Biting patterns of mosquitoes, the dynamics of malaria transmission and the existence of the knockdown resistance (kdr) gene in parts of the central forest belt of Ghana

By

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DECLARATION

I, Ayimbire Abonuusum, hereby declare that this submission is my own work towards the PhD and that, to the best of my knowledge, it contains no material previously published by another person nor material that has been accepted for the award of any other degree of the university, except where due acknowledgement has been made in the text.

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DEDICATION

These theses is dedicated to my parents – Mr. Abonuusum Ayimbire and Mrs. Ayimbire Abugre – for their doting love and care that, working with God's grace, have brought me to this stage.

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ABSTRACT

Malaria is presumed to be holoendemic in the forest belt of Ghana, though no comprehensive transmission studies have been conducted. Therefore this study aimed to estimate several entomological parameters of mosquitoes in the forest belt of Ghana and also collect epidemiological baseline data on the vectors of malaria.

Four sites were selected for the study: rural Afamanaso and Kona in the then Afigya Sekyere District as well as peri-urban Boadi and Anwomaso in the Kumasi Metropolis. Mosquito collection was by human-landing-catches. *Anopheles* caught were examined for *Plasmodium* infections either microscopically by dissection and/or by Enzyme Linked Immuno-Sorbent Assay (ELISA). Three types of Polymerase Chain Reaction (PCR) methodologies were used to identify the following: a) sibling species of the *A. gambiae* complex (conventional), b) *Plasmodium* species (Real-Time) and c) the presence of the knock-down resistance (kdr) gene (Fluorescence Resonance Energy Transfer - FRET). The entomological data collected were compared with secondary data from a parallel clinical study to ascertain whether there was any relationship between malaria incidence and entomological inoculation rate (EIR).

A bell-shaped hourly biting pattern was observed for both *Anopheles* and *Culex* species The hourly biting pattern of *A. gambiae s.l.* at Boadi, peaked at 02.00 hours, which was significantly different from that at Afamanaso and Kona where it peaked around midnight. A total of 80, 12-hour human landing catches were undertaken which yielded 5608 mosquitoes from Afamanso and Kona, 4636 *Anopheles* species and 972 *Culex* species There were 3479 *A. gambiae s.l* caught and 324 of them were infected to give a sporozoite rate of 10.11± 1.37%.The number of *A. funestus* collected was 1157, of which 57 were infected by *Plasmodium* species, giving a

sporozoite rate of 6.17 ±1.70%. Annual transmission by *A. gambiae s.l* was very high at Afamanaso and Kona with EIRs of 699.42 ± 119.93 and 433.06 ± 85.65 respectively, while *A. funestus* contributed EIRs of 202.05 ± 56.87 and 42.49 ± 23.08 ib/p/yr, respectively. At Boadi a total of 10,409 mosquitoes were caught in 14 12-hour human landing catches: 2684 *Anopheles* species and 7725 *Culex* species were caught. In all, 18 out of 1161 or 1.55 ± 0.94% *A. gambiae s.l.*, were infected by *Plasmodium* species; whereas none of 6 *A. funestus* caught was infected. Few (10) out of a total of 1504 or 0.66 ± 0.27% *A. ziemanni* were infected by *P. falciparum*. Average monthly EIR by *A. gambiae s.l.* was 4.82 ± 1.93 ib/p/m at Boadi. The potential EIR of *A. ziemanni* was estimated at 5.21 ± 2.33 ib/p/m. At Anwomaso, 7 12 hour human landing collections yielded 1867 mosquitoes, 345 *Anopheles*, and 1522 *Culex* species. Few (4) out of 71 or 5.63 ±1.59% *A. gambiae s.l.* were infected by *P. falciparum*. An average monthly EIR of 37.47 ± 7.12 ib/p/yr was recorded at that site. Analysis of graphs showed that entomological parameters (entomological inoculation rate (EIR), biting and sporozoite rates) preceded malaria incidence rates by a time lag of about two months. Sixty-eight percent (68%) *A. gambiae s.l.* were caught in pens fenced with the ITN and 32% in pens without ITN. Only *A. gambiae s.s.* was identified from 135 members of *A. gambiae* complex analyzed. The kdr gene was absent in only two percent (2%) (3 out of 135) specimens of *A. gambiae s.l.* analyzed.

The very high and varied EIRs indicate very high and heterogeneous malaria transmission in the forest belt of Ghana. There was a relationship between entomological parameters (e.g. EIR) and malaria incidence rate. ITN did not provide protection against the endophilic *A. gambiae s.s.* The study provided important baseline data for the evaluation and implementation of intervention measures such as integrated vector control strategies.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Brief Background on Malaria in Ghana

Malaria persists as the most important insect-borne disease in tropical Africa and remains one of the biggest public health problems that mankind has faced (Nwanji *et al.*, 2003). Ghana had an estimated 7.2 million malaria cases in a population of 20 million in 2006; representing 3% of the total malaria cases in the WHO African Region (WHO, 2008). The disease is responsible for 13.8% and 9.4% of all reported cases and deaths respectively in pregnant women in Ghana. The proportion of reported deaths due to the disease is 13.2% in the general population but in children under five years it is as high as 22% (Antwi and Marfo, 1998; Asante and Asenso-Okyere, 2003). It is estimated to account for 40-60% of daily OPD (out patient department) attendance and 2.2 million of OPD recorded cases annually. Records show that the disease is persistent and increasing over the years despite efforts on intervention strategies (WHO, 2008). Of all malaria cases in Ghana, it is estimated that *Plasmodium falciparum* is responsible for 80%-90%, *P. malariae* for 20%-36% and *P. Ovale,* 0.15%. These parasite species are carried by members of the *Anopheles gambiae complex* and *A. funestus* (MOH, 1991). Other species of *Plasmodium* that infect man include *P. vivax* and *P. knowlesi;* these have not been recorded in Ghana. The former occurs mainly in temperate regions while the latter, previously commonly misidentified as *P. malariae,* frequently infects man in Southeast Asia. Monkeys are, however, the natural reservoirs of *P. knowlesi* (Cook and Zumla, 2008).

1.2 Malaria Control Efforts in Ghana

The Ghanaian government has made considerable effort at malaria control through the WHO-recommended approaches. Facilities have been provided for the effective testing and diagnosis of malaria patients. Chloroquine-resistance in the infective malaria parasites has also been minimized by the use of the WHO recommended artesunate-amodaquine combination for treatment. In the area of prevention, the Ghana National Malaria Control Programme has prioritized the use of insecticide-treated net (ITN) materials as a key strategy for malaria vector control (Yawson *et al.,* 2004). There is an on-going focal distribution of ITNs to pregnant women and lactating mothers. However, the occurrence of the Knock-down resistance (kdr) gene in Ghanaian samples of *A. gambiae s.l.* and *A. funestus* (Kristan *et al.*, 2003; Yawson; *et al.,* 2004; Coetzee *et al.*, 2005) poses a serious challenge to the efforts of government at controlling the vector and invariably, the disease. This gen\e has been linked to resistance to DDT and pyrethroids in the malaria vectors *A. gambiae s.l.* and *A. funestus* (Chandre *et al.*, 1995; Abdoulaye *et al.,* 2003; Coetzee *et al.*, 2005). There has not been an extensive investigation on the degree of occurrence and potential effect of this gene on malaria control efforts in Ghana.

1.3 Justification for this Work

Malaria transmission dynamics have been shown to vary greatly across Africa (Beier *et al.*, 1999, Elissa *et al.,* 2003). Female *Anopheles* species mosquitoes (and never the males) have long been known for transmitting malaria parsites through their bites as they feed on humans, requiring the blood to mature their eggs. However, the actual relationship between the entomological parameters and malaria incidence rate has not been thoroughly documented in the forest region of Ghana. Such parameters include the:

a) biting rate (the number of mosquitoes captured as they land on a person to bite over a defined period of time), b) sporozoites rate (the proportion of the captured mosquitoes in a) that are infected by the malaria parasite) and c) Entomological Inoculation Rate (EIR) i.e. the product of a) and b). (EIR is expressed in terms of the average number of infective mosquitoes biting a person over a defined period of time. Thus EIR is a more accurate estimate of malaria transmission in a population (MacDonald, 1957; Trape and Rogier, 1996; Beier *et al*., 1999; Elissa *et al.*, 2003).

Data exist on malaria transmission in the savannah areas of Ghana where 94.3% of the infective *Anopheles* species were *A. gambiae s.l.* and *A. funestus*, 5.4% were *A. pharoensis* and 0.3% *A. rufipes.* Of the *A. gambiae* complex 97.7% were *A. gambiae s.s.* and transmission was highly seasonal with microecological variations, being heaviest from June to October. About 60% of the EIRs occurred indoors during the second half of the night, peaking at daybreak between 4 and 6 am (Appawu *et al.*, 2004). However, no comprehensive study has been done in the forest belt of Ghana to collect transmission data. Limited data in the forest belt exist in Kumasi (Afrane *et al.*, 2004) and Obuasi (Coetzee *et al.*, 2005). These, however, did not determine EIR in detail. Therefore, a comprehensive study in the forest belt of Ghana was required to collect transmission data to assist in control efforts. This entomological work was therefore carried out in parallel to a clinical study in the same study villages in order to gather enough data for comprehensive analysis of the malaria situation in the forest belt of Ghana.

Bauer *et al.* (2006) found that fencing kraals of dairy cattle with a 150 cm high lambda-cyhalothrin-treated mosquito netting in Kenya successfully protected animals against tsetsefly-transmitted trypanosomiasis. Observations of the participating farmers also indicated a reduction of the mosquito populations. Studies on the Cattle Farm at Boadi in 2005 and 2006, were follow-ups to assess the impact of permethrin-impregnated bed-nets on mosquitoes in the presence and absence of cows respectively and so determine whether the results of Bauer *et al.*, 2006 could somewhat be replicated.

1.4 The Main Objective of this Work

The main objective was to assess the dynamics of malaria transmission and the biting patterns of culicine and *Anopheles* mosquitoes in the forest belt using two large rural settlements in the AFigya Sekyere District of Ashanti Region of Ghana; Afamanaso and Kona and two peri-urban study sites, a) a cattle farm of KNUST, at Boadi and b) Anwomaso to establish a comparative baseline for the study in the AFigya Sekyere District.

1.4.1 Specific Objectives

To assess the biting patterns of mosquitoes and the mode of malaria transmission in the forest region of Ghana by:

i. Analyzing the species of mosquitoes in the forest belt, their abundance and respective biting patterns.

ii. validating the empirical assertion that *A. gambiae s.l.* is anthropophilic by comparing its biting rates in human densely populated villages of Afamanaso and Kona with its biting rate on a cattle farm at Boadi devoid of human habitation.

iii. using both Enzyme-Linked-Immuno-Sorbent Assay (ELISA) and salivary gland dissection in determining sporozoite infection of the anopheles mosquitoes.

iv. determining the sporozoite rates of the malaria vectors in the study areas.

v. determining the sibling species of *A. gambiae* complex population using conventional PCR and their contributions to malaria transmission.

vi. determining the species of *Plasmodium* using real-time PCR and their infection of the malaria vectors.

vii. assessing the daily, monthly and annual entomological inoculation rates (EIR).

viii. assessing the presence of the knock-down resistance (kdr) gene in the study areas using Fluorescence Resonance Energy Transfer (FRET).

ix. assessing the effect of a 1m high deltamethrin-impregnated net fence around a partially roofed 2.5m high pen for cattle on mosquitoes.

x. analzing the relationship between three entomological parameters (human biting rate (HBR), sporozoite rate (SR) and entomological inoculation rate (EIR) and malaria incidence rate.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Mosquitoes

It has been reported that mosquitoes are the most important insects affecting human health (Woodbridge and Edward, 2006; Okwa *et al.*, 2007) chiefly in the spread of malaria, the most common lethal disease second only to HIV/AIDS (Rowton, 2005). There are approximately 3,500 species of mosquitoes placed in 41 genera. The commonly occurring mosquitoes are classified as follows:

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta or Hexapoda

Order: Diptera

Family: Culicidae

Subfamily: Anophelinae, Culicinae, etc.

Genus: *Anopheles, Culex, Aedes*, etc.

Species: *Anopheles gambiae s.l., Anopheles funestus, Culex quinquefasciatus, Culex decens, Aedes aegypti, Aedes albopictus.*

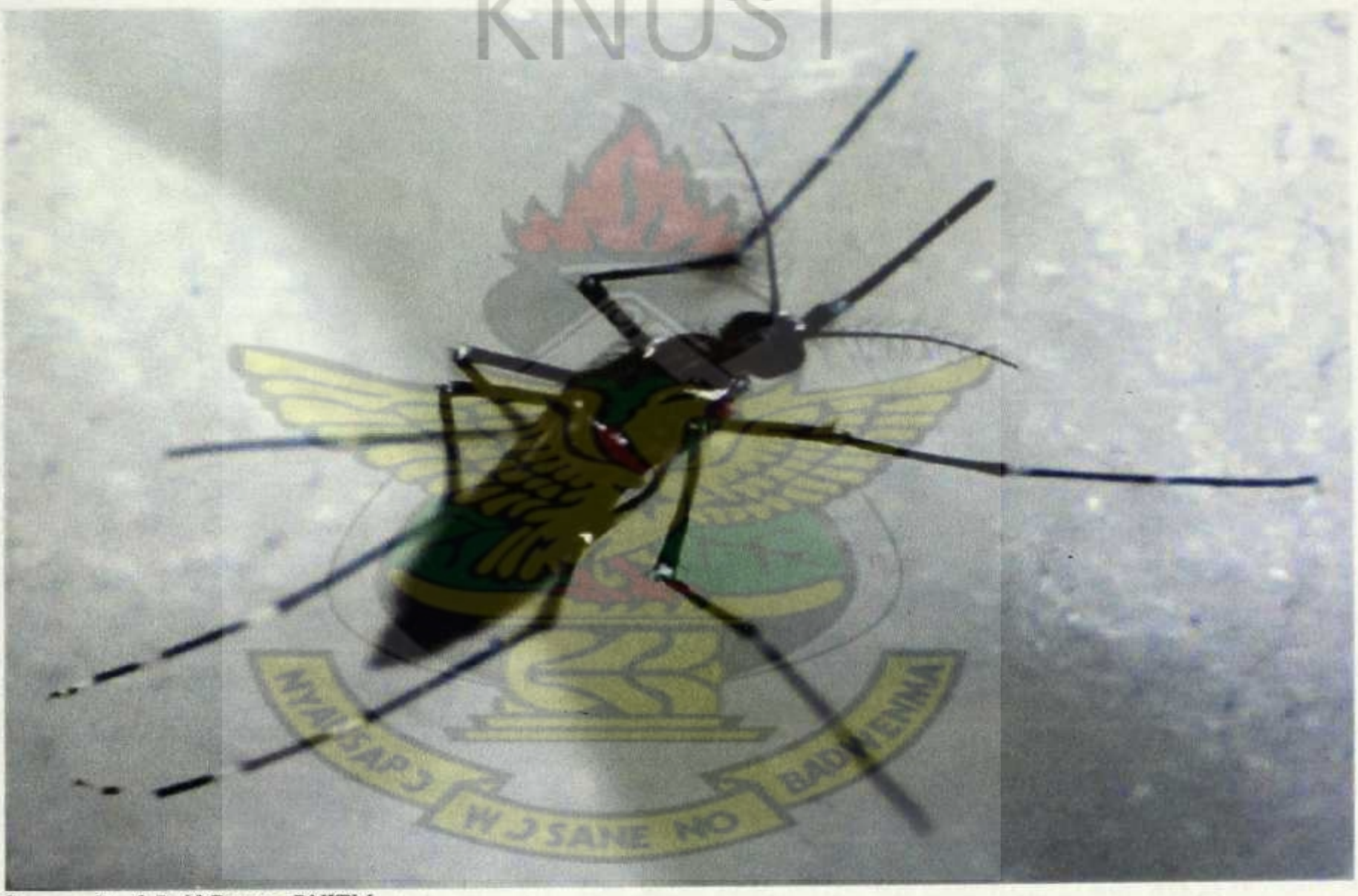
(Service, 2000; Cook and Zumla, 2008).

2.1.1 *Aedes* species

These are widespread transmitting various insect-borne diseases. For instance, *Aedes aegypti* (Plate A), an anthrophagic mosquito, is the major vector of dengue as well as the yellow fever virus. It is widely adapted to varied urban and semi-urban habitats

(Figueiredo, 2003). The Asian "tiger mosquito", *Aedes albopictus* from South-East Asia (Plate B), is a major biting pest throughout much of its range of occurrence. This secondary vector of dengue fever is generally known to inhabit forest-fringe areas. It has been found to be a competent laboratory vector of at least 22 arboviruses, including many viruses of public health importance including the Cache Valley and eastern equine encephalomyelitis viruses (Moore and Mitchell, 1997; Hiriyan *et al.*, 2003).

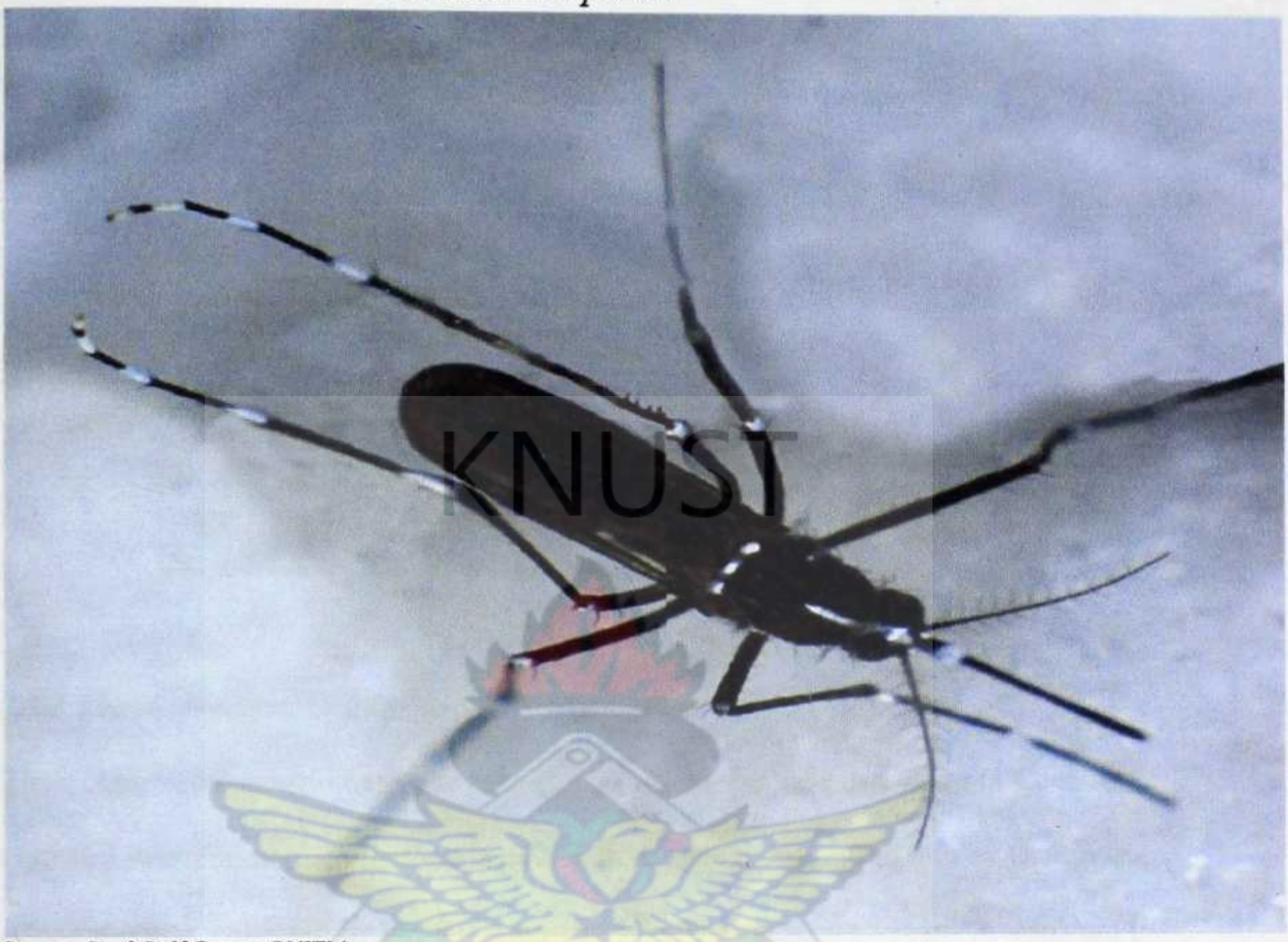
Aedes aegypti



Source: Prof. Rolf Garms, BNITM.

Plate A: *Aedes* aegypti.

Aedes albopictus



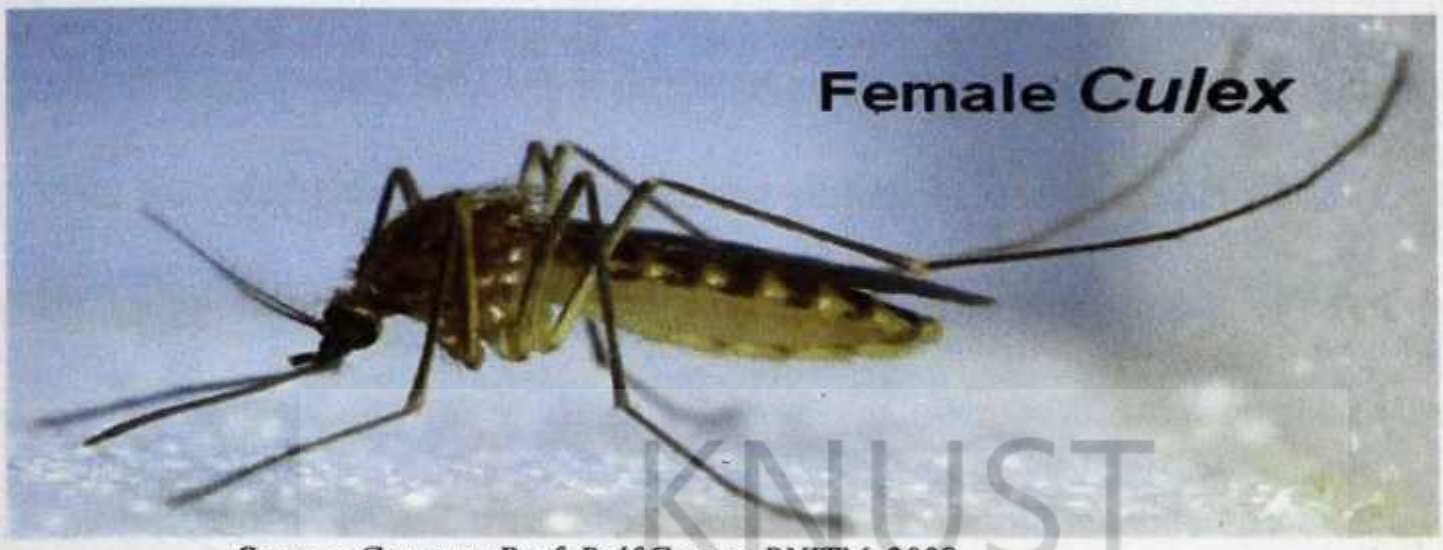
Source: Prof. Rolf Garms, BNITM.

Plate B: *Aedes albopictus* (Asian tiger mosquito)

2.1.2 *Culex* species

Occurring in many parts of the world, these mosquitoes are also efficient vectors of various insect-borne diseases (Plate C). The West Nile virus, for example, is known to be transmitted by *Culex* mosquitoes while feeding on birds and humans. Other diseases transmitted by *culex* mosquitoes include filariasis and encephalitis (Syed and Leal1, 2009). However, in West Africa, filariasis is transmitted by *Anopheles* species with no *Culex* species implicated in the spread of this disease. *Culex caudelli* transmits the arbovirus (Chadee and Tikasingh, 2008). Other *Culex* species occurring in certain parts of

Africa and Ghana include *Culex tigripes, C. decens, C. Duttoni* and *Culex quinquefasciatus* (Cornelius, 1962; Appawu *et al.*, 2001).



Source: Courtesy Prof. Rolf Garms, BNITM, 2008.

Plate C: A *culex pipiens* female.

2.1.3 The *A. coustani* Group

These *Anopheles* mosquitoes were named *A. coustani,* because according to Coetzee (1994), Laveran described these mosquitoes from specimens sent to him by Dr. Coustan of Montpellier, collected by Dr. Rasamimanana in Madagascan swamps. *A. coustani* is widespread, occurring in Africa, Madagascar, Mauritius, and Reunion. They are known to constitute a group of mostly outdoor biting mosquitoes whose members include: *A. coustani* Laveran complex, *A. tenebrosis* Dönitz, *A. symesi, A. ziemanni* Grünberg, *A. namibiensis* Coetzee and *A. paludis* Theobald. Ribeiro and Ramos (1975) reported that adult members of the group are mainly exophilic and bite both man and cattle. According to him examinations of salivary glands for malaria parasites in Angola were always negative.

2.1.3.1 *Anopheles coustani* Laveran Complex

Coetzee (1994), his earlier studies, (Coetzee, 1983), on polytene chromosomes and cross-mating characteristics of the South African populations of *A. coustani* revealed the existence of 2 cryptic species, referred to as species A and B. The publication of Coetzee (1994) provided the current names of the sibling species A and B of the *A. coustani* complex as *Anopheles* (*Anopheles*) *coustani* Laveran and *Anopheles* (*Anopheles*) *crypticus* new species respectively. Recently, Coetzee (2008) reported that examination of the polytene chromosomes of the fourth instar larvae provided evidence for the existence of two sibling species within the taxon *Anopheles* (*Anopheles*) *coustani* Laveran. He contended further that crosses between the two chromosomal types produced sterile male offspring.

2.1.3.2 *A. paludis* Theobald

This member of the *A. coustani* group is reported to occur in many parts of Africa, but of interest is its role in Zaïre as an important malaria vector (Karch and Mouchet, 1992). During an entomological study carried out on the transmission of malaria in the Bandundu region, Zaïre, *A paludis* was found to be the dominant species representing 55.1% of the mosquitoes caught. It thus had the highest average biting rate of 4.2 b/p/n. This mosquito had a sporozoite rate of 6.2% and EIR of 0.26 ib/p/n, or one infective bite every four days. *A. paludis* was therefore a major vector of malaria with *A. gambiae s.l.* in this region (Karch and Mouchet, 1992).

2.1.3.3 *A. ziemanni* (Grünberg)

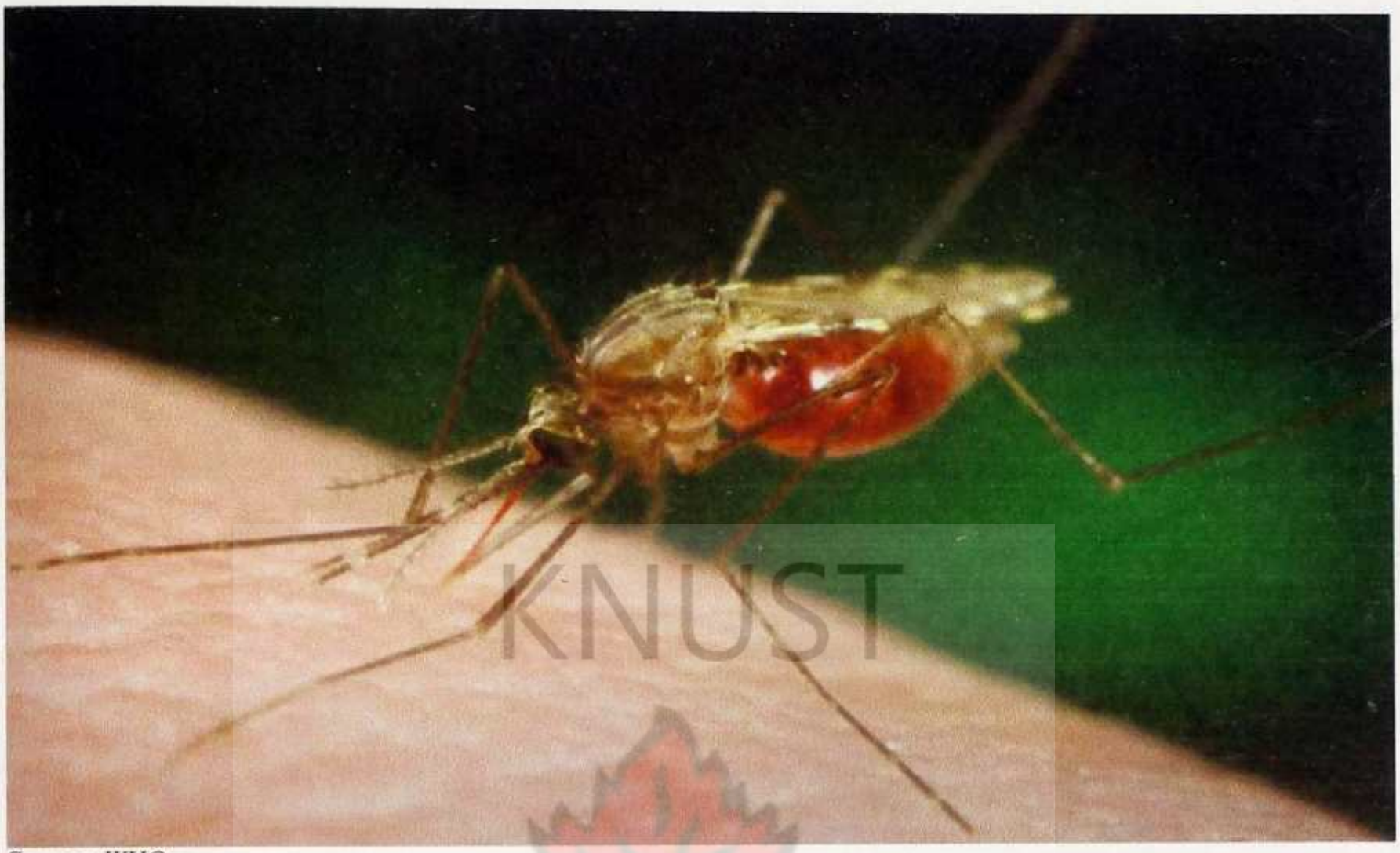
Another potential vector for malaria transmission is *A. ziemanni* (Grünberg) which is a member of the *A. coustani* group, which are generally exophilic and zoophilic. *A. ziemanni* occurs in a wide range of habitats extending from Senegal to Ethiopia (Gillies and De Meilon, 1968). To assess its potential to transmit malaria, Ribeiro *et al.* (1964), and Ribeiro and Ramos (1975), dissected a total of 846 specimens all negative for sporozoites in Angola. In Kenya, the human blood index (HBI) in females caught in light traps was 0.09; most of the feeds being on bovids. Biting activity in this area was mainly in the early part of the night and practically ceasing after 01 hours (Chandler *et al.*, 1975). In Uganda, biting activity was almost exclusively at ground level (Haddow and Ssenkubuge, 1974). However, recent studies by Antonio-Nkondjio *et al.* (2006), and Kerah-Hinzoumbé *et al.* (2009), found malaria parasites or their circumsporozoite proteins in *A. ziemanni* samples. Gillies and De Meillon (1968) report of earlier studies in Ethiopia and Cameroon in the 1950s in which *A. ziemanni* were found infected with sporozoites.

2.1.4 Vectors of Malaria

A malaria vector is any insect, usually an *Anopheles* mosquito, that fully supports the sexual phase of development of the malaria parasite, *Plasmodium* species, and transmits the developed parasite to its victim – the human host (Cook and Zumla, 2008). An anopheles mosquito seeking blood to help develop her eggs, inadvertently takes up *Plasmodium* species gametocytes from the bloodstream of an infected person. The gametocytes develop into gametes which fuse to form zygotes, the latter go through developmental stages from, a) ookinet to, b) oocyst and finally to c) infective sporozoites

that migrate to the salivary gland of the mosquito. In the process of taking its next blood-meal, the insect would transmit the sporozoites to the person by pumping sporozoites-laden saliva into the bloodstream to act as an anticoagulant during the biting process (Touray *et al.,* 1992; Robert *et al.,* 1994; Barillas-Mury and Kumar, 2005).

The discovery in 1899 by Ronald Ross, a British officer in the Indian Medical Service, which revealed that the malaria parasites are transmitted from person to person by mosquitoes, solved the mystery of malaria transmission (Bynum and Overy, 1998; Brey, 2003; CDC, 2004). Malaria is transmitted by mosquitoes of the genus *Anopheles*. This genus contains over 400 species of which only about 70 transmit *Plasmodium* species Out of this, 40 are important malaria vectors with 25 species considered as primary vectors. Usually there are 4-5 primary vectors adapted to a particular geographical region (White, 1974; Service, 2000; WHO, 2005). In some parts of West and Central Africa the main vector is *Anopheles funestus* (Plate D) but in most parts of sub-Saharan Africa, it is the extremely efficient *A. gambiae s.l.* (Plate E) that remains the main vector (Elissa *et al*., 1999; WHO Africa Report, 2003; Okech *et al.,* 2008).



Source: WHO

Plate D: *A. funestus* taking a blood-meal.

Source: Courtesy Prof. Rolf Garms, BNITM, 2008.

Plate E: *A. gambiae s.l.* resting on a surface.

Climatic conditions determine the distribution of *Anopheles* mosquitoes. Tropical areas of the world have the best combination of adequate rainfall, high temperature and high humidity allowing for the breeding and survival of the malaria vectors (WHO, 2005).

2.1.4.1 *A. funestus sensu lato* (*s.l.*)

A. funestus s.l. is the third major malaria vector in Africa belonging to a group of nine species with morphologically similar characteristics. Morphological identification of members of the *A. funestus* group is difficult because of 1) the overlap of distinguishing characteristics in adult or immature stages and 2) the necessity to rear iso-female lines to examine larval and egg characters (Koekemoer *et al.*, 2002; Cohuet *et al.*, 2003). Members of the group include: *A. funestus s.s.* (Giles), which is the most anthropophilic and endophilic member of the group; and a highly efficient vector of malaria. It is widespread over most of the malarious areas of Africa, extending from northern Sudan to South Africa and across West Africa to northern Mali and Senegal. It is also common in Madagascar (Koekemoer *et al.*, 2002). The other members of the group are mainly zoophilic and only *A. rivulorum* (Leeson) has been implicated as a minor vector in Tanzania. *A. rivulorum* (Leeson) together with *A. leesoni (*Evans) are widespread, occurring from Ethiopia to the northern parts of South Africa and across West Africa (Koekemoer *et al.*, 2002; Cohuet *et al.*, 2003). *A. vaneedeni* (Giles and Coetzee) has been shown to be a vector under laboratory conditions but has so far not been shown to be associated with malaria transmission in nature. It occurs only in Mpumalanga and other areas of the Northern Province in South Africa. *A. parensis* (Giles) and *A. confusus* (Evans and Leeson) are found in eastern Africa from Kenya and Tanzania in the North to KwaZulu/Natal Province in South Africa. The other members of the group are localized:

A. aruni (Sobti) from Zanzibar, *A. fuscivenosus* (Leeson) from Zimbabwe, and *A. brucei* (Service) from Nigeria (Koekèmoer *et al.*, 2002; Cohuet *et al.*, 2003).

2.1.4.2 *A. funestus s.s.*

A. funestus s.s., like *A. gambiae s.s.*, is also undergoing evolution through chromosomal inversion. It has two West African chromosomal forms (Folonzo and Kiribina) that clearly differ in their degree of association with humans and therefore have quite different vectorial potentials. These forms, as in *A. gambiae s.s.,* are adapted to different breeding sites; Folonzo prefers permanent water bodies while Kiribina survives in intermittent ones. These different breeding habitats have consequences for malaria transmission and control as in *A. gambiae s.s.* (Coluzzi, 1984; della Torre *et al.,* 2002; Michel *et al.,* 2006). Research on Kiribina and Folonzo so far has revealed slight but significant differentiation between the two forms, consistent with the hypothesis of incipient speciesecіation between them (Michel *et al.*, 2005; Guelbeogo *et al.*, 2005), though this may not be the case everywhere (Dia *et al.,* 2000; Boccolini *et al.,* 2005).

2.1.4.3 *Anopheles gambiae* Complex

Discovered in 1899 (National centre for Zoonotic Diseases, 2004), *A. gambiae s.l* is now thought to be a complex of morphologically identical sibling species made up of *A. gambie s.s, A. arabiensis, A. melas, A. merus, A. quadriannulatus, A. bwambae* and *A. quadrianulatus* species B. These are found in Africa, South-Western Arabia and the oceanic islands of Africa from Cape Verde to Mauritius. Widespread in Africa, they are the world's most efficient vectors of malaria and filariasis and the most difficult to control. In effect they can be regarded as Africa's most dangerous animals (Lehmann *et*

al., 2003; Nwanji *et al*., 2003; WHO, 2003; Rebecca *et al*., 2004; WHO, 2005). Being sibling species and often sympàtric taxa that are reproductively isolated but difficult to distinguish morphologically (Lincoln *et al.,* 1998), they are adapted to different ecological niches and exhibit diverse behavioural patterns. These adaptations equip them with great abilities to evade control measures by varying their breeding places, resting sites, host preferences, etc. (White, 1974; Rebecca *et al*., 2004). The complex include two saltwater-breeding species: a) *A. merus* (Döitz) and b) *A. melas* (Evans) from the coasts of East and West Africa respectively and one mineral water breeder: c) *A. bwambae* from the Semliki forest area of the Uganda/Zaire border. The two remaining ones are fresh water breeders: d) *A. gambiae* (Giles) *sensu stricto* (*s.s.*) and e) *A. arabiensis* (Patton) both of which are distributed sympatrically all over Africa, though the latter is more adapted to arid regions and the former to humid regions (Rebecca *et al.,* 2004). The sixth species is f) *A. quadriannulatus* (Theobald) occurs in Zanzibar, Ethiopia and South Africa. The seventh unnamed species of the complex is called *A. quadriannulatus* species B, discovered in Ethiopia in 1998 (Coetzee *et al*., 2000)

2.1.4.4 *A. arabiensis* (Patton)

Anopheles arabiensis (Patton) (formally species B of the *A. gambiae* complex) and *A. gambiae s.s.* (Giles) are the principal vectors of malaria in sub-Saharan Africa. However, *A. arabiensis* is distributed in over 70% of sub-Saharan Africa, dominating in arid zones and some highland areas where it is the predominant malaria vector species (White, 1974; Onyabe and Conn, 2001; Mutero *et al.,* 2004; Mahandel *et al., 2007*). Multiple chromosome inversion polymorphisms in *A. arabiensis* endow this member of the *A. gambiae* complex with greater ecophenotypic plasticity than is known for any other

mosquito, enabling it to exploit various alternative niches, a feature that makes control of this particular malaria vector difficult. What makes this mosquito versatile is that when majority of its hosts are domestic and indoor at night, the bulk of *A. arabiensis* females become endophagic and partially or completely endophilic. However, when a high proportion of hosts are available outdoors at night engorged *A. arabiensis* becomes exophagic and exophilic though such *A. arabiensis* females may enter houses postprandially to shelter for the day (White and Rosen, 1973; White, 1974; Curtis, 1978; Githeko *et al.* 1996; Lindsay *et al.*, 1998; Minakawa *et al.*, 2002; Nyanjom *et al.*, 2003).

In response to indoor residual spraying (IRS), *A. arabiensis* becomes completely exophilic (Coluzzi *et al.*, 1979; Ameneshewa *et al.*, 1996; Mendis *et al.*, 2000; Mahandel *et al.*, 2007). In experimental release/recapture trials conducted at the Mabogini verandah huts in Tanzania, *A. arabiensis* was found to have much higher exophilic tendency (80.7%) compared to *A. gambiae s.s.* (59.7%) (Mahandel *et al.*, 2007). Working in the same country, White *et al.* (1974) observed that in Segera, *A. arabiensis* displayed a 2.3 times greater tendency to occur outdoors compared to *A. gambiae s.s.* In Kenya, Highton *et al.* (1979), reported that *A. arabiensis* in the Kisumu area, showed a tendency to occur outdoors 2.2 times more frequently than indoors, similar to the 2.8 times higher outdoor tendency for the same mosquito that Joshi *et al.* (1975), had found in the same place. *Anopheles arabiensis* is not only anthropophilic but probably more zoophagic. In experiments conducted in Northern Tanzania, it was found that odours from cattle attracted 90.3% compared to odours from human, which attracted 9.7% of *A. arabiensis*. *Anopheles arabiensis* has a low human blood index (HBI) and shows a marked preference for cattle and other warm-blooded animals. Estimation of HBI in *A. arabiensis*

collected from houses in three Tanzanian villages indicated lower ratios for mosquitoes collected from houses with cattle compared to those without cattle. HBI was also lower in mosquitoes collected outdoors (0.1–0.3) compared with indoors (0.4 – 0.9) (Habtewold *et al.,* 2004; Mahande1 *et al.,* 2007).

2.1.4.5 *A. gambiae sensu stricto* (s.s.)

There have been findings of further subdivision of the species of the *A. gambiae s.s.* Studies on the gene arrangements of the 2R chromosomal arm (differing by inversions) show five chromosomal forms in *A. gambiae s.s* and these are: i) Mopti ii) Bamako iii) Forest iv) Savanna and v) Bissau (Coluzzi *et al.,* 1985; Toure *et al.*, 1994, 1998; Coluzzi *et al.*, 2002). Molecular studies of these "forms" have confirmed the existence of genetic discontinuity within this sibling species. Diagnostic differences in the intergenic sequence (IGS) and internal transcribed sequence (ITS) regions of ribosomal DNA (rDNA) on the X chromosome distinguishes only two "molecular forms", called M and S (Favia *et al.,* 1997; Mukabayire *et al.,* 2001; Gentile *et al.,* 2001, 2002). The correspondence between chromosomal and molecular forms is not completely resolved and it varies in different areas of the geographic range of the species In some areas of West Africa, for example, Mali and Burkina Faso, there is a one-to-one correspondence between the M molecular form and the Mopti chromosomal form. Similarly, the S molecular form always corresponds to the Savanna or Bamako chromosomal form. In other areas of West Africa, this clear correspondence breaks down (della Torre *et al.*, 2002). The molecular M and S forms of *A. gambiae s.s.* were found to occur in sympatry in southern Ghana. The S form predominated throughout its distribution in the coastal savannah, except at one location in the strand and mangrove zone where rice was

cultivated. The M form was the only form collected in northern Ghana and it was also the predominant form in Burkina Faso. No M/S hybrids have been detected (Yawson *et al.*, 2004). Although interbreeding between M and S forms in the laboratory is possible in principle and yields fertile progeny, M-S hybrids are rarely observed in nature. Where these forms overlap in time and space, the rate of heterogamous insemination is very low (Tripet *et al.*, 2001), clearly demonstrating the existence of a premating barrier, albeit an incomplete one. Nucleotide substitutions in intron 1 of the voltage-gated sodium channel gene located on the second chromosome have also been shown to segregate according to the M and S molecular forms (Weill *et al.*, 2000; Gentile *et al.*, 2004). The result is that the knock-down resistance (kdr) allele in the *para* sodium channel gene, which confers resistance to pyrethroid insecticides, is found in the S form populations from some West African countries where both M and S forms are sympatric., but not detected in the M form populations from the same locales (Chandre *et al.*, 1999; della Torre *et al.*, 2001; Gentile *et al.*, 2001; Wondji *et al.*, 2002; Awolola *et al.*, 2005). Exceptions are, however, found in Benin, Nigeria, and Cameroon (Reimer *et al.*, 2005; Santolamazza *et al.*, 2008). Thus, both indirect and direct genetic evidence indicates incomplete but substantial barriers to gene flow between different *A. gambiae* s.s. molecular forms. The data suggest that we are observing speciesеciation at its very earliest stages, with the persistence of variation shared because of recent common ancestry and with low levels of gene flow continuing to homogenize regions of the genome not directly involved in the speciesеciation process (della Torre *et al.*, 2002). These evolutionary trends in *A. gambiae s.s.* have clear implications for malaria epidemiology, given that both the chromosomal and M-S molecular forms are adapted to different ecological habitats

(Touré *et al.*, 1998; Calzetta *et al.*, 2008). In particular, the ongoing speciesseciation process leading to the M form has extended the transmission potential of this vector in space and time (Toure *et al.*, 1994; 1998). In dry areas of West Africa where malaria is hyper- to holoendemic, this taxon is able to exploit breeding opportunities due to human activities (e.g. irrigation) that would otherwise be available only to *A. arabiensis.* It is likely that in *A. gambiae s.s.* chromosomal inversions allow for more specialized and efficient exploitation of both spatial and temporal environmental heterogeneity. This is expected to enhance such traits as survival probability of individual mosquitoes and stability of vector populations, both important features of malaria epidemiology. Furthermore, increased vector diversity due to these evolutionary trends, leads to evasion of control measures. The experience gathered from various malaria vector control efforts support an inverse relationship between success of the control strategy and the diversity of the vectorial system. The most successful eradication attempts against *A. gambiae s.s.* or *A. arabiensis* involved more or less monomorphic populations of these species as was the case in Mauritius and the forested areas of Cameroon. On the other hand, non-uniform exposure to indoor residual spraying with propoxur against highly polymorphic sympatric populations of *A. gambiae s.s.* and *A. arabiensis* in the WHO-led Garki project of Northern Nigeria was not successful (Molineaux and Gramiccia, 1980; Coluzzi, 1984; della Torre *et al.,* 2002; Yawson *et al.,* 2004).

2.2 Malaria Control

Like all other diseases, efforts at eliminating or controlling malaria began before the causative parasite and transmitting vector were discovered. However, unlike a disease such as measles which has been controlled or even irradicated, malaria continues to

persist with high morbidity and mortality rates in the tropics, especially, in sub-Saharan Africa (Nwanji *et al.*, 2003; WHO Africa Report, 2003; WHO World Report, 2005; 2008).

2.2.1 Early Diagnosis and Treatment of Malaria

The first strategy was to kill the malaria parasites in the patients. Physicians in the United States Public Health Service (PHS) began mass administration of quinine in some states in southern U.S.A. in the 1920 (Bleakley, 2007). Then a cheap, safe and effective drug, chloroquine, was discovered. Its widespread use led to an enormous decline in malaria incidence rates in the 1950s (WHO, 1997; Basu, 2002). However, chloroquine-resistant strains of *Plasmodium* evolved after years of successful drug therapy, intensifying in the late 1980s (Basu, 2002). New treatments using amodiaquine, pyrimethamine, the sulphonamides, pyrimethamine/sulfadoxine, mefloquine hydrochloride, pyrimethamine/sulfadoxine/mefloquine, primaquine, etc. were introduced. These decreased resistance in the early 1990s (NIAID, 1997; Basu, 2002). Unfortunately, these newer drugs too did not elude the evolution of resistant strains of *Plasmodium* for as long as did chloroquine (Strobel, 1999). Basu, (2002), reported that mefloquine was introduced in South East Asia in the mid 1980s but complete resistant strains were observed only after four years of its use. Resistance to atovaquone (a hydroxy-1, 4-naphthoquinone) developed so quickly that doctors observed resistant strains of *Plasmodium* during clinical trials (Strobel, 1999: Basu, 2002). Research on drug-resistance has shown that the malaria parasite frequently mutates and can therefore become immune to nearly any drug therapy. As the basic molecular genetics of the

parasite is still rudimentary, drugs designed to disrupt the genetic profiles of the parasites that would not be susceptible to resistance are far from production lines (NIAID, 1997; Basu, 2002).

2.2.2 Developing a Malaria Vaccine.

The development of a safe, inexpensive and easily administered malaria vaccine with a reasonable long shelf life and conferring life-long immunity to the disease, according to Basu (2002), is the alternative effective control measure being worked on. It is believed that developing a malaria vaccine is feasible because a) Partial, natural immunity has been shown to develop in adults living in malaria endemic areas (Wirtz and Cattani, 1997; Aide *et al.*, 2007; Sharma and Pathak, 2008). Therefore, it is possible to evoke immunity by causing the development of antibodies that would protect one against malaria (Good and Doolan, 1999). b) Again research has proven that adults treated with a radiated form of the parasite can be completely protected against malaria. Patients treated in this manner are immunised against a variety of strains of the parasite and their immunity last for long periods of time (Miller and Hoffman, 1998). However, though the development of a vaccine against malaria is evidently feasible, there are difficult challenges to overcome:

1) Technically, the malaria parasite has many strains, a complex life cycle and high mutability, presenting a myriad of antigens that vary throughout the different stages of its life cycle (Aide *et al.*, 2007). Therefore, an effective malaria vaccine will likely be highly complex, possibly containing five or more antigens to be able to

attack the parasite at multiple stages in its life cycle and overcome allelic and antigenic variations.

2) There is no appropriate animal model so the only way of testing the efficacy of a vaccine is to carry out logistically complex and expensive clinical trials in malaria endemic areas (Basu, 2002).

3) There is no sufficient support for research into malaria vaccine because it affects almost exclusively the poorer tropics where people cannot afford to pay for medicine for treatment let alone for research (Sachs, 1999).

Notwithstanding these obstacles, four general categories of malaria vaccine candidates, each representing a different stage of intervention is being researched into:

A. Pre-erythrocytic (or sporozoite) vaccines are directed against the sporozoite and liver stages of the parasite. A sporozoite vaccine could prevent infection either by blocking invasion of liver cells (antibody response) or destroying infected liver cells (cell mediated response) thereby preventing the development and release of merozoites into the bloodstream. An example is the RTS, S/AS02A vaccine candidate (Basu, 2002; Alonso *et al.*, 2004). The Kintampo Health Research Centre (KHRC), in collaboration with GSK Biologicals, the PATH Malaria Vaccine Initiative (MVI), and the Kumasi Centre for Collaborative Research/School of Medical Sciences, KNUST, Ghana (KCCR/SMS) has successfully started a Phase IIb RTS,S vaccine trial in Ghana to evaluate the administration and safety of RTS,S candidate malaria vaccine in children aged between 5 and 17 months old. About 540 children will be involved in this phase of the trial. KHRC will recruit and

coordinate half of the children in Kintampo while the other half will be recruited in Agogo, Ghana, under the coordination of KCCR/SMS.

B. Asexual blood-stage (or erythrocytic) vaccines are directed against the merozoite stage of the parasite, which invades and replicates in the red blood cell. A blood stage vaccine is expected to reduce both the severity and duration of the disease by decreasing the blood parasite density (Dicko *et al.*, 2007; 2008).

C. Transmission-blocking vaccines are designed to raise antibodies in humans against the gamete stage of the parasite present in the mosquito gut. Such antibodies, taken up by the mosquito during a blood meal should block further parasite development, resulting in the mosquito becoming a non-infectious vector (Wu *et al.*, 2008).

D. An anti-disease vaccine will be designed to identify parasite toxins that contribute to the development of diseases. Such a vaccine might be more effective and its immunity longer lasting given that due to the problems of antigenic diversity and variation, redundancy in parasite-host invasion pathways, host immune evasion strategies and genetic restriction in the immune response to parasite antigens, anti-parasite immunity is easily lost though it takes many more years to develop (Schofield *et al.*, 2002).

2.2.3 Malaria Vector Control

The discovery of the malaria parasite and its transmission by mosquitoes at the end of the nineteenth century raised hopes that technology would soon solve the problem of malaria. Even in those early days, there were fierce debates regarding whether control strategies should be targeted at the parasite or the vector. However, some of the early successes

such as the control of malaria during the construction of the Panama Canal required an integrated malaria control requiring the use of a combination of anti-parasite drugs, vector control through spraying and improved housing conditions in which mosquito screens reduced access to resting places options for gravid mosquitoes (National Centre for Zoonotic Diseases, 2004).

2.2.3.1 Early Efforts at Malaria Vector Eradication

It has been reported in publications by CDC, (2004) and Hoyt (2007), that in the early days, the realization that malaria incidence could be significantly reduced and brought under control by eliminating its vector, the *Anopheles* mosquito, led to a concerted vector control effort. In the Americas, for instance, eradication programmes consisted primarily of:

1) DDT application to the interior surfaces of rural homes or entire premises in areas where malaria was reported to have been prevalent
2) drainage of water bodies within all villages with distances of about 100 yards to individual houses.
3) removal of mosquito breeding sites by filling them with sand or concrete
4) mass spraying (occasionally from air crafts) with insecticides
5) the screening of houses
6) use of larvicides (mixtures of carbolic acid, resin and caustic soda), oiling surfaces of large bodies of water that could not be drained and
7) cutting bushes and grasses within 100 yards from individual houses and maintaining them at less than one foot high. The rationale was the assertion that mosquitoes would normally not cross open areas over 100 yards.

There is an assertion that it was such campaigns that led to successful eradication of malaria in Europe, the USA and other countries in the temperate zone by the 1950s and which consequently prompted WHO to launch its global eradication campaign at the World Health Assembly in 1955 (de Zulueta, 1973). However, the WHO campaign achieved moderate and only temporary successes in tropical countries due to:

1) the efficiency of the local vectors of malaria, such as *A. gambiae s.l.* and *A. funestus*
2) the favourable warm climate for the survival and breeding of these mosquitoes all year round and
3) the relatively poor social and economic conditions which differ from those prevailing in the temperate zone (de Zulueta, 1973; Greenwood, 2008).

2.2.3.2 Indoor Residual Spraying (IRS) and Use of DDT in Malaria Control

Brookel *et al.* (2001), reported of a 1936 publication by Park Ross suggesting that malaria vector control directed at indoor resting mosquitoes began in the 1930s with the use of pyrethrum extracted from flowers. DDT and dieldrin with their longer residual effects replaced the use of pyrethrum as an indoor vector control strategy shortly after the World War II. The success in the use of these organochlorine insecticides to control outbreaks of malaria further encouraged the focus on vector control to prevent malaria. Garrett-Jones *et al.* (1969), and Killeen *et al.* (2000), both reported that in 1955, the World Health Assembly launched a malaria eradication campaign based largely on the use of DDT to control the mosquito vectors; and as a consequence of this action, sporozoite rates were reduced four fold and human biting rates was reduced by approximately three fold at Kankiya by spraying with DDT.

However, in spite of its success, Basu (2002) reported that due to the negative ecological impact of organochlorines notably DDT on the environment, and the evolution of mosquito species resistant to it, DDT and other organochlorines were banned. However, DDT, in particular, still remains a most potent vector control insecticide to the extent that some workers called for its limited usage particularly in indoor residual spraying (IRS) (Pearson, 2004). Most South American countries, in lieu of the ban, abandoned DDT use except Ecuador. Therefore, the international agreement known as the Stockholm Convention on Persistent Organic Pollutants, which controls the production and use of DDT, has given a specified and limited exemption for the production and public health use only of DDT for indoor application to vector-borne diseases; mainly because of the absence of equally effective and efficient alternatives (Rehwagen, 2006; WHO, 2007).

2.2.3.3 Current Efforts at Malaria Vector Control in Ghana

Malaria vector control at the moment is diverse, depending on the continent, country or even region. In Africa, integrated vector management is advocated with each country adopting such measures as are conducive to their local situation (Najera and Zaim, 2003; Chanda *et al.*, 2008). In Ghana indoor residual spraying (IRS) protected 134,000 and 154,000 households in 2006 and 2007 respectively in selected areas. The national malaria control programme (NMCP) distributed 3.6 million long lasting insecticide treated nets (ITNs) in 2006–2007. In 2006, 30% of households owned at least one mosquito net, but only 19% owned one or more of insecticide treated nets (WHO, 2008).

2.2.3.4 Use of Insecticide-Treated Bed-Nets (ITN)

National Centre for Zoonotic Diseases, (2004), of CDC reported that Ronald Ross recommended the use of bed nets to protect against infective bites of anopheline vectors a century ago (1910). Bed nets give protection not only against infective mosquitoes but also from their painful bites as well as many other biting insects, thereby providing comfort to the user. However, untreated bed nets have been found not to be very effective in decreasing the risk of malaria infection and morbidity in highly endemic areas (Snow *et al*., 1988); but impregnation of bed nets with 'knock-down acting' pyrethroid insecticides such as deltamethrin; permethrin, etc. with a long residual effect improved the efficiency of these nets in reducing the risk of malaria. The advantage of insecticide treated bed nets (ITNs) is that apart from the direct protection of sleepers, they have great potential for mass vector control when used by most of the community members in which case they act as traps baited by sleepers, selectively attracting and killing anthropophilic mosquitoes. Mosquitoes are killed before biting unlike in the case of indoor residual spraying where they may blood-feed and inadvertently transmit the malaria parasite before getting killed on attempting to rest on the walls. ITNs therefore are important tools for a) breaking the man/vector contact, b) reducing malaria vector populations, and above all, c) reducing the mean life span of mosquitoes (Robert *et al*., 1989). Thus ITNs appear to be the most promising cost-effective and readily available method of reducing malaria by directly controlling the vector. These are effective, relatively cheap and easy to implement compared to other control tools (Curtis *et al*., 1998). Though ITN has limitations such as low acceptability in some communities, low re-impregnation rate and the emergence of resistant mosquitoes (Hargreaves *et al*., 2000; Matambo *et al*., 2007; John *et al*., 2008) the strategy of their use has achieved successes. For example, on the

Kenyan coast, the introduction of ITNs led to significant reductions of 33% in infant mortality, and severe, life-threatening malaria among children aged 1-59 months (44%) (Nevill *et al.*, 1996). Binka *et al.* (2002), reported of a 17% efficacy in preventing all-cause mortality in children aged 6-59 months in northern Ghana from July, 1993 to June, 1995. A follow-up until the end of 2000 found no indication in any age group of increased mortality in the ITN group after the end of the randomized intervention. A series of entomological studies (Darriet *et al.*, 1984; Ranque *et al.*, 1984; Lines *et al.*, 1987; Snow *et al.*, 1987a) have shown that insecticide-impregnated nets reduce feeding on man by vector mosquitoes and increased mortality among those that have fed. In Mali, nets treated with deltamethrin reduced the prevalence of splenomegaly (Ranque *et al.*, 1984) and in Papua New Guinea treated bed nets had some effect on parasitaemia in children aged 0-4 years (Graves *et al.*, 1988). In The Gambia, treatment of individual nets with permethrin reduced the incidence of clinical attacks of malaria among children sleeping under these nets, compared with children in the same village sleeping under untreated nets, but treatment had little effect on the level of asymptomatic malaria parasitaemia (Snow *et al.*, 1987b). In the same country, the use of permethrin-impregnated bed nets had a dramatic effect on mosquito populations and reduced the blood-feeding success of vectors by 90%. These entomological changes were accompanied by a 63% reduction in the incidence of episodes of fever accompanied by heavy malaria parasitaemia in children who slept under treated nets over the course of the rainy season (Snow *et al.*, 1988).

2.2.3.5 Controlling Malaria Vectors by Treating Cattle with Insecticides

In areas where malaria vectors are exophagic and/or zoophilic, interventions such as ITN and IRS are not effective. Consequently, there is increasing interest in developing methods to control such mosquitoes. One potential method is the use of insecticide-treated cattle (Habtewold *et al.,* 2004).

The feasibility and efficacy of this method form the subject of a number of research works. In one such experiment, Rowland *et al.* (2001), investigated whether domestic livestock treated with deltamethrin (applied by a sponging method) could prove toxic to mosquitoes and therefore aid in malaria control. They found from clinic records that the incidence of malaria caused by *P. falciparum* decreased by 56% and *P. vivax* by 31% in livestock-treated villages in Pakistan. Cross-sectional surveys showed comparable decreases in parasite prevalence. Furthermore, the density and life expectancy of *A. stephensi* and *A. culicifacies* populations were reduced in treated villages. The efficacy in reducing malaria using insecticide treatment of livestock was similar to that achieved through indoor spraying but the campaign in the former costs 80% less compared to the latter. When applied in a highly endemic settlement, the incidence of falciparum malaria decreased from 280 episodes per 1000 person-years to nine episodes per 1000 person-years (Rowland *et al.*, 2001). Earlier, Hewitt and Rowland, (1999) had conducted a series of investigations to assess the potential of cattle treated with pyrethroid to control zoophilic mosquitoes. The possibility that treatments might cause diversion of host-seeking vectors from cattle to people nearby was also investigated. At the doses tested, deltamethrin had the most pronounced and longest-lasting effect, reducing the proportion of blood-fed survivors by over 50% for the first two weeks. The majority of mosquitoes

affected by the insecticide were killed before feeding. Insecticidal impact was generally lower on grazing than on sheltered animals. There was no diversion of host-seeking mosquitoes from treated cattle to nearby humans at any stage after treatment. Field experiments have been conducted in Southern Ethiopia to establish whether Zebu cattle (*Bos indicus* L.) treated with a pour-on pyrethroid formulation of 1% deltamethrin, widely used to control ticks and tsetse flies, would be effective against *A. arabiensis* or cause the female mosquitoes to feed more frequently on humans, due to behavioural avoidance of insecticide-treated cattle. Contact bioassays (3 min exposure) showed that the insecticide remained effective for about one month (kill rate >50%) against mosquitoes feeding on the flanks of treated cattle. A novel behavioural assay demonstrated that *A. arabiensis* readily fed on insecticide-treated cattle and were not deflected to human hosts in the presence of treated cattle. DNA fingerprinting of blood meals revealed that *A. arabiensis* naturally feeds most frequently on older animals; thus consistent with the established practice of applying insecticide only to older cattle, while allowing younger untreated animals to gain immunity against infections transmitted by ticks. These encouraging results were tempered by the finding that over 90% of *A. arabiensis, A. pharoensis* and *A. tenebrosus* females feed on the legs of cattle, farthest from the site of pour-on application along the animal's back and where the treatment may be least residual due to weathering. Observations of mosquitoes feeding naturally on insecticide-treated cattle showed that the majority of wild female anophelines alighted on the host animal for less than 1 min to feed, with significantly shorter mean duration of feeding bouts on insecticide-treated animals, and the effective life of the insecticide was only a week. Thus the monthly application of deltamethrin to cattle, typically used to

control tsetse flies and ticks, is unlikely to be effective against *A. Arabiensis* populations or their vectorial capacity. Even so, it seems likely that far greater impact on anopheline mosquitoes could be achieved by applying insecticide selectively to the legs of cattle (Habtewold *et al.*, 2004).

2.2.3.6 Limitations of Adulticide Based Vector Control Strategies

Macdonald's work in the 1950s (MacDonald, 1957) showed that adulticiding was more effective in reducing the basic reproductive rate than larviciding of anophelines. Therefore many vector control measures against malaria transmission such as ITNs and IRS target adult female mosquitoes to reduce their longevity and human-feeding frequency. However, suppression of transmission over large areas depends upon population-level exposure of vectors to the intervention and this, in turn, depends upon the level of coverage within the human community. Adult vectors are highly mobile flying insects that can readily detect and avoid many commonly used insecticides. Therefore effective coverage of an intervention strategy may not necessarily be equivalent to the absolute coverage of humans and may be considerably less if vectors evade it. Thus by avoiding covered humans, vectors may redistribute their biting activity towards those who are not covered by personal protection measures such as treated bed-nets. Therefore the control of adult but not immature aquatic-stage mosquitoes is compromised by the ability of the former to avoid interventions such as excito-repellant insecticides. The failure of the global eradications of malaria by solely relying on indoor residual sprayings during the period of 1960s to 1970s demonstrate the need for integrated mosquito management for which MacDonald, (1957) had earlier expounded on and for which later workers have confirmed need of (Gimnig *et al.*, 2001; Killeen *et*

al., 2002; Weidong *et al.*, 2005). The effectiveness of malaria control programmes is crucially dependent upon not only the extent of coverage but also the ability to target the most intense foci of transmission. Adulticide-based control may be limited because of constantly shifting distributions of biting vectors. A number of field studies (Muirhead-Thomson, 1960, Charlwood and Graves, 1987; Magesa *et al.*, 1991; Bogh *et al.*, 1998) have shown that vectors prevented from feeding upon individuals protected by treated nets are not diverted to unprotected humans in the same dwelling or those immediately nearby. However, excito-repellent bed net treatments and indoor residual sprays are known to lower human blood indices in vector populations when applied at the community level so mosquitoes that are deterred from covered homes probably do feed elsewhere upon whatever unprotected humans and alternative hosts are available. Thus it seems that vector biting density may be redistributed to unprotected humans and livestock but over longer distances than have been tested thus far. In many situations, the majority of female mosquitoes in a focal area are likely to emerge from prolific habitats, which might account only for a small proportion of habitats (Bogh *et al.*, 1998).

2.2.3.7 Larval Control of Malaria Vectors

On the other hand, the egg, larval and pupal stages of mosquitoes are of relatively low mobility compared with flying adults. They are confined within relatively small aquatic habitats and cannot readily escape control measures. It is the humans that must bring the intervention to them rather than vice versa. The application of measured quatities of microbial larvicides containing *Bacillus sphaericus* strain (*Bs*) and *Bacillus thuringiensis* var.*israelensis* (*Bti*), for instance, on the open water surfaces of the breeding sites of mosquitoes, has been shown to cause larval mortality in all species of between 90%-

100% on post-treatment day in Turkey (Aldemir, 2007). In The Gambia the major malaria vectors were found to be highly susceptible to both microbials (Majambere *et al.*, 2007). Fillinger *et al.* (2003), in Western Kenya and Seyoum and Abate (1999), in Ethiopia reported similar findings to buttress the efficacy of larval control of mosquitoes and the need for the employment of integrated vector management strategies for the control of malaria and other mosquito-borne diseases.

Targeted larval interventions have a great potential to maximize limited resources and should be vigorously pursued in integrated malaria management in Africa. Targeted interventions require quantification of habitat productivity based on sampling data, e.g. larval density and surface size of habitats. The guiding principle of integrated malaria control in any area is to tailor interventions to the local entomological and epidemiologic characteristics. One of the key local determinants of transmission is abundance, distribution and adult productivity of larval habitats. Elimination of mosquito larval habitats in one's homestead is not sufficient for reducing mosquito densities in a community because adult mosquitoes in a house may have originated from larval habitats of several hundred meters apart. Therefore, inventory of aquatic habitats regarding their productivity can provide critical information for characterizing species-specific oviposition habitat selection and planning of integrated mosquito managements (Killeen *et al.*, 2002; Minakawa *et al.*, 2002; Weidong *et al.*, 2005).

2.2.3.8 Mosquito Resistance to Insecticides

Some reports on the evolution of mosquito strains resistant to DDT and pyrethroids dates back to the 1960s. However, according to Lynd *et al.* (2005), the increasing prevalence

of pyrethroid insecticide resistance in malaria vectors, especially *A. gambiae s.l.*, the major vector of malaria in sub-Saharan Africa, threatens to compromise the successful use of insecticide-treated materials. Resistance to pyrethroid insecticides was first detected in *A. gambiae s. s.* in Burkina Faso, West Africa (Elissa *et al.,* 1993) then was subsequently detected in Kenya, East Africa (Vulule *et al.,* 1994) and is now widespread. This threatens the success of insecticide-treated nets (ITN) in reducing the morbidity and mortality of malaria that led to their widespread use in malaria endemic areas, being incorporated into national control programmes.

Resistance is based on several mechanisms that could segregate according to their operational impact on vector biology and control. DDT and pyrethroids act by modifying the voltage-gating kinetics of the sodium ion channel. Knock-down resistance (kdr) mutation in the sodium ion channel gene results in reduced sensitivity of the sodium ion channel along nerve axons to these insecticides, causing resistance to them. Knock-down resistance (kdr) to DDT and pyrethroids in the major Afro-tropical vector species, *A. gambiae s.s.* is associated with two alternative point mutations at amino acid position 1014 of the voltage-gated sodium ion channel gene, resulting in either the substitution of leucine (TTA) by phenylalanine (TTT), designated (L1014F), or leucine (TTA) by serine (TCA) designated (L1014S). In *A. gambiae* S-form populations, the former mutation appears to be widespread in West Africa though it has been recently reported in Uganda. The L1014S mutation, originally recorded in Kenya, has been recently found in Gabon, Cameroon and Equatorial Guinea. In M-form populations surveyed to date, only the L1014F mutation has been found, although less widespread and at lower frequencies than in sympatric S-form populations. The L1014F allele coexist with the L1014S one in

samples from Cameroon, Gabon and north-western Angola (Brooke1 *et al.* 2001; Etang *et al.* 2004, Etang *et al.* 2006; Pinto *et al.*, 2006; Verhaeghen *et al.*, 2006; John *et al.*, 2008; Santolamazza *et al.*, 2008). Investigations using WHO recommended bioassays and polymerase chain reaction amplification of specific allele diagnostic test have found resistance to be associated with the presence of the kdr mutation. The strong correlation between kdr allelic frequency and resistance to DDT or pyrethroids indicate that kdr is a resistance factor for insecticides (Abdoulaye *et al.* 2003; Chandre *et al.*, 1995; Coetzee *et al.*, 2005). Kdr alleles were found to be more frequent in resistant mosquitoes (L1014F: 16.7%; L1014S: 5.6%) than in susceptible ones (L1014F: 4.6%; L1014S: 1.8% (Moreno *et al.*, 2008). In trying to assess the long-term efficacy of ITNs in Ghana and Nigeria, Kristan *et al.* (2003), found in a bioassay that susceptibility of *A. gambiae s.l.* to DDT was 94-100% and 72-100% respectively, indicating low levels of DDT resistance in Ghana compared to Nigeria. Deltamethrin gave the highest mortality rates in both countries: 97-100% in Ghana, 95-100% in Nigeria. Ghanaian samples of *A. gambiae s.l.* were fully susceptible to permethrin, whereas some resistance to permethrin was detected at 4 out of 5 Nigerian localities with survivors including both *A. arabiensis* and *A. gambiae s.s.* identified by PCR assay. Even so, the mean knockdown time was not significantly different from a susceptible reference strain, indicating absence or low frequency of kdr-type resistance. Therefore they concluded that such low levels of pyrethroid resistance are unlikely to impair the effectiveness of pyrethroid-impregnated bed-nets against malaria transmission. However, a year later Yawson *et al.* (2004) observed that in Ghana the kdr mutation occurred at very high frequencies (98–100%) within the S form but reached a maximum of only 3.38% in the M form in a population at

an irrigation scheme in a coastal savannah zone. Similarly, kdr mutation occurred in both S and M forms in southern Ghana but with higher frequencies in the former. A year after the finding of Yawson *et al.* (2004), a correlation was established between the kdr gene and bioassay results in Ghana: sequencing of the IIS6 domain containing the kdr mutation from nine surviving mosquitoes in a bioassay showed that eight were homozygous resistant while one was heterozygous. This correlated with the bioassay results and with previous studies on West African *A. gambiae* (Coetzee *et al.*, 2005). However, it has been shown that nets impregnated with permethrin or deltamethrin provided good levels of protection against *kdr* homozygous strains of *A. gambiae* (RR) from Kou valley, Burkina Faso, as well as Tola in Côte d'Ivoire (RR), and heterozygous field population (94% *kdr* frequency) from Yaokoffikro, also in Côte d'Ivoire (Chandre *et al.*, 2001). The explanation seems to be that high proportions of *kdr* females are killed by prolonged contact with pyrethroids through diminished sensitivity to the usual irritant and repellent effects, though relatively few *kdr* females take advantage of this prolonged contact to ingest a blood meal (Chandre *et al.*, 2001). There is also strong evidence for metabolic-based resistance mechanisms in the African malaria vectors. Three major enzyme families (esterases, glutathione S transferases and cytochrome P450 oxidases) are involved in insect detoxification. Elevation of their activity usually results in resistance to insecticides such as pyrethroids. In Cameroon, elevated esterase, oxidase or glutathione S transferase activities were reported as the main resistance mechanisms in many populations of the *A. gambiae* complex (Etang *et al.*, 2004). A lower correlation observed between kdr frequency and resistance to 4 of 5 pyrethroids tested was attributed to another mechanism being involved, likely a metabolic detoxification (Chandre *et al.*,

1995). In other findings in Ghana, molecular analysis of the IIS5-IIS6 segment of the sodium channel gene gave no indication of any kdr-type mutations associated with resistance phenotypes. However, biochemical analysis suggests that DDT and pyrethroid resistance may be metabolically mediated, although there were no clear correlations between enzyme levels/activities and insecticide resistance across families. Furthermore, an altered acetylcholinesterase conferring carbamate resistance was evident (Okoye *et al.*, 2008).

2.2.3.9 Biological Control of Malaria Vectors

Biological control is defined as the action of predators, parasites (parasitoids) or pathogens in maintaining the density of another organism at a lower average than would occur in their absence (Ghosh *et al.*, 2005). At present, researchers are inclined to renewed interest in the use of biocontrol agents and bio-insecticides rather than chemical ones due to concerns about a) the residual effects of chemical insecticides, b) widespread resistance in target insects, c) high refusal rate for indoor spray, d) soaring price of chemicals, etc. (Ghosh *et al.*, 2005). For now biological control methods are only operational against non-adult aquatic stages of mosquitoes.

2.2.3.10 Use of Larvivorous Fishes

An effective biocontrol method is the use of larvivorous fish in appropriate water bodies in mosquito control, which has been the most successful in many parts of the world. There are certain traits that make a particular fish species a good candidate for mosquito control; a) fish must eat mosquito larvae as a main source of food, b) have behavioural and physical adaptation for surface feeding, c) be of small size, d) have high fecundity with short periods of maturation, e) disease resistant , f) good adaptability to its new

environment and g) have the ability to utilize alternative foods from diverse waters when prey population decreases (Ghòsh *et al*., 2005; Howard *et al*., 2007). Almost 200 fish species are known to feed on mosquito larvae, including *Aphanius chantrei, Cyprinus carpio, Ctenopharyngodon idella, Oreochromis niloticus, Gambusia affinis, Poecilia reticulata, Carassius auratus* and *Clarias gariepinus* (Kusumawathie *et al*., 2006). Many of these larvivorous fishes have been assessed as potential mosquito control agents both under laboratory and field conditions and found to be effective. For instance *O. niloticus* (first called *Tilapia nilotica* and then re-classified *Sarotherodon niloticus*) - a native African fish, under laboratory conditions, has been shown to be larvivorous (Kusumawathie *et al*., 2006) with a 'marked interest in mosquito larvae' (Asimeng *et al*., 1993). The fry (young fish) actively pursue mosquito larvae and pupae. However, when they achieve a length greater than 150 mm they prefer eating macrophytes (el Safi *et al*., 1985). This behaviour of the larger fish is complimentary to the larval control ability of its fry and fingerlings because as the larger fish eat the plant material in which the mosquito immatures hide, the larvae are exposed for the fry to find them. In a field trial it was found that after *O. niloticus* introduction, mosquito densities immediately dropped in the treated ponds but increased in a control pond. The results showed that after 15 weeks the fish caused a more than 94% reduction in both *A. gambiae s.l.* and *A. funestus* in the treated ponds, and more than 75% reduction in culicine mosquitoes. There was a highly significant reduction in *A. gambiae s.l.* numbers when compared to pre-treatment levels. *O. niloticus*, commonly farmed by people in western Kenya as a source of protein and income, is a prolific breeder spawning every few weeks. The efficacy of *O. niloticus* as a strong biocontrol agent against *A. stephensi* was proven under field conditions along

with *Cyprinus carpio, Ctenopharyngodon idella* and *Clarias gariepinus* where larval mosquito abundance decreased significantly at 30 and 45 days after the introduction of each fish species in the natural habitat of larval mosquitoes. There was also a steady increase in the larval abundance after the removal of fishes which suggested that the reduction was due to the presence of the larvivorous fish species *Clarias gariepinus* was found to be the most voracious and therefore efficient. *Gambusia affinis* at a stocking rate of 5 fishes/square metre significantly reduced the larval and pupal densities in experimental fields as compared to control fields during an entire observation period of 42 days (Das and Prasad, 1991; Ghosh *et al.* 2005; Howard *et al.*, 2007; Yıldırımand and Karacuha, 2007).

Larvivorous fish therefore offer advantages as biocontrol agents: they feed on mosquito pupae as well as larvae and are generally self-sustaining, so in most cases do not require repeat applications. They can also serve as a source of income and protein.

However, the main disadvantage of using lavivorous fish is that they can only be used under certain conditions conducive to their survival; thus it is, for example, difficult to maintain effective numbers of fish in running water and some breeding habitats of important vectors such as temporary waters that are created after rain, which *A. gambiae* prefer to use. Such water bodies may neither be accessible nor suiTable for the fish (Howard *et al.*, 2007).

2.2.3.11 Use of Bio-Insecticides

The long residual action and toxicity of the chemical insecticides have brought about serious environmental problems such as;

a) the emergence and spread of insecticide resistance in many species of vectors

b) high mammalian and avian toxicity, and

c) accumulation of insecticide residues in the food chain.

These problems have necessitated the need for alternative bio-insecticide. The best known of these are the entomo-pathogenic *Bacillus thuringiensis* var. *israelensis* (*Bti*) and *Bacillus sphaericus* (*Bs*) which are both effective and safe biological control agents. They have attracted considerable interest as possible replacements for the chemical insecticides (Magda *et al.*, 2006; Howard *et al.*, 2007). Laboratory and field trials of these larvicidal bacteria have demonstrated high potency and great potential for use in mosquito control. Fillinger *et al.* (2006) reported an overall reduction of *Anopheles* larval density by 95% and human exposure to bites from adults by 92% in a field trial of Bti in rural Kenya. In the Golbası district of Ankara, in Turkey, an increase in larval mortality was detected in all treatments with bio-insecticide as dose rates increased (Mulla *et al.*, 1999; Aldemir, 2007). In Ouagadougou, Burkina Faso, it has been reported that different levels of larval mortality were obtained depending on such factors as the method of estimation, the formulation of the larvicide, the mosquito species and the type of breeding site (Skovmand and Sanogo, 1999). Pupae development was reduced by 94% at weekly re-treatment intervals of mosquito breeding sites with bio-insecticide in The Gambia (Majambere *et al.* 2007). In Western Kenya, under laboratory conditions, the larvae of *A. gambiae* were found to be more susceptible to *Bs* than to the *Bti* formulations, as had been reported from studies in natural breeding sites in the Democratic Republic of Congo (Karch *et al.*, 1991), and Burkina Faso (Majori *et al.*, 1987; Lacey *et al.*, 1988). Results from open field trials with *Bti* water dispersible granules (WDG) showed that just a very low dosage of 200 g/ha is required to effectively suppress late instars and therefore the

resulting pupae. Such low application dosages offer the possibility to keep operational costs low even if weekly treatments, caused by the absence of residual activity, have to be considered (Fillinger *et al.*, 2003).

Advantages provided by microbial larvicides are that they a) have high efficacy, b) are environmentally safe, and c) are harmless to non-target organisms including humans thus they can be applied in drinking water with no adverse effect (WHO, 1999). Furthermore, they are effective even when extremely low dosages are applied. This advantage offers the possibility to keep operational costs low even if weekly treatments, caused by the absence of longer residual activity, have to be considered as with *Bti.* Bio-larvicides are very species-specific, and appear not to induce long-term permanent resistance (Fillinger *et al.*, 2003; Magda *et al.,* 2006; Majambere *et al.*, 2007).

However the drawbacks of microbial larvicides are that they are a) effective against mosquito larvae only but cannot control the pupal stage which does not feed, b) frequent repeat applications are required due to absence of long enough residual activity and this makes it c) more expensive than they ought to be (Magda *et al.,* 2006; Howard *et al.*, 2007)

2.2.3.12 Genetic Manipulations

Mosquitoes are obligatory though inadvertently passive vectors for malaria parasite transmission. Therefore the spread of malaria could be curtailed by rendering the mosquito vector incapable of transmitting the parasites. Foreign genes can be introduced

into the germ line of these mosquitoes, and these transgenes can be expressed in a tissue-specific manner (Catteruccia *et al.*, 2000).

The feasibility of using such genetic-based control technologies to limit the size of vector populations (population reduction) or to alter the populations so that they are resistant to pathogens (population replacement) is one promising strategy being considered. However, one factor worth noting is the separation of the target species into reproductively isolated panmictic units. Any attempt to introduce a) sterility, b) lethal factors, or c) genotypes selected for refractoriness to the parasite into a natural vector population should be based on evidence that the released mosquitoes are fully compatible with the mating recognition system of the target population (Coluzzi, 1984).

Sterile insect technique (SIT), for instance, is the mass production, sterilization, and subsequent release of sterile male insects to swamp a target population in an area-wide (entire insect populations within a delimited geographical area), and usually integrated, pest management strategy. The released males would hopefully compete fully with the wild males to inseminate wild females with sterile sperm. The females would subsequently fail to produce viable offspring leading to an overall size reduction or eradication of the target population (Helinski *et al.*, 2008). Over the years, SIT has proven to be a safe, effective and environmentally sound method to suppress, eliminate or contain any targeted insect pest populations (Dyck *et al.*, 2005).

A transgene is a foreign gene (effector gene or molecule) introduced into an insect to cause a desired transformation of the latter, e.g. refractoriness to a parasite. Genetically engineered mosquito vectors for refractoriness to malaria parasites is a strategy for

reducing disease transmission. For instance, transgene expression reduced *Plasmodium berghei* oocyst formation by 87% on average and greatly impaired transmission of the parasite to naive mice (Moreira *et al*., 2002). Success of the population replacement strategy is contingent in part on engineering effector genes that impair replication or development of the pathogens within the vectors (Collins *et al.*, 1986).

However, many challenges remain to achieve the long-term goal of controlling malaria transmission by genetic modification of the mosquito. A major obstacle is to devise safe means of spreading foreign genes across mosquito populations in the field. Another potential obstacle is the genetic diversity and mutability of *Plasmodium*. Because development of the parasite in transgenic mosquitoes is not completely blocked, the possibility exists that 'resistant' variants will be selected. To address this concern, it will be important that mosquitoes be modified with multiple genes, each of which inhibits parasite development by a different mechanism (Ito *et al.*, 2007).

2.2.3.13 Integrated Vector Management (IVM)

Africa is home to various insect vector-borne diseases; and certainly, a cost-effective integrated approach of the vector will be beneficial to the control of these diseases. With the current global concern, it is imperative for scientists, clinicians, policy-makers, health managers and health-care providers in Africa, to act in concert with other players to reduce the burden of malaria and other vector-bone diseases on the continent, using an integrated approach (Okenu, 1999). Integrated vector management (IVM), also referred to as integrated vector control (IVC), and has been defined diversely as:

1. the utilisation of all appropriate technical and management techniques to bring about an effective degree of vector suppression in a cost-effective manner (WHO, 1983).
2. the targeted use of different vector control methods alone or in combination to prevent or reduce human-vector contact cost-effectively, while addressing sustainability issues (Chanda *et al.*, 2008).
3. a rational decision-making process for the optimal use of resources for vector control (Beier *et al.*, 2008).

IVM relies on an understanding of the entomology of the disease and its vectors; for example how;

a) environmental factors affect the distribution and densities of different species of vectors.
b) effective control measures reduce vector-human-contact.
c) vector survival relates to the overall intensity of pathogen transmission.

The essential requirement for IVM is the availability of more than one method of control or the ability to use one method that enhances or in the least does not obstruct the action of another method; e.g. the choice of a pesticide which has no detrimental effect on naturally occurring biological control agents (Beier *et al.*, 2008). The development of the long lasting residual chemical insecticide during the 1940s, for instance, presented a single, extremely high cost effective method of vector control. However, a number of factors including, a) the development of resistance to insecticide in vectors, b) the concern about environmental contamination, c) human safety and ,d) the increased cost of alternative insecticides made blanket and singular use of insecticides unsuiTable and

led again to the development and use of several techniques, simultaneously or sequentially in IVM (WHO, 1983).

When IVM was used in the Republic of Korea in the form of periodic complete water drainage from irrigated rice fields for 2-3 days and repeated within the intervals of 10-15 days with selective use of pesticides, there was a drastic reduction of mosquito density. Random use of pesticides led to resistance in the vectors while natural predators of larvae of the vectors were killed by the insecticide. Selectivity in pesticide use caused suppression of vector proliferation by allowing natural biological control agents (predators) to survive and feed on them. This choice of pesticides for maximum effectiveness against the vector but minimum impact on the biological control agents makes the Korea programme a model IVM strategy (WHO, 1983).

In situations where source reduction is not possible, use of biolarvicides such as Bti and Bs has been recommended. Insecticide treatment of pigsties and cowsheds could form part of an IVM component given that some vector mosquitoes are partially or completely zoophagic. Menial or small magnitude measures such as filling of tree-holes, destruction of discarded containers, frequent scrubbing of washrooms and renewal of water in domestic containers could form an essential part of IVM (WHO, 1983, Beier *et al.*, 2008). The objective of IVM is to effectively reduce adult and larval vector populations and invariably pathogen transmission by use of interventions that are ecologically, environmentally, socially, economically and politically acceptable. To achieve this, IVM should not create adverse side effects such as environmental contamination or the development of resistance, nor have a negative impact on non-target organisms, including beneficial insects, humans, domestic animals and wildlife (Beier *et al.*, 2008). A

successful implementation of IVM requires an assessment and strengthening of the following components of the vector control programme:

a) Policy: A policy framework must be in place to provide the enabling environment for a national IVM programme implementation.

b) Institution building: There should be collaboration and coordination between and among institutions involved to facilitate vector control.

c) Managerial development: The establishment of clear criteria and decision-making procedures to manage the vector control programme.

d) Technical strengthening: Development of technical facilities to support IVM programmes.

e) Human-resource development: The training of personnel in the relevant disciplines and skills to work on the IVM project.

f) Community participation; the citizens should be educated to play active roles in the programme to ensure the success and sustainability of control strategies.

Assessment and strengthening of vector control capability must identify strategies that will ensure that the various components of the IVM programme are properly and efficiently integrated into routine efforts of the national malaria control programmes (Beier *et al.*, 2008).

Recent reports by Chanda *et al.* (2008; 2009), describe a comprehensive and highly successful IVM programme in Zambia that effectively reduced malaria related morbidity and mortality in the general population and malaria related OPD cases at Chongwe rural health facility in particular. A similar work at Mwea division, Kenya recorded decreased malaria cases in the district hospital and parasitaemia in school children (Okech *et al.*,

2008). Beier *et al.* (2008), reported that historically over the years, IVM have significantly reduced vector populations and malaria transmission across a range of transmission settings in Africa as they quote the work of Wiseman *et al.*, 1939, in Kenya, Gilroy *et al.,* 1945 in Nigeria, Bang *et al.,* 1975 in Tanzania, Utzinger *et al.,* 2001 in Zambia and Caldas de Castro *et al.*, 2004, also in Tanzania). Nevertheless, still in most parts of Africa, malaria control programmes lack adequate vector control components (WHO, 1983). As a result, progress in the implementation of vector control activities have been very limited (WHO, 2000). Some of the factors for the limited vector control implementation include, a) insufficient guidance on vector control implementation, b) low priority to vector control with insufficient resource allocation, c) lack of appropriate infrastructure, d) lack of adequate number of technically skilled, competent staff, e) and a high attrition of the few staff available (Beier *et al.,* 2008). Other reasons for the low progress in the implementation of IVM in Africa are:

a) Exaggeration of the requirements of resources needed to implement IVM programmes.
b) Over-sophistication of IVM: For many mosquito species, enough is already known to base the introduction of integrated control so that IVM does not necessarily have to be preceded by costly, complex vector studies.
c) Insufficient conviction of the advantages of IVM.
d) Misconceptions about the use of pesticides in integrated control. IVM use does not mean exclusion of pesticides, but their more rational and correct use.

e) There is not enough consciousness of evaluating IVM programmes to ensure that their cost effectiveness and benefits exceeds risks (WHO, 2004, Decision; UNEP, 2006, Chanda *et al.*, 2008).

However, since 2001, the World Health Organization has been promoting IVM as the new strategic approach to vector control (Rafatjah, 1982; 2001).

2.2.3.14 Why Vector Borne Diseases Still Persist in Spite of IVM

There are many reasons why vector-borne diseases remain a major public health problem, especially, in tropical Africa in spite of vigorous attempts at their suppression. These include:

a) the inability of specialist agents of IVM (entomologists and vector-control specialists) to convince decision-makers on the importance and feasibilty of vector control and further persuade such decision-makers of their ethical duty to control mosquitoes and the pathogens they transmit.

b) the difficulty in achieving intersectoral collaboration aimed at improving mosquito control.

c) IVM technocrats have not been able to use models and adaptive management effectively to quantify the effect of disease and vector control interventions on the economic and social development of communities and nations.

d) health education and enforcement of laws on vector control have not been adequately done.

e) the status quo of concentrating efforts on active and passive case-detection and treatment at the expense of preventive vector control measures has not been overcome (Beier *et al.*, 2008).

f) the unique combination of optimal environmental condition that promotes the proliferation of an adapTable highly efficient vector makes IVM less effective than it should be on paper.

g) however, recent results from the implementation of IVM show that the Fight against vector-borne diseases, especially, malaria is making progress (Okech *et al.*, 2008; Chanda *et al*., 2008, 2009).

2.2.3.15 Obstacles to Current Efforts at Malaria Vector control in the Tropics

Malaria vector control measures in the tropical world, especially, in sub-Saharan Africa have not been as successful as in the temperate zone. The result is the continuous escalation in the rate of malaria infection in sub-Saharan Africa and the frequent export of malaria sub-Saharan Africa to the "free zone", i.e. Europe and parts of America where malaria has been controlled. The factors that make malaria control in sub-Saharan Africa difficult include:

The high efficiency of the species of vector mosquitoes in the tropics in transmitting malaria compared to those in the temperate regions. In those regions where malaria has been eradicated, mosquitoes spend their winter in hibernation or a non-reproductive state. Effective implementation of control programmes during these periods was very successful. In tropical zones where mosquitoes do not hibernate and breeding sites are widespread, including many temporary water pools after rains, the frequent use of insecticide to attempt to bring numbers down only resulted in the evolution of resistance (Basu, 2002; WHO, 2003).

Furthermore, the clearing of breeding sites has been impractical given that water collected even in foot-prints in the forest floor, for instance, are good sites for *A. gambiae s.l.* to breed in (Minakawa *et al.,* 2005).

Inhabitants in the tropics therefore receive continuous and multiple malaria infections resulting in the development of non-sterilising immunity to the disease. Such immunity suppresses clinical symptoms thereby allowing those infected persons to appear healthy while malaria parasites develop and circulate in their blood so that they become a reservoir for mosquitoes to use to infect healthy patients. Mosquitoes in temperate regions, on the other hand, re-infect individuals rarely. People in these regions exhibit decipherable symptoms of malaria upon infection and can be treated promptly (Hamoudi and Sachs, 1999).

The high vectorial capacity (ability to spread the parasites from one human to others) of members of the *A. gambiae* complex is another obstacle to the control of malaria in endemic sub-Saharan Africa. This enables the vector to cause malaria infections in several individuals even when only one infected person is part of the population. High vectorial capacity also enables the *A. gambiae* complex to sustain malaria infection in the population with extremely low vector population. An example is the WHO-led "Garki Project" in Garki, Nigeria. In the project, WHO coordinated extensive insecticide spraying and mass drug administration in an attempt to totally eradicate malaria in 164 villages (Molineaux and Gramiccia, 1980). This reduced the biting rate of mosquitoes by 90%. However, prevalence of the malaria parasite among villagers did not significantly change. The vectorial capacity of the surviving mosquitoes was simply too high to overcome (Hamoudi and Sachs, 1999). Thus the difficulty in

controlling malaria stems from the unusual nature of the parasite and of its vector. Both are highly complex genetically and even more so with the vector as a sexually reproducing animal capable of mixing genes during reproduction to enable quick evolution to acquire resistance to insecticides.

It is also believed that the malaria parasite co-evolved with the human species in Africa so the two organisms are well adapted to one another.

The other important drawback in the control of malaria is inadequate funding. Malaria Research and Development (R&D) Alliance (2005), reported that malaria currently receives only about 9% of the over US $3.3 billion in annual R&D funding. The low funding attributed to lack of market for malaria drugs and other products retards scientific efforts at controlling the disease. However, there has been progressive increase over the years in malaria support, with the USA government and other agencies playing important roles (Malaria Research and Development Alliance, 2004). The lack of total understanding of the spatial distribution of the molecular and chromosomal forms of tropical mosquitoes has important epidemiologic implications for the control of these most efficient vector species because ecologic and behavioural studies have shown that these forms may differ in their ecology and possibly transmission capacity (Santolamazza, *et al.* 2004).

2.3 Trends in Malaria Morbidity and Mortality in Ghana

The data of WHO on Ghana covering the period 2001 to 2007 (Tables 1 and 2), show that the trend in malaria morbidity and mortality in the country, though not exactly consistent, is escalating gradually on annual basis (WHO, 2008).

Table1: Reported malaria cases (morbidity)

Year	2001	2002	2003	2004
All ages	3 044 844	3 140 893	3 552 896	3 416 033
< 5 years	856 872	705 288	920 140	1 289 874

Year	2005	2006	2007
All ages	3 452 969	3 511 452	3 123 147
< 5 years	2 876 465	789 952	1 056 331

Source: WHO, 2008.

Table 2: Reported malaria deaths (mortality).

Year	2001	2002	2003	2004	2005	2006	2007
All ages	1 717	1 917	1 680	1 260	1 759	2 832	4 622
< 5 years	1 441	1 360	1 376	1 354	922	805	1 241

Source: WHO, 2008.

2.4 Relationship between Entomological Parameters and Malaria Transmission

Mosquito studies to help in malaria control are set out to define quantitatively a) mean annual entomological inoculation rate (EIR), b) sporozoites rate (SR) and c) biting rates (BR). It has been established that malaria transmission is effective even if the EIR vary from less than 1 to over 1000 ib/p/yr and sporozoite rates is within the range of 1% to 20%. These findings demonstrate the complexity of malaria transmission and the difficulty in controlling the disease effectively. It does not seem to matter much whether disease suppression is by an integrated vector control, anti-malaria drug therapy, or vaccine development. The degree of control will clearly differ in situations where residents are exposed to different levels of EIR (Trape *et al.,* 1996; Beier, *et al*., 1999; Hay *et al.,* 2000; Appawu *et al*., 2004; Bigoga *et al*., 2007). Other studies also show a direct relationship between EIR and malaria prevalence, mortality, clinical malaria attack incidence and therefore transmission intensity in a given area (McElroy *et al*., 1994; Bull *et al.,* 1998; Meunier *et al*., 1999; Ofosu-Okyere *et al.,* 2001; Smith *et al*., 2001; Bonnet

et al., 2002; Elissa *et al.,* 2003). The basic relationship between EIR and malaria transmission in a given population shows that small increases in annual EIR results in large increases in *P. falciparum* malaria prevalence (Fig. 2). The dependence of prevalence on EIR is such that any detectable EIR is associated with prevalence rates of *P. falciparum* malaria large enough to seriously impact public health. Many reports suggest that there are sites in Africa where high prevalence rates are associated with extremely low or hardly detectable EIR. For example, on the Kenyan coast, a site with an annual EIR of 0.001ib/p/yr and another site where no infected mosquitoes were caught had *P. falciparum* malaria prevalence rates of 44.7% and 49.3%, respectively (Mbogo *et al.,* 1995; Beier *et al.,* 1999). These reports also cited other older and recent findings that collectively and consistently point to the fact that substantial reductions in transmission intensity are necessary to reduce the prevalence of malaria infection in human populations (Boyd, 1949; Macdonald *et al.,* 1957; Najera, 1989; Oaks *et al.,* 1991; Lines, 1996; Molineaux, 1997; Lengeler *et al.,* 1998; Lengeler, 1998). In some holoendemic areas, a 95% reduction in EIRs or more is required to record any decreases in the prevalence of *P. falciparum* infection (Farid, 1980; Molineaux and Gramiccia, 1980; Modiano, *et al.,* 1998).

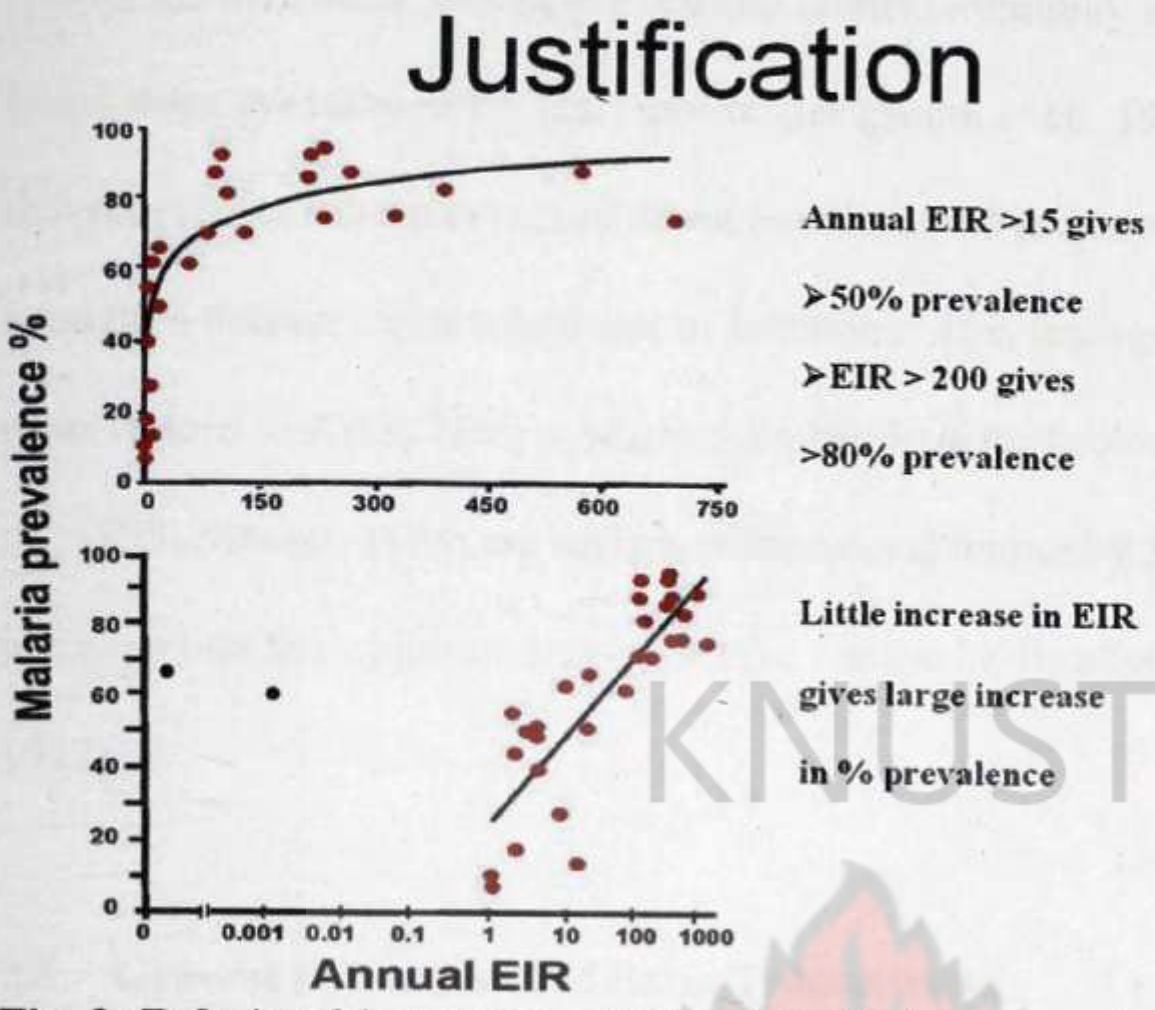


Fig. 2: Relationship between EIR and *P. falciparum* malaria prevalence in Africa. (Beier *et al.*, 1999)

Therefore, vector control measures which have historically had the biggest impact on malaria control remained the most effective means to prevent malaria transmission (Coluzzi *et al.*, 2002; WHO, 2005). Accurate information on the distribution of EIR on the ground permits interventions to be targeted towards the foci of transmission. Such targeting greatly increases the effectiveness of control measures. On the other hand, the inadvertent exclusion of these locations of high transmission causes potentially effective control measures to fail (Carter *et al.*, 2000). However, whereas some schools of thought have suggested that reducing transmission may counter-intuitively lead to greater mortality due to loss of immunity (Snow *et al.*, 1996; 1997; 1998), others think there is no direct evidence from longitudinal studies to support this assertion (Greenwood, 1997; Molineaux, 1997). Loss of immunity due to decreasing transmission intensity may not be of clinical concern until the point where control measures are achieving significant

reductions in malaria prevalence because clinical immunity is determined by exposure to blood stage parasites rather than sporozoites (Smith *et al.*, 1998). Fig. 2 further shows that prevalence rates can exceed 40–60% even at the lowest observed EIRs, which is enough to sustain the development of immunity. This is supported by data from direct observations in Kilifi, Kenya, where even barely detecTable low-level EIRs (Mbogo *et al.*, 1993; Mbogo, 1995) are sufficient for natural immunity in children to develop early in life so that few children develop severe disease or die after the age of 6 years (Marsh, 1992).

2.5 General Dynamics of Malaria Transmission

Dynamics of malaria transmission refers to the changing patterns in the transmission of malaria in a population over time (Smith and McKenzie 2004); consequently the dynamics has an impact on malaria epidemiology and control (CDC, 2004). Malaria transmission has been constantly reported to be variable; seasonal in some places, perennial in others and there is even a difference in transmission intensity between and even within places (Greenwood, 1989; Appawu *et al.*, 2004). Thus the intensity of malaria transmission, infection and morbidity within one village may be substantially different from neighbouring villages (Thomas and Lindsay, 2000; Clarke *et al.*, 2002). This observed variability in malaria transmission from one location to another is usually determined by a combination of both intrinsic and extrinsic factors. The intrinsic factors include, 1) the genetics and physiology of the different vector species, 2) the anti-parasite defence systems of host and vector, 3) the immune status of the local human population whereas the extrinsic factors include, 1) biting and resting behaviour of vector mosquitoes, 2) spatial heterogeneity of vector habitats and 3) the mosquito-control

strategies employed by the local communities. These factors determine the competence of the malaria vectors which in turn influence the variability. For instance, the important roles of *A. gambiae s.s.* and *A. arabiensis* as vectors of malaria in Africa, are due to the high competence of these mosquito species in the uptake, development and transmission of *P. falciparum*. Thus, the extent of malaria distribution depends on whether, a) temperatures are high or low or b), rainfall is adequate, inadequate or even absent with a consequent bearing on the level of humidity as these affect the development of both parasite and vector and therefore vectorial competence. These factors can create areas with *Anopheles* mosquitoes without malaria; for instance, unusually high temperatures in some areas may not allow for development of the *Plasmodium* parasite within the mosquitoes (Lindsay *et al.* 1991). Similarly, lack of rain that leads to the drying up of vector breeding sites will lead to the lack of vector mosquitoes to carry the malaria parasite (Shililu *et al.*, 2003). According to Takken and Lindsay, (2007), under harsh or unusual climatic and ecological events such as unexpected drought, mortality rate of the vectors may be very high, causing a sudden reduction in malaria morbidity and mortality.

Spatial and temporal variations in vector distribution, vector population dynamics and behaviour also affect the distribution of malaria. For example, in tropical Africa, it is not the presence of an infectious reservoir in humans that is the greatest contributing factor to the highly endemic status of malaria but rather the ubiquitous nature of the *A. gambiae s.l* which is known to have extremely high vectorial competence, focused anthropophagy and relatively long survival rate (Takken ana Lindsay, 2007). Other factors such as the cultivation methods used in farming also determine the distribution of the vector and therefore malaria in time and space. Thus if there are overlaps between several

agricultural cycles (e.g. caused by irrigation) and deforestation, malaria vectors breed throughout the year due to creation and sustenance of breeding sites (Manga *et al.*, 1995; Koudou *et al.*, 2005; Yasuoka and Levins, 2007). Therefore given its high anthropophily, favourable all year breeding grounds provision; together with the precarious living conditions such as lack of mosquito-proof screened windows or doors, open eaves and the low percentage of people sleeping under ITNs, the malaria vector is able to access and transmit the disease to man all year round (Lengeler, 2004; Koudou *et al.*, 2005).

2.5.1 Dynamics of Malaria Transmission in the Tropics other than Africa

There is uniqueness in the transmission of malaria in different parts of the world.

a) Latin America

Gil *et al.* (2003), working in the Brazilian Amazonia, reported that determination of hourly biting rates disclosed seasonal variations in two distinct endemic areas; Urupá and Portuchuelo. In Portuchuelo, mosquito density was found to peak at the height of the rainy season, whereas at Urupá it peaked in the dry season. The increase in mosquito density and peak incidence of malaria cases were coincident in both cases. Similarly in north-western Argentina, malaria transmission is seasonal, starting in October or November in Salta and Jujuy Provinces, in November or December in Tucumán, Santiago del Estero and Catamarca Provinces, and in December and January in La Rioja and Córdoba Provinces. These periods, however, get delayed if the previous winter had been very cold or the spring was intensely rainy. The transmission season could last until May or June for the whole area, longer during mild winters. In El Oculto there was no marked seasonality in vector abundance while at Aguas Blancas, high abundance was detected at the end of spring and the beginning of summer. In Aguas Blancas again,

peaks of mosquito abundance preceded peaks of malaria cases by three months (Dantur Juri *et al.,* 2009). The main malaria vector was *A. pseudopunctipennis.* In these regions, *P. falciparum*, *P. vivax* and *P. malariae* are aetiological agents of malaria. The years with normal rainfall caused high malaria morbidity. In contrast, very rainy or very dry years caused a lower morbidity. In the former, flowing streams wash away the breeding places, while during the latter the breeding sites dry up; thus both scenarios result in a decrease in adult mosquito population and hence the incidence of malaria. The fluctuation in the main vector population of *A. pseudopunctipennis* and consequent fluctuation in malaria cases also seem to be related to the mean temperature. Thus if the ambient temperature increases, the population of the species and cases of malaria will similarly increase (Dantur Juri *et al.,* 2009). Another study in the northern Peruvian Amazon showed changes in malaria incidence over time. It was, however, found that spatial patterns of cumulative malaria risk existed in the same geographic areas within communities, revealing micro-high risk areas for infection. The micro-high risk areas and spatial malaria clusters found were linked to proximity of disturbed secondary growth forest which increased both breeding sites; and as a result of human activity in these forests it facilitated the proximity and accessibility of residents to the breeding habitats and the vector (Bautista *et al.,* 2006). In Colombia, malaria transmission is variable; highest in the upper Sinú River and lowest in Cauca River regions, in Urabá, and at the Pacific Coast (WHO, 2008).

b) Asia

In San Dulakudar, a village in Sundargarh District in the state of Orissa in eastern India, malaria transmission is perennial with *P. falciparum*, accounting for greater than 80% of malaria cases. Transmission intensity varies with season with high transmission after the monsoon rains in autumn and winter, low transmission in summer, and intermediate transmission in spring. The anthropophagic mosquito *A. fluviatilis* was identified as the main vector for malaria transmission (Sharma *et al.*, 2004). Dash *et al.* (2007) observed that five other mosquito species implicated as important malaria vectors in India include *A. culicifacies, A. dirus, A. minimus, A. sundaicus* and *A. stephensi*. The following mosquitoes, *A. philippinensis-nivipes*, *A. varuna*, *A. annularis* and *A. jeyporiensis* are considered secondary yet important malaria vectors in India.

Trung *et al.* (2004), reported of a study in three villages of the hilly forested areas of Cambodia and central Vietnam, where the vector *A. dirus* type A maintained perennial malaria transmission inside the villages despite its low density while in a northern study site in the same region *A. minimus* A and C were found in all collections, but no malaria transmission was recorded.

2.5.2 Dynamics of Malaria Transmission in Africa

In Africa, where the disease is endemic in large areas of the continent, malaria transmission varies widely according to region, country and even locality; ranging from perennial to seasonal as well as high to low levels (Beier *et al.*, 1999; Owusu-Agyei *et al.*, 2009).

a) East Africa

Working in western Kenya, Beier *et al.* (1990), reported variable malaria transmission with mean sporozoite rates by dissection ranging from 2.2% to 5.4% in Kisian and from 9.9% to 13.6% in Saradidi. In Kenya, more than 90% of the infections were *P. falciparum*, either alone or mixed with *P. malariae* or *P. ovale*. Heaviest transmission from April to July coincided with the end of the long rainy season. Entomological inoculation rates (EIR) averaged 0.82 ib/p/n which approximates to a monthly infective bites of about 25 ib/p/m inside houses in Kisian and 0.65 ib/p/n or about 20 ib/p/m in Saradidi. Outdoors, EIRs averaged 0.09 ib/p/n or about 3 ib/p/m in Kisian and 0.52 ib/p/n or 16 ib/p/m in Saradidi.

Muturi *et al.* (2008), observed in central Kenya that malaria transmission was sustained by *A. arabiensis* and *A. funestus*. The sporozoite rate and EIR for *A. arabiensis* was 1.1% and 3.0 ib/p/yr. respectively, with no significant differences among villages and depicting a fairly constant transmission. Overall, 3.0% of *A. funestus* samples tested positive for *P. falciparum* sporozoites. An annual EIR of 2.21 estimated for this species in a non-irrigated village was significantly higher than 0.08 for an irrigated rice village. Thus in many parts of Africa, reports vary on the impact of irrigation on malaria transmission. Many workers, however, contend that irrigation increases malaria transmission (Appawu *et al.*, 2004).

b) West Africa.

In a study in rural area of southern forested Cameroon, Manga *et al.* (1995), reported of a permanent malaria transmission particularly near a deforested area because of the

secondary vector relay role played by *A. gambiae s.l.,* when transmission due to the primary vector *A. moucheti* was interrupted. According to the study, this situation was similar to that noticed by Carnevale *et al.* (1992), when in certain riverside villages of the Sanaga River in Southern Cameroon; *A. gambiae s.l.* augmented the transmission of the main vector *A. nili* to maintain high and perennial transmission. Bonnet *et al.* (2002), reported in the same South Cameroon, in the district of Mengang, that malaria transmission is perennial but variable, ranging from moderate to high with seasonal variations. They found that entomological inoculation rates (EIR) were estimated as varying from 17 to 175 ib/p/yr according to the village considered. They observed what was similar to the findings of Meunier *et al.*(1999), that a) a rapid increase in the anopheline population at the beginning of the short rainy season was immediately followed by a parallel increase in the EIR in both regions, b) there were several months apparently with no transmission (EIR = 0) despite constant parasite prevalence and c) increases in EIR were associated with significant increases in morbidity and high parasitaemia, especially in young children (0–10 years). In a study by Bigoga *et al.* (2007), in Tiko in coastal south-western Cameroon, it was found that malaria transmission was perennial with direct correlation between increasing intensity on one side and the prevalence of parasitaemia and increasing amount of rainfall on the other. *A. gambiae s.s.* M molecular form, *A. funestus* and *A. nili* were the main malaria vectors in this survey. They asserted in their publication that their results were different from that reported by Manga *et al.* (1993), in the rural areas of southern-forested Cameroon suggesting that rather *A. gambiae* plays a secondary role with *A. moucheti* as the major malaria vector.

A recent study in Goulmoun, a rural city in south-western Chad (Kerah-Hinzoumbé *et al.*, 2009), revealed that the level of malaria transmission was seasonal and correlates with rainfall patterns, consistent with observations in some other tropical regions. In Goulmoun, vector biting and consequently malaria transmission started soon after dusk and continued till daybreak. The peak of transmission was observed in the second half of the night. Almost all the transmission was recorded during the rainy season. With an annual EIR of 311 ib/p/yr *A. arabiensis* was the main vector, accounting for 84.5% of the EIR recorded during the full year of the study period. It was followed by *A. pharoensis* which was responsible for 12.2%, *A. funestus* for 2.5% and *A. ziemanni,* 0.8%.

In a similar study on malaria transmission dynamics in central la Côte d'Ivoire, Koudou *et al.* (2005), found levels of transmission were high at Zatta. The highest EIRs occurred concurrently with a high biting rate. On the contrary, in Tiémélékro, high transmission coincided with periods of low biting rates. Highest biting rates of *A. gambiae s.l.* occurred towards the end of the main rainy season. However, in Zatta, high biting rates of this vector were observed due to continuous mosquito breeding in irrigated rice field which made the vector to proliferate independent of rainfall pattern. The study contended that this was consistent with previous entomological investigations in the humid African savannah (Robert *et al.*, 1985; Dossou-Yovo *et al.,* 1995). In Koudou *et al.* (2005), high biting frequencies of *A. funestus* were observed between August and October; a dry period between a long and short rainy seasons. In the same vein, in Tiémélékro, high biting rates of *A. funestus* occurred during the dry season between February and April. Koudou *et al.* (2005), showed that malaria vectors had high EIRs in rural areas with irrigated rice agriculture. When rice irrigation was interrupted in Zatta in 2003, malaria

transmission was restricted to the second half of the main rainy season. The estimated EIR was very high during the period of irrigated rice cultivation, but it decreased significantly towards the end of the rice growing cycle. They observed that their findings agreed with previous observations made in Bobo Dioulasso, Burkina Faso in the West African savannah (Robert *et al.*, 1985) where irrigation enhanced malaria transmission. They further asserted that in Burundi it was found that the vectorial capacity of *A. arabiensis* (Patton) was 150 times higher in rice cultivating areas when compared to cotton plantations (Coosemans, 1985). Other surveys carried out confirmed these trends. In northern Côte d'Ivoire, intensification of shallow rice cultivations was accompanied by higher malaria incidence (De Plaen *et al.*, 2003). However, other workers have at the same time observed no impact of irrigation on malaria transmission (Dossou-Yovo, 1999; Ijumba and Lindsay, 2001; Henry *et al.*, 2003; Keiser *et al.*, 2005; Koudou *et al.*, 2005).

2.5.3 Malaria Transmission Dynamics in Ghana

Malaria transmission dynamics in Ghana is no different from other parts of Africa where results are mixed. Various researchers report on diverse forms of transmission. According to Banda *et al.* (2004), malaria transmission is hyperendemic in Ghana with perennial transmission to people of all ages throughout the country. The geo-epidemiological transmission of malaria in Ghana is classified as: a) tropical rainforest (middle belt), b) coastal lagoons/mangrove swamps (south-western part of the country), and c) coastal savanna (South eastern) and northern savannah. The principal vectors transmitting malaria parasites are the females of *A. gambiae s.l.* complex of which *A. gambiae s.s* predominates. *A. melas* is wide-spread in the swampy south-west coast and

A. arabiensis in the savannah northern part of the country. *A. funestus* is the other major vector aside those of the complex in the country. On their part, Klinkenberg *et al.* (2005), observed that malaria transmission can no longer be regarded only as a rural phenomenon because although levels of transmission in urban areas may be lower than in rural areas, high population densities in cities, irrigated vegetable farming and other practices result in nearly the same disease impact in urban settings as in the rural areas. The irrigated urban agriculture has helped to increase the risk for malaria by providing suiTable breeding sites. Klinkenberg *et al.* (2008), recently observed in Accra that EIR seemed to be on the increase in urban areas where irrigated farming took place. They further found that annual EIR, was 19.2 and 6.6 in areas practising urban agriculture and areas without agriculture respectively, showing high but varied transmission of the disease. Biting rate and therefore malaria transmission was markedly heterogeneous across the urban landscape. In northern Ghana, Appawu *et al.* (2004) reported that in the irrigated areas of the Kasena-Nankana District, mosquito vectors were in constant contact with inhabitants throughout the year because of the availability of vector breeding sites in the irrigation system even during the dry season. They also found that with an overall annual EIR of 418 ib/p/yr, malaria transmission in the Kasena-Nankana District was high and seasonal, the highest transmission occurring during the peak rains from June to October. In their report they asserted again that there were microecological variations in transmission, with values of EIRs varying from 228 ib/p/yr in areas of rocky highlands and 360 in lowland areas to 630 in irrigated areas. Generally malaria transmission occurred mostly during the wet season and fell to very low levels during the long dry season.

A similar pattern is associated with malaria prevalence and episodes in some locations in Ashanti Region of Ghana. It was found that the occurrence of early malaria parasite infections was dependent on the season. The infection prevalence was 88% higher in the rainy season than in the dry season while the diversity of parasites was significantly higher in the dry season than in the rainy season (Kobbe *et al.*, 2006). *P. falciparum* infections peaked in August (29.0%) and was lowest in February (6.4%). The highest parasite densities were observed in April and the lowest in May (Kobbe *et al.*, 2006). Working in the same localities in Ashanti, Kreuels *et al.* (2008), reported of malaria incidence being heterogeneous between villages with ecological analyses showing strong correlations with village area and population size. Their results demonstrated pronounced spatial heterogeneity of malaria incidence in a region of vegetational and altitudinal homogeneity. Varying malaria attack rates were observed in villages which were at most 18km distant from each other.

These variations and intensive malaria transmission result in diverse complications resulting in mortality, especially, in children and pregnant women in the country. For instance, recently, Ofori *et al.* (2009), found that symptomatic infections in pregnant women rose sharply from the first trimester to the last, consistent with an earlier study to assess the symptoms of severe malaria and their contribution to mortality in 290 children in northern Ghana where Mockenhaupt *et al.* (2004), found that circulatory collapse, impaired consciousness, hypoglycemia, and malnutrition independently predicted death. In response to these, therefore, some workers have explored the possibility of controlling either the malaria parasite or its vector and/or at least reducing the vector-host contact that leads to control of the disease. Thus in a study to verify the impact of intermittent

preventive treatment in pregnancy with sulphadoxine-pyrimethamine (IPTp-SPECIES) in southern Ghana, Hommerich *et al.* (2007), found that placental malaria and maternal anaemia declined substantially while birth weight increased after the implementation of IPTp-SPECIES In order to determine how far the protective efficacy of intermittent preventive treatment in infants (IPTi) in the Ashanti Region depends on variations of the prevailing incidence of malaria, Kobbe *et al.* (2007), showed that the extent of protection provided by IPTi increased almost linearly with malaria incidence. Working in the same Ashanti Region, Marks *et al.* (2005) assessed the prevalence of markers of *P. falciparum* resistance to chloroquine (CQ) and pyrimethamine-sulfadoxine (PYRSDX) where the use of these drugs is negligible. They found a high frequency of CQ, PYR, and SDX resistance markers, with the frequency being higher in 2003. Binka *et al.* (1996), on the other hand, conducted a community-based randomized, controlled trial of permethrin impregnated bed nets in a rural area of northern Ghana to assess the impact on the mortality of young children in an area of intense transmission of malaria with no history of bed net use. They reported that the use of permethrin impregnated bed nets was associated with 17% reduction in all-cause mortality in children aged 6 months to 4 years. They observed that the reduction in mortality was confined to children aged 2 years or younger, and was greater during the wet season, a period when malaria mortality is likely to be increased, than in the dry season.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study sites

A major part of the study was conducted in two small towns; Afamanaso (population: 2,508) and Kona (population: 5,853) and in two smaller hamlets close to Afamananso, in the AFigya Sekyere District (ASD), whose district capital, Agona (population 9321) is 27km from Kumasi, the regional capital (Fig. 3). The other part of the study was conducted in two peri-urban settlements which form part of the Kumasi metropolis namely Boadi and Anwomaso. All these sites located in the Ashanti Region in the forest zone of the country (Fig. 4), like most parts of Ghana are malaria holoendemic.

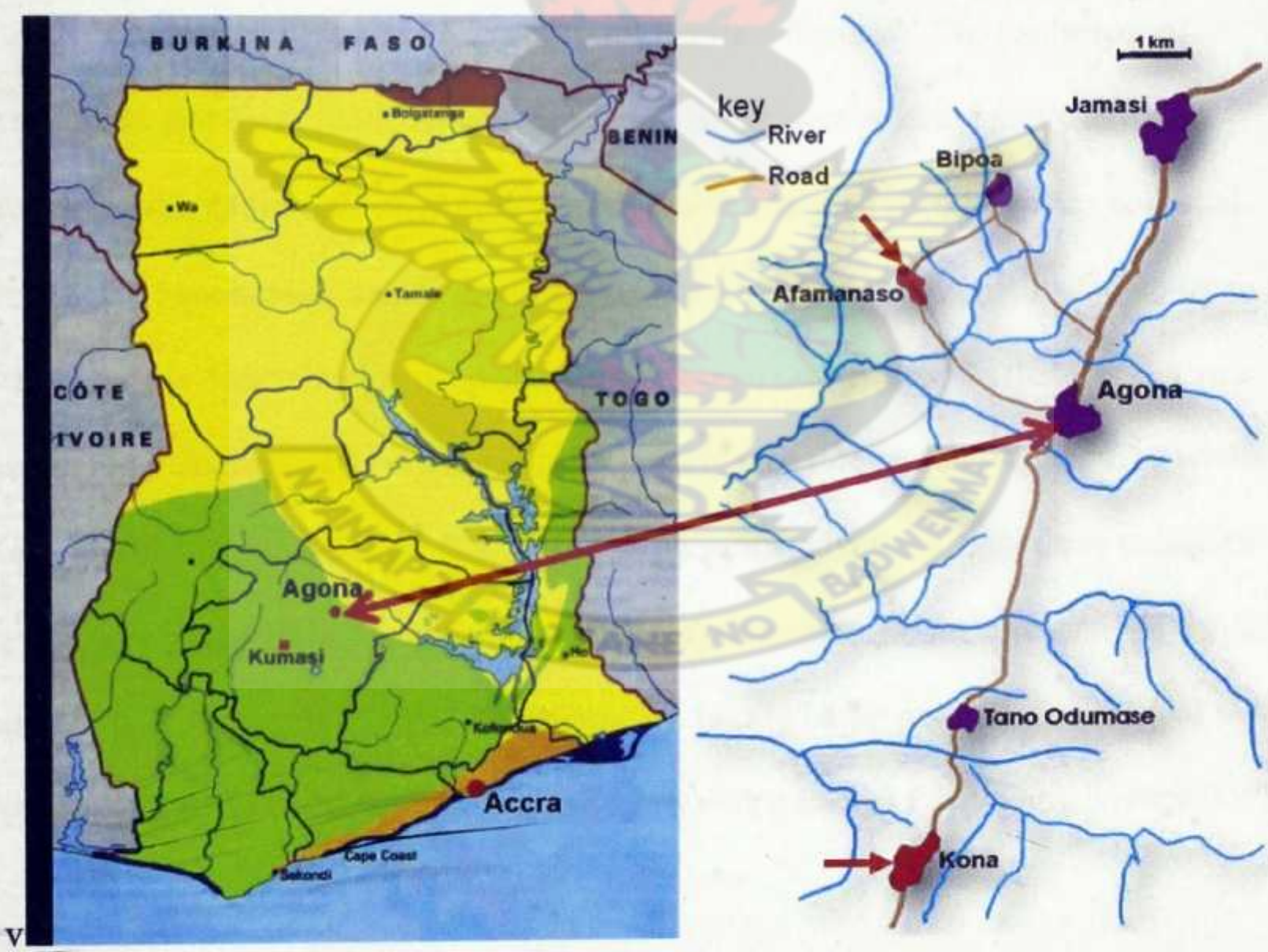


Fig. 3: Maps of Ghana (left) and study sites (right) in AFigya Sekyere District, capital Agona.

The AFigya Sekyere district covers a land area of 714 km^2 and supports a population of 131,658 (Ghana Statistical Service Report, 2000). The vegetation is mostly rain forest of which denuded parts may have large patches of grassland. There are roughly four seasons in the year, and for this study, each was estimated to last for four months, with August added to the minor rainy season. The seasons include the major rains of April-July, minor rains of August –November and the dry spell of December to February. The River Offin meanders through the district, providing pools of water and flooded marshy areas in the rainy seasons. A forest reserve, the Aboma forest reserve, transects the district from Mampong to Agona. Average monthly rainfall during the two year study period (2004-2005) was 258 mm, with the highest of 435 mm in November 2004 and the lowest of 13 mm in January 2005 (Ghana Meteorological Service, Kumasi). The two towns of Afamanaso and Kona in the AFigya Sekyere District were selected purposely after studying data obtained from an earlier work which analysed blood samples collected from 3 to 15-month-old babies from many communities in the district including these two towns, for the determination of malaria parasitaemia prevalence. In that work, it was realised that Afamanaso had a crude malaria incidence rate ratios (IRR) of 1.20 while Kona had 0.56 (Kreuels *et al.*, 2008). In this present study, there were three mosquito-catching points in Kona and nine in Afamanaso (three sites for malaria analysis and six outdoor sites for comparison of *A. gambiae s.l.* hourly biting pattern). Positions of town centres and vector collection points were taken with a Global Positioning System (GPS).

3.1.1 Afamanaso (06° 56' 45"N, 1° 30' 30"W)

Afamanaso is a rural settlement with a population estimate of 2,508 people by the 2000 census (Ghana Statistical Service Report, 2000) consisting mainly of mixed and cash food crop farmers of cocoa, plantain, maize, fruits, etc. Some inhabitants indulge in animal husbandry, rearing poultry and livestock such as goats, sheep, etc. The settlement comprises a cluster of houses concentrated along 2 main roads, the intersection of the roads being the town centre.

Afamanaso is built on a highland surrounded by lowlands of farms with rivulets and streams whose tributaries flow into River Offin. During the rainy season, these water bodies overflow their banks leading to the creation of marshy areas as well as temporary pools of water that serve as putative breeding sites for mosquitoes (Plate G). Normally each homestead consists of a central quadrangle enclosed by adjoining rooms providing accommodation on one side and storage and service areas on the other. The accommodation may occupy two sides, the kitchen, bath and toilet, the other and the fourth side provides a roofed entrance and an open patio which is a rest/meeting area. All homes have their private bath facilities but not all of them have private toilets. Those without their own toilets use communal ones at the ends of the town. However, not all the homes have this complete picture; in one of our collection sites at Afamananso, which was a developing homestead, the quadrangle was not complete (Plate H). There was a row of sleeping rooms at one end faced by the service area of kitchen and bath at the other. In time the owner would complete the quadrangle. All the houses were roofed with corrugated iron sheets but not all of them had ceiling; particularly so with those in which the quadrangle was not completed.

Plate G: Prospecting for larvae and pupae in temporary pools of water which serve as putative breeding sites for mosquitoes at Afamanaso.

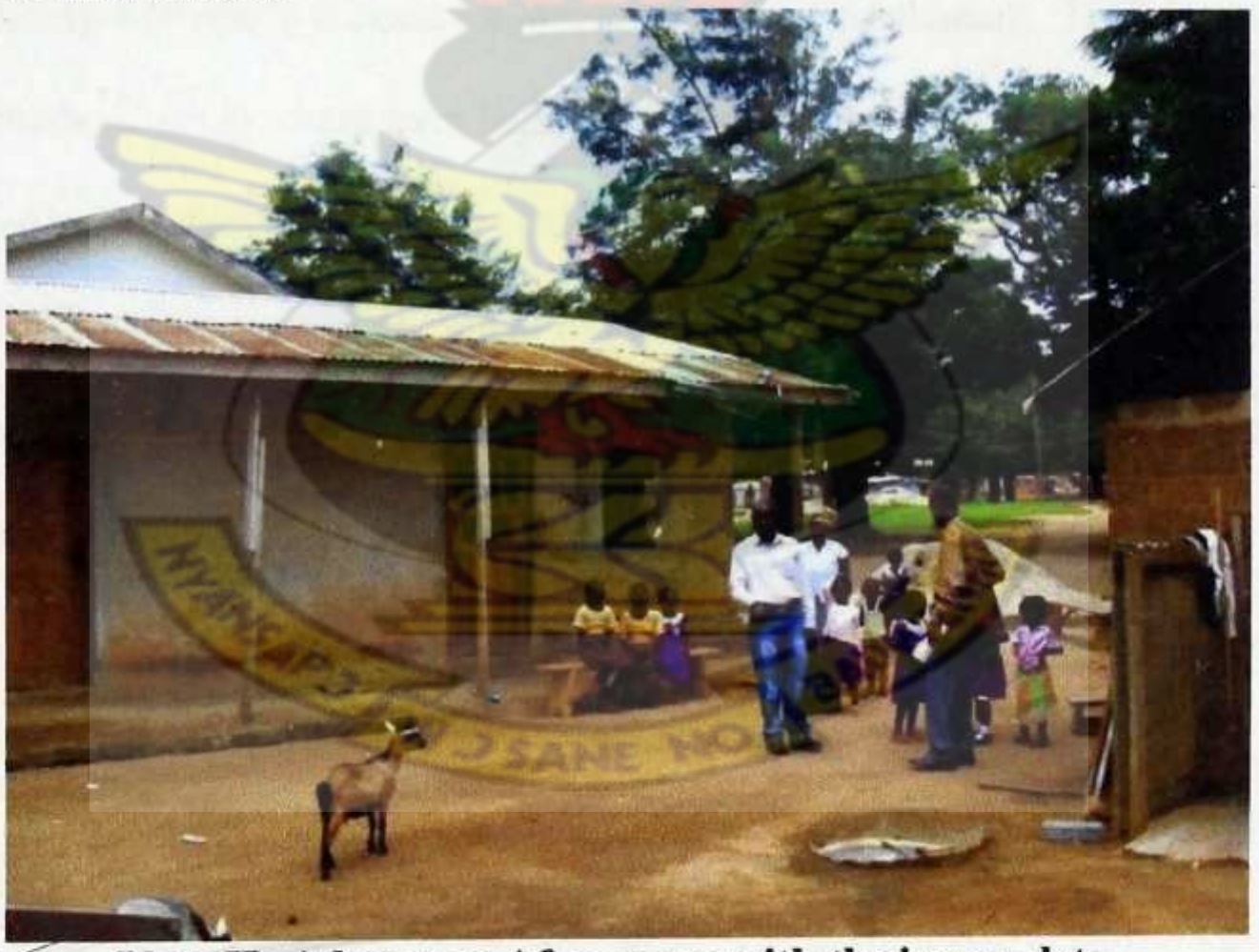


Plate H: A house at Afamanaso with the incomplete quadrangle where mosquitoes were caught.

The residents did not seem to worry too much about exposure to mosquito bites as majority of houses were not screened with mosquito-proof nets on doors and windows

and the few individuals who used bed-nets did not treat them with any insecticide at the time of the study. Indoor vector collections were done in a room in an incomplete quadrangle house (plate H) and in a classroom of a school block. In all such indoor collections, windows and doors were left open throughout the 12 hour collection cycle. Outdoor mosquito catches were done in the courtyard of a completed house with a quadrangle. Mosquitoes were also caught outdoor only from Afamanaso town centre as well as two farming hamlets: Afamanaso and Morso hamlets, to compare the *A. gambiae s.l.* hourly biting activities at different locations of the Afamanaso town.

3.1.2 Afamanaso hamlet (06° 57' 00"N, 01° 30' 842"W)

This is a farming settlement located a kilometre away, of Afamanaso. The houses were temporary sheds (Plate I) where farm hands lived, though new buildings were being put up at the time of the study. The farmers cultivated cocoa, cassava, plantain, maize, etc. (Plates J and K). Mosquitoes were caught outdoor only from two points purposely to compare the biting activity of *A. gambiae s.l.* at this cottage with those in the other parts of Afamanaso where mosquitoes were caught.

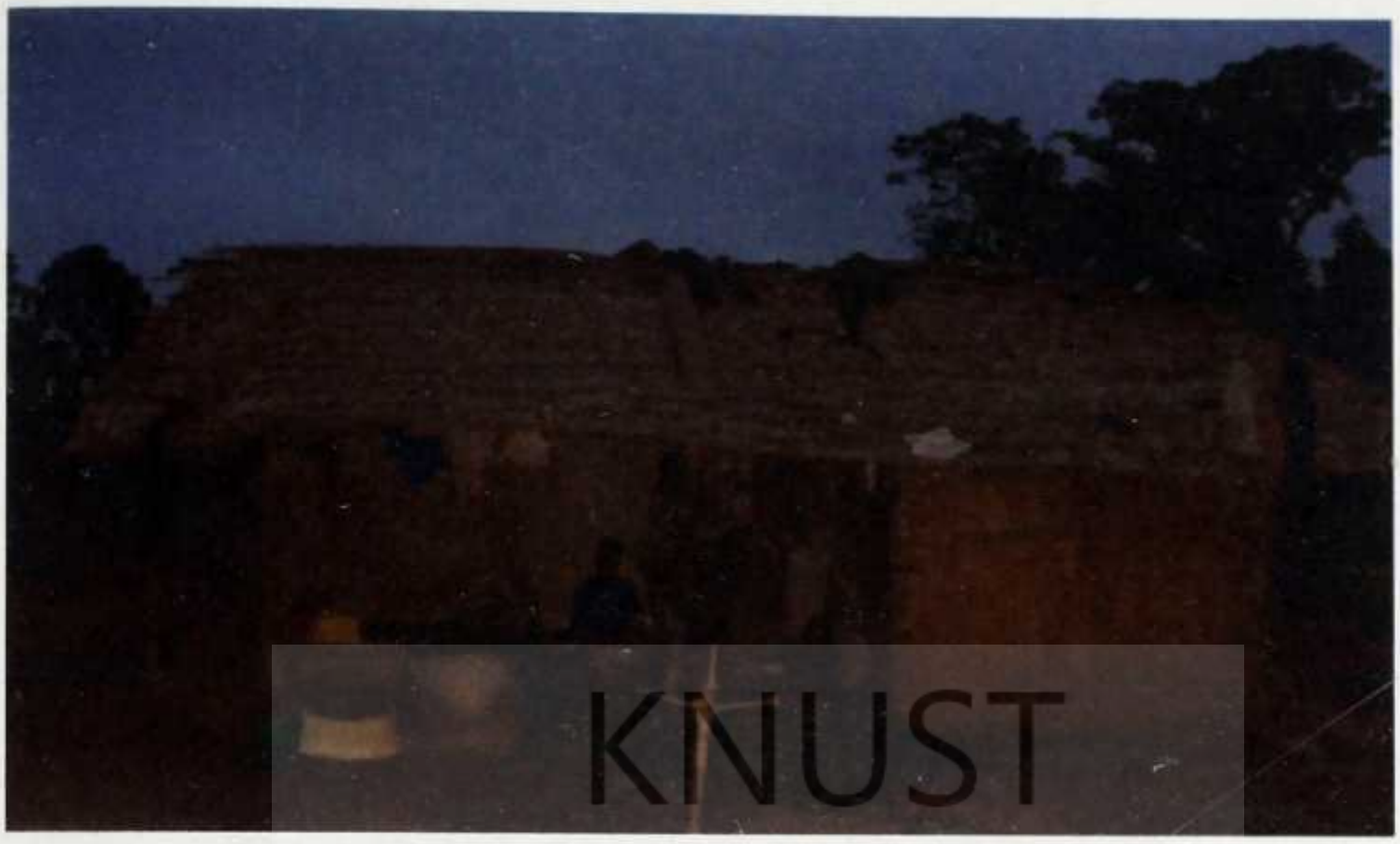


Plate I: Temporary structures that housed farm-hands at both Afamanaso and Morso hamlets.

3.1.3 Morso hamlet (06° 56' 925"N, 01° 31' 193"W)

This is a cocoa farming settlement similar to the Afamanaso Cottage but located about one kilometre further west of the Afamanaso Cottage and about two kilometres from Afamanaso town. The houses were temporary structures (Plate I) with no sign of permanent houses being put up. Cash crops such as cocoa and oranges were grown. Food crops such as plantain, cassava and maize were also cultivated. Mosquitoes were also caught from two points for comparing the biting activity of *A. gambiae s.l.* at the cottage with those obtained for the other parts of Afamanaso.

3.1.4 Kona (6° 52' 25"N, 1° 30' 34"W)

Kona is a small town about twice the size of Afamanaso with a population of 5,853 according to the 2000 census (Ghana Statistical Service Report, 2000). The occupation of the people is similar to those of the peuple Afamanaso, being mainly engaged in farming

cocoa, plantain, maize, fruits, etc. However, unlike Afamanaso, some of the citizens earn their living by trading and working in a number of cottage industries including weaving (Plate L).

Plate J: A farmer at Kona drying his cocoa beans in the foreground. At the top right corner is plantain farm, and top left is a section reflecting typical houses.

Plate K: A section of cassava and oil palm plantation at Afamanaso

Plate L: A citizen of Kona in his weaving shop. A number of weavers work in this shop.

The town, like Afamanaso, is surrounded by low-lying farmlands permeated by tributaries of the Offin River. Kona, however, is built along a first class asphalted major road linking Kumasi to Tamale through Yeji via the Mampong scarp. The structures of the house-holds are similar to those of Afamanaso (Plates F and H). Mosquitoes were caught indoors in a completely sheltered classroom whose doors opened into a narrow corridor. The second indoor collection was done in one room in a complete house with a quadrangle. Outdoor collection was done under an open shed which served as a classroom during the day for a local public school. The shed had a concrete floor, but no walls. No other mosquito collection was done here to compare the biting activities of *A. gambiae s.l.*

3.1.5 Boadi (N 6° 41' 00"; W 1° 32' 00")

The study was conducted on the KNUST's Cattle Research Farm located at the south-east of the University. The population of Boadi; a peri-urban settlement of Kumasi (Fig. 4), at the time of the study was 3196. The Boadi settlement is built on level ground with typical traditional houses as described for Kona and Afamanaso. A larger and new site has modern screened buildings springing up, eclipsing the otherwise smaller, typical Asante settlement (found in the old site) in what used to be a small idyllic village. The most common activity in the town is trading. Remaining farmlands that have not yet been sold as building plots are a few kilometres outside the town where food crops such as plantain, cassava, maize, etc. are grown.

Fig. 4: Map of Boadi Cattle Farm (6o 41' N; 1o 32' W) showing locations of experimental enclosures A to D constructed along tributaries of the Subin River and Boadi town. Pens were separated from each other by a distance of about 500m.

On the Cattle Farm where the mosquito collection was done, workers come to the farm during the day and close in the evening, leaving a few security personnel to take care of the place. The rest of the farm is made up of large pastoral plots for cattle grazing and scrubland. On the farm, mosquitoes were collected twice a week for four weeks over two consecutive years covering the transition between the end of the rainy season (October) and the beginning of the dry season (November). Two mosquito collectors worked at each of the four experimental pens during the same night, one always collected inside the pen and the second one about 20 m outside. The four experimental pens A to D (Fig. 4) constructed for the study, measured 6 m x 7 m; had concrete floors, were half-roofed with corrugated iron sheets and fenced by a 1 metre high wire-mesh (Plate M).

Plate M: The enclosure (Pen) constructed for mosquito collection at Boadi

During the work on the cattle farm, in order to assess the effect of a deltamethrin-treated nets on numbers of mosquitoes entering the enclosure and landing on the collectors, one of the four pens was permanently protected by the treated net (Plate M) which was fixed

permanently on the wire mesh in pen D whereas the other three A, B and C had none. However, to confirm whether the reduced number of mosquitoes collected at pen D during this first part was mainly due to the impact of the ITN and not differences in prevailing conditions in the four locations, in the second half of the study, the ITN was rotated from one pen to the next after each mosquito collection.

3.1.6 Anwomaso (06° 41' 63"N 01° 31' 74"W)

Anwomaso is as settlement adjacent to the Cattle Farm, about a kilometre away to the North East (Fig. 4). Like Boadi town, it is built on a level ground with modern screened buildings springing up eclipsing the otherwise smaller typical Asante settlement. Though the most common activity in the town is trading, a good number of the 5410 citizens (Census, 2000) here are food crop farmers. To compare the biting activity of *A. gambiae s.l.* on the Cattle Farm with its biting activity in this town, mosquitoes were collected from two sites in the town and three sites on a farm (E in Fig. 4) about a kilometre out of the town. Collections were done outdoor in both sites in the town, in the courtyards of walled bungalows to the west of the town and another to the east, respectively. On the farm, one of the mosquito-catching points was located near the entrance to the farm; the second was near a fish pond while the last was about 100m from the entrance into the farm.

3.2 Data Recording Forms

Field forms were designed for recording data on the number of mosquitoes collected in each hour, hourly temperature, and weather variables such as rainfall and wind. These were sent to the field on each collection day. Dissecting forms were designed for

recording data on mosquito species, infection status, hour of collection, parity, etc. during dissection.

3.3 Mosquito Collection

Mosquitoes were collected over a twelve- hour cycle, starting from 6 pm and ending at 6 am, both indoors and outdoors. The frequency of collection depended on what it was needed for. In Kona and Afamanaso, there were a regular twice- a- month collection over a period of 21 months from December 2003 to August 2005 at Kona and 18 months from March 2004 to August 2005 at Afamanaso. The Collections in the centre of Afamanaso town as well as the hamlets of Afamanaso and Morso were done once in each case in October 2007, for the purpose of comparing biting patterns. In Boadi, collections were done twice a month for two (2) months in October and November 2005 and then weekly for a month in October/November 2006. In the Anwomaso settlement, collections were done twice a week for a month in October/November 2006 whereas in the Anwomaso farm, three collectors were put at different locations to make a one-off twelve- hour overnight catch in October 2007.

3.3.1 Method of Mosquito Collection

Mosquito collections were done by human-landing catches in which a trained collector or I was trained to sit still on a stool and expose the lower limbs. Using a flash light in one hand, a periodic beam was scanned over the exposed feet and legs for landing mosquitoes. Whenever a mosquito was found on the skin or perhaps a bite was felt, the individual kept still and gently focused the beam of the flash light on the mosquito. Using the opened end of a plastic collection tube, the mosquito was trapped and the tube quickly

closed as the mosquito flew invariably to the base. The captured mosquito in the tube was placed in an appropriately labelled hourly plastic bag. For each collector, there were 12 of such bags appropriately labelled for the overnight work (Plate N).

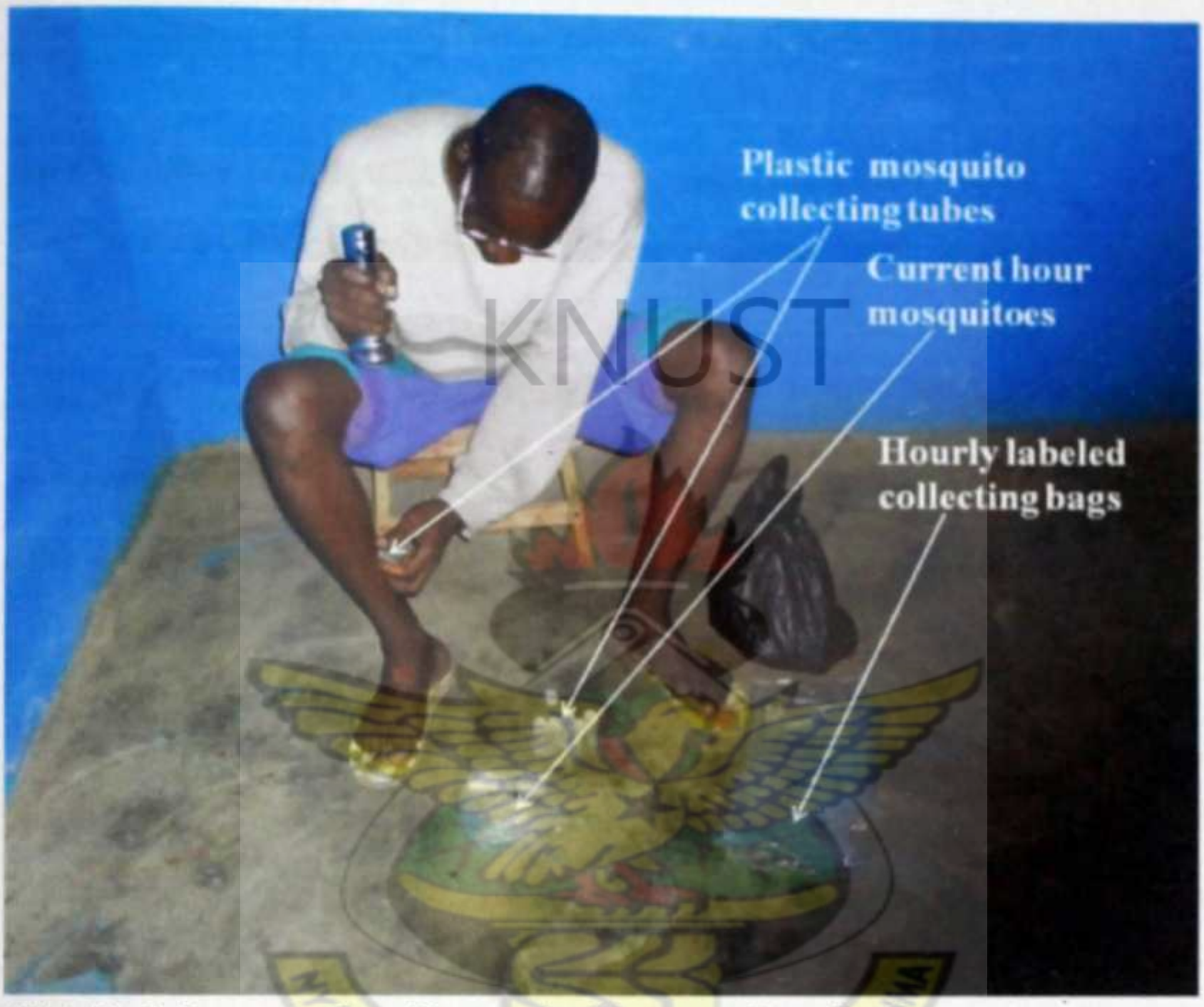


Plate N: A demonstration of human-landing mosquito collection.

At the end of each hour's vigil the plastic bag containing mosquitoes caught within that hour was tied and placed in a cooling box with ice draped over with towels to provide the needed humidity and temperature to keep the mosquitoes alive for dissection. Two mosquito collectors worked at each site during the same night to allow them to take turns to rest. In that regard one collected from 6.00 p.m. to midnight, the second from midnight to 6.00 am the following morning. Collectors were rotated from site to site to compensate

for differences in their respective attraction to mosquitoes. The vector collectors were put on prophylaxis and were promised to receive prompt and immediate access to treatment in case of any infection. Weather variables such as temperature and rainfall were recorded on field forms during the period of collection at the different sites.

3.4 Laboratory Investigations

All laboratory investigations were carried out at the Kumasi Centre for Collaborative Research in Tropical Medicine - KCCR (a set up between KNUST and Bernhard Nocht Institute for Tropical Medicine (BNITM) in Hamburg Germany. Further analyses were done using Real-time PCR and the fluorescence resonance energy transfer technique (FRET) in the laboratories of BNITM.

At the end of each collection cycle mosquitoes caught were immediately brought to the laboratory where they were sorted into genera of *Anopheles*, *Culex* and *Aedes* by direct visual observation. All the *Anopheles* species were further sub-sorted into *A.gambiae s.l.* and *A. funestus,* using the keys of Gillies and Coetzee (1987), stored in the refrigerator at about 4°C, and were dissected in turns for sporozoites of *Plasmodium* species and consequently parity. During dissection, certain body parts, e.g. head/thorax, were stored in phosphately-buffered saline (PBS) solution in specific wells of micro-titre plates and later used in ELISA (Enzyme-Linked Immunio-sorbent Assay) to identify the presence of *Plasmodium* species Other parts such as leg and wing, were similarly stored dry in wells corresponding to those containing the head/thorax parts of the mosquito; e.g. well A1 of one plate would contain the head/thorax parts of a mosquito whose leg and wing parts are stored in well A1 of another plate, both plates were labelled appropriately to contain

mosquitoes of a particular collection. The leg and wing parts were later used in PCR to identify the proportion of the sibling species of *A. gambiae s.l.* that was *A. gambiae s.s.* Positive salivary gland homogenate solutions were afterwards used in Real-time PCR to identify the proportion of *Plasmodium* species that was *P. falciparum*. Fig. 5 is a schematic representation of the processes that the caught *Anopheles* species were taken through in the laboratory for the purposes of identifying the infective mosquitoes, parity, *A. gambiae s.l.* sibling species *Plasmosdium* species and the kdr gene.

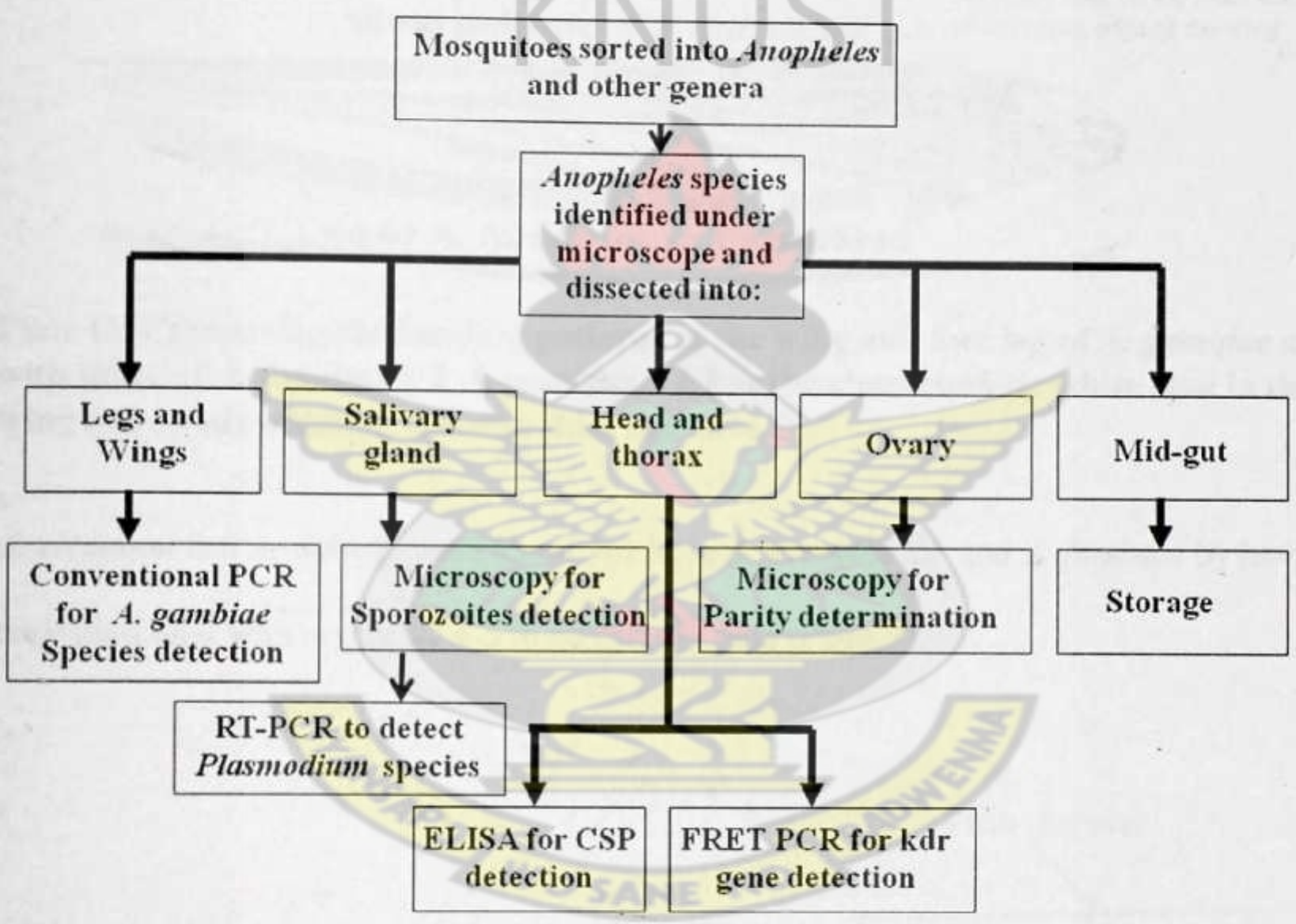


Fig. 5: Schematic diagram of the processes the collected *Anopheles* species were taken through in the laboratory.

3.4.1 Identification of the *Anopheles* Mosquitoes

Using a dissecting microscope and the keys of Gillies and Coetzee (1987), Anopheles mosquitoes were identified to species level. The presence of a white band on the inner vein of the upper edge of the wing of *A. gambiae s.l.* together with its speckled legs

differentiated it from *A. funestus* which has dark smooth legs and no interruption at equivalent spot on the inner vein of the upper edge of its wing (Plate O).

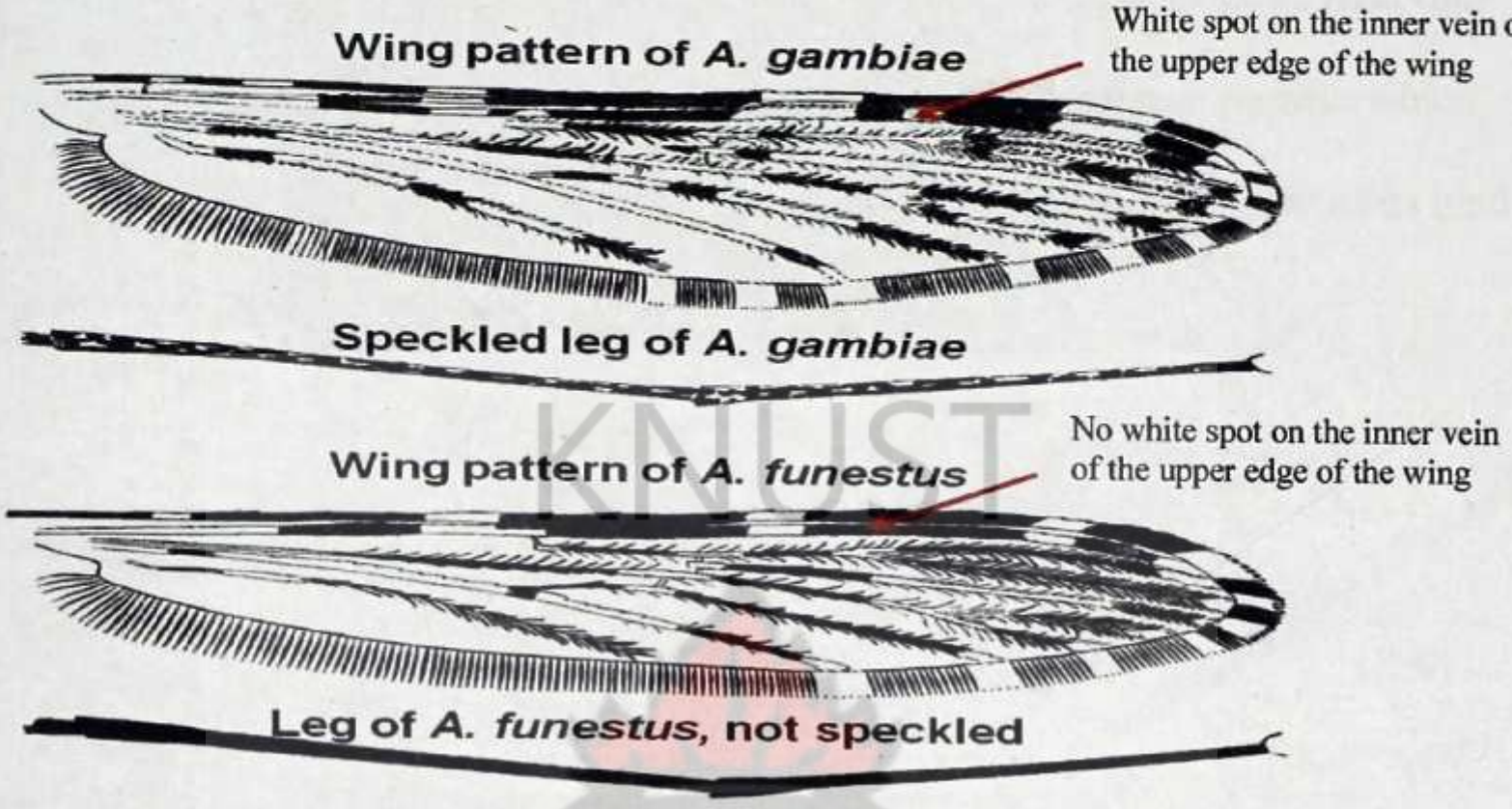


Plate O: Comparing the banding patterns of the wing and fore leg of *A. gambiae s. l.* with those of *A. funestus s. l. A. gambiae s. l.* has the characteristic white spot in the wing (arrowed) which *A. funestus* does not have

A. ziemanni and *A. paludis* were identified from *A. gambiae s.l.* and *A. funestus* by having their hind tarsi with segments 4-5 to be entirely pale (Plate P).

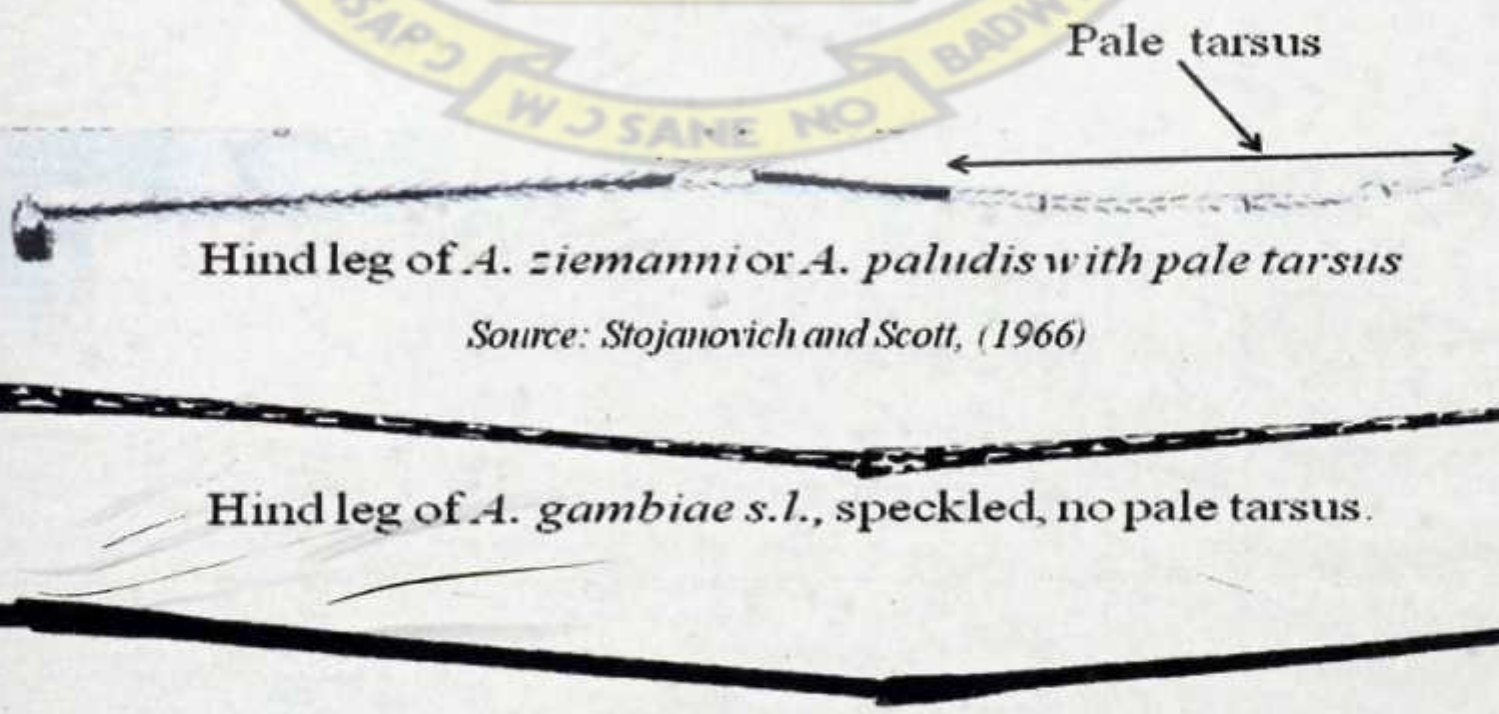


Plate P: Differences between the *Anopheles* species caught during the study.

Then *A. ziemanni* was differentiated from the other member of the *A. coustani* group that were caught on the cattle farm, *A. paludis,* by the presence on the apex of its hind tibia and base of its hind tarsal segment l, of a broad pale band as against *A. paludis* which does not have such broad pale band on the apex of its hind tibia and the base of its hind tarsal segment l (Plate Q) (Stojanovich and Scott, 1966).

A. ziemanni *A. paludis*

Source: Stojanovich and Scott, (1966).

Plate Q: Difference between *A. ziemanni* and *A. paludis* caught during the study.

After this preliminary identification, the legs and wings were used later in conventional PCR to identify the sibling species of the *A. gambiae s.l* complex.

Large numbers of *Culex* species were caught from all the sites (Plate C). These were, however, not identified to species level and were discarded. They were counted and their numbers were used to determine their biting patterns.

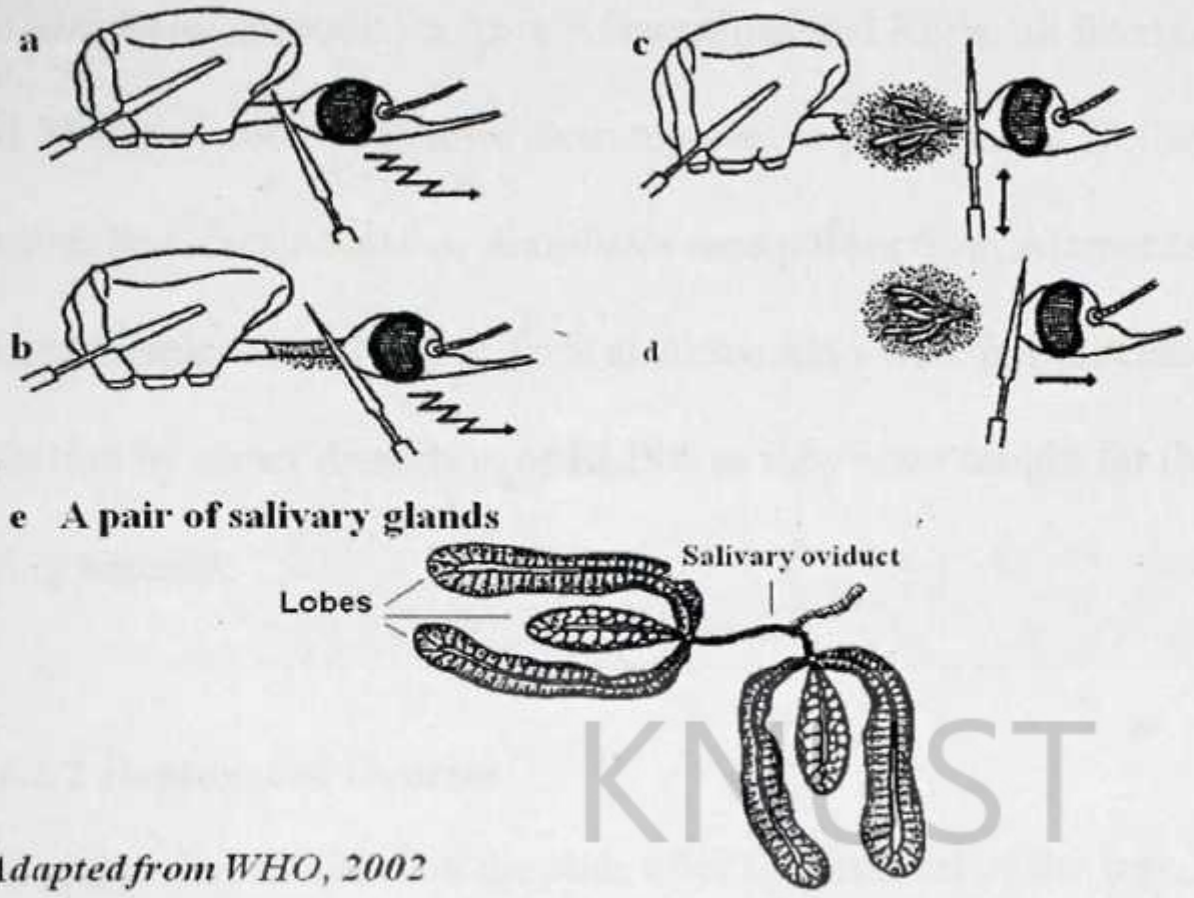
3.4.2 Dissecting *Anopheles* Mosquitoes

The equipment used to dissect mosquitoes include dissecting and compound microscopes as well as dissecting tools consisting of needles, fine forceps, slides, cover slips, micro-titre plates and two droppers, one each for distilled water and 10% PBS (phosphately buffered saline) respectively. To reduce denaturation of both mosquito and *Plasmodium*

proteins, the plastic tubes containing the mosquitoes being dissected are immersed in dry ice pellets contained in a trough. Data on the *Anopheles* mosquitoes being dissected were recorded on forms designed for the purpose.

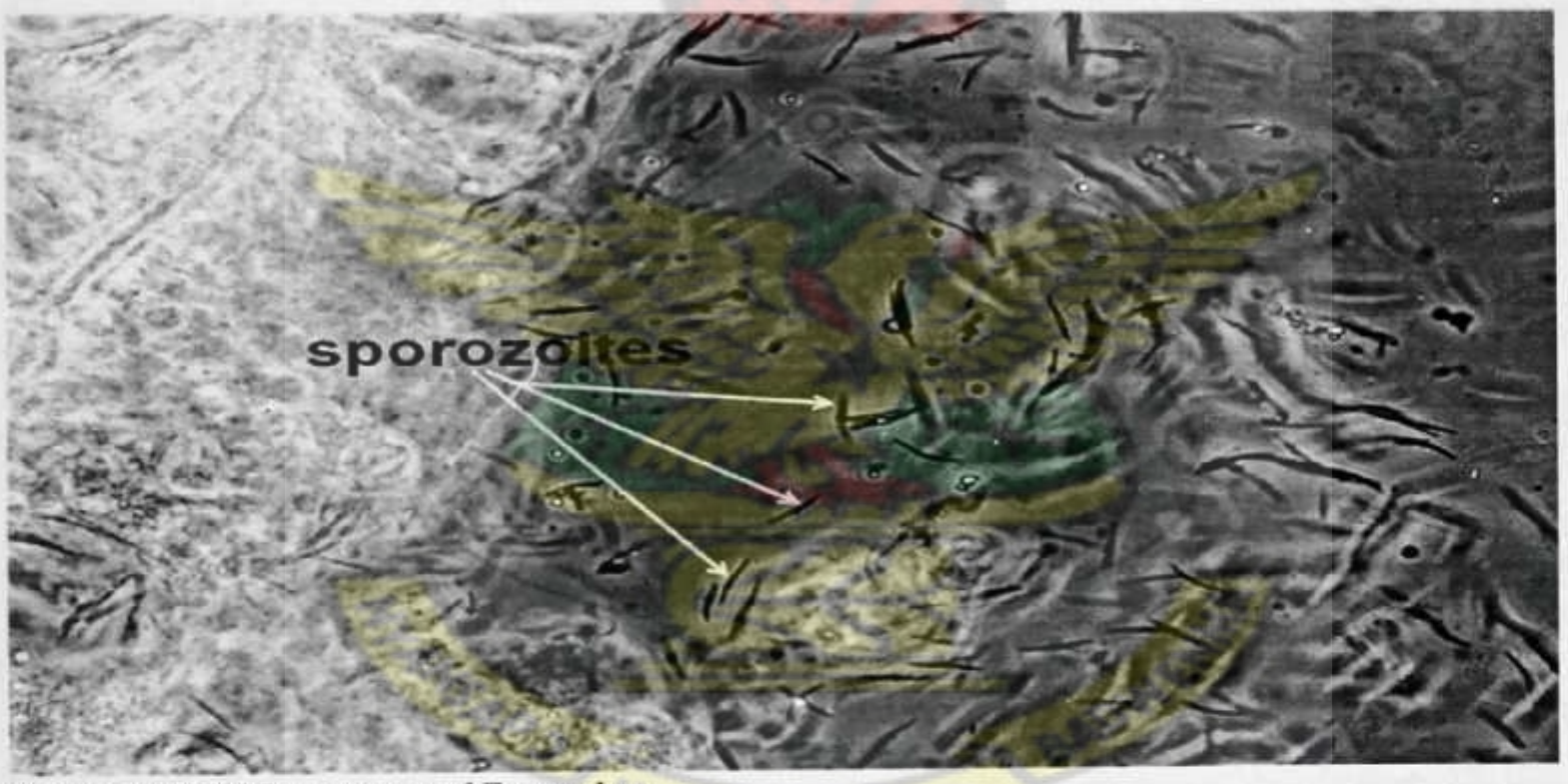
3.4.2.1 Removal and Observation of Salivary Glands

Placing an *Anopheles* species mosquito clipped off its wings and legs on its side with the head pointing to the right (Figure 6 a-d), a small drop of the PBS was put close to the front of the thorax. Holding the thorax firmly with a dissecting needle in one hand, the head of the mosquito was gently pulled away from the thorax with another pin on the other hand. In the process, the salivary glands came out of the thorax, attached to the head. In a few cases where the glands failed to come out, they were obtained by gently pressing the thorax with the pin. The salivary glands (Figure 6 e) were isolated into a smaller drop of PBS. With a cover slip placed over them, they were crushed by gently pressing the cover slip with the tip of a forceps to release the sporozoites which were seen moving under high-power (x40 objective) as slender spindle-shaped organisms similar to that shown in Plate R.



Adapted from WHO, 2002

Figure 6: a-d are the steps involved in removing the salivary glands of a mosquito; e is a pair of salivary glands (WHO, 2002).



Source: BNITM Department of Entomology.

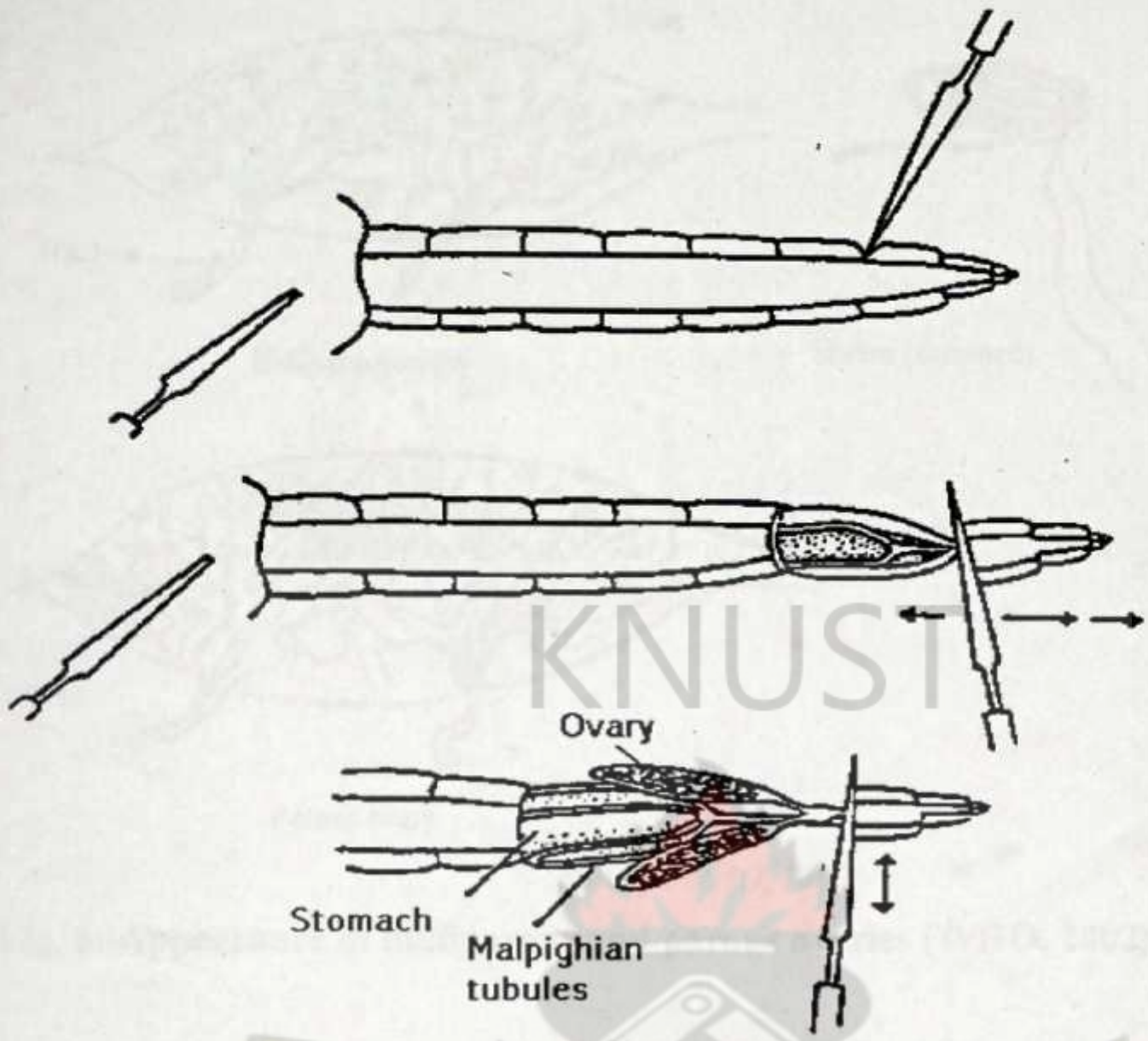
Plate R: An infected salivary gland of *A. gambiae s.l.* showing sporozoites.

Salivary glands of 2858 mosquitoes, about 62% of the *A. gambiae s.l. and A. funestus* caught at Afamananso and Kona as well as all the *A. ziemanni* collected in the second year from Boadi were dissected and observed under microscope for infective sporozoites (WHO, 2002). Notwithstanding the result of salivary gland dissection, all (except 2) of

the *Anopheles* mosquitoes from Afamananso and Kona, all from the cattle farm at Boadi, and 71 out of 266 from Anwomaso town were processed in ELISA to determine their sporozoite-infection status. *Anopheles* mosquitoes from Afamanaso centre and Hamlet, Morso Hamlet as well as the farm at Anwomaso were not processed for sporozoite infection by either dissection or ELISA as they were caught for the purpose of assessing biting patterns.

3.4.2.2 Removal of Ovaries

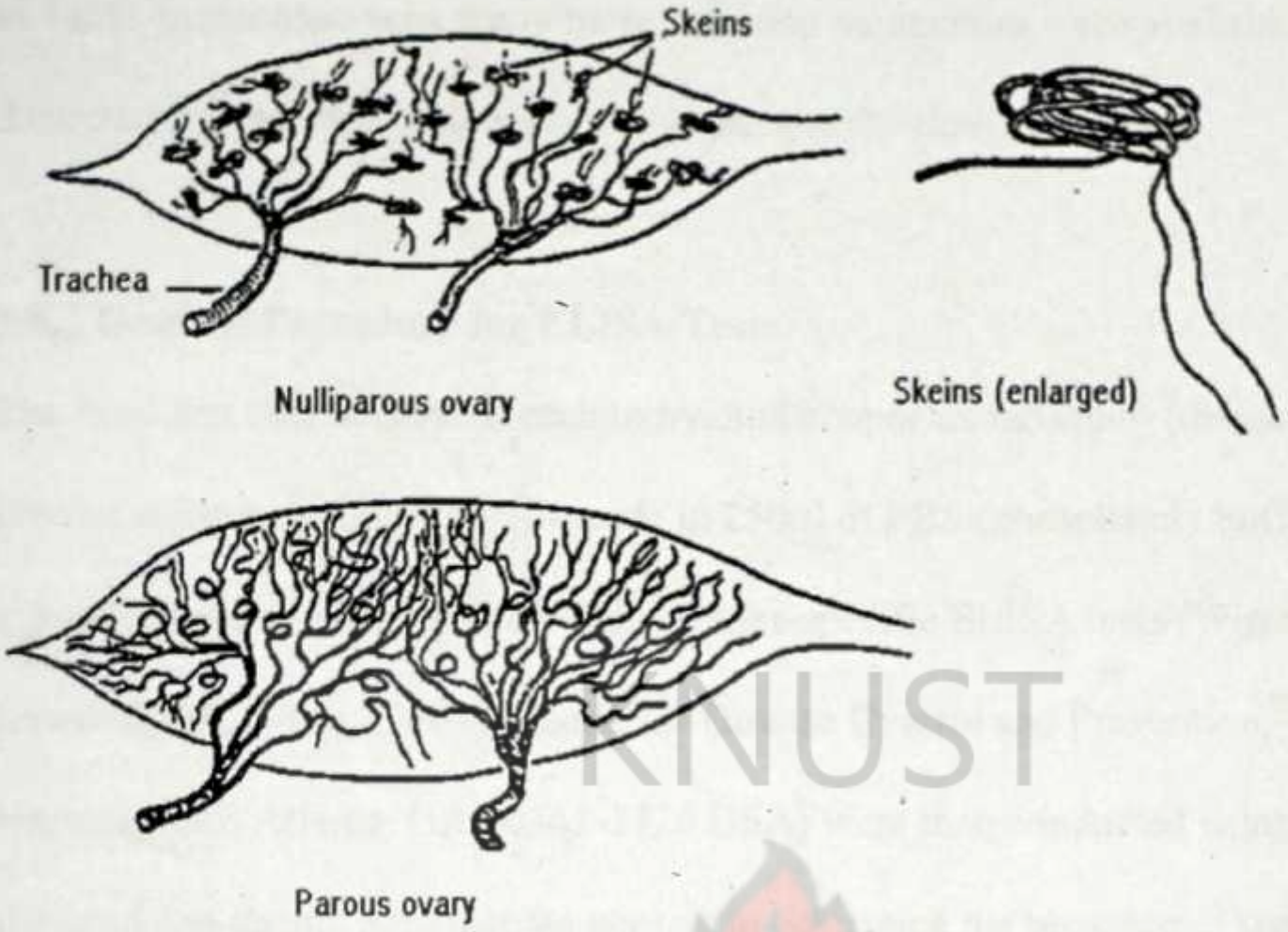
The abdomen remained on the slide after the removal of the legs, wings, head and thorax of the mosquito being dissected. A drop of PBS was placed on it, and while one needle was placed firmly on the thorax-end of the abdomen, its tip at the opposite end was pulled away from the rest of the abdomen with another needle held in the other hand. In this process, the ovaries normally come out of the abdomen (Fig. 7). A cut through the common oviduct freed the ovaries from the entrails of the abdomen. The ovaries were transferred into a drop of distilled water on another part of the slide and allowed to dry (WHO, 2002).



Source: WHO, 2002

Fig. 7: The steps involved in dissecting the ovary of a mosquito (WHO, 2002).

The dried ovaries were examined under a compound microscope using the x40 objective. Females in which the ovaries had coiled tracheolar skeins were nulliparous. Ovaries in which the tracheoles had become stretched out meant those mosquitoes had laid eggs and therefore were parous (Fig. 8). Ovaries contained eggs at the time of disection were also parous.



Source: WHO, 2002.

Fig. 8: Appearance of nulliparous and parous ovaries (WHO, 2002).

3.5 Immunological and molecular studies

These methods were used to determine a) sporozoite infectivity of the *Anopheles* mosquitoes, b) identify the *A. gambiae s.l.* sibling species, c) *Plasmodium* species and d) characterise the knock-down resistance (kdr) gene in the *Anopheles* species in the study areas.

3.5.1 Immunological studies – Enzyme-Linked immune-Sorbent Assay (ELISA) Tests for the Determination of Infectivity of the *Anopheles* Species by *Plasmodium* Species

The *Anopheles* mosquitoes caught were taken through ELISA processing to determine whether they contained the circum-sporozoite protein (CSPECIES) of *P. falciparum* (that is whether they were seen to be infected directly by dissection or they were missed,

and also to establish how many more infective mosquitoes were available but were not dissected for the sheer volume of the work and the time available).

3.5.2 General Procedure for ELISA Tests

The head and thorax parts of each individual anopheles mosquito (dissected or not) were ground using a sterilised micro-pestle in 250µl of PBS (phosphately buffered saline) in 1.5ml Eppendorf tubes. *Plasmodium* species-specific ELISA tests (Wirtz, *et al.*, (unpublished), Wirtz, (2004), Centre for Disease Control and Prevention, 4770 Buford Highway, NE Atlanta, GA 30341-3724 USA) were then conducted using 100µl each of the head and thorax homogenate per test to determine the presence of the CSP of *Plasmodium* species

Each well of a 96-well ELISA plate consisting of 7 positive and negative control wells each and 80 sample wells (two wells were always kept empty) was coated with 0.20µg capture monoclonal antibody (capture Mab) directed against the circumsporozoite protein (CSP) of the specific *Plasmodium* species being tested for in 50µl of PBS. The coated ELISA plate was incubated for an hour or over night, according to the manufacturer's instructions (Wirtz, 2004). This procedure created complementary binding sites in each well specific to those of the CSP of the specific *Plasmodium* species being tested for. Unspecific binding sites in each well were blocked by incubating each well with 200µl of blocking buffer (BB) made of 200µl PBS, pH 7.4, 2µg of 1.0% bovine serum albumen (BSA) and 1µg of 0.5% casein for an hour. This prevented the binding of other molecules to other binding sites in cross reactions that may interfere with the desired and expected reaction. The first 7 wells of the first two columns of each coated ELISA plate (also

called micro-titre plate), A1 to G1 and A2 to G2 were used for positive and negative control experiments respectively. In the positive control column, 0.1ng of the specific *Plasmodium* species CSP in 50µl BB was placed in A1. B1 to G1 had a 1:2 serial dilution of the content of A1 to create a decreasing concentration gradient of 0.05, 0.025, 0.0125, 0.00625, 0.00313 and 0.00156ng CSP in 50µl BB respectively. In the negative control column, head and thorax homogenates of different nulliparous mosquitoes (one mosquito per two wells) were used to inoculate two adjacent wells (A2 and B2, C2 and D2, E2 and F2) while the 7th, i.e. G2, was inoculated in single using homogenate of a fourth nulliparous mosquito. Each of the rest of the 80 wells (excluding H1 and H2) was inoculated with 50µl homogenate of a test sample, two wells per mosquito as in the first six wells of the negative control column (Plate S).

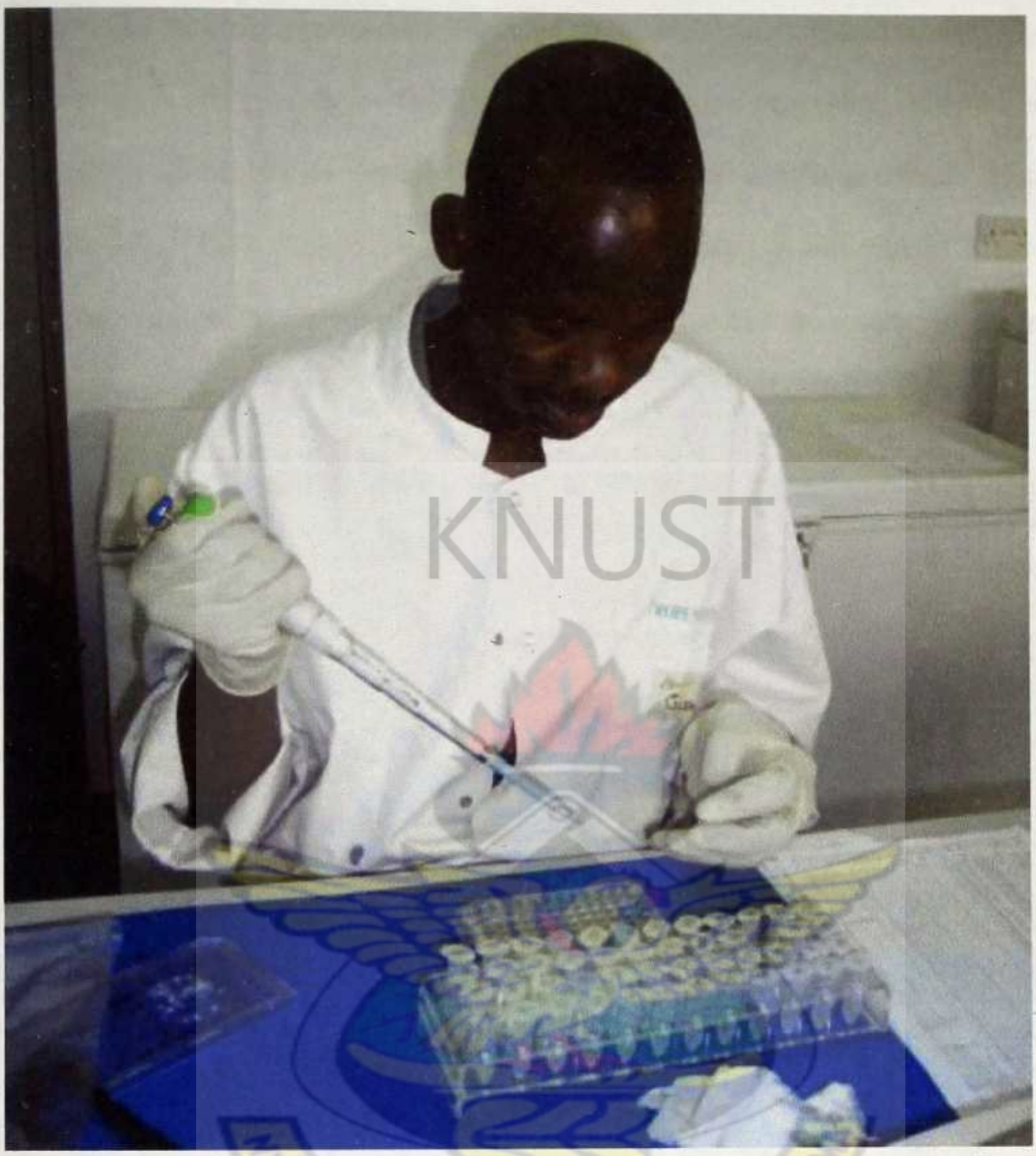


Plate S: Inoculating the ELISA micro-titre plate with homogenates of the mosquito samples. Each 1.5ml Eppendorf tube in the rack contains the head/thorax homogenate of one mosquito. 50µl of this homogenate is put into each of two adjacent wells of the micro-titre plate.

The test plate was incubated for two hours at room temperature. Unbound material was then washed away with PBS-Tween. CSP-capture Mab complexes were formed in wells containing infected mosquito homogenates. Inoculating each well with 0.05µg Mab-peroxidase in 50µl BB and incubating for an hour at room temperature resulted in CSP-

capture Mab-Mab-peroxidase complexes in wells containing infected mosquito homogenate. When washed with PBS-tween and 100µl of peroxidase substrate added to each, the latter complexes were detected visually by their deep blue colours (Plate T) (Wirtz *et al.*, 1987). Results were read out at 405nm using an automated ELISA Micro-titre plate reader. The reader is connected to a printer so that the results were printed (Plate T).

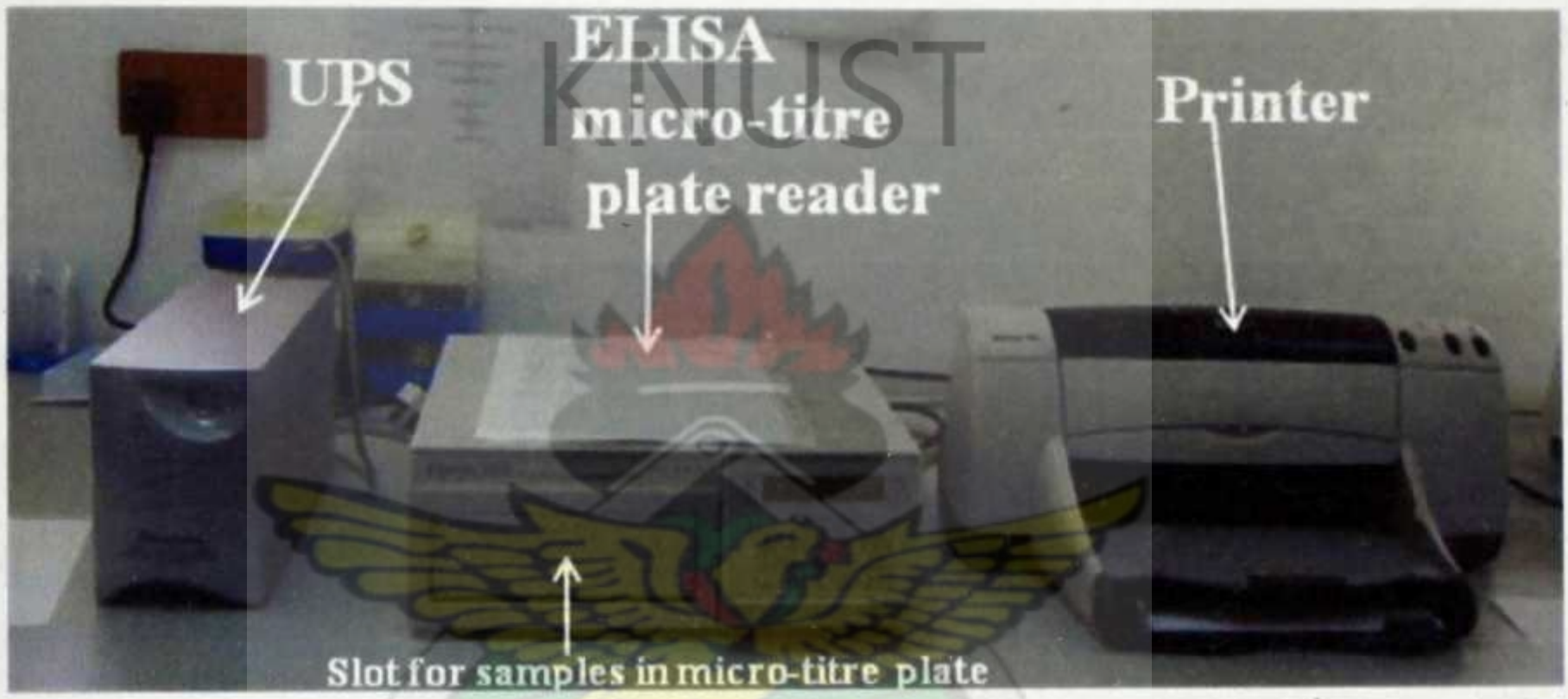


Plate T: The ELISA reader attached to a printer and UPS.

3.5.3 ELISA Tests for the Determination of Infectivity of the *Anopheles* Species by *P. falciparum.*

The general procedure outlined above was used. In this case, each of the 96 wells of each ELISA plate used was coated with 0.20µg capture monoclonal antibody (capture Mab) directed against the circumsporozoite protein (CSP) of *P. falciparum* in 50µl of PBS.

Positive results were obtained by establishing a cut-off optical density (OD) value according to the manufacturer's instruction, so that samples giving OD values of at least

the cut-off values were considered to be infected by *P. falciparum.* This cut-off was calculated from the OD values of the negative control experiment as follows:

Assuming that the average of the optical density values of the seven wells of the negative control column is x, then 2x was the cut-off OD value for positive samples. Therefore a mosquito was considered as infected if the average OD value of its two wells was at least 2x.

3.6 Molecular Studies - DNA extraction and PCR Assays

Various DNA extraction methods (depending on the state of the samples, i.e. whether the extraction is taken from body tissue such as head, thorax, wings, etc. or from body fluids such as haemocoel, saliva, etc.) and PCR assays (of which there were three types- Conventional, Real-time (RT-PCR) and Fluorescence Resonance Energy Transfer (FRET)) were used to process the mosquito samples in order to achieve three main objectives:

a) Identification of sibling species of *A. gambiae* complex using body tissue DNA extraction and Conventional PCR.
b) Identification of *Plasmodium* species using body fluid (mosquito saliva) DNA extraction and Real-time PCR.
c) Determination of the knockdown resistance (kdr) gene in the study sites using body tissue DNA extraction and FRET.

3.6.1 General Precautions in PCR Assays

The under-listed general precautions were undertaken to avoid contamination during DNA extraction:

1. Sterilised Eppendorf tubes were always used.
2. Sterilised pipette tips were always used.
3. A new pipette tip was always used each time a volume of sample or chemical was taken.
4. Pipettes were designated for use in DNA extraction only.
5. All chemicals and samples were appropriately labelled.
6. Disposable hand gloves were always used and changed as and when necessary.

In order to avoid contamination and obtain accurate PCR results from samples under investigation, the following general precautions were always strictly taken during the preparation of each PCR reaction mixture:

1. Sterilized PCR tubes of the required volumes, each appropriately labeled were used.
2. Sterilized pipette tips of appropriate volumes were used.
3. A new pipette tip was used to pipette each single volume of reaction mixture, reagent and sample. Pipettes designated for PCR reactions only were used and were never used in any other reaction, such as DNA extraction, etc.
4. The samples (DNA extracts), requisite PCR reagents and primers for the reaction were put in dry ice while PCR reaction mixtures were being prepared.
5. Disposable hand gloves were always used and changed as and when necessary.
6. A master-mix containing all the common reagents in their respective volumes was prepared first during each PCR procedure to avoid delay during pippeting.
7. The taq polymerase enzyme was always the last reagent to be added to the master-mix.

8. A positive control (containing the actual DNA e.g.DNA of the species being identified) and negative control containing distilled water experiments were included in each PCR experiment.

3.6.2 General Procedures and Requirements of a PCR Assay

In PCR assays, primers bearing base pair sequences that are directly complementary to the specific region of the DNA strand of interest, example, in the present study, those specific to each sibling species of the *A. gambiae* complex are used. The sequences of primers used for characterizing *A. gambiae s.s.* and *A. arabiensis* in a conventional PCR assay are shown below:

UN (universal primer): GTG TGC CCC TTC CTC GAT GT

GA (*A. gambiae s.s*): CTG GTT TGG TCG GCA CGT TT

AR (*A. arabiensis*): AAG TGT CCT TCT CCA TCC TA

Samples were processed in batches of between 5 and 30 at a time depending on the capacity of the PCR cycler, the number of samples available to be tested and convenience to have effective control of the process. The reaction mixtures were prepared by pippeting the appropriate volumes of the PCR reagents and primers and DNA extracts (shown under each type of PCR technique below). Certain reagents, however, were common to all PCR procedures. These were:

1) dNTPs
2) $MgCl_2$
3) 10x buffer
4) Taq polymerase and
5) H_2O

To the stipulated values of these standard reagents were added also specific volumes of the appropriate primers. Specific volumes of these common reagents were pippetted into a labelled composite master-mix Eppendorf tube, depending on the type of PCR assay. Unit volumes of these common reagents and the appropriate primers were pipetted in required multiples. An additional unit volume of each reagent was always added as a contingency against losses and errors and subsequently shared equally into the sample tubes, taking into account the extra volume added.

3.6.3 DNA Extraction for Conventional PCR

Using the Phenol-chloroform-Isoamyl alcohol protocol (Powell and Gannon, 2002), DNA was extracted from 140 samples, in some cases, using the remnant 50µl of the 250µl head/thorax homogenate originally prepared from each mosquito (with 200 µl used earlier for ELISA), or leg and wing parts that were removed during dissection and stored. The extracted DNA was used in conventional PCR to determine the sibling species of the *A. gambiae* complex (Scott *et al.*, 1993). The 140 samples were processed for convenience in batches of 10 at a time.

1. To each 1.5ml eppendorf tube containing the 50µl of the mosquito homogenate was added 50µl of PBS to make it up to 100µl. In the case of the leg and wings, 100µl PBS was added.
2. 100µl of phenol-chloroform-Isoamyl alcohol was then added and mixed well by vortexing (Putting on a mechanical shaker) for about 20 seconds.
3. The 10 tubes of mixtures were centrifuged together at 14000 rpm for 2 minutes.

4. 80µl of the supernatant was carefully transferred, without touching the phenol phase, to a new tube.
5. 200µl of absolute ethanol was added to each sample and mixed well by vortexing.
6. All 10 samples were subsequently incubated at -80°C for at least 20 minutes and then centrifuged at 14000 rpm for 10 minutes.
7. The supernatant in each tube was manually decanted and the residue in each tube was washed with 200µl of 70% ethanol by centrifuging at 13000 rpm for 5 minutes.
8. The supernatant in each tube was again decanted and the residue in each tube which was the extracted DNA pellets were dried by leaving the tubes open at room temperature.
9. 100µl of aqua (specialised sterile water) was then added to each DNA pellet and mixed well by vortexing to make a homogenous solution of the extracted DNA. The homogenates were ready for conventional PCR assay immediately or stored at –20°C for later assay as and when necessary.

3.6.4 Identification of sibling species of *A. gambiae* complex using body tissue DNA extraction and Conventional PCR.

This assay was used to determine the sibling species of *A. gambiae* complex. The following procedure was followed:

a) Five PCR tubes appropriately labelled and numbered 1-5 (could vary from 5-30) were set up in a rack for the experiment.
b) The unit volumes of the common reagents with the appropriate primers were pippetted in the required multiples (in this case 6X of each unit volume) into a

master-mix tube before being divided by 6 and shared as a composite mix into each of the five tubes. The unit volumes are presented in Table 3.

Table 3: Conventional PCR Reagents, Concentrations and Volumes

Reagents/Primers	Manufacturer's stock conc.	Volume in PCR tube (µl)
dNTPs	4 mM	1 µl
$MgCl_2$	80 mM	4 µl
10x buffer (KCl, Tris-HCl)	50 mM 10 mM	5 µl
Taq polymerase		0.25 µl
Universal (UN)	500 µg/µl	2 µl
A. arabiensis (AR)	750 ng/µl	2 µl
A. gambiae (GA)	250ng	2 ul
DNA extract from mosquitoes		5 µl
Water		28.75 µl
Final volume		50 µl

c) Once the master-mix has been prepared and shared into the 5 tubes appropriately labelled from 1 -5 then

- 5µl of the DNA of an *A. gambiae s.s.* (positive control) was pipetted into tube 1,
- 5µl of the DNA extracted from each of the three mosquitoes to be tested, stored in tubes labelled correspondently as 2, 3, and 4, were subsequently pipetted into their respective labelled tubes 2, 3 and 4.

- Finally, 5µl of distilled water was put in tube 5 (negative control) to give a total final volume of 50µl in each PCR tube.

The tubes were immediately placed in PCR cycler, which has been programmed to run in sequence repeatedly to conclude all reaction effectively according to the temperature regime below (Plate U).

1. 95 °C for 3 minutes to activate the taq polymerase.
2. 95 °C for 1 minute to denature DNAs.
3. 50 °C for 1 minute to anneal primers.
4. 72 °C for 1 minute for strand extension or replication.
5. 72 °C for 10 minutes for completion of all replications.

Steps 2- 4 were automatically repeated 35 times by the cycler to allow bulk reproduction of DNA strands.

Plate U: Conventional PCR cyclers with one showing sample slots.

The PCR products from the cycler were subsequently electrophoresed on a 1.5% agarose gel containing ethidium bromide at 65V for 50 minutes and visualised under UV light. The position of the matching base pair-bands of the samples with positive control bands indicated positive identification of the sibling species (Plate Y).

The base pairs of *A. arabiensis* and *A. gambiae s.s* which are two members of the *A. gambiae* complex are 315 bp and 390 bp respectively.

3.6.5 General DNA Extraction Procedure for Real-Time and Fluorescent Resonance Energy Transfer Technique PCR Assays.

Salivary glands that were found to be infected during dissection were washed in 100µl of PBS and stored in a 200µl Eppendorf tube each at -20°C. DNA was extracted from these homogenates using the blood and body fluid speciesin protocol of the QIAamp DNA Mini Kit and QIAamp DNA Blood Mini Kit Hand book and used in real-time PCR (Mangold *et al.*, 2005) to determine the species of *Plasmodium* in the salivary glands. The steps in the extraction process are:

1. The extraction began with the addition of 100µl of PBS to the 100µl salivary gland homogenate making up the volume to 200µl.
2. 20µl of the QIAGEN protease enzyme (Proteinase K) was then added to each test sample to digest the proteins to liberate the DNA.
3. 200µl of Buffer AL was added to each test sample
4. The samples were then incubated at 56°C for 10 minutes for lysis to take place.
5. Then 200µl of ethanol (96–100%) was added to each sample and each of the test mixtures at this stage was carefully applied to a QIAamp spin column (placed in a

sterile 2ml collection tube) to commence the process of filtration that results in the washing and purification of the liberated DNA. The caps of the QIAamp spin columns containing the test mixtures were closed and the latter were centrifuged at 6000 x *g* (8000 rpm) for one minute.

6. Each of the QIAamp spin columns (containing the sample) was again placed in a sterile 2ml collection tube and each of the tubes containing the filtrate that was collected from the test mixtures during centrifugation was discarded.
7. In cases where the lysate failed to completely pass through the column after centrifugation, centrifugation was repeated at higher speed until the QIAamp spin column was empty.
8. The QIAamp spin column was carefully opened and 500µl of Buffer AW1 added. The caps were closed and samples centrifuged again at 6000 x *g* (8000 rpm) for another minute.
9. Once again the QIAamp spin columns were each placed in new sterile 2ml collection tubes as those containing the filtrate were discarded.
10. Carefully opening the QIAamp spin columns, 500µl of Buffer AW2 was added to each. The caps were closed and the mixtures centrifuged, this time, at full speed (20,000 x *g*; 14,000 rpm) for three minutes.
11. To eliminate any chance of possible Buffer AW2 remaining in the QIAamp spin columns, these were placed in new sterile 2ml collection tubes and centrifuged again at full speed (20,000 x *g*; 14,000 rpm) for one minute.
12. Finally the QIAamp spin columns were placed in new sterilized 1.5ml micro-centrifuge tubes, the collection tubes, containing filtrate or not were discarded.

13. The QIAamp spin columns were carefully opened and 200µl of Buffer AE added. These were incubated at room temperature for five minutes to dissolve the DNA pellets and then centrifuged at 6000 x *g* (8000 rpm) for a minute.
14. The filtrate contained the extracted DNA in Buffer AE which was ready for use immediately; otherwise it was stored at –20°C for use in the future as and when needs arise.

3.6.6 Real Time PCR Primers

In Real time PCR assay, research has shown that DNA extract containing any *Plasmodium* species to act as a template can be amplified by the two oligonucleotide primers PL1473F18 and PL1679R18 (Mangold *et al.*, 2005). Unlike conventional PCR where each *A. gambiae s.l.* species needed a primer for its identification, these two are sufficient for the identification of the four medically important species of *Plasmodium* that cause malaria in humans, namely *P. falciparum, P. malariae, P. ovale* and *P. vivax.* The sequences of these primers are:

PL1473F18:- 5-TAA CgA ACg AgA TCT TAA-3

PL1679R18:- 5-gTT CCT CTA AgA AgC TTT-3

3.6.7 Identification of *Plasmodium* Species Using Body Fluid (saliva) DNA Extraction and Real-Time PCR Assay.

Real-time PCR procedure is essentially similar to conventional PCR procedure, yet with some distinct differences between the two. The main difference between Real-time and

conventional PCR is how the results are visualised. In the latter, the PCR results were read off after the amplification with the help of a separate procedure - electrophoresis. In Real-time PCR, however, amplified sections of the DNA strands (amplicons) were simultaneously melted into fluorescent curves characteristic of each *Plasmodium* species the peaks of these melted curves corresponded to different temperature regimes so that the results can be read off directly on a computer attached to the PCR cycler (Plate V).

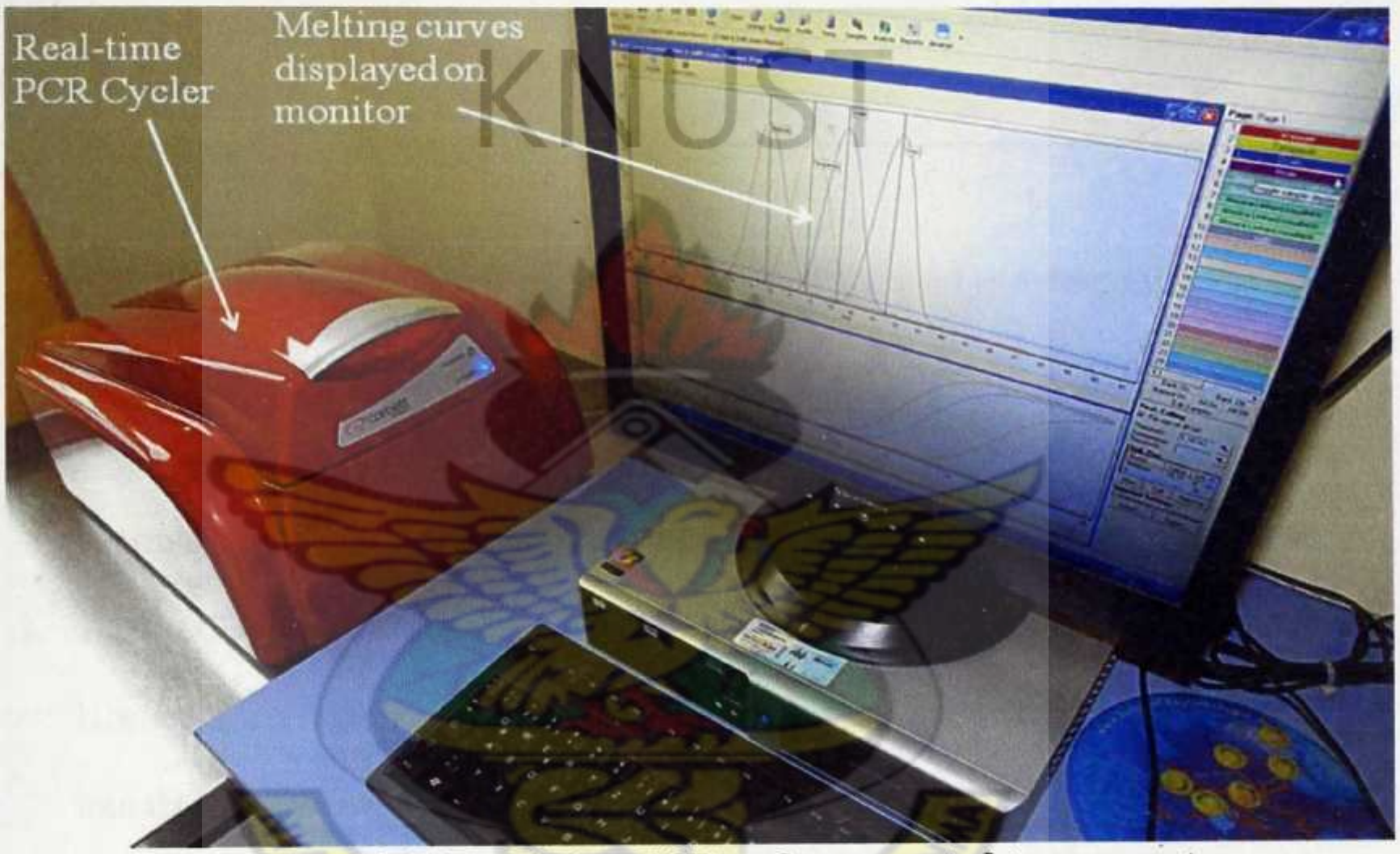


Plate V: The real-time PCR cycler connected to a computer.

All the four standard reagents used in conventional PCR were also used in real time PCR except the quantities and details in the procedure differed. To begin with the dNTPs, 10x buffer and taq polymerase were all mixed already from the manufacturer to form the SyBR Green Mix. Therefore the master-mix in this case contains the 1) SyBR Green Mix, 2) $MgCl_2$, 3) the special primers: i) PL 1473F18 and ii) PL1679R18 and 4) H_2O as shown in Table 4 with their respective unit volumes.

Table 4: Real-time PCR reagents, concentrations and volumes.

Reagents	Manufacturer's stock conc.	Volume in PCR tube (µl)
SyBR Green Mix		2.00
$MgCl_2$	25 mM	4.40
PL 1473F18	10 pmol/µl	1.00
PL1679R18	10 pmol/µl	1.00
DNA		10.00
H_2O		1.60
Total Volume		20.00

As in conventional PCR once the master-mix has been prepared and shared into the appropriately labelled test sample tubes, then

1. 10µl of the DNAs of *P. falciparum, P. malariae, P. ovale* and *P. vivax* (positive controls) were pipetted into correspondingly labelled positive control tubes and
2. 10µl of the DNA extracted from each of the samples to be tested, stored in tubes labelled to correspond to labels on test sample tubes, were subsequently pipetted into their respective labelled tubes to give a total volume of 20µl in each PCR tube.

The tubes were immediately placed in the PCR cycler as was done in conventional PCR to run according to the temperature regime shown below. However, in real time PCR, there were additional steps to melt the amplicons into fluorescent curves tha would characterise each *Plasmodium* species that could be visualised and printed, as explained below.

1. 95°C for 10 minutes to activate the taq polymerase enzyme
2. 95°C for 10 seconds to denature the double stranded DNA

3. 60°C for 5 seconds for primers to anneal and touch down by 0.5 °C for 11 cycles
4. 72°C for 20 seconds to extend or replicate and acquire fluorescence.
5. 95°C for 2 minutes
6. 68°C for 30 seconds
7. 40°C for 20 seconds to cool

Steps 2-4 were repeated automatically, in this case, for 40 cycles by the cycler in continuous replication for mass production of the synthesized strands, with fluorescence acquisition at the end of each extension step. Amplification was immediately followed by a melt programme consisting of steps 5 and 6 and a stepwise temperature increase by 0.6 °C from 68°C to 90°C with fluorescence acquisition at each temperature transition during which the synthesized strands separate to the species-specific ones at about 75°C, 76°C and 78°C for *P. malariae, P. falciparum and P. ovale* respectively (Fig 39).

3.6.8 The Fluorescence Resonance Energy Transfer (FRET) PCR Amplification Technique.

FRET PCR was used to determine the presence of the knockdown resistance gene (kdr) in *A. gambiae s. l.* The procedure for FRET PCR is similar to the Conventional and Real Time PCRs except that in FRET PCR, the whole process is automated so machines performed everything from pipetting to cycling. As in the case of real time PCR, the results were visualised as temperature-dependent, fluorescent melted curves (Plate W), one corresponding to the mutation and the other to susceptible parts of the DNA strand as explained below.

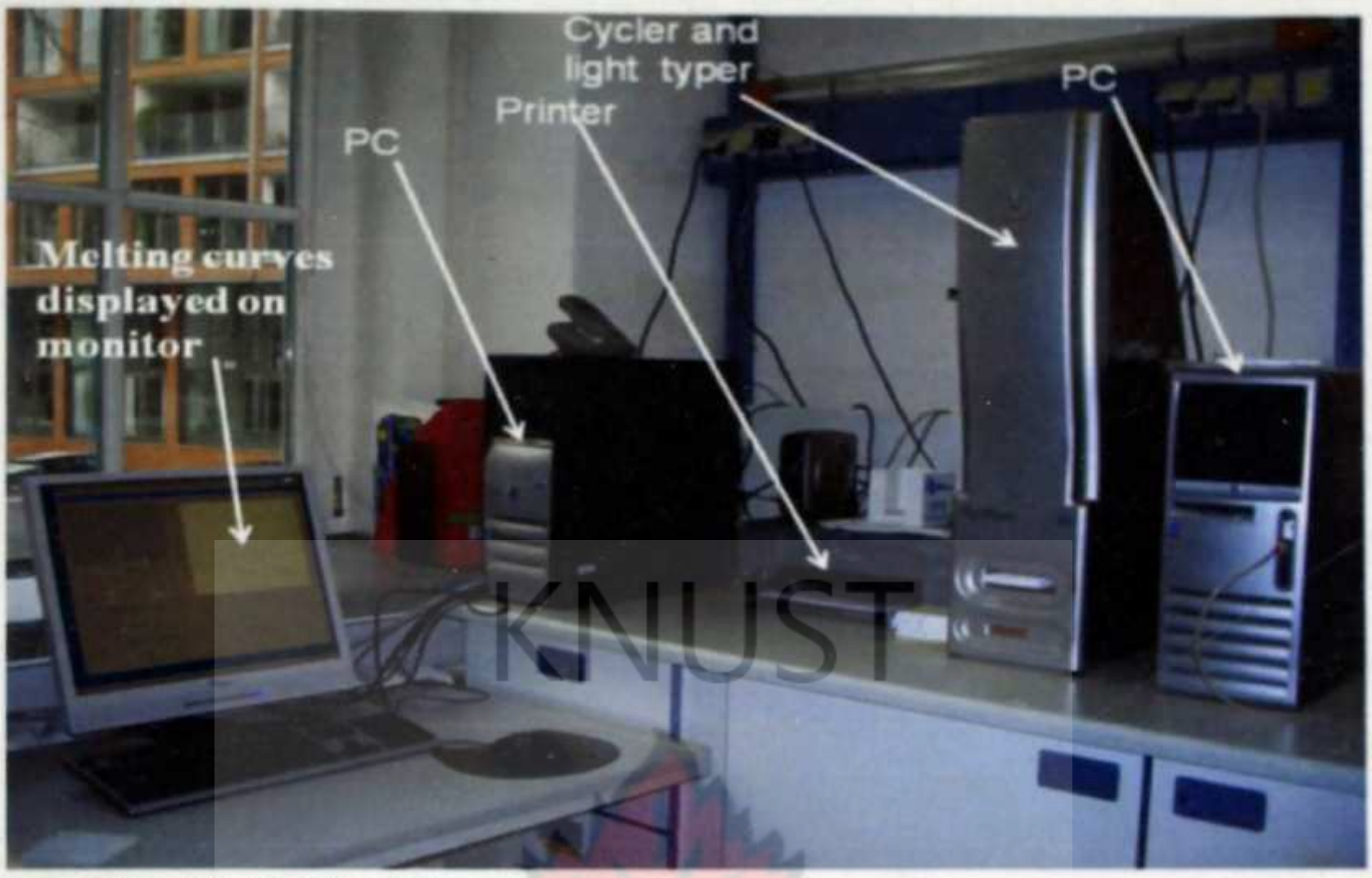


Plate W: FRET PCR equipment

3. 6.10 DNA Extraction Procedure for FRET

The same precautions as those described under DNA extraction for real time PCR were used here. The only difference between both DNA extraction methods is that a blood / body fluid protocol was used in the DNA extraction for real time PCR while for FRET, the tissue protocol was used. The extraction procedure was as follows:

1. The samples were remnants of mosquito body parts in 50µl of PBS kept in a 1.5ml micro-centrifuge tubes to which 150µl of PBS was added to make up the volume to 200µl for extraction. The other samples were whole mosquitoes, each of which was placed in 1.5ml micro-centrifuge tube for the extraction.
2. To each of the 1.5ml micro-centrifuge tubes containing the 200µl of head/thorax homogenate sample prepared as described above 20µl of Proteinase K was added

but to each of the whole mosquito samples was added 180µl of Buffer ATL and then 20µl of Proteinase K.

3. These were then incubated at 56°C overnight on a shaking water bath.
4. 200µl of Buffer AL was added to each sample mixture and the latter were subsequently incubated at 70°C for 10 minutes.
5. 200µl ethanol (96–100%) was next added to each sample.
6. The mixtures were then carefully applied to the QIAamp spin column in a 2 ml collection tube. From that stage, the same procedure was used as in the extraction of DNA for real time PCR (from steps 6-14) to obtain the DNA extracts.

3.6.11 FRET PCR Primers

In the FRET PCR assay, the primers Agd1 and Agd2 amplifying the mutation and susceptible sections of the DNA strands were used. Their base pair sequences are shown below:

Agd1 5′ ATAGATTCCCCGACCATG 3′

Agd2 5′ AGACAAGGATGATGAACC 3′

3.6.12 Determination of the Presence of kdr Gene Using Body Tissue DNA Extraction and FRET Assay.

The concentration of the reagents used in FRET PCR assay are shown in Table 5.

Table 5: Concentrations of the reagents used in FRET PCR assay.

Reagents	Stock concentration	Volume in PCR tube (µl)
10x Buffer B, without $MgCl_2$		1.2
$MgCl_2$	25 mM	1.2
Agd 2	10 pM/µl	1.0
Agd 1	10 pM/µl	0.2
kdr-Anchor	4 pM/µl	0.5
kdr-Sensor	4 pM/µl	0.5
dNTPs	10 mM	0.2
FirePol Taq	5 U/µl	0.2
DNA	5 ng/µl	2.0
H_2O		3.0
Total volume		10.00

In FRET PCR, machines performed everything from pipetting to cycling. In the set up equipment, there is a separate machine (not shown) for the pipetting of the samples after which the samples were taken to the PCR cycler which was attached to a lighttyper, computer and printer. As in the case of real-time PCR, the results were visualised as temperature-dependent, fluorescent melted curves; one corresponding to the kdr gene and the other to the wild type gene.

The FRET PCR conditions shown below were similar to those of the previous PCR assays.

1. 94°C for 3 minutes to activate the enzyme
2. 94°C for 20 seconds to denature the double stranded DNA
3. 50°C for 30 seconds for primers to anneal
4. 72°C for 30 seconds to extend or replicate the DNA strand
5. 72°C for 5 minutes for all replications to complete.

As in real time PCR, steps 2-4 were repeated automatically for 40 cycles by the cycler in continuous replication for mass production of the amplicons, with fluorescence at the end of each step. The results, displayed on a computer were then printed for analysis.

3.7 Preview of Results

The rainfall data used to construct the monthly biting rate and EIR are those recorded at Kona during the study, not those collected from the Meteorological Service.

The presentation of results largely followed the same order as the materials and methods, as outlined below:

1. Mosquitoes were collected for the estimation of their prevalence and biting rate; i.e. the number of mosquito bites that a person receives at a given locality over a specified period of time (an hour, a night, a month or a year).
2. Dissection was done to remove the salivary glands of *Anopheles* mosquitoes to determine the proportion of them that were infected by *Plasmodium* species in the study sites
3. ELISA tests were used to determine the proportion of *Anopheles* mosquitoes infected by *P. falciparum* in the study sites.
4. Dissection was done to remove the ovaries *Anopheles* mosquitoes to determine their parity in the study sites

5. Sporozoite rate that estimates the proportion of the *Anopheles* species infected by the *Plasmodium* species was calculated as a ratio of the infected out of the total anopheles mosquitoes tested.
6. Parity or parous rate which estimates the proportion of the mosquitoes that have laid eggs meaning that they have had previous blood-meals and therefore could be infected by *Plasmodium* species was calculated as the proportion of the dissected anopheles mosquitoes that were parous out of the total dissected.
7. Analysis was conducted using the Boadi cattle project site as a bench mark against the others to test the hypothesis that *A. gambiae* is largely anthropophilic and that *A. ziemanni* is zoophilic with preference for cattle.
8. The Boadi site was also used to test the hypothesis that deltamethrin-impregnated nets fenced round cattle pens were effective against mosquitoes including malaria vectors.
9. Afamanaso, Anwomaso and Boadi were used to verify whether the biting pattern of *A. gambiae s.l.* in each of these sites differed from the typical.
10. Analysis was provided in the use of Conventional PCR to determine the sibling species of *A. gambiae* complex in the study sites
11. Real Time PCR was used to determine the species of *Plasmodium*
12. FRET PCR was used to determine the prevalence of the kdr gene in the study sites.
13. Entomological inoculation rate (EIR) which estimates the level of malaria transmission in terms of the number of infective bites over a specified period of time, was calculated as the product of the biting and sporozoite rates.

14. Secondary data from the work on malaria incidence rates among 1069 three month-old infants from Afamanaso and Kona that have been collected by a clinical team (Kobbe *et al.*, 2006) as a parallel study to the present entomological one were used to assess the extent of correlation between direct estimation of malaria prevalence by the clinical study and the present study of entomological parameters.

Unless otherwise stated, data analysis and statistics were done using Wilcoxon rank-sum (Mann-Whitney) test in STATA 10.0 software for Windows (StataCorp LP, College Station, USA). P-values <0.05 were considered significant. 95% confidence intervals were used

CHAPTER FOUR

4.0 RESULTS

The results presented here are an attempt to provide both field and laboratory evidence on behavioural and population dynamics of some species of mosquitoes caught by human landing catches in some selected localities in rural and peri-urban sites. Of additional interest is how the dynamics of *Anopheles* species may ultimately impact the dynamics of malaria transmission in the forest belt of Ghana.

Table 6 presents an overview of all the places where the species of mosquitoes collected and the locations of the mosquito collector, i.e. indoor or outdoor.

Table 6: The places and locations where the various species of mosquitoes were collected.

Places	Outdoor	Indoor	*A. gambiae*	*A. funestus*	*A. ziemanni*	*Culex* species
Afamanaso	Yes	Yes	Yes	Yes	Yes	Yes
Afamanaso Hamlet	Yes	No	Yes	No	No	Yes
Morso Hamlet	Yes	No	Yes	No	No	Yes
Kona	Yes	Yes	Yes	Yes	No	Yes
Boadi	Yes	No	Yes	Yes	Yes	Yes
Anwomaso	Yes	No	Yes	No	No	Yes
Anwomaso (Nana's Farm)	Yes	No	Yes	No	Yes	Yes

"Yes" to indoor at any place means the species of mosquitoes marked "yes" at that place were all collected indoor. Similarly, "yes" to outdoor means species of mosquitoes indicated were collected outdoor. "Yes" to both indoor and outdoor means mosquitoes specified were collected both indoor and outdoor. "No" to indoor means specified mosquitoes were not caught indoor but only outdoor.

4.1 Prevalence and Biting Rates of the Mosquitoes in the Rural AFigya Sekyere Sites (Afamanaso and Kona).

Tables 7 and 8 show that in all, there were 5608 mosquitoes caught during 80 human landing night collections from Afamanaso and Kona (excluding the collection points at

Afamanaso town centre as well as the hamlets of Afamanaso and Morso where mosquitoes were caught solely to compare the biting pattern of *A. gambiae s.l.* These data are presented and analyzed separately). Out of this total, 4636 were *Anopheles* species: - *A. gambiae s.l.* and *A. funestus* (Table 9) and 972 *Culex* species the numbers of each mosquito species caught *A. gambiae s.l., A. funestus* and *Culex* were significantly different (P = 0.04). However, the total number of mosquitoes caught at Afamanaso was not significantly different from that collected at Kona (P = 0.14). The results show that *A. gambiae s.l.* and *Culex* species were common in the study areas (Table 6).

Table 7: The numbers and proportions of each mosquito species collected indoor and outdoor at Afamanaso and Kona.

Numbers caught				Total	Proportion caught (%)		
Town	*A. gambiae*	*A. funestus*	*Culex*		*A. gambiae*	*A. funestus*	*Culex*
Indoor							
Afamanaso	714^{a_i}	345^{e_o}	254^{qu}	1313	54.38^{a_i}	26.28^{e_k}	19.35^{qu}
Kona	$1,049^{b_k}$	144^{f_m}	107^{rv}	1300	80.69^{b_m}	11.08^{f_o}	8.23^{rv}
Outdoor							
Afamanaso	$1,241^{c_j}$	648^{g_p}	249^{su}	2138	58.04^{c_j}	30.31^{g_l}	11.65^{su}
Kona	475^{d_l}	20^{h_n}	362^{tw}	857	55.43^{d_n}	2.33^{h_p}	42.24^{tw}
Total	3,479	1,157	972	5608	62.04	20.63	17.33

$P^{ab} = 0.00 \Rightarrow 0.01 > P^{ab} < 0.05$, $P^{cd} = 0.00 \Rightarrow 0.01 > P^{cd} < 0.05$, $P^{ef} = 0.00 \Rightarrow 0.01 > P^{ef} < 0.05$,
$P^{gh} = 0.00 \Rightarrow 0.01 > P^{gh} < 0.05$, $P^{ij} = 0.00 \Rightarrow 0.01 > P^{ij} < 0.05$, $P^{kl} = 0.00 \Rightarrow 0.01 > P^{kl} < 0.05$,
$P^{mn} = 0.00 \Rightarrow 0.01 > P^{mn} < 0.05$, $P^{op} = 0.00 \Rightarrow 0.01 > P^{op} < 0.05$, $P^{qr} = 0.01 \Rightarrow 0.01 = P^{qr} < 0.05$,
$P^{st} = 0.01 \Rightarrow 0.01 = P^{st} < 0.05$, $P^{uu} = 0.12 \Rightarrow P^{uu} > 0.05$, $P^{vw} = 0.00 \Rightarrow 0.01 > P^{vw} < 0.05$.

Different letters of the superscripts, e.g. ab, suggest a significant difference in the proportion of mosquito species caught indoor and outdoor between Afamanaso and Kona.
Same letters of the superscripts, e.g. uu, suggest no significant difference in the proportion of mosquito species caught indoor and outdoor between Afamanaso and Kona.

1. ab suggest significant difference between the number or proportion of *A. gambiae s.l.* caught indoor at Afamanaso and that at Kona.
2. cd suggest significant difference between the number or proportion of *A. gambiae s.l.* caught outdoor at Afamanaso and that at Kona.
3. ef suggest significant difference between the number or proportion of *A. funestus* caught indoor at Afamanaso and that at Kona.
4. gh suggest significant difference between the number or proportion of *A. funestus* caught outdoor at Afamanaso and that at Kona.
5. ij suggest significant difference between the number or proportion of *A. gambiae s.l.* caught indoor and outdoor at Afamanaso.

6. kl suggest significant difference between the number or proportion of *A. gambiae s.l.* caught indoor and outdoor at Kona.
7. mn suggest significant difference between the number or proportion of *A. funestus* caught indoor and outdoor at Kona.
8. op suggest significant difference between the number or proportion of *A. funestus* caught indoor and outdoor at Afamanaso.
9. qr suggest significant difference between the number or proportion of *Culex* species caught indoor at Afamanaso and that at Kona.
10. st suggest significant difference between the number or proportion of *Culex* species caught outdoor at Afamanaso and that at Kona.
11. uu suggest no significant difference between the number or proportion of *Culex* species caught indoor at Afamanaso and outdoor at Afamanaso.
12. vw suggest significant difference between the number or proportion of *Culex* species caught indoor at Kona and outdoor at Kona.

Table 8: The numbers and proportions of the various species of mosquitoes caught at Afamanaso and Kona.

Town	Total number of each species caught				Proportion out of total caught ((%))		
	A. gambiae	*A. funestus*	*Culex*	Totals	*A. gambiae*	*A. funestus*	*Culex*
Afamanaso	1,955	993	503	3,451	56.65^{a}	28.77^{c}	14.58^{e}
Kona	1,524	164	469	2,157	70.65^{b}	7.60^{d}	21.74^{e}
Totals	3,479	1,157	972	5,608	62.04	20.63	17.33

$P^{ab} = 0.00 => 0.01 > P^{cd} < 0.05$, $P^{ee} = 0.10 => P^{cc} > 0.05$

Different letters of the superscript, ab suggest a significant difference in the proportion of mosquitoes collected at Afamanaso and that at Kona.
Same letters of the superscript, cc, suggest no significant difference in the proportion of mosquitoes collected at Afamanaso and that at Kona

1. ab suggest significant difference between the number or proportion of *A. gambiae s.l.* caught at Afamanaso and that at Kona.
2. cd suggest significant difference between the number or proportion of *A. funestus* caught at Afamanaso and that at Kona.
3. ef suggest significant difference between the number or proportion of *Culex* species caught at Afamanaso and that at Kona.

Table 9: The numbers and proportions of *Anopheles* species collected at Afamanaso and Kona for malaria analysis.

Town	Number caught		Total	Proportion (%)	
	A. gambiae	*A. funestus*		*A. gambiae*	*A. funestus*
Afamanaso	$1,955^{a}$	993^{c}	2,948	42.17^{a}	21.42^{c}
Kona	$1,524^{b}$	164^{d}	1,688	32.87^{b}	3.54^{d}
Total	3,479	1,157	4,636	75.04	24.96

$P^{ab} = 0.00 => 0.01 > P^{ab} < 0.05$, $P^{cd} = 0.00 => 0.01 > P^{cd} < 0.05$.

Difference in letters of the superscripts, e.g. ab, suggest a significant difference in the proportion of mosquitoes collected at Afamanaso and Kona.

1. ab suggests a significant difference in the proportion of *A. gambiae s.l.* caught at Afamanaso and Kona.
2. cd suggests significant difference in the proportion of *A. funestus* caught at Afamanaso and Kona.

As shown in Table 9 the 4636 *Anopheles* mosquitoes comprised 75% *A. gambiae s.l.*, significantly more than the 25% *A. funestus* (P = 0.00). Of these *Anopheles* mosquitoes, 2948 were obtained by 36 human landing catches at Afamanaso. These consisted of 66% *A. gambiae s.l.* which was not significantly different from the 34% *A. funestus* (P = 0.2). At Kona 1688 anopheles mosquitoes were collected by 44 human landing catches. *A. gambiae s.l.* occurred more abundantly in Kona (P = 0.00), comprising 90% of the collection compared to the 10% of *A. funestus*.

Fig 10 shows the numbers of mosquitoes collected from different places in Afamanaso to compare the biting patterns of *A. gambiae s.l.* The number of *A. gambiae s.l.* was the largest in all three places. However, *A. funestus,* the second most abundant mosquitoes caught at Afamanaso for almost the two years period of collections, was not found in any of the other places where only a single night outdoor collection was made. Again, *Culex* species were caught in the Afamanaso Hamlet alone.

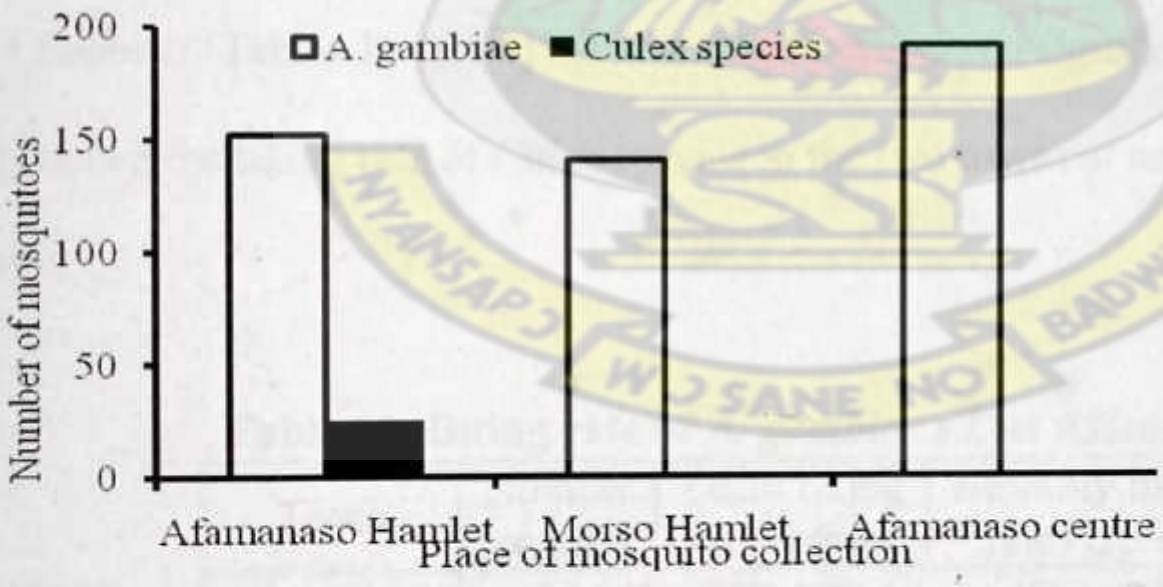


Fig 10: Mosquitoes collected from other parts of Afamanaso to compare the biting patterns of *A. gambiae s.l.*

4.1.1 Biting rate at Afamanaso and Kona:

Biting rate is the average number of mosquitoes biting a person at a given location in a specified period of time. The average daily biting rate is the number of mosquitoes collected as they land on, and attempt, to bite the collectors within the time period, divided by the number of collectors. Monthly and annual biting rates are obtained by multiplying the daily biting rate by the appropriate number of days in the month or year whereas the hourly biting rate is obtained by dividing by the number of hours in a day. Thus, the transmission of mosquito-borne diseases such as malaria is dependent on the frequency of biting: the higher the biting rate, the higher the transmission rate. Each of Tables 10 -12 presents the average daily, monthly and annual biting rates of the three mosquito species at Afamanaso and Kona. The average calculated daily biting rate of *A. gambiae s.l.* at Afamanaso was 20.80 bites per person per night (b/p/n) but at Kona it was 12.00 b/p/n. These high biting rates may signify high transmission of malaria. There were significant differences in biting rates between the two towns of both *A. gambiae s.l.* and *A.funestus* (Tables 10 and 11) (P = 0.00). However, no significant differences existed between the biting rate of *Culex* species in the two towns at any level (Table 12) as P>0.05

Table 10: Biting rate of *A. gambiae s.l.* at Afamanaso and Kona

Town	Number caught	Daily biting rate (b/p/n)	Monthly biting rate (b/p/m)	Annual biting rate (b/p/yr)
Afamanaso	1955	20.80^{a}	623.94^{a}	7596.42^{a}
Kona	1524	12.00^{b}	360.00^{b}	4383.00^{b}
Total	3479	15.74	472.26	5749.80

P^{ab} = 0.00 => 0.01>P^{ab} <0.05

Different letters of the superscript, ab, suggest a significant difference in biting rate at Afamanaso and Kona.

ab suggests significant difference in the biting rate of *A. gambiae s.l.* at Afamanaso and Kona.

Table 11: Biting rate of *A. funestus* at Afamanaso and Kona

Town	Number caught	Daily biting rate (b/p/n)	Monthly biting rate (b/p/m)	Annual biting rate (b/p/yr)
Afamanaso	993	10.5638^{a}	316.915^{a}	3858.44^{a}
Kona	164	1.29134^{b}	38.7402^{b}	471.661^{b}
Total	1157	5.23529	157.059	1912.19

$P^{ab} = 0.00 \Rightarrow 0.01 > P^{ab} < 0.05$

Different letters of the superscript, ab, suggest a significant difference in biting rate at Afamanaso and Kona.

ab suggests significant difference in the biting rate of *A. funestus* at Afamanaso and Kona.

Table 12: Biting rate of *Culex* species at Afamanaso and Kona.

Town	Number caught	Daily biting rate (b/p/n)	Monthly biting rate (b/p/m)	Annual biting rate (b/p/yr)
Afamanaso	503	5.35^{a}	160.53^{a}	1954.48^{a}
Kona	469	3.69^{b}	110.79^{b}	1348.84^{b}
Total	972	4.40	131.95	1606.44

$P^{ab} = 0.04 \Rightarrow 0.10 < P^{a} < 0.05$

Different letters of the superscript, ab, suggest a significant difference in biting rate at Afamanaso and Kona.

ab suggest significant difference in the biting rate of *Culex* species at Afamanaso and Kona.

Tables 13-15 display the indoor and outdoor biting rates of the three mosquito species at Afamanaso and Kona. It can be seen from Table 13 that *A. gambiae s.l* switched from being endophagic, biting mainly indoor at Kona to being exophagic, biting more outdoor at Afamanaso. A similar trend was displayed by *A. funestus,* (Table 14) biting more indoor than outdoor at Kona but the reverse at Afamanaso. *Culex* species, however, demonstrated a clear opposite pattern by biting slightly more indoor at Afamanaso but largely outdoor at Kona (Table 15).

Table 13: Biting rates of *A. gambiae s.l.* at Afamanaso and Kona

Town	Number caught	Daily biting rate (b/p/n)	Monthly biting rate (b/p/m)	Annual biting rate (b/p/yr)
Indoor				
Afamanaso	714	12.31^{ae}	374.70^{ae}	4496.35^{ae}
Kona	1049	11.79^{bg}	358.89^{bg}	4306.30^{bg}
Outdoor				
Afamanaso	1241	34.47^{cf}	1049.25^{cf}	12590.98^{cf}
Kona	475	16.02^{dh}	487.59^{dh}	5851.02^{dh}

$P^{ab} = 0.04 \Rightarrow 0.01 < P^{ab} < 0.05$, $P^{cd} = 0.00 \Rightarrow 0.01 > P^{cd} < 0.05$, $P^{ef} = 0.00 \Rightarrow 0.01 > P^{ef} < 0.05$,
$P^{gh} = 0.00 \Rightarrow 0.01 > P^{gh} > 0.05$

Different letters of the superscript, ab, suggest a significant difference between the parameters being compared

1. ab suggest significant difference between the indoor biting rate at Afamanaso and that at Kona.
2. cd suggest significant difference between the outdoor biting rate at Afamanaso and that at Kona.
3. ef suggest significant difference between the indoor and outdoor biting rates at Afamanaso.
4. gh suggest significant difference between the indoor and outdoor biting rates at Kona.

Table 14: Biting rates of *A. funestus* at Afamanaso and Kona

Town	Number caught	Daily biting rate (b/p/n)	Monthly biting rate (b/p/m)	Annual biting rate (b/p/yr)
Indoor				
Afamanaso	345	5.95^{ae}	181.05^{ae}	2172.61^{ae}
Kona	144	1.45^{bg}	44.13^{bg}	529.61^{bg}
Outdoor				
Afamanaso	648	18^{cf}	547.88^{cf}	6574.50^{cf}
Kona	20	0.75^{dh}	22.83^{dh}	273.94^{dh}

$P^{ab} = 0.00 \Rightarrow 0.01 > P^{ab} < 0.05$, $P^{cd} = 0.00 \Rightarrow 0.01 > P^{cd} < 0.05$,
$P^{ef} = 0.01 \Rightarrow 0.01 = P^{ef} < 0.05$, $P^{gh} = 0.00 \Rightarrow 0.01 > P^{gh} < 0.05$.

Different letters of the superscript, ab, suggest a significant difference between the parameters being compared.

1. ab suggest significant difference between the indoor biting rate at Afamanaso and that at Kona.
2. cd suggest significant difference between the outdoor biting rate at Afamanaso and that at Kona.
3. ef suggest significant difference between the indoor and outdoor biting rates at Afamanaso.
4. gh suggest significant difference between the indoor and outdoor biting rates at Kona.

Table 15: Biting rates of *Culex* species at Afamanaso and Kona

Town	Number caught	Daily biting rate (b/p/n)	Monthly biting rate (b/p/m)	Annual biting rate (b/p/yr)
Indoor				
Afamanaso	254	2.70^{ad}	81.06^{ad}	986.95^{ad}
Kona	107	0.84^{be}	25.2^{be}	307.73^{be}
Outdoor				
Afamanaso	249	2.65^{cd}	79.47^{cd}	967.52^{cd}
Kona	362	2.85^{cf}	85.51^{cf}	1041.11^{cf}

$P^{ab}= 0.00 => 0.01>P^{ab}<0.05$, $P^{cc}= 0.06=> P^{cc}>0.05$, $P^{dl}= 0.1 => P^{dl}>0.05$, $P^{ef}= 0.00 => 0.01>P^{ef}<0.05$.

Different letters of the superscript, e.g. ab, suggest a significant difference between the parameters being compared.
Same letters of the superscript, e.g. cc, suggest no significant difference between the parameters being compared.

1. ab suggests significant difference between the indoor biting rate at Afamanaso and that at Kona.
2. cc suggests no significant difference between the outdoor biting rate at Afamanaso and that at Kona.
3. dd suggests no significant difference between the indoor and outdoor biting rates at Afamanaso.
4. ef suggests significant difference between the indoor and outdoor biting rate at Kona.

Tables 16 and 17 present the daily, monthly and annual biting rates of the two malaria vectors; *A. gambiae s.l.* and *A. funestus* respectively in the various sites within each town where mosquitoes were collected. It turned out that at Afamanaso, the biting activity of *A. gambiae s.l.* was significantly higher at House 1 than House 2 and School while the biting rate was not significantly different between House 2 and School. At Kona, on the other hand, no significant difference was found between the three sites School 1, School 2 and House.

Table 16: The average biting rates of *A. gambiae s.l.* at the various vector collection sites at Afamanaso and Kona.

Town	Collection site	Number caught	Daily biting rate (b/p/n)	Monthly biting rate (b/p/m)	Annual biting rate (b/p/yr)
Afamanaso	House 1	1,241	34.47^{ac}	1049.25^{ac}	12590.98^{ac}
	House 2	307	13.96^{be}	424.74^{be}	5096.90^{be}
	School	407	11.31^{de}	344.11^{de}	4129.35^{de}
Kona	House	485	13.27^{gh}	403.95^{gh}	4847.39^{gh}
	School 1	564	10.49^{fh}	319.40^{fh}	3832.84^{fh}
	School 2	475	16.02^{fg}	487.59^{fg}	5851.02^{fg}

P^{ab} = 0.00 => 0.01>P^{ab}<0.05, P^{cd} = 0.00 => 0.01>P^{cd}<0.05, P^{ee} = 0.38=> P^{ee}>0.05,
P^{ff}= 0.06 => P^{ff}>0.05, P^{gg} = 0.63=> P^{gg}>0.05, P^{hh} = 0.09 => P^{hh}>0.05.

Difference in letters of the superscripts, e.g. ab, suggest a significant difference in biting rates between the sites indicated by the labelled Figures.
Same letters of the superscripts, e.g. ee, suggest no significant difference in biting rates between the sites indicated by the labelled Figures.

1. ab suggest a significant difference in biting rate between House 1 and House 2.
2. cd suggest a significant difference in biting rate between House 1 and School.
3. ee suggest a significant difference in biting rate between House 2 and School.
4. ff suggest a significant difference in biting rate between School 1 and School 2.
5. gg suggest a significant difference in biting rate between House and School 2.
6. hh suggest a significant difference in biting rate between House and School 1.

Table 17: The average biting rates of *A. funestus* at the various vector collection sites at Afamanaso and Kona.

Town	Collection site	Number caught	Daily biting rate (b/p/n)	Monthly biting rate (b/p/m)	Annual biting rate (b/p/yr)
Afamanaso	House 1	648	0.86^{bd}	26.21^{ab}	314.52^{ab}
	House 2	110	0.18^{ae}	5.53^{ac}	66.41^{ac}
	School	235	0.47^{ac}	14.37^{de}	172.48^{de}
Kona	House	38	0.09^{hi}	2.61^{gh}	31.31^{gh}
	School 1	106	0.17^{fj}	5.14^{fh}	61.64^{fh}
	School 2	20	0.077^{gh}	2.34^{fh}	28.10^{fh}

P^{aa} = 0.41 => P^{aa}>0.05, P^{bc} = 0.00 => 0.01>P^{bc}<0.05, P^{de} = 0.00 =>0.01>P^{de}>0.05,
P^{fg}= 0.00 =>0.01>P^{fg}>0.05, P^{hh} = 0.35=> P^{hh}>0.05, P^{ij} = 0.00 =>0.01>P^{ij}>0.05.

Different letters of the superscripts, e.g. bc, suggest a significant difference in biting rates between
the sites indicated by the labelled Figures.
Same letters of the superscripts, e.g. aa, suggest no significant difference in biting rates between the sites indicated by the labelled Figures.

1. aa suggest a significant difference in biting rate between House 2 and School.
2. bc suggest a significant difference in biting rate between House 1 and School.
3. de suggest a significant difference in biting rate between House 2 and School.
4. fg suggest a significant difference in biting rate between School 1 and School 2.
5. hh suggest a significant difference in biting rate between House and School 2.

6. ij suggest a significant difference in biting rate between House and School 1.

4.1.2 Biting patterns of mosquitoes in AFigya-Sekyere sites

The hourly biting patterns of the various mosquito species were obtained by plotting the percentage proportion of the mosquitoes collected at a given hour on the ordinate axis against the specific hour that the particular proportion of mosquitoes was collected on the abscissa axis. Monthly biting rate graphs were obtained by plotting the biting rate value of each month on the ordinate axis against the corresponding month on the abscissa axis.

In general the hourly biting patterns of all three species of mosquitoes show a bell-shaped curvature. Biting activities always started at the beginning of the 12-hour collection cycle at 18 hours GMT and continued throughout the night and progressively declined till 06 hours the following morning. Differences in biting activity between the species of mosquitoes were found in the times of peak biting activity. Whereas the biting activities of *Anopheles* species normally peaked by midnight, those of the *Culex* species begin to peak earlier. Figs. 11 and 12 show the hourly biting activities of all three mosquito species: *A. gambiae s.l., A. funestus* and *Culex* species in both Afamanaso and Kona. At Afamanaso (Fig. 11) while the hourly biting activities of the *Culex* species began to peak at 21 hours, climaxing at about 22 hours, that of the *Anopheles* species peaked together between 23 and 01 hours with the highest biting activity at midnight. The biting patterns of the *Anopheles* species formed the actual bell-shape: a very small proportion of mosquitoes (<1%) began biting by 18 hours and a very high proportion of about 15% were biting during the climax with about 3% still biting by 06 hours. That of the *Culex* species on the other hand, gave a similar but more open curvature, with about half (6%) of the percentage of mosquitoes that bit at the peak (12%) already biting at 18 hours,

increasing only gradually to the peak and, declining even more gradually to 05 hours when about 4% of the mosquitoes were still biting. The biting activities of all three species of mosquitoes were about the same between 22 and 04 hours (Fig. 11).

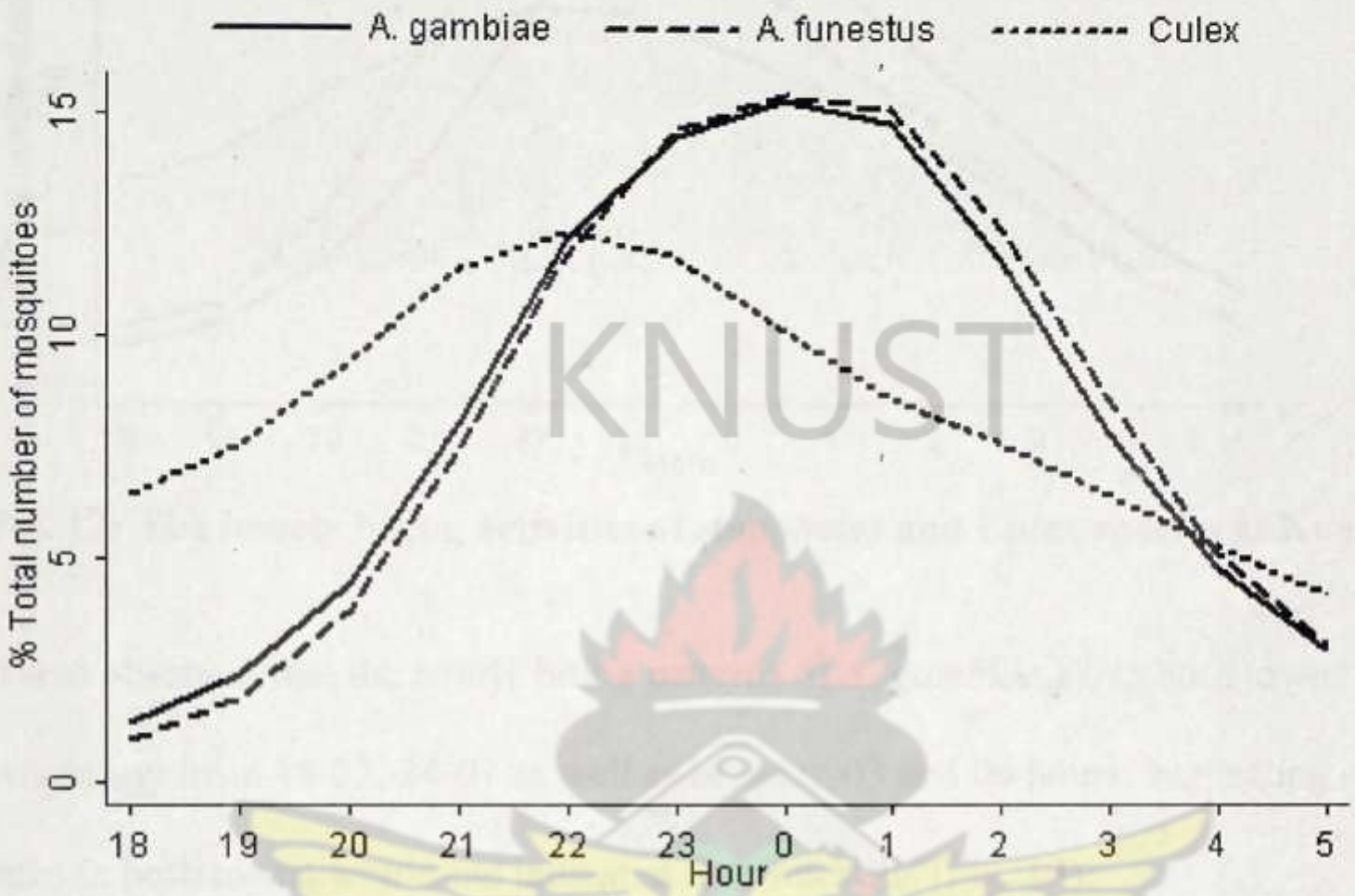


Fig. 11: The hourly biting activities of *Anopheles* and *Culex* species at Afamanaso.

At Kona (Fig. 12), a similar trend was observed, except that the peak of hourly biting activity of the *Culex* species coincided with that of *A. funestus*, at 22 hours. On the other hand, the biting activity of *A. gambiae s.l.* peaked at 23 hours. At both Afamanaso and Kona, however, the peaks of biting activities of the *Anopheles* species were higher than those of the *Culex* species (Figs 11 and 12).

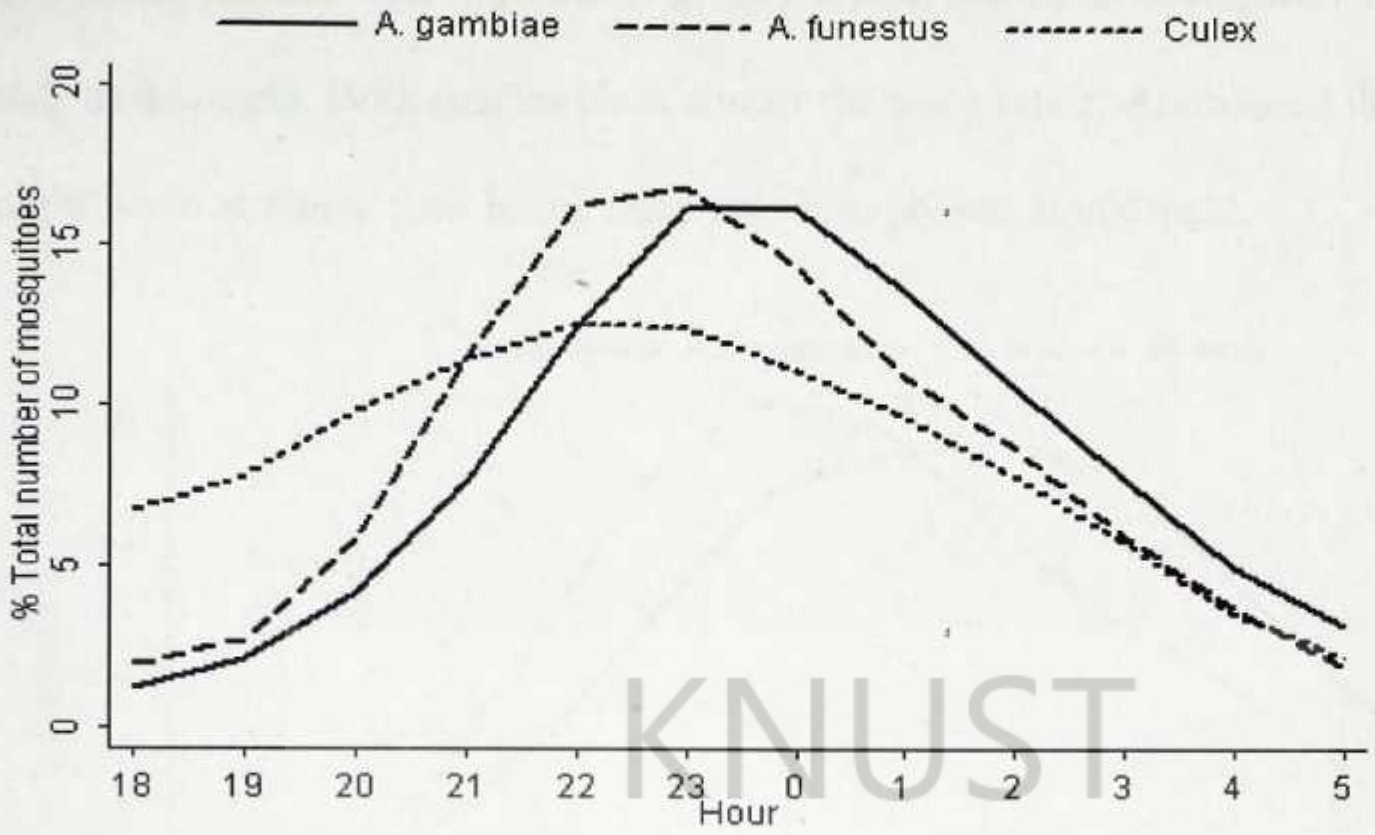


Fig. 12: The hourly biting activities of *Anopheles* and *Culex* species at Kona.

It was observed that the hourly biting patterns of *A. gambiae s.l.* in both towns were in synchrony from 18-22, 24-01 as well as between 03 and 06 hours, suggesting equal biting rates in both towns within the indicated time intervals (Fig. 13).

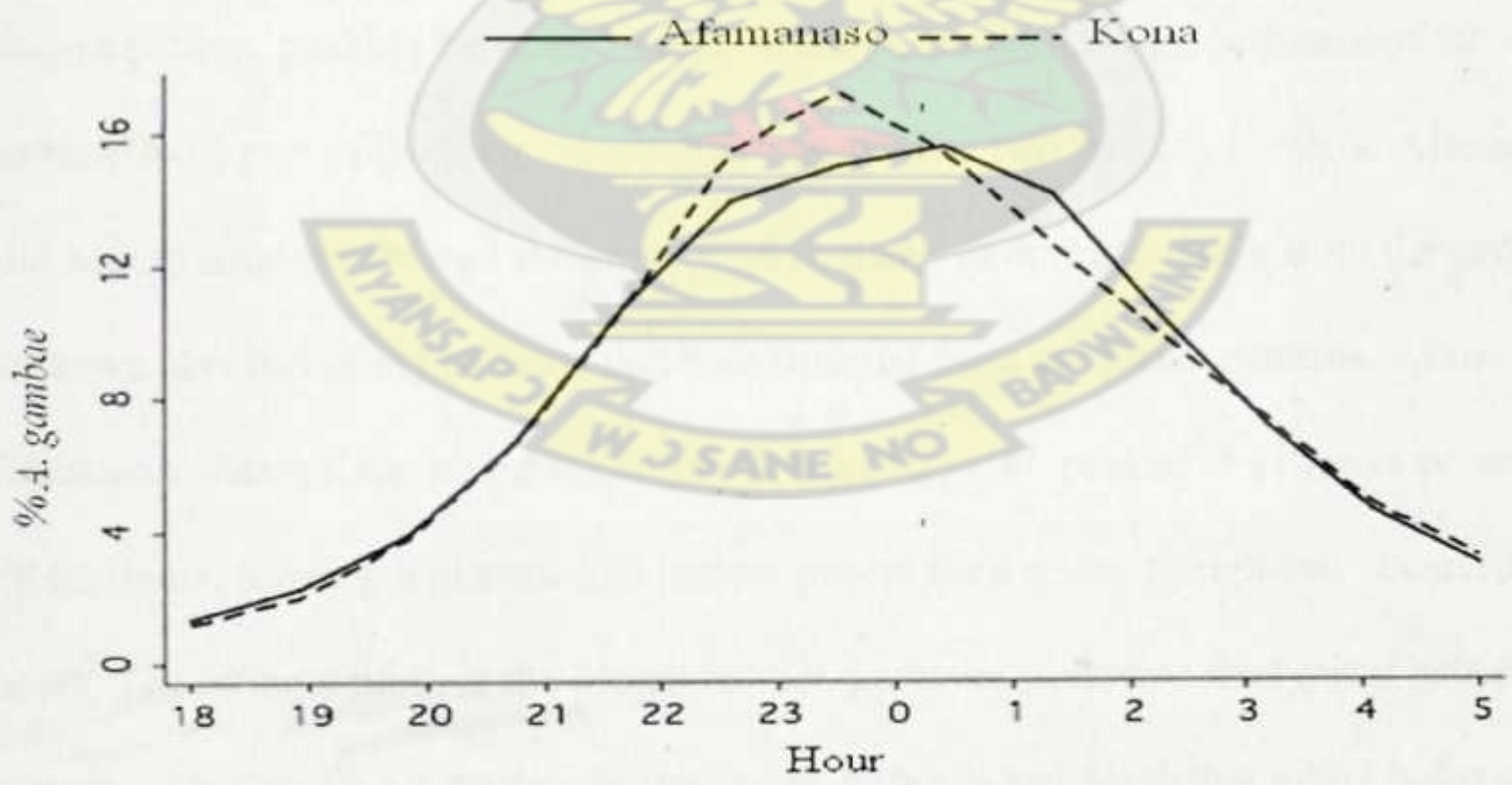


Fig. 13: The hourly biting activities of *A. gambiae s.l.* at Afamanaso and Kona.

The biting patterns of *A. funestus* (Fig. 14) in both towns, however, were in synchrony only at midnight. Both species bit at almost the same rate at Afamanaso throughout the night while at Kona, their biting rates only interphased at midnight.

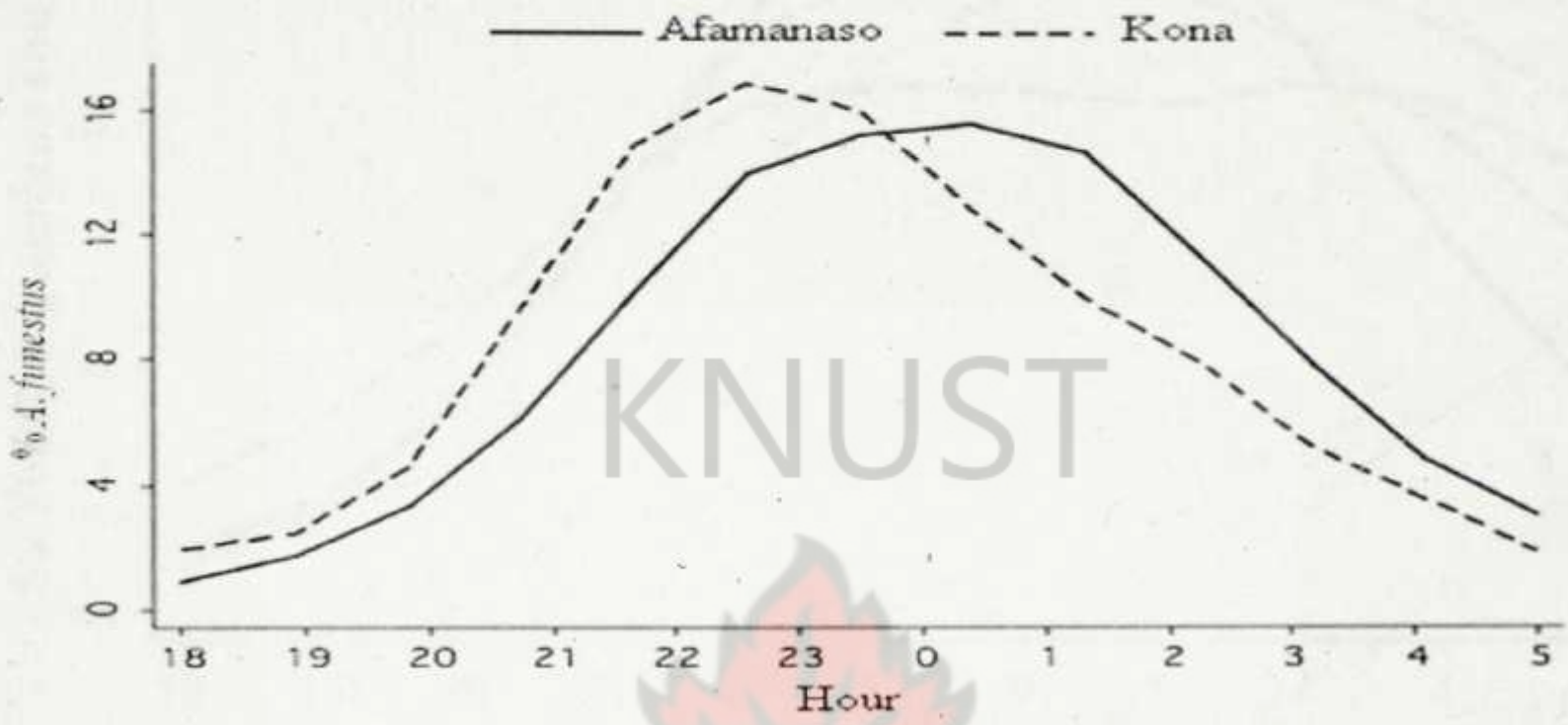


Fig. 14: The hourly biting activities of *A. funestus* at Afamanaso and Kona.

Fig. 15 compares the hourly biting activities of *A. gambiae s.l.* at different parts of Afamanaso. In the central part of the town, biting activities followed the typical bell-shaped pattern, peaking between 22 and 02 hours, similar to what pertained at the south-eastern (S-E) part of the town, about 500m away. The two farming hamlets; Afamanaso and Morso hamlets, located at distances of 1km and 2km respectively from the centre of the town also had biting patterns that both differed from the above patterns. At the Afamanaso hamlet, the biting activities of *A. gambiae s.l.* peaked at 21 hours persisting till 03 hours, forming a plateau-like pattern except for a minor trough that occurred at 01 hours. The biting pattern at the Morso hamlet, however, followed the typical bell-shaped pattern with peak biting activity beginning at 23 hours and persisting till 03 hours as in Boadi. Biting aetivities of *A. gambiae s.l.* in all the locations except the Morso Hamlet were found to be in synchrony by 21 hours (Fig. 15).

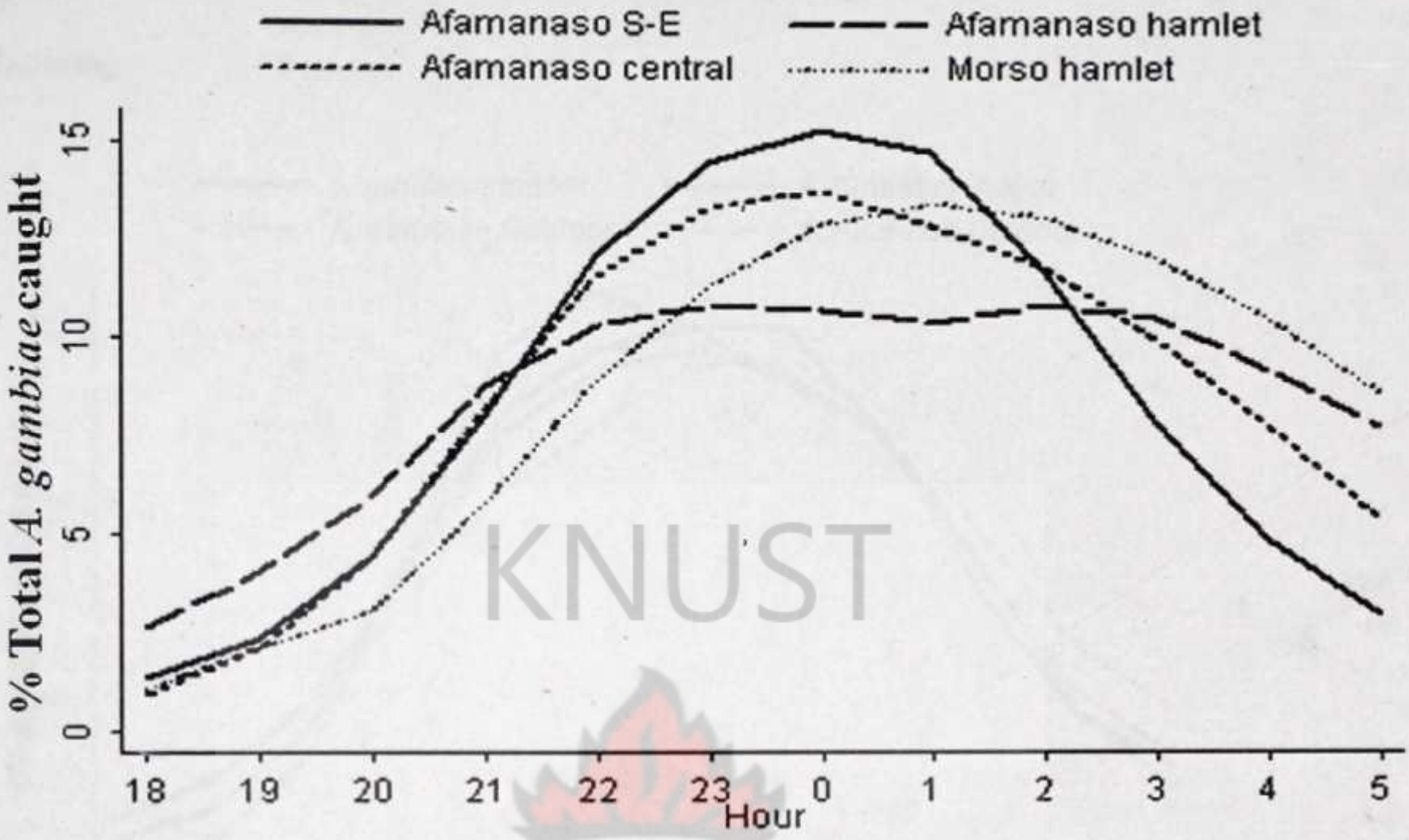


Fig. 15: Hourly biting patterns of *A. gambiae s.l.* at different collection sites at Afamanaso.

4.1.3 Indoor and Outdoor Biting Activities of Mosquitoes in ASD

The indoor and outdoor hourly biting patterns of both *A. gambiae s.l.* and *A. funestus* ascribed well to the bell-shaped curvature pattern (Fig. 16) known to be typical for *A. gambiae s.l.*, with peak biting activities taking place at midnight when their victims were dead asleep. A close observation shows that at Afamanaso, the indoor and outdoor biting patterns of *A. gambiae s.l* were very similar, beginning rigorous biting with a first peak at 22 hours. Intense outdoor biting activities peaked at midnight while indoor biting activities peaked an hour later at 01 hours. Both peaks declined gradually till 02 hours, after which biting activities progressively decreased to cease at 06 hours. Indoor biting activity of *A. funestus* rose to peak between 22 and 23 hours before declining slightly till

02 hours. Outdoor biting activities peaked between midnight and 02 hours, before declining.

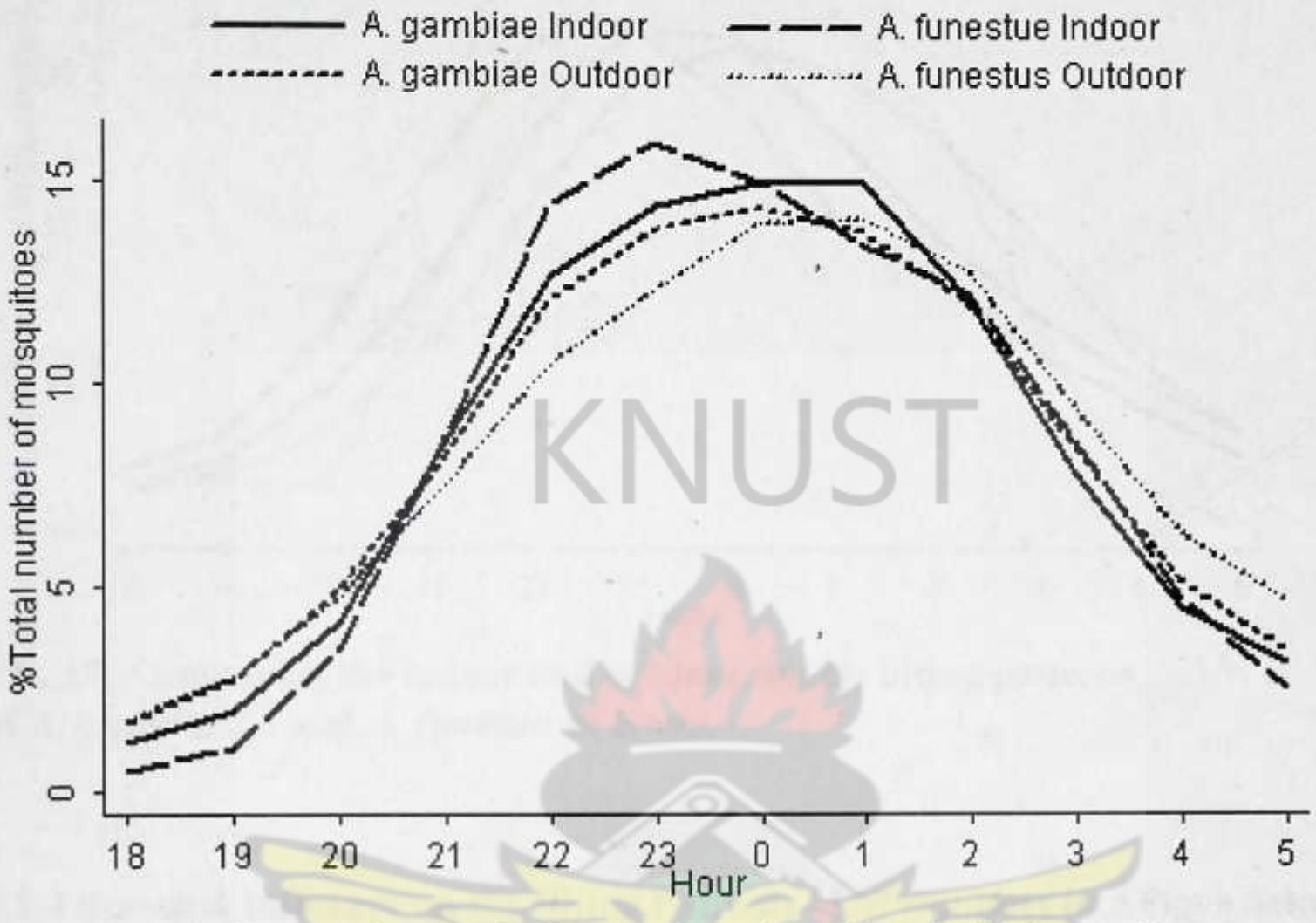


Fig. 16: Comparing the indoor and outdoor hourly biting patterns of *A. gambiae s.l.* and *A. funestus* at Afamanaso.

At Kona the indoor biting activities of *A. funestus* peaked an hour earlier than *A. gambiae s.l.* at 22 hours (Fig. 17). This peak was maintained for only an hour before declining at 23 hours, by which time *A. gambiae s.l.* reached its peak of biting which was maintained for one hour, before declining slightly at midnight. Even though there appeared to be slight variations in the time of peak biting activities, it could be observed from Figs. 16 and 17 that the most intensive biting activity by the two malaria vectors, began by 22 hours both indoor and outdoor. This rigorous biting activity was sustained till 02 hours when a gradual but progressive decline in biting was observed.

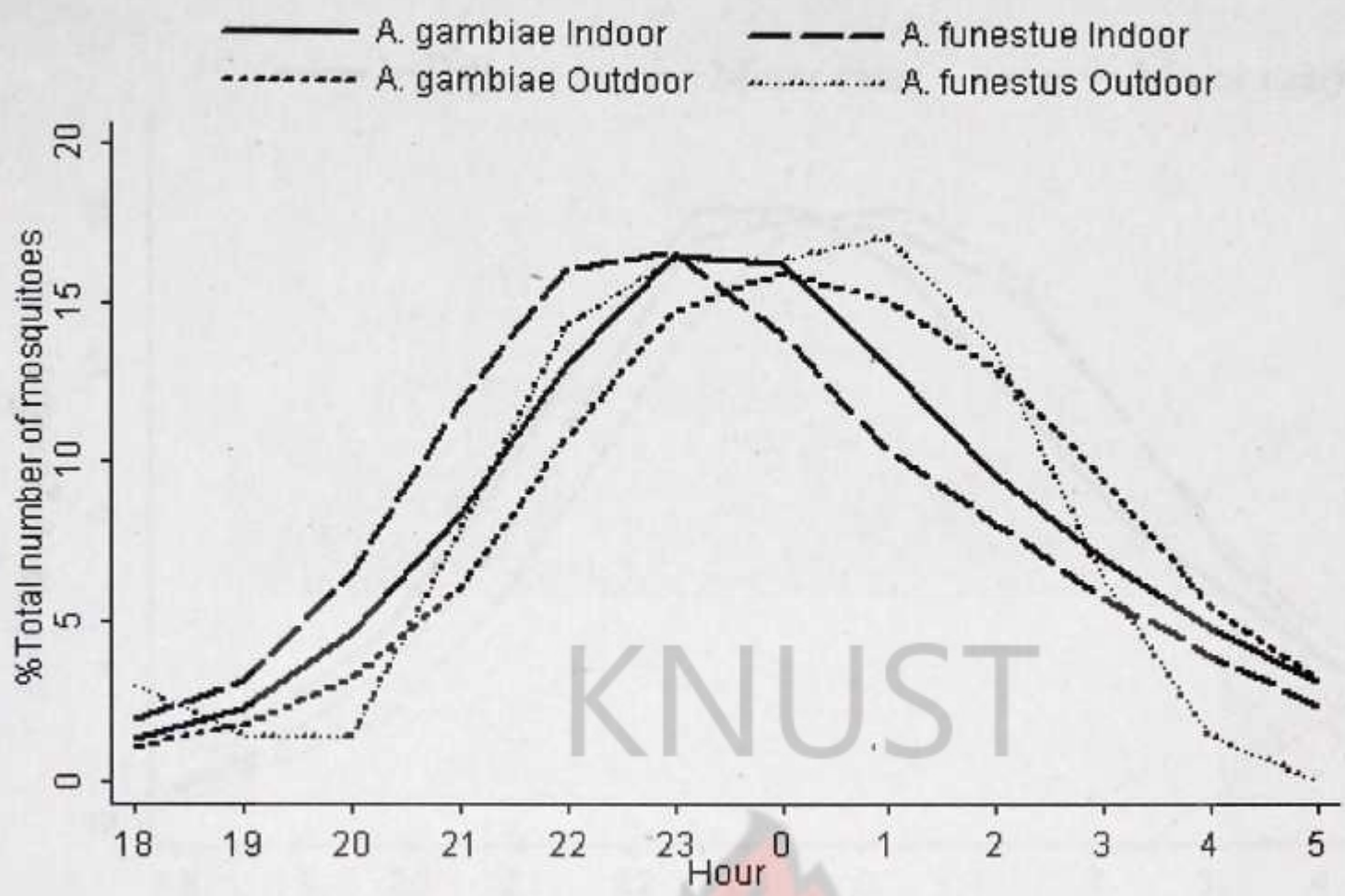


Fig. 17: Comparing the indoor and outdoor hourly biting patterns of *A. gambiae s.l.* and *A. funestus* at Kona.

4.1.4 Seasonal Influence on the Biting Patterns of Mosquitoes in AFigya Sekyere District.

The hourly biting pattern of *A. gambiae s.l.* at Afamanaso and Kona, shown in Fig. 18 was generally the same during the dry season, the minor rainy season as well as the major rainy season. *A. gambiae s.l.* was found to have peak biting activity at about the same rate at about midnight during the dry, minor and major rainy seasons. The biting pattern of *A. funestus* was largely similar to that of *A. gambiae s.l.*

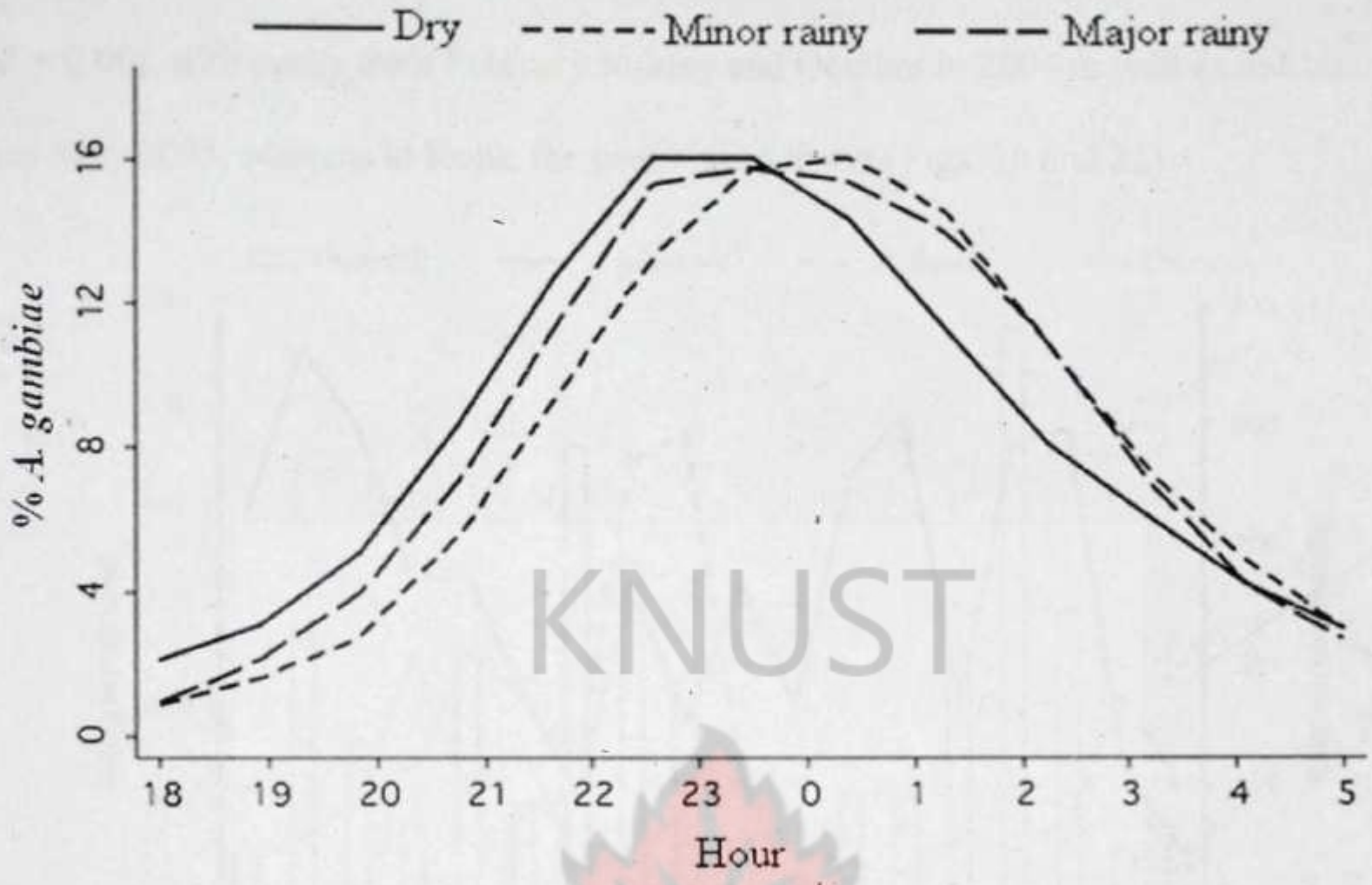


Figure 18: Hourly biting activities of *A. gambiae s.l.* at Afamanaso and Kona during the dry, major and minor rainy seasons in a year's cycle.

Monthly biting activities of all three species of mosquitoes monitored over 20 months were compared in Afamanaso and Kona as shown in Figures 20 and 21. Though biting activity was perennial for all three species, - biting activities of *A. gambiae s.l.* and *A. funestus* were higher compared to that of the *Culex* species at Afamanaso. Biting peaks of these mosquitoes either coincided with or were preceded by peaks of rainfall (Figs. 19-22). At Afamanaso, monthly biting peaks of the two malaria vectors; *A. gambiae s.l.* and *A. funestus,* coincided in July and October, 2004 (Fig. 19). Out of the three mosquito species, *A. gambiae s.l.* was the one that showed the least seasonal dip in biting activity during the period in both towns. The *Culex* species and *A. funestus* showed a clear dip in biting activity during the dry months of December to March. In general, *A. gambiae s.l.* had higher biting rates at Afamanaso than at Kona (P = 0.00); (Fig. 21). Similarly, the

monthly biting activity of *A. funestus* at Afamanaso was significantly higher than at Kona (P = 0.00), with peaks from February to May and October in 2004 as well as in March and May 2005, whereas at Kona, the peaks were lower (Figs. 20 and 22).

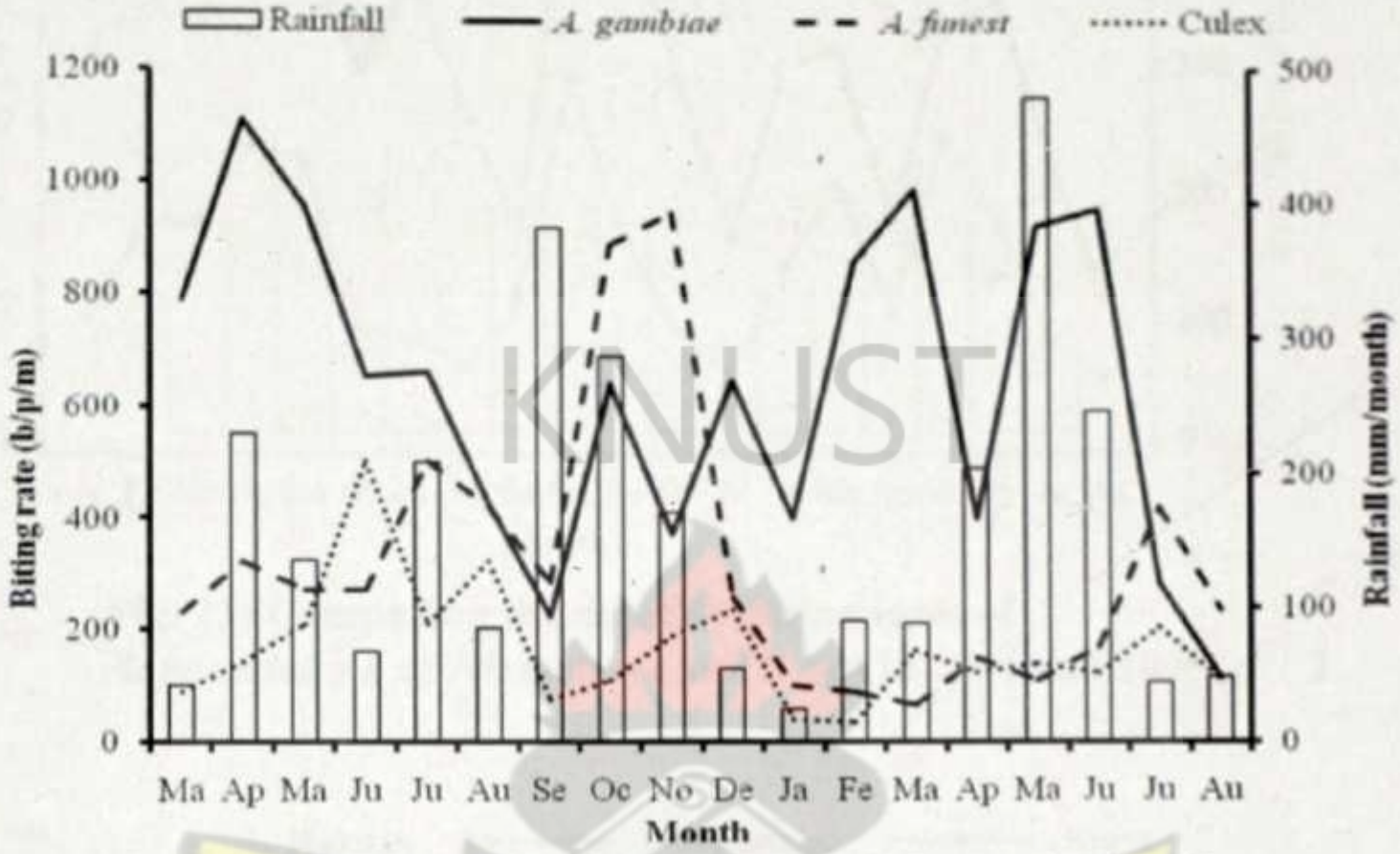


Fig. 19: Comparing the monthly biting activities of mosquito species at Afamanaso in 2004 and 2005.

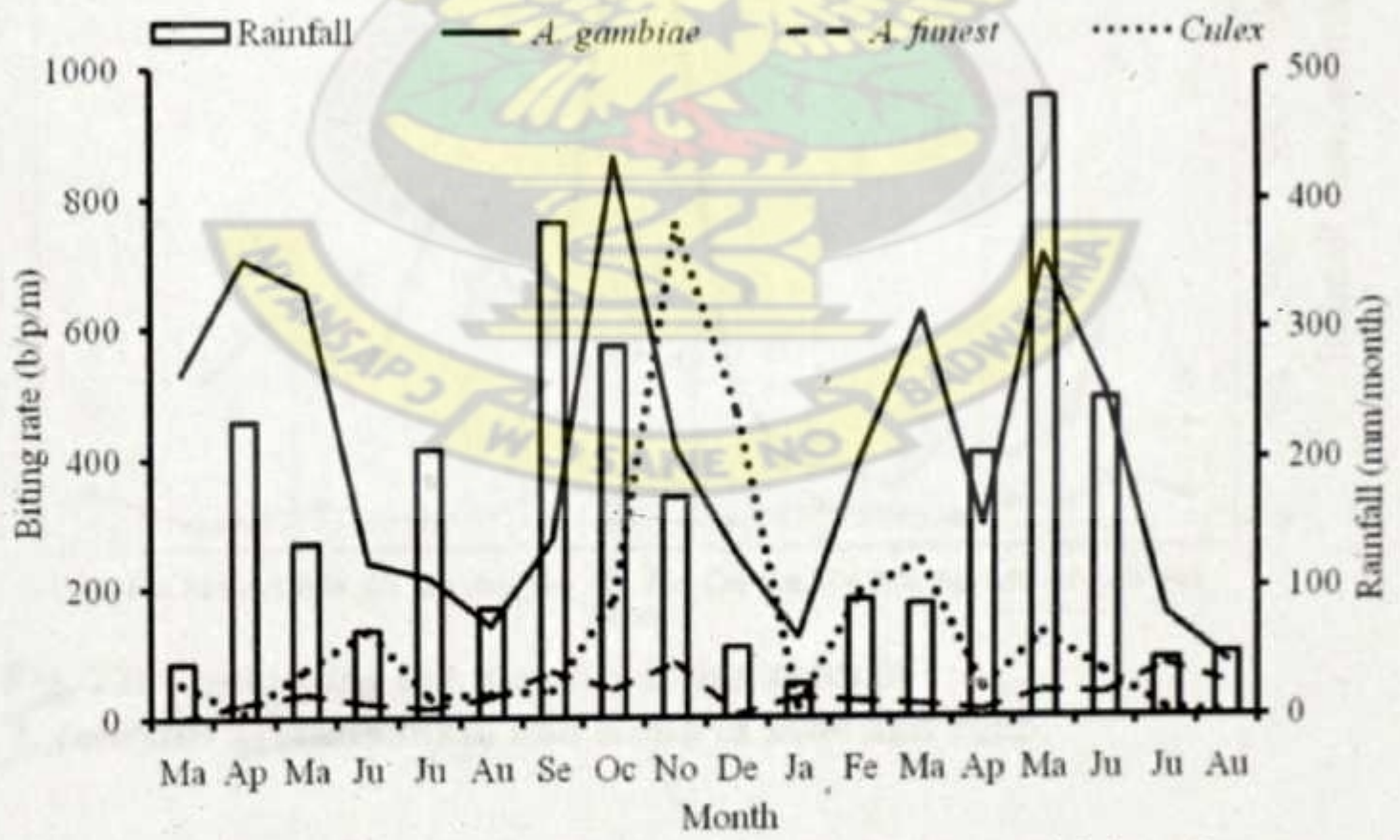


Fig. 20: Comparing the monthly biting activities of mosquito species at Kona in 2004 and 2005.

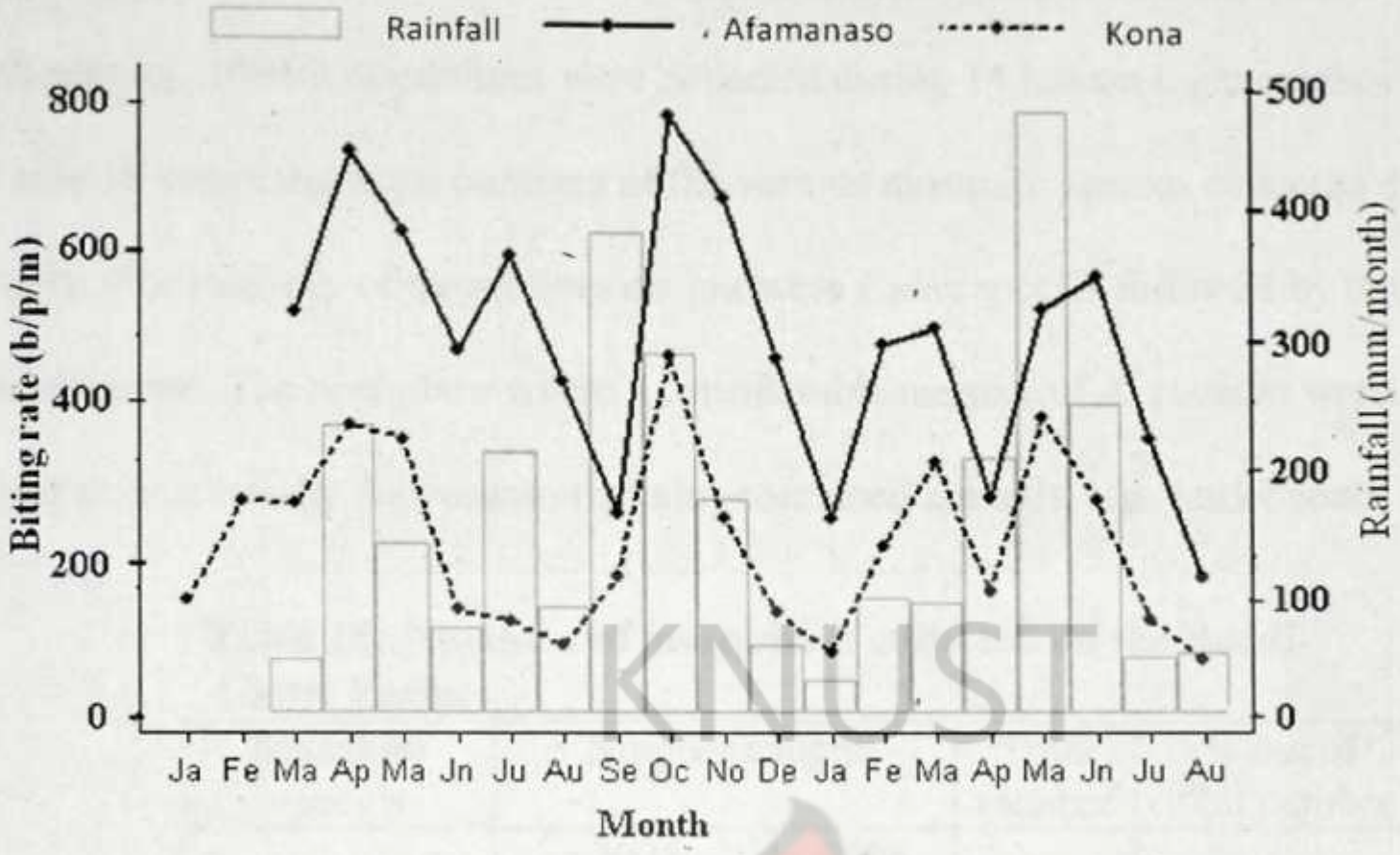


Fig. 21: Comparing the monthly biting rates of *A. gambiae s.l.* at Afamanaso and Kona in 2004 and 2005. 2

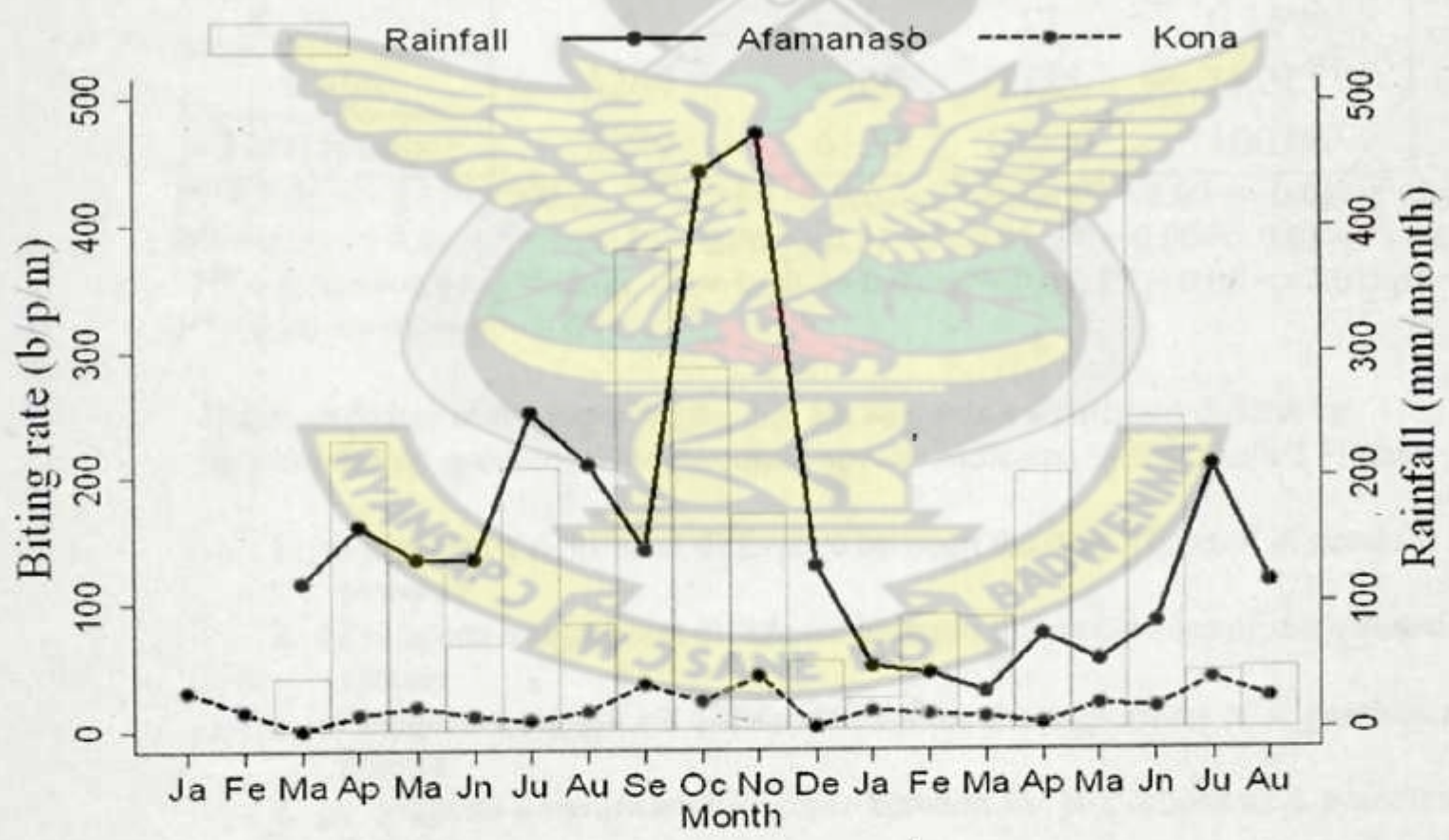


Fig. 22: Comparing the monthly biting rates of *A. funestus* at Afamanaso and Kona in 2004 and 2005.

4.2 Mosquitoes Collected at Peri-Urban Kumasi (Boadi and Anwomaso) sites

Altogether, 10,409 mosquitoes were collected during 14 human night catches at Boadi. Table 18 summarises the numbers of the various mosquito species collected during the study. The majority of mosquitoes caught were *Culex* species followed by the zoophilic *A. ziemanni.* The next place where a considerable number of *A. ziemani* were caught was on a farm at nearby Anwomaso that also contained animals, e.g. cattle, goats, etc.

Table 18: Numbers of mosquitoes collected on the Boadi Cattle Farm.

Mosquito species	Number caught		Total number	% out of total number
	2005	2006		
A. gambiae	388	773	1161	$11.15^{ae_{im}}$
A. ziemanni	630	874	1504	$14.45^{bc_{ko}}$
A. paludis	0	2	2	0.02^{gjls}
A. funestus	6	11	17	0.16^{hnpq}
Culex	3265	4460	7725	$74.21^{df_{rt}}$
Total number	4289	6120	10409	100.00

$P^{ab} = 0.00 => 0.01 > p^{ab} < 0.05$, $P^{cd} = 0.00 => 0.01 > p^{cd} < 0.05$, $P^{ef} = 0.00 => 0.01 > p^{ef} < 0.05$, $P^{gh} = 0.00 => 0.01 > p^{gh} < 0.05$, $P^{ij} = 0.00 => 0.01 > p^{ij} < 0.05$, $P^{kl} = 0.00 => 0.01 > p^{kl} < 0.05$, $P^{mn} = 0.00 => 0.01 > p^{mn} < 0.05$, $P^{op} = 0.00 => 0.01 > p^{op} < 0.05$, $P^{qr} = 0.00 => 0.01 > p^{qr} < 0.05$, $P^{st} = 0.00 => 0.01 > p^{st} < 0.05$.

Different letters of the superscripts, e.g. ab, suggests a significant difference between the proportions of the mosquito species indicated by the labelled Figures.

1. ab suggests a significant difference between the proportions of *A. gambiae* and *A. ziemanni*
2. cd suggests a significant difference between the proportions of *A. ziemanni* and *Culex* species
3. ef suggests a significant difference between the proportions of *A. gambiae* and *Culex* species
4. gh suggests a significant difference between the proportions of *A. paludis* and *A. funestus*
5. ij suggests a significant difference between the proportions of *A. gambiae* and *A. paludis*
6. kl suggests a significant difference between the proportions of *A. ziemanni* and *A. paludis*
7. mn suggests a significant difference between the proportions of *A. gambiae* and *A. funestus*
8. op suggests a significant difference between the proportions of *A. ziemanni* and *A. funestus*
9. qr suggests a significant difference between the proportions of *A. funestus* and *Culex* species

[10] st suggests a significant difference between the proportions of *A. paludis* and *Culex* species

Fig. 23 shows the numbers of mosquitoes collected from Anwomaso and the neighbouring Nana Siaw's farm. It is observed that the *Culex* species was the most abundant from these sites.

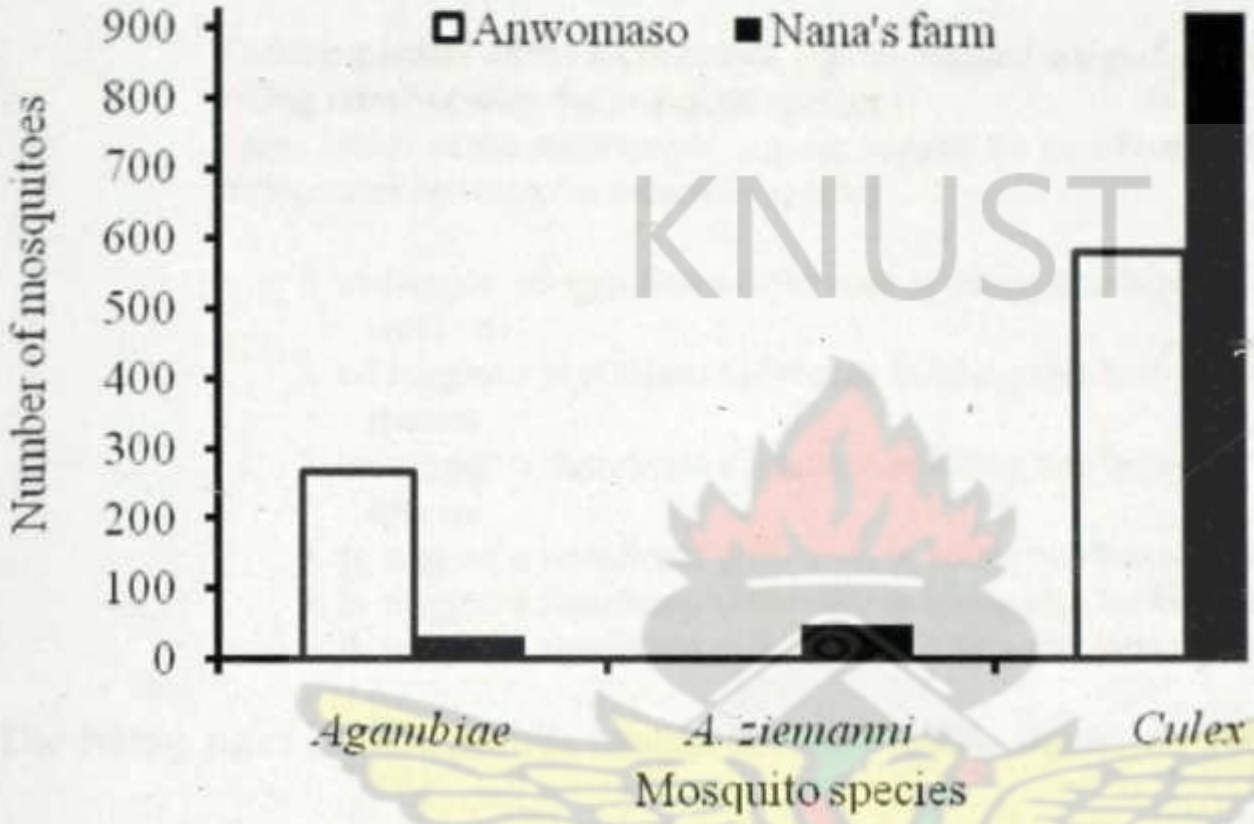


Fig. 23: The numbers of mosquitoes collected from Anwomaso sites

4.2.1 Biting Rate of Mosquitoes at Boadi and Anwomaso

Table 19 shows the average daily and monthly biting rates of all the mosquito species caught on the Boadi Cattle Farm. The biting rate of *A. gambiae s.l.* was lower than that of *A. ziemanni* while only 28 *A. funestus* were caught. The problem of nuisance, sleeplessness, etc. was very serious on the cattle farm as testified by the security guards who sleep there. This is caused by the very high biting rates of the *Culex* species and *A. ziemanni*.

Table 19: Biting rates of all mosquito species on the Boadi Cattle Farm

Mosquito species	Number caught	Daily biting rate (b/p/n)	Monthly biting rate (b/p/m)
A.gambiae	1161	10.37	315.62^{ad_j}
A.ziemanni	1504	13.37	406.93^{ab_g}
A.funestus	17	0.15	4.66^{fhk}
Culex species	7725	68.97	2069.20^{cc_i}

P^{aa} =0.11 => p^{aa}>0.05, P^{bc} =0.00 => 0.01>p^{bc}<0.05, P^{de}=0.00 => 0.01>p^{de}<0.05,
P^{fg} = 0.00 => 0.01>P^{fg}<0.05, P^{hi} = 0.00 => 0.01>P^{hi} <0.05, P^{jk} = 0.00 => 0.01>P^{jk}<0.05.

Different letters of the superscripts, e.g. ab, suggest a significant difference in biting rates between the mosquito species
Same letters of the superscripts, e.g. aa, suggest no significant difference in biting rates between the mosquito species

1. aa suggest no significant difference in biting rate between *A. gambiae* and *A. ziemanni*
2. cd suggest a significant difference in biting rate between *A. ziemanni* and *Culex* species
3. be suggest a significant difference in biting rate between *A. gambiae* and *Culex* species
4. fg suggest a significant difference in biting rate between *A. ziemanni* and *A.funestus*
5. hi suggest a significant difference in biting rate between *A.funestus* and *Culex* species
6. jk suggest a significant difference in biting rate between *A. gambiae* and *A.funestus*

The biting rates of mosquitoes caught at Anwomaso, shown in Table 20, gave a similar trend as on the Boadi Cattle Farm, with *Culex* species having the highest biting rates. However, due to the absence of cattle in Anwomaso town, no *A. ziemanni* was caught. Nevertheless, on Nana's farm, 1 km away from Anwomaso town, where there was livestock, *A. ziemanni* had higher biting rates than *A. gambiae s.l.* though on both farm and town, *Culex* species had the highest biting rate as on the Boadi Cattle Farm.

Table 20: Biting rates of mosquitoes collected at Anwomaso

Place	Daily biting rate (b/p/n)			Monthly biting rate (b/p/m)		
	A. gam -biae	*A. zie-manni*	*Culex*	*A. gam -biae*	*A. zie-manni*	*Culex*
Anwomaso	22.17^{a}	0	97.50^{c}	665.00^{a}	0	2925^{c}
Nana's farm	10.33^{b}	16	312.33^{d}	310^{b}	480	9370^{d}

$P^{ab} = 0.00 => 0.01 > p^{ab} < 0.05$, $P^{cd} = 0.00 => 0.01 > p^{cd} < 0.05$

Different letters of the superscripts, e.g. ab, suggests a significant difference in biting rates between the places where the mosquitoes were caught.

1. ab suggests a significant difference in biting rate of *A. gambiae s.l.* between Anwomaso and Nana' farm
2. cd suggests a significant difference in biting rate of *Culex* species between Anwomaso and Nana' farm

4.2.2 Biting Patterns of Mosquitoes in Boadi and Anwomaso

The hourly biting activities of *A. gambiae s.l.,* shown in Fig. 24, commenced at 18 hours to peak between 01 and 03 hours. The hourly biting activities of *A. ziemanni,* on the other hand, rose gradually from 18 hours and peaked by 01 hours, earlier than that of *A. gambiae s.l.,* then declined steadily but continued till 05 hours. The biting activities of the *Culex* mosquitoes, however, rose sharply from 18 hours to an early peak at 20 hours, which was maintained until 3 hours, then declined till 05 hours.

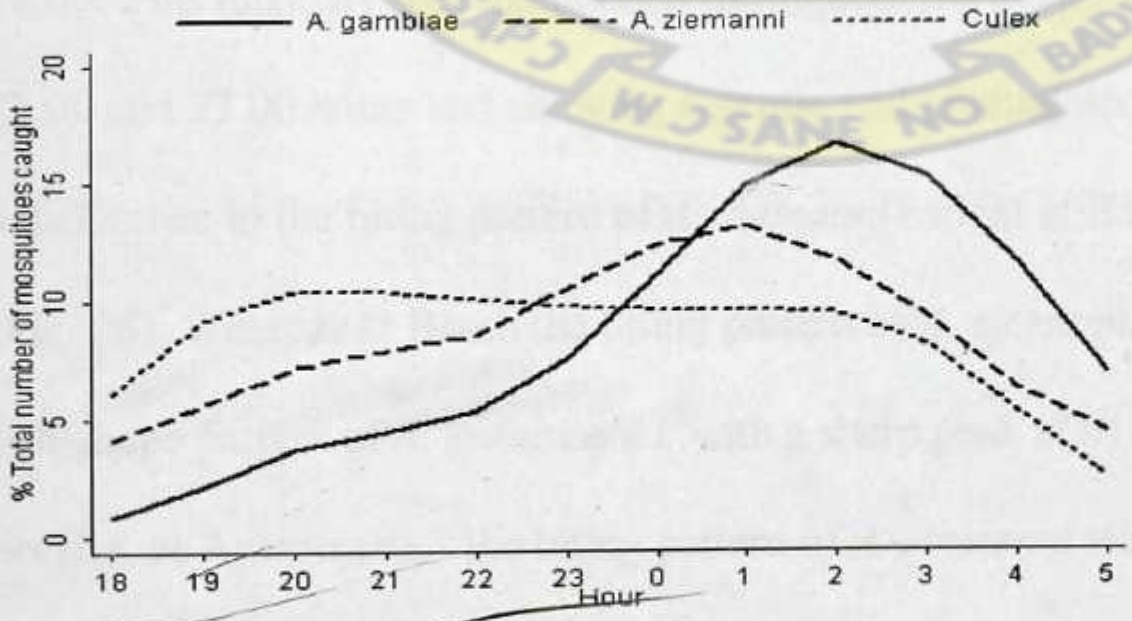


Fig. 24: Hourly biting patterns of *A. gambiae s.l.*, *A. ziemanni* and *Culex* on the Boadi Cattle Farm.

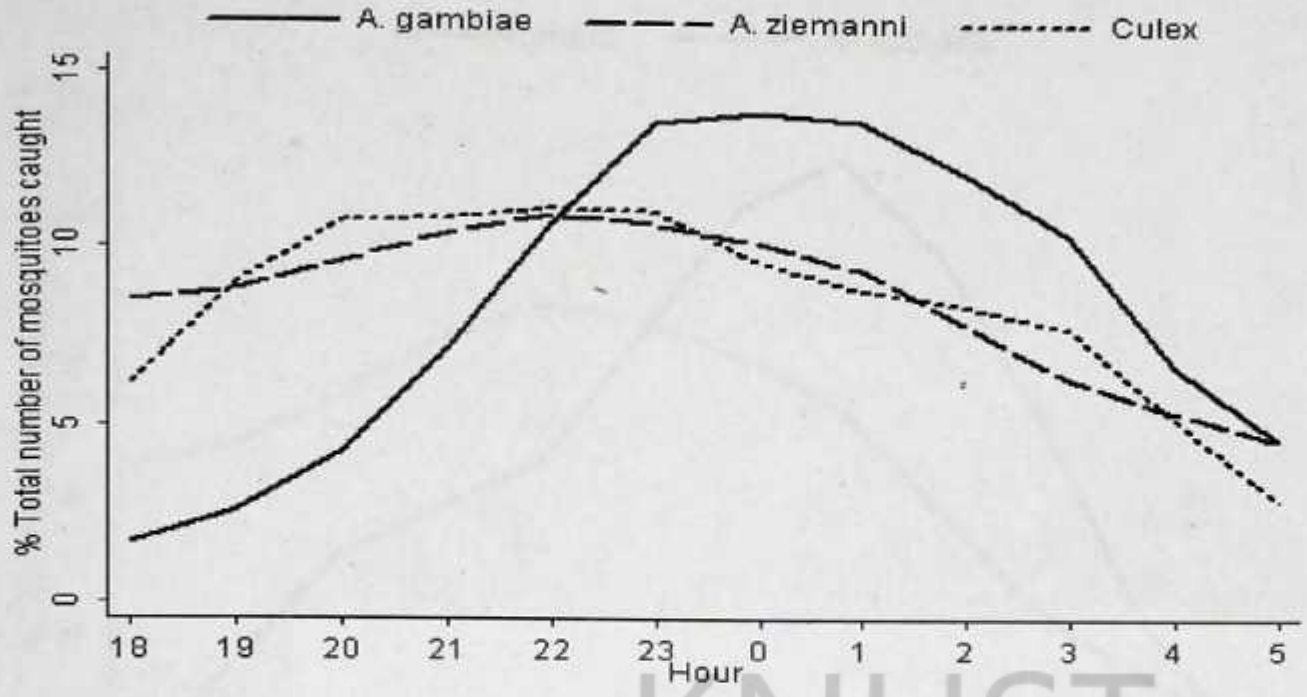


Fig. 25: Hourly biting patterns of *Anopheles* and *Culex* at Anwomaso.

Fig 25 shows the hourly biting pattern of *Anopheles* and *Culex* species at Anwomaso. The typical bell-shaped biting pattern of *A. gambiae s.l.* was similar to what was observed at Afamanaso and Kona, located at about 45km away. The hourly biting activities of both *A. ziemanni* and *Culex* species, on the other hand, appeared to have a similar pattern in terms of bites per hour. However, the biting activities of the *Culex* first peaked at 20.00 hours, maintaining a 03 hour biting plateau before declining steadily with a minor plateau till 3.00 hours before going into a sharp decline thereafter. *A. ziemanni* showed the highest biting intensity right from the beginning of the collection cycle at 18.00. This intensity was maintained throughout the cycle, with a minor peak between 22.00 and 23.00 hours and showing a gentle and gradual decline till morning. There was a difference in the biting pattern of *A. ziemanni* caught at Boadi from that at Anwomaso (Fig. 26). Whereas at Boadi the biting pattern of *A. ziemanni* was similar to the typical bell-shape pattern of *A. gambiae s.l.* with a sharp peak at 01 hours, proceeded by a sharp decline, at Anwomaso, the biting pattern of *A. ziemanni* was broader with a minor peak by 22.00 hours and followed by a gradual decrease in biting activity.

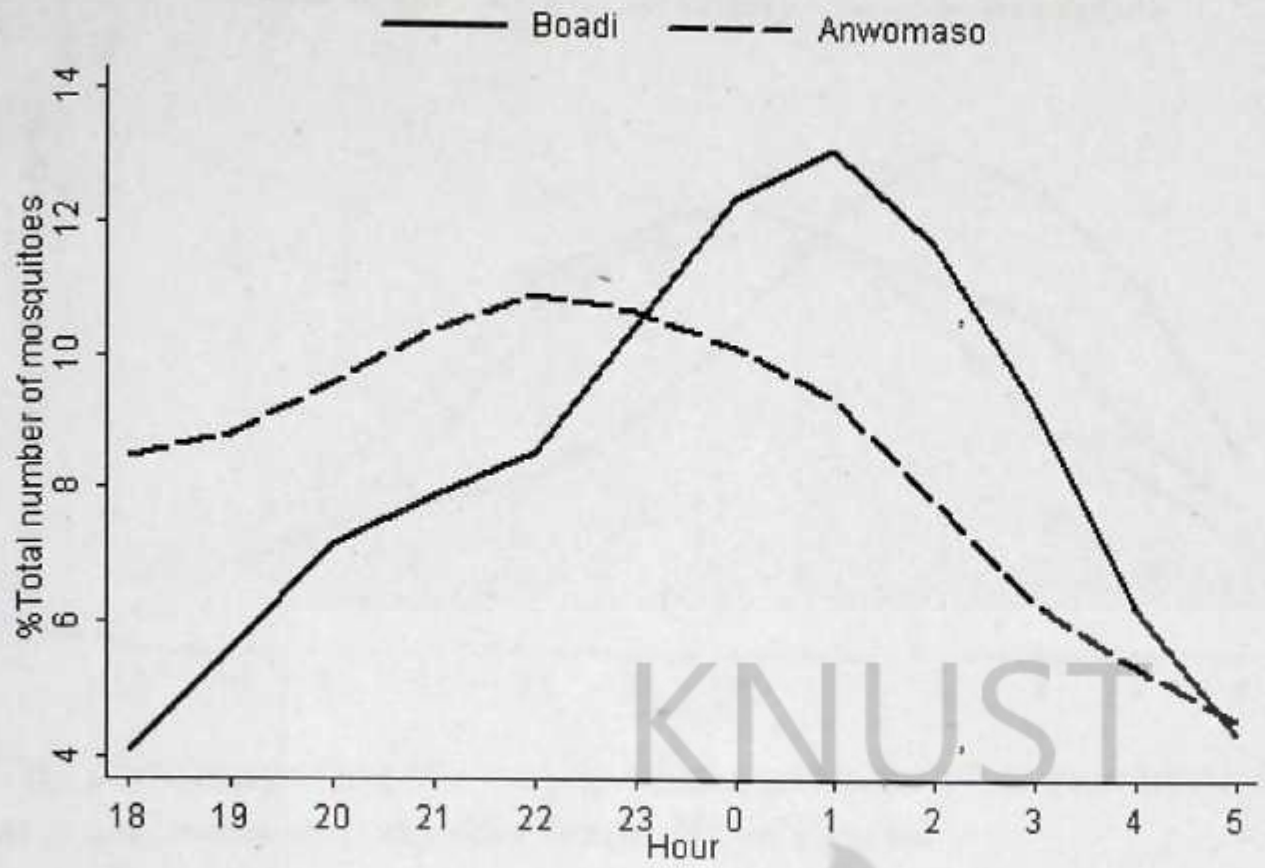


Fig. 26: Hourly biting patterns of *A. ziemanni* at Boadi and Anwomaso

Fig. 27 is a comparison of the hourly biting patterns of *A. gambiae s.l.* on the Boadi Cattle Farm with its biting patterns at Anwomaso and another farm (Nana's farm). These are located at 1 km and 2 km away from the Boadi Cattle Farm. The biting patterns at Anwomaso and Nana's farm more similar to the typical bell-shaped biting pattern of this species as was also observed at Afamanaso and Kona where peak biting occurs at midnight than that observed at Boadi.

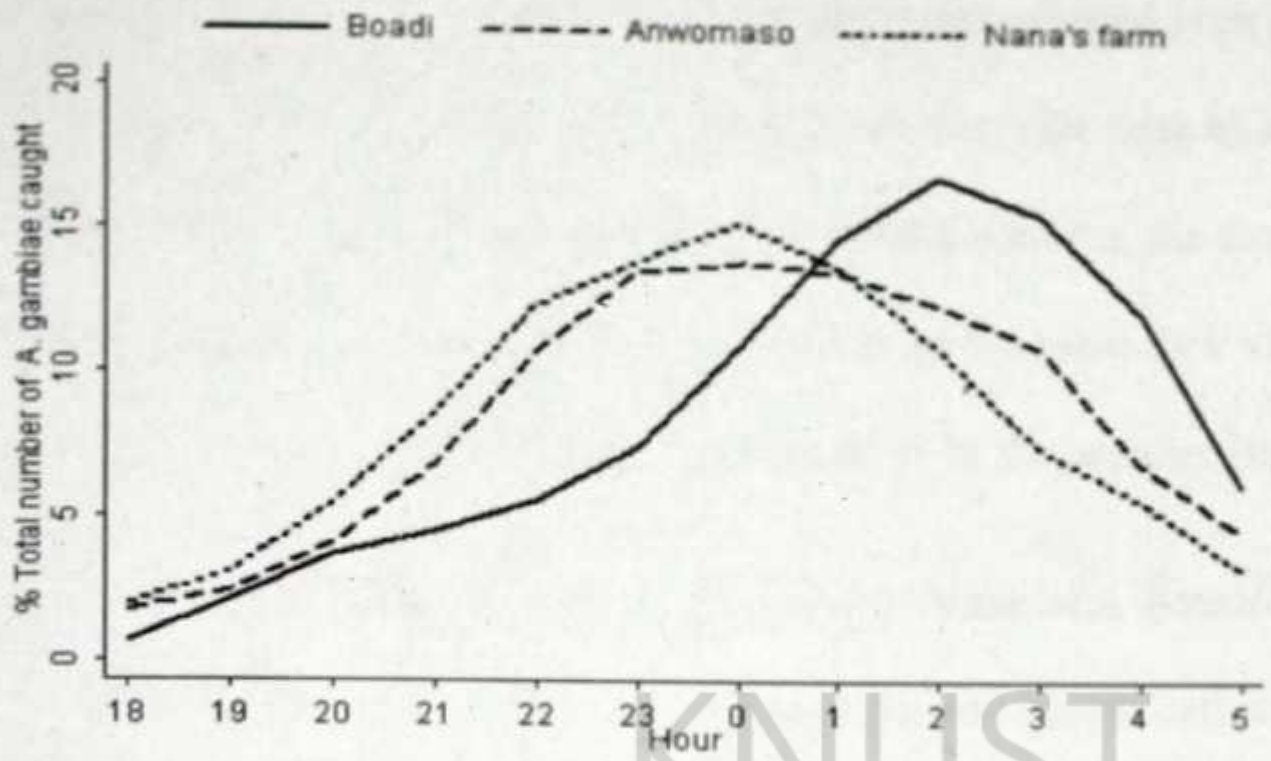


Fig. 27: Comparing the hourly biting patterns of *A. gambiae s.l.* at Boadi with those at Anwomaso and another farm; Nana's farm.

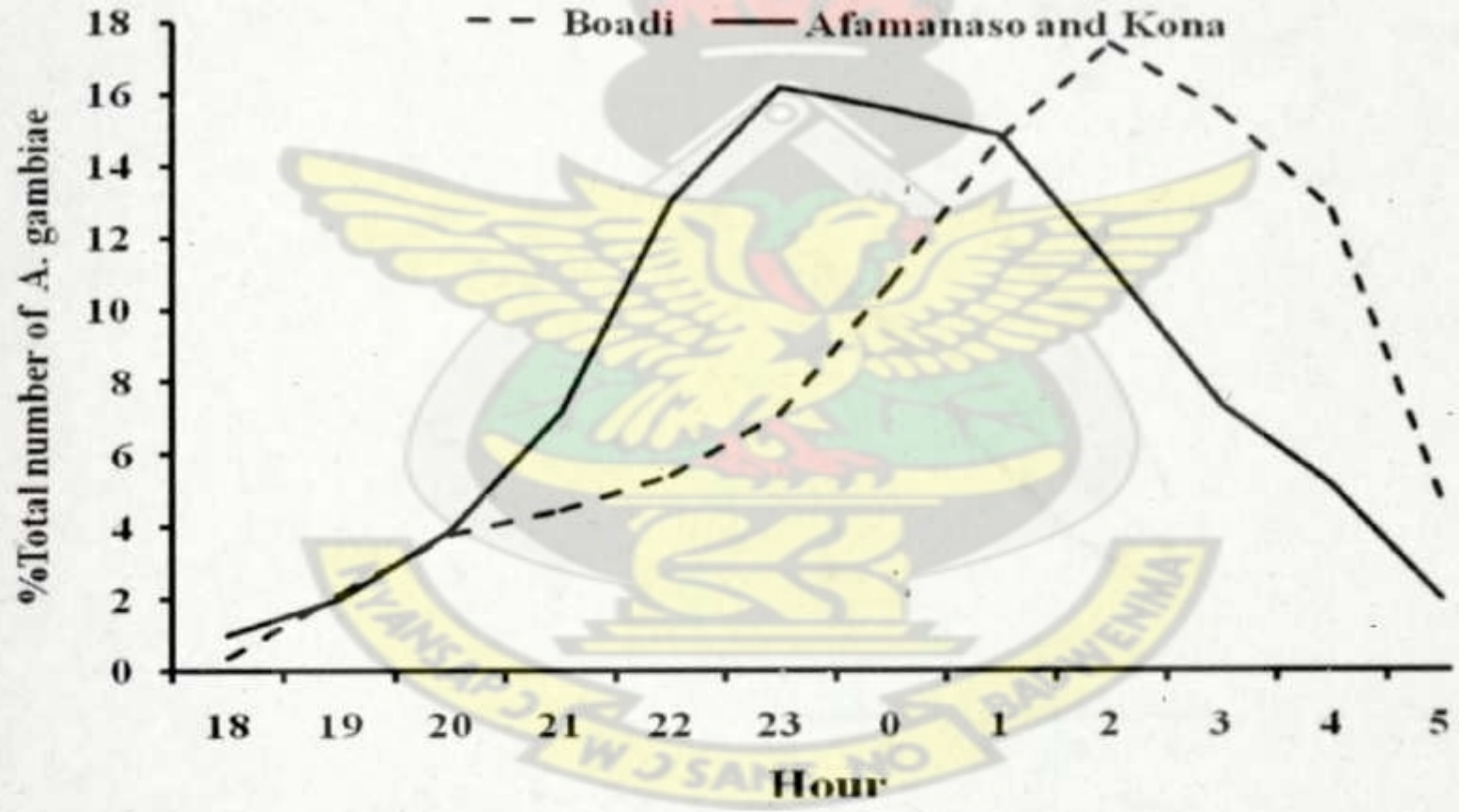


Fig. 28: Comparing the hourly biting pattern of *A. gambiae s.l.* at the Boadi Cattle Farm with those at Afamanaso and Kona.

Fig. 28 shows how the biting pattern of *A. gambiae s.l.* on the Boadi Cattle Farm differs from the typical bell-shaped curvature characteristic of this mosquito. From the beginning, both patterns were similar, coinciding between 18.30 and 20 hours. Then as the biting activity in the typical pattern took a sharp ascent to peak at 23 hours in a time

interval of three hours, the peculiar Boadi pattern progressed with a gradual ascent, taking seven hours to reach its peak at 02 hours when the peak biting activity in the bell-shaped pattern began to decline. However, in both patterns, the duration of peak biting activity was about three hours and occurred at the time that their victims were asleep, i.e. 23 to 01 hours in the typical pattern and 01 to 03 in the peculiar Boadi pattern.

In Fig. 29 one observes that the hourly biting patterns of *A. gambiae s.l.* at Afamanaso, Kona and Anwomaso were all well-correlated to the typical bell shape curvature with peaks between 23 and 01 hours whereas that on the Boadi Cattle Farm was totally different, peaking at 02 hours.

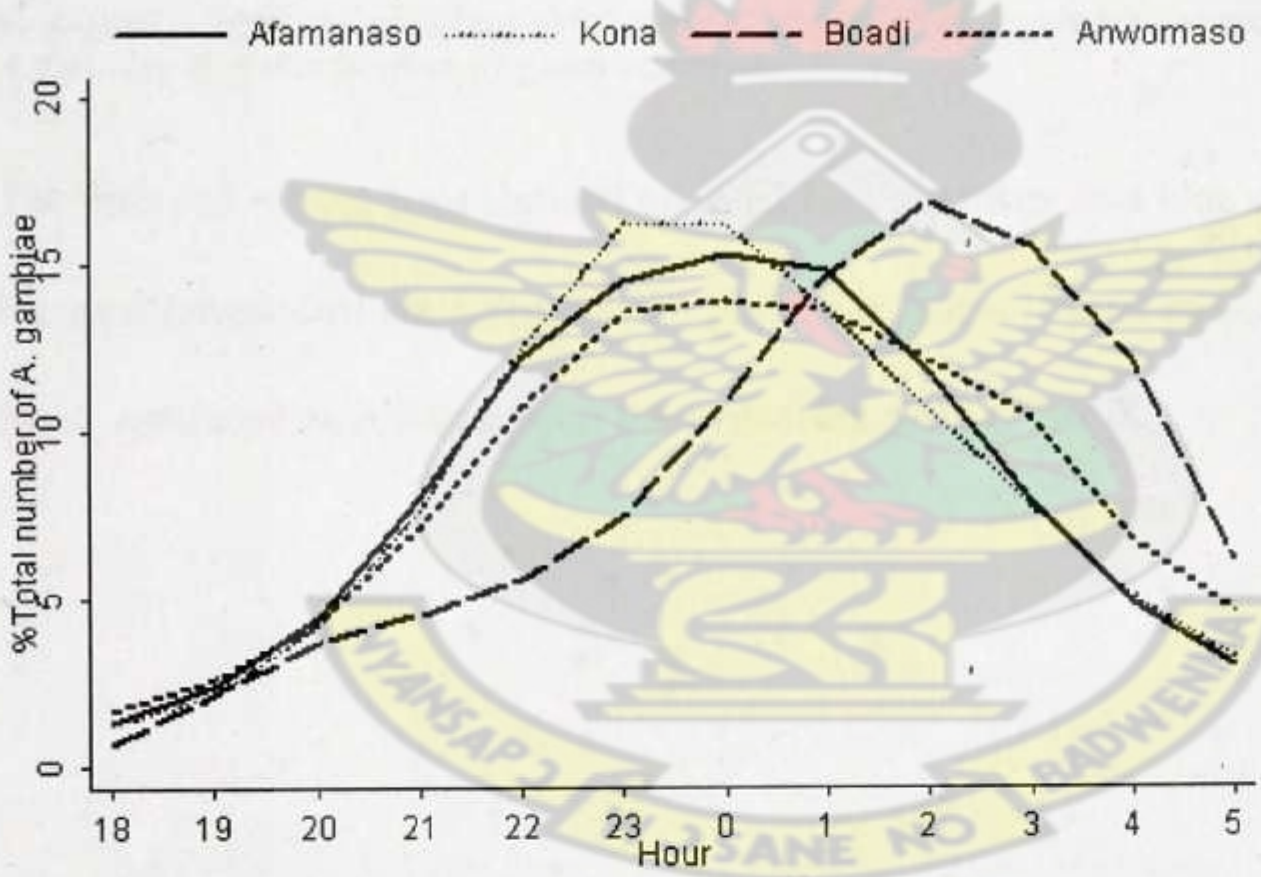


Fig. 29: Hourly biting pattern of *A. gambiae s.l.* at the four study sites: Afamanaso, Kona, Boadi and Anwomaso.

4.3. Salivary Gland Dissection and ELISA Tests to Determine Sporozoite Rate

In this study, an attempt was made to identify mosquitoes with infective sporozoites either by dissection or by ELISA. In dissection, living sporozoites oozed as spindle-shaped "wrigglers" when the dissected salivary glands were gently pressed under cover-

slips on a slide and observed at high power (x400) under the microscope (plate R). Salivary gland dissection of 2858 *Anopheles* species yielded 168 infected (5.88%) mosquitoes (Table 21).

Table 21: Summary of results of salivary gland dissections.

species	Gland positive	Gland negative	Total	% Positive
A. gambiae	149	2154	2303	6.47[a]
A. funestus	19	536	555	3.42[b]
Grand Total	168	2690	2858	5.88

P^{ab} = 0.04, =>0.01< P^{ab}<0.05

Different letters of the superscripts, e.g. ab, suggests a significant difference between the proportions of the mosquito species found to be positive by gland dissection.

ab suggest a significant difference between the proportion of *A. gambiae s.l.* and *A. funestus* that was positive by gland dissection.

The infected mosquitoes showed up in ELISA positives as a blue colouration similar to the positive control on the microtitre plate. The non-infected mosquitoes, on the other hand, appeared as colourless on the microtitre plate (Plate X).

ELISA results

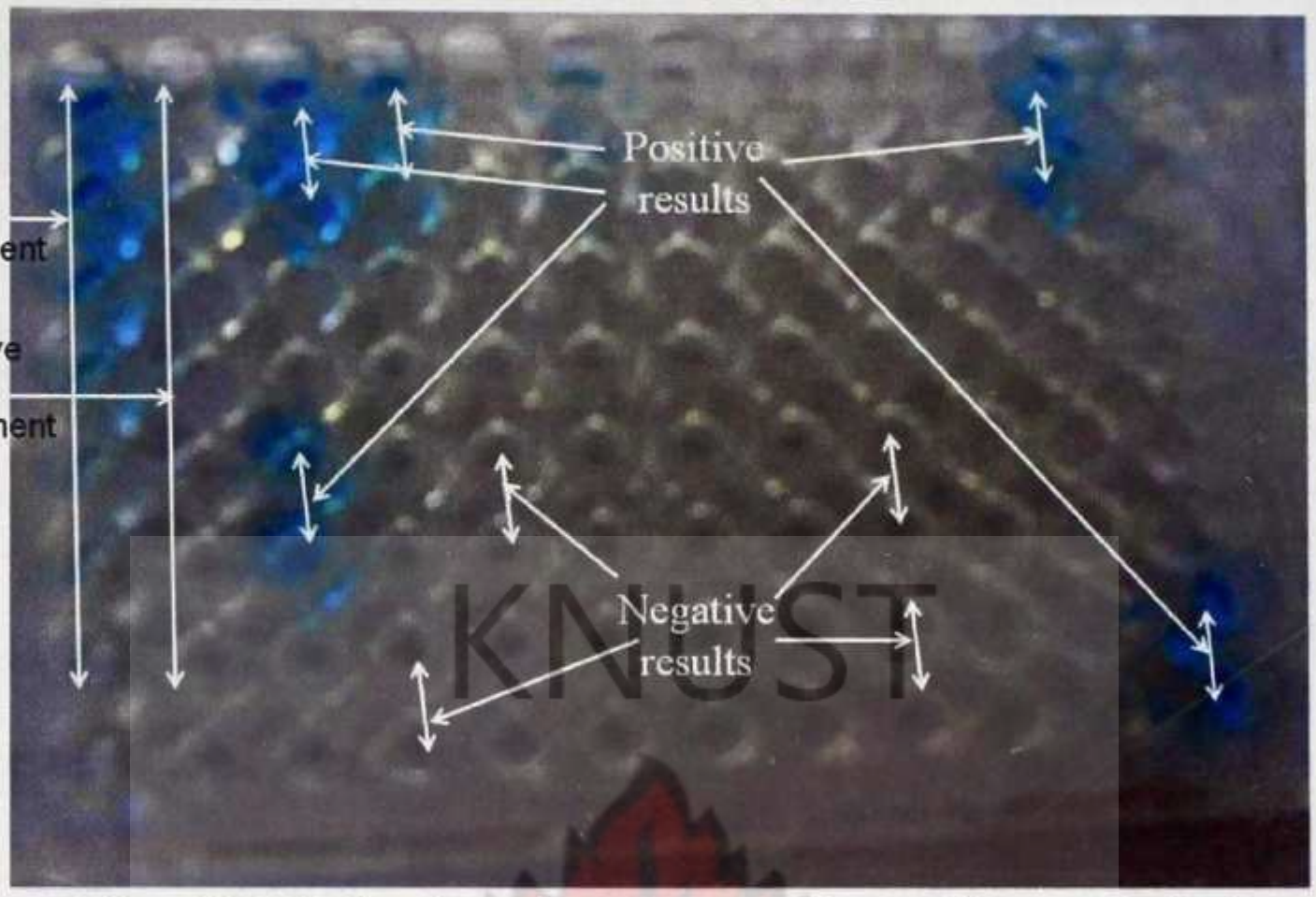


Plate X: ELISA micro-titre plate showing positive results in deep blue. Extreme left column shows results of positive control with decreasing colour intensity as CSP concentration decreased. The next column is the negative control with no positive result.

ELISA tests were carried out on 4,634 *Anopheles* mosquitoes and 6.99% of them were found to be infected by *P. falciparum* (Table 22).

Table 22: Summary of results of ELISA tests.

	ELISA			
species	Positive	Negative	Total	% Positive
A. gambiae	267	3,211	3,478	7.68[a]
A. funestus	57	1,099	1,156	4.93[b]
Grand total	324	4,310	4,634	6.99

P^{ab} $0.04 => 0.01 < P^{ab} < 0.005$

Different letters of the superscripts, e.g. ab, suggests a significant difference between the proportions of the mosquito species found to be positive by ELISA.

ab suggests a significant difference between the proportions of *A. gambiae s.l.* and *A. funestus* that was positive by ELISA.

In a comparative analysis of ELISA positive and dissected salivary gland positive results, it was observed that where salivary glands have been removed from *Anopheles*

mosquitoes during dissection, the number of *A. gambiae s.l.* infected by *P. falciparum* using ELISA tests was lower than those whose salivary glands had not been removed (Table 23). Similar analysis of ELISA results of *A. funestus* gave a significant difference in infection between mosquitoes whose salivary glands were removed and those with intact glands (Table 24).

Table 23: Effect of salivary gland removal from *A. gambiae s.l.* on the ability of ELISA to detect their infection by P. *falciparum*.

Glands	Removed	not removed
Total tested	2302	1175
ELISA 1 positive	162	105
% positive	7.03[a]	8.94[a]

$P^{aa} = 0.06 \Rightarrow p^{aa} > 0.05$

aa Suggest that the ability of ELISA to detect the presence of circumsporozoite proteins in *A.gambiae s.l.* whose salivary glands had been removed was not significantly lower than in *A. gambiae s.l.* whose salivary glands had not been removed.

Table 24: Effect of salivary gland removal from *A. funestus* on the ability of ELISA to detect their infection by *P. falciparum*.

Glands	Removed	not removed
Total tested	554	602
ELISA 1 positive	19	38
% positive	3.423[a]	6.312[b]

$P^{ab} = 0.02 \Rightarrow 0.01 < p^{ab} < 0.05$

ab Suggest that the ability of ELISA to detect the presence of circumsporozoite proteins in *A. funestus* whose salivary glands had been removed was significantly lower than in *A. funestus* whose salivary glands had not been removed.

In a similar analysis, it was found that only 3.11% (67 out of 2154) of members of *A.gambiae s.l.* were infected by *Plasmodium* species using ELISA but was negative by salivary gland dissection, whereas 35.14% (52 out of 148) of members of this species were infected by salivary gland dissection that were negative by ELISA (Table 25).

Table 25: Number of *A. gambiae s.l.* found to be infected by *Plasmodium* Species using either salivary gland dissection or ELISA test.

	ELISA		
Gland	Positive	Negative	Total
Negative	67	2087	2154
Positive	96	52	148
Total	163	2139	2302

In the same vein, it was observed that 1.86%, (10 out of 536) of *A. funestus* were infected by *Plasmodium* species using ELISA which was negative by salivary gland dissection whereas 50% (9 out of 18) of members of this species was infected by salivary gland dissection that was negative by ELISA (Table 26)..

Table 26: Number of *A. funestus* found to be infected by *Plasmodium* species using either salivary gland dissection or ELISA test.

	ELISA 1		
Gland	Positive	Negative	Total
Positive	9	9	18
Negative	10	526	536
Total	19	535	554

Table 27: Sensitivity and specificity of ELISA using salivary gland dissection as the gold standard:

	Gland dissection	
ELISA	Positive	Negative
Positive	A 105	B 77
Negative	C 61	D 2613

The sensitivity of ELISA, with salivary gland dissection as the gold standard, calculated from Table 27 was [A/(A+C)]x100= [105/(105+61)]*100 = 63.25%. Similarly, specificity was [D/(B+D)]x100 = [2613/(77+2613)]*100 = 97.14%.

4.4 Sporozoite Rate

Which is defined as the proportion of the mosquitoes carrying infective sporozoites of *Plasmodium* species, the sporozoite rate is the ratio of the number of mosquitoes infected to the total number caught, usually expressed as a percentage.

a) Afamanaso and Kona

The combined sporozoite rate of *A. gambiae s.l.* at Afamanaso and Kona was 10.11% (95% CI: ±1.38). The sporozoite rate of *A. gambiae s.l.*, indoor was 10.66% (95% CI: ± 1.84) whereas its outdoor sporozoite infection rate was 9.25 (P = 0.51; CI: ± 4.11). The sporozoite rate of *A. funestus* was 6.17 (95% CI: ± 1.70). Indoors the sporozoite rate was 7.37% (95% CI: ± 5.26) while outdoors, it was 4.75% (P = 0.90; 95% CI: ± 2.02). The sporozoite rates of the two malaria vectors at Afamanaso and Kona as well as the places where mosquitoes were collected are shown in Tables 28 and 29.

Table 28: The sporozoite rates of *A. gambiae s.l.* and *A. funestus* at the various sites of mosquito collection in Afamanaso and Kona.

Town	Site	Number caught		Sporozoite rate (%)	
		A. gambiae	*A. funestus*	*A. gambiae*	*A. funestus*
Afamanaso	House 1	1,241	648	10.14^{ab}	4.26^{hi}
	House 2	307	110	11.64^{ac}	3.65^{gi}
	School	407	235	9.57^{bc}	7.63^{gh}
Kona	House	485	38	10.82^{de}	10.61^{kl}
	School 1	564	106	10.90^{df}	8.80^{jl}
	School 2	475	20	7.49^{ef}	10.53^{jk}

$P^{aa} = 0.57 \Rightarrow P^{aa} > 05$, $P^{bb} = 0.08 \Rightarrow P^{bb} > 05$, $P^{cc} = 0.42 \Rightarrow P^{cc} > 05$, $P^{dd} = 0.23 \Rightarrow P^{dd} > 05$,
$P^{ee} = 0.23 \Rightarrow P^{ee} > 05$, $P^{ff} = 0.18 \Rightarrow P^{ff} > 05$, $P^{gg} = 0.10 \Rightarrow P^{gg} > 05$, $P^{hh} = 0.43 \Rightarrow P^{hh} > 05$,
$P^{ii} = 0.19 \Rightarrow P^{ii} > 05$, $P^{jj} = 0.88 \Rightarrow P^{jj} > 05$, $P^{kk} = 0.89 \Rightarrow P^{kk} > 05$, $P^{ll} = 0.985 \Rightarrow P^{ll} > 05$

Same letters of the superscripts, e.g. aa, suggest no significant difference in sporozoite rates between the sites indicated by the superscripts.

1. aa suggest no significant difference in the sporozoite rate of *A. gambiae s.l.* between House 1 and House 2.
2. bb suggest no significant difference in sporozoite rate of *A. gambiae s.l.* between House 1 and School.

3. cc suggest no significant difference in sporozoite rate of *A. gambiae s.l.* between House 2 and School.
4. dd suggest no significant difference in sporozoite rate of *A. gambiae s.l.* between House and School 1.
5. ee suggest no significant difference in sporozoite rate of *A. gambiae s.l.* between House and School 2.
6. ff suggest no significant difference in sporozoite rate of *A. gambiae s.l.* between School 1 and School 2.
7. gg suggest no significant difference in the sporozoite rate of *A. funestus* between House 2 and School.
8. hh suggest no significant difference in sporozoite rate of *A. funestus* between House 1 and School.
9. ii suggest no significant difference in sporozoite rate of *A. funestus* between House 1 and House 2.
10. jj suggest no significant difference in sporozoite rate of *A. funestus.* between School 1 and School 2.
11. kk suggest no significant difference in sporozoite rate of *A. funestus* between House and School 2.
12. ll suggest no significant difference in sporozoite rate of *A. funestus* between House and School 1.

Table 29: Sporozoite rate of *A.gambiae s.l.* and *A. funestus* at Afamanaso and Kona.

Town	Number caught		Sporozoite rate (%)	
	A. gambiae s.l.	*A. funestus*	*A. gambiae s.l.*	*A. funestus*
Afamanaso	1955	993	10.25^{a}	5.12^{b}
Kona	1524	164	9.97^{a}	9.54^{b}

$P^{aa} = 0.16 \Rightarrow P^{aa} > 0.05$, $P^{bb} = 0.80 \Rightarrow P^{bb} > 0.05$

Same letters of the superscripts, e.g. aa, suggest no significant difference in sporozoite rates between the towns indicated by the superscripts.

1. aa suggest no significant difference in the sporozoite rate of *A. gambiae s.l.* between Afamanaso and Kona.
2. bb suggest no significant difference in sporozoite rate of *A. funestus* between Afamanaso and Kona.

b) Boadi and Anwomaso

The sporozoite rate of *A. gambiae s.l.* was significantly higher at Anwomaso than that on the Boadi Cattle Farm (Table 30). Though in the first part of the study, some of the *A. ziemanni* caught (10 out of 630) were infected by *P. falciparum* with a sporozoite rate of 1.59%, none of the 874 specimens of this mosquito collected during the second study, dissected for sporozoites and subsequently tested in ELISA was found to be infected.

Table 30: Average sporozoite rate of *A. gambiae s.l.* at Anwomaso and Boadi.

Place	Mosquitoes caught	Number tested (ELISA)	Number infected	Sporozoite rate (%)
Anwomaso	266	71	4	5.63^{a}
Boadi	1161	1161	18	1.55^{b}

$P^{ab} = 0.02 \Rightarrow 0.01 < P^{ab} < 05$

Different letters of the superscripts, ab, suggests a significant difference between the sporozoite rate of *A. gambiae s.l.* at Boadi and Anwomaso.

4.5 Entomological Inoculation Rate (EIR)

EIR was calculated as the product of the sporozoite and biting rates. The average monthly and annual EIRs are obtained by multiplying the daily EIR by the appropriate number of days in the month or year whereas the hourly EIR is obtained by dividing the daily EIR by the number of hours in a day. EIR estimates the intensity of transmission of mosquito-borne diseases such as malaria. Therefore, the higher the EIR is, the higher the transmission of malaria will be.

4.5.1 EIR at Afamanaso and Kona

The annual EIRs at Afamanaso was 699.42 (95% CI: ±119.94) infective bites per person per year (ib/p/yr) about 1.6 times higher than what was recorded at Kona (433.06, P = 0.00, 95% CI: ± 85.65). Disparities in EIRs between the towns and locations within each town were found (Tables 31 and 32). *A. gambiae s.l.* contributed significantly higher EIRs (561.46 95% CI: ± 73.02) than *A. funestus*, 119.41 (95% CI: ± 30.06, P = 0.00) making it the dominant vector in the study area

Table 31: The EIR of *A. gambiae s.l.* at the various mosquito catching sites at Afamanaso and Kona.

Town	Collection site	Number caught	Daily EIR (ib/p/n)	Monthly EIR (ib/p/m)	Annual EIR (ib/p/yr)
Afamanaso	House 1	1241	3.06^{ac}	93.00^{ac}	1116.04^{ac}
	House 2	307	1.55^{be}	47.04^{be}	564.48^{be}
	School	407	1.00^{de}	30.44^{de}	365.25^{de}
Kona	House	485	1.27^{fg}	38.70^{fg}	464.39^{fg}
	School 1	564	1.23^{fh}	37.48^{fh}	449.71^{fh}
	School 2	475	1.00^{gh}	30.44	365.25^{gh}

$P^{ab} = 0.00 \Rightarrow 0.01 > P^{ab} < 05$, $P^{cd} = 0.00 \Rightarrow 0.01 > P^{cd} < 05$, $P^{ee} = 0.37 \Rightarrow P^{ee} > 05$,
$P^{ff} = 0.93 \Rightarrow P^{ff} > 05$, $P^{gg} = 0.35 \Rightarrow P^{gg} > 05$, $P^{hh} = 0.38 \Rightarrow P^{hh} > 05$.

Differences in letters of the superscripts, e.g. ab, suggest a significant difference in EIR between the sites indicated by the superscripts.
Same letters of the superscripts, e.g. ee, suggest no significant difference in EIR between the sites indicated by the superscripts.

1. ab suggest a significant difference in EIR between House 1 and House 2.
2. cd suggest a significant difference in EIR between House 1 and School.
3. ee suggest no significant difference in EIR between House 2 and School.
4. ff suggest no significant difference in EIR between House and School 1.
5. gg suggest no significant difference in EIR between House and School 2.
6. hh suggest no significant difference in EIR between School 1 and School 2.

Table 32: The EIR of *A. funestus* at the various mosquito catching sites at Afamanaso and Kona.

Town	Collection site	Number caught	Daily EIR (ib/p/n)	Monthly EIR (ib/p/m)	Annual EIR (ib/p/yr)
Afamanaso	House 1	648	0.86^{bd}	26.21^{bd}	314.52^{bd}
	House 2	110	0.18^{ac}	5.53^{ac}	66.41^{ac}
	School	235	0.47^{ad}	14.37^{ad}	172.48^{ad}
Kona	House	38	0.086^{fh}	2.61^{fh}	31.31^{fh}
	School 1	106	0.17^{eh}	5.14^{eh}	61.64^{eh}
	School 2	20	0.08^{ef}	2.34^{ef}	28.10^{ef}

$P^{aa} = 0.07 \Rightarrow P^{aa} > 05$, $P^{bc} = 0.00 \Rightarrow 0.01 > P^{bc} < 05$, $P^{dd} = 0.08 \Rightarrow P^{dd} > 05$,
$P^{ee} = 0.15 \Rightarrow P^{ee} > 05$, $P^{ff} = 0.65 \Rightarrow P^{ff} > 05$, $P^{gg} = 0.25 \Rightarrow P^{gg} > 05$.

Differences in letters of the superscript, e.g. bc, suggest a significant difference in EIR between the sites indicated by the superscripts.
Same letters of the superscripts, e.g. aa, suggest no significant difference in EIR between the sites indicated by the superscripts.

1. aa suggest no significant difference in EIR between House 2 and School.

2. bc suggest a significant difference in EIR between House 1 and House 2.
3. dd suggest no significant difference in EIR between House 1 and School.
4. ee suggest no significant difference in EIR between School 1 and School 2.
5. ff suggest no significant difference in EIR between House and School 2.
6. gg suggest no significant difference in EIR between House and School 2.

There were higher EIRs (P = 0.01) outdoor at Afamanaso than indoor, the reverse being the case at Kona (P = 0.00) (Tables 33 and 34) *A. gambiae s.l.* and *A. funestus*.

Table 33: Indoor and outdoor EIR of *A. gambiae s.l.* at Afamanaso and Kona

Town	Number caught	Daily EIR (ib/p/n)	Monthly EIR (ib/p/m)	Yearly EIR (ib/p/yr)
Indoor				
Afamanaso	714	1.21^{ad}	36.73^{ad}	440.82^{ad}
Kona	1049	1.25^{cd}	38.05^{cd}	456.56^{cd}
Outdoor				
Afamanaso	1,241	3.06^{be}	93.00^{be}	1116.04^{be}
Kona	475	1.00^{cf}	30.44^{cf}	365.25^{cf}

$P^{ab} = 0.00 \Rightarrow 0.01 > P^{ab} < 05$, $P^{cc} = 0.320 \Rightarrow P^{cc} > 05$, $P^{dd} = 0.25 \Rightarrow 0.01 < P^{dd} < 05$, $P^{ef} = 0.00 \Rightarrow 0.01 > P^{ef} < 05$.

Differences in letters of the superscript, e.g. ab, suggest a significant difference in EIR between the indoor or outdoor positions in the towns indicated by the superscripts.
Same letters of the superscripts, e.g. cc, suggest no significant difference in EIR between the indoor or outdoor positions in the towns indicated by the superscripts.

1. ab suggest a significant difference in *A. gambiae s.l.* EIR between the indoor and outdoor positions at Afamanaso.
2. cc suggest no significant difference in *A. gambiae s.l.* EIR between the indoor and outdoor positions at Kona.
3. dd suggest no significant difference in *A. gambiae s.l.* indoor EIR between Afamanaso and Kona.
4. ef suggest a significant difference in *A. gambiae s.l.* outdoor EIR between Afamanaso and Kona.

Table 34: Indoor and outdoor EIR of *A. funestus* at Afamanaso and Kona

Town	Number caught	Daily EIR (ib/p/n)	Monthly EIR (ib/p/m)	Yearly EIR (ib/p/yr)
Indoor				
Afamanaso	345	0.36^{ac}	11.02^{ac}	132.25^{ac}
Kona	144	0.13^{bd}	3.96^{bd}	47.48^{bd}
Outdoor				
Afamanaso	648	0.86^{ac}	26.21^{ac}	314.52^{ac}
Kona	20	0.08^{bf}	2.34^{bf}	28.10^{bf}

$P^{aa} = 0.05 \Rightarrow P^{aa} = 05$, $P^{bb} = 0.27 \Rightarrow P^{bb} > 05$, $P^{cd} = 0.03 \Rightarrow 0.01 < P^{cd} < 05$, $P^{ef} = 0.00 \Rightarrow 0.01 > P^{ef} < 05$.

Differences in letters of the superscript, e.g. ab, suggest a significant difference in EIR between the indoor or outdoor positions in the towns indicated by the superscripts.
Same letters of the superscripts, e.g. cc, suggest no significant difference in EIR between the indoor or outdoor positions in the towns indicated by the superscripts.

1. aa suggest no significant difference in *A. funestus* EIR between the indoor and outdoor positions at Afamanaso.
2. bb suggest no significant difference in *A. funestus* EIR between the indoor and outdoor positions at Kona.
3. cd suggest a significant difference in *A. funestus* indoor EIR between Afamanaso and Kona.
4. ef suggest a significant difference in *A. funestus* outdoor EIR between Afamanaso and Kona.

Malaria transmission, in the main, did not differ significantly between the seasons (Tables 35 and 36).

Table 35: Seasonal EIR of *Anopheles* species at Afamanaso.

Seasonal biting rate		Seasonal EIR	
A. gambiae s.l.	*A. funestus*	*A. gambiae s.l.*	*A. funestus*
Dry season (December to March)			
1727.33^{ce}	1061.51^{ik}	136.97^{no}	68.48^{qr}
Minor rainy season (August to November)			
2552.19^{af}	559.29^{gl}	226.00^{mo}	31.96^{pr}
Major rainy season (April to July)			
1165.76^{bd}	1427.52^{hj}	136.97^{mn}	60.88^{pq}

$P^{ab} = 0.00 => 0.01 > P^{ab} < 05$, $P^{cd} = 0.00 => 0.01 > P^{cd} < 05$, $P^{ef} = 0.00 => 0.01 > P^{ef} < 05$
$P^{gh} = 0.00 => 0.01 > P^{gh} < 05$, $P^{ij} = 00 => 0.01 > P^{ij} < 05$, $P^{kl} = 0.01 => 0.01 = P^{kl} < 05$
$P^{mm} = 0.20 => P^{mm} > 05$, $P^{nn} = 1.00 => P^{nn} > 05$, $P^{oo} = 0.20 => P^{oo} > 05$
$P^{pp} = 0.47 => P^{pp} > 05$, $P^{qq} = 0.69 => P^{qq} > 05$, $P^{rr} = 0.53 => P^{rr} > 05$

Difference in letters of the superscript, e.g. ab, suggests a significant difference in EIR between the seasons indicated by the superscripts.
Same letters of the superscripts, e.g. mm, suggest no significant difference in EIR between the seasons indicated by the superscripts.

1. ab suggest a significant difference in *A. gambiae s.l.* biting rate between the minor and major rainy seasons at Afamanaso.
2. cd suggest a significant difference in *A. gambiae s.l.* biting rate between the dry and major rainy seasons at Afamanaso.
3. ef suggest a significant difference in *A. gambiae s.l.* biting rate between the dry and minor rainy seasons at Afamanaso.
4. gh suggest a significant difference in *A. funestus* biting rate between the minor and major rainy seasons at Afamanaso.
5. ij suggest a significant difference in *A. funestus* biting rate between the dry and major rainy seasons at Afamanaso.
6. kl suggest a significant difference in *A. funestus* biting rate between the dry and major rainy seasons at Afamanaso.

7. mm suggest no significant difference in *A. gambiae s.l.* EIR between the minor and major rainy seasons at Afamanaso.
8. nn suggest no significant difference in *A. gambiae s.l.* EIR between the dry and major rainy seasons at Afamanaso.
9. oo suggest no significant difference in *A. gambiae s.l.* EIR between the dry and minor rainy seasons at Afamanaso.
10. pp suggest no significant difference in *A. funestus.* EIR between the minor and major rainy seasons at Afamanaso.
11. qq suggest no significant difference in *A. funestus.* EIR between the dry and major rainy seasons at Afamanaso.
12. rr suggest no significant difference in *A. funestus* EIR between the dry and minor rainy seasons at Afamanaso.

Table 36: Seasonal EIR of *Anopheles* species at Kona.

Seasonal biting rate		Seasonal EIR	
A. gambiae s.l	*A. funestus*	*A. gambiae s.l.*	*A. funestus*
Dry season (December to March)			
908.28^{cd}	121.06^{gh}	68.48^{jk}	7.61^{mn}
Minor rainy season (August to November)			
1620.18^{ae}	77.74^{fh}	155.48^{ik}	7.40^{ln}
Major rainy season (April to July)			
930.80^{bc}	156.12^{fg}	94.26^{ij}	17.67^{lm}

$P^{ab} = 0.03 \Rightarrow 0.01 < P^{ab} < 05$, $P^{cc} = 0.30 \Rightarrow P^{cc} > 05$, $P^{de} = 0.02 \Rightarrow 0.01 < P^{de} < 05$
$P^{ff} = 0.20 \Rightarrow P^{ff} > 05$, $P^{gg} = 0.45 \Rightarrow P^{gg} > 05$, $P^{hh} = 0.39 \Rightarrow P^{hh} > 05$
$P^{ii} = 0.18 \Rightarrow P^{ii} > 05$, $P^{ij} = 0.30 \Rightarrow P^{ij} > 05$, $P^{kk} = 0.12 \Rightarrow P^{kk} > 05$
$P^{ll} = 0.23 \Rightarrow P^{ll} > 05$, $P^{mm} = 0.23 \Rightarrow P^{mm} > 05$, $P^{nn} = 0.75 \Rightarrow P^{nn} > 05$

Differences in letters of the superscript, e.g. ab, suggest a significant difference in EIR between the seasons indicated by the superscripts.
Same letters of the superscripts, e.g. cc, suggest no significant difference in EIR between the seasons indicated by the superscripts.

1. ab suggest a significant difference in *A. gambiae s.l.* biting rate between the minor and major rainy seasons at Kona.
2. cc suggest no significant difference in *A. gambiae s.l.* biting rate between the dry and major rainy seasons at Kona.
3. de suggest a significant difference in *A. gambiae s.l.* biting rate between the dry and minor rainy seasons at Kona.
4. ff suggest no significant difference in *A. funestus* biting rate between the minor and major rainy seasons at Kona.
5. gg suggest no significant difference in *A. funestus* biting rate between the dry and major rainy seasons at Kona.
6. hh suggest no significant difference in *A. funestus* biting rate between the dry and major rainy seasons at Kona.
7. ii suggest no significant difference in *A. gambiae s.l.* EIR between the minor and major rainy seasons at Kona.
8. jj suggest no significant difference in *A. gambiae s.l.* EIR between the dry and major rainy seasons at Kona.
9. kk suggest no significant difference in *A. gambiae s.l.* EIR between the dry and minor rainy seasons at Kona.

10. ll suggest no significant difference in *A. funestus.* EIR between the minor and major rainy seasons at Kona.
11. mm suggest no significant difference in *A. funestus.* EIR between the dry and major rainy seasons at Kona.
12. nn suggest no significant difference in *A. funestus* EIR between the dry and minor rainy seasons at Kona.

A larger number of infective bites occurred before 21 hours and after 04 hours though the highest infective biting activities took place between 21 and after 04 hours (Tables 37 and 38).

Table 37: EIR by *A. gambiae s.l.* before (18-22), during (22-04) and after bedtime (04-06) at Afamanso and Kona.

Hour	Number caught	Annual biting rate (b/p/yr)	Sporozoite rate (%)	Annual EIR (ib/p/yr)
Afamanaso				
Before bed (18-21)	139	2160.42^{ce}	12.33^{op}	62.67^{vw}
Bedtime (21-04)	1678	11177.3^{af}	10.64^{mp}	1032.47^{tx}
After bed (05-06)	138	3217.31^{bd}	5.57^{no}	233.14^{vu}
Kona				
Before bed (18-21)	104	1366.98^{ik}	10.24^{rs}	159.12^{AB}
Bedtime (21-04)	1311	6901.52^{gl}	9.77^{qs}	609.09^{yC}
After bed (05-06)	109	2017.92^{hj}	11.25^{qr}	227.83^{zA}

$P^{ab} = 0.00 => 0.01 > P^{ab} < 05$, $P^{cd} = 0.02 => 0.01 < P^{cd} < 05$, $P^{ef} = 0.00 => 0.01 < P^{ef} < 05$
$P^{gh} = 0.00 => 0.01 > P^{gh} < 05$, $P^{ij} = 0.01 => 0.01 < P^{ij} < 05$, $P^{kl} = 0.02 => 0.01 < P^{kl} < 05$
$P^{mn} = 0.01 => 0.01 = P^{mn} < 05$, $P^{oo} = 0.29 => P^{oo} > 05$, $P^{pp} = 19 => P^{pp} > 05$
$P^{qq} = 0.58 => P^{qq} > 05$, $P^{rr} = 0.98 => P^{rr} > 05$, $P^{ss} = 0.55 => P^{ss} > 05$
$P^{tu} = 0.00 => 0.01 > P^{tu} < 05$, $P^{vv} = 0.73 => P^{vv} > 05$, $P^{wx} = 0.00 => 0.01 > P^{wx} < 05$
$P^{yz} = 0.00 => 0.01 > P^{yz} < 05$, $P^{AA} = 0.35 => P^{AA} > 05$, $P^{BC} = 0.00 => 0.01 > P^{BC} < 05$

Different letters of the superscripts, e.g. ab, suggests a significant difference in *A. gambiae s.l.* annual biting rate between the hours as indicated by the superscripts.
Same letters of the superscripts, e.g. oo, suggest no significant difference in *A. gambiae s.l.* annual biting rate between the hours as indicated by the superscripts.

1. ab suggest a significant difference in *A. gambiae s.l.* annual biting rate between the hours of bedtime and after bed at Afamanaso.
2. cd suggest a significant difference in *A. gambiae s.l.* annual biting rate between the hours before bedtime and after bed at Afamanaso.
3. ef suggest a significant difference in *A. gambiae s.l.* annual biting rate between the hours before bed and bedtime at Afamanaso.

4. gh suggest a significant difference in *A. gambiae s.l.* annual biting rate between the hours of bedtime and after bed at Kona.
5. ij suggest a significant difference in *A. gambiae s.l.* annual biting rate between the hours before bedtime and after bed at Kona.
6. kl suggest a significant difference in *A. gambiae s.l.* annual biting rate between the hours before bed and bedtime at Kona.
7. mn suggest a significant difference in *A. gambiae s.l.* sporozoite rate between the hours of bedtime and after bed at Afamanaso.
8. oo suggest no significant difference in *A. gambiae s.l.* sporozoite rate between the hours before bedtime and after bed at Afamanaso.
9. pp suggest no significant difference in *A. gambiae s.l.* sporozoite rate between the hours of before bed and bedtime at Afamanaso.
10. qq suggest no significant difference in *A. gambiae s.l.* sporozoite rate between the hours of bedtime and after bed at Kona.
11. rr suggest no significant difference in *A. gambiae s.l.* sporozoite rate between the hours before bedtime and after bed at Kona.
12. ss suggest no significant difference in *A. gambiae s.l.* sporozoite rate between the hours before bed and bedtime at Kona.
13. tu suggest a significant difference in *A. gambiae s.l.* EIR between the hours of bedtime and after bed at Afamanaso.
14. vv suggest no significant difference in *A. gambiae s.l.* EIR between the hours before bedtime and after bed at Afamanaso.
15. wx suggest a significant difference in *A. gambiae s.l.* EIR between the hours before bedtime and bedtime at Afamanaso.
16. yz suggest a significant difference in *A. gambiae s.l.* EIR between the hours of bedtime and after bed at Kona.
17. AA suggest no significant difference in *A. gambiae s.l.* EIR between the hours before bedtime and after bed at Kona.
18. BC suggest a significant difference in *A. gambiae s.l.* EIR between the hours before bed and bedtime at Kona.

Table 38: EIR of *A. funestus* before (18-22), during (22-04) and after bedtime (04-06) at Afamanso and Kona.

Hour	Number caught	Annual biting rate (b/p/yr)	Sporozoite rate (%)	Annual EIR (ib/p/yr)
Afamanaso				
Before bed (18-21)	54	839.298^{ce}	8.78^{mn}	77.71^{tu}
Bedtime (21-04)	868	5781.83^{af}	4.75^{ln}	293.08^{rv}
After bed (05-06)	71	1655.28^{bd}	4.70^{lm}	69.94^{st}
Kona				
Before bed (18-21)	11	151.886^{ij}	0.00^{pq}	0^{yA}
Bedtime (21-04)	148	717.58^{gk}	10.76^{oq}	72.84^{wB}
After bed (05-06)	5	43.396^{hi}	0.00^{op}	0^{xz}

$P^{ab} = 0.00 \Rightarrow 0.01 > P^{ab} < 05$, $P^{cd} = 0.02 \Rightarrow 0.01 < P^{cd} < 05$, $P^{ef} = 0.00 \Rightarrow 0.01 < P^{ef} < 05$
$P^{gh} = 0.00 \Rightarrow 0.01 > P^{gh} < 05$, $P^{ii} = 0.15 \Rightarrow P^{ii} > 05$, $P^{jk} = 0.00 \Rightarrow 0.01 > P^{jk} < 05$
$P^{ll} = 0.43 \Rightarrow P^{ll} < 05$, $P^{mm} = 0.41 \Rightarrow P^{mm} > 05$, $P^{nn} = 0.76 \Rightarrow P^{nn} > 05$
$P^{oo} = 0.58 \Rightarrow P^{oo} > 05$, $P^{pp} = 0.98 \Rightarrow P^{pp} > 05$, $P^{qq} = 0.55 \Rightarrow P^{qq} > 05$
$P^{rs} = 0.01 \Rightarrow 0.01 = P^{rs} < 05$, $P^{tt} = 0.88 \Rightarrow P^{tt} > 05$, $P^{uv} = 0.004 \Rightarrow 0.01 > P^{uv} < 05$
$P^{wx} = 0.04 \Rightarrow 0.01 > P^{wx} < 05$, $P^{yz} = 0.01 \Rightarrow 0.01 = P^{yz} < 05$, $P^{AB} = 0.00 \Rightarrow 0.01 > P^{AB} < 05$

Different letters of the superscripts, e.g. ab, suggest a significant difference in *A. funestus* annual biting rate between the hours as indicated by the superscripts.
Same letters of the superscripts, e.g. oo, suggest no significant difference in *A. funestus* annual biting rate between the hours as indicated by the superscripts.

1. ab suggest a significant difference in *A. funestus* annual biting rate between the hours of bedtime and after bed at Afamanaso.
2. cd suggest a significant difference in *A. funestus* annual biting rate between the hours before bedtime and after bed at Afamanaso.
3. ef suggest a significant difference in *A. funestus* annual biting rate between the hours before bed and bedtime at Afamanaso.
4. gh suggest a significant difference in *A. funestus* annual biting rate between the hours of bedtime and after bed at Kona.
5. ii suggest no significant difference in *A. funestus* annual biting rate between the hours before bedtime and after bed at Kona.
6. jk suggest a significant difference in *A. funestus* annual biting rate between the hours before bed and bedtime at Kona.
7. ll suggest no significant difference in *A. funestus* sporozoite rate between the hours of bedtime and after bed at Afamanaso.
8. mm suggest no significant difference in *A. funestus* sporozoite rate between the hours before bedtime and after bed at Afamanaso.
9. nn suggest no significant difference in *A. funestus* sporozoite rate between the hours of before bed and bedtime at Afamanaso.
10. oo suggest no a significant difference in *A. funestus* sporozoite rate between the hours of bedtime and after bed at Kona.

11. pp suggest no significant difference in *A. funestus* sporozoite rate between the hours before bedtime and after bed at Kona.
12. qq suggest no significant difference in *A. funestus* sporozoite rate between the hours before bed and bedtime at Kona.
13. rs suggest a significant difference in *A. funestus.* EIR between the hours of bedtime and after bed at Afamanaso.
14. tt suggest no significant difference in *A. funestus* EIR between the hours before bedtime and after bed at Afamanaso.
15. uv suggest a significant difference in *A. funestus* EIR between the hours before bedtime and bedtime at Afamanaso.
16. wx suggest a significant difference in *A. funestus* EIR between the hours of bedtime and after bed at Kona.
17. yz suggest a significant difference in *A. funestus* EIR between the hours before bedtime and after bed at Kona.
18. AB suggest a significant difference in *A. funestus* EIR between the hours before bed and bedtime at Kona.

The hourly graphs (Figs. 30-32) and Tables 40 and 41 show that transmission began as early as 18 hours and persisted till 06 hours. The hourly infective biting activities followed the same pattern as those of the biting activities. The hourly EIRs of *A. gambiae s.l* were the same in both towns from 19-22 hours, midnight and between 4and 5 hours (Fig. 30). Those of *A. funestus* in both towns were about the same after 19 and 23 hours (Fig. 31). The hourly EIRs of the two vectors were also about the same at 19, 23 and after 03 hours at Afamanaso (Fig. 32). At Kona, the hourly EIRs of the vectors were about the same by 20 and 00 hours (Fig. 30).

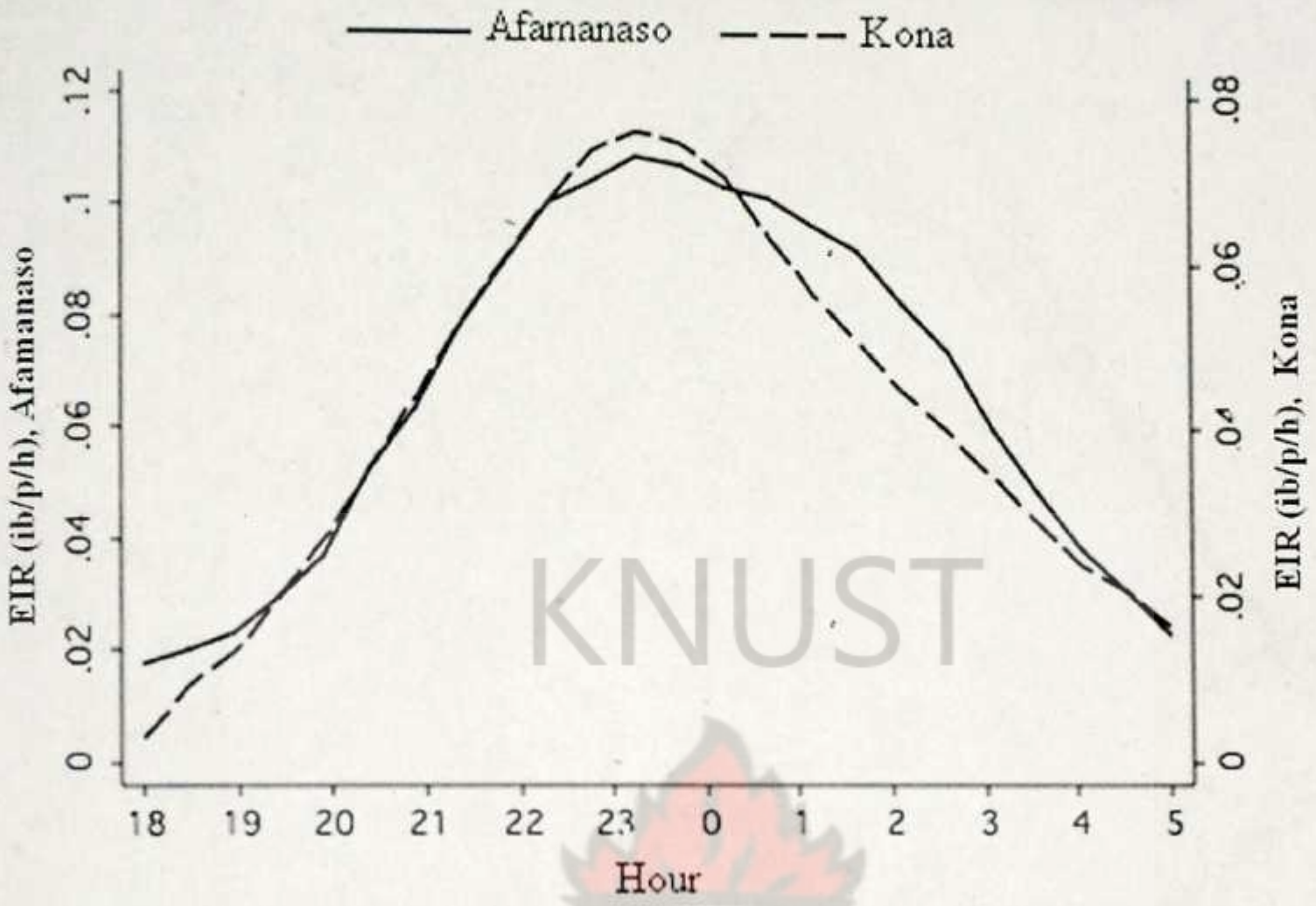


Fig. 30: Hourly EIR of *A. gambiae s.l.* at Afamanaso and Kona in 2004 and 2005.

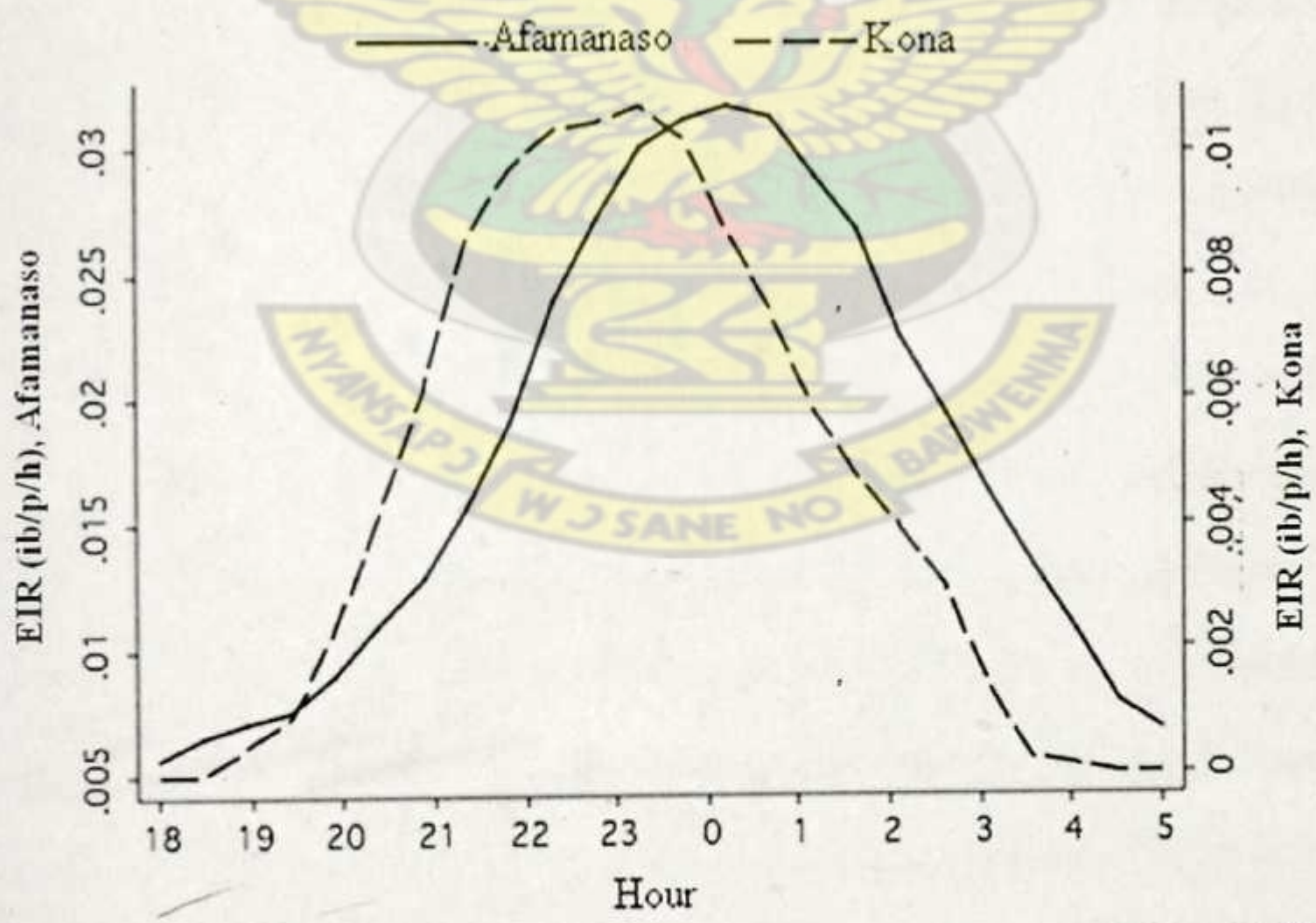


Fig. 31: Hourly EIR of *A. funestus* at Afamanaso and Kona in 2004 and 2005.

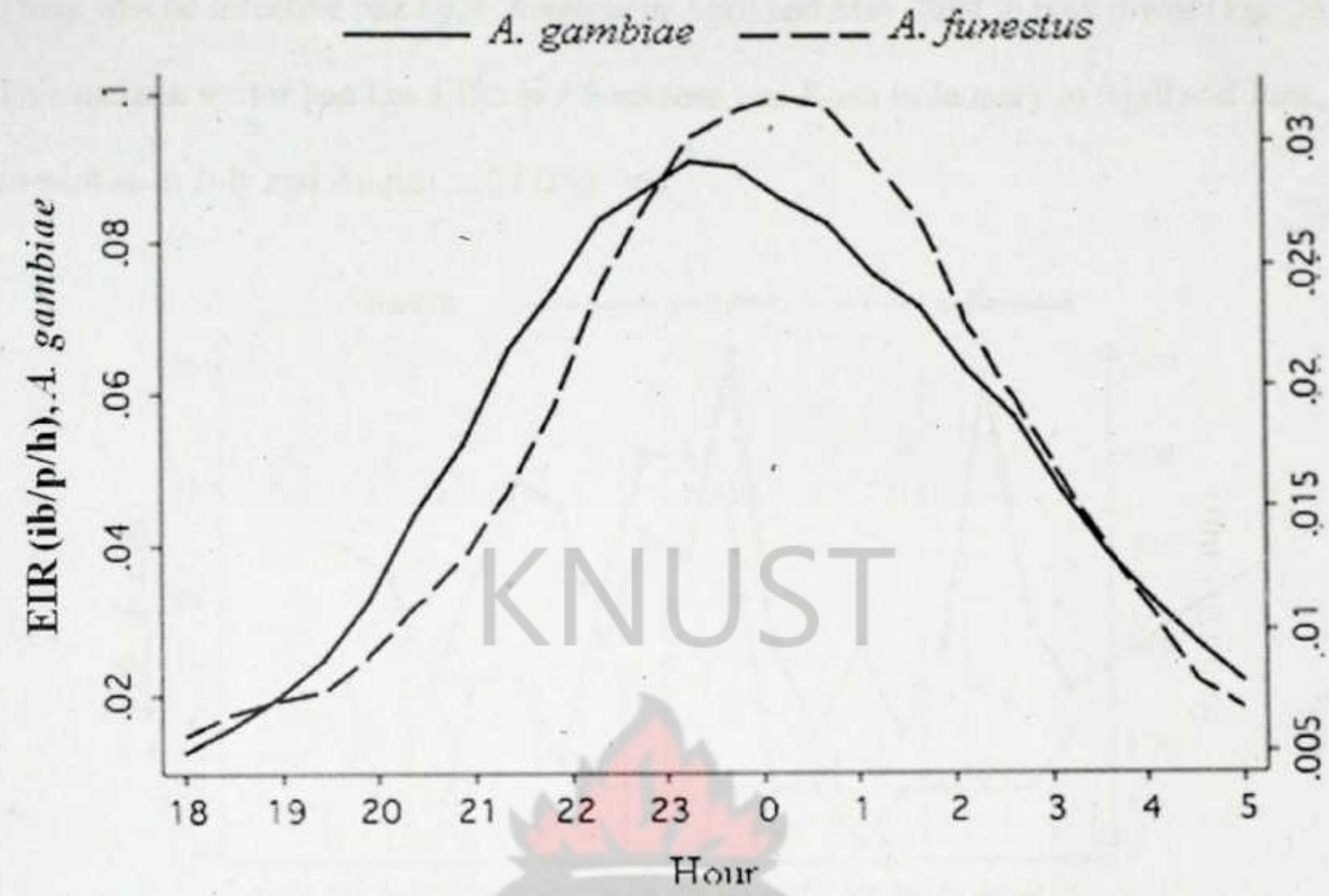


Fig. 32: Hourly EIR of *Anopheles* species at Afamanaso in 2004 and 2005.

Monthly EIRs of both vector species showed a similar trend, climaxing during or after periods of rainfall (Figs. 35 and 36). The EIRs of both species were about the same at Afamanaso in September and November 2004 at about 7 and 15 ib/p/m respectively (Fig. 33). Equal EIRs of the two species occurred at Kona in January 2005 at 3 ib/p/m and July at 8 ib/p/m (Fig. 34). In Fig. 35 it is observed that the same EIRs of about 10 and 11 ib/p/m were recorded for *A. gambiae s.l.* in April and June 2004 respectively at Afamanaso and Kona. The proficient malaria vector again contributed the same EIRs of about 5 ib/p/m in April and about 12 ib/p/m in May 2005 in Kona and Afamanaso. Apart from May 2004 and 2005 when the monthly EIRs of *A. gambiae s.l.* were higher at Kona than at Afamanaso, the vector generally had higher monthly EIRs at Afamanaso than at Kona.

There was no infective bite by *A. funestus* in April and May 2004 in both towns (Fig. 35). This malaria vector had low EIRs in Afamanaso and Kona in January to April and June, as well as in July and August 2005 (Fig. 36).

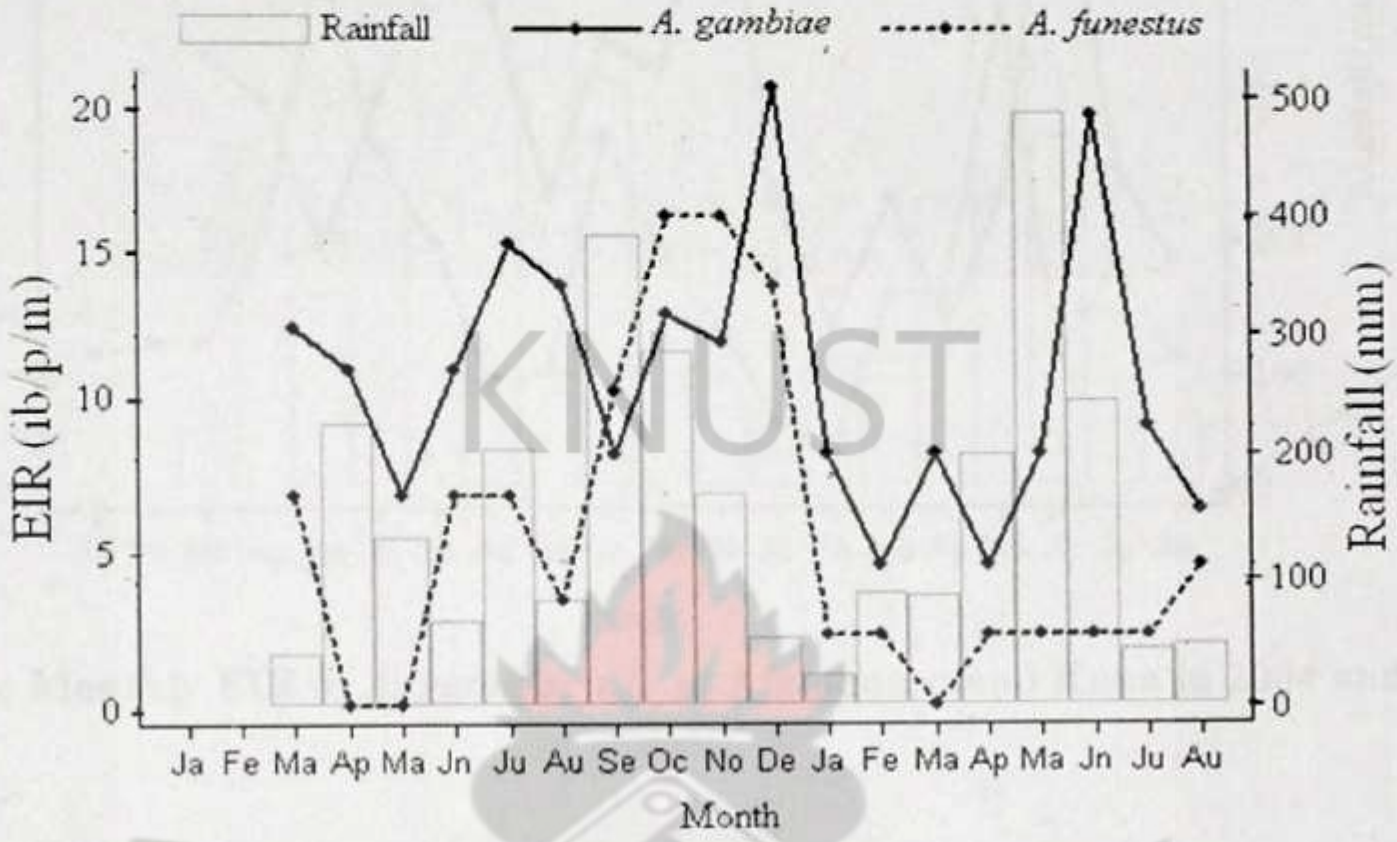


Fig. 33: Comparing the EIRs of the two malaria vectors at Afamanaso.

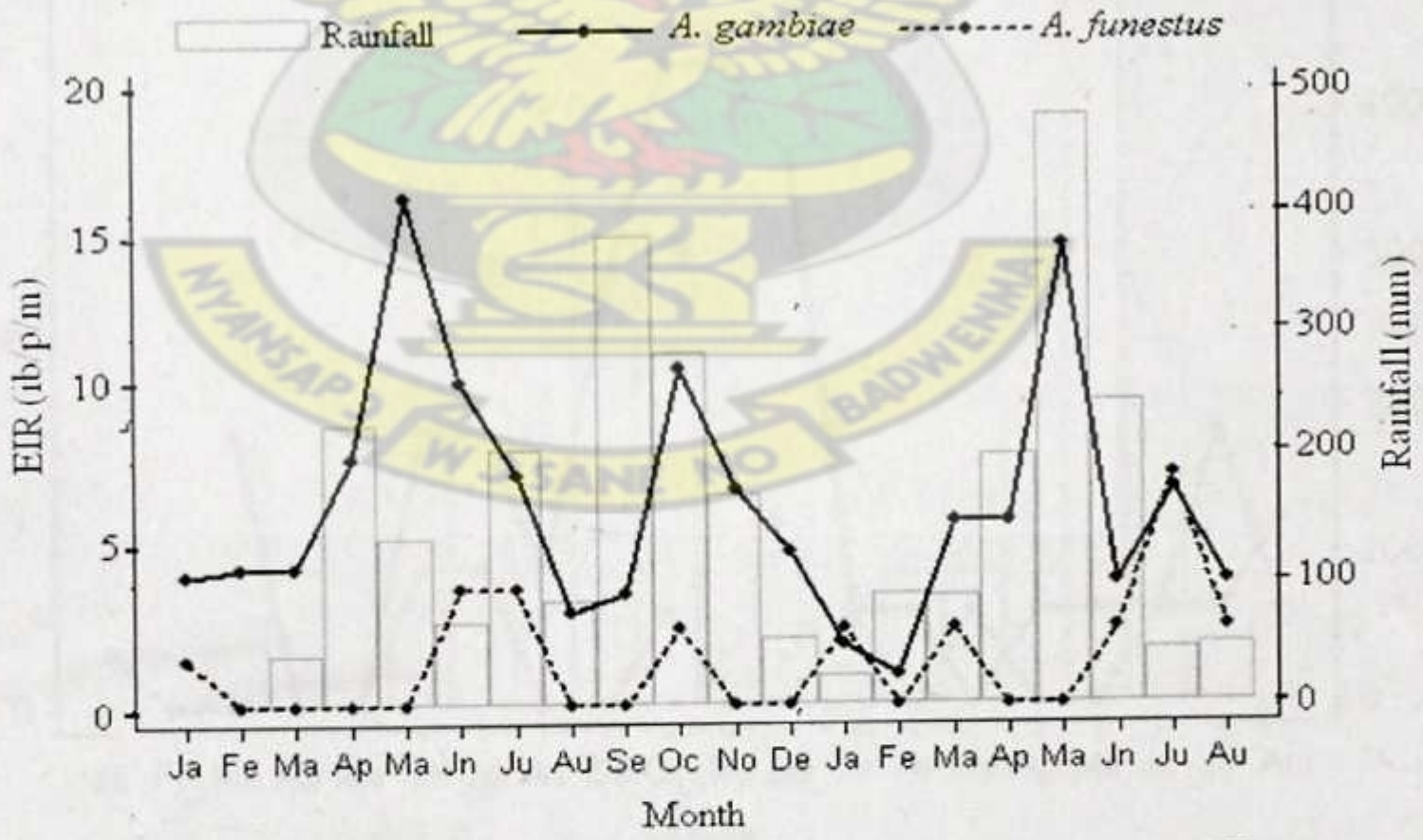


Fig. 34: Comparing the EIRs of the two malaria vectors at Kona.

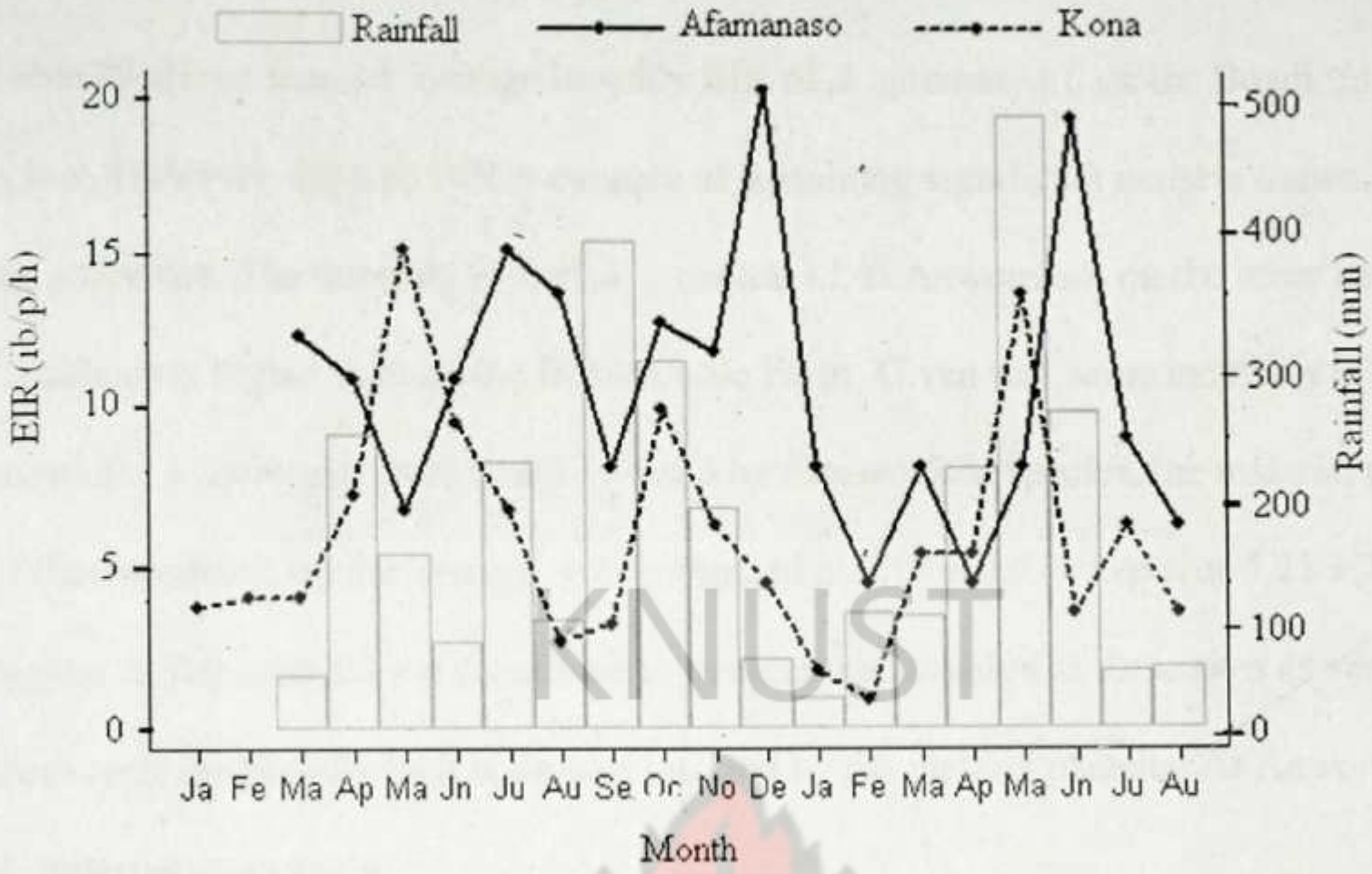


Fig. 35: Monthly EIR of *A. gambiae s.l.* at Afamanaso and Kona in 2004 and 2005.

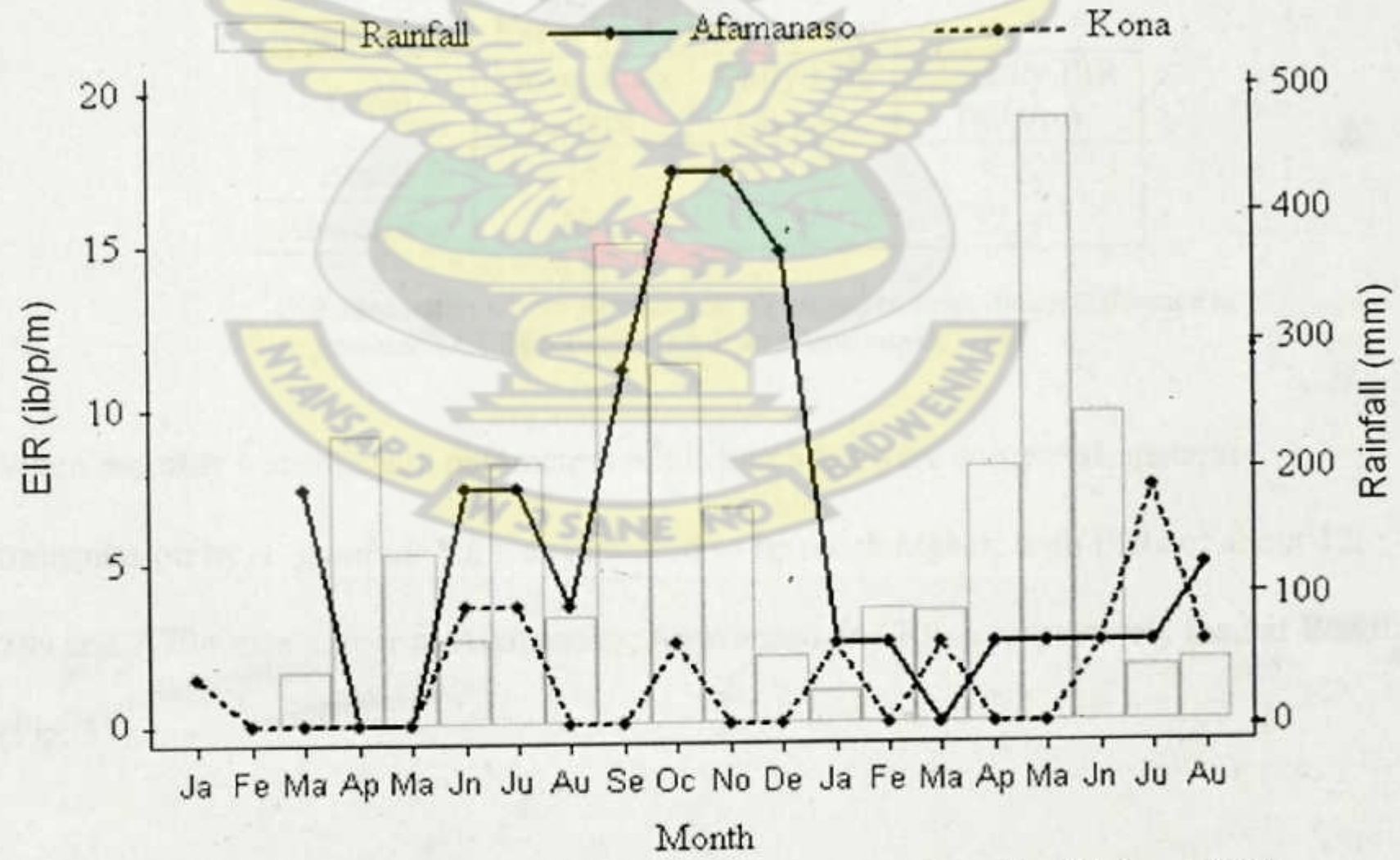


Fig. 36: Monthly EIR of *A. funestus* at Afamanaso and Kona in 2004 and 2005.

4.5.2 EIR at Boadi and Anwomaso

Table 39 shows that the average monthly EIR of *A. gambiae s.l.* on the Boadi Cattle Farm is low. However, such an EIR is capable of sustaining significant malaria transmission at the study site. The monthly EIR of *A. gambiae s.l.* at Anwomaso, on the other hand, was significantly higher than on the Boadi Cattle Farm. Given that some members of the zoophilic *A. ziemanni* were found infected by *Plasmodium* species, the malarial potential of this mosquito, on the average, was estimated at 0.17 ± 0.077 ib/p/n or 5.21 ± 2.33 ib/p/m. *A. funestus* did not contribute to malaria transmission at these sites as very few of them were caught of which none was infected by the malaria parasite. At Anwomaso, no *A. funestus* was caught.

Table 39: Average EIR of *A. gambiae s.l.* on the Boadi Cattle Farm and at Anwomaso.

Town	Mosquitoes caught	Daily EIR (ib/p/n)	Monthly EIR (ib/p/m)
Boadi	1161	0.16^{a}	4.82^{a}
Anwomaso	266	2.5^{b}	37.47^{b}

$P^{ab} = 0.00 \Rightarrow 0.01 > P^{ab} < 05$,

Different letters of the superscripts, ab, suggests a significant difference in *A. gambiae s.l.* EIR between Boadi and Anwomaso.

When monthly transmission parameters of all four sites were compared, malaria transmission by *A. gambiae s.l.* was observed to be much higher; with EIRs of about 12, 7.99 and 7.70 times higher at Afamanaso, Anwomaso and Kona respectively than at Boadi (Fig. 37).

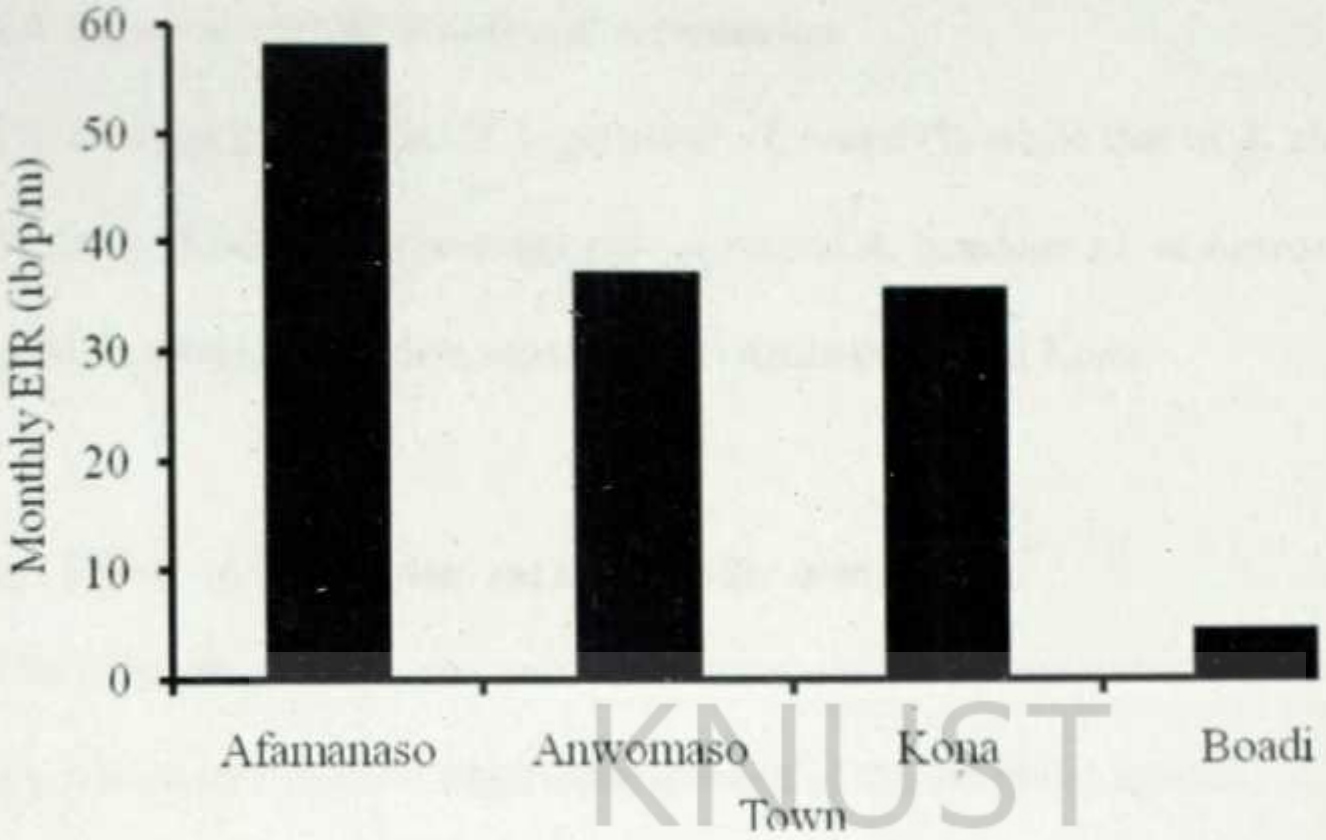


Fig. 37: Comparing monthly EIR at the four sites.

4.6 Parous Rate at Afamanaso and Kona

Parous rate is the proportion of the mosquitoes that had laid eggs before to those that had not, usually expressed as a percentage. Fig 38 shows that throughout the study period *Anopheles* species had an average monthly porous rate of 84%. The average monthly parous rates of *A. gambiae s.l.* and *A. funestus* were 83% and 85 respectively %.

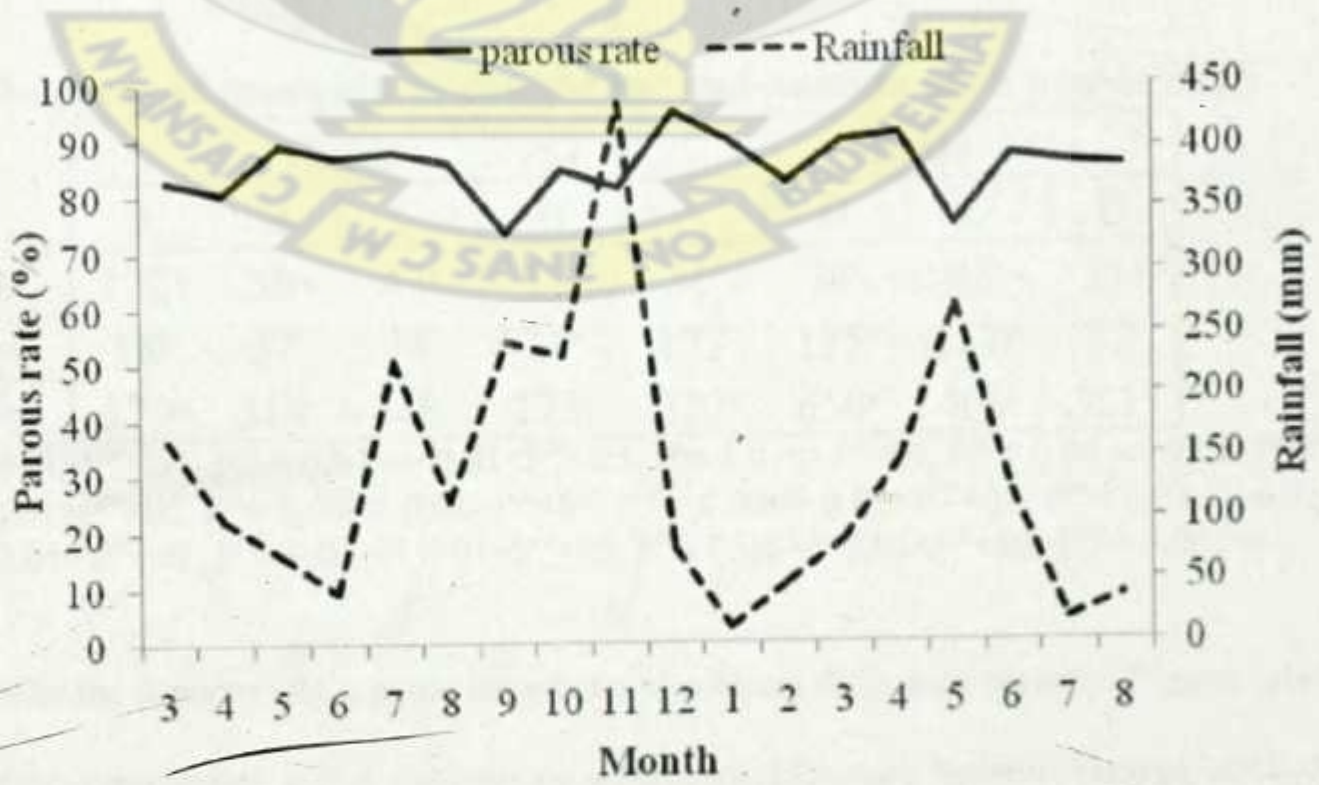


Fig. 38: Average monthly parous rates of *Anopheles* species at Afamanaso and Koná.

4.6.1 Parous rate at Boadi and Anwomaso:

The average parous rate of *A. gambiae s.l.* was 67% while that of *A. ziemanni* was 65.55%. However, the average parous rate of *A. gambiae s.l.* at Anwomaso was as high as 90%, even higher than those at both Afamanaso and Kona.

4.7 Effect of ITN on the anthropophilic mosquitoes.

The numbers of each mosquito species caught inside and outside the four pens in 2005 are shown in Table 40. Significantly more of the exophilic species: *A. ziemanni* and *Culex* species were caught attempting to feed outside each of the pens than inside. Out of the numbers of these species collected, 71% (77 out of 108) of the *A. ziemanni* ($P = 0.03$) and 72% (128 out of 321) of the *Culex* species ($P = 0.00$) were attempting to feed outside pen D. On the other hand, significantly more members of the endophilic malaria vector, *A. gambiae s.l.* were collected inside than outside the pens (except Pen C). It was observed that 63% (36 out of 57) of this vector were caught inside the ITN-surrounded pen as against 37% (21 out of 57) that bit outside it ($P = 0.04$).

Table 40: Numbers of mosquitoes caught in- and outside each pen in 2005

	Inside				Outside			
	A	B	C	D	A	B	C	D
A. gambiae	122^{a}	58^{c}	43^{e}	36^{f}	31^{b}	34^{d}	43^{e}	21^{g}
A. ziemanni	18^{h}	57^{j}	28^{l}	31^{n}	177^{i}	125^{k}	117^{m}	77^{o}
Culex species	379^{p}	514^{r}	348^{t}	128^{v}	520^{q}	650^{s}	405^{u}	321^{w}

$P^{ab} = 0.00 \Rightarrow 0.01 > P^{ab} < 05$, $P^{cd} = 0.04 \Rightarrow 0.01 < P^{cd} < 05$, $P^{e} = 1.0 \Rightarrow P^{e} > 05$, $P^{fg} = 0.04 \Rightarrow 0.01 < P^{fg} < 0.05$, $P^{hi} = 0.00 \Rightarrow 0.01 > P^{hi} < 05$, $P^{jk} = 0.00 \Rightarrow 0.01 > P^{jk} < 05$, $P^{lm} = 0.00 \Rightarrow 0.01 > P^{lm} < 05$, $P^{no} = 0.03 \Rightarrow 0.01 < P^{no} < 05$, $P^{pq} = 0.01 \Rightarrow 0.01 = P^{pq} < 05$, $P^{rs} = 0.01 \Rightarrow 0.01 = P^{rs} < 05$, $P^{tu} = 0.04 \Rightarrow 0.01 < P^{tu} < 05$, $P^{vw} = 0.00 \Rightarrow 0.01 > P^{vw} < 05$.

Different letters of the superscripts, e.g. ab, suggests a significant difference between Figures labelled by the letters.
Same letters of the superscripts, e.g. e, suggests no significant difference between Figures labelled by the letters.
ab suggest significant difference between numbers of *A. gambiae s.l.* caught inside and outside pen A.
cd suggest significant difference between numbers of *A. gambiae s.l.* caught inside and outside pen B

e suggest no significant difference between numbers of *A. gambiae s.l.* caught inside and outside pen C
fg suggest significant difference between numbers of *A. gambiae s.l.* caught inside and outside pen D
hi suggest significant difference between numbers of *A. ziemanni* caught inside and outside pen A
jk suggest significant difference between numbers of *A. ziemanni* caught inside and outside pen B
lm suggest significant difference between numbers of *A. ziemanni* caught inside and outside pen C
no suggest significant difference between numbers of *A. ziemanni* caught inside and outside pen D
pq suggest significant difference between numbers of *Culex species* caught inside and outside pen A
rs suggest significant difference between numbers of *Culex species* caught inside and outside pen B
tu suggest significant difference between numbers of *Culex species* caught inside and outside pen C
vw suggest significant difference between numbers of *Culex species* caught inside and outside pen D

The impact or otherwise of the ITN on moquitoes sampled in 2005 is shown in Table 41.

In general, there was a reduction in the numbers of mosquitoes caught inside and outside the ITN-surrounded pen compared with the pens not surrounded by ITN. Inside these latter pens, 52% *A. gambiae s.l.*,10% *A. ziemanni* and 69% *Culex* species, were collected which, with the exception of *A. ziemanni,* were significantly more than those caught in pens surrounded by the ITN. On the average, signicantly more *A. gambiae s.l.* were caught inside than outside the pens irrespective of whether they were surrounded by ITN ($P = 0.04$) or not ($P = 0.03$). However, the avaerage numbers of the exophilic mosquitoes collected outside the pen that was not surrounded by the ITN were significantly larger than those collected inside it (Table 41).

Table 41: Numbers of mosquitoes caught inside and outside experimental pens with and without deltamethrin-impregnated net (ITN) in 2005.

	Inside			Outside		
	Ag	*Az*	*Culex*	*Ag*	*Az*	*Culex*
ITN on pen	36^{em}	31^{im}	128^{qr}	21^{lv}	77^{ip}	321^{fs}
No ITN ON 3 pens/3	74^{gn}	34^{jm}	414^{hq}	36^{kv}	140^{jp}	525^{ft}
Totals	110	65	542	57	217	846
Change (%)	-52	-10	-69	-42	-45	-39

Ag = A. gambiae s.l, Az = A. ziemanni, Af = A. funestus, Culex =Culex species
$P^{ab} = 0.04 \Rightarrow 0.01<P^{ab}<05$, $P^{cd} = 0.03 \Rightarrow 0.01<P^{cd}<05$, $P^{ef} = 0.00 \Rightarrow 0.01>P^{ef}<05$,
$P^{gh} = 0.03 \Rightarrow 0.01<P^{gh}<0.05$, $P^{ij} = 0.00 \Rightarrow 0.01>P^{ij}<05$, $P^{kl} = 0.04 \Rightarrow 0.01<P^{kl}<05$,
$P^{mn} = 0.03 \Rightarrow 0.01<P^{mn}<0.05$, $P^{op} = 0.12 \Rightarrow P^{op}>05$, $P^{qs} = 0.00 \Rightarrow 0.01>P^{qs}<05$.
$P^{rs} = 0.04 \Rightarrow 0.01<P^{rs}<0.05$, $P^{tu} = 0.00 \Rightarrow 0.01>P^{tu}<05$, $P^{vw} = 0.04 \Rightarrow 0.01<P^{vw}<05$.

Different letters of the superscripts, e.g. ab, suggests a significant difference between Figures labelled by the letters.
Same letters of the superscripts, e.g. o, suggests no significant difference between Figures labelled by the letters.

1. ab suggest significant difference between numbers of *A. gambiae s.l.* caught inside and outside the pen surrounded by the deltamethrin-impregnated net (ITN).
2. cd suggest significant difference between numbers of *A. ziemanni* caught inside and outside the pen surrounded by the deltamethrin-impregnated net (ITN).
3. ef suggest significant difference between numbers of *Culex* species caught inside and outside the pen surrounded by the deltamethrin-impregnated net (ITN).
4. gh suggest significant difference between numbers of *A. gambiae s.l* caught inside and outside the pen that was not surrounded by the deltamethrin-impregnated net (ITN).
5. ij suggest significant difference between numbers of *A. ziemanni* caught inside and outside the pen that was not surrounded by the deltamethrin-impregnated net (ITN).
6. kl suggest significant difference between numbers of *Culex* species caught inside and outside the pen that was not surrounded by the deltamethrin-impregnated net (ITN).
7. mn suggest significant difference between numbers of *A. gambiae s.l.* caught inside the pen that was surrounded and one that was not surrounded by the deltamethrin-impregnated net (ITN).
8. o suggest no significant difference between numbers of *A. ziemanni* caught inside the pen that was surrounded and one that was not surrounded by the deltamethrin-impregnated net (ITN)
9. pq suggest significant difference between numbers of *Culex* species caught inside the pen that was surrounded and one that was not surrounded by the deltamethrin-impregnated net (ITN)
10. rs suggest significant difference between numbers of *A. gambiae s.l.* caught outside the pen that was surrounded and one that was not surrounded by the deltamethrin-impregnated net (ITN).
11. tu suggest significant difference between numbers of *A. ziemanni* caught outside the pen that was surrounded and one that was not surrounded by the deltamethrin-impregnated net (ITN)
12. vw suggest significant difference between numbers of *Culex* species caught outside the pen that was surrounded and one that was not surrounded by the deltamethrin-impregnated net (ITN)

The numbers of all mosquito species collected inside or outside the control pens, i.e.pens that were not surrounded by ITN (A, B and C), were the same as shown in Table 40.

The numbers of each species of mosquitoes caught inside and outside the four pens in 2006 are shown in Table 42. Generally pen D, recorded the least numbers of mosquitoes, 2005, whether or not it was surrounded by ITN, since the latter rotated from pen to pen throughout the study. Again, as was the case in the 2005 samples, significantly more of the exophilie species (*A. ziemanni* and *Culex* species) were caught attempting to feed

outside the pens than inside while significantly more of the endophilic malaria vector, *A. gambiae s.l.* were collected inside than outside the pens (Table 42).

Table 42 : Numbers of mosquitoes caught in- and outside each pen in 2006 with or without ITN.

	Inside				Outside			
	A	B	C	D	A	B	C	D
A. gambiae	132^{a}	89^{c}	180^{e}	86^{g}	96^{b}	45^{d}	82^{f}	63^{h}
A. ziemanni	24^{i}	50^{k}	32^{m}	19^{o}	275^{j}	251^{l}	154^{n}	69^{p}
Culex species	444^{q}	569^{s}	423^{u}	194^{w}	824^{r}	959^{t}	649^{v}	398^{x}

$P^{ab} = 0.04 \Rightarrow 0.01<P^{ab}<05$, $P^{cd} = 0.03 \Rightarrow 0.01<P^{cd}<05$, $P^{ef} = 1.0 \Rightarrow P^{cf}>05$, $P^{gh} = 0.04 \Rightarrow 0.01<P^{gh}<0.05$, $P^{ij} = 0.00 \Rightarrow 0.01>P^{ij}<05$, $P^{kl} = 0.00 \Rightarrow 0.01>P^{jk}<05$, $P^{mn} = 0.00 \Rightarrow 0.01>P^{mn}<05$, $P^{op} = 0.00 \Rightarrow 0.01>P^{op}<05$,
$P^{qr} = 0.00 \Rightarrow 0.01>P^{qr}<05$, $P^{st} = 0.00 \Rightarrow 0.01>P^{st}<05$, $P^{uv} = 0.00 \Rightarrow 0.01>P^{uv}<05$, $P^{wx} = 0.00 \Rightarrow 0.01>P^{wx}<05$.

Different letters of the superscripts, e.g. ab, suggests a significant difference between Figures labelled by the letters.

1. ab suggest significant difference between numbers of *A. gambiae s.l.* caught inside and outside pen A.
2. cd suggest significant difference between numbers of *A. gambiae s.l.* caught inside and outside pen B
3. ef suggest significant difference between numbers of *A. gambiae s.l.* caught inside and outside pen C
4. gh suggest significant difference between numbers of *A. gambiae s.l.* caught inside and outside pen D
5. ij suggest significant difference between numbers of *A. ziemanni* caught inside and outside pen A.
6. kl suggest significant difference between numbers of *A. ziemanni* caught inside and outside pen B
7. mn suggest significant difference between numbers of *A. ziemanni* caught inside and outside pen C
8. op suggest significant difference between numbers of *A. ziemanni* caught inside and outside pen D
9. qr suggest significant difference between numbers of *Culex species* caught inside and outside pen A.
10. st suggest ssignificant difference between numbers of *Culex species* caught inside and outside pen B
11. uv suggest significant difference between numbers of *Culex species* caught inside and outside pen C
12. wx suggest significant difference between numbers of *Culex species* caught inside and outside pen D

The numbers of each mosquito species caught inside and outside the three control pens (without ITN) in 2006 are shown in Table 43. Whereas significant larger numbers of *A. gambiae s.l.* were caught inside than outside the pens (except pens A and D), *A. ziemanni* and *Culex* species were significantly larger in numbers outside than inside the pens.

Table 43: Numbers of mosquitoes caught in- and outside the control pens in 2006.

	Inside				Outside			
	A	B	C	D	A	B	C	D
A. gambiae	66^{a}	69^{b}	122^{d}	55^{f}	73^{a}	35^{c}	55^{e}	46^{f}
A. ziemanni	22^{g}	41^{i}	25^{k}	19^{m}	212^{h}	204^{j}	120^{l}	48^{n}
Culex species	365^{o}	504^{q}	338^{s}	143^{u}	678^{p}	717^{r}	440^{t}	303^{v}

$P^{a} = 0.10 \Rightarrow P^{a} > 05$, $P^{bc} = 0.04 \Rightarrow 0.01 < P^{bc} < 05$, $P^{de} = 0.01 \Rightarrow 0.10 = P^{de} > 05$, $P^{f} = 0.09 \Rightarrow P^{f} > 0.05$, $P^{gh} = 0.00 \Rightarrow 0.01 > P^{gh} < 05$, $P^{ij} = 0.00 \Rightarrow 0.01 > P^{ij} < 05$, $P^{kl} = 0.00 \Rightarrow 0.01 > P^{kl} < 05$, $P^{mn} = 0.04 \Rightarrow 0.01 < P^{mn} < 05$,
$P^{op} = 0.00 \Rightarrow 0.01 > P^{op} < 05$, $P^{qr} = 0.00 \Rightarrow 0.01 > P^{qr} < 05$, $P^{st} = 0.00 \Rightarrow 0.01 > P^{st} < 05$, $P^{uv} = 0.00 \Rightarrow 0.01 > P^{uv} < 05$.

Different letters of the superscripts, e.g. bc, suggests a significant difference between Figures labelled by the letters.
Same letters of the superscripts, e.g. a, suggests no significant difference between Figures labelled by the letters.

1. a suggest no significant difference between numbers of *A. gambiae s.l.* caught inside and outside pen A.
2. bc suggest significant difference between numbers of *A. gambiae s.l.* caught inside and outside pen B
3. de suggest significant difference between numbers of *A. gambiae s.l.* caught inside and outside pen C
4. f suggest no significant difference between numbers of *A. gambiae s.l.* caught inside and outside pen D
5. gh suggest significant difference between numbers of *A. ziemanni* caught inside and outside pen A.
6. ij suggest significant difference between numbers of *A. ziemanni* caught inside and outside pen B
7. kl suggest significant difference between numbers of *A. ziemanni* caught inside and outside pen C
8. mn suggest significant difference between numbers of *A. ziemanni* caught inside and outside pen D
9. op suggest significant difference between numbers of *Culex species* caught inside and outside pen A.
10. qr suggest significant difference between numbers of *Culex species* caught inside and outside pen B
11. st suggest significant difference between numbers of *Culex species* caught inside and outside pen C
12. uv suggest significant difference between numbers of *Culex species* caught inside and outside pen D

Numbers of mosquitoes caught inside and outside the pens whether or not they were surrounded by ITN are presented in Table 44. While there was a significant reduction in numbers of *A. ziemanni* (50%) and *Culex* species (38%) entering the pens with ITN as against those entering pens not surrounded by ITN, numbers of *A. gambiae s.l.* caught in

pens with ITN were even 68% higher than numbers collected in pens without ITN (P = 0.03). Collections of the exophilic mosquito species were not significantly higher outside pens with or without ITN than inside (**Table 44**).

Table 44: Numbers of mosquitoes caught inside and outside experimental pens with and without deltamethrin-impregnated net (ITN) in 2006.

	Inside			Outside		
	Ag	*Az*	*Cx*	*Ag*	*Az*	*Cx*
ITN on pen	175^{am}	18^{co}	280^{eq}	77^{bs}	165^{dt}	692^{fv}
No ITN on 3 pens/3	104^{gn}	36^{ip}	450^{kr}	70^{hs}	195^{ju}	713^{lw}
Totals	279	54	730	147	360	1405
Change (%)	68	-50	-38	10	-15	-2.9

Ag = A. gambiae s.l, Az= A. ziemanni, Af = A. funestus, Cx=Culex species

$P^{ab} = 0.02 \Rightarrow 0.01<P^{ab}<05$, $P^{cd} = 0.00 \Rightarrow 0.01>P^{cd}<05$, $P^{ef}= 0.00 \Rightarrow 0.01>P^{ef}<05$,
$P^{gh} = 0.04 \Rightarrow 0.01<P^{gh}<05$, $P^{ij} = 0.00 \Rightarrow 0.01>P^{ij}<05$, $P^{kl}= 0.00 \Rightarrow 0.01>P^{kl}<05$.
$P^{mn} = 0.03 \Rightarrow 0.01<P^{mn}<0.05$, $P^{op} = 0.03 \Rightarrow 0.01< P^{op}>05$, $P^{qr}= 0.00 \Rightarrow 0.01>P^{qr}<05$.
$P^{s} = 0.10 \Rightarrow 0.01<P^{s}<0.05$, $P^{tu} = 0.04 \Rightarrow 0.01<P^{tu}<05$, $P^{vw}= 0.04 \Rightarrow 0.01<P^{vw}<05$.

Different letters of the superscripts, e.g. ab, suggests a significant difference between Figures labelled by the letters.
Same letters of the superscripts, e.g. s, suggests no significant difference between Figures labelled by the letters.

1. ab suggest significant difference between numbers of *A. gambiae s.l.* caught inside and outside the pen surrounded by the deltamethrin-impregnated net (ITN).
2. cd suggest significant difference between numbers of *A. ziemanni* caught inside and outside the pen surrounded by the deltamethrin-impregnated net (ITN).
3. ef suggest significant difference between numbers of *Culex* species caught inside and outside the pen surrounded by the deltamethrin-impregnated net (ITN).
4. gh suggest significant difference between numbers of *A. gambiae s.l* caught inside and outside the pen that was not surrounded by the deltamethrin-impregnated net (ITN).
5. ij suggest significant difference between numbers of *A. ziemanni* caught inside and outside the pen that was not surrounded by the deltamethrin-impregnated net (ITN).
6. kl suggest significant difference between numbers of *Culex* species caught inside and outside the pen that was not surrounded by the deltamethrin-impregnated net (ITN).
7. mn suggest significant difference between numbers of *A. gambiae s.l.* caught inside the pen that was surrounded and one that was not surrounded by the deltamethrin-impregnated net (ITN).
8. op suggest significant difference between numbers of *A. ziemanni* caught inside the pen that was surrounded and one that was not surrounded by the deltamethrin-impregnated net (ITN)
9. qr suggest significant difference between numbers of *Culex* species caught inside the pen that was surrounded and one that was not surrounded by the deltamethrin-impregnated net (ITN)
10. s suggest no significant difference between numbers of *A. gambiae s.l.* caught outside the pen that was surrounded and one that was not surrounded by the deltamethrin-impregnated net (ITN).
11. tu suggest significant difference between numbers of *A. ziemanni* caught outside the pen that was surrounded and one that was not surrounded by the deltamethrin-impregnated net (ITN)

12. vw suggests significant difference between numbers of *Culex* species caught outside the pen that was surrounded and one that was not surrounded by the deltamethrin-impregnated net (ITN)

4.8 Molecular analysis of data:

Three different PCR techniques were used to process the mosquitoes to obtain various results as outlined below.

4.9 Identification of sibling species of *A. gambiae* complex using conventional PCR:

When 135 members of *A. gambiae s.l.* were tested in PCR to determine the sibling species of the *A. gambiae* complex, 132 were identified as *A. gambiae sensu stricto* (*s.s.*) and 3 failed to amplify. Plate Y is a 3-sample gel, one of the results.

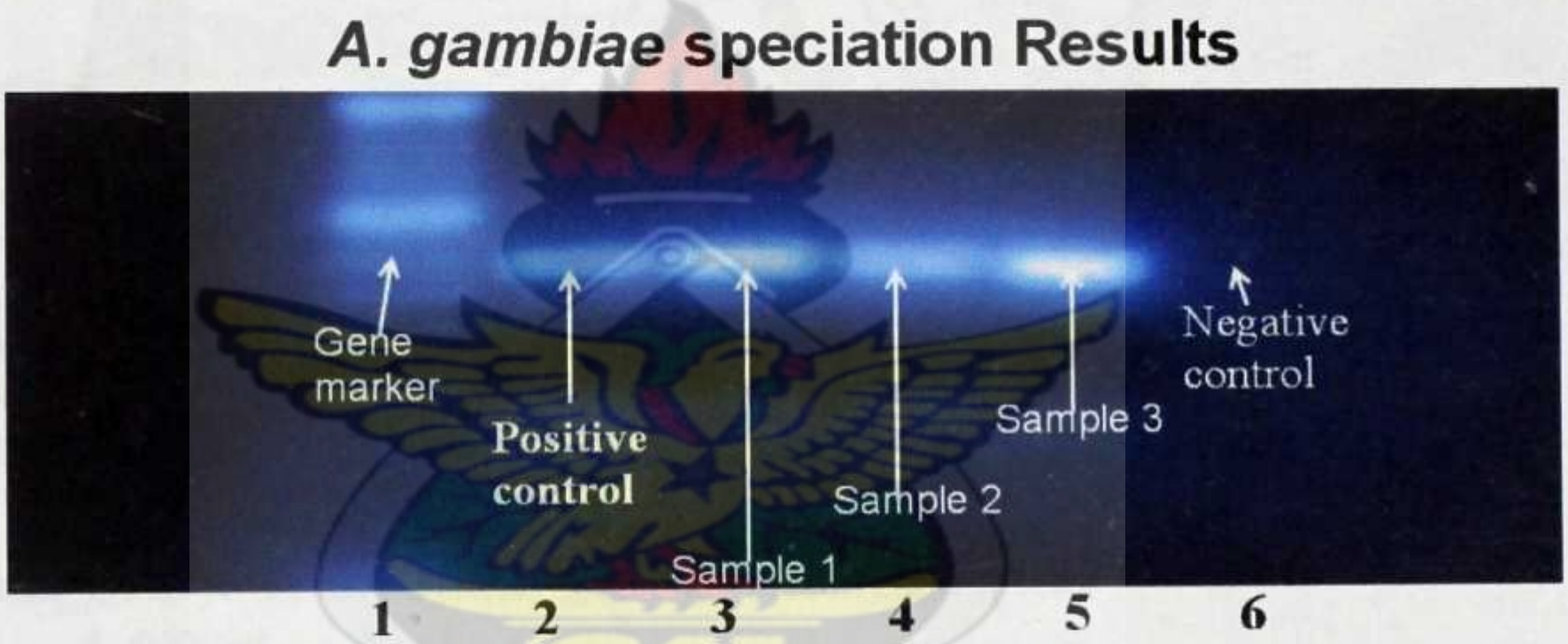


Plate Y: A gel showing PCR results of *A. gambiae* sibling species differentiation. 1 is a gene marker, 2 is an *A. gambiae s.s.* positive control, 3-5 are samples identified as *A. gambiae s.s.*, 6 is a negative control.

4.10 Identification of *Plasmodium* species using Real time PCR:

The 144 infected salivary glands processed yielded 109 *P. falciparum,* seven (7) *P. malariae,* seven (7) *P. ovale,* one *P. falciparum /P. malariae* mixed infection and twenty (20) negatives. Fig. 39 shows the melting curves from one of the experiments while Table 45 summarises the results of identification of *Plasmodium* species in mosquito salivary gland homogenates.

A. funestus was infected by *P. falciparum* only; unlike *A. gambiae s.l.* Which was infected by all three tropical species of *Plasmodium* (*P. Falciparum, P. Malariae* and *P. Ovale6*).

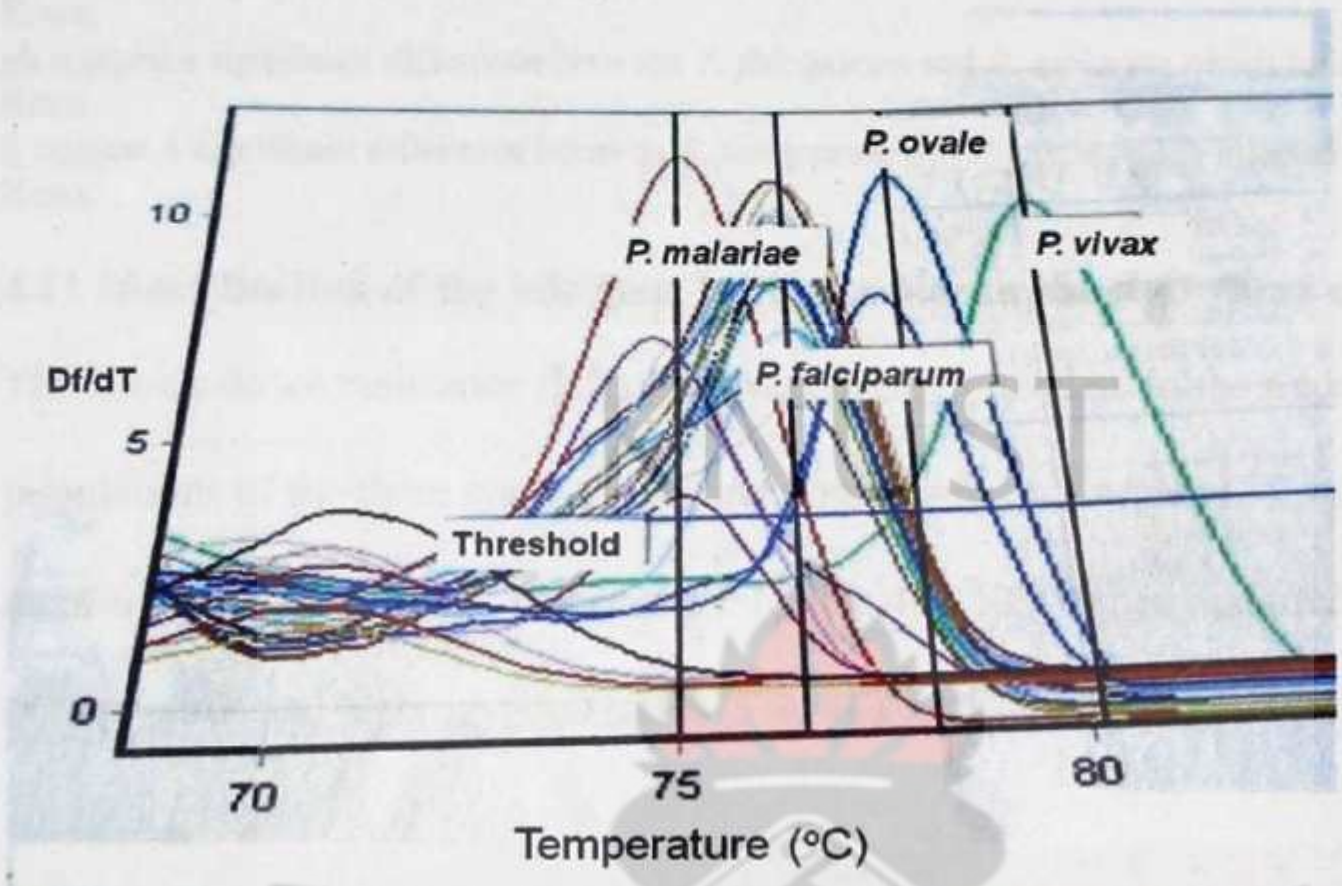


Fig. 39: Real-Time PCR melt curves of *Plasmodium* species as identified in DNA extracted from salivary gland homogenates of field collected mosquitoes from the Agona study area. Curves with peaks below the threshold are negative.

Table 45: Infection of *Anopheles* species by *Plasmodium* species

A. gambiae

Town	*P. falciparum*	*P. malariae*	*P. ovale*	*P. falciparum & P. malariae*	No amplification	tot
Afamanaso	51 (77.30%)^{bd}	3 (4.5%)^{ac}	3 (4.5%)^{ae}	1 (1.5%)	8 (12.1%)	66
Kona	46 (78.0%)^{gi}	4 (6.8%)^{fh}	4 (5.1%)^{fj}	0 (0%)	5 (8.5%)	59
Total	97 (77.3%)	7 (4.5%)	7 (4.5%)	1 (1.5%)	13 (12.1%)	12

A. funestus

Afamanaso	7 (63.60%)	0	0	0	4 (36.4%)	11
Kona	5 (62.50%)	0	0	0	3 (37.5%)	8
Total	12 (63.20%)	0	0	0	7 (36.8%)	19

P^{aa} = 0.99 => P^{aa} >0.05, P^{bc} = 0.00 => 0.01>P^{bc}<05, P^{de} = 0.00 => 0.01>P^{de}<05.
P^{ff}> = 0.99 => P^{ff}>0.05, P^{gh} = 0.00 => 0.01>P^{gh}<0.05, P^{ij} = 0.00 => 0.01>P^{ij}<0.05.

Different letters of the superscripts, e.g. bc, suggests a significant difference between the *Plasmodium* species indicated by the superscripts.
Same letters of the superscripts, e.g. aa, suggests no significant difference between the *Plasmodium* species indicated by the superscripts.

aa suggest no significant difference between *P. malariae* and *P. ovale* which infected *A. gambiae s.l.* at Afamanaso
bc suggest a significant difference between *P. falciparum* and *P. malariae* which infected *A. gambiae s.l.* at Afamanaso
de suggest a significant difference between *P. falciparum* and *P. ovale* which infected *A. gambiae s.l.* at Afamanaso
ff suggest no significant difference between *P. malariae* and *P. ovale* which infected *A. gambiae s.l.* at Kona.
gh suggest a significant difference between *P. falciparum* and *P. malariae* which infected *A. gambiae s.l.* at Kona.
ij suggest a significant difference between *P. falciparum* and *P. ovale* which infected *A. gambiae s.l.* at Kona.

4.11 Identification of the kdr gene in *A. gambiae* in the study area using FRET PCR

The knock-down resistance (kdr) gene was highly prevalent in the malaria vector populations of the three study towns and absent only in 3 of the 135 successfully amplified specimens of *A. gambiae s.l.* (Table 46). Resistance occurred both in the homozygous and heterozygous states (Fig. 40).

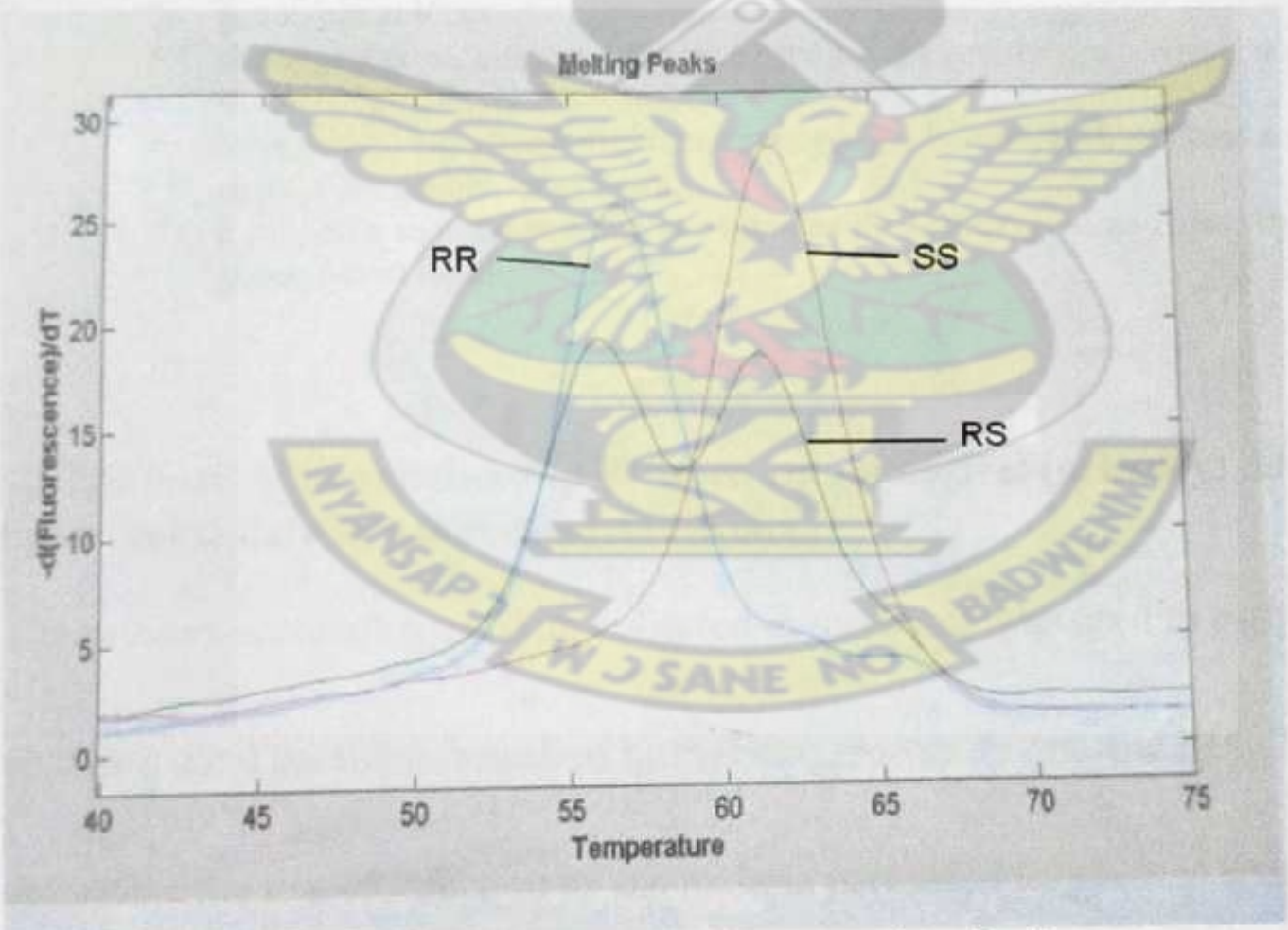


Fig.40: Melting curves of the FRET PCR showing the homozygous resistant (RR), susceptible (SS) and heterozygous resistant (RS) peaks.

Table 46: Presence of the kdr gene in *A. gambiae s.s.* in the study area.

Town	Number amplified	Homozygous resistant (RR)	Heterozygous resistant (RS)	Homozygous susceptible (SS)	Not amplified
Afamanaso	61	53 (86.9%)^{ad}	6 (9.8%)^{bc}	2 (3.3%)^{ce}	5
Kona	48	45 (93.8%)^{fi}	2 (4.2%)^{gh}	1 (2.1%)^{hi}	13
Boadi	26	25 (96.2%)^{j}	1 (3.8%)^{j}	0 (0%)	0
Totals	135	123 (91.1%)	9 (6.7%)	3 (2.2%)	18

$P^{ab} = 0.00 \Rightarrow 0.01 > P^{ab} < 0.05$, $P^{cc} = 0.05 \Rightarrow 0.01 < P^{cc} < 0.05$, $P^{de} = 0.02 \Rightarrow 0.01 < P^{de} < 0.05$
$P^{fg} = 0.02 \Rightarrow 0.01 < P^{fg} < 0.05$, $P^{hh} = 0.22 \Rightarrow P^{hh} > 0.05$, $P^{ii} = 0.09 \Rightarrow P^{ii} > 0.05$
$P^{jj} = 0.10 \Rightarrow P^{jj} > 0.05$.

Different letters of the superscripts, e.g. ab, suggests a significant difference between the genotypes indicated by the superscripts.
Same letters of the superscripts, e.g. cc, suggests no significant difference between the genotypes indicated by the superscripts.

1. ab suggest a significant difference between the homozygous and heterozygous resistant genotypes at Afamanaso.
2. cc suggest no significant difference between the heterozygous resistant and homozygous susceptible genotypes at Afamanaso.
3. de suggest a significant difference between the homozygous resistant and homozygous susceptible genotypes at Afamanaso.
4. fg suggest a significant difference between the homozygous and heterozygous resistant genotypes at Kona.
5. hh suggest no significant difference between the heterozygous resistant and homozygous susceptible genotypes at Kona.
6. ii suggest no significant difference between the homozygous resistant and homozygous susceptible genotypes at Kona.
7. jj suggest a significant difference between the homozygous and heterozygous resistant genotypes at Boadi.

4.12 Assessing the relationship between entomological (primary) data and malaria (secondary) data in Afamanaso and Kona.

The primary entomological data collected in this study was used to estimate entomological parameters such as biting rate, sporozoite rate and EIR. These parameters determine the risk of infection by the malaria parasite, *Plasmodium* species as well as the level of transmission of this parasite by the malaria vectors, *Anopheles* species Clinical parameters such as malaria incidence, prevalence and attack rates indicate the state of the disease in the human population. An attempt was therefore made to determine whether or

not any relationship existed between the entomological parameters (primary data) on one hand and the clinical parameters (data from a secondary source) on the other. The clinical data were collected concurrently with the entomological study in the same study sites of Afamanaso and Kona by a team of medical personnel (Kobbe *et al.*, 2006; Kreuels *et al.*, 2008).

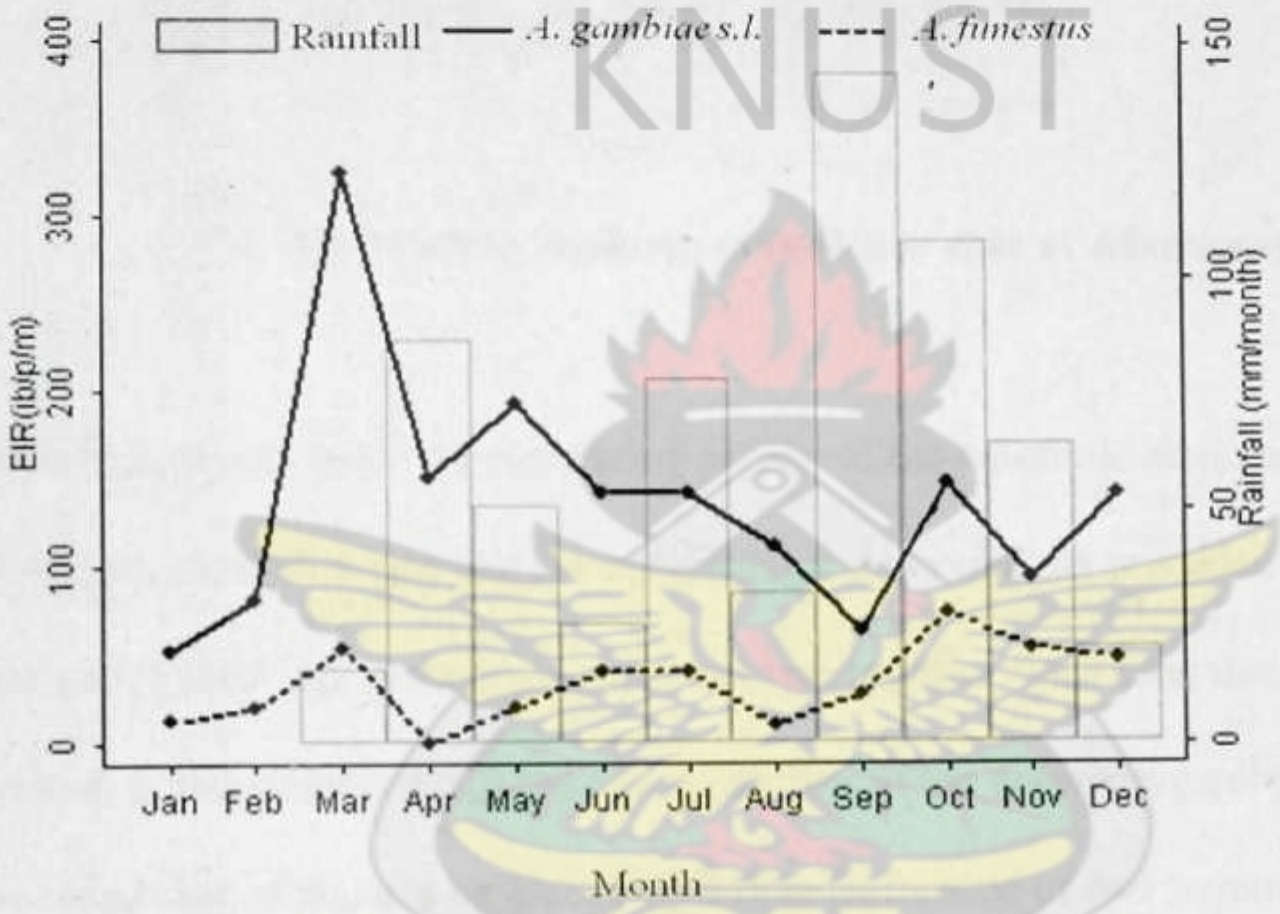
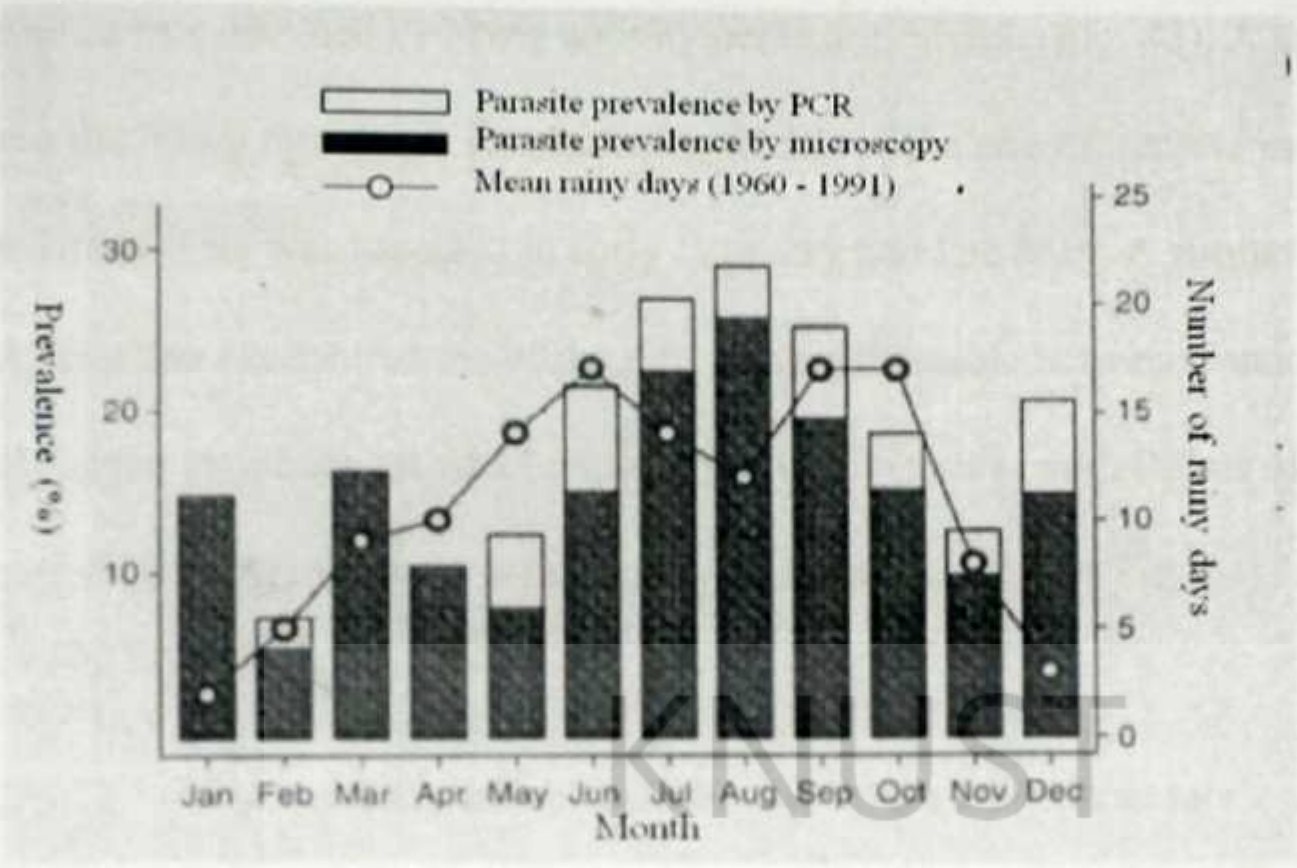


Fig. 41: Monthly EIR at Afamanaso and Kona

There was a general congruence in pattern when entomological parameters such as EIR, biting and sporozoite rates were compared with malaria incidence rates. Whereas in Fig 41, malaria transmission (EIR) peaked towards the end of the dry season and the beginning of the major rainy season, Fig. 42 shows that malaria prevalent rates in the people was highest towards the end of the major and at the beginning of the minor rainy seasons.



Source: Kobe et al., 2006

Fig. 42: Monthly malaria prevalence rate at Afamanaso and Kona.

Therefore, it was found by comparing graphs of the entomological parameters with those of malaria incidence rate that the entomological parameters preceded malaria incidence rate with a time lag, in the main, of about two months. Each time that both parameters peaked, it was observed that the rate of decline of the entomological parameters, e.g. EIR, exceeded that of malaria incidence by a time difference of two months in most cases, resulting in an earlier anti-climax and subsequent climax of the EIR than the incidence rate. There were, however, points of equilibrium when the rate of decline of one parameter equals that of ascent of the other.

4.12.1 Anopheline biting and malaria incidence rates:

The time lag of about two months was observed between monthly peaks of anopheline biting and those of malaria incidence rates at Kona. While biting rate was highest in October, malaria incidence rate climaxed in December when biting rate was low. The

incidence rate declined as biting activity peaked in March (Fig. 43). A point was reached where the biting rate graph intersected with that of the rate of malaria incidence in the population. This was repeated in early February and late May. A similar trend was obtained at Afamanaso except that the biting rate was rather stable between January and April whereas the incidence rate declined sharply within that period. Points of equilibrium occurred here (Afamanaso) in December, February and May (Fig. 44).

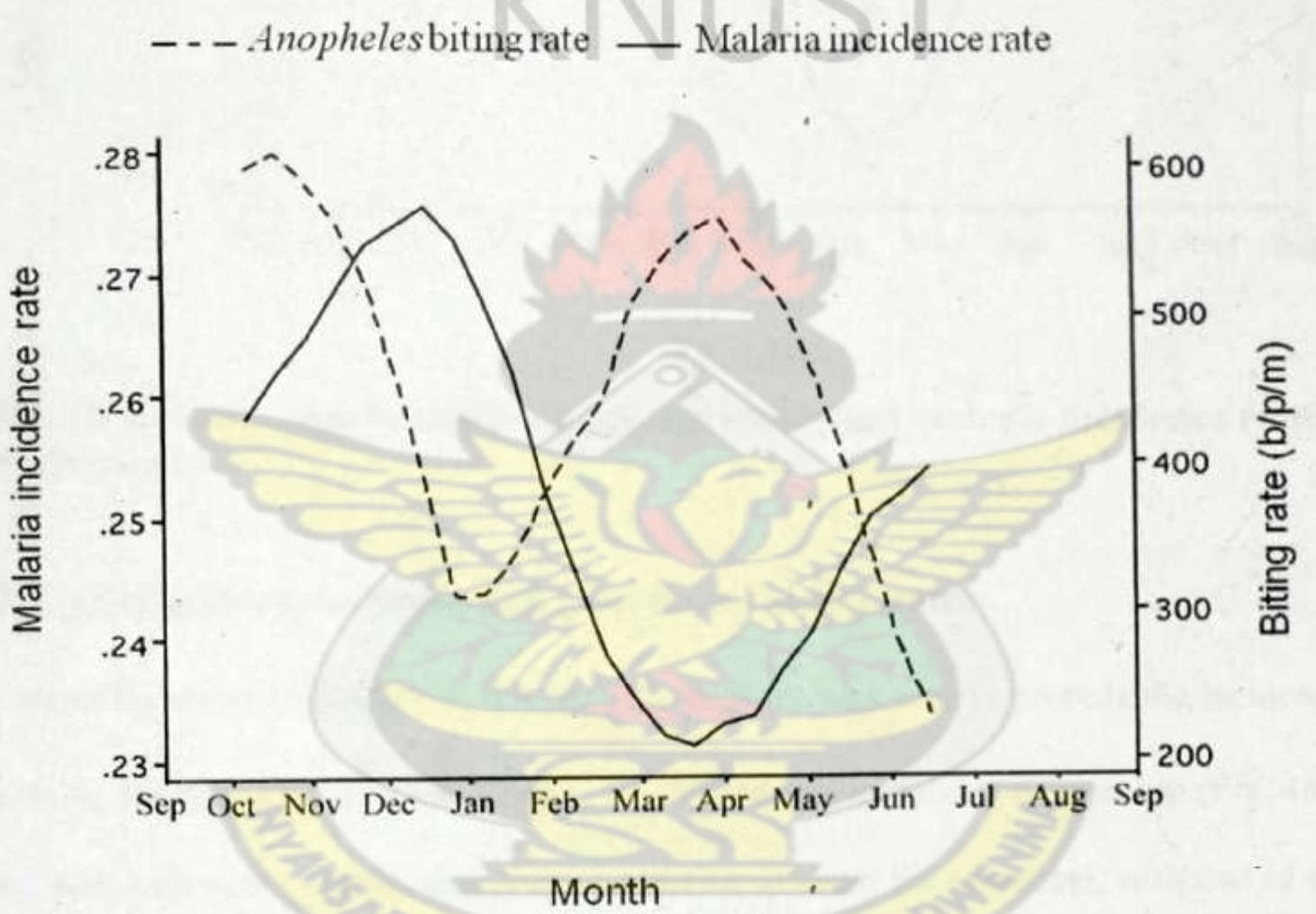


Fig. 43: Relationship between *Anopheles* biting and malaria incidence rates at Kona.

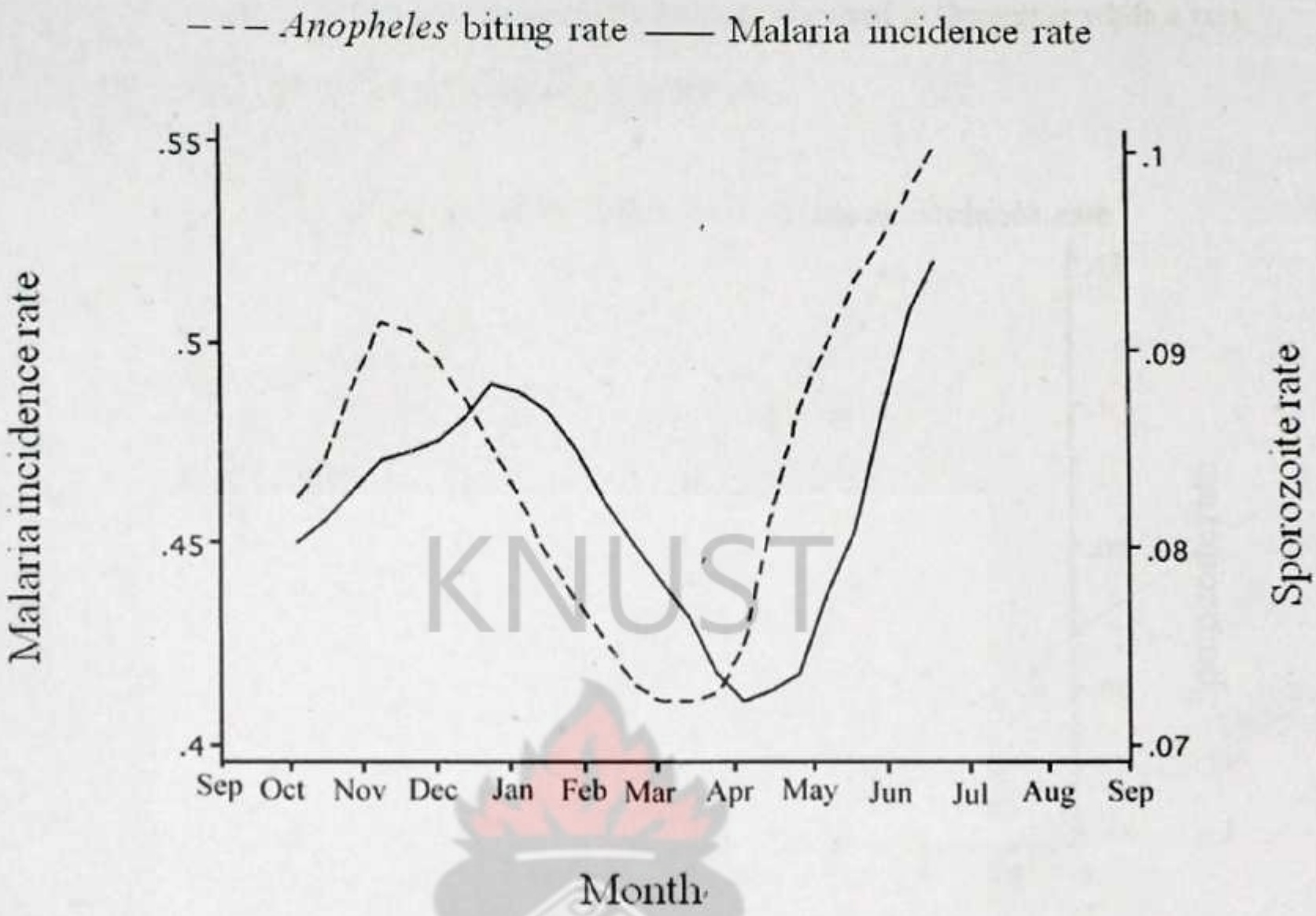


Fig. 44: Relationship between *Anopheles* biting and malaria incidence rates at Afamanaso.

4.12.2 Anopheline sporozoite and malaria incidence rates:

The monthly sporozoite rate of the malaria vectors was seen to precede the incidence rate of malaria by between one month at Kona (Fig. 45) and two months at Afamanaso (Fig. 46). At Kona, both parameters started to increased together at about the same rate, with that of sporozoite rate of *Anopheles* species peaking earlier in November while the malaria incidence rate did so in December. In a similar vein, sporozoite rate declined steadily to its lowest level in February as the rate of malaria incidence was least in March. In early March, an equilibrium rate was established as the sporozoite rate of the mosquitoes equalled the rate of malaria incidence in the population. On the other hand, high sporozoite rate and malaria incidence rates were recorded in November and January respectively, at Afamanaso, with both parameters decreasing in March

and April respectively. In this town, a high equilibrium rate occurred in December while a very low one was recorded for the two parameters late in March.

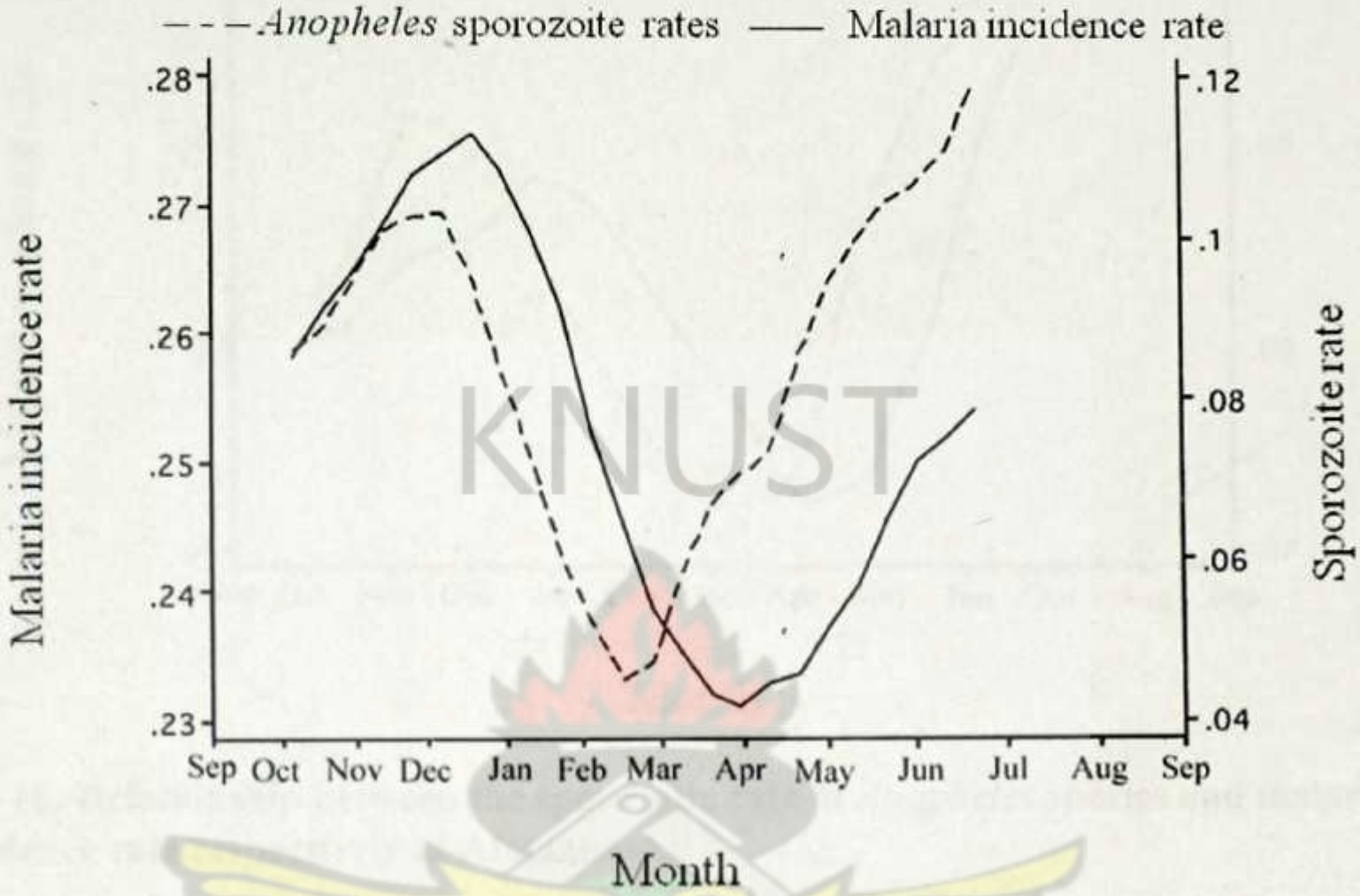


Fig. 45: Relationship between the sporozoite rates of *Anopheles* species and malaria incidence rate at Kona.

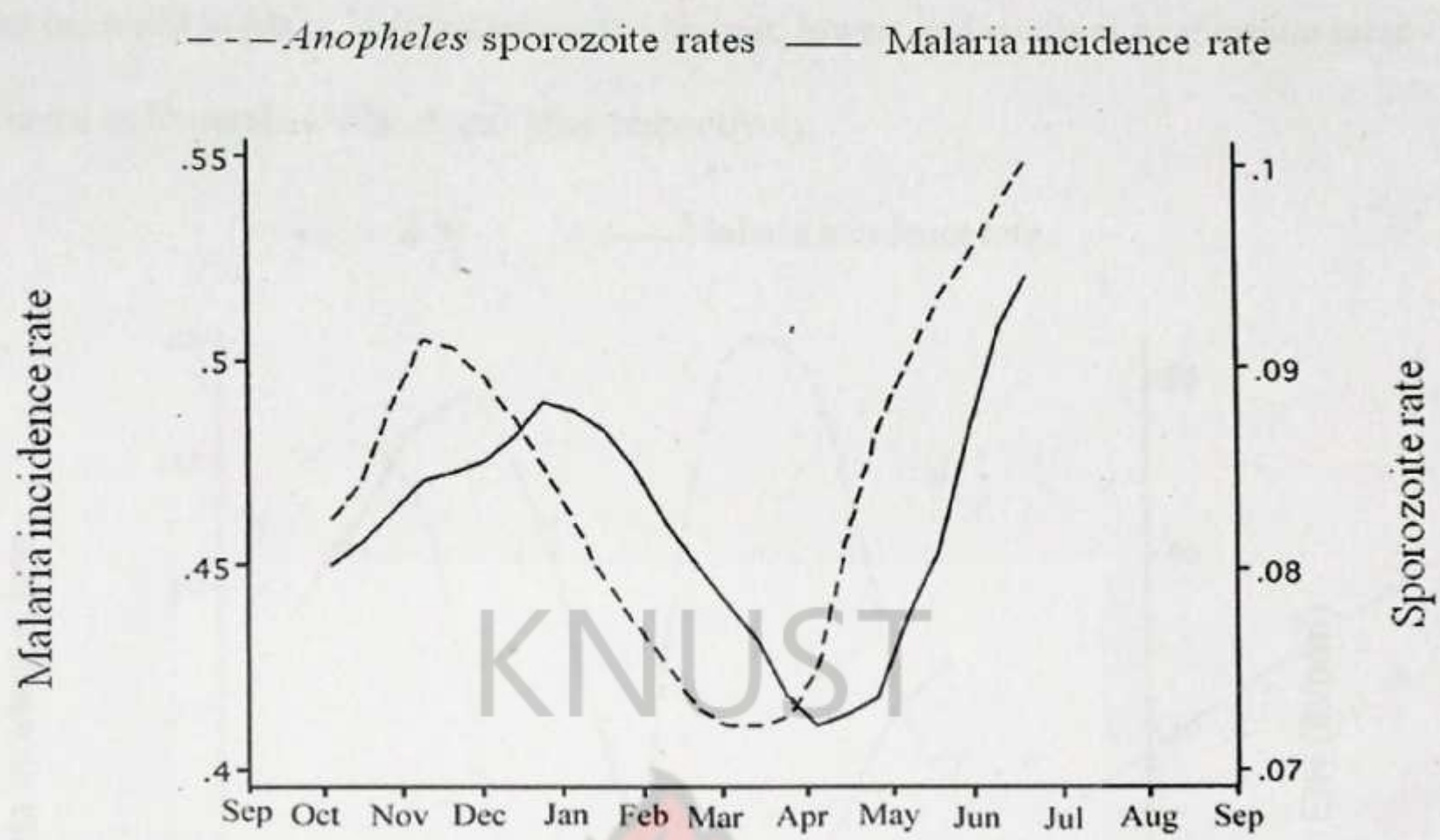


Fig. 46: Relationship between the sporozoite rate of *Anopheles* species and malaria incidence rate respectively at Afamanaso.

4.12.3 Anopheline EIR and malaria incidence rate:

The monthly EIR and malaria incidence rate followed a similar trend as biting or sporozoite and malaria incidence rates. Peaks of EIR and malaria incidence rate occurred in October and December, declining in January and March at Kona on one hand (Fig. 47), but peaking in November and December to decline in February and April at Afamanaso on the other (Fig. 48), with the same time lag of about one to two months. The highest point of equilibrium at Kona was in November when the graph of decreasing EIR intersected with that of increasing rate of malaria incidence in the population. In February, the lowest equilibrium was reached with EIR increasing at the same rate as the decreasing rate of incidence of malaria in the population, whereas a medium equilibrium

point occurred in May. At Afamanaso, the highest, lowest and medium equilibrium rates occurred in December, March and May respectively.

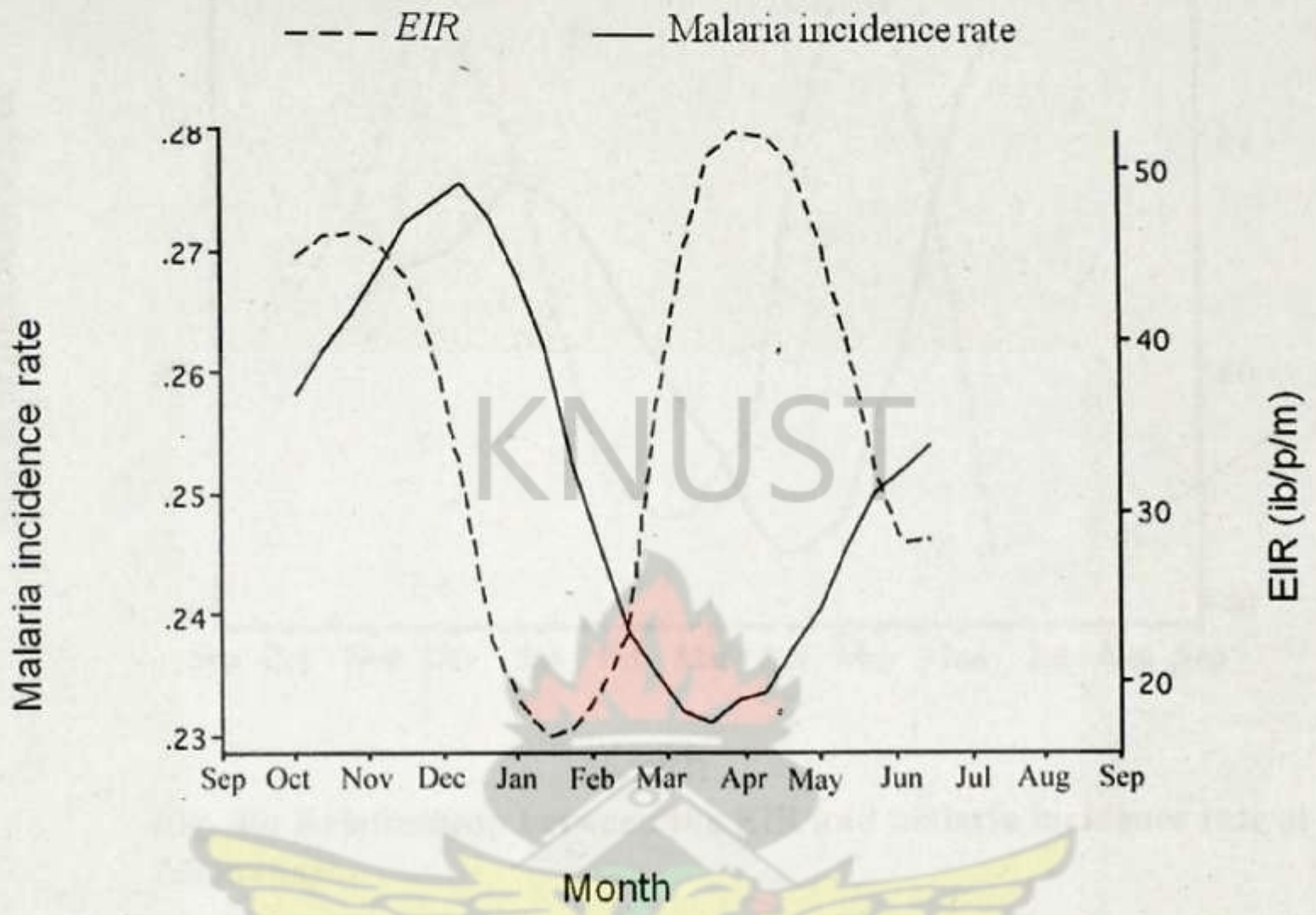


Fig. 47: Relationship between the EIR and malaria incidence rate respectively at Kona.

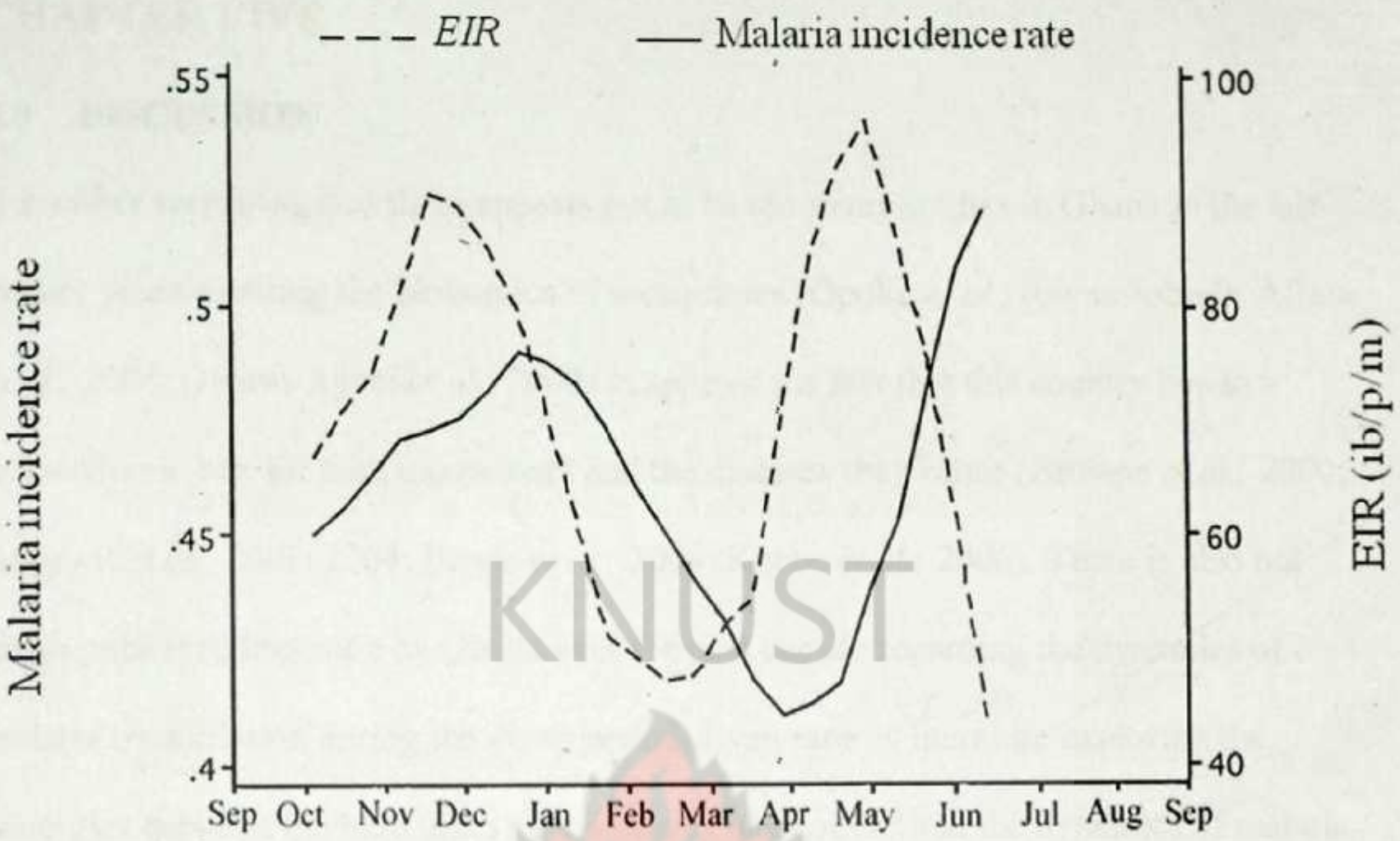


Fig. 48: Relationship between the EIR and malaria incidence rate at Afamanaso.

CHAPTER FIVE

5.0 DISCUSSION

It is rather surprising that there appears not to be too many studies in Ghana in the last twenty years covering the bionomics of mosquitoes (Opoku *et al.,* (unpublished); Afrane *et al.,* 2004; Owusu-Agyei *et al.,* 2009) in spite of the fact that this country lies in a holoendemic belt for both mosquitoes and the diseases they cause (Browne *et al.,* 2000; Appawu *et al.,* 2001; 2004; Banda *et al.,* 2004; Kobbe *et al.,* 2006). There is also not much published literature on Ghana over the past decade regarding the dynamics of malaria transmission during the given period. Even rarer is literature exploring the synergies between the bionomics of *Anopheles* mosquitoes and the dynamics of malaria transmission in the last decade.

The little data available, however, demonstrate the general endemicity of malaria in Ghana because of a suitable climate and the absence of intensive control efforts (Owusu-Agyei *et al.,* 2009). The combinations of these two factors provide suitable conditions for the spread of the disease which are even more favourable in the forest heartland in the middle of Ghana, where, as a result, malaria is holoendemic (Kobbe *et al.*, 2006). Within this forest belt, available data in the last five years is limited to two main sources; a) In Kumasi where verification was made in order to find out the possible impact of irrigated urban agriculture on malaria transmission in cities. Afrane *et al.* (2004), compared city locations without irrigated agriculture with locations with irrigated urban vegetable production, and also with peri-urban locations with rain-fed agriculture. They assessed entomological parameters, self-reported malaria episodes, and household-level data in the city. b) The other source of data was work done in Obuasi, a gold mining town, where in

preparation for the implementation of an integrated malaria control programme, Coetzee *et al.* (2005), studied insecticide resistance in malaria vector mosquitoes and the implications for malaria control. Neither work determined EIR which is the standard entomological parameter used to estimate malaria transmission. Therefore, the current study perhaps represents the first comprehensive study in the forest belt of Ghana to relate entomological parameters to the dynamics of malaria transmission.

The current study found four *Anopheles* species in the study area: *A.gambiae s.l., A. funestus, A. ziemanni* and *A. paludis*. A lot of *Culex* species and only few *Aedes* species were also collected, but were not identified to species level. All samples of *A. gambiae s.l.* randomly processed in conventional PCR turned out to be *A. gambiae s.s.* corroborating an earlier work by Afrane *et al.* (2004), in Kumasi, who similarly analyzed members of *A. gambiae s.l.* in conventional PCR and found all the specimens processed were *A. gambiae s.s.* Though studies on the diversity of species of mosquitoes in Ghana appear to be limited, available data show that the two efficient malaria vectors, *A.gambiae s.s.* and *A. funestus* as well as *Culex* species are the most widespread. In a study to assess mosquito species occurrences and the effects of some ecological characteristics on their breeding in Accra, Opoku *et al.* (unpublished) recorded five species of mosquitoes: *A.gambiae s.l., A. melas, Culex decens, Culex quinquefasciatus*, and *Aedes aegypti*, occurring in a wide variety of places. He observed that *Culex decens* was the most abundant and widely distributed, occurring sympatrically with *Culex quinquefasciatus* in rice fields and fishponds. Whereas the *Culex* species occurred largely in pools of water with high nutrient levels, the *Anopheles* species occurred largely in pools of water of low nutrient levels but high dissolved oxygen levels. Working in the

same Accra, Klinkenberg *et al.* (2008), also found the predominant *Anopheles* species to be *A. gambiae s.s.* with the proportion of molecular forms being 86% S-form and 14% M-form in their study sites. This result agrees with a report from the Guinea savannah area of Upper East Region, where Appawu *et al.* (2001), conducted an entomological study to document the effects of irrigation on the vectors and transmission dynamics of lymphatic filariasis. Collecting mosquitoes by indoor spraying of houses in a cluster of communities located around irrigation projects (Tono and Vea) and control community without reservoirs (Azoka), they reported that *A. gambiae s.s.* was the dominant species and major vector, followed by *A. funestus*. They observed that though *A. arabiensis* constituted 9–14% of the *A. gambiae* complex for malaria transmission, none was found to be infected by the filarial worm suggesting a non-vectorial role in that area as is the case for other members of the complex for filarial transmission in West Africa. True to form though the *Culex quinquefasciatus* caught was as expected, it was not infected by the worm in these communities. Similarly, a later study in the same savannah area in the Kassena-Nankana District, a yearlong intensive mosquito sampling using human landing catches in three micro-ecological sites (irrigated, lowland and rocky highland) yielded 18,228 mosquitoes. *A. gambiae s.l.* and *A. funestus* were the commonest *Anopheles* species collected, constituting 94.30% of the total collection. A small number of *A. pharoensis* (5.4%) and *A. rufipes* (0.3%) were also collected. Molecular analysis of 728 *A. gambiae s.l.* identified *A. gambiae s.s.* as the most dominant sibling species (97.70%) of the *A. gambiae* complex from the three ecological sites with *A. arabiensis* present in fewer numbers (2.3%). Further PCR analysis identified 78.7% M and 21.3% S molecular forms in the *A. gambiae s.s.* population (Appawu *et al.*, 2004). A recent report by

Owusu-Agyei *et al.* (2009), in the drier forests of Central Region also recorded *A. gambiae s.s., A. arabiensis* and *A. funestus* as *Anopheles* species caught, though *A. arabiensis* was not found infected by the malaria parasite. When all these reports covering the coastal savanna, the guinea savanna, the drier forests and the present study in the wetter forest are put together, they further confirm that *Anopheles gambiae s.s.* is the most widespread and most important malaria mosquito followed by *A. funestus* in Ghana. This study further concurs with Owusu-Agyei *et al.* (2009), contention that the climate is well suited to the survival of these *Anopheles* species with many suitable breeding sites throughout the year.

The present study also asserts that the relatively large numbers of *A. gambiae s.s.* caught in both the rural and the peri-urban sites and *A. funestus* in the rural sites generally suggest a high potential for the prevalence of malaria in the study areas. This assertion is further supported by the high malaria prevalence and episodes reported by Kobbe *et al.* (2006), and Kreuels *et al.* (2008), in the AFigya-Sekyere District where two of the study sites Afamanaso and Kona are located and by Browne *et al.* (2000), in parts of the Ashanti region where all the study sites are located. The biting activities of all mosquito species captured during the current study at all the sites covered were found to be very high and perennial with *Anopheles* species showing higher biting activities than *Culex* at Afamanaso and Kona. It is generally agreed that in rural areas where there is less organic pollution of water bodies *Culex* species are less of á menace compared with *Anopheles* species (Klinkenberg *et al.,* 2008) and the present study supported the trend. At Boadi, however, larger numbers of *Culex* species were caught than *Anopheles* species. It must be noted that the Boadi site is a cattle farm with no permanent human settlement. The

larger numbers of *Culex* species may be that the cattle wastes that are cleaned out and find their way into some of the water bodies (Cornelius, 1962; Lisa *et al.,* 2007; Opoku *et al.,* unpublished).

The bell-shaped curves produced by the hourly biting patterns of both *Anopheles* and *Culex* species, are characteristic of *A. gambiae s.s.* (Gillies and De Meillon, 1968; Aniedu *et al*., 1993; Geissbühler *et al*., 2007). These curves were generally similar at Afamanaso and Kona, and suggest biting activity began at dusk and progressively increased but they peaked at different times of the night, and dropped progressively, but persisted till 06. hours the next morning. It was interesting to observe that the biting activities of the *Culex* species on the Boadi Cattle Farm peaked as early as 19 hours, maintained this peak till 04 hours resulting in the formation of a plateau before declining thereafter. This plateau curve was different from the pattern of this same mosquito species at Afamanaso and Kona where single biting peaks occurred between 22 and 23 hours to inscribe a typical Anopheles species bell-shaped curve. The plateau-shaped biting pattern of the *Culex* mosquitoes at Boadi, however, compares favourably with that in a report by, Sucharit *et al.* (1981), in Bangkok on *Culex quinquefasciatus*, where there were two biting peaks with a short dip of 02 hours in between. In that work, the first peak occurred between 22 and 23 hours and the latter peak, after midnight between 01 and 04 hours. In the present study at Boadi, the first peak at the beginning of the plateau occurred earlier at 19 hours whereas the second peak ending the plateau occurred at 04 hours; similar to the report of Sucharit *et al.* (1981). Similar bimodal biting pattern is also reported by Forattini *et al.* (1988) working in Brazil ~~on *Culex ribeirensis*~~. In essence, however, the results from Boadi rather bears a closer resemblance to the work of Davies (1975), that showed

Culex (Melanoconion) portesi and *C. (M.) taeniopus* showed two peaks of biting activity, one in the early evening and the other at dawn. Perhaps the shallow trough reported by Sucharit *et al.* (1981), was missed from Boadi because the period of the trough was only 2 hours long. Further focused work may be required to validate the existence of this trough. On the other hand, the Boadi pattern, shows even much closer similarity to a unimodal biting pattern reported in the work of Chadee and Tikasingh (2008), in Trinidad, West Indies, in which he found biting activities of *Culex caudelli* as one well-defined peak between 22 and 04 hours; broad enough, in my considered opinion, to form a well defined plateau. The period of peak biting activity of *Culex* species, at Afamanaso and Kona, however, were both similar to the first peak of Sucharit *et al.* (1981), occurring between 22 and 23 hours. One study perhaps, is not enough to propound any theories yet; but perhaps a few more studies in the peri-urban localities where *Culex* species predominate, should allow one to explore the reasons behind the plateau in the biting pattern of *Culex* in the urban areas as compared to the bell- shape in the rural areas.

The biting patterns of *Anopheles* species generally peaked between 22 and 01 hours at Afamanaso and Kona. This bell-shaped hourly biting pattern is considered typical of *A. gambiae* s.s. (Gillies and De Meillon, 1968; Aniedu *et al.*, 1993; Geissbühler *et al.*, 2007). In Nigeria Awolola *et al.* (2003), observed similar patterns in *A. gambiae s.s.*, with peak indoor and outdoor biting activities occurring at 24 and 01 hours respectively. In the mount Cameroon region peak biting activity occurred between 01 and 02 hours (Wanji *et al.*, 2003). There is an interesting disparity, however, in this typical *A. gambiae s.l.* biting pattern described in this work and also reported in literature that was observed on the cattle farm at Boadi in the Kumasi Metropolis; where peak biting activity occurred at a

much later time of between 02 and 03 hours, climaxed at 02 hours. To validate the peculiarity of this pattern on the cattle farm, *A. gambiae s.s.* mosquitoes were collected from Anwomaso, a peri-urban town located about 1 km away from the Boadi Cattle Farm and another farm (Nana's farm) located at a distance of 2km from the Boadi Cattle Farm. It was found that the biting pattern of *A. gambiae s.s.* at both Anwomaso sites correlated well with the typical bell- shape of Afamanaso and Kona, about 45km away. The reason for this skewed shape at Boadi remains unexplained except to say that the peak activity at a later time of 2.00 bears a resemblance to the findings of Geissbühler *et al.* (2007), in Tanzania in which peak activity occurred between the hours after 12 midnight and 3.00 am. The hourly biting activity of *A. funestus* at Afamanaso and Kona were strikingly similar to the pattern inscribed by *A. gambiae s.s.*, with peak biting activity also occurring between 23 and 02 hours. This observation is supported by the work of Awolola *et al.* (2003), in Nigeria, who recorded a similar biting pattern for *A. funestus s.s.* with both indoor and outdoor peaks at 23 hours.

The peaks of monthly biting rates plotted over a year for *Anopheles* and *Culex* species collected in Afamanaso and Kona showed undulating patterns. These peaks almost coincided with the peaks of rainfall. The troughs inscribed times of less rain. Detailed observation showed that the peak biting rates occurred slightly after the peak rainfall. This observation is in line with literatures which suggest that mosquitoes, particularly. *Anopheles* species proliferate in moderate rains which preserve breeding sites or thrive some days after a heavy rain washes out their breeding sites and new temporary pools are re-created after such down pours, (Paaijmans *et al.*, 2007; Krijn *et al.*, 2007; Rozendaal, 2008). Furthermore, the biting rates of *Anopheles* species at the rural sites of Kona and

Afamanaso were consistently higher than those of the *Culex* species suggesting that in rural areas, *A. gambiae* may be more efficient in causing nuisance with their bites than that of the *Culex* species. Again *A. gambiae s.l.'s* peak biting activity occurs deep in the night (Gillies, 1957) when most rural community victims would have been fast asleep and been most vulnerable to bites. These two observations complement each other to perhaps help explain why *A. gambiae s.l.* has become the most efficient vector of malaria (WHO, 2003; 2005). Since no report has been found comparing the biting activities of the *Anopheles* with that of the *Culex* species, the current study perhaps may be one of the few, reporting the activities of the two mosquitoes in tandem; and in future it may be worth exploring how, perhaps, their relative abundance may influence each other's biting patterns. It was observed in the present study that the biting pattern of the *Culex* species assumed the bell-shaped pattern of their *Anopheles* counterparts in the rural sites where their numbers were smaller. However, in the peri-urban sites where their numbers are much larger than their *Anopheles* species counterparts, the biting pattern of *Culex* species shows an early peak and a sustained plateau for several hours before declining.

The current study also confirmed the sibling species of *A. gambiae s.l.* as the primary vector of malaria parasites in the forest zone of Ghana with *A. gambiae s.l.* as the major player while *A. funestus* is a minor but equally important player. All the study sites with the exception of Boadi Cattle Farm, consistently recorded higher overnight catches of *A. gambiae s.l.* than *A. funestus.* At Kona it comprised as high as 90% of the mosquitoes caught in overnight catches. The study by Coetzee *et al.* (2005), who collected more *A. gambiae s.l.* than *A. funestus* in the forest zone at Obuasi, corroborates these results. This result is in contrast to the situation in the drier grassland areas of the country where the

results are mixed. Whereas Klinkenberg *et al.* (2008), working in the Coastal savannah area of Accra confirms this work by reporting A. *gambiae s.l.* as the major malaria vector, Appawu *et al.* (2004), found that both *A. gambiae s.l. and A. funestus* contributed approximately the same to malaria transmission in the Guinea savannah areas of northern Ghana. Interestingly, Elissa *et al.* (1999), working in the forested Gabon in Central Africa asserted that *A. funestus* is the primary vector. Similarly, in Cameroon, Manga *et al.* (1995), found that *A. gambiae s.s.* played a minor relay role only when transmission due to the main vector *A. moucheti* was interrupted.

The results of the current study, together with what was reported in literature, thus reveal how complex the bionomics of malaria transmission can be, regarding the interplay between the plasmodia parasites and their mosquito vectors. It appears that minute micro-ecological changes that are not totally understood, determine which the major vectors or parasites will be in a locality. To begin, in a rainforest zone of Nigeria, 45.50% of the total *A. gambiae s.l.* analyzed by PCR were identified as *A. gambiae* s.s. while 54.5% were *A. arabiensis* (Oyewole *et al.*, 2005). Yet Okwa *et al.* (2007), studying in the same Nigeria, reported that *A. gambiae s.s.* was the most widespread and competent vector in all six areas studied in Nigeria, with *A. funestus* being the second predominant and competent vector. Then, in far away, Dar es Salaam, Tanzania, Geissbuhler *et al.* (2007), found *A. gambiae s.s.* as the most important vector with *A. funestus* making minor contribution to transmission just as in Ghana. Other works that showed *A. gambiae s.s.* as the major malaria vector include that in Burkina Faso (Boudin *et al.*, 1991), Tanzania (Magesa *et al.*, 1991), Western Kenya (Shililu *et al.*, 2003), Nigeria (Awolola *et al.*, 2003); WHO, (2003) and that of WHO, (2005).

It stands to reason from the foregoing that it would be presumptuous to prescribe a single formula for bionomics of malaria transmission for any large region such as a country. Rather, a patch work of bionomics from various localities may eventually build a montage for understanding malaria transmission in a country. This current study, may perhaps be establishing for the first time a patch work for the forest belt of Ghana in suggesting that *A. gambiae s.s.*, the most proficient malaria vector, is mainly responsible for malaria transmission in that region.

However, the proportion of *A. gambiae s.s.* was as high as 56.65% at Afamanaso and even higher at 70.65% at Kona because this mosquito species is anthropophagic (Awolola *et al.*, 2002; 2005; Okwa *et al.*, 2007) and abounds in human dwellings. The proportion of *A. funestus* of 28.77% and 7.60% at Afamanaso and Kona respectively are also much higher than the 0.17% observed on the Boadi Cattle Farm with no human habitation and again because this malaria vector too, is anthropophilic (Awolola *et al.*, 2002; 2005; Okwa *et al.*, 2007).

The number of *A.ziemani* collected in this study also provides interesting information only two (2) mosquitoes caught at Afamanaso were thought to be members of *A. ziemanni* (data not presented) and none at all in Kona. No specimen of *A. ziemanni* was caught at Anwomaso, perhaps due to the absence of livestock. And 4.72% of mosquitoes caught on Nana's Farm were *A. ziemanni* where there were livestock. The peculiar low numbers *of A. gambiae s.s.*, and the relatively larger numbers of *A. ziemanni* and *Culex* species on the Boadi Cattle Farm with no human habitation, simultaneously confirm three interesting observations from literature. 1) Gillies and Wilkes, (1972) reported *A. ziemanni* as being zoophilic with particular preference for cattle. In agreement with this

assertion, *A. ziemanni* constituted 14.45% out of the total number of mosquitoes caught on the Boadi Cattle Farm; more than the 11.15% for *A. gambiae s.s.* 2) That *A. gambiae s.s.* is anthropohilic (Okwa *et al.*, 2007) was demonstrated by the relatively low ratio of *A. gambiae s.s.* caught in Boadi Cattle Farm compared to collections elsewhere in the study. 3) That *Culex* species has become an urban nuisance mosquito due to increasing organic pollution of water bodies is also amply demonstrated here. The organic cattle dander produced by cattle are ultimately washed into the water bodies creating the optimal organically rich environment that provides food supply for the *Culex* species while at the same time create unfavourable environments for mosquito predators due to the diminished oxygen tensions (Calhoun *et al.*, 2007; Chaves *et al.*, 2009). The unusually large proportions of 74.22% for the *Culex* species on the Boadi Cattle Farm attest to this. In sharp contrast to the situation at peri-urban Boadi and Anwomaso where *Culex species* were caught in larger proportions out of the total caught, is the situation at rural Afamanaso and Kona, where there were no cattle. For that reason, the proportion of the *Culex* species was as low as 14.58% in the more rural farming village, (Afamanaso) and 21.74% at the slightly urbanised Kona with relatively more organic pollution of water bodies. In peri-urban Anwomaso with considerable polluted water collections, 31.26% of the town collections were *A. gambiae s.l.* with a larger 68.74% being *Culex* species However, on the adjacent Nana's farm, even a much lower 3.05% was recorded for *A. gambiae s.l.* with a very high 92.22% for the *Culex* species with the remaining 5% being *A. ziemani;* because the wastes from the livestock that end up in the water bodies create suitable breeding sites for the *Culex* species (Calhoun *et al.*, 2007; Chaves *et al.*, 2009).

Furthermore, results of the present study seem to suggest that some kind of association has been established between increasing numbers of *A. ziemanni* as a result of the presence of livestock and increasing numbers of *Culex* species, perhaps brought about by the organic waste washed from these farms into water bodies. This may perhaps be the first time this association between the two species is being reported. Additionally, two key findings that may be receiving their first mention ever in Ghana also occurred at the Boadi Cattle Farm; and these are, a) the discovery of infective *P. falciparum* sporozoites in 10 out of the over 600 *A. ziemanni* caught in the first part of the study and, b) capture and subsequent identification of a few members of A. *paludis* belonging to the *A. coustani* group, during the second part of the study.

The hourly biting pattern of the zoophilic mosquito, *A. ziemanni,* on both the Boadi Cattle Farm and Nana's Farm inscribed the typical, bell-shaped pattern of *A. gambiae s.s.* Whereas the biting activity of this mosquito species at Boadi peaked by midnight (just like those of *A. gambiae s.l.* and *A. funestus*), declining progressively thereafter, the situation on Nana's farm resembled the description of the behaviour of *A. ziemanni* in Kenya, where biting activities began in the early part of the night, practically ceasing after 01 hours (Chandler *et al.*, 1975).

There were more outdoor biting activities of *A. gambiae s.l.* and *A. funestus* than indoor at Afamanaso, the reverse of which occurred at Kona. The results at Afamanaso is similar to a finding in two communities in Ibadan, south-western Nigeria (Awolola *et al.*, 2003) as well as another on the Atlantic Coast of Lagos, Nigeria, where outdoor biting mosquito numbers were significantly larger than that observed indoors (Afolabi *et al.*, 2006). In São Tomé, Charlwood *et al.* (2003), had a similar finding that biting of *A. gambiae s.s.*

took place primarily outside at ground level, with less than one third of the biting occurring inside houses.

The occurrence of more biting *A. funestus* indoor at Kona than outdoor, on the other hand, corresponds with the findings of Githeko *et al.* (1996), who worked in Western Kenya and reported that *A. funestus* was 6.6–8.2 times more likely to bite people indoors than outdoors, while *A. gambiae s.l.* females were only 2 times as likely. Interestingly, the same mosquito species (*A. funestus*) bit more frequently outdoor at Afamanaso than indoor and that is inconsistent with the work of Githeko *et al.* (1996). Nevertheless, these findings are consistent with that in Tanzania where *A. gambiae s.s.* and *A. funestus* were found to be exophagic in urban Dar es Salaam but endophagic in rural Kilombero Valley (Geissbuhler *et al.*, 2007).

The tendency toward outdoor feeding by the endophilic vectors is noteworthy. In areas with houses screened with mosquito-proof nets and/or where indoor residual spraying and/or ITNs were used in vector control, such exophilic behaviour was attributed to adaptation to avoid contact with the control agent (de Zulueta, 1959; Pates and Curtis, 2005; Geissbuhler *et al.*, 2007). Afamanaso and Kona had no history of the use of these control measures except that pyrethroid insecticides were used for agricultural purposes. The citizens of the relatively more urbanised Kona, however, stayed outdoors up to midnight either weaving or selling and this could serve to attract outdoor biting. These mixed reports from literature of the behaviour of the same species of mosquito acting differently in different locations suggest these mosquitoes may rapidly alter their behaviour to respond to both conducive and adverse conditions.

The use of salivary gland dissection to determine sporozoite infection has been considered as the gold standard method for estimating malaria transmission (Lochouam and Fontenille, 1999). Two main reasons have been given for this assertion: 1) the sporozoites are clearly seen which reduces the possibility of false positives by ELISA due to contamination on any part of the mosquito's body; and, 2) the sporozoites are in the salivary gland of an infected *Anopheles* mosquito suggesting the mosquito is actually infective. However, due to the practical difficulty of dissecting large numbers of mosquitoes, coupled with the possibility of failing to detect sporozoites in mosquitoes whose salivary glands may contain just a few sporozoites, other methods such as ELISA have been developed (Burkot *et al.,* 1984; Wirtz *et al.,* 1987, Collins *et al.,* 1988; Wirtz *et al.,* 1992). In my considered opinion, the two methods i.e. salivary gland dissection and ELISA, when used in tandem, provide a good quality control for each other. In the present study, the sporozoite rate determined by ELISA was 15.88% times higher than that by dissection which was not significantly different (P = 0.06); suggesting minimal possibility of scoring for false ELISA positives or missing out on small number of sporozoites in dissected salivary glands. This finding from the current study is different from the results obtained by Beier *et al.* (1990), in which ELISA infection rates were about 43% higher than dissection sporozoite rates. However, given the much higher cost required to run an ELISA, dissection may still be the most practical and useful method in the determination of malaria parasite infections of mosquito vectors particularly in Sub-Saharan Africa.

An attempt was also made in the present study to determine whether or not the removal of the salivary gland from a mosquito during dissection has any effect on the detection of

P. falciparum infection of that mosquito using ELISA tests. It was observed that where salivary glands have been removed from *A. gambiae s.l.* samples during dissection, the number of those mosquitoes found to be infected by *P. falciparum* using ELISA tests was lower than those whose salivary glands had not been removed, though the difference between the two groups was not statistically significant (P=0.06). The reason why ELISA tests could bring no distinction between falciparum infections in the two groups may perhaps be due to the high proficiency of *A. gambiae s.l.* as a vector of *P. falciparum.* This means larger numbers of the parasites develop within the mosquito so that removing the salivary glands may still leave higher circumsporozoite proteins (CSP) concentration in the thorax and head than would be the case in less competent malaria vectors. This assertion is supported by the fact that where salivary glands have been removed from *A. funestus* during dissection, the number of these gland-removed *A. funestus* found to be infected by *P. falciparum* using ELISA tests was significantly lower than in those whose salivary glands were intact (P=0.02).

Furthermore, when the head and thorax parts of members of *A.gambiae s.s.* that were found to be negative by dissection were tested in ELISA, only 3.11% turned out positive. This is in accord with Beier *et al.* (1990), where in dissection-negative *Anopheles,* ELISA test detected circumsporozoite protein (CSPECIES) in 5.20% salivary gland samples and in 12.20% thorax samples.

However, when the head and thorax parts of members of *A. gambiae s.s.* that were found to be positive by salivary gland dissection were tested in ELISA, 35.14% of them turned negative in ELISA test. A similar result was observed with members of *A. funestus* in which 50% of dissection-positives were found negative in ELISA test. It would be

expected that almost all dissection-positives be ELISA-positives while some dissection-negatives may be ELISA-positives. It thus appears that removing the salivary gland from a mosquito may perhaps reduce the concentration of CSPECIES in the infected mosquito to a lower level than could be detected in ELISA test. Given that no report has so far been found on this observation, the current work perhaps may be one of the few reporting it for the first time.

The sporozoite rates were considerably high in rural Afamanaso (10.25%) and Kona (10.00%) compared to the peri-urban towns Boadi (1.55%) and Anwomaso (5.63%). confirming the hypothesis that malaria transmission is more of a rural phenomenon. The overall results also corroborate reports of endemicity of malaria in the forest belt (Browne *et al.*, 2000; Kobbe *et al.*, 2006; 2007; Kreuel *et al.*, 2008).

Sporozoite rates of *A. gambiae s.s.* were not significantly higher than those of *A. funestus* at Afamanaso (P = 0.20). This finding is in accord with Appawu *et al.* (2004), who found no significant difference in *P. falciparum* sporozoite rates of 7.2% and 7.1% for *A. gambiae s.l.* and *A. funestus*, respectively in northern Ghana. *A. gambiae s.l.* however, contributed a significantly higher sporozoite infection rates at Kona (P = 0.00), than *A. funestus*. This contrasting finding nevertheless agrees with the results of Okwa *et al.* (2007), in six areas of Lagos, Nigeria, where *A. gambiae s.l.* had significantly higher sporozoite rates and human blood indices in all six areas. The results at Kona were at variance with the finding by Shililu *et al.* (1998), in Western Kenya where *P. falciparum* sporozoite rates of 6.30% for *A. gambiae s.l.* and 9.5% for *A. funestus* were obtained. Given the contrasting reports from literature and the closeness of Kona and Afamanaso (and therefore the expectation that they would yield similar results), the observations so

far underscore the danger of making generalised statements about similar behaviour of mosquitoes within a locality. It appears that within the same settlement the behaviour of the mosquitoes may change from site to site depending on prevailing conditions. In the present study, sporozoite rates of both *A. gambiae s.l.* and *A. funestus* varied from one study site to the other significantly (P = 0.00).

The infection of the zoophagic *A.ziemanni* by *P. falciparum* on the Boadi Cattle Farm during the first part of the study gives credence to the work of Kamau *et al.* (2006), in Western Kenya where a human blood index of 0.09 in the species compared well with those of other malaria vectors. It also affirms an earlier report of sporozoite infection in this mosquito in Cameroon (Antonio-nkondjio *et al.*, 2006), suggesting its susceptibility to, and its potential as *P. falciparum* vector. The absence of an infected *A. ziemanni* in the second year when 874 specimens were dissected for sporozoites and subsequently tested in ELISA corresponds with an earlier report of 846 dissections of this same species all of which all proved negative for sporozoites in Angola (Ribeiro *et al.*, 1964; Ribeiro and Ramos, 1975). Granted that *A. ziemanni* is not a competent vector of malaria, it could well be that just a few sporozoites managed to develop fully and fewer still were able. The result is that mosquitoes infected by very few sporozoites are neither detected by dissection nor by subsequent ELISA test. This may perhaps be the reason for the failure of the present work to detect infected *A. ziemanni* during the second part of the study, though infected ones were found during the first part of the study. To definitely confirm that *A.ziemanni* in Boadi carries infective *P. falciparum* may perhaps be best investigated in a follow-up study.

The entomological inoculation rates (EIRs) (which are good indicators of the level of malaria transmission) calculated in this study for the various sites were generally very high and therefore suggest very high malaria transmission. At Afamanaso and Kona malaria transmission can be considered perennial though seasonal in intensity as revealed by EIR values calculated from data collected for over twenty months. A study found that sites with annual EIRs of 5, 15 and 200 ib/p/yr were associated with *P. falciparum* prevalence of 40%, 50% and 80% respectively (Beier *et al.*, 1999). Therefore, the very high annual EIRs of *A. gambiae s.l* at Afamanaso (699.42 ib/p/yr), and Kona (433.06 ib/p/yr) are indications of very high malaria transmission. The projected annual EIR of 449.58 ib/p/yr at Anwomaso and 56.28 ib/p/yr on the Boadi Cattle Farm were much higher than the annual EIRs of 19.20 and 6.60 ib/p/yr estimated for urban agricultural and non-agricultural areas of Accra respectively (Klinkenberg *et al.*, 2008). All these findings further affirm the middle forest belt as a malaria holoendemic zone (Kobbe *et al.*, 2006).

The annual EIRs recorded at Anwomaso, Kona and Afamanaso were all higher (much higher in the latter town) than the value of 418.00 ib/p/yr reported by Appawu *et al.* (2004), in the savannah area of the Kasena-Nankana District in northern Ghana. In addition, Appawu *et al.* (2004) observed generally high biting and sporozoite rates of the malaria vectors which varied between the study locations, being significantly higher in an irrigated area than in non-irrigated lowland and rocky highlands, confirming that the intensity of malaria transmission was not only very high, but was also seasonal and varied from place to place. On their part, Owusu-Agyei *et al.* (2009) reported high average annual EIR of 269 ib/p/yr in the relatively drier forest of the Central Region of Ghana, which again, suggest very high malaria transmission. Afari *et al.* (1995) also

recorded high average annual biting rate of *A. gambiae s.l.* of 562.49 b/p/yr at Prampram and 288.55 b/p/yr at Dodowa with the average sporozoite rate being higher at Dodowa than at Prampram.

Reasons for this high malaria transmission in the central forest belt of Ghana could be the prevalence of rivers and tributaries permeating the study areas and which provide putative mosquito breeding sites, accounting for the high vector densities, biting rates and EIRs in these places. A study undertaken in Senegal (Trape *et al.*, 1992), Kenya (Minakawa *et al*., 2002), Ghana (Appawu *et al.*, 2004; Klinkenberg *et al*., 2008), and Equatorial Guinea, (Cano *et al*., 2006) all showed a correlation between the abundance of breeding sites to high malaria transmission due to high vector density and biting rate. Kreuels *et al*. (2008), reported from the same study area as the current study that malaria incidence rates (IRs) steadily decreased by 0.20±0.06 episodes/per person at risk (E/PYAR) (P = 0.00) with every 50m increase in distance from the village-forest border toward the Centre of the village suggesting an association between malaria incidence rates on one hand and distance of households from putative mosquito breeding sites at the village-forest border on the other. Staedke *et al*. (2003), reported a strong association between incidence of clinical episodes of malaria and proximity of residence to potential mosquito breeding sites in Uganda.

The widespread occurrence of malaria vector mosquitoes in the forest belt, as this study suggests therefore supports the reports of concomitant high prevalence of malaria parasitaemia and clinical attack incidence rates in Ghana as a whole. Owusu-Agyei *et al.*(2009) observed that the burden of malaria was very high in the forest/savannah transition of central Ghana. In an earlier report, Owusu-Agyei *et al.* (2002), in an age-

stratified cluster sample of 308 individuals from Kassena-Nankana District of northern Ghana, it had been suggested that the overall prevalence of *P. falciparum* by microscopy was as high as 70%, with the maximum among children whose age ranged between 5 and 9 years old. These findings support an even earlier report of Afari *et al.* (1995), which indicated a considerably high and variable rates of clinical malaria incidence and prevalence of patent parasitaemia when they conducted a survey to compare malaria infection, morbidity and transmission patterns between a coastal savannah community along the coast of Ghana (Prampram) and one other inland community (Dodowa) on the forest fringes bordering the coastal savannah zone in south-eastern Ghana. The findings also agree with claims of high malaria burden and transmission in Ghana by Adams *et al.* (2004), when they reported that while there is an overall consistent decrease of other infectious and parasitic diseases (from 31.80% in 1985 to 19.50% in 2003), there has been an increase in malaria cases (from 37.10% in 1985 to 44.70% in 2003).

Time of peak malaria mosquito biting activity and therefore presumably malaria transmission was observed to occur deep in the night when victims are fast asleep in a normal rural setting where bedtime is 8 pm when night has fallen. A twelve-hour distribution of EIR (6 p.m. – 6 a.m.) indicated that the hourly malaria transmission that peaked at 11 pm in both Afamanaso and Kona while that of *A. funestus* peaked almost an hour earlier at Kona (between 10pm and 11 pm) than at Afamanaso (between 11 pm and 12 midnight). In contrast, transmission by both *A. gambiae s.l.* and *A. funestus* was found to peak at day-break between 5 am and 6 am in northern Ghana (Appawu *et al.*, 2004).

That malaria transmission by the two mosquito species in the forest belt in the current study peaked by midnight indicates that ITN use could be effective in reducing malaria

transmission in the study sites (Snow *et al.*, 1988; Alonso *et al.*, 1991; Binka *et al.*, 1996; Gimnig *et al.*, 2003), as potential victims of malaria, given the earlier time they sleep would still be protected under an insecticide-treated net long before peak biting activity of the malaria mosquitoes. Even though ITNs can help in reducing malaria pressure on the citizens, we need to put into perspective its limitations i.e. there would be considerable effective inoculations before 7 pm and after 4 am. In African communities where women return late from farms and markets to prepare late supper, even children who have ITNs may not be in bed before 7 pm. In the same vein, by 4 am, some women are out with children on their backs, preparing food for sale in the bars or on their way to the farms or markets, further exposing them to infective bites. Nevertheless, intervention measures such as use of ITNs need to be enhanced by widespread use in the communities to cause mass vector killing through contact with the multitudes of ITNs. It has been reported that high coverage of ITNs is associated with a community-wide suppression of mosquito populations that even result in protection against malaria vectors in neighbouring villages lacking ITNs (Magesa *et al.*, 1991; Gimnig *et al.*, 2003; Pates *et al.*, 2005; Carnevale *et al.*, 1988; Karch *et al.*, 1993; Jaenson *et al.*, 1994; Quinones *et al.*, 1998). For holoendemic and perennial malaria transmission areas such as this work has shown in the central forest belt of Ghana, widespread use of ITN alone to reduce malaria may not be sufficient; rather widespread ITN use as part of an integrated scheme may be more prudent.

The observation in this study that perennial malaria transmission was also seasonal in intensity, and that it correlated well with rainfall pattern is consistent with a finding by Bigoga *et al.* (2007), in Cameroon that malaria transmission was perennial and rainfall-

dependent. In the central forest belt, there are two rainfall peaks; a major one starting in March and peaking in May and tailing off in June and a minor one starting in July peaking in August/September and tailing off by the end of October. In the present study, the infective biting rate of *A. gambiae s.l.* climaxed in the rainy periods of the year with peak biting activity in March (major), July, October (minorr) and December (dry) 2004 and June (major), 2005 at Afamanaso. At Kona, the peaks of *A. gambiae s.l.* were recorded in May (major) and October, 2004 and May, 2005. These patterns are attributed to the abundant availability during these periods of temporary pools of clean rain water, the preferred breeding sites of *A. gambiae s.l.*, This observation is consistent with the work of Boudin *et al.* (1991), in Burkina Faso where transmission by *A. gambiae s.l* was higher in the rainy season. The infective biting rate of *A. funestus* peaked in June, July, October, November and December 2004 at Afamanaso. The infective biting peaks of this vector at Kona occurred in June and July, 2004 and July, 2005. These peaks coincided with or occurred after heavy rains as this vector requires permanent water bodies with vertical vegetations, uninterrupted by frequent fluctuation (Gillies and De Mellon, 1968). Therefore, intense malaria transmission by *A. funestus* often occur during weeks of drought after heavy rains or in the dry season when only permanent water bodies are present (Boudin *et al.* 1991). This observation is supported by Coetzee *et al.* (2005), who reported a marked decrease in numbers of *A. funestus* collected in the rainy season as against high numbers in the dry season in Obuasi, Ghana.

At Afamanaso, outdoor EIR was significantly (1.6 times) higher than indoor (P = 0.00). In contrast, EIR was rather significantly (3.6 times) higher indoor at Kona than outdoor (P = 0.00). While the observation at Afamanaso contrasts the finding by Appawu *et al.*

(2004), that 60% of the EIR occurred indoor in northern Ghana, the observation in Kona, corroborates with the finding of Appawu *et al*. (2004).

Transmission was heterogeneous in the study sites. Combined EIR of both vectors was 1.90 times higher in Afamanaso (901.47) than in Kona (475.55); giving credence to the findings of Kreuels *et al*.(2008), of 2.20 malaria episodes per person per year at risk (PYAR) in Afamanaso and 1.0 malaria episode PYAR in Kona. Such differences in EIRs between and within each of the two towns corroborate early findings of heterogeneous transmission in northern Ghana (Appawu *et al*., 2004) as well as the much earlier observation on West Africa by Greenwood (1989). The difference in transmission intensity between Afamanaso and Kona, settlements only a few kilometres apart, may simply be due to the fact that Afamanaso is relatively more rural than Kona. Furthermore, Afamanaso has more potential vector breeding sites relative to Kona. Afamanaso is built on a high ground surrounded by low land areas permeated by tributaries of the Offin River creating the putative breeding sites such as temporary pools of clean water and marshy areas caused by the flooding during the rainy seasons. Furthermore, studies have shown that the main factor in malaria prevalence variations is vector density. Limitation in the dispersion of *Anopheles* from breeding sites tend to localize vector densities, such that in certain locations, by virtue of their proximity to vector breeding sites are exposed to more vector bites, and therefore, concomitant increases in sporozoite rates and EIRs. Such variations in turn cause differences in transmission (Smith *et al.,* 2004). The observation in the current study also consistent with work done in Western Kenya (Minakawa *et al*., 2002; Zhou *et al*., 2007) and Equatorial Guinea (Cano *et al*., 2006)

where a positive correlation was found between adult vector population densities and larval habitats. In Senegal, Trape *et al.* (1992) found that vector density was 5 and 20 times lower at a distance of 500 and 800 metres respectively, from a larval habitat (marsh) than at a distance of 0-160 meters. In the same study it was found that children had a parasite rate that was on average three times higher in the immediate vicinity of the breeding sites than at 600 metres away. According to Smith *et al.* (2004), when the rate at which adult mosquitoes emerge from breeding sites increases, mosquito density and biting rate increase to a peak, which is followed later by the peak in EIR and sporozoite rate because it takes time for an infectious agent to spread through the human and mosquito populations. EIR varies spatially because larval habitat and blood-meal hosts are heterogeneously distributed across a landscape. (Kreuels *et al.*, 2008; Staedke *et al.*, 2003). These findings show that the level of transmission at any point in a town depends on the nearness of that point to a vector breeding site. Given that households in all towns and villages cannot all be equidistant from larval sites, there will always be disparities in vector densities, biting rates, sporozoite rates, EIRs and therefore malaria transmission within and between towns and villages. This heterogeneity was clearly demonstrated in the present study within Afamanaso and Kona settlements as well as sites within each of them.

The relatively lower malaria transmission on the Boadi Cattle Farm compared to Anwomaso, Afamanaso and Kona, in spite of the high anopheline density may be due to low vector-human host contact. The Scientific investigations made in southern Ghana, (Afari *et al.*, 1995), northern Ghana, (Appawu *et al.*, 2004), western Kenya, (Githeko *et al.*, 1996), Nigeria, (Awolola *et al.*, 2003), Cameroon, (Wanji *et al.*, 2003), and Tanzania

(Geissbuhler *et al.*, 2007) show that *Anopheles* mosquitoes bite mainly during late night to dawn.. However, workers are on the cattle farm during the day. At night, at most, two security personnel provide over night cover for the farm. Therefore, the mosquitoes on the farm have reduced access to human hosts at night. The nearest human dwellings for the farm workers are about a kilometre away from the collection sites given that the farm is a university cattle research centre, there is significant malaria awareness for which the people tried to provide some mitigating factors for their households. This observation is in keeping with the study that people who can afford preventive measures such as good housing; i.e. with windows and trap doors screened with mosquito-proof nettings, use of ITNs, repellents, etc. and can also afford to seek prompt treatment when they experience malaria symptoms ultimately ameliorate malaria transmission in their vicinity. (Ijumba and Lindsay, 2001; Geissbuhler *et al.*, 2007). Another possible factor contributing to the lower malaria transmission on the Boadi Cattle Farm is the lower parous rates of *A. gambiae s.l.* and *A. ziemanni* than those at Afamanaso and Kona. This suggests a younger generation of mosquitoes than could have higher infection rates under conditions of low vector-host-contact (Robert *et al.*, 1992).

It is argued that a tendency exists for *Anopheles* mosquitoes that have significantly high parous or sporozoite rates to bite during the early hours of the night (Pates and Curtis, 2005). However, it was observed in this study that at Afamanaso, though the sporozoite rates of both vectors were not significantly different (P = 0.20), the parous rate of *A. gambiae s.l.* was significantly higher than that of *A. funestus* (P = 0.00). Apparently, in accordance with Pates and Curtis, (2005), the infective biting rate of *A. gambiae s.l.* peaked at about 23.30 hours, an hour earlier than that of *A. funestus* which peaked an

hour later at about 24.30 hours. Though both vectors had similar biting patterns with about the same peak, there was what seems to be just a slight skewing of the *A. gambiae s.l.* biting pattern to the left in favour of an early biting regimen.

At Kona, on the other hand, both the sporozoite (P = 0.00) and parous (P = 0.01) rates of *A. gambiae s.l.* were significantly higher than those of *A. funestus,* but the infective biting pattern of *A. funestus* was skewed to the left with the peak beginning at 22 hours and reaching its climax at 23 hours, coinciding with the peak of *A. gambiae s.l.* Again the biting activities of *A. funestus* peaked a clear hour earlier than usual between 22 and 23 hours with those of *A. gambiae s.l.* peaking an hour later between 23 and 24 hours. These observations at Kona, unlike what pertained at Afamanaso, do not seem to be in agreement with Pates *et al.* (2005). Therefore, in the middle forest belt of Ghana, further studies may be necessary to validate this finding.

The results presented in the present study on ITN show that a deltamethrin-impregnated net (ITN) 1m high surrounding an enclosure of 6m x 7m does not provide sufficient protection against the competent, endophilic malaria vector, *A. gambiae s.l.* This is in contrast to a previous observation in East Africa where a decline in malaria infection was attributed to the use of ITN nets of similar design (Bauer *et al.*, 2006). However, the location of the pen seems to have an impact on the numbers caught with or without ITN. Whether the ITNs were mounted simultaneously in sites A-D or moved in turns through the sites, pen D came out with the least numbers suggesting some environmental conditions around pen D may not be conducive to mosquitoes. *A. gambiae s.l.* is a highly endophilic mosquito which prefers to enter houses for blood-feeding and to rest in houses after the blood-meal (Faye *et al.*, 1997; Mahandel *et al.,* 2007; Snow, 2008). In contrast,

members of the *A. coustani* group, including *A. ziemanni* and several *Culex* species which are exophilic and normally feed outside houses (Chandler *et al.,* 1975; Snow, 2008) were thus caught in relatively larger numbers both inside and outside pens compared to *A. gambiae s.l.*; an observation in agreement with literature. In the main, ITN was shown to be effective against the exophilic mosquitoes and not the endophilic *A. gambiae*. Therefore the number of *A. ziemanni* caught inside pens with ITN, were 50% less than those caught in pens without the ITN fence. For the *Culex* species, there was a decrease of 38% Culex mosquitoes entering the pens fenced with ITN compared to those without ITN. However, the differences between numbers of mosquitoes caught outside pens with or without ITN were not significant suggesting that the 1m high ITN apparently had little effect on the numbers of mosquitoes collected outside the pens. Looking at these findings, it has to be questioned whether the changes in numbers of mosquitoes caught inside and outside the pens can be attributed to the deltamethrin-treated net. It could perhaps be that in 2005 Pen D recorded lower numbers of mosquitoes than the other pens not because the ITN had any impact but because the position of the pen (D) is in an area of low mosquito density. It can safely be concluded that the 1m high ITN fence did not provide sufficient protection against the malaria vector *A. gambiae s.l.* and only had a limited effect on the *A. ziemanni* and *Culex* species It may perhaps be concluded that the partially roofed pens enclosed by the mosquito netting in addition to the rather open wire mesh mimicked conditions attractive for the endophilic *A. gambiae s.l.* but were less attractive for the exophilic *A. ziemanni* and *Culex* species The results of this study show that *P. falciparum* is the main malaria parasite in the forest belt, making up 77.30% of the species of *Plasmodium* detected in the *Anopheles* mosquitoes. This is in accord with the

findings of Afari *et al.* (1995), in the coastal savanna of Ghana, at Prampram and Dodowa in 1992 that *P. falciparum* infection contributed 78-85% of the parasitaemia in April (major rains) and 93-99% in August (minor rains).

The finding that 97-100% of *A. gambiae s.l.* from three of the four study sites possessed the kdr gene confirms the report of Yawson *et al.* (2004), who found in a kdr characterisation experiment in Ghana that the kdr mutation occurred at very high frequencies (98–100%). The similar finding in this study further supports the conclusion of Yawson *et al.* (2004) that the occurrence of the kdr gene at such high frequencies has implication for malaria vector control given that the national malaria control programme prioritizes the use of ITNs as a key strategy for malaria vector control. Recently, Okoye *et al.* (2008) published that molecular analysis of the sodium channel gene gave no indication of any kdr-type mutations associated with resistance phenotypes in Ghana. Their finding agrees, with that of Kristan *et al.* (2003), who found in a bioassay that susceptibility of *A. gambiae s.s.* to DDT was 94-100% in Ghana (suggesting little or no resistance) and 72-100% in Nigeria. However, Coetzee *et al.* (2005) established a correlation between the kdr gene and insecticide-resistance in Ghana. They reported that of nine mosquitoes which survived insecticide treatment in a bioassay, eight were homozygous and one was heterozygous for the kdr mutation. The correlation was established by sequencing the part of the rDNA of each of the nine mosquitoes containing the kdr mutation after the failure of the standard PCR assay to detect the kdr mutation in the mosquitoes. This finding raises concern on the efficiency of standard PCR as a tool to detect the kdr gene and questions the reliability of PCR-based recommendations for the use of ITNs.

Given these conflicting reports by Kristan *et al.* (2003), and Yawson *et al.* (2004), as well as the thought provoking findings by Coetzee *et al.* (2005), one has to admit that the susceptibility or otherwise of malaria vectors to insecticides in Ghana requires further in depth study with specialised PCR as was done with FRET PCR in the current work to be fully established.

Analysis of monthly clinical and entomological data from Afamanaso and Kona, showed a relationship between entomological parameters; biting rate, sporozoite rate and EIR on one hand, and malaria incidence rate on the other. Peak manifestations of these entomological parameters preceded those of malaria incidence rate with a time lag of between one and two months. The analysis showed that a two months' time lag has the highest percentage of the variation of malaria incidence rate explained by the EIR (R^2 = 40.70%).

When a similar analysis was done in Gabon, Elissa *et al.* (2003), found that variations of EIR preceded malaria attack incidence by 1-month time lag in Dienga and 2-month time lag in Benguia in the two villages studied. The time lag, they explained as corresponding to the pre-patency period that differed in the two populations, possibly according to differences in immunity related to parasite transmission. They, however, reported that EIRs did not relate to malaria parasite prevalence because anopheline mosquitoes were present throughout the year, and variations in EIRs in their study areas were of too short duration to have a measurable impact on the malaria prevalence rates in the human population (Smith *et al.*, 1993).

The rate at which humans are infected by the malaria parasite depends on the anopheline biting rate, sporozoite rate and EIR. Thus a high biting rate may lead to a high sporozoite rate and a subsequent high EIR. The latter in turn results in a high malaria incidence rate in the human population. The concomitant high parasitaemia causes a high sporozoite rate. The result is a dynamic cycle of entomological parameters and malaria incidence rate in an interdependent fashion. It is interesting to observe the various points of equilibrium during which entomological parameters interphase with malaria incidence.

The occurrence of the time lag between the two parameters may be attributed to the time taken for the parasite to develop and spread through the human and mosquito populations (Elissa *et al*., 2003; Smith *et al*., 2004). The extrinsic development of the malaria parasite (e.g. *P. falciparum*) in its definitive host, the vector mosquito (sporogony) takes between 8 and 13 days depending on temperature. The intrinsic development (schizogony) in man requires 15 to 18 days comprising; hepatocytic or exo- or pre-erythrocytic stage which takes 6 days, erythrocytic phase, 2 days and gametocytogony, 7 to 10 days (Service, 2000; Cook and Zumla, 2008). This sum up to a time lag of about a month (23 to 31 days). The additional month may be due to delays caused by the various biochemical and physiological processes (peritrophic matrix, enzyme action, pH, , etc.) as well as the immune system of both definitive and secondary hosts which are barriers opposed to the development of the parasite in both the mosquito and the human victim (Shahabuddin and Castero, 2001). The efficiency of these and other factors may limit the likelihood that gametocyte ingestion results in spontaneous sporozoite formation in the mosquito and sporozoite inoculation originates a parasite hepatic cycle which leads to blood infection (Beier *et al*., 1994; Dieye *et al*., 1997; Elissa *et al*., 2003). Immunity against erythrocytic

parasite stages is able to extend the time necessary for the parasite density to reach the pyrogenic threshold (Rogier and Trape, 1993).These may explain the low R^2 of only about 41%.

6.0 CONCLUSIONS

6.1 Confirmation of Existing Findings

1. The current study found four *Anopheles* species in the study area: the majority being *A. gambiae s.l.* but also significant numbers of *A. funestus* and *A. ziemanni,* with a few *A. paludis.* A lot of *Culex* and few *Aedes* species were also captured. All the samples of *A. gambiae s.l.* processed in PCR turned out to be *A. gambiae s.s.*

2. *A. gambiae s.l.* (presumably all *A. gambiae s.l.* by statisitical projection) was more common than *A. funestus* comprising 66% and 90% of the malaria vectors collected at Afamanaso and Kona respectively. *A. gambiae s.l.* contributed 3.8 and 9 times more infective bites than *A. funestus* at Afamanaso and Kona respectively. At Boadi, three *Anopheles* species: *A. ziemanni, A.gambiae s.l.* and *A. funestus* were collected with *A. ziemanni* having the highest proportion of 56.08% to 43.29% for *A.gambiae s.l.* At Boadi *A.gambiae s.l* was the main contributor of EIR and the only infected mosquito at Anwomaso. At the latter sites, the mosquito population consisted of 86.09% *A.gambiae s.l.* and 13.91% *A. ziemanni.* Therefore *A.gambiae s.l.* was found to be the main malaria vector in all the sites studied in the middle forest belt of Ghana.

3. The biting activities of all mosquito species captured during the study at all the sites covered were found to be very high and perennial, with *Anopheles* species showing higher biting activities than *Culex* in the rural sites with the reverse occurring in the peri-urban settlements where *Culex species* showed higher biting activity.

4. The hourly biting activities of all the *Anopheles* species inscribed a bell-shaped pattern in all sites. The *Culex* species also formed the bell-shaped pattern in all but the Boadi Cattle Farm where a plateau-shaped pattern was observed.

5. A deviation from the typical peak biting time of *A. gambiae s.s* at about midnight was observed on the Boadi Cattle Farm in the Kumasi Metropolis; where peak biting activity occurred at a much later time of 02 hours.

6. The results of this study reveal how complex the bionomics of malaria transmission can be regarding the interplay between the plasmodia parasites and their mosquito vectors. It appears minute micro-ecological changes that are for now, not totally understood would reshuffle who the major vectors or parasites will be in a locality.

7. It appears that removing the salivary gland from a mosquito may perhaps reduce the concentration of CSPECIES (circumsporozoite protein) in the infected mosquito to a lower level than could be detected in ELISA test.
   The sporozoite rates were relatively higher at rural Afamanaso (10.25%) and Kona (10.00%) compared to the peri-urban towns Boadi Cattle Farm (1.55%) and Anwomaso (5.63%), confirming the fact that intense malaria transmission is more of a rural phenomenon. EIRs of *A. gambiae s.l.* were observed to be about 12, 7.99 and 7.70 times higher at Afamanaso, Anwomaso and Kona respectively than at Boadi

8. Malaria transmission was very high, perennial, heterogeneous and seasonal in intensity, correlating well with rainfall pattern.

9. The study found considerable per hour EIR values before and after peak transmission range of 21 and 04 hours implying significant transmission occurs while people are out of beds so ITN needs to be augmented by other integrated vector control measures in order to control malaria.

10. Despite the high anopheline biting rate recorded during the two months of investigation, there was a lower malaria transmission on the Boadi Cattle Farm than at all the other sites.

11. The identification of sporozoites revealed that 77.30% tested positive for *P. falciparum* the rest being *P. malariae*, and *P. ovale*.

12. It was observed that the one metre high deltamethrin-impregnated net (ITN) surrounding a pen of 6m x 7m was no intact barrier against the endophilic malaria vector, *A. gambiae s.l.* which still made it into the relatively more endophilic atmosphere prevailing inside the protected pens.

13. The kdr gene was found to be present in the homozygous and heterozygous states in 91.1% and 6.7% respectively of the *A. gambiae s.l.* tested, with only 2.2% of the mosquitoes being susceptible.

14. There was a correlation between malaria transmission and malaria incidence rate. Peak malaria transmission preceded malaria incidence rate by a time lag of up to two months.

6.2 New Findings

1. The abundance of the zoophagic *A. ziemanni* and its subsequent infection by *P. falciparum* in the first part of the study on the cattle farm may perhaps be the first report of its presence and potential involvement in malaria transmission in the forest belt of Ghana.

2. The identification of a few numbers of *A. paludis,* a member of the *A. coustani* group on the Boadi Cattle Farm, may also be the first report of its presence in the forest belt of Ghana.

3. It was found that *A.gambiae s.l* is so obligately endophilic that a deltamethrin-impregnated net (ITN) fenced round a pen could not deter it from entering into the relatively more endophilic conditions created inside the pen by the ITN. This may be the first report on the degree to which *A.gambiae s.l* is endophilic.

4. Removing the salivary glands from an infected mosquito may reduce the concentration of CSPECIES in the infected mosquito to a lower level than could be detected in ELISA test. This was more evident in *A. funestus* than in *A. gambiae s.l.,* probably due to the high proficiency of *A. gambiae s.l.* as a vector of *P. falciparum* which means higher numbers of the parasites develop in it so that removing the glands may still leave higher CSPECIES concentration in the thorax and head than in less competent malaria vectors. This assertion is supported by the fact that where salivary glands have been removed from *A. funestus* during dissection, the number of this secondary malaria vector found to be infected by *P. falciparum* using EI ˙SA tests was significantly lower than in those whose

salivary glands had not been removed (P=0.02). This may perhaps be the first report of this observation.

5. All the samples of *A. gambiae s.l.* processed in PC*R* turned out to be *A. gambiae s.s.* The in-depth EIR study carried out in the current work may thus be the first to confirm that *A. gambiae s.l.,* the most proficient malaria vector, is the principal malaria vector followed by *A. funestus* in the middle forest belt of Ghana.

6. A kind of association may be established between increasing numbers of *A. ziemanni* as a result of the presence of cattle on which they feed and increasing numbers of *Culex* species which breed in organic nutrient-rich sites, perhaps created by the organic waste washed from these farms into water bodies. This association may just be reported for the first time.

7. As yet no report has been found comparing biting patterns of *Anopheles* with *Culex* species, this study perhaps may be one of a few reporting the activities of the two mosquito species for the first time.

6.3 Limitations of this Study

1. Attempts made to sample *Anopheles* larvae to confirm breeding sites of the mosquitoes were successful mainly on the Boadi Cattle Farm, since only a few *A. funestus* larvae and abundance of *Culex* larvae were found at Afamanaso and Kona.

2. The ELISA test was specific for *P. falciparum* only. However, the results of *Plasmodium* species identification revealed that 77.30% of sporozoite infections were by *P. falciparum* Therefore 22.70% of the mosquitoes were infected by *P. ovale* only and/or *P. malariae* only which could not be detected by the ELISA test.

If these mosquitoes were not dissected, then such infections were missed. However, over 60% of the mosquitoes were dissected so these losses were minimised, given that sporozoite rate determined by dissection was not significantly different from that determined by ELISA.

3. The outdoor collection sites consisted of either a roofed classroom that had no walls or the central court yard of an enclosed traditional house. The results of the collection of substantial numbers of *A. gambiae s.l* in 1m high ITN used on the Boadi Cattle Farm show that these mosquitoes already recognize the stilled conditions within these structures as partially indoor.

6.4 Recommendations

1. It is observed in this study that the biting pattern of the *Culex* species assumed the bell-shaped pattern of their Anopheles counterparts in the rural sites. However, in the peri-urban sites the biting pattern of *Culex* species showed an early peak and a sustained plateau for several hours before declining. One study perhaps, is not enough to pointificate any theories yet, perhaps a few more studies in the peri-urban localities where *Culex* species predominate, should allow one to explore the reasons behind the plateau in the biting pattern of *Culex* in the urban areas as compared to the bell-shaped in the rural areas.

2. Granted the current work did not identify Culex mosquitoes to species level, therefore a follow-up study that identifies and determines the biting patterns of specific *Culex* species may be more revealing.

3. In future, it may be worth exploring perhaps the relative abundance of the two members of the *A. coustani* group; *A. ziemanni* and *A. paludis* on the Boadi Cattle Farm and Nana's Farm at Anwomaso. A follow-up study is required to definitely confirm their potential or otherwise as vectors of the malaria parasites - *Plasmodium* species

4. It is argued that a tendency exists for *Anopheles* mosquitoes that have significantly high parous or sporozoite rates to bite during the earlier hours of the night (Pates *et al.*, 2005). Results of the current study agree with this at Afamanaso but not at Kona. Therefore, in the middle forest belt of Ghana, a further study may be necessary to confirm this finding or otherwise.

5. The finding that the kdr gene was present in the homozygous and heterozygous states in 91.1% and 6.7% respectively of the *A. gambiae s.l.* tested, with only 2.2% of the mosquitoes being susceptible from three of the four sites confirm the report of Yawson *et al.* (2004), who found in a kdr characterisation experiment in Ghana that the kdr mutation occurred at very high frequencies (98–100%). Given conflicting reports by: a) Okoye *et al.* (2008), that molecular analysis of the sodium channel gene gave no indication of any kdr-type mutations associated with resistance phenotypes in Ghana and Kristan *et al.* (2003), who found in a bioassay that susceptibility of *A. gambiae s.s.* to DDT was 94-100% in Ghana suggesting little or no resistance on one hand and b,) the thought provoking findings by Coetzee *et al.* (2005), who established a correlation between the kdr gene and insecticide-resistance in Ghana using only nine mosquitoes, one has to admit that the susceptibility or otherwise of malaria vectors to insecticides in Ghana requires

further study using WHO-approved insecticide susceptibility, molecular and biochemical assays in order to understand the resistant mechanisms that might be involved in Ghanaian malaria vectors. Again given a recent report (Munhenga *et al.,* 2008) that patterns of insecticide resistance may undergo rapid change over time, it is important to have an active entomological surveillance system (for periodic and ongoing insecticide susceptibility testing of malaria vector populations) as part of a malaria vector control programme.

6. The significant number of infective bites our results show for outdoor as well as before and after bedtime shows that ITN offers good but incomplete protection against malaria parasite infection and transmission in the forest zone. Effectiveness of ITN could be enhanced by high population coverage at community level to reduce vector population through insecticidal effect of high numbers of ITNs. The principle here is that when almost everyone in a community is using an ITN, then mosquitoes attracted to the odour of ITN occupants may be killed as they land on the net in sufficient numbers leading to a decline in vector population density so that few mosquitoes survive long enough to support the complete development of malarial sporozoites in them. This could lead to a major reduction in the vectorial capacity of the malaria mosquito populations and therefore reduction in transmission. This could be augmented by effective integrated vector control measures such as screening of windows and doors of houses with mosquito-proof ITNs, ceiling of rooms, indoor residual spraying, treatment of curtains with insecticides, use of bio-larvicides on putative vector breeding sites and

environmental management to reduce malaria transmission in the forest belt of Ghana.

6.5 Epilogue

The study has provided baseline data useful for epidemiological purposes such as the planning of malaria management strategies. The very high malaria transmission recorded in the Ghanaian forest belt together with the significant number of infective bites outdoor as well as before and after bed time show that ITN offers good but incomplete protection against malaria parasite infection and transmission in the forest zone and the need for effective integrated vector control measures for the dream of malaria control to be realized. Again, the data on the high infection rate of the primary vector, *A. gambiae* s.*l.* by the most prevalent and dangerous parasite species, *P. falciparum* may be useful information for any future malaria vaccine trial in the study area.

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