THE EFFECT OF MALARIA PARASITE INFECTION ON THE FEEDING BEHAVIOUR AND GONOTROPHIC CYCLE OF ANOPHELINE MOSQUITOES IN KISUMU AREA, WESTERN KENYA.

BY

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE OF THE UNIVERSITY OF NAIROBI,

1990.

DECLARATION.

This is my original work and has not been presented for a degree in any other university.

JOSEPH WAKOLI WEKESA

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

PROF. RICHARD W. MWANGI.

DR. ROBERT S. COPELAND.

DEDICATION.

This thesis is dedicated to

Joinah Nekesa and Mackenzie Wekesa.

Both who nurtured me,

For their Kindness, understanding and

guidance.

And above all

For being my parents.

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THE EFFECT OF MALARIA PARASITE INFECTION ON THE FEEDING BEHAVIOUR AND GONOTROPHIC CYCLE OF ANOPHELINE MOSQUITOES IN KISUMU AREA, WESTERN KENYA.

ABSTRACT

Studies were carried out in Kisian area with the objective of observing the influence of malaria parasite infection on the feeding behaviour and fecundity of wild anopheline mosquitoes. Five anopheline species; *Anopheles gambiae s.l., An. funestus, An. ziemanni, An. coustani* and *An. pharoensis* were identified in the study area. Hungry females captured by human bait were placed in small cages and offered a blood meal from anaesthetised hamsters. Mosquito behaviour was recorded for a period of 10 minutes. Thirty-five percent of hungry females probed. Feeding behaviour was compared between infected and uninfected groups of *An. gambiae s.l.* and *An. funestus. Plasmodium falciparum*-infected *Anopheles gambiae s.l.* made significantly more probes than their uninfected counterparts. Infected *An. gambiae s.l.* probed nearly twice as often as uninfected *An. gambiae s.l.* and also had a significantly higher mean total probing time than uninfected females. This behaviour was not correlated with the intensity of the infection. Sporozoite infection of *An. gambiae s.l.* did not affect the time taken by the mosquito to feed to repletion. Like *Anopheles gambiae s.l.*, infected *An. funestus* probed more often and for a longer time than did uninfected females. However, these differences were not

statistically significant. Among uninfected females, probing and feeding behaviour of *An.gambiae s.l.* was not related to parity status.

Infection did not influence the amount of blood *An. gambiae s.l.* took during feeding after probing.

Anopheles gambiae s.l. and *An. funestus* were the only species that were sporozoite positive either by dissection or a *Plasmodium falciparum* ELISA test during the study period. The estimated sporozoite rate for *Anopheles funestus* was 6.57% estimated by dissection while that for *An. gambiae s.l.* was 4.88%. *An. gambiae s.l.* and *An. funestus* of Kisumu area were found to prefer humans to other hosts. On the other hand *An. ziemanni* had a broader host range and fed on a variety of hosts including bovids, goat/sheep, dog and chicken. The high sporozoite rates for *An. gambiae s.l.* and *An. funestus* could be associated with their short gonotrophic cycle. *Anopheles gambiae s.l.* took 1.91 $\pm$ 0.52 days to lay eggs after a blood meal. While *An. funestus* females laid eggs in 2.05 $\pm$ 0.55 days after a blood meal.

ACKNOWLEDGEMENTS

This study was conducted while I was in receipt of a DAAD scholarship and it recieved material assistance in terms of space and equipment from Kenya Medical Research Institute (KEMRI) and United States Army Medical Research Unit - Kenya (USAMRU-K/WRAIR).

I am greatly indebted to Professor Richard W. Mwangi and Dr. Robert S. Copeland for all the favours they accorded me and for their friendly supervision. I sincerely thank Dr. Davy K. Koech, the Director of KEMRI, for his timely assistance at the early stages of this work. The development of this scientific concept was the conception of Dr. Robert S. Copeland from KEMRI and USAMRU-K/WRAIR. For his unfaltering and unimpeachable interest in the gradual unfolding and conlusion of this work, I am sincerely grateful. It was with his great assistance that I first learned how to use Statistix II software and Microsoft Word computer programs. I give my sincere appreciation to Professor Richard W. Mwangi for his interest and inspiring suggestions. I thank Professors John D. Edman and Phillippe A. Rossignol for providing the relevant literature and their important suggestions.

My further acknowledgement goes to Mr. David Chumo, the Administrator of Walter Reed Project field station in Kisumu, his generosity kept me working in the field. I wish to thank Mr. David B. Wati for providing me with the necessary accommodation during the field study. I thank Mr. Christopher

Oyaro for polishing my dissection skills. Also deserving many thanks are the laboratory personnel (Malaria) of the Walter Reed Project both at KEMRI, Kisumu and Nairobi, for assisting me with the dissection, ELISA test and DNA probe procedures. They include Joseph Koros, Charles Asiago, Michael Ouko, Nduati Mwangi, George Oyare and Alex Masinya. I thank Mr. Joseph M. Sagwa for typing this work. I wish to pass my sincere gratitude to Mr. Isaac Ouma for his great effort and sacrifice in ensuring that this thesis was printed on time and at high precision. I wish to thank the people of Kisian for their assistance and cooperation with which this study was made possible. I thank Eliakim Onyango and David Otieno, the mosquito collectors, for their loyalty and dedication to work. Finally, my parents for their patience and encouragement, my success is theirs.

CHAPTER ONE.

INTRODUCTION.

Malaria transmission in nature is dependent on various factors which include the mosquito vector, the human population and associated physiological and environmental factors (Burkot, 1988). Understanding the epidemiology of malaria requires among others detailed studies on the vectorial efficiency, behaviour and ecology of mosquitoes of *Anopheles* species. Of primary importance in malaria transmission is the rate of infective mosquito bites received per man per day. This parameter is commonly known as the daily entomological inoculation rate (Pull and Grab, 1974). Any factor that enhances the inoculation rate increases the vectorial capacity of a vector.

Recent laboratory studies have suggested that the behaviour of insect vectors and vertebrate hosts of various tropical diseases is affected by certain parasite infections (Burkot, 1988). For instance, rodents infected with malaria parasites have been found to exhibit reduced defensive behaviour leading to their being preferentially fed upon by mosquitoes (Day and Edman, 1983; 1984; Day *et al.*, 1983; Rossignol *et al.*, 1985; Walker and Edman, 1985; 1986). With respect to insect vectors, it has been shown that invasion of mosquito salivary glands by malaria sporozoites can induce pathology that impairs the ability of mosquitoes to

locate a blood vessel and therefore to engorge (Ribeiro *et al.*, 1984; 1985). This may result in increased probing time. Increased probing duration may prolong the period of vector-host contact, and, allergic reactions and irritation of the host by vector saliva may result in interrupted blood meals (Grimstad *et al.*, 1980; Rossignol *et al.*, 1986). A disturbed vector may feed again on the same or different host thus increasing the chance of parasite transmission. This suggests that parasites have probably developed a host-pathogen relationship that tends to enhance their transmission and survival by maximising their chances of being passed from one host to the other. Mosquitoes that are infected may also take partial blood meals, while a full blood meal is required for ovarian development (Waage and Nondo, 1982; Rossignol *et al.*,1986). Thus infection may directly affect the fecundity of the mosquito vector since the amount of blood taken is directly related to the number of eggs laid (Clements, 1963; Gillet, 1971).

In this study, the possibility that infection with *Plasmodium* species could modify the feeding behaviour, fecundity and other related parameters of wild *Anopheles* mosquitoes was investigated in Kisumu area of Western Kenya.

1.1. The objectives of the study were as follows:-

1.1.1. To confirm the potential malaria vector species in Kisumu area.

1.1.2. To determine malaria sporozoite infection rates in anopheline vectors.

1.1.3. To investigate the effect of sporozoite infections on mosquitoes probing behaviour, blood engorgement and fecundity.

1.1.4. To establish the feeding patterns and man-biting rates of anopheline species.

1.2. LITERATURE REVIEW.

1.2.1 Malaria vectors in Kenya.

The distribution of malaria vectors in Africa, south of the Sahara, was comprehensively reviewed by Gillies and de Meillon (1968). In their review, they observed that *Anopheles gambiae s.s.* Giles (species A) and *An. arabiensis* Patton (species B) occur over a wide area of tropical Africa, while *Anopheles funestus* Giles is widespread in the highland areas of the savanna region. *Anopheles melas* Theobald and *An. merus* Donitz were limited to salt water areas of the west African and east African coasts, respectively. Three sibling species of the *An. gambiae* complex; *An. gambiae s. s.*, *An. arabiensis* and *An. merus*, together with *An. funestus* are the major malaria vectors in E. Africa (White, 1974). The other three sibling species of *An. gambiae s.l.* namely, *An. melas*, *An. quadriannulatus* Theobald and *An. bwambwae* (species D) have not been reported locally. White (1972) while studying the *An. gambiae* complex in the Kisumu area of Western Kenya, showed that *An. gambiae s.s.* and *An. arabiensis* were present in varying proportions, while *An. funestus* was more abundant than *An. gambiae s.l.* Service (1970) studied the ecology of *An. gambiae s.s.* and *An. arabiensis* in the same area. He found that *An. arabiensis* was more exophilic than *An. gambiae s.s.*

Studies on the distribution of the *Anopheles gambiae s.l.* in western Kenya have also been undertaken by Davidson *et al.* (1967) and Joshi *et al.* (1975). Examination of chromosomal preparations has shown that *An. gambiae s.s.* and *An. arabiensis* are the only two species of the *An. gambiae* complex present in the Kisumu area (Coluzzi and Sabatini, 1967; Davidson *et al.*, 1967; White, 1972; 1974; Joshi *et al.*, 1975; Fontaine *et al.*, 1976). The role of each species in the transmission of malaria in Nyanza province was further examined by Highton *et al.* (1979). Prevalence and infection rates of the two species were studied by Joshi *et al.* (1975) before a large scale fenitrothion (OMS-43) trial by WHO (Fontaine *et al.*, 1976). Studies on the distribution and ecology of the sibling species along the coast of Kenya include those of Mosha and Mutero (1982) and Mosha and Petrarca (1983).

Studies involving anopheline species other than *An. gambiae s.l.* and *An. funestus* are limited. Gillies and Furlough (1964) described the behaviour of *An. parensis* Gillies, at Malindi, on the Kenyan Coast. Surtees (1970) studying the distribution and abundance of adult and larval mosquito population at a rice irrigation scheme in Nyanza province, recorded 4 anopheline species, *An gambiae s.l.* Giles, *An. funestus* Giles, *An. coustani* Laveran and *An. pharoensis* Theobald. Also *An. ziemanni* Grunberg and *An. pharoensis* Theobald were observed on Kano plains by Chandler *et al.* (1975). In Mwea Irrigation Scheme, *An. funestus, An.*

rufipes, An. coustani, An. pretoriensis and *An. maculipalpis* were identified by Ijumba (1988) and Mukiama and Mwangi (1989). Foote and Cook (1952) suggested that *An. pharoensis* Theobald could be an important vector of malaria in the rice growing areas of Kenya, based on its relative abundance. Indeed, Ijumba (1988), Mukiama and Mwangi (1989) showed that *An. pharoensis* Theobald was the most common mosquito in Mwea rice irrigation scheme after *An. arabiensis* Patton. *An. pharoensis* was shown to be a potential malaria vector in Mwea irrigation scheme, and some workers have suggested that it could contribute to the epidemiology of malaria disease in the area (Ijumba, 1988; Mukiama and Mwangi, 1989).

1.2.2 Mosquito feeding behaviour.

Most female mosquitoes require blood to develop their eggs. Mosquitoes are blood vessel feeders (Lavoipierre, 1965). They have evolved a mechanism of location and penetration of blood vessels in the host skin that leads to successful blood feeding (Gordon and Lumsden, 1939; Pappas *et al.*, 1986). Salivation during feeding by mosquitoes is of great importance facilitating blood vessel location and enhancing blood flow (Clement, 1963; Hudson, 1964; Mellink and Van Den Bovenkamp, 1981; Ribeiro *et al.*, 1984; Ribeiro, 1987).

Several laboratory studies have demonstrated that some parasites induce behavioural changes in their vectors which

may enhance their transmission by prolonging vector-host contact. The normal blood feeding behaviour of insect vectors has been shown to be affected by protozoan parasites (Killick-Kendrick *et al.*, 1977; Molyneux and Jenni, 1981; Anez and East, 1984; Beach *et al.*, 1984; Rossignol *et al.*, 1984; Beach *et al.*, 1985; Rossignol *et al.*, 1986), bacteria (Wheeler and Douglas, 1941; Cavanaugh, 1971; Egerter *et al.*, 1988) and viruses (Grimstad *et al.*, 1980; Mitchell *et al.*, 1980). In the latter case for example, La Crosse virus (Grimstad *et al.*, 1980) and St. Louis encephalitis virus (Mitchell *et al.*,1980) were shown to modify the feeding success of their mosquito vectors. *Aedes triseriatus* infected by La Crosse virus probed several times in order to take a blood meal, and viruses were transmitted with each probe. The same observation was made for *Culex pipiens* infected by St. Louis encephalitis virus. *Lambornella clarki* (Cilliophora: Tetrahymenidae) infection has been shown to inhibit blood feeding in *Aedes sierrensis* (Egerter *et al.*, 1988). The mechanisms leading to modified feeding behaviour of many vectors by viruses and bacteria due to parasite pathology are not known (Rossignol *et al.*, 1986). However, in the Oriental rat flea, *Xenopsylla cheopis,* the plague bacillus induces an embolus in the foregut preventing ingestion of blood (Cavanaugh, 1971).

Studies on the modification of the feeding behaviour of vectors by parasite infection has not been limited to mosquito related infections. Work on other vectors has

produced results similar to those for mosquitoes. Killick-Kendrick *et al.* (1977) observed that when *Lutzomyia longipalpis* was experimentally infected with *Leishmania mexicana amazonensis*, individual flies frequently probed many times and took no blood or only a small quantity, yet on these occassions transmission occurred. Beach *et al.* (1985) reported similar results while working on laboratory-reared *Phlebotomus duboscqi* infected with *Leishmania major*. Killick-Kendrick (1979) observed that *Leishmania* interferes with the cibarial sensilla which monitors the obstruction of blood feeding behaviour. The tsetse fly, *Glossina morsitans morsitans* was observed to probe more frequently than uninfected controls when infected with *Trypanosoma brucei* (Molyneux and Jenni, 1981; Jenni *et al.*, 1980) or *T. congolense* (Roberts, 1981). Such behaviour was attributed to parasite interference with mechanoreceptors monitoring blood flow rate in the proboscis. However, Moloo (1983) failed to observe changes in the feeding behaviour of *Glossina m. morsitans* infected with *T. brucei*. Triatomine bugs (*Rhodnius prolixus*), which are vessel feeders (Lavoipierre, 1965), have been reported to show significant modification of their feeding behaviour when infected with *Trypanosoma rangeli* (Anez and East, 1984). Infected *R. prolixus* probed more frequently without taking any blood or taking only a small quantity yet on these occassions transmission occurred.

Other studies have focused on feeding behaviour modification of mosquitoes infected with malaria parasites. Females of *Aedes aegypti* infected with *Plasmodium gallinaceum* were unable to take a blood meal despite initiating frequent probes (Ribeiro *et al.*, 1985). This occurs due to inability of the infected mosquitoes to locate a blood vessel. Sporozoites cause lesions in the apical acini of *Ae. aegypti* (L) mosquito salivary glands resulting in reduced levels of salivary apyrase (Rossignol *et al.*, 1984). This critical enzyme inhibits platelet aggregation, thus lowering host haemostasis and shortening the duration of probing (Sterling *et al.*, 1973; Ribeiro *et al.*, 1984; Ribeiro, 1987). Damage to the salivary glands results in a decrease in the quantity of salivary apyrase without a corresponding decrease in volume of the saliva (Rossignol *et al.*, 1986). Thus, the function of saliva is to ensure that a mosquito takes a blood meal in the shortest period possible. Rossignol *et al.* (1984; 1986) observed that infection resulted in increased intradermal probing time and increased biting rate. The tendency of mosquitoes to transmit sporozoites by probing without blood feeding enhances their vectorial capacity. According to Jeffery *et al.* (1959), 100% transmission of *Plasmodium falciparum* could be achieved through probing alone so long as the vector had a high sporozoite load. Shute *et al.* (1976) and Ungureanu *et al.* (1976) also reported the same results when they used *Plasmodium vivax*.

Several authors have shown that increased intradermal probing time and increased biting rates may lead to interrupted blood meals with the mosquito eventually taking a partial blood meal (Edman and Downe, 1964; Edman and Kale, 1971; Edman *et al.*, 1974; Klowden and Lea, 1978; Waage and Nondo, 1982). Partial blood meals usually lead to low fecundity (Clements, 1963; Gillet, 1971; Grimstad *et al.*, 1980). Thus, parasite-induced pathology may increase the frequency of interrupted blood meals and, thereby, cause an increase in the relative number of hosts contacted by an infected mosquito. This may result from an arousal of host defenses as a consequence of lengthened duration of contact (Edman and Kale, 1971; Webber and Edman, 1972; Waage and Nondo, 1982). It may also increase the likelihood of the vector to cease feeding due to interruptions (Ribeiro *et al.*, 1985). Modification of vector probing behaviour by pathogens may represent an adaptation of the parasite to lengthen infective contact of the vector with the host. Such a prolonged host-vector contact with multiple hosts is evolutionarily significant as it enhances the vectorial capacity of such a vector (Rossignol *et al.*, 1984).

Previous studies examining the influence of malaria parasite infection on mosquito feeding behaviour have used culicine species that were artificially infected in laboratories. No report however is available on naturally infected anopheline mosquitoes in the field. Investigation

on the effect of malaria parasites on field populations of anopheline species form the basis of the present work.

1.2.3 Host selection by mosquitoes.

Of primary importance in malaria transmission is the proportion of mosquitoes in a population that feed on humans, and the proportion of blood meals taken from infected individuals. The Human Blood Index (HBI) is defined as the proportion of freshly fed mosquitoes found to contain human blood (Garret-Jones, 1964; Burkot, 1988). In malaria control programmes, the HBI is an important epidemiological tool for assessing the success of measures to interrupt transmission through vector control. The vectorial efficiency of a vector population depends to a great extent on its feeding pattern and host selection.

Several techniques have been developed and used to analyse vector blood meals (Weitz, 1956; Tempelis, 1975; Burkot *et al.*, 1981; Beier *et al.*, 1988). The techniques can be classified as simple in the case of a single meal or mixed when two or more meals are involved. Mixed blood meals were classified as patent if they originated from two or more host species or cryptic when from two or more individuals of the same species (Boreham and Garrett-Jones, 1973). The proportion of blood meals derived from two or more hosts depends on the probability of a blood meal being interrupted, for example, through host defensive behaviour

(Waage and Nondo, 1982; Edman and Kale, 1971; Edman and Scott, 1987) and the likelihood of each host being selected by the mosquitoes (Tempelis, 1975). Boreham and Garrett-Jones (1973) showed that there is a higher proportion of cryptic than patent mixed blood meals, the former ranging between 2.7-5.7% for *An. gambiae* and 8-9% for *An. sacharovi*, respectively. Burkot (1988) commented that due to clustered distribution of humans in houses, a mosquito interrupted while feeding on a person sleeping in a house is more likely to complete its feed on another person in the house than on an alternative host species.

Selection of hosts by various mosquito species in nature is diverse. Some species, such as *Aedes taeniorhynchus* Wiedermann, are opportunistic and feed on a variety of animal species (Day and Edman, 1984). Others, such as *An. gambiae s.s.* Giles, are nearly totally restricted to humans (Garrett-Jones, 1964; Garrett-Jones *et al.*, 1980). Several reasons for the observed feeding patterns of mosquitoes in nature have been proposed and reviewed by Day and Edman (1984). However, Gillies (1964) showed that host preference is genetically determined in *An. gambiae s.s.* The host preference patterns of the six sibling species of the *An. gambiae* complex were discussed by White (1974). Global data on host selection patterns in anopheline mosquitoes as indicated by precipitin test of individual blood meals were summarised and published by Garrett-Jones *et al.* (1980). In Kenya, studies on host

feeding patterns of the *Anopheles* include those of Joshi *et al.* (1975), Fontaine *et al.* (1976), Service *et al.* (1978), Highton *et al.* (1979) and Beier *et al.* (1988). All these studies present data for three principal malaria vectors, namely *An. gambiae s.s.* Giles, *An. arabiensis* Patton and *An. funestus* Giles.

In the present study I sought to assess the epidemiological implications of the feeding behaviour of naturally infected anopheline vectors infected by *Plasmodium* sp.

CHAPTER TWO

MATERIALS AND METHODS.

2.1 Study site.

The studies were conducted in Kisian which is located about 10 km to the west of Kisumu ($0^{o}05'S$ and $34^{o}40'E$) along the shores of Lake Victoria, in Nyanza Province. Kisian comprises three villages: Karombo, Kodawo and Kanyuto (Figure 1). It is bordered to the west by the Kisian river, by Kisumu airport to the east and by the Nyahera hills (an extension of Nandi escarpment) to the north. The climate of Kisumu with special reference to mosquitoes and their microclimate has been described by Haddow (1942). Kisumu receives a mean annual rainfall of 1240mm with maximum precipitation occurring from February to mid June (Haddow, 1942; Surtees, 1970; White, 1972; Chandler *et al.*, 1975). An erratic smaller peak of precipitation falls in November-December. The driest period is August and September. The average monthly maximum temperature ranges from $27^{o}C$ in June and July to $29^{o}C$ in November-January with a relative humidity (1400 hours) ranging between 41% and 57%.

The study area has an altitude of about 1150m, and is characterised by savannah-type vegetation dominated by low scrub thorn trees and *Euphorbia* spp, *Lantana* hedges and woods of *Eucalyptus* trees. However, much of the area is

bare scrub without any extensive tree growth. The area is covered with fertile alluvial deposits from the surrounding highlands. Kisian has a flat terrain with extensive papyrus swamps that fringe the margins of Lake Victoria and these may provide the major breeding sites for most local species of mosquitoes throughout the year. Poor drainage of the area leads to further mosquito breeding during the wet season.

The human population density of Kisian is high with varying types of settlement where houses are concentrated in homesteads, each homestead is occupied by members of an extended family. Scattered homesteads of about 7 to 16 houses are typical of this area (Figure 1). The homestead comprises of houses that range from temporary grass thatched and mud walled huts to semipermanent iron roofed and mud walled houses. Most houses have large eaves between the roof and the wall providing easy entrance and exit for mosquitoes. The villagers own small pieces of land, normally ranging between 0.08 hectares and 6.9 hectares. Cultivation of the land is not intensive. Several homes grow some subsistence crops, mainly millet, sorghum, maize, bananas and sweet potatoes. The land is communally grazed outside the cultivated areas by cattle, sheep and goats. Most homesteads had cattle and sheep or goat. Cattle are corralled near the houses at night, but young calves, sheep, goats and chicken are often kept inside human sleeping quarters during hours of darkness. Donkeys are rare. Oxen

are used as beasts of burden in the area. Every homestead had poultry, a few had cats and dogs. Some rabbits and guinea pigs were observed in some homes at Kanyuto and Karombo villages. Pigs were only observed in Kanyuto village near Otonglo market (Figure 1). Wild ducks, egrets, herons, kingfishers, hawks, and many other bird species were common in the area.

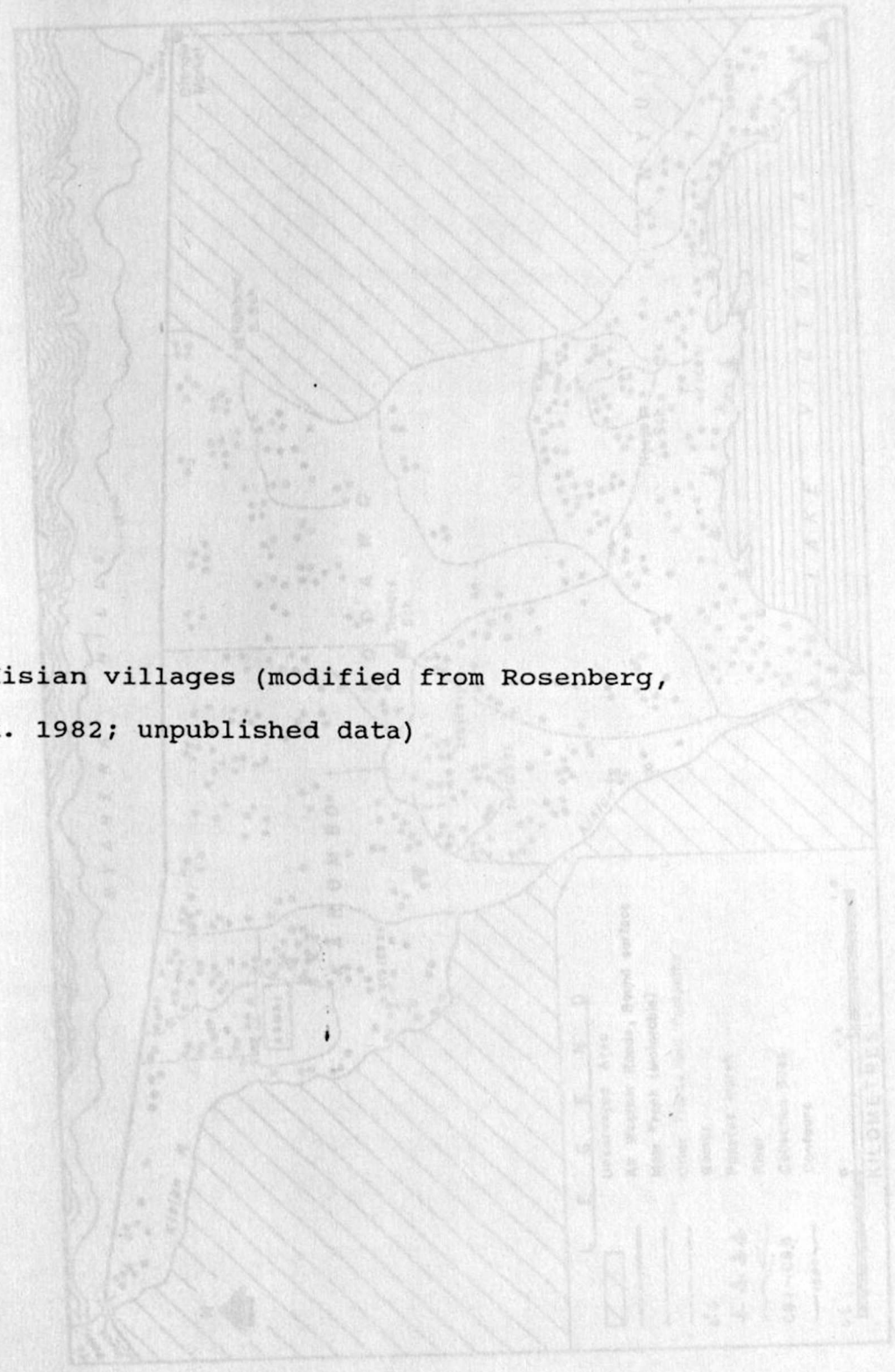
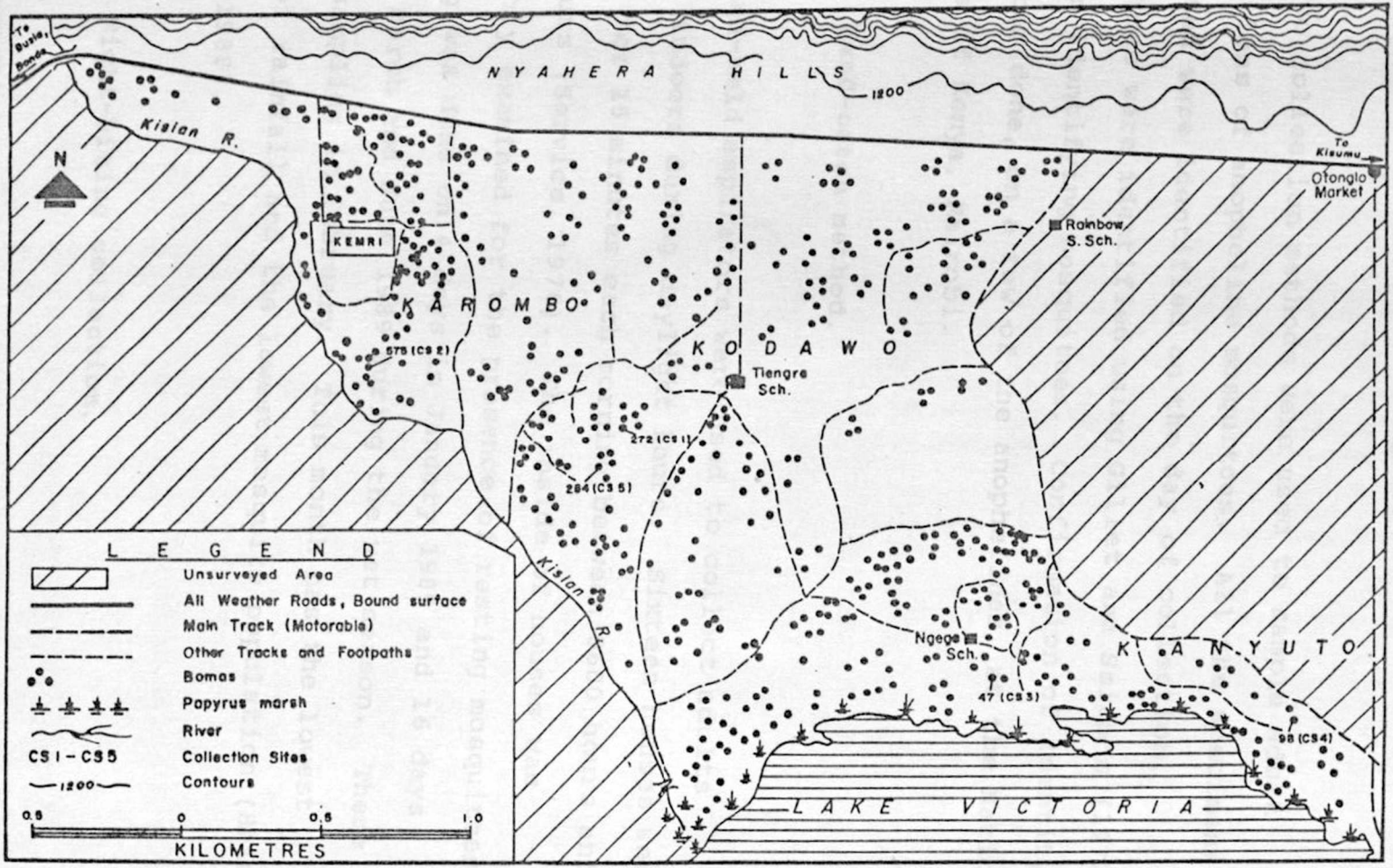


Figure 1. Kisian villages (modified from Rosenberg, R. 1982; unpublished data)


To Busia, Bondo
NYAHERA HILLS
1200
Kisian R.
N
To Kisumu
Otongla Market
KEMRI
Rainbow S. Sch.
KARAMBO
KODAWO
575 (CS 2)
Tiengre Sch.
272 (CS 1)
284 (CS 5)
Kisian R.
Ngege Sch.
KANYUTO
47 (CS 3)
98 (CS 4)
LAKE VICTORIA
LEGEND
Unsurveyed Area
All Weather Roads, Bound surface
Main Track (Motorable)
Other Tracks and Footpaths
Bomas
Papyrus marsh
River
CS 1 - CS 5 Collection Sites
1200 Contours
0.5 0 0.5 1.0
KILOMETRES

2.2 Mosquito collection techniques.

Two collection methods were used to sample adult populations of anopheline mosquitoes. All the specimens collected were identified on the day of collection. Mosquitoes were identified using Gillet and Smith's (1972) key for identifying mosquitoes. Confirmation of identity was later done, on a few of the anophelines, at the National Museums of Kenya, Nairobi.

2.2.1 Hand-catch method.

Hand-held aspirators were used to collect adults resting indoors during daylight hours. Sixteen houses were sampled for 15 minutes each morning between 0600 hours and 0900 hours (Service, 1976). The inside of houses was thoroughly examined for the presence of resting mosquitoes. Sampling was done on 4 days in January 1989 and 16 days between March and June 1989 during the wet season. There was no sampling in February. This month has the lowest amount of rainfall and the lowest mosquito population (Beier *et al.*, 1988).

2.2.2. Night-biting collection.

All-night human bait collections (Service, 1976) were performed three times a week between December 1988 and June

1989. The author and one village volunteer were used as human baits between 2200 hours and 0600 hours twice a week. The same collection was done between 1800 hours and 0600 hours once a week. Collections were rotated among five houses. The types of houses used for night-biting collections were either semipermanent or temporary. Temporary houses were of two categories, those with smoke and those without. All the houses used for collections had night occupants.

Blood-seeking females were caught using hand-held aspirators with the help of flash lights (WHO, 1975). Mosquitoes were identified at capture and all identifications were confirmed the following morning before dissection.

2.3 Assessment of mosquito probing and feeding behaviour.

Unfed anopheline mosquitoes collected by the human-baited method were used in the probing and feeding experiments. Hamsters were used as a source of blood meals. The hamsters were anaesthetised by intraperitoneal injection (I.P.) of 0.15mg/kg of sagatal (sodium pentobarbitone B.P.) before being exposed to *Anopheles* females. The abdomen of the rodents was shaved to allow mosquitoes to feed easily (Rossignol *et al.*, 1986). The anesthetist hamster was laid on its back on an observation bench with limbs spread out

(Plate 1). Captured blood seeking *Anopheles* mosquitoes were gently aspirated into transparent (2.5 by 2.5 by 5.0 cm) perspex cages covered with a nylon net on two of the long sides so that mosquitoes could probe through them. The cage wall had a small opening of 1.5 cm diameter at the square end through which mosquitoes were introduced. The opening was plugged by a cork. After identification, mosquitoes were allowed to acclimatize for 5-10 minutes. The cage was then placed on the shaved abdomen (belly) of the anaesthetised hamster. Mosquito behaviour was observed for 600 seconds (10 min.) and all the observations were recorded on a cassette tape. Field observations were made in torch light or dim light from a hurricane lamp. A stop watch was used to record time in seconds.

Data was transcribed in the laboratory and the following variables noted: the number of probes, time taken before the first probe (aggressiveness), total probing time, time from the beginning of probing until engorgement (engorgement time) and total time spent between probes (time between probes). Females not responding to the host during this 10 minute period were considered inhibited in blood feeding. There were female anophelines that probed and never fed (NF), while others took either a partial (PF) or a full (FF) blood meal. Each insect was used only once. Individual mosquitoes were retained in labelled cages until the following morning when they were transported to the Walter Reed Project field laboratory at Nyanza Provincial

Hospital, Kisumu. Here, the specimens were kept in the refrigerator at 4°C until dissection for sporozoites and parity was performed later in the day. After dissection all specimens were stored in a REVCO (a refrigerator that mantains ultra low temperatures; REVCO Inc.,USA) at -70°C for Enzyme Linked Immunosorbent Assay (ELISA) for sporozoite species identification. Mosquitoes with midgut oocysts but no sporozoites in their salivary glands and those that tested positive by ELISA without corresponding positive salivary glands were both considered sporozoite negative. Frozen conditions for the specimen's preservation are necessary to keep circumsporozoite (CS) in detectable state (Collins *et al.*, 1988; Beier *et al.*, 1988).

2.4 Parity determination.

The method of Detinova (1962) and a modified method of Polovadova (WHO, 1975) were used to estimate parous rates. Ovaries of females collected by the different techniques were dissected. Those found to be in Christopher's stage I or II were covered with a cover slip and left to dry out. Ovaries with coiled tracheolar skeins were classified as nulliparous (Detinova, 1962). The ovaries in Christopher's stage II or late stage III were classified as parous. Also ovaries with mature eggs retained in the ovarioles were classified as parous.

2.5 Determination of the gonotrophic cycle and fecundity.

Fifty to 60 freshly fed females collected by aspiration inside houses once per week were kept individually in 50 ml vials. Wet cotton wool covered by filter paper was put at the base of the vials to serve as a substrate for oviposition. The vials were covered by a mosquito netting held in position by a self locking cover and left at ambient temperature (WHO, 1975). Regular observations were made on the digestion of blood meals in relation to the development of eggs. The interval between observations was six hours on the first day, and 3 to 4 hours on subsequent days. The approximate time at which eggs were laid was recorded.

Females whose eggs did not develop beyond Christopher's stage I and II were dissected for parity. Those found to be nulliparous were classified as pre-gravid (with ovaries in Christopher's stage I or II). Parous females whose eggs did not develop beyond Christopher's stage II were classified as showing gonotrophic discordance (Gillies, 1955). Females that became engorged during night-biting collections were also kept for the same purpose until eggs were laid.

The salivary glands of all females used in this experiment were dissected for sporozoites, while body parts were kept for confirmation of *Plasmodium* infection using ELISA test.

2.6 Blood meal identification and malaria sporozoite detection.

2.6.1. Blood meal identification.

Fully fed female mosquitoes collected from indoor resting sites by the hand-catch method had their blood meals smeared on filter papers using the head of a paper pin (WHO, 1975). A single pin was used for smearing a single blood meal. The blood smears were preserved dry until they were prepared for testing by ELISA for host identification (Beier *et al.*, 1988). In the ELISA test, blood smears were cut out of the filter paper and soaked individually in 250 ul of 0.01M Phosphate Buffered Saline (PBS) for 12 hours

overnight. They were then frozen at -20°C until tested. The ELISA test procedures were described by Beier *et al.* (1988). Samples were initially screened for human and bovine blood meals. Only those which did not react were retested against antisera of other hosts. The other host antisera included anti-goat/sheep, anti-equine, anti-dog, anti-cat and anti-chicken. From the sample, 50 ul volumes were added individually to wells of 96-well polyvinyl microtitre plates. The plates containing the samples were incubated at room temperature for 3 hours. Each well was washed twice with PBS containing 0.5% Tween 20 (PBS-TW20). Having washed the wells, 50 ul of host-specific conjugate was diluted at 1:2000 (or 1:250 for bovids) in 0.5% boiled casein containing 0.025% tween 20 was added. After 1 hour, wells were washed 3 times with PBS-TW20 and 100 ul of ABTS (2, 2 azino-di [3-ethyl benzthiazoline sulfonate]) Peroxidase substrate was added to each well. Absorbance at 414 nm was determined by an ELISA reader 30 minutes after the addition of substrate. Samples were considered positive if absorbance values exceeded the mean plus three times the standard deviation of four negative controls consisting of unfed female mosquitoes. To determine the second host source (cow as the second host) in the same plate, a second conjugate Phosphate-labelled anti-human IgG solution was employed. After reading the absorbance at 30 minutes, the wells were washed 3 times with PBS-TW20, and 100 ul of Phosphatase substrate were added to each well. Plates were

read after 1 hour and overnight to determine positive bovine reactions. The readings from overnight incubated samples were used in the final analysis.

After screening samples for human and cow blood using the two-step procedure described above, negative samples were tested for goat/sheep, equine, dog, cat and chicken. For each test 1:500 dilutions of heterologous serum (seven hosts) were added to the conjugate solution to reduce background absorbance. Each plate contained control serum samples (1:500 dilutions in PBS) of human, cow, goat/sheep, horse/donkey, dog, cat and chicken. Each plate also contained four field-collected unfed female *Anopheles* ground in PBS at the same dilution as test samples.

2.6.2 Sporozoite detection.

Salivary glands of parous females were dissected and examined for sporozoites. The number of sporozoite was estimated as 1-10, 10-100, 100-1000 and above 1000 sporozoites per female mosquito. These number of sporozoites were recorded as 1, 2, 3, and 4, respectively. Any remaining undissected females were preserved in labelled vials for sporozoite ELISA testing. The specimens preserved for testing by ELISA were not sorted out into parous and nulliparous females. They were cut at the thorax and abdomen into three parts; head, thorax and abdomen. Only the thorax was used in the test to reduce the likelihood of

false positives (Beier *et al.*, 1987). Glands of females which were sporozoite positive by dissection were preserved in Non-indet P-40 (NP-40) (Sigma Chemical Co, United Kingdom.) and kept in a REVCO at -70°C to be tested by ELISA for *Plasmodium falciparum* infection (Wirtz *et al.*, 1987).

The falciparum ELISA employs the 2A10 monoclonal antibody (MAb). It recognizes the circumsporozoite (CS) tetrapeptide, Asn-Ala-Asn-Pro, which is highly conserved for *Plasmodium falciparum* strains throughout the world (Weber and Hockmeyer, 1985).

Individual thoraces were placed in labelled PVC 1.5 ml centrifuge tube and the other body parts were returned in the original vial and stored at -70°C. 50 ul blocking buffer (BB) : NP-40 was added to each tube and ground with a pestle attached to motor driven grinder. The pestle was rinsed with two 100 ul volumes of blocking buffer, catching the rinse in the tube to make the total volume, 250 ul. The pestle was then rinsed in PBS-Tween 20 twice and dried with a paper towel to prevent contamination between mosquitoes. The ground mosquito triturate was then stored overnight at -20°C. The test procedure modified from that of Wirtz *et al.* (1987) involved placing 50 ul of monoclonal antibody (MAb) solution in each well of a 96-well microtitre plates which was then incubated for 30 minutes at room temperature. After aspirating the MAb solution, each well was filled with BB and the plate incubated for 1 hour. The contents of the wells were aspirated and 50 ul of individually ground

mosquito thorax was added to each well. Positive and negative controls were also added. After 2 hours of incubation, wells were washed twice with 200 ul/well of PBS-TW20 per wash. 50 ul of horse-radish peroxidase (HRP) conjugate (MAb) was then added to each well. After 1 hour of incubation, plates were washed 4 times with PBS-TW20, and 100 ul of peroxidase substrate was added per well. Plates were read after 30 minutes at 414 nm with a Tirtertek Multiskan ^{C}MCC ELISA reader.

Laboratory-reared, known uninfected *Anopheles* females mosquitoes were used as negative controls. The positive controls consisted of recombinant *Plasmodium falciparum* as protein produced in *E. coli* (R32tet32) (Wirtz *et al.*, 1987). Eight positive controls ranged in concentration from 100 ug of R32tet32 per 10 ul of BB to 1.5 ug/10 ul and eight negative controls were also run on each plate. Mosquitoes were considered infected if ELISA absorbance values, at 30 minutes, exceeded the mean plus five times the standard deviation of eight negative control mosquitoes on the same plate. All positive samples were retested for confirmation.

2.7 Meteorological data.

Temperature, rainfall and relative humidity data were acquired from the Kisumu meteorological station, located a short distance (2 Km) from Kisian railway station. The mean temperature and relative humidity for the period were

calculated from the summary sheet. The mean of maximum and minimum temperature for each day was taken to be the temperature for that day. The mean of the maximum and minimum relative humidity was also taken to be the relative humidity for that day. The total amount of rainfall received per month was recorded as the rain for that month.

2.8 Statistical analysis.

The statistical analysis compared different parameters of feeding behaviour between sporozoite-positive and negative mosquitoes. Student t-test, chi-square, Fisher's exact test, Kruskal-wallis test and Yates chi-square corrected for continuity were some of the tests used in the analysis. Data was analysed with Statistix II software (NH Analytical Software, 1987) on an IBM XT micro-computer.

CHAPTER THREE

RESULTS

3.1 Mosquito species composition

Five anopheline species *An. gambiae s.l.*, *An. funestus*, *An. ziemanni*, *An. coustani* and *An. pharoensis* were identified in the study area (Table 1). *An. gambiae s.l.* was the most abundant species in all collections comprising 70.22% of the total collection (n=2290).

Anopheles funestus was the second most abundant species after *An. gambiae s.l.* in hand-catch collections comprising 22.31% (n=189) of the catch. However, *An. funestus* was the third most abundant species after *An. gambiae s.l.* and *An. ziemanni* in night-biting collection. *An. funestus* constituted 10.12% of night-biting catch in comparison with *Anopheles gambiae s.l.* and *An. ziemanni* both having 69.23% and 17.95% respectively. *An. coustani* and *An. pharoensis* were the least abundant species in all collections. All the five species were represented in collections made indoors by the two techniques.

Anopheles ziemanni and *An. coustani* were more abundant in night-biting collections than in hand-catch collections. The two species were collected more in houses that had bovids, goat/sheep, chickens and other domestic animals than those with human beings only.

Table 1. Species composition and percentages of adult anopheline mosquitoes collected indoors in Kisian village by all-night biting and day resting collections.

	Collection Techniques				
	Hand Catch		Human-biting Catch		Total number
Species	N	% of Total	N	% of Total	collected
An. gambiae s.l.	609	71.90	999	69.23	1608
An. funestus	189	22.31	146	10.12	335
An. ziemanni	44	5.20	259	17.95	303
An. coustani	3	0.35	23	1.59	26
An. pharoensis	2	0.24	16	1.11	18
Total	847	100.00	1443	100.00	2290
Culicines	279		893		1172

3.2 Probing and feeding responses.

3.2.1 Comparisons of species probing and feeding behaviour.

Of the 1,443 adult anopheline mosquitoes collected in human-biting collections, 487 specimens were used in the probing and feeding experiment (Table 2). *Anopheles gambiae s.l.* accounted for 81.52% of the anopheline mosquitoes used in the probing experiment. Table 2 shows a summary of the probing and feeding events of hungry anophelines exposed to anaesthetised hamsters.

A total of 397 hungry *Anopheles gambiae s.l.* were exposed to anaesthetised hamsters. Of these, only 34.30% (136) probed. Of the 136 *An. gambiae s.l.* that probed, 11.03% (15) probed and never fed, 23.53% (32) probed but took a partial blood meal and 65.44% fed and engorged. A total of 44 hungry *Anopheles funestus* were exposed to anaesthetised hamsters and of these only 34% probed. All the specimens that probed took a blood meal. Of these, 20.00% (3) took a partial meal and 80% (12) took a full blood meal. Thirty one hungry *An. ziemanni* were collected and exposed to anaesthetised hamsters. Of these, only four (13%) females probed and took a blood meal. Only 1 of 12 *Anopheles coustani* and none of the 3 *An. pharoensis* probed and these species were not considered further. There was no significant difference between *An. gambiae s.l.* and *An.*

Table 2. Blood feeding summary of Anopheles species.

Species	Total[1] number probed	Feeding results[1] NF	PF	FF	mean number of probes	mean time between probes (sec.)	mean probing time (sec.)	mean time to engor. (sec.)	Gland[2] +ves	ELISA
An. gambiae s.l.	136	15	32	89	2.75	41.69	225.13	192.96	33	28/361
An. funestus	15	0	3	12	2.62	36.31	214.54	166.54	6	5/34
An. ziemanni	4	0	1	3	1.25	98.00	125.75	126.25	0	0
An. coustani	1	0	1	0	7.00	148.00	188.00	0.00	0	0

[1]Mosquitoes probed at least once. NF=Did Not Feed; PF= Partially Fed; FF= Replete (Fully Fed).
[2]Gland sporozoite positive by dissection.

funestus that did and those that did not respond to the anaesthetised hamsters ($x^2 = 2.16$, $P > 0.05$).

Only specimens that were offered a host and probed at least once were included in the analysis of feeding behaviour following host contact. The mean number of probes made by hungry *An. gambiae s.l.* females was 2.75 probes per female, while *An. funestus* averaged 2.62 probes per female. There was no significant difference in the number of probes elicited by the two malaria vectors ($t = 0.49$, $df = 149$, $p > 0.05$). *Anopheles ziemanni* probed an average of 1.25 probes per female.

Average total probing time varied among species. *Anopheles gambiae s.l.* females probed for an average of 225.13 seconds, followed by *An. funestus* (214.54 seconds) and *An. ziemanni* (125.75 seconds). However, there was no significant difference in total probing time between *An. gambiae s.l.* and *An. funestus* ($t = 0.43$, $df = 149$, $p > 0.05$). Observations for *An. gambiae s.l.* and *An. funestus* showed that the total probing time was higher than the mean engorgement time. Also most specimens that probed and never engorged took a longer time attempting to get a blood meal than those that took a full blood meal. This difference was observed with *An. gambiae s.l.* and *An. funestus* but not with *An. ziemanni*. The mean time between probes did not vary significantly between *An. gambiae s.l* and *An. funestus* ($t = 0.278$, $df = 149$, $p > 0.05$). There was no variation in mean engorgement time between the three species. In general

An. gambiae s.l. and *An. ziemanni* took a longer time before probing than *An. funestus*.

3.2.2 Association of mosquito probing behaviour and Plasmodium infection.

Of the 397 *An. gambiae s.l.* exposed to hamsters, 33 specimens were shown by dissection to have sporozoites in their salivary glands. Of the 397 *An. gambiae s.l.* tested by ELISA, a total of 35 specimens were positive for *P. falciparum*. Two of these had been negative by dissection. However, among the 136 specimens that probed 26 of them were positive by dissection and 28 were positive for *P. falciparum* by ELISA test (Table 2). Of the 44 *An. funestus* used in the feeding experiment, 6 specimens were found on dissection to have sporozoites. Five of the 34 specimens of *An. funestus* that were tested by ELISA were positive for *P. falciparum*. Only 2 of the 15 specimens that probed were positive both by dissection and ELISA test (Table 2). Since specimens that test positive by ELISA do not necessarily have sporozoites in their glands (Beier *et al.*, 1987) ELISA results were not used in the analysis of the effect of sporozoite infection on probing and feeding behaviour of mosquitoes.

Table 3 shows that, of the 136 *An. gambiae s.l.* which probed, 26 specimens were positive by salivary gland dissection. There was a significant difference between the

able 3. The effect of *Plasmodium falciparum* on blood feeding behaviour of *Anopheles gambiae s.l.*

ariable	Salivary gland condition	Sample size(N)	Mean (x) ± S.E.	p[1]
umber of robes	uninfected	110	2.37 ± 0.205	t = 3.05
	infected	26	4.00 ± 0.675	p = 0.0029
otal Probing ime (sec.)	uninfected	110	214.10 ± 11.88	t = 2.28
	infected	26	276.80 ± 26.37	p = 0.0232
ime to full ngorgement(sec)	uninfected	107	182.10 ± 15.84	t = 1.80
	infected	26	248.70 ± 37.08	p = 0.07(ns)
Mean time(sec) between probes	uninfected	60	38.04 ± 6.57	t = 0.89
	infected	20	27.44 ± 6.05	p = 0.38(ns)

[1]Probability that there was no difference between means for uninfected and infected mosquitoes; t-test (ns= not significant).

mean number of probes made by infected *An. gambiae s.l.* compared to uninfected mosquitoes ($t = 3.05$, $df = 134$, $p < 0.05$). Infected specimens probed nearly twice as often as uninfected mosquitoes. Table 3 shows that infected *An. gambiae s.l.* also had a significantly higher mean total probing time than uninfected specimens ($t = 2.28$, $df = 134$, $p < 0.05$). Infected *An. gambiae s.l.* probed an average of 276.80 ± 26.37 seconds compared to 214.10 ± 11.88 seconds for uninfected specimens. Out of the 26 infected *An. gambiae s.l.* 26.9% (n=7) probed without engorging while 29.09% of the uninfected (n=110) specimens probed without engorging. There was no effect of sporozoite infection of *An. gambiae s.l.* on the time taken for total engorgement. The time to engorge between infected and uninfected was not significantly different ($t = 1.80$, $df = 131$, $0.1 > p > 0.05$). Of the 136 *An. gambiae s.l.* that probed only 80 specimens made more than one probe. Infected females spent an average of 27.44 ± 6.05 seconds between probes compared to 38.04 ± 6.57 seconds for uninfected females. There was no significant difference between the two means ($t = 0.89$, $df = 134$, $p > 0.05$).

Table 4 shows that 11.54% (3) of infected *An. gambiae s.l.* probed once while 88.46% (23) elicited multiple probes. On the other hand 47.27% (52) of the uninfected *An. gambiae s.l.* probed once while 57.73% (58) probed more than once. Being infected was observed to have a significant influence on whether *An. gambiae s.l.* made single or multiple probes

Table 4. Contingency table to show the effect of sporozoite infection on the number of probes made by *Anopheles gambiae s.l.*

Salivary gland condition	Mean	One-Probe	Multiple-probe	X^2	p
infected	4.96	3	23	=9.71	<0.05
uninfected	2.27	52	58		

X^2 is the Yates Chi-square corrected for continuity.

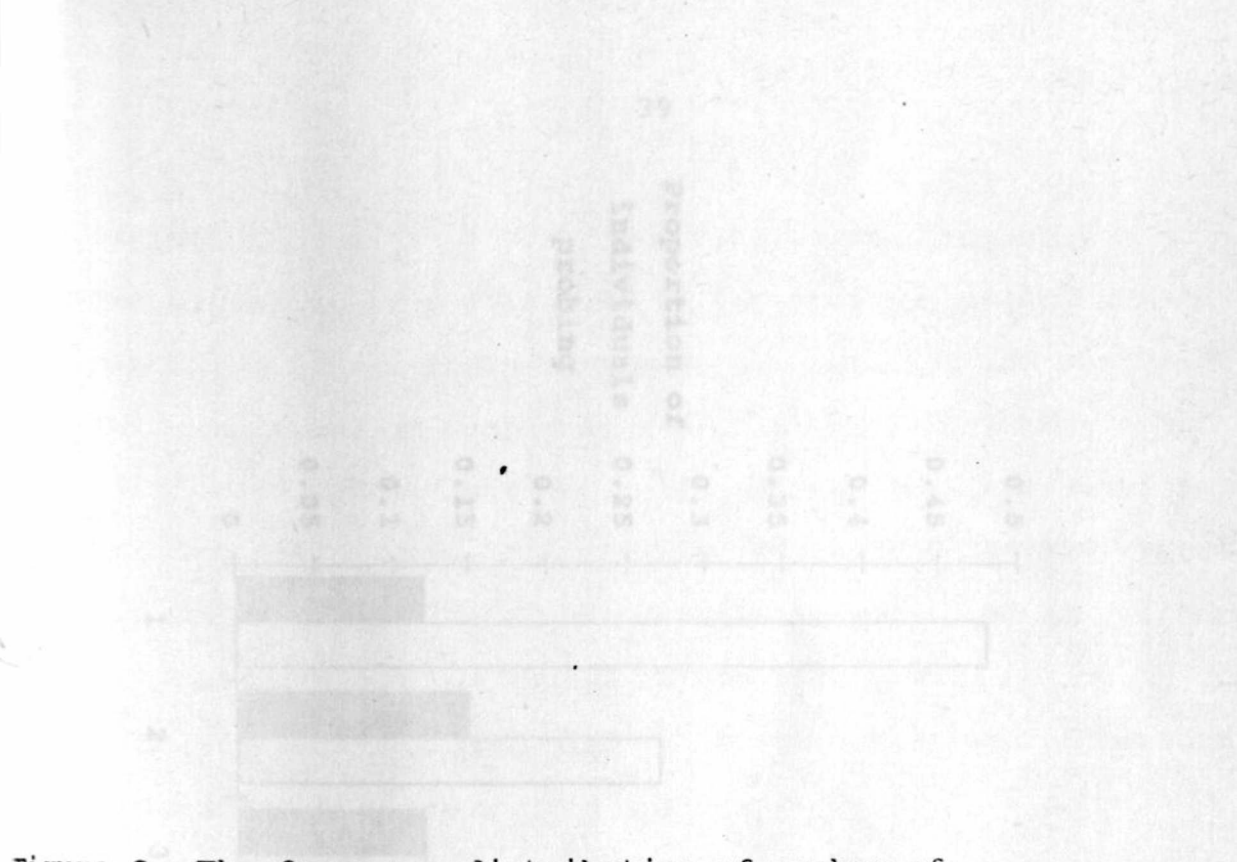
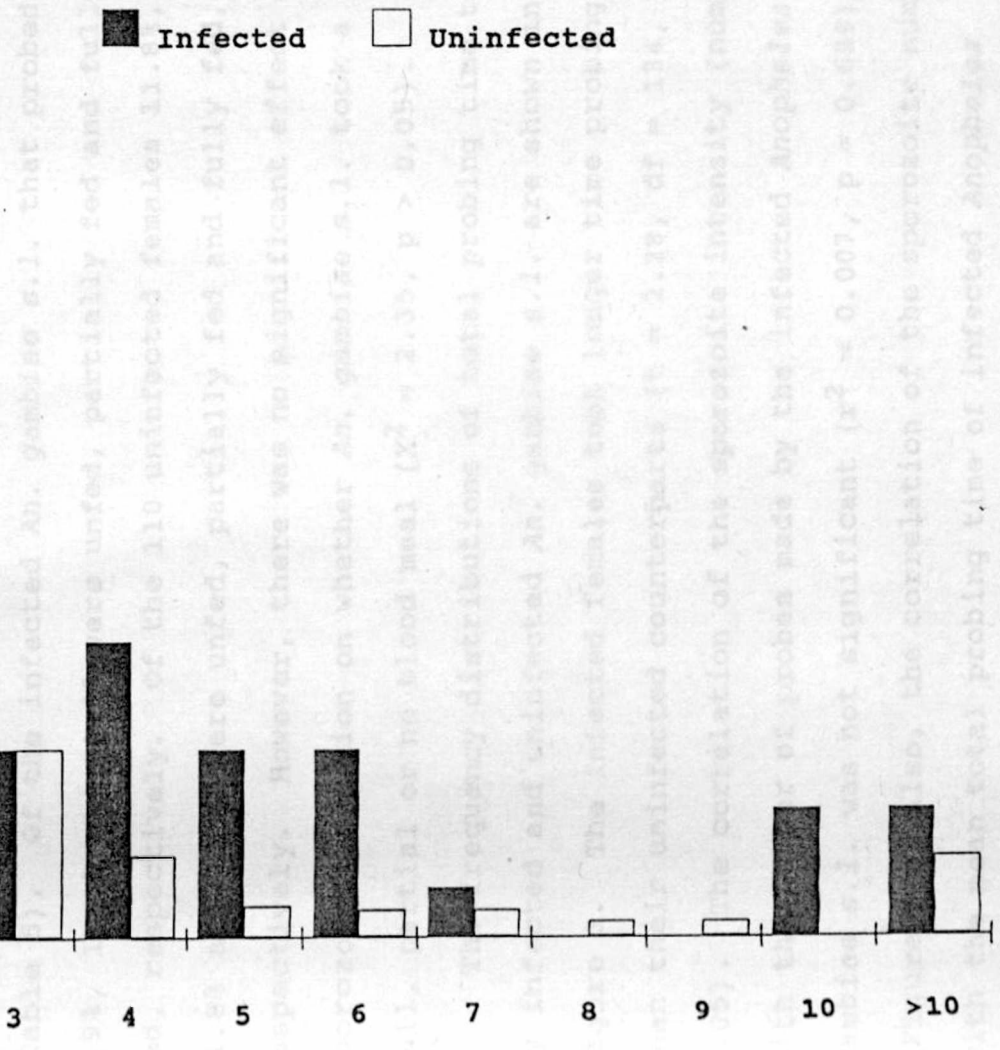


Figure 2. The frequency distribution of number of probes per individual infected and uninfected *Anopheles gambiae s.l.*

Proportion of individuals probing
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
1
2


Infected
Uninfected
3
4
5
6
7
8
9
10
>10
Number of probes

during feeding ($X^2 = 9.71$, $p < 0.05$). The frequency distributions of probes made by infected and uninfected *An. gambiae s.l.* are shown in Figure 2. Infected *An. gambiae s.l.* had a median number of 4 probes while that of uninfected females was two. 74% (81) of the uninfected *An. gambiae s.l.* probed once or twice compared to 26.92% (7) of the infected specimens. Sporozoite infection of *An. gambiae s.l.* also influenced whether a mosquito probed or not. Infected individuals tended to locate a host and probe more frequently than uninfected ones ($x^2 = 16.87$, $p < 0.05$) (Table 5). Of the infected *An. gambiae s.l.* that probed 3.9%, 15.4% and 80.8% were unfed, partially fed and fully fed, respectively. Of the 110 uninfected females 11.8%, 21.8% and 66.4% were unfed, partially fed and fully fed, respectively. However, there was no significant effect of sporozoite infection on whether *An. gambiae s.l.* took a full, partial or no blood meal ($X^2 = 2.35$, $p > 0.05$).

The frequency distributions of total probing time taken by infected and uninfected *An. gambiae s.l.* are shown in figure 3. The infected females took longer time probing than their uninfected counterparts ($t = 2.28$, $df = 134$, $p < 0.05$). The correlation of the sporozoite intensity (number) with the number of probes made by the infected *Anopheles gambiae s.l.* was not significant ($r^2 = 0.007$, $p = 0.689$) (Figure 4). Also, the correlation of the sporozoite number with the mean total probing time of infected *Anopheles gambiae s.l.* was not significant ($r^2 = 0.029$, $p = 0.501$)

Table 5. Contingency table to show the effect of malaria infection on the probing response of *An. gambiae s.l.*

	PROBING RESPONSE					
Salivary gland condition	Probed		Not probed			
	N	%	N	%	X^2	p
infected	26	77.14	8	22.86	=16.87	<0.05
uninfected	110	37.85	179	62.15		

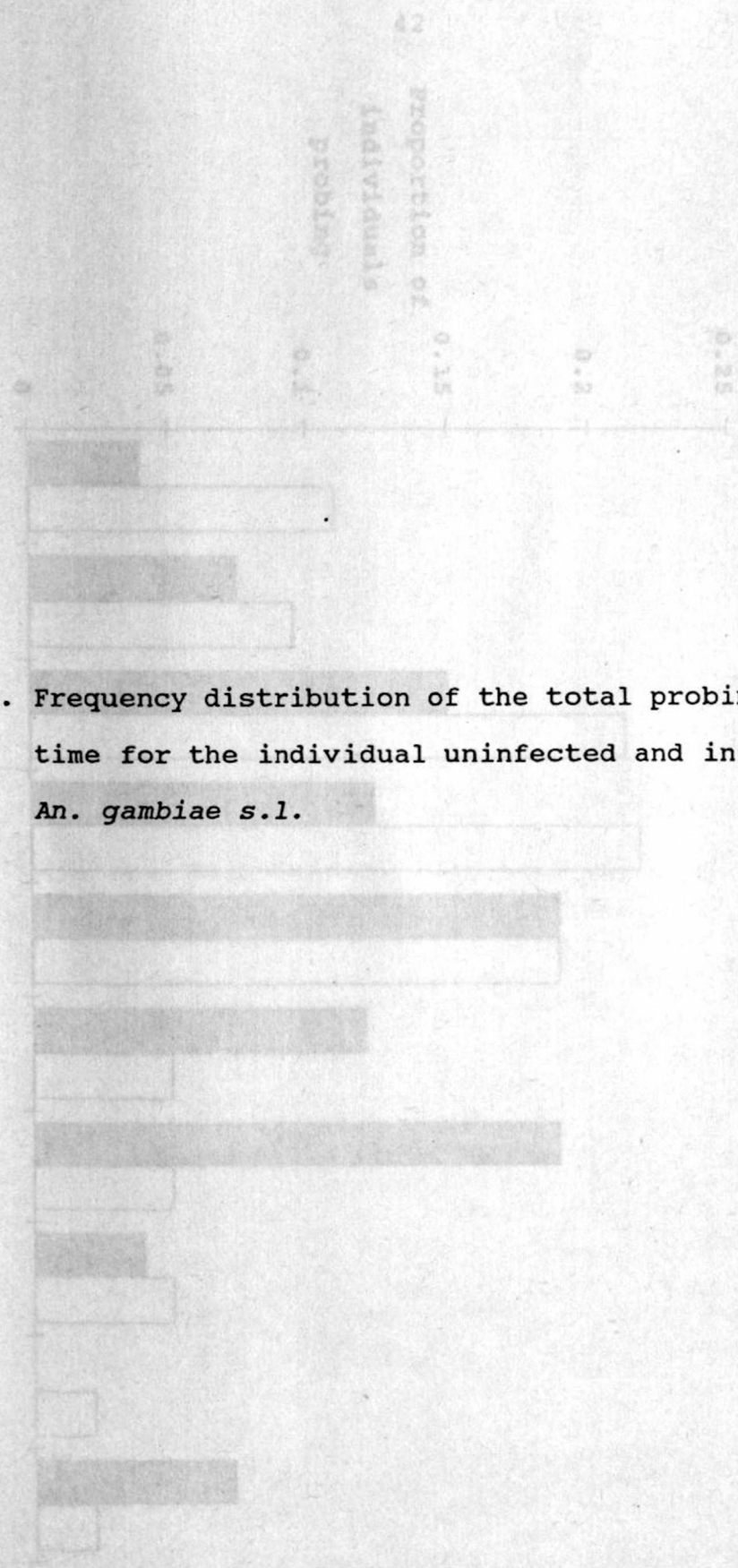
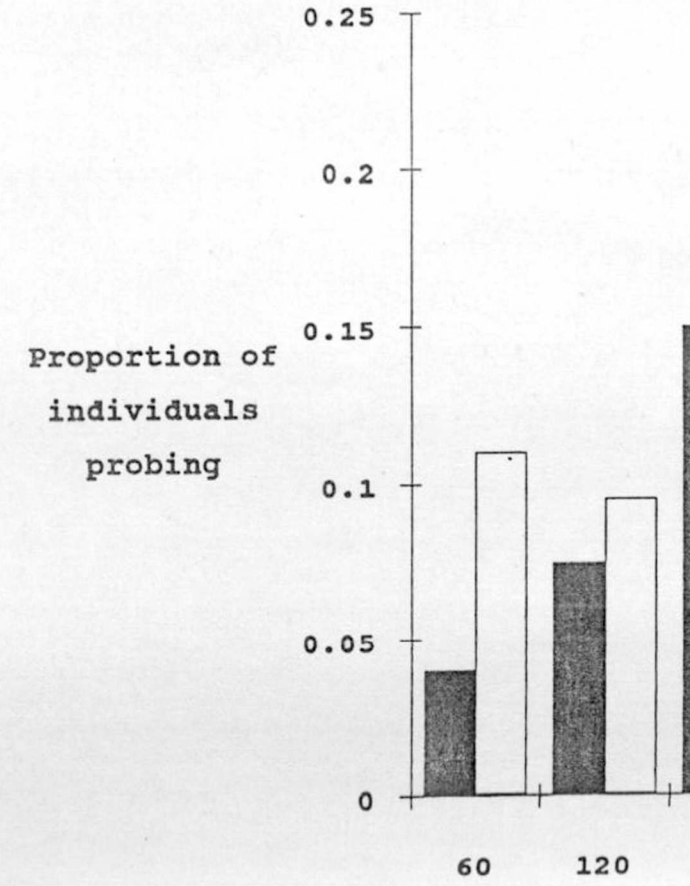


Figure 3. Frequency distribution of the total probing time for the individual uninfected and infected *An. gambiae s.l.*


Proportion of individuals probing
0.25
0.2
0.15
0.1
0.05
0
60
120

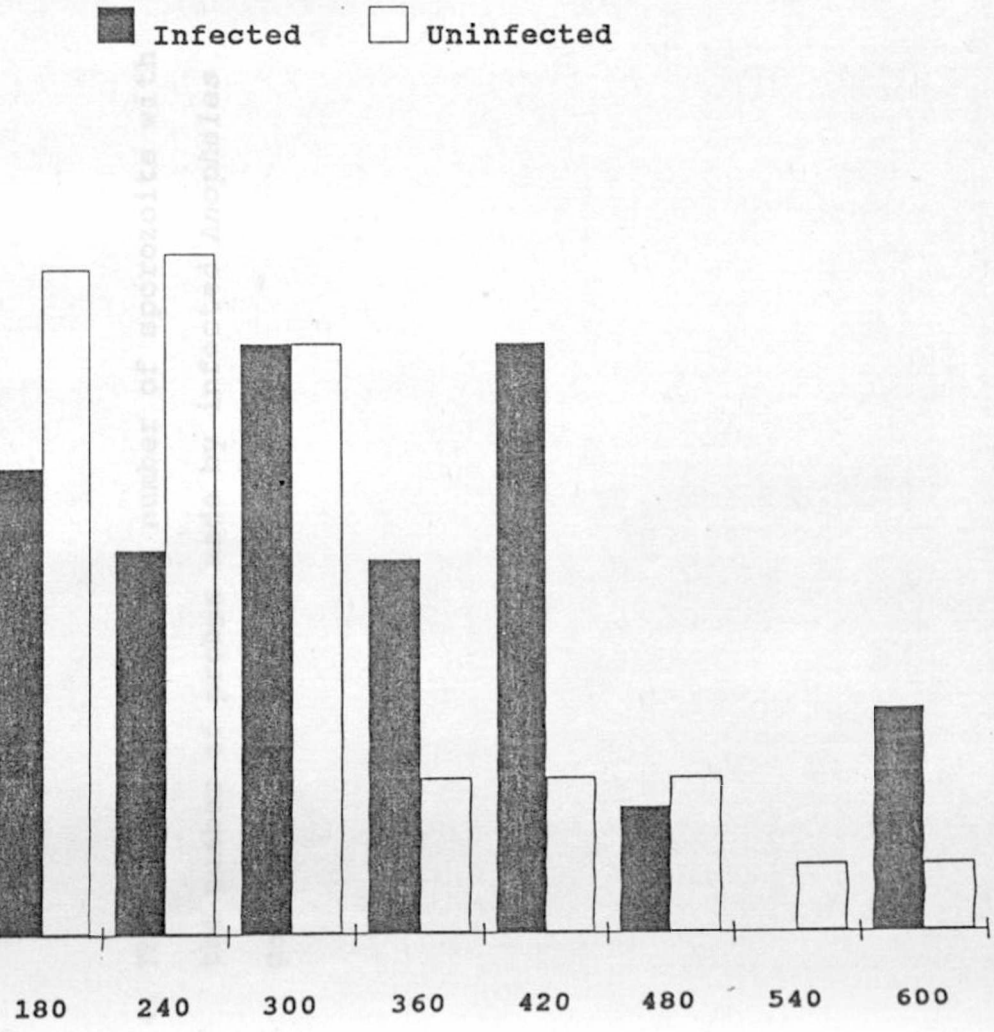
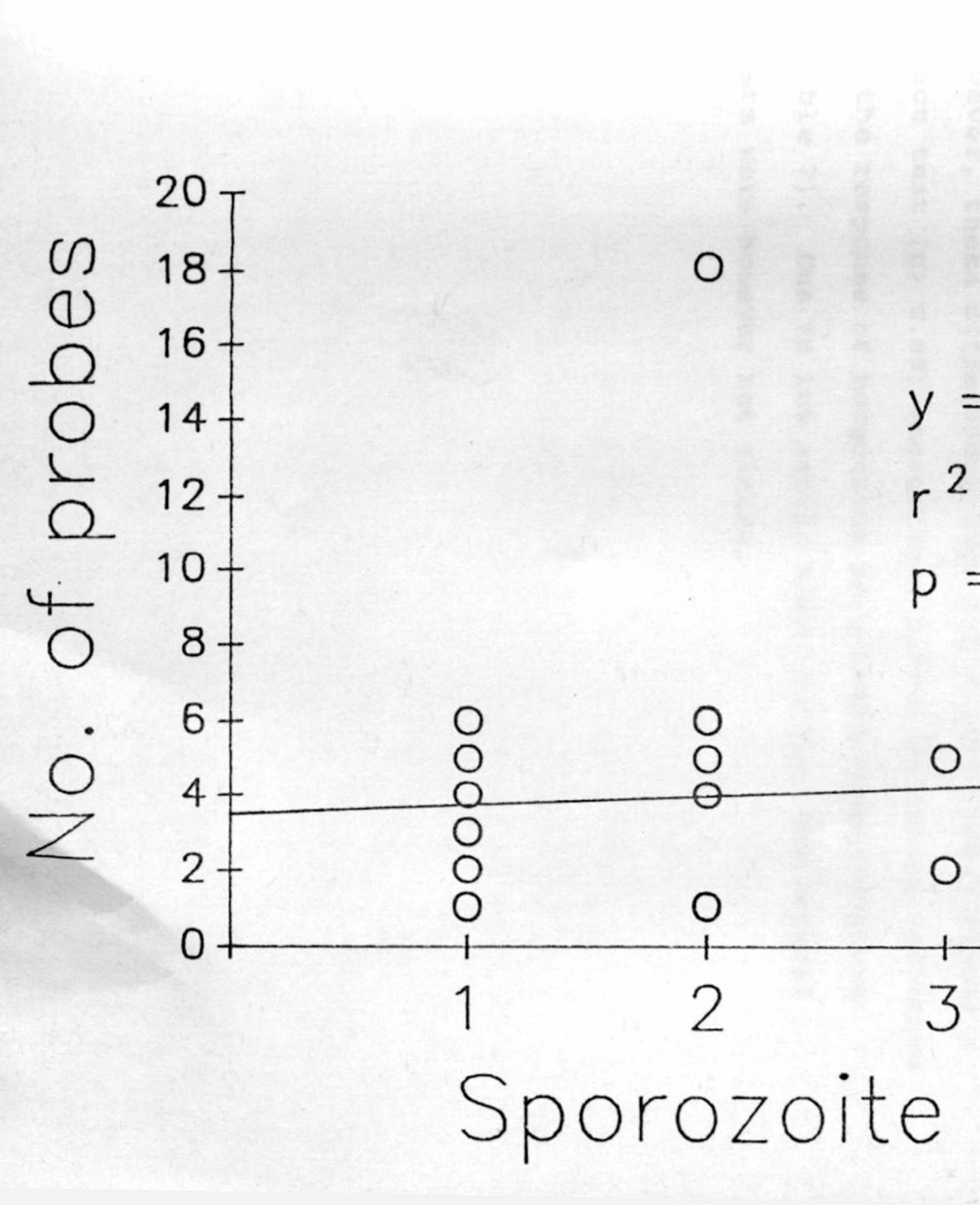

Infected
Uninfected
180
240
300
360
420
480
540
600
Time (60 second intervals)

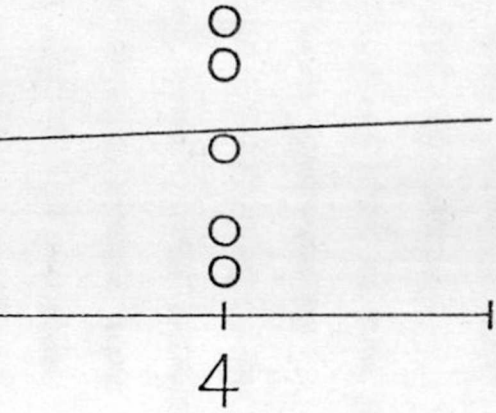
Figure 4. The correlation of the number of sporozoite with the number of probes made by infected *Anopheles gambiae s.l.*


No. of probes
20
18
16
14
12
10
8
6
4
2
0
1
2
3
Sporozoite
y =
r 2
p =

$= (x/2.51) + 3.48$

$= .007$

$= .689$



load

(Figure 5). This demonstrated that the effect of parasite infection on the vector feeding behaviour is also expressed at low parasites densities. Of the 44 *An. funestus* that were used in the probing experiment only 15 females eventually probed (Table 2). Table 6 shows that of the 15 *An. funestus* that probed, 2 (13.33%) were positive by salivary gland dissection, and both were confirmed to be positive by ELISA for *Plasmodium falciparum*. Like infected *Anopheles gambiae s.l.*, *An. funestus* females probed more often and for a longer time than did uninfected females. However, these differences were not significant. Fisher's exact test ($p > 0.05$) showed that infection had no influence on the response of mosquitoes to anaesthetised hamsters (Table 7). Due to low sample size, further statistical tests were however not viable.

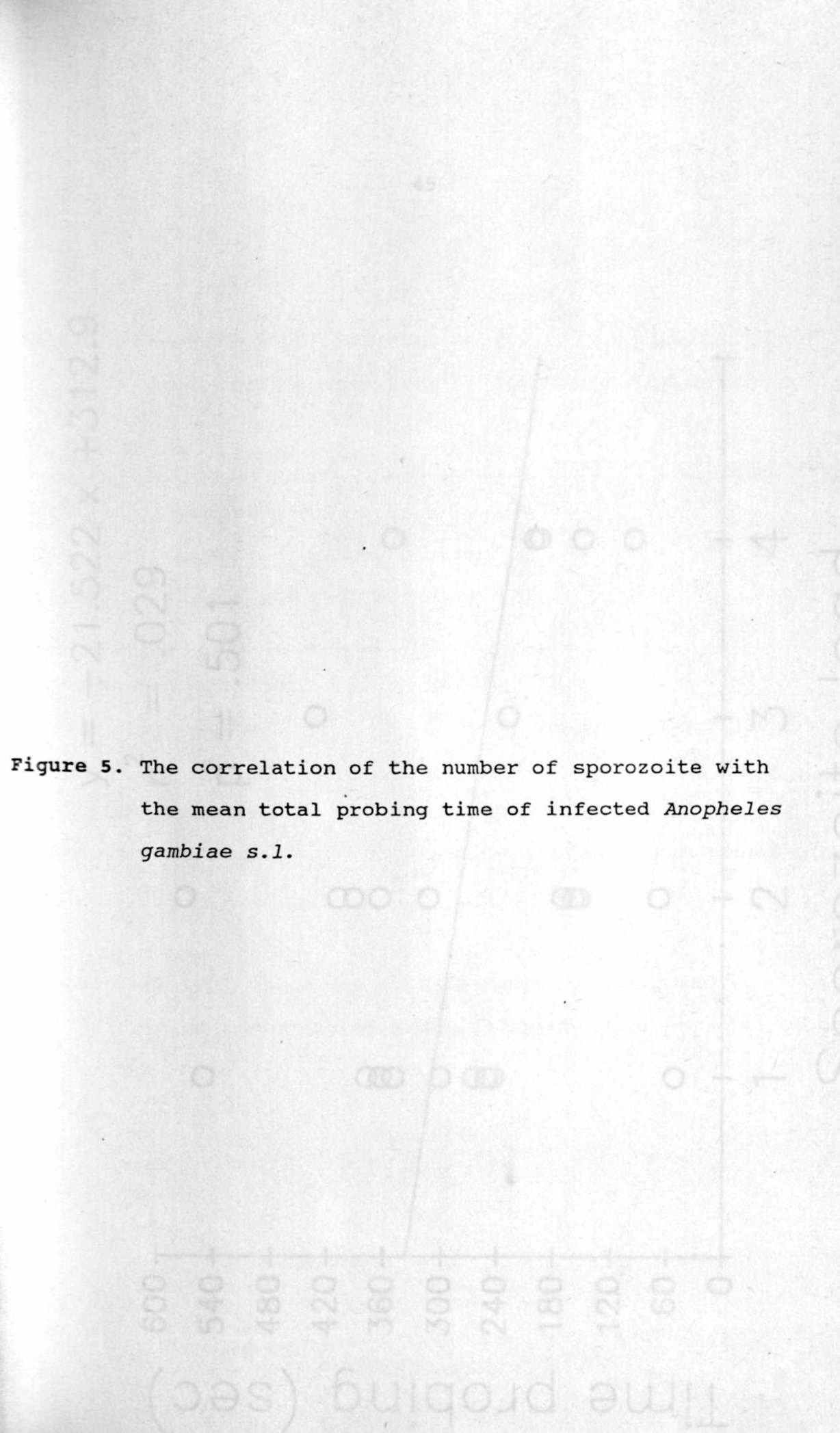
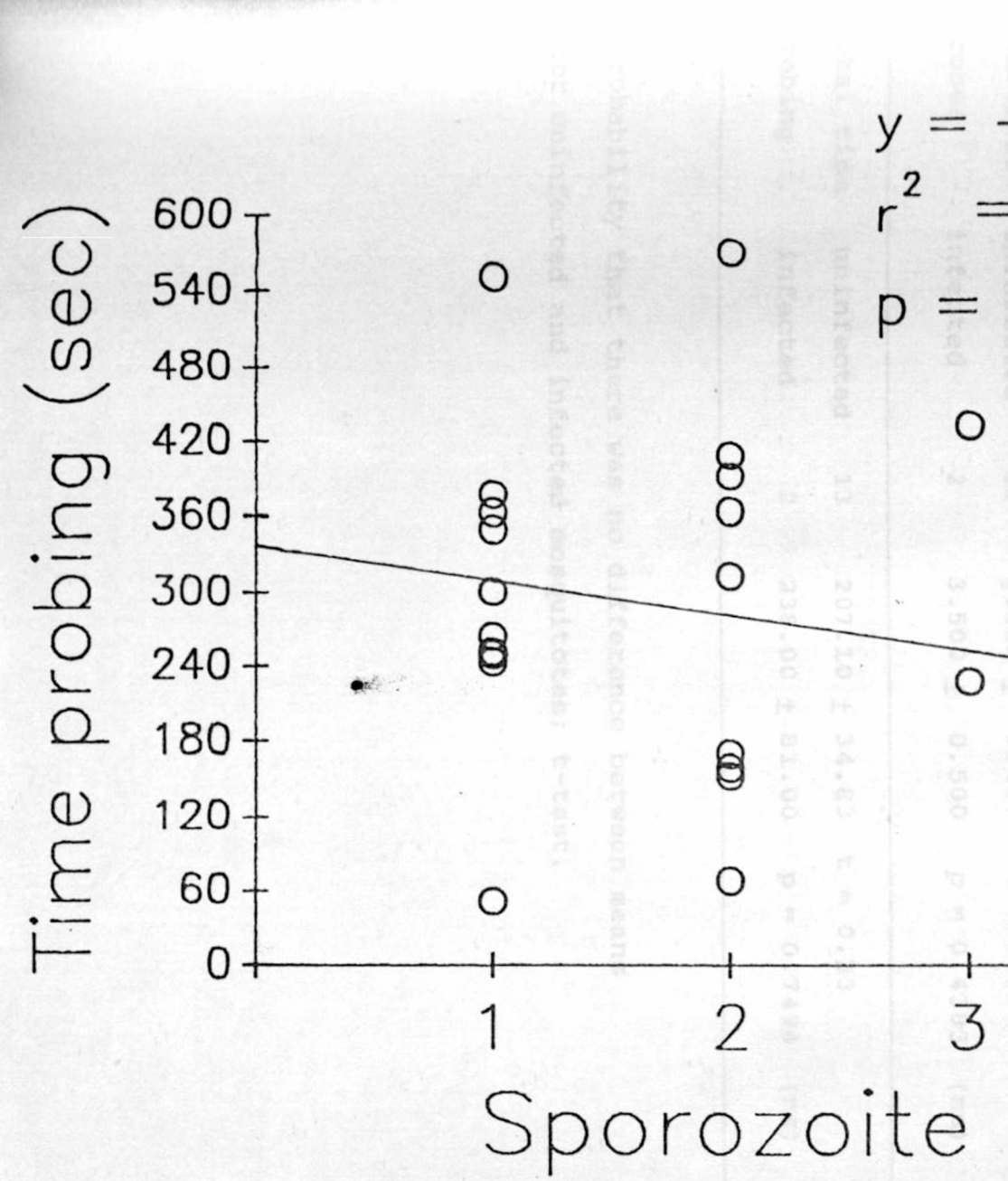


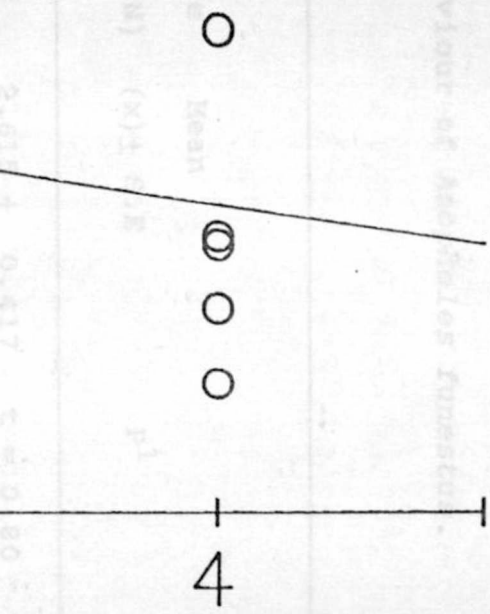
Figure 5. The correlation of the number of sporozoite with the mean total probing time of infected *Anopheles gambiae s.l.*


y =
r²
p =
Time probing (sec)
600
540
480
420
360
300
240
180
120
60
0
1
2
3
Sporozoite

$-21.522 \times +312.9$

$= .029$

$.501$



load

Table 6. The effect of infection of *Plasmodium falciparum* on blood feeding behaviour of *Anopheles funestus*.

Variable	Salivary gland condition	Sample size(N)	Mean (x)± S.E	P[1]
Number of Probes	uninfected	13	2.615 ± 0.417	t = 0.80
	infected	2	3.500 ± 0.500	p = 0.4383 (ns)
Total time Probing	uninfected	13	207.10 ± 34.83	t = 0.33
	infected	2	238.00 ± 81.00	p = 0.7494 (ns)

[1]Probability that there was no difference between means for uninfected and infected mosquitotes; t-test.

Table 7. Contingency table to show the effect of *Plasmodium* infection on the probing response of *An. funestus*.

Gland infection	Probed N	Probed %	Not probed N	Not probed %	X^2	P[1]
infection	2	40.00	3	60.00	=0.72	<0.001
uninfected	13	34.21	25	65.79		

[1]Fisher's Exact test.

3.3 Sporozoite rates.

Table 8 shows the sporozoite rates of anopheline species and their variation between dissection and *Plasmodium falciparum* ELISA. *An. gambaie s.l.* and *An. funestus*, unlike *An. ziemanni*, *An. coustani* and *An. pharoensis* were found to be positive both by dissection and ELISA test for *P. falciparum*.

Of the 983 *An. gambiae s.l.* dissected during the study period 48 were positive through dissection. Only 649 *An. gambiae s.l.* females were tested by ELISA for *P. falciparum* throughout the study period. Of these, 35 were confirmed to be positive. The sporozoite rate for *An. gambiae s.l.* determined by dissection was estimated at 4.88% while that determined by ELISA was 5.39% throughout the study period. Of the 137 *An. funestus* females dissected, 9 were positive for sporozoites during the study period. Of the 74 females of *An. funestus* tested by ELISA, 8 were positive for *P. falciparum*. All but one of *An. funestus* females that were sporozoite positive by dissection were later confirmed by ELISA to be positive for *P. falciparum*. The sporozoite rate for *An. funestus* was 6.57% as determined by dissection while the sporozoite rate by ELISA was 10.81% during the study period.

None of the 89 specimens of *An. ziemanni* and 12 females of *An. coustani* dissected during the study period were positive for sporozoites. Sixteen *An. ziemanni* and 10 *An.*

Table 8. Sporozoite rates of anopheline species caught in Kisian between December to February and March to June 1988-89 as determined by *Plasmodium falciparum* sporozoites detected by ELISA and dissection.

Species	no. positive by dissection	no. positive by ELISA	overall % positive
An. gambiae s.l.	48/983	35/649	5.14
An. funestus	9/137	8/74	8.01
An. ziemanni	0/89	0/16	0.00
An. coustani	0/12	0/10	0.00
An. pharoensis	0/7	0/2	0.00

coustani were tested by ELISA and all were found to be negative for *Plasmodium falciparum*. The seven specimens of *An. pharoensis* dissected during the study period were negative. They were confirmed negative by ELISA for *P. falciparum*.

3.4 Parity.

The interspecific variation in the parous rates of anopheline species showed that *An. funestus* had the lowest values (0.47) followed by *An. ziemanni* (0.49) and lastly *An. gambiae s.l.* (0.57). There was no significant difference between the number of probes made by parous and nulliparous uninfected *Anopheles gambiae s.l.* during the study period ($F = 0.45$, $df = 251$, $p > 0.05$). Also, there was no significant difference in probing time between uninfected parous and nulliparous *An. gambiae s.l.* ($F = 0.53$, $df = 96$, $p > 0.05$). Nulliparous *An. gambiae s.l.* probed for an average of 198.6 seconds compared to 222.8 seconds for parous females. Whether *An. gambiae s.l.* was parous or nulliparous did not influence whether or not an individual initiated probing ($x^2 = 0.34$, $p > 0.05$) (Table 9).

3.5 Blood meal identification.

Table 10 shows the feeding pattern and host range of anopheline species. Both *An. gambaie s.l.* and *An. funestus*

Table 9. The contingency table to show the relationship of parity status of *Anopheles gambiae s.l.* to the probing response.

Parity	number of probes probed	not probed	chi-square	p
Nulliparous	34	60		
			=0.34	=0.56
Parous	63	95		

Table 10. Identification of blood meals of *Anopheles* species by a direct ELISA test (percentage).

Host	*ANOPHELES* SPECIES				Total meals +ve per Host.
	gambiae s.l.	*funestus*	*ziemanni*	*coustani*	
Human	77.23	92.59	26.09	0	126
Bovine	10.57	3.70	26.09	50.00	21
Human/Bov.	5.69	3.70	8.70	0	10
Goat/Sheep	3.25	0	13.04	0	7
Cat	0.81	0	0	0	1
Dog	0.81	0	4.34	0	2
Chicken	0	0	17.39	50.00	5
Donkey	0	0	0	0	0
Unknown	1.63	0	4.34	0	3
Total no. tested	123	27	23	2	175

females preferred human to bovid hosts. *Anopheles ziemanni* fed equally on bovids and humans.

Of the 123 *An. gambiae s.l.* blood meals tested, 77.23% were human, 10.57% were bovid. Mixed blood meals (human/bovid) accounted for 5.7%. Blood meals from other hosts were 4.50% with goat/sheep having been 3.20% while cat and dog were 0.80%. Unidentified blood meals or those which had deteriorated beyond identification accounted for 1.63%.

A total of 27 blood meals of *An. funestus* were tested. Of these 92.59% were human, and 3.70% were bovid. Mixed blood meals (human/bovid) accounted for 3.70%. None of the blood meals tested was positive for other hosts. None of the blood meals were unidentified. A total of 23 blood meals of *An. ziemanni* were tested. Of these 26.09% were human, 26.09 were bovids, 13.04% were goat/sheep, 4.34% were dog and 17.39% were chicken. Mixed blood meals (human/bovid) accounted for 8.70%. Unidentified blood meals or those which had deteriorated beyond identification amounted to 4.34%. The analysis of *An. coustani* was inconclusive due to low sample size (Table 10).

3.6 Night-biting Collections.

In the months of December, *Anopheles gambiae s.l.* and *An. funestus* were the only anopheline species collected on human baits. The average man-biting rate/night was 14, 10 and 102 bites of *An. gambiae s.l.*, *An. funestus* and

culicines, respectively. In January, the number of bites/man/night decreased, and *Anopheles gambiae s.l.*, *An. funestus* and culicine species were biting at the rate of 7, 2, and 31 bites/man/night, respectively. *Anopheles ziemanni*, *An. coustani* and *An. pharoensis* appeared in the night-biting collection for the first time. The man-biting rates of these species were 1, 2 and 1 bite(s)/man/night, respectively.

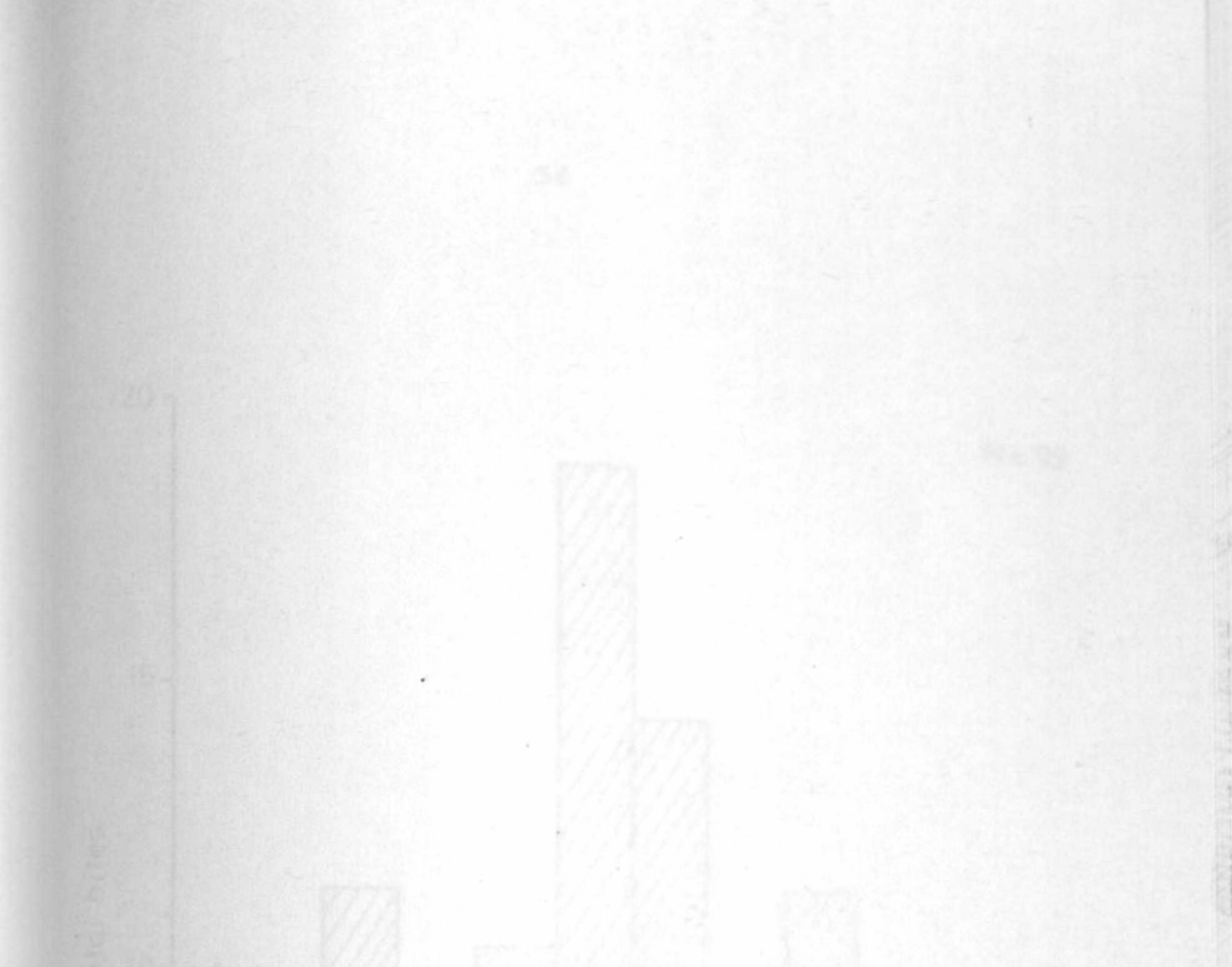
Between March and June (during long rains) four anopheline species were collected. During this period the man-biting rates among the species increased with the peak being in the month of May. *Anopheles gambiae s.l.*, *An. funestus*, *An. ziemanni* and *An. coustani* were biting at the rate of 48, 7, 12 and 2 bites/man/night respectively for the month of May.

The number of *An. gambiae s.l.* collected by the two human baits did not differ significantly throughout the study period ($t = 0.81$, $df = 27$, $p > 0.05$). The numbers for the other anopheline species was too low for any conclusive analysis.

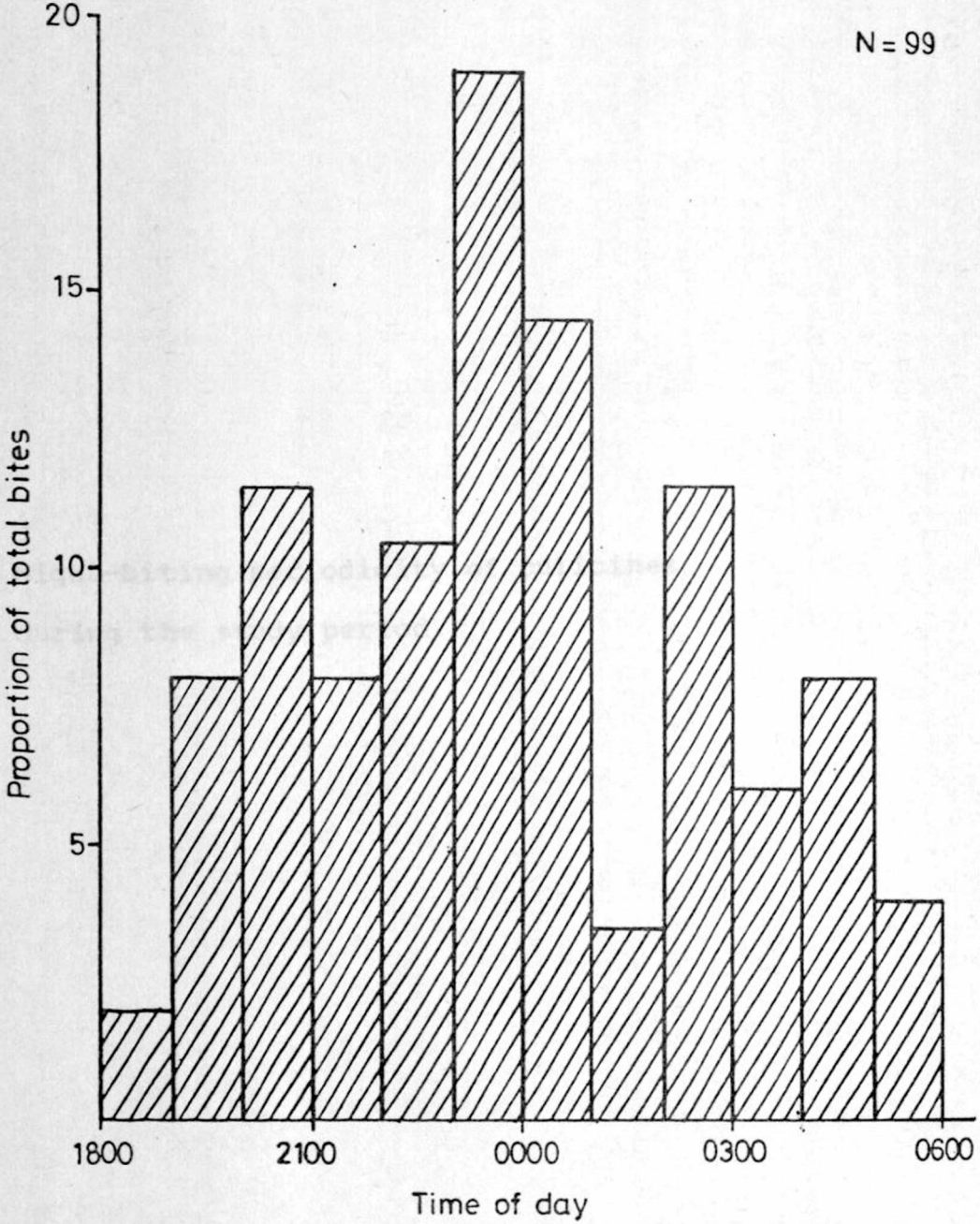
Figures 6 and 7 show the night-biting periodicity of *Anopheles gambiae s.l.* and culicine mosquitoes of Kisian villages throughout the study period. Females of *Anopheles gambiae s.l.* were biting in large numbers between 2300 and 0600 hours (Figure 6). The peak was between 0300 and 0600 hours throughout the study period. *Anopheles gambiae s.l.* females were biting almost continuously after midnight.

Females of *An. funestus* were biting intermittently throughout the night. The peak of activity of *Anopheles funestus* occurred between 0200 and 0600 hours throughout the study period. A high number of females were also biting between 2000 and 2100 hours; the biting rate decreased to the lowest level between midnight and 0200 hours.

The biting activity for culicines was low at 1800 hours and increased to the highest level at midnight then dropped off consistently to the lowest at 0600 hours (Figure 7). The peak of activity occurred between 2200 and 0100 hours throughout the night. The night-biting periodicity of other species were low and erratic. Insignificant numbers of *An. ziemanni, An. coustani* and *An. pharoensis* did not allow for analysis of night biting frequency.



e 6. Night-biting periodicity of *Anopheles gambiae s.l.* during the study period.


20
15
10
5
N = 99
Proportion of total bites
1800
2100
0000
0300
0600
Time of day

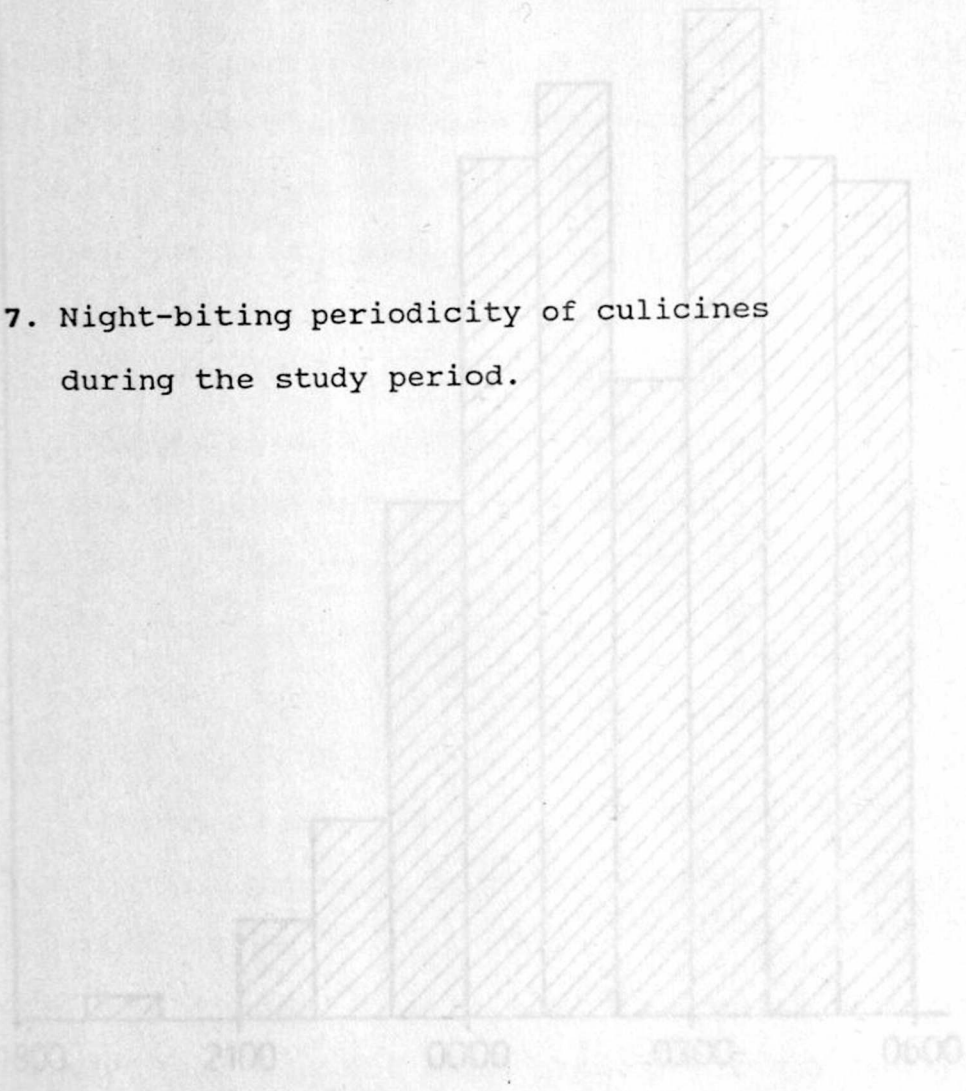
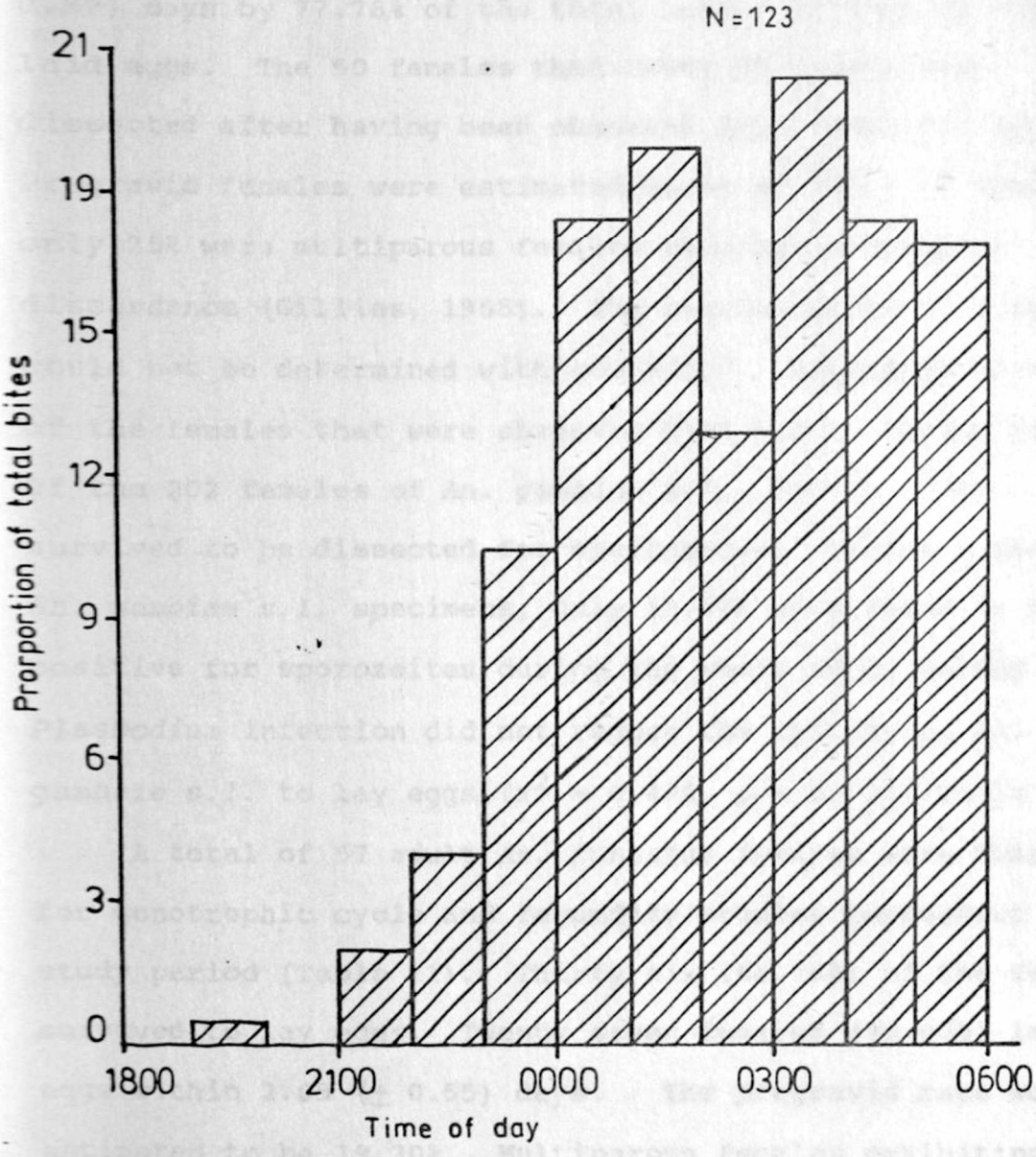


Figure 7. Night-biting periodicity of culicines during the study period.


N = 123
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Prorportion of total bites
1800
2100
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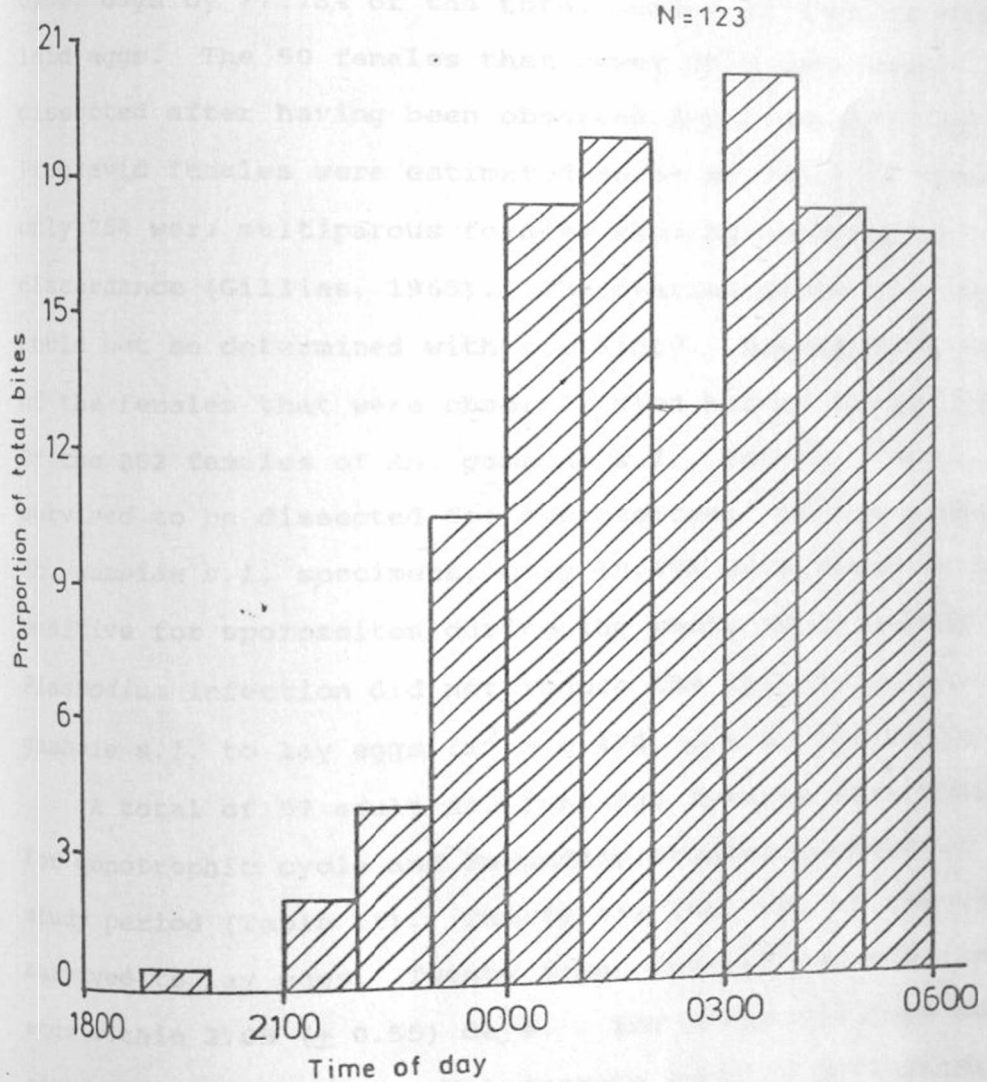
Time of day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Prorportion of total bites
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3.7 Gonotrophic cycle, Parity rate and fecundity.

A total of 202 adult females of *An. gambiae s.l.* were observed for gonotrophic cycle and fecundity studies during the study period (Table 11). Of the 202 females, 66.83% of the mosquitoes laid eggs. Eggs were laid within 1.91 (± 0.52) days by 77.78% of the total number of females that laid eggs. The 50 females that never laid eggs were dissected after having been observed for 4 days (96 hours). Pregravid females were estimated to be 25.74%. Of these, only 25% were multiparous females showing gonotrophic discordance (Gillies, 1955). The ovarian state of 6.23% could not be determined with certainty. Seventeen (8.42%) of the females that were observed died before laying eggs. Of the 202 females of *An. gambiae s.l.*, 185 (91.58%) survived to be dissected for sporozoites. Of the dissected *An. gambiae s.l.* specimens, only 10.89% were found to be positive for sporozoites during the whole study period. *Plasmodium* infection did not reduce the ability of *An. gambaie s.l.* to lay eggs ($x^2 = 0.478$, $p > 0.05$) (Table 12).

A total of 57 adult *An. funestus* females were observed for gonotrophic cycle and fecundity studies throughout the study period (Table 11). Thirty six (63.16%) of the females survived to lay eggs. Twenty seven females (75.00%) laid eggs within 2.05 (± 0.55) days. The pregravid rate was estimated to be 19.30%. Multiparous females exhibiting gonotrophic discordance accounted for 22.22% of the pre-

Table 11. The gonotrophic cycle in relationship with malaria infectoin of Anopheles species in the Kisian villages of Kisumu area. (note: percentage in parenthesis).

	A n o p h e l e s s p e c i e s.			
specimens	gambiae s.l	funestus	ziemanni	coustani
number observed	202	57	34	8
number +ve (sporozoites)	22	6	0	0
no. females laid eggs	35 (66.8%)	36 (63.16%)	17 (50%)	6 (75%)
gonotrophic cycle (days) ± S.E	1.91 ± 0.52	2.01 ± 0.55	2.69 ± 0.81	1.96 ± 0.04
pregravid rate	50 (25.7%)	14 (19.30%)	8 (23.53%)	0
gonotrophic discordance	25.00%	22.2%	33.3%	1%
no. died in 4 days	15 (7.43%)	10 (17.54%)	8 (23.53%)	3(37.5%)

gravid females. Seven females (12.28%) that were under observation died before laying eggs. Fifty (87.72%) *An. funestus* females were dissected for sporozoites, and a sporozoite rate of 12.0% was estimated for the study period. *Plasmodium* infection was found not to have any significant effect on the ability of *An. funestus* to lay eggs (x^2 = 0.138, $p > 0.05$) (Table 12).

A total of 34 females of *An. ziemanni* were observed for gonotrophic studies during the study period. Only 17 females survived to lay eggs. Of these 83.33% (15) laid eggs within 2.69 ($\pm$ 0.81) days. It was not possible to determine the pregravid rate with certainty. Eight (23.53%) of the females that were observed died before laying eggs. None of the females observed was positive for sporozoites after dissection.

Eight engorged females of *An. coustani* were observed for gonotrophic cycle studies during the study period. Of the six females that survived to lay eggs, 66.67% laid eggs within 1.99 ($\pm$ 0.26) days. It was not possible to determine the pregravid rate with certainty. The sample size of *An. coustani* was small for analysis for other variables.

Table 12. Contingency table showing the relationship of *Plasmodium* infection and the ability to lay eggs by *Anopheles* mosquitoes.

Species	Salivary gland condition	laid eggs N	laid eggs %	not laid eggs N	not laid eggs %	Chi-square	p
Anopheles gambiae s.l.	uninfected	103	62.4	62	37.6	=0.478	>0.05(ns)
	infected	16	69.6	7	30.4		
Anopheles funestus	uninfected	36	76.6	11	23.4	=0.138	>0.05(ns)
	infected	5	83.3	1	16.7		

3.8 Meteorological data.

The meteorological data was summarised as follows. The mean monthly temperature was 23.13 ± 0.18°C (average of daily minimum and maximum temperature) during the study period. The range of daily means was between 22.48°C to 24.06°C throughout the study period. The total monthly rainfall had a range between 24.5 mm in August 1988 to 202.3 mm in March 1989. The rainfall recorded for the period between June 1988 to May 1989 was 1307.5 mm as the total (annual) rainfall.

The mean monthly minimum relative humidity was 47.19 ± 1.21 (taken at 1200 hours) and the mean monthly maximum relative humidity was 68.53 ± 1.75 (taken at 0600 hours) between June 1988 and May 1989.

CHAPTER FOUR

DISCUSSION

Only five anopheline species were present in the study area. *Anopheles gambiae s.l.* was the most abundant species in all collections made by the different techniques. Both *Anopheles gambiae s.s.* and *An. arabiensis* have been identified in this area. According to the findings of Copeland (personal communication) *An. gambiae s.s.* comprises about 80% of *An. gambiae s.l.* population in Kisian and *An. arabiensis* makes up the rest. Kisumu area seems to be ideal for the breeding of *An. gambiae s.l.* with a small flood plain and numerous other transient sites.

Anopheles funestus was also abundant in the area. Both *Anopheles gambiae s.l.* and *An. funestus* have been shown to be important vectors of malaria in Kisian (Beier *et al.*, 1988). In the present study it was also found by both dissection and ELISA that both species were important vectors in this area. The small proportion of other anopheline species found in the study area and the fact that they had a higher preference for non-human hosts suggest that they are of minor or of no significant importance in the transmission of malaria in Kisian. This was confirmed both by failure to find sporozoites in their salivary glands after dissection and by negative ELISA tests.

The number of *An. ziemanni* collected by human-biting collection was higher than the sample collected by the hand-catch method. *Anopheles ziemanni* tended to feed indoors but rest outdoors due to its exophilic nature. *Anopheles ziemanni* seemed to be an opportunistic feeder considering that in a house with chicken, goat/sheep, guinea pigs and cats, mosquitoes were found to contain blood from most of these animals as well as cows and humans. In houses that were inhabited by humans alone, the blood meal attributed to humans was higher.

The rate of initiating probing activity among anopheline mosquitoes to anaesthetised hamsters was low. This was not surprising in the light of the fact that the frequency of blood feeding by confined wild *An. gambiae s.l.* and *An. funestus* is often low (R.S. Copeland and J. Beier, personal communication). Three explanations could be offered for the low probing and blood feeding responses. Firstly, wild mosquitoes select their hosts in a natural environment where there is no confinement. An acclimatization period of 5-10 minutes might not be long enough to provide a natural simulated environment for the mosquito. Friend and Smith (1977) reported that the second generation of wild mosquitoes reared in the laboratory were more acclimatized than their first generation parents. Therefore, the profound effect of caging and confining mosquitoes might tend to reduce their probing and feeding response. Secondly, lighting conditions during the test may

not have been optimal. Light is a component of mosquito feeding behaviour (Pilitt and Jones, 1972). Thirdly, hamsters are not a natural host for either *An. gambiae s.l.* and *An. funestus.*

Plasmodium falciparum-infected *An. gambiae s.l.* were observed to make significantly more probes than their uninfected counterparts. In addition, infected females probed for a longer time than did uninfected females. This study also showed that sporozoite-infected *An. gambiae s.l.* tended to locate a host and initiate probing more frequently than uninfected females. However, they were less efficient at getting blood than their uninfected counterparts.

This is the first report of parasite caused changes in feeding behaviour among wild insect vectors as far as the author is aware. Increased probing and increased probing time of mosquitoes has been observed previously in the laboratory. *Aedes aegypti* (L) infected by *Plasmodium gallinaceum* was observed by Rossignol *et al.* (1984, 1986) to make significantly more probes with a longer duration of probing than their uninfected counterparts. The mechanism of this behavioural alteration was demonstrated by Ribeiro *et al.* (1984) and Ribiero (1987). This workers observed that sporozoites of *P. gallinaceum* cause lesions in the apical acinus of *Ae. aegypti* (L) salivary glands responsible for secretion of salivary apyrase. Consequently, the effect results in reduced levels of salivary apyrase without, however, a corresponding decrease in the volume of saliva.

This enzyme has been shown to inhibit host haemostasis and shorten the duration of blood location by essentially improving the ability of mosquitoes to locate a blood vessel, thus, ensuring continuous feeding and engorgement (Ribeiro *et al.*, 1984). Sporozoite infection could reduce the probability of blood vessel location in host skin, thus, forcing other probing attempts or intiating events leading to a search for a new host for a blood meal. This could explain why infected *An. gambiae s.l.* had significantly more probes than uninfected females when exposed to hamsters and took a longer time with the proboscis in the host skin.

Increased probing and total probing time prolongs the duration of host-vector contact and thus enhances the chances of a vector transmitting the parasites to the host. Such an increased duration of contact may also lead to host irritation and disruption of a blood meal (Boreham and Garrett-Jones, 1973 ; Burkot, 1988). Rossignol *et al.* (1986) asserted that, in nature, a host with prior exposure to mosquito saliva will react to further contact by experiencing local irritation due to an immediate hypersensitivity reaction. Increased number of probes and intradermal probing time lead to irritation of the host, and the host in its defense may disrupt blood feeding by the vector. Walker and Edman (1986) and Edman *et al.* (1987), also provided data that supports host irritation as a cause of blood feeding interruption. In nature, mosquitoes search for different hosts in order to feed to repletion (Webber

and Edman, 1972). Since it has been established that a single probe can cause sporozoite transmission (Jeffery *et al.*, 1959; Shute *et al.*, 1976; Ungureanu *et al.*, 1976), then mosquitoes that elicit multiple probes from one or more hosts enhance the chances of malaria transmission. Data presented in this work seem to show that *An. gambiae s.l.* is a vector whose competence is enhanced by the infection of the salivary glands with sporozoites.

The feeding to repletion of sporozoite-infected *An. gambiae s.l.* and uninfected mosquitoes was not significantly different. This implies that once a vector has located a blood vessel there is no further hinderance to the mosquito from taking blood to repletion. Additionally, in this study *Plasmodium* infection did not affect the amount of blood the mosquito took. However, malaria infection significantly affected the initiation of probing by *An. gambiae s.l,* as infected females were more likely to initiate probing. The impaired ability of the infected female *An. gambiae s.l.* to feed to repletion in nature, due to defensive reactions by the host, may make infected females more avid in intiating probing on a second or third host.

Anopheles funestus feeding behaviour followed a similar trend as those of *An. gambiae s.l.* However, the small sample size probably obscured any significant differences between infected and uninfected females. Further studies on *An. funestus* are necessary.

Only two anopheline species were harbouring malaria parasites (*P. falciparum*) as indicated by ELISA test. *An. gambiae* had lower sporozoite rate (4.88%) than *An. funestus* (8.01%). These results agree with previous studies by Beier *et al.* (1987). Also, there was a high percentage of gametocyte carriers in the human population. A routine blood smear check of house occupants of the houses where the day resting collection was done showed almost 100% of the house occupants to be asymptomatic gametocyte carriers (Beier J., personal communication). This provides an ideal environment for the maintenance of intense malaria transmission.

Anopheles gambiae s.l. is probably the primary transmitter of malaria in the study area, since it was the most abundant species found indoors, had a high sporozoite rate, and showed a preference for human blood over other hosts. The high sporozoite rate was possibly enhanced by a short gonotrophic cycle and high parous rate. The gonotrophic cycle of 1.91 ± 0.52 days shows that *An. gambiae s.l.* took approximately two days to seek another blood meal after the first one. This meal was more likely to be from the human host, thus lending more support to the suggestion that *An. gambiae s.l.* is the primary transmitter of malaria in the study area. Regardless of their survival rate, sporozoite-infected *An. gambiae s.l.* had an increased contact with the host.

The mean number of probes per *An. funestus* female was as high as *An. gambiae s.l.* *An. funestus* also had an even higher sporozoite rate despite the small sample size. This demonstrates that during the study period, transmission of malaria attributable to *An. funestus* was probably high. *Anopheles ziemanni* and *An. coustani* had lower number of probes than *An. gambiae s.l.* and *An. funestus*. *An. ziemanni* had a long gonotrophic cycle and a low preference for human hosts. These observations among others suggest that there is little chance of this species being a malaria vector. *Anopheles ziemanni* and *An. coustani* have never been observed to be malaria vectors (Highton *et al.*, 1979).

Unlike in the laboratory, the wild mosquito population varies in age, as emergent mosquitoes join the population at various times of the year. The rate of hatching is higher in the wet season. There is a slight difference of age between infected and uninfected insects since more of the emergent specimens belong to the latter category. In this study, it was assumed that age did not have effect on the probing and feeding behaviour of *Anopheles* females. This assumption was supported by evidence which showed that the parity status of uninfected *An. gambiae s.l.* females did not affect probing and total probing time. Chamberlain and Sudia (1961) and Grimstad *et al.* (1980) showed that vector competence and behaviour of *Aedes aegypti* was not affected until after the mosquitoes were of the age of 4-5 weeks.

Of the 35 females of *An. gambiae s.l.* that were positive through dissection only 33 tested positive by ELISA for *P. falciparum*. This could be explained in two ways. Firstly, these specimens could have had *Plasmodium* infection that was not *P. falciparum*. This could have been of either *P. malariae* or *P. ovale* (Beier *et al.*, 1987). Secondly, the two specimens that tested negative by ELISA had oocysts in their gut but no corresponding sporozoites in their glands. For these specimens to test negative may imply that CS proteins had not migrated through the haemocoel into the thorax. Nine of the 259 females of *An. gambiae s.l.* that were negative through dissection when tested by ELISA for confirmation were found to be positive for *P. falciparum*. Beier *et al.* (1989) demonstrated that ELISA test overestimates malaria infection. Therefore this could be the case in this study. *An. ziemanni* and *An. coustani* were not infected by malaria during the study period. The same was true for *An. pharoensis*. *An. pharoensis* is known to be a secondary vector elsewhere in Africa (Foote and Cook, 1952; Ijumba 1988; Mukiama and Mwangi, 1989). It was unlikely that this species contributed to malaria transmission during the study period.

The results of the direct ELISA test on blood meals from *An. gambiae s.l.* and *An. funestus* suggest that humans were preferred as a source of blood over other hosts. The proportion of female anophelines of different species feeding on humans was higher than those feeding on bovids

and other hosts. Since only indoor resting mosquitoes were collected, such results were expected. The fact that a high proportion of indoor-resting females of *An. ziemanni* had fed on bovids implied exophagy because cattle were mainly kept outdoors. These species also showed high percentage of chicken and goat/sheep blood meals. These results were expected since the houses in which *An. ziemanni* were predominant had large numbers of sheep, cats, dog, guinea pigs and chicken, with cattle kept next to the house entrance. The blood meal results of *An. ziemanni* show the opportunistic feeding nature of the species. Chandler *et al.* (1975) observed that *An. ziemanni* is an opportunistic feeder.

The sporozoite rates for the sub-sample anopheline mosquitoes used in the gonotrophic cycle and fecundity study were higher than those observed during the entire study period. This could be explained by the observations made by Macdonald (1957). In his study he showed that keeping the mosquitoes in the laboratory after capture increases the number of sporozoite-positive mosquitoes since it allows the sporogony process to occur before dissection. *An. ziemanni* and *An. coustani* were not positive for malaria. Between 50%-75% of observed gravid anopheline females laid eggs. The mean number of eggs laid per female varied according to species of the vector, and the deposition of eggs was not affected by the amount of blood meal ingested. These observations are similar to the findings of Clements (1963).

However, these observations differ from the findings of Klowden and Lea (1978), and Leprince and Bigras-poulin (1988). They reported that fecundity was affected by the amount of blood meal taken by the vector and the vector size. Gillet (1967) showed that 3 ul of imbibed blood was the minimum amount necessary to initiate the maturation of the maximum number of eggs a mosquito could produce in one gonotrophic cycle. The 3 ul of blood is the threshold level and any blood volume above this does not increase the number of eggs laid per female. However, mosquitoes may lay fewer eggs than their potential with a blood meal of less than 3 ul. The partial blood meal category observed in this study may be above 3 ul threshold level reported by Gillet (1967). Also, *Plasmodium* infection did not affect whether or not anopheline mosquitoes laid eggs. However, Rossignol *et al.* (1986) observed that malaria infection reduces fertility in *Aedes aegypti*. They reported that the observed reduction of fertility did not occur due to parasite drain on the food reserves but rather was due to the inability of individual mosquitoes to take a full blood meal. They also demonstrated that infected *Aedes aegypti* (L) when allowed to engorge showed no difference in the mean number of eggs laid from the uninfected females. Clements (1963) also believed that the number of eggs laid per female decreased with the increase in age. Tesfa-Yohannes (1982) showed that *Aedes malayensis* decreases in egg productivity with increase in the number of gonotrophic cycles. Leprince and Begras-

Poulin (1988) observed that this decrease in fecundity is closely associated with the decrease and degeneration of the number of ovarioles. From the foregoing, reduction in mosquito fertility is probably caused by a number of factors acting in concert; it is yet to be shown how such knowledge will affect the epidemiology of malaria. Since infected mosquitoes are older, age and parasite related factors may both contribute to reduced fecundity. This is an area of research that is worth pursuing in the assessment of malaria transmission. Comparison of the egg output for infected and uninfected specimens was not done in this study.

The epidemiological implication of the modified frequencies of probing and probing time of mosquitoes by *Plasmodium* infection is not yet known. This study has established that as for *Plasmodium gallinaceum* in the laboratory, *P. falciparum* affects the probing and feeding behaviour of wild anopheline mosquitoes by increasing the number of probes and the total probing time. These effects on feeding behaviour are as pronounced in mosquitoes with light infections as in those with heavy infections indicating that the consequences of infection may be felt equally irrespective of sporozoite intensity. Since the current model of malaria transmission assumes a constant biting rate over the life of a mosquito irrespective of infection, the modification of feeding behaviour of infected vectors probably alters malaria dynamics. These results suggest that increased duration of contact of an infected

vector with a host probably limits feeding success, and increases the rate of host-vector contact per gonotrophic cycle. Any increase in host-vector contact associated with malaria infection of *Anopheles* probably serves to enhance the likelihood of malaria transmission. These modifications of vector-host interactions, therefore, suggests a reevaluation of the current methods of estimating vectorial efficiency of mosquitoes.

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