STUDIES ON THE MALARIA VECTOR MOSQUITOES (DIPTERA: CULICIDAE) IN KISUMU AREA, KENYA IN RELATION TO MALARIA CONTROL USING IMPREGNATED BEDNETS.''

By

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DECLARATION

I hereby declare that this thesis is my original work and has not been presented for a degree in any other university.

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This thesis has been submitted for examination with our approval as the University Supervisors.

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DEDICATION

This thesis is dedicated to my parents William and Dinah, sons Eric & Philemon and daughter Margaret.

LIST OF ABBREVIATIONS AND SYMBOLS USED IN THE TEXT.

a.s.l	Above sea level
Appr.	Approximately
App.	Appendix
BB	Blocking Buffer
BC	Boiled casein
BC-Tw	Boiled casein tween
BSA	Bovine serum antibody
CS	Circum-sporozoite
gms.	grammes
gms/ml.	grammes per millilitre
HBC	Human bait catch
HCL	Hydrochloric acid
HRP	Heparin
L or l	Litre
LD	Lethal Dose
LC	Lethal Concentration
M	Molarity
Mabs	Monoclonal antibodies
ml.	millilitre
mgs.	milligrammes
mg/ml	milligrammes per millilitre
N	Molar or Normal
nm	nanometre
NaoH	sodium hydroxide
$Ø_C$	degrees centigrade
PBS	phosphate buffered saline
PBS-Tween	phosphate buffered saline

PSC	pyrethrum spray catch
pH	Acidity or Alkalinity
s. l.	senso lato
s. s.	senso stricto
ug	microgramme
ul	microlitre
ug/ul	microgramme per microlitre

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ABSTRACT

Malaria continues to be a health problem in Kenya, due to the growing resistance to insecticides among the mosquito vectors (WHO, 1970) and increasing resistance to drugs by the malaria parasites. Parasite resistance to chloroquine as a common anti-malaria drug was first reported in Kenya in 1982. These two facts, coupled with inadequate national resources for mounting massive malaria control programme calls for a simple, cheap, sustainable and an appropriate vector control measure such as using mosquito nets.

A field trial with mosquito bednets impregnated with 1% permethrin was carried out against malaria vectors in Kisumu to assess the potential of this method as a means of reducing vector densities thus reducing the risk of transmission of malaria. For effective control programmes to be conducted, the ecology and behaviour of the vector should also be understood. Therefore their breeding sites, seasonality and biting habits i. e. peak biting rates were investigated. Host preference was determined by bloodmeal analysis. The sporozoite rates were also determined by dissection and ELISA for sporozoites methods.

Six species of anopheline mosquitoes were identified in the area namely; *Anopheles gambiae sensu stricto* Giles, *Anopheles arabiaensis* Patton, *Anopheles funestus* Giles, *Anopheles pharoensis* Theobald, *Anopheles pretoriensis* Theobald and *Anopheles maculipennis*

Theobald. Collected data showed that the predominant mosquitoes in the region were *An. gambiae s. s.* and *Anopheles funestus*, and were singled out as the main malaria vectors in the area as earlier sited by (Surtees, 1970). *Anopheles pretoriensis* and *An. maculipenis* appeared only during the rainy seasons. The two species together with *An. pharoensis* were of no importance in malaria transmission.

Use of permethrin treated bed nets led to a marked decline in the mosquito densities when compared to the use of untreated bed nets or the no-nets situation. There was statistically, a significant difference found in the house resting densities of mosquitoes in the three study sites. Using the Analysis of variance F was 0.63, at degree of freedom (2,24), at probability level $P < 0.05$, based on Pyrethrum Spray Catch data.

Rainfall had an influence on mosquito house resting densities. Data collected by both the PSC and night catch indicated a marked increase of both *An. gambiae s.l.* and *An funestus* in site 2 and 3 with increased rains while in site 1 increase was negligible. This increase in sites 2 and 3 was attributed to the increased mosquito breeding sites. There was a decrease in the mosquito densities during the short rains and the dry season due to the limited breeding grounds in all the three sites.

The bioassay experiments on the permethrin treated nets showed that the insecticide had a long lasting residual effect as it was found effective up to

9 months after impregnation i.e. it could knock down and kill 70% of the exposed test mosquitoes and according to WHO this would be the correct time for re-impregnation.

The malaria parasites found among 92 children examined were predominantly *Plasmodium falciparum* Welch, 1897, found in 56 children (64%), *Plasmodium malariae* Grassi and Feletti, 1890, found in 27 children (30%), and *Plasmodium ovale* Stephens, 1922, found in 8 children (6%). Children with *P. malariae* and *P. ovale* infections' also had concurred infections of *P. falciparum*. Blood smears also showed a great decline of parasitaemia amongst children between 0-10 years of age in site 1 (the permethrin treated bed net site). At site 1 *Plasmodium falciparum* percentage prevalence was reduced from the original 98% during the baseline data to an average of 20% throughout the 11 subsequent months of the data collection period. Site 2 and 3 also had a reduction from 83% to an average 40% and from 90% to an average 60% respectively. There was a significant difference $P < 0.05$ noted between the percentage prevalence of *P. falciparum* in the three study sites where by the Chi square X^2 was 6.55, and degree of freedom 2.

CHAPTER ONE

1 INTRODUCTION AND LITERATURE REVIEW

1.1 General introduction.

Malaria is one of the five parasitic diseases which is of great concern to the world as it claims many lives each year especially children below five years. The disease is caused by a protozoan parasite of genus *Plasmodium* which infects the liver parenchyma cells and the red blood cells of man. There are four species which are known to infect man but only three are of great medical importance in Kenya. *Plasmodium falciparum* which is the predominant species in holo-endemic areas causes malignant tertian malaria (severe and complicated malaria) a more severe malaria with high fever after every 48 hours that contributes to high child mortality rate. *Plasmodium malariae* causes quartan malaria that is characterised by intermittent fever with paroxysms occurring every 72 hours. While *P. ovale* causes benign tertian malaria which is mild and less severe. These parasites are transmitted by various species of *Anopheles* mosquitoes whose distribution varies in different ecological zones. The commonest malaria vectors in the Afro-tropical region are *Anopheles gambiae s.l.* complex and *Anopheles funestus* as earlier sited by (Garnham, 1938; Surtees, 1970).

1.1.1 Malaria problem in Kenya particularly in Kisumu and the surrounding areas.

Malaria continues to be the leading vector-borne disease in Kenya in terms of high morbidity and significant mortality in many parts of the country. The population at risk of getting malaria was estimated as 19 million in 1988 and the number of cases reported in that year was 4 million, Kenya Annual report (WHO, 1988).

Kenya has both seasonal and perennial malaria transmission. Malaria transmission is determined by altitude, which determines average temperature and the rainfall pattern as follows: Endemic regions are below 1300m where there is stable malaria with transmission occurring for more than 6 months of the year. Between 1300-1700m transmission is less stable and more seasonal, following the rainfall pattern. Transmission in the area is for 3-6 months of the year. Epidemic regions are between 1700-2500m where there is very unstable transmission, in form of epidemics and sporadic transmission for 1-3 months. At 2500m and above is known as a malaria free zone where there is no transmission.

The map on Figure 1 shows the endemicity of malaria in Kenya according to the number of malaria cases (DVBD Annual Report, 1983). Stable malaria is found in the coastal districts of Kwale, Kitui and Mombasa, and in the districts bordering the Lake

Victoria (Kisumu, Siaya and South Nyanza), where it is the primary cause of morbidity. The disease is also highly endemic in semi-arid zone of Tana River. Seasonal malaria occurs in parts of the central highlands and in discrete pockets within the rift valley. While unstable malaria is generally restricted to the highlands areas of Nandi, Kericho, Kisii and Elgeyo and to the dry areas which have exceptional rainfall, such as masailand and Londiani. Surtees (1970) surveyed and reviewed general mosquito fauna and their ecology in the Nyando valley and found that the main malaria vectors were *An. gambiae sensu lato* and *An. funestus* whose hut index remained consistently high throughout the year due to the available breeding sites. The two above vectors are responsible in the maintaining holo-endemic malaria in Kisumu area. They breed on the periphery of papyrus swamps, slow moving streams and in the seasonal ponds which result from heavy rains. Studies on malaria vectors and parasites in Kisumu can be traced as early as 1929, (Garnham, 1929; 1945) who investigated anophelines at several localities in the surrounding highlands in relation to malaria epidemics. There is more additional data in unpublished annual reports of Division of insect-borne diseases, Kenya (DIBD reports). The aim of the studies was to understand the ecology and the population dynamics of the vectors so as to lay down concrete control strategies.

1.1.2 Malaria control.

Intergrated approaches of mosquito control including residual insecticide spraying in huts (WHO, 1959; 1960; DIBD Annual reports, 1988), larvicide spraying in the rice irrigation scheme, use of larval predators as *Gambusia spp.* of fish are some of the control methods that have been conducted in Kisumu within the irrigation scheme in Kano plains to reduce the vectors. A specially developed bacteria *Bacillus thuringiensis* with a half-life of 24 hours is a recent biological control measure that is frequently applied to kill the larval stages of the mosquito (DVBD Annual reports, 1988). Chemotherapy and chemoprophylaxis are targeted on the parasites. Despite previous attempts to control malaria the aim is far from achieved and hence the need of other vector and parasite control measures.

Mass residual insecticide spraying although originally successful in western Kenya (DIBD reports) has encountered serious setbacks which will be looked at later in this chapter. It has become clear that a single universally applicable method would never become available for the control of all vector borne diseases. Consequently studies are required in each locality to be able to select the appropriate methods and adopt them according to the existing transmission conditions. More emphasis should be placed on organisational, economic and cultural aspects whereby innovative cost-effective vector control measures can be used by communities.

Vector control methods entailing the use of mosquito nets and repellents have recently received more attention. For many years mosquito nets have been used to protect man against mosquitoes and other biting insects (Lindsay and Gibson, 1988). Ross (1910) advocated the use of bed nets as a protective measure against malaria. Mosquito nets are used in many countries and the extent of usage depends on the mosquito nuisance, availability and affordability.

Impregnated mosquito nets treated with either repellent or killing insecticide have shown some positive results in malaria control in many parts of the world e.g. Mali (Raque *et. al.* 1984); Burkina Faso (Carnvale *et. al.* 1988, Manjori *et. al.* 1987); Suriname (Rozendaal *et. al.* 1989); Malaysia (Hii *et. al.* 1987); Papua (Graves *et. al.* 1987); Hainam (Li Zuzi *et. al.* 1987); United states (Shreck *et. al.* 1987); The Gambia (Snow *et. al.* 1987, 1988); Tanzania (Curtis *et. al.* 1989) and Kenya (Sexton *et. al.* 1990). This technique has an advantage over the untreated bednets in that once the net is treated it protects the sleeper whether it is in bad condition with tears since the chemical repels or kills the vectors before they feed.

Results of this study have shown that man's activities and occupation contributes to the malaria prevalence. For instance quarries and sand gorges found in the area acts as seasonal breeding sites for the vectors especially during the long rains.

Parasitaemia levels in children of ages between Ø-1Ø years were investigated, and the Bioassays were made on the treated nets. It was noted that cultural practice of warming around the fire to about 22hrs. before sleeping increased the chances of mosquito bites hence malaria prevalence in the area.

In this study a twelve month project was conducted in the malarious area of Kanyawegi (Figure 2) Sub-location, Kisumu District, (Western Kenya) inorder to find the effectiveness of the permethrin treated mosquito net as a means of controlling mosquitoes and reducing malaria prevalence in children of ages between Ø-1Ø years. The area which lies along the shores of lake Victoria is known for high infant mortality as a result of malaria (DIBD reports). The nets which were given to the people were made of cheap nylon material treated with 1% permethrin insecticide. One long-term objective was to encourage the community to purchase their own nets and insecticide for subsequent use if the method was found effective. Permethrin is expensive but only a small quantity is required to impregnate several nets. The chemical which has low mammalian toxicity has a long-lasting residual effect and can be active for nine months killing 7Ø% of the vectors as sited by (WHO, 1989).

1.2 LITERATURE REVIEW

1.2.1 General review of malaria vectors.

The distribution of malaria vectors in Africa South of Sahara was comprehesively reviewed by Gillies and de Mellion (1968). The distribution of *An. gambiae s.l.* complex has been investigated by Davidson (1964b) who found that *An. gambiae senso stricto* and *An. arabiensis* which he referred to as species A and B respectively in his study are widespread in Africa. They occur in an area stretching from the central part of South Africa to the Southern boarders of Sahara in the North, and between Western and Eastern Africa coasts including the Indian ocean Islands. The sibling species of the *An. gambiae* complex together with *An. funestus* are the major malaria vectors in East Africa (White, 1976a).

Omer and Dukeen (1986), conducted on ecological study of *Anopheles arabiensis* Patton in the Nile; Sudan. Larval collections in Arabia have been conducted by Leeson (1948), in the White and Blue Nile by Lewis (1948; 1958), to determine the breeding sites and their population densities. He found that breeding of *An. gambiae s.l.* occurred in the reservoir among the *Naja pectinata* swamps, and along the residual pools in the river-bed in the dry seasons. This conducive breeding factors increased vector populations and hence, malaria transmission is all the year round.

Studies on breeding of *Anopheles gambiae s.l.* complex and *Anopheles funestus s.l.* have been conducted world-wide. Kinoti (1971) made observations of breeding sites of mosquitoes in the L. Manyara, a saline lake in East Africa and found that *An. gambiae s.l.* and *An. rhodesiensis* were the only anophelines that breed in both fresh and brackish water. Observations made on two papyrus swamps in Kampala (Goma 1960) reveals that *An. gambiae s.l.* does not breed in the interior of papyrus swamps in their natural undisturbed state, but rather breed at the periphery. Breeding also occurs outside the papyrus zone particularly in hoof prints, cattle drinking places and in open natural pools. Therefore, swamps are not potent sources of malaria (Hopkin, 1940; Senior-white, 1939).

1.2.2 Malaria vectors in Kenya, their ecology, distribution and breeding.

Many studies have been conducted in Kenya on the ecology and behaviour of anopheline mosquitoes, as well as their relationship to the epidemiology of malaria (Symes 1972; Heisch, 1949; Teesdale, 1959; Van Someren *et al* 1958). Service (1971, 1976, 1984, 1986) has conducted many studies on the ecology of larvae in the Kano plains of Kenya with an emphasis on the *Anopheles gambiae s.l.* complex. Further studies on the ecology and behaviour of mosquitoes in the Kano plains have been published by Khamala (1971), who recognised six natural

terrestrial mosquito breeding habitat i.e. temporary or permanent, presence or absence of emergent plants and by the chemical and physical characteristics of water. Mosquito collections in irrigated and non-irrigated area using both animal and human baits have been established by Chandler (1975). Succession of mosquito species in rice fields of Kisumu and their possible control methods have been done by Highton (1979). Surtees (1959, 1970 a) conducted studies on general mosquito fauna in the Nyando valley and larval population influence on mosquito numbers and mosquito preliminary studies in Kano plains and found that the main malaria vector as being *An. gambiae s.l.* and *An. funestus* whose hut index remained consistently high throughout the year. Garnham (1929) studied malaria epidemics at Londiani a place of exceptionally high altitude 2270-2570 metres above sea level, where breeding of the malaria vectors here are governed by the human carriers, such as those moving between the lake region and the highland, carrying both the vector and parasite. Furthermore, the range of rainfall and suitable temperature too favour vector breeding.

Garnham (1945) investigated anophelines at several localities in relation to malaria epidemics in Nandi Hills, he found that epidemics transmitted by *An. gambiae s. l.* flare up regularly from May to August during the long rains. While epidemics transmitted by *An. funestus* occurred in March to June. Matson (1957)

reviewed malaria history in the same area. Odhiambo *et. al.*, 1980) made a comparative study of malaria transmission rates in *An. gambiae s.l.* complex and *An. funestus* in Kisumu area.

Mukiama and Mwangi (1989) studied the breeding of *Anopheles arabiensis* larvae as well as the seasonal population changes and malaria transmission potential of *Anopheles pharoensis* and minor anophelines in Mwea irrigation scheme. Ijumba and Mwangi (1990) investigated transmission of malaria in the same area by *Anopheles* mosquitoes. Further studies show that *An. gambiae s.s.* predominates in humid situations while *An arabiansis* is relatively more successful in arid zones (Davidson *et. al.*, 1967; White *et. al.*, 1972).

1.2.3 Malaria vectors sampling methods.

In nature, maintenance of malaria involves the interaction of the vector, the human host and the disease pathogen. Breaking the vector-host contact is a potential line of stopping disease infection and subsequent transmission. Detailed knowledge of patterns of contact between a vector and a host are essential since it is the assessment of such vector-host contacts at any given time and place, that makes it possible to predict epidemiologically dangerous situations and to take adequate measures of malaria prevention and vector control.

Many sampling techniques have been used by various investigators to collect larval/adult mosquitoes in order to study their populations. Larval sampling detects the larval habitats and gives an assessment of change in their densities (Southwoods, 1961). Adult collection is an important aspect in the population estimation of mosquitoes either indoor or outdoor resting densities. There are many methods of capturing adult stages of mosquitoes as clearly elaborated by Service (1976). The techniques for collecting adults include human and animal baits, exit and entry traps fitted in huts, CDC light traps, direct catches using aspirators and spraying houses with knock down Pyrethrum Spray Catch (PSC) technique. Each of these methods can be applied depending on the objectives of a particular experiment.

Light traps have been used for many years to catch insects in America, Japan and other parts of the world. Smith (1956 a, 1956 b) used varendah light traps to determine the house frequenting habits of mosquitoes and observed that 51% of *An. gambiae s.l.* in all gonotrophic stages left the hut each night with 85% of the egress occurring by the window traps and 15% by the eaves. (White and Megayuka 1972; White and Rosen 1973) used this method and others in a comparative study on sibling species of the *Anopheles gambiae s.l.* complex in Kaduma Nigeria and Segera, Tanzania. There are many other studies which have been conducted using light

traps as reported by Bradley and McNeel (1935) in Florida. Breeland (1974) reported correlation between light traps and larval collection.

Night catch using human baits in houses, sampling was originally developed by Kerr (1933) who carried out a detailed study on biting of *An. gambiae s.l.* when working on yellow fever in West Africa. This was later modified by other workers (Service, 1976). The purpose was to monitor changes in numbers of biting female mosquitoes from 1800 to 0600hrs.

1.2.4 Feeding habits of Malaria vectors.

As stressed by Gillies (1956), it is important to distinguish between these types of behaviour, namely exophagy & endophagy and exophily & endophily, in relation to epidemiology and control of malaria vectors. Exophagy and endophagy as defined by Senior-White (1954) refer to the tendency by a mosquito to feed outside or inside human habitations respectively. Endophily is the tendency by a mosquito to rest inside man made shelters while exophily is the tendency to rest outside human habitations.

Anthropophily refers to preference for man while zoophily refers to preference for other animal hosts. Of all the *An. gambiae s.l.* complex only species A is most endophagic and anthropophillic the same as *An. funestus* and will be normally caught biting inside houses (Davidson & Draper, 1953; White, 1974a). Species

B, *An. arabiensis* is the most opportunistic it feeds where most hosts are abundant indoors or outdoors (Smith & Draper, 1959).

Further work on feeding habits have been conducted by Haddow (1942) in local mud walls and grass thatched huts in Kisumu, Kenya. His observations on females of *An. gambiae s.l.* showed that peak biting hours were between 1600hrs and 2400hrs. Hocking (1947) investigated on bionomics of *An. gambiae s.l.* and *An. funestus* in East Africa and found that both species enter the buildings throughout the night to feed, but more *An. gambiae s.l.* entered between 1600 and 2400hrs. *An. funestus* however entered just before dawn. The males of both species entered only just before dawn. Various other workers have carried out night catches of *An. gambiae s.l.* in widely scattered parts of Africa and all work to date has confirmed that it is basically a nocturnal species and that the biting activity reaches maximum between midnight and sunrise. (Mattingly, 1949; Van Someren, 1955; Haddow, 1973).

1.2.5 Malaria vector control in parts of Western Kenya.

Studies have been conducted on Anopheline mosquitoes as vectors of malaria and as a result ways of controlling them and the diseases have led to methods that target both the vector and the parasite, as attack on the vector or parasite alone has proved insufficient (WHO, 1973). The main method that has been used

previously is the residual insecticide spraying in dwelling places and breeding sites, which has been widely practised in the Kisumu area (WHO, 1973). Until mid-1930s, 4000-6000 huts were sprayed annually with dieldrin by the Kisumu town council (DIBD reports 1958).

In the hills surrounding the town DDT Bi-annual residual spray rounds was applied to about 4000 huts per annum over 70 square miles around Kericho in 1945-1949 which interrupted malaria transmission until the epidemics of 1980s. The campaigns in the hills caused a very successful control of the anophelines, to the point of virtual eradication. Six thousand huts in Nandi areas were sprayed annually with dieldrin in the period of 1955-1957 (Odhiambo, 1980) which also interrupted transmission for nine years. In 1969, the WHO team (ACRU II) commenced the evaluation of fenitrothion (OMS-43) as a residual house-spraying against anophelines over a 23.3 square miles per area stretching a 2-3 miles wide strip from the Nandi foothills almost to the lake shores (WHO, 1970; Fontain *et. al.* 1978). This was very successful against *An. funestus* and *An. gambiae s.l.* since only 3 *An. gambiae s.l.* females were obtained from houses in the treated area during the following survey (White, 1972). Susceptibility studies by Bransby-Williams in 1958 gave no indication that anophelines collected around Kisumu were resistant to dieldrin, Benzene hexachlorine (BHC) or DDT (DIBD Reports, 1958). However more recent tests

have shown that *An. funestus* and *An. gambiae s.l.* from both the valley and foothills are now resistant to dieldrin.

Residual spraying has proved unsuccessful due to various limitations: such as inadequate resources for maintaining the activities, prohibitive costs of insecticides, poor co-operation by the population and as mentioned above the development of resistance by some strains of mosquitoes to some insecticides. Larval control has equally failed in the area due to vast breeding sites which remain permanent all year round. This method can only be applicable in areas with seasonal breeding sites, as stated by Alfonso *et. al.* (1991).

1.2.6 Malaria vector control by impregnated bed nets.

Impregnation of mosquito nets with fast-acting insecticide either repels or kills the mosquitoes before they have time to find a hole to enter and feed on the bait. The deliberate treatment of netting with insecticidal or repellent compounds seems to have been invented independently in the USSR in the 1930's using lysol and plant products (Blgoveschensky *et. al.*, 1945). In American (Harper *et. al.*, 1947; Gouck *et. al.* 1967 a and b, 1971) treated wide mesh netting together with screening of buildings and were found effective in keeping mosquitoes from entering the small enclosures. Later Grothaus *et. al.* (1976) studied insect repellent

jackets and their potential in mosquito bite control.

Bed nets and other forms of screening for personal protection are popular in many parts of the world used by various social groups, ranging from traditional Amerindians communities to tourists on camping safaris in Africa (WHO, 1989). Smith and Gratz (1983) provided figures for bed net usage in important urban areas in tropical countries as 25%. Bed nets impregnated with pyrethroid insecticides are being used increasingly to control malaria in many parts of the tropics (Curtis *et. al.* 1989; Rozendaal *et. al.*, 1989; WHO, 1989). The control strategy has been most effective at combating malaria in areas of low seasonal transmission as in the Gambia Alfonso *et. al.* (1991).

Brun and Sales (1976) were the first researchers to document the value of cotton mosquito nets treated with several organophosphates against malaria vectors. This was done following a successful application of tsetse fly traps impregnated with deltamethrin studies. Photostable pyrethroid developed as molecular analogues of natural pyrethrum were highly successful not only against agricultural pests but also on clothing against mosquitoes and ticks (Elliots *et. al.* 1973). Schreck *et. al.* (1978, 1982) found that permethrin treated clothing protects man against blood sucking arthropods. Tests with repellent-treated netting against black salt-marsh mosquitoes were found to be effective in keeping mosquitoes from entering small enclosures.

Impregnation of bed netting has also been successful against *culicoides*. Studies using other materials than nets against other vectors and pests have been done. Quisenberry *et. al.* (1984) observed that permethrin impregnated ear-tags were effective in reducing horn-flies (*Haematobia irritans*) during the fly season as compared to control.

Studies by Hervy and Sales (1980); Chao (1984) and Raque *et. al.* (1984) show that the photostable pyrethroid was appropriate for impregnation of bed nets because they are relatively safe to humans and are with rapid insecticidal effect and low volatility with long persistence on netting and lack of odour. Pyrethroids belong to the safest category of insecticides to human, a WHO Expert Committee (1988) stated the following about the safety of permethrin impregnated nets, " Properly treated, bed nets should pause no hazard to those who use them". The acute oral toxicity of Permethrin in aqueous solution for rats is very low, the LD50 being over 4000mg/kg. Dermal toxicity is so low that it could not be demonstrated, it has a very low vapour pressure and hardly evaporates. Loong *et. al.* (1985); Li zuzi *et. al.* (1989); Snow and Jawara (1987) reported that one year after impregnation, the residual effect was still sufficient to kill more than 95% of the mosquitoes exposed to impregnated nets. Results such as the above mentioned have led to the use of impregnated bed nets in many countries.

Permethrin-treated bed nets protect people from mosquito bites in many ways. Firstly, the vectors may be killed by landing on the fabric (Darriet *et. al.* 1984). Secondly, some which come into contact with treated netting may became physically irritated by the insecticide, hence reducing the time spent searching for a bloodmeal. Lastly a proportion of the mosquitoes will be deterred from entering the huts with permethrin treated bed nets. Studies in experimental huts suggest possible effects on mosquitoes such as deterring them from entering houses; inhibition of feeding; repelling the mosquitoes outside after contact with impregnated netting as well as mosquito mortality (Darriet *et. al.* 1984; Majori *et. al.* 1987; Lines *et. al.* 1987; Rozendaal *et. al.* 1989). Village-scale studies have included those which have measured the individual protection against malaria conferred on children when the nets of a proportion of the village population are impregnated (Snow *et. al.* 1987; Li Zuzi, 1988).

In Mali, Raque *et. al.* (1984) were the first to carry out a village-scale trial using impregnated nets and found that the use of these bed nets reduced splenomegaly. In China Li Zuzi *et. al.* (1989), found that the nets reduced malaria prevalence while in the United Republic of Tanzania Lines *et. al.* (1987) found that the treated net made even a torn one effective. In The Gambia Snow *et. al.* (1987a) found permethrin impregnated bed nets to lower prevalence of heavy

parasitaemia in children which led to fewer clinical episodes of fever. Other similar reports come from studies in Papua, New Guinea Charlwood and Graves (1987); Suriname Rozendaal *et. al.* (1989) and Malaysia (Loong *et. al.* 1985; Hii *et. al.* 1987). Although many problems were encountered, the results were considered sufficiently promising for the planned large-scale introduction of treated bed nets for example China, where over five million persons sleep under impregnated bed nets. In Papua New Guinea, Solomon Islands and Vietnam, the use of impregnated bed nets has already reached operational scale where tens of thousands of people use bed nets.

In the American region, about thirty four countries in Central and South America use bed nets and hammock nets traditionally against the malaria vector *Anopheles darlingi* which bites late in the night (WHO, 1989). Countries such as Mexico, Venezuela, have trial studies on impregnated mosquito nets and curtains carried out by the Ministry of Health while in Surinam due to the weekly washing of the nets by the local population trials with impregnated strips in the eaves openings are being carried out (WHO, 1989).

In the Eastern Mediterranean region, due to increasing insecticide resistance in the target insects, high costs of newer insecticides and environmental pollution, efforts are being made to identify alternative control methods. The use of impregnated

materials for reducing vector-man contact and thereby reduce disease incidence and prevalence is being explored in several countries in the region. These include, Pakistan, Afghanistan and Egypt (WHO, 1989).

In the South East Asia region, where the main vector borne diseases include malaria, dengue, haemorrhage fever (DHF), Japanese encephalitis (JE), filariasis and leishmaniasis, malaria is of major public concern; in eight out of the nine countries in the region. Due to the vector resistance and/or refractory behaviour of some of the vectors to the commonly used insecticides, extensive population movement and unwillingness of the community to accept spraying of residual insecticides, the only apparent solution left is the impregnated bed nets. The region includes countries such as Indonesia, Bangladesh, and India.

In the African region, the malaria control strategy adopted for more than 45 countries is the reduction of mortality and morbidity by the rational use of drugs (WHO, 1989). However this approach has been threatened by the appearance and spread of chloroquine resistant *Plasmodium falciparum*. There is therefore a need to use vector control methods such as the use of impregnated bed nets and curtains to overcome this situation. The main African malaria vectors are *Anopheles gambiae s.l.* and *Anopheles funestus* that bite indoors and at night and are therefore particularly vulnerable to the effects of impregnated bed nets.

Reports of trials with impregnated nets and other materials in the Africa region are available from Burkina Faso where a village trial with deltamethrin treated bed nets has been conducted by Carnevale *et. al.* (1988). Entomological evaluation showed that there was an appreciable reduction in man biting densities of *An. gambiae s.l.* after the introduction of the nets and mosquito survival and sporozoite rates were greatly reduced.

More studies with these nets are being carried out in Cameroon, Congo, Gambia, in village scale trials. In Gambia for instance reduction in febrile episodes with parasitaemia in children have been recorded due to the use of bed nets (Snow *et. al.* 1987, 1988). In the united republic of Tanzania village scale trials with impregnated bed nets has shown evidence of reduction of mosquito survival and sporozoite infection rates and of prevalence of high levels of parasitaemia in children (Curtis *et al*, 1989).

In Kenya, the first trial of permethrin impregnated curtains and bed nets as malaria control measure was evaluated in Uriri in 1988 (Sexton *et. al.* 199Ø) who found a reduction in parasitaemia levels, episodes of fever or chills and a marked reduction in the house resting densities. Since then some village-scale trials were began by the Kenya Medical Research Institute (KEMRI) in collaboration with the Centre for Disease Control (CDC), Atlanta USA using Permethrin

impregnated bed nets and sisal strips. Reports on the findings have not been published yet.

Despite the many studies that have been conducted in various areas in the world, interpretation of variable results obtained is complicated by variations in the epidemiology of malaria in different areas where trials have been conducted. Moreover the variation in design of these trials and the different clinical and parasitological measurements used make comparisons between studies difficult WHO (1987). Only one study has attempted to evaluate the impact of this form of malaria control on the child mortality and on the mortality attributable to malaria. The study was carried in The Gambia, and reported reductions in overall mortality and in malaria-specific mortality of 63% and 70% respectively; in children aged 1-4 years sleeping under insecticide treated bed nets (Lindsay *et. al.* (1992).

1.2.7 Mosquito vector Bloodmeal Analysis.

Over the years, serological approach has been preferred to other methods of identification of the source of bloodmeals (Weitz, 1956), since these methods have been found to be accurate and consistent. The approach is based on the concept that each vertebrate possesses one or more plasma proteins with antigen determinants unique to the species. Thus identification of the sources of bloodmeals will depend on the ability

of the antisera to recognise only the unique protein of the host's blood.

The necessity for sensitivity and specifity of these techniques has led to development of several serological methods including: Precipitin method including the ring test, Capillary tubes and Ouchterlony gels have been described by Phelps and Pennington (1968); Templis and Lofy (1975); and Crans (1969). Also Haemoglobin crystallisation has been proposed as an adjunct to the precipitin tests by Washino and Else (1977). Passive haemogglutination inhibition has been conducted by Templis and Rodnek (1972).

Immunohistochemical method of immunofluorescence has been advocated by Gentry *et. al.* (1967) and Mckinney *et. al.* (1972). Immunofluorescence techniques require sophisticated equipments and have been evaluated only against a limited number of blood sources (Roger 1972).

The precipitin techniques have proven neither sensitive nor specific. Haemoglobin agglutination method although specific is unreliable in identifying the blood of animals with soluble haemoglobin and accuracy in the identification varies with mosquito species (Washino & Else, 1977). Like precipitin test, latex agglutination could not distinguish between closely related hosts (Boorman *et. al.* 1977). Passive agglutination inhibitions is sensitive to the generic level but is an elaborate and difficult test and does not lend itself to direct blood meal testing (Templis,

1975).

Enzyme linked immunosorbent assay (ELISA) is the only technique which has been found to be more sensitive than agglutination techniques such as; quantitative precipitation, haemagglutination and immunofluorescent assays (Buxton *et. al.* 1980). According to Carlson *et. al.* (1972), ELISA technique is specific and reliable.

1.2.8 Cytogenetic studies using polytene chromosomes.

In the field of genetics some of the greatest advances have been made through studies of the polytene ("giant") chromosomes of fruit flies (*Drosophila* spp). Workers engaged in cytogenetic studies of vectors of disease are therefore fortune because similar polytene chromosomes bearing characteristic coded banding patterns are also present in certain tissues of black flies (Rolhfels and Dunbar, 1953; Dunbar, 1969; 1972); tsetse flies (Riordan, 1968); Mosquitoes (Sultan, 1942; Frizzi, Coluzzi, 1968) and other Diptera.

The pioneering work by Frizzi (1947) on the palearctic *Anopheles maculipennis* complex and the studies of Kitsmiller and his collaborators (1966) on the Nearctic members of this group demonstrated the value of cytogenetics in identifying members of sibling species complex while tracing cytogenetics have been published by Coluzzi and Sabatini (1967) and Coluzzi (1970). The practical value of mosquito cytogenetics has increased in recent years, particularly in

connection with the problem of identification of members of *An. gambiae s.l.* complex in Africa.

Coluzzi and Sabatini (1968) found good polytene chromosomes present also in ovarian nurse cells of adult female mosquitoes. This finding has made practicable the direct and easy application of cytogenetics to the study of Anopheline female vector populations. Cytogenetic studies have also lessened the laborious and time-consuming methods of identification of the sibling species either through crossing experiments (Davidson, 1964) or by examination of salivary gland chromosomes from larval progeny of female mosquitoes caught in the wild.

1.3 Justification of the study.

Unlike other prior methods the use of bed nets have an advantage in that they can be used in any type of house and even by outdoor sleepers such as herdsmen. Bed nets are portable since they are made of light nylon or cotton materials. However nets alone, when not well fitted are easily torn and also mosquitoes can feed through the net if part of the body is in contact with it during the night.

Impregnating bed nets with fast acting insecticides kills or repels mosquitoes before they can find a hole to enter and feed. This gives impregnated bed nets an advantage over non-impregnated bed nets.

The amount of insecticide used in the impregnation of nets is minimal as compared to the amount used in house spraying and that makes it a less expensive practice for the users. The spraying of insecticides as a control method has failed since the programme is too expensive for the government.

The permethrin treated bed nets as a mosquito control method can be considered to be of great advantage as it actively involves the community unlike the earlier methods where the community has been passive.

The intensely used malaria control method by chemotherapy which targets on the parasite has been challenged by the strains of *Plasmodium* that are becoming resistant to the formally reliable anti-malaria drug, chloroquine.

Treated bed nets have been found to reduce the overall survival rate of the vector population to such an extent that few live long enough for the parasites to mature to the stage at which they can be transmitted.

The use of impregnated bed nets or curtains kills many vectors other than mosquitoes e.g. bedbugs and insect pests which is of great benefit to the net user.

Kisumu areas have conditions that favour the continuous breeding of malaria vectors throughout the year, this malaria contributes to the high infant and child mortality. Many methods have been tried in the area including house spraying, larviciding, biological control but have not curbed the problem to the expected level. Therefore it is necessary to evaluate the impact that treated nets have on mortality in the area.

Therefore basing on the results attained during the study conducted in this part of Kisumu, the project was worth pursuing. Treated bed net method is the only alternative that acts as a barrier between man and the vector hence reducing man-vector contact by reducing the biting rate and therefore the malaria transmission.
Since the method is proving to be successful the community has been encouraged to purchase their own nets and permethrin for future use once the current ones tear.

1.4 Objectives of the study.

1. To find out the breeding sites of malaria causing *Anopheles* species in the area.
2. To investigate the effect of permethrin impregnated mosquito nets as a mosquito and malaria incidence and prevalence control method.

Parameters examined included:

(a) The effect of the treated bed nets on the house resting densities of *An. gambiae* and *An. funestus*.

(b) The effect of the use of the impregnated bed nets on the sporozoite rates.

(c) The effect of the nets on the mosquito biting behaviour (peak biting rates) by Human bait catch.

(d) Effect of the nets on the host preference of the mosquito by bloodmeal analysis.

(e) The effect of the nets on the malaria prevalence in children of ages Ø-1Ø years (Parasitaemia levels).

3. To investigate the residual effect of insecticide treated mosquito nets (Bioassay).

4. To investigate the effect of weather on mosquito house resting densities (Effect of long-rains, short-rains dry seasons.

CHAPTER TWO

MATERIALS AND METHODS

2. 1 Study site

The study was conducted at Kanyawegi-Osiri Sub-locations, South west Kisumu Location in West Kenya. The site is about 18 km South west of Kisumu town off the Kisumu - Bondo main road. It lies between Ø.Ø55S-Ø.1ØØS latitude, 34.35E- 34.4ØE longitude and stands at an altitude of between 114Ø- 12ØØ metres above sea level. The area is occupied by the Luo ethnic group.

2.2.1 Topography and social organisation

Kanyawegi - Osiri area lies along the lake shores in the Winam gulf of Lake Victoria. The lake lies 372Ø feet a.s.l. with annual fluctuations of water levels during and after long rains which vary yearly. Figure 2 shows the sketch map of the area the project was conducted. This area lies within the malaria holoendemic area see Fig. 1. The soils are sandy and clayey however some few parts are very rocky, hence the area being utilised for quarrying. There are only two seasonal rivers draining the region. The area is characterised by two rainy seasons, the long rains from March to July and short rains from October to November, the area remains dry and dusty most times of the year with temperatures ranging between 18^{o}C - 39^{o}C.

Figure 1. A map showing endemicity of malaria in Kenya (adopted from DVBD Annual report 1983).

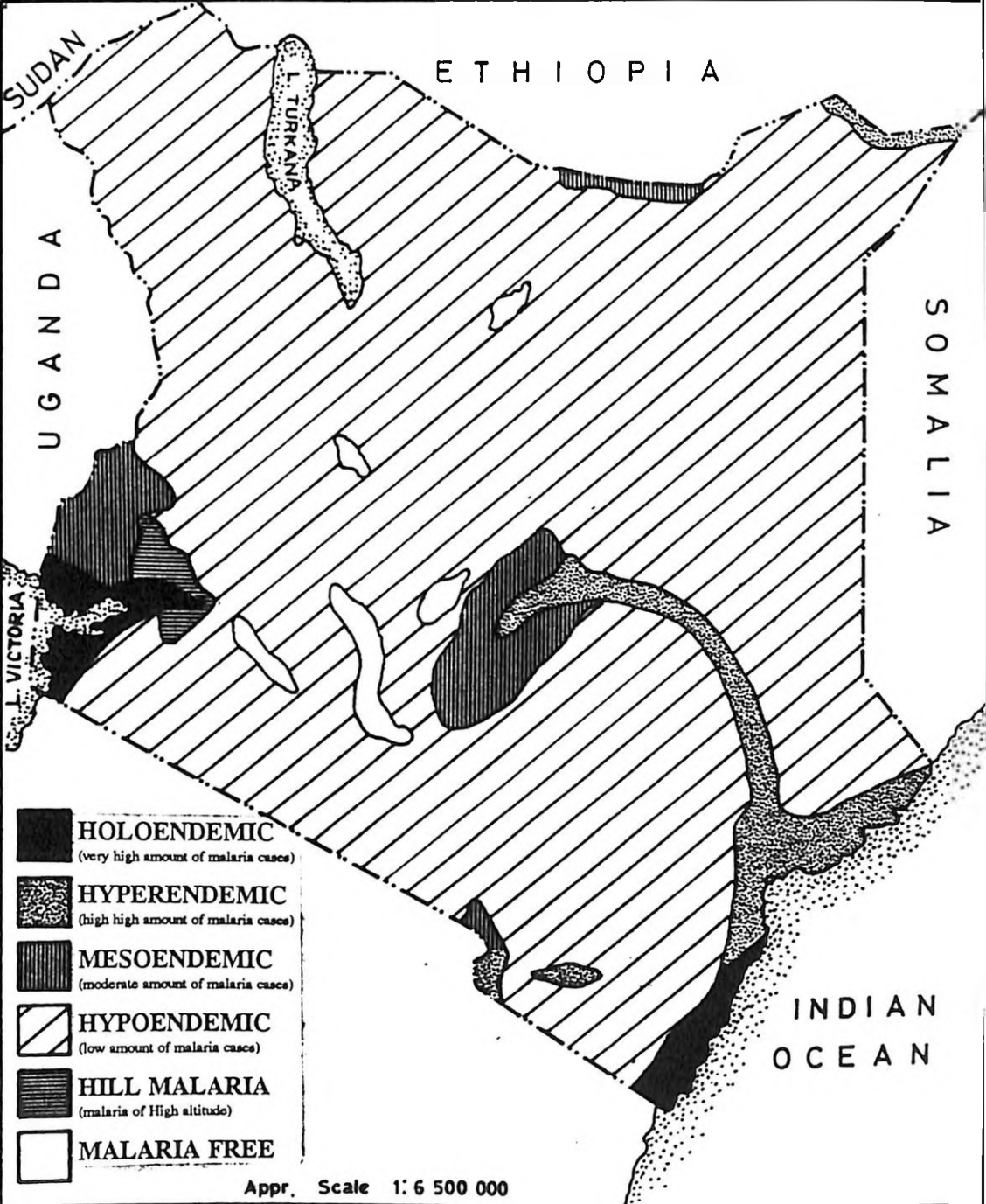
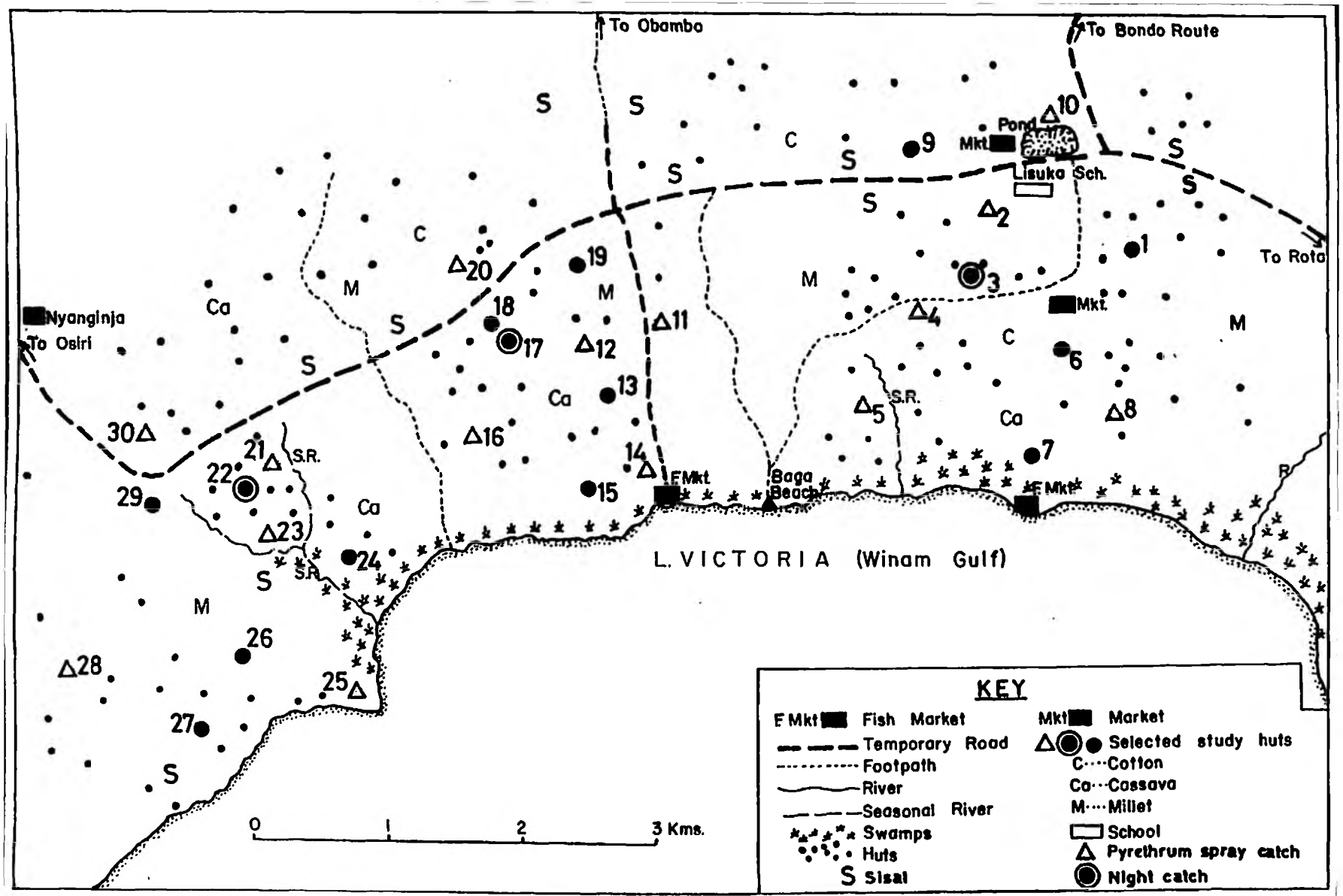

SUDAN
ETHIOPIA
L. TURKANA
UGANDA
SOMALIA
L. VICTORIA
INDIAN OCEAN
HOLOENDEMIC
(very high amount of malaria cases)
HYPERENDEMIC
(high high amount of malaria cases)
MESOENDEMIC
(moderate amount of malaria cases)
HYPOENDEMIC
(low amount of malaria cases)
HILL MALARIA
(malaria of High altitude)
MALARIA FREE
Appr. Scale 1: 6 500 000

Figure 2. A sketch map of Kanyawegi showing the three study sites, the surveyed and selected houses for the measurement of various parameters.


To Obambo
To Bondo Route
To Rota
Nyanginja
To Osiri
Pond
Mkt
Lisuka Sch.
Mkt.
S.R.
F Mkt
Baga Beach
L. VICTORIA (Winam Gulf)
0
1
2
3 Kms.
KEY
F Mkt Fish Market
Mkt Market
Temporary Road
Selected study huts
Footpath
C····Cotton
River
Ca···Cassava
Seasonal River
M····Millet
Swamps
School
Huts
Pyrethrum spray catch
S Sisal
Night catch

The vegetation is mainly Savannah Shrubs of *Lantana camara* with a few scattered trees and some finger euphobia which are used as windbreaker hedges. The area studied covered about $9Km^2$, and as per August 1991 the resident population of the area was 696 persons of whom 45% were children below 11 years. The occupation of the people is mainly all year round fishing in the lake, mat making and pottery. Subsistence farming is practised during the long rains in the area. The food crops grown include; cassava, maize, millet and bananas while cotton and sisal are the cash crops. Most homesteads keep cattle, sheep and goats as a source of milk and meat and oxen for ploughing.

Another characteristic of the area is the papyrus vegetation along the shores where during long rain seasons there is common swamping which causes some inhabitants to migrate due to these floods. These swamps act as good breeding sites for the mosquitoes. Preliminary survey done on search for breeding sites indicated, large larval populations of *An. gambiae s.l.* at the periphery of the swamps. The breeding grounds remained all the year round, a major contributing factor to all year round malaria transmission in the area.

The area has quarries and ponds, locally called "yao", which store water during rainy seasons and act as breeding grounds too. A primary school, Lisuka Primary School, is within the study area and a Health Centre

Ober-Kamoth is about 8 Km from the study site.

Most families in the study area know malaria as a disease transmitted by mosquito though they do not use nets or insecticides. A few people have been using traditional herbs in controlling the population of mosquitoes especially during rainy seasons. They burn *Lantana camara* branches or cowdung when going to bed. It was however noted that most families slept late (i.e. 10-11 pm.). They spend most part of the evening chatting outdoor around a fire.

2.2.2 Climatic conditions.

A climatic report of Kanyawegi from July 1991-July 1992 shows that the short rains occurred during the end of September reaching a total of 108.2mm in 12 rainy days, the period continuing to mid-November, see Figure 20 Appendix VIII. The long rains started in mid-March which saw the peak amount of 219.9mm in 20 rainy days in June. The highest mean monthly rainfall was recorded in June 1992 (7.33mm), and the lowest in January 1992 (14.9mm). Highest maximum daily temperatures were in December 1991 (30.3^{0}C) and January 1992 (33.4^{0}C. Lowest maximum temperatures were in June 1992 (27.9^{0}C while the lowest minimum daily temperatures were in July 1992 (15.7^{0}C). The relative humidity ranged between 22% in January to 76% in June at 0600hrs. The mean monthly temperature was 22.7 $\pm$ 2^{0}C during the short rains, 23.7 $\pm$ 3^{0}C during the dry season and in the long rains it

increased to 23.7 ± 3$^{\emptyset}$C. These temperatures were taken daily at 15ØØhrs. The mean monthly relative humidity was 65 ± 1% in the short rains, 55 ± 5% in the dry season and 72.9 ± 1.5% during the long rains. These were taken at 15ØØhrs.

2.3 Selection of experimental huts

A survey was first made to choose an area which was divided into 3 areas having buffer zones of about 2km in between them. Forty-nine (49) homesteads of a total of 1Ø7 houses were covered, in a period of two weeks 5th - 19th August during which time the demographic data was collected using a questionnaire (Appendix iv). The presence of a herd in the home was also an important factor to be considered. Data collected using the questionnaire showed (99) 93% of the round huts were grass thatched with mud wall and eaves ranging between 1Ø - 3Ø cms wide. Only (8) 7% of houses were iron roofed and with mud walls. Most of the houses had two rooms, with some of the bedrooms containing beds for the heads of the household and the outer room where mats were placed at bedtime for the children to sleep on.

The study area was divided into three (3) blocks; Site 1, 2 and 3 which were a distance of approximately 2km apart, see Figure 2. They all had similar geographical features and their distance from the shores was about uniformly one and a half kilometres. During

Plate 2. A one room hut selected for Night catch to determine the biting rates.

hut selection there were three main factors which were considered: (i) the size of the house, a small size hut for easy spraying; (ii) type of house, a mud walled and grass-thatched huts were selected for collection of data of the different parameter that were studied see Plate 2; and (iii) presence of a cowshed for studies on host preference. Most huts had large eaves for more ventilation during the hot dry season. The ages of the children who slept in the houses were also noted. The selected houses had at least children of ages Ø - 1Ø years from whom blood samples were taken for determining malaria prevalence. All houses selected for the study at least met these three conditions.

In each block, Twelve (12) houses were sampled, houses in site 1 were distributed with permethrin treated bed nets while site 2 had untreated bed nets. Block C the control had no bed nets. All children ages Ø-1Ø years in these houses were fortnightly pricked to attain blood smears and those found infected were cured with pyrimethamine/2-sulphanilamimido-3-methoxy-pyrazine (Metakelvin).

2.4 Treatment of bed nets.

Sixty (6Ø) cone - shaped bed nets of nylon polyester material were purchased from different local retailers in Kisumu town. They were 2 types of nets, small size, for single bed with a surface area of $7m^2$ (Plate 3) and large nets of $9m^2$ which were being used by

children sleeping on mats (Plate 4). Sometimes in July thirty (30) bed nets 15 small and 15 large nets were treated with the 20% permethrin insecticides which was purchased from the Wellcome Laboratory limited, Kenya. The total quantity of permethrin used per net depended upon the surface area of the net. All in all a concentration of 0.5 g/m^2 was used. Trials were conducted before dipping began to determine how much water different sizes of nets absorbs without wastage whilst ensuring that the whole surface area was covered. On average small nylon polyester nets took up 750mls of water while the large size nets took up to 1250mls of water. One percent (1%) concentration of the solution was made by adding 50ml of 20% permethrin in 1000ml water.

$$\frac{50 \times 20}{1000 \quad 1} = 1\% \text{ Concentration}$$

A higher concentration of permethrin was chosen because the nylon/synthetic bed nets unlike the cotton bed nets do not absorb more solution into the fibre hence retaining most of the active ingredient on the surface. The nets were therefore dipped into 1% permethrin solutions prepared in basins and buckets and dripped to get rid of excess chemical. The nets were then spread in the room on polythene, this was to avoid photodegradation and loss of chemical unto the ground. They were turned frequently for equal distribution of the chemical, till they were totally dry.

Plate 3. A small size bed net of surface area $7m^2$ to fit a single bed.

Plate 4. A large size bed net of surface area 9m^2 to fit mats of area 6 x 5 feet.

The dry nets were then labelled with water-soluble ink in order to double-check whether the users had washed them. They were packed in polythene bags and kept till they were distributed in block A study area. The rest of the 30 nets were washed, dried in the sun labelled, packed and distributed to block B. The C block houses which were the control without intervention of nets or insecticide were not provided with nets but were visited for entomological and parasitological data collection.

2.5 Estimation of house resting densities of *Anopheles gambiae s.l.* and *An. funestus.* using the Pyrethrum spray catch method.

Over a three-day period (during the month of August) all the thirty-six (36) houses, Twelve (12) from each of the three (3) blocks were sprayed using Pyrethrum Spray Catch method (PSC) to obtain the baseline data of mosquito densities, before the subsequent PSC catches which took day per site. This was repeated forthnightly. Houses were first emptied of all edibles, drinking water and furniture then doors and windows closed. Clean white sheets were then spread on the floor and beds. The insecticide was then sprayed using a flit-pump. The spray solution was made of 5ml crude extract of pyrethrum synergised by 2 ml pyperonyl-butoxide in 5 litres of paraffin as a solvent. The spraying was done along the eaves from the inside of

the houses. Then the knockdown effect was after 10 minutes when the sheets were carefully folded, taken outside the house and mosquitoes picked using sharp forceps, kept in petri-dishes, moistened with soaked filter paper, and were then transported to the laboratory.

In the laboratory the mosquitoes were first sorted into groups depending on the condition of the abdomen i.e. gonotrophic stages. The four abdominal conditions were categorised as empty or unfed, engorged (blood fed), half-gravid, or gravid. In this study interest was only on the female *Anopheles funestus* and *Anopheles gambiae s.l.* which the study identified as malaria vectors in the area. For both species of mosquitoes 80% of the total collected were processed for sporozoite rates by dissection and 20% were preserved and later processed using ELISA technique for sporozoites 0.20 ug/50ul PBS (section 2.7 procedure). Blood-fed females were also preserved and later processed for blood meals. Those *Anopheles gambiae s.l.* found in the Christopher's stage III were used for the preparation of polytene chromosomes for the purpose of speciation using cytogenetic technique (section 2.10). Their total population was also recorded to determine the effect of the permethrin treated bed nets on house densities.

Pyrethrum spray catch data collected in the subsequent months from the month of September, this was made in five (5) houses only out of the twelve (12)

selected from every site, due to unforeseen logistic problems. However scientifically the 15 huts selected were also a good size sample to represent the population for data collection.

The other seven (7) houses in each block that were not selected for pyrethrum spray catch, one house was used for night catch which was conducted to determine the man biting rates (MBR), one house for bioassays in site 1 and 2 (see experiment section 2.11). All the 12 houses in each site were used for parasitological data collection.

2.6 Sampling of adult malaria vectors using the Night catch (Human bait catch) method.

After the baseline data collection, three houses, one from each study site were selected, these were small size houses 2 or one room, and with at least 4 children sleeping in them. Twelve hour human bait catches were conducted in the three huts, in each hut a team of four trained collectors divided into two groups of two each collected on a continuous 6 hour shift. During the six hour collection each of the collectors sat with legs and feet exposed, and aspirated any mosquito biting them with the aid of a torch light, and aspirators. Mosquitoes were collected hourly from 1800hrs to 0600hrs and were transported to the laboratory in the morning where they were sorted into species, their abdominal conditions, counted and recorded. 80% of the

catch was dissected for sporozoites while 20% were kept for ELISA for sporozoites.

2.7 Sporozoite rates

The primary objective of the investigation was to compare malaria sporozoite rates in the three study sites and to determine the effect of permethrin treated bed nets on malaria transmission. Sporozoite rates were determined from the processed (PSC) mosquitoes using two methods, the manual dissection of 80% of the mosquitoes collected from the field by (PSC) and ELISA for the other 20%.

2.7.1 Determination of sporozoite rates by dissection

Materials and Procedure.

(a) The mosquitoes used were female *An. gambiae s.l.* and *An. funestus* collected from the field by pyrethrum spray catch method of collection. The mosquito was held by one wing while the legs and other wings were pulled out.

(b) The mosquito was then placed on the slide on it's side with the head on the right.

(c) The needle held by the left hand was gently inserted on the thorax just below the region where the glands are situated.

(d) The neck was cut off close to the head with the needle in the right hand.

(e) A drop of saline was placed close to the neck

section (the size of a pin-head).

(f) The right hand needle was then pressed gently on the thorax a little above the left hand needle in order to express the gland from the thorax. As soon as this occurred the left hand needle was dipped into the saline and brought into contact with the glands.

(g) The slide containing salivary glands was then transferred to the platform of the microscope and examined with low power lens.

(h) A coverslip was placed on the glands and examined at magnification x400 using just enough saline (0.65%) so that the coverslip presses the glands to rupture the cells thus releasing the sporozoites from the gland cells if the cells are infected. When the glands are not ruptured enough by the coverslip pressure, they were pressed gently with the needle on the coverslip in order to disrupt them.

2.7.2 Determination of sporozoite rates by ELISA

Processing specimen for sporozoite ELISA.

1. Mosquitoes about 20% of those collected from the field by pyrethrum spray catch method were arranged in vials each according to the species, house number in which they were caught date of collection with an identification number labelled on it. The mosquitoes were then kept to dry in

the dessicator after which were stored at room temperature.

2. During processing for ELISA the whole mosquito was cut, separating head and thorax from the abdomen. The head and thorax were kept in a labelled starstedt PVC microfuge tube and then processed for ELISA for sporozoites. The abdomen were also placed in separate microfuge tubes and processed for direct ELISA for bloodmeals (Appendix 1 for ELISA processing reagents).

3. For sporozoite ELISA, 50uls. blocking Buffer: NP40 was added to each microphage tube containing the head and the thorax and was incubated for 1hr. After which using the pestle this was ground to form the triturates. The pestle was then rinsed with two 100ul volumes of blocking Buffer Catching the rinse in the tube, (total volume = 250ul).
The pestle was again rinsed in PBS-Tween twice and dried to prevent contamination between samples.
The ground mosquitoes were then stored overnight at -20^{o}C.

4. 50uls. of Monoclonal Antibody solution were placed in each well of the ELISA plate: *Plasmodium falciparum* = 0.02mg/50ml PBS cover plate disposable polystylene: 96 well plates and incubated for 30 minutes at room temperature.

5. The MAb solution was dumped by banging the well plates on paper towels. The wells were with then

filled with blocking buffer (approx. 200ul/well) and incubated for 1 hour.

The Blocking Buffer was also dumped and 50uls. of the mosquito triturates added into each of the 90 wells. Into the remaining 6 wells, 2 were added positive controls and into 4 negative controls. This was incubated for 2 hours (App. IX diagram).

The mosquito triturates were then dumped and the wells washed twice with PBS-Tween. Each wash with approximately 200ul/well.

50uls. of Mab-peroxidase conjugate solution was added in each well. (i.e. approx. 0.05mg/50mls. Blocking Buffer for *P. falciparum*). and incubated for 1 hour.

The Mab-peroxidase conjugate were dumped and the wells washed 4 times with PBS-Tween after which 100mls. peroxidase substrate per well and incubated for 30 minutes.

The plates were then read using an ELISA reader machine at 414nm.

2.8 Protocol for direct ELISA on Human and Bovine host bloodmeal.

Materials and Procedure.

1. 5Øul. per well of eight negative controls were added to column 1 of the PVC flex plate.
2. 5Øul. per well of seven (human) positive controls were added to column 2 of the PVC flex plates, one well was left as a blank.
3. The remaining 8Ø wells of the plate received 5Øul. per well of mosquito bloodmeal samples and the plate was incubated overnight.
4. The plate was washed three times with PBS-Tween (wash solution) and 5Øul. of prepared enzyme conjugate solution (Appendix IE) added into each well. The plates were incubated for 1hr.
5. The plates were then washed with PBS-Tween three times and into each well 1ØØul. of peroxidase substrate was added (5mls. per plate of solution A and B) Appendix IE. This was incubated for 3Ø mins.
6. The plates were read by an ELISA reader spectrophotometer at an absorbance of 414nm.
7. After reading for human blood meals the plates were washed with PBS-Tween (wash solution) three times. Phosphatase substrate for the detection of bovine blood meals was then added and the plates incubated for 5hrs.
8. The plates were then again read using the spectrophotometer machine at an absorbance 414nm.

2.9.1 Parasitaemia (Infection rates).

A total of 95 children (50boys and 45 girls) aged between 0 - 10 years were identified during the hut selection survey. They were to be monitored for the transmission of malaria by looking at the effect of the permethrin treated bed nets on the parasitaemia levels. During the sixteen visits, which were carried out fortnightly from mid-August 1991, blood smears were taken from each of the 95 children. Thick smear for parasite count and thin smear for identification of the parasite. The slides were stained with Giemsa stain (see Appendix IID for preparation of stain).

2.9.2 Staining of the slides and treatment of the children.

Both the thick and thin smears were stained in 5% of Giemsa stain for 30 minutes. The slides were then washed in water, dried on a slide rack and then observed under oil immersion magnification x100. Parasite counts were made out of every 300 white blood cells and the species of the parasite were identified.

All the children who were found positive were treated with metakelvin tablets (see Appendix IID).

2.10 Technique for squash preparation of the polytene chromosomes.

The technique used here is one adapted from Coluzzi (1968) for adult female mosquitoes with christophers' stage III ovaries.

Collection of mosquito specimen.

A mid-morning pyrethrum flit catch indoors yielded freshly fed female anopheline with Christophers' stage - III ovaries in excellent condition for chromosome preparation. Freshly fed anopheline collected in the early morning from indoors were held alive at 25-30°C until at least midday before the Christophers' stage III was reached; for obtaining well differentiated chromosomes in the ovarian nurse cells. Pre-gravid females were also used in cytogenetical study by keeping them alive, or to re-feed them and wait for ovarian development to proceed to stage III while they are held for 3-12 hours.

Dissection of mosquito for removal of chromosomes.
A step by step procedure of processing them as adapted by Colluzi (1968).

(a) To prepare mosquito specimen for dissection first the legs and wings were removed.

(b) The mosquito was placed on the slide with 5% dilute Carnoy's fixative.(see Appendix IIB)

(c) With one needle holding the thorax, the point of the other needle was inserted at the tip of the last abdominal segment and the two ovaries pulled out.

(d) A small drop (2 mm in diameter) of concentrated Carnoy's fixative (acetic acid fixative) was placed off the centre of a siliconized cover slip. Then a large drop of stain placed next to it in the centre of the coverslip. [Clean and dry coverslips were immersed into siliconising fluid in a sealed jar for 1-2 minutes with GE SC-87 or a few hours with Repelcote. The coverslips were then transfered into soapy water, washed then removed and dried thoroughly with tissue paper].

(e) The tissues of the ovaries were transferred to the concentrated Carnoy's fixative with the point of a needle.

(f) The specimen (two lobes of ovaries) were left in the concentrated Carnoy's fixative for 2 minutes.

(g) After which the Carnoy's fixative was wrapped with blotting paper and the drop of stain was moved into the middle of the coverslip and mixed with the droplet containing the tissues of the ovaries.

(h) The tissues were macerated with a needle tip and left to stain for 3 minutes.

(i) A clean microscope slide was inverted over and picked up the coverslip by touching the slide unto the stain on the coverslip.

(j) The slide was then turned over so that the coverslip is on the upper side taking care not to slip the coverslip.

(k) The slide was examined under microscope to see whether the nurse cells nuclei were well stained and in good condition.

(l) The slide was wrapped in a blotting paper to soak extra stain from the edges of the coverslip. The coverslip was tapped gently for some time and then pressed firmly to squash the nurse cells nuclei to release the chromosomes.

(m) The preparation was examined under microscope to verify proper spreading of the chromosomes.

(n) Once there was optimal spreading of the chromosomes the slides were sealed at the coverslip edges with clear nail varnish.

2.11 Bioassays

2.11.1 Rearing of mosquitoes in the laboratory.

Adult female mosquitoes, resting inside houses were searched by using ordinary battery operated torches and aspirated into glass tubes of 1.2cm. diameter and 30cm. length attached to 20cm. rubber tubing of similar diameter. From the aspirator, the mosquitoes were gently blown out by mouth into a cage from which engorged, half-gravid and gravid females of *An. gambiae s.l.* were sorted out and aspirated into the rearing cages. The cages were made of metal frames measuring

40x40 cm. enclosed with a white nylon mosquito netting. The mosquitoes were fed on sucrose solution 10% (weight per volume) soaked onto a cotton wool supported inside a beaker. Petri-dishes lined with moist cotton wool covered with filter paper (Whatman NO.1 of 15cm. diameter.) were placed in the cages for oviposition by the gravid females. The eggs were washed into plastic trays using distilled water and incubated in a room at a temperature of $26\pm1^{0}C$ where they were left to hatch into larvae. The laboratory reared larvae were fed on dog biscuits and yeast (Excel Kenya LTD) powder till they pupated. The pupa were transferred into a beaker where they emerged into adults. These adults were used for the bioassay only 48 hours after emergence before taking a bloodmeal as recommended by the WHO standards (WHO, 1989).

2.11.2 Bioassay experiments.

Bioassay test was conducted to assess the effectiveness of the insecticide left in the nets with time. In this experiment two types of mosquitoes were used, the laboratory reared *Anopheles gambiae s.l.* unfed females (see section 2.11.1 above) and wild aspirated *Anopheles gambiae s.l.* unfed females. Once a month batches of 10 laboratory bred and 10 wild aspirated were aspirated into clean uncontaminated papercups and taken to the experimental houses in the two study sites for bioassay experiments. The wild ones were caught in the same morning from the study houses in

the area, sorted out in tens (10) of unfed females then allowed to rest for 1 hour before being used for the test. Two sites on the nets were tested; the top part which is the rarely
touched part of the conical bed net, and the lower (bottom) part, which is usually dirty due to handling.

Four plastic cones were attached onto the permethrin treated bed net in site 1 two cones on the top part and 2 cones at the bottom part. Into one cone on the top laboratory reared *Anopheles gambiae s.l.* were aspirated and onto the other wild aspirated the same was repeated for the two bottom cones on the lower part of the net. The four batches of mosquitoes were all exposed for 10 minutes, according to WHO standards bioassay test time procedure (WHO, 1989). After the exposure the mosquitoes were then carefully transferred into clean and uncontaminated papercups and provided with a piece of cotton wool dampened with 10% sucrose solution, so as not to die from hunger.

A batch of 10 wild unfed female *Anopheles gambiae s.l.* and 10 laboratory bred unfed females were also exposed to the control, untreated bed net for 10 minutes, transferred into papercups then transferred into the laboratory with some sucrose dampened cotton wool. The mosquito mortality rate was determined after 24 hours from the time of exposure to the nets. Note that once the mortality is found to be below the 70% limit then the nets are to be re-impregnated (WHO, 1989).

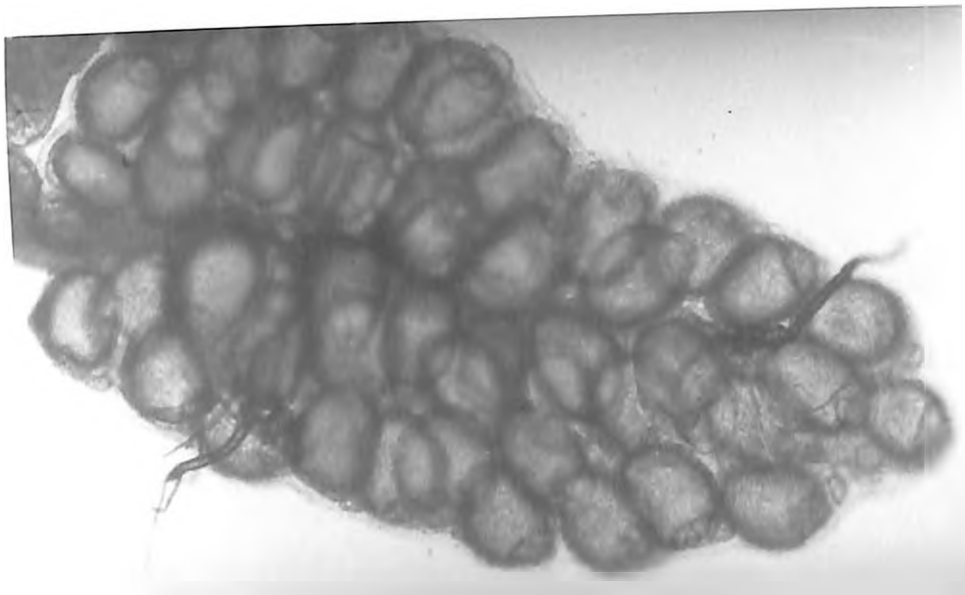
CHAPTER THREE

3 RESULTS.

3.1 Species composition.

Six (6) anopheline species were identified namely: *Anopheles gambiae s.s., Anopheles arabiensis, Anopheles pharoensis, An. funestus, An. pretoriensis.* and *An. maculipennis. Anopheles gambiae s.s.* and *An. arabiensis* were the only members of the *Anopheles gambiae* complex found in the area. Of the 50 chromosome preparations for identification of the *Anopheles gambiae s.l.* complex, only 35 were readable. They were identified against the standard maps (Coluzzi and Sabatini, 1967). Only 1 (2%) of the preparations were for *An. arabiensis.* The other 34 (98%) were for *An. gambiae s. s.* (Plate 7).

Out of the 6 anophelines identified *An. funestus,* and *An. gambiae s.s.* were found to be of importance as malaria vectors, though *Anopheles gambiae s.l.* was the most abundant species caught using Pyrethrum spray catch, and the human bait catch (Night catch) methods (Table 1). During the twelve months study period a total of 8384 anopheline mosquitoes were collected by both methods (PSC) and (NBC). A total of 6911 *An. gambiae s.l.* was collected by PSC which accounted for 92% of the total collected. *An. funestus*



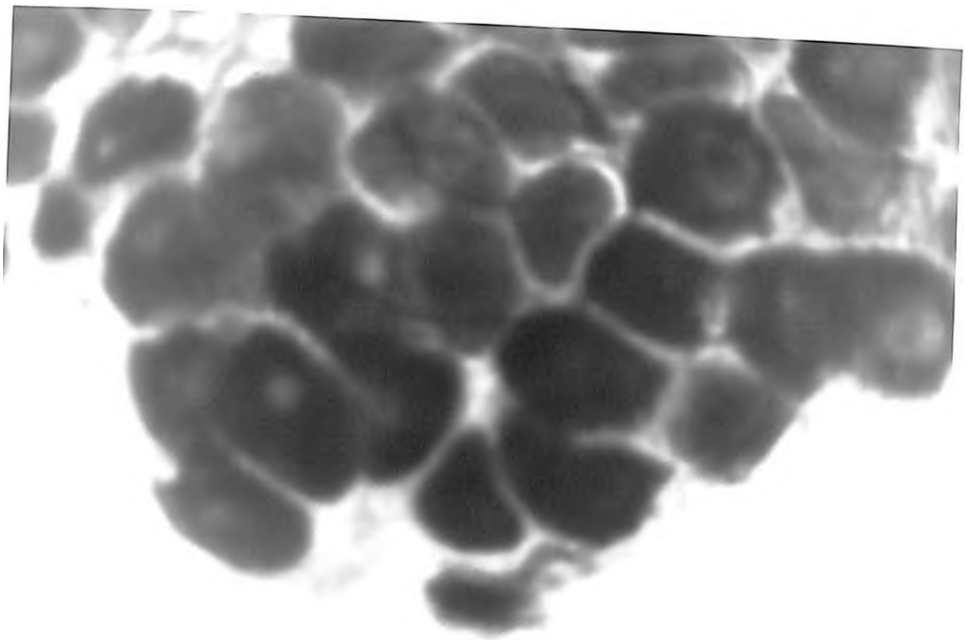


Plate 6. A photomicrograph showing the nurse cells of the ovary where chromosomes are found (magnification x600).

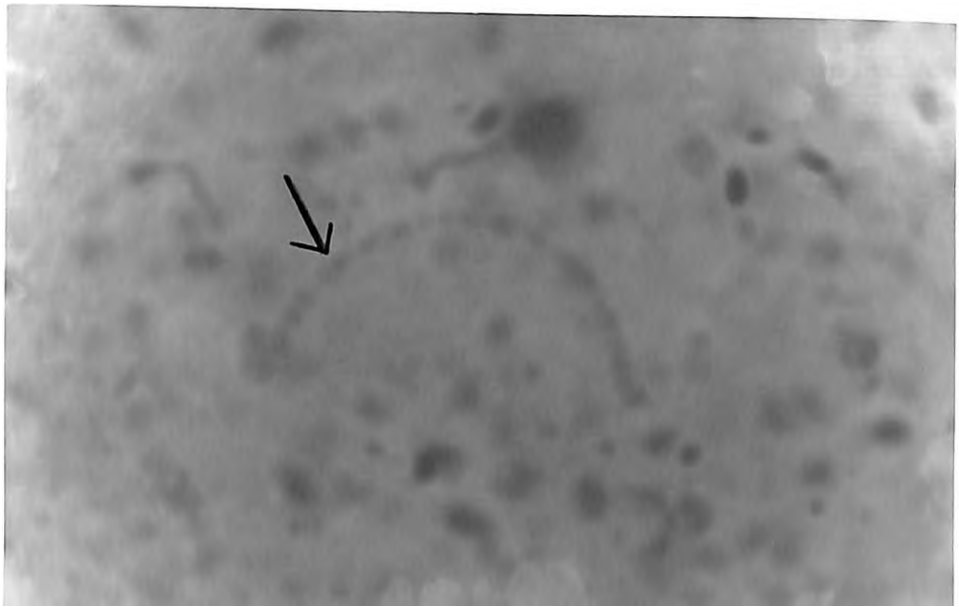


TABLE 1. Species composition and sex ratios of adult anopheline mosquitoes collected in Kanyawegi sub-location in three study sites (1,2,3).

	Site 1		Site 2		Site 3		Total collection per species
Methods of collection	PSC	HBC	PSC	HBC	PSC	HBC	
Species							
An. gambiae s.l.	454	57	29Ø7	233	3550	475	7535
An. funestus	227	11	2Ø5	2Ø	154	47	664
An. pharoensis	3	Ø	1	Ø	Ø	Ø	4
An. pretonensis	12	Ø	11	Ø	13	Ø	36
An. maculipennis	Ø	Ø	2	Ø	Ø	Ø	2
Totals	696	68	3126	253	3717	522	8386
Sex ratio:	m:f	m:f	m:f	m:f	m:f	m:f	m:f
An. gambiae s.l.	1:1.8	-	1:2	-	1:1.9	-	1:2
An. funestus	1:4.2	-	1:2.5	-	1:7.2	-	1:4.2

PSC - Pyrethrum spray catch.

HBC - Human Bait catch.

m:f - male: female.

comprised the rest 661 (8%). 765 (90.3%) of HBC were *An. gambiae s. l.* while the rest 78 (9.7%) were *An. funestus*. *Anopheles pharoensis* accounted 4 (0.05%), *An. pretoriensis* 36 (0.5%) and *An. maculipennis* were 2 (0.02%). The number of mosquitoes collected and the male to female ratios are shown on (Table 1). The male:female ratios have not been indicated in the (NBC) method because during the collections there were no males of either species caught by this method. *An gambiae s. l.* and *Anopheles funestus* females are true house haunting species, they are both endophillic and endophagic. However their males are exophagic and endophillic, they feed outside in the night on plant juices and rest indoors in the day.

An. gambiae s.l. and *An. funestus* were in large numbers during the wet season (April-June) but *An. gambiae s.l.* was higher during short rains in September and October as compared to *An. funestus*. In the dry season November-March only *An. gambiae s. l.* was caught. The general observation made was that numbers of *An. funestus* were lower as compared to *An. gambiae s. l.* throughout the study period. The overall male: female ratio of *An. gambiae s. l.* was not significant while that of *An. funestus* was highly significant for the PSC method (Table 1).

3.2 Mosquito house resting densities based on pyrethrum spray catch method (PSC).

Figures 3 & 4 (Table 2 & 3 see Appendix VII) shows the monthly mean numbers of the house resting densities of the *Anopheles gambiae s.l.* and *Anopheles funestus* in the three sites collected during study period. When comparing the house resting densities in the three sites, the lowest numbers of *An. gambiae s.l.* was recorded in site 1, 9.5%. Whereas site 2 and 3 had large house resting collections i.e. 41% and 45% respectively.

A statistical test was conducted to find if there was a difference between the house resting densities of *Anopheles gambiae s. l.* in the three study sites, according to the test $F = 0.63$, with d. f. = (2, 24), at $P > 0.05$. The pooled standard deviation was 31.98. The means for the three sites 1, 2 and 3 were 4, 20 and 20 respectively. This shows that site 1 was significantly different from the 2 and 3. Site 1 had bed nets treated with permethrin insecticide there was intervention and insecticide which reduced the house resting densities. Site 2 which had bed nets, intervention alone without permethrin had same mean with site 3 which was the control. *An. funestus* population was low as compared to *An. gambiae s. l.* except during the month of August when the baseline data was collected that *An. funestus* predominated. (Appendix VII, Table 3)

Figure 3. Monthly mean percentages of house resting densities of adult *Anopheles gambiae s.l.* in three study site.

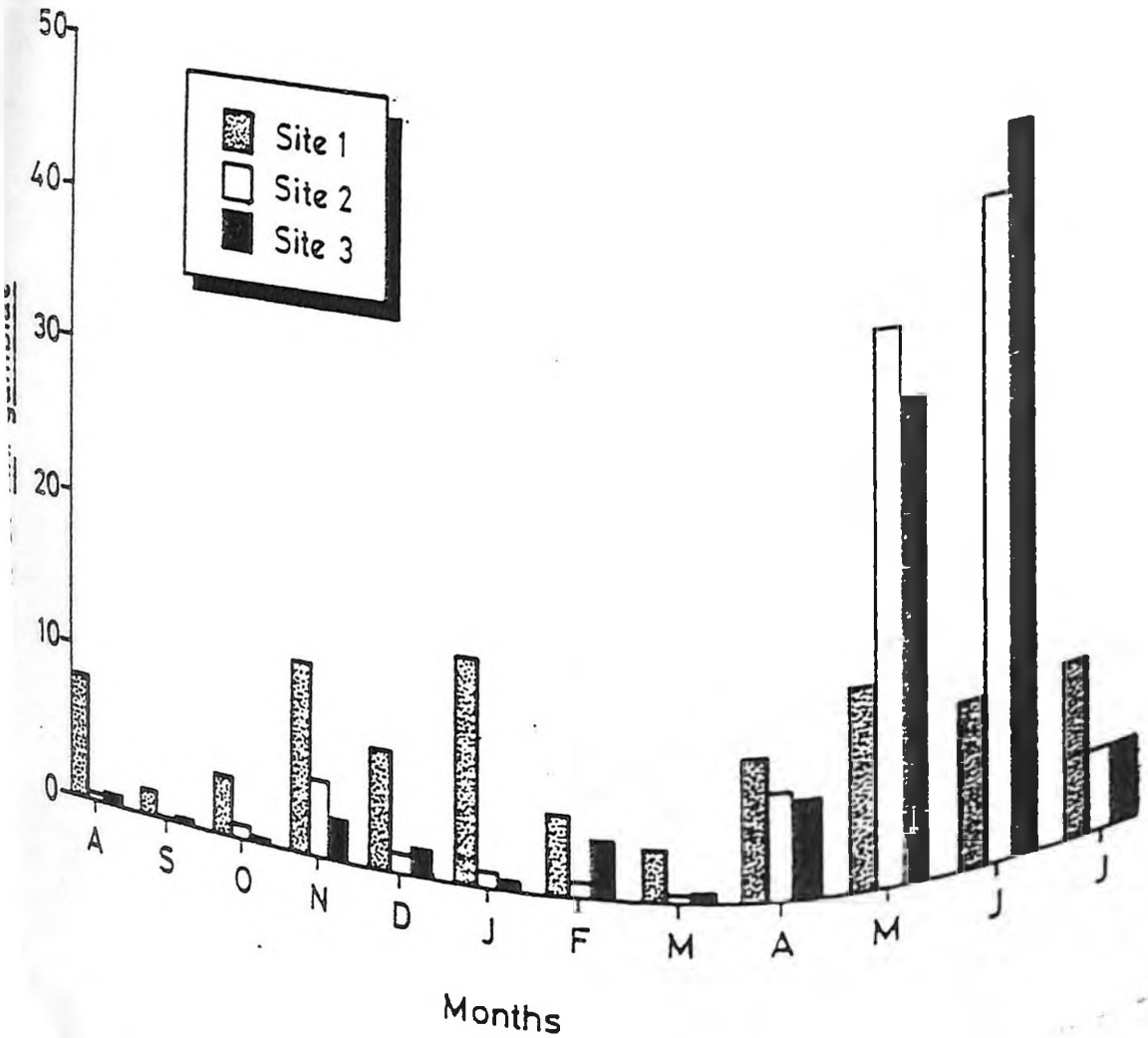
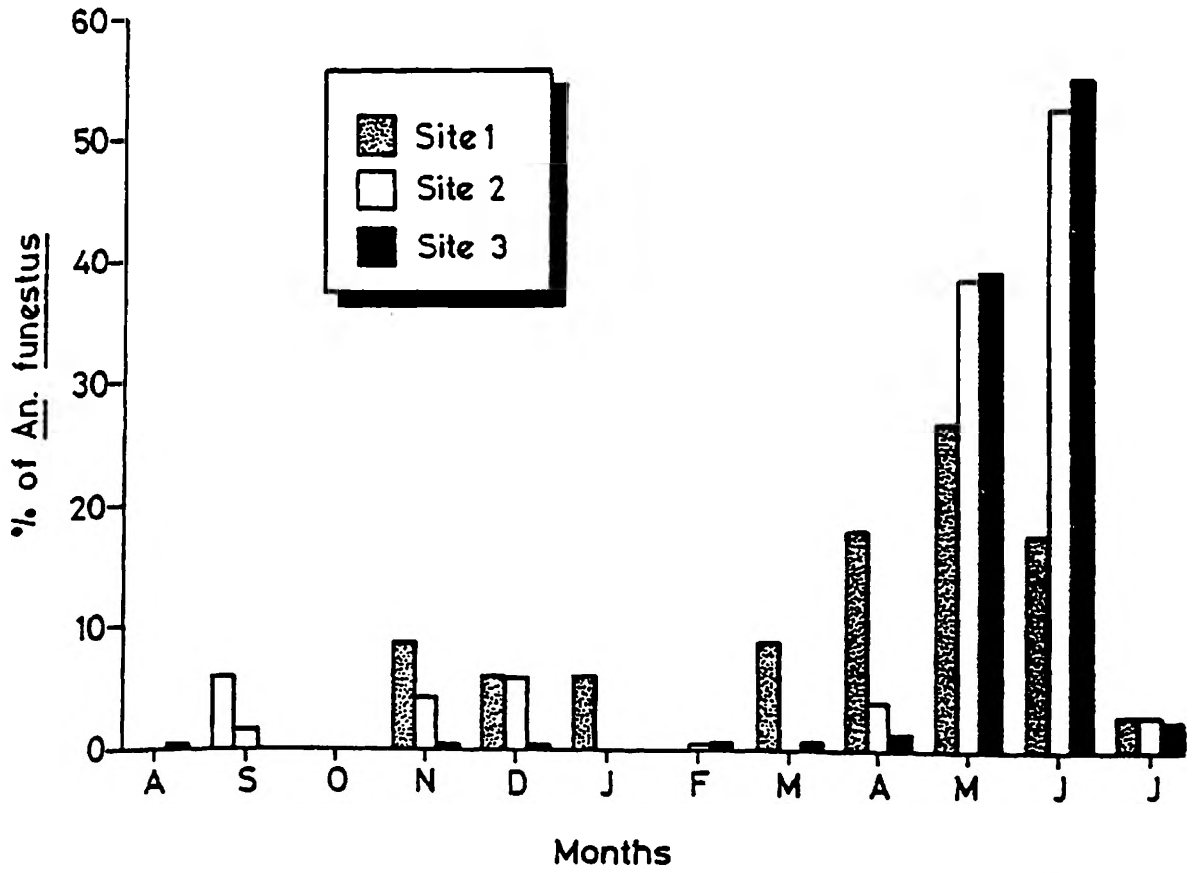

50
40
30
20
10
0
Site 1
Site 2
Site 3
A
S
O
N
D
J
F
M
A
M
J
J
Months

Figure 4. Monthly mean percentages of house resting densities of adult *An. funestus* in three sites.


60
50
40
30
20
10
0
% of An. funestus
Site 1
Site 2
Site 3
A
S
O
N
D
J
F
M
A
M
J
J
Months

A homogeneity test was conducted to compare the densities of *An. funestus* in the three sites for the 12 months of study and results showed F = 4.95, with d. f. = (11, 24), at p < Ø.Ø5. The month of August was significantly different from the rest of the months in site 1 it had the highest number of mosquitoes recorded.

In site 1, after the introduction of treated bed nets the population of *An. funestus* was reduced to mean number of 4 mosquitoes/house/month whereas site 2 and 3 had 17 & 19 respectively. A test was conducted to compare the house resting densities of the two species in the three sites, F = Ø.66, with d. f. = (2, 24), at P < Ø.Ø5. The pooled standard deviation was 6.33Ø, with means of 1.5, 4.5, and 5.5 in sites 1, 2, and 3 respectively. Statistically there was no difference found between the house resting densities of *An. funestus* using the pyrethrum spray catch method.

A test of homogeneity was conducted to compare indoor resting densities of *An. gambiae s. l.* and *An. funestus* in the three sites gave this result F = 5.28, d. f. = (2, 24) and P > Ø.Ø5. The pooled standard deviation was 12.69 and the means for sites 1, 2 and 3 were 5, 12 and 25 respectively. The difference was highly significant.

Figures 5a, 6a, 7a and 8a show the monthly proportions of house resting densities of the various physiological status of *An. gambiae s. l.* collected by PSC method (Appendix VII, Tables 4a, 5a, 6a, and 7a).

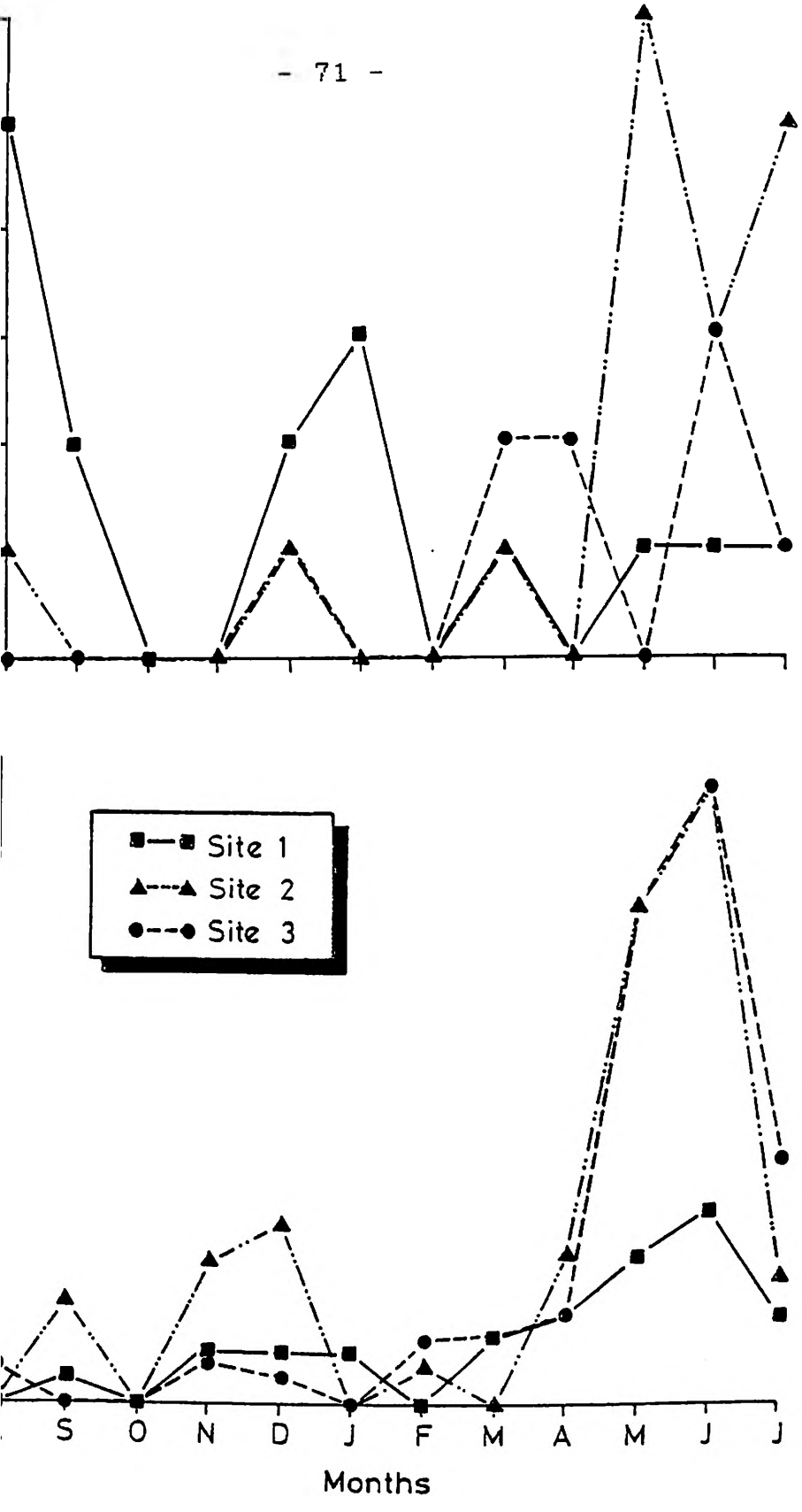
The four stages compared were empty (unfed), blood-fed (engorged), half-gravid, and gravid. According to Figure 5b which shows the house resting densities of unfed females of *An. gambiae s.l.*, there was a decrease in numbers of unfed female mosquitoes during the study period. There is however a slight increase during the long rains, April-June (Appendix VII, Table 4a). There was a decrease in numbers of unfed females in sites 2 and 3 during the dry season, December-March followed by great increase during the long rains, April-June (see Appendix VII, Table 4a).

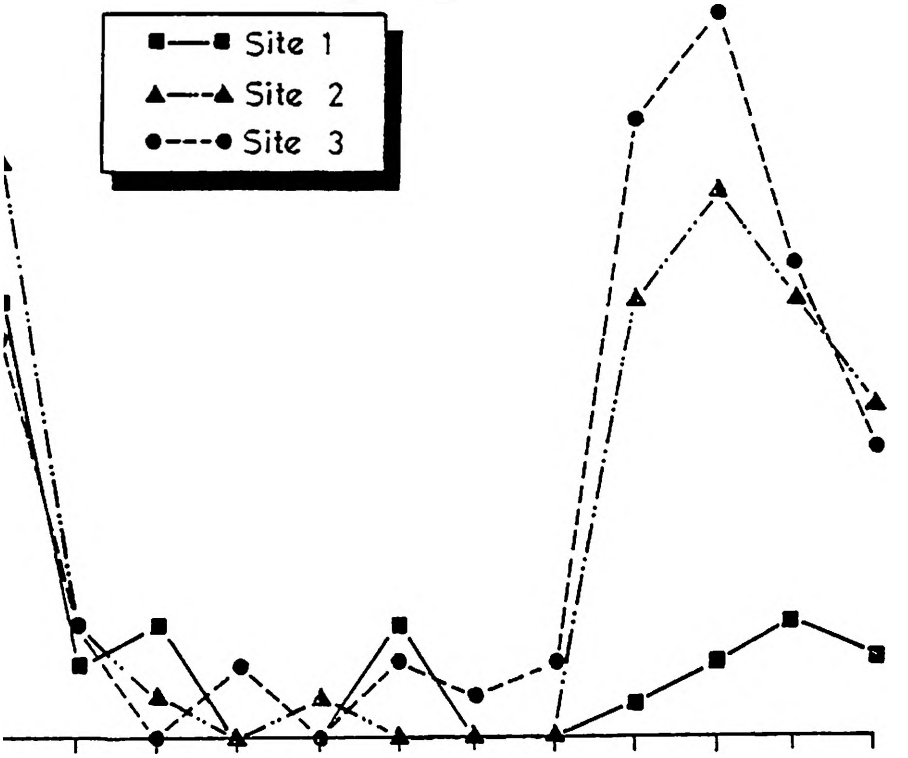
The trend in the house resting densities shown by the unfed mosquitoes was also seen in the bloodfed mosquitoes (Figure 6a). Site 1 shows a decrease in the number of blood-fed females during the short rains October-November and in the dry season, January-March followed by a slight increase during the long rains, April-June. In sites 2 and 3 there was a decrease during the short rains and dry season followed by an alarming increase in population during the long rains.

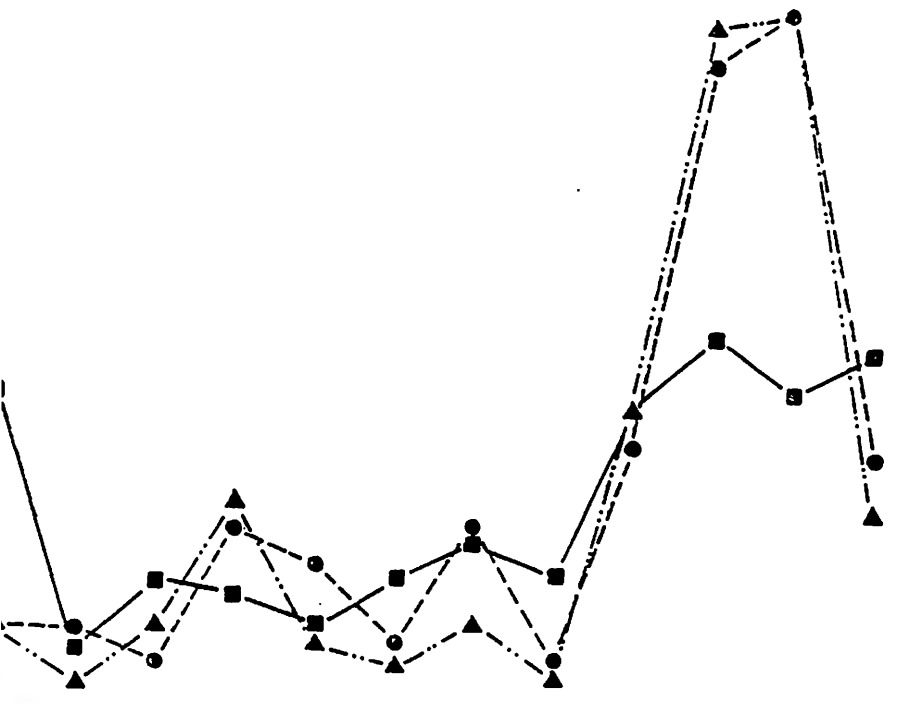
Figure 7a shows the monthly proportions of half-gravid females of *An. gambiae s.l.* from the three study sites. There was a drop in the number from 20% to 6% which occurred immediately the bed nets were introduced and was maintained through the short rains and dry season and it is during the long rains that the numbers increased to 10%. In sites 2 & 3 there was a tremendous increase of house resting densities in the

Figure 5a. Mean monthly numbers of empty (unfed) *An. gambiae s.l.* females in three study sites.

Figure 5b. Mean monthly numbers of unfed (empty) females of *An. funestus* females in three study sites.


Site 1
Site 2
Site 3
S
O
N
D
J
F
M
A
M
J
J
Months


Site 1
Site 2
Site 3



long rains from an average of 10% in each to 40% and 50% respectively.

Figure 8a shows the monthly proportions of gravid female *An. gambiae s.l.* In site 1 there was an increase during the short and long rains, however during the dry season February and March no mosquito was caught (Appendix VII, Table 7a). In sites 2 and 3 however there was an increase during the short and long rains with 5% collections in the dry season.

Figures 5b, 6b, 7b and 8b show the mean numbers of house resting densities of the various physiological status of *An. funestus* in the sites 1, 2 and 3; collected by the PSC method. Figure 5a, shows the unfed females of *An. funestus* whereby in site 1 the species disappeared during the short rains and dry periods reappeared in small numbers during the long rains. In sites 2 and 3 the species reappeared in large numbers during the long rains (Table 4a).

Figure 6b shows that there was a marked decrease of the blood fed females during the short rains and the dry season but an increase was witnessed during the long rains.

As shown in Figure 7b for Half-gravid and Figure 8b for gravid females of *An. funestus* the numbers in site 1 dropped from 17 to 2 in the half-gravid stage and from 22 to 0 in the gravid stage respectively after the introduction of treated bed nets. The numbers for 8b (gravid) were maintained through the short rains, the

dry season and during the long rains there was an increase in numbers to 2. The observation made here was contrary to that of site 2 & 3 where the mosquito numbers remained low an average of 4 during both the short rains and the dry season but greatly increased in the long rains.

Figure 9a and 9b shows the house resting densities of *Anopheles gambiae s. l.* and *An. funestus* during the short rains September-November 1991. Results indicate a general increase of the mosquito population densities of the *An. gambiae s. l.* in all the three study sites. However site 1 had a lower population increase. There was a decrease of the *An. funestus* with site 1 having no mosquitoes at all by the end of the short rains. The only explanation being that the treated bed nets had some effect on the mosquito population which either repelled, deterred them from entering the houses or killed them whenever they were in contact with the nets. Statistically there was no difference found between the house resting populations of *An. gambiae s. l.* in the three sites during the short rains $F = 1.\emptyset5$, d. f. = (2, 9) at $p > \emptyset.\emptyset5$, similarly with *An. funestus* $F = 1.\emptyset6$, d. f. = (2, 9), at $p > \emptyset.\emptyset5$.

Figure 7a. Mean monthly proportions of half-gravid *An. gambiae* collected from three sites.

Figure 7b. Mean monthly proportions of *An. funestus* from three sites.

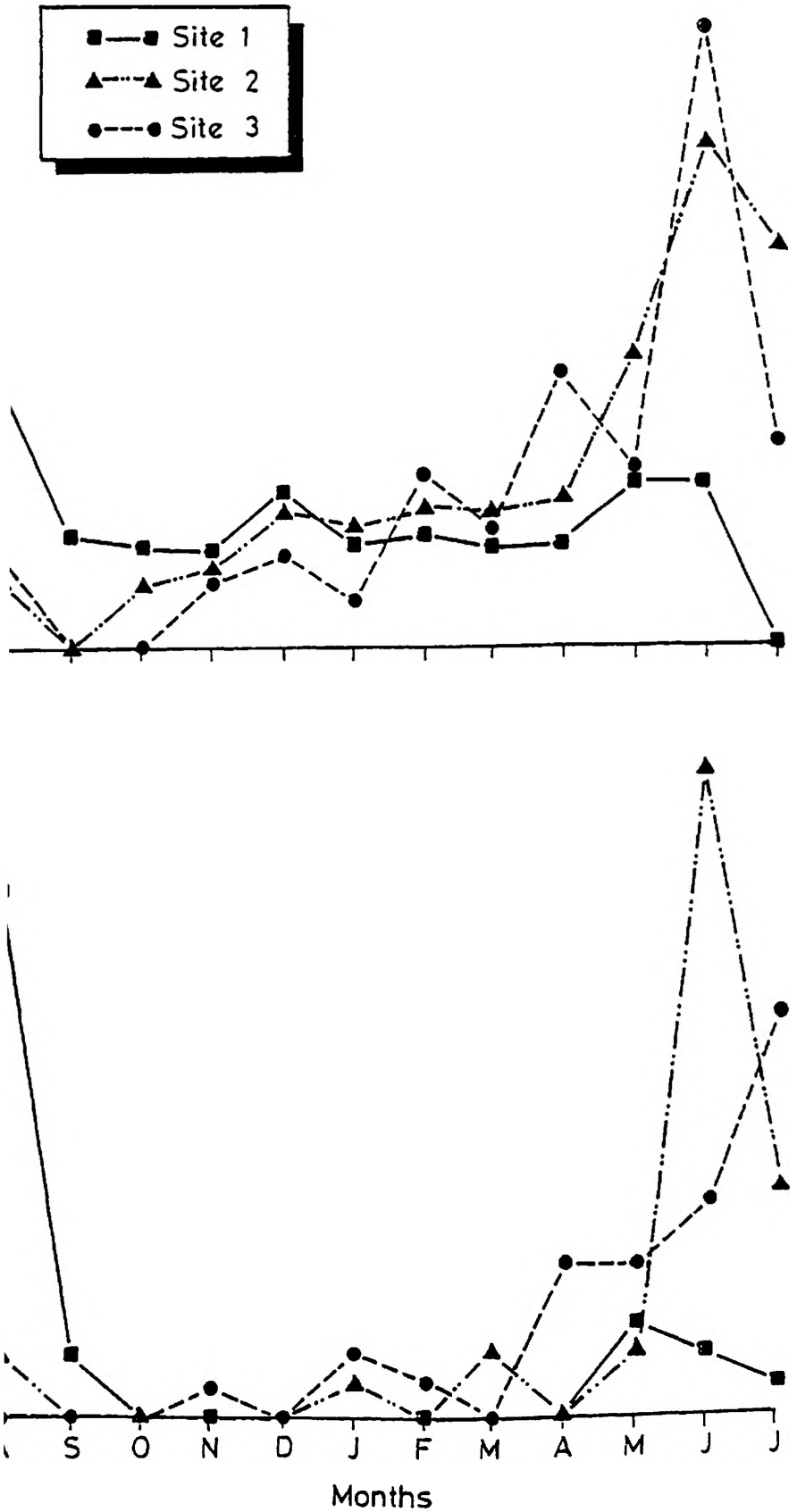
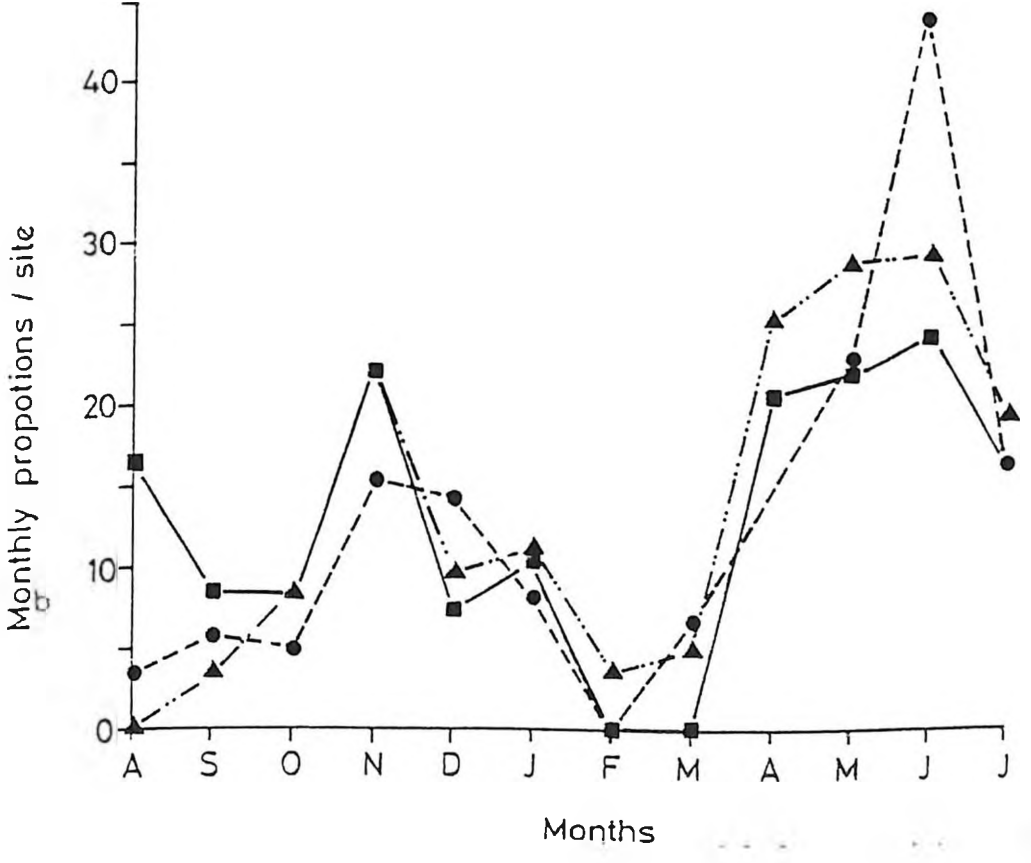
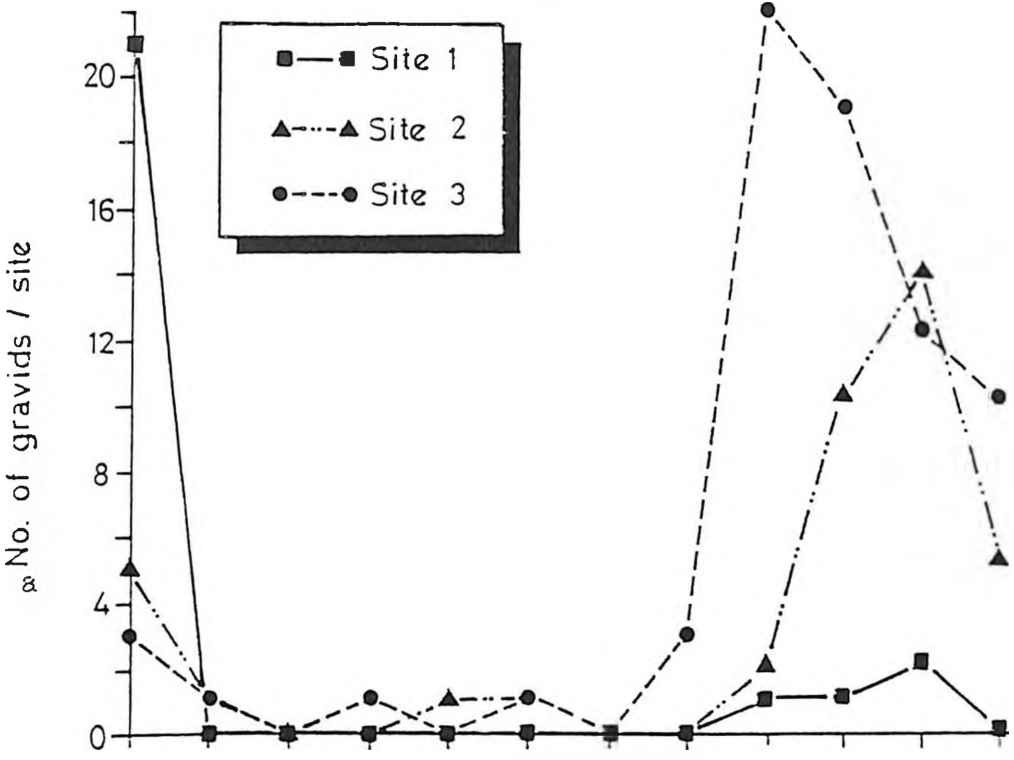

Site 1
Site 2
Site 3
S
O
N
D
J
F
M
A
M
J
J
Months

Figure 8a. Mean monthly proportions of gravid *An. gambiae s.l.* from three sites.

Figure 8b. Mean monthly proportions of gravid *An. funestus* collected from three sites.


Monthly propotions / site
40
30
20
10
0
A
S
O
N
D
J
F
M
A
M
J
J
Months


Site 1
Site 2
Site 3
20
16
12
8
4
0
No. of gravids / site

Figures 10a and 10b represent the house resting densities of *An. gambiae s. l.* and *An. funestus* during the dry season December-March collected by the pyrethrum spray catch method. Figure 10a for *An. gambiae s.l.* shows a constant population of an average of 5 mosquitoes/month in site 1. Sites 2 and 3 maintained an average population of 10 mosquitoes/month. The house densities of *An. funestus* during the dry season as shown in Figure 10b, remained lowest with site 1 no collection in February which was the driest month. Sites 2 & 3 had an average of 6 and 10 mosquitoes.

Figure 9a. House resting densities of *An. gambiae s.l.* collected by pyrethrum spray catch method during the short rains.

Figure 9b. House resting densities of *An. funestus* collected by (PSC) method during the short rains.

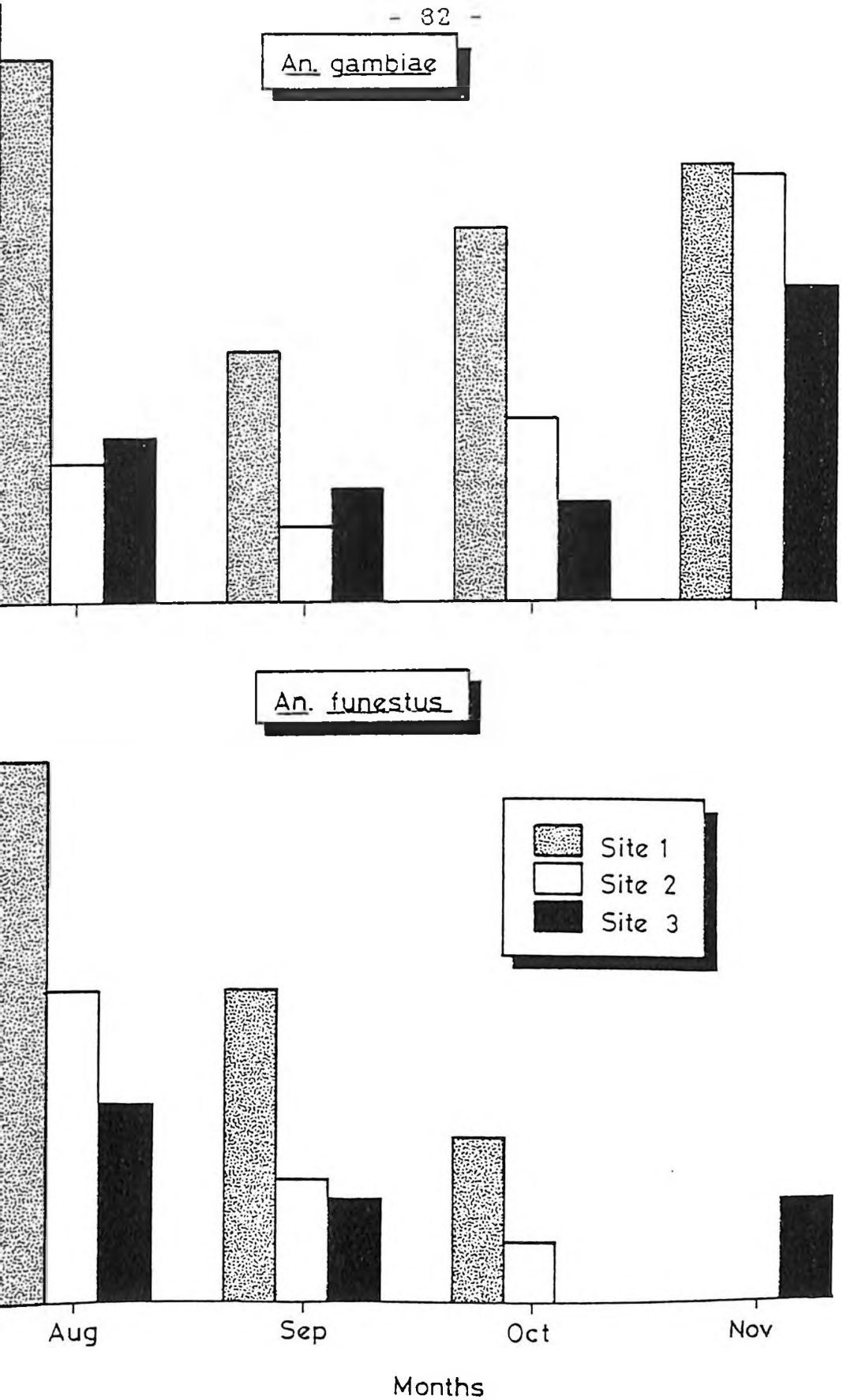
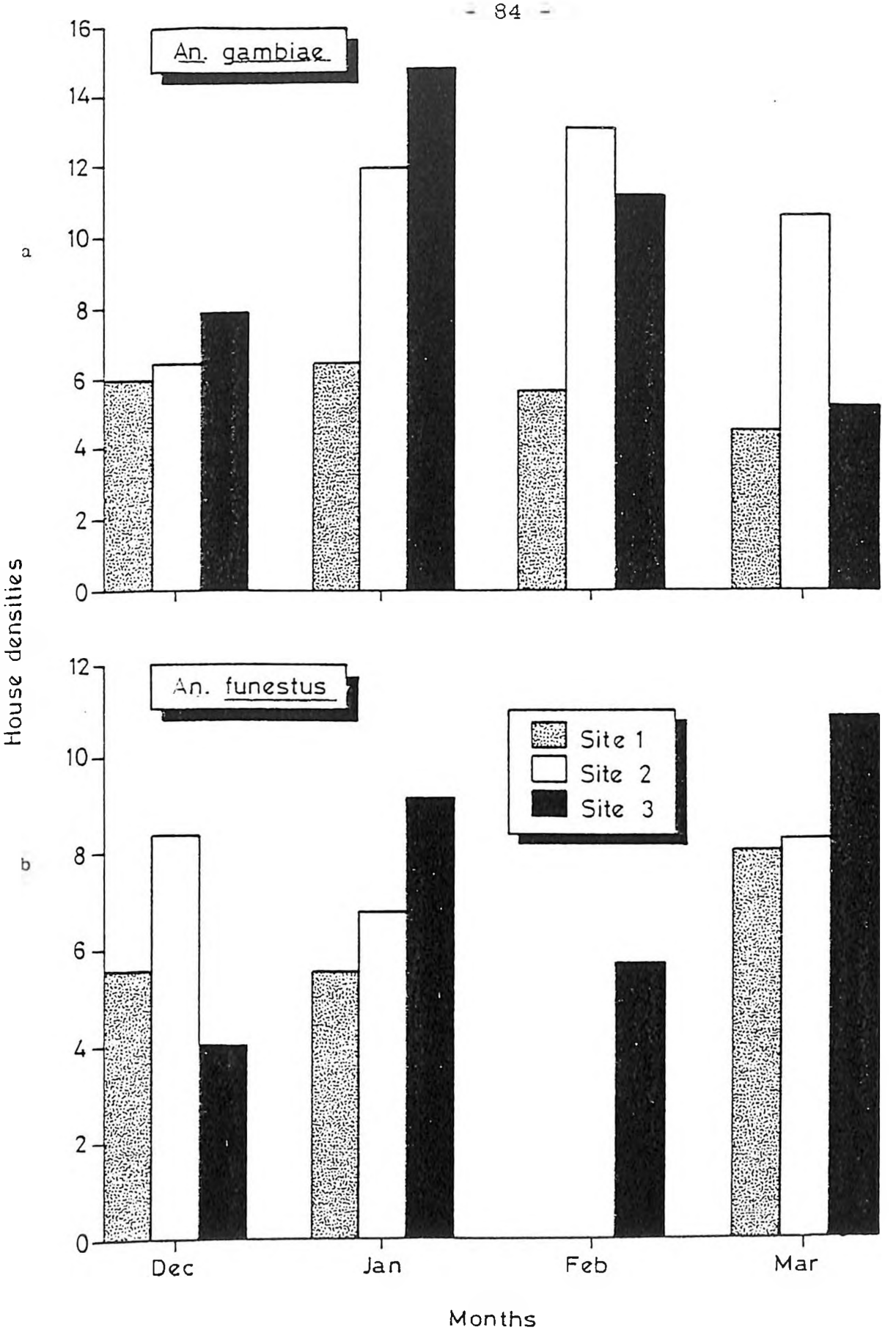

An. gambiae
An. funestus
Site 1
Site 2
Site 3
Aug
Sep
Oct
Nov
Months

Figure 10a. House resting densities of *An. gambiae s.l.* collected by pyrethrum spray catch method during the dry season.

Figure 10b. House resting densities of *An. funestus* collected by (PSC) method during the dry season.


a
An. gambiae
16
14
12
10
8
6
4
2
0
b
An. funestus
12
10
8
6
4
2
0
Site 1
Site 2
Site 3
House densities
Dec
Jan
Feb
Mar
Months

Figures 11a and 11b show the house resting densities of *An. gambiae s.l.* and *An. funestus* during the long rains April-July. As shown in Figure 11a, there was an increase in numbers of *An. gambiae s.l.* resting in houses in the three sites. In site 1 the increase was from an average of 3 mosquitoes in the dry season to 15 mosquitoes per month during the long rains. Sites 2 and 3 however had an an increase to 35 and 40 mosquitoes/month respectively.

There was similarly an increase in the densities of *An. funestus* in all the three sites with a higher increase in sites 2 and 3 which had densities of 20 and 25 mosquitoes/month respectively. Statistical test conducted to compare the house densities of *An. gambiae s.l.* in the thee sites showed a significant difference at 0.05 level. $F = 11.7$, with d. f. = (2, 9), at $p < 0.05$, site 1 was found to be different from site 2 and 3. There was also a significant difference found with *An. funestus*.

Figures 12a & 12b show the mean numbers and standard errors (SE) of *An. gambiae s.l.* and *An. funestus* collected by the (PSC) method in the three study sites through the three seasons of the year.

Figure 11a. House resting densities of *An. gambiae s.l.* collected by pyrethrum spray catch method during the long rains.

Figure 11b. House resting densities of *An. funestus* collected by (PSC) method during the long rains.

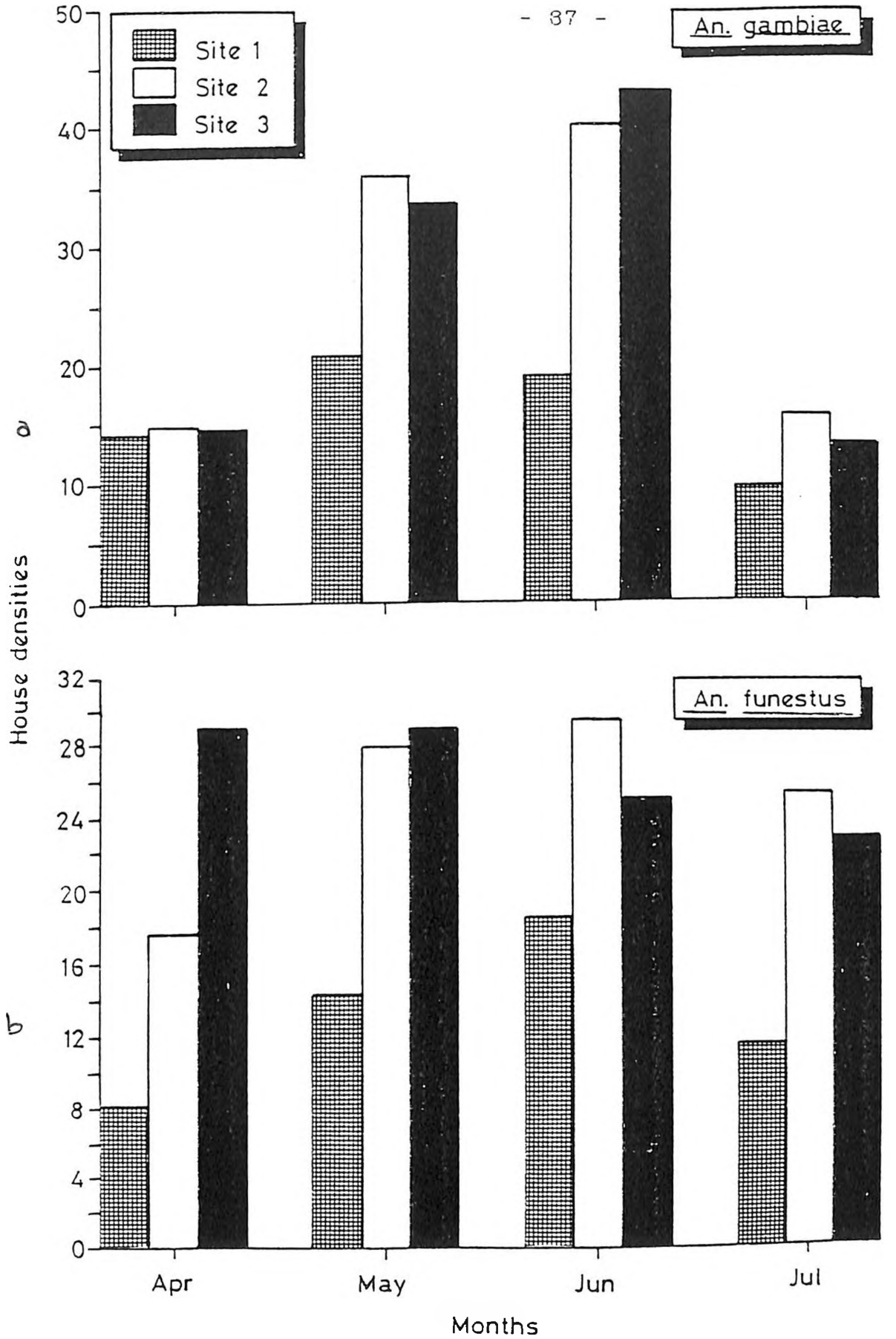

An. gambiae
Site 1
Site 2
Site 3
50
40
30
20
10
0
a
An. funestus
32
28
24
20
16
12
8
4
0
b
House densities
Apr
May
Jun
Jul
Months

Figure 12a shows that during the short rains and dry season the mean numbers of *An. gambiae s.l.* in site 1 were higher, 11 as compared to site 2 and 3 which had a mean of 7 and 5 respectfully. During the long rains the population increased in all the three sites however the mean number of mosquitoes in site 1 were lower than site 2 and 3. Site 1 had mean number of 20 per house while site 2 and three had a mean of 28.

Figure 12b shows that the mean numbers of An. funestus was 19 in site 1 during the short rains as compared to sites 2 and 3 which had 9 and 7 respectively. During the dry season the mean numbers for site 1 decreased to 5 while in site 2 and 3 the decreased to 7 and 8 respectively. During the long rains however the numbers for site 1 were 12, site 2 had 27 and site 3 had 28.

3.3 Mosquito densities based on night- biting collection (NBC)

Night catches commenced before the onset of the short rains that's in August 1991. During this season, both *An. gambiae s. l.* and *An. funestus* were collected on human baits. The average man biting rates/ night for the two species was 8 and 16 respectively in site 1, 3 and 15 in site 2, and 5 and 15 in site 3. Following the short rains in October-November the number of bites/man/night slightly increased in sites 2 and 3 but not a single mosquito caught in site 1.

Figure 12a. The mean numbers of *An. gambiae s.l.* collected from three study sites during the three study seasons of the year.

Figure 12b. The mean numbers of *An. funestus* collected from three study sites during the three study seasons of the year.

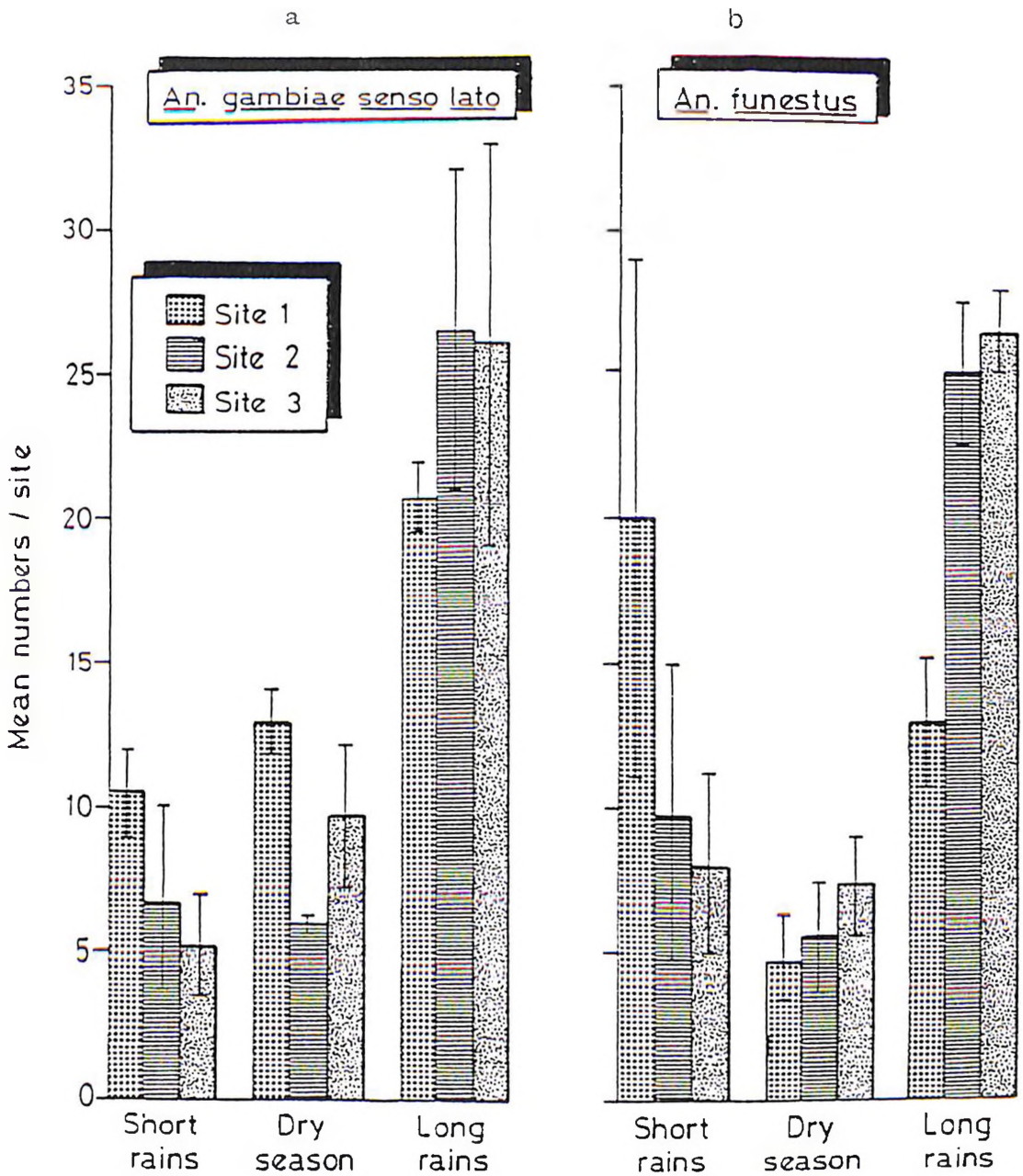

a
b
An. gambiae senso lato
An. funestus
Site 1
Site 2
Site 3
Mean numbers / site
35
30
25
20
15
10
5
0
Short rains
Dry season
Long rains
Short rains
Dry season
Long rains

Table 8a and 8b show data on monthly night biting collections of *An. gambiae s. l.* and *An. funestus* in the three study sites respectively with man biting rates indicated. During the night catch period a total of 830 mosquitoes were collected of which 755 (88%) were *An. gambiae s. l.* and 75 (12%) were *An. funestus*. Site 1 had 68 (12%) of the collection 57 *An. gambiae s.l.* and 11 *An. funestus* while site 2 & 3 had 253 (27%) and 522 (61%) i.e. 233 and 475 *An. gambiae*; 20 and 47 *An. funestus* respectively.

Figure 13a and 13b show the mean numbers and standard Errors of human biting rates of *An. gambiae s.l.* and *An. funestus* in three seasons of the study. During the dry season (December - mid-march) only a few female *Anopheles gambiae s.l.* were collected in the three sites, site 3 had 10 mosquitoes while site 1 had none. No *Anopheles funestus* caught. During the long rains, (April-May) the number of bites/man/night for *An. gambiae s.l.* increased especially in sites 2 and 3. Site 1 had an average of 2 *An. funestus* and 14 *An. gambiae s.l.* respectively; site 2 had 2 and 43; while site 3 had 6 and 136 respectively. There clear indication that the control site had the most bites as compared to site 2 and site 1.

Table 8a. Monthly night collections of *An. gambiae s.l.* by the Human bait catch method in three study sites. (Man biting rates per month in parentheses).

	Site 1	Site 2	Site 3	Totals
Month				
August	20 (5)	15 (3.8)	16 (4)	41
September	0 (0)	3 (0.8)	4 (1)	7
October	0 (0)	6 (1.5)	5 (1.3)	11
November	0 (0)	9 (2.3)	8 (2)	17
December	0 (0)	16 (4)	4 (1)	20
January	1 (0.3)	3 (0.8)	3 (0.8)	7
February	2 (0.5)	2 (0.5)	1 (0.3)	5
March	4 (1)	14 (3.5)	10 (2.5)	18
April	8 (2)	24 (6)	63 (15.8)	95
May	11 (2.8)	61 (15.3)	209 (52.3)	291
June	7 (1.8)	69 (17.3)	111 (27.8)	187
July	4 (1)	11 (2.8)	41 (10.3)	56
Total	57	233	475	755

Table 8b. Monthly night collections of *An. funestus* by the Human bait catch method in three study sites. (Man biting rates per month in parentheses).

	Site 1	Site 2	Site 3	Total
Month				
August	5 (1.3)	3 (Ø.8)	8 (2)	16
September	Ø (Ø)	1 (Ø.3)	1 (Ø.3)	2
October	Ø (Ø)	1 (Ø.3)	Ø (Ø)	1
November	Ø (Ø)	2 (Ø.5)	1 (Ø.3)	3
December	Ø (Ø)	Ø (Ø)	Ø (Ø)	Ø
January	Ø (Ø)	Ø (Ø)	Ø (Ø)	Ø
February	Ø (Ø)	Ø (Ø)	Ø (Ø)	Ø
March	Ø (Ø)	Ø (Ø)	2 (Ø.5)	2
April	1 (Ø.3)	2 (Ø.5)	5 (1.3)	8
May	3 (Ø.8)	4 (1)	7 (1.8)	13
June	2 (Ø.5)	5 (1.3)	19 (4.8)	26
July	Ø (Ø)	2 (Ø.5)	4 (1)	6
Total	11	2Ø	47	75

Figure 13a. Mean numbers of human biting *An. gambiae s.l.* collected by the human bait catch method during the three study seasons.

Figure 13b. Mean numbers of human biting *An. funestus* collected by the human bait catch method during the three study seasons.

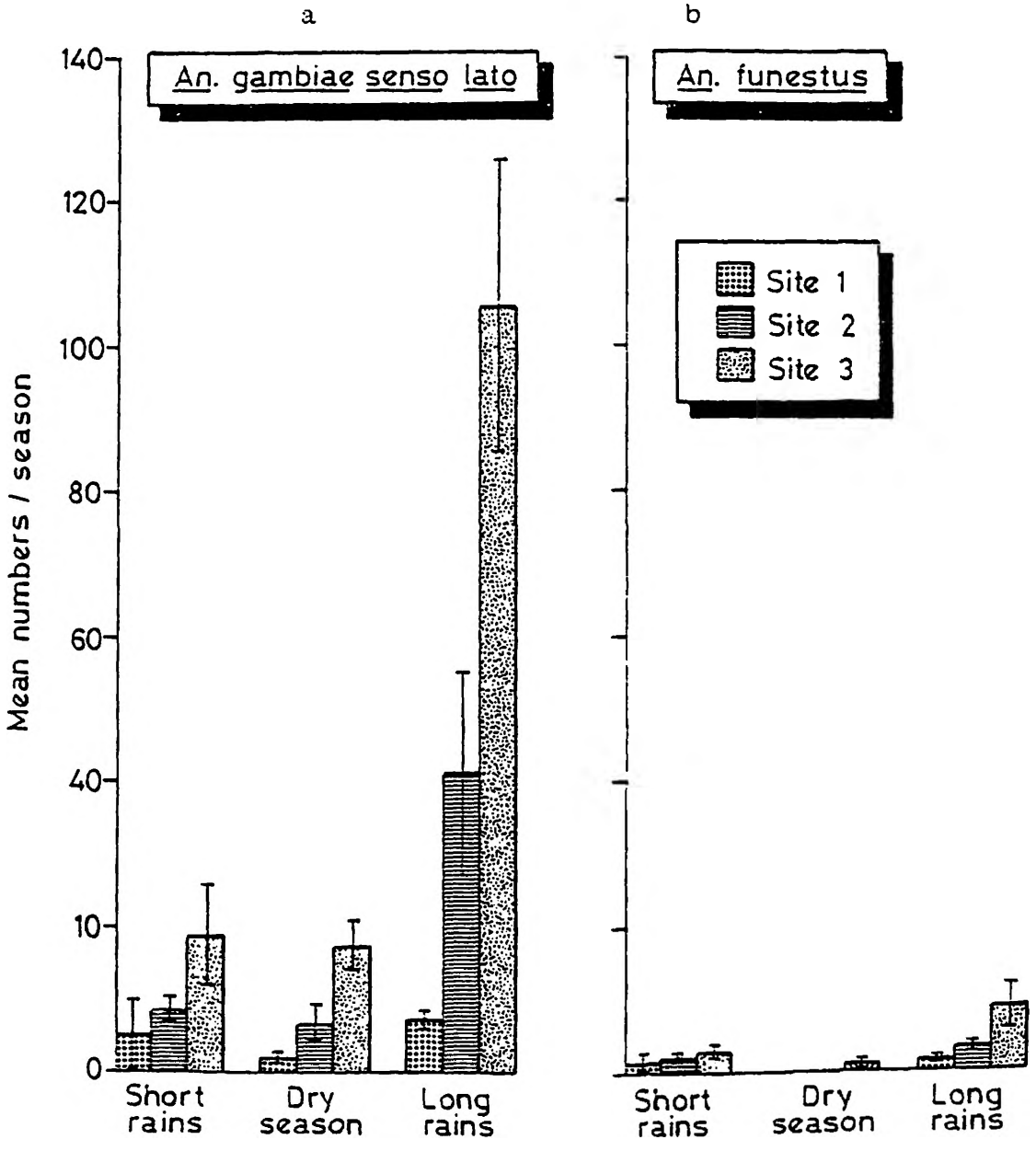

a
b
An. gambiae senso lato
An. funestus
Site 1
Site 2
Site 3
Mean numbers / season
140
120
100
80
60
40
10
0
Short rains
Dry season
Long rains
Short rains
Dry season
Long rains

Figure 14 shows the monthly percentage night collections of *An. gambiae s.l.* and *An. funestus* combined in the three sites during the study period. At the start of the study in August site 1 had the highest night catch of 38% while sites 2 and 3 had 10% and 5% respectively. The catch however decreased to 0% in site 1 for the subsequent 7 months when the numbers started increasing. In sites 2 and 3 low populations were maintained of average 10% till the long rains between April-July when populations increased to over 65% in both sites.

The figure 15 shows the night biting rates of both *An. gambiae s. l.* and *An. funestus*. Results indicates that site 1 has peak biting hours between 1800hrs - 2200hrs when the highest numbers of catches were made an average of 17 mosquitoes/hour. A Chi Square test shows a significant difference in the peak biting hours in the three sites as shown $X^2 = 9.46$, d. f. = 3, $p < 0.05$.

Figure 14. The mean monthly night collection percentages of *Anopheles gambiae s.l.* and *Anopheles funestus* collected in three sites.

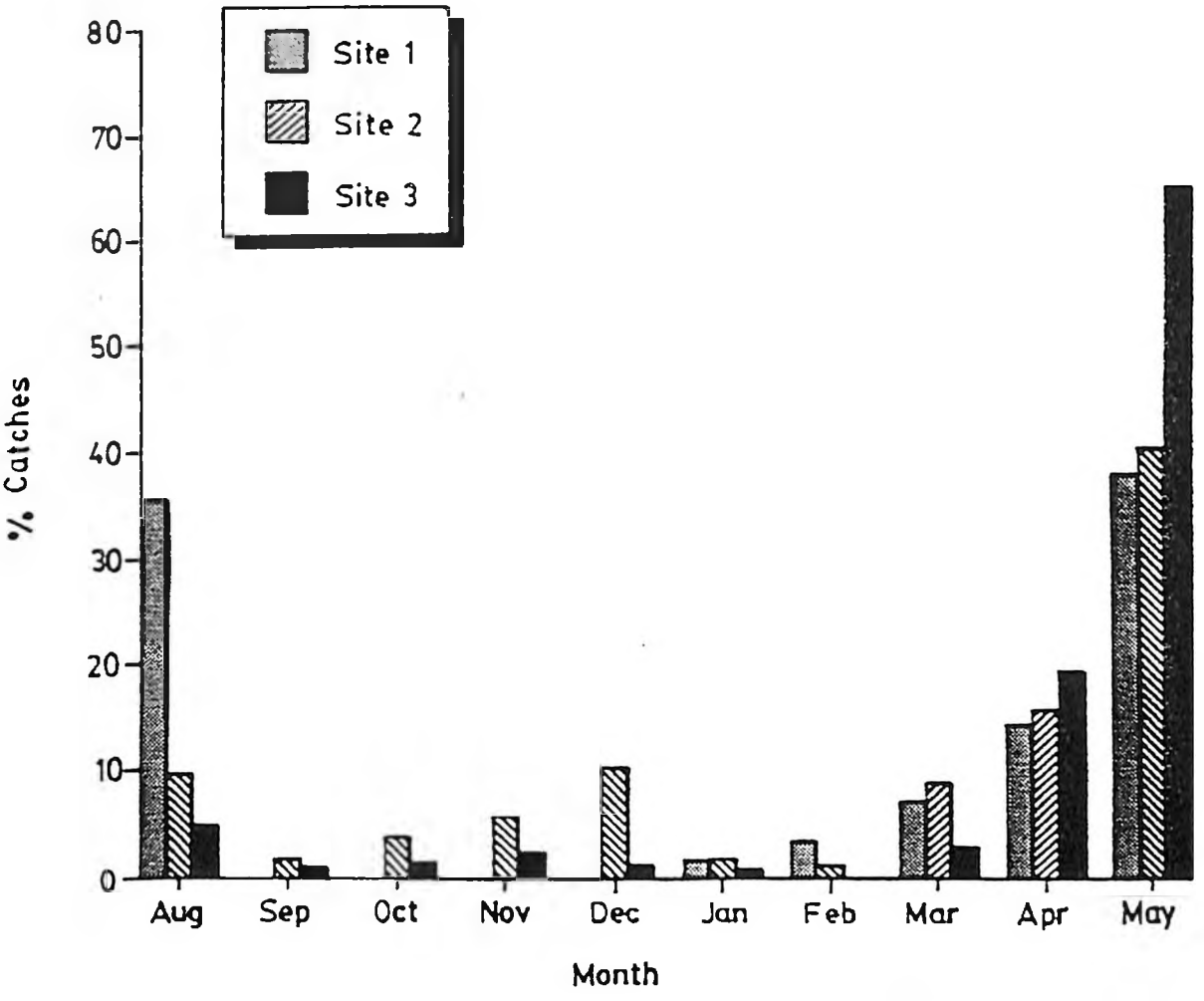
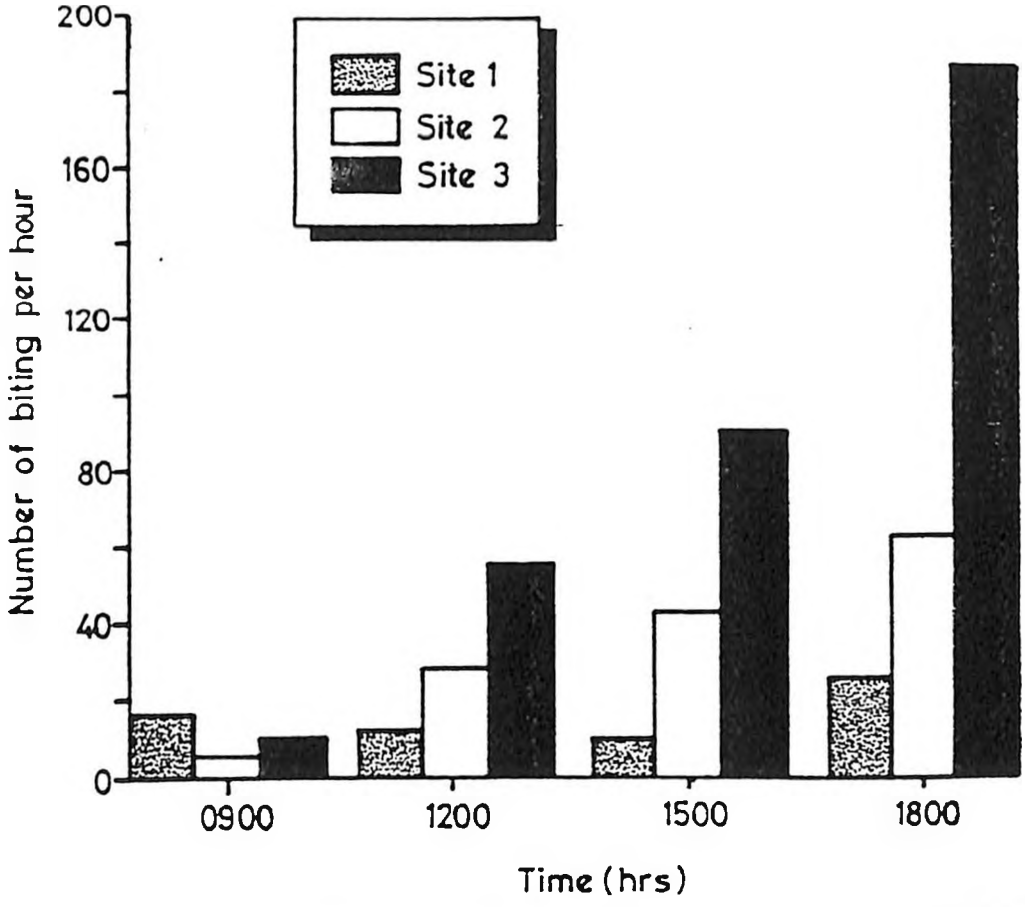

Site 1
Site 2
Site 3
% Catches
80
70
60
50
40
30
20
10
0
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Month

Figure 15. The night biting rates of both *An. gambiae* and *An. funestus* collected from three study sites.


Site 1
Site 2
Site 3
Number of biting per hour
200
160
120
80
40
0
0900
1200
1500
1800
Time (hrs)

3.4 SPOROZOITE RATES.

3.4.1 Sporozoite rates by dissection.

Table 9 shows the sporozoite rates seasonal variation of *Anopheles gambiae s. l.* and *Anopheles funestus* in three (30 study sites over a 12 months study period. Of the total population 6021 females of both species were collected by the two methods, 5192 by PSC and 829 by HBC method. Of these *An. gambiae s. l.* accounted 5368 (87%) while *An. funestus* was 1559 (13%).

A total of 1020 females of both species, 771 (76%) *Anopheles gambiae s.l.* and 249 (24%) of *An. funestus* were dissected for sporozoites. Plate 8 (see page 105) shows the six lobes of salivary glands of an infected mosquito and see Plate 9 for the sporozoites.

Only 13 *An. gambiae s. l.* and 8 *An. funestus* were found positive for *Plasmodium* sporozoites respectively. Test of goodness of fit was conducted to find if a difference existed between the sporozoite loads of the two species and results showed that no difference existed in the sporozoite loads in the two species, X^2 = 0.63, d. f. = 1, $p > 0.05$.

Observations made showed that most of the positive cases were found during August to November during the short rains and also in March to May during the onset of the long rains. It was during these periods that mosquitoes were most abundant in houses.

3.4.2 Sporozoite rates by ELISA

Of the total catch 20% of the population caught were preserved for sporozoite Elisa. A total of 580 mosquitoes comprising of both *Anopheles gambiae s. l.* and *Anopheles funestus* were tested. Those found positive for *P. falciparum* at an observance of 405nm, the absorbance (O.D) ranged between 0.075 (lowest) to over 2.500 (highest). This data was gathered in March at the onset of long rains. Twenty four (24) mosquitoes tested positive for *Plasmodium falciparum* sporozoites 17 (71%) were *An. gambiae s. l.* and 7 (29%) were *An. funestus*. Statistical analysis by the goodness of fit test (G-test) was conducted to find if a difference existed between the sporozoite loads of the two species of mosquito vector results were as shown X^2 = 40.48, d. f. = 6, at $p < 0.05$. There was a great significant difference between the sporozoite loads in *An. gambiae s. l.* and *An. funestus* with ELISA for sporozoites.

Table 9. Sporozoite rates of *An. gambiae s.l.* and *An. funestus* collected by pyrethrum spray catch method and processed by dissection and *Plasmodium falciparum* sporozoite ELISA.

	Site 1
An. gambiae s.l.	
No. Diss.	107
No. +ve.	3
% Positive	2.80
No. Process (ELISA)	120
No. +ve. (ELISA)	3
% Positive (ELISA)	2.50
Overall % +ve	2.64
An. funestus	
No. Diss.	111
No. +ve.	1
% Positive	0.90
No. Process (ELISA)	68
No. +ve. (ELISA)	2
% Positive (ELISA)	2.94
Overall % +ve	1.63
Overall % +ve (for both spp.)	1.64

No.+ve (Diss.) = number posit

No.+ve ELISA = number posit

%+ve = percentage p

Total +ves = total positi

	Site 1	Site 2	Site 3	Total/ % ves.
An. gambiae s.l.				
No. Diss.	107	213	297	617
No. +ve.	3	2	5	10
% Positive	2.80	0.94	1.68	1.62
No. Process (ELISA)	120	140	138	398
No. +ve. (ELISA)	3	7	6	16
% Positive (ELISA)	2.50	5.00	4.35	4.02
Overall % +ve	2.64	2.55	2.53	2.56
An. funestus				
No. Diss.	111	50	83	244
No. +ve.	1	1	2	4
% Positive	0.90	2.00	2.43	1.64
No. Process (ELISA)	68	61	58	182
No. +ve. (ELISA)	2	5	2	9
% Positive (ELISA)	2.94	8.20	3.45	4.95
Overall % +ve	1.68	5.41	2.84	3.05
Overall % +ve (for both spp.)	1.64	5.10	2.94	3.30

No.+ve (Diss.) = number positive by dissection per species.

No.+ve ELISA = number positive by ELISA per species.

%+ve = percentage positive by both dissection & ELISA

Total +ves = total positives in three sites 1,2,3.

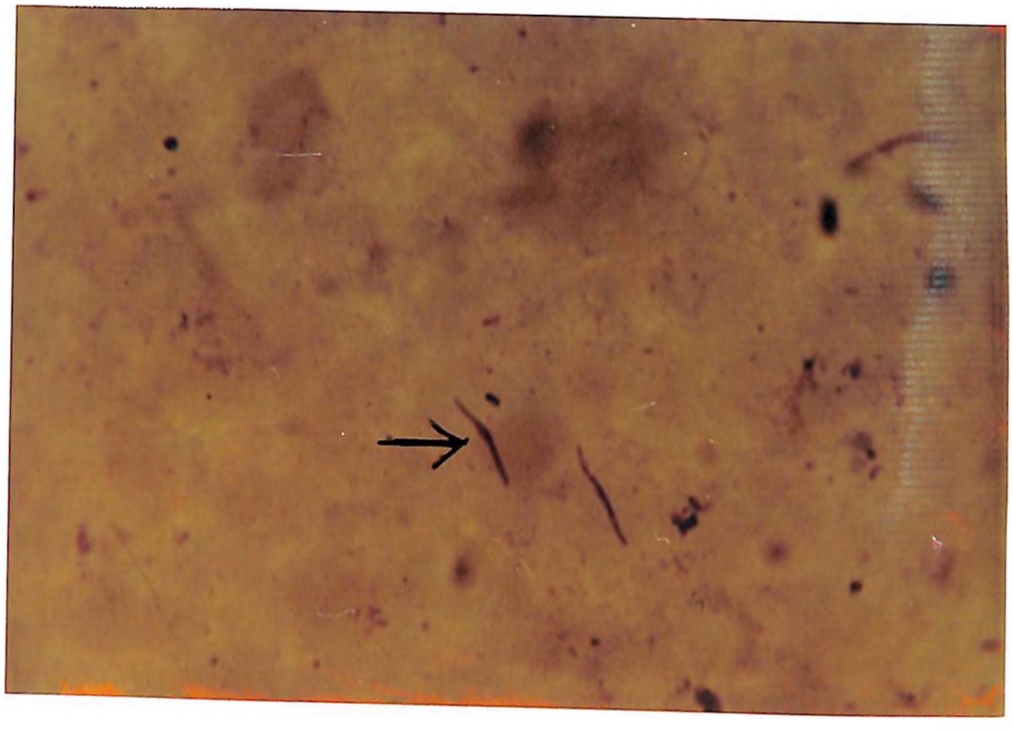


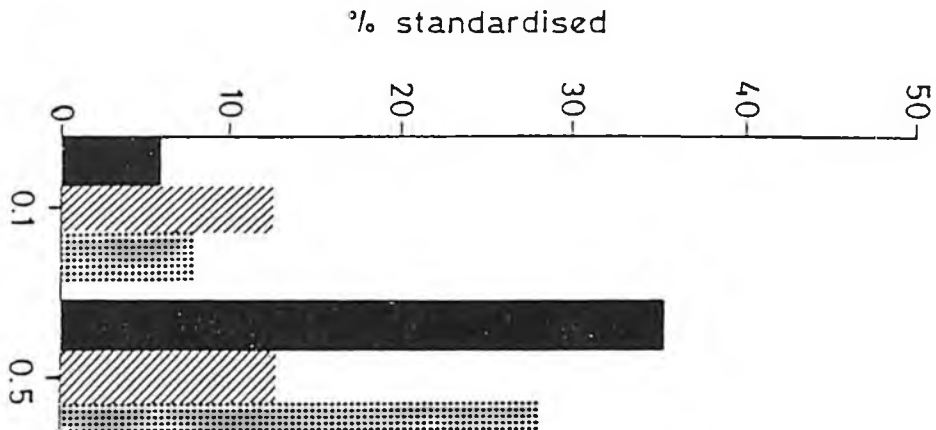
Plate 8. A photomicrograph of sporozoites extracted from infected glands of *An. gambiae s.l.* female stained with Giemsa. (mgf. x1000)

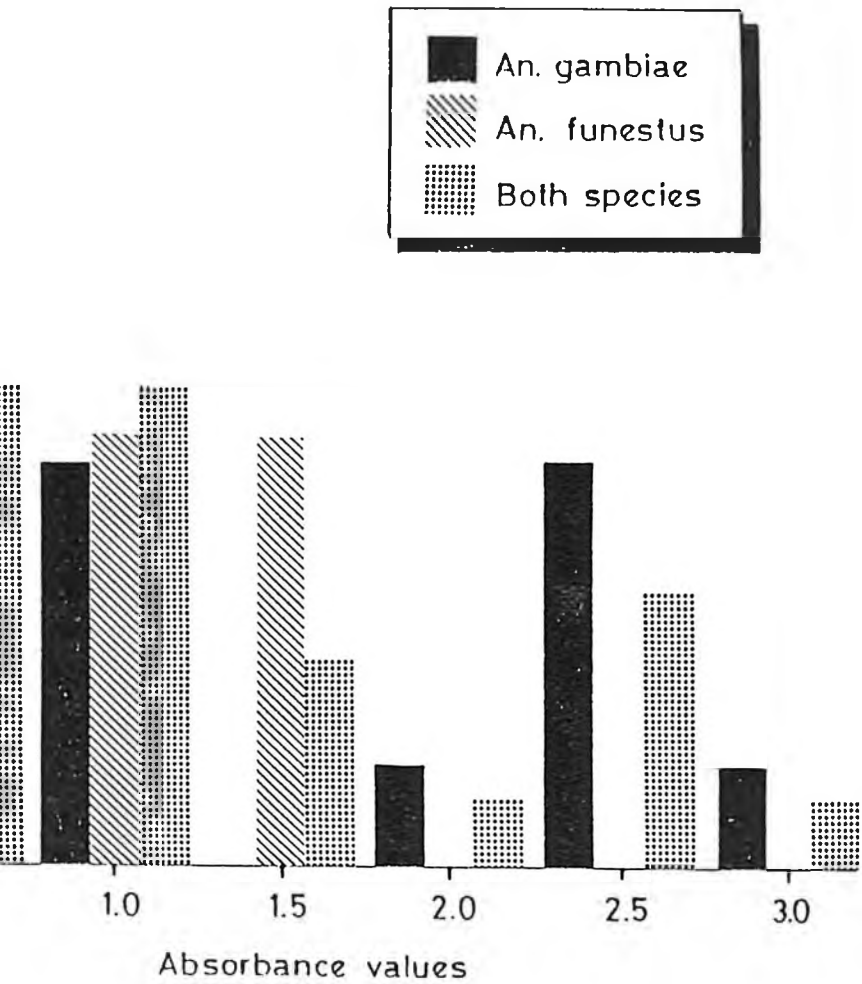
Figure 16 shows that *An. gambiae s. l.* has higher sporozoite loads > 1.5 O.D, with a range between Ø.1-2.5 O.D. While *An. funestus* has a lower sporozoite loads ranging between Ø.1- 1.5 O.D. Seventeen (17) *An. gambiae s. l.* tested positive for *P. falciparum* of which 1Ø were collected during the short rain and dry seasons. However of the 7 *An. funestus* that tested positive 5 were collected in August during the onset of the short rains. During the dry season no *An. funestus* were represented for ELISA because there were none present in any of the three sites.

Another observation made was that of the 24 that tested positive for *P. falciparum* sporozoites, 6 (25%) were from site 1, 1Ø (41%) from site 2, and 9 (33.3%) from site 3.

Figure 16 shows the standardised percentages of absorbance values representing *P. falciparum* sporozoite loads of *An. gambiae s. l.* and *An. funestus* during the whole period of study. Most mosquitoes were collected in August, April and May. The sporozoite rates by dissection was 2.Ø5% while the rates by ELISA were 4.1% the sporozoite rates based on both methods was 3.Ø8%.

Figure 16. Standardized percentages of absorbance values representing *Plasmodium falciparum* loads of *Anopheles gambiae s.l.*, *An. funestus* and others.


% standardised
0
10
20
30
40
50
0.1
0.5

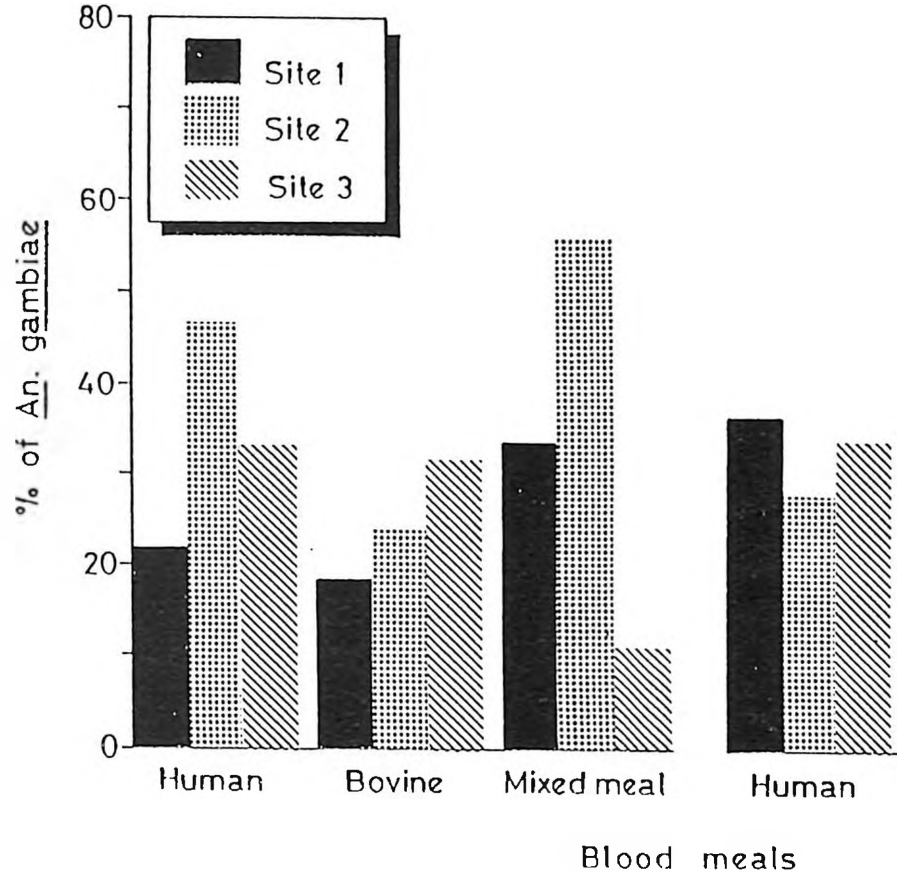

An. gambiae
An. funestus
Both species
1.0
1.5
2.0
2.5
3.0
Absorbance values

3.5 Blood meal identification

Blood meal identification were conducted to find out the feeding pattern and host preference of *An. gambiae s.l.* and *An. funestus* during the 12 months study period from the short rains through the dry seasons to heavy rains. As indicated in chapter two, the mosquitoes used were those caught using the pyrethrum spray catch for sporozoites and abdomens of the blood-fed kept for blood meal analysis by direct ELISA.

Figures 17 & 18 shows the human and bovine bloodmeals identification for *An. gambiae s.l.* and *An. funestus* in sites 1, 2 and 3 in three seasons; short rains, dry season and long rains respectively. Of the 438 blood fed female mosquitoes of both species tested 246 were identified by direct ELISA as the human or bovine blood-meals. From the initial screening of *An. gambiae s. l.* and *An. funestus* bloodmeal using the human-peroxidase and bovine-phosphatase two-step procedure, 184 (72%) were identified as human, 46 (18%) as bovine and 16 (6%) as mixed human and bovid. The remaining 192 have not been identified, they may have been of other hosts e.g. goat, sheep, chicken or other vertebrate.

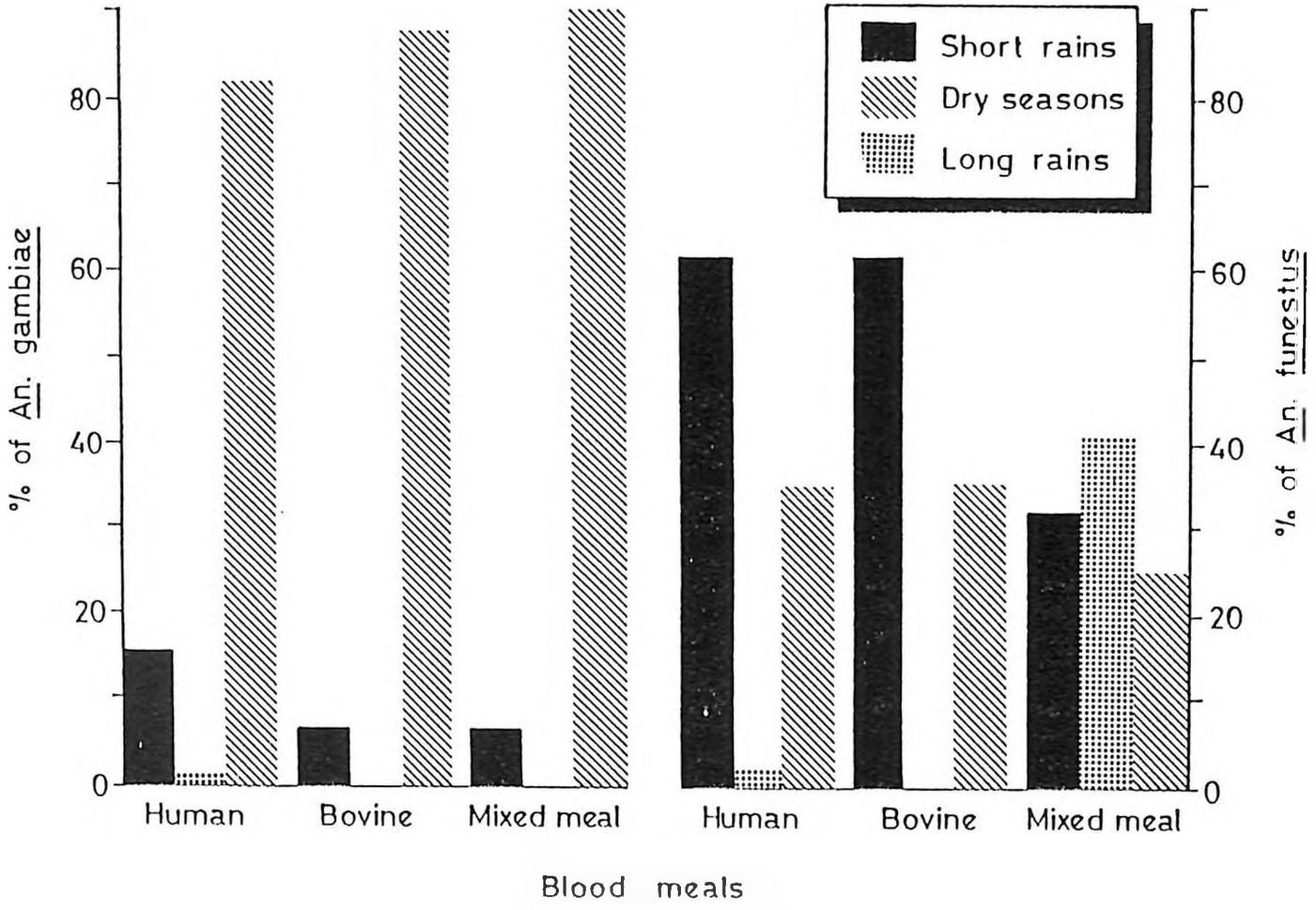
Observations made from each site show that human bloodmeals of *An. gambiae* were mainly from sites 2 & 3 that is 65 (37%) and 70 (40%) respectively while site 1


80
60
40
20
0
% of An. gambiae
Site 1
Site 2
Site 3
Human
Bovine
Mixed meal
Human
Blood meals

80
60
40
20
0
% of An. funestus
Bovine
Mixed meal

Figure 17. Identification of blood-meals of *An. gambiae s.l.* and *An. funestus* from three study sites.

Figure 18. Identification of blood-meals of *An. gambiae s.l.* and *An. funestus* from three seasons of the year.


Short rains
Dry seasons
Long rains
% of An. gambiae
% of An. funestus
0
20
40
60
80
Human
Bovine
Mixed meal
Human
Bovine
Mixed meal
Blood meals

had 44 (24%) human bloodmeals. However *An. funestus* had 61 (35%), 43 (24%) and 54 (32%) for sites 1, 2 and 3 respectively. A statistical analysis was conducted to compare the host preference of *An. gambiae s.l.* and *An. funestus* mosquitoes in the three study sites by goodness of fit test indicated a great significant difference X^2 = 6.4, d. f.= 2, p < Ø.Ø5. This difference was attributed by the different treatments given to the three sites. However *An. funestus* being more anthropophagic tends to feed on humans still despite the repellency unlike *An. gambiae s. l.* As shown in figure 17 *An. funestus* sparingly feed on bovine in site 2 & 3. *Anopheles gambiae s.l.* site 1 had 32 (2Ø%) of the bovid bloodmeals, while site 2 & 3 had 42 (26%) and 6Ø (35%) respectively. According to the Chi square test conducted there was also a significant difference between bovine blood-meals in the three sites X^2 = 6, d. f.= 2, p < Ø.Ø5.

For mixed blood-meals in site 1, *An. gambiae s.l.* had 57% while site 2 & 3 had 55% and 1Ø% respectively. There was also significant difference mixed blood-meals in *An. gambiae s.l.* in the three sites X^2 = 11.6, d. f. = 2, p < Ø.Ø5. *Anopheles funestus* had in site 1 72%, while site 2 & 3 had 15% each.

Seasons were found to have an effect on the feeding pattern of the vectors. Of the 184 human bloodmeals identified in both *An. gambiae s.l.* and *An funestus* 51.8% were collected during the long rains,

47.3% during the short rains and only 0.9% was collected during the dry season. Blood-meals positive for bovids, 87% were collected during the long rains, 13% during short rains and none during the dry season. However for mixed bloodmeals 76% was collected during the long rains, while the short rains and dry season had 12% each.

Of the 184 mosquitoes detected positive for human blood meal 25 (14%) were positive for *P. falciparum* circumsporozoite antigen. Whereas of the 46 identified as bovid blood meal only 1 (2%) was positive for *P. falciparum* circumsporozoite antigen.

3.6 Infection rates - Parasitaemia

A total of 95 children aged 0-10 were followed for the whole study and their blood smears were made and parasite counted out of every 300 white blood cells. Plate 10 (see page 115) shows a photomicrograph of smear made from a child infected with *P. falciparum*. The first smears taken during baseline data, showed that 87 (92%) of the children had parasitaemia ranging between 1 -2560 parasites per 300 white blood cells. It was observed that high parasitaemia was recorded mainly in children 0 - 3 years mainly as compared to children between 5 - 10 years. Three different *Plasmodium* species were identified, *Plasmodium falciparum* was the most abundant which was found in 59 (64%) of the children, while *P. ovale* had 8 (6%) of the children, 14 (15%) of

the children had multiple infection of *P. falciparum* and *P. malariae*, 3 (3%) had *P. falciparum* and *P. ovale* while only 1 (1%) had an infection of all the three i.e. *P. falciparum*, *P. malariae* and *P. ovale*.

During the study period of 10 months (August-May) a total of 16 visits once every two weeks were made to these children and blood smears were taken in every visit (Appendix IIIA, IIIB,IIIC for Tables 11, 12 and 13). Those found infected with *Plasmodium* parasites were treated with the drug namely pyrimethamine/2-sulphanilamido-2-methoxy-pyraxine (Metakelvin, see Appendix IID for composition and dosage). One child however died due to malnutritional complications coupled with high parasitaemia as ascertained by clinician from the research station.

As shown in Figure 19a & 19b there was a general decrease of the percentage of infection from an average of 90% to 50% in site 3 (the control) i.e. the area which was without intervention but had chemotherapy. There was also a drop in the percentage of infection in site 2 from an average of 85% to 45% where both chemotherapy and intervention were practised. In site 1 where intervention with permethrin treated bed nets and chemotherapy were applied there was a drop of *P. falciparum* percentage prevalence from 95% to an average of 20% and this was maintained throughout the study except in the first month after the treatment i.e. September when the parasitaemia levels were too low 9%.

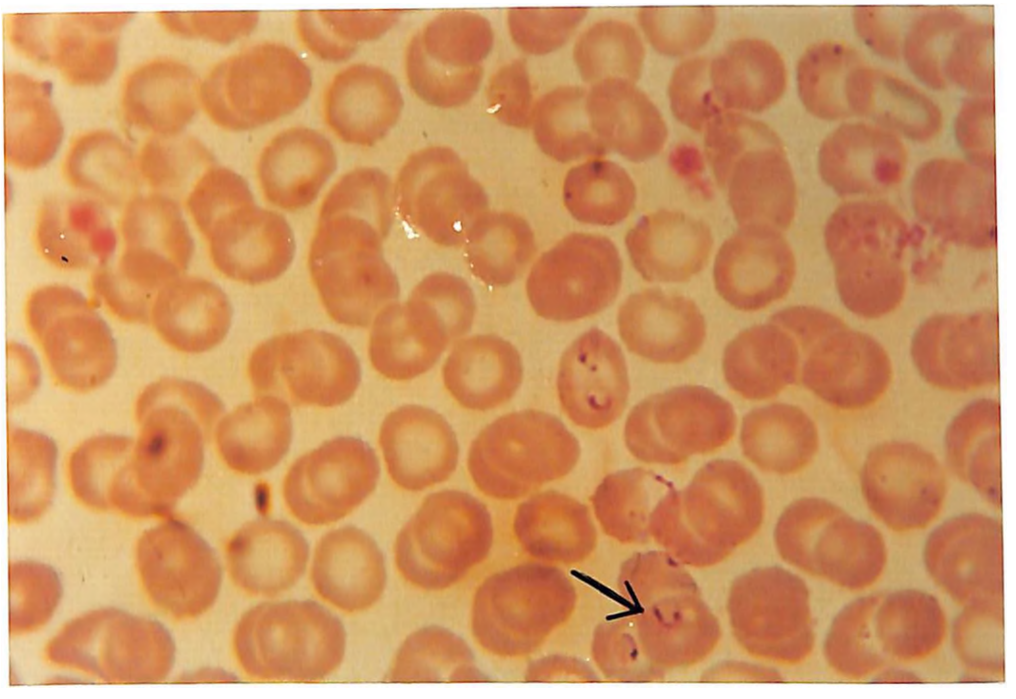


Plate 9. A photomicrograph of blood smear of a child infected with *Plasmodium falciparum* parasites. Note the ring forms of the parasite inside the red blood cell (magnification x 1000).

There was one untreated case that was negative for *Plasmodium* parasites during the baseline data collection period. In general these results indicate that the percentage prevalence is higher in sites 2 and 3 which remain at an average of 40% and 55% respectively as compared to site 1 which had an average of 20%.

A statistical test was conducted to find if there existed a difference between the *P. falciparum* prevalence in the three study sites. Results shows a significance difference between the percentage prevalence in the 3 sites whereby $X^2 = 6.55$, with d. f. = 2 , at $p < 0.05$.

3.7 Bioassays

Bioassays were conducted once a month in 1 and 2. In site 1, one net treated with permethrin insecticide was used and in site 2, one net not treated was used to act as a control. Throughout this period the insecticide was found effective and able to knock down at least 70% of the exposed mosquitoes. This was in according to the recommendation by (WHO, 1989). By the end of 10 months the nets were therefore ready for re-impregnation.

Two points on the net were tested, the upper part of the net rarely touched or folded and the lower site that was always touched, folded and dirty. A statistical test was conducted to find if there existed

Figure 19a. Monthly parasitaemia levels of Plasmodium falciparum data collected from three study sites (represented in bars).

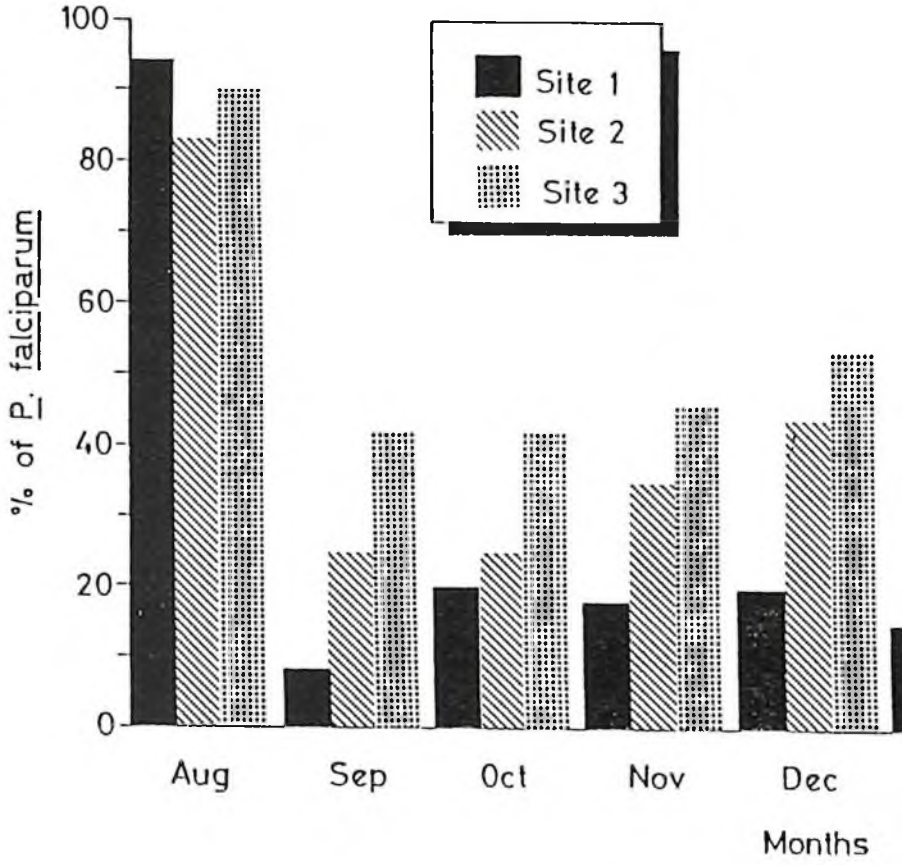
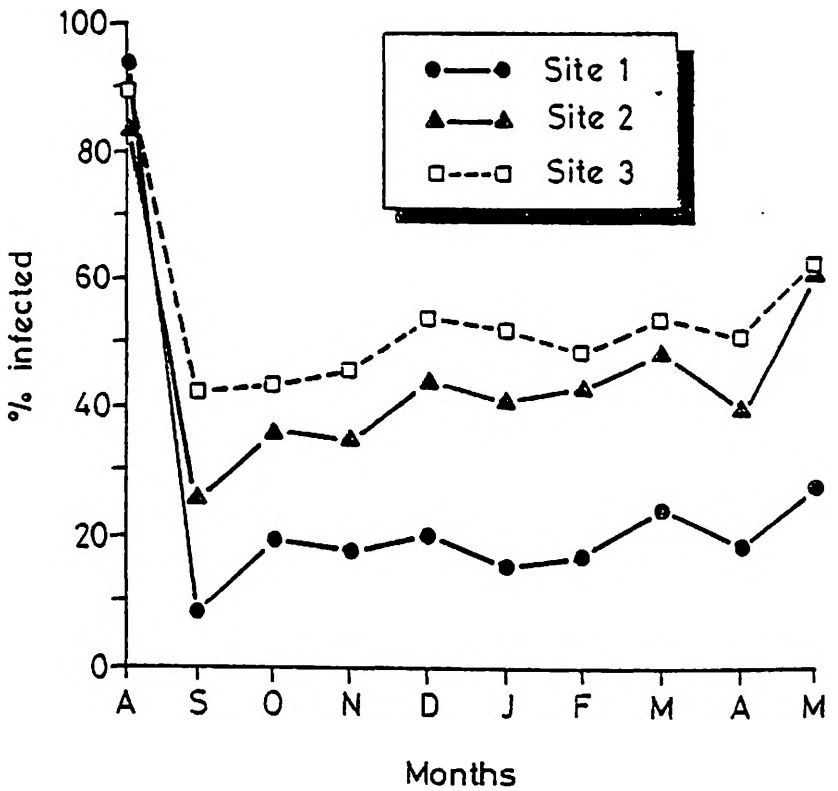

100
80
60
40
20
0
% of P. falciparum
Site 1
Site 2
Site 3
Aug
Sep
Oct
Nov
Dec
Months

Figure 19b. Monthly parasitaemia levels of Plasmodium falciparum data collected from three study sites (represented in lines).


100
80
60
40
20
0
% infected
Site 1
Site 2
Site 3
A
S
O
N
D
J
F
M
A
M
Months

a difference between the upper and lower points of the net in retaining of the insecticide. There was no significant difference between the two points the upper and the lower zones of the bed net, as was t = Ø.29, with d. f. = 18, at p > Ø.Ø5. This indicates that touching and dirt had minimal effect on the potency of the insecticide in the net. A t-test was also conducted to find if there was a difference between the effect of permethrin on the two mosquito batches used in the test. The difference between laboratory-reared and wild aspirated mosquitoes and there reaction to the insecticide, the results were as shown t = 2.6Ø, with the d. f. = 2, at p < Ø.Ø5. This indicates a difference between the two batches of mosquitoes.

Another t-test was conducted to find if there was a difference between the mortality rate of the mosquito batch exposed to permethrin treated bed net and that of untreated net. The t-test was highly significant between two mosquitoes batches exposed whereby t = 2Ø.56, with d. f. = 1, at p < Ø.Ø5.

During the bioassay experiment it was observed that the wild aspirated mosquitoes were more irritated by insecticide as compared to the laboratory bred type. The wild type could not rest on the nets but kept flying till they were knocked down. The laboratory bred mosquitoes on the other hand were at ease with the treated nets and were less irritable but were knocked down fast even before the exposure time was over 1Ø minutes as recommended by WHO (1989).

CHAPTER FOUR

4.1 Discussion

4.1.1 Species composition

Only 6 species of anopheline were present in the study area Table 1. The most abundant species in all collections made by different techniques was *An. gambiae s.l.* There were two members of the *An. gambiae s.l.* i.e. species A is now referred to as *An. gambiae sensu stricto* as described by Giles (1902) from a material collected in Gambia, West Africa and species B which was named *An. arabiensis* by Patton in (1905). This finding agreed with the prognosis of White (1974a).

Thirty four (34) chromosome preparations of *An. gambiae* complex were made of which (n = 25) were identified as those of *An. gambiae sensu stricto* and only one was *An. arabiensis* while eight (8) got spoilt. *An. funestus* was next in abundance to *An. gambiae sensu lato* . During the dry season *An. arabiensis* predominated over *An. gambiae sensu stricto* whilst in the rainy seasons April to July 1992 *An. gambiae sensu stricto* was the only complex species found with *An. funestus.* The proportions of other anopheline species found in the study area suggest that *An. pretoriensis, An. pharoensis* and *An. maculipennis* are of minor or no significance in malaria transmission in the area.

Therefore from the study it can be suggested that only *An. gambiae sensu lato* complex and *An. funestus*

can be considered of great medical importance in malaria transmission in the area. Figure 12a show that during the short rains the population of *An. funestus* is higher as compared to *An. gambiae s.l.* which is contrary in the long rains whereby the population of both species is high though *An. gambiae s.l.* is slightly higher. The reason for this is the different breeding habits of the two vectors. *An. funestus* breeds in shallow dirty waters while *An. gambiae s.l.* breeds in clean waters and in slow moving streams which are available during the long rains.

4.1.2 House resting densities

4.1.2.1 Pyrethrum spray catch (PSC)

Results from pyrethrum spray catch on house resting densities indicated a marked decrease in the house resting densities of *An. gambiae* and *An. funestus* in site 1 where permethrin treated bed nets were being used as compared to site 2 and 3 where untreated bed nets and no nets were applied respectively. The population of *An. funestus* resting indoors decreased drastically to an average of 5% for the 8 subsequent months i.e. September to April in site 1. There numbers started increasing in May when the long rains commenced and it was at this time that bioassay tests indicated a reduction in the insecticide potency.

Permethrin treated bed nets had a dramatic effect on the mosquito populations by either repelling or killing the vectors especially the exclusively anthropophagic and endophilic as *An. funestus*, and also reduced their blood feeding success. The nets gave protection during the short rains and the dry seasons as compared to the long rains. A possible explanation is that the nets were less effective during the long rains because the number of infective mosquitoes were at their peak. This agrees with observations made by Snow *et.al.* (1988) in the Gambia and also in Burkina Faso by Carnavale *et.al.* (1988) whereby insecticide treated bednets were not found as effective in reducing malaria morbidity. Graves *et.al.* (1987) in Papua, New Guinea where pressure of malaria transmission is higher than in the Gambia made the same conclusion. Therefore insecticide treated bed nets may prove to be most useful as malaria control measure in areas with low or moderate levels of transmission. This observation was made by Sexton *et.al.* (1990) during the study on permethrin treated bednets and curtains in Western Kenya.

Results showed that large numbers of adult mosquitoes rest indoors during early hours of the day. The discrepancy between the indoor-resting density and the man-biting densities implied that not all female mosquitoes found resting indoors in the morning participated in the man-biting activity the previous night. Apart from the females leaving human dwellings

for oviposition, others were probably females in late stages of egg development proceeding to rest outdoor prior to oviposition. This was the reason as to why there were usually gravids and pregravids caught in the houses during the pyrethrum spray catch. Others too search for an alternative meal outside then come to rest indoors i.e. they are zoophagic but endophillic. This results confirm to observations made elsewhere by Krafsur (1977) and Okedi (1988) in Mwea-Tebere.

Field trials by Lines *et.al.* (1987) and have shown that people sleeping under insecticide-treated bed nets in villages did not get bitten as frequently indoors by malaria vectors as could be the case if only untreated nets were used in the community. Similarly the persons sleeping without nets in the huts with treated nets get the benefit of no or few bites. It has also been shown that the presence of insecticide repels or deterrs vectors from entering the human habitations (Darriet *et.al.*, 1984) achieving the overall reduction of human-vector contact. Therefore the nets do protect the users and to a low degree the non-users sleeping in these huts.

This shows that the usage of treated nets reduces malaria transmission within a village due to a fall in the vector numbers and a decrease in the proportion of vectors with sporozoites as confirmed by Sexton *et. al.* (1990). This finding is supported from entomological studies carried out in The Gambian village where most

people sleep under permethrin treated bed nets (Lindsay *et. al.* unpublished data). Reports indicate that use of permethrin treated bed nets, reduced the vector infection rates due to the reduced chances of sucking infective blood. This therefore reduces the vectorial capacity of the vector and hence the malaria transmission.

4.1.2.2 Night catch (Human bait catch)

Night catch results conforms with Haddow's (1942) and Hocking (1948) findings that there was low population collection of *An. gambiae s.l.* females between 0600-1100hrs and this was due to low numbers of mosquitoes entering into houses. However the peak entry falls between 1200-0500hrs in the morning when the vectors entered to look for a bloodmeal and this was the period that the greatest catches were made. The night biting collections were mainly newly hatched unfertilised and unfed females which had mites on their scales. A peak observed at dawn was however made up of already fed females and analysis of most of these bloodmeals were bovid and not human. These bloodmeal results which were especially from site 1 contradicted with White, (1974a) and with those found by Gillies (1954a) whereby they stated that the above species is almost exclusively endophagic. Results above shows that an exclusively endophillic vector can be exophagic once it has missed a blood meal indoors therefore not

all endophillic vectors are endophagic. This indicated that the above species was both indoor and outdoor-feeder once they missed a meal indoors they fed outdoor and entered into houses in the morning to rest in the day and digest the meal.

Fewer *An. funestus* were collected on human baits in site 1 especially as compared to sites 2 and 3. Large numbers of unfed females were found resting inside human shelters as shown by the pyrethrum spray catch method (see Table 1). This conforms to the anthropophilic and endophilic behaviour of the species well established in literature (Gillies 1954 b an c) and also the findings in Ethiopia by Krafsur, (1977). As was expected the number of *An. funestus* were very low in site 1 after the introduction of the treated bed nets as compared to the previous season before the nets were introduced, and as compared to *An. gambiae s.l.*. The anthropophillic and endophillic feeding behaviour of *An. funestus* which is well documented by Gillies (1954b & c) is one of the reasons for this species being wiped out in malaria control programme in some parts of the world a good example is Madagascar (Gillies 1954 a & b; White 1974a).

An. gambiae s.l. population reduced in site 1 too but not to very low numbers the reason being, though the species is endophilic it is both anthropophagic and zoophagic. Therefore during the introduction of treated bed nets the vectors were deterred from feeding on humans but were able to feed outside on other

vertebrates and only a few entered the huts to rest and digest the bloodmeal while majority remained outside. This was contrary to site 2 and 3 where there was no effect on the mosquito densities.

4.1.3 Sporozoite Rates

Both *An. gambiae s.l.* and *An. funestus* were harbouring *Plasmodium falciparum* malaria parasites as indicated by the ELISA test results. The sporozoite rates for *An. gambiae s.l.* was 4.Ø2% an average for the three sites however their specific rates were 2.5Ø%, 5.ØØ% and 4.35% for the three site 1, 2 and 3 respectively based on ELISA. The sporozoite rates based on dissection overall was 1.62% on all the three sites however the specific rates were 2.8Ø%, Ø.94% and 1.68% for sites 1, 2 and 3 respectively. The average sporozoite rates for *An. funestus* based on ELISA in the 3 sites was 4.95%, while for respective sites was 2.94%, 8.2Ø% and 3.45%. Based on dissection average was 1.64% and were Ø.9Ø%, 2.ØØ% and 2.43% in sites 1, 2 and 3 respectively. Site 1 had the lowest overall sporozoite rates of 1.64% in both species based on both methods. Site 2 had the highest 5.1Ø% and site 3 had 2.94%. The reason for such an occurrence can be attributed to the treated bed nets in site 1 which reduced the man-vector contact by repelling or killing the vectors thereby reducing the transmission rate. In site 2 and 3 which had the high positive mosquitoes for sporozoites

indicates that despite the intervention in site 2 there was little or no reduction of man-vector contact.

Nets alone had no effect on mosquito mortality or repellency though they interrupted their feeding patterns which sometimes resulted into incomplete bloodmeals from the bait sleeping under untreated bed net. Therefore untreated bed nets do protect the user to a very low degree. These nets just like the control maintains high vectorial capacity and infection rate but are a disadvantage to the vectors in interrupting the feeding. It can therefore be concluded that treated bed nets have an advantage over untreated bed nets in the mosquito population control and reduction of malaria episodes.

4.1.4 Bloodmeals

Analysis of bloodmeals from captured females showed that the majority of *An. gambiae senso lato* and *An. funestus* had fed on humans than bovids in sites without treated bed nets. However in the site with permethrin treated bed nets the vectors fed on bovid and other blood meals than humans. This was an indication that the females searched for a meal outside the dwelling places due to repellency by the insecticide; then flew into the houses in the early hours of the morning to rest and digest the bloodmeal hence the high numbers of females reported in site 1 at 0600hrs. A comparison of feeding patterns of *An. gambiae sensu lato* and *An. funestus* on seasonal basis showed that more

females of both species were feeding on humans during the short rains and long rains than during the dry season. There was statistically a great significant difference between bovid meals of *An. gambiae s.l.* collected during the long and short rains in the three sites. Mosquitoes fed on bovids more during long rains as compared to the short rains the most probable reason being that there was a large population of mosquitoes which led to competition for a meal.

4.1.5 Infection rates

The study showed that children who slept under permethrin impregnated bed nets experienced lower parasitaemia levels and significantly fewer clinical episodes of malaria than those who slept under untreated mosquito nets or those who had no nets at all. There was statistically a significant difference in the parasitaemia levels in children of ages between Ø-1Ø years which was collected during the sixteen visits in the three study site whereby $F = 6.72$, $df = (2, 32)$, $p < Ø.Ø5$. This observation agreed with that of Snow *et.al.* (1987) during a study in the Gambia. The prevalence study showed that the use of bed nets was associated with a reduced incidence of splenomegaly and malaria. A similar observation was made in a study undertaken in Mali by Raque *et.al.* (1984), where there was a reduction in parasitaemia levels and spleen rates among children who slept under nets impregnated with

permethrin.

However despite the encouraging results of a drop in the parasitaemia levels from 90% to 20% in site 1 there was a general increase in parasitaemia levels in all the 3 sites during the long rains (April-July). During the long rains the average proportions of parasitaemic children were as follows; site 1, 9/32; site 2, 18/32 and site 3, 18/29 as compared to the short rains which was 5/32, 12/32 and 14/29 respectively. The increased parasitaemia could be attributed to the increased breeding sites which led to an increase of house resting densities of the vectors even in the huts with permethrin treated bed nets, and subsequently increased sporozoite rates as compared to the dry and short rains season.

The other reason why there was presence of parasites in site 1 children is they were getting some mosquito bites before sleeping when the vectors were searching for a bloodmeal outside the huts. There was evidence to this since there was a cultural practice of staying up late outside houses warming around fire place till about 11 PM. Children in the area had a habit of visiting their relatives far from home this too could have contributed to the positive cases in this site since they were exposed to vector bites by not using the nets.

Another possible reason for presence of parasites in site 1 children was bites inside the huts, this could

be possible too because according to bioassay reports the potency of the permethrin had greatly reduced by this season as it was already 9 months since the previous treatment. However the reduction in the time the mosquito proboscis is inserted might have reduced the number of the sporozoites injected into the host, thus increasing the length of time required to reach a high parasitaemia and allowing the host to mount an immune response. It's possible that both groups received a similar number of infective bites children sleeping under treated bed nets recieved a lower sporozoite innocula and hence were less prone to malaria episodes. The children sleeping under untreated bed nets and the control received larger sporozoite innocula and hence were prone to malaria episodes. This was the reason as to why children sleeping under treated nets were also experiencing significantly fewer episodes of excessive sweating and headaches than those sleeping under untreated bed nets or the control.

4.2 CONCLUSION.

The present study revealed information that could significantly contribute in planning of control measures for the malaria vectors *An. gambiae s.l.* and *An. funestus* populations and the malaria incidence in Kanyawegi. The high degree of anthropagy exhibited by the later vector could suggest that contact with it could be avoided through the use of impregnated bed nets which will repel them from the hosts.

The situation as regards the natural outdoor resting sites and larvae breeding sites in Kanyawegi facilitates implementation of control measure directed to the adults than larval populations. This is because the area is along the lake shores and breeding occurs throughout the year.

A permethrin treated bed net had dramatic effect on the mosquito populations by either deterring them from successful feeding or by killing them. Despite the encouraging results the nets were very effective during the drier seasons of the year when the population of the vector was low or moderate. During the long rains however even the protected population is prone to mosquito bites.

A bed net's effectiveness as a physical barrier to mosquitoes is undoubtedly reduced as it becomes worn, torn and tattered. Impregnation with permethrin insecticide has the potential of returning the

effectiveness of such a net to, or above that of an intact net. Results obtained in subsequent months after the introduction of treated bednets into study huts most stages collected were mainly the unfed females of both species *An. gambiae s.l.* and *An funestus* (see Table 4a and Figure 3a) and afew blood feds. The unfed females were either newly emerged females seeking for a blood meal in the habitations unsuccessfully or those that had had the first meal and were seeking for a second. The blood fed that were collected from the huts in this site were found by the meal analysis by direct ELISA that majority had fed on bovine meal and had only entered the huts to rest and digest the meal.

Children of ages ranging between Ø-1Ø years old, who slept under permethrin treated bed nets had a pronounced reduction in the parasitaemia levels, malaria episodes, fewer episodes of excessive sweating and headaches. This therefore concludes that the use of permethrin treated bed nets is a principal idea for the reduction of malaria incidence in Kanyawegi and as a control measure in malaria endemic zones.

The use of permethrin treated bed nets is an ideal measure for reducing the human-vector contact through community participation. They are easy to use; durable (if handled properly); reasonably priced (in most cases) and do not involve strenuous labour or excessive time to hang and maintain.

The community compliance to use of the bednets was not a problem since the permethrin treated nets were not

only for the control of mosquitoes but also protected the users against bites from ticks, cockroaches, centipedes, bedbug and fleas hence allowed uninterrupted sleep.

The potency of the permethrin was found by the bioassay tests to last effectively for 9 months before re-impregnation of the nets since it was able to kill 70% of the test vectors according to WHO standards.

Results obtained from the study are encouraging and calls for an intervention programme to be considered for the area. The good results at the individual level encourage the use of permethrin treated nets at the whole village as a protective measure against malaria vectors and parasites.

4. 3 RECOMMENDATIONS.

1. Permethrin treated bed nets were found effective as vector and malaria control measure in the malaria endemic zone. This was because of the repellency and killing effect of the insecticide which reduced the house resting densities of the vectors, their vectorial capacity and infection rate. Permethrin is photosensitive therefore after the impregnation by the dipping method the bed nets should be left to dry under shed or indoor preferably on the mattresses since the insecticide also kills other vectors as bedbugs.
2. Net re-impregnation can be done after 9 months of use and that is only if the nets are not washed in between this period. If washing is done in between the 9 months then re- impregnation will be done within less 9 months that is 3 or 6 months depending on the frequency of the washing since it has been found that washing reduces the potency of the insecticide.
3. To avoid the frequent washing dull colours of the bed nets can be used as opposed to white since the dull colours do not show dirt especially in huts where there is smoke.
4. For the nets to remain effective the users should avoid vector bites outside the huts by withdrawing the cultural practise of staying late outside

around the fire warming and chatting and sleep earlier before the vectors start biting that is from about 1100hrs. in the night.

5. Children of this age should be therefore given the first priority whenever there is a treated net available. Adults may get some protection by sleeping inside those huts with treated bednets.

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APPENDICES

APPENDIX I: REAGENT PREPARATION

IA. Phosphatase buffered saline (pbs plain).

The reagent is used for the dilution of capture monoclonal antibody (mab) before the plates are coated and when preparing the blocking buffer and pbs-tween.

1 litre distilled water, whole content of Dulbecco's pbs bottle (9.7gm.Ø). Mix on magnetic stirrer for 1Ø min. to dissolve.

IB. pbs-tween 2Ø. (Wash solution for plates)

1 litre pbs plain, 5ØØ ul tween-2Ø. Mix with magnetic stirrer.

IC. Blocking buffer. (used for blocking plates)

1 litre pbs plain, 1Øgm. Bovine albumen (BSA), 5 gm. casein, Ø.1 gm. thermosol, Ø.Ø1 gm. phenol red. Mix with magnetic stirrer.

ID. 2A1Ø Monoclonol antibody dilution (capture Mab).

6 mls. pbs plain, 24 ul capture Mab., Mix and dispense 5Ø ul into each well of PVC plate.

IE. Peroxidase subtrate solution (enzyme).

Equal parts (1:1) of solution A and solution B.

Solution A: (ABTS) (2,2 azino-di (-ethyl-benzthiazoline)).

Solution B: Hydrogen peroxidase.

APPENDIX II

IIA. Giemsa stain.

3.8gms. Giemsa powder, 250 mls. methanol and 250 mls. glycerol. Mix and filter with Whatman's paper No. 1. Dilute 5 mls. concentrated Giemsa with 95 mls. distilled water.

IIB. Concentrated Carnoy's fixative:

2 parts absolute ethanol, 1 part glacial acetic acid.

IIC. Aceto-lactic orcein concentrated stain:

2% by weight of concentrated synthetic orcein powder, in 1 part 85% lactic acid and 1 part glacial acetic acid. 50mls acetic acidis added onto 2 gms. synthetic powder, then 50mls. 85% lactic acid.

IID. Metakelvin tablets:

The drug is for therapy and prophylaxis of malaria caused by *P. falciparum, P. malariae, P. vivax, and P. ovale.*

Composition: 2-sulphanilamido-2-methoxy-pyrazine 500 gms., pyrimethamine 25 gms.

APPENDIX IIIA

Table 10. Infection rates of *P. falciparum* parasite counts in children (N=33) ofages between 0-10 years in site 1. Counts made per every 300 white blood cells.

M = Males (n=14)

F = Females (n=19)

V = Number of visits (n=16)

- = Negative (No parasite in the smear)

. = No count made (child was absent)

PARASITE COUNTS / VISIT

AGE	SEX	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16
0.5	F	11	-	-	-	174	-	-	211	-	50	--	-	-	-	310	258
0.5	M	2560	-	786	-	3730	-	9	-	44	-	-	230	-	-	92	-
0.5	M	365	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.
0.5	F	260	-	-	-	-	-	-	64	-	-	-	-	-	-	-	390
1.5	F	35	-	-	-	-	12	-	-	-	-	4	-	-	8	-	-
2.0	F	91	-	-	-	288	-	-	-	-	9	-	156	-	21	-	-
2.0	M	1250	-	-	344	-	-	846	-	125	-	-	365	-	-	-	-
2.0	F	133	-	-	21	-	-	107	-	76	-	-	1620	-	-	115	-
3.0	M	54	-	-	47	-	-	-	-	19	-	41	-	-	15	-	35
4.0	M	24	-	-	-	-	-	-	33	-	-	20	-	-	-	31	-
4.0	F	36	-	-	-	-	-	-	-	-	-	-	-	-	-	-	74
4.0	F	16	-	-	-	-	10	-	-	-	-	-	-	-	-	-	31
5.0	M	104	-	30	-	-	174	-	22	-	29	-	6	-	4	-	-
5.0	F	51	-	-	90	-	-	-	14	-	-	14	-	-	-	-	-
6.0	F	1	-	-	-	-	-	226	-	-	-	121	-	-	-	-	44
6.0	M	47	-	-	-	-	28	-	-	-	-	-	-	-	-	27	-
6.0	F	15	-	-	45	-	-	-	-	-	-	-	-	-	-	-	-
6.0	F	107	-	-	-	-	-	-	-	-	-	-	-	-	-	-	330
6.0	M	--	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-
7.0	F	39	-	-	-	-	-	-	-	-	-	-	-	121	-	41	-
7.0	F	22	-	-	-	27	-	-	-	-	-	-	-	112	-	-	-
8.0	M	61	-	-	-	134	-	-	-	10	-	-	-	10	-	-	99
8.0	M	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9.0	F	1	-	-	-	435	-	-	-	-	15	-	122	-	-	-	-
9.0	M	8	-	-	-	-	-	-	-	-	-	6	-	-	-	-	-
9.0	F	20	-	11	-	-	-	-	-	192	-	-	28	-	9	-	-
9.0	F	182	-	-	-	-	15	-	-	-	-	-	-	-	14	-	-
10.0	M	25	-	-	-	-	14	-	-	8	-	-	-	-	8	-	27
10.0	M	23	-	14	-	-	-	-	18	-	21	-	-	-	121	-	-
10.0	M	61	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10.0	F	58	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10.0	F	113	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TOTAL POSITIVE		31	1	4	6	7	6	5	6	7	5	6	7	4	7	6	9
TOTAL NEGATIVE		32	32	28	26	25	26	27	26	25	27	26	25	28	25	26	23

APPENDIX IIIB

Table 11. Infection rates of *P. falciparum* parasite counts in children (N=33) ofages between Ø-1Ø years in site 2. Counts made per every 3ØØ white blood cells.

M = Males (n=2Ø)

F = Females (n=13)

V = Number of visits (n=16)

- = Negative (No parasite in the smear)

. = No count made (child was absent)

PARASITE COUNTS / VISIT

AGE	SEX	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16
.5	F	192	-	-	-	-	-	1	-	-	18	-	77	-	1	-	-
1.5	F	150	124	140	-	1	-	-	1050	-	18	-	196	-	21	-	21
1.5	M	5	-	9	-	-	-	160	-	1	1	-	2	-	-	-	10
1.5	M	500	-	465	-	-	-	84	-	30	-	-	18	-	31	-	-
1.5	F	321	-	5	-	-	159	-	105	570	-	-	-	51	-	4	40
1.5	F	22	-	45	-	-	-	-	-	-	210	1	-	1	-	36	-
2.0	M	1055	-	1	2	-	-	121	-	76	-	44	-	-	21	51	310
2.0	M	89	-	-	-	161	-	-	-	-	1	-	184	1	-	114	56
3.0	M	725	-	-	-	279	3	-	43	1	59	-	69	-	1	-	-
3.0	F	20	-	-	-	-	5	-	45	-	12	-	4	14	-	-	11
4.0	M	103	-	4	-	-	-	33	-	127	-	89	-	21	-	-	1
4.0	F	312	-	2	-	-	141	1	-	5	-	-	36	-	15	-	18
4.0	M	-	-	-	-	-	1	-	151	-	-	121	-	4	-	-	1
5.0	F	35	-	5	-	423	-	28	-	-	-	41	-	-	5	-	77
5.0	F	20	-	2	41	-	-	137	-	-	39	-	1	-	17	2	10
6.0	F	19	-	-	-	10	-	-	116	-	-	-	-	-	-	-	2
6.0	F	9	-	-	145	1	9	-	-	11	-	-	13	-	192	-	-
6.0	M	17	-	-	141	-	21	-	-	149	-	132	-	111	-	21	-
7.0	M	161	-	-	16	-	-	14	-	-	8	17	-	-	25	-	1
7.0	M	240	-	3	-	22	-	17	-	-	-	94	2	-	-	-	7
8.0	M	1	-	-	3	-	121	-	-	-	1	-	1	-	8	-	1
9.0	M	52	-	-	1	-	-	1	-	-	-	128	-	15	-	-	19
9.0	M	81	-	-	-	19	-	43	-	22	-	-	2	-	1	8	-
9.0	M	1	-	1	-	1	151	-	52	10	-	-	-	-	141	-	12
9.0	M	1	-	-	14	-	141	-	5	-	197	-	-	8	-	465	46
10.0	F	48	-	-	-	2	-	-	-	-	1	21	-	3	-	51	-
10.0	F	-	1	-	-	-	-	-	1	-	4	-	-	310	-	4	-
10.0	M	50	-	2	-	-	-	1	111	1	-	-	3	-	8	42	-
10.0	M	48	-	-	16	18	-	-	4	1	142	-	2	-	-	35	2
10.0	M	55	-	-	-	4	-	-	13	1	-	5	-	-	19	8	13
10.0	F	-	425	-	-	13	-	-	2	-	1	-	41	-	19	-	-
10.0	F	23	-	1	-	2	-	-	1	1	-	-	1	17	-	-	13
10.0	M	408	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TOTAL POSITIVE		30	3	13	11	14	10	13	14	10	14	12	15	15	16	13	19
TOTAL NEGATIVE		3	30	19	21	18	22	19	18	22	18	20	17	17	15	19	13

APPENDIX IIIC

Table 12. Infection rates of *P. falciparum* parasite counts in children (N=29) ofages between Ø-1Ø years in site 1. Counts made per every 3ØØ white blood cells.

M = Males (n=15)

F = Females (n=14)

V = Number of visits (n=16)

- = Negative (No parasite in the smear)

. = No count made (child was absent)

PARASITE COUNTS / VISIT

AGE	SEX	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16
0.5	M	48	-	-	19	5650	11	150	-	14	4	540	8	612	-	25	-
1.5	M	163	2	107	-	246	-	-	342	1	-	-	141	600	4	-	93
1.5	M	326	-	-	41	-	-	-	10	-	18	-	111	-	-	10	-
1.5	M	315	13	1	-	639	3	-	138	-	-	23	-	327	-	1410	121
1.5	F	4	-	73	-	120	-	41	465	3	-	59	-	42	-	15	-
2.0	F	72	-	1	-	342	-	41	780	4	11	310	19	-	105	11	217
3.0	M	56	-	-	-	102	-	-	149	-	19	19	-	1	-	49	-
3.0	F	32	1	-	-	66	-	2	46	-	19	-	-	48	-	-	36
3.0	F	2	2	-	-	1	-	72	73	4	-	788	11	265	25	42	215
4.0	M	164	-	10	2	-	-	-	43	28	-	35	-	-	119	44	182
4.0	M	1	-	2	-	-	19	-	144	-	42	-	219	3	-	-	2
4.0	M	198	2	-	-	-	91	-	34	-	-	21	-	39	-	2	38
5.0	F	-	-	32	182	-	-	1	-	4	14	-	14	-	9	-	14
5.0	F	16	-	-	-	2	-	-	-	1	-	-	-	1	-	605	111
5.0	M	61	-	1	4	-	14	-	50	-	121	-	-	1	-	21	159
6.0	M	18	-	4	-	147	-	4	-	21	-	26	1	-	14	-	-
6.0	F	14	-	-	-	2	-	-	1	-	19	-	12	-	21	-	-
6.0	F	25	-	-	3	74	-	-	2	-	-	34	11	-	31	-	3
7.0	F	17	-	74	-	-	113	-	-	17	4	-	-	63	-	27	-
8.0	F	-	-	4	-	-	191	-	62	-	12	-	-	1	-	-	68
9.0	M	10	1	-	4	-	-	55	-	40	-	4	-	180	41	-	21
10.0	M	2	12	-	1	-	-	19	-	-	-	8	-	-	4	-	-
10.0	F	-	-	44	-	1	41	-	-	19	3	4	-	-	19	-	45
10.0	M	1	-	-	1011	-	-	45	11	-	44	-	51	-	-	141	1
10.0	M	1	-	40	-	-	1	-	-	-	91	-	14	-	8	-	-
10.0	F	44	1	-	98	-	19	-	26	1	-	4	-	-	26	-	-
10.0	M	14	-	-	1	-	3	-	-	19	-	-	117	-	19	2	-
10.0	F	373	128	38	-	480	-	-	219	4	-	1	-	35	-	7	118
10.0	F	57	1	-	-	48	-	3	35	-	-	13	-	-	4	-	54
TOTAL POSITIVE		26	10	13	10	14	11	11	19	15	12	16	13	14	16	14	18
TOTAL NEGATIVE		3	19	16	19	15	18	18	10	14	17	13	16	15	13	15	11

APPENDIX IV. TREATED BED-NETS STUDY QUESTIONEER.

HOMESTEAD NO._____ NUMBER OF HOUSES___ DATE_____

NAME OF HH HEAD_____

NAMES of household	SEX 1=Male 2=Female	AGE 1=Years 2=Months	EDUC.	SLEEP ON 1=Bed 2=Mat	NETS 1=Yes 2=No
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

HOUSE TYPE:
No. of houses with
Iron roof _____
Grass roof _____
Mud walls _____
Stone wall _____

EDUCATION:
Ø = None
1 = Some primary
2 = completed prim
3 = Secondary
4 = University

APPENDIX V. (PSC) DATA COLLECTION FORMAT.

SITE: ..... MONTH: ..... TYPE OF CATCH: PSC .....

VILLAGE	DATE	HOUSE NO.	A. GAMBIAE							A. FUNESTUS							OTHER ANOPHELINES							CULICINES				
			O	E	B	HG	G	T	P/D	O	E	B	HG	G	T	D/P	O	E	B	HG	G	T	P/D	O	E	B	HG	G
T																												

O-Male E-Empty B-Blood Fed HG-Half Gravid G-Gravid T-Species Total $\frac{P}{D}$ - Number positive over number Dissected.

APPENDIX VI. NIGHT CATCH DATA COLLECTION FORMAT.

LOCALITY: ____________________

DATE: ______________ METHOD: ______________

HOUSE NO.	TIME	SPECIES							
		An. gambiae	An. funestus	An. ziemanni	Ma. africana	Ma. uniformis	Cx. fatigans	Misc. Aedes sp.	Misc. Culex sp.
	1900-2100 hrs								
	2100-2400 hrs								
	2400-0300 hrs								
	0300-0600 hrs								
	TOTAL								
	1900-2100 hrs								
	2100-2400 hrs								
	2400-0300 hrs								
	0300-0600 hrs								
	TOTAL								
	1900-2100 hrs								
	2100-2400 hrs								
	2400-0300 hrs								
	0300-0600 hrs								
	TOTAL								
	1900-2100 hrs								
	2100-2400 hrs								
	2400-0300 hrs								
	0300-0600 hrs								
	TOTAL								

REMARKS: ______________________________

DATE: ______________ SIGNATURE: ______________
( )

APPENDIX VII. TABLES

Table 2. Monthly indoor resting densities of adult *An. gambiae* females collected by pyrethrum spray catch method in selected houses in three study sites. (Transformed data (arcsine) in Parentheses).

Month	Site 1	Site 2	Site 3	Totals
August	23 (16.3)	10 (4.1)	17 (4.9)	50
September	5 (7.5)	3 (2.2)	8 (3.4)	16
October	11 (11.2)	18 (5.4)	6 (2.9)	35
November	35 (13.0)	97 (12.9)	62 (9.4)	194
December	22 (15.9)	25 (6.5)	44 (7.9)	91
January	41 (22.0)	21 (5.9)	15 (14.8)	77
February	15 (13.0)	19 (5.6)	87 (11.2)	121
March	10 (10.6)	12 (4.6)	19 (5.2)	41
April	49 (24.2)	132 (15.0)	147 (14.6)	328
May	37 (20.8)	680 (35.9)	702 (33.6)	1419
June	31 (19.0)	821 (40.1)	1071 (43.1)	1926
July	33 (19.6)	1071 (15.7)	121 (13.3)	259
Totals	234	1013	1107	2463
Mean	16.08	12.80	13.70	
S.E	± 5.11	± 3.64	± 3.58	

S.E = Standard Error.

TABLE 3. Monthly house resting densities of adult *An. funestus* females collected by pyrethrum spray catch method.

	Site 1	Site 2	Site 3	Total
Month				
August	45 (42.5)	24 (24.4)	14 (15.5)	173
September	12 (24.5)	4 (9.8)	4 (8.2)	20
October	5 (13.0)	1 (4.8)	0 (0.0)	6
November	0 (0.0)	0 (0.0)	4 (8.2)	4
December	4 (11.6)	3 (8.4)	1 (4.1)	11
January	4 (11.6)	2 (6.8)	5 (9.2)	21
February	0 (0.0)	0 (0.0)	2 (5.8)	2
March	2 (8.2)	3 (8.4)	7 (10.9)	12
April	2 (8.2)	13 (17.7)	46 (29.0)	61
May	6 (14.3)	31 (28.0)	46 (29.0)	83
June	10 (18.5)	34 (29.4)	35 (25.0)	79
July	4 (11.6)	26 (25.4)	30 (23.0)	60
Total	99	141	196	449
Mean	13.65	13.59	13.59	
S.E	± 3.28	± 3.13	± 2.91	

Transformed data (arcsine) in Parentheses.

Site 1 = study area with permethrin treated bednets.

Site 2 = study area with non-treated bednets.

Site 3 = study area without treated or non-treated bednets.

TABLE 4a. Monthly indoor resting empty *An. gambiae s.l.* Giles collected by pyrethrum spray catch method (PSC).(Transformed data (arcsine) in Parentheses).

	Site 1	Site 2	Site 3	Total
Month				
August	Ø (Ø.Ø)	Ø (Ø.Ø)	2 (3.1)	2
September	2 (14.Ø)	1 (8.1)	Ø (Ø.Ø)	3
October	Ø (Ø.Ø)	Ø (Ø.Ø)	Ø (Ø.Ø)	Ø
November	3 (17.3)	3 (11.5)	2 (3.1)	8
December	2 (14.Ø)	6 (14.2)	1 (2.1)	9
January	2 (14.Ø)	Ø (Ø.Ø)	Ø (Ø.Ø)	2
February	Ø (Ø.Ø)	2 (3.1)	4 (4.3)	6
March	3 (17.3)	Ø (Ø.Ø)	5 (4.8)	8
April	6 (25.1)	23 (17.9)	1Ø (6.9)	49
May	9 (31.3)	228 (38.7)	274 (38.8)	511
June	6 (25.1)	311 (46.7)	385 (47.9)	7Ø2
July	1 (1Ø.Ø)	18 (9.9)	17 (8.9)	36
Totals	44	557	7ØØ	1336
Mean	4.Ø1	11.99	9.9	111.33
S.E	± 2.98	± 4.44	± 4.6Ø	± 68.Ø2

S.E = Standard Error.

TABLE 5a. Monthly indoor resting blood-fed *An. gambiae s.l.* Giles collected by pyrethrum spray catch method (PSC). (Transformed data (arcsine) in Parentheses).

	Site 1	Site 2	Site 3	Total
Month				
August	14 (18.4)	8 (5.5)	10 (5.7)	32
September	1 (14.2)	1 (2.0)	5 (3.8)	7
October	2 (8.2)	8 (5.5)	4 (3.6)	14
November	13 (17.3)	41 (12.5)	37 (11.1)	101
December	11 (15.8)	6 (4.6)	24 (8.9)	36
January	15 (18.5)	3 (3.4)	7 (4.7)	25
February	5 (9.9)	8 (5.5)	38 (11.2)	51
March	3 (8.3)	2 (2.8)	4 (3.6)	9
April	13 (17.3)	79 (17.6)	70 (15.3)	162
May	19 (21.1)	332 (38.3)	349 (36.0)	700
June	14 (17.9)	346 (39.9)	398 (38.9)	758
July	28 (20.3)	32 (11.1)	62 (14.4)	122
Total	148	866	1008	2017
Mean	15.60	12.33	13.23	168.1
S.E	± 1.30	± 3.80	± 3.47	± 77.1

S.E = Standard Error.

TABLE 6a. Monthly indoor resting half-gravid *An. gambiae s.l.* collected by pyrethrum spray catch (PSC). (Transformed data (arcsine) in Parentheses).

	Site 1	Site 2	Site 3	Total
Month				
August	5 (19.7)	2 (5.3)	4 (7.1)	11
September	1 (8.7)	Ø (Ø.Ø)	Ø (Ø.Ø)	1
October	4 (17.6)	4 (7.4)	Ø (Ø.Ø)	8
November	2 (12.3)	3 (6.5)	2 (5.1)	8
December	6 (21.7)	7 (9.9)	4 (7.1)	17
January	4 (17.6)	6 (9.1)	1 (3.6)	11
February	1Ø (28.6)	8 (1Ø.5)	12 (12.9)	3Ø
March	4 (17.6)	8 (1Ø.5)	6 (8.7)	18
April	4 (17.6)	9 (11.2)	35 (21.4)	48
May	2 (12.3)	34 (22.3)	38 (23.5)	72
June	2 (12.3)	94 (39.Ø)	145 (47.8)	241
July	Ø (Ø.Ø)	62 (3Ø.8)	17 (14.7)	79
Totals	44	237	264	3Ø5
Mean	15.49	13.32	11.82	
S.E	± 2.Ø6	± 3.33	± 3.74	

S.E = Standard Error.

TABLE 7a. Monthly indoor resting gravid An. gambiae collected by pyrethrum spray catch (PSC). (Transformed data (arcsine) in parantheses).

	Site 1	Site 2	Site 3	Totals
Month				
August	4 (16.4)	Ø (Ø.Ø)	1 (3.4)	5
September	1 (8.3)	1 (3.4)	3 (5.8)	5
October	5 (18.4)	6 (8.3)	2 (4.8)	13
November	17 (22.Ø)	41 (22.5)	21 (15.3)	79
December	3 (14.2)	8 (9.6)	17 (14.2)	28
January	6 (2Ø.3)	11 (11.2)	6 (8.1)	23
February	Ø (Ø.Ø)	1 (3.4)	Ø (Ø.Ø)	1
March	Ø (Ø.Ø)	2 (4.8)	4 (6.6)	6
April	6 (2Ø.3)	52 (25.Ø)	32 (2Ø.3)	1ØØ
May	7 (22.Ø)	67 (28.7)	43 (22.8)	117
June	9 (24.4)	7Ø (29.4)	145 (43.9)	224
July	4 (16.4)	32 (19.4)	25 (14.7)	61
Total	52	291	297	64Ø
Mean	15.2Ø	13.74	13.45	
S.E	± 2.39	± 3.Ø6	± 3.45	

S.E = Standard Error.

TABLE 4b. Monthly indoor resting empty *An. funestus* collected by pyrethrum spray catch (PSC). (Transformed data (arcsine) in parentheses).

	Site 1	Site 2	Site 3	Total
Month				
August	5 (34.Ø)	1 (14.Ø)	Ø (Ø.Ø)	6
September	2 (2Ø.3)	Ø (Ø.Ø)	Ø (Ø.Ø)	2
October	Ø (Ø.Ø)	Ø (Ø.Ø)	Ø (Ø.Ø)	Ø
November	Ø (Ø.Ø)	Ø (Ø.Ø)	Ø (Ø.Ø)	Ø
December	2 (2Ø.3)	1 (14.Ø)	1 (19.5)	4
January	3 (25.8)	Ø (Ø.Ø)	Ø (Ø.Ø)	3
February	Ø (Ø.Ø)	Ø (Ø.Ø)	Ø (Ø.Ø)	Ø
March	1 (14.4)	1 (14.Ø)	2 (28.1)	4
April	Ø (Ø.Ø)	Ø (Ø.Ø)	2 (28.1)	2
May	1 (14.5)	6 (36.6)	Ø (Ø.Ø)	7
June	1 (14.5)	3 (24.9)	3 (35.3)	7
July	1 (14.5)	6 (36.6)	1 (19.5)	1
Total	16	17	9	42
Mean	13.26	11.35	1Ø.87	
S.E	± 3.27	± 3.96	± 4.Ø5	

S.E = Standard Error.

TABLE 5b. Monthly indoor resting blood-fed *An. funestus* collected by pyrethrum spray catch method. (Transformed data (arcsine) in parentheses).

	Site 1	Site 2	Site 3	Total
Month				
August	12 (41.0)	16 (28.8)	11 (21.9)	69
September	2 (15.3)	3 (12.1)	3 (11.2)	10
October	3 (19.7)	1 (6.9)	0 (0.0)	4
November	0 (0.0)	0 (0.0)	2 (9.2)	2
December	0 (0.0)	1 (6.9)	0 (0.0)	4
January	3 (19.7)	0 (0.0)	2 (9.2)	8
February	0 (0.0)	0 (0.0)	1 (6.5)	1
March	0 (0.0)	0 (0.0)	2 (9.2)	2
April	1 (10.8)	12 (24.7)	17 (27.6)	30
May	2 (15.3)	15 (27.8)	20 (30.2)	37
June	3 (19.7)	12 (24.7)	13 (23.9)	28
July	2 (15.3)	9 (21.2)	8 (18.6)	19
Total	28	69	79	176
Mean	13.0	12.74	13.95	
S.E	± 3.49	± 3.44	± 2.97	

S.E = Standard Error.

TABLE 6b. Monthly indoor resting half-gravid *An. funestus* collected by pyrethrum spray catch method. (Transformed data (arcsine) in parentheses).

	Site 1	Site 2	Site 3	Total
Month				
August	17 (54.3)	2 (14.Ø)	Ø (Ø.Ø)	19
September	2 (16.4)	Ø (Ø.Ø)	Ø (Ø.Ø)	2
October	Ø (Ø.Ø)	Ø (Ø.Ø)	Ø (Ø.Ø)	Ø
November	Ø (Ø.Ø)	Ø (Ø.Ø)	1 (9.9)	1
December	Ø (Ø.Ø)	Ø (Ø.Ø)	Ø (Ø.Ø)	Ø
January	1 (11.5)	1 (9.8)	2 (14.Ø)	4
February	Ø (Ø.Ø)	Ø (Ø.Ø)	1 (9.9)	1
March	Ø (Ø.Ø)	2 (14.Ø)	Ø (Ø.Ø)	2
April	Ø (Ø.Ø)	Ø (Ø.Ø)	5 (22.6)	5
May	3 (2Ø.3)	1 (9.8)	5 (22.6)	9
June	2 (16.4)	21 (51.8)	7 (27.Ø)	3Ø
July	1 (11.3)	7 (27.Ø)	13 (38.2)	21
Total	26	34	34	94
Mean	1Ø.86	1Ø.55	12.ØØ	
S.E	± 4.57	± 4.4Ø	± 3.77	

S.E = Standard Error.

TABLE 7b. Monthly indoor resting gravid *An. funestus* collected by pyrethrum spray catch method. (Transformed data (arcsine) in parentheses.

	Site 1	Site 2	Site 3	Total
Month				
August	21 (66.4)	5 (21.Ø)	3 (12.9)	79
September	Ø (Ø.Ø)	1 (9.21)	1 (7.4)	3
October	Ø (Ø.Ø)	Ø (Ø.Ø)	Ø (Ø.Ø)	2
November	Ø (Ø.Ø)	Ø (Ø.Ø)	1 (7.4)	1
December	Ø (Ø.Ø)	1 (9.2)	Ø (Ø.Ø)	3
January	Ø (Ø.Ø)	1 (9.2)	1 (7.4)	5
February	Ø (Ø.Ø)	Ø (Ø.Ø)	Ø (Ø.Ø)	Ø
March	Ø (Ø.Ø)	Ø (Ø.Ø)	3 (12.9)	4
April	1 (11.6)	2 (13.1)	22 (37.3)	26
May	1 (11.6)	1Ø (3Ø.4)	9 (22.8)	19
June	2 (16.4)	14 (36.8)	12 (26.6)	28
July	Ø (Ø.Ø)	5 (21.Ø)	8 (21.4)	13
Total	25	39	6Ø	124
Mean	8.83	12.49	13.Ø1	
S.E	± 5.53	± 3.58	± 3.42	

S.E = Standard Error.

APPENDIX VIII.

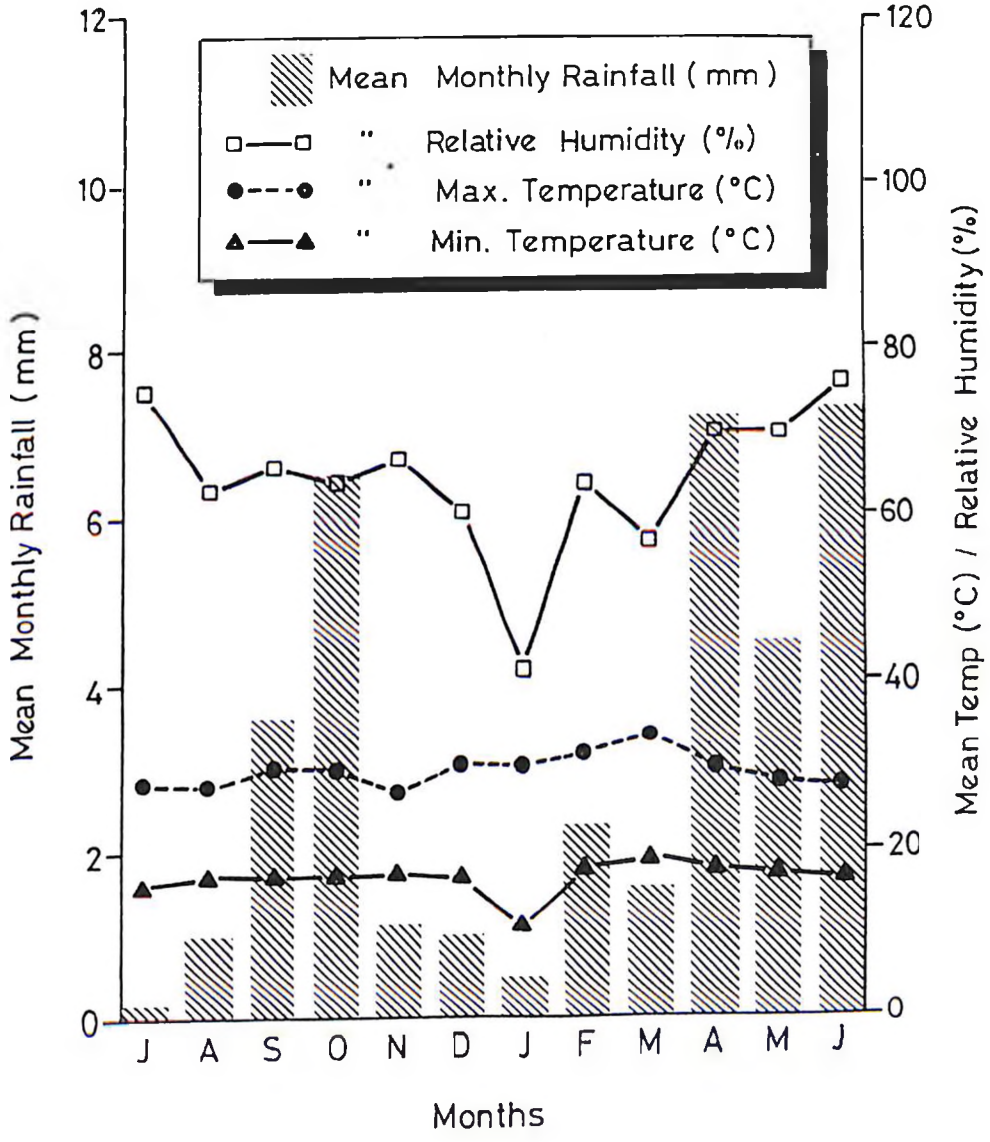

Figure 20. The weather report of Kanyawegi between July 1991 to July 1992.