COMPARATIVE STUDIES ON MALARIA TRANSMISSION POTENTIAL AT A LAKESHORE AND A NEARBY INLAND SITE IN WESTERN KENYA

BY

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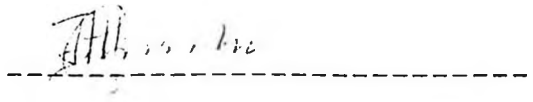
A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN THE UNIVERSITY OF NAIROBI

1992

DECLARATION

THIS THESIS IS MY ORIGINAL WORK AND HAS NOT BEEN PRESENTED FOR A DEGREE IN ANY OTHER UNIVERSITY.



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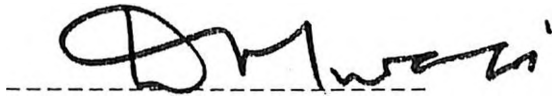

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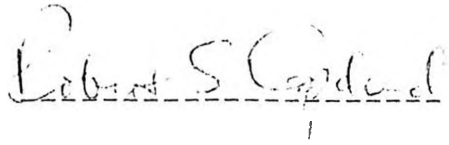

DR. ROBERT S. COPELAND

DEDICATION

To J. & M. & M.

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ACKNOWLEDGEMENTS

This research was conducted while I was under a WHO/TDR scholarship. It also received material assistance from the Kenya Medical Research Insititute (KEMRI)/Walter Reed research project of Kenya.

I sincerely and unreservedly thank my supervisors, Professor R.W. Mwangi of the University of Nairobi, and Doctor R.S. Copeland of Walter-Reed, for their wonderful supervision. I wish to extend my heart felt gratitude to Professor T.K. Mukiama, of the University of Nairobi for sparing his time to look at my polytene chromosome preparations.

A special word of gratitude goes to the Onyona family, and the Asiagos in particular, for having warmly received and accepted me to live among them as one of their very own. I am of course most grateful to the people of Kaksingri location in whose homes we carried out this research and who so patiently cooperated with us. Also deserving my many thanks are reseach assistants of Kaksingri for their dedication to mosquito catching, especially M.O. Agawo and J.A. Oswago for their help in various areas of endeavor. I thank E.O. Onyango of Kenya Medical Research Institute (KEMRI)-Kisumu, for helping with polytene chromosome preparations. I also thank the laboratory staff of the KEMRI/Walter Reed Project.

With no exception I extend my heart felt gratitude to all my classmates, more so to Betti, P.K. and Rotich, M.K.

Finally, from its inception up to its realisation, this research was ably guided by the stern task master and good friend Dr. R.S. Copeland. To him I shall for ever be indebted.

ABSTRACT

This study was conducted in Kaksingri location west of Homabay and south of Mbita point. It is located in Mbita division, South Nyanza district, Nyanza province, Kenya

The general objectives of the study centred around comparing the potentials of malaria transmission by Anopheles species between two sites located close to each other, but with different *Anopheles* breeding characteristics. *Anopheles* species found in the study area were identified, their seasonal abundancies determined, infection rates measured, and an attempt to determine their vector bionomics was made. In addition, nonhuman hosts for these vectors were identified. Malaria infection rates in the human population were also examined. *Anopheles* larval habitats were more directly dependent on rainfall at the inland site, Kaksingri East (KE), than at the lakeshore site, Kaksingri West (KW). In all collections, first instars predominated and pupae were the least found. KW represented a more stable larval breeding situation than KE. The lake played a major role in the breeding of mosquitoes in the study sites.

Five *Anopheles* species, *An. arabiensis*, *An. gambiae*, *An. funestus*, *An. pharoensis*, and *An. ziemanni* were identified at KW, while at KE, only 3 species were found; *An. arabiensis*, *An. gambiae* and *An. funestus*. *An. gambiae* was the most abundant species taken by all collection techniques, from both sites. Species identification following DNA amplification by polymerase chain reaction (PCR) showed that *An. arabiensis* and *An. gambiae* were the only members of the *An. gambiae* s.l. complex present in the two study areas.

There was a remarkable difference in the distribution of mosquito species between the two sites. The relative abundance of *An. gambiae* compared to *An. arabiensis* was higher in KE than in KW. Monthly population densities per house of *An. arabiensis* and *An. gambiae* were significantly different between the two sites. *An. funestus* was found in higher densities in KW than in KE, and comparison between sites was not possible for this species. Lagged total monthly rainfall was significantly correlated to the house resting density of *An. arabiensis* and *An. gambiae* in both sites. *An. funestus* showed positive but not significant correlation to the rainfall, in this site.

Night biting collections were only analysed for KW, because only a few specimen were captured at KE. For KW, there were seasonal differences in the night biting rates between species. *An. funestus* was biting more at night in August to November 1991, and by March to April 1992, this species was not found at all. *An. gambiae* on the other hand showed lower night biting rates in the period August to Novermber 1991, and highest in March to April 1992.

The only hosts for *Anopheles* mosquitoes identified in the study areas were humans and bovids. From blood meals smeared on filter papers, tests on other hosts were negative. From blood meal pellets, *An. arabiensis* and *An. gambiae* were more anthropophilic in both sites. *An. funestus* caught in KW were more zoophilic, based, however, on very little data. Feeding patterns were however different between the three *Anopheles* species within sites. Feeding patterns between sites were similar.

An. arabiensis and *An. funestus* were negative for *P. falciparum* Circumsporozoite Protein (*P. falciparum* CSP) by Enzyme Linked

immunosorbent Assay (ELISA) in KE. In the same site, *An. gambiae* infection rates by ELISA were generally high, and the peak followed the long rains. In KW, *An. gambiae* showed the same pattern of infection as in KE. In KW, *An. arabiensis* was infected more at the end of the two rainy seasons. *Plasmodium* infection rates by ELISA were significantly higher in KE than in KW. Sporozoites were not found by dissection in either site.

Parasitological investigations showed that the two study sites are malaria endemic areas. Prevalence rates were high in both sites ranging above 94% in children and above 80% in adults. There were high gametocyte rates in both age groups in both sites, which increased the chances of infecting feeding mosquitoes.

This study showed a difference in the malaria transmission potentials between the two sites. It is more risky to reside a short distance inland as opposed to residing on the lake shore itself.

INTRODUCTION

The relationship between mosquitoes and some diseases such as malaria, filariasis, and some arboviruses, has been known for a long time. Of all diseases transmitted by mosquitoes, malaria is by far the most important cause of both morbidity and mortality in man.

Despite the large number of mosquito species that occur throughout the world, only a small number of them transmit diseases to man. Those that carry and transmit malaria are only a few species of *Anopheles*. In Africa the most important malaria vectors are *An. gambiae* Giles *sensu lato* and *An. funestus* Giles. For transmi

ssion to occur, the female mosquito must feed on the blood of two different people; the first feed to acquire the parasite and the second to transmit it.

Malaria parasites belong to the genus *Plasmodium*. *Plasmodium falciparum* is responsible for most of the morbidity and mortality that is attributed to malaria, especially in Africa. Human *Plasmodium* species which are not as important epidemiologically as *P. falciparum*, are *P. malariae*, *P. ovale*, and *P. vivax*.

In nature, maintenance of malaria transmission involves a complex interaction of the mosquito vector, the human host, the disease organisms, and the environment. An understanding of this relationship is key to the control and eventual eradication of malaria. Control programmes must be based on sound knowledge of how these factors relate to one another. From an entomological point of view,

information on vector biology and the environment is crucial in drawing up genuine plans of action for interruption of malaria transmission. Such information must include identification and distribution of the vector in the concerned area. It should also include a good background knowledge of environmental factors bearing upon questions of when, where, and how these measures must be applied in order to break malaria transmission, with least disruption to other biological systems. An understanding of patterns of contact between a vector and the human host is essential. Assessment of vector-host contact at any given time and place makes it possible to predict epidemiologically important situations and be able to carry out corrective measures in good time.

In Kenya, many studies have been conducted on various aspects of malaria transmission. These studies have been carried out in different parts of the country. Western Kenya is one of the malaria endemic areas in Kenya. Vectors so far incriminated in the transmission of malaria in this region are, two members of the *An. gambiae complex* (*An. gambiae* Giles *sensu stricto*, and *An. arabiensis* Patton) and *An. funestus*. Other species also occur, but none have been incriminated in the transmission of malaria.

This study was conducted in Kaksingri location, Mbita division, Homa Bay district, South Nyanza province, Kenya (0° 30′ S, 34° 10′ E). This is a site on the shores of Lake Victoria. It receives marginal rainfall, confined only to certain months of the year. Droughts are common. Weather is influenced by the lake to a large extent. Temperatures are high, and humidity follows rainfall pattern.

Seasonality plays an important role in the transmission of malaria. This area appears prone to pronounced differences in malaria challenge due to the seasonal nature of vector breeding conditions. There has been no research carried out in this locality on malaria vectors prior to this study. The main aim of this study was to compare and contrast malaria transmission potentials between a site on the shore of Lake Victoria and a site three kilometres inland.

In this study, an attempt was made to identify *Anopheles* species found in the study area, vectors of malaria, seasonal changes in their abundance, their sporozoite rates, their resting, feeding, and breeding places. An attempt was also made to identify nonhuman hosts for these vectors. A survey was conducted to determine malaria parasite infection rates in the human population. Specific objectives were;

1. To identify *Anopheles* in two localities situated at varying distances from Lake Victoria

2. To determine and compare the relative densities and malaria parasite infection rates among *Anopheles* populations in the two localities

3. To study and compare the malaria vectors' breeding habits as well as resting and feeding behaviour

4. To determine the human-blood index among the various *Anopheline* mosquitoes

5. To compare *Plasmodium* infection rates between the human populations in the two localities

LITERATURE REVIEW

In 1902, Giles working in the Gambia, West Africa, identified and described *Anopheles gambiae* and *Anopheles funestus* Giles (Giles, 1902). Davidson and Jackson (1962), established through crossing experiments that *An. gambiae* was actually a species complex. These experiments were necessitated by the by the marked differences observed in different populations of these mosquitoes and their varied responses to control measures. Crossing experiments was the method used then, to establish the existence of a species, on the basis of reproductive compatibility. Following these experiments, they then split it into *An. gambiae* species A and B. A third species which was called C, had been discovered and described by Theobald in 1911 (Patterson, 1964). This was in addition to two salt water breeding species *An. melas* and *An. var gambiae* which had already been described (Davidson, 1962). It brought the number of *An. gambiae* species to five distinct species (Davidson, 1964; Mattingly, 1964; Patterson, 1964). White (1973), found and described yet another species. He found it breeding in mineral water swamps in Bwamba county, Uganda and called it species D. All the species A, B, C, and D have now been given full names (Mattingly, 1977). The specimen which was described by Giles (1902), from West Africa and later designated as species A, has retained the name *An. gambiae sensu stricto.* Species B is called *An. arabiensis* as it was described by Patton (1905). Species C has retained Theobald's (1911) name, *An. quadriannulatus.* Species D is called *An. bwambae*, as it was named by White (1973). *An. melas* Theobald, is still named as such since

Theobald described it in 1903. It is the salt water breeding species in West Africa and else where. Donitz (1902) described the member of the *An. gambiae* complex breeding in salt water in East Africa and called it *An. merus*.

Distribution of the *An. gambiae* complex and *An. funestus* in Africa south of the Sahara is fairly well known (Davidson, 1964; Giles and De Meillon, 1968). They occur from southern borders of the Sahara desert, down to central parts of South Africa, and between the East African coast, including the adjacent Indian ocean islands, and the West African coast. Within this area, various *An. gambiae* complex distribution patterns have been noted by different workers (Davidson *et al.*, 1967; Omer and Cloudsley-Thompson, 1970; Service, 1970a; White *et al.*, 1972; White, 1974; Chandler *et al.*, 1976; Krafsur, 1977; Service *et al.*, 1977; Highton *et al.*, 1979; Mosha and Subra, 1982; Mosha and Petracca, 1983; Petracca *et al.*, 1983; Miles *et al.*, 1983; Bafort, 1985; Ralisoa Randrianasolo and Coluzzi, 1987; Lombardi *et al.*, 1983).

In East Africa, the major malaria vectors are *An. funestus* and some members of the *An. gambiae* complex. In Kenya, three sibling species of the *An. gambiae* complex (*An. gambiae s.s.*, *An. arabiensis*; and *An. merus*) together with *An. funestus* are the major vectors of malaria. The other three species, *An. melas*, *An. quadriannulatus*, and *An. bwambae*, have not been found in Kenya (White, 1974). In Kisumu district, White (1972) and Petracca *et al.* (1991) worked on distribution patterns of sibling species of the *An. gambiae* complex. Highton *et al.* (1979), (In Nyanza province) examined the role of each

species in the transmission of malaria. Chandler and Highton (1975) worked on seasonal variations in the species composition in the rice fields in Kisumu. Service (1970b) identified *An. gambiae* complex species A and B by cytological means and noted their resting and host preferences. He found no difference in larval habitats between the two species, but that adults of species B were more exophilic than those of A. Prevalence and infection rates of *An. gambiae* by *Plasmodium falciparum* were studied by Joshi *et al.* (1975). Taylor *et al.* (1990) working in Western Kenya found that *P. falciparum* sporozoite infection rates, as determined by Enzyme Linked Immunosorbent Assey (ELISA), were, 0.4% for *An. arabiensis*, 9.6% for *An. gambiae*, and 6.1% for *An. funestus*. Beier *et al.* (1990a), while collecting baseline data for a malaria vaccine trial in the same area, found that the peak malaria transmission period in this area occured between April and July, during and immediately following the long rains. Ma *et al.* (1990) worked on differentiation of *An. gambiae* and *An. arabiensis*, comparing ELISA and cytotaxonomic techniques. They found that there was 98.4 % agreement between the two methods. Collins *et al.* (1988) found a 97.0% concordance between ELISA and cytotaxonomic methods.

Other *Anopheles* species found in Kenya are of less importance as vectors of malaria and have been described by various workers. Gillies and Furlough (1964) studied the behaviour of *An. parensis* Gillies in Malindi on the Kenyan coast. Mosha and Mutero (1982), Mosha and Subra (1982), Mosha and Petracca (1983), and Mutero *et al.* (1984) carried out ecological studies on *An. gambiae* complex along

the Kenya coast, and found *An. merus* to be markedly exophilic and exophagic. They found no *Plasmodium*-infected females of this species. This led them to conclude that this species is unlikely to be of any epidemiological importance in malaria transmission, under normal conditions. Surtees (1970) examined mosquito distribution and abundance in the rice growing scheme of Western Kenya. He recorded among other Anopheles species; *An. coustani* Laveran and *An. pharoensis* Theobald. Chandler *et al.* (1976) recorded *An. ziemanni* Grunberg and *An. pharoensis* in Kano plains. Foote and Cook (1952) in Mwea irrigation scheme had suggested that *An. pharoensis* could be an important malaria vector in rice growing schemes in Kenya, by virtue of its relative abundance. Mukiama and Mwangi (1989a) and Ijumba *et al.* (1990) found that *An. pharoensis* was the most abundant mosquito in Mwea after *An. arabiensis*. Other Anophelines identified in the same area by these workers were: *An. coustani*, *An. pretoriensis* Theobald, *An. rufipes* Edwards and *An. maculipalpis* Giles. White (1972) showed in Kisumu area that, *An. gambiae* and *An. arabiensis* were present in differing proportions, and that *An. funestus* was more abundant than *An. gambiae s.l.* Service (1970) in the same area studied the ecology of *An. gambiae* and *An. arabiensis*. He showed that *An. arabiensis* was more exophilic than *An. gambiae*. In the same area, Joshi *et al.* (1973) looking at the causes of occasional high counts in pyrethrum spray catches in huts sprayed with a residual insecticide, found that these high counts were due to animal-fed mosquitoes which had entered the sprayed houses at dawn for day time resting, after having fed outdoors. Due to the short time of exposure to insecticides between time of entry and

collection time, there were many mosquitoes still surviving. Joshi *et al.* (1975) surveyed *An. gambiae* species A and B prior to insecticide application in Kisumu area. He found that species A dominated throughout the year, but that populations of species B decreased relatively less in drier months than those of species A. A post insecticide-application survey for the same programme was carried out by Service *et al.* (1978) who found species B predominating in outdoor collections, while species A was highly endophilic, and that application of insecticides had caused an increase in both the degree of exophily in species B and in its relative numbers with respect to species A.

McCrae (1983) studied oviposition behaviour of fresh water *An. gambiae* in a coastal region and found that oviposition patterns in most cases depended on local conditions of blood feeding, temperature and distance from day resting sites, not on endogenous activity rythms of any circadian nature other than day time inhibitions. In Western Kenya, Beier *et al.* (1990 b) reared *Anopheles* larvae from dry soil collected from potential breeding places such as hoof prints and edges of temporal and permanent pools. They concluded that egg viability in dry soil may represent a significant survival mechanism for two species of the *An. gambiae* complex (*An. gambiae* and *An. arabiensis*). Mukiama and Mwangi (1989a) carried out larval studies on *An. arabiensis* in Mwea, where they estimated pupal productivity to be only 1% of total immature population. Wekesa (1990) studied the effects of *P. falciparum* on the feeding behaviour of naturally infected *An. gambiae* in Western Kenya. He found that infected female mosquitoes

tend to probe more frequently and longer than the uninfected ones.

Various workers have shown that *Anopheline* mosquitoes feed only during the night, and show distinct biting peaks which are characteristic for the species, locality, and prevailing weather conditions (MacClelland 1959). Members of the *An. gambiae* complex show peak biting times between midnight and sunrise (Mattingly 1977, Muirhead-Thompson 1956, Shelly 1973, Haddow and Ssenkubuge 1974). The exception to this pattern is *An. quadriannulatus* which exhibits a different feeding pattern by having its peak biting times before midnight followed by a decline to dawn (White 1974).

Garret-Jones (1964) defined the human blood index (HBI) as "The proportion of bites on man by a population of blood sucking insects". Gillies (1964b) reported that the preference for hosts in *An. gambiae* complex is genetically determined. Fontaine *et al.* (1961), Joshi *et al.* (1973), Service *et al.* (1978), Highton *et al.* (1979), and Beier *et al.* (1988), all carried out studies on feeding patterns of the principal malaria vectors: *An. gambiae*, *An. arabiensis* and *An. funestus*. They all found that, feeding by mosquitoes depended on various climatic factors such as temperature, rainfall, humidity, breeding sites, resting sites, etc.

Senior-White (1954) classified *Anopheles* mosquitoes according to their resting places. He introduced the term exophily to imply outside resting, and endophily to mean resting indoors. Gillies (1956) introduced the terms obligatory exophily or endophily to mean resting

outdoors or indoors exclusively, facultative exophily or endophily where resting outdoors or indoors is optional, and deliberate exophily or endophily where by mosquitoes are forced by external factors to rest either indoor or outdoors. Gillet (1971) defined anthropophily and zoophily, as vectors feeding on humans and other nonhuman vertebrates respectively. These resting and feeding behaviour patterns are important as they have a profound effect on the control of malaria vectors. It should be noted that failure to interrupt malaria transmission in most vector control programmes has been attributed partly to the deliberate avoidance of insecticide-sprayed-surfaces by members of the *An. gambiae* group (Pampana 1969, White 1974, Coluzzi *et al.*, 1977, Molineaux and Gramicia 1980, Man *et al.*, 1984, Mutero *et al.*, 1984, el Said *et al.*, 1986, Snow 1987, Marquetti *et al.*, 1990, Petracca *et al.*, 1991).

2. MATERIALS AND METHODS

2.1. STUDY SITE

This study was carried out in Kaksingri location which is situated approximately 40 Kilometres west of Homa Bay town, and about 12 Km south of Mbita point. It is found in Mbita division South Nyanza district, Nyanza province, Kenya. Kaksingri location is situated on the north-eastern shore of lake Victoria, 0° 30′ S and 34° 10′ E at 1302 M above sea level (Figure 1). The area receives marginal rainfall and soils are of volcanic ash type. Kaksingri is a flatland surrounded by hills on three sides and by water on the fourth side. It is bounded in the north by the Mukende and Gembe hills, Sumba hills in the east, and Ran'gwa hills in the south. It faces the Kaksingri bay to the west.

The area receives relatively little rainfall, most of it falling between March and June, during the long rains. Short rains occur in October. Temperatures are high for most of the year. Humidity depends on rainfall and lake influence.

The vegetation of Kaksingri is of the Savannah type, characterised by thorny shrubs, herbs, suffrutices, climbers and a few scattered Balanites and Acacia trees. The family Euphorbiaceae predominates in thickets. The ground is covered mostly by *Aloe* Spp. Grass species are found in few places especially in the hills. Dominant grass species are *Hyperrhenia* and *Setaria* Spp. In cultivated and abandoned fields, usual common farm land weeds such as *Lantana camara*, *Bidens pilosa* and *Gynandra* Spp. are common. On

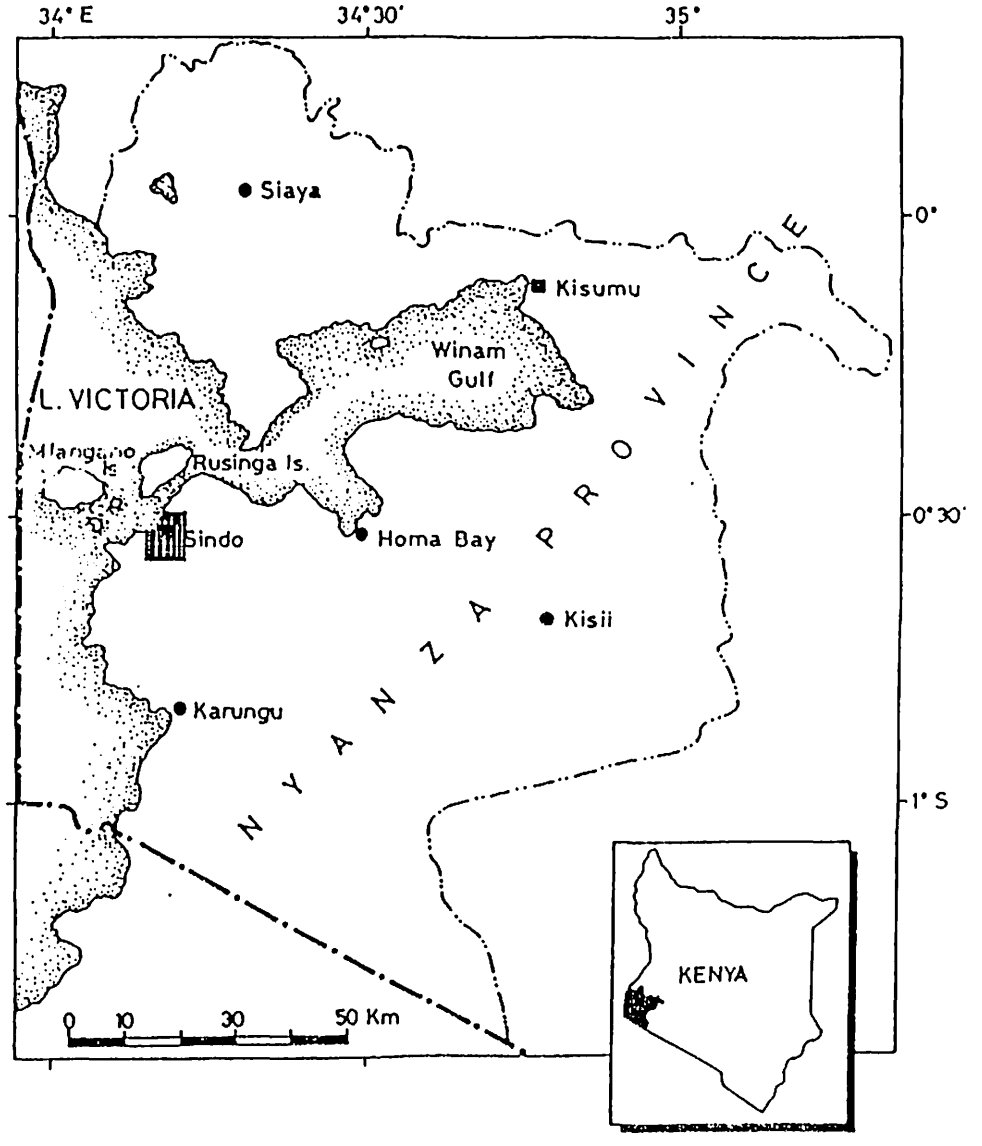
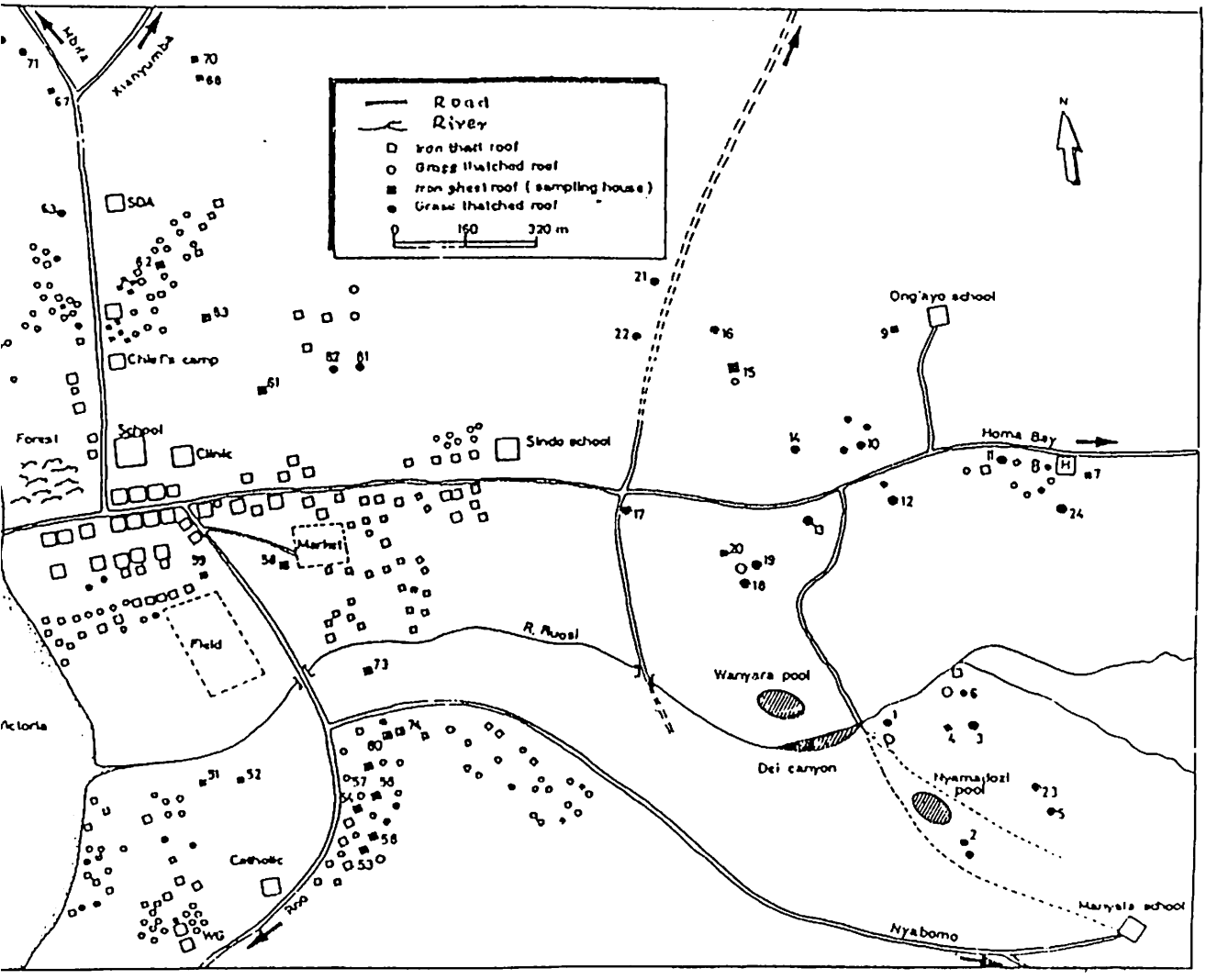


Figure 1. Map showing study site
(After Barthromew, 1985)

the lake shores where the soil is salty, the ground is covered by *Cynodon dactylon*, *Typha* Spp. and *Phragmites* Spp.

Soils are rich volcanic ash which resulted from a volcanic erruption in the Sumba hill system. The area slopes towards the lake making soil erosion a serious problem in the area. There are no permanent rivers in the area. However, there are two small springs where water trickles from rocks throughout the year. One of the springs is in the Gembe and the other in the Sumba hill systems. These springs do not produce enough water to flow down to the lake.

In comparison to other parts of the same district, human population density in Kaksingri is still relatively low, though there is a higher density in the Central parts of Kaksingri, particularly near the lake shore. People are commonly housed in mudwall-grass thatch type of houses. Other types of human dwellings are brickwall-iron roof, or brickwall-grass thatch, or mudwall-iron roof. This latter type is common at the commercial centre. All types of houses have large eaves between the roof and the wall. Eaves have an important bearing on malaria epidemiology, since mosquitoes can enter or leave the house, without obstruction. Homesteads are organised in family clusters called Bomas. Neighbouring bomas may be clustered together or seperated by up to 100 M. Calves, kids, lambs, and chickens are sometimes found in human sleeping quarters. At night goats and sheep are kept in enclosures made of wooden poles and thorns. Cattle are tethered in front of houses, whereas donkeys and dogs are left free to roam in the compound at night. Most people in Kaksingri are subsistence farmers, cultivating the same piece of land every year.



ure 2. Map showing location of adult mosquito sampling houses.

The most common crops grown are sorghum, millet, maize, and some cassava. Partial crop failures are not uncommon. Sisal is planted as hedge around Bomas and farms. Most families own cattle, sheep, and goats on a subsistence level. Donkeys, dogs, cats, turkeys and ducks are kept to a lesser extent. Fishing is done on a small scale. However, there is a small community which carries out fishing commercially. There is a fish trading organisation for women. Otherwise, most of the fish is sold to traders from outside the area. Social amenities available to the community are a health centre, schools, a market, and some shops. Infrastructure includes telephone lines and roads going in three directions. Water for all purposes is obtained from the lake or from local boreholes to a lesser extent.

The study was centred around two sites approximately three kilometres apart, one on the lake shore and the other inland. For the convenience of this study, these two areas were called Kaksingri east (KE) and Kaksingri west (KW). KW was located along lake Victoria shore, while KE was situated 3 Km inland (Figure 2).

2.2. MOSQUITO COLLECTION TECHNIQUES

Five techniques were used for sampling adult *Anopheles* mosquitoes; hand-catch method, night-biting collections, pyrethrum-spray catch, light traps, and exit window traps. Mosquitoes were identified as far as was possible on the day of collection. Some specimens were identified using Gillet's (1972) keys for mosquito identification. Confirmation was later done in Nairobi at Kenya

Medical Research Institute Laboratories.

2.2.1. Hand-catch method

Indoor collections were made using Hand-held aspirators to sample resting adults during daytime as described by Service (1976). Once each week, a total of 24 houses in each site were searched for 15 minutes each by a group of 4 searchers, each carrying an aspirator and a flash light. The search was conducted in KW on Mondays, and in KE on Tuesdays, between March 1991 and April 1992. Mosquitoes were placed into paper cups and transported to a central place. Houses were sampled in the morning between 0600 and 0900 hours local time.

From September 1991 to April 1992, Two pit shelters were also searched weekly for outdoor resting adults for 15 minutes. One in KW Mondays, and the other in KE on Tuesdays, at about 0800 hours local time. Hand-held aspirators were also used to sample mosquitoes in Kingfisher holes along a temporary river bank in Kaksingri west, in the morning around 0800 hours local time. The aspirator was sucked as it was being inserted into the hole. Eight holes were sampled from August to October, 1991. This procedure was discontinued after the birds migrated away from the site in October 1991.

2.2.2. Night-biting collections

All-night biting collections (Human bait collections) were conducted as described by Sevice (1976). Collections were made once

fortnightly in each location between September, 1991 and April, 1992. Four houses were selected, two in each site. The type of houses were selected such that all types were represented. In each house, two volunteers sat facing each other, each with a flash light and a test tube (WHO, 1975a). They collected mosquitoes which landed on them in the first 30 minutes of every hour. Additionally a similar collection was done outside one of the houses at each site. Collected mosquitoes were placed into pre-marked paper cups. At the end of the hour, the cup was put aside and left until morning, when the mosquitoes were either dissected or desiccated. Collections were made between 1800 and 0600 hours of the following day. All houses searched in this exercise had humans sleeping in them.

2.2.3. Pyrethrum-spray catch

Pyrethrum spray collections (PSC) were carried out in 8 houses, 4 in each site, following the method described by WHO, (1975a). Pyrethrum was diluted with kerosene to 0.3% and synergised with 0.1% piperonyl butoxide. PSC was conducted between 0700 and 0900 hours local time.

Two hand-held spray pumps with a cone spray nozzle were used to spray the insecticide in the houses. White calico sheets were used to cover all surfaces in the houses. After spraying a house, there was an allowance of 10 minutes before removal of sheets to ensure the knock down effect. Mosquitoes knocked down were picked up using a fine-tipped pair of forceps and placed into a petri dish lined with

moist filter paper. Mosquitoes were then transported to a central place for identification and processing. This exercise was carried out every week at both sites from November, 1991 to April, 1992. The same exercise was conducted in granaries, 4 in each site in Bomas where PSC was carried out. Granaries were only sampled in April and May , 1992; once every week. The same procedure as for PSC in houses was followed for granaries.

2.2.4. Light traps

Dry-cell operated CDC light traps were used in 4 houses, two in each site to trap mosquitoes which were attracted to light. Trapping was carried out monthly in each site. They were not used during the dry season (January and February, 1992). Traps were placed in rooms where people slept at night. They were hung from a string tied to roof rafters. Traps were operated for 12 hours from 1800 to 0600 hours, local time (WHO, 1975a; Service, 1976).

2.2.5. Exit window trap

Exit window traps were used as described by WHO, (1975a). They were used to sample adult mosquitoes exiting from houses. Eight traps were used, in 4 houses per site. Traps were set up at 1800 hours and removed the following day at 0600 hours, local time. Mosquitoes were removed with an aspirator. This exercise was conducted from August 1991 to April 1992.

2.3. Host census

Apart from the human population, almost every household in Kaksingri keeps some cattle which are usually tethered in front of houses at night. Sheep and goats are common. Donkeys are also found in some Bomas. Cats and dogs are present in some homesteads. Poultry is common in most households. Ducks and turkeys were only observed in KW. Wild animals such as Hyenas, antelopes, hares, and porcupines are common in Kaksingri. Others were tortoises, snakes, lizards, monitors and frogs. Wild birds include wild ducks, egrets, kingfishers, herons, kites, nightjars, hawks, falcons, doves, guineafowls, partridges, and many others.

2.4. Blood meal identification

Blood from engorged females from all collections was smeared onto a filter paper, using the head of an insect pin. One pin was used for a single mosquito (WHO, 1975a). Smears were desiccated and kept until they were tested for host identification by ELISA using the method of Beier(1988).

2.5. Sporozoite detection

Salivary glands were dissected out in phosphate buffered saline from female mosquitoes collected between September, 1991 and March, 1992. After examination, glands were washed into a 1.5 ml

tube, using 10 µl of saline buffer. The thorax of the same mosquito was cut and also placed into the same tube. Contents of tubes were sun-dried before being transfered to the desiccator. These were later used for sporozoite testing by ELISA (Beier, 1987).

To dried specimens in individual tubes, 50 µl of blocking buffer (BB) with Non-Ident P-40 (NP-40) was added. After one hour, salivary gland material was ground manually using a plastic pestle (one pestle per specimen). To the ground material, 200 µl of blocking buffer was added to bring the volume in each tube to 250 µl. Mosquitoes were then stored overnight at -20°C. Fifty microlitres of anti-sporozoite monoclonal antibody (MAb) solution (Pf2A10) was coated onto each well of an ELISA plate (u-shaped, 96-well microtitre plate). The plate was covered and incubated for 30 minutes at room temperature. MAb solution was removed by banging the plate onto absorbent paper. Wells were loaded with blocking buffer (approximately 200 µl per well). The plate was incubated for 1 hour at room temperature. Blocking buffer was removed and wells loaded with 50 µl of mosquito triturate. Seven positive controls consisted of two-fold serial dilutions of R_{32} Tet_{32} ranging from 100 picograms to 1.5 picograms. Eight negative controls (male *An. gambiae* mosquitoes) were also loaded. Plates were covered and incubated for 2 hours at room temperature. After this time, the triturate was removed and wells washed twice with PBS-Tween (polyoxyethylene-sorbitan monolaurate Tween-20). Then, 50 µl of *Pf* 2A10 Mab-peroxidase enzyme conjugate solution was added to each well, and the plate incubated for 1 hour at room temperature. The mab-peroxidase conjugate was then removed, and

wells washed 4 times with PBS-tween. To each well 100 µl of peroxidase substrate was added and incubated at room temperature for 30 minutes. The samples were then read at wavelength 414 nanometres (nM), using an ELISA reader (Dynatech-MR-5000, Quernsey Channel Islands).

2.6. Chromosome preparation

Polytene chromosomes were prepared from freshly collected mosquitoes whose ovaries in the abdomens were in Christopher's stage III-IV. Ovaries with eggs in Christopher's stage III-IV were dissected out in carnoy's fixative solution. They were transferred to orcein stain and squashed by thumb and tapped with the end of a pencil. The coverslip was sealed onto the slide using clear nail polish. Slides were then examined under a binocular microscope. The X-chromosome was used to identify members of the *An. gambiae* complex. Comparison was made against standard chromosome maps of Coluzzi and Sabatini (1967) (WHO 1975b). Confirmation of species identity was carried out at the University of Nairobi.

2.7. Polymerase chain reaction (PCR)

Mosquitoes collected and identified to be *An. gambiae s.l.* had their legs and wings removed, placed into a 1.5 ml tube, and desiccated. These samples were later processed by Polymerase chain reaction (PCR) for identification of *An. gambiae* sibling species (Paskewitz and Collins 1990).

2.7.1. DNA extraction

From preserved specimens of *An. gambiae s.l.*, DNA was extracted by adding 100 µl of a mixture of homogenization and lysis buffers in the ratio 4:1, in a 1.5 ml microfuge tube. Homogenisation buffer consisted of: 0.1 M NaCl 0.5g, 0.3 M sucrose 6.84g, 0.01 M EDTA 0.37g, 0.03 M trizima base 0.36g, and 100 ml sterile water pH 8.00. Lysis buffer comprised: 0.25 M EDTA 9.28g, 2.5% (W/V) SDS 1.88g, 0.5 M Trizima base 0.36g, and 100 ml sterile water pH 9.2. The material was ground manually using plastic pestles. This was then incubated at 65 over a water bath, for 30 minutes. Fourteen µl of potassium acetate was added, and samples were cooled on ice for 30 minutes. Specimens were then centrifuged at 15,000 RPM for 10 minutes. The supernatant was carefully decanted into another sterile microfuge tube. To the supernatant, 100 µl of cold 95 % ethanol was added and then, samples were cooled at -20°C for over 20 minutes. Samples were centrifuged at high speed for 20 minutes. The supernatant was discarded and 200 µl of cold 70 % ethanol was added, then poured off. A further 200 µl of 95 % ethanol was added and poured off. Tubes and contents were allowed to dry completely. Pellets were then suspended into 100 µl of sterile water for atleast 15 minutes.

2.7.2. DNA amplification

Nucleotide tri-phosphates (200 µl each), primers (0.7mg/µl

each), magnesium chloride (0.4M) and Taq polymerase (0.05 units/µl) were combined into a master mixture. To microtiter plate wells, 9 µl of the master mix was pipetted, followed by 1 µl of the mosquito triturate. Controls (extracted DNA of *An. gambiae* and *An. arabiensis)* were also loaded into wells. Wells were covered with mineral oil, and sealed with tape. The plate was placed into a DNA programmable thermal cycler (M J Research corporation). The cycler takes samples through 30 cycles of Denaturing (94 °C for 1 minute), Annealing (60 °C for a minute) and Polymerisation (extension)(74 °C for 1 minute). At the end of this process, the samples were held at 4°C.

Agarose gels (3%) containing ethidium bromide were used to electrophorese the amplified DNA. The loading-dye bromophenol blue was added to DNA samples which were then pipetted onto the gel. Electrophoresis was allowed to proceed until the bromophenol blue indicator had migrated at least 3 cm from the origin. Gels were read on a transilluminator. Individual samples were identified as *An. gambiae* or *An. arabiensis* by comparing the migrational distance of unknown with control samples of these two species.

2.8. Larval survey

Larval searches were made once every week within and around the study sites. Possible breeding sites were sampled using a standard 400 ml, plastic dipper; white in colour, with a wooden handle (1.5 M long). Intensive searches were made weekly in sites with standing water to determine the distribution of larval instars. Ten scoops were

made per potential breeding site, and results were noted (Service 1976). This exercise was conducted in both KE and KW between August 1991 and April 1992.

2.9. Malaria parasite rates

A Survey to determine malaria parasite rates in the human population was conducted at the end of the dry season in March 1991 in four primary schools. Thin and thick blood smears were collected by finger prick from school children (6-14 years). Smears were dried and later stained with Giemsa, before being examined for malaria parasites.

2.10. Weather

Rainfall was recorded using two types of rain gauge, one reading in millimetres and the other reading in inches in both sites. Recordings were made at the end of each day at about 1700 hours local time.

Temperature measurements were made every day at 16.00 hours local time, using a minimum/maximum mounted under the eaves of a house in the study areas.

A wet and dry bulb hygrometer was employed to make relative humidity measurements. Humidity was registered twice per day, at 0800 hours and at 1600 hours local time.

Both temperature and humidity measurents were made under eaves of a grass thatched house, only in KE.

3. RESULTS

3.1. Larval survey

Weekly immature *Anopheles* collections made in KE and KW during the study period are given in Table 1.

In KE there were 7 sites sampled every week (Figure 3). There were no immature stages of *Anopheles* caught, until October 1991, when 1.43 larvae per breeding site were captured. This represented 7.8% of the total catch in this site, throughout the study period. The next positive collection was recorded during April 1992, when 92% (N= 16.8) of the collection was made. In this site, pupae were only caught in April 1992. The pupal density was 0.57 pupae per breeding site (Table 1).

In KW, 6 sites were searched once every week (Figure 3). Larvae were caught from the breeding sites from August 1991 to April 1992, with an exception of two months (November 1991 and February 1992). March 1992 represented the lowest catch per breeding site (0.70), apart from the two months when none were found. The bulk of immature stages were caught in the months September 1991 and April 1992, when 13% (N= 2.14) and 52% (N= 9.76) were captured respectively. Pupae were only found in April 1992, giving the number of pupae per breeding site to be 0.83 (Table 1).

A total of 128 larvae were collected at KE, and 112 larvae were

Table 1. Number of *Anopheles* larvae and pupae per habitat collected in KE and KW from August 1991 through April 1992.

SITE	STAGE	AUG 1991	SEP 1991	OCT 1991	NOV 1991	DEC 1991	JAN 1992	FEB 1992	MAR 1992	APR 1992	TOTAL
K.E.	LARVE	0	0	1.43	0	0	0	0	0	16.86	18.29
	PUPAE	0	0	0	0	0	0	0	0	0.57	0.57
K.W.	LARVE	1.5	2.14	1.33	0	1.33	2.17	0	0.17	9.76	18.4
	PUPAE	0	0	0	0	0	0	0	0	0.83	0.83

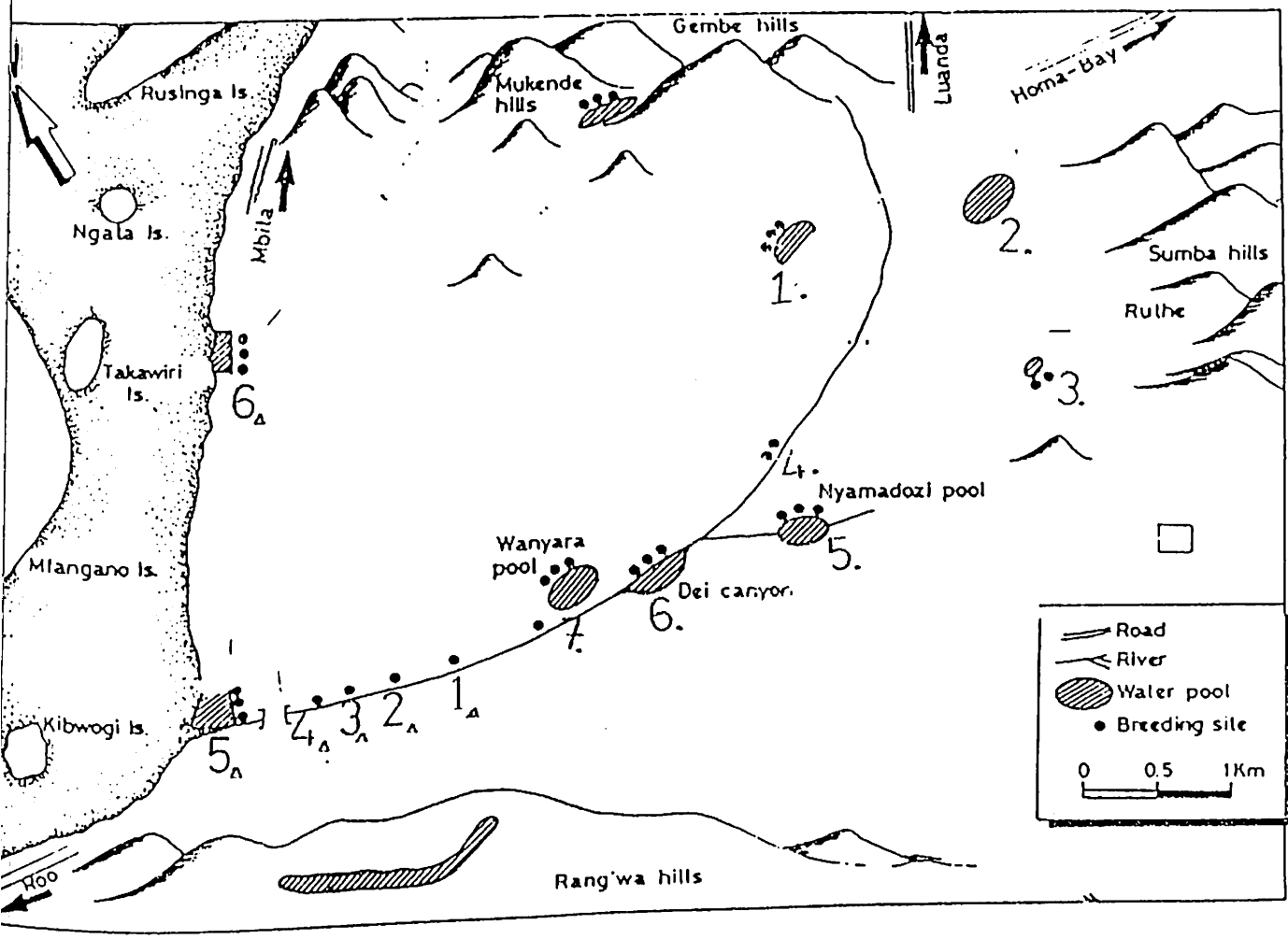


Figure 3. Anopheles larvae breeding sites.

collected at KW. The frequency distributions (as percentages) of different larval instars per site are given in Figure 4. In both sites more first larval instars were caught followed by second, third, fourth instars and pupae in that order. Greater than 10 times more first instars than pupae were found.

3.2.Species composition

Five *Anopheles* species, *An. arabiensis*, *An. gambiae*, *An. funestus*, *An. pharoensis*, and *An. ziemanni* were identified in the study area (Table 2). Two members of the *An. gambiae s.l.* complex (*An. arabiensis* and *An. gambiae*) were the only ones caught during the study period. Members of the complex were determined by two methods; PCR DNA amplification technique and cytotaxonomic method. In a few specimens (N=37), polytene chromosomes were used to confirm some of the identities by PCR. Out of these, 89% (33) squashes were readable. All the cytotaxonomic identifications agreed with PCR identifications.. All *An. gambiae* s.l.,were tested by ELISA for the presence of *P. falciparum* circumsporozoite protein(*P. falciparum* CSP). Mosquitoes which tested positive for the *P. falciparum* CSP were identified by PCR amplification of sample DNA, as either *An. arabiensis* or *An. gambiae* . Out of *An. gambiae* S.L.,which were negative for *P. falciparum* CSP,a subsample of atleast 20 individuals per site per week were identified to species by PCR. The numbers of *An. arabiensis* and *An. gambiae* captured per house in both sites were therefore estimated by extrapolation of the proportions of each

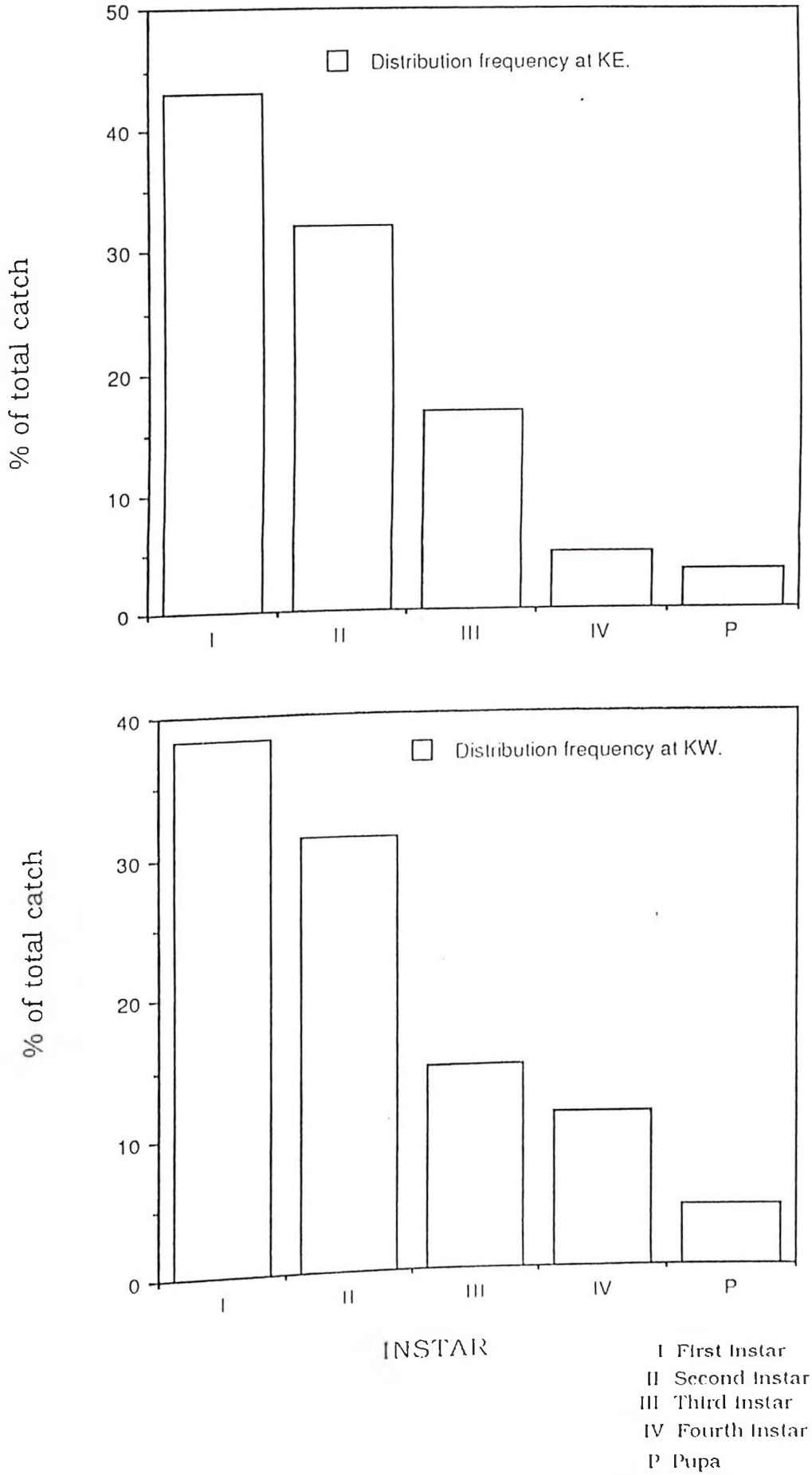


Figure 4. Distribution frequency of immature stages of Anopheles at KE and KW.

Table 2. Species composition of adult mosquitoes collected from February 1991 to April 1992 per site.

		COLLECTION TECHINIQUE								
SITE	SPECIES	RC	PSC	NBC	PIT	WINDOW	LIGHT	GRANARY	K/FISHER	TOTAL/ SPP
KE	An. arabiensis	107	1	0	0	0	0	0	-	108
	An. gambiae	1361	2	2*	1*	1*	1*	2*	-	1363
	An. funestus	3	0	0	0	0	0	0	-	3
	An. pharoensis	0	0	0	0	0	0	0	-	0
	An. ziemanni	0	0	0	0	0	0	0	-	0
	TOTAL	1471	3	2	1	1	1	2	-	1474
	Culicine	392	80	32	17	7	1	0	0	529
KW	An. arabiensis	369	26	2	0	0	0	0	0	397
	An. gambiae	836	48	8*	0	7*	4*	5*	2*	884
	An. funestus	104	42	8	0	1	0	5	0	160
	An. pharoensis	9	0	0	1	0	0	0	0	10
	An. ziemanni	2	0	0	0	0	0	0	0	2
	TOTAL	1320	116	18	1	8	4	10	2	1453
	Culicine	1303	658	584	91	24	161	0	0	2821

* = *An. gambiae sl.* not identified to species
RC=resting collection, PSC=Pythrethrum spray-catch,
NBC=Night biting collection

species from each site each week onto the total *An. gambiae* s.l. per week, for both *P. falciparum* and uninfected adults.

An. gambiae was the most abundant species in both study areas, making up 92.5% (N=1361) of *Anopheles* mosquitoes caught (N=1471), and 63.33% (N=836) of 1320 of all day resting collections in KE and KW respectively. *An. gambiae* made up 66.67% (N=3) and 41.38% (N=48) of the total PSC collections in KE and KW respectively. Human bait catches conducted from August 1991 to April 1992, yielded 2 *Anopheles* mosquitoes in KE, while in KW collections, 18 *Anopheles* were captured (Table 2).

An. arabiensis was the second most abundant species. It made up 7.23%, of day resting collections in KE. In KW it comprised 27.95% of day resting collections, and 22.41% of PSC catches, and 11.11% of human bait catches (Table 2).

An. funestus made up only 0.2% of day resting collections in KE. It was absent in all other catches in this site. In KW *An. funestus* comprised 7.88% of 1395 *Anopheles* from day resting collections, 36.21% of PSC and 42.86% of night biting collections (Table 2).

Two other *Anopheles* species were captured, and only in KW. *An. pharoensis* was caught from houses in day resting collections, of which it made up 0.68% of the catch. A single specimen was captured in an outdoor pit-shelter in the same site (Table 2). *An. ziemanni* was found resting indoors, where it made up 0.15% of the total *Anopheles* catches (Table 2).

Few mosquitoes were caught by other techniques. These were in numbers too small for meaningful analysis. In KE four exit window traps caught one *Anopheles* as did two light traps. PSC in granaries yielded two *Anopheles*. In KW exit window traps captured 8 mosquitoes, 7 *An. gambiae s.l.* and 1 *An. funestus*. Two light traps caught 4 *An. gambiae s.l.*, while 5 *An. gambiae s.l.* and 5 *An. funestus* were caught by PSC from granaries. In addition, two *An. gambiae s.l.* were caught by a special trap designed to catch mosquitoes emerging from Kingfisher underground nests. There was a significant difference in the total numbers of mosquitoes caught resting per house, between the two sites (X^2= 4.09, df= 1, $P<0.05$).

3.3. Population dynamics

Mosquito numbers captured per house per day during day resting collections are shown by month and site in Table 3.

In KE, a total of 1471 *Anopheles* resting in human dwellings were caught. From morphologic characteristics, 1468 (99.60%) were identified as *An. gambiae s.l.*, and 3 (0.20%) were *An. funestus*. Out of 1468 *An. gambiae s.l.*, 568 were tested by PCR. By extrapolation, *An. arabiensis* made up 7.29% and 92.71% were *An. gambiae*. *An. arabiensis* was found to have an average daily house resting density for the entire study of 0.03 mosquitoes per house and *An. gambiae* had 1.12 mosquitoes resting per house. *An. funestus* house resting density was determined directly, without extrapolation and was found to be 0.001 females resting per house. Daily house resting densities for each species are shown by month in figure 5.

In KW, 1320 *Anopheles* mosquitoes were captured from indoor resting collections. They were morphologically identified into 1205 (82.93%) *An. gambiae s.l.* and 104 (7.16%) *An. funestus*. The average daily house resting density for *An. funestus* was 0.08. In this site, 484 *An. gambiae s.l.* were identified by PCR. By extrapolation, 30.60% (N= 369) were *An. arabiensis* and 62.38% (N= 836) *An. gambiae*. For the entire study, average daily house resting densities of *An. arabiensis* and *An. gambiae* were 0.17 and 0.86 mosquitoes respectively. Monthly house resting densities per site are shown in figure 6.

Trends in mosquito numbers in KE over time shows that the

Table 3. Monthly mean daily catches of indoor resting *Anopheles* collected from February 1991 through February 1992 from human dwellings in KE and KW

SITE	MONTH	An. arabiensis	An. gambiae	An. funestus
KE	FEB. 91	0	0	0
	MAR. 91	0	0	0
	APR. 91	0.100	0.03	0.02
	MAY 91	0.445	0.375	0
	JUN. 91	0.305	4.015	0.01
	JUL. 91	0.295	6.885	0.01
	AUG. 91	0.010	2.96	0.01
	SEP. 91	0	0.29	0
	OCT. 91	0	0	0
	NOV. 91	0	0	0
	DEC. 91	0	0.01	0
	JAN. 92	0	0	0
	FEB. 92	0	0	0
KW	FEB. 91	0.049	0.024	0
	MAR. 91	0.230	0.163	0.01
	APR. 91	1.205	0.665	0.175
	MAY 91	0.88	2.930	0.070
	JUN. 91	1.10	4.415	0.185
	JUL. 91	0.605	0.805	0.160
	AUG. 91	0.125	0.170	0.160
	SEP. 91	0	0.075	0.105
	OCT. 91	0.031	0.010	0.01
	NOV. 91	0.016	0.026	0.01
	DEC. 91	0	0.052	0.01
	JAN. 92	0	0.042	0.01
	FEB. 92	0	0	0

bulk of the three major species were captured in the period April to July 1991. *An. gambiae* was most abundant followed by *An. arabiensis*. *An. funestus* was almost non-existent in this site. By September 1991, all species went down in numbers considerably. In fact from September to February 1992, only 3 *An. gambiae* were found. The other two *Anopheles* species were not caught.

In KW Figure 6 shows that most mosquitoes were caught between March and August 1991. There was a general drop around October to November 1991 in all three species. Numbers picked up again around mid-November 1991 to January 1992. By February 1992, there was another drop in the numbers. For individual species in this site;

An. arabiensis density was highest in the month of April 1991, and a smaller peak was again noted in July 1991. From September 1991 to February 1992, it was the least common species. The numbers of *An. gambiae* per house were higher than the other two species throughout the study period. Two peaks were observed; One spread over 5 months (March - July 1991), and the other a shorter surge in numbers was seen in September 1991. Numbers reduced to a low level for the remainder of the study period (October 1991- February 1992), though there was a small increase in December 1991. The numbers of *An. funestus* in this site was relatively lower than the other two species. Most individuals of this species were found between May and August 1991..

The relative proportions of *An. arabiensis* and *An. gambiae* were

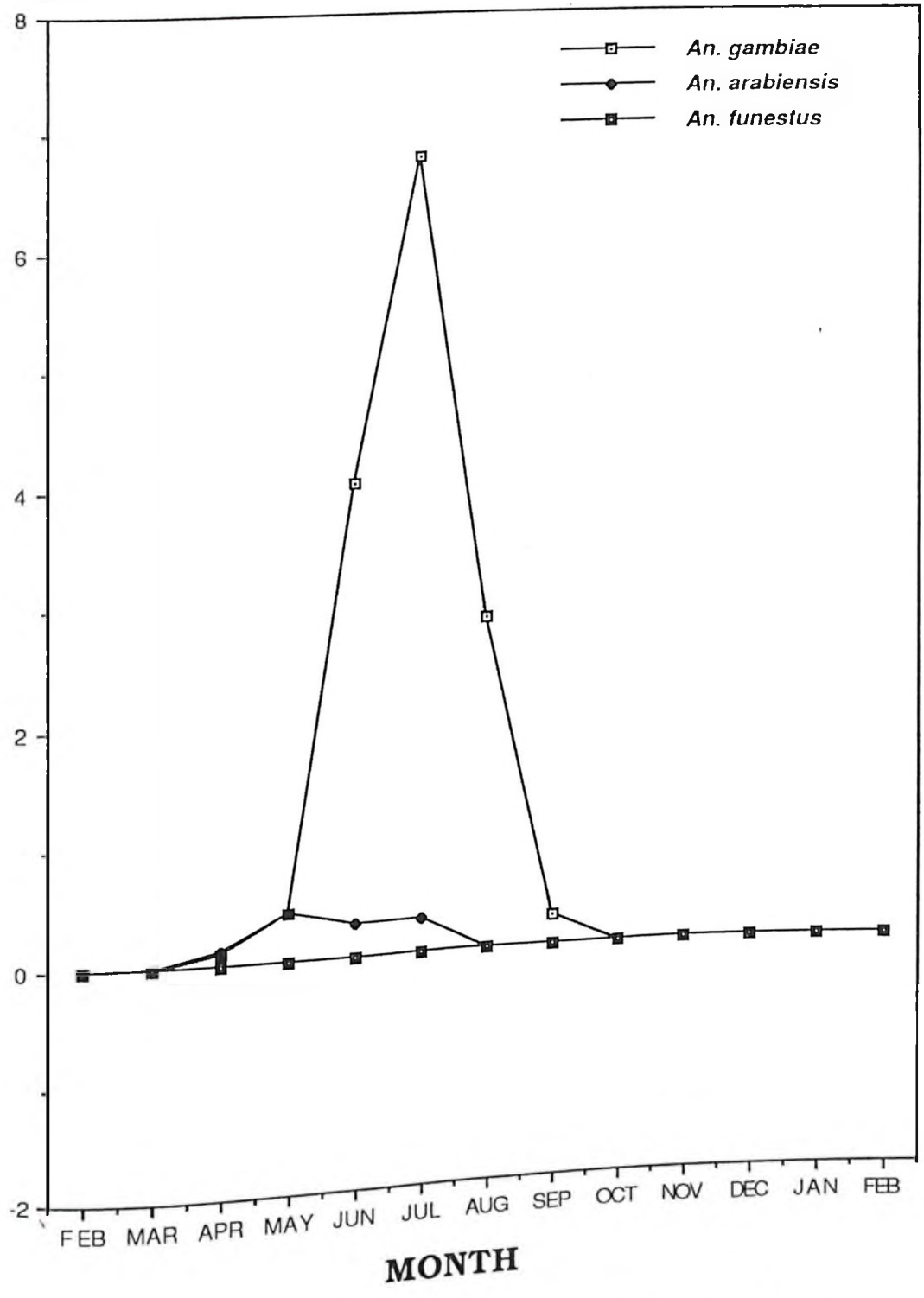


Figure 5. Mothly mean number of *Anopheles* collected per house at KE.

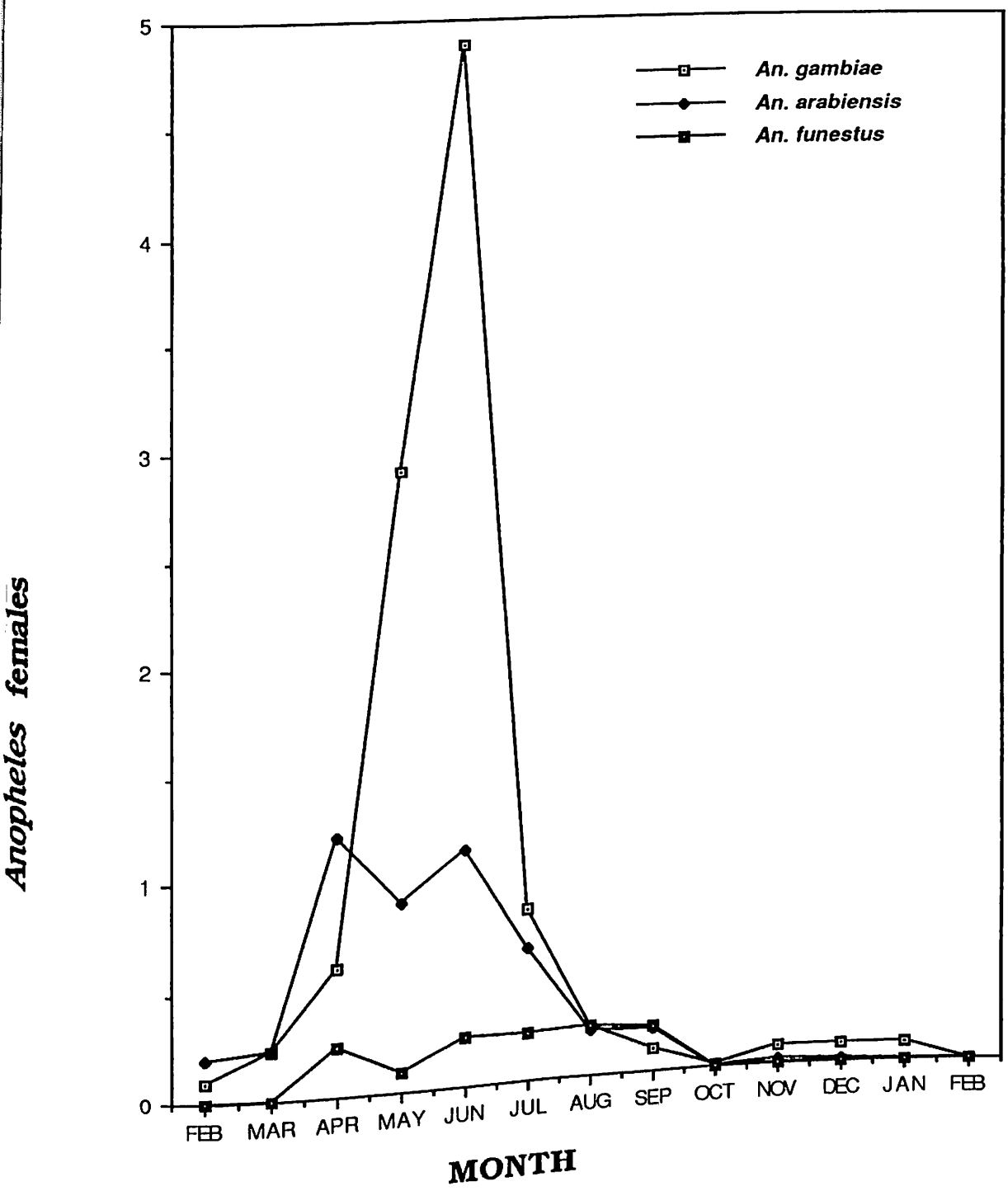


Figure 6. Monthly mean number of *Anopheles* females collected per house at KW.

significantly different between sites (X^2= 24.62, df= 1, P<0.05). *An. funestus* was found in low numbers, and so could not be included in this test.

The relationship between mosquitoes resting per house and the total monthly rainfall per site was examined by regressing mean daily resting density per month on the previous month's rainfall. This was to compensate for the time delay between commencement of the rains and the build up of mosquito densities. Comparisons were made for collections made between March 1991 and February 1992. Before analysis ,mosquito numbers of all species . except *An. funestus* in KW were transformed into logarithms to base 10 to normalise the data. The lagged comparisons were made in both sites for the period including the long rains up to the start of the short rains (Marh to Octobe 1991).

In KE, *An.gambiae* was positively and significantly correlated to the lagged monthly rainfall (r^2=0.64, P=0.020). *An. arabiensis* had a positive, but only marginally significant correlation to the rainfall (r^2=0.52, P=0.057). *An. funestus* , density was not correlated with rainfall, (r^2=0.096, P=0.620)

In KW, a similar situation was observed. *An. arabiensis* and *An. gambiae* had positive and significant correlations with monthly rainfall, whereas, density of *An. funestus* was not significantly correlated with rainfall. Correlation coefficient values for the three species in this site were; r^2=0.54, P= 0.048, r^2=0.70, P=0.012, r^2=0.18, P= 0.411, for *An. arabiensis*, *An. gambiae* and *An. funestus* respectively.

3. Gonotrophic condition

An examination of females found resting indoors in KE and identified as *An. arabiensis*, showed that, of a total of 39 captured, 57.89% were bloodfed, 18.42% each were half gravid and gravid respectively, and 5.26% were unfed. Of individuals identified as *An. arabiensis* and collected in KW, 47.46% were bloodfed, 26.73% were gravid, 21.66% half gravid and 4.15% were unfed (Table 4).

Day time collections of *An. gambiae* resting indoors showed 56.48% were bloodfed, 23.70% were gravid, 17.96% half gravid, and 1.85% unfed, in KE. In KW resting percentages were 54.13%, 27.07%, 16.24%, and 2.56% for bloodfed, half gravid, gravid, and unfed females respectively (Table 4).

In KW 136 female *An. funestus* were caught indoors. Of these, 53.68% were bloodfed, 23.53%, were gravid, 18.38%, were half gravid, and 4.41% were unfed (Table 5). In KE only 3 *An. funestus* females were caught.

Comparing the gonotrophic conditions, there was no significant difference in the indoor resting condition of either *An. arabiensis* or *An. gambiae* females between sites (X^2= 2.18, df= 3, P> 0.05 and X^2= 5.875, df= 3, P> 0.05) respectively. Only 3 *An. funestus* were found in KE, making a comparison with KW impossible.

Comparison of the gonotrophic condition of indoor resting mosquitoes within sites between the *Anopheles* species were not significantly different in KE (X^2= 5.65, df= 6, P> 0.05). In KW, there

was a significant difference in the indoor resting gonotrophic condition (X^2= 14.03, df= 6, P< 0.05).

Table 4. Gonotrophic condition of indoor day resting *Anopheles* females by site (*An. gambiae* s.l identified to species)

Site	Method	*An. arabiensis*					*An. gambiae*					*An. funestus*				
		E	BF	HG	G	T	E	BF	HG	G	T	E	BF	HG	G	T
KE	R.C	2	22	7	7	38	10	305	97	128	540	0	1	2	2	5
	P.S.C	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0
	TOTAL	2	23	7	7	39	10	305	97	128	540	0	1	2	2	5
KW	R.C	5	101	37	49	192	5	182	68	53	308	5	53	13	26	97
	P.S.C	4	2	10	9	25	4	8	27	4	43	1	20	12	6	39
	TOTAL	9	103	47	58	217	9	190	95	57	351	6	73	25	32	136

E =Unfed
BF=Bloodfed
HG =Half gravid
G =Gravid
RC =Indoor day resting collection
PSC =Pyrethrum spray catch
T = Total

3.6. Night biting collections

A total of 10 *An. gambiae s.l.*, 7 *An. funestus* and 584 Culicines were captured in 15 12-hour human bait catches between August 1991 and April 1992 in KW (Table 6), while only 2 *An. gambiae s.l.* and 32 Culicines were caught feeding on man in KE during the same period (Table 6). Because of low numbers of *Anopheles* caught, no meaningful analysis could be made out of the KE collections. Biting catches from KW were pooled into seasonal collections. Months August to November 1991 were considered as the short rainy season, December 1991 to February 1992 as the dry season, and March to April 1992 as the long rainy season.

There were no *Anopheles* caught feeding outdoors in either site. Man-biting rates per night were determined by dividing the total number caught per night by the number of houses from which collections were made per site (2 sites), and by the number of collections made per season.

During August to November 1991, one specimen of *An. gambiae s.l.*, 6 of *An. funestus*, and 230 Culicine species were caught in KW (Table 6). Man biting rates in this season were, 0.0625, 0.375, and 18.000 bites of *An. gambiae, s.l. An. funestus* and culicines respectively. In the following season (December 1991 to February 1992) The number of bites/man/night changed, and *An. gambiae s.l.*, *An. funestus*, and Culicines were biting at the rate of 0.167, 0.083, and 23.75 bites/man/night, respectively. In the long rains (March-April 1992)

Table 5. Seasonal night biting collections made between August 1991 and April 1992 in KW.

TIME (Hr)	AUG-NOV 1991			DEC 91-FEB.92			MAR-APR.1992		
	G	F	C	G	F	C	G	F	C
1830-1900	0	0	19	1	0	30	4	0	4
1930-2000	0	0	23	0	0	34	1	0	6
2030-2100	0	2	18	0	0	37	0	0	8
2130-2200	0	0	16	0	0	30	0	0	6
2230-2300	0	0	16	0	0	22	0	0	6
2330-0000	0	0	16	0	0	20	0	0	10
0030-0100	0	2	32	1	0	19	0	0	11
0130-0200	0	1	27	0	0	19	0	0	5
0230-0300	0	0	22	0	0	21	1	0	2
0330-0400	1	0	12	0	1	20	0	0	7
0430-0500	0	1	18	0	0	17	1	0	2
0530-0600	0	0	11	0	0	16	0	0	2
TOTAL	**1**	**6**	**230**	**2**	**1**	**285**	**7**	**0**	**69**

G =An. gambiae s.l; F =An. funestus; C =Culicines

the biting rates became 0.875, 0.00, and 11.25 for *An. gambiae s.l.*, *An. funestus* and Culicines respectively.

Figures 7 shows night biting periodicity of *Anopheline* and Culicine mosquitoes throughout the study period, although for *Anopheles* these were based on the behaviour of a few mosquiotes. Females of *An. gambiae s.l.* were starting to bite at about sunset and stopped feeding an hour before sunrise. A sharp increase in biting activity occured around sunset, and went on for about two hours, after which activity decreased until midnight, building up again to a second peak at about midnight. Subsequently, biting activity reduced to a low level and ceased altogether at 0500 hours local time (Figure 7).

Biting periodicity of *An. funestus* was generally similar to that of *An. gambiae s.l.* Peak biting time occured two hours after sunset, until 2100 hours. There was a reduction in the number of bites up to midnight. Biting activity remained high till about 0200 hours when it reduced and continued at a low level until dawn (Figure 7).

Biting activity of Culicines remained high throughout the night. Activities were highest between 1900 and 2100 hours. There was a decline before mid night, biting activity increased again and levelled out gradually until sunrise at 0600 hours (Figure 7).

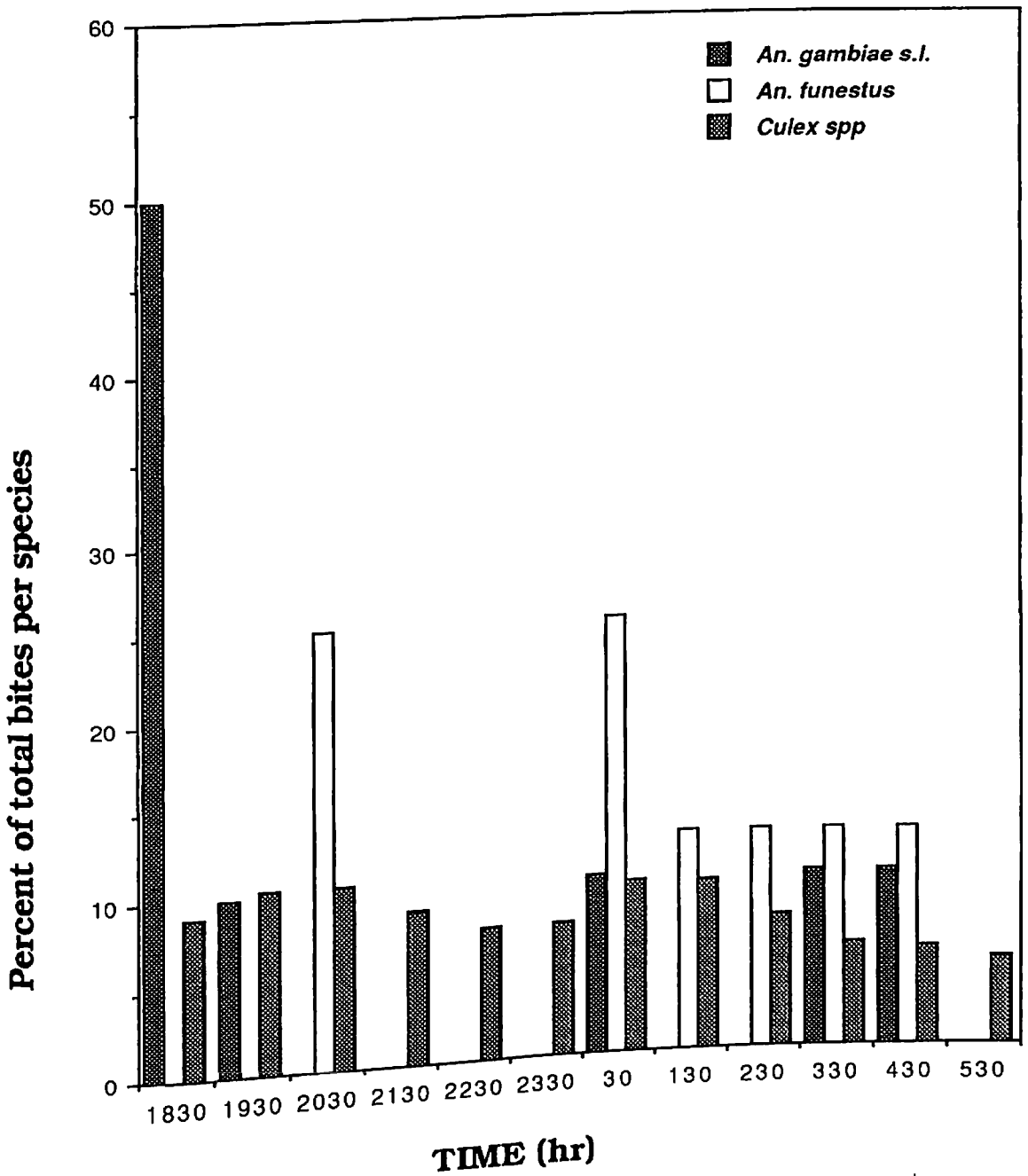


Figure 7. Night biting periodicity during the study period (August 1991- April 1992) at KW.

3.7. Blood meal identifications

Feeding patterns and host range for *An. gambiae s.l.* and *An. funestus* for Kaksingri are shown in Table 6. Thirty four (34) blood smears were tested. *An. gambiae s.l.* accounted for 38.26%, and 61.76% were *An. funestus*. The host range tested was human bovine, goat/sheep, dog and chicken. For *An. gambiae s.l.*, of 13 females, 53.85% had fed on man, 23.08% on bovines, while 23.08% blood meals were negative. *An. funestus* on the other hand had taken 71.43% blood meals from human, 23.81% from bovines, and 4.76% were negative (Table 7). Two *An. gambiae s.l.* tested were from KE out of which one had fed on man, while the other failed to react with antisera to any of these hosts.

Blood feeding patterns in *Anopheles* females examined at both study sites by direct ELISA consisted of protein pellets remaining after processing of mosquitoes for DNA extraction (Table 8). These pellets were tested by direct ELISA for the identification of mosquito hosts.

In KE of 14 *An. arabiensis*, 78.6% had fed on humans and the remainder on cows. There were no human-cow mixed blood meals by this species in this site. Humans made up 93.60% of the blood meals of *An. gambiae* from this site, while, 4.81% had cow blood and 1.60% mosquitoes had blood from both hosts. Two *An. funestus* females caught from this site had human blood (Table 7).

In KW, of 75 *An. arabiensis* females, 53.3% had human blood and

Table 6. Identification of Blood meal source by direct ELISA

HOST	*An. gambiae s.l*	*An. funestus*	TOTAL TESTED
HUMAN	7(53.85)	15(71.43)	22
BOVINE	3(23.08)	5(23.81)	8
GOAT/SHEEP	0(0)	0(0)	0
DOG	0(0)	0(0)	0
CHICKEN	0(0)	0(0)	0
OTHER	3(23.08)	1(4.76)	4
TOTAL	13	21	34

*Percentages are shown in parentheses
Filter paper blood smears

46.7% had cow blood. This species showed no mixed feeding. Of 168 bloodfed *An. gambiae* 94.1% had human blood, 4.76% had cow blood, and 1.2% had mixed blood meals. Of 9 *An. funestus* 33.3% contained human blood, while 66.7% had cow blood (Table 8).

Within site comparisons of human and cow feeding by *An. arabiensis* and *An. gambiae* were significantly different in both sites (X^2= 6.92, df= 1, P<0.05 and X^2= 61.71, df= 1, P< 0.05, for KE and KW respectively). There was no significant difference in blood feeding between the sites, for *An. arabiensis* (X^2= 3.07, df= 1, P>0.05 or *An. gambiae* (X^2= 1.04, df= 1, P>0.05). *An. funestus* was not included in the analysis because of small numbers of this species captured, especially at KE (Table 7).

Table 7. Percentage of human, cow, or mixed blood meals for *Anopheles* species collected by hand catch method indoors.

SITE	SOURCE	An. arabiensis	An. gambiae	An. funestus	Total
K.E.	HUMAN	11(78.57)	292(93.59)	2(100)	305
	COW	3(21.43)	15(4.81)	0(0)	18
	MIXED	0(0)	5(1.60)	0(0)	5
	TOTAL	14	312	2	328
K.W.	HUMAN	40(53.33)	158(94.05)	3(33.33)	201
	COW	35(46.67)	8(4.76)	6(66.67)	49
	MIXED	0(0)	2(1.19)	0(0)	2
	TOTAL	75	168	9	252

* Percentages are shown in parentheses

Blood meal prepared as pellets when preparing mosquitoes for PCR DNA extraction.

3.8. *Anopheles* infection rates

During the entire study period, all *Anopheles* which had been collected resting indoors and then desiccated, were tested by ELISA for the presence of *P. falciparum* CSP. *An. arabiensis* and *An. gambiae* infection rates over time for each species and their relation to mosquito densities per house per month from February 1991 to February 1992 were estimated by extrapolation. All *P. falciparum* ELISA positive *An. gambiae s.l.* were identified by PCR to species. A weekly subsample of *P. falciparum* ELISA negative *An. gambiae s.l.* were identified to species, and total uninfected females for each species each web was then determined by extrapolation.

In KE, 19.9% (N=1361) *An. gambiae* tested positive by ELISA for *P. falciparum CSP.* None of 107 *An. arabiensis* and 3 *An. funestus* tested positive for *P. falciparum* CSP.

In KW, 6/375 (1.60%) *An. arabiensis* tested by ELISA were positive for *P. falciparum* CSP. For *An. gambiae*, 68/836 (8.24%) were infected. Out of 104 *An. funestus* 6 were positive, giving an infection rate of 5.77%. None of 9 *An. pharoensis* and 2 *An. ziemanni* tested positive for *P. falciparum* CSP.

In KE, Figure 8 shows the monthly mosquito densities per house and monthly infection rates for *An. gambiae*. It was not possible to make the same comparison for *An. arabiensis* and *An. funestus*, since

there were no infected females found in these two species in this site.

Figures 9, 10, and 11 show monthly mosquito densities and infection rates in KW for *An. arabiensis*, *An. gambiae*, and *An. funestus*.

P. falciparum CSP infection rates in *An. gambiae* were statistically different between the two sites (X^2 = 53.12, df= 1, P<0.001). There was a significant difference in the infection rates between the 3 *Anopheles* species in KW (X^2= 19.30, df= 2, P< 0.001). Also in KW, infection rates between *An. gambiae* and *An. arabiensis*, without *An. funestus* were significantly different (X^2 = 19.26, df= 1, P<0.001).

Infection rates of *Anopheles* mosquitoes were also estimated by dissection and later the same specimen were confirmed by ELISA test. None of 4 *An. arabiensis* and 6 *An. gambiae* dissected in KE were positive for *P. falciparum* sporozoites. Similary, in KW, 36 *An. arabiensis* 50 *An. gambiae*, and 61 *An. funestus* dissected were all negative for sporozoites. Salivary gland material from dissection slides and thoraces were preserved, and later tested by ELISA for *P. falciparum* CSP. Only 1 (1.64%) *An. gambiae* captured in KW negative by dissection tested positive for *P. falciparum* CSP. *An. arabiensis* and *An. funestus* in this site were negative by ELISA. In KE, both *An. arabiensis* and *An. gambiae* which were the only *Anopheles* species dissected were negative by ELISA for *P. falciparum* CSP.

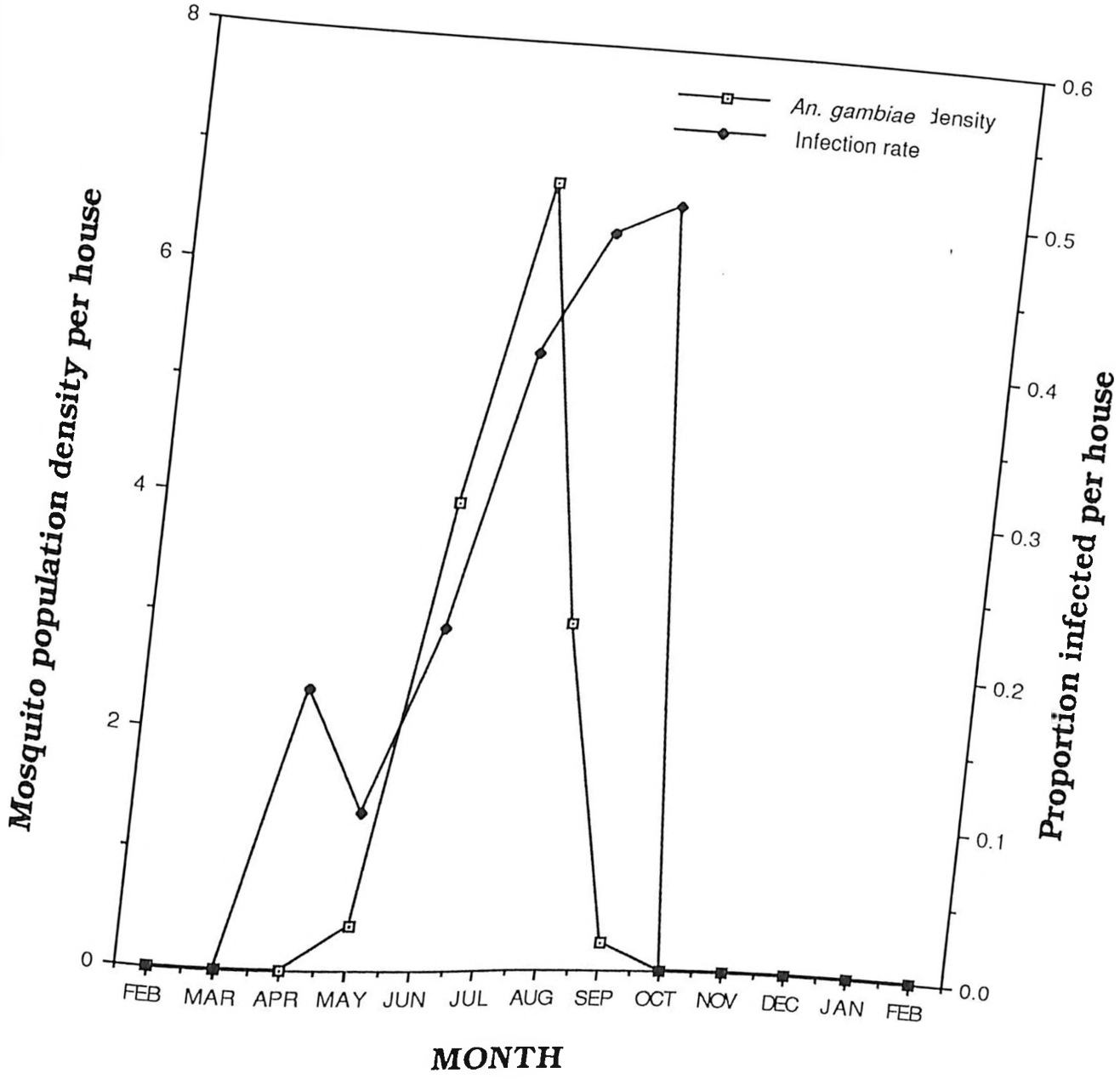


Figure 8. Monthly *An. gambiae* mean densities per house and infection rates at KE.

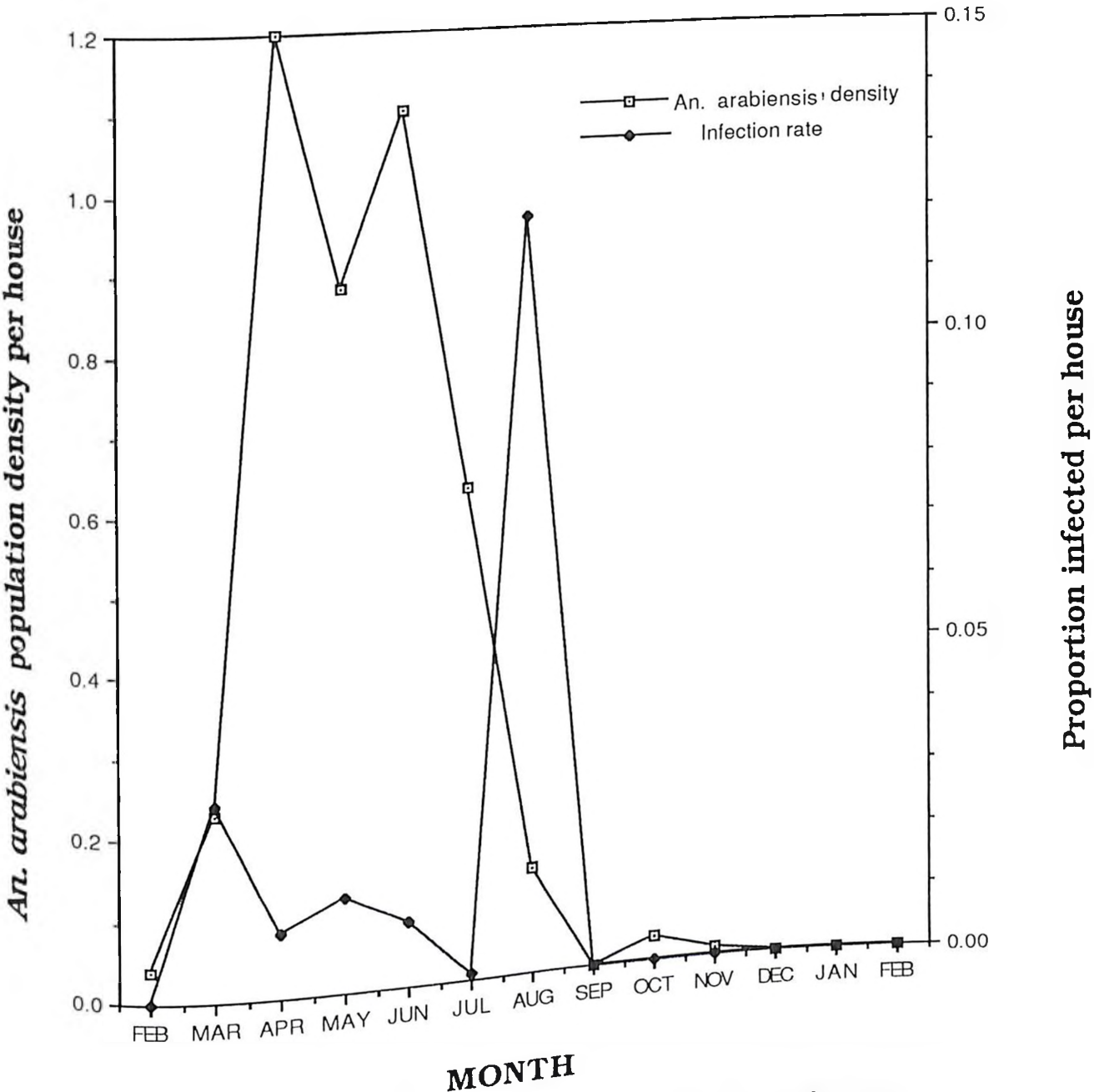


Figure 9. Monthly *An. arabiensis* mean density per house and proportion infected at KW.

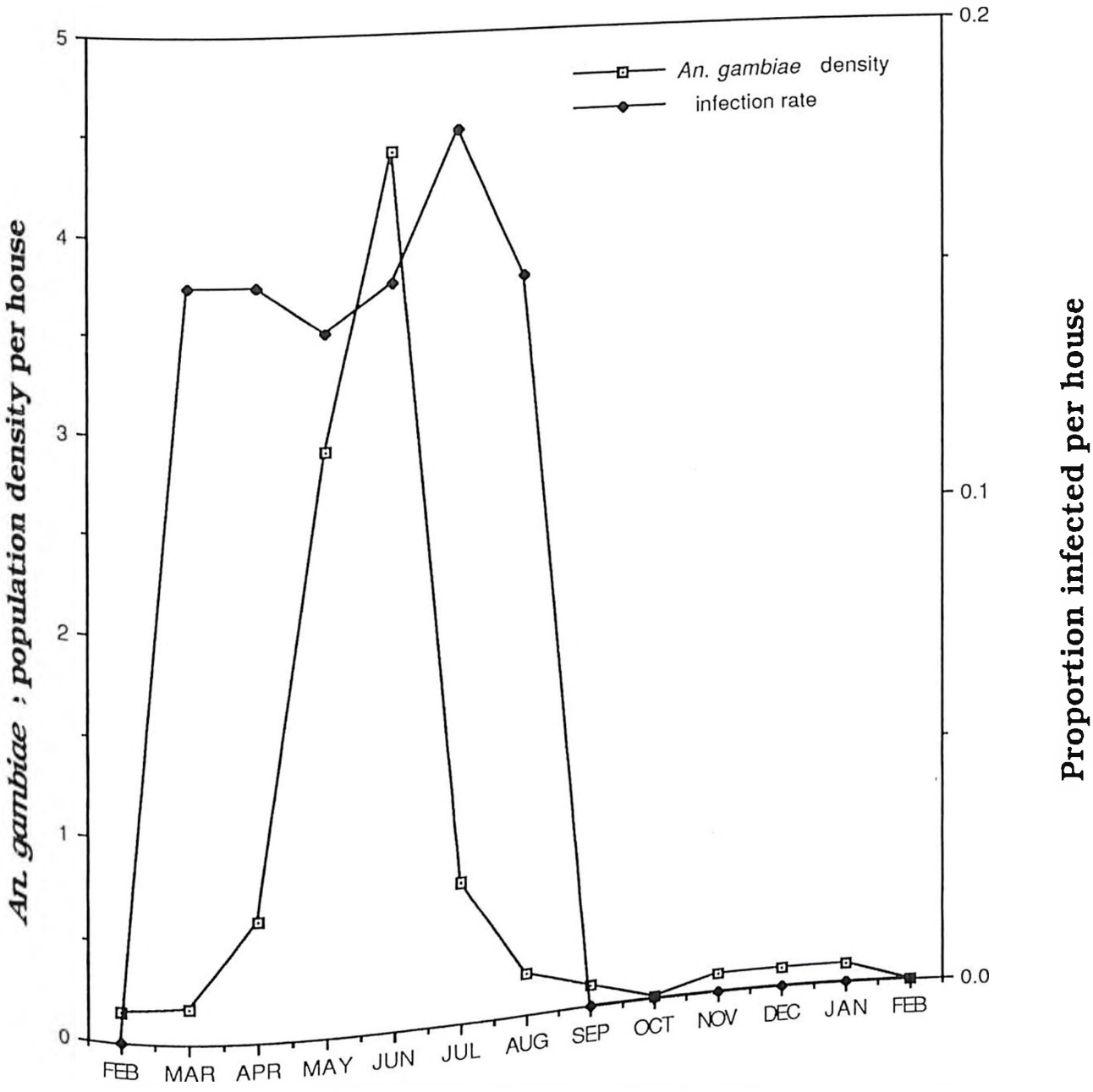


Figure 10. Monthly *An. gambiae* infection rates and mean densities per house at KW.

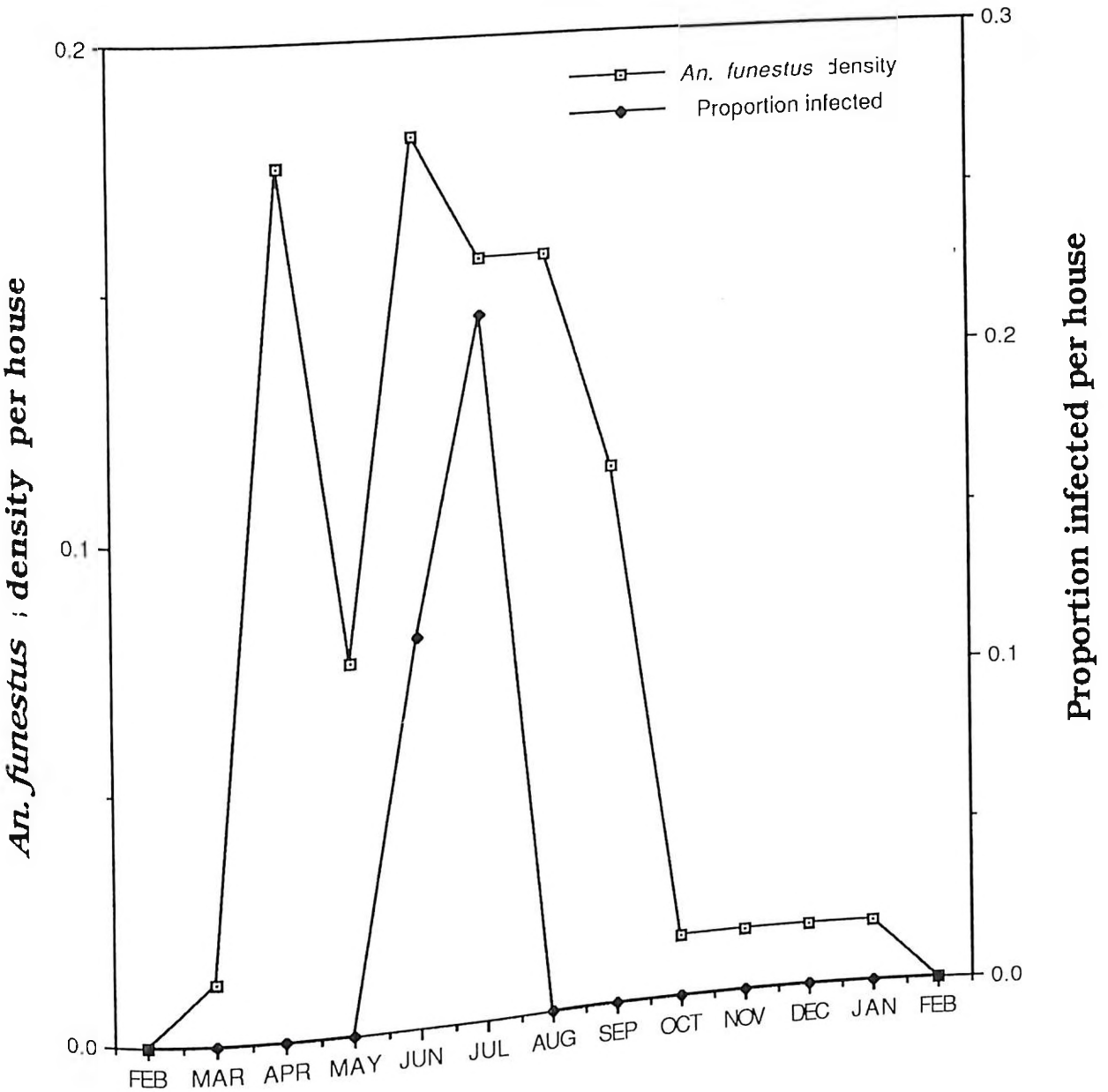


Figure 11. monthly *An. funestus* infection rates and mean density per house at KW.

3.9 *Plasmodium* infection rates in primary school children

Plasmodium infection rates in school children were determined in March 1991, at the end of the dry season. Children's ages ranged from 3-14 years, with a mean of 8 years. Total infection rates were 97.07% (N= 232) in KE, and 97.15% (N= 239) in KW. A number of adults (age > 15 years) were also examined. This latter group had *Plasmodium* prevalence rates of 91.67 % (N= 33) in KE and 83.93 % (N= 47) in KW.

The parasite seen in greater frequency in both areas was *P. falciparum* (Table 8). Prevalence rates in exess of 94 % were observed in both sites. *P. malariae* was second most common parasite 7.64 % (N=21) in KE, and 5.63 % (N=17) in KW. *P. ovale* was the least frequently seen parasite with infection rates of 5.1 % (N =14) in KE and 1.99 % (N=6) in KW (Table 9).

All infections due to *P. malariae* and *P. ovale* appeared as mixed infections. They were all found together with *P. falciparum*. There were 4 triple infections involving all three *Plasmodia* species found only in KE (Table 8).

Parasitological prevalences within the two age groups were similar between the two sites (X^2= 0.003, df= 1, P> 0.05, and X^2= 1.16, df= 1, P>0.05) for children and adults respectively.

Within sites, trophozoite densities of *P. falciparum* between children and adults were significantly different (X^2= 66.34, df= 2, P<

0.05, and X^2= 51.56, df= 2, P< 0.05), for KE and KW respectively. Comparison of trophozoite densities between sites for children and adults were not significantly different (X^2= 1.25, df= 2, P> 0.05, and X^2= 0.92, df= 2, P> 0.05), for children and adults respectively. Gametocytes were found in children in 8.2% (N= 18) of *P. falciparum* positive slides in KE and 12.6% (N= 21) of *P. falciparum* positive slides examined in KW. Adult gametocyte rates were 3.03% (N= 36) in KE and 4.39% (N= 32) in KW (Table 9). Gametocyte densities between children and adults within sites were similar (X^2= 0.21, df= 1, P> 0.05, and X^2= 0.21, df= 1, P> 0.05), for KE and KW respectively. Gametocyte densities between age groups within sites were also similar (X^2= 0.84, df= 1, P> 0.05 and X^2= 0.89, df= 1, P> 0.05), for KE and KW respectively.

Table 8 Prevalence of malaria parasitemia by *Plasmodium* species.in the human population

SITE	SPECIES	EXAMINED	POSITIVE	% POSITIVE
KE	*P. falciparum*	275	265	96.36
	P. falciparum + *P. malariae*	275	21	7.64
	P. falciparum + *P. ovale*	275	14	5.09
	P. falciparum + *P. malariae* + *P. ovale*	275	4	1.46
KW	*P. falciparum*	302	286	94.70
	P. falciparum + *P. malariae*	302	17	5.63
	P. falciparum + *P. ovale*	302	6	1.99
	P. falciparum + *P. malariae* + *P. ovale*	302	0	0

Table 9. *P. falciparum* trophozoite and gametocyte density ranges in different age groups,by site.

SITE	AGE(Years)	TROPHOZOITE RANGE			GAMETOCYTE RANGE	
		1- 75	76- 150	151+	0- 2	3 +
KE	≤14	96	4	7	15	3
	≥15	7	8	21	33	3
KW	≤14	97	2	9	20	1
	≥15	17	15	15	28	4

3.11. Rainfall, temperature and humidity

Rainfall (mm) in Kaksingri location is given in Figure 12, while mean monthly temperature (°C) is presented in Figure 13, and average morning and afternoon relative humidity (%), are shown in Figure 14.

In both sites, March to July 1991 was the period of long rains, and short rains fell in October and November 1991. December 1991 to February 1992 was the dry season. March 1992 was the begining of the long rains for 1992, and relatively more rain fell compared to the amount which fell during the same period in the previous year (Figure 12). Monthly rainfall totals were consistently higher in KE than in KW, though not significantly.

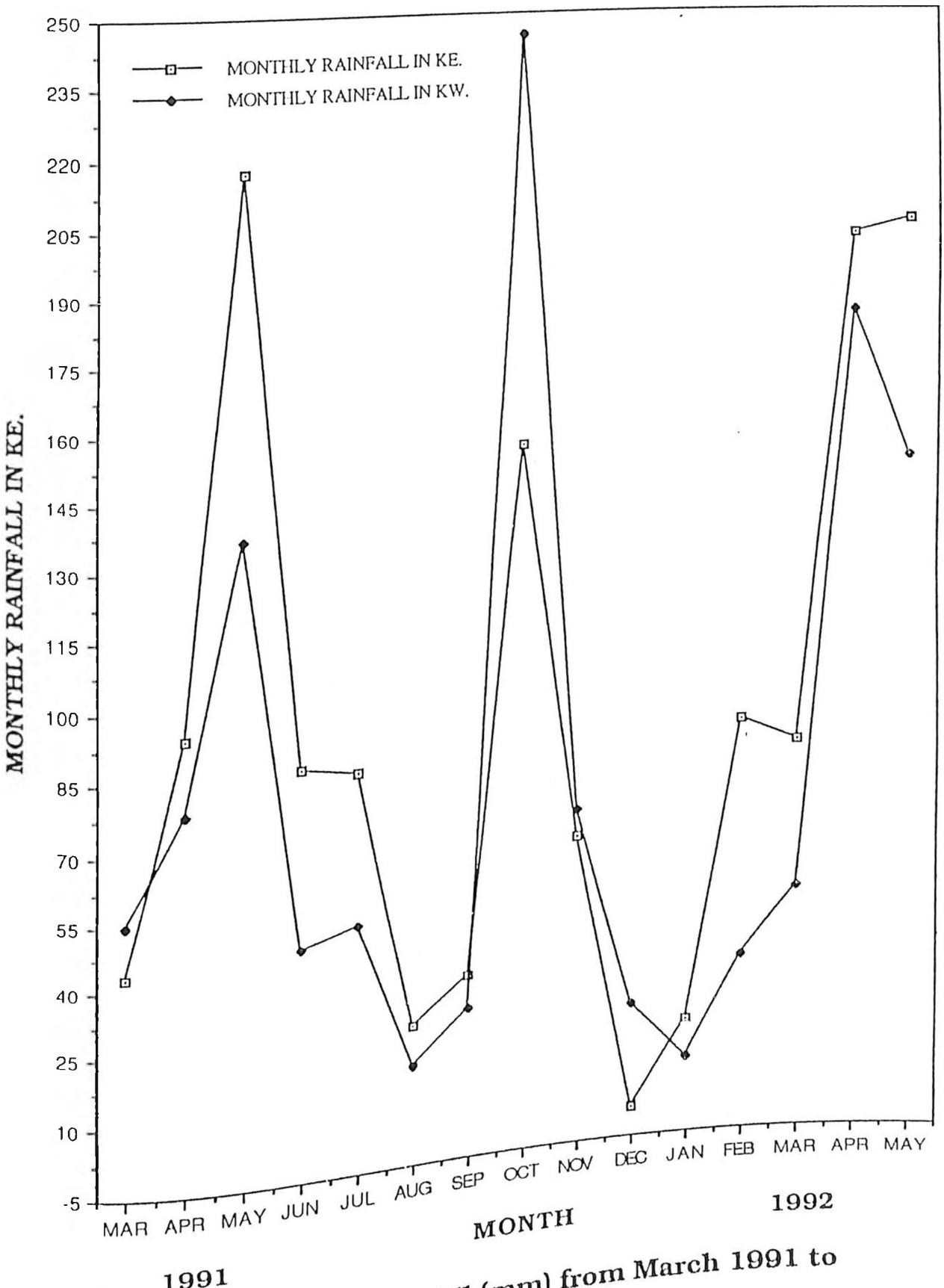


Figure 12. Monthly rainfall (mm) from March 1991 to May 1992 in the study site.

Temperature readings were taken from August 1991 to May 1992. Readings were done at KE. Figure 13 shows the temperature patterns in the whole location. Maximum daily temperatures were highest during mid- January to mid-March 1992, averaging 31- 34°C. Lowest daily maximum during the same time was 30°C. Minimum daily temperatures recorded were 20.3°C in May 1992. Highest min monthly temperatures were 22.1°C in February 1992. Monthly mean temperatures (Maximum + minimum)/2, fluctuated from 23.9°C in May 1992, to 27.9°C in March 1992 (Figure 13).

Relative humidity was also determined only in one site. Recordings were made in KE. Monthly averages of 62.5- 86.9% at 0800 hours local time, and 50.5- 71.3% at 1600 hours local time (Figure 14). Mean monthly relative humidity (r.h. at 0800 hrs + r.h. at 1600 hrs)/2, was lowest in August 1991 (53%) and highest in October 1991 (79.1%). Monthly fluctuations of r.h. at 0800 hrs were generally higher compared to those at 1600 hrs which were consistently lower. (figure 14).

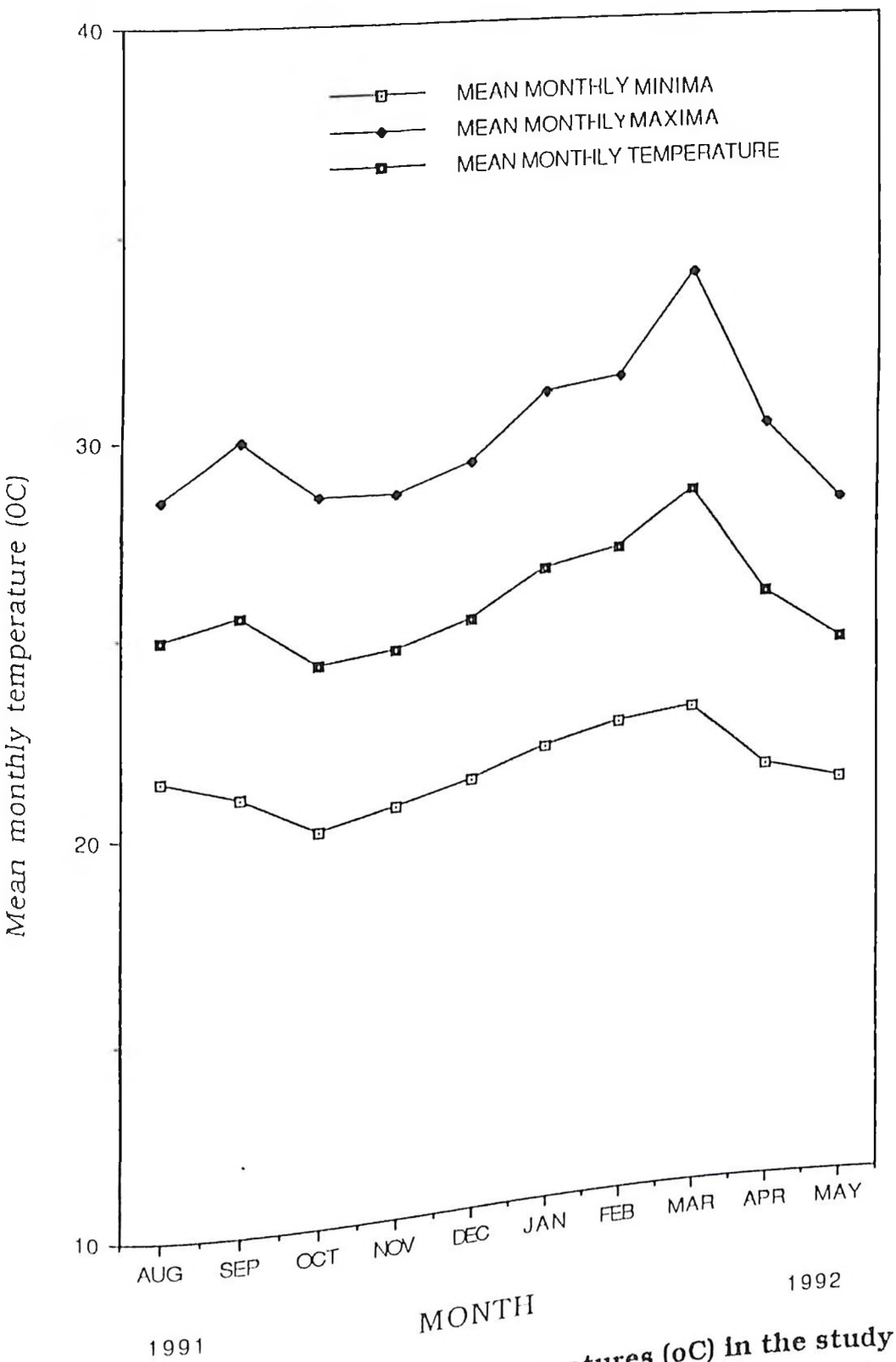
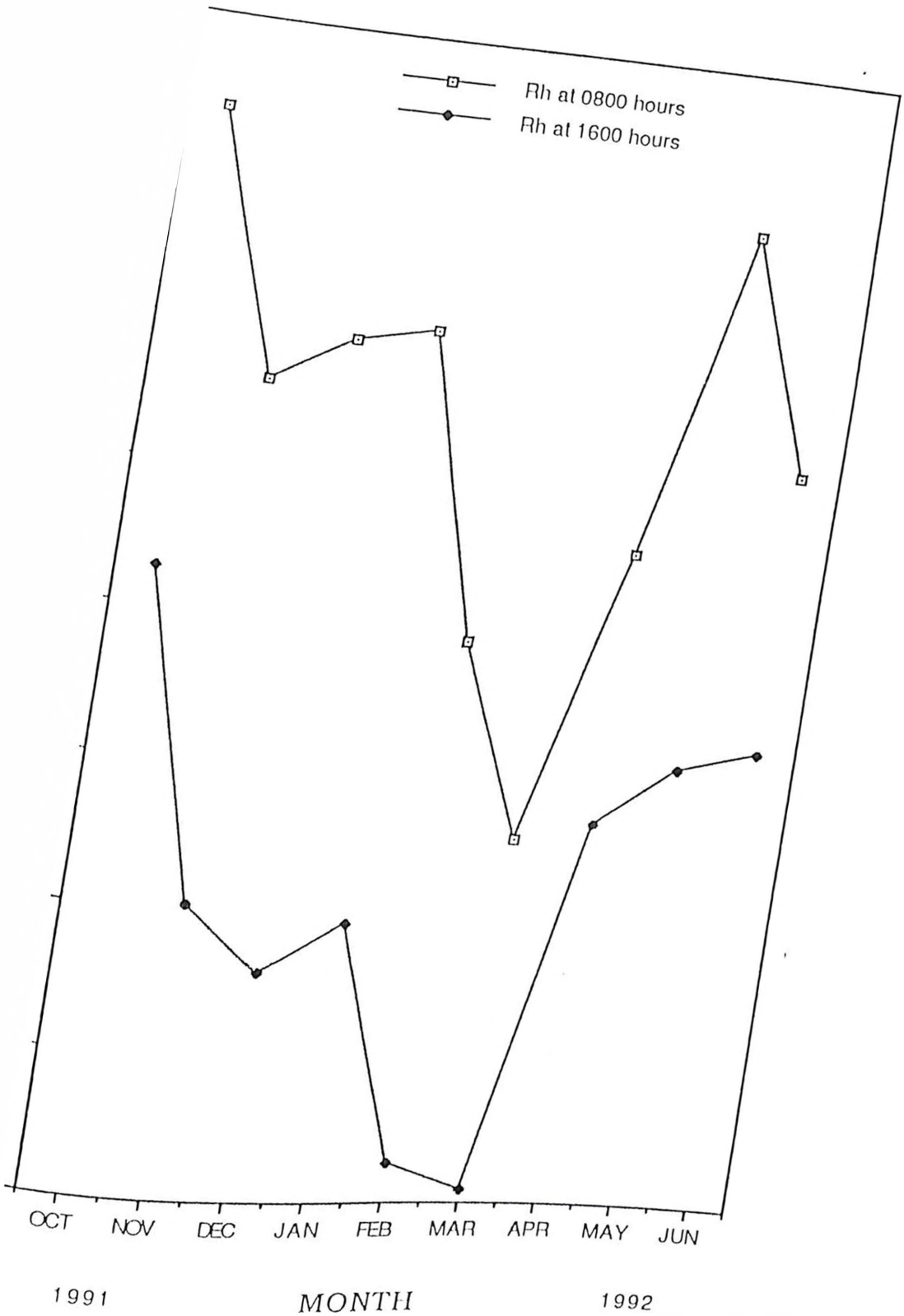


Figure 13. Mean monthly temperatures (oC) in the study sites from August 1991 to May 1992.



re 14. *Relative humidity (%) at 0800 and at 1600 hours in the study site from October 1991 to June 1992.*

DISCUSSION

Results describing the immature stages collected in the study area from August 1991 to April 1992 show that in KE, October 1991 and April 1992 were the only months when larvae were found. These catches coincided with short rains in October, and the begining of long rains in April. Breeding of mosquitoes in this site was clearly dependent on rainfall. This site was generally dry during the study period. *Anopheles* breeding sites were located along Ruosi river (a temporory stream). There were depressions in the river bed and along the sides of the river. It is within these depressions that water collects. During time of heavy rains, water could remain in these places, for up to 3 weeks. These water pools in most cases were used for domestic purposes as well as being drunk by domestic and wild animals. Footprints and hoofprints on the edges of these water bodies presented an important breeding site for *Anopheles*. Larvae collected were not identified into species, and so preference for breeding sites by different species was not ascertained.

In KW, there was some breeding of *Anopheles* mosquitoes throughout the study period. In this site, Lake Victoria provided water all the time. Mosquitoes were found breeding on the fringes of the lake, confined to some points along the water margins. During days of high tides, water from the lake spilled onto land. In certain cases the spillage stayed for a number of days in hoofprints and other similar depressions. When tides were infrequent, some of these places

retained water long enough for *Anopheles* breeding to take place. However, when this water remained for a long time, debris and sometimes animal excreta polluted them. When this happened, *Anopheles* breeding ceased and was replaced by Culicine breeding. When the lake spilled over again, the same sites would be flooded again, with the water becoming clean enough for *Anopheles* breeding to occur once more. On the other hand, when spillages were less frequent, these places would dry up completely and mosquito breeding ceased. In KW, perrenial breeding of mosquitoes also took place in some springs located in the surrounding hills (Figure 3). Water comes out of rocks and accumulates into nearby depressions, giving rise to permanent mosquito breeding sites. Rainfall in KW affected the seasonal magnitude of mosquito breeding as it supplemented lakeside breeding. Breeding of mosquitoes in KW represented a more stable situation compared to KE where large fluctuations were observed.

Determination of the proportion of each larval instar collected showed that in both sites, more first instars were found, followed by second, third, and fourth instars and finally pupae. This result compares well with the findings of Mukiama and Mwangi (1988) in Mwea for the breeding of *An. pharoensis*, and by Service (1970a) in Kisumu area in the breeding of *An. gambiae s.l.* First instars were difficult to notice during larval searches, mainly due to their small size. It was common to find larger aggregations of this life history in small bodies of water. On the other hand, later stages together with pupae, though relatively easy to recognise, were often less clumped in their

distribution per breeding site. Mortality due to predation as well as abiotic factors probably caused the reduction in numbers of these later life stages.

Five Anopheles species were identified in the two study sites. *An. arabiensis*, *An. gambiae*, *An. funestus*, *An. pharoensis*, and *An. ziemanni*. *An. arabiensis* and *An. gambiae* were the only members of the *An. gambiae s.l.* sibling species identified. *An. pharoensis* and *An. ziemanni* were only found in KW. *An. arabiensis*, *An. gambiae* and *An. funestus* were the three known vectors of human malaria found in the study areas. These findings agree with the reports of other workers in the same province (Service, 1970a, service *et al.* 1978, Fontaine *et al.* 1978, Highton *et al.* 1979, Wekesa 1990, Petracca *et al.* 1991). In the present study, members of the *An.gambiae* complex were identified by PCR DNA amplification (Paskewitz and Collins 1990). Some specimens were confirmed by cytotaxonomic means (Coluzzi and Sabatini 1967).

Species diversity was higher in KW, where in addition to *An. arabiensis*, *An. gambiae*, and *An. funestus*; two other Anopheles species were caught, *An. pharoensis* and *An. ziemanni*. *An. pharoensis* has been described as a vector of malaria in other places, such as Ethiopia (Krafsur 1977). But in Kenya, it has not yet been incriminated as a vector of human malaria (Gillies and de Meillon 1968, Mukiama and Mwangi 1989, Ijumba *et al.* 1990). The present study provides no evidence that *An. pharoensis* is involved in the transmission of malaria, as no infected females were found. In rice growing areas, studies have shown this species to be exophilic (Mukiama and Mwangi 1989,

Ijumba *et al.* 1990). *An. ziemanni* was only found resting indoors in KW. It was the least abundant *Anopheles* species caught during the study. Too few specimens were caught of these two species for any meaningful analysis.

In Kenya, many studies have been conducted on the *Anopheles gambiae* species complex. Western Kenya, particularly around Kisumu is one such area where many studies on various aspects of *Anopheles* mosquitoes have been conducted. Service (1970a) in Kisumu area found that though *An. gambiae* and *An. arabiensis* were common in huts, *An. arabiensis* predominated in the exophilic populations, making up 80.6% of those collections. Service *et al.* (1978) in the same area carried out a survey on the impact of spraying houses with fenitrothion on the population of *An. arabiensis* and *An. gambiae*. They found that adults of *An. arabiensis* predominated in outdoor collections, while *An. gambiae* was highly endophilic. Highton *et al.* (1979) working in Kisumu noted that the proportions of *An. arabiensis* and *An. gambiae* differed markedly in different localities. They found that on valley floors, *An. gambiae* made up only 5.4%, while *An. arabiensis* made up 94.6% of *An. gambiae s.l.* collections. In contrast *An. gambiae* predominated in the foothills. They reasoned that differences in rainfall distribution was responsible with valley floors receiving less rainfall than the foothills. Petracca and others (1991) at two sites in Western Kenya from September to November 1987, found a higher prevalence of *An. gambiae* than *An. arabiensis*. In day resting collections they caught 84.8% *An. gambiae* and 15.2% *An. arabiensis* at one site, and 81.2% *An gambiae* and 18.8% *An. arabiensis*, at the other

site. Wekesa (1990) in the same area, at a different time of the year (December 1988 to June 1989), found that *An. gambiae* made up 80% while *An. arabiensis* was 20% of the total collections. In the same Kisumu area, Joshi *et al.* (1975) carried out a survey on *Anopheles* species A and B, prior to fenitrothion application on a large scale. The survey was conducted from January to December 1972. They found the overall mean percentage *of An. gambiae* to be 75.3% of the total catches per year, while *An. arabiensis* made up 24.7% of the total catches.

In the present study, *Anopheles* species were distributed differently between the two sites. In KE the proportions of *An. gambiae* per month were always higher than *An. arabiensis*. In fact, *An. arabiensis* was only present in the overall collection from April to August 1991. In KW on the other hand, although *An. gambiae* was still dominant in the collections, *An. arabiensis* was found in much higher proportions than in KE. *An. arabiensis* predominated during February to April 1991. This was the period preceding and into the beginning of the long rains. From April to August 1991, numbers of *An. arabiensis* were high. This species was virtually absent by September 1991. A smaller peak of this species was observed during the month of October 1991 (Short rains). *An. gambiae* numbers built up rapidly from February to April 1991. The peak occured over a 4 month period (April to July 1991). By August 1991, numbers of this species had become very low. In KE, *An. arabiensis* and *An. gambiae* reached their peak in population densities at the same time; i.e during the long rains, with *An. gambiae* being more abundant. These differences

could be attributed to the exophilic nature of *An. arabiensis*. Since these were only indoor collections, it is possible that more *An. arabiensis* was resting outdoors, where there might have been better resting sites.

In KW there was a seasonal variation in the indoor resting densities of the two *An. gambiae* sibling species. *An. arabiensis* was dominant in the early part of the long rains, but as the rainfall progressed, *An. gambiae* increased and overtook *An. arabiensis* in the house resting densities. This pattern has been commonly observed in East African populations of *An. gambiae* and *An. arabiensis*.

There was a significant difference in the number of mosquitoes resting per house, between the two sites and also between *An. arabiensis* and *An. gambiae*. This observations could be due to a number of reasons. Either there were better outside resting sites in KE than in KW, or houses in KW presented better resting places than houses in KE. It is also possible that there were two different *An. arabiensis* populations between the two sites, with different behavioural patterns. Also considering the fact that the land slopes towards KE, immaure stages might have been taken from KW towards KE. This could have had the effect of increasing the mosquito populations at KE. *An. gambiae* on the other hand presented a reverse of the situation to that observed in *An. arabiensis*. There were significantly more females resting indoors in KE than in KW. This might have been due to opposite effects to those described for *An. arabiensis*. In addition, *An. gambiae* could have been irritated out of the houses in KW by the use of insecticides, or they could have been

leaving houses early in the morning to rest outdoors. The relationship between monthly rainfall and *Anopheles* house resting densities were carried out with a one month difference between the two parameters. In KE, transformed data showed that *An. gambiae* positively and significantly correlated with the rainfall. *An. arabiensis* was also positively, but only marginally significantly correlated with rainfall. *An. funestus* on the other hand, although positively correlated, the relationship was not significant. The same result was observed at KW. These findings in both sites are evidence for the known behaviour of *An. funestus*, that, its breeding cycle is longer than those of *An. arabiensis* and *An. gambiae*. It probably took longer to build up its numbers in this study. Perhaps if a longer lagging period was considered, the relationship may have been significant. These findings compare well with those by Shelly (1972), else where that, the build up of the density of *An. arabiensis* was delayed by one month following the start of the rains. Apart from day resting collections, NBC, and PSC, other methods resulted in the capture of very few mosquitoes. Window traps caught almost nothing in KE and only 8 *Anopheles* mosquitoes in KW. The possible explanation for this observation may be that those mosquitoes leaving their resting sites to go and oviposit were doing so through eaves rather than through the windows. In most houses under normal conditions windows, stayed closed. Eaves on the other hand, were permanently open. Light traps in both areas also captured negligible numbers of mosquitoes. This could possibly be explained by the reason given by Lines *et al.* (1991), in Tanzania that, when light traps are used on their own without mosquito nets (Bed nets), only a small proportion of mosquitoes,

seeking a blood meal will ever come near enough to the trap to be attracted and caught by it. The observation may also have been largely because mosquito densities were low during trapping.

There was a general tendency for bloodfed female mosquitoes to remain indoors at least in the morning, in both sites. *An. arabiensis* and *An. funestus* were not different in their resting habits between the two sites. *An. gambiae* however, showed a significant difference in the gonotrophic condition between KE and KW. This difference could have been due to the difference in the resting densities between KE and KW. There was no significant difference in the resting gonotrophic condition within sites between different species. In the collections from both areas, and for all species, approximately 50% of females caught indoors were bloodfed. The remaining half was divided equally between halfgravid and gravid females which made up 20% each of the indoor resting population. What this reflects is probably that mosquitoes were feeding throughout the night, as seen in NBC collections. Those that feed early in the night will become gravid earlier than those that feed later, thus, the approximate 50% bloodfed becomes about 25% each for half gravid and gravid respctively later. Also, bloodfed females resting indoors may have been higher because, the resting female mosquitoes which were feeding indoors remained inside after taking a bloodmeal to complete the gonotrophic process. In addition to this, a proportion of those feeding outside also entered houses to rest and develop their eggs. Part of the endophagic group would leave houses probably one day after taking a bloodmeal, and continue their gonotrophic cycle elsewhere. The group that continues

the cycle indoors will only be a fraction of what was resting indoors when they were freshly fed. This is the group which will convert to half gravids, and eventually to gravids before flying off in search of oviposition sites. Females after ovipositing would either head directly to the houses in search of another bloodmeal, or would go and look for a bloodmeal elsewhere, or mortality factors would operate.

Mosquitoes biting man per night were estimated by NBC from August 1991 to April 1992. Negligible numbers were captured in KE, and therefore, could not be included in the analysis of the data. In KW also, NBCs were quite low and as a result, were pooled into seasonal biting activities. August to November 1991, December 1991 to February 1992, and March to April 1992. For *An. gambiae s.l.* the biting rates per man per night were lowest in August to November 1991, and highest in March to April 1992. For *An. funestus*, the reverse of the situation observed in *An. gambiae s.l.* was noted. Biting rates were highest in August to November 1991, and lowest in March to April 1992. Probably because of the delay between rains and appearance of *An. funestus* which is known to have a very long larval development period.

During the night, hourly collections, of *An. gambiae s.l.* were seen divided into 3 peaks. The first took place soon after dusk and went on for two hours. The second after midnight for an hour and the third occured after 0200 hours, which remained constant until just before dawn. *An. funestus* also showed 3 biting phases. Phase one taking place soon after sunset, followed by a decline to midnight, at which biting bacame intense for 2 hours before declining until again

just about sunrise. Results of biting activities of Culicine species was spread throughout the night. Peak biting time was early in the evening for three hours and again from midnight for three hours before reduction to dawn. Although these results reflect the typical biting patterns of the mosquitoes considered, the results cannot be used conclusively because of the few specimens caught throughout the study period, especially for *An. gambiae s.l.* and *An. funestus.*

In the present study, *An. gambiae s.l.* was more likely than *An. funestus* to transmit malaria throughout the period under consideration. During the short rains, *An. funestus* was more likely to transmit malaria compared to *An. gambiae s.l.* since more of *An. funestus* was feeding on man per night. At this time, *An. funestus* was biting 6 times higher than *An. gambiae s.l.* In the dry season. December 1991 to February 1992, there was more *An. gambiae* s.l. biting per night than *An. funestus. An. gambiae s.l.* was biting at twice the rate of *An. funestus.* At the start of the long rains (March- April 1992), there was no *An. funestus* caught, but there was a large increase in the biting activity of *An. gambiae s.l.* It was biting at the rate of 0.85 bites/man/night. The results show that while, there were seasonal fluctuations in the night biting rates of *An. gambiae s.l.* There was always some biting by this species. *An. funestus* on the other hand was found to bite only in the short rains and early in the dry season. During the long rains, this species was not posing any danger to the community as far as malaria transmission was concerned. Small numbers of both *An. gambiae s.l.* and *An. funestus* cannot be ruled out to having affected the interpretation of these results.

The biting patterns of Culicines cannot be ignored altogether. They were a nuisance mosquito, and as seen from the results, they had higher man biting rates throughout the study period. In the short rains they were biting at the rate of 18 bites/man/night. This changed to 24 bites/man/night, in the dry season, and at the start of the long rains, Culicines were biting at the rate of 11 bites/man/night.

The only hosts for *Anopheles* mosquitoes identified in the study areas were human and bovids. From mosquito blood meals smeared onto filter paper strips, tests for other hosts; goats/sheep, dog, and chicken were negative. In addition, there were 23.08% of *An. gambiae s.l.* and 4.76% of *An. funestus* whose blood meals were not identified. This may have been due to sample degradation. Only 2 *An. gambiae s.l.* tested by this method were from KE out of which one had fed on man and the other did not react. From these blood meals, *An. gambiae s.l.* was feeding more on humans than on bovines. The same was observed for *An. funestus*. Blood smears whose source could not be identified, had either fed on hosts other than those tested, or the blood meal smears had deteriorated during processing and or storage and as such did not react appropriately.

Blood meal pellets prepared following DNA extraction showed that *An. arabiensis* and *An. gambiae* fed more on humans than cows. Blood meal feeding by *An. arabiensis* and *An. gambiae* mosquitoes on human and bovids were significantly different within sites. The tendency to feed on human hosts is known to be higher in *An. gambiae* than in *An. arabiensis* (Shelly 1972, Highton *et al.*, 1979, Mukiama and Mwangi 1990, Petracca *et al.*, 1991). *An. funestus* is particularly

established in literature to be highly anthropophilic (Gillies 1954). The findings here generally agree with those in literature in the case of *An. gambiae* and *An. arabiensis*. *An. gambiae* was found to be more anthropophilic basically due to its endophilic nature, compared to *An. arabiensis*. The fact that *An. gambiae* rested more indoors than outside, increased its chances of contact with man, and this encouraged it to feed more on man than other hosts. The fraction which was feeding outside was small and this could have represented the zoophagic, but endophilic population. *An. arabiensis* on the other hand was slightly more anthropophilic in KE. and more zoophilic in KW. Generally, this species had a higher fraction of mosquitoes feeding on bovids, especially so in KW, where nearly half of the population was feeding on bovids. This different feeding pattern by the same species in different localities may be attributed to the availability of hosts or to being more outdoor resting in one site, and slightly more endophilic in the other.

Contrary to the findings elsewhere that *An. funestus* is highly anthropophilic (Gillies, 1954), it was found in this study to be more zoophilic. This similar observation was made by Ijumba *et al.* (1990) in Mwea. This finding suggests that other members of the *An. funestus* group may be present in the study area. Negligible numbers of this species were caught at KE, and so could not be included in the analysis.

Wekesa (1990) in Kisumu area reported that, other *Anophelines* like *An. ziemanni* fed on other hosts such as chicken, goat/sheep, dog, guinea pig, or cat. The single specimen of this species tested here,

had neither fed on human nor cow. *An. pharoensis* also had fed on hosts other than human or cow.

The fact that there were mixed bloodmeals (human and cow), in both sites in *An. gambiae*, implied that, this species once interrupted from feeding to repletion on one host, could complete the meal on the next available host of the same or different species. *An. arabiensis* and *An. funestus* were more restricted in their host ranges, since they showed no mixed feeding.

The seasonal nature of *Plasmodium* infection rates in *Anopheles* has often been documented (Krafsur 1977, Ijumba *et al.*, 1990, Taylor *et al.*, 1990). Results reported here show that *P. falciparum* infection rates varied throughout the study period, in different *Anopheles* species. In KE only *An. gambiae* was infected. In this species, infection rates peaked at the end of the long rains. There was another smaller peak in during the dry season in December 1991. In KW, *An. gambiae* showed high *Plasmodium* infection rates over the same period as in KE. In this site, *An. arabiensis* was infected more in the dry season (February 1991), and again at the end of the long rains in July 1991. *An. funestus* also showed high infection rates at the end of the long rainy season, and during the dry period (November 1991 to January 1992).

A comparison of infection rates in *An. gambiae* was made between the two sites, KE showed significantly higher infection rates than KW. Infection rates were significantly different between the 3 *Anopheles* species within KW. Comparison of *An. arabiensis* and *An.*

gambiae infection rates within KW without *An. funestus*, were also significantly different. Joshi *et al.* (1975) in Kisumu, and Service (1970 b) elsewhere, found no significant difference in the infection rates between *An. arabiensi* and *An. gambiae*. Petracca *et al.* (1991) on the other hand, found *An. gambiae* to have significantly higher infection rates compared to *An. arabiensis*. Service (1970a) in Kisumu also noted a different in the infection rates between the two species. In the same area, Wekesa (1990) compared *Plasmodium* infection rates between *An. gambiae* and *An. funestus*. He found that *An. funestus* had higher infection rates compared to *An. gambiae*. He also noted that *An. pharoensis* and *An. ziemanni* were negative for *P. falciparum* CSP.

Sporozoites were not found by dissection in this study. The same situation was also found by Ijumba *et al.* (1990) in Mwea. This finding could either be because the sporozoite loads in the salivary glands of the mosquitoes were low, or there was a significant error in the dissection techiniques (Beier *et al.*, 1990).

Infection rates were generally higher, at the end of the long rains, and again after the short rains in all 3 *Anopheles* species, in KW and only *An. gambiae* in KE. This finding may be attributed to the fact that, at the end of the long rains or start of the dry season, there was a sudden drop in adult emergences (Nulliparous group). Older mosquitoes therefore made up an increasing proportion of the remainder of the population, and a higher proportion of these, will have lived long enough for the completion of the sporogonic cycle. The converse was also true. Infection rates were lowest at the start of

the wet period. This could be accounted for by the high influx of young female mosquitoes into the population. During the dry season, few mosquitoes were found resting indoors. Considering the reduction in the quality and quantity of outside resting places (especially vegetation) at this time of the year, and the low numbers of mosquitoes found, a large proportion of these mosquitoes were resting indoors where they had maximum contact with humans, and these were more likely to be infected.

An. gambiae had higher infection rates in KE than in KW. Factors such as rainfall, temperature and humidity were reasonably similar between the two sites and as such, other factors could have been responsible for this difference. Though mosquitoes were breeding throughout the study period in KW, and only during rains in KE, there could have been migration of mosquitoes upwind from the lake (KW) to inland (KE). If so, proportinately older females would be found in KE, and some of those had fed on gametocytemic individuals before migrating. Infection rates may have been affected in both sites, increasing in KE and decreasing in KW. This migration phenomenon could have also been triggered by the increased use of mosquito coils indoors at KW. Differences in infection rates could also be due to due to different mortality rates in mosquitoes between the two sites.

Infected *An. arabiensis* were only found in KW. No *Plasmodium* infected females of this species were found in KE. A similar situation was observed in *An. funestus*. This species was present in the collections from KW. Small numbers of both species were caught in KE. Despite the number of *An. funestus* collected in KW, most of the

females were feeding on bovines. The small proportion that was anthropophilic were infected. It was unlikely that this species was migrating between the two sites considering the few numbers found in KE.

During the study, in both sites, *An. gambiae* was probably the most important malaria vector. In KE it was the only infected mosquito species. In KW, *An. gambiae* was the major vector of malaria. *An. funestus* was second and *An. arabiensis* was least in apparent importance as a vector. However, the role of An. arabiensis in the transimission of malaria, as Taylor et al (1990) put it, should be defined locally. KE appeared to have greater overall malaria challenge and a higher risk of transmission than did KW. KW however, appeared to provide conditions allowing continuous, low-level transmission through the dry season. Since sporozoites were not found by dissection, it was not possible to estimate entomological inoculation rates (EIR). Considering man biting rates, *An. gambiae* was the most important mosquito in malaria transmission in both sites.

Results of parasitological investigations show a situation of endemic malaria, in the study sites (Pampana 1969). Parasite rates were high especially in children, less than 15 years of age. A slightly lower parasite frequency was noted in the adult population (above 15 years old). Adults who have been exposed to infections many times have acquired some immunity, and so show reduced infection rates. McGregor (1964) and Spencer *et al.* (1987) made similar observations that, in an endemic area, infants (0-1 year) have reduced parasitemias probably because they have maternal antibodies which are reduced as

the child grows older, so much so that at about 3 years of age, protection is very low. The process of building up immunity continues until the child is about 9 years. In the age group 9-14 years, a child has reasonably strong immunity to *P. falciparum* malaria. Beyond age 15, the person is fully immunised and can withstand the parasites much more effectively.

Gametocyte rates were found to be similar between the two age groups in the two sites. Gametocyte rates indicated a proportion of the human population infected with the parasite which was capable of infecting the vector during bloodmeal feeding.

The determination of infection rates in the human population was conducted in March 1991, at the end of the dry season. This was the time when mosquito numbers as well as mosquito infection rates were just starting to build up. Since infection rates were determined from children and teachers at schools, asymptomatic malaria cases were high in the human population. This observation may be important in the epidemiology of malaria. The fact that vector numbers were just starting to build up, and the parasite reservoir was already high meant that the vector efficiency was going to be high, and more people in the population were going to be infected, particularly young children and non-immune adults. This could result in epidemic type episodes of malaria infections leading to higher rates of morbidity and mortality, which were suspected in these sites.

This study shows that there were different malaria transmission potentials between KE and KW. From the results it appears to be

more of a risk to reside a short distance inland than on the lake shore itself. It should be noted however, that more sampling time should be allowed and more such sites to be considered in order to be sure about these general conclusions.

REFERENCES

Bartholomew, J.C. (1985) *The times concise atlas of the world.* Times book limited. london. 88P.

Bafort, J.M. (1985) *An. marshallis* s.l. A secondary vector of malaria in Africa (Letter). *Trans. Roy. Soc. Trop. Med. Hyg.* **79** (4): 566-567.

Bailey, N.T. (1979) *Statstical methods in biology.* 2nd edition Edward Arnold publishers. London. 216 P.

Beier, J.C., Perkins P.V., Wirtz, R.A., Whitmire, R.E., Mugambi, M., Hockmeyer, W.T. (1987) Field evaluation of Enzyme Linked Immunosorbent Assey (ELISA) for *Plasmodium falciparum* sporozoite detection in *Anopheline* mosquitoes from Kenya. *Am. J. Trop. Med. Hyg;* **36** (3): 459-468.

Beier, J.C., Perkins, P.V., Wirtz, R.A., Koros, J., Diggs, D., Gargan, T.P., Koech, D.K. (1988) Blood meal identification by direct ELISA, tested on *Anophelines* (Diptera: Culicidae) in Kenya. *J. Med. Entomol.* **25** (1): 9-16.

Beier, J.C., Perkins, P.V., Onyango, F.K., Gargan, T.P., Oster, C.N., Whitmire, R.E., Koech, D.K., Roberts, C.R. (1990a) Characterisation of malaria transmission by *Anophelines* (Diptera: culicidae) in Western Kenya, in preparation for malaria vaccine trials. *J. Med. Entomol.* **27** (4): 570-577.

Beier, J.C., Copeland, R.S., Oyaro, C., Masinya, A., Odago, W.O., Oduor, S., Koech, D.K., Roberts, C.R. (1990b) *An. gambiae* complex egg-stage survival in dry soil from larval development sites in Western Kenya. *J. Am. Mosq. Control. Assoc.* **6** (1): 105-109.

Chandler, J.A., Highton, R.B. (1975) The succesion of mosquito species (Diptera: Culicidae) in rice fields in Kisumu area, of Kenya., and their possible control. *Bull. Entomol. Res.* **65**: 295-302.

Chandler, J.A., Highton, R.B., Hill, M.N. (1976) Mosquitoes of the Kano plain, Kenya, II. Results of outdoor collections in irrigated and nonirrigated areas using human and animal bait and light traps. *J. med. entomol.* **13**: 202-207.

Collins, F.H., Petracca, V., Mpofu, S., Brandling-Bennett, A.D., Were, J.B., Rasmussen, M.O., Finnerty, V. (1988) Comparison of DNA probe and cytologocal methods for identifying field collected *An. gambiae* complex mosquitoes. *Am. J. trop. Med. Hyg;* **39** (6): 545-550.

Coluzzi, M., and Sabatini, A. (1967) Cytogenetic observations of species A and B of the *An. gambiae* complex. *Parassitologia* **9:** 73-88.

Coluzzi, M., Petracca, V., and Di deco, M.A. (1977) Behavioural divergencies between mosquitoes with different inversion karyotypes on polymorphic populations of the *An. gambiae* complex. *Nature.* **266**: 832-833.

Davidson, G. (1955) Further studies of the basic factors concerned in the transmission of malaria. *Trans. Roy. soc. Trop. Med. Hyg.* **49**: 4 339- 350.

Davidson, G. (1962) Anopheles gambiae complex. *Nature* **196**: 907

Davidson, G. (1964) The five mating types in the *An. gambiae* complex. *Revista di malarologia* **43** : 167- 183.

Davidson, G. and Jackson, C.E. (1962) Incipient speciation in *An. gambiae* Giles. *Bull. Wld. Hlth. Org.* **27** : 303-305.

Davidson, G., Patterson, H.E., Coluzzi, M., Mason, G.E., Micks, T.W. (1967) The *An. gambiae* complex. *In genetics of insect vectors of disease.* Ed. Wright, J., pal R. 211-250. Amsterdam. Elservier.

El Said, S., Beier, J.C., Kenaway, M.A., Morsy, Z.S., Mardan, A.I. (1986) *Anopheles* population dynamics in two malaria endemic villages in Fayum governorate, Egypt. *J. Am. Mosq. Control. Assoc.* **2** (2) 156-163.

Fowler, J.M. and Cohen, L. (1986) *Statistics guide.* British trust for ornithology. London. 175P.

Fontaine, R.E., Najjar, A.E., Prince, J.S. (1961) The 1958 malaria epidemic in Ethiopia. *Amer. J. Trop. Med. Hyg.* **10**: 795-803.

Fontaine, R.E., pull, J.H., Payne, D., Pradhan, G.D., Joshi, G.P., Pearson, J.A., Tymakis, M.K., Ramos, C., (1978) Evaluation of fenitrothion for the control of malaria. *Bull. WHO.* 56: 3 445- 452.

Foote, R.H., and Cook, D.R. (1952) *Mosquitoes of Medical importance. Agriculture hand book #152.* Agricultural Research service, U.S. Department of Agriculture. Washington D.C.for ELISA development. *Bull. Wld. Hlth. Org.* **65:** (1) 39-45.

Garret-Jones, C. (1964) The human blood index of malaria vectors in relation to epidemiological assessment. *Bull. Wld. Hlth. Org.* **30:** 241-261.

Giles, A.M. (1902) *A handbook of the gnats or mosquitoes.* 2nd Ed. London. John Bale sons and Danielson.

Gillet, J.D. (1971) *Mosquitoes.* Weidenfield and Nicholson. 5 Wisley St. London W1. 274 PP.

Gillies, M.T. (1954) Studies in the house leaving and outside resting in *An. gambiae* and *An. funestus* Giles in East Africa-II. The exodus from the houses and the house resting population. *Bull. Ento. Res.* **45:** 375-387.

Gillies, M.T. (1956) The problem of exophily in *An. gambiae. Bull. Wld. Hlth. Org.* **15**: 437-449

Gillies, M.T. (1964) Selection for host preference in *An. gambiae. Nature.* **203:** 852-854.

Gillies, M.T. and Wilkes, T.J., (1965) A study of the age composition of populations of *An. gambiae* Giles and *An. funestus* Giles in North-eastern Tanzania. *Bull. Ent. Res.* **56:** 237- 262.

Gillies, M.T., de Meillon, B. (1968) *The Anopheline* of Africa south of

the Sahara (Ethiopian zoogeographical region). *South Africa Insititute of medical research.* Publication # 54, 2nd edition.

Gillies, M.T., Furlogh, M. (1964) An investigation into the behaviour of *An. parensis* Gillies at Malindi, on the Kenya coast. *Bull. Ent. Res.* **55**: *1-16.*

Haddow, A.J., (1942) The mosquito fauna and climate of native huts at Kisumu, Kenya. *Bull. ent. Res.* **33:** 91- 142.

Haddow, A.J., Ssenkubuge, Y., (1974) The mosquitoes of Bwamba county Uganda. Observations on the biting behaviour of *Anopheles* species other than *An. gambiae* Giles with notes on the behaviour of these species in the Entebe area. *Bull. Ent. Res.* **64:** 45-51.

Highton, R.B., Bryan, J.H., Boreham, P.F.L., and Chandler, J.A. (1979) studies on the sibling species of *An. gambiae* Giles and *An. arabiensis* Patton, (Diptera: Culicidae) in Kisumu area. *Bull. Ent. Res.* **69**: 43-53.

Ijumba, J.N., Mwangi, R.W., Beier, J.C. (1990) Malaria transmission potential of *Anopheles* mosquitoes in the Mwea-Tebere irrigation scheme. *Med. Vet. ent. Res.* **4**: 425-432.

Joshi, G.P., Fontaine, R.E., Thymakis, K., Pradhan, G.D. (1973) The cause of occassional high counts of *An. gambiae* in morning pyrethrum-spray collections in huts sprayed with fenitrothion, Kisumu, Kenya. *Mosquito news.* **33:** 29-38.

Joshi, G.P., Service, M.W., Pradhan, G.D. (1975) A survey of species A and B of the *An. gambiae* Giles complex in the Kisumu area of Kenya, prior to insecticidal spraying with OMS-43 (Fenitrothion). *Ann. Trop. Med. Parasit.* **69**: 91-104.

Krafsur, E.S. (1977) The bionomics and relative prevalence of *Anopheles* species with respect to transmission of *Plasmodium* to man in Western Ethiopia. *J. Med. entomol.* **14**: 180-194.

Lombardi, S., Esposito, F. (1983) Enzyme Linked Immunosorbent Assay (ELISA) for the identification of mosquito blood meals. *Parasitologia.* **25**: (1) 49-56.

Ma, M., Beier, J.C., Petraca, V., Gwadz, R., Zhang, J., Song, Q., Koech, D. (1990) Differentiation of *An. gambiae* and *An.arabiensis* (Diptera: culicidae) by ELISA using Immunoaffinity-purified Antibodies to vitellogenin. *J. Med. Ent.* **27**: #4 564-577.

MacClelland, G.H.A., (1959) Observations on the mosquito *Aedes* (Stegomyia) *aegypti* (L) in east Africa. I. The biting cycle in an outdoor population at Entebe, Uganda. *Bull. ent. Res.* **50**: 227-235.

Mc Gregor. I.A., (1964) studies in the acquisition of immunity to *P. falciparum* infections in Africa. *Trans. Roy. Soc. Trop. Med. Hyg.* *58*: 1.

Miles, C.J., Green, C.A., Hunt, R.H., (1983) Genetic observations on the taxon *Anopheles* (Cellia) *pharoensis* (Teobald) (Diptera: Culicidae). *J. Trop. Med. Hyg.* **86**(4): 153- 157.

Mani, T.R., Tewari, S.C., Reuben, R., Devaptra, M. (1984) Resting behaviour of *Anophelines* and sporozoite rates in vectors of malaria along the river Thenpennai (Tamil Nadu). *Indian. J. Med. Res;* **80**: 11-17.

Marquetti, M.C., Navarro, A., Bisset lazcano, J.A., Garcia, F.A. (1990) Comparison of three methods for collecting adult *Anophelines* in a risk area of malaria transmission. *Rev. Cubana. Med. Trop;* **42** (2): 247-253.

Mattingly, P.F. (1964) The *An. gambiae* complex. Some introductory notes. *Riv. Malar.* **43**: 165-166.

Mattingly, P.F. (1977) Names of the *An. gambiae* complex. *Mosquito systematics* **9** *: 323-328.*

McCrae, A.W. (1983) Oviposition by African malaria vector mosquitoes. I. Temporal activity patterns of caged, wild-caught, fresh water *An. gambiae* Giles s.l. *Ann. Trop. Med. Parasitol;* **77** (6): 615-625.

Miles, S.J., Green, C.A., Hunt, R.H. (1983) Genetic observations on the taxon *An.* (Cellia) *pharoensis* Theobald (Diptera: Culicidae). *J. Trop. Med. Hyg.* **86** (4): 153-157.

Molineaux, l., and Gramiccia, G. (1980) *The Gark project. Research on the epidemiology and control of malaria in the Sudan savannah of West Africa. WHO,* Geneva.

Mosha, F.W. and Mutero, C.M. (1982a) The influence of salinity on

larval development and population dynamics of *An. merus* Donitz (Diptera: Culicidae). *Bull. Ent. Res.* **72**: 119-128.

Mosha, F.W., and Subra, R. (1982b) Ecological studies on *An. gambiae* complex sibling species in Kenya I. Preliminary observations on their geographical distribution and chromosomal polymorphic anversions. Unpub. *WHO/VBC/82.* 876.

Mosha, F.W., Petraca, V. (1983) ecological studies on *An. gambiae* complex sibling species on the Kenya coast. *Trans. Roy. soc. Trop. Med. Hyg;* **77** (3): 344-345.

Muirhead-Thompson, R.C., (1951) *Mosquito behaviour in relation to malaria transmission and control in the tropics.* london. Edward Arnold publishers. 219 P.

Mukiama, T.K., Mwangi, R.W. (1989a) Seasonal population changes in malaria transmission potential of *An. pharoensis* and other minor *Anophelines* in Mwea irrigation scheme, Kenya. *Acta. Trop.* **46** (3): 181-190.

Mukiama, T.K., Mwangi, R.W. (1989b) Field studies of larval *An. arabiensis* Patton of Mwea irrigation scheme, Kenya. *Insect. Sci. Applic.* **10**: 55-62.

Mukiama, T.K., Mwangi, R.W. (1992) Temporal population fluctuations and notes on the gonotrophic cycle of *Culex quinquefasciatus* an dthe minor culicines in Mwea rice irrigation rice scheme, kenya. *Unpublished.*

Mutero, C.M., Mosha, F.W., Subra, R. (1984) Biting activity and resting behaviour of *A. merus* Donitz (Diptera: Culicidae) on the Kenyan coast. *Ann. Trop. Med. Parasitol.* **78** (1): 43-47.

Omer, S.M., Cloudsley-Thompson, J.L. (1970) Survival of the females of *An. gambiae* Giles through a 9-month dry season in Sudan. *Bull. Wld. Hlth. Org.* **42:** 319-330.

Pampana, E.Y. (1969) *A text book of malaria eradication* 2nd Ed. London., New york., Toronto., Oxford Unv. Press. *Parasitol*; **78** (1): 43-47.

Paskewirtz, S.M., Collins, F.H. (1990) Use of the polymerase chain reaction to identify mosquito species of the *An. gambiae* complex. *Med. Vet. Entomol.* **4:** 367-373.

Patterson, H.E. (1964) Direct evidence for the specific distinctiveness of forms A, B and C., of the *An. gambiae* complex. *Riv. Malar.* **43**: 191-196.

Petracca, V., Beier, J.C., Onyango, F., Koros, J., Asiago, C., Koech, D.K. (1991) Species composition of the *An. gambiae* complex (Diptera: Culicidae) at two sites in Western Kenya. *J. Med. Entomol.* **28** (3): 307-313.

Ralisoa, Randrianasolo, B.O., Coluzzi, M. (1987) Genetical investigations on zoophilic *An. arabiensis* from Antananarivo area (Madagascar). *Parasitologia.* **29 (1)** 93-97.

Senior White, R.A. (1954) Adult *Anopheline* behaviour patterns:

Suggested classification. *Nature.* **173:** 730.

Spencer, H.C., Kaseje, D.C.O., Koech, D.K. (1987) Community based malaria control in Saradid. *Ann. Trop. Med. parasit.* **81**: 1 13-23.

Service, M.W. (1970a) Ecological notes on species A and B of the *An. gambiae* complex in the Kisumu area of Kenya. *Bull. Ento. Res.* **60**: 105-108.

Service, M.W. (1970b) Identification of *An. gambiae* complex in Nigeria by larval and adult chromosomes. *Ann. Trop. Med. Parasit.* **64:** 2 131- 136.

Service, M.W. (1976) *Mosquito ecology: Field sampling methods.* Applied science publishers, London. 583 PP.

Service, M.W., Joshi. G.P., Pradhan, G.D. (1977) A survey of *An. gambiae* (species B) of the *Anopheles gambiae* Giles complex in the Kisumu area of Kenya, following insecticidal spraying with OMS-43 (Fenitrothion). *Ann. Trop. Med. & Parasit.* **72**(4): 10 377-385.

Shelly, A.J. (1973) Observations on the behaviour of *An. gambiae* species B in Kambole, Zambezi valley, Zambia. *Ann. Trop. Med. Parasit.* **67**: 237-248.

Surtees, G. (1970) Large scale irrigation and arbovirus epidemiology Kano plain, Kenya I. description of the area and preliminary studies on the mosquitoes. *J. Med. entomol.* **7:** 509-517.

Snow, W.F. (1987) Studies of house entering habits of mosquitoes in Gambia, West Africa: Experiments with prefabricated huts with varied wall apertures. *Med. Vet. Entomol.* **1** (1): 9-21.

Taylor, K.A., Koros, J.K., Nduati, J., Copeland, R.S., Collins, F.H., Brandling-Bennett, A.D. (1990) *Plasmodium falciparum* infection rates in *An. gambiae, An. arabiensis,* and *An. funestus* in Western Kenya. *Am. Trop. Med. Hyg.* **43** (2) 124-129.

Wekesa, J.W., (1990) Effects of *Plasmodium falciparum* on the feeding behaviour of naturally infected *Anopheles* mosquitoes. M.Sc. Thesis.

White, G.B. (1972) The *Anopheles gambiae* complex and malaria transmission around Kisumu, Kenya. *Trans. Roy. Soc. Trop. Med. Hyg.* **66** *(4): 572-581.*

White, G.B. (1973) Comparative studies on sibling species of *An. gambiae* complex (Diptera: Culicidae) III. The distribution ecological behaviour, and vectorial importance of species D in Bwaba county, Uganda, with an analysis of biological, ecological, and cytogenetical relationships of Ugandan species D. *Bull. Ent. Res.* 63: 65- 97.

White, G.B. (1974) *An. gambiae* complex and disease transmission in Africa. *Trans. Roy. Soc. Trop. Med. Hyg.* **68**: 278-298.

White, G.B., Magayuka, S.A., Boreham, P.F.L. (1972) Comprehensive studies on the sibling species of the *An. gambiae* Giles complex (Diptera: culicidae) : Bionomics and vectorial activities of species

A and species B, at Segera, Tanzania. *Bull. Ent. Res.* **62**: 295-317.

WHO. (1975a) *Mannual on practical entomology in malaria.* Part II. WHO, Geneva.

WHO. (1975b) Cytogenetic studies of insect vectors. *WHO/MAL/***75**. 849.

Wirtz, R.A., Zavala,F., Charoenvit, Y., Campbell, G.H., Burkot,T.R., Schneder,I., Esser, Roberts, D.R. (1987) Comparative testing of monoclonal antibodies againist *Plasmodium falciparum* sporozoites for ELISA development. *Bull. Wld. Hlth. Org.* **65**: 20-25.