., 2003). The success of the members of the *An. gambiae* Gilles s.l. includes the fact that they are highly anthropophilic and are adapted to a wide variety of micro- and macro-environmental conditions (Gimnig *et al*., 2001; Ye-Ebiyo *et al*., 2003; Edillo *et al*., 2006; Diabete' *et al*., 2005; Sibomana, 2002; Kabulah, 2007).

5.2.1 Anopheles hancocki Edwards

The presence of *An. hancocki* species in the study sites is of interest. Although *An. hancocki* has only rarely been found infected with *Plasmodium* parasites (Vaucel & Campourcy 1943; Fontenille *et al.* 2000), a recent study in the Mount Cameroon Region showed that *An. hancocki* had a relatively high sporozoite rate (5%) in the dry season and playing a significant role in malaria transmission (Wanji *et al*., 2003). In the present study, however, *An. hancocki* occurred in relatively low frequencies in each study site and the sporozoite rate was nil. Previous entomological survey also indicated the presence of only one mosquito of the *An. hancocki* species in the coastal forest of Dodowa, southern Ghana with zero sporozoite rate (Appawu *et al*., 2001). Thus, *An. hancocki* may not play any role in malaria transmission in Ghana.

5.2.2 Anopheles coustani Leveran

Four *An. coustani* mosquitoes were identified from Hwidiem in the Asutifi District during the rainy season with none infected with *Plasmodium.* Previous studies undertaken in two ecological zones of southern Ghana indicated the presence of two *An. coustani* Laveran in the coastal forest of Dodowa with negligible sporozoite rates

(Appawu *et al*., 2001). The role of *An. coustani* in malaria transmission in Ghana at large appears to be negligible, seeing that most of the entomological studies undertaken in the country did not report the presence of this *Anopheles* mosquito (see e.g. Appawu *et al*., 2004; Afrane *et al*., 2004; Yawson *et al*., 2004; Stiles-Ocran, 2003; Stile-Ocran *et al*., 2007; Kabulah, 2007).

5.2.3 Anopheles gambiae sensu lato Gilles

The *An. gambiae* is a complex of seven sibling species that are very much related and morphologically indistinguishable from each other but are distinct with regard to ecological and behavioral characteristics, and vectorial competence (White 1974). In West Africa, *An. arabiensis* and *An. gambiae* s.s. are the two most important members of the *An. gambiae* complex that transmit malaria, with the latter being the more efficient malaria vector because it possess a higher degree of anthropophily [Pates *et al*. 2001; Coetzee *et al*. 2000; Besansky *et al*. 2004]. The risk of malaria infection also differ significantly between the M and S molecular forms of the *An. gambiae* s.s. (Okoye *et al*. 2005). Other studies have also indicated variations in the distribution of insecticide resistance genes among these molecular forms of the *An. gambiae* s.s. (Yawson *et al*. 2004; Adasi & Hemingway, 2008).

5.2.3.1 Anopheles gambiae sensu stricto Gilles

In the present study, *An. gambiae* s.s. was the only member of the *An. gambiae* s.l. found in the study areas. This observation is consistent with those of previous studies undertaken in some communities in the Greater Accra Region along the coast of southern Ghana (Yawson *et al.* 2004), in Ankasa, Tarkwa and the coastal parts of the Western Region in the south-western Ghana (Kristan *et al.* 2003) as well

as in Kumasi in the middle belt of Ghana (Yawson *et al.* 2004; Afrane *et al.* 2004) where *An. gambiae* s.s was the only sibling species of *An. gambiae* s.l. found. Elsewhere in Ghana and Africa at large, *An. gambiae* s.s has been found in sympatry with *An. arabiensis* as were the case in the Kassena-Nankana District and other areas in northern Ghana (Appawu *et al.* 2004; Kelly-Hope *et al.* 2003). *Anopheles gambiae* s.s. has also been found in sympatry with *An. arabiensis* in the Greater Accra and Volta Regions along the coast of southern Ghana and in the southern parts of Burkina Faso (Yawson *et al.* 2004; Kelly-Hope *et al.* 2003) and in Kenya (Gimnig *et al.* 2001). *Anopheles gambiae* s.s also occurs in sympatry with relatively small numbers of *An. melas* in southern Ghana (Kristan *et al.* 2003; Yawson *et al.* 2004; Appawu *et al.* 2001). The presence of the *An. melas* in the coastal zone of southern Ghana and its absence in the middle and northern belt of Ghana was to be expected because this sibling species of the *An. gambiae* s.l. is reported to be confined to the coastal areas because they prefer to breed in salt water bodies (Coluzzi & Sabatini, 1968; Coluzzi, 1984; Akogbeto, 1995, 2000; Fonseca *et al.* 1996; Diop *et al.* 2002). On the other hand, the absence of *An. arabiensis* in the present study areas where significant deforestation and urbanisation have taken place was inconsistent with the earlier observation that *An. arabiensis* penetrates areas after deforestation and urbanisation (Coluzzi *et al.* 1979). *Anopheles gambiae* s.s appears to dominate in most areas where it occurs in sympatry with other sibling species of the "gambiae complex". For example, *An. gambiae* s.s was found to be the predominant sibling species of the complex in the coastal zone in the Greater Accra Region (Appawu *et al.* 2001; Adeniran, 2002; Sibomana, 2002; Otieno, 2004; Achonduh, 2005; Kabulah, 2007), South-western Region (Kristan *et al.* 2003; Yawson *et al.* 2004), rainforest middle belt in the Ashanti Region (Yawson *et al.* 2004; Afrane *et al.* 2004; Stiles-Ocran,

2003; Stiles-Ocran *et al.* 2007) and the Guinea Savannah zone of northern Ghana (Appawu *et al.* 2004; Yawson *et al.* 2004). However, in the southern coastal zone of Ghana, Chinery (1984) found *An. arabiensis* to be dominant over *An. gambiae* s.s and suggested that *An. arabiensis* had replaced *An. gambiae* s.s as a result of urbanisation. Lastly, it is important to mention that all the larvae that were collected from Sunyani and Ahafo Kenyasi were found to be *An. gambiae* s.s. This was also consistent with studies undertaken in the middle belt (Yawson *et al.* 2004) and southern belt (Kristan *et al.* 2003) of Ghana. *Anopheles gambiae* s.s dominated the current study sites and most of the areas explored in Ghana and other part of Africa not only because it has adapted to a wide range of ecological conditions but also transient larval habitats adequate for this species are available throughout the two rainfall regimes. Coluzzi *et al.* (1979); Chinery, (1984); Appawu *et al.* (1994, 2001) and Coetzee *et al.* (2000) showed that this species prefers and dominates in this type of ecological zone.

5.2.3.1.1 The molecular forms of the *An. gambiae* s.s.

In the present study, the two molecular forms namely, M and S forms of *An. gambiae* s.s were found to be sympatric in all sampling areas. These findings are consistent with the study conducted in the Kassena-Nankana District in northern Ghana (Appawu *et al.* 2004) and in several areas of the Central and the Greater Accra Regions in the southern part of Ghana (Yawson *et al.* 2004; Kabulah, 2007) where both molecular forms of *An. gambiae* s.s were reported. The results also agree with the results of the studies conducted in Mali, where M and S forms were sympatric in several larval breeding sites (Edillo *et al.* 2002). Although the M and S molecular forms were sympatric at the study sites, they were not adapted to these areas to the same extent. This is evidenced by the difference in their relative abundance in the

various breeding grounds or study communities surveyed. In the present study, the S molecular form predominated in all the study communities with the exception of Chiraa where the frequencies of the S and M forms were not significantly different ($p > 0.05$). Studies undertaken in southern Ghana also found higher proportions of the *An. gambiae* s.s. being S forms (Yawson *et al.* 2004; Kabulah, 2007; Adasi & Hemingway, 2008). For example, Yawson *et al.* (2004) recorded high proportions of S forms of *A. gambiae* s.s. including 95.1% in Mampong, 95% in Okyereko, 96.9% in Abia and 98.8% in Osurogba. The S form has also been found to be predominant in some areas in the south-eastern part of Ghana (>95%) [Adasi & Hemingway, 2008]. The present sites are characterised largely by rain-dependent temporary breeding sites, which dry up when rainfall was low or nil. The S form of *An. gambiae* s.s. is reportedly found mainly in temporary, rain-dependent areas (Toure' *et al.* 1994).

The observation of both the M and S molecular forms in this study differs from the results of other studies conducted in Kumasi in the middle belt of Ghana, where only the S molecular form was found, or in Korania and Bonia in northern Ghana and Kobri in southern Burkina Faso where only the M form was reported (Yawson *et al.* 2004). It appears the presence of permanent breeding conditions in the latter areas provided through urban agricultural activities offer suitable habitats for the M forms to prevail. This is because the M forms have been associated with urban environment and flooded or irrigated sites, characterised by extensive crop cultivation (Toure' *et al.* 1998; Diabate' *et al.* 2003b) in several areas in West Africa except for Foumbot in Cameroon, where *An. gambiae* s.s larvae sampled from an area of intensive gardening (man-made habitat) were found to be S forms (Etang *et al.* 2006). In the present study sites, the M and S forms occurred in sympatry possibly as a result of the availability of both permanent and temporary breeding sites especially in the

rainy season. At Chiraa, virtually no temporary breeding sites containing aquatic stage mosquitoes were found throughout the present study. Hence, there is the likelihood that both forms have adapted considerably to breeding in permanent water bodies.

The results of the present study also showed that no hybrid of M and S molecular forms of *An. gambiae* s.s. were present in the study areas. The absence of hybrid M/S form in this study is consistent with some previous reports of studies conducted in several communities in Ghana (Yawson *et al.* 2004; Kabulah, 2007; Stiles-Ocran *et al.* 2003), Cameroon (Wondji *et al.* 2002; Etang *et al.* 2006) and Angola (Cuamba *et al.* 2006). This absence of M/S hybrids in the field has been hypothesised to be due to some reproductive barriers between the molecular forms, with gene flow occurring only in some geographical locations or at some seasons (Black and Lanzaro, 2001). Such a pre-mating isolation mechanism may include the alternative swarming habits of the molecular forms observed in an area where these molecular forms occur in sympatry (Diabete *et al.* 2003a). On the other hand, Appawu, (2005) observed possible hybrids between the M and S molecular forms from the rocky highland areas of the Kassena-Nankana District of northern Ghana. Cases of low level hybridisation between M and S molecular forms have also been observed elsewhere in Africa at frequencies ranging from 0.26% (della Torre *et al.* 2001) through 0.30% (Tripet *et al.* 2001) to 0.71% (Taylor *et al.* 2001). Hybridisation between the M and S forms may be due to the strong assortative mating within these forms (Tripet *et al.* 2001), although contamination during the experimentation has also been suggested as a possible cause of some occurrence of M/S hybrid patterns in *An. gambiae* s.s form identifications (della Torre *et al.* 2001).

5.3 Biting rates, biting habits and feeding behaviour of the *An. gambiae* s.s.

The vectorial capacity of *Anopheles* mosquito species is greatly influenced by their behaviour; knowledge of such behaviour is important in determining the suitability of the available malaria vector control options (CDC, 2004a, b, WHO, 2003). Factors such as insecticide usage could influence the behaviour of *Anopheles* species. The dominant feature is the change in behaviours which underlies mating, dispersion, host seeking, occupation of day resting places and oviposition (Charlwood & Jones, 1979; WHO, 2003). The present study explored the human biting rate and feeding habit of the main malaria vector in the study communities through human-landing collections. The feeding habit in this study refers to whether the vectors under consideration prefer to feed indoors or outdoors (endophagy and exophagy) and the times of feeding during the night (night biting cycle). However, the human-biting rate refers to average number of bites per person per night by a vector species, and its estimation involves both the feeding habit of the vector and the night-time habits of the local populace (WHO, 1975, 2003). The human-landing collection method was used as it gives a direct measure of all human biting mosquitoes in a given area (WHO, 2003; Faye *et al.* 1992; Hii *et al.* 2000; Davis *et al.* 1995).

5.3.1 Biting rates and biting habits of the An. gambiae s.s.

The results of the present study showed some spatial, temporal and seasonal variation in biting rates and biting habits of mosquitoes within and between areas short distance apart. These findings are not unusual of anopheline vectors since other studies have also compared transmission among different ecological zones and even among villages in the same area, and have reported significant variations in transmission intensity among communities short distances apart and even between

rural and urban settings in the same area (Fontenille *et al.* 1997 Appawu *et al.* 2001, 2004; Robert *et al.* 2003). The results of the present study showed higher human-biting rates at Sunyani and Ahafo Kenyasi than the comparatively less urbanised Chiraa and Hwidiem, although there were no significant differences between Sunyani, Ahafo Kenyasi and Hwidiem. These findings appear to contradict some previous observations. For example, according to Bruce-Chwatt, (1985), there are significantly lower hygienic conditions in more urbanised localities which lead to the contamination of breeding places with polluted materials, making them unsuitable for the development of *Anopheles* larvae. In the present study, however, several *Anopheles* larvae were collected from polluted, highly turbid, muddy water. The larvae either occurred along the clearer banks of the stagnant water or in the entire water body where they mostly assumed the colour of the water. In fact, in most breeding grounds where *Anopheles* larvae were collected during the surveys, culicines especially those belonging to the genus *Culex* were also collected. In Accra, southern Ghana, entomological study indicated that *An. gambiae* s.l. was adapted to breeding in organically polluted waste water habitats (Chinery, 1984). This mosquito species has also been found in sewage ponds and in sewage extremely polluted with organic matter in Dar es Salaam, Tanzania (Sattler *et al.* 2005). Lastly in Lahore, Pakistan, *Anopheles* species were found in waste water (Mukhtar *et al.* 2003). These findings could indicate a change of *Anopheles* species breeding requirements in the urban environment or their adaptation to contaminated water and are inconsistent with the long-established view that the larvae of *Anopheles* mosquito species only flourish in clean or uncontaminated water (Bruce-Chwatt, 1985; WHO, 2003; CDC, 2004b). These imply that malaria vector control interventions, such as those employing

environmental measures and insecticide application, must deem all open water bodies as potential breeding grounds for *Anopheles* mosquito vectors (Sattler *et al.* 2005).

The Figures 4.22 to 4.25 illustrate the hourly biting pattern of the *An. gambiae* in the rainy season. These figures show that this mosquito species bites throughout the night with the biting intensity peaking during the third quarter (00:00 – 03:00 hours) or the fourth quarter (03:00 – 06:00 hours) of the night. The fact that a high degree of biting occurred during the early hours of the morning (05:00 – 06:00 hours) is of interest. This observation does not concur to the situation in Prampram in the coastal savannah zone and in Dodowa in the coastal forest zone of southern Ghana (Appawu *et al.* 2001) where significantly low biting occurred in the early mornings but agrees with the situation in the Kassena-Nankana District of the northern savannah zone of Ghana (Appawu *et al.* 2004) where significant bites occur in the early hours of the morning. This observation has important epidemiological implications for the Roll Back Malaria Initiative in Ghana and the Ghana National Malaria Control Programme. A key strategy of these two bodies is the use of insecticide-impregnated bednet (ITN) for combating malaria transmission (Yawson *et al.* 2004; RBM, 2005). The present study did not characterise the distribution of infectivity of biting mosquitoes with regard to the hours of night but the reports of Appawu *et al.* (2004) and (Appawu, 2005) indicated that in the Kassena-Nankana District, 76.5% infective mosquitoes (*An. gambiae* s.s. and *An. funestus*) were found biting after midnight in the wet season with biting peaks occurring at dawn between 04:00 and 06:00 hours. In the present study communities as was observed in the Kassena Nankana District of nothern Ghana, most of the adult inhabitants were usually found to be awake during the period of 05:00 to 06:00 hours and may be out of their bednets, thereby increasing their exposure to infective bites from *An. gambiae* s.s (Appawu *et al.* 2004).

The results of the present study also showed that in the dry season, *An. gambiae* was more aggressive during the second (21:00 – 00:00 hour) and third (00:00 – 03:00 hour) quarters of the night, with the climax occurring between 23:00 and 04:00 hours. The implication for the peak biting activities occuring during deep hours of the night in the dry season is that insecticide-treated bed-nets may be effective in preventing sporozoite infective bites from *An. gambiae* in both adults and children. The results of the present study also show that unlike in the rainy season, significantly higher biting occurs in the first quarter (18:00 to 21:00) of the night than in the fourth quarter (03:00 to 06:00) as illustrated in Figures 4.26 to 4.29. This may be attributed to the differences in temperature and other weather conditions during these two quarters of night. It was also observed in the present study that sometimes outdoor biting stops before the 05:00 hours when the temperaure was low (~18°C or less). The low density of mosquitoes in these hours seemed to concur with the low density observed in southern Ghana (Appawu *et al.* 2001). According to the study by Martens, (1995) blood feeding frequency declines with temperature, leading to less host vector contact and resulting in decreased proportions of infective mosquito bites.

Figure 4.15 to 4.21 also illustrate the association between monthly biting rates and climatological factors observed in the present study. The results suggest a strong positive correlation between biting rates and rainfall at Sunyani, Chiraa and Hwidiem. Similarly, there was a strong positive correlation between biting rates and relative humidity at 0600 hours and 1500 hours at Sunyani. Lastly, there was a weak negative correlation between biting rates and maximum temperature and a strong positive correlation between biting rates and minimum temperature at Sunyani. Previous studies in northern Ghana showed that biting rates were generally high in the rainy season and declined greatly in the dry season (Appawu *et al.* 2004). Similarly,

average daily rainfall was found to be significantly correlated with the *An. gambiae* abundance at Jaribuni in the coastal zone of Kenya in 2001. Also, the association between relative humidity and *An. gambiae* biting rates observed in this study agrees with the observation at Jaribuni in 2001 where relative humidity was strongly correlated with the abundance of *An. gambiae* (Midega, 2008). It should also be said that the association between rainfall, relative humidity, temperature and biting rates is not unique to *An. gambiae* as a strong positive correlation has been observed between these factors and abundance of *An. funestus* (Midega, 2008). Similarly, a strong positive correlation has been observed between biting rates of *Culicoides fulvithorax*, and rainfall and relative humidity in Nigeria (Agbolade *et al.* 2006. The findings of the present study and the several others as indicated imply that climatic factors may be significant determinants of human-biting rates (ma) which is one of the key parameters used in determining the vectorial capacity of mosquito species. According to Molineux, (1988); Le Sueur & Sharp, (1991) and WHO, (1990, 2003) climatic factors such as rainfall determine the availability of breeding habitats.

5.3.2 Feeding behaviour of the An. gambiae s.s.

The results of the present study show that higher proportion of the human biting *An. gambiae* mosquitoes was caught indoor in both the rainy and the dry seasons. The high tendency of endophagy demonstrated by *An. gambiae* is consistent with the studies conducted in the irrigated and lowland areas of the northern savannah zone, Ghana (Appawu *et al.* 2004; Appawu, 2005) and at Dodowa in the coastal forest zone of southern Ghana (Appawu *et al.* 2001) where significantly higher proportion of the man-biting anopheline mosquitoes were found biting indoors. Especially important is the observation that 60% of the infective *P. falciparum* sporozoite

inoculations in the northern Ghana occurred indoors (Appawu, 2005). Such highly endophagic behaviour has also been reported in the mount Cameroon region (Wanji *et al.* 2003) and is known to be typical of the *An. gambiae* species (CDC, 2004b). Protection against infective bites from endophagic mosquitoes is mostly achieved through residual insecticide spraying and insecticide treated materials (WHO, 2003; CDC, 2004b). Therefore, the comparatively higher indoor biting rates observed in the present study communities imply that malaria transmission could be prevented to a significant extent with these malaria vector control methods.

Although on the whole, there was a significant difference in the indoor and outdoor biting rates in the present study sites, outdoor biting rates ranged from 45.6% at Chiraa to 47.2% at Hwidiem in the rainy season and 37.5% at Ahafo Kenyasi to 46.1% at Chiraa in the dry season. In the rocky highlands of the Kassena-Nankana District of northern Ghana, the proportion of *An. gambiae* s.s. which were found biting outdoors was even significantly higher than indoors (Appawu, 2005). At Prampram in the coastal savannah zone of southern Ghana, *An. gambiae* species also displayed no significant difference in endophagic and exophagic behaviours (Appawu *et al.* 2001). The outcome of outdoor biting rates of at least about 38% observed in this study as well as the significantly high exophagic behaviours observed elsewhere in the southern and northern Ghana is that a good number of *An. gambiae* will bite outside as long as there are people outside. This poses risk to those who spend long hours outdoors especially for business purposes such as food vendors who sit by the roadside and night market squares to sell their products. Prevention of malaria transmission in this case may depend considerably on the use of mosquito repellents, such as synthetic chemical repellents and/ or natural botanical repellents as well as the use of protective clothing.

5.4 Parity, daily survival rates and longevity of the *An. gambiae* s.s.

The probability of daily survival of the *Anopheles* mosquitoes is important in malaria epidemiology, especially in determining their life expectancy and vectorial capacity (WHO, 1975, 2003). The mosquito must not only be able to take a blood meal from man but should also survive long enough for the sporogonic cycle of malaria parasite to take place. If the mosquito survives for a long time, it will be able to take several blood meals and have a higher possibility of being infected with malaria parasites (WHO, 1975, 2003; Gilles, 1988). According to Gilles, (1988) bites from the older sections of an *Anopheles* vector population which are the parous ones are therefore, especially risky due to the fact that they are most likely to be infective with malaria parasites. It has been observed that quite small changes in daily survival rate could have a tremendous influence on *Anopheles* vectorial capacity (Macdonald, 1952; WHO, 2003). *Anopheles* mosquitoes can live for more than 3-4 weeks in nature (Service, 1993a; WHO, 2003; White, 1982), athough the mean survival in nature ranges from 6-9 days. These observations seem to suggest that only a proportion of the *Anopheles* vector population normally survive long enough for the reproductive cycle of the malaria parasites to be completed in them in order to be transmitted to the human host. The duration of the reproductive cycle of the malaria parasites depends on the *Plasmodium* species under consideration but irrespective of the species, it varies inversely with environmental temperature. The minimum duration of the sporogonic cycle has been reported widely to be 10 days (White, 1982; Gillies, 1988; WHO, 2003; Buynavanich & Landrigan, 2003). However, according to Macdonald, (1957) the sporogonic cycle on average lasts approximately 10 days but shortens to about five days as temperature increases to over 30°C. It is therefore necessary to investigate the age-grading of a female *Anopheles* mosquito population in a given

community in order to determine the fraction which survives long enough to transmit malaria parasites.

5.4.1 Parity rates of the An. gambiae s.s.

The most essential parameter with respect to the longevity of the female *Anopheles* vectors is the parity rates, which refers to the proportion of adults that are nulliparous (that is, have not laid any batch of eggs) and/ or are parous (that is, have laid at least a batch of eggs) [Davidson, 1954; Clement & Patterson, 1981; WHO, 2003]. According to Garrett-Jones & Grab, (1964); Clement & Patterson, 1981), parity rate can be used to obtain a direct estimate of the survival rates at each gonotrophic cycle. The present study explored the parity rates of the main malaria vector *An. gambiae* in both the rainy and the dry seasons in the present study areas.

The results of the present study indicated there were no significant variations in parous rates between the study sites. Similarly, Afrane *et al.* (2004) did not find any spatial variation in parity rates within fifteen different sites in the Kumasi Metropolis in the middle belt of Ghana. The lack of variations in parity rates between these study sites may be due to similarities in the climatological factors in these study sites. For example, the present sudy showed that there were no significant differences in rainfall pattern between Sunyani and Chiraa from 2005 to 2007 (p = 0.695). Also, there were no significant differences in monthly rainfall between Sunyani, Chiraa and Hwidiem in the year 2006 when the entomological surveillance was undertaken (p = 0.710).

The results of the present study also revealed a strong negative correlation between monthly parous rates and monthly rainfalls at Sunyani and Hwidiem but a weak negative correlation between parous rates and rainfall at Chiraa. This implies that there was a considerable seasonal variation in parity rates of *An. gambiae* with

parity rates being higher in the dry season than in rainy season. Previous studies in southern Ghana showed that parity rates did not differ significantly between Dodowa and Prampram during an entomological survey conducted in 1992 but there were significant differences in parous rates between these communities during the surveys undertaken in 1993 when a significant variation in rainfall was also observed between these two communities, suggesting some association between rainfall and parity rate (Appawu *et al.* 2001). Furthermore, the studies by Appawu *et al.* (2001) at Dodowa in the coastal forest and Prampram in the coastal savannah zones of southern Ghana and Afrane *et al.* (2004) in Kumasi in the rainforest middle belt of Ghana showed that parous rates and survival rates were significantly higher in the dry season. The low parous rates of *An. gambiae* in the rainy season at the present study sites and other areas surveyed in Ghana may be due to the influx of newly emerged, nulliparous female mosquitoes from their breeding sites in this season (Vythilingam *et al.* 2003).

The results of the present study also suggested there was no significant correlation between parous rates and mean daily relative humidity at 0600 and 1500 hours. There was also no significant association between parous rates and mean daily maximum temperature but there was a strong association between parous rates and mean daily minimum temperature at Sunyani. The strong negative correlation between parous rates and minimum temperature is of interest because very low temperatures are known to lengthen the gonotrophic cycle but reduce survival rate of mosquito vectors (De Meillon, 1934; MARA, 1998). This might be the reason why *An. gambiae* for example survives only in frost-free regions or where absolute minimum temperatures in winter remain above 5°C (Leeson, 1931; De Meillon, 1934; MARA, 1998).

5.4.2 Daily survival rates and longevity of the An. gambiae s.s.

The probability of *An. gambiae* surviving a day was high, ranging from 87% in the rainy season to 94% in the dry season. Life expectancy (in days) ranged from about seven to eight in the rainy season but was higher in the dry season, ranging from approximately 12 – 17. Similar estimates of *Anopheles* survival have been found in the coastal savannah of Prampram and coastal forest of Dodowa in southern Ghana where the probability of *An. gambiae* surviving a day was 94% in 1992 when rainfall was relatively low and ranged from 74 to 76% in 1993 when rainfall was relatively high at the respective sites (Appawu *et al.* 2001). Similarly, the parity data from Afrane *et al.* (2004) also indicated that the daily survival rates of the *An. gambiae* in the rainy season were 87, 88 and 89% in urban areas without agricultural activities (UW), urban areas with agricultural activities (UA) and peri-urban areas with agricultural activities (PU) respectively in the Kumasi Metropolis. The respective life expectancies in days were approximately seven, eight and nine. Survival rates was high in the dry season, ranging from 92% in UW through 95% in UA to 96% in PU areas and their respective estimated life expectancies were approximately 13, 22 and 26 days respectively. The relatively low survival rates of *An. gambiae* populations observed during the rainy season in Ghana differ from the results of the study by Wanji *et al.* (2003) who estimated a comparatively high survival rate of 93% and life expectancy of about 14 days for *An. gambiae* in the rainy season at mount Cameroon region. Daily survival rate of up to 95% was also estimated for *An. gambiae* s.l. populations along coastal Kenya in Jaribuni and Mtepeni (Midega *et al.* 2007). However, comparatively similar estimates of survival rates to those reported in Ghana have been found in several other parts of Africa. For example, daily survival probabilities of 67.0 – 82.0% were estimated for *An. gambiae* s.l. in Burkina Faso

(Costantini *et al.* 1996). Charlwood *et al.* (1997) also estimated daily survival rates of 81.3 – 83.9% for the *An. gambiae* s.l. in Ifakara, Tanzania. Similarly, Takken *et al.* (1998) estimated daily survival probabilities of 63.0 – 78.0% for the *An. gambiae* s.l. in Namawala, Tanzania. It has been established that if the daily mortality of an anopheline mosquito population averages 50%, then less than one percent (<1%) of the females are to be expected to survive to the minimum of 10 days necessary for the extrinsic cycle of *P. falciparum* (White 1982; WHO, 2003). Moreover, for any female *Anopheles* mosquito population to have a considerable vectorial capacity, their daily survival probability must be at least 60%, usually 80 – 90% (White 1982). The results of the present study together with the available literature seem to suggest that the *An. gambiae* populations in the explored areas have high survival rates and are therefore good at transmitting malaria.

5.5 Sporozoite rates of the *An. gambiae* s.s.

The vectorial capacity of a particular female *Anopheles* species with reference to malaria transmission can be defined as the ability of that mosquito to bite a human host, ingest the parasite, promote the development of the parasite until the sporozoite stage and live long enough to infect another and/ or the same human host with the parasite (Failloux *et al.* 1995). This means that the *Anopheles* vectors of malaria must be able to support the development of the ingested parasite to the minimum of 10 days required for the parasite to get to the infective stage (White, 1982). One of the parameters for assessing such vectorial capacity of *Anopheles* mosquitoes in a given area is the *Plasmodium* species sporozoite rates in the salivary gland of the mosquito population (White, 1982; WHO, 1975, 2003). In this work, the infectivity rates of *An. gambiae* population were based on the presence of *P. falciparum* circumsporozoite

antigens in the head and thorax. *Plasmodium falciparum* was tested because it has been reported to be responsible for up to 90 to 98% of the malaria cases in Ghana (Binka *et al.* 1994; Assenso-Okyere & Dzator, 1997). Studies have also been conducted to explore the utility of circumsporozoite enzyme-linked immunosorbent assay for determining sporozoite rates in mosquitoes instead of the traditional method which involves the dissection of the salivary gland (Wirtz *et al.* 1985, 1987a, b, 1992; Beier & Koros, 1991; Adungo *et al*,. 1991; Fontenille *et al.* 2001).

The infectivity test results of the present study showed that there was high *P. falciparum* sporozoite rates in *An. gambiae* ranging from 1.89% (-0.70 – 4.48) at Hwidiem to 4.05% (-0.44 – 8.54) at Chiraa. There were no significant differences in infectivity rates between study sites as well as between the rainy and the dry seasons. The infectivity of *An. gambiae* has also been found to be high in other communities in Ghana where entomological surveillance has been conducted. Previous studies have reported high infectivity rates of *An. gambiae* from the Kassena-Nankana District in the northern savannah zone as well as in the coastal forest and coastal savannah zones in southern Ghana (Appawu *et al.* 2001; 2004; Appawu, 2005). For example, the studies by Appawu, *et al.* (2004) estimated *P. falciparum* sporozoite rates of 7.2% and 7.1% for *An. gambiae* s.s. and *An. funestus*, respectively. Other studies conducted on malaria transmission in several other parts of Africa endemic for malaria found sporozoite rates to usually range from 1 to 20% (Wirtz & Burkot, 1991). This high sporozoite rates associated with the major malaria vectors in the surveyed areas in Ghana and Africa at large imply that a good proportion of this mosquito species live long enough to enable the *Plasmodium* parasite to develop up to the infective stage.

5.6 Entomological inoculation rates of the *An. gambiae* s.s.

The entomological inoculation rates, which refer to the product of the human biting rates and the sporozoite rates at a given time point is usually used to express the intensity of malaria transmission. It provides a standard and relatively simple means of measuring levels of exposure to infected mosquitoes and the suitability of vector control methods among others (Macdonald, 1957; Fontenille *et al.* 1997, Burkot & Graves, 1995; Onori & Grab, 1980). The entomological inoculation rates in the present study describe the average daily and monthly risk of an inhabitant of the study communities receiving a *P. falciparum* infected bite from *An. gambiae* s.s. The overall average inoculation rate was 2.864 infective bites per man per night (ib/m/n) in the rainy season, which adds up to 85.92 infective bites per man per month (ib/m/m). Similarly, the overall average inoculation rate was 0.468 ib/m/n in the dry season, which adds up to 14.07 ib/m/m. Previous studies have estimated EIR of 6-21 ib/m/n for Dodowa in the coastal forest zone and Prampram in the coastal savannah zone in southern Ghana with *An. gambiae* s.l and *An. funestus* group being the main vectors (Appawu *et al.* 2001). Studies undertaken in the Kassena Nankana District in northen Ghana also estimated daily EIRs of 2.4, 3.2 and 1.5 ib/m/n in the irrigated, lowland and rocky highland areas, respectively during the rainy season. During the dry season at the same locality, daily EIRs of 0.4 was recorded in the irrigated area, and 0.1 in the lowland area with virtually no transmission recorded in the rocky highland areas (Appawu *et al.* 2004). The implication of such findings is that malaria transmission in the present study areas and the areas surveyed in Ghana may occur in both the rainy and the dry seasons, although the intensity is seasonal with higher transmission occurring in the rainy season ($P > 0.05$). Studies conducted in other parts of Africa have also showed that malaria transmission intensity in Africa is highly

heterogeneous with annual *P. falciparum* EIRs ranging from < 1 to > 1,000 infective bites per man per year (Beier *et al.* 1999). The urban areas in Africa had an ovearall mean of 14 (range: 0 – 43), those in areas where irrigated rice cultivation are practised had a mean of 99 (0 - 601) followed by the the rural community with a mean of 146 (range: 0 – 884). The EIRs in the areas explored were not only highly dependnent on locality as illustrated but also on the seasons of the year [Hay *et al.* 2000].

5.7 Pyrethroid insecticide resistance status of the *An. gambiae* s.s.

The strategy of the Ghana National Malaria Control Programme is based on effective case management and the use of insecticide treated bed nets among vulnerable groups especially children less than five years of age and pregnant women (MoH, 2008). There is renewed interest in the use of insecticides for malaria vector control due to the effectiveness of insecticide treated materials that show promise in reducing malaria transmission intensity and morbidity. The use of the pyrethroid insecticides is also spreading fast in the public health sector due to its success in ITNs, insecticide-treated curtains, house-spraying and so on (Greenwood & Pickering, 1993; Choi *et al.* 1995; Binka *et al.* 1996; Lengeler, 2004). A disadvantage of the wide use of pyrethroids is cross-resistance resulting from pyrethroids formulated for use in agriculture (Chandre *et al.* 1999b; Fanello *et al.* 2003; Diabate *et al.* 2002, 2003; 2004; Stile-Ocran *et al.* 2007). This highlights the need to generate insecticide resistance related data that will be useful in planning, implementation and evaluation of pyrethroid insecticides and their role in the control of malaria vectors in Ghana. The present study determined the resistance status of *An. gambiae*, the main malaria vector to permethrin and deltamethrin in some communities in two districts of the Brong Ahafo Region. The study made use of the WHO Bioassays on the vector and

the determination of the leucine to phenylalanine knockdown resistance *(kdr*) gene mutation that the vector might have evolved over the years.

The insecticide resistance test results show high permethrin and deltamethrin resistance in the populations of *An. gambiae* in the study areas. The analysis of the results were based on the WHO criteria for characterising insecticide susceptibility and/ or resistance, where susceptibility is defined as mortality rates greater than 98% 24 hours post-expossure; marginal susceptibility as death rates between 80-97% and resistance as mortality rates less than 80% 24 hours post-exposure (WHO, 1998a; 2003). In the present study, mortality rates observed were less than 80% ranging from 27 – 35% for the samples exposed to 0.75% permethrin impregnated papers to 45 – 59% for samples exposed to 0.05% deltamethrin and 1.5% permethrin impregnated papers. Such low mortality rates which suggest high resistance rates in the *An. gambiae* populations in the present study communities appear to be consistent with the levels of insecticide resistance reported from other communities in the Greater Accra Region of Ghana (Adasi, 2000; Adeniran, 2002; Achonduh, 2005; Kabulah, 2007; Adasi & Hemingway, 2008). Adasi & Hemingway, (2008) recorded mortality rates of 47.5% and 56.4% for *An. gambiae* s.s. exposed to 0.75% permethrin at Dodowa and Accra respectively. These low mortality rates less than 80% recorded for *An. gambiae* s.s are indications of the existence of insecticide resistance in this mosquito for the insecticide in question (WHO, 1998a, 2003). Insecticide resistance in malaria vectors to pyrethroids have also been detected through bioassays or molecular analysis in several neighbouring countries of Ghana (Elissa *et al.* 1993, 1994; Akogbeto & Yakoubou, 1999; Chandre *et al.* 1999a, b; Kolaczinski *et al.* 2000; Weill *et al.* 2000; Diabate *et al.* 2002, 2003, 2004; Fanello *et al.* 2003). However, the results of the current study contrast with those of Kristan *et al.* (2003) who showed that in

spite of pyrethroid insecticide use for agricultural purposes in south-western Ghana, *An. gambiae* s.s. remained susceptible to deltamethrin and permethrin.

The exact cause of insecticide resistance in the present study sites is not known but insecticide resistance development has been mainly attributed to insecticide usage. The present study did not establish insecticide usage pattern in the study areas but the study of practices at the rice and vegetable farms in Okyereko and Mampong revealed heavy usage of three pyrethroid insecticides throughout the growing season to control a wide range of agricultural pests (Yawson *et al.* (2004). Studies conducted in the Greater Accra Region in southern Ghana (Adasi, 2000) and in the Ashanti Region of the middle belt of Ghana (Stiles-Ocran *et al.* 2007) have established a positive correlation between insecticide use and the development of insecticide resistance in *An. gambiae* species. Elsewhere in Africa, pyrethroid insecticide resistance has also been attributed to the intensive use first of DDT and, since the 1970s, of pyrethroid insecticides for crop protection purposes (Chandre *et al.* 1999b; Fanello *et al.* 2003; Diabate *et al.* 2002, 2003; 2004). Thus, the insecticide resistance observed in the present study sites may be due to insecticide usage because insecticides and/ or insecticide treated materials are used throughout Ghana for public health and agricultural pest control purposes (Gerken *et al.* 2001; Obeng-Ofori *et al.* 2002; RBM, 2005; MoH, 2008)). However, further studies would be necessary to determine the association between insecticide usage patterns in these study sites and the development of pyrethroid resistance in the main malaria vector *An. gambiae* s.s.

The current study also determined the distribution of the leucine to phenylalanine knockdown resistance (*kdr*) gene mutation in the M and S molecular forms of the *An. gambiae* s.s. The results obtained suggest high frequency of the *kdr* mutation ranging from 60.24% to 70.21%. This high frequency of the *kdr* gene

mutation observed is most probably accountable for the low level of mortalities observed in field populations with permethrin and deltamethrin insecticides. These results are consistent with earlier studies done in the Greater Accra Region (Adasi *et al.* 2000; Adeniran, 2002; Achonduh, 2005; Kabulah, 2007) and in the Ashanti Region (Stiles-Ocran *et al.* 2003) which also discovered high levels of *kdr* gene mutation. For example, *kdr* gene mutation frequencies of 88.20% and 91.49% were observed at Dodowa and Accra respectively in the Greater Accra Region of the south-eastern Ghana (Adasi & Hemingway, 2008). Similarly, *kdr* gene mutation frequencies ranging from 98 – 100% were observed in the S form of *An. gambiae* s.s in Kumasi in the Ashanti Region, middle belt of Ghana (Yawson *et al.* 2004). In the present study, the *kdr* gene mutation was found in both the M and S molecular forms of the *An. gambiae* s.s even though it was more frequent in the S molecular forms. This high frequency of *kdr* mutations in the S molecular form and its low/absence in the M molecular form has been reported in previous studies in some areas in Ghana (Adasi *et al.* 2000; Adeniran, 2002; Achonduh, 2005; Kabulah, 2007; Yawson *et al.* 2004), Burkina Faso (Diabete' *et al.* 2003, 2004; Yawson *et al.* 2004), Côte d' Ivoire (della Torre *et al.* 2001), Nigeria (Awolola *et al.* 2003) and Mali (Fanello *et al.* 2003). On the other hand, high *kdr* mutation frequency of 63.60% has been recorded recently in the M molecular form of *An. gambiae* s.s in a community in the Greater Accra Region of Ghana (Kabulah, 2007). Also, Corbel *et al.* (2004) recorded 78% of *kdr* mutation frequency in the M molecular form in the Benin Republic. Moreover, previous studies in the urban area of Cotonou indicated *kdr* mutation in both the M and S forms of *An. gambiae* s.s at the same frequency (Akogbeto, 2002). The precise cause of this spatial variation in *kdr* mutation frequencies in the M molecular form of *An. gambiae* s.s is not known. Some studies have suggested that the *kdr* gene mutation reached the M

molecular form from the S molecular form through genetic introgression (Weill *et al.* 2000; Diabete' *et al.* 2004). However, a separate origin of the *kdr* gene mutation in the M molecular form of *An. gambiae* s.s other than the genetic introgression has been reported on the island of Bioko, Equitorial Guinea (Reimer *et al.* 2005). Two earlier studies have also reported separate origin of the *kdr* gene mutation in some member species of the *An. gambiae* s.l. (Diabate *et al.* 2004; Ranson *et al.* 2000). The independent origin of *kdr* gene mutation observed by Reimer *et al.* (2005) may have recently arisen separately in the M molecular form on Bioko due to recent and intensive pyrethroid insecticide application. In this setting, the mutation could not have been said to have reached the M molecular form through introgression because of a complete absence of *kdr* allele in the S form there (Reimer *et al.* 2005).

Toxicologically, pyrethroids are the preferred choice for treating bed nets due to their high efficacy, rapid rate of knockdown, residual and strong mosquito excito-repellent properties and low mammalian toxicity (Diabate *et al.* 2002). It was thought that pyrethroids would not produce resistance due to their rapid toxicological action. In the current study however, resistance was considerably high against permethrin and deltamethrin, which are common active ingredients for treated bednets, aerosols and coils on the Ghanaian market. The full impact of insecticide resistance development in the malaria vectors on malaria vector control efforts is not clear (Takken, 2002). For example, studies in Côte d'Ivoire indicated that even where there is a high frequency of *kdr* mutation in the *An. gambiae,* deltamethrin or permethrin-treated nets give good protection (Darriet *et al.* 1998; Chandre *et al.* 2000). This observation can be attributed to the fact that the reduced excito-repellent effect causes prolonged contact with the insecticide (Chandre *et al.* 2000) and so mosquitoes may still be killed. Thus, while the effect of insecticide resistance development on morbidity and

mortality following continuing use of ITNs in insecticide resistant areas is yet to be fully studied and understood (Takken, 2002), insecticide resistance found in the present study should not hinder the current promotion and use of treated nets for protection against malaria transmission in these areas. Rather, this awareness could guide the choice of right doses of pyrethroid insecticides for intervention strategies such as curtain and net treatments. However, the situation could be different if there is a combination of *kdr* gene mutation and metabolic resistance within individual mosquitoes. Already, a study in the Hohoe District of the Volta Region of Ghana has demonstrated that esterases could be implicated in resistance to pyrethroid insecticide in the *An. gambiae* s.l. (Charo 2005). Additionally, pyrethroid resistance due to enhanced P450 monooxygenase activity has also been observed in the *An. gambiae* s.s. M molecular form from Odumasi in southern Ghana (Müller *et al*. 2007). Monitoring of the *kdr* allele in diverse *An. gambiae* complex populations in addition to surveillance studies on other mechanisms for insecticide resistance will generate valuable data on the resistance status to pyrethroids. Such information may also be useful in establishing the full impact and/or implication of the reported pyrethroid insecticide resistance status in the *An. gambiae* s.l. for the current malaria vector control operations in the current study sites and in the country at large.

CHAPTER SIX

CONCLUSION, LIMITATIONS OF STUDY AND RECOMMENDATIONS FOR FUTURE STUDIES

6.1 Conclusion

The results of the present study demonstrate that *An. gambiae* s.l Gilles is the major human biting *Anopheles* species with the *An. gambiae* s.s. as the sole sibling species of the complex. The molecular forms of the *An. gambiae* s.s. designated the M and S molecular forms are sympatric at all the study communities but the S molecular form was the predominant form; no M/S hybrid was present there. The absence of M/S hybrids implies that the two molecular forms are reproductively isolated.

The human-biting rate of *An. gambiae* s.s is highly seasonal and may differ spatially at areas short distances apart. The biting pattern of *Anopheles* species over a night period differ between the rainy and the dry seasons. *Anopheles gambiae* bites throughout the night in the rainy season but the intensity of biting is time dependent, such that most of the biting occur after midnight and is sustained until daybreak. The biting by *An. gambiae* in the dry season is also higher from midnight to daybreak than from dusk to midnight. The implication of these biting pattern observed is that the risk of malaria parasite transmission may be higher during the night hours after midnight. In the dry season, *An. gambiae* is less aggressive in the fourth quarter of the night (03:00 – 06:00 hours) than in the first quarter with biting being occasionally nil after the 5:00 hour especially when the weather is relatively humid and cold. This means that in the dry season, the risk of malaria parasite transmsion may be higher in the early hours of the night than in the early hours of the morning. Behavioural change communication (BCC) or public education on malaria transmission in the study areas

emphasising the biting habit of the main man biting *Anopheles* mosquito species *Anopheles gambiae* s.s may be instrumental in malaria control efforts.

The results of the present study also indicated that on the whole, a higher proportion of the *An. gambiae* s.s. tends to bite inside human dwellings. This implies that malaria parasite transmission prevention strategies which target endophagic (indoor biting) mosquitoes such as the insecticide treated bednets may be significantly useful in the present study communities. Although a significantly higher proportion of bites occurred indoors, the proportion of the outdoor biting observed cannot be overlooked especially in communities where people tend to spend long hours outdoor. Public education on the outdoor feeding behaviour observed in these study areas may also be useful in encouraging resident to adopt personal protective measures that target outdoor biting such as the use of repellent and protective clothing.

The expectant life of the *An. gambiae* tends to be higher in the dry season than in the rainy season but entomological inoculation rates were higher in the rainy than in the dry season. This notwithstanding, *An. gambiae* s.s is long-lived and has a capacity to be an efficient malaria vector in both the rainy and the dry seasons. This observation may be useful as a baseline data especially during the future expansion of the indoor residual spraying strategy planned by the Ministry of Health, Ghana.

The results obtained in the present study show that the Sunyani and Ahafo Kenyasi populations of *A. gambiae* s.s are resistant to deltamethrin and permethrin insecticides that were tested against them and therefore vector control effort using any of these insecticides may be compromised by insecticide resistance. The results obtained in the present study, therefore, would enable informed choice of insecticides for use in vector control programmes. In addition, the data obtained would provide researchers with information required to carry out further investigations into the

dynamics of insecticide resistance development that would in the long run help in understanding and management of insecticide resistance in the study areas. The results also suggest that *kdr* mutation is responsible for insecticide resistance at all the study communities. This *kdr* mutation is present in both the M and S forms of the *An. gambiae* s.s but is more predominant in the S molecular forms. The evolution of *kdr* mutation in the *An. gambiae* s.s. in these study sites may be due to the recent widespread use of pyrethroids for agricultural and domestic insect control purposes. It seems the *kdr* mutation which was at first associated with the S form of *A. gambiae* s.s. has probably reached the M form through introgressive hybridisation but since no M/S hybrid forms were observed, the chances of introgressive hybridisation being responsible for *kdr* mutation in the M forms may be appreciably small. On the other hand, it may be that the *kdr* mutation in the M forms of *An. gambiae* s.s has arisen independently in response to insecticide use in the study areas.

6.2 Limitations and Recommendation for Further Studies

6.2.1 Malaria transmission

Not much entomological investigations have been done in the Brong Ahafo Region on the diversity of malaria vectors and malaria transmission. The present study has unveiled some findings on malaria transmission that will assist in providing a better malaria control programme in the study areas and possibly the neighbouring communities with similar climatological and ecological conditions. Therefore, there is still the need for further surveys on malaria vectors in all the two main ecological zones of the region. Such future studies should seek to compare entomological data with parasitological and clinical data such as parasite and spleen rates in order to determine the indepth malaria transmission situation in these sites. The Brong Ahafo

Region consists of a degenerating southern semi-deciduous rainforest zone and northern guinea savannah woodlands. The present study was undertaken in the southern part of the region. Studies show that malaria transmission intensity may differ between ecological zones of a particular region. For example, Appawu *et al.* (2004) showed a micro-geographical variation in malaria transmission intensity in a single district in northern Ghana. Also, malaria prevalence differs between the two islands that comprise the archipelago of São Tomé and Príncipe (Pinto *et al.* 2000). A study of the pre-gravid behaviour of female *An. gambiae* from these islands (Charlwood *et al.* 2003) showed that differences in the biology of *An. gambiae*, which happens to be the only vector on the islands, contributed to differences in malaria transmission intensity. Therefore, these recommendations for further studies into the bionomics of *Anopheles* mosquito species between the degenerating southern semi-deciduous rainforest zone and the northern guinea savannah woodlands of the Brong Ahafo Region, Ghana are justified.

6.2.2 Insecticide resistance

Further studies comparing insecticide susceptibility and/ or resistance status of the malaria vectors in the two main ecological zones of the Brong Ahafo Region may also be needed. The outcome of such studies may confirm the observations made on insecticide resistance status and the distribution of the *kdr* mutation in the districts and the region at large. Future research should also establish the origin of the *kdr* mutation in the M forms of *An. gambiae* s.s. It is important to note that the present study similar to most of the studies done in West Africa and Western Kenya, surveyed only for the presence of the *kdr* gene mutation to the exclusion of other possible insecticide resistance mechanisms known to exist. However, it would be necessary to obtain data

on the distribution of other insecticide resistance mechanisms in the present study sites. The future studies should seek to explore the presence of the monooxygenase-based resistance mechanism (Müller *et al.*, 2007) reported in Odumasi in southern Ghana, the esterase-based resistance type found in Hohoe District (also in southern Ghana) [Charo, 2005], the elevated non-specific esterases (Oppenoorth 1985, Roberts and Andre 1994, Scott *et al.* 1998, Vulule *et al.* 1999; Brooke *et al.* 2001), and elevated gluthathione S-transferases (Lagadic *et al.* 1993, Ranson *et al.* 2001) which might also be responsible for some level of pyrethroid resistance in the *An. gambiae* complex. Such an exploration would not only provide data on the distribution of these mechanisms in Ghana but also the possibility of the existence of cross-resistance and multiple insecticide resistance in the malaria vectors. The outcome of such studies will be instrumental in the planning and implementation of large-scale insecticide-based malaria vector contol programmes in the study areas.

REFERENCES

ABUAKU, B.K. KORAM, K.A. & BINKA, F.N. (2005) Antimalarial prescribing practices**:** A challenge to malaria control in Ghana. *Medical Principles and Practices,* **14,** 332-337**.**

ACHONDUH, O.A. (2005) Insecticide resistance in *Anopheles gambiae s.l.* Gilles (Diptera: Culicidae) in cabbage growing areas associated with pyrethroid and organophosphate use in Accra, Ghana. *Master of Philosophy in Entomology Thesis, University of Ghana, Legon,* pp 59-84 & 111-114.

ADAMS, I. DARKO, D. & ACCORSI, S. (2004) Malaria: A burden explored. *Bulletin of Health Information* **1**(1). Retrieved on January 20, 2005 from the website:*www.ghanahealthservice.org/includes/upload/publications/Malaria%20A%20Burden%20Explored.pdf#search=%22Malaria%3A%20A%20burden%20explored.%20Bulletin%20of%20Health%20Information%22.*

ADASI, K. & HEMINGWAY, J. (2008) Susceptibility to three pyrethroids and detection of knockdown resistance mutation in Ghanaian *Anopheles gambiae* sensu stricto. *Journal of Vector Ecology,* **33(2),** 255-262.

ADASI, K. (2000) Studies on insecticide usage and pyrethroid resistance in populations of *Anopheles gambiae* sensu stricto (Diptera: Culicidae) in the Greater Accra Region of Ghana. *A Master of Philosophy Thesis Submitted to the University of Ghana, Legon-Accra.*

ADASI, K. WILSON M.D. & BOAKYE, D.A. (2000) Studies on insecticide usage and pyrethroid resistance in populations of *Anopheles gambiae* s.s. (Diptera: Culicidae) in the Greater Accra Region of Ghana. *WHO-MIM Conference on Insecticide Resistance, Harare, Zimbabwe.*

ADENIRAN, T.A. (2002) Studies on insecticide usage and pyrethroid resistance in populations of *Anopheles gambiae* s.s (Diptera: Culicidae) in the Greater

Accra Region, Ghana. *Master of Philosophy in Entomology Thesis, University of Ghana, Legon*, pp 63-83.

ADJEI, G.O. KURTZHALS, J.A.L. RODRIGUES, O.P. LOTTE M.A. HOEGBERG, C.G. KITCHER, E.D. BADOE, E.V. LAMPTEY, R. & GOKA, B.Q. (2008) Amodiaquine-artesunate *vs* artemether-lumefantrine for uncomplicated malaria in Ghanaian children: a randomized efficacy and safety trial with one year follow-up. *Malaria Journal*, Available from the website of the Malaria Journal http://www.malariajournal.com/content/pdf/1475-2875-7-127.pdf

ADUNGO, N. I. MAHADEVAN, S. MULAYA, N.L. SITUBI A.P. AND GITHURE J.I. (1991) Comparative Determination of *plasmodium falciparum* Sporozoite Rates in Afrotropical *Anopheles* from Kenya by Dissection and ELISA. *Annals of Tropical Medicine and Parasitology*, **85,** 387-394.

AFRANE, Y.A. KLINKENBERG, E. DRECHSEL, P. OWUSU-DAAKU, K. GARMS, R. & KRUPPA, T. (2004) Does irrigated urban Agriculture influence the transmission of malaria in the city of Kumasi, Ghana? *Acta Tropica*, **89:** 125-134.

AFU, S. (1999) Incidence of substandard drugs in developing countries (letter). *Tropical Medicine and Internal Health*, 4, 73.

AGBOLADE, O.M. AKINBOYE, D.O. OLATEJU, T.M. AYANBIYI, O.A. KULOYO, O.O. & FENUGA O.O. (2006) Biting of anthropophilic *Culicoides fulvithorax* (Diptera: Ceratopogonidae), a vector of *Mansonella perstans* in Nigeria. *Korean Journal of Parasitology*, **44(1),** 67 - 72.

AHORLU, C.K. DUNYO, S.K. AFARI, E.A. KORAM, K.A. & NKRUMAH, F.K. (1997) Malaria-related beliefs and behaviour in southern Ghana: implications for treatment, prevention and control. *Tropical Medicine and International Health*, **2,** 488-499.

AKOGBETO, M. & ROMANO, R. (1999) Infectivity of *Anopheles melas* vis-à-vis *Plasmodium falciparum* in the coastal lagoon area of Benin. *Bulletin de la Socite Pathologie Exotique,* **92**(1), 57-61.

AKOGBETO, M. & YAKOUBOU, S. (1999) Resistance of malaria vectors to pyrethroids used for impregnated bednets in Benin, West Africa. *Bulletin de la Socite Pathologie Exotique,* **92,** 123–130.

AKOGBETO, M. (1995) Entomological study on the malaria transmission in coastal and lagoon areas: the case of a village built on brackish lake. *Ann. Soc. Belg. Med. Trop.* **75**(3), 219-227.

AKOGBETO, M. (2000) Lagoonal and coastal malaria at Cotonou: entomological findings. *Sante* **10**(4), 267-275.

AKOGBÉTO, M. (2002) *Pyrethroid resistance in Anopheles gambiae in West Africa (Communication orale n°569).* The third MIM Pan-African Malaria Conference, Arusha, Tanzanie, pp 319-320.

ALONSO, P.L. LINDSAY S.W. ARMSTRONG, J.R. CONTEH, M. HILL, A.G. DAVID, P.H. FEGAN, G. DE FRANCISCO, A. HALL, A.J. SHENTON, F.C., GREENWOOD, B.M., CONTEH, M., CHAM, K., HILL, A.G., DAVID, P.H., FEGAN, G. & HALL, A.J. (1991) The effect of insecticide-treated bed nets on mortality of Gambian children. *Lancet,* **337,** 1499-1502.

ALONSO, P.L. LINDSAY, S.W. ARMSTRONG SCHELLENBERG, J.R. KEITA, K. GOMEZ, P. SHENTON, F.C. HILL, A.G. DAVID, P.H. FEGAN, G. CHAM, K. & **GREENWHOOD, B.W. (1993)** A malaria control trial using insecticide-treated bed nets and targeted chemoprophylaxis in a rural area of The Gambia, west Africa. 6. The impact of the interventions on mortality and morbidity from malaria. *Transactions of the Royal society of Tropical Medicine and Hygiene,* **87(Suppl 2)**, 37-44.

ALTO, B.W. & JULIANO, S.A. (2001) Temperature effects on the dynamics of *Aedes albopictus* (Diptera: Culicidae) populations in the laboratory. *Journal of Medical Entomology*, **38**, 548-556.

ANTONIO-NKONDJIO, C. AWONO-AMBENE, P. TOTO, J.C. MEUNIER, J.Y. ZEBAZE-KEMLEU, S. NYAMBAM, R. WONDJI, C.S. TCHUINKAM, T. FONTENILLE, D. (2002) High malaria transmission intensity in a village close to Yaounde, the capital city of Cameroon. *Journal of Medical Entomology*, **39,** 350–355.

APPAWU, M. OWUSU-AGYEI, S. DADZIE, S. ASOALA, V. ANTO, F. KORAM, K. ROGERS, W. NKRUMAH, F. HOFFMAN, S.L. FRYAUFF, D.J. (2004) Malaria transmission dynamics at a site in northern Ghana proposed for testing malaria vaccines. *Tropical Medicine and International Health*, **9,** 164-170.

APPAWU, M.A. (2005) Genetic diversity of the *Anopheles* gambiae s.s. (Diptera: Culicidae) and malaria transmission dynamics at selected sites in the Kasena Nankana District. *Doctor of Philosophy in Entomology Thesis, University of Ghana, Legon-Accra.*

APPAWU, M.A. BAFFOE-WILMOT, A. AFARI, E.A. DUNYO, S. KORAM, K.A. & NKRUMAH, F.K. (2001) Malaria vector studies in two ecological zones in southern Ghana. *African Entomology*, **9,** 59–65.

APPAWU, M.A. BAFFOE-WILMOT, A. AFARI, E.A. NKRUMAH, F.K. & PETRARCA, V. (1994) Species composition and inversion polymorphism of the *Anopheles gambiae* sensu lacto in some sites of Ghana, West Africa. *Acta Tropica*, **56**, 15-23.

ASANTE, F.A. & ASENSO-OKYERE, K. (2003) Economic burden of malaria in Ghana. *A Technical Report Submitted to the World Health Organisation,*

(WHO), African Regional Office (AFRO). Institute of Statistical, Social and Economic Research (ISSER), University of Ghana, Legon.

ASENSO-OKYERE, W.K. & DZATOR, J.A. (1997) Household cost of seeking malaria care. A retrospective study of two districts in Ghana. *Social Science and Medicine*, **45(5)**, 659-667.

AWOLOLA, T.S. BROOKE, B.D. KOEKEMOER, L.L. & COETZEE, M. (2003) Absence of the *kdr* mutation in the molecular 'M' form suggests different pyrethroid resistance mechanisms in the malaria vector mosquito *Anopheles gambiae* sensu stricto. *Tropical Medicine and International Health*, **8**(**5**), 420-422.

AWOLOLA, T.S. ODUOLA, O.A. STRODE, C. KOEKEMOER, L.L. BROOKE, B. RANSON H. (2008) Evidence of multiple pyrethroid resistance mechanisms in the malaria vector *Anopheles gambiae* sensu stricto from Nigeria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, doi:10.1016/j.trstmh.2008.08.021.

AWOLOLA, T.S. OKWA, P. HUNT, R.H. OGUNRINADE, A.F. COETZEE, M. (2002) Dynamics of the Malaria-vector Populations in Coastal Lagos, South-western Nigeria. *Annals of Tropical Medicine and Parasitology*, **96(1),** 75-82

AWONO-AMBENE, H. KENGNE, P. SIMARD, F. ANTONIO-NKONDJIO, C. FONTENILLE, D. (2004) Description and bionomics of *Anopheles* (Cellia) ovengensis (Diptera: Culicidae), a new malaria vector species of the *Anopheles nili* group from south Cameroon. Journal of Medical Entomology, 41: *in press*.

BAIRD, J.K. (1995) Host age as a determinant of naturally acquired immunity to *Plasmodium falciparum*. *Parasitology today* **11(3)**, 105-111.

BANERJEE, A. & NAYAK, B. (2001) Epidemiological and Entomological correlation of Malaria transmission in an Air Force station. *Medical Journal of the Armed Forces of India,* **57**(3), 191-3.

BANERJEE, A. NAYAK, B. (2002) Deltamethrin Impregnated Mosquito Nets: An Experimental Study in an Air Force Station in Central India. *Medical Journal of the Armed Forces of India*, **58**(1), 3-4.

BARBER, M. A. & RICE, J. B. (1937) A survey of malaria in Egypt. *American Journal of Tropical Medicine and Hygeine* **17,** 413.

BASCO, L.K. (2004) Molecular epidemiology of malaria in Cameroon. XIX. Quality of antimalarial drugs used for self-medication. *American Journal of Tropical Medicine and Hygeine*, **70(3)**, 245-250.

BEEBE, N.W. & COOPER, R.D. (2000) Systematics of malaria vectors with particular reference to *Anopheles punctulatus group. International Journal for Parasitology,* **30,** 1-17.

BEIER, J.C. & KOROS, J. (1991) Visual assessment of sporozoite and blood meal enzyme-linked immunosorbent assay (ELISA) samples in malaria field studies. *Journal of Medical Entomology,* **28,** 805-808.

BEIER, J.C. ASIAGO, C.M. ONYANGO, F.K. & KOROS, J.K. (1988) ELISA absorbance cut-off method affects malaria sporozoite rate determination in wild Afrotropical *Anopheles. Medical and Veterinary Entomology*, **2(3)**, 259-64.

BEIER, J.C. KILLEEN, G.F. & GITHURE, J.I. (1999) Short report: entomologic inoculation rates and *Plasmodium falciparum* malaria prevalence in Africa. *American Journal of Tropical Medicine and Hygeine***, 61(1)**, 109-13.

BEIER, J.C. PERKINS, P.V. WIRTZ, R.A. KOROS, J. DIGGS, D. GARDAN, T.P. & KOECH, D.K. (1988) Blood meal identification by direct enzyme

linked immunosorbent assay (ELISA) tested on *Anopheles* (Diptera: Culicidae) in Kenya. *Journal of Medical Entomology,* **25,** 9-16.

BESANSKY, N.J. HILL, C.A. & COSTANTINI, C. (2004) No accounting for taste: host preference in malaria vectors. *Trends Parasitology,* **20,** 249-251.

BESANSKY, N.J. LEHMANN, T. FAHEY, G.T. FONTENILLE, D. BRAACK, L.E.O. HAWLEY, W.A. & COLLINS, F.H. (1997) Patterns of mitochondrial variation within and between African malaria vectors, *Anopheles gambiae* and *Anopheles arabiensis*, suggest extensive gene flow. *Genetics,* **147,** 1817-1828.

BINKA, F.N. KUBAJE, A. ADJVIK, M. WILLIAMS, L.A. LENGELER, C. MAUDE, G.H. ARMAH, G.E. KAJIHARA, B. ADIAMAH, J.H. & SMITH P.G. (1996) Impact of permethrin impregnated bed nets on child mortality in Kassena-Kankana district, Ghana: a randomised controlled trial. *Tropical Medicine and International Health*, **1**, 147-54.

BINKA, F.N. MORRIS, S.S. ROSS D.A. ARTHUR, P. & ARYEETEY, M.E. (1994) Patterns of malaria morbidity and mortality in children in northern Ghana. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **88(4)**, 381–385.

BLACK, W.C. & LANZARO, G.C. (2001) Distribution of genetic variation among the chromosomal forms of the *Anopheles gambiae s.s.:* introgressive hybridisation, adaptive inversions, or recent reproductive isolation? *Insect Molecular Biology,* **10**, 3-7.

BOUMA, M.J. DYE, C. & VAN DER KAAY, H.J. (1996) *Falciparum* malaria and climatic change in the northwest frontier province of Pakistan. *American Journal of Tropical Medicine and Hygiene,* **55**, 131-137.

BREAUD, T.P. (1993) Insecticide resistance in Florida mosquitoes. *Journal of Florida Mosquito Control Association,* **64**, 14-21.

BREMAN, J.G. ALILIO, M.S. & MILLS, A. (2004) Conquering the intolerable burden of malaria: what's new, what is needed: A summary. *American Journal of Tropical Medicine and Hygeine,* **71(2),** 1-15.

BROGDON, W.C. & MCALLISTER, J.C. (1998) Insecticide resistance and vector control. *Emerging Infectious Diseases*, **4,** 605-613.

BROGDON, W.G. & BARBER, A.M. (1990) Fenitrothion-deltamethrin cross resistance conferred by esterases in Guatemalan *Anopheles albimanus*. *Pesticides Biochemistry & Physiology,* **37,** 130–139.

BROGDON, W.G. BEACH, R.F. STEWART, J.M. & CASTANAZA, L. (1988b) Microplate assay analysis of the distribution of organophosphate and carbamate resistance in Guatemalan *Anopheles albimanus*. *Bulletin of the World Health Organisation,* **66,** 39-46.

BROGDON, W.G. HOBBS, J.H. ST. JEAN, Y. JACQUES, J.R. & CHARLES, L.B (1988a) Microplate assay analysis of reduced fenitrothion susceptibility in Haitian *Anopheles albimanus. Journal of America Mosquito Control Association,* **4**, 152-8.

BROGDON, W.G. HOBBS, J.H. ST. JEAN, Y. JACQUES, J.R. & CHARLES, L.B. (1988) Microplate assay analysis of reduced fenitrothion susceptibility in Haitian *Anopheles albimanus. Journal of American Mosquito Control Association,* **4,**152-8.

BROOKE, B.D. KLOKE, G. HUNT, R.H. KOEKEMOER, L.L. TEMU, E.A. TAYLOR, M.E. SMALL, G. HEMINGWAY, J. & COETZEE, M. (2001) Bioassay and Biochemical Analyses of Insecticide Resistance in Southern African *Anopheles funestus* (Diptera:Culicidae). *Bulletin of Entomological Research,* **91(4),** 265-72.

BRUCE-CHWATT, L.J. & DE ZULUETA, J. (1981) The Rise and Fall of Malaria in Europe. *A Historico-Epidemiological Study. Oxford University Press, New York*

BRUCE-CHWATT, L.J. (1985) Essential Malarialogy, William Heinemann Medical Books, London.

BRYAN, J.H. (1983) *Anopheles gambiae* and *Anopheles melas* at Brefet, the Gambia, and their role in malaria transmission. *Annals of Tropical Medicine and Parasitology,* **77(1),** 1-12.

BRYAN, J.H. DI DECO, M.A. PETRARCA, V. & BULLINI, L. (1982) Inversion polymorphism and incipient speciation in *Anopheles gambiae* s.s. in the Gambia, West Africa. *Genetica, 59,* 167–176.

BURKOT, T.R. & GRAVES, P.M. (1995) The value of vector-based estimation of malaria transmission. *Annal of Tropical Medicine and Parasitology, 89,* 125-134.

BURKOT, T.R. WILLIAMS, J.L. & SCHNEIDER, I. (1984) Identification of Plasmodium falciparum-infected mosquitoes by a double antibody enzyme-linked immunosorbent assay. *American Journal of Tropical Medicine and Hygeine*, **33,** 783–788.

BUYNANVANICH, S. & LANDRIGAN, C.P. (2003) ‘‘The impact of climate change on child health”. *Ambulatory Pediatrics*, **3(1)**, 44-52.

CACCONE, A. GARCIA, B.A. & POWELL, J.R. (1996) Evolution of the mitochondrial DNA control region in the *Anopheles gambiae* complex. *Insect Molecular Biology*, **5**, 51-59.

CARINO, F. KOENER, J.F. PLAPP, J.R. F.W. FEYEREISEN, R. (1994) Constitutive overexpression of the cytochrome p450 gene CYP6A1 in a house fly strain with metabolic resistance to insecticides. *Insect Biochemistry and Molecular Biology*, 24, 411–418.

CARLSON, D.A. & SERVICE, M.W. (1980) Identification of mosquitoes of *Anopheles gambiae* species complex a and b by analysis of cuticular components. *Science,* **207,** 1089-1091.

CARNEVALE, P. LE GOFF, G. TOTO, J.C. ROBERT, V. (1992a) *Anopheles nili* as the main vector of human malaria in villages of southern Cameroon. *Medical and Veterinary Entomology*, **6,** 135–8.

CARNEVALE, P. ROBERT, V. AND MOUCHET, J. (1992b) The Biology of Vectors and Malaria Control. Laveran Journal No. 1, Les Pensières, Veyrier-du-Lac, France.

CARRARA, G., PETRARCA, V. NIANG, M. & COLUZZI, M. (1990) *Anopheles pharoensis* and transmission of *Plasmodium falciparum* in the Senegal River Delta. *Medical and Veterinary Entomology,* **4,** 421–424.

CASIMIRO, S., COLEMAN, M., HEMINGWAY, J., SHARP, B. (2006) Insecticide resistance in *Anopheles arabiensis* and *Anopheles gambiae* from Mozambique. *Journal of Medical Entomology*, **43(2),** 276–282**.**

CDC, CENTERS FOR DISEASE CONTROL AND PREVENTION, (2004a) *Frequently Asked Questions about Malaria*. National Center for Infectious Diseases, Division of Parasitic Diseases. Retrieved on October 21, 2004 from the website: *http://www.cdc.gov/malaria/faq.htm*

CDC, CENTRE FOR DISEASE CONTROL AND PREVENTION, (2004b) *Anopheles* mosquitoes. Retrieved on December 29, 2004 from the website: http:www.cdc.gov/malaria/biology/mosquito/

CDC, CENTRE FOR DISEASE CONTROL AND PREVENTION, (2004c) Malaria. Retrieved on December 29, 2004 from the website: http:www.cdc.gov/malaria/diagnosis-treatment/diagnosis.htm/

CHANDRE, F. DARRIET, F. DARDER, M. CUANY, A. DOANNIO, J.M.C. PASTEUR, N. & GUILLET, P. (1998) Pyrethroid resistance in Culex

quinquefasciatus from West Africa. *Medical and Veterinary Entomology,* **12(4),** 359-366.

CHANDRE, F. DARRIET, F. DUCHON, S. FINOT, L. MANGUIN, S. CARNEVALE, P. & GUILLET, P. (2000) Modification of pyrethroid effects associated with kdr mutation in *Anopheles gambiae. Medical and Veterinary Entomology*, **14**, 81–88.

CHANDRE, F. DARRIET, F. MANGUIN, S. BRENGUES, C. CARNEVALE, P. & GUILLET, P. (1999a) Pyrethroid cross-resistance spectrum among populations of *Anopheles gambiae* s.s. from Cote d'Ivoire. *Journal of American Mosquito Control Association,* **15**, 53–59.

CHANDRE, F. MANGIUN, S. BRENGUES, C. DOSSOU YOVO, J. DARRIET, F. DIABETE, A. CARNEVALE, P. & GUILLET, P. (1999b) Current distribution of a pyrethroid resistance gene in *Anopheles gambiae* complex from West Africa and further evidence for reproductive isolation of the Mopti form. *Parasitologia,* **41,** 319-322.

CHARLWOOD, J. D. SMITH, T. BILLINGSLEY, P. F. TAKKEN, W. LYIMO, E.O.K. & MEUWISSEN. J.M.E.T. (1997) Survival and Infection probabilities of anthropophagic anophelines from an area of high prevalence of *Plasmodium falciparum* in humans. *Bulletin of Entomological Research,* **87**, 453-455.

CHARLWOOD, J.D. & JONES, D.R. (1979) Mating behaviour in the mosquito, *Anopheles gambiae* s.l. I. close range and contact behaviour. Physiological Entomology, **4,** 111-120.

CHARLWOOD, J.D. PINTO, J. SOUSA, C.A. FERREIRA, C. PETRARCA, V. & DO E ROSARIO, V. (2003) 'A mate or a meal' – Pre-gravid behaviour of female *Anopheles gambiae* from the islands of São Tomé and Príncipe, West

Africa. *Malaria Journal*, Retrieved on June 4, 2003 from the malaria journal website: *http://www.malariajournal.com/content/2/1/9*.

CHARO, S.K. (2005) Resistance mechanisms and susceptibility to organophosphates, carbamates, and pyrethroids in *Anopheles gambiae* s.l. Giles (Diptera: Culicidae) in Hohoe District, Ghana. *Master of Philosophy in Entomology Thesis, University of Ghana, Legon-Accra*.

CHINERY, W.A. (1984) Effect of ecological changes on the malaria vectors *Anopheles funestus* and the *Anopheles gambiae* complex of mosquitoes in Accra, Ghana. *Journal of Tropical Medicine and Hygiene,* **87,** 75-81.

CHOI, H.W. BREMAN, J.G. TEUTSCH, S.M. LIU, S. HIGHTOWER, A.W. SEXTON, J.D. (1995) The effectiveness of insecticide-impregnated bed nets in reducing cases of malaria infection: a meta-analysis of published results. *American Journal of Tropical Medicine and Hygiene,* **52,** 377-382.

CLEMENTS, A.N. & PATERSON, G.D. (1981) The analysis of mortality and survival rates in wild population of mosquitoes. *Journal of Applied Ecology,* **18,** 373-399.

COCKBURN, A.F. & SEAWRIGHT, J.A. (1988) Techniques for mitochondrial and ribosomal DNA analysis of anopheleine mosquitoes. *Journal of American Mosquito Control Association,* **4(3)**, 261-265.

COETZEE, M. CRAIG, L.E. & SUEUR, D. (2000) Distribution of African malaria mosquitoes belonging to the *Anopheles gambiae* complex. *Parasitology Today,* **16**, 74–77.

COHUET, A. SIMARD, F. TOTO, J.-C., KENGNE, P. COETZEE, M. FONTENILLE, D. (2003) Species identification within the *Anopheles funestus* group of malaria vectors in Cameroon, and evidence for a new species. *American Journal of Tropical Medicine and Hygiene,* **69,** 200–205.

COLEMAN, M. SHARP, B. SEOCHARAN, I. HEMINGWAY, J. (2006) Developing an evidence-based decision support system for rational insecticide choice in the control of African malaria vectors. *Journal of Medical Entomology*, **43,** 663-8.

COLLINS, F.H. & PASKEWITZ S.M. (1996) A review of the use of ribosomal DNA (rDNA) to differentiate among cryptic *Anopheles* species. *Insect molecular Biology*, **5(1),** 1-9.

COLLINS, F.H. & PASKEWITZ, S.M. (1997) PCR Amplification of Insect Ribosomal DNA. Crampton J.M. Beard C.B. Louis C, Eds. The Molecular Biology of Insect Disease Vectors. A methods manual. London: Chapman and Hall, 374-383.

COLLINS, F.H. FINNERTY, V. & PETRARCA, V. (1988) Ribosomal DNA probes differentiate five cryptic species in the *Anopheles gambiae* complex. *Parasitologia,* **30,** 231-240.

COLLINS, F.H. MENDEZ, M.A. RAZMUSSEN, M.O. MEHAFFEY, P.C. BESANSKY, NJ, & FINNERTY, V (1987) A ribosomal gene differentiates Members species of the *Anopheles gambiae* complex. *American Journal of Tropical Medicine and Hygeine,* **37**, 37-41.

COLLINS, F.H. SAUNDERS, R.D. KAFATOS, F.C. ROTH, C. KE Z. WANG, X. DVMBROWSKI, K. TOM, L. & HOGAN, J. (1999) Genetics in the study of mosquito susceptibility to *Plasmodium*. *Parassitologia,* **41**, 163-168

COLUZZI, M. (1984) Heterogeneities of the malaria vectorial system in tropical Africa and their significance in malaria epidemiology and comtrol. *Bulletin of World Health Organisation,* **62,** *107-113.*

COLUZZI, M. & SABATINI, A. (1968) Cytogenetic observations on the salt-water species *Anopheles merus* and *Anopheles melas* of the *gambiae* complex. *Parasitologia*, **10,** 155-156**.**

COLUZZI, M. SABATINI A. PETRARCA V. AND DI DECO M. A. (1977) Behavioural divergences between mosquitoes with different inversion karyotypes in polymorphic populations of the *Anopheles gambiae* s.l. *Nature,* **266**, 832-833.

COLUZZI, M. SABATINI, A. PETRARCA, V. & DI DECO, M.A. (1979) Chromosomal differentiation and adaptation to human environments in the *Anopheles gambiae* complex. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **73**, 483–497.

COLUZZI, M.A. PETRARCA, V. & DI DECO, M.A. (1985) Chromosomal inversion intergradation and incipient speciation in *Anopheles gambiae*. *Bolletino di Zoologia,* **52**, 45–63.

CONN, J.E, BOLLBACK, J.P, ONYABE, D.Y. ROBINSON, T.N, WILKERSON, R.C & PO'VOA, M.M. (2001) Isolation of polymorphic microsatellite markers from the malaria vector *Anopheles darlingi*. *Molecular Ecology Notes,* **1,** 223-225.

CONWAY, G.R. TRPIS, M. & MCCLELLAND, G.A.H. (1974) Population parameters of the mosquito *Aedes aegypti* (L.) estimated by mark-release-recapture in the suburban habitat in Tanzania. *Journal of Animal Ecology,* **43,** 289-304.

CORBEL, V. CHANDRE, F. BRENGUES, C. AKOGBETO, M. LARDEUX, F. HOUGARD, J.M. & GUILLET, P. (2004) Dosage-dependent effects of permethrin-treated nets on the behaviour of *Anopheles gambiae* and the selection of pyrethroid resistance. *Malaria Journal,* **3,** 22.

CORBEL, V. N'GUESSAN, R. BRENGUES, C. CHANDRE, F. DJOGBENOU, L. MARTIN, T. AKOGB´ETO, M. HOUGARD, J.M. ROWLAND M. (2007) Multiple insecticide resistance mechanisms in *Anopheles gambiae* and *Culex quinquefasciatus* from Benin, West Africa. *Acta Trop,* **101**, 207-16.

COSTANTINI, C. S. G. LI, A. DELLA TORRE, N. SAGNON, M. COLUZZI, & C. TAYLOR. (1996) Density, survival and dispersal of *Anopheles gambiae* complex mosquitoes in a West African Sudan Savanna village. *Medical and Veterinary Entomology*, 10: 203-219.

COTRAN, R.S. COLLINS, T. & KUMAR, V. (1998) Robbins Pathological Basis of Disease. Elsevier Science.

COX, F.E.G. (1991) Systematics of parasitic protozoa. In: Krier, J.P. & Baker, J.R. (eds). *Parasitic Protozoa.* Academic Press, San Diego, **1**, 55-80.

COX-SINGH, J., DAVIS, T.M., LEE, K.S., SHAMSUL, S.S., MATUSOP, A., RATNAM, S., RAHMAN, H.A., CONWAY, D.J., SINGH, B. (2008) *Plasmodium knowlesi* malaria in humans is widely distributed and potentially life threatening. *Clinical Infectious Diseases*; **46**,165–71.

CRANS, W.J. (1973) Experimental infection of *Anopheles gambiae* and *Culex pipiens* fatigans with *Wuchereria bancrofti* in coastal East Africa. *Journal of Medical Entomology*, **10**, 189–193.

CUAMBA, N. CHOI, K.S. & TOWNSON, H. (2006) Malaria vectors in Angola: distribution of species and molecular forms of the *Anopheles gambiae* complex, their pyrethroid insecticide knockdown resistance *(kdr)* status and *Plasmodium falciparum* sporozoite rates. *Malaria Journal*, **5, 2**

D'ALESSANDRO, U. OLALEYE, B. LANGEROCK, P., BENNETT, S., CHAM, K., CHAM, B. & GREENWOOD, B.M.ET AL. (1997) The Gambian National Impregnation Bed Net Programme: evaluation of effectiveness by means of case-control studies. *Transactions of the Royal society of Tropical Medicine and Hygiene*, **91,** 638-42.

D'AVANZO, N.J. MORRIS, V.M. CARTER, T.R. MAILLARD, J.M. SCANLON, P.M. STENNIES, G.M. WILSON, M. MACDONALD, P.D.M. & NEWMAN, R.D. (2002) Congenital Malaria as a Result of

Plasmodium malariae. Journal of the American Mosquito Control Association, **287,** 1520-1521.

DARRIET, F. GUILLET, P. N'GUESSAN, R. DOANNIO, J.M. KOFFI, A. KONAN, L.Y. & CARNEVALE, P. (1998) Impact of resistance of *Anopheles gambiae* s.s. to permethrin and deltamethrin on the Efficacy of impregnated mosquito nets. *Med Trop*, **58(4),** 349-54.

DAVIDSON, G. & HUNT, R.H. (1973) The crossing characteristics of a new sixth species in the *Anopheles gambiae* complex. *Parasitologia* **15,** 121-128.

DAVIDSON, G. (1954) Estimation of the survival rate of anopheline mosquitoes in nature. *Nature,* **174,** 792–793.

DAVIS, J.R. HALL, T. CHEE, E.M. MAJALA, A. MINJAS, J. & SHIFF, C.J. (1995) Comparison of sampling anopheline mosquitoes by light-trap and human-bait collection indoors at Baramoyo, Tanzania. *Medical and Veleterinaty Entomology,* **9,** 249-255.

DAVIS, J.R. HALL, T. CHEF, E.M. MAJALA, A. MINJAS, J. SHIFF, C.J. (2008) Comparison of Sampling anopheline mosquitoes by light-trap and human-bait Collections Indoors at Bagamoyo, Tanzania. *Medical and Veterinary Entomology,* **9(3),** 249-255.

DE MEILLON, B. **(1934)** Observation on *Anopheles funestus* and *Anopheles gambiae* in the Transvaal. *Publications of the South African Institute for Medical Research,* **6**, 195.

DE MEILLON, B. VAN EEDEN, G.J. COETZEE, L. COETZEE, M. MEISWINKEL, R. DU TOIT, C.L.N. HANSFORD, C.F. (1977) Observation on a species of the Anopheles funestus subgroup, a suspected exophilic vector of malaria parasites in northeastern Transvaal, South Africa. *Mosquito News,* **37,** 657–61.

DE MERIDA, A.M. PALMIERI, M. YURRITA, M. MOLINA, A. MOLINA, E. & BLACK, W.C. (1999) Mitochondrial DNA variation among *Anopheles albimanus* populations. *American Journal of Tropical Medicine and Hygiene,* **61,** 230–39.

DE MOOR, P.P. & STEFFENS, F.E. (1970) A computer-simulated model of an arthropod-borne virus transmission cycle, with special reference to chikungunya virus. *Transactions of the Royal society of Tropical Medicine and Hygiene*, **64,** 927-934.

DELLA TORRE, A. FANELLO, C. AKOGBETO, M. DOSSOU-YOVO, J. FAVIA, G. PETRARCA, V. & COLUZZI, M. (2001) Molecular evidence of incipient speciation within *Anopheles gambiae s.s.* West Africa. *Insect Molecular Biology*, **10**, 9-18.

DIA, I., KONATE, L., SAMB, B., SARR, J.B., DIOP, A., ROGERIE, F., FAYE, M., RIVEAU, G., REMOUE, F., DIALLO, M., FONTENILLE, D. (2008) Bionomics of malaria vectors and relationship with malaria transmission and epidemiology in three physiographic zones in the Senegal River Basin. *Acta Tropica*, **105**,145-153.

DIABATE A, BALDET T, CHANDRE F, AKOGBÉTO M, GUIGUEMDE RT, DARRIET F, BRENGUES C, GUILLET P, HEMINGWAY J, GRAHAM JS, HOUGARD JM (2002) The role of agricultural use of insecticides in resistance to pyrethroids in *Anopheles gambiae s.l.* in Burkina Faso. *American Journal of Tropical Medicine and Hygiene,* **67,** 617-622.

DIABATE, A. BALDET, T. BRENGUES, C. KENGNE, P. DABIRE, K.R. SIMARD, F. & CHANDRE, **F. (2003a)** Natural swarming behaviour of the molecular M form of *Anopheles gambiae. Transactions of the Royal society of Tropical Medicine and Hygiene,* **97**, 713–716.

DIABATÉ, A. BALDET, T. CHANDRE, C. DABIRE, K.R. KENGNE, P. GUIGUEMDE, T.R. SIMARD, F. GUILLET, P. HEMINGWAY, J. & HOUGARD, J.M. (2003b) KDR mutation, a genetic marker to assess events of introgression between the molecular M and S forms of *Anopheles gambiae* (Diptera: Culicidae) in the tropical savannah area of West Africa. *Journal of Medical Entomology*, **40,** 195–198.

DIABATÉ, A. BRENGUES, C. BALDET, T. DABIRÉ, K.R. HOUGARD, J.M. AKOGBETO, M. KENGNE, P. SIMARD, F. GUILLET, P. HEMINGWAY, J. & CHANDRE, F. (2004) The spread of the Leu-Phe *kdr* mutation through *Anopheles gambiae* complex in Burkina Faso: genetic introgression and de novo phenomena. *Tropical Medicine and International Health*, **9(12)**, 1267-1273.

DIABATÉ, A. DABIRÉ, R.K. KIM, E.H. DALTON, R. MILLOGO, N. BALDET, T. SIMARD, F. GIMNIG, J.E. HAWLEY, W.A. & LEHMAN, T. (2005) Larval development of the molecular forms of *Anopheles gambiae* (Diptera: Culicidae) in different habitats: A transplantation experiment. *Journal of Medical Entomology*, **42(4),** 548-553.

DIETZ, K. MOLINEAUX, L. & THOMAS, A. (1974) A malaria model tested in the African savannah. *Bulletin of the World Health Organisation*, **50,** 347-357.

DIOP, A. MOLEZ, J.F. KONATE, L. FONTENILLE, D. GAYE, O. DIOUF, M. DIAGNE, M. & FAYE, O. (2002) Role of *Anopheles melas* Theobald (1930) in malaria transmission in a mangrove swamp in Saloum (Senegal). *Parasite*, **9(3),** 239-246.

DONNELLY, M.J. PINTO, J. GIROD, R. BESANSKY, N.J. & LEHMANN, T. (2004) Revisiting the role of introgression vs shared ancestral polymorphisms as key processes shaping genetic diversity in the recently separated sibling species of the *Anopheles gambiae* complex. *Heredity*, **92**, 61–68.

EDILLO, F.E. TRIPET, F. TOURÉ, Y.T. LANZARO, G.C. DOLO, G. & TAYLOR, C.E. (2006) Water quality and immatures of the M and S forms of *Anopheles gambiae* s.s and *Anopheles arabiensis* in a Malian village. *Malaria Journal,* **5,** 35.

EDILLO, F.E. TRIPET, F. TOURÉ, Y.T. LANZARO, G.C. DOLO, G. & TAYLOR, E.T. (2002) Spatial distribution of *Anopheles gambiae* and *Anopheles arabiensis* (Diptera: Culicidae) in Bahaman village, Mali. *Journal of Medical Entomology*, **39,** 70-77.

ELISSA, N. MOUCHET, J. RIVIERE, F. MEUNIER, J.Y. & YAO K (1993) Resistance of *Anopheles gambiae* s.s. to pyrethroids in Cote D'Ivoire. *Annales de la Societe Belge de Medecine Tropicale,* **73**, 291–294.

ELISSA, N. MOUCHET, J. RIVIERE, F. MEUNIER, J.Y. & YAO, K. (1994) Susceptibility of *Anopheles gambiae* to insecticides in the Ivory Coast . *Sante,* **4,** 95–99.

ETANG, J. FONDJO, E. CHANDRE, F. MORLAIS, I. BRENGUES, C. NWANE, P. CHOUAIBOU, M. NDJEMAI, H. & SIMARD, F. (2006) Short report: first report of knockdown mutations in the malaria vector *Anopheles gambiae* from Cameroon. *American Journal of Tropical Medicine and Hygiene,* **74(5),** 795–797.

FAILLOUX, A.B. RAYMOND, M. UNG, A. GLAZIOU, P. MARTIN, P.M. & PASTEUR, N. (1995) Variation in the vector competence of *Aedes polynesiensis* for *Wuchereria bancrofti*. *Parasitology,* **111**, 19–29.

FAILLOUX, A.B. RAYMOND, M. UNG, A. GLAZIOU, P. MARTIN, P.M. & PASTEUR, N. (1995) Variation in the vector competence of *Aedes polynesiensis* for *Wuchereria bancrofti*. *Parasitology* **111**, 19–29.

FANELLO, C. PETRARCA, V. DELLA TORRE, A. SANTOLAMAZZA, F. DOLO, G. COULIBALY, M. ALLOUECHE, A. CURTIS, C.F. TOURÉ,

Y.T. & COLUZZI, M. (2003) The pyrethroid knock-down resistance gene in the *Anopheles gambiae* complex in Mali and further indication of incipient speciation within *Anopheles gambiae* s.s.. *Insect Molecular Biology*, **12(3),** 241-245.

FANELLO, C. SANTOMALAZZA, F. & DELLA TORRE, A. (2002) Simultaneous identification of species and molecular forms of the *Anopheles gambiae* complex by PCR-RFLP. *Medical and Veterinary Entomology*, **16**, 461–464.

FAVIA, G. DELLA TORRE, A. BAGAYOKO, M. LANFRANCOTTI, A. SAGNON, N'F. TOURE´, Y.T. & COLUZZI, M. (1997) Molecular identification of sympatric chromosomal forms of *Anopheles gambiae* and further evidence of their reproductive isolation. *Insect Molecular Biology*, **6**, 377–383.

FAVIA, G. DIMOPOULOS, G, DELLA TORRE, A. TOURE Y.T. COLUZZI, M. & LOUIS, C. (1994a) Polymorphisms detected by random PCR distinguishes between different chromosomal forms of *Anopheles gambiae*. *Proceedings of the National Academy of Science*, **91**, 10315–10319.

FAVIA, G. LANFRANCOTTI, A. SPANOS, L. SIDÉN-KIAMOS, I & LOUIS, C. (2001) Molecular characterisation of ribosomal DNA polymorphisms discriminating among chromosomal forms of *Anopheles gambiae* s.s. *Insect Molecular Biology*, **10**, 19-23.

FAYE, O. DIALLO, S. GAYE, O. NDIR, O. & FAYE, O. (1992) Efflcacite comparee de l'utilisation des pieges lumineux du type CDC et des sujets humains pour l'échantillonnage des nonulations anonhéliennes. Resultats obtenus dans la zone de Big nona (Sénégal). *Annales de la SocGd Belge de Midecine Tropicale*, **85, 185-189.**

FAYE, O. GAYE, O. FONTENILLE, D. SY, N. KONATE, L. HEBRARD, G. HERVE, J.P. TROUILLET, J. DIALLO, S. & MOUCHET, J. (1995) Comparaison de la transmission du paludisme dans deux facies epidemiologiques au Senegal: la zone cotiere sahelienne et la zone meridonale soudaninenne. *Dakar Medical,* **40,** 201-207.

FEYEREISEN, R. (1995) P-450 mediated resistance in the house fly. pp. 192. In: Toward the genetic manipulation of insects. Keyston Symposium on Molecular Cellular Biology. Tammarron, Coloroda, USA.

FFRENCH-CONSTANT, R.H., ANTHONY, N., ARONSTEIN, K., ROCHELEAU, T. & STILWELL, G. (2000) Cyclodiene insecticide resistance: from molecular to population genetics. *Annual Review of Entomology*, **45**, 449–466.

FFRENCH-CONSTANT, R.H., PARK, Y. & FEYEREISEN, R. (1999) Molecular biology of insecticide resistance, *pp*. 531-551. *In* A. Puga and K. B. Wallace [eds.], Molecular biology of the toxic response. Taylor and Francis, Philadelphia, PA.

FINNEY, D.J. (1971) Probit Analysis, Cambridge University press. Cambridge, United Kingdom.

FISHER, R.A. & FORD, E.B. (1947) The spread of a gene in natural conditions in a colony of the moth *Panaxia dominula* L. *Heredity*, **1,** 143-174.

FONSECA, L.F. DI DECO, M.A. CARRARA, G.C. DABO, I. DO ROSARIO, V. & PETRARACA, V. (1996) *Anopheles gambiae* complex (Diptera: Culicidae) near Bissau City, Guinea Bissau, West Africa. *Journal of Medical Entomology*, **33(6),** 939-945.

FONTENILLE, D. LOCHOUARN, L. DIAGNE, N. SOKHNA, C. LEMASSON, J.J. DIATTA, M. KONATE, L. FAYE, F. ROGIER, C. & TRAPE, J.F. (1997) High annual and seasonal variations in malaria transmission by

anophelines and vector species composition in Dielmo, a holoendemic area in Senegal. *American Journal of Tropical Medicine and Hygiene,* **56,** 247–253.

FONTENILLE, D. MEUNIER, J.Y. NKONDJIO, C.A. TCHUINKAM, T. (2001) Use of circumsporozoite protein enzyme-linked immunosorbent assay compared with microscopic examination of salivary glands for calculation of malaria infectivity rates in mosquitoes (Diptera: Culicidae) from Cameroon. *Journal of Medical Entomology,* **38(3),** 451-454.

FONTENILLE, D. WANJI, S. DJOUAKA, R. & AWONO, A.H.P. (2000) *Anopheles hancocki* vecteur secondaire du paludisme au Cameroon. *Bulletin de liaison et de documentation de l'OCEAC,* **33,** 23–26.

FOOTE, R.H. & COOK, D.R. (1959) Mosquitoes of Medical Importance in West Africa. United State Department of Agriculture, Washington.

FOSTER, S. (1995) Treatment of malaria outside the formal health services. *Journal of Tropical Medicine and Hygiene,* **98,** 29–34.

GALE, K.R. & CRAMPTON, J.M. (1987) DNA probes for species identification of mosquitoes in *Anopheles gambiae* complex. *Medical and Veterinary Entomology,* **1(2),** 127-36.

GALLUP, J.L. & SACHS, J.D. (2001) The economic burden of malaria. *American Journal of Tropical Medicine and Hygiene,* **64(1, 2)** S, pp 85-96.

GARNHAM, P.C.C. (1966) *Malaria parasites and other haemosporidia.* Blackwell Scientific publications, Oxford. In: *Medical and Veterinary Entomology.* 2nd Edn. Oxford CAB international, University Press, Cambridge, UK. pp 559-90.

GARNHAM, P.C.C. (1988) Malaria parasites of man: life-cycles and morphology (excluding ultrastructure). In: Wernsdorfer, W.H. & McGregor, I. (eds) *Malaria Principles and Practice of Malariology.* Churchill Livingstone, Edinburgh, **1,** 61-96.

GARRET-JONES, C. (1970) Problems of epidemiological entomology as applied to malariology. *Miscellaneous publications of Entomological Society of America***, 7,** 168- 177.

GARRETT-JONES, C. & GRAB, B. (1964) The assessment of insecticidal impact on the malaria mosquito's vectorial capacity from data on the proportion of parous females. *Bulletin of the of the World Health Organization*, **31,** 71–86.

GARRETT-JONES, C. & SHIDRAWI, G.R. (1969) Malaria vectorial capacity of a population of *Anopheles gambiae*, an exercise in epidemiological entomology. *Bulletin of the of the World Health Organization*, **40,** 531–545.

GATTI, M. SANTINI, G. PIMPINELLI, S. & COLUZZI, M. (1977) Fluorescence banding techniques in the identification of sibling species of the anopheles gambiae complex. *Heredity*, **38,** 105-108.

GENTILE, G. DELLA TORRE, A. MAEGGA, B. POWELL, J.R. & CACCONE, A. (2002) Genetic differentiation in the African malaria vector, *Anopheles gambiae s.s.* and the problem of taxonomic status. *Genetics*, **161,** 1561–78.

GERKEN, A. SUGLO, J-V. & BRAUN, M. EGYIR, I. FLEISCHER, G. (2001) Pesticides Use and Policies in Ghana. An Economic and Institutional Analysis of Current. Practice and Factors Influencing Pesticide Use. A Publication of the Pesticide Policy Project. Hannover, May 2001. Publication Series, No. 10.

GILLIES, M.T. & COETZEE, M. (1987) Supplement to the Anophelinae of Africa South of Sahara. Johannesburg: *Publication of South African Institute of Medical Research*, **55**, 1-143.

GILLIES, M.T. & DE MEILLON, B. (1968) The Anophelinae of Africa South of Sahara. Johannesburg: *Publication of South African Institute of Medical Research*, **54**, 1-343.

GILLIES, M.T. & WILKES, T.J. (1965) A study of the age-composition of populations of *Anopheles gambiae* Gilles *and Anopheles funestus* Gilles in North-Eastern Tanzania. *Bulletin of Entomological Reseach,* **56,** 237-62.

GILLIES, M.T. (1988) *Anopheles* mosquitoes: vector behaviour and bionomics. *In: Malaria. Principles and Practice of Malariology.* W.H. Wernsdorfer and Sir McGregor Eds. Churchill Livingstone City- Great Britain. pp. 453-485.

GIMNIG, J.E. OMBOK, M. KAMAU, L. & HAWLEY, W.A. (2001) Characteristics of larval anopheline (Diptera: Culicidae) habitats in western Kenya. *Journal of Medical Entomology,* **38,** 282-288.

GITHEKO, A.K. SERVICE, M.W. MBOGO, C.M. ATIELI, F.K. & JUMA, F.O. (1993) *Plasmodium falciparum* sporozoite and entomological inoculation rates at the Ahero irrigation scheme and the Miwani sugar-belt in western Kenya. *Annals of Tropical Medicine Parasitology,* **87,** 379–391.

GOMPERTZ, B. (1825) On the nature of the function expressive of the law of human mortality and on a new mode of determining life contingencies. *Philosophical Transactions of the Royal Society of London,* **115**, 513–585.

GRANT, D.F. & MATSUMURA, F. (1989) Glutathione *S*-transferase 1 and 2 in susceptible and insecticide resistant *Aedes aegypti*. *Pesticide Biochemistry and Physiology,* **33,** 132-143.

GRANT, D.F. DIETZE, E.C. HAMMOCK, B.D. (1991) Glutathione Stransferase isozymes in *Aedes aegypti*: purification, characterization, and isozyme-specific regulation. *Insect Biochemistry,* **21,** 421–433.

GREEN, C.A. & HUNT, R.H. (1980) Interpretation of variation in Ovarian Polytene Chromosomes of *Anopheles Funestus* Gilles, *Anopheles parensis* Gillies and *A. aruni. Genetica,* **51,** 187-195.

GREEN, C.A. (1982) Cladistic Analysis of Mosquito Chromosome Data (*Anopheles*) Cellia Myzomyia, *Heredity,* **73,** 2-11.

GREENWOOD, B.M. BRADLEY, A.K. GREENWOOD, A.M. BYASS, P. JAMMEH, K. MARSK, K. TULLOCH, S. OLDFIELD, F.S. & HAYES, R. (1987) Mortality and Morbidity from Malaria in a Rural Area of the Gambia, West Africa *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **81(3),** 478-486.

GREENWOOD, B.M. PICKERING, H. (1993) A malaria control trial using insecticide-treated bed nets and targeted chemoprophylaxis in a rural area of The Gambia, West Africa. A review of the epidemiology and control of malaria in the Gambia, West Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **87,** 3-11.

GUENGERICH, F.P. YUN, C-H. & MACDONALD, T.L. (1996) Evidence for a One-Electron Mechanism in N-Dealkylation of N,N-Dialkylanilines by Cytochrome P450 2B1. Kinetic Hydrogen Isotope Effects,Linear Free Energy Relationships with Biomimetic Models, Comparisons with Horseradish Peroxidase, and Studies with Oxygen Surrogates. *Journal of Biological Chemistry*, **271,** 27321-27329.

GUILLET, P. (2001) Insecticide-treated nets in Africa: where do we stand? *Africa Health*, **23,** S22–S24.

GUILLET, P. N'GUESSAN, R. DARRIET, F. TRAORE-LAMIZANA, M. CHANDRE, F. & CARNEVALE, P. (2001) Combined pyrethroid and carbamate 'two-in-one' treated mosquito nets: field efficacy against pyrethroid-resistant *Anopheles gambiae* and *Culex quinquefasciatus*. *Medical and Veterinary Entomology*, **15,** 105–112.

GULLAN, P.J. & GRANSTON, P.S. (1994) The insects; an outline of entomology. London: Chapman & Hall. Pp 491

HACKETT, B.J. GIMNIG, J. GUELBEOGO, W. COSTANTINI, C. KOEKEMOER, L.L. COETZEE, M. COLLINS, F.H. & BESANSKY,

N.J. (2000) Ribosomal DNA internal transcribed spacer (ITS2) sequences differentiate *Anopheles funestus* and *Anopheles rivulorum*, and uncover a cryptic taxon. *Insect Molecular Biology*, **9,** 369–374.

HAMILTON, R.J. & SERVICE, M.W. (1983) Value of cuticular and internal hydrocarbon for the identificationo f larvae of *Anopheles gambiae* Giles, *An. arabiensis* Patton and *An. melas* Theobald. *Annals of Tropical Medicine and Parasitology*, **77**, 203-210.

HAMON, J. SUBRA, R. SALES, S. & COZ, J. (1968) *Présence dans le SudQuest de la Haute Volta d'une population d'Anopheles gabiae* 'A' *resistante au DDT*. Organisation Mondiale de la Sante, WHO/Mal/68.657, 10p.

HAMON. J. MOUCHET, J. (1961) Les vecteurs secondaires du paludisme humain en Afrique. *Med Trop,* **21,** 643–60.

HARBACH, R.E. (1994) Review of the internal classification of the genus *Anopheles* (Diptera: Culicidae): the foundation for comparative systematics and phylogenetic research. *Bulletin of Entomological Research,* **84,** 331 – 42.

HARGREAVES, K. HUNT, R.H. BROOKE, B.D. MTHEMBU, J. WEETO, M.M. AWOLOLA, T.S. COETZEE, M. (2003) Occurrence and significance of DDT resistance in two members of the *Anopheles gambiae* complex in South Africa. *Medical and Veterinary Entomology,* **17,** 417–422.

HARGREAVES, K. KOEKEMOER, L.L. BROOKE, B.D. HUNT, R.H. MTHEMBU, J. COETZEE, M. (2000) *Anopheles funestus* resistant to pyrethroid insecticides in South Africa. *Medical and Veterinary Entomology,* **14,** 181–189.

HAY, S.I. ROGERS, D.J. TOOMER, J.F. & SNOW, R.W. (2000) Annual *Plasmodium falciparum* entomological inoculation rates (EIR) across Africa: literature survey, internet access and review. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **94,** 113-127.

HAYES, J.D. WOLF, C.R. (1988) Role of glutathione transferase in drug resistance. In: Sies, H. Ketterer, B. (Eds.), Glutathione conjugation. *Academic Press*, pp. 315.

HEGGENHOUGEN, H.K. HACKETHAL, V. & VIVEK, P. (2003) The behavioural and social aspects of malaria and its control. UNDP/World Bank/WHO Special Programme for Research & Training in Tropical Diseases. TDR/STR/SEB/VOL/03.1 , pp 1 – 228.

HEMINGWAY J. (1999) Insecticide resistance in malaria vectors: A new approach to an old subject. *Parassitologia*, In press

HEMINGWAY, J. & CRAIG A. (2004) Medical Biology: On the Control of Malaria. *Science*, **303**, 1984.

HEMINGWAY, J. & KARUNARATNE, S.H.P.P. (1998) Mosquito carboxylesterases: a review of the molecular biology and biochemistry of a major insecticide resistance mechanism. *Medical and Veterinary Entomology*, **12**, 1-12.

HEMINGWAY, J. & RANSON, H. (2000) Insecticide resistance in insect vectors of diseases. *Annual Review of Entomology*, **45,** 371-391.

HEMINGWAY, J. (1981) Genetics and biochemistry of insecticide resistance in Anophelines. Ph.D Thesis. University of London. pp. 310. In: HEMINGWAY, J. (1982) The biochemical nature of malathion resistance in *Anopheles stephensi* from Pakistan. *Pesticides Biochemistry and Physiology*, **17,** 149-155.

HEMINGWAY, J. (1982) The biochemical nature of malathion resistance in *Anopheles stephensi* from Pakistan. *Pesticides Biochemistry and Physiology*, **17,** 149-155.

HEMINGWAY, J. (1983) The genetics of malathion resistance in *Anopheles stephensi* from Pakistan. *Transactions of Royal Society of Tropical Medicine and Hygiene,* **77,** 106-108.

HEMINGWAY, J. (1985) Malathion carboxylesterase enzymes in *Anopheles arabiensis* from Sudan. *Pesticide Biochemistry and Physiology,* **23**, 309–13.

HEMINGWAY, J. (2000) The molecular basis of two contrasting mechanisms of insecticide resistance. *Insect Biochemistry and Molecular Biology,* **30,** 1009–1015.

HEMINGWAY, J. FIELD, L. & VONTAS. J. (2002) An overview of insecticide resistance. *Science,* **298,** 96-7.

HEMINGWAY, J. HAWKES, N.J. MCCARROLL, L. & RANSON, H. (2004) The molecular basis of insecticide resistance in mosquitoes. *Insect Biochemistry and Molecular Biology,* **34,** 653–665.

HERATH, P.R.J. HEMINGWAY, J. WEERASINGHE, I.S. JAYAWARDENA, K.G.I. (1987) The detection and characterisation of malathion resistance in field populations of *Anopheles culicifacies* B in Sri Lanka. *Pesticide Biochemistry and Physiology,* **29,** 157–162.

HII, J.L.J. SMITH, T. MAI, A. IBAM, E. & ALPERS, M.P. (2000) Comparison between anopheline mosquitoes (Diptera: Culicidae) caught using different methods in a malaria endemic area of Papua New Guinea. *Bulletin of Entomological Research,* **90,** 211-219.

HOMBHANGE, F.N. (1998) *Plasmodium ovale* species in Papua New Guinea – lest we forget. *Papua New Guinea Medical Journal,* **41 (3-4),** 116- 118.

http://en.wikipedia.org/wiki/*Anopheles*

HUNT, R.H. COETZEE, M. & FETTENE, M. (1998) *Anopheles gambiae* complex: a new species from Ethiopia. *Transactions of Royal Society of Tropical Mediciene and Hygiene,* **92,** 231 - 235.

JAMBOU, R. RANAIVO, L. RAHARIMALALA, L. RANDRIANAIVO, J. RAKOTOMANANA, F. MODIANO, D. PIETRA, V. BOISTER, P. RABARIJAONA, L. RABE, T. RAVELOSON, N. & DE GIORGI, F. (2001) Malaria in the highlands of Madagascar after five years of indoor spraying of DDT. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **95,** 14-18.

JONES, G. STEKETEE, R.W. BLACK, R.E. BHUTTA, Z.A. & MORRIS. S.S. (2003) Bellagio Child Survival Study Group: How many child deaths can we prevent this year? *Lancet*, **362,** 65-71.

KABULAH, I.B. (2007) Characterisation of *An. gambiae* s.l. Gilles (Diptera: Culicidae), their insecticide resistance profile and physiochemical properties of their breeding water within the Accra Metropolis. *Master of Philosophy in Entomology Thesis, University of Ghana, Legon-Accra.*

KAGER, P.A. (2002) Malaria control: constraints and opportunities. *Tropical Medicine and International Health* **7,** 1042–1046.

KARUNARATNE, S.H.P.P. JAYAWARDENA, K.G.I. HEMINGWAY, J. KETTERMAN, A.J. (1993) Characterisation of a B-type esterase involved in insecticide resistance from the mosquito *Culex quinquefasciatus*. *Biochemistry Journal*, **294,** 575–579.

KEAS, B.E., (1999A) Taxonomic Classification. Retrieved on June 1, 2009 from the website: https://www.msu.edu/course/zol/316/pspptax.htm.

KEAS, B.E., (1999b) Geographic distribution of *Plasmodium* species. Retrieved on June 1, 2009 from the site: https://www.msu.edu/course/zol/316/psppgeo.htm.

KELLY-HOPE, L.A. DIGGLE, P.J. ROWLINGSON, B.S. GYAPONG, J.O. KYELEM, D. COLEMAN, M. THOMSON, M.C. OBSOMER, V. LINDSAY, S.W. HEMINGWAY, J. & MOLYNEUX, D.H. (2006) Short communication: Negative spatial association between lymphatic filariasis and

malaria in West Africa. *Tropical Medicine and International Health,* **11**, 129–135.

KENGNE, P. AWONO-AMBENE, P. ANTONIO-NKONDJIO, C. SIMARD, F. FONTENILLE, D. (2003) Molecular identification of the *Anopheles nili* group of African malaria vectors. *Medical and Veterinary Entomology,* **17,** 67–74.

KETTLE, D.S. (1995) Culicidae (Mosquito). In: *Medical and Veterinary Entomology*. 2nd Edition. Oxford CAB international, pp 109-151.

KILIAN, A.H. LANGI, P. TALISUNA, A. & KABAGAMBE, G. (1999) Rainfall pattern, El Nino and malaria in Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **93**, 22-23.

KOEKEMOER, L. KAMAU, L. HUNT, R.H. & COETZEE, M. (2002a) A cocktail polymerase chain reaction assay to identify members of the *Anopheles funestus* (Diptera:Culicidae) group. *American Journal of Tropical Medicine and Hygiene,* **6(6),** 804-811.

KOEKEMOER, L.L. HARGREAVES, K. HUNT, R.H. COETZEE, M. (2002b) Identification of *Anopheles parensis* (Diptera: Culicidae) using ribosomal DNA internal transcribed spacer (ITS2) sequence variation. *African Entomology,* **10,** 235–239.

KOLACZINSKI, J.H. FANELLO, C. HERVE´, J.-P. CONWAY, D.J. CARNEVALE, P. & CURTIS, C.F. (2000) Experimental and molecular genetic analysis of the impact of pyrethroid and non-pyrethroid insecticide-impregnated bednets for mosquito control in an area of pyrethroid resistance. *Bulletin of Entomological Research,* **90,** 125–132.

KORAM, K.A. BENNET, S. ADIAMAH, J.H. & GREENWOOD, B.M. (1995) Socio-economic risk factors for malaria in a peri-urban area of the Gambia. *American Journal of Tropical Medicine and Hygiene,* **89,** 146-150.

KORAM, K.A. OWUSU-AGYEI, S. FRYAUFF, D.J. ANTO F. ATUGUBA, F. HODGSON, A. HOFFMAN, S.L. & NKRUMAH F. (2003) Seasonal profiles of malaria infection, anemia and bednet use among age groups and communities in northern Ghana. *Tropical Medicine and International Health,* **8,** 793–802.

KRAFSUR, E.S. (1977) The bionomics and relative prevalence of *Anopheles* species with respect to the transmission of *Plasmodium* to man in western Ethiopia. *Journal of Medical Entomology,* **14,** 180–194.

KREBS, C.J. (1972) *Ecology. The Experimental Analysis of the Distribution and Abundance.* Harper & Row, New York.

KRISTAN, M. FLEISCHMANN, H. DELLA TORRE, A. STICH, A. & CURTIS, C.F. (2003) Pyrethroid resistance/susceptibility and differential urban/rural distribution of *Anopheles arabiensis* and *Anopheles gambiae* s.s. malaria vectors in Nigeria and Ghana. *Medical and Veterinary Entomology,* **17,** 326–332.

KUMAR, R. (1984) Insect pest control with special reference to African Agriculture. English Langauge Society/Edward Arnold.

LAGADIC, L. CANNY, A. BERGE, J.B. & ECHUABARD. (1993) Purification and partial characterisation of GSTs from insecticide resistant and lindane-induced susceptible Spodoptera littolaris (Biod.) larvae. *Insect Biochemistry and Molecular Biology,* **7,** 467-474.

LANZARO, G.C. TOURE, Y.T. CARNAHAN, J. ZHENG, L. DOLO, G. TRAORE, S. PETRARCA, V. VERNICK, K.D. & TAYLOR, C.E. (1998) Complexities in the genetic structure of *Anopheles gambiae* populations in West Africa as revealed by microsatellite DNA analysis. *Proceedings of National Academy of Science, USA,* **95,** 14260–14265.

LANZARO, G.C. ZHENG, L. TOURE, Y.T. TRAORE, S.F. KAFATOS, F.C. & VERNICK, K.D. (1995) Microsatellite DNA and isozyme variability in a west African population of *Anopheles gambiae*. *Insect Molecular Biology*, **4**, 105-12.

LAUBACH, H.E. VALIDUM, L. BONILLA, J.A. AGAR, A. CUMMINGS, R. MITCHELL, C. CUADRADO, R.R. & PALMER, C.J. (2001) Identification of *Anopheles aquasalis* as a possible vector of malaria in Guyana, South America. *West Indian Medical Journal,* **50(4),** 319-321.

LE GOFF. G. CAMEVALE, I. FONDIO. E. & ROBERT, V. (1997) Comparison of three sampling methods of man-biting anophelines in order to estimate malaria transmission in a village of south Cameroon. *Parasite,* **4,** 75-80.

LE SUEUR, D. & SHARP, B.L. (1991) Temperature-dependent variation in merus larval head capsule width and adult wing length: implications for anopheline taxonomy. *Medical and Veterinary Entomology,* **5,** 55-62.

LEESON, H.S. (1931) Anopheline mosquitoes in Southern Rhodesia. The London School of Hygiene and Tropical Medicine, London, UK.

LEHMANN, T. BESANSKY, N.J. HAWLEY, W.A. FAHEY, T.G. KAMAU, L. & COLLINS, F.H. (1997) Microgeographic structure of *Anopheles gambiae* in western Kenya based on mtDNA and microsatellite loci. *Molecular Ecology,* **6,** 243–53.

LEHMANN, T. LICHT, M. ELISSA, N. MAEGA, B.T. CHIMUMBWA, J.M. WATSENGA, F.T. WONDJI, C.S. SIMARD, F. & HAWLEY, W.A. (2003) Population Structure of *Anopheles gambiae* in Africa. *Journal of Heredity,* **94,** 133–147.

LENGELER, C. (2004) Insecticide-treated bed nets and curtains for preventing malaria. *Cochrane Database System Review*, **2:**CD000363.

LENGELER, C. CATTANI, J. & DE SAVIGNY (EDS) (1996) Net gain: A new method for preventing malaria deaths. *International Development Research Centre* Ottawa and World Health Organization, Geneva

LIEHNE, P.F.S. (1998) Climatic influence on mosquito-borne diseases in Australia. *In: Greenhouse: Planning for Climate Change* (Pearlman GI, ed). Melbourne, Australia: *CSIRO*, 1998.

LINDBLADE, K.A. WALKER, E.D. ONAPA, A.W. KATUNGU, J. & WILSON, M.L. (1999) Highland malaria in Uganda: prospective analysis of an epidemic associated with El Nin˜ o. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **93,** 480–487.

LINDSAY, M.D. **& MACKENZIE, J.S. (1997)** Vector-borne viral disease and climate change in the Australia region; major concerns and public health response. *In: Climate Change and Human Health in the Asia-Pacific region* (Curson P, Guest C, Jackson E. eds). Canberra, Australia: *Australia Medical Association and Greenpeace International*, 47-62.

LINDSAY, S.W. & BIRLEY, M.H. (1996) Climate Change and Malaria Transmission. *Annals of Tropical Medicine and Parasitology,* **90: ,** 573-588.

LINDSAY, S.W. & MARTENS W.J. (1998) "Malaria in African highland: past, present and future". *Bulletin of the World Health Organisation,* **76(1),** 33-45.

LINDSAY, S.W. BODKER, R. MALIMA, R. MSANGENI, H.A. & KISINZA, W. (2000) Effect of 1997-98 El Nino on highland malaria in Tanzania. *Lancet,* **355,** 989-990.

LINDSAY, S.W. PARSON, L. & THOMAS, C.J. (1998) Mapping the ranges and relative abundance of the two principal African malaria vectors, *Anopheles gambiae* sensu stricto and *Anopheles arabiensis*, using climate data. *Proceedings of Royal Society of London, Biochemistry and Biological Science,* **265,** 847-854.

LINES, J.D. & ZAIM, M. (2000) Insecticide products: treatment of mosquito nets at home. *Parasitology Today* **16**, 91-2

LINES, J.D. (1988) Do agricultural insecticides select for insecticide resistance in mosquitoes? A look at the evidence. *Parasitology Today*, **4,** S17-S20.

LINES, J.D., CURTIS, C.F., WILKS, T.J., NJUNWA, K.J. (1991) Monitoring human biting mosquitoes (Diptera: Culicidae) in Tanzania with light traps hung beside mosquito nets. *Bulletin of Entomology Research*, **81**, 77-84.

LJUNGSTRÖM, I. PERLMANN, H. SCHLICHTHERLE, M. SCHERF, A. & WAHLGREN, M. (2004) Methods in malaria research. MR4/ATCC Manassas, Virginia. Pp 110-111.

LOEVINSOHN, M.E. (1999) Climatic warming and increased malaria incidence in Rwanda. *Lancet,* **343**, 714-718.

LUND, A.E. & NAHARASHI, T. (1983) Kinetics of sodium channel modification as the basis for the variation in the nerve membrane effects of pyrethroid and DDT analogs. *Pesticide Biochemistry and Physiology*, **20** 203-216.

MACDONALD, G. (1952) The analysis of the sporozoite rate. *Tropical Diseases Bulletin*, **49,** 569-585.

MACDONALD, G. (1957) The Epidemiology and Control of Malaria. London: Oxford University Press.

MACDONALD, P.T. (1977a) Population characteristics of domestic *Aedes aegypti* (Diptera: Culicidae) in villages on the Kenya Coast. I. Adult survivalship and population size. *Journal of Medical Entomology*, **14,** 42-48.

MADWAR, S. (1936) A preliminary note on *Anopheles pharoensis* in relation to malaria in Egypt. *Journal of Egypt Medical Association,* **19,** 616.

MAGBITY, E.B. LINES, J.B. MARBIAH, M.T. DAVID, K. & PETERSON, E. (2002) How reliable are light-traps in estimating biting rates of adults

Anopheles gambiae s.l. (Diptera: Culicidae) in the presence of treated bed nets? *Bulletin of Entomological Research,* **92,** 71-76.

MAHON, R.J. GREEN, C.A. & HUNT, R.H. (1976) Diagnostic allozymes for routine identification of adults of the *Anopheles gambiae* complex (Diptera: Culicidae). *Bulletin of Entomological Research*, **66,** 25-31.

MALCOLM, C.A. (1988) Current status of pyrethroid resistance in anophelines. *Parasitology Today,* **4**, S13-S6.

MANGA, L. TOTO, J.C. LE GOFF, G. & BRUNHES, J. (1997) The Bionomics of *Anopheles funestus* and its role in malaria transmission in a forested area of southern Cameroon. *Transactions of the Royal society of Tropical Medicine and Hygiene,* **91,** 387–388.

MARA MAPPING MALARIA RISK IN AFRICA TOWARDS AN ATLAS OF MALARIA RISK IN AFRICA, (2002) *Ghana: Distribution of Endemic Malaria.* Retrieved on December 20, 2004 from the website http://www.mara.org.za/

MARA, MAPPING MALARIA RISK IN AFRICA TOWARDS AN ATLAS OF MALARIA RISK IN AFRICA, (1998) *Medical Research Council,* South Africa.

MARQUETTI FERNANDEZ, M.C. NAVARRO ORTEGA, A. BISSET LAZCANO, J. & GARCIA, F. (1991) Physiological age of 2 populations of *Anopheles* (N.) *albimanus* Wied, 1821 (Diptera: Culicidae) and its importance in malaria transmission *Rev Cubana Medica Tropica,* **43(3),** 174-177.

MARTENS, W.J. (1995) Climate change and malaria: exploring the risks. *Medical War,* **11,** 202-213.

MARTIN, P.H. & LEFEBVRE, M.G. (1995) Sensitivity of Malaria Potential Transmission to Climatic Changes. *Ambio,* **24 (**4).

MARTINEZ-TORRES, D. CHANDRE, F. WILLIAMSON, M.S. DARRIET, F. BERGE J.B. & DEVONSHIRE, A.L. GUILLET, P. PASRUER, N. & PAURON, D. (1998) Molecular characterisation of pyrethroid resistance in the malaria vector *Anophles gambiae*. *Insect Molecular Science,* **7,** 179-84.

MARTINEZ-TORRES, D., DEVONSHIRE, A.L., WILLIAMSON, M.S. (1997) Molecular studies of knockdown resistance to pyrethroids: cloning of domain II sodium channel sequences from insects. *Pesticide Science*, **51**, 265–270.

MBOGO, C.M. J.M. MWANGANGI, J. NZOVU, W. GU, G. YAN, J. GUNTER, C. SWALM, J. KEATING, J.L. REGENS, J.I. SHILILU, J.I. GITHURE, J.I. & BEIER, J.C. (2003) Spatial and temporal heterogeneity of *Anopheles* mosquitoes and *Plasmodium falciparum* transmission along the Kenyan coast. *American Journal of Tropical Medicine and Hygeine,* **68,** 734 - 742.

MBOGO, C.N.M. GLASS, G.E. FORSTER, D. KABIRU, E.W. GITHURE, J.I. OUMA, J.H. & BEIER, J.C. (1993a) Evaluation of light traps for sampling anopheline mosquitoes in Kilifi, Kenya. *Journal of American Mosquito Control Association*, **9,** 260–263.

MCCARROLL, L. & HEMINGWAY, J. (2002) Can insecticide resistance status affect parasite transmission in mosquitoes? *Insect Biochemistry and Molecular Biology*, **32,** 1345–1351

MCCARROLL, L. PATON, M.G. KARUNARATNE, S.H.P.P. JAYASURYIA, H.T.R. KALPAGE, K.S.P. & HEMINGWAY, J. (2000) Insecticides and mosquito-borne disease. *Nature,* **407**, 961–962.

MCCARTHY, D.F. WOLF, H. & WU, Y. (2000) The growth costs of malaria. World Bank, Georgetown University and NBER.

MCEWEN, P. (2000) Report: Harmful Species of Mosquito- A worldwide focus. Brandenburg UK Limited by Insect Investigations Ltd.

MCGREEVY, P.B. KOLSTRUP, N. TAO, J. MCCREEVEY, M.M. & MARSHALL, T.F. (1982) Ingestion and development of *Wucheria bancrofti* in *Culex quinquefasciatus*, *Anopheles gambiae* and *Aedes aegypti* after feeding on humans with varying densities of microfilaria. *Trans R Soc Trop Med Hyg* 76, 288–296.

MIDEGA, J.T. (2008) Distribution patterns, dispersal and population genetics of anopheline mosquitoes along the Kenyan coast. *Doctor of Philosophy in Entomology Thesis, University of Ghana*, pp 74-113.

MIDEGA, J.T. MBOGO, C.M. MWAMBI, H. WILSON, M.D. OJWANG, G. MWANGANGI, J.M. NZOVU, J.G. GITHURE, J.I. YAN, G. & BEIER, J.C. (2007) Estimating Dispersal and Survival of *Anopheles gambiae* and *Anopheles funestus* Along the Kenyan Coast by Using Mark–Release–Recapture Methods. *Journal of Medical Entomology*, **44(6)**, 923-929.

MILANI, R. (1954) Comportamento medeliano della resistenza alla azione abbatante del DDT: correlazione tran abbatimento e mortalia in *Musca domestica* L. *Review of Parasitology*, **15,** 513-542.

MILES, S.J. (1978) Enzyme variation in the *Anopheles gambiae* Gilles group of species (Diptera: Culicidae). *Bulletin of Entomology Research,* **68,** 85-96.

MILES, S.J. GREEN, C.A. HUNT, R.H. (1983) Genetic observations on the taxon *Anopheles* (Cellia) pharoensis Theobald (Diptera: Culicidae). *Journal of Tropical Medicine and Hygiene,* **86,** 153–157.

MILLER, L.H. DVORAK, J.A. SHIROISHI, T. & DUROCHER, J. (1973) Influence of erythrocyte membrane components on merozoite invasion. *Journal of Experimental Medicine,* **138,** 1597-1601.

MITTAL, P.K. ADAK, T. & SHARMA, V.P. (1991) Acute toxicity of certain organochlorine, organophosphorus, synthetic pyrethroid and microbial

insecticides to the mosquito fish *Gambusia affinis* (Baird and Girard). *Indian Journal of Malariology*, **28**, 167–170.

MIYAZAKI, M. OHYAMA, K. DUNLAP, D.Y. & MATSUMURA, F. (1996) Cloning and sequencing of the para-type sodium channel gene from susceptible and kdr-resistant German cockroaches (*Blatella germanica*) and house fly (*Musca domestica*). *Molecular Genetics,* **252**, 61-8.

MOERMAN, F. LENGELER, C. CHIMUMBWA, J. TALISUNA, A. ERHART, A. COOSEMANS, M. & D'ALESSANDRO, U. (2003) The contribution of health-care services to a sound and sustainable malaria-control policy. *Lancet Infectious Diseases,* **3,** 99–102.

MOH – MINISTRY OF HEALTH, (2008) National Malaria control monitoring and evaluation plan 2008 – 2015 (Draft). National malaria control programme (NMCP). Ghana Health Service.

MOLINEUX, L. (1988) The epidemiological of human malaria as an expression of its distribution including some implications for its control. Werndofer WH, McGregor IA, Eds. *Malaria, Principles and Practice of Malariology.* Volume 2. London Churchill, Livingstone, 913-998.

MOLINEUX, M.E. TAYLOR, T.E. WIRIMA, J.J. & BORGSTEIN, A. (1989) Clinical features and prognostic indicators in pediatric cerebral malaria: A study of 131 comatose Malawian children. *Quarterly Journal of Medicine,* **71(265),** 441-459.

MOUCHET, J., P. CARNEVALE, M. COOSEMANS, J. JULVEZ, S. MANGUIN, D. RICHARD-LENOBLE, & J. SIRCOULON, (2004) Biodiversité du paludisme dans le monde Editions. John Libbey Eurotext. Montrouge, France.

MÜLLER, P. DONNELLY, M.J. & RANSON, H. (2007) Transcription profiling of a recently colonised pyrethroid resistant *Anopheles gambiae* strain from

Ghana. Retrieved on 22 June, 2007 from the websites: http://www.biomed central .com/1471-2164/8/36.

MUKABAYIRE, O. CARIDI, J. WANG X. TOURE, Y.T. COLUZZI, M. & BESANSKY, N.J. (2001) Patterns of DNA sequence variation in chromosomally recognized taxa of *Anopheles gambiae*: evidence from rDNA and single-copy loci. *Insect Molecular Biology*, **10,** 33-46.

MUKHTAR, M. HARREL, M. AMERASINGHE, F.O. ENSINK, J. VAN DER HOEK, W. KONRADSEN, F. (2003) Role of waster water irrigation in mosquito breeding in South Punjab, Pakistan. *Southeast Asian Journal of Tropical Medicine and Public Health*, **34,** 72-80.

MULLIS, K. FALOONA, F. SCHARF, S. SAIKI, R. HORN, G. & ERLICH, H. (1986) Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. *Cold Spring Harbor Symposium on Quantitative Biology*, **51,** 263-273.

N'GUESSAN, R. CORBEL, V. AKOGBETO, M. ROWLAND, M. (2007) Reduced efficacy of insecticide-treated nets and indoor residual spraying for malaria control in pyrethroid resistance area, Benin. *Emerging Infectious Diseases*, **13**, 199-206.

NABARRO, D.N. & TAYLER, E.M. (19982000) The 'roll back malaria' campaign. *Science*, **280,** 2067-2068.

NAJERA, J. A. & ZAIM, M. (2003) Malaria Vector Control. Decision making criteria and procedures for judicious use of insecticides. WHO/CDS/WHOPES /2002.5Rev.1 pp 22

NARANG, S.K. SEAWRIGHT, J.A. & KITZMILLER, J.B. (1981) Linkage relationships and assignment of esterase-4 and esterase-8 loci to chromosome 3 in *Anopheles allbimanus*. *Journal of Heredity*, **72,** 157-160.

NARANG, S.R., KAISER, P.E. & SEAWRIGHT, J.A. (1989a) Dichotomous electrophoretic key for the identification of sibling species A, Band C of the *Anopheles quadrimaculatus* Say complex (Diptera: Culicidae). *Journal of Medical Entomology,* **26**, 94-99.

NARANG, S.R., KAISER, P.E. & SEAWRIGHT, J.A. (1989b) Identification of' species D, a new member of the *Anopheles quadrimaculatus* species complex. A biochemical key. *Journal of American Mosquito Control Association,* **5**, 317-324.

NEVILL, C.G. SOME, E.S. MUNG'ALA, V.O. MUTEMI, W. NEW, L. MARSH, K. LENGELER, C. & SNOW, R.W. (1996) Insecticide-treated bed nets reduce mortality and severe morbidity from malaria among children on the Kenyan coast. *Tropical Medicine and International Health,* **1,** 139-46.

NIH – NATIONAL INSTITUTES OF HEALTH, (2001) *NIAID Global Health Research Plan for HIV/AIDS, Malaria, and Tuberculosis*. National Institute of Allergy and Infectious Diseases.

NIJHOUT, M.M. & CARTER, R. (1978) Gamete development in malaria parasites: Biocarbonate-dependent stimulation by p^H in vitro. *Parasitology,* **76,** 39–53.

NIJHOUT, M.M. (1979) *Plasmodium gallinaceum*: Exflagellation stimulated by a mosquito factor. *Experimental Parasitology,* **48,** 75–80.

OBENG-OFORI, D. OWOUSU, E.O. & KAIWA, E.T. (2002) Variation in the level of carboxylesterase activity as an indicator of insecticide resistance in populations of the diamondback moth *Plutella xylostella* (L.) attacking cabbage in Ghana. *Journal of the Ghana Science Association,* **4(2),** 52–62.

OKOYE, B.N. WILSON, M.D. BOAKYE, D.A. BROWN, C.A. (2005) The impact of the Okyereko irrigation project in Ghana on the risk of human malaria infection by *Anopheles species* (Diptera: Culicidae), *African Entomology,* **13(2), 249-253.**

ONORI, E. & GRAB, B. (1980) Indicators for the forecasting of malaria epidemics. *Bulletin of the World Health Omanization*, **58,** 91-98.

OPPENOORTH, F.J. (1985) Biochemistry and genetics of insecticide resistance. In: Kerkut, G.A. & Gilbert, L.I. (eds.), *Comprehensive insect physiology, biochemistry and pharmacology*, **12,** 731-773. Pergamon, Oxford.

OSEI TUTU, E. (2009) IntermittentIntermittent preventive treatment of malaria in pregnancy; its effect on maternal morbidity and neonatal health in Offinso District of Ashanti Region, Ghana. *Doctor of Philosophy in Biological Sciences (Medical Parasitology) Thesis, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana).*

OTIENO, J.O. (2004) Efficacy of deltamethrin-treated recycled net/curtain materials against different populations of *Anopheles gambiae s.l.* Gilles (Diptera: Culicidae) in Southern Ghana. *Master of Philosophy in Entomology Thesis. University of Ghana,* pp 77-90.

PANYIM S. YASOTHORNSRIKUL, S. TUNGPRADUBKUL, S. BAIMAI, V. POSENBERG, R. ANDRE, R.G. & GREEN, C.A. (1988) Identification of isomorphic malaria vectors using a DNA probe. *American Journal of Tropical Medicine and Hygeine,* **38(1)**, 47-49.

PARK, Y. & TAYLOR, M.J.F. (1997) A novel mutation L1029H in sodium channel gene *hscp* associated with pyrethroid resistance fopr Heliothis virescens (Lepidoptera Noctuidae). *Insect Biochemistry & Molecular Biology*, **27**, 9-13.

PASTEUR, N. & RAYMOND, M. (1996) Insecticide resistance genes in mosquitoes: their mutation, migration and selection in field populations. *Journal of Heredity,* **87,** 444-449.

PATERSON, H.E. PATERSON, J.S. & VAN EDEN, G.J. (1964) Records of the breeding of salt-water *Anopheles gambiae* in inland localities in southern Africa. *Nature,* **201,** 524.

PATES, H. & CURTIS, C. (2005) Mosquito behaviour and vector control. *Annual Reviewa of Entomology*, **50**, 53-70.

PATES, H.V. TAKKEN, W. , STUKE, K. & CURTIS, C.F. (2001) Differential behaviour of *Anopheles gambiae* sensu stricto (Diptera: Culicidae) to human and cow odours in the laboratory. *Bulletin of Entomological Research,* **91,** 289-296.

PATIL, N.S. LOLE, K.S. & DEOBAGKAR, D.N. (1996) Adaptive larval thermotolerance and induced cross-tolerance to propoxur insecticide in mosquitoes *Anopheles stephensi* and *Aedes aegypti*. *Medical and Veterinary Entomology*, **10**, 277-82.

PATTERSON, H.E.H. (1981) The recognition concept of species. In: Vrba ES, editor. Species and speciation. Pretoria, Transvaal Museum: 21-29.

PAURON, D. BARHANIN, J. AMICHOT, M. PRALAVORIO, M. BERGÉ, J.B. & LAZDUNSKI, M. (1989) Pyrethroid receptor in the insect sodium channel: alteration of its properties in pyrethroid-resistant flies. *Biochemistry*, **28**, 1673-1677.

PENILLA, R.P. RODRIGUEZ, A.D. HEMINGWAY, J. TORRES, J.L. ARREDONDO-JIMENEZ, J.I. RODRIGUEZ, M.H. (1998) Resistance Management strategies in malaria vector mosquito control. Baseline data for a large-scale field trial against *Anopheles albimanus* in Mexico. *Medical and Veterinary Entomology*, **12,** 217–233.

PERERA, M.D.B. HEMINGWAY, J. & KARUNARATNE, S.H.P.P. (2008) Multiple insecticide resistance mechanisms involving metabolic changes and insensitive target sites selected in anopheline vectors of malaria in Sri Lanka. *Malaria Journal*, retrieved on October 20, 2008 from the website http://www.malariajournal.com/content/7/1/168

PERSIANA, A. DI DECO, M.A. & PETRANGELI, G. (1986) Laboratory observation of inversion polymorphisms originating from the crossing of various populations of *Anopheles gambiae* sensu stricto. *Ann. Ist. Super. Sanita* **22**, 221-223.

PETERS, W. & GILLES, H.M. (1995) *A Colour Atlas of Tropical Medicine and Parasitology.* Mosby-Wolfe, London.

PETRARCA, V. CARRARA, G.C. DI DECO, M.A. & PETRANGELI, G. (1983) II Complesso *Anopheles gambiae* in Guinea Bissau. *Parassitologia*, **25**, 29-39.

PHARMACIA, L.K.B. (1989) Electrophoresis Cataloque. Pharmacia LKB Biotechnology, Uppsala, Sweden.

PHILLIPS, A. & MILLIGAN, P. (1986) Cuticular hydrocarbons distinguish sibling species of vectors. *Parasitology Today,* **2,** 180-181.

PHILLIPS, A. MILLIGAN, P.J.M. BROOMFIELD, G. & MOLYNEUX, D.H. (1988) Identification of medically important Diptera by analysis of cuticuar hydrocarbons. Pp. 39-59.

PINTO, J. SOUSA, C.A. GIL, V. FERREIRA, C. GONÇALVES, L. LOPES, D. PETRARCA, V. CHARLWOOD, J.D. & DO ROSÁRIO, V.E. (2000) Malaria in São Tomé and Príncipe: Parasite prevalences and vector densities. *Acta Tropica,* **76,** 185-193.

POLOVODOVA, V.P. (1949) The determination of the physiological age of female *Anopheles*, by the number of the gonotrophic cycles completed. *Meditsinskaya Parazitologiya I Parazitarnye Bolenni,* **18,** 352-355.

POTHIKASIKORN, P. OVERGAARD, H. KETAVAN, C. VISETSON, S. BANGS, M.J. & CHAREONVIRIYAPHAP, T. (2007) Behavioural responses of malaria vectors, *Anopheles minimus* complex, to three classes of agrochemicals in Thailand. *Journal of Medical Entomology*, **44(6)**, 1032-1039.

PRAPANTHADARA, L. HEMINGWAY, J. & KETTERMAN, A.J. (1995) DDT-resistance in *Anopheles gambiae* (Diptera: Culicidae) from Zanzibar, Tanzania, based on increase DDTdehydrchlorinase activity of glutathione S-transferases. *Bulletin Entomology Resistance*, **85**, 267 - 274.

PRAPANTHADARA, L. HEMINGWAY, J. KETTERMAN, A.J. (1993) Partial purification and characterization of glutathione S-transferases involved in DDT resistance from the mosquito *Anopheles gambiae*. *Pesticide Biochemistry and Physiology*, **47,** 119–133.

PRAPANTHADARA, L. KOOTTATHEP, S. PROMTET, N. HEMINGWAY, J. & KETTERMAN, A.J. (1996) Purification and characterization of a major glutathione S-transferase from the mosquito *Anopheles dirus* (species B). *Insect Biochemistry and Molecular Biology*, **26**, 277-85.

PRINGLE, G. (1965) A count of the sporozoites in an oocyst of *Plasmodium falciparum*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **59,** 289-290.

PROFT, J. MAIER, W.A. & KAMPEN, H. (1999) Identifcation of six sibling species of the *Anopheles maculipennis* complex (Diptera: Culicidae) by a Polymerase Chain Reaction Assay. *Parasitology Research*, **85,** 837-843.

RAI, K.S. & BLACK, W.C. (1999) Mosquito genomes: structure, organization, and evolution. *Advances in Genetics*, **41,** 1–33.

RAMASAMY, R. RAMASAMY, M.S. WIJESUNDERA, D.A. WIJESUNDERA A.P. DEWIT, I. & RANASINGHE, C. (1992) High seasonal malaria transmission rates in the intermediate rainfall transmission zone of Sri Lanka. *Annals of Tropical Medicine and Parasitology*, **86,** 591-600.

RANSON, H. JENSEN, B. VULULE, J.M. WANG, X. HEMINGWAY, J. & COLLINS F.H. (2000a) Identification of a point mutation in the voltage-

gated sodium channel gene of Kenyan *Anopheles gambiae* associated with resistance to DDT and pyrethroids. *Insect Molecular Biology,* **9,** 491-497.

RANSON, H. ROSSITET, L. ORTELLI, F. JENSON, B. WANG X. ROTH, C.W. COLLINS F.H. & HEMINGWAY, J. (2001) Identification of a novel class of insect glutathione S-transferases involved in resistance to DDT in the malaria vector *Anopheles gambiae. Biochemistry Journal,* **15,** 295-304.

RBM – Roll Back Malaria, (2005) WHO/RBM, United Nations Children Fund (UNICEF): World Malaria Report. WHO, Geneva, Switzerland.

REIDY, G.F., ROSE, H.A., VISETSON, S. & MURRAY, M. (1990) Increased glutathione S–transferase activity and glutathione content in an insecticide-resistant strain of *Tribolium castaneum* (Herbst). *Pesticide Biochemistry and Physiology*, **36**, 269–276.

REIMER, L.J. TRIPET, F. SLOTMAN, M. SPIELMAN, A. FONDJO, E. & LANZARO, G.C. (2005) An unusual distribution of the *kdr* gene among populations of *Anopheles gambiae* on the island of Bioko, Equatorial Guinea. *Insect Molecular Biology,* **14,** 683-688.

REITHINGER, R, (2001) Bogus antimalarials: a forgotten tale. *Trends in Parasitology,* **17,** 359.

RIVET, Y. RAYMOND, M. RIOUX, J.A. DELALBRE, A. & PASTEUR, N. (1994) Resistance monitoring in *Culex pipiens* (Dipteria: Culicidae) from central-eastern France. *Journal of Medical Entomology*, **31**, 231-9

ROBERT, V. MACINTYRE, K. KEATING, J. TRAPE, J.F. DUCHEMIN, J.B. WARREN, M. & BEIER, J.C. (2003) Malaria transmission in urban sub-Saharan Africa. *American Journal of Tropical Medicine and Hygiene,* **68,** 169 - 176.

ROBERTS, D.R. & ANDRE, R.G. (1994) Insecticidal resistance issues in vector-borne disease control. *American Journal of Tropical Medicine and Hygiene*, **50** (**6suppl)**, 21-34.

RUEDA, L.M., PATEL, K.J., AXTELL, R.C. & STINNER, R.E. (1990) Temperature dependent development and survival rates of *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). *Journal of Medical Entomology*, **27,** 892-898.

SACHS, J. D. (2002) A new global effort to control malaria. *Science*, 298, 122-4.

SADASIVAIAH, S. TOZAN, Y. & BREMAN, J.G. (2007) Dichloro-diphenyl-trichloroethane (DDT) for indoor residual spraying in Africa: How can it be used for malaria control? *The American Society of Tropical Medicine and Hygiene,* **77(6),** 249-263.

SAKYI, K.Y. (2004) Investigation into the fitness costs of *kdr* insecticide resistance in *Anopheles gambiae* sensu stricto malaria vectors. *MPhil. Entomology Thesis submitted to ARPPIS, University of Ghana, Legon-Accra.*

SAMARAWICHREMA, W.A. (1967) A study of the age composition of natural populations of *Culex pipiens fatigans* Wiedemann in relation to the transmission of filariasis due to *Wucheria bancrofti* (Cobbold) in Ceylon. *Bulletin of the World Health Organisation,* **37,** 117-137.

SAMARAWIEKREMA, W.A. (1962) Changes in the ovariole of Mansonia (Mansonioides) mosquitoes in relation of age determination. *Annals of Tropical Medical Parasitology*, **56,** 110-26.

SAMBROOK, J. FRITSCH, F. & MANIATIS, T. (1989) *Molecular Cloning: A Laboratory Manual.* 2 Edn. Cold Spring Harbour Laboratory, New York.

SATTLER, M.A. MTASIWA, D. KIAMA, M. PREMJI, Z. TANNER, M. KILLEEN, G.F. & LENGELER, C. (2005) Habitat characterisation and

spatial distribution of *Anopheles* species mosquito larvae in Dar es Salaam (Tanzania) during an extended dry period. *Malaria Journal*, **4,** 4.

SCHMITZ, B, & GELFAND, M. (1976) A study of the clinical features of malaria in Rhodesia Part 1. *Central African Journal of Medicine*, **22,** 83-88.

SCOTT, J.A. BROGDON, W.G. & COLLINS, F.H. (1993) Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. *American Journal of Tropical Medicine and Hygiene*, **49,** 520–529.

SERVICE M.W. (1993b) *Mosquito Ecology: Field sampling methods*, 2nd edition. Elsevier Applied Science Publishers Ltd. London

SERVICE, M.W. (1985) *Anopheles gambie*: Africa's principal malaria vector, 1902-1984. *Bulletin of the Entomological Society of America*, **31**, 8-l2.

SERVICE, M.W. (1993a) The *Anopheles* vector. In: Gilles H.M. Warrell D.A. (eds) Bruce-Chwatt's Essential Malariology. 3rd edition. Boston: Edward Arnold, Pp. 97-123

SHARAKOV, I.V. BRAGINETS, O. MBOGO, C.N. & YAN, G. (2001a) Isolation and characterisation of trinucleotide microsatellites in African malaria mosquito *Anopheles funestus. Molecular Ecology Notes*, **1,** 289-92.

SHEPPARD, P.M., MACDONALD, W.W., TONN, R.J. & GRAB, B. (1969) The dynamics of an adult population of *Aedes aegypti* in relation to dengue haemorrhagic fever in Bangkok. *Journal of Animal Ecology*, **38**, 661–702.

SHIFF, C. (2002) A call for integrated approaches to controlling malaria. *Parasitology Today*, **3(3),** 125.

SHILILU, J.I. MAIER, W.A. SEITZ, H.M. & ORAGO, A.S. (1998) Seasonal density, sporozoite rate and entomological inoculation rates of *Anopheles gambiae* and *Anopheles funestus* in a high altitude sugarcane growing zone in western Kenya. *Tropical Medicine and International Health* **3,** 706–710.

SHORTT, H.E. & GARNHAM, P.C.C. (1948) The pre-erythrocytic development of *Plasmodium cynomolgi* and *Plasmodium vivax. Transactions of the Royal Society of Tropical Medicine and Hygiene,* **41,** 785-795.

SIBOMANA, I. (2002) Investigation into the ecological determinants of distribution of *Anopheles gambie* s.s (Diptera: Culicidae) larval populations in urban Accra, Ghana. *Master of Philosophy in Entomology Thesis, University of Ghana, Legon,* Pp. 93-99.

SIDHU, A.B. VERDIER_PINARD, D. & FIDOCK. D.A. (2002) Chloroquine resistance in *Plasmodium falciparum* malaria parasites conferred by pfcrt mutations. Science 298, 210-3.

SINDEN, R.E. & SMALLEY, M.E. (1976) Gametocytes of *Plasmodium falciparum*: phagocytosis by leucocytes *in vivo* and *in vitro*. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **70,** 344–345.

SINDEN, R.E. (1975) The sporogonic cycle of *Plasmodium yoelii nigeriensis*: a scanning electron microscope study. *Protistologica,* **11**, 31-39. In: Kettle, D.S. (1995) Culicidae (Mosquito). In: *Medical and Veterinary Entomology.* 2nd Edition. Oxford CAB international, pp 109-151.

SMITH, T. (1995) Proportionality between light trap catches and biting densities of malaria vectors. *Journal of America Mosquito Control Association,* **11,** 377-378.

SNOW, R.W. BASTOS, D. AZVEDO, I. LOWE, B.S. KABIRU, E.W. NEVILL, C.G. MWANKUSYE, S. KASSIGA, G. MARSH, K. & TEUSCHER, T. (1994) Severe childhood malaria in two areas of markedly different *falciparum* transmission in East Africa. *Acta Tropica* **57(4),** 289-300.

SNOW, R.W. SCHELLENBERG, J.R.M. PESHU, N. FORSTER, D. NEWTON, C.R. WINSTANLEY, P. MWANGI, I. WARUIRU, C. WARN, P.A. NEWBOLD, C. & MARSH, K. (1993) Periodicity and space time clustering

of severe childhood malaria on the coast of Kenya. *Transactions of the Royal society of Tropical Medicine and Hygiene* **87(4),** 386-390.

SODERLUND, D.M. & KNIPPLE, D.C. (2003) The molecular biology of knockdown resistance to pyrethroid insecticides. Insect *Biochemistry and Molecular Biology* **33,** 563–577.

STAFFORD III K.C. (1999) Tick Bite Prevention. Connecticut Department of Public Health.

STEVENSON, M.M. & RILEY, E.M. (2004) Innate immunity to malaria Nature Reviews Immunology **4,** 169-180.

STILES-OCRAN, J.B. (2003) Preliminary studies on the distribution of molecular markers in *Anopheles gambiae* s.s in the Ashanti Region, Ghana. *Bachelor of Science in Chemistry Project. Kwame Nkrumah University of Science and Technology, Ghana.*

STILES-OCRAN, J.B. BOAKYE, D.A. COETZEE, M. HUNT, R. (unpublished) Bionomics of malaria vectors in the Obuasi Municipality. Monitoring and evaluation data of the Obuasi Malaria Control Programme (OMCP) obtained from the Obuasi Malaria Control Centre on September 28, 2008.

STILES-OCRAN, J.B. WILSON, M.D. FRIMPONG, M.T. OWIREDU, W.K. & BOAKYE, D.A. (2007) Studies on insecticide usage pattern and resistance status of *Anopheles gambiae* s.s. in the Ashanti Region of Ghana. *American Journal of Tropical Medicine and Hygiene,* **77(5-Suppl),** 170-257.

STRICKLAND, G.T. (1991) Malaria In: Strickland, G.T. (ed.) *Hunter's Tropical Medicine.* Saunders, Philadelphia, pp. 586-615.

TAKKEN, W. (2002) Do insecticide-treated bednets have an effect on malaria vectors? *Tropical Medicine and International Health,* **7,** 1022-1030.

TAKKEN, W. CHARLWOOD, J. D. BILLINGSLEY, P.F. & GORT G.G. (1998) Dispersal and survival of *Anopheles funestus* and *Anopheles gambiae* s.l.

(Diptera: Culicidae) during the rainy season in Southeast Tanzania. *Bulletin Entomology Research,* **88,** 561-566.

TAYLOR, C.E. TOURÉ, Y.T. CARNAHAN, J. NORRIS, D.E. DOLO, G. TRAORÉ, S.K. EDILLO, F.E. & LANZARO, G.C. (2001) Gene flow among population of the malaria vector *Anopheles gambiae*, in Mali, West Africa. *Genetics*, **157,** 743–750.

TEKLEHAIMANOT, H.D. SCHWATRZ, J. TEKLEHAIMANOT, A. & LIPSITCH, M. (2004) Alert threshold algorithms and malaria epidemic detection. *Emerging Infectious Diseases,* **10,** 1220-1226.

THELWELL, N.J. HULSMAN, R.A. HARBACH, R.E. & BUTLIN, R.K. (2000) Evidence for mitochondrial introgression between *Anopheles bwambae* and *Anopheles gambiae. Insect Molecular Biology,* **9,** 203-210.

TOMITA, T. & SCOTT, J.G. (1995) cDNA and deduced protein sequence of Cyp6D1: the putative gene for a cytochrome P450 responsible for pyrethroid resistance in house fly. *Insect Biochemistry and Molecular Biology,* **25,** 275-83.

TOMITA, T. LIU, N. SMITH, F.F. SRIDHAR, P. & SCOTT, J.G. (1995) Molecular mechanisms involved in increased_expression of a cytochrome P450 responsible for pyrethroid resistance in the housefly, *Musca domestica*. *Insect Molecular Biology* 135-140.

TORRES, E.P. FOLEY, D.H. & SAUL, A. (2000) Ribosomal DNA sequence markers differentiate two species of the *Anopheles maculatus* (Diptera: Culicidae) complex in the Philippines. *Journal of Medical Entomology,* **37,** 933-37.

TOURE, Y.T. PETRARCA, V. & TRAORE, S.F. COULIBALY, A. MAIGA, H.M. SANKARE, O. SOW, M. DI DECO, M.A. & COLUZZI, M. (1994)

Ecological genetic studies in the chromosomal form Mopti of *Anopheles gambiae* s.s. in Mali, West Africa . *Genetica,* **94,** 213–223.

TOURE, Y.T. PETRARCA, V., & TRAORE, S.F., COULIBALY, A., MAIGA, H.M., SANKARÉ, O., SOW, M., DI DECO, M.A., COLUZZI, M. *ET AL.* (1998) Distribution and inversion polymorphism of chromosomally recognised taxa of the *Anopheles gambiae* complex in Mali, West Africa. *Parasitologia*, **40**, 477–511.

TRAPE, J.-F. & ROGIER, C. (1996) Combating malaria morbidity and mortality by reducing transmission. *Parasitol Today*, **12,** 236–240.

TRIGG, P.I. & KONDRACHINE, A.V. (1998) Commentary: Malaria control in the 1990s. *Bulletin of the World Health Organization*, **76,**11-16.

TRIPET, F. TOURE, Y.T. TAYLOR, C.E. NORRIS, D.E. DOLO, G. & LANZARO, G. (2001) DNA analysis of transferred sperm reveals significant levels of gene flow between molecular forms of *Anopheles gambiae*. *Molecular Ecology*, **10,** 1725–1732.

VAIS, H. WILLIAMSON, M.S. HICK, C.A. ELDURSI, N. DEVONSHIRE, A.L. & USHERWOOD, P.N. (1997) Functional analysis of a rat sodium channel carrying a mutation for insect knock-down resistance (*kdr*) to pyrethroids. *FEBS Lett*, **413**, 327-32.

VAUCEL, M. & CAMPOURCY, A. (1943) L'anophelisme au Cameroun franc¸ais. Revue des Sciences Me´dicales, Pharmaceutiques et Ve´terinaires de l'Afrique Libre **2,** 85–88.

VONTAS, **J.G. McCARROLL**, **L. KARUNARATNE**, **S.H.P.P. LOUIS**, **C. HURD**, **H.** & **HEMINGWAY J. (2004)** Does environmental stress affect insect-vectored parasite transmission? *Physiological Entomology*, **29(3),** 210–213.

VULULE, J.M. BEACH, R.F. ATIELI, F.K. MOUNT, D.L. ROBERTS, J.M. & MWANGU, R.W. (1999) Reduced susceptibility of *Anopheles gambiae* to permethrin associated with the use of permethrin impregnated bednets and curtains in Kenya. *Medical and Veterinary Entomology,* **8,** 71–75.

VULULE, J.M. BEACH, R.F. ATIELI, F.K. ROBERTS, J.M. MOUNT, D.L. & MWANGI, R.W. (1994) Reduced susceptibility of *Anopheles gambiae* to permethrin associated with the use of permethrin impregnated bednets and curtains in Kenya. *Medical and Veterinary Entomology,* **8,** 71–75.

VYTHILINGAM, I. PHETSOUVANH, R. KEOKENCHANH, K. YENGMALA, V. VANISAVETH, V. PHOMPIDA, S. & HAKIM S.L. (2003) The prevalence of *Anopheles* (Diptera: Culicidae) mosquitoes in Sekong Province, Lao PDR in relation to malaria transmission. *Tropical Medical and International Health,* **8(6),** 525-535.

WANJI, S. TANKE, T. ATANGA, S.N. AJONINA, C. NICHOLAS, T. & FONTENILLE, D. (2003) *Anopheles* species of the mount Cameroon region: biting habits, feeding behaviour and entomological inoculation rates. *Tropical Medical and International Health,* **8(7),** 643–649.

WARE, G.W. & WHITACRE, D.M. (2004) The Pesticide Book, 6th Ed. 496 pp. Meister Media Worldwide, Willoughby, Ohio. (ISBN 1892829-11-8)

WARE, G.W. (1978) The pesticide book. W.H. Freeman Company, Pp 175. Ware, G.W. & Whitacre, D.M. (2004) An introduction to insecticides. 4th edition. Retrieved on August 16, 2005, from: *http://ipmworld.umn.edu/chapters/ware.htm.*

WEETO, M.M. KOEKEMOER, L.L. KAMAU, L. HUNT, R.H. COETZEE, M. (2004) Evaluation of a species-specific PCR assay for the *Anopheles funestus* group from eleven African countries and Madagascar. *Transactions of the Royal Society for Tropical Medicine and Hygiene*, **98,** 142–147.

WEIDHAAS, D.E. BREELAND, S.G. LOFGREN, C.S. DAME, D.E. & KAISER, R. (1974) Release of chemosterilised males for the control of *Anopheles albimanus* in El Salvador. IV. Dynamics of the test population. *American Journal of Tropical Medicine and Hygeine,* **23,** 298-308.

WEILL, M. CHANDRE, F. BRENGUES, C. MANGUIN, S. AKOGBETO, M. PASTEUR, N. GUILLET, P. & RAYMOND, M. (2000) The *Kdr* mutation occurs in the Mopti form of *Anopheles gambiae* s. s. through introgression. *Insect Molecular Biology*, **9**, 541- 455.

WELLEMS, T. E. (2002) *Plasmodium* chloroquine resistance and the search for a replacement antimalarial drug. *Science,* 298, 124-6.

WELLEMS, T.E. & FAIRHURST, R.M. (2005) Haemoglobin C protects against *Plasmodium falciparum* malaria. *Nature Genetics,* **37(11),** 1160-1162.

WERNSDORFER, W.H. & MC GREGOR, I. (1986) Malaria: Principles and practice of malariology: Churchill Livingstone.

WERNSDORFER, W.H. (1980) The importance of malaria in the world. In: *"Malaria" Vol. 1. Eddited by Kreier, J.P.* Academic Press, New York. 1-93.

WHITE, G. B. (1974) *Anopheles gambiae* complex and disease transmission in Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **68,** 278-301.

WHITE, G.B. (1977) The place of morphological studies in the investigation of *Anopheles* species complexes. *Mosquito Systematics,* **9,** 1-24.

WHITE, G.B. (1982) Malaria vector ecology and genetics. *Br. Med. Bull.* 38(2), 207-212.

WHO/AFRO/MAL/MIP.IPT/05

WILKERSON, R.C. PARSONS, T.J. ALBRIGHT, D.G. KLEIN, T.A.& BRAUN, M.J. (1993). Random amplified polymorphic DNA (RAPD)

markers readily distinguish Cryptic mosquito species (Diptera Culicidae: *Anopheles*). *Insect Molecular Biology,* 205-211.

WILKERSON, R.C. PARSONS, T.J. KLEIN, T.A. DAFFIGAN, T.V. BERGO E. & CONSOLIM, J. (1995) Diagnosis by Random amplified polymorphic DNA polymerase chain reaction of four cryptic species related to *Anopheles* (Nyssorhynchus) *albitarsis* (Diptera: culicidae) from Paraguay, Argentina, Brazil. *Journal of Medical Entomology,* **32,** 697-704

WILKES, T.J. MATOLA, Y.G. CHARLWOOD, J.D. (1996) *Anopheles rivulorum*, a vector of human malaria in Africa. *Medical and Veterinary Entomology,* **10,** 108–110.

WILLIAMSON, M.S. MARTINEZ-TORREZ, D. HICK, C.A. & DEVONSHIRE, A.L. (1996a) Identification of mutations in the housefly para-type sodium channel gene associated with knockdown resistance (*kdr*) to pyrethroid insecticides. *Molecular and General Genetic,* 252, 51-60.

WIRTZ, R.A. & BURKOT, T.R. (1991) Detection of malarial parasites in mosquitoes. *Advances in Disease Vector Research.* New York: Springer-Verlag.

WIRTZ, R.A. BURKOT, T.R. ANDRE, R.G. ROSENBERG, R. COLLINS, W.E. & ROBERTS, D.R. (1985) Identification of *Plasmodium vivax* sporozoites in mosquitoes using enzyme-linked immunosorbent assay. *American Journal of Tropical Medicine and Hygiene,* **34(6),** 1048-54.

WIRTZ, R.A. BURKOT, T.R. GRAVES, P.M. & ANDRE, R.G. (1987a) Field evaluation of enzyme-linked immunosorbent assays for P. falciparum and P. vivax sporozoites in mosquitoes (Diptera: Culicidae) from Papua, New Guinea. *Journal of Medical Entomology,* 24, 433–437.

WIRTZ, R.A. SATTABONGKOT, J. HALL, T. BURKOT, T.R. & ROSENBERG, R. (1992) Development and evaluation of an enzyme-linked

immunosorbent assay for *Plasmodium vivax* -VK247 sporozoites. *Journal of Medical Entomology*, **29,** 854-857.

WIRTZ, R.A. ZAVALA, F. CHAROENVIT, Y. CAMBELL, G. H. BURKOT, T.R. SCHNEIDER, I. ESSER, K.M. BEAUDIN, R.L. & ANDRE, G.R. (1987b) Comparative testing of *Plasmodium falciparum* sporozoite monoclonal antibodies for ELISA development. *Bulletin of the World Health Organisation*, 65, 39-45.

WISEMAN, V. SCOTT, A. CONTEH, L. MCELROY, B. STEVENS, W. (2008) Determinants of provider choice for malaria treatment: experiences from The Gambia. *Social Science and Medicine,* **67,** 487–496.

WONDEMAGEGNEHU E, (1999) *Counterfeit and Substandard Drugs in Myanmar and Vietnam*. Report of a study carried out in cooperation with the Governments of Myanmar and Vietnam. Geneva: World Health Organization. WHO/EDM/QSM/99.3.

WONDJI, C. SIMARD, F. & FONTENILLE, D. (2002) Evidence for genetic differentiation between the molecular forms M and S within the forest chromosomal form of *Anopheles gambiae* s.s. in an area of sympatry. *Insect Molecular Biology*, **11**, 11- 19.

WORLD HEALTH OGANISATION, (2008) World Malaria Report. Retrieved on February 2009 from the websites: http://malaria.who.int/wmr2008/malaria2008.pdf.

WORLD HEALTH ORGANISATION, (1975) Manual on Practical Entomology. Part II. Methods and Techniques. Geneva: World Health Organization.

WORLD HEALTH ORGANISATION, (1980) *Environmental Management for Vector Control,* Technical Report Series No. 649, World Health Organization, Geneva.

WORLD HEALTH ORGANISATION, (1987) Entomological findings and their epidemiological implications. In: Kondrashin AV, Rashid KM, editors.

Epidemiological considerations for planning malaria control in South-east Asia region. World Health Organisation Regional Office for South-east Asia, New Delhi, 367-87.

WORLD HEALTH ORGANISATION, (1990) Potential heath effects of climatic change. *WHO/PEP.90.10*, Geneva.

WORLD HEALTH ORGANISATION, (1992) Vector resistance to insecticides. Fifth Report of the World Health Organisation Expert Committee on Vector Biology and Control. *World Health Organisation Technical Report Series* **818**, 1-62.

WORLD HEALTH ORGANISATION, (1993) Implementation of the Global Malaria Control Strategy. Geneva. : World Health Organization, *Technical Report Series*, no. 839.

WORLD HEALTH ORGANISATION, (1998a) Roll Back Malaria Geneva: World Health Organization. Fact Sheet, number 203.

WORLD HEALTH ORGANISATION, (2003) Malaria entomology and vector control. Learner's Guide. HIV/AIDS, Tuberculosis and Malaria, Roll Back Malaria. WHO/CDS/CPE/SMT/2002.18 Rev.1

WORLD HEALTH ORGANISATION, (2005a) Malaria Fact Sheet. Retrieved on May 2, 2006 from the site: http://www.unicef.org/ media/files/MalariaFact Sheet.pdf.

WORLD HEALTH ORGANISATION, (2005b) World Malaria Report. Retrieved on May 1, 2007 from the site: www.rbm.who.int/wmr2005/profiles/ghana.pdf

WORLD HEALTH ORGANISATION, (2006) *Pesticides and their application*. 6th edition. Geneva: WHO/CDS/NTD/ WHOPES/GCDPP/2006.1.

WORLD HEALTH ORGANIZATION (1986) Severe and complicated malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 80 (Supplement): 1-50.

WORLD HEALTH ORGANIZATION, (1993) Counterfeit drugs. *Bulletin of World Health Organisation,* **71,** 464–466.

WORLD HEALTH ORGANIZATION, (1998a) Test procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticide on treated surfaces, Geneva. *Ref:WHO/CDS/CPC/MAL/98.12.*

WORLD HEALTH ORGANIZATION, (1998b) Techniques to detect insecticide resistance mechanisms (field and laboratory manual). Geneva 1998.

WORLD HEALTH ORGANIZATION, (1999) "Roll Back Malaria". World Health Report. Geneva, Switzerland.

WORLD HEALTH ORGANIZATION, (2000) Report of the fourth WHOPES Working Group meeting, Geneva. Retrieved from on January 22, 2005: whqlibdoc.who.int/hq/2001/WHO_CDS_WHOPES_2001.2.pdf.

WORLD HEALTH ORGANIZATION, (2006a) WHO guidelines for treatment of malaria. WHO, Geneva, Switzerland.

WORLD HEALTH ORGANIZATION, (2006b) Malaria vector control and personal protection. WHO technical report series 936. Geneva, Switzerland.

WORLD HEALTH ORGANIZATION, (2006c) Pesticides and their application for the control of vectors and pests of public health importance. Six edition. *WHO/CDS/NTD/WHO PES/GCDPP/2006.1*

WORLD HEALTH ORGANIZATION, (2006d) Malaria. Retrieved on December 21, 2006 from the website: http:/who.int/topics/malaria/en/.

WOUBE, M. (1997) Geographical distribution and dramatic increases in incidences of malaria: consequences of the resettlement scheme in Gambela, S.W. Ethiopia. *Indian Journal of Malariology,* **34,** 140-163.

www.mara.org.za/pdfmaps/GhaDistribution.PDF

www.newmontghana.com/

www.who.int/bulletin/volumes/86/2/07-050633/en

YASOTHORNSRIKUL, S. PANYIM, S. & ROSENBERG, R. (1988) Diagnostic restriction fragment patterns of DNA from the four isomorphic species of *Anopheles dirus*. *Southeast Asian Journal of Tropical Medicine and Public Health* **19(4),** 703-708.

YAWSON, A.E. MCCALL, P.J. WILSON, M.D. & DONNELLY, M.J. (2004) Species abundance and insecticide resistance of *Anopheles gambiae* in selected areas of Ghana and Burkina Faso. *Medical and Veterinary Entomology* **18**, 372-377.

YE-EBIYO, Y. POLLACK, R.J. KISZEWSKI, A.E. & SPIELMAN, A. (2003) A componet of maize pollen that stimulates larval mosquitoes (Diptera: Culicidae) to feed and increases toxicity of microbial larvicides. *Journal of Medical Entomology*, **40,** 860-864.

ZAIM, M. AITIO, A. & NAKASHIMA, N. (2000) Safety of pyrethroid-treated nets. *Medical and Veterinary Entomology*, **14**, 1-5

ZHENG, L. BENEDICT, M.Q. CORNEL, A.J. COLLINS, F.H. & KAFATOS, F.C. (1996) An integrated genetic map of the African human malaria vector mosquito, *Anopheles gambiae*. *Genetics*, **143,** 941-952.

ZHENG, L. CORNEL, A.J. WAND, R. ERFEL, H. VOSS, H. ANSORGE, W. KAFA-TOS, F.C. & COLLINS, F.H. (1997) Quantitative trait loci for the refractoriness of *Anopheles gambiae* to *Plasmodium cynomolgi* B. *Science* **276,** 425-428.

ZHOU, Z. & SYVANEN, M. (1997) A complex glutathione transferase gene family in the housefly Musca domestica. *Molecular and General Genetics*, **256,** 187-194.

APPENDICES

APPENDIX I: MOLECULAR BIOLOGY TECHNIQUES

Preparation of Standard Solutions Used in Molecular Biology Studies

The following standard solutions used in in this study were prepared using sterile distilled water (sddH_20). Where appropriate, the solutions were autoclaved at 121 1b/sq in. for 15 minutes in Eyela Autoclave (Rikikakki, Tokyo). The distillation apparatus and the autoclave machine were acquired from the Bacteriology Department, NMIMR-UG, Accra. All other equipment or facilities used for the molecular biology studies were obtained from the Parasitology Department, NMIMR.

a. Solutions for deoxyribonucleic acid (DNA) extraction

Bender buffer (pH 8.0): 0.1 M NaCl, 0.2 M sucrose, 0.1 M Tris-HCl pH 7.5, 0.05 M EDTA pH 9.1, 0.5% SDS. The solution was then stored at 4°C.

0.5 M EDTA (pH 8.0): 186.1 g/l of EDTA was dissolved in sterile double distilled water; the pH was adjusted with NaOH pellet to 8.0, the volume was made to 1000 ml with sddH_2O and stored at room temperature.

KAc (5 M K, 8 M Acetate): 60 ml of 5 M KAc and 11.5 ml glacial acetic acid in 28.5 ml sterile double distilled water (sddH_20).

TE (pH 8.0): 10 mM Tris-HCl, 1 mM EDTA (pH 8.0). The solution was then stored at room temperature.

Primers for PCR: Primers used in PCR reaction were reconstituted and diluted according to the manufacturers recommendations (Oswell Laboratories).

b. Solutions for electrophoresis

Agarose Gels

10 X TAE buffer: 242 g Tris Base, 57.1 ml glacial acetic acid, 100 ml 0.5 M EDTA, pH was adjusted to 7.7 (with glacial acetic acid) and the volume made to 1000 ml with sddH_20.

EtBr (10 mg/ml): 1 g of EtBr was completely dissolved in 100 ml sddH_20 and stored in a dark bottle in the dark at room temperature.

c. Gel loading buffers

6 X Bromophenol blue: 0.25 % bromophenol blue was added to 40 % sucrose in water and stored at 4°C.

Bromophenol blue xylene cyanol: 1 volume of bromophenol blue xylene cyanol and four volumes of cyanide.

5X orange G: 20 % w/v Ficoll, 25 mM EDTA, 2.5 % (w/v) orange G. Stored at room temperature.

DNA molecular weight size marker

The 100 bp molecular weight size marker was obtained from sigma and was diluted according to the manufacturer's recommendations and used as standard for the experimentation. For the 100 bp ladder, the first band size is 100 bp, the next ones measure 200, 300, 400, 500, 600, 700, 800, 900 and 1000 base pairs.

Casting Gels

a. Preparation of Agarose solution

Two percent Agarose solution was prepared in this manner: Two grams of agarose was weighed out in 80 ml 50 x TAE buffer and melted in a microwave oven for 2 minutes. The molten agarose was allowed to cool to about 60°C 1 µl ethidium bromide added. This was mixed thoroughly and the conical flask placed in a hot water bath at 50°C until gel was ready to be poured. Gloves were worn throughout the process since ethidium bromide is known to be a casinogenic substance. To avoid warping the unit agarose was not poured at temperature hotter than 50°C.

b. Pouring of agarose solution and well preparation

The casting tray was placed on a level surface and the running plate kept inside the casting tray. A comb of desired thickness was placed across the rim of the casting tray to form sample wells. The height of the teeth of the comb was adjusted so that they penetrated the gel but left about 1 mm of gel between the teeth and the running plate. The tendency of the gel seeping under the running plate while being

poured was reduced by fitting the handles of the running plate properly. The gel was poured into the casting tray on the running plate. After the gel was poured, it set or solidified after at least 30 minutes. When the gel was well hardened, the comb was carefully removed to avoid breaking the peripheries between wells. The running plate was lifted out of the casting tray. Where some gel adhered to the base of the plate, it was peeled off. The running plate plus the gel was then transferred to the main unit.

c. Running the gel

Deoxyribonucleic acid sample was carefully loaded after mixing with one third volume of the loading dye, using a Gilson pipette. The lid was placed on the unit so that the cathode (black cord) was nearest the sample wells, since nucleic acid samples migrate towards the anode (red cord). Electrodes were connected to the gel and electrophoresis conducted towards the anode (+) at 85 or 100V for 1 hour.

d. Visualising the DNA bands

DNA bands were visualised under UV light box inside a dark room. DNA is usually visualised by florescence of bound ethidium bromide. The gel was removed from the main unit and transferred to a black Perspex sheet. The DNA bands were observed fluorescing under short wavelength UV light in a dark room.

Goggles were worn to protect the eyes from UV radiation damage. Gloves were always used to handle gels or buffers containing ethidium bromide, which is a powerful mutagen. Photographs were taken of the gels using type 667 film on a Polaroid camera fitted with an orange filter for permanent record of result. Photographs were also taken with a Kodak Camera fitted to the UV light chamber and connected to a computer.

APPENDIX II: BIOCHEMICAL/IMMUNOLOGICAL METHODS

Solutions Used in Immunology/Parasitology Studies

a. Mosquito/Plasmodium Antigen (MPA) preparation

I. Grinding solution (GS)

Blocking buffer (BB) and Nonidet P – 40 (NP – 40) were mixed in the ratio of 5 µl NP – 40: 1 ml BB. The resulting solution was then stored at stored at 4°C until it was required for experimentation.

II. MPA Preparation

Test mosquito sample was put in a pre-labelled 1.5 ml micro-centrifuge (Eppendorf) tube containing 50 µl GS. It was then crushed with a pestle to obtain a homogenous suspension. The pestle was rinsed with more GS into the suspension to obtain a total of 200 µl of MPA suspension. MPA extract was tested immediately or stored frozen.

b. Dulbecco's Phosphate Buffered Saline (DPBS) Preparation

I. Calibration of flask

A litre of tap water was poured into a 5 l capacity flat bottom conical flask. A stirring rod was gently slid along the side of the flask into the bottom. The final volume of water in the flask was then marked.

II. Preparation of buffer

The tap water in step I. above was discarded and the flask rinsed with distilled water. Seven hundred and fifty millilitres of distilled water was poured into the calibrated flask. The stirring rod was rinsed with distilled water and slid into the flask. The flask was placed on an electronic magnetic stirrer and stirred. A bottle of DPBS powder was emptied into the flask while the content was still stirring. The bottle was rinsed thoroughly and the content was added to the flask. The pH of the solution was adjusted to 7.2. More distilled water was added to the content of the flask to the level of the calibrated mark. The solution was stored at 4°C and used within two weeks.

a. Washing Buffer [PBST (tween)] Preparation

The washing buffer was made up of PBS and 0.05 % Tween 20. The washing buffer was prepared following the method for the preparation of PBS above to the stage of pH adjustment. Zero point five millilitres of Tween 20 was added to one litre PBS while the content was being stirred. The Tween 20 was released slowly, a litre at a time, but continuously into the content of the flask to ensure prompt dissolution. More distilled water was added to the level of the calibrated mark.

b. Blocking buffer (BB) preparation

BB can be mixed in two ways, a. Boiled casein BB and b. unboiled casein BB. It was the former methodology that was used in this work. Casein was suspended in 0.1 M NaOH and the resulting solution brought to a boil. After the dissolution of the casein, the PBS was slowly added to the content. The content was allowed to cool and the pH adjusted to 7.2 with hydrochloric acid.

Boiled casein BB method

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

c. Substrate Solution Preparation

Two solutions, namely, solution A, which consists of KP&LABTS and solution B, which consists of hydrogen peroxide, were mixed in a ratio of 1:1 immediately before use. The Substrate solution was incubated at 100 µl per micro-titre well.

d. Preparation of Solution for coating one micro-titre plate

The stock captured monoclonal antibody (MAb) was 0.5 µg/µl. The coating rate was 0.1 µg/50 µl/well. One micro-titre plate contains 96 wells. This was approximated to 100 wells, the extra 4 wells being provision against spillage. 10 (100 x 1) µg MAb was required to coat one plate. Since the stock captured MAb was 0.5 µg/µl, 10 µg MAb was contained in 10 x 1/ 0.5 = 20 µl solution. Since the coating rate of the MAb was 0.1 µg/50 µl/well, 5000 (50 x 100) µl of MAb solution was needed to coat one micro-titre plate. Four thousand nine hundred and eighty micro-litres (4, 980 µl) of PBS was added to 20 µl (10 µg MAb) solution. The plate was coated immediately and incubated at 4°C overnight and used the following day. Each well was coated with 50 µl (0.1 µg MAb).

APPENDIX III: HUMAN LANDING COLLECTION DATA

Table I Indoor human landing collection data during rainy period at Sunyani

Number of *Anopheles gambiae* sensu lacto Caught Indoors									
		May		June		July		October	
HOUR	Total	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
18.00-19.00	25	1	0	2	5	4	6	7	0
19.00-20.00	52	8	0	5	12	5	8	9	5
20.00-21.00	76	13	4	11	14	8	6	11	9
21.00-22.00	124	11	17	21	21	10	13	15	16
22.00-23.00	137	16	23	21	13	13	14	21	16
23.00-00.00	210	21	28	27	31	28	26	28	21
00.00-01.00	208	29	30	22	29	25	24	32	17
01.00-02.00	210	33	26	15	31	35	23	26	21
02.00-03.00	229	26	32	16	45	27	20	31	32
03.00-04.00	219	22	35	21	31	25	32	27	26
04.00-05.00	236	35	20	34	37	31	28	33	18
05.00-06.00	208	27	20	23	32	26	26	25	29
Total	1934	242	235	218	301	237	226	265	210

Table II Outdoor human landing collection data during rainy season at Sunyani

Number of *Anopheles gambiae* sensu lacto Caught Outdoors									
		May		June		July		October	
HOUR	Total	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
18.00-19.00	35	5	6	4	0	0	6	8	6
19.00-20.00	41	3	4	4	0	6	8	4	12
20.00-21.00	96	9	11	12	14	14	6	16	14
21.00-22.00	110	10	15	9	13	18	11	9	25
22.00-23.00	128	16	20	14	14	22	16	14	12
23.00-00.00	144	20	17	15	20	16	19	12	25
00.00-01.00	146	11	18	13	18	20	24	21	21
01.00-02.00	217	25	28	31	26	25	33	20	29
02.00-03.00	218	27	23	25	23	33	23	27	37
03.00-04.00	177	20	17	11	29	25	26	19	30
04.00-05.00	190	24	23	30	26	18	23	21	25
05.00-06.00	182	24	24	22	18	22	26	20	26
Total	1684	194	206	190	201	219	221	191	262

Table III Indoor human landing collection data during dry season at Sunyani

Number of *Anopheles gambiae* sensu lacto Caught Indoors									
		August		November		December		January	
HOUR	Total	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
18.00-19.00	9	2	0	2	3	1	0	0	1
19.00-20.00	12	2	1	4	1	1	2	0	1
20.00-21.00	19	4	2	5	4	0	0	2	2
21.00-22.00	23	7	4	4	0	2	2	2	2
22.00-23.00	39	3	7	9	6	5	4	2	3
23.00-00.00	38	6	6	9	10	4	0	0	3
00.00-01.00	41	8	7	9	5	5	2	4	1
01.00-02.00	36	7	4	12	3	6	0	2	2
02.00-03.00	45	3	5	10	11	12	0	2	2
03.00-04.00	39	8	4	13	7	3	4	0	0
04.00-05.00	19	0	4	9	3	1	2	0	0
05.00-06.00	2	0	0	2	0	0	0	0	0
Total	322	50	44	88	53	40	16	14	17

Table IV Outdoor human landing collection data during dry season at Sunyani

Number of *Anopheles gambiae* sensu lacto Caught Outdoors									
		August		November		December		January	
HOUR	Total	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
18.00-19.00	4	2	0	1	0	0	1	0	0
19.00-20.00	6	1	1	1	1	0	0	2	0
20.00-21.00	13	1	2	2	3	3	1	1	0
21.00-22.00	25	3	0	5	2	6	5	0	4
22.00-23.00	30	4	2	6	4	4	4	2	4
23.00-00.00	28	6	2	6	6	4	2	0	2
00.00-01.00	34	3	4	8	7	4	2	2	4
01.00-02.00	37	2	6	9	6	4	2	4	4
02.00-03.00	31	5	3	6	4	4	3	4	2
03.00-04.00	24	3	3	6	4	2	4	2	0
04.00-05.00	6	0	0	2	4	0	0	0	0
05.00-06.00	1	0	0	1	0	0	0	0	0
Total	239	30	23	53	41	31	24	17	20

Table V Indoor human landing collection data during rainy season at Chiraa

Number of *Anopheles gambiae* sensu lacto Caught Indoors									
		May		June		July		October	
HOUR	Total	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
18.00-19.00	28	5	9	6	0	0	0	7	1
19.00-20.00	38	7	9	5	0	4	3	9	1
20.00-21.00	54	6	12	9	7	3	7	6	4
21.00-22.00	79	13	11	14	6	5	12	7	11
22.00-23.00	83	11	14	9	12	1	16	10	10
23.00-00.00	117	16	17	18	14	10	14	10	18
00.00-01.00	146	14	16	20	26	14	20	16	20
01.00-02.00	167	16	20	24	21	18	24	20	24
02.00-03.00	162	18	20	22	22	20	18	20	22
03.00-04.00	170	20	22	27	16	18	27	22	18
04.00-05.00	154	18	24	18	22	18	18	16	20
05.00-06.00	152	19	24	20	14	25	20	18	12
Total	1350	163	198	192	160	136	179	161	161

Table VI Outdoor human landing collection data during rainy season at Chiraa

Number of *Anopheles gambiae* sensu lacto Caught Outdoors									
		May		June		July		October	
HOUR	Total	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
18.00-19.00	21	5	0	2	4	0	6	0	4
19.00-20.00	32	4	6	2	0	4	6	4	6
20.00-21.00	48	6	2	8	12	8	0	2	10
21.00-22.00	91	11	12	10	10	10	14	12	12
22.00-23.00	98	12	10	8	15	14	16	11	12
23.00-00.00	105	9	13	11	17	13	14	13	15
00.00-01.00	108	10	12	18	18	8	20	12	10
01.00-02.00	123	13	17	18	15	18	11	17	14
02.00-03.00	140	17	20	21	17	20	15	20	10
03.00-04.00	138	21	17	20	11	18	14	18	19
04.00-05.00	120	18	8	14	16	16	20	8	20
05.00-06.00	106	16	12	17	9	14	12	6	20
Total	1130	142	129	149	144	143	148	123	152

Table VII Indoor human landing collection data during dry season at Chiraa

Number of *Anopheles gambiae* sensu lacto Caught Indoors									
		August		November		December		January	
HOUR	Total	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
18.00-19.00	6	1	0	3	0	2	0	0	0
19.00-20.00	7	0	0	2	3	0	0	2	0
20.00-21.00	8	3	2	3	0	0	0	0	0
21.00-22.00	22	5	2	4	2	2	1	3	3
22.00-23.00	31	8	2	7	4	4	0	2	4
23.00-00.00	30	5	7	4	6	0	0	3	5
00.00-01.00	40	6	5	12	9	0	2	4	2
01.00-02.00	35	5	3	15	4	0	2	2	4
02.00-03.00	41	4	4	12	10	3	5	2	1
03.00-04.00	32	4	3	11	8	0	2	4	0
04.00-05.00	17	2	4	4	2	0	3	0	2
05.00-06.00	7	2	2	2	0	1	0	0	0
Total	276	45	34	79	48	12	15	22	21

Table VIII Outdoor human landing collection data during dry season at Chiraa

Number of *Anopheles gambiae* sensu lacto Caught Outdoors									
		August		November		December		January	
HOUR	Total	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
18.00-19.00	8	2	0	2	2	0	2	0	0
19.00-20.00	9	0	3	1	5	0	0	0	0
20.00-21.00	15	3	0	0	4	2	2	3	1
21.00-22.00	21	5	4	5	3	0	0	2	2
22.00-23.00	26	1	3	8	6	0	2	2	4
23.00-00.00	25	0	3	7	5	0	1	5	4
00.00-01.00	30	5	2	8	2	3	4	4	2
01.00-02.00	26	4	2	5	0	7	2	2	4
02.00-03.00	29	6	0	7	5	1	4	4	2
03.00-04.00	30	6	3	6	5	3	5	0	2
04.00-05.00	16	0	5	3	0	2	0	3	3
05.00-06.00	1	0	0	0	1	0	0	0	0
Total	236	32	25	52	38	18	22	25	24

Table IX Indoor human landing collection data during rainy season at Ahafo Kenyasi

Number of *Anopheles gambiae* sensu lacto Caught Indoors									
		May		June		July		October	
HOUR	Total	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
18.00-19.00	23	6	0	5	2	2	8	0	0
19.00-20.00	40	8	6	9	1	5	3	3	5
20.00-21.00	74	9	7	14	5	9	8	8	14
21.00-22.00	103	14	13	12	14	11	18	5	16
22.00-23.00	150	20	17	19	20	15	25	13	21
23.00-00.00	158	17	21	24	18	16	22	16	24
00.00-01.00	190	23	19	27	18	25	31	23	24
01.00-02.00	224	22	32	19	24	34	27	32	34
02.00-03.00	222	26	37	33	18	35	20	27	26
03.00-04.00	237	18	38	38	24	37	18	32	32
04.00-05.00	235	22	31	37	28	38	24	28	27
05.00-06.00	212	27	28	23	27	31	25	26	25
Total	1868	212	249	260	199	258	229	213	248

Table X Outdoor human landing collection data during rainy season at Ahafo Kenyasi

Number of *Anopheles gambiae* sensu lacto Caught Outdoors									
		May		June		July		October	
HOUR	Total	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
18.00-19.00	24	2	0	8	5	0	4	5	0
19.00-20.00	64	12	10	8	9	3	10	6	6
20.00-21.00	98	10	6	11	15	10	17	11	18
21.00-22.00	128	22	10	18	19	12	11	13	23
22.00-23.00	140	20	14	21	17	13	17	16	22
23.00-00.00	144	22	20	17	15	11	17	15	27
00.00-01.00	188	28	17	22	27	29	20	21	24
01.00-02.00	208	31	24	20	25	34	27	18	29
02.00-03.00	198	28	23	27	21	26	24	13	36
03.00-04.00	211	30	18	26	20	30	26	21	40
04.00-05.00	198	26	30	22	17	27	22	26	28
05.00-06.00	195	19	24	25	15	24	23	27	38
Total	1796	250	196	225	205	219	218	192	291

Table XI Indoor human landing collection data during dry season at Ahafo Kenyasi

Number of *Anopheles gambiae* sensu lacto Caught Indoors									
		August		November		December		January	
HOUR	Total	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
18.00-19.00	6	0	1	2	2	1	0	0	0
19.00-20.00	10	0	4	2	0	1	3	0	0
20.00-21.00	16	2	2	4	3	0	2	2	1
21.00-22.00	36	1	3	6	6	4	6	5	5
22.00-23.00	33	3	5	7	8	3	4	3	0
23.00-00.00	39	6	5	12	2	3	9	1	1
00.00-01.00	45	6	4	9	6	8	5	5	2
01.00-02.00	41	8	6	12	5	4	4	2	0
02.00-03.00	44	8	4	9	11	4	4	4	0
03.00-04.00	33	5	7	7	7	3	0	3	1
04.00-05.00	30	3	1	12	4	3	3	2	2
05.00-06.00	13	1	3	7	2	0	0	0	0
Total	346	43	45	89	56	34	40	27	12

Table XII Outdoor human landing collection data during dry season at Kenyasi

Number of *Anopheles gambiae* sensu lacto Caught Outdoors									
		August		November		December		January	
HOUR	Total	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
18.00-19.00	4	2	0	2	0	0	0	0	0
19.00-20.00	4	1	0	2	1	0	0	0	0
20.00-21.00	10	2	0	3	1	1	2	0	1
21.00-22.00	19	3	2	6	3	0	2	1	2
22.00-23.00	19	3	3	6	5	1	0	1	0
23.00-00.00	29	5	4	9	8	1	1	0	1
00.00-01.00	30	6	3	8	8	2	0	2	1
01.00-02.00	36	8	5	9	10	1	0	1	2
02.00-03.00	29	6	4	6	9	2	2	0	0
03.00-04.00	17	1	3	3	5	0	3	1	1
04.00-05.00	11	1	1	5	3	0	1	0	0
05.00-06.00	0	0	0	0	0	0	0	0	0
Total	208	38	25	59	53	8	11	6	8

Table XIII Indoor human landing collection data during rainy season at Hwidiem

Number of *Anopheles gambiae* sensu lacto Caught Indoors									
		May		June		July		October	
HOUR	Total	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
18.00-19.00	22	3	6	4	5	1	0	3	0
19.00-20.00	48	5	8	7	11	5	4	1	7
20.00-21.00	64	8	6	11	11	5	5	9	9
21.00-22.00	90	3	5	19	9	16	10	11	17
22.00-23.00	122	9	11	26	9	21	16	9	21
23.00-00.00	130	17	13	20	11	17	18	13	21
00.00-01.00	180	23	13	23	20	30	26	11	34
01.00-02.00	192	20	18	28	11	32	28	18	37
02.00-03.00	215	28	30	18	23	25	28	30	33
03.00-04.00	226	31	28	26	31	22	32	27	29
04.00-05.00	220	29	25	31	22	23	34	24	32
05.00-06.00	201	26	28	21	28	18	31	20	29
Total	1710	202	191	234	191	215	232	176	269

Table XIV Outdoor human landing collection data during rainy season at Hwidiem

Number of *Anopheles gambiae* sensu lacto Caught Outdoors									
		May		June		July		October	
HOUR	Total	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
18.00-19.00	43	6	6	11	2	6	8	4	0
19.00-20.00	63	11	8	16	0	2	9	8	9
20.00-21.00	79	9	8	13	6	16	9	11	7
21.00-22.00	90	9	11	12	6	14	14	12	12
22.00-23.00	101	12	11	7	10	18	15	9	19
23.00-00.00	115	9	18	13	16	12	13	16	18
00.00-01.00	150	19	20	23	9	16	22	18	23
01.00-02.00	177	22	27	25	17	18	21	18	29
02.00-03.00	172	16	25	31	18	20	24	18	20
03.00-04.00	190	19	29	33	23	20	21	22	23
04.00-05.00	177	21	24	29	18	22	19	18	26
05.00-06.00	169	16	26	31	26	16	14	18	22
Total	1526	169	213	244	151	180	189	172	208

Table XV Indoor human landing collection data during dry season at Hwidiem

Number of *Anopheles gambiae* sensu lacto Caught Indoors									
		August		November		December		January	
HOUR	Total	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
18.00-19.00	7	0	2	1	2	0	2	0	0
19.00-20.00	13	0	1	3	3	3	1	2	0
20.00-21.00	15	0	0	5	2	2	0	4	2
21.00-22.00	29	4	6	4	4	6	3	0	2
22.00-23.00	35	3	4	9	4	7	0	2	6
23.00-00.00	41	4	2	7	6	4	5	4	9
00.00-01.00	40	2	7	6	11	6	4	0	4
01.00-02.00	43	6	5	11	7	4	2	6	2
02.00-03.00	34	4	4	9	6	0	5	4	2
03.00-04.00	42	6	3	11	12	5	2	1	2
04.00-05.00	28	6	5	4	7	4	2	0	0
05.00-06.00	3	0	0	2	1	0	0	0	0
Total	330	35	39	72	65	41	26	23	29

Table XVI Outdoor human landing collection data during dry season at Hwidiem

Number of *Anopheles gambiae* sensu lacto Caught Outdoors									
		August		November		December		January	
HOUR	Total	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
18.00-19.00	8	1	2	3	2	0	0	0	0
19.00-20.00	12	2	0	4	2	0	2	0	2
20.00-21.00	20	5	3	4	0	0	2	2	4
21.00-22.00	22	4	7	5	2	2	2	0	0
22.00-23.00	25	1	2	7	6	3	1	1	4
23.00-00.00	28	5	4	7	4	3	1	0	4
00.00-01.00	33	3	4	9	4	5	4	2	2
01.00-02.00	32	4	0	6	10	4	4	2	2
02.00-03.00	29	3	0	12	4	4	4	0	2
03.00-04.00	25	1	2	5	2	5	4	2	4
04.00-05.00	10	2	0	3	2	1	0	2	0
05.00-06.00	0	0	0	0	0	0	0	0	0
Total	244	31	24	65	38	27	24	11	24

APPENDIX V: RAW DATA ON PYRETHROID INSECTICIDE RESISTANCE OR SUSCEPTIBILITY IN *ANOPHELES GAMBIAE* FROM THE STUDY SITES

Table XVII Raw data on 0.05% deltamethrin insecticide susceptibility or resistance status in *A. gambiae* s.l from Sunyani, Ghana conducted using the bioassay method of WHO, (1998a, 2003).

Number of replicates	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate (total)	Control
Number Exposed	25	25	25	25	100	25
Knockdown time						
5 minutes	0	0	0	0	0	0
10 minutes	2	3	3	3	11	0
15 minutes	7	3	4	5	19	0
30 minutes	13	6	8	10	37	0
30 minutes	14	8	9	11	42	0
40 minutes	17	7	10	12	46	0
50 minutes	17	10	11	13	51	0
60 minutes	17	8	11	13	49	0
80 minutes	19	9	11	14	53	0
Mortality (%) after 24hrs	19	12	13	15	59	0

Table XVIII Raw data on 0.05% deltamethrin insecticide susceptibility or resistance status in *Anopheles gambiae* s.l from Ahafo Kenyasi, Ghana conducted using the bioassay method of WHO, (1998a, 2003).

Number of replicates.	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate (Total)	Control
Number exposed	25	25	25	25	100	25
Knockdown time						
5 minutes	0	0	0	0	0	0
10 minutes	3	0	1	2	6	0
15 minutes	4	3	3	2	12	0
30 minutes	4	3	4	3	14	0
30 minutes	5	6	4	3	18	0
40 minutes	6	5	4	6	21	0
50 minutes	8	7	4	6	25	0
60 minutes	8	7	4	5	24	0
80 minutes	10	7	4	6	27	0
Mortality (%) after 24hrs	8	5	6	16	35	1

Table XX Raw data on 0.75% permethrin insecticide susceptibility or resistance status in *Anopheles gambiae* s.l from Sunyani, Ghana conducted using the bioassay method of WHO, (1998a, 2003).

Number of replicates	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate (Total)	Control
Number exposed	25	25	25	25	100	25
Knockdown time						
5 minutes	0	0	0	0	0	0
10 minutes	0	0	0	0	0	0
15 minutes	1	2	4	2	9	0
30 minutes	3	4	9	5	21	0
30 minutes	8	4	18	10	40	0
40 minutes	10	8	20	14	52	0
50 minutes	17	19	20	18	74	0
60 minutes	20	19	20	19	78	0
80 minutes	20	19	20	19	78	0
Mortality (%) after 24hrs	11	9	12	13	45	0

Table XXI Data on 0.75% permethrin insecticide susceptibility or resistance status in *Anopheles gambiae* s.l from Ahafo Kenyasi, Ghana conducted using the bioassay method of WHO, (1998a, 2003).

Number of replicate	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate (Total)	Control
Number exposed	25	25	25	25	100	25
Knockdown time						
5 minutes	0	0	0	0	0	0
10 minutes	0	2	0	1	3	0
15 minutes	1	3	2	3	9	0
30 minutes	3	7	5	6	21	0
30 minutes	5	9	7	6	27	0
40 minutes	12	15	13	10	50	0
50 minutes	15	15	13	10	53	0
60 minutes	16	19	15	10	60	0
80 minutes	16	19	15	11	61	0
Mortality after 24hrs	12	13	10	11	46	0

Table XXII Data on 1.50% permethrin insecticide susceptibility or resistance status in *Anopheles gambiae* s.l from Ahafo Kenyasi, Ghana conducted using the bioassay method of WHO, (1998a, 2003).

Number of replicates	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate (Total)	Control
Number exposed	25	25	25	25	100	25
Knockdown time						
5 minutes	0	0	0	0	0	0
10 minutes	0	0	0	0	0	0
15 minutes	2	0	0	0	2	0
30 minutes	3	1	0	1	5	0
30 minutes	3	2	3	2	10	0
40 minutes	4	4	5	4	17	0
50 minutes	5	4	5	4	18	0
60 minutes	6	6	7	5	24	0
80 minutes	8	8	7	8	31	0
Mortality after 24hrs	8	7	5	7	27	0

Table XXIII Data on 0.05% deltamethrin insecticide susceptibility or resistance status in *Anopheles gambiae* s.l from Kisumu, Kenya using the bioassay method of the World Health Organisation, (1998a, 2003).

Number of replicate	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate (Total)	Control
Number exposed	20	20	20	20	20	100	20
Knockdown time							
5 minutes	0	0	0	0	0	0	0
10 minutes	0	2	0	0	0	2	0
15 minutes	2	2	1	0	0	5	0
20 minutes	9	10	7	6	4	36	0
30 minutes	14	17	12	19	10	72	0
40 minutes	15	19	19	19	20	92	0
50 minutes	19	20	19	20	20	98	0
60 minutes	20	20	20	20	20	100	0
Mortality after 24hrs	20	20	20	20	20	100	0
%Mortality	100	100	100	100	100	500	0

Table XXIV Data on 1.5% permethrin insecticide susceptibility or resistance status in *Anopheles gambiae* s.l from Kisumu, Kenya using the bioassay method of World Health Organisation, (1998a, 2003).

Number of replicate	Replicate. 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate (Total)	Control
Number exposed	20	20	20	20	20	20	20
Knockdown time							
5	1	1	0	0	0	2	0
10	2	1	1	0	1	5	0
15	3	3	2	3	6	17	0
20	8	10	8	8	10	44	0
30	10	17	16	12	15	70	0
40	19	19	18	19	18	93	0
50	19	20	18	19	19	95	0
60	20	20	19	20	19	98	0
Mortality after 24hrs	20	20	20	20	20	100	0
%Mortality	100	100	100	100	100	500	0

Table XXV Data on 0.75% permethrin insecticide susceptibility or resistance status in *Anopheles gambiae* s.s from Kisumu, Kenya using the bioassay method of WHO, (1998a, 2003).

No. of replicate	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Rep. 5	(Total)	Control
Number exposed	20	20	20	20	20	20	20
Knockdown time							
5	0	1	0	0	0	1	0
10	11	10	6	2	2	31	0
15	15	15	11	9	9	59	0
20	17	20	18	12	15	82	0
30	20	20	19	19	18	96	0
40	20	20	20	20	18	98	0
50	20	20	20	20	19	99	0
60	20	20	20	20	20	100	0
Mortality after 24hrs	20	20	20	20	20	100	0
% Mortality	100	100	100	100	100	100	0