VECTOR ECOLOGY AND MALARIA TRANSMISSION IN SOUTHERN SIERRA LEONE

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THIS WORK IS DEDICATED TO THE MEMORY OF

UNCLE GUY KOSIA

AND ALL THOSE WHO LOST THEIR LIVES DURING THE WAR IN PENDEMBU

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ABSTRACT

Studies on the ecology of Anopheles gambiae s.s. and An. funestus and the transmission of malaria were undertaken in four villages in a high rainfall forested area in the Bo District of southern Sierra Leone. Malaria was found to be hyperendemic with transmission taking place throughout the year. The prevalence of Plasmodium falciparum in both the dry and the wet seasons was about 62%. The prevalence of P. malariae and P. ovale were 12% and 1% respectively. Plasmodium vivax was not recorded.

Anopheles gambiae s.s., identified by chromosomal techniques as the Forest form, was the most important vector of malaria in the study area. Surprisingly, rice fields or swamps were not favoured breeding places for this species; breeding was mainly taking place in temporary pools. Anopheles funestus which was found in relatively low numbers was mainly a dry season vector. The mean annual sporozoite rates of An. gambiae s.s. and An. funestus determined by ELISA were 7.4 and 11.4% respectively. Similar sporozoite rates were found using the dissection technique. Pyrethrum spray, human-bait, and exit trap collections, as well as identification of mosquito blood-meals using the ELISA method, showed that An. gambiae s.s. and An. funestus were highly anthropophagic and that An. gambiae s.s. was also highly exophilic. The numbers of An. gambiae s.s. caught in CDC light-traps placed in houses correlated strongly with the numbers caught by indoor human-bait collections. Geographically the study area was not homogenous and the pattern of malaria transmission was different in two groups of villages situated in areas having different landscape and vegetation. The annual average man-biting rates of An. gambiae s.s. and An. funestus, for the combined villages, were 1.1 and 0.1 bites/man/night respectively. The corresponding annual inoculation rates were 0.08 and 0.007 infective bites/man/night.

Further ecological studies on An. gambiae s.s. carried out in a fifth, nearby village (Bayama) revealed a daily survival rate of 0.85 and a vector life expectancy of 6.1 days.

The small area variations observed in the pattern of malaria transmission underline the importance of planning vector control at the village level. Deliberate exophily, surprisingly exhibited by An. gambiae s.s., would seem to preclude vector control by residual house spraying.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 MALARIA AND VECTORS

1.1.1 The global malaria situation

Malaria affects 267 million people in 103 countries, and is widespread throughout most of the tropics, but also occurs in some temperate regions. It is estimated that there are 107 million clinical cases and that 1-2 million people die of the disease every year (Liisberg, 1991). The death toll is heaviest amongst children in endemic areas. Agricultural and other development projects have, in many areas of the world, aggravated existing malaria problems. For example, rice irrigation has in some areas increased malaria prevalence and the large numbers of mosquitoes breeding in the rice fields have been responsible for extending the transmission season, sometimes over an entire year (Service, 1989a). Development projects and settlement schemes, have also sometimes created malaria problems where formerly none existed (Nakajima, 1991; Najera, 1989). The resistance of malaria parasites to drugs, in particular the resistance of Plasmodium falciparum to chloroquine, has spread to over 40 countries (Brinkmann & Brinkman, 1991; Payne, 1987). In Africa chloroquine resistance first appeared in 1979 in Tanzania and Kenya, and has now been reported from 31 countries including five countries in West Africa (Payne, 1987). In many, but not

all areas, important malaria vectors have developed resistance to various insecticides. Presently there are at least 57 species of Anopheles that have developed resistance to one or more groups of insecticides (WHO, 1986).

During the euphoric DDT-era of the 1950's and 1960's it was believed that global malaria eradication was technically possible, although subSarahan Africa was not included in the global malaria eradication programme, which was initiated by the World Health Organization in 1955, because of the problems of intense transmission of Plasmodium falciparum Welch (Zahar, 1984). However, it is now realised that because of drug resistance in addition to resistant strains of mosquito vectors, and social and economic reasons the realistic goal can only be malaria control. Even this will prove to be a major challenge in some countries.

1.1.2 The malaria situation in Africa

In Africa south of the Sahara, about 100 million cases of malaria may occur every year (Liisberg, 1991). Endemicity reaches its highest levels in regions of West and Central Africa, where there is holoendemic malaria, and decreases to lower levels of endemicity in many eastern and southern African countries. In these latter regions there are often epidemic situations, e.g in Ethiopia, Kenya, Swaziland and Namibia. It is estimated that one million infants die of the disease every year (Benzerroug, 1991). In The Gambia, malaria specific mortality is 6.3 per 1000 per year in infants and 10.7 per 1000 per year in children 1-4 years old (Greenwood et al., 1987). Recent studies in West Africa have also shown that the prevalence of malaria can

vary widely between neighbouring villages and within different parts of the same village (Greenwood, 1989). Local variations in the epidemiology of malaria can be very important.

1.1.3 The Anopheles vectors and malaria transmission

Human malaria can be transmitted only by Anopheles mosquitoes, of which there are about 416 species, but only about 70 species are vectors of malaria under natural conditions, and of these probably only about 40 can be considered important vectors.

Although most Anopheles prefer to feed at night, their activities are not necessarily confined to hours of darkness. Most species will bite readily in the evenings or early mornings around sunrise. Some Anopheles have a tendency to feed out of doors (exophagic) whereas others prefer to feed indoors (endophagic). After blood-feeding engorged females of some species rest in houses (endophilic), whereas in other species blood-fed individuals will rest outdoors (exophilic) in a variety of natural shelters such as amongst vegetation, on trees and in rodent borrows, in cracks and crevices in the ground and in other dark and damp situations. The resting behaviour of Anopheles can have a direct bearing on control. For example, in some areas in Africa the interruption of malaria transmission by residual house-spraying has proved unattainable because a significant proportion of the vectors are exophilic. Chromosomal studies by Coluzzi et al. (1977) have shown that exophily in An. gambiae is at least partially under genetic control. Few Anopheles feed exclusively on either man or non-human hosts, most feed on both, but the degree of

anthropophagism and zoophagism varies according to species, and is a very important factor in malaria epidemiology. The extent of zoophagic behaviour can vary considerably within a species according to local conditions, such as the relative number of other hosts e.g cattle, in relation to the human population.

All four species of human malaria, Plasmodium falciparum Welch, P. vivax Grassi & Feletti, P. ovale Stephens and P. malariae Grassi & Feletti are transmitted in sub-Saharan Africa, but P. falciparum is the most common parasite - as well as the most lethal. In West Africa, the population is protected from P. vivax because the people lack the Duffy group of antigens (Miller et al., 1976).

The life cycle of all species of human malaria is essentially the same. Malaria parasites are inoculated by the bite of an infective Anopheles. Following the inoculation of the infective sporozoites, the parasites multiply in the parenchyma cells of the liver and develop into pre-erythrocytic schizonts. The process of development and multiplication in the liver is called pre-erythrocytic schizogony. Mature schizonts burst to release thousands of merozoites which are set free into the blood circulation where they invade the red blood cells and develop into trophozoites. Trophozoites undergo a dividing process called erythrocytic schizogony and give rise to schizonts which in turn give rise to merozoites. Red blood cells burst to release merozoites which invade fresh red blood cells. After several generations of merozoites have been produced, some develop into sexually differentiated gametocytes.

Anopheles vectors become infected by feeding on the blood of infected people. In

the mosquito stomach the gametocytes develop into micro- and macrogametes which fuse to produce the zygote. The zygote develops into the ookinete which penetrates the stomach wall to form the oocyst. Mature oocysts burst to produce sporozoites which invade the salivary glands of the mosquito.

1.1.4 Current status of malaria vectors in Africa south of the Sahara

The main malaria vectors in Africa are species of the An. gambiae complex and An. funestus Giles, both of which have a wide distribution. There are at least six species in the An. gambiae species complex; An.gambiae s.s. Giles, An.arabiensis Patton, An.quadriannulatus, Theobald, An. bwambae White, An.melas Theobald and An.merus Dönitz.

Anopheles gambiae s.s. is undoubtedly the most important vector in Africa and probably the world's most efficient malaria vector (Service, 1985), although in some countries, such as Ethiopia and South Africa, An. arabiensis is the main vector. Anopheles quadriannulatus is a highly zoophagic and exophilic species which has little or no direct role in the transmission of malaria. Both An. melas and An. merus are salt water breeding species. The former occurs in coastal areas along the West African coast, whilst An. merus is restricted to East and Southern Africa.
Anopheles bwambae is restricted to the forest areas of the Rift Valley, west of the Ruwenzori mountains in Uganda.

There are several secondary vectors such as An. nili Theobald, An. pharoensis

Theobald, An. hargreavesi Evans and An. moucheti Evans that may be of local importance in some countries. In Zaire, for example, An. nili maintains a high level of transmission (Carnevale and Zoulani, 1975), as it is reported to do in southwest Ethiopia (Krafsur, 1970). Anopheles moucheti could be a principal vector in certain localities in Gabon (Service et al., 1977). Further details on the vectorial status of African anophelines are given by Gillies and de Meillon (1968) and Gillies and Coetzee (1987).

1.2 HISTORY OF MALARIA STUDIES IN SIERRA LEONE

1.2.1 Review of studies on malaria in Sierra Leone: 1899-1990

Sierra Leone became known as " The white-man's grave " in it's early history because of the high mortality rate amongst Europeans. It is believed that by transmitting malaria the mosquito helped prevent the 'white-man' from making a permanent settlement in the country. In fact the 'Medal of the Mosquito', is a military honour awarded for gallantry in Sierra Leone.

In 1898 the Secretary to the British Colonies, Mr. Joseph Chamberlain, addressed a circular to the governors of all British Colonies expressing his concern over the unhealthy state of West African colonies. Part of this circular read:

" The great mortality among Europeans in such climates as those of West African Colonies and Protectorates has not failed to attract my notice ..... my attention was more definitely directed to the importance of scientific enquiry into the causes of malaria."

The result of this circular was the official formation of the Liverpool and London Schools of Tropical Medicine in 1898 and 1899, respectvely. On the 29th July 1899, Major Ronald Ross a lecturer at the Liverpool School of Tropical Medicine, led a Malaria Expedition to Freetown. This was the beginning of scientific enquiry into the causes and control of malaria in Africa.

Before 1899

In 1812 an act was passed in Freetown preventing people from allowing the formation of **"stagnant pools which generate disease and mosquitoes over the town".** A comprehensive account of the legislative acts concerning public health and anti-malarial activities in Freetown during the period 1800 to 1870 is given by Kennan (1910). When Mary Kingsley visited Sierra Leone in 1893 the idea that malaria was caused by miasmata, the putrid vapour from swamps, was rife. Rankin (1836) suggested a medical project for sterilising miasmata by discharging carbonic acid gas from lime-kilms erected in the mangrove swamps of Freetown. His recommendation was never carried out. Studies on mortality and morbidity due to malaria was carried out by the army in Freetown between 1890 and 1898. In 1895, the malaria parasite was observed in Sierra Leone for the first time (Thin, 1896; Duggan, 1897). Wilson (1898) looked at the prevalence of fever among the troops in Sierra Leone between 1892 and 1897. He noted that fever was more common in the white troops than in the black troops. According to his survey, the annual death rates of white soldiers from malaria fever was 42.9 per 1000 as against 5.5 per 1000 for black soldiers.

1899-1920

Members of the Liverpool Malaria Expedition working in Freetown collected two species of Anopheles which were identified by Giles (Austen, 1900; Ross et al.,1900, addendum I). One of the species was An. gambiae at that time known as Anopheles costalis Loew. The other species had not been previously described and at the request of one member of the expedition (Austen) it was named Anopheles funestus. A natural infection rate of 4.5% was found for An. gambiae which also harboured

infective stages of Wuchereria bancrofti. Wild caught Anopheles mosquitoes were also fed on fever patients and later dissected for oocysts and sporozoites. Anopheles mosquitoes were found breeding in small pools scattered throughout the town, but a few including An. gambiae, also bred in domestic containers. Anti-malaria measures recommended by the Malaria Expedition included the treatment of such pools with tar and the use of bed-nets and window screens. As a consequence, sanitary authorities in Freetown applied tar regularly to stagnant pools, but as soon as these measures stopped Anopheles larvae reappeared. Ross (1900) also suggested segregation as a means of protecting Europeans from infection.

In 1900 the Malaria Committee of the Royal Society sent J.W.W. Stephens and S.R. Christophers to continue studies on malaria and its control in Sierra Leone. Stephens and Christophers (1900) observed that Anopheles breeding sites were more widely distributed in Freetown than Ross and others had discovered. Anopheles larvae were found in streams, small drains, rock pools and gardens. They also showed that Africans attracted mosquitoes more readily than Europeans. This cast some shadow over the previous suggestion by King and Laveran that the immunity of Africans may be due to the fact that they are bitten less frequently, either because of an odour noxious to mosquitoes or from the possession of thicker skins (Nuttal 1899, cited by Stephens and Christophers 1900). Other investigations carried out by Stephens and Christophers in Freetown established the connection between blackwater fever (tropical haemoglobinuria) and malaria. Their studies on the antilarval effect of larvicidal substances and drainage revealed that the only adequate method of treating streams, drains and rock pools was by surface drainage and the use of well made

triangular gutters. The value of the segregation of Europeans was reemphasized by them (Christophers and Stephens, 1900a,b).

Following the report of the Malaria Committee, intensive vector control measures were started in Freetown in 1901. A team of 20 men was divided into two gangs. A small gang of six men called the Culex gang collected from private houses all calabashes, and other containers in which Culex and Aedes mosquitoes bred. A larger gang called the Anopheles gang was concerned with draining pools and puddles in streets and the backyards of the houses in which Anopheles were recorded breeding. After three months the Culex gang had cleared 6,500 houses and had removed more than 1000 cartloads of rubbish (Ross 1901).

In March 1902 the government took over operations and started to drain the streets. Larger pools were treated with larvicides, mainly crude kerosene. Construction of isolated European bungalows were started in the same year at Hill Station situated 900 ft above sea level. In 1904 a delegation led by Dr. Robert Boyce, Dean of the Liverpool School of Tropical Medicine, visited Freetown to evaluate the impact on health of the anti-mosquito and sanitary measures implemented since the visit of the Malaria Expedition (Boyce et al., 1905). The delegation which expressed satisfaction over the progress of anti-malaria activities, found that many drains had been completed, although a large number were not constructed properly. They recommended the reconstruction of the bed of streams crossing the town to prevent the formation of breeding places. The government continued its sanitary measures at levels proportionate to available funds, but unfortunately until 1930 such funds

were never sufficient to allow any large undertaking. Results of sanitary measures taken between 1904 and 1930 are in the Annual report of the Medical and Sanitary Department published by the government printing department, Freetown.

According to Blacklock and Evans (1926) the anti-mosquito activities between 1899 and 1920 had greatly reduced the density of biting mosquitoes in Freetown. To justify their claim they quoted Bacot (1916) who wrote about the apparent absence and actual rarity of mosquitoes in Freetown. No scientific data, however exist to ascertain these observations. Before 1920 the assessment of many workers of the mosquito situation was based on casual examination (Gordon et al., 1932). Rankin (1836) wrote that in Freetown "mosquitoes are not frequent.....the climate of Sierra Leone is too deadly even for these persecutors of the human race". Ross (1900) wrote that he was "once actually informed that there are no mosquitoes in Sierra Leone".

Anti-malaria measures were concentrated in the capital Freetown and its environs. Muirhead-Thomson (1947) who worked in Freetown for nearly two years wrote that " however important the interland (provinces) may be, as far anti-malaria work is concerned it is the coast on which the capitals of the four (British West African) colonies are located that will be our main concern for some time to come". In the provinces, malaria related activities were limited to a few parasitological and entomological surveys. Between March and November 1912, Simpson (1913) travelled extensively in the provinces visiting almost all major towns. He noted that malaria was by far the most prevalent insect-borne disease and that An.gambiae and

An. funestus were very common. Butler (1915) made a list of blood-sucking Diptera in the Koinadugu District in the Northern Province. In this area the sporozoite rate of An. gambiae and An. funestus were 8.8 and 11.0%, respectively (Wood, 1915). Butler (1916) examined thin blood films of 75 healthy looking boys at Bo school in the Southern Province, and found the prevalence rate was 37.3% for students with an average age of 10.5 years. Government reports on health and sanitation from 1900-1920 were published annually in the Annual Reports of the Medical Department, printed by Waterloo and Sons Limited, Printers, London Wall, London.

1920 - 1930

The period 1920 - 1930 was the decade for systematic research aimed at providing base-line information for the scientific monitoring of malaria control. The Alfred Lewis-Jones Laboratory, a field laboratory of the Liverpool School of Tropical Medicine, was opened at Tower hill in 1920. Professor Blacklock, the first director of the laboratory had as his initial task, to ascertain whether the streams referred to by Stephens and Christophers (1900) were still breeding places of anopheline mosquitoes. He found anopheline larvae in large numbers especially at the lower end of the streams. Anopheles gambiae was the commonest species in Freetown. Anopheles funestus was found rarely in water courses on the eastern and southern sides of the town (Blacklock, 1921; Blacklock and Evans, 1926). Blacklock & Evans (1926) described the breeding places of all the anophelines found in Freetown and provided a key for the identification of fourth-stage larvae. Evans (1925) described a new variety of Anopheles marshalli, namely An. marshalli var. freetownensis, and also pin-pointed certain distinguishing characters in the An. funestus group (Evans,

1930). Gordon (1929) undertook a survey of man-biting mosquitoes in Mabang in the Northern Province, while Gordon and Macdonald (1930) provided a list of all anophelines known to occur in Sierra Leone, together with notes on their habits and malarial infection rates. A list of culicine mosquitoes occurring in Freetown was made by Evans (1926), and included the description of two new species: Aedes (Aedimorphus) apicoannulata and Toxorhynchites aeneus, originally ascribed to the genus Megarhinus.

Studies on the indoor-resting densities of mosquitoes in houses in Freetown and Kissy were started in 1930 (Gordon et al., 1932). Anopheline density was 34 times greater in Kissy than in Freetown, although the sporozoite rate remained similar. Sporozoite rates for An. gambiae and An. funestus in Freetown were 8.2 and 4.1%, respectively. Out of a total of 22 An. nili dissected only 1 (9.1%) was infected. A method for calculating inoculation rate from the density of infected vectors was introduced by Davey and Gordon (1933).

Between July 1925 and March 1926, Macdonald (1926) examined 1059 children aged 3-12 years, in the schools of Freetown, in order to determine the amount of malarial infection and its effect on the health of the children. The spleen rates in the 'endemic' and 'hyperendemic' areas identified by him were 50 and 72%, respectively. The parasite rates were 41 and 72 percent respectively. The Sierra Leone Government Printing Department published Annual Medical and Sanitary Reports for the years 1921- 1928. Reports for 1929 and 1930 were published in the Annual Reports of the Medical and Sanitary Department.

1930 - 1939

In 1930 a very comprehensive scheme of malaria control was commenced. In Freetown, pools, gutters and cesspits were drained or oiled, with intensified vigour. Work on the canalization of Sander's Brook, a stream running through Freetown, was started. The aim was to canalize the brook by means of a concrete channel from the municipal boundary to its outfall in Kroo Bay. This was to provide permanent surface drainage for an area of about 540 acres draining into the brook. The canal was completed in 1935. Details of anti-malaria measures carried out between 1930 and 1939 are given in the Annual Reports of the Medical and Sanitary Department, published by the Government Printers Department, Freetown.

In Kissy, near Freetown, Gordon et al. (1932) collected 28 adult An. nili from 582 rooms during the wet season. Although 9.1% of them were infective it was not considered an important vector in the area.

In November and December 1935, Peaston and Renner (1939) carried out spleen and blood examinations on school children in Freetown which showed a marked decrease in the incidence of malaria in the hyperendemic area as a result of vector control measures. Infection with P. malariae was becoming very common in Freetown (Gordon and Davey 1932, 1933). Examination of blood films from 200 children aged 3-4 years showed a parasite rate of 50.8% for P. falciparum, 68.6% for P. malariae, and 1.9% for P. vivax. It is now agreed that P. vivax is absent from most of West Africa, the identification of this parasite in Freetown by Macdonald (1926), Gordon and Davey (1932) and Peaston and Renner (1939) could be related to

members of the West African forces returning from Asia after the first world war. Turner and Walton (1946), however, thought that there had been considerable confusion between P. vivax and P. ovale especially when some investigators only used thick films for identification.

1939 - 1945

The renewed use of the port and the arrival of service personnel during the Second World War gave rise to serious malarial problems. Malaria affected the crews and troops on the ships at anchor in the harbour to an extent which threatened to disorganize the whole convoy system.

In 1940 Blacklock was asked to revisit Freetown to investigate the high incidence of malaria in the port and organize control measures. He, together with Wilson, conducted a survey on malaria in and around Freetown during 1940 and 1941, and showed that some of the sea-going ships brought mosquitoes with them into the harbour. In addition launches and lighters in the harbour carried mosquitoes from shore to ship. The report of the survey (Blacklock, 1941; Blacklock and Wilson, 1942c) formed the basis for subsequent control measures undertaken jointly by the civil and military medical departments. Launches and lighters were sprayed with pyrethrum insecticide and so also were public rooms and alley-ways on ships. These measures resulted in a dramatic reduction of the parasite rate of personnel on vessels permanently stationed in the harbour (Anon, 1946; Tredre, 1946).

To deal with the problem of preventing mosquitoes from moving from the shore to

the ships, studies were conducted in the surrounding villages and control measures recommended (Blacklock, 1942; Blacklock and Wilson, 1942a,c). As an immediate and temporary measure regular insecticidal spraying of houses in the estuarine villages was undertaken. To try and get a permanent reduction of adult mosquitoes, Blacklock and Wilson (1941) surveyed the breeding places of anopheline mosquitoes on the shores of the estuary. They suspected that An. melas, at that time regarded as a variety of An. gambiae, was a vector of great importance in the whole harbour area. Out of 30 An. melas dissected two (6.6%) had infected glands. Ribbands (1944a, b, c, 1946) working on anopheline mosquitoes in western Freetown was convinced that An. melas, whose breeding places were uncontrolled was a vector of malaria on the Aberdeen peninsular. Muirhead-Thomson (1945) studied the larval habitats and control of An. gambiae and An. melas in the coastal districts. He showed that out of 1000 An. melas dissected 42, (4.2%) had infected glands. He also made the important discovery that the eggs of An. melas were morphologically different from those of An. gambiae. It was Ribbands (1944a,b), however, who showed that the larvae of the two species could be distinguished by the structure of their pectens and by the greater tolerance of An. melas larvae to salinity.

Between 1943 and 1944 embankments (bunds) were constructed at Wellington and Aberdeen to surround the egg-laying zones and prevent them from becoming flooded with tidal waters. The construction of these bunds apparently resulted in a big reduction in the indoor resting densities of female anophelines at Aberdeen (Elliot, 1949). Tredre (1946) also investigated the role of An. melas in the transmission of malaria in the vicinity of Freetown estuary and confirmed that it was an efficient

vector. Larvae of Anopheles brunnipes Edwards and Anopheles flavicosta Edwards were first described from specimens collected from Sierra Leone by Davey (1941, 1942).

Muirhead-Thomson (1945) found An. nili to be a malaria vector of local importance in the Orugu Valley, which runs just behind the range of hills which forms the background of Freetown. In the driest months of the year, January, February and March, adult female An. nili were found to be 10 times more abundant in the houses than An. gambiae. Out of 106 An. nili dissected, 34 (3.2%) had infected glands. Over 20 years later An. nili was also found in large numbers in the Bombali District in the Northern Province where a sporozoite rate of 3.4% was recorded (Storey, 1967).

Towards the end of 1943 all administrative measures in malaria control had become effective and plans were ready for an all-out anti-mosquito campaign in the Western Area. A civilian Malaria Control Unit was formed which started an intensive campaign based on a policy of mosquito eradication in the eastern part of the Western Area, which included Freetown and Kissy. The weekly treatment was with larvicides, mainly 'malariol' (a high-spreading oil containing DDT) but Paris Green was used in the streams, of every potential breeding place and was co-ordinated with extensive collections of adult mosquitoes from houses. The Royal Army Medical Corps was responsible for malaria control in the western part of Freetown area i.e Aberdeen and Murray Town. By 1945 only 5-7% of African infants at the age of two years were carrying malaria infections in Freetown (Walton 1947, 1948 & 1949).

A comprehensive account of the malaria situation in Freetown from 1939 -1945 is given by Turner and Walton (1946).

Brian Maegraith, former Dean of the Liverpool School of Tropical Medicine, came to prominence in the field of tropical medicine when he was recruited as a pathologist for the army in Sierra Leone in 1940, and where his work on malaria and its effect on the kidney attracted the attention of many eminent scientists (Maegraith, 1944; Maegraith and Findlay, 1944; Maegraith & Havard, 1944).

1945 - 1980

After the World War the civilian Malaria Control Unit had total responsibility for malaria control. In Freetown and its environs, control activities were supervised by the Medical Entomologist, while in other parts of the country they became the responsibility of the Health Officers and District Medical Officers. After field trials with gammexane, HCH and DDT by Davidson (1947a,b), residual house-spraying was embarked on in some areas, but anti-larval measures remained the main line of attack. The use of HCH for house spraying was discontinued in 1958, but DDT continued to be used for both house-spraying and larviciding (Boardman, 1959).

The transport of malaria vectors into Freetown by means of the railway was investigated by Thomas (1960b). He concluded that trains might be important in malaria transmission in eastern Freetown where the incidence of malaria and malaria vectors was higher than in the remainder of the urban area. Trains passed through this area on their way to the terminus, and moreover railway workshops and wagons

were located in this area. ıas (1951) also studied the feeding behaviour of adult An. gambiae. A review malaria work in Freetown from 1900 to 1964 was presented by Storey (197

Anti-larval operations in th greater Freetown area continued into the 1970's with temephos (Abate) and mala on as the main insecticides. In 1975 a parasitological survey revealed that cont measures were unsuccessful, because of lack of supervision, poor techniq and a possible resistance to malathion. Dieldrin resistance had already bee ported in An. gambiae in Freetown in 1966 (Storey, 1967). Results of the na malaria survey carried out between 1977 and 1979 showed no difference in arasite rates of 2-9 year olds in Freetown (64.4%) where malaria control was g practiced, and the provinces (65.7%) where virtually no control activities were rtaken (Giri & Colusa, 1980).

In 1980 bed-nets were tried a malaria control measure in five peninsular villages. Unfortunately an evaluation of the trial in 1981 came to no conclusions regarding their effectiveness, despite general acceptance by the population (97.9%) of the bed-nets supplied (Bulengo, 19

Occasionally anti-malaria a ities were carried out in the provinces, mainly in the Northern Province. In 194 the Royal Air Force sprayed certain villages around Lungi airfield with DDT. When the airport attained international status, the Malaria Control Unit took over responsibility for mosquito control. Villages within a three mile radius of the airport were sprayed with HCH at regular intervals. Thomas

(1960a) investigated the breeding places of Aedes aegypti Linnaeus and also carried out studies on Culex quinquefasciatus Say (Thomas, 1956) in Freetown.

Lewis (1956) carried out an entomological survey of the Tonkolili valley in Northern Province. He considered An. hancocki Edwards, as a minor vector of malaria, because out of 115 mosquitoes dissected one (0.9%) had infected glands. Mills (1967) undertook a malaria survey in Lunsar, also in Northern Province. A chemoprophylaxis campaign consisting of bimonthly treatment with chloroquine was started in the Bombali District in the Northern Province, in October 1979. An evaluation a year later showed a regression in parasite indices in the treated population (Bespiatov et al., 1984).

In addition to the above, Medical and Health Officers posted to the provinces have at various times made attempts to control malaria, but such activities were on a small scale and lasted only while the officers concerned were in the areas. After 1940, government health reports were published in the Report of the Medical and Health Services, printed by the Government Printing Department.

1.2.2 Recent and continuing investigations

A National Antimalaria Strategy for a Malaria Action Programme was formulated in 1981 by the Ministry of Health, Freetown (Anon, 1982). According to this document malaria in Sierra Leone is the commonest single cause of mortality and morbidity, especially in children under the age of 9 years. The estimated malaria specific child

mortality rate was 40 % for the under-fives. The main objective of the programme was to protect children and pregnant women by chemoprophylaxis and curative treatment using chloroquine; to strengthen larviciding operations in Freetown and to expand this to the provinces. A National Malaria Control Committee was formed in 1980 to pursue these objectives.

MacCormack (1984) briefly mentions the use of traditional country cloth to prevent mosquito bites along the coast of Sierra Leone. The traditional concepts of the causes and treatment of malaria among the Mende ethnic group of Sierra Leone have been discussed by Bledsoe and Goubaud (1985). Also Barnish and Samai (in press) have carried out a survey of the use of traditional medicinal plants in the treatment of malaria. Kandeh (1986) compiled information about the causes of infant and early childhood (1-4 years) deaths from hospital records and demographic surveys, which showed fevers among the leading causes of infant mortality. Recent studies have also been carried out on malaria in children in Freetown (Morgan and Sherunkeh-Sawyarr, 1988) and the An. gambiae complex (Morgan, 1990). The first longitudinal studies of malaria in the provinces of Sierra Leone were carried out in Bo District in the Southern Province (Barnish et al., 1990, 1992; Bockarie et al., 1992a,b,c). Subsequently, studies on the use of insecticide-impregnated bed-nets for malaria control began in 1991 in Bo in the Southern Province.

Finally, a proposal from the National Malaria Control Committee to undertake studies on malaria control in Freetown has been technically supported by WHO, and preparations are now in progress for malaria control studies to resume in Freetown.

1.3 THE PRESENT STUDY

1.3.1 Aim of the study

The general objective of the present study was to investigate those aspects of the ecology and behaviour of adult anophelines which govern the transmission dynamics of malaria in a high rainfall forested area of Sierra Leone.

The study was undertaken in the Bo area, and the specific aims were to:-

1. Identify all Anopheles species in the area

2. Identify the malaria vectors

3. Determine the man-biting rates and indoor resting densities of malaria vectors

4. Determine the extent to which Anopheles exit bedrooms soon after a blood-meal and identify their outdoor resting sites

5. Determine sporozoite rates, human blood indices, and inoculation rates of malaria vectors

6. Determine the duration of the gonotrophic cycle, survival rates and vectorial capacities of the malaria vectors.

1.3.2. Plan of the study

The study, which was planned to last 24 months (December 1989 to November 1991), was carried out by the author and six field assistants. The entomological data were collected in two main areas. From December 1989 to April 1991, collections were made in four villages in the Bo northeast constituency some 20 km from the town of Bo, and formed the entomological component of a bigger study on the epidemiology of malaria in Southern Sierra Leone. However, as few Anopheles were collected by any of the various sampling techniques in any of the villages in the area, it was considered essential that another area be identified which had much larger anopheline populations. Such a place was the village of Bayama just 3 km north of Bo. Consequently entomological data were collected from Bayama from November 1990 to November 1991, but at the same time collections continued in the other villages until April 1991, at which time all research was focused on Bayama village.

The first two weeks in December 1989 involved negotiating with the village authorities in the Bo northeast constituency for permission to carry out the study, explaining the aims of the study and soliciting the cooperation of the villagers. For the next two weeks trial catches were made in 15 villages, from which four villages were selected for entomological investigations. Selection of villages were mainly based on availability of suitable houses for the entomological techniques proposed. Regular mosquito collections in these four villages started in January 1990 and continued until April 1991. Collections at a fifth village, Bayama commenced in November 1990 and continued for 13 months.

1.3.3 Vegetation and climate of Sierra Leone

Sierra Leone is situated on the west coast of Africa between Guinea and Liberia in the West African subregion. It has 341 km of coastline along the Atlantic ocean. It's population of 4.2 million (1986 census) live on 72,326km^2 of land. Although small in territory, Sierra Leone is one of the most densely populated countries in West Africa. The country is divided into three provinces: the Northern Province, the Eastern Province and the Southern Province, and the Western Area. The provinces are subdivided into 12 districts which include 150 chiefdoms. Sierra Leone comprises a number of interior plateaux and swampy, low-lying coastal plains. Over half the country is less than 153m above sea level. The drainage network is dense; it is composed of nine main river systems as well as a number of minor coastal creeks and intra-coastal tidal streams.

According to Clarke (1966) there is ample evidence that much of Sierra Leone was originally covered with primary forest, but as a result of extensive human activities, the country is now largely covered by old farm bush characterised by low secondary growth (oil-palm bush). In the far north a narrow band of the land is covered by upland savanna woodland (Gwynne-Jones et al., 1978).

Seasonal variation in rainfall is well marked in Sierra Leone. The wet season begins in April with sudden rain storms often coming from the east, and accompanied by high winds, thunder and lightning. It is usually warm at the beginning of the rainy season and the air is often dry. By July it becomes very cloudy and comparatively

cool, there is frequent rain, which is sometimes heavy, but little or no thunder. The wet season finishes earliest in the western and northern parts of the country in about October and latest in the south-east, in November. The dry season usually begins in November and lasts until April. The relative humidity can be very high (>90%) at the beginning of the dry season but by January the air becomes very dry due to the Harmattan wind blowing down from the Sahara. Rain tends to come a few weeks earlier in the south-east, but may not reach some coastal areas before May. The average annual rainfall varies between 200cm on the northern plateaux to 350cm in the coastal region. The mean annual temperature is 26.5 C.

The meteorological data for the study areas are presented in Table 1.1.

1.3.4 Description of the study area

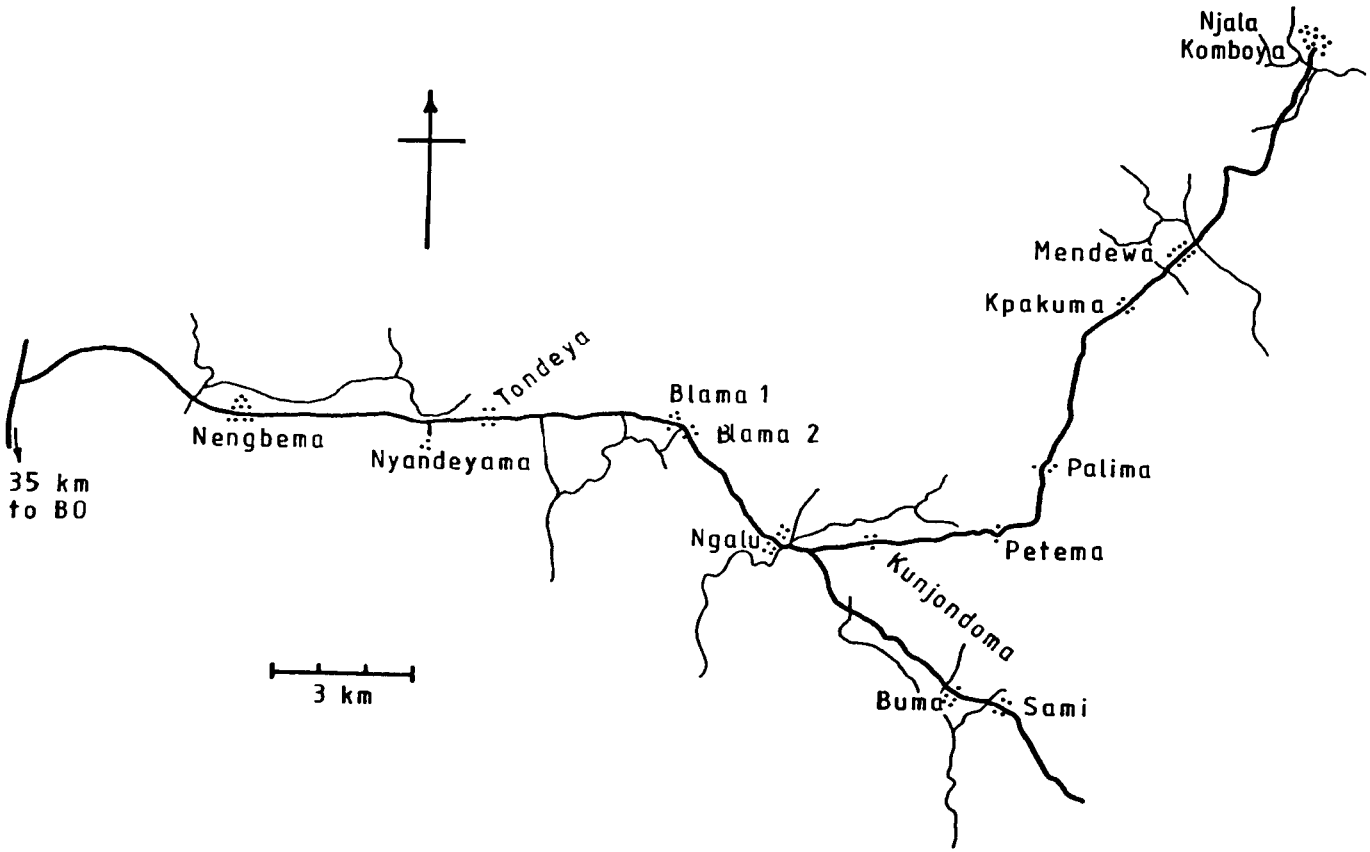
All five "entomological" villages are in the Bo District, Southern Province. This District covers an area of 5,100 km^2, mostly in the interior lowlands and mostly 150m above sea level. About 50km from Bo town in the north-east of Bo District, the land rises to a 300m plateaux (interior plateaux). In the interior lowlands the vegetation is typical oil-palm bush, but in the interior plateaux the vegetation can best be described as 'derived savanna'- the original primary forest, however, has been replaced mostly by the elephant grass Perinisetum purpureum Schum. Rain forest trees survive in sacred areas, along some streams and on steep hills (Gwynne-Jones et al., 1978).

Table 1.1 Summary of meteorological data for Bo, 1900-1991

Month	Mean daily temperature (°C)				Relative humidity (%)		Rainfall in mm
	Mean max.	Mean min.	Absolute max.	Absolute Min.	At 0900	At 1500	
Jan	35.1	23.6	36.2	26.3	84.1	65.0	0.0
Feb	34.2	23.4	36.2	26.3	83.4	67.0	9.2
Mar	35.1	23.6	36.5	26.5	80.1	63.0	20.3
Apr	33.4	24.4	36.5	26.0	87.3	66.2	63.6
May	32.3	24.3	35.0	26.0	89.4	67.2	160.7
Jun	31.0	24.0	33.5	26.0	90.0	66.2	306.2
Jul	28.6	23.5	33.5	24.5	89.8	73.5	401.4
Aug	28.6	23.1	31.5	24.0	94.3	78.0	644.9
Sep	30.9	23.2	34.0	24.0	90.0	71.5	435.5
Oct	32.2	23.3	39.0	25.0	86.1	69.5	237.4
Nov	31.6	23.7	33.5	26.5	89.1	70.1	200.0
Dec	32.1	23.9	34.2	27.1	84.2	69.2	2.7

Figure 1.1
A map of Sierra Leone showing the study area and some major towns.

Figure 1.2
A map of the study area.



There are many rivers and streams fringed with dense but narrow growths of riverine vegetation. In the dry season the volume of water is greatly reduced in rivers, and many of the streams and smaller rivers completely dry up. Soil consists mostly of reddish-brown so called laterites with granite outcrops occurring mainly in the interior plateaux. It might be noted here that the term laterite was originally used to describe roads in India which are soft when dug out but which harden to a rock-like consistency when dry and exposed to the air. In fact the laterite is derived from the Latin word later- a brick. However, the term laterite has been commonly used in much of Africa for almost any type of red soil- soft or hard (Pomeroy & Service,1986). The laterite roads passing through all the villages provide numerous water-filled pools that become breeding places for mosquitoes during the wet season.

Three of the study villages, Bayama, Nengbema and Nyandeyama are situated in the interior lowlands, while the other two, Mendewa and Njala-Komboya, are located on the interior plateaux. Nengbema, Nyandeyama, Mendewa and Njala-Komboya will from now on be referred to together as project villages, to distinguish them from Bayama. Maps of the study area are shown in Figures 1.1 and 1.2.

1.3.5 Data recording and statistical analysis

Data collected in the field and laboratory were recorded on forms that had been precoded for entry into a computer. The information on each form was entered twice, once by the author and again by a data entry clerk. The two data sets were compared for errors during the entry process and edited where appropriate.

The design of the data entry forms allowed for alternative groupings of time, space or vector population using a data base program (dbase 3.0). Analysis of the relationships between different variables were carried out using statistical programmes e.g. SPSS/PC and MINITAB.

CHAPTER 2

VECTOR ABUNDANCE AND MAN-BITING RATES

2.1. INTRODUCTION

In quantitative epidemiology of malaria, the man-biting rate is represented by the term **ma**. It is the product of the absolute female mosquito density per person (**m**), and the man-biting habit (**a**), that is the rate at which a single mosquito bites humans. Human bait collections effectively yield **ma** -the man-biting rate, usually expressed as the number of bites per man per night. With other sampling techniques, the parameters **a** and **m** have to be determined independently. The parameter **a** is a composite of host preference, host availability and oviposition cycle duration; it is often calculated by dividing the human blood index by the duration of the gonotrophic cycle in days. Parameter **m** can sometimes be attained by dividing the total number of freshly blood-fed adults of an endophilic mosquito caught resting in bedrooms by the number of sleepers. When man-biting rates (**ma**) are estimated directly from human bait collections, the analysis of blood-meals becomes unnecessary. Biting rates estimated from human bait collections are usually higher than those derived from indoor pyrethrum spray-sheet collections, and therefore poses the question as to which sampling method represents the true incidence of mosquito-man contact.

Vector abundance measurements and man-biting rates are often difficult to appraise.

Estimates of man-vector contact are subject to biases that must be carefully assessed for particular situations. The adult baits normally used to monitor vector populations are on average bitten more frequently than children (Bryan & Smalley, 1978; Carnevale et al., 1978; Port et al. 1980) consequently the man-biting rate estimated on adults is an overestimate of the average man-biting rate. The time that people normally spend outdoors before going to bed also affects the estimates of indoor man-biting rates. Attractiveness to vectors as well as ability to catch them also varies significantly between collectors. Room densities, that is the number of females resting in bedrooms, as determined by early morning pyrethrum spray-sheet collections, is another method that is commonly used to measure seasonal changes in vector abundance. In general unfed mosquitoes are attracted to rooms in numbers related to the number of human occupants (Haddow, 1942), but no simple arithmetic relationship has been established between catch size and number of occupants.

The use of light-traps to sample African endophagic and endophilic Anopheles became a useful tool after Odetoyinbo (1969), working in The Gambia, showed that if CDC miniature light-traps were placed in houses, they could sample populations of the An. gambiae complex. Highton (1981) working in Kenya showed that CDC-type traps placed inside houses gave reliable and unbiased collections of An. arabiensis. Such light-traps catch a proportion of hungry unfed mosquitoes before they have succeeded in biting an occupant, and also a proportion of those that have taken a blood-meal, and are either seeking a resting surface in the house or trying to leave the house. Semi-gravid and gravid females flying out of the house may also be caught. Increased catches in light-traps usually result when people sleep under bed-

nets, because this extends their flying time in houses, in search of a blood-meal and consequently increases their likelihood of being caught in the traps.

In this Chapter, I will assess the applicability of different sampling techniques to determine man-vector contact and seasonal variations in mosquito abundance.

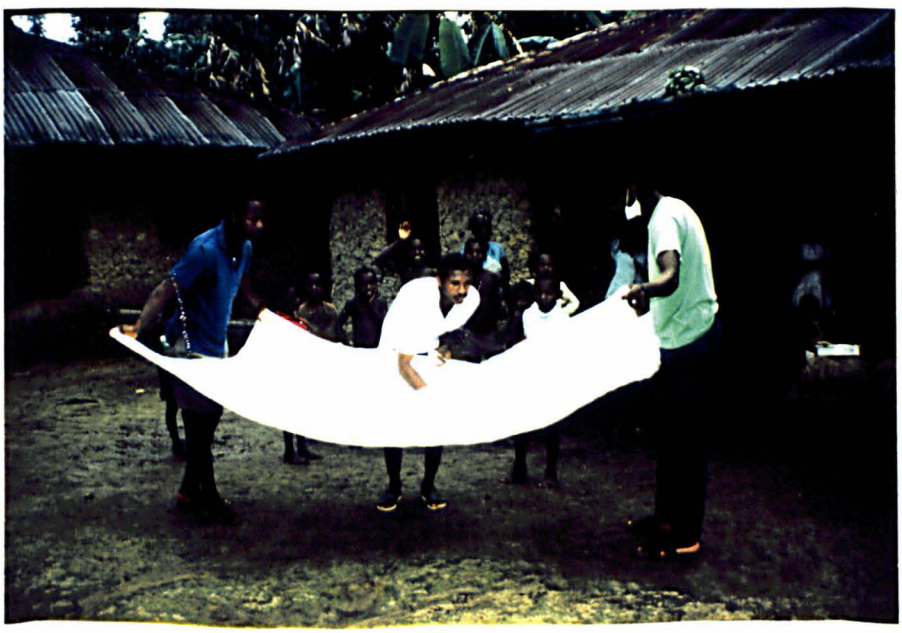
2.2 MATERIALS AND METHODS

2.2.1 Pyrethrum spray collections (PSC)

To obtain information on seasonal abundance of the malaria vectors, pyrethrum spray sheet collections were carried out in at least six houses every fortnight in the villages of Nengbema, Nyadeyama, Mendewa and Njala-Komboya, from January 1990 to April 1991. In Bayama similar collections were made in six houses once a week from April 1991 to October 1991. The routine in all villages was the same, namely inhabitants were asked to leave their rooms and all easily removable objects such as small tables, chairs, exposed food and drinking water removed. Then in each room, two field assistants carefully laid white cotton sheets (2x4m) over the entire floor as well as over beds, large furniture and miscellaneous objects that were not easily removable. All doors and windows were closed and the bedroom sprayed by three people, two of whom remained inside, while the other went outside and sprayed along the open eaves gaps (Figure 2.1) and other potential escape routes such as closed doors and windows. The spray was directed towards the roof or ceiling of a bedroom for about three minutes, after which the sprayers left the room and closed the door. Houses were sprayed early in the morning, between 0630 and 0900 hr, with PYCON 819e (5% w/vol pyrethrins synergised with 25% w/vol piperonyl butoxide), which was formulated with an emulsifier and thus enabled water to be used as the diluent. A dilution of 1:4 was always freshly prepared in the field. The concentrate was shaken before dilution and again after dilution prior to spraying. After 10 min two people re-entered the bedroom and collected knocked-down mosquitoes from the floor

Figure 2.1
A field assistant spraying along eave gaps on a typical thatch house to prevent the escape of mosquitoes during indoor pyrethrum spray collections.

Figure 2.2
Spray sheet brought outdoor and searched for knocked down mosquitoes.



sheets, starting at the door and moving to the centre. But on dry days, the sheets were carefully removed by lifting them by the four corners and shaking them gently so that the mosquitoes collected in the middle (Figure 2.2). All mosquitoes were picked up with forceps and placed in plastic petridishes lined with damp filter paper, and then placed in a cool box for transportation to the laboratory in Bo.

After the completion of the spray collections furniture and other objects removed from the bedrooms were returned to their original positions. The name of the village, house number, time of spraying, and the number of people who slept in the room were recorded on forms.

In the laboratory, mosquitoes were identified to species and sex and their gonotrophic condition recorded. Samples from appropriate age-categories were used for chromosomal identification, blood-meal identification, sporozoite rates, age-grading to nulliparous or parous condition, while some were preserved for identification by DNA probes.

2.2.2 Human bait collections

To obtain information on seasonal variations in man-biting rates and the biting cycles of anthropophagic species, human bait catches were carried out at Nyandeyama and Mendewa from December 1989-April 1991, and at Bayama from November 1990 to October 1991.

Mosquitoes were collected by a team of six people from 1800 - 0600 hr. Two people seated on benches or chairs collected mosquitoes for two hours before they were replaced by another set of two people. To avoid sampling bias in personal attraction the same collecting pair did not catch mosquitoes during the same time on successive sampling occasions. The collectors, aided by the light from a small hurricane lamp and torches which were used intermittently, captured in test tubes all mosquitoes coming to bite. Hourly collections were put in a labelled cotton bag which was placed in a cool box containing ice packs. Initially collections were performed out of doors from 1800-2200 hr to coincide with outdoor activities of the local people, and then continued from 2200 - 0600hr in a bedroom. At Nyandeyama and Mendewa, such catches were performed once a fortnight. In addition all-night out of door biting collections were performed in each of the these two villages at least once a month. At Bayama, indoor and out of door collections were made on alternate weeks from November 1990 to March 1991. No collections were made for three weeks in April 1991 because of political disturbances in the area. From May 1991 to October 1991 catches were performed in a bedroom six times a month and out of door twice a month.

Mosquito collectors were semi-immunes and were normally exposed to mosquito bites in Bo, and so were not given malaria prophylaxis. Diagnostic and therapeutic services for malaria, were, however, freely available.

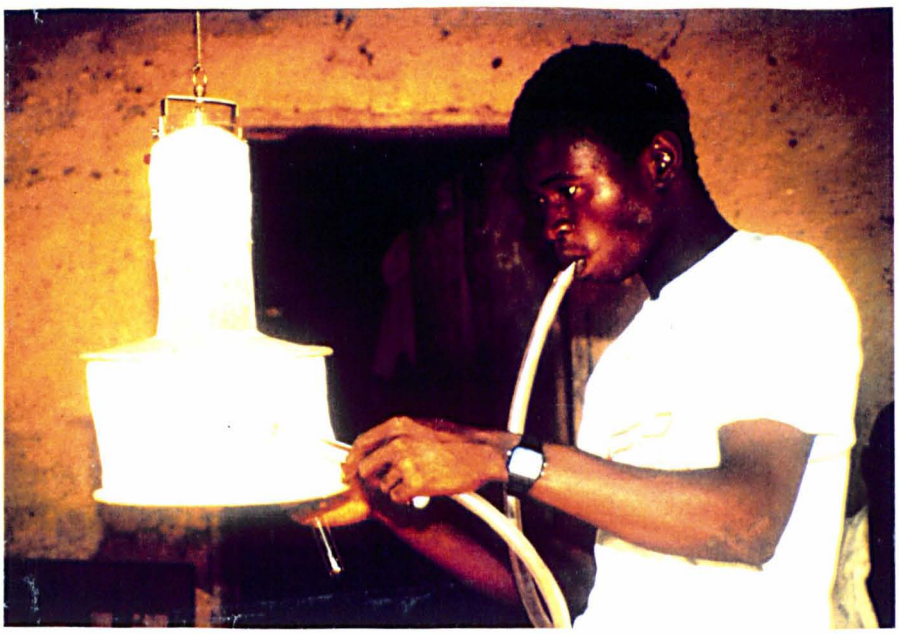
In the laboratory, mosquitoes were identified as in the pyrethrum catches and samples used for various specific purposes, such as age-grading and sporozoite determination.

2.2.3 Light-trap collections

CDC light-traps operated from 6-V rechargeable gell-cell batteries were used to sample indoor mosquitoes. The trap used in this study has been described and illustrated by Sudia and Chamberlain (1962). The body of the trap was made from a 15-cm length of 8.25-cm internal diameter clear perspex (plexiglas) tubing. A slot on each side permitted the insertion of the motor support bracket for holding the motor. A 4-V bulb was mounted directly above the motor at the top of the trap body. Colour-coded binding posts and 'snap-on' terminals permitted easy connection of leads from the battery and motor assembly. A detachable wire mesh screen was placed over the entrance of the trap to exclude large insects. A fine mesh cloth collecting bag with a narrow neck was fitted to the end of the trap body. In bedrooms without a ceiling, the trap was suspended so that the light bulb was on the same level as the eaves and as close to the sleeper as possible. In bedrooms with ceilings, it was suspended about 15 cm below the ceiling, opposite a window and close to sleeper.

Once every fortnight, light-traps were placed in 3-5 bedrooms at Nyandeyama and at Mendewa. At Nyandeyama, three of the rooms had bed-nets while one of the rooms at Mendewa had a bed-net. At Bayama, trap collections were made twice a week from April 1991 to October 1991. The traps were switched on at 1800 hr and switched off at 0700 hr. Figure 2.3 shows a field assistant collecting mosquitoes from a CDC light-trap in the morning.

Figure 2.3
A field assistant collecting mosquitoes from a CDC light-trap.



In the laboratory, all Anopheles caught were classified according to species, and gonotrophic condition as in the pyrethrum spray catches. Also samples were used for age-grading, sporozoite and blood-meal determination. Culicines were in general classified by sex and genus and then pinned for identification at a later date.

2.2.4 Survey of mosquito breeding sites

Vector abundance usually depends on the availability of breeding sites. Surveys were carried out intermittently in all the villages to determine mosquito species breeding in different places. Natural collections of water in transient rain pools, rice swamps and the edges of seasonal streams, were carefully checked for mosquito larvae. Temporary pools were found in rocks, roads, foot paths and in children's playgrounds in the middle of the villages. Collections of water in domestic containers, and plant receptacles such as the axils of pineapples and bananas were also checked for mosquito larvae.

Soup ladles were used to collect larvae from small collections of water such as pools, while plastic bowls were used to collect larvae from swamps and streams. Larvae were placed in labelled glass tubes and transported in cool boxes to the laboratory in Bo where they were identified either as larvae or as emerged adults, using anopheline keys by Gillies and Coetzee (1987) and culicine keys by Hopkins (1952) and Service (1990).

2.3 RESULTS

The results of collections from the project villages, that is Nengbema, Nyandejama, Mendewa and Njala-Komboya, will be presented together, and separately from results for Bayama village. Only Anopheles gambiae and Anopheles funestus are considered here. Other mosquito species will be discussed in chapter 7.

2.3.1 Pyrethrum spray collections in the project villages

A total of 748 spray collections were carried out in 39 houses in the four project villages from January 1990 to April 1991. A summary of the PSCs and number of mosquitoes collected in the villages is given in Table 2.1.

The total number of female An. gambiae and An. funestus collected were 1776 (91.7%) and 161 (8.3%) respectively. Both species were caught in all the villages, but An. funestus was more common in the high altitude villages (Mendewa and Njala-Komboya), especially Mendewa, than in the low altitude villages (Nengbema and Nyandeyama). Although the number of collections in the two groups of villages were similar, 378 and 370 in the low and high altitude villages respectively, the number of An. gambiae caught in the low altitude villages was twice the number caught in the high altitude villages, whereas the number of An. funestus caught in the high altitude villages was over five times greater than caught in the low altitude villages. Female An. gambiae was caught in 32 out of 39 houses sprayed. Four of the seven houses which yielded no female An. gambiae were sprayed only once. The

TABLE 2.1
Numbers of An. gambiae and An. funestus caught in pyrethrum spray collections in the different villages.

Village	Number of houses	Number of PSCs	Number of mosquitoes (%)		
			Anopheles gambiae	Anopheles funestus	Total
Nengbema	10	207	595 (97.9)	13 (2.1)	608
Nyandeyama	7	171	620 (98.1)	12 (1.9)	632
Mendewa	11	183	205 (65.1)	110 (34.9)	315
Njala	11	187	356 (93.2)	26 (6.8)	382
Total	39	748	1776 (91.7)	161 (8.3)	1937

other three were each sprayed less than five times. Female An. funestus were caught in 23 houses, 13 of which were from the high altitude villages. A total of 66 male An. gambiae were caught in PSCs, and were caught in all the villages, but only two male An. funestus were caught, both in Mendewa. The highest numbers of An. gambiae and An. funestus collected from one room were 61 and 14 respectively, and that was in Nyandeyama and Mendewa during the months of July and April respectively.

On the whole, the number of female mosquitoes collected were few. About half the number the of PSCs in 39 houses (49.7%) yielded neither An. gambiae nor An. funestus. Only 43 out of the 748 spray collections produced more than 10 mosquitoes. Table 2.2 gives a frequency distribution of different catch groups of An. gambiae classified as no females, 1-10 females and more than 10 females caught per room. The number of females caught did not follow a normal distribution because of the high number of zero values. The non-parametric Mann-Whitney rank test was therefore used to compare abundance in different populations. This test showed that the number of female An. gambiae caught in the wet season (May to November) was significantly higher than in the dry season from December to April ($Z = -9.849$, $p < 0.0001$). In the case of An. funestus, there was no significant difference at the 95% confidence level in the number of females caught during the dry and wet seasons.

The geometric mean of the number of indoor-resting female mosquitoes per bedroom was calculated to estimate indoor-resting densities. The maximum number of possible collections from any house was 32, but some houses were not sprayed

TABLE 2.2
Frequency distribution of different catch groups of female An. gambiae and An. funestus (pyrethrum spray collections), all villages combined.

Vector species	Catch group	Frequency	%
Anopheles gambiae	No mosquitoes	394	52.7
	1-10 mosquitoes	315	42.1
	>10 mosquitoes	39	5.2
Anopheles funestus	No mosquitoes	674	90.1
	1-10 mosquitoes	72	9.6
	>10 mosquitoes	2	0.3
Anopheles gambiae + Anopheles funestus	No mosquitoes	372	49.7
	1-10 mosquitoes	333	44.5
	>10 mosquitoes	43	5.7

throughout the period of study because of occupant's disapproval or their emigration to another village, leaving the house empty. Only houses sprayed 20 times or more were considered for analysis for indoor-resting densities. A total of 608 PSCs in 23 houses was therefore considered. Indoor-resting densities of 1.0 and <0.1 females/room were estimated for An. gambiae and An. funestus respectively, for the whole period of study. In the different villages, indoor-resting densities of An. gambiae ranged from 0.7 females/room at Mendewa to 1.6 females/room at Nyandeyama. Densities of An. funestus were never more than 0.3 females/room in any of the villages. Indoor-resting densities for the different villages are given in Table 2.3. The indoor-resting densities for the two seasons are given in Table 2.4 for An. gambiae and An. funestus.

The relationship between the time of collection, rainfall and number of sleepers in the rooms, and the indoor-resting density of female An. gambiae was investigated for the wet season. Table 2.5 gives a summary of room densities of An. gambiae according to the time of spray sheet collections and whether it rained the previous night. The difference in the densities estimated when PSC were performed before (2.1 females/room) and after (1.5 females/room) 0800 hr was not significant ($Z=1.37$ and $p=0.17$). However, significantly more mosquitoes were caught on mornings following nights when it did not rain (2.5 females/room) than when it did (1.5 females/room) ($Z= -2.59$ and $p=0.0095$). Table 2.6 gives the wet season room densities of An. gambiae according to the number of sleepers in the rooms on the night preceding the PSC. In all the villages, the room densities increased between one and three sleepers, but this trend was not apparent when there were four or more

TABLE 2.3
Indoor-resting densities (females/room) of An. gambiae and An.funestus in the different project villages

Village	Number of houses	Number of PSCs	Room densities	
			An. gambiae	An. funestus
Nengbema	6	168	1.0	0.0
Nyandeyama	6	167	1.6	0.1
Mendewa	6	146	0.7	0.3
Njala-Komboya	5	127	1.3	0.1
Total	23	608	1.1	<0.1

TABLE 2.4

Indoor-resting densities (females/room) of An. gambiae and An. funestus according to season in the combined project villages.

Season	No. PSCs	An. gambiae	An. funestus
Dry	270	0.4	0.2
Wet	338	1.9	0.1
Annual	608	1.1	<0.1

TABLE 2.5
Geometric mean An. gambiae/room in the wet season according to : A) time of spray and B) whether it rained or not on night preceding PSC, all villages combined. Numbers in parentheses indicate number of times PSC was performed.

A) Time of spray (hr)		B) Precipitation	
Before 0800 (204)	After 0800 (134)	Rain (190)	No rain (148)
2.1	1.6	1.5	2.5

TABLE 2.6
Geometric mean number of female An. gambiae/room (wet season) in the different villages, according of the mean number of sleepers/room. Numbers in parentheses indicate number of times PSC was performed.

Village	Number of sleepers			
	1	2	3	4 or more
Nengbema	0.6(26)	2.4(21)	3.8(25)	2.6(15)
Nyandeyama	1.3(18)	3.9(26)	4.3(35)	2.0(9)
Mendewa	0.7(31)	0.8(37)	2.2(9)	2.6(9)
Njala-Komboya	0.6(28)	2.1(20)	3.3(7)	3.3(21)

sleepers, except in Mendewa, but the number of rooms sprayed (9) were not large and the increase from 2.2 to 2.6 females/room was not significant ($P>0.05$). Seasonal variations in room densities of An. gambiae and An. funestus, and rainfall are shown in Figure 2.4 for all villages combined and in Figures 2.5 and 2.6 for the different villages. The An. gambiae indoor-resting densities for the dry (December - April) and wet (May - November) seasons were 0.4 and 1.9 females/room, respectively. The corresponding values for An. funestus were 0.2 and 0.1 females/room (Table 2.4). The indoor-resting density of An. gambiae increased explosively, very early in the wet season and attained it's peak in three villages in July when it started raining heavily, but in Mendewa such a unimodal peak was not apparent. In all villages, the numbers caught rapidly declined after July and remained low for the rest of the year. Anopheles funestus was found in reasonable numbers only in the dry and early wet season at Mendewa (Figure 2.6A), reaching a peak in April.

Indoor man-biting rates were estimated from the number of blood-fed females (freshly-feds and late-feds) caught in PSC, divided by the number of sleepers in the room the previous night. Using this approach, the man-biting rates for An. gambiae and An. funestus for all villages, extended over the entire period were 1.2 and 0.1 bites/man/night, respectively. Table 2.7 gives a summary of the man-biting rates in the different villages, and shows that the rates for An. gambiae varied from 0.5 bites/man/night at Mendewa to 1.3 bites/man/night at Nyandeyama. The An. funestus biting rate was 0.2 bites/man/night or less in all the villages. Seasonal variations in the estimated man-biting rates of the two vector species (Figure 2.7)

TABLE 2.7
Number of bites/man/night of An. gambiae and An. funestus in the different project villages, based on pyrethrum spray catches.

Village	Mean sleepers per room	Bites/person/night	
		An. gambiae	An. funestus
Nengbema	2.3	1.1	0.0
Nyandeyama	2.4	1.3	0.0
Mendewa	1.9	0.5	0.2
Njala-Komboya	2.7	0.7	0.0
All villages	2.3	1.2	0.1

Figure 2.4

Seasonal variation in indoor-resting density of An. gambiae and An. funestus, all villages combined. Rainfall data included for comparison.

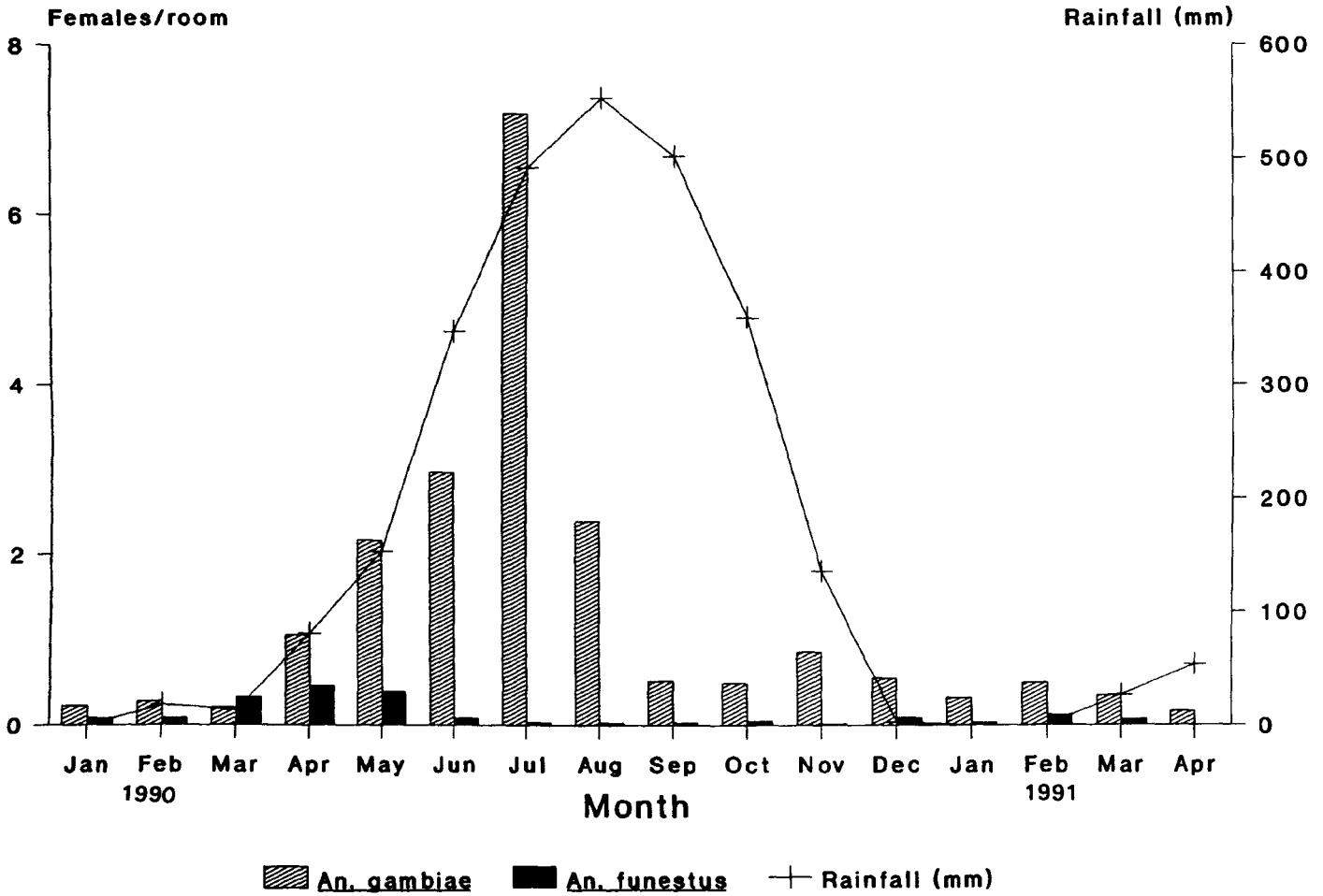
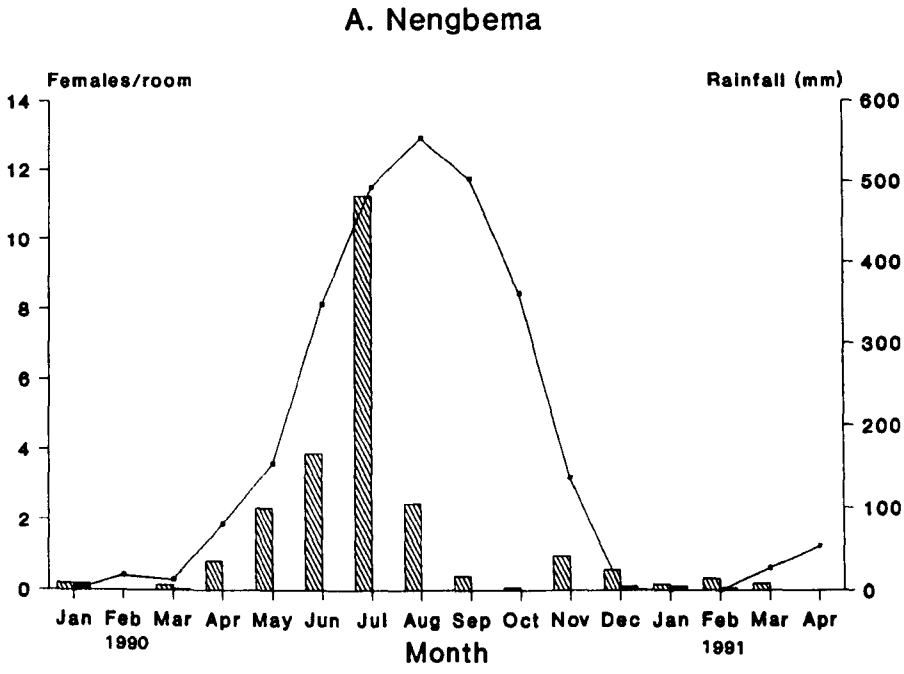


Figure 2.5

Seasonal variation in indoor-resting densities of An. gambiae and An. funestus in the low altitude villages: A) Nengbema B) Nyandeyama.



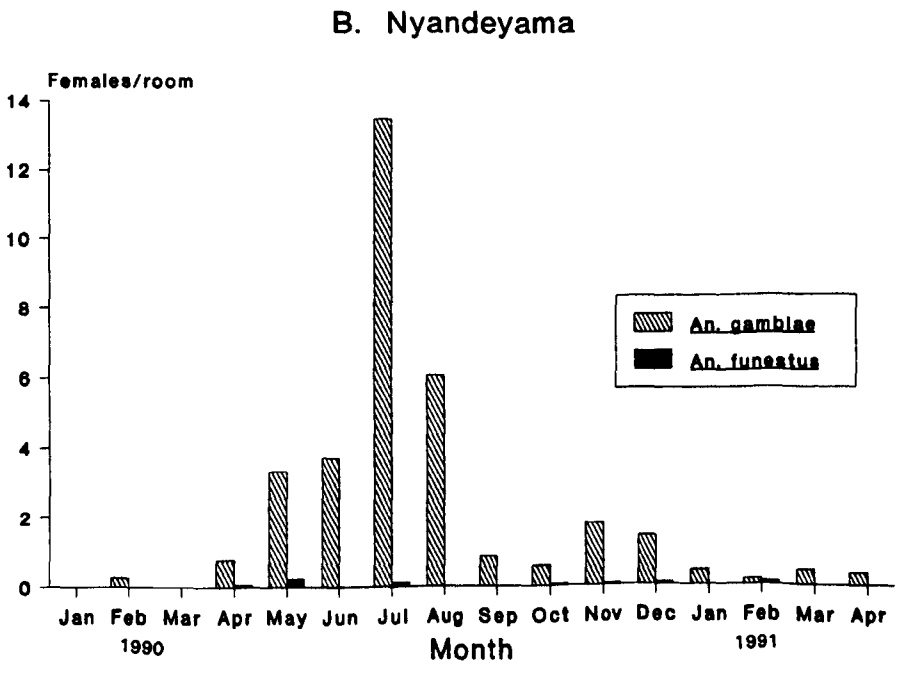
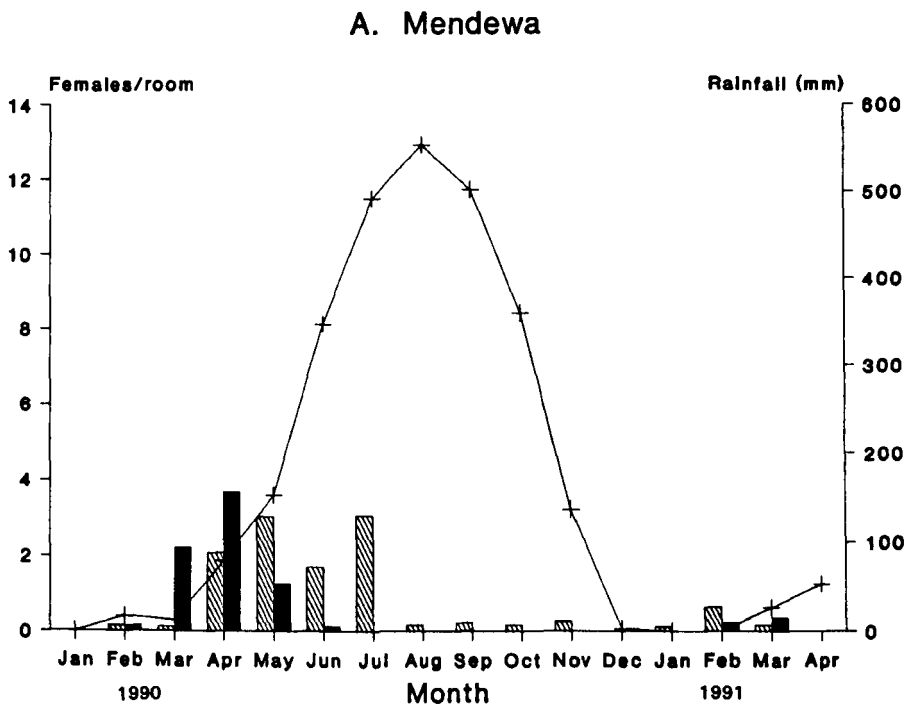


Figure 2.6

Seasonal variation in indoor-resting densities of An. gambiae and An. funestus in the high altitude villages: A) Mendewa B) Njala-Komboya.



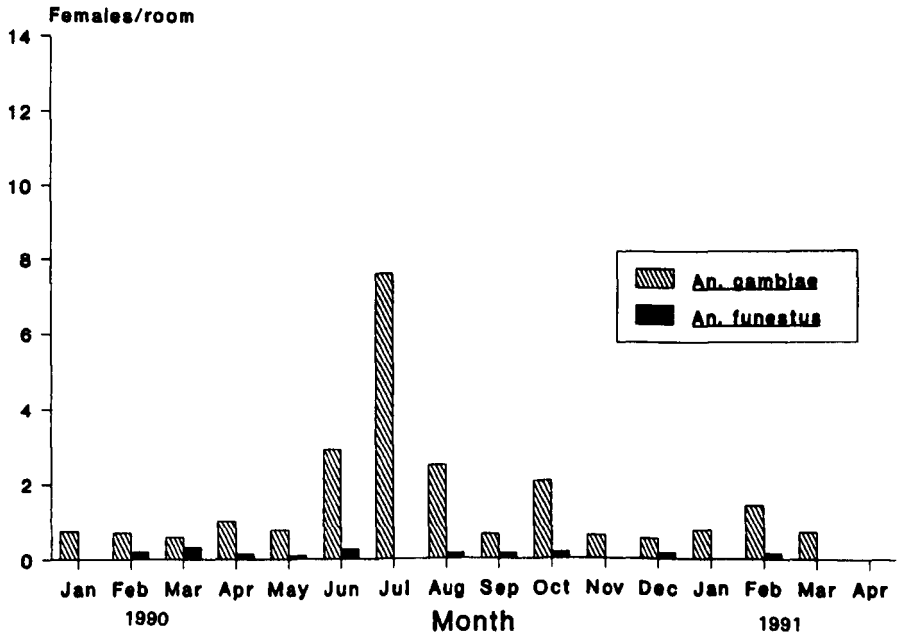
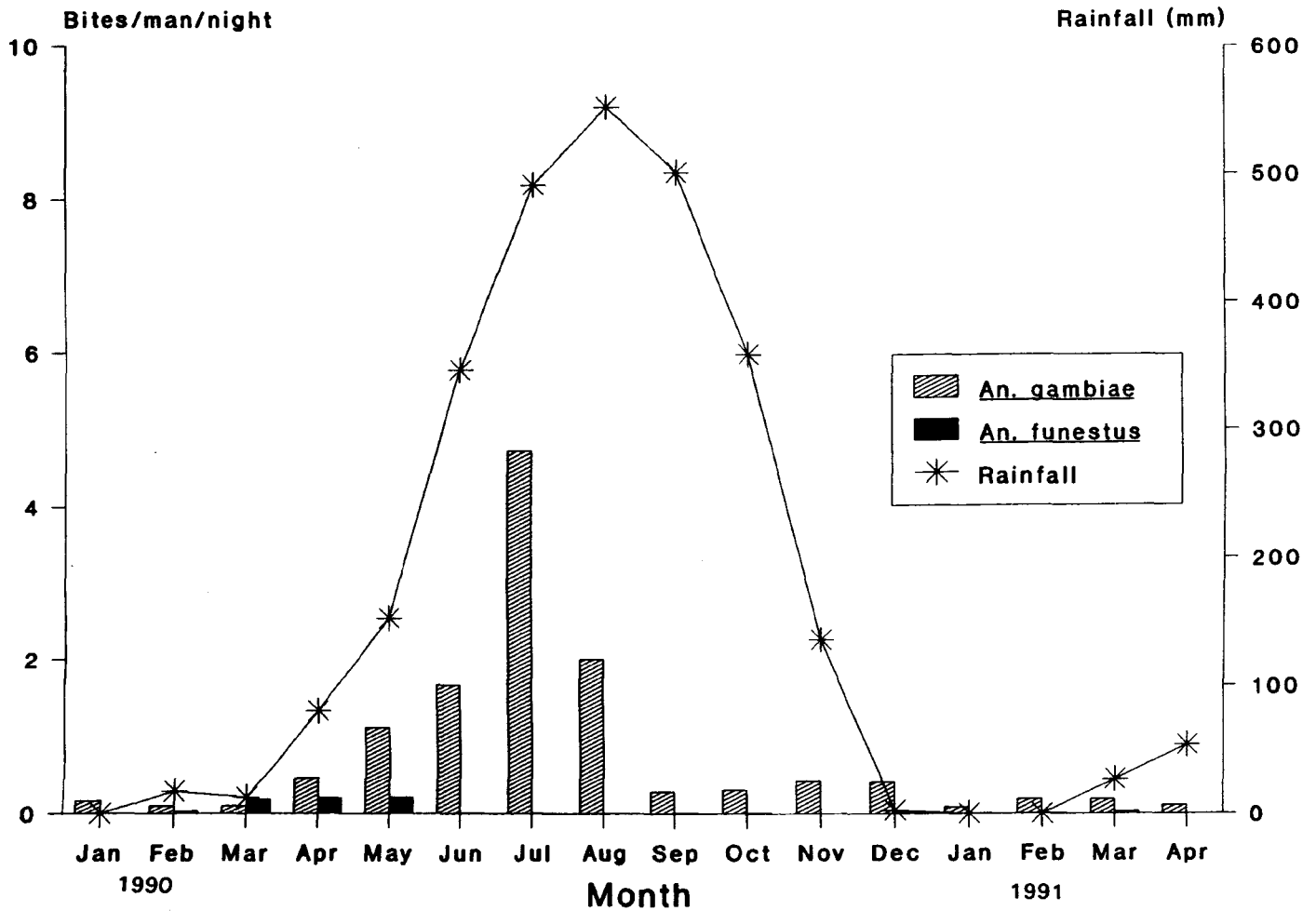


Figure 2.7

Seasonal variation in man-biting rates of An. gambiae and An. funestus estimated from PSC, all villages combined.



show that in July a person could receive an estimated 4.7 bites a night from An. gambiae. A peak man-biting rate of 0.2 bites/man/night was estimated for An. funestus in April and May.

2.3.2 Light-trap collections in the project villages

A total of 256 light-trap collections were performed in five houses each at Nyandeyama and Mendewa. The number of times traps were hung in individual rooms varied from 20 to 31. Four of the ten rooms used for collections had bed-nets which, for most of the time, remained folded up and were not used when people went to bed. For example, out of a total of 111 collections from such rooms, the bed-nets were used only nine times. Bed-net usage in the villages will be discussed in chapter 6. The geometric mean number of female An. gambiae and An. funestus per trap-night was less than the indoor-resting density, but for An. gambiae both indices followed the same trend in seasonal variation (Figure 2.8). The proportion of female An. funestus caught in Nyandeyama and Mendewa in light-traps (12.3%) was more than in PSC (8.3%). Also, light-traps caught An. funestus in 80% of the houses in which they were placed, as compared to PSC which produced the same species in only 58% of the houses sprayed. Table 2.8 gives a summary of light-trap collections.

During the wet season, the number of An. gambiae caught in bedrooms when bed-nets were in use was more than five times greater than when bed-nets were not used (Table 2.9). Light-traps hung in rooms when sleepers were using bed-nets, caught

Figure 2.8

Seasonal variation in mean number of An. gambiae in bedrooms at Nyandeyama, estimated from PSC and light-traps collections.

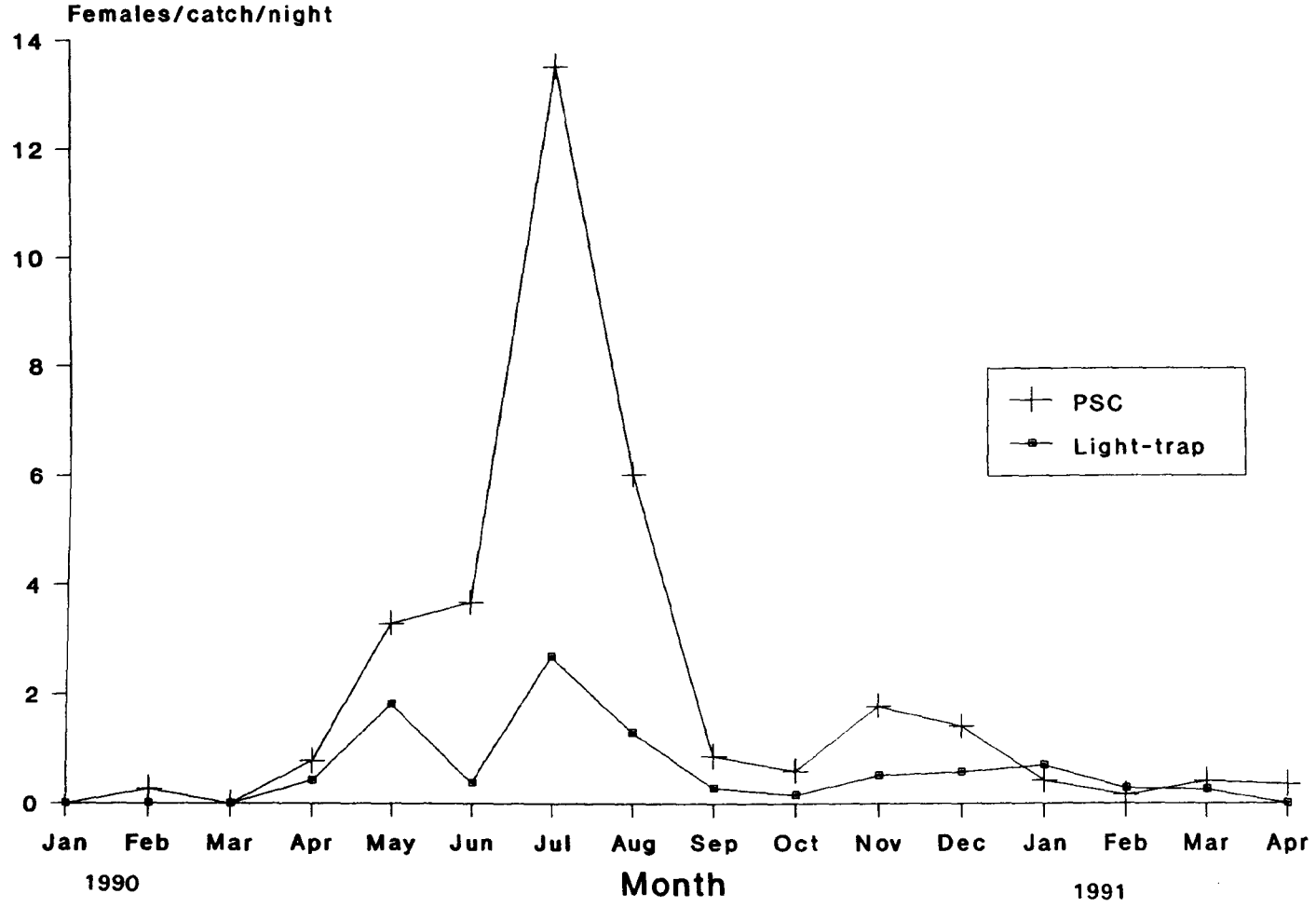


TABLE 2.8
Numbers of An. gambiae and An. funestus caught in light-traps at Nyandeyama and Mendewa

Village	Number of light-nights	Number of females		Total
		An. gambiae(%)	An. funestus(%)	
Nyandeyama	132	159 (94.1)	10 (5.9)	169
Mendewa	124	48 (71.6)	19 (28.4)	67
Total	256	207 (87.7)	29 (12.3)	236

overnight a maximum of 26 female An. gambiae, whereas in rooms where bed-nets were not used the maximum catch was 10 female An. gambiae. Comparisons were not made for An. funestus because few adults were caught in villages where bed-nets were used. Throughout the entire catching period only 15 male An. gambiae and 1 male An. funestus were collected in light-traps.

2.3.3 Human-bait collections in the project villages

A total of 67 human-bait collections were carried out at Nyandeyama and Mendewa, 52 indoors and 12 outdoors, but only 165 An. gambiae and 11 An. funestus were caught (Table 2.10). Although bait catches were performed from January to December 1990, An. gambiae was caught only during the wet season (Figure 2.9) when up to 9.5 and 3.8 bites/man/night were recorded in bedrooms at Nyadeyama and Mendewa, respectively. Peak monthly biting rates in both villages were experienced in July. The peak outdoor man-biting rate at Nyandeyama was 16 bites/man/night, also in July, but at Mendewa, the highest outdoor man-biting rate was 2 bites/man/night in May, at the beginning of the wet season. The highest man-biting rate recorded for An. funestus was 1 bite/man/night indoors at Mendewa in March. Table 2.11 gives a summary of man-biting rates estimated from combined human-bait in Nyandeyama and Mendewa. Mean annual indoor and outdoor biting rates were similar for both species. The man-biting rates (indoor and outdoor combined) for An. gambiae and An. funestus were 1.1 and 0.1 bites/man/night respectively.

TABLE 2.9
Geometric mean numbers of females/light-trap-night according to bed-net usage.

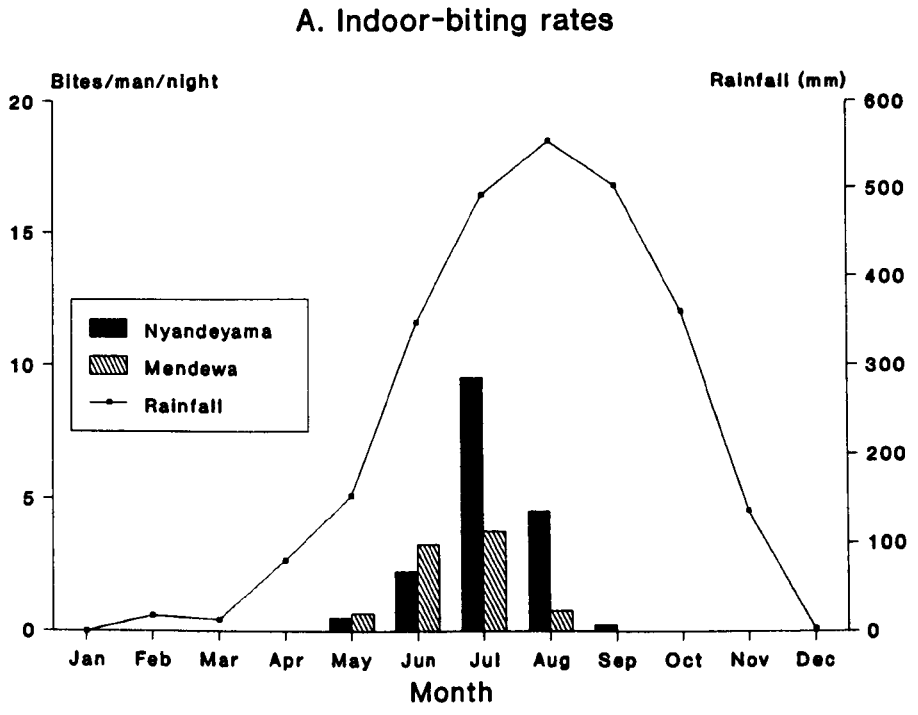
Net usage	Trap-nights	An. gambiae	An. funestus
Net	9	2.2	0.0
No net	247	0.4	0.1

TABLE 2.10
Number of An. gambiae and An. funestus caught at human-bait catches at Nyandeyama and Mendewa

Village	An. gambiae		An. funestus		Total
	Indoor	Outdoor	Indoor	Outdoor	
Nyandeyama	77	46	0	1	124
Mendewa	35	7	9	1	52
Total	112	53	9	2	
Grand Total	165		11		176

Figure 2.9

Seasonal variation in man-biting rates (combined indoor and out of door human-bait catches) of An. gambiae at Nyandeyama and Mendewa.



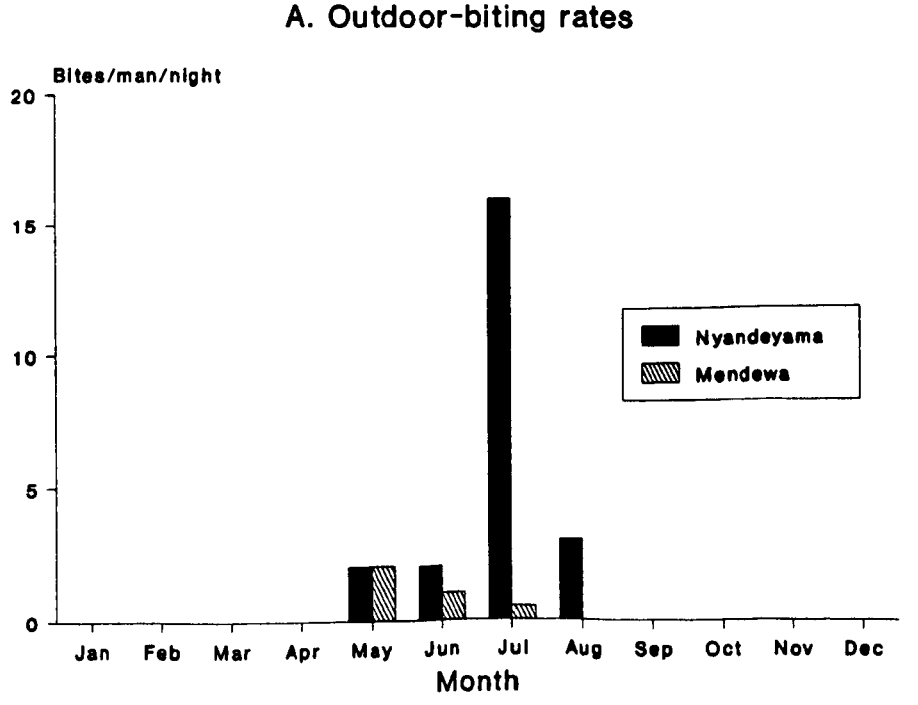


TABLE 2.11
Man-biting rates of An. gambiae and An. funestus estimated from combined human-bait catches at Nyandeyama and Mendewa

Season	An. gambiae		An. funestus	
	Indoor	Outdoor	Indoor	Outdoor
Dry	0.0	0.0	0.2	0.0
Wet	1.9	1.6	0.1	0.1
Annual	1.1	1.1	0.1	0.1

TABLE 2.12
Summary of entomological indices for An. gambiae estimated from pyrethrum spray collections (PSC) at Bayama

Month	Number of PSC	Number of females	Indoor-resting density[+]	Man-biting rate[++]
June	22	153	4.4	2.1
July	12	112	5.1	2.5
August	21	138	4.1	2.1
September	19	53	1.5	1.1
October	10	41	2.7	1.3
Jun-Oct.	84	497	3.4	1.8

[+] Mean number of females/room
[++] Bites/man/night

In marked contrast, at Bayama, the small village 22 km south of Nengbema, human-bait catches estimated up to 325 An. gambiae bites/man/night. Pyrethrum spray and light-trap catches were also higher than in the project villages, although not to the same degree. Anopheles gambiae accounted for more than 99% of the vector population. The following results are for Bayama village.

2.3.4 Pyrethrum spray collections at Bayama

A total of 84 PSCs were carried out in five out of the six dwelling houses in the village from June to October 1991, and 497 female An. gambiae but only three An. funestus were caught. The mean indoor-resting density and man-biting rate of An. gambiae from the period June to October were 3.4 females/room/night and 1.8 bites/man/night respectively. Table 2.12 gives the geometric mean monthly room densities of An. gambiae and the man-biting rate estimated from the number of fed females and the number of sleepers. As in the project villages, the highest room density was recorded in July (Figure 2.10), but the increase in mean density from June to July was not as explosive as in the project villages (Figure 2.4).

About half of the PSCs (44) were performed after 0800 hr, but the room densities estimated from these relatively late collections (3.7 females/room) did not differ significantly from estimates for collections before 0800 hr (3.0 females/room (Mann-Whitney $p > 0.05$). Whether it rained or not on the night preceding PSC also did not significantly affect the number of females caught: Indoor resting densities on mornings following wet and dry nights were 3.1 and 3.8 females/room respectively.

Figure 2.10

Geometric mean monthly indoor-resting densities of An. gambiae at Bayama. PSCs were only performed during five months of 1991.

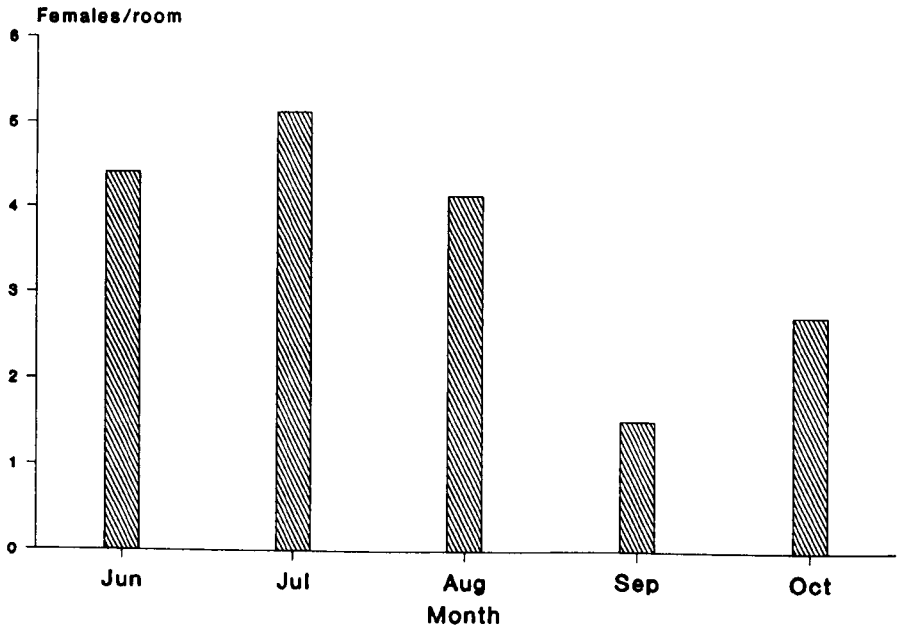
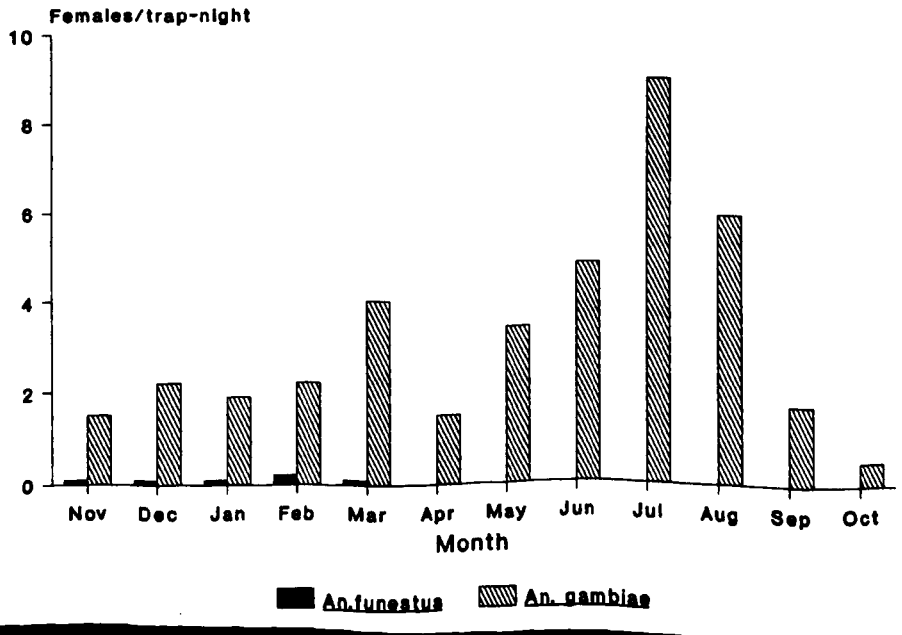


Figure 2.11

Seasonal variation in light-trap collections of An. gambiae and An. funestus at Bayama.



Tables 2.13 gives indoor-resting densities according to the time of spray and whether it rained or not on nights preceding collections.

There was a house to house variation in the mean indoor-resting densities of An. gambiae, ranging from 1.1 - 7.1 females/room (Table 2.14). There was no clear-cut relationship between the number of An. gambiae caught in rooms and the number of people sleeping in them (Table 2.15).

2.3.5 Light-trap catches at Bayama

A total of 235 light-trap collections yielded 2085 An. gambiae (99.4%) and 13 An. funestus (0.6%). The number of females caught in a single trap varied from 8 to 183 and the annual mean number of female An. gambiae /trap-night was 3.0. The averages for the dry and wet season were 2.3 and 3.4 females/trap night respectively (Table 2.16). Anopheles funestus were only caught in the dry season (Figure 2.11). Catches of An. gambiae in different bedrooms varied from 1.6 to 11.2 females/trap-night, both estimates being for two rooms in the same house (Table 2.17). The geometric means of female An. gambiae caught when traps were hung in rooms with bed-nets in use, and also in rooms where bed-nets were not used, were 3.3 and 3.0 females/trap night respectively (Table 2.18). The difference was, however, not significant (Mann-Whitny Z= -0.48 and P=0.628). Rooms without bed-nets but with windows located close to the vegetation surrounding the village (Room 602 and 201) yielded the most mosquitoes in light-traps.

TABLE 2.13
Geometric mean numbers of An. gambiae/room according to time of spray and whether it rained or not on night preceding PSC. Numbers in parentheses indicate number of times PSC was performed.

Time of spray (hr)		Precipitation	
Before 0800 hr (40)	After 0800 hr (44)	Rain (53)	No rain (31)
3.0	3.7	3.1	3.8

TABLE 2.14
Indoor-resting densities (IRD) and man-biting rates (MBR) estimated from spray collections in the different rooms at Bayama. All rooms were in different houses.

Room	Number PSC	Mean sleepers/room	IRD	MBR
103	13	2.9	3.8	2.2
201	15	3.9	6.2	2.2
302	16	3.3	1.1	0.4
502	16	1.0	1.8	3.5
603	15	1.3	7.1	7.8
All rooms	75	2.4	3.4	2.4

TABLE 2.15
Geometric mean number of An. gambiae/room for rooms with different number of sleepers

	Number of sleepers				
	1	2	3	4	>4
Number of PSCs	32	6	27	16	3
Mean females/room	3.2	1.4	3.1	5.5	3.4

TABLE 2.16
Geometric mean numbers of females/trap-night in the wet and dry seasons at Bayama.

Season	Trap-nights	An. gambiae	An. funestus
Dry	84	2.3	0.1
Wet	151	3.4	0.0
Annual	235	3.0	<0.1

TABLE 2.17
Geometric mean numbers of female An. gambiae/light-trap-night in different rooms at Bayama

Room number	Trap-nights	Females/trap-night
103*	27	2.0
102*	52	2.4
201	20	4.5
301	50	2.2
501	18	2.6
601**	33	1.6
602**	29	11.2

* Both rooms in house No. 1
** Both rooms in house No. 2

TABLE 2.18
Geometric mean numbers of females/trap-night according to net usage in Bayama village.

Net usage	Trap-nights	Females/trap-night
Net	20	3.3
No net	211	3.0

TABLE 2.19
Geometric mean number of female An.gambiae/trap-night for the different light-traps.

Trap number	Number of collections	Females /trap night
1	42	2.4
2	39	3.8
3	40	4.3
4	37	3.0
5	41	2.1
6	36	2.8

To test whether individual light-traps, which were the same ones used earlier in the project villages, varied in their efficiency to catch mosquitoes, a Chi-Square test was performed on the relationship between different traps and the number of female An. gambiae they caught. Table 2.19 shows the mean catch per trap, and that the variation in numbers caught by the different traps was more than could be explained by chance alone (χ^2=13.97, P= 0.16). That is, some traps were intrinsically better than others.

2.3.6 Human-bait catches at Bayama

A total of 17113 An. gambiae and 16 An. funestus were collected from 64 human-bait catches performed outdoors and 24 performed indoors. The mean indoor and outdoor man-biting rates estimated from the 12 months collections were 56.8 and 103.5 bites/man/night respectively. Monthly indoor biting rates varied from 8.5 - 146 bites/man/night and outdoor biting rates from 5.5 to 192/bites/man/night (Figure 2.12). Biting increased considerably in May and peaked during July. There were no records for indoor collections in April and May because of military activity in the village at the time made spare bedrooms unavailable for the mosquito collectors. Man-biting rates varied from 0.2 to 325 bites/man/night outdoors and 4.5 to 244.5 bites/man/night indoors.

Figure 2.12 illustrates seasonal variation in indoor and outdoor man-biting rates which followed the same trend. The lowest biting rates in both cases were in January when it did not rain at all and the highest rates in July when the heavy rains (>350 mm)

Figure 2.12

Seasonal variation in indoor and out of door human-bait catches of An. gambiae at Bayama

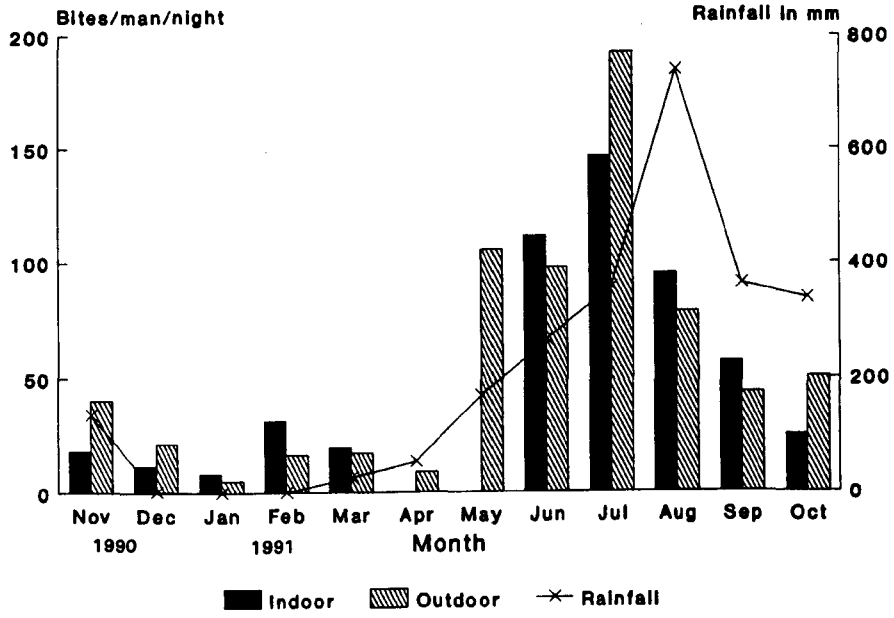
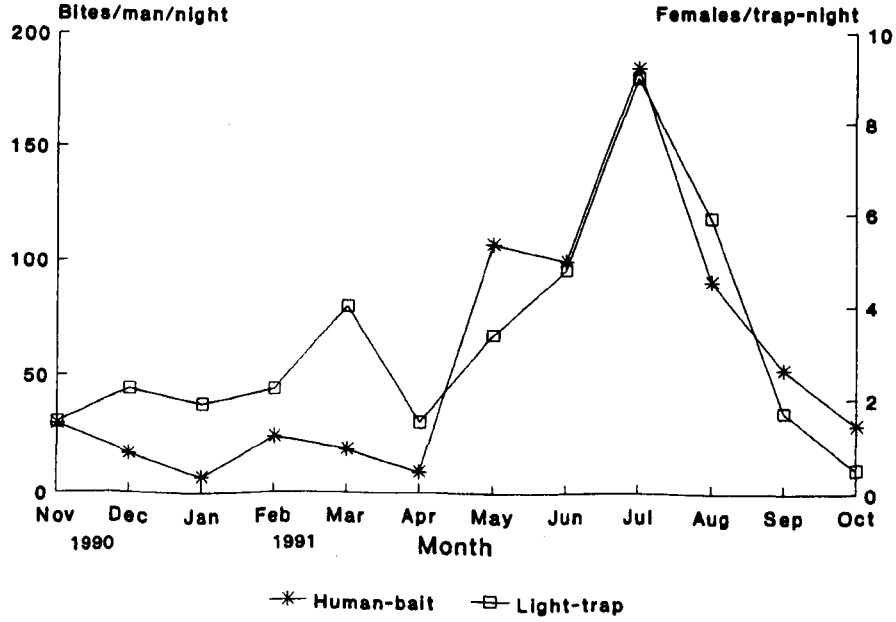


Figure 2.13

Seasonal variation in combined indoor and out of door human-bait and light-trap catches of An. gambiae at Bayama.



started. Although the annual average for the indoor man-biting rate was about half that of the outdoor biting rate, the two rates showed similar seasonal trends with the indoor biting-rates usually being greater, but in the months of November, December and the following July and October, more An. gambiae were caught biting outdoors than indoors. Seasonal variations in the combined man-biting rates (indoor and outdoor) were very similar to the variation in light-trap catches (Figure 2.13). The correlation coefficient r between the two was 0.86 at P=0.001. The only real discrepancy was in March, when light-trap catches increased whereas biting catches decreased.

On 43 out of 55 nights of outdoor catches, it rained during catches and sometimes heavily. The man-biting rates on the dry and wet nights were 90.9 and 82.1 bites /man/night respectively, but the difference was not significant.

2.3.7 Larval surveys

Anopheles gambiae was found breeding in transient pools in all the villages, but not in rice swamps. Only a single An. funestus larva was found, along the edge of a swamp in Mendewa.

2.4 DISCUSSION

Considerable information exists on the bionomics of An. gambiae and An. funestus of the Western Area of Sierra Leone (Blacklock, 1921, 1941; Blacklock & Wilson, 1942b; Gordon et al., 1932; Muirhead-Thomson, 1945; Ribbands, 1944a,b,c, 1946; Ross et al., 1900; Tredre, 1946;), but apart from the present study there is very little information on the ecology of anophelines in the provinces, and certainly no information exists on the ecology of malaria vectors in the Southern Province. On the west coast of Africa, most of the information on the biology of malaria vectors comes from The Gambia (Bryan, 1983; Bryan et al., 1984, 1987) and Nigeria (Molineux & Gramiccia, 1980; Service, 1963) often in regions of forest-savanna mosaic having relatively low rainfall and relative low humidities, and a severe dry season.

The rainfall in Sierra Leone is abnormally high for West Africa (Kowal, 1979) and in our study area the relative humidity at 0900 hr was never below 80%. The only species of the Anopheles gambiae complex identified in our study area was An. gambiae s.s which is more common in humid forest zones such as encountered in the Bo area.

The mean annual density of An. gambiae (1.1 females/room), for all villages combined, was very low, so low that it was not caught in human-bait collections carried out inside bedrooms, in the dry season. However,

Pyrethrum spray concentrates miscible with water proved to be very efficient in

'knocking down' indoor-resting mosquitoes in bedrooms. In the dry season when An. gambiae was not caught biting human-bait in bedrooms, the species was none-the-less caught in spray collections. This seems to suggest, contrary to expectations, that at least, in areas of low vector abundance, the more sensitive sampling method can be pyrethrum spray collections. In the wet season when An. gambiae indoor-resting density was much higher (1.9 females/room) than in the dry season (0.4 females/room) more females were caught biting indoors than in pyrethrum spray collections, which tends to indicate an exophilic behaviour of the An. gambiae population in our study area.

Anopheles gambiae was by far the most abundant mosquito species found in the study area and there were large seasonal variations in the indoor-resting densities of this species in all the villages; it was about five times more common in the wet season than in the dry season. Anopheles gambiae was also more abundant in the low altitude villages (1.3 females/room than in the high altitude villages (0.8 females/room)

On the contrary, An. funestus, with an annual mean room density of density of 0.08/females/room, was more common in the dry season (0.2 females/room) than in the wet season (0.1 females/room). The indoor-resting density of An. funestus also varied according to locality, and was more common in the high altitude villages (0.2 females/room) were the grassland vegetation was relatively more abundant, than in the low altitude villages (0.05 females/room).

Other observations in Sierra Leone have shown An. funestus to be more common in the dry season than the wet season (Blacklock, 1942) and also more abundant in savanna type vegetation in the north of the country (Bespiatov et al., 1984). Kuhlow & Zielke (1978) working in the forest and savanna areas of Liberia showed that An. funestus was more common in the savanna than in the forest villages. Studies in the savanna areas of Kenya (Githeko, 1992) have also showed that An. funestus was most abundant in the dry season.

In all the project villages, indoor-resting vector (An. gambiae and An. funestus) densities were below 14 females/room and the man-biting rates estimated from human-bait catches never exceeded 17 bites/man/night. In this area of low vector abundance, there was a clear positive relationship between the number of sleepers in a bedroom and the number of female An. gambiae caught in morning spray collections. However, at Bayama where the man-biting rate of An. gambiae sometimes exceeded 300 bites/man/night this positive relationship between number of sleepers indoor-resting density was not established. The reason for this was not clear, but at Bayama the degree of exophily of An. gambiae was high (see chapter 3) and many of the females entering houses in search of a blood-meal failed to feed and left before dawn to seek blood-meals outdoors where at least some people could be found throughout the night. The outdoor feeding behaviour of An. gambiae at Bayama is further discussed in Chapter 3. In the project villages, An. gambiae 'insisted' on taking its blood-meal indoors before leaving, even when smoke from naked fires sometimes tended to make the indoor environment unsuitable for resting. In Nigeria Molineux and Gramiccia (1980) showed that at low to medium densities

of An. gambiae s.l. (0.1-4 and 4.1-16 females/hut/night) the indoor-resting densities increased slightly with an increasing number of sleepers, but at higher vector densities (>16.1 females/hut/night, they found no demonstrable effect of the number of sleepers.

According to Haddow (1942) working in Kenya, unfed mosquitoes are in general attracted to huts in numbers related to the number of human occupants, nevertheless no general arithmetic relationship has been established between catch size and the number of occupants. Another variable which affected the number of females resting in bedrooms in our study area was the presence of smoke from naked fires. For example, at Nyandeyama, there was a higher proportion of blood-fed An. gambiae caught in exit traps fitted to rooms adjacent to rooms filled with smoke, than in bedrooms with no nearby fire (see Chapter 3). Spencer (1965) found few An. farauti Laveran in huts with fires in Papua New Guinea,
however, this was considered to be due to reduced humidity rather than irritation from smoke.

Rain on the nights before spray collections also affected the number of female An. gambiae caught in the project villages but, again not at Bayama. In the project villages there were significantly fewer females caught in the mornings following a night of rain, but at Bayama, there was no significant difference in indoor-resting densities or the number of females An. gambiae caught at bait on a wet or dry night, despite it raining for 43 out of 98 nights of human bait collections. It rained very heavily in our study area and at the beginning of the wet season, in May, rain storms

were accompanied by high winds, thunder and lightning, and according to Service (1978a), wind and heavy rain drastically reduce the number of mosquitoes caught biting. At Bayama, An. gambiae readily resumed biting in high numbers when the rain ceased, because adults were mostly resting in vegetation near the houses. The high recapture rate (9.5%) of marked unfed An. gambiae on the day after release (see chapter 4) indicated that the adults did not rest far away from the houses.

In all the villages, no significant differences were observed between the numbers of An. gambiae caught during pyrethrum sprays performed at different times between 0630 and 0900 hr. This finding is in agreement with Service (1964), who working in Nigeria observed no significant differences between the numbers of An. gambaie and An. funestus caught in huts sprayed at different times from 0400-0730 hr, and with Joshi et al. (1973) in Kenya who found no significant differences in spray catches of An. gambiae performed at 0730 and as late as 1400 hr.

Seasonal variations in light-trap and pyrethrum spray sheet catches and human-bait collections followed the same trend in all villages, thus lending further support to the suggestion of Highton (1981) that light-traps could replace human-baits in monitoring changes in vector densities. There was a very strong correlation ($r=0.86$, $P=0.001$) in the variation of An. gambiae density estimated from human-bait and light-trap collections. Similarly, in Tanzania a comparison of the numbers of An. gambiae and An. funestus caught in CDC light-traps hung in a bedroom where the sleeper was protected under a bednet with those caught during other nights by human-baits also showed a clear correlation between the two methods (Lines et al., 1991).

However, pyrethrum spray collections were more productive than light-traps when vector densities were low and were moreover, easier to organise. For best results from light-traps, they had to be strategically hung and batteries had to be in fully charged, conditions which were not always met. For example, there was some variation in the placing of light-traps in bedrooms because of different sleeping arrangements in relation to eave gaps and the use of cotton bed screens in some rooms, especially in 'women's rooms' having more than one bed.

The efficiency of light-traps in collecting indoor flying mosquitoes improves when they are placed in rooms with people sleeping under bed-nets (Charlwood et al., 1986; Githeko, 1992). In my study, traps placed in rooms with bednets, in the project villages caught significantly more mosquitoes than traps placed in rooms without bednets. However, this was not the case at Bayama where there were large house to house variations in trap catches. At Bayama, the location of a bedroom window opening near a shrubby vegetation appeared to be more important than the presence of a bednet in increasing the numbers of female An. gambiae caught in a light-trap. Also, indoor resting densities of An. gambiae in Bayama were significantly higher in rooms with windows facing the surrounding vegetation, than in rooms with windows located elsewhere.

In the project villages, the annual An. gambiae man-biting rates estimated from human-bait catches (1.1 bites/man/night) was similar to that based on the numbers of blood-fed females caught in spray collections (1.0 bites/man/night); and the same was true for An. funestus. However, at Bayama, the ratio between the man-biting rate

estimates from human-bait and pyrethrum spray collections was 20:1 in the wet season; spray collections were only carried out in the wet season. Estimates of man-biting rates from human-bait and spray collections were therefore similar in the project villages which were characterised by low vector abundance, but differed considerably in Bayama with its relatively high vector abundance. But even in the project villages, as vector populations increased in the wet season, estimates from bait catches became larger than estimates derived from indoor-resting blood-fed females.

The differences, in the bionomics of malaria vectors, between Bayama and the project villages and between high and low altitude villages suggests that entomological factors could be very important in explaining small area variations observed in the epidemiology of malaria in Papua New Guinea (Cattani et al., 1986) and The Gambia (Greenwood, 1989).

CHAPTER 3

FEEDING AND RESTING BEHAVIOUR

3.1 INTRODUCTION

Man becomes infected with malaria parasites when an Anopheles mosquito infected with sporozoites of one of the four species of human malaria takes a blood-meal from him. The human blood index (HBI) is the proportion of blood-meals of a mosquito population obtained from man (Garrett - Jones, 1964). In malaria epidemiology, the index can be used with the sporozoite rate to estimate the entomological inoculation rate, and is required to calculate the malaria reproductive rate and the mosquito vectorial capacity (see chapter 4). It is incorporated as the man-biting habit (**a**) in the expression for man-biting rate, namely **ma.** The man-biting habit can be calculated by dividing the HBI by the gonotrophic cycle duration (from blood-meal to oviposition).

Vector blood-meals can be classified as simple or mixed. A simple blood-meal is obtained from a single meal while a mixed blood-meal obtained from two or more host species is referred to as patent. If obtained from two or more individuals of the same species it is called cryptic (Boreham and Garrett-Jones, 1973).

Several, mainly serological, techniques have been developed to analyze vector blood-

meals. The precipitin test usually performed as the precipitin ring test in small glass tubes has been the most widely used. The test makes use of the precipitation that occurs between serum eluted from an insect blood-meal, and specific antibodies to that serum raised in a convenient laboratory animal, often a rabbit. Counter-current immunoelectrophoresis has been found useful for the detection of small blood-meals and mixed meals (Dhanda & Gill, 1982). Haemagglutination assays offer greater sensitivity and specificity but are much more elaborate than precipitin tests, and have been used mainly to distinguish blood-meals from closely related host species (Tempelis & Rodrick, 1972). A simple, rapid and inexpensive technique is the latex agglutination test because it does not require any sophisticated equipment and the results can be read in two minutes (Boorman et al., 1977). However, the test has not been widely used due to the problem of stability of the polystyrene particles coated with antibodies, which means that it becomes difficult to get reproducible results. Immunofloresecence tests for blood-meal identification require sophisticated equipment and technology (Gentry et al., 1967; McKinney et al., 1972). None of the tests described above satisfies the requirements of a simple, yet sensitive, technique which can replace the precipitin test. However, simple and sensitive enzyme linked immunosorbent assays (ELISAs) have been developed for the detection of blood-meals in mosquitoes (Beier & Koros, 1991; Beier et al., 1988a & b; Service et al., 1986) and are now used in many laboratories in preference to the precipitin test.

No reliable measurements of HBI can be made unless care is taken to obtain unbiased samples, together with information for interpreting the index correctly once the blood-meals have been analyzed (Garrette-Jones, 1964). Since most mosquito species are

meals have been analyzed (Garrette-Jones, 1964). Since most mosquito species are entirely or partly exophilic, the collection of outdoor resting adults gives a much broader spectrum of species and usually a more representative estimate of the HBI than most other sampling methods. However, the principal African malaria vectors, such as An. gambiae s.s., An. funestus and An. arabiensis are characteristically highly endophilic, especially the first two species. Nevertheless, even with these species a proportion of the population rests out of doors. Searches for outdoor resting populations of mosquitoes is difficult and time-consuming because mosquitoes are usually dispersed over wide areas, although they may show marked preferences for specific biotypes. Even if representative samples have been collected from different biotypes the computation of the vector HBI may still be an involved process where several distinct ecological niches have to be separately considered.

The gonotrophic conditions of mosquitoes leaving houses provide specific information on their feeding and resting behaviour. For example, the presence of blood-fed females in exit traps denotes deliberate exophily.

Polytene chromosomes of An. gambiae s.s. and An. arabiensis show polymorphism for paracentric inversions, the frequencies of which vary with resting behaviour (Coluzzi et. al., 1979). To determine local characteristics, the behaviour of the different populations should be studied for each area.

3.2. MATERIALS AND METHODS

3.2.1 Exit trap collections

In order to provide information on both the numbers and gonotophic conditions of mosquitoes leaving houses, exit traps of the Muirhead-Thomson (1948) type were fitted to windows of houses to catch a proportion of mosquitoes flying out at dawn (Figure. 3.1).

Three bedrooms each in Nyandeyama and Mendewa used for pyrethrum, collections were fitted with window type exit traps. The traps were designed according to the description by Service (1963). The 30-cm^3 metal frame was made by welding together four 30-cm lengths of 5-cm diameter galvanised steel. This cube cage was covered with fine cotton mosquito netting, and had an inwardly projecting funnel sewn on one face, it's larger opening being about 15 cm in diameter tapering to about 4 cm in diameter. The smaller opening was reinforced with a metal ring from which four pieces of fine wire were tied and connected at the corners of the cage to support the funnel. The metal ring was at least 7 cm from the opposite face which had a small sleave of netting to enable mosquitoes to be removed using an aspirator. The tube was knotted to prevent the escape of mosquitoes. Wooden frames had to be made to hold the traps in place in the windows. A window had to be made for one bedroom which had none.

Exit trap collections were made fortnightly at Nyandeyama and Mendewa from April

Figure 3.1 A window exit trap

1990 to April 1991. At Bayama, exit trap collections started in May 1991 and were carried out weekly until September 1991. The traps were fitted into the windows at 1800 hr and removed at 0700 hr when adults were aspirated from the traps and put into polystyrene cups for transportation to the laboratory, where they were sorted according to species and gonotrophic condition.

3.2.2 Outdoor collections of resting adults

Outdoor resting mosquitoes were collected once a fortnight at Nyandeyama and Mendewa from January to April 1991. Weekly collections at Bayama started in November 1990 and continued through to November 1991. Collections were carried out in the morning from 0700 - 0900 hr by one person who searched a variety of both natural and man-made shelters such as, rice barns, empty houses, earth banks, buttress roots of trees, empty and abandoned vehicles, bricks piles, under the eaves of thatch roofs of houses. Mosquitoes were collected with aspirators or tubes, with the aid of a torch. The gonotrophic conditions of the mosquitoes were recorded and blood-smears made from freshly fed females for blood-meal analysis.

3.2.3 Biting cycles

To obtain information on the biting cycles and the effect of moonlight and rainfall on the feeding behaviour of mosquitoes, human-bait collections were performed at Bayama from November 1990 to October 1991. During the human-bait collections, moon phase, cloud cover, and rainfall were recorded. The organisation of human bait

collections have already been described in chapter 2.

3.2.4 External examination of abdomen

The abdomens of female mosquitoes collected from light-traps, exit traps, pyrethrum spray collections and outdoor aspirator catches were examined to obtain information on their resting and man-biting habits. The abdominal appearance was classified as unfed, freshly fed, semi-gravid and gravid (Table 3.1).

3.2.5 Enzyme linked immunosorbent assay (ELISA) for identifying mosquito blood-meals

An indirect sandwich ELISA technique (Service et al., 1986) was used to identify blood-meals in Anopheles mosquitoes. Freshly fed mosquitoes collected by all sampling methods were used for the ELISA. The abdomens of the blood-engorged females were squashed onto small areas on filter paper (Whatman no.1) and each filter paper with 16 smears was sandwiched between thin typing paper and stored in a desiccator with silica gel crystals, which were checked once a week for decoloration. Crystals loosing their blue coloration were heated and put back in the desiccator.

To test for blood-meal source, the blood smeared sections on the filter paper were cut out and placed in 1.0ml PBS/Tween 20 in labelled plastic tubes for a period of at least 60 mins at room temperature. Smears were first tested for human and

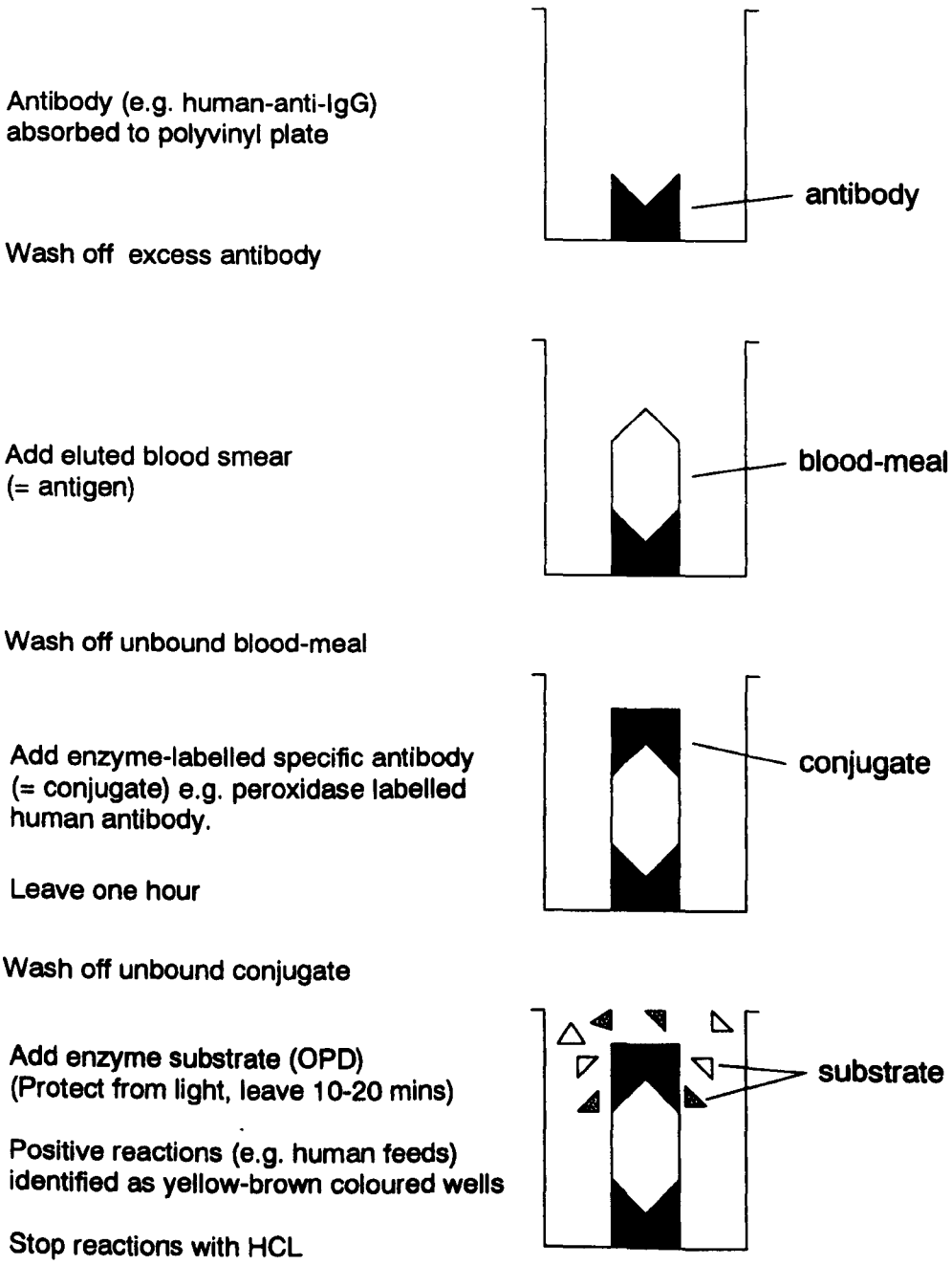
TABLE 3.1

Classification of gonotrophic conditions according to abdominal appearance

Abdomonal appearnce	Description	Illustration
Unfed	The abdomen is collapsed, the stomach empty and the ovaries occupy only one third or less of the abdomen.	
Fed	Stomach with red blood, ovaries occupying not more than 2-3 segments ventrally and up to one dorsally.	 
Semi-gravid	Blood dark red, ovaries occupying from 4-7 segments ventrally and up to a segment dorsally.	 
Fully Gravid	Blood completely digested or present as a black trace.	

sheep/goat antigens. Those negative for these two hosts were then tested for dog and pig antigens. On some occasions smears were tested for all four hosts to identify mixed blood-meals. Polyvinylchloride (PVC) plates (Dynatech Laboratories, UK) coated with the appropriate host antibody were used. To each test well, 100μl of eluted blood smear was added. On each plate, two wells were used for negative controls and contained just PBS/Tween 20, and one well was the positive control and consisted of the host serum diluted in PBS (1/500). The control wells each received 100μl of the control preparations. The plates were incubated at room temperature for 60 min on wet paper towelling under a plastic box. After incubation the plates were washed four times with PBS/Tween 20 and shaken dry on a towel. Then 100μl of appropriate congugates (anti host peroxidase in host diluent) were added to each well and incubated at room temperature for 60 min. At the end of the incubation period the plates were again washed four times in PBS/Tween 20 and dried. To each well was then added 100μl of freshly prepared substrate solution (4 tablets of ortho-phenylene diamine in 12ml distilled water for a 96 well plate). The plates were incubated for 10-20 minutes under a cardboard box to keep them in the dark. The peroxidase enzyme reacted with the substrate to give a yellow coloration, the reaction was then stopped by adding 1 drop of 2.5 M HCl to each well. Positive wells were read visually. Details of the procedure are shown diagrammatically in Figure 3.2.

Figure 3.2 ELISA Double antibody sandwich technique for identification of vector blood-meals



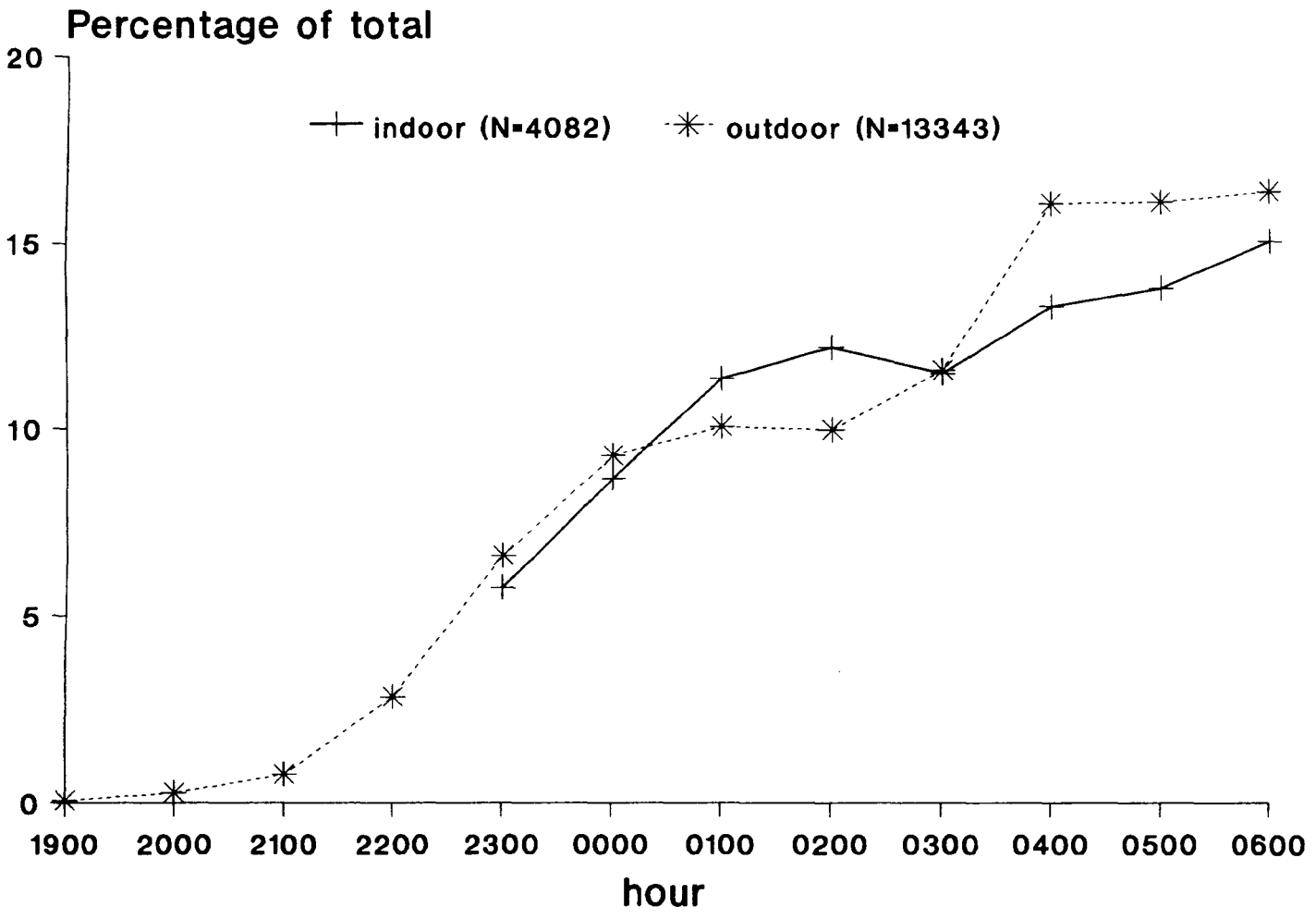
3.3 RESULTS

3.3.1 Biting cycle of Anopheles gambiae

Analysis for biting cycles were made for An. gambiae only, because the total number of An. funestus caught in human bait collections (16) was too small. The number of females caught per hour, expressed as a percentage of the total caught during the 12-hour collection period, is given in Figure 3.3 for indoor and outdoor human-bait collections. What has been referred to as indoor biting collections actually included catches performed out of doors from 1800 to 2200 hr. All collections inside houses started at 2200 hr because that was about when people went into their rooms to sleep. The indoor biting cycle in Figure 3.3 therefore started at 2200 hr and the first hourly score was at 2300 hr. Although outdoor collections started at 1800 hr, score for the hour 1800 hr - 1900 hr was made at 1900 hr.

The biting cycles for both indoor and outdoor human-bait collections showed that An. gambiae was biting mainly in the second half of the night after 0000 hr. The biting pattern from the indoor biting females showed a gradual increase in the percentage of females biting from before 2200 hr to 0600 hr with a slight peaks at 0200 hr. The biting cycle for the outdoor biting females also increased gradually from before 1900 hr to peak at 0400 hr and then almost maintain a plateaux to 0600 hr. Biting took place mostly in the last two hours from 0400 to 0600 hr. During this period, the percentage of females biting outdoors was significantly higher than that biting indoors (χ^2, $p<0.0001$, $df=1$).

Figure 3.3 Indoor and outdoor biting cycles of An. gambiae s.s., Bayama



The biting cycles of parous and nulliparous females are shown in Figure 3.4. Nulliparous females had a bimodal biting cycle with peaks at 0000 and 0400 hr. The biting cycle of the parous females had a single peak at 0400 hr. The number of nulliparous and parous females caught between 0500 and 0600 hr was 394 and 801 respectively. The corresponding values for the period 1800 to 2300 hr were 264 and 200, indicating that the number of nulliparous females was 32% higher than the number of parous females. In the hourly collections from 1800 to 2300 hr the number of nulliparous females was always higher than the number of parous females.

The influence of the moon on the biting cycle of An. gambiae was also investigated. The biting cycles during different phases of the moon are illustrated in Figure 3.5. The biting cycles at new moon and the last quarter were similar, with two small peaks. The biting cycles during full moon and the first quarter were similar compared to the cycles in the other phases, in that there was only one peak which occurred at 0400 hr. Biting was more intense in the first half of the night during new moon and the last quarter, with 20.1 and 22.7% of the females caught during this period than during full moon (14.0%) and first quarter (15.8%). During new moon there was a peak at 0100 and 0500 hr. During last quarter there was a peak at 0000 and 0400 hr.

Figure 3.4 Biting cycles of parous and nulliparous An. gambiae s.s., Bayama

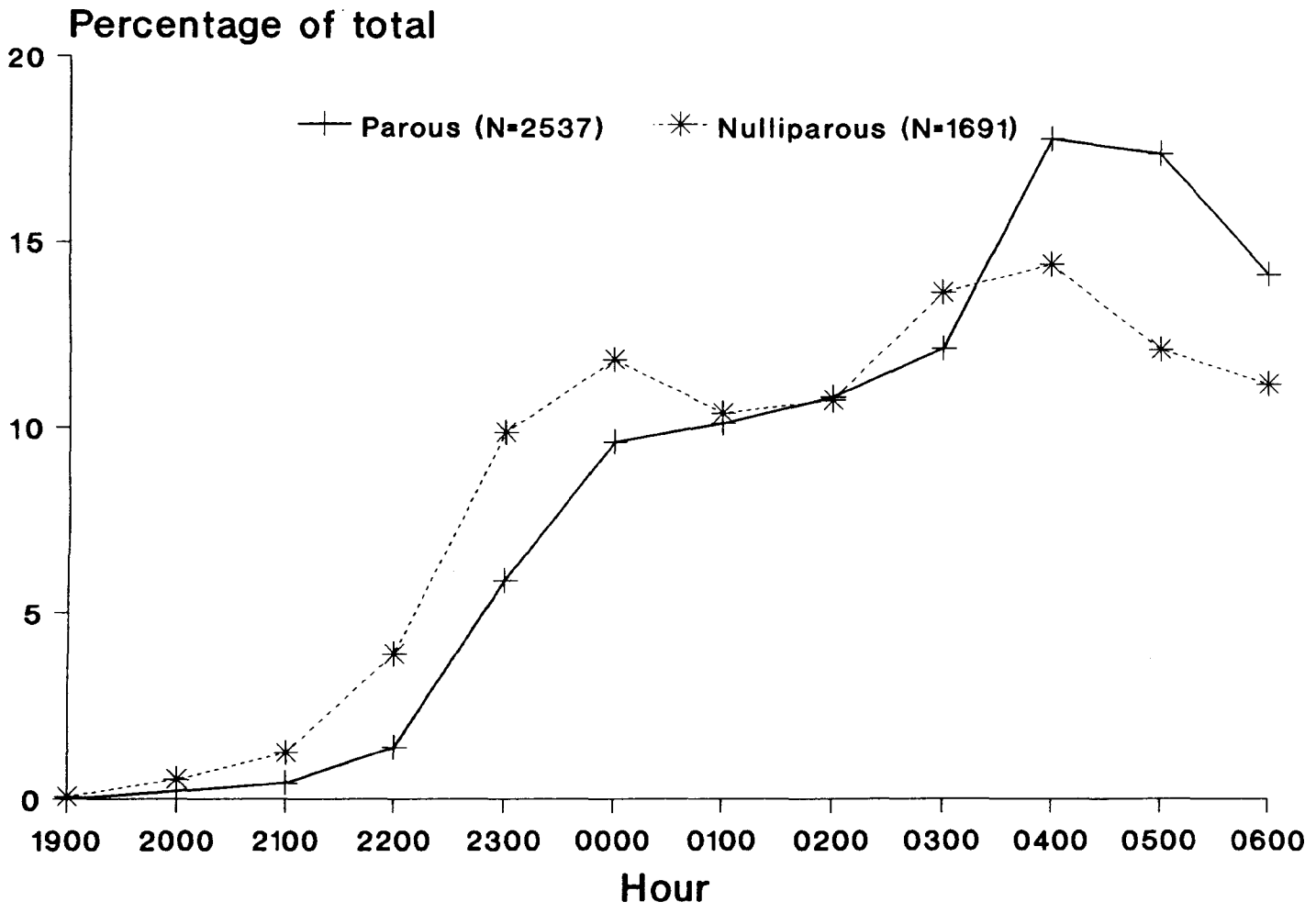
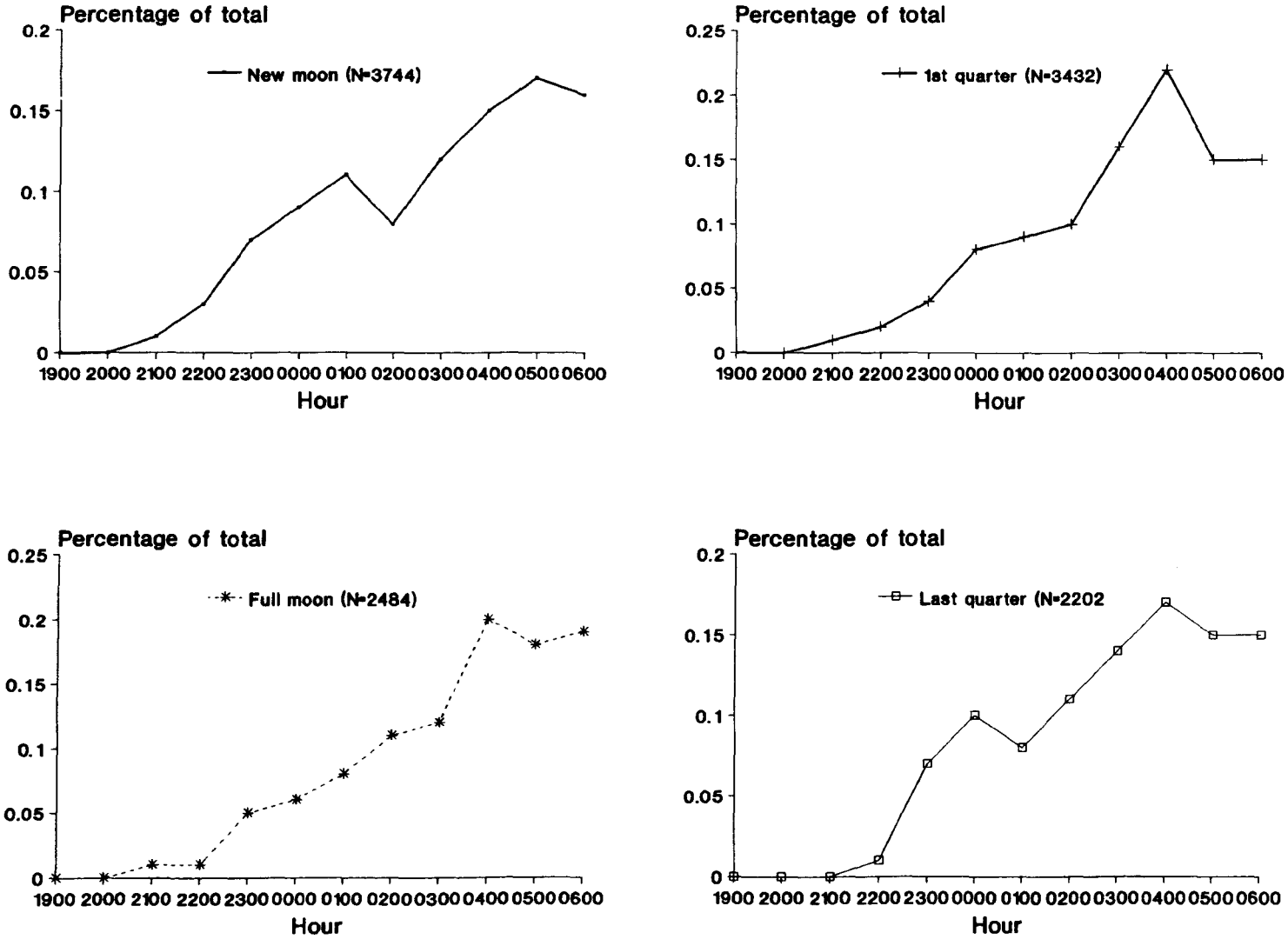


Figure 3.5 Biting cycles of An. gambiae s.s. during different phases of the moon, Bayama.



3.3.2 Gonotrophic conditions of An. gambiae and An. funestus in the pyrethrum spray, light-trap and exit trap collections in the project villages.

Female An. gambiae and An. funestus that were collected were classified according to abdominal appearance, into four different gonotrophic conditions: unfed, fed, semi-gravid and fully gravid. The fed group included both freshly and late feds (Table 3.1) The newly engorged females probably fed during the night immediately preceding the morning of collection, while semi-gravid and gravid females had likely fed on the previous night because freshly fed females from morning collections, kept in polystyrene cups in domestic houses in Bo town, took about 12 hours to reach the half-gravid condition. Table 3.2 gives the distribution by gonotrophic condition of An. gambiae and An. funestus in the different collections. Anopheles gambiae in spray collections comprised of 0.9% unfeds, 80.0% feds, 13.6% semi-gravid and 5.5% fully gravid. The ratio of fed:gravid for An. gambiae was 4.2:1, suggesting that a higher proportion of fed females (76.1%) left the house before they reached the gravid stage. Most (51.1%) An. gambiae caught in the exit traps were blood-fed, and the high proportion of fed:gravid females (1.5:1) corroborates the findings in the spray collections that some blood-fed females leave bedrooms after feeding. The unfeds made up 14.5% of female An. gambiae in the exit traps but only 0.9% in the spray collections, indicating that most of the females which were unsuccessful in feeding left the bedroom before dawn. In the case of An. funestus, no unfeds were found in spray collections but five out of the 10 females found in exit traps were unfed. Light-trap collections which are not normally used in determining exophily also contained blood-fed An. gambiae in a ratio to gravids of 1.2:1, rather similar to

TABLE 3.2
Numbers of An. gambiae s.s. and An. funestus in different gonotrophic conditions, all villages combined. Precentages are shown in parentheses.

Method of collection	Number of collections	Species	Gonotrophic condition				Total
			Unfed (%)	Fed (%)	Semi-gravid (%)	Fully gravid (%)	
PSC	748	An. gambiae	16 (0.9)	1421 (80.0)	241 (13.6)	98 (5.5)	1776
		An. funestus	0 (0.0)	97 (60.2)	48 (29.8)	16 (9.9)	161
Exit trap	148	An. gambiae	32 (14.5)	113 (51.1)	30 (13.6)	46 (20.8)	221
		An. funestus	5 (50.0)	1 (10.0)	3 (30.0)	1 (10)	10
Light-trap	265	An. gambiae	71 (34.0)	75 (35.9)	29 (13.9)	34 (16.3)	209
		An. funestus	21 (72.4)	4 (13.8)	2 (6.9)	2 (6.9)	29

the exit trap collections. The ratios of fed:gravid for An. funestus in spray and exit-trap collections were 1.5:1 and 1:1 respectively, suggesting that it also had the tendency to leave the same night after feeding, but to a lesser degree compared to An. gambiae. In other words, An. funestus was more endophilic than An. gambiae.

The gonotrophic conditions of An. gambiae and An. funestus in spray collections in the different villages is given in Table 3.3. The fed:gravid ratios suggests that in all the villages, fewer An. gambiae than An. funestus remain in the rooms for long enough to become gravid. The fed:gravid ratio for An. gambiae in the different villages varied from 2.4:1 at Mendewa to 5.7:1 at Nengbema. For An. funestus the fed:gravid ratio varied from 1.3:1 at Nengbema to 1.8:1 at Njala-Komboya. The extent to which fed females exit rooms is similar in the low and high altitude villages. The total numbers of female An. funestus caught in exit traps (10) and light-traps (29) were too small for further analysis by village.

Table 3.4 gives a summary of the gonotrophic conditions of An. gambiae according to season. The ratio of fed:gravid in spray collections was higher in the wet season (4.7:1) than in the dry season (2.2:1), indicating that An. gambiae was more endophilic in the wet season. Surprisingly, in the exit trap collections, the fed:gravid ratio for the wet season (1.6:1) was also higher than the ratio for the dry season (0.9:1), contradicting the spray collection results . The percentage of blood-feds in the spray collections in the wet and dry seasons were 81.4 and 68.3% respectively. The corresponding percentages in the exit trap collections were 52.3 and 42.3%.

TABLE 3.3
Numbers of An. gambiae s.s. and An. funestus in different gonotrophic conditions, in spray collections, in the different villages. Percentages are shown in parentheses.

Village	Number of collections	Species	Gonotrophic condition				Total
			Unfed (%)	Fed (%)	Semi-gravid (%)	Fully gravid (%)	
Nengbema	207	An. gambiae	2 (0.3)	507 (85.2)	63 (10.6)	23 (3.9)	595
		An. funestus	0 (0.0)	8 (61.5)	3 (23.1)	2 (15.4)	13
Nyandeyama	171	An. gambiae	7 (1.1)	490 (78.8)	96 (15.4)	29 (4.7)	620
		An. funestus	0 (0.0)	7 (58.3)	5 (41.7)	0 (0.0)	12
Mendewa	183	An. gambiae	2 (1.0)	144 (70.2)	32 (15.6)	27 (13.2)	205
		An. funestus	0 (0.0)	65 (59.6)	34 (31.2)	10 (9.2)	110
Njala-Komboya	187	An. gambiae	5 (1.4)	282 (79.2)	50 (14.0)	19 (5.3)	356
		An. funestus	0 (0.0)	17 (65.4)	6 (23.1)	3 (11.5)	26

TABLE 3.4
Numbers An. gambiae s.s. in different gonotrophic conditions according to season, all villages combined. Percentages are shown in parentheses.

Season	Collection method	Gonotrophic condition				
		Unfed (%)	Fed (%)	Semi-gravid (%)	Fully gravid (%)	Total
Dry deason	PSC	0 (0.0)	140 (68.3)	47 (22.9)	18 (8.8)	205
	Light-trap	22 (66.7)	4 (12.1)	5 (15.2)	2 (6.1)	33
	Exit trap	3 (11.5)	11 (42.3)	2 (7.7)	10 (38.5)	26
Wet season	PSC	16 (1.0)	1281 (81.4)	194 (12.3)	80 (5.1)	1571
	Light-trap	49 (27.8)	71 (40.3)	24 (13.6)	32 (18.2)	176
	Exit trap	29 (14.9)	102 (52.3)	28 (14.4)	36 (18.5)	195

In order to investigate the effect of smoke on the indoor resting behaviour of female An. gambiae, exit trap collections from two rooms in houses in Nyadeyama which did not have naked fires in them throughout the period of study, were compared with collections from a room (No. 3009) in another house which regularly had a naked fire that produced a lot of smoke. Tables 3.5 and 3.6 give the number of females in different gonotrophic conditions caught in exit traps and spray collections in the different houses. All the rooms where about the same size (approximately 3.5 m^2); they had mat ceilings and were in houses built from mud and sticks and with open eaves. Room 3011, however, had a thatch roof while the others had corrugated iron roofs.

The percentage of blood-feds caught in exit-traps fitted to room 3009 was 78.3% compared to 25.0 and 21.1% for rooms 3011 and 3014 respectively. Despite variations in the efficiency of exit traps fitted to different rooms, the difference in the number of females caught in room 3009 compared to the other rooms was particularly striking. Although the number of exit trap collections (25) from room 3009, which was regularly filled with smoke at night, was slightly less than that for the other rooms (27, 28) there were twice as many female An. gambiae caught in this room (No. 3009) than room 3014 which, in turn had more females than room 3011. The number of blood-fed females (90) caught leaving room 3009 was at least seven times more than those caught leaving either of the other two rooms. The difference in the efficiency of different exit traps could not , alone, account for the large difference in numbers leaving room 3009 as compared to the other rooms, because the different exit traps were rotated between rooms.

TABLE 3.5

Numbers of female An. gambiae s.s. in different gonotrophic conditions in exit trap collections from three rooms at Nyandeyama. Percentages are given in parentheses.

Bedroom number	Indoor fire	Number of collections	Gonotrophic condition				
			Unfed (%)	Fed (%)	Semi-gravid (%)	Fully gravid (%)	Total
3009	+	25	7 (6.1)	90 (78.3)	10 (8.7	8 (7.0)	115
3011	-	28	9 (25.0)	9 (25.0)	7 (19.4)	11 (30.6)	36
3014	-	27	14 (24.6)	12 (21.1)	11 (19.3)	20 (35.1)	57

When the fed:gravid ratio in the spray and exit trap collections were recalculated omitting collections from room 3009, the ratios in the spray collections for the dry and wet seasons were 2.1:1 and 4.4:1 respectively. The corresponding ratios for exit-trap collections were 0.7:1 and 0.4:1, values which are more to be expected. The percentage of blood-fed females in the spray collections in the dry and wet seasons were 67.8 and 81.0%, and in the exit traps the corresponding percentages were 35.3 and 19.1 %. The surprising results obtained when all houses were considered was clearly due to the irritating effect of smoke on mosquitoes in room 3009.

Smoke, however, did not appear to deter feeding and some females (7.0% in exit traps) were able to stay in room 3009 until they were fully gravid. Despite the high percentage of blood-feds (78.3%) leaving room 3009, 94.% of indoor-resting females in the same room were blood-fed (Table 3.6). This suggests that finding a high proportion of blood-feds in a room is no evidence against exophily.

To investigate the effect of bed-nets on the feeding behaviour of An. gambiae, the gonotrophic conditions of females in light-trap catches from rooms with people sleeping under bed-nets were compared with conditions in catches from rooms where people did not sleep under bed-nets. Table 3.7 gives the numbers of female An. gambiae in different gonotrophic conditions in light-trap catches from rooms where people were sleeping under bed-nets and rooms without bed-nets. A Mann-Whitney test showed, rather surprisingly, no significant difference in the mean number of blood-feds in light-traps in the two situations ($Z = -2.11$ and $p = 0.03$). A Chisquare

TABLE 3.6
Numbers of female An. gambiae s.s. in different gonotrophic conditions in spray collections from three rooms at Nyandeyama. Percentages are shown in parentheses.

Bedroom number	Indoor fire	Number of collections	Gonotrophic condition				
			Unfed (%)	Fed (%)	Semi-gravid (%)	Fully gravid (%)	Total
3009	+	32	2 (2.8)	68 (94.4)	0 (0.0)	2 (2.8)	72
3011	-	27	0 (0.0)	21 (53.8)	12 (30.8)	6 (15.4)	39
3014	-	32	1 (0.4)	170 (73.9)	51 (22.2)	8 (3.5)	230

TABLE 3.7
Numbers female An. gambiae s.s. in different gonotrophic conditions in light-trap collections depending on whether or not bednets were used. All villages combined. Percentages are shown in parentheses.

Bednet usage	Number of collections	Gonotrophic condition				
		Unfed (%)	Fed (%)	Semi-gravid (%)	Fully gravid (%)	Total
Used	9	14 (30.4)	15 (32.6)	7 (15.2)	10 (21.7)	46
Not used	275	57 (34.9)	60 (36.8)	22 (13.5)	24 (14.7)	163

test showed no significant difference in the proportion of females caught in light-traps that were blood-fed, semi-gravid or fully gravid.

3.3.3 Gonotrophic conditions of An. gambiae caught in pyrethrum spray, light-trap and exit trap collections at Bayama village.

At Bayama village exit-trap and pyrethrum spray collections were carried out during the wet season only, from May to October in 1991. The total numbers of An. funestus in spray collections numbered just two fed and one semi-gravid, and only a single unfed An. funestus was caught in the exit traps.

Table 3.8 gives the numbers of An. gambiae caught in spray, exit and light-trap collections, according to gonotrophic condition. Of those caught resting in houses, 75.6% were blood-fed and the ratio of fed:gravid in spray collections was 5.4:1, indicating that in Bayama also, most of the blood-feds did not stay in bedrooms long enough to become gravid. A large proportion left the rooms after taking a blood-meal, as is indicated by the fact that 34.8% of those in exit traps were fed, and also by the high ratio of fed:gravid in the exit traps (4.1:1). However, most exiting An. gambiae consisted of unfeds (56.7%). The ratio of females (unfed and fed) which left the room after seeking a blood-meal (exit trap collections) to those that remained the following morning (spray collections) was 6:1. The number of females, irrespective or gonotrophic condition, caught in a single exit trap varied from 1 -260. The highest numbers of feds and unfeds caught in a single exit trap were 83 and 251 respectively. These maximum figures were obtained in July from a room occupied

by a single adult. During one period, from 29th June to 3rd July, we were consistently collecting over 80% unfeds from one exit trap fixed to a room occupied by one adult. Upon investigation it was found that the occupant, an elderly man, had been sick with fever and therefore went to bed fully dressed and at the same time covered himself with a cloth of thickly cotton material, traditionally woven in the villages.

Light-trap collections also contained females in all the different gonotrophic conditions. The percentage of blood-feds and gravids were 19.7 and 16.0% respectively. Most of the females were unfed (56.8%).

Exit trap collections were made from three houses in the village, two of which (rooms 103 and 201) had walls built of mud and sticks. These two houses had wide eave gaps and the rooms from which exit trap collections were made had no ceilings. The other house (502) was built of mud bricks and the walls plastered with cement. There were no eave gaps in this house and the room which had the exit trap had it's walls painted white about 20 years ago. Table 3.9 gives a summary of the exit trap collections from the three rooms including information on the gonotrophic conditions of the female An. gambiae caught. The ratio of fed:gravid from rooms 103 and 201 were 0.5:1 and 0.7:1 respectively, and 11.4:1 for room 502 indicating that the degree of exophily was very much higher in the room compared to the others.

To investigate the degree of unsuccessful feeding in rooms in the village in the wet season (June - September), the numbers of unfeds caught in exit traps were expressed

TABLE 3.8
Numbers of female An. gambiae s.s. in different gonotrophic conditions in the different collections at Bayama. Percentages are shown in parentheses.

Method of collection	Number of collections	Gonotrophic condition				
		Unfed (%)	Fed (%)	Semi-gravid (%)	Fully gravid (%)	Total
PSC	84	51 (10.3)	376 (75.6)	14 (2.8)	56 (11.3)	497
Exit trap	96	1572 (56.7)	965 (34.8)	52 (1.9)	184 (6.6)	2773
Light-trap	235	1060 (56.8)	367 (19.7)	141 (7.6)	298 (16.0)	1866

TABLE 3.9
Numbers of female An. gambiae s.s in different gonotrophic conditions in exit trap collections from three bedrooms at Bayama. Percentages are shown in parentheses.

Bedroom number	Eave gap	Number of collections	Gonotrophic condition				
			Unfed (%)	Fed (%)	Semi-gravid (%)	Fully gravid (%)	Total
103	+	16	77 (36.7)	47 (22.4)	19 (9.0)	67 (31.9)	210
201	+	34	63 (33.2)	53 (27.9)	3 (1.6)	71 (37.4)	190
502	-	46	1432 (60.3)	865 (36.5)	30 (1.3)	46 (1.9)	2373

as percentages of feds and unfeds combined (Table 3.10). The proportion of unfeds was highest in July (72.1%), the coldest month of the year. Collections made in May were not considered because few (15) were caught during this month.

3.3.4 Outdoor resting collections of An. gambiae and An. funestus

A total of 155 An. gambiae and seven An. funestus were collected from various outdoor resting shelters in the four project villages. Anopheles gambiae was found resting in rice barns and on earth banks (Figure 3.6) from where 114 (73.5%) and 39 (25.2) females were collected respectively. One female each was collected from a metal drum and a wooden mortar. Only three female An. funestus each were found resting in rice barns and on earth banks. One female An. funestus was found resting in an old bucket. A total of 70 male An. gambiae and four male An. funestus were found resting outdoors in the various shelters.

The gonotrophic conditions of the outdoor resting females are given in Table 3.11. The proportions of unfeds, feds, and semi-gravids and gravids combined, of the An. gambiae outdoor resting population were 9.0, 15.5 and 75.5% respectively. Three unfeds, three feds and one fully gravid An. funestus were found resting outdoors.

In Bayama village, outdoor resting populations of An. gambiae were found on palm fences, large tree roots and under the eaves of thatch-roofed houses. The total number of females found at these sites were 114 , 12 and 85 respectively. The proportions of unfeds, feds and gravids were 12.3, 58.8 and 28.9% respectively

TABLE 3.10
Monthly variation in the percentage of unfed females exiting bedrooms. Unfeds were expressed as a percentage of combined blood-feds and unfeds.

Month	Unfed	Fed	Total	% unfed
June	496	477	973	50.9
July	974	377	1351	72.1
August	22	42	64	34.4
September	56	27	83	67.5
Total	1548	923	2471	62.6

Figure 3.6 A typical rice barn; the main outdoor resting shelter for An. gambiae s.s. in the project villages

TABLE 3.11
Numbers of An. gambiae s.s. and An. funestus in different gonotrophic conditions, all project villages combined. Percentages of females in the different conditions are given in parentheses.

Species	Gonotrophic condition					Males
	Unfed	Fed	Semi-gravid	Fully-gravid	Totals	
An. gambiae	14 (9.0)	24 (15.5)	50 (32.3)	67 (43.2)	155	70
An. funestus	3(42.9)	0(0.0)	3(42.9)	1(14.3)	7	4

TABLE 3.12
Numbers of An. gambiae s.s. in different gonotrophic conditions in outdoor resting collections at Bayama.

Resting site	Gonotrophic condition					Males
	Unfed	Fed	Semi-gravid	Fully gravid	Totals	
Palm fence	19	79	3	13	114	76
Tree roots	3	5	4	0	12	2
Eaves	4	40	1	40	85	11
Totals (%)	26 (12.3)	124 (58.8)	8 (3.8)	53 (25.1)	211	89

(Table 3.12). A total of 89 males were found resting outdoors.

3.3.5 Blood-meal analysis

Anopheles gambiae s.s. which fed mainly on humans, also fed on large domestic animals such as goats, sheep, pigs and dogs which were present in or around the study villages. An ELISA test blood for cattle blood was not employed because cattle were not present in or near any of the villages. Large domestic animals were most common in the smaller villages such as Bayama, Nyandeyama and Mendewa where the human to domestic animal ratio varied from 6.3:1 (Nyandeyama) to 2.5:1 (Bayama). In the larger villages of Nengbema and Njala-Komboya, the human to domestic animal ratios were 19.5:1 and 25.6:1 respectively.

The human blood indices (HBI) of An. gambiae and An. funestus in all the project villages combined were 0.99 (1043/1049) and 1.0 (78/78) respectively (Tables 3.13 and 3.14). The HBIs for An. gambiae in the smaller villages of Nyandeyama and Mendewa were 0.99 and 0.97 respectively. In the two bigger villages, Nengbema and Njala-Komboya, An. gambiae had fed only on humans.

On very few occasions, An. gambiae fed on pigs (0.2%), goats (0.4%) and dogs (0.1%). Three out of the four blood-meals positive for goats were also positive for human IgG. The only An. funestus blood-meal positive for goat IgG was also positive for human blood. The proportions of mixed blood-meals for An. gambiae and An. funestus were therefore 0.3% (3/1049) and 1.2% (1/78).

TABLE 3.13
Numbers of An. gambiae s.s. with positive blood-meal ELISA for various hosts in the different villages. Numbers in parentheses, in the non-human host columns, indicate those that were also positive for humans.

Village	Number tested	Host				Mixed
		Human	Pig	Goat	Dog	
Nengbema	348	348	0	0	0	0
Nyandeyama	376	375	0	(3)	1	3
Mendewa	135	132	2	1	0	0
Njala-Komboya	188	188	0	0	0	0
Combined villages	1047	1043	2	1(3)	1	3

TABLE 3.14
Numbers of An. funestus with positive blood-meal ELISA for various hosts in the different villages. Numbers in parentheses, in the non-human host columns, indicate those that were also positive for humans.

Village	Number tested	Host				Mixed
		Human	Pig	Goat	Dog	
Nengbema	3	3	0	0	0	0
Nyandeyama	7	7	0	0	0	0
Mendewa	59	59	0	(1)	0	1
Njala-Komboya	9	9	0	0	0	0
Combined villages	78	78	0	(1)	0	0

In Bayama village the HBI for An. gambiae was 0.98 (163/166); Table 3.16 gives the results of the blood-meal ELISA for Bayama village. Three of the five blood-meals positive for dog were also positive for human indicating a mixed blood-meal percentage of 0.02 (3/166). There was no blood-fed An. funestus in any of the Bayama collections.

Blood-meal analysis according to methods of collection are given in Table 3. 15 for the project villages, and in Table 3.16 for Bayama. There were females with mixed blood-meals in all the collections except those from light-traps. Despite extensive searches, few blood-fed females were found out of doors, of the few (17) tested from the project villages, 13 had fed on man, and one also on a goat. Out of a total of 31 females tested from outdoor collections in Bayama, 30 had fed on man.

3.3.6 Feeding index of Anopheles gambiae s.s.

It is often assumed that the results from blood-meal analysis reflect host preferences, which may well not be true (Boreham & Garrette-Jones, 1973). The human blood index (HBI) does not provide information on the numbers and distribution of alternative hosts . Kay et al. (1979) introduced the term **feeding index**, to take into account the number of alternative hosts, in analyzing blood-meal identification results. **Feeding index** was defined by Kay et al. (1979) as the "proportion of feeds on one host with respect to another divided by the comparative proportion of feeds on those two hosts". It may be expressed mathematically as

TABLE 3.15
Numbers of An. gambiae s.s. with positive blood-meal ELISA for various hosts, according to collection method, in the different project villages. Numbers in parentheses, in the non-human host columns, indicate those that were also positive for humans.

Collection method	Number tested	Host				Mixed
		Human	Pig	Goat	Dog	
PSC	974	973	0	1(1)	0	1
Exit trap	37	37	0	(1)	0	1
Light-trap	20	20	0	0	0	0
Outdoor collections	16	13	2	(1)	1	1
Total	1047	1043	2	1(3)	1	3

TABLE 3.16
Numbers of An. gambiae s.s. with positive blood-meal ELISA for various hosts, according to collection method, in Bayama village. Numbers in parentheses, in the non-human host columns, indicate those that were also positive for humans.

Collection method	Number tested	Host				Mixed
		Human	Pig	Goat	Dog	
PSC	82	82	0	0	(2)	2
Exit trap	30	30	0	0	0	1
Light-trap	22	22	0	0	1	0
Outdoor collections	31	30	0	0	1(1)	1
Total	165	163	0	0	2(3)	3

$$FI = \frac{Ne/Ne'}{Ef/Ef'}$$

where

FI= Feeding index

Ne= Number of feeds on host I

Ne'= Number of feeds on host II

Ef = Expected proportion of feeds on host I

Ef'= Expected proportion of feeds on host II

Thus an index of 1.0 indicates equal feeding on the two hosts being compared while figures less than one and greater than one indicate a decrease or increase in feeding on the first host relative to the second.

In order to substantiate the interpretation of my blood-meal analysis, the feeding index was calculated for alternative hosts in Bayama, the village with the highest number of domestic animals relative to the human population. In Bayama, there were 48 humans, 14 goats and 5 dogs, but positive ELISAs were only observed for humans (163/165) and dogs (5/165). The human-dog host feeding pattern of An. gambiae s.s. was therefore compared using the feeding index. If feeding occurred equally on humans and dogs, the expected ratio of positive ELISAs would have been 9.6:1, or 9.6. The observed proportion (Ne/Ne') from the blood-meal analysis was 163/5 or 32.6 giving a feeding index of 32.6/9.6 or 3.4. This result indicates greater feeding on humans than dogs at Bayama.

3.3.7 Man-biting rates estimated from pyrethrum spray, exit traps and human-bait collections in the same room

Human-bait, pyrethrum spray and exit trap collections were carried out at least once a month in a back veranda room (5002) in a house in the centre of Bayama village. It was the most isolated house in terms of occupants and neighbouring houses. It was occupied by one person and for most of the time, and only one other person stayed in the other room in the house.

Table 3.17 gives the man-biting rates of An. gambiae s.s. estimated using different collection methods in this one room, during the wet season. Although only a proportion of exiting females were caught in exit traps, the mean number of blood-fed females or bites/man/night (19.2), estimated from exit trap collections was five times higher than estimates from spray collections (3.5 bites/man/night). This indicates that even though An. gambiae s.s. was highly endophagic with an indoor man-biting rate of 68.8 bites/man/night, most of the females left the room, on the same night, after taking a blood-meal.

TABLE 3.17
Man-biting rates of An. gambiae s.s. calculated from females caught using different collection methods, in the same room in Bayama, in the wet season.

Method of collection	Number of collections	No. of females	Number of bites/man/night
Human-bait	52	3578	68.8
Pyrethrum spray*	16	54	3.5
Exit trap*	45	865	19.2

* Only blood-fed females were considered; assuming they all fed on the single adult male who occupied the room. HBI for Bayama was about 99%.

3.3 DISCUSSION

Anopheles gambiae and An. funestus fed almost exclusively on humans in villages where domestic animals roamed freely and slept on the verandas of houses at night. The human blood index (HBI) of 0.97 estimated for An. gambaie s.s. from outdoor resting collections at Bayama, where the human:domestic animal ratio was 2.5:1, was exceptionally high. In the Kisumu area of western Kenya where the HBI estimated from house collections at various times were 0.96 (Joshi et al., 1975) and 0.80 (Service et al., 1978b) the estimates from outdoor collections, were 0.07 and 0.02 respectively. White et al. (1972) working in Segera, Tanzania estimated an HBI of 0.91 from house collections but only 0.02 from outdoor collections. However, Service (1963) working in northern Nigeria also found a high HBI (0.89) from outdoor collections of An. gambiae s.l. In the present study, three An. gambiae s.s. caught resting inside houses had mixed blood-meals (human and goat) indicating that after feeding on goats outside, An. gambiae s.s. went inside houses to feed on man, probably to complete an interrupted blood-meal. One other female with a mixed blood-meal from human and goat was caught in an exit trap, suggesting that it probably just went into the room to complete the blood-meal and not to rest. In Kenya An. arabiensis has been reported to move into houses after feeding outside on cattle (Githeko, 1992; Petrarca, et al. 1991). One female An. gambiae s.s. which had fed on goat/sheep was caught in a morning spray collection carried out inside a bedroom, implying that the mosquito moved into the room to rest after feeding on a goat/sheep outside. Animal blood-fed An. gambiae s.s. reported inside bednets in The Gambia (Boreham & Port, 1982) were considered to have entered the bednets to rest,

probably when the occupant rose in the morning.

Anopheles gambiae s.s. was both endophagic, and exophagic but exophagy appeared to be more common when host seeking indoors was mostly unsuccessful i.e. when most females caught in exit traps were unfed. Females which failed to feed indoors did not rest but left houses in search of another host. Port & Boreham (1982) working in The Gambia observed that unfed An. gambiae s.s. usually left huts the same night, probably in search of alternative food sources. This might explain why the outdoor biting cycle of An. gambiae s.s. in the study area was shifted more towards the latter part of the night than the indoor biting cycle, with more biting taking place outdoors than indoors in the last three hours before dawn (Figure 3.3).

The difference in the resting behaviour of unfed An. gambiae s.l. in the humid coastal zones of West Africa and drier savanna areas of East Africa was pointed out by Ribbands (1946). In comparing the indoor resting density of unfed females in humid coastal areas of Sierra Leone to those that Haddow (1942) recorded in the savanna area of Kenya, he found there were 60 times more unfed An. gambiae s.l. resting in empty houses in Kenya than in Sierra Leone. In recent studies in Kenya, Githeko (1992) found that 17% of An. gambiae s.l. caught in pyrethrum spray collections were unfed, whereas in the Bo area, I found that just 0.1% of the females resting in houses were unfed.

A deficit of gravid females in morning pyrethrum spray collections in houses is a common phenomenon for An. gambiae s.l. in West Africa. In Burkina Faso,

Brengues & Coz (1973) recorded a fed:gravid ratio of 1.5:1 in one area, and in another area Brun (1973) recorded a ratio of 3:1. In northern Nigeria, it was estimated that about half the blood-meals taken on man are followed by resting indoors (Molineaux and Gramiccia, 1980). However, the large percentage of freshly fed females found in exit-trap, and outdoor collections in our study area was remarkable. Githeko (1992) working in Kenya found 16.3% of freshly fed females in exit traps in an area where An. gambiae s.s. and An. arabiensis were biting. Also whereas in Sierra Leone 33.9% of adult An. gambiae s.s. in light-traps were blood-fed, in Kenya only 10.3% of those caught in indoor placed light-traps were blood-fed, irrespective of whether or not the people used bed-nets (Githeko, 1992). The finding by Brun (1973) in Burkina Faso of 40% blood-fed An. gambiae s.l. in artificial shelters was considered exceptional by Gillies & Coetzee (1987). The high percentage of freshly blood-feds in outdoor collections at Bayama (58.8%) compared to the project villages (15.5%) was probably due to feeding on people outdoors, because of their activities at night around a police road barrier in the village. The palm fence from which most of the freshly fed An. gambiae s.s. were collected was actually built by the police to act as a screen when they searched travellers passing through the village. The barrier was manned throughout the night and some village people stayed around the police area till about 2300 hr selling cigarettes, whereas in the project villages, outdoor activities virtually ceased after 2200 hr. However, the high level of outdoor night activities at Bayama did not appear to encourage more outdoor biting because for five of the nine months when human-bait collections were carried out indoors, the indoor and outdoor biting rates were similar, but with the indoor rate being slightly higher.

The sudden upsurge in the outdoor biting rate on the catches in July was probably due to unsuccessful indoor biting on the villagers caused by most people at this time covering themselves completely with heavy cotton material when they went to bed- it was the coldest time of the year. A similar high outdoor biting rate in July was also experienced in the project villages. In the cold month of the year, June to August, when rainfall is very heavy, the Mende ethnic group, which was the main group in our area, used the traditional 'country' cloth - a very heavy locally spun and woven cotton material - to cover themselves at night. MacCormark (1984) observed that along the swampy coast of Sierra Leone, people -especially children- completely enveloped themselves in country cloth at night when they went to bed. A specimen of the cloth tested at the London School of Tropical Medicine and Hygiene was shown to be too thick for mosquitoes to penetrate (MacCormark, 1984).

The collection of a low percentage (<20%) of freshly fed An. gambiae s.s. in exit-traps from room 502 (Bayama) for four consecutive nights when the sole occupant was sick and covered with more cloth than usual, lends some support to the idea that the increase in the proportion of unfeds encountered in exit-traps during the cold month of July might be due to the prevention of successful feeds by cloths. Normally the percentage of freshly fed An. gambiae s.s. in exit-traps varied between 50 and 100%, and on one night as many as 83 blood-fed females were caught from a single exit-trap.

Results of human-bait, pyrethrum spray and exit-trap collections from the same room in Bayama village suggests that the An. gambiae s.s. population was very endophagic

and also very exophilic, leading to low catches of blood-feds in indoor pyrethrum spray collections which did not reflect the number of females that took a blood-meal during the night. This might explain the low values of man-biting rates, estimated from blood-feds in pyrethrum spray collections, in studies in humid coastal areas of Sierra Leone (Gordon et al., 1932) and Southern Nigeria (Baber & Olinger, 1931) were malaria was hyperendemic. The indoor-resting densities of Anopheles mosquitoes in the malaria endemic Kissy village, near Freetown in 1931 were 10.5 and 1.1 females/room in the wet and dry seasons respectively (Gordon, et al., 1932). It is very difficult to reconcile the low indoor-resting densities in the project villages and the P. falciparum prevalence rate of over 60% in 0-7 year olds in the dry and wet season. Results of the malaria survey in the project villages are given in chapter 6

Anopheles gambiae s.s. was the main vector in our study area where no other member of the An. gambiae complex was identified. Evidence from the present studies suggest that An.gambiae s.s. was highly exophilic, a behavioral characteristic that is not normally associated with the species (Gillies & Coetzee, 1987; Gillies & De Meillon, 1968). Comparative studies on the ecology of An. gambiae s.s. and An. arabiensis in a savanna area of Nigeria and in Segera, Tanzania suggested that An. gambiae s.s. was highly endophilic (White et al., 1972; White & Rosen, 1973). The evidence for the endophilic nature of this species, however, comes from observations of the relative abundance of An. gambiae s.s. and An. arabiensis in indoor resting collections. Coluzzi (cited by Molineaux & Gramiccia, 1980) after a careful study in Nigeria concluded that significant differences in resting behaviour of the two

species cannot be determined from their relative abundance in indoor resting collections. He found that where both species were sympatric, An. gambiae s.s. was more anthropophagic than An. arabiensis, but there was no clear cut selection in favour of an increasing proportion of An. arabiensis resting in houses following residual house spraying, as might have been expected if An. gambiae s.s. was more endophilic. He found that resting behaviour was related more to the frequency of certain chromosomal inversions within the cytospecies population, than to the relative abundance of the two species. Chromosomal arrangements which are more frequent in outdoor collections are those adapted to relatively more humid environment (Coluzzi et al., 1979).

The chromosomal variant of An. gambiae s.s. in our study area is characterised by the standard arrangement of chromosome 2R and 2L which have been found to be most frequent in outdoor collections of An. gambiae s.s. (Coluzzi et al., 1979). Exophily is therefore an inherent behaviourial characteristic of the chromosomal variant of An. gambiae in our study area. However, contrary to what one might expect, An. gambiae s.s. in our area tended towards a lower degree of exophily in the wet season when it was more humid.

The number and proportion of blood-fed An. gambiae s.s. which exited rooms were higher in rooms with plenty of wood smoke, it is important that such houses are not used in exit-trap studies designed to determine feeding and resting behaviour.

The biting cycle of An. gambiae s.s. in the Bo area was similar to what has been

observed for the species in other places, with most biting taking place in the second half of the night (Kuhlow & Zielker, 1978; Molineaux & Gramiccia, 1980). However , while in most other places the man-biting rate dropped rapidly after 0500 hr, in our study area it remained high until 0600 hr. The biting cycle of An. gambiae s.s. was influenced by the phase on the moon. The observation that more biting took place in the first half of the night during new moon and during the last quarter, than during the other phases of the moon was also made by Ribbands (1946) for An. funestus in the Freetown area of Sierra Leone. The similarity of the biting cycles during new moon and last quarter, and also how together they differed from the other two phases was also observed by Rosenberg & Maheswary (1982) for An. dirus Peyton & Harrison in Bangladesh.

The feeding and resting behaviour of the forest chromosomal variant of An. gambiae s.s. was in many ways different from what has been recorded for unspecified chromosomal forms of An. gambiae s.s. elsewhere in Africa. This might have very important implications for the epidemiology of malaria in the high rainfall forest areas of West Africa, especially when evidence now exists from studies in Kenya (Petrarca & Beier, 1992) that the sporozoite rate of the forest chromosomal form could be higher than that of the savanna variant.

CHAPTER 4

VECTOR INOCULATION RATES AND VECTORIAL CAPACITIES

4.1 INTRODUCTION

The entomological inoculation rate, which is the number of infective mosquito bites a person can receive in a unit time, is a measure of the intensity of malaria transmission. It is estimated by multiplying the sporozoite rate or the proportion of infective Anopheles species (those whose salivary glands contain sporozoites) by the man-biting rate (**ma**) - see chapter 2. In areas where malaria transmission is seasonal, the time during which sporozoites are found in Anopheles mosquitoes marks the transmission season and therefore the period when protection from mosquitoes is required to prevent infections. The determination of sporozoite rates is therefore essential in the evaluation of intervention methods. It has been considered by some workers (Wirtz et al., 1987), as the most important entomological factor in the epidemiology of human malaria.

Traditionally, infective mosquitoes are identified by dissecting out the salivary glands of individual mosquitoes and examining them for sporozoites. In a given situation, the sporozoite rate varies with time, vector species and parasite species. Sporozoites of the four human species of malaria are impossible to distinguish from each other

morphologically and also from more than 100 other malaria parasites species which infect rodents, birds, reptiles and non-human primates. This can pose a problem in certain areas, such as Malaysia where some of the suspected vectors of human malaria may also transmit non-human malaria. In Africa, however, this is not a problem because the Anopheles gambiae complex, An. funestus and many less important vectors are very rarely infected with non-human malaria. Nevertheless, detection of sporozoite-positive mosquitoes by dissection can be time-consuming, and as stated sporozoites of the different human malarias cannot be distinguished morphologically. For these and other reasons there have been vigorous attempts to replace the dissection method with immunological methods and this has been made possible because of the sporozoite protein envelope which has antigens unique for each species.

The external surface of a mature sporozoite is covered by a proteinaceous layer - circumsporozoite (CS) proteins. Circumsporozoite proteins are heat stable and are found on all species of sporozoite so far studied. They consist of an immunodominant central region made up of repeated amino-acid sequences. The length and amino acid sequence which makes up the repeats are unique for each species of malaria. The development of high affinity monoclonal antibodies directed against the species-specific repeat region of the circumsporozoite proteins has resulted in immunoassays which identify infected mosquitoes and the parasite with which they are infected. One of these immuno assays, the enzyme-linked immunosorbent assay (ELISA), has been developed as a useful tool in the identification of malaria-infected mosquitoes. Monoclonal antibodies have been developed for all four species of

human malaria (Beier et al., 1988; Burkot et al., 1984; Collins et al., 1988; Wirtz et al., 1985).

Recent developments in molecular biology have made the production of vaccines against the sporozoites of human malaria a possibility (Zavala et al., 1982; Patarroyo et al., 1988; Halloran & Struchiner, 1992). It is therefore essential that there is a better understanding of the rate at which the human population in malaria endemic areas is inoculated with sporozoites, and the relationship between inoculation rates and prevalence rates. For sporozoite vaccines, inoculation rate is a measure of the challenge.

A more complicated but useful method of estimating transmission intensity is by measuring the vectorial capacity of the vectors. It is the average number of inoculations from a single case of malaria in unit time, usually a day, that the vector population transmits to man, where all vectors biting an infected person become infective. The concept of vectorial capacity evolved from the early quantification of entomological and malariometric data, by Macdonald (1952). He was concerned with estimating the basic malaria reproduction rate (R_o), which is the average number of secondary cases of a disease arising from each primary infection in a defined population of susceptible individual hosts. In other words, vectorial capacity is the entomological component of the malaria reproduction rate. The usual formula for vectorial capacity shown below, was derived by Garrett-Jones (1964) in terms of daily a rate.

$$C = \frac{ma^2p^n}{-\log_e p}$$

C = Vectorial capacity, that is potential new infections disseminated per person per day by each mosquito assuming that all infected females become infective.

ma = the man biting rate in bites/man/night

a = the man-biting habit, that is the proportion of females feeding on man divided by the duration of the gonotrophic cycle in days. **a** is multiplied by **ma** to give ma^2 because refeeding is necessary for transmission.

p = probability of daily survival, estimated vertically from the population age structure if the duration of the gonotrophic cycle is known, or horizontally from the daily loss rate of identified cohorts, e.g marked females, over time. Thus vector life expectancy = $1/-\log_e p$

n = time from infection to infectivity in days and is usually estimated from the ambient temperature using a degree relationship. Thus p^n = probability of a mosquito surviving to become infective and the duration of infective life in days = $p^n/-\log_e p$.

4.2 MATERIALS AND METHODS

4.2.1 Vector infection rates

a) Sporozoite detection by salivary gland dissection

Two techniques were used for the extraction of the salivary glands using a pair of dissecting needles (WHO, 1975). In the first technique an identified female mosquito was placed close to a drop of 0.65% saline. One needle was gently placed on the thorax slightly below the region where the salivary glands were located and the neck cut close to the head with the other needle. The salivary glands were slowly squeezed out of the thorax by gently pressing with a needle. The glands which usually came out in a mass of muscle tissue were separated from the tissues using the other needle while still pressing the thorax. Glands were easier to locate against a dark background. Separated glands were placed in a drop of saline on another slide and then covered with a square coverslip so that they remained in one corner of the coverslip for easy location. The glands were located under a compound microscope using a X10 objective and then the coverslip was gently pressed to rupture and flatten the glands, and so release the sporozoites. The glands were then examined using a 40X objective and reduced illumination. The sporozoites were visible as elongated, needle-like structures.

In the other technique, one needle was gently placed on the thorax slightly below the region where the salivary glands are situated and the other needle placed on the neck of the mosquito without cutting. The head of the mosquito was then detached by

gently pulling it into a drop of saline. The salivary glands were pulled out of the thorax still attached to the head. They were then severed from the head and examined under a compound microscope as before. If the neck severed before the glands came out they were extracted from the thorax as in the first technique.

b. Detection of P. falciparum sporozoites by ELISA

Mosquitoes for sporozoite determination by ELISA were obtained in all study villages, from human-bait, pyrethrum spray and aspirator catches (indoor and outdoor), and from light-trap and exit trap collections. They were transported in cool boxes to the MRC Laboratories in Bo where they were sorted according to species. Abdomens were removed and the head-thorax portions put in 88 out of the 96 wells of a polyvinyl microtitre plate leaving 8 wells for negative and positive controls, in four of which male An. gambiae were put for negative controls. The plates were carefully wrapped in cling film and placed in a desiccator containing silica gel. The desiccator was kept at 4°C in a fridge and the silica gel crystals checked every 3 days for decoloration. The history of the mosquito, e.g. date of collection, collection method, village and house number, and well number were recorded on a form coded for entry into a computer . All enzyme - linked immunosorbent assays (ELISA) for the detection of Plasmodium falciparum sporozoites was performed at the Medical Research Council (MRC) Laboratories, Fajara The Gambia. Between May 1990 and October 1991 the author made two journeys to The Gambia with a total of 10,050 Anopheles mosquitoes for ELISA testing. In the MRC Laboratories Fajara, the specimens were stored at 4°C in desiccators as soon as they arrived.

Individual head-thoraces were tested for P. falciparum circumsporozoite antigen using 2A10 monoclonal antibody according to the methods described by Wirtz et al. (1987). Each specimen was prepared for ELISA by grinding in 20μl of blocking buffer containing nonindet P-40 in wells in a labelled PVC plate (Dynatech Laboratories UK). The specimens were ground with a pestle which was rinsed with 2 x 65μl volumes of blocking buffer, putting the rinses in the wells (Total final volume/well = 150μl). After grinding each mosquito head-thorax the pestle was rinsed in PBS/Tween twice and once in PBS and dried before using it to grind another specimen. Ground mosquitoes were kept at -20°C until samples were ready for testing.

ELISA plates were coated with the monoclonal antibody and incubated for 30 min at room temperature. After incubation the contents of the wells were aspirated, filled with blocking buffer (305μl/well) and incubated at room temperature for one hour. After incubation the well contents were aspirated and 25μl blocking buffer added to each test well followed by 25μl sample supernate (Total final volume being 50μl/well). Two positive controls on each plate (50μl/well) consisted of 100 and 10pg of a recombinant P. falciparum circumsporozoite protein (Wirtz et al., 1987a). To four negative control wells were added 25μl/well of supernates from male An. gambiae. Plates were incubated for 2 hours and washed 6 times in PBS/Tween. The conjugate, a peroxidose - linked monoclonal antibody (MA66) was added (50μl/well) and incubated for 1 hour at room temperature. The washing procedure was repeated after incubation. After drying the plates on a paper towel a clear peroxidase substrate solution was added (50μl/well). The peroxidase enzyme reacted

with the substrate to give a dark green product, the intensity of which varied according to the amount of circumsporozoite protein present in the test sample. Plates were read by an ELISA reader (MR700 Microplate - a Dynatech product). Samples were considered positive if absorbance values (range 0 -2.00) at 414nm exceeded twice the mean of the negative control values (Beier et al., 1988b).

All positive samples were again tested using 7 positive controls: 100, 50, 25, 12, 6, 3 and 1.5pg of recombinant circumsporozoite protein.

4.2.2 Visual assessment of sporozoite ELISA results

In order to evaluate the accuracy of sporozoite ELISA results by visual examination, a double blind comparison of results determined by eye and by the ELISA reader was carried out. The ELISA plates, containing test samples, were first read visually by the author, two minutes before they were scanned by the ELISA plate reader. The reader was operated by an entomological assistant, who had previously performed ELISAs on over 60,000 mosquitoes from The Gambia. Positive samples determined by both methods were retested using the ELISA reader alone to confirm the positivity of samples.

4.2.3 Statistical significance of sporozoite rates

To test the significance of sporozoite rates, the standard error was calculated using the method of Gillies et al. (1961). According to this method, the standard error for

sporozoite rates of 1% or more can be calculated using the formula

$$SE = \sqrt{\frac{\%\ positive \times \%\ negative}{Number\ examined}}$$

4.2.4. Survival rates and gonotrophic cycle duration

Two methods were used to estimate the survival rate and gonotrophic cycle duration of Anopheles. One method is based on parous rates determined from ovary dissections. The other method involved mark - recapture studies.

Ovary dissection

Anopheles ovaries were dissected out to establish parity according to the method of Detinova (1962). Dissected ovaries were transferred to a film of water on a labelled slide and allowed to dry, so that trachoelar skeins used to determine parity could be seen. Dry ovaries were examined immediately or at a later date using a compound microscope with a X10 eyepiece and X40 objective. When kept in boxes with camphor to prevent the degradation of the organic matter by fungi. Slides remained in good condition for over 15 months. Nulliparous females were identified by the presence of coiled tracheolar skeins on the ovaries. Ovaries of parous females had uncoiled tracheolar skeins.

Mark - recapture experiments

Human-bait caught mosquitoes were identified to species in the field, and then placed

in plastic cups at densities of 50 - 60 per cup. Catching was terminated at 0500 hr so that females could be marked and released at 0600 hr. Mosquitoes were marked using 'Fiesta' daylight fluorescent colours. The two colours used, together with their commercial codings in parentheses were yellow (A27) and red (A3). For marking, females were held in cups and fluorescent powders puffed into the cups using 5 ml hypodermic syringes. Marked mosquitoes were released into the vegetation outside the village. All females were released as unfeds. Starting on the evening following the first release, human bait collections were carried out at two outdoor sites within the village, by two groups of collectors.

Fluorescent dusts were used because they have been shown to have little or no effect on mosquito survivorship (Reisen & Aslankhan, 1979). Nevertheless, to check whether powders used in the present experiment had any detrimental effect on survival par cup containing another 30 unmarked females was used as a control. Mortality in both cups was recorded for one week.

4.2.5 Pre-gravid rate estimation

In some individuals, ovarian development in Anopheles may not proceed beyond Christophers stage II after the first blood-meal, making a second blood-meal necessary for complete ovarian development. The proportion of mosquitoes which require more than one complete blood-meal for the ovaries to fully mature is called the pre-gravid rate (Gillies, 1954).

To determine the pre-gravid rate of Anopheles gambiae s.l. fully engorged females were collected from exit traps at Bayama village and kept in polystyrene cups (10/cups) for 48 hours. Those whose abdomens appeared to revert to the unfed condition were dissected and the ovaries examined, and if they failed to develop beyond Christophers stage II, they were recorded as being pre-gravid.

4.3. RESULTS

4.3.1. Parous rates of An. gambiae s.s. in the project villages

Few anophelines were caught biting, and only during the wet season in some project villages. Therefore only 148 female An. gambiae s.s. from human-bait catches carried out at Nyandeyama and Mendewa were dissected for parity determination. Parous rates of 48.0 and 58.0% were observed for indoor and outdoor catches respectively (Table 4.1), the difference, was, however, not statistically significant (χ^2 = 0.964, p=0.326, df=1). Due to the low number of females caught, parous rates could not be analyzed at village or monthly levels.

The small number of An. funestus dissected (6) could not provide any reliable information on parity, and are therefore not discussed.

Since only unfed females brought alive to the laboratory were dissected for parity, pyrethrum spray, light-trap and exit-trap catches which contained few live females, were not used for parity determination.

4.3.2 Parous rates of An. gambiae s.s. at Bayama

At Bayama a total of 6940 female An. gambiae s.s. were dissected for parity determination and 4258 (61.4%) were parous. Table 4.2 gives the mean monthly parous rates which varied from 73.1% at the end of the dry season to 46.7 % in April

TABLE 4.1
Mean parous rates (%) of indoor and outdoor human-bait collections of An. gambiae s.s. at Nyandeyama and Mendewa combined

Catching site	Number dissected	Number parous	Parous rate (%)
Indoor	98	47	48.0
Outdoor	50	29	58.0
Total	148	76	53.4

TABLE 4.2
Mean monthly parous rates (%) of unfed An. gambiae s.s. caught in human-bait collections at Bayama

Month 1990/1991	Number caught	Number dissected	Number parous	Parous rate (%)
Nov	235	150	106	70.7
Dec	133	105	60	57.1
Jan	56	43	27	62.8
Feb	192	164	107	65.2
Mar	148	104	76	73.1
Apr	18	15	7	46.7
May	4506	2129	1223	57.4
Jun	2987	1629	1044	64.1
Jul	6266	1152	683	59.3
Aug	1453	526	290	55.4
Sep	946	605	432	71.4
Oct	455	318	203	63.8
Total	17395	6940	4258	61.4

at the beginning of the wet season but the last figure is unreliable due to the small sampling size (15). Overall, there was no obvious trend in the variation of parous rates throughout the study period. The monthly mean parous rate varied around the annual mean of 61.4% in a regular fashion (Figure 4.1).

Table 4.3 gives the parous rates of An. gambiae s.s. according to season and catching site (indoor or outdoor). There was no significant difference in the parous rates of the indoor (60.8%) and outdoor (61.6%) biting populations ($\chi^2=0.399$, $p=0.528$, $df=1$), or the dry (64.3%) and wet (61.2%) biting populations ($\chi^2=1.518$, $p=0.218$, $df=1$).

For the same reasons put forward for the project villages, An. funestus and An. gambiae s.s. obtained from pyrethrum spray and light-trap collections were not used for parity determination. However, since exit-traps often contained large numbers of freshly blood-fed An. gambiae s.s , collections from these traps in Bayama, were used to estimate the pre-gravid rate.

4.3.3 Pre-gravid rate of An. gambiae s.s. at Bayama

Results of dissections from exit-trap collection, used to calculate the pre-gravid rate of An. gambiae s.s. are given Table 4.4. A total of 151 unfed females were dissected from five days collections in June and July, giving a nulliparous rate of 60.2%, and consequently a parous rate of 39.8% which was significantly less ($p<0.0001$) than the parous rate of 63.2% observed in human bait collections from June and July

FIGURE 4.1 Seasonal variation in parous rates of An. gambiae s.s. at Bayama

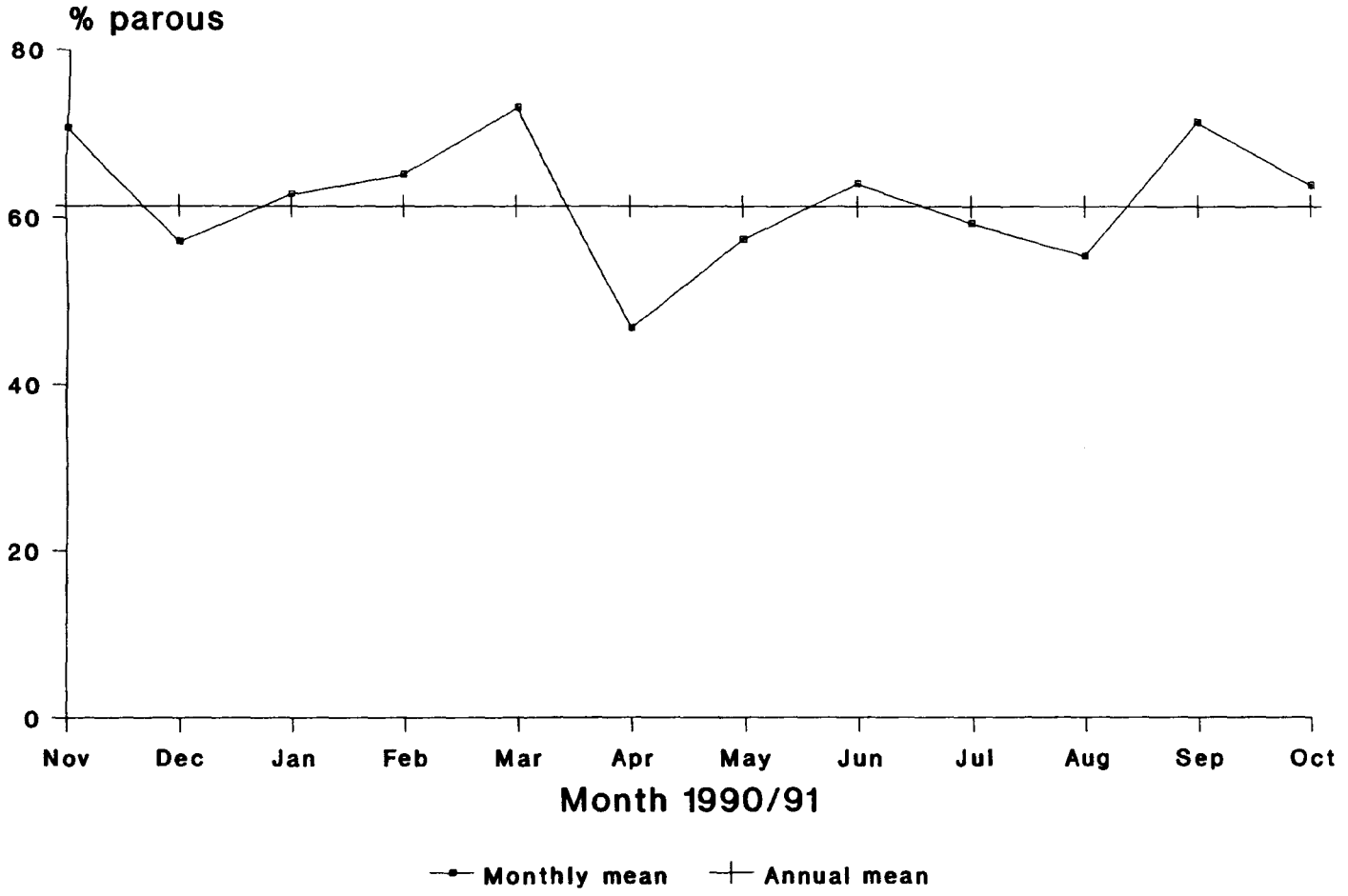


TABLE 4.3
Mean parous rates of indoor and outdoor biting populations of An. gambiae s.s. in the dry and wet seasons at Bayama

Catching site	Dry season		Wet season		Total	
	No. diss.	% pars.	No. diss.	% pars.	No. diss	% pars
Indoor	233	66.5	1785	60.0	2018	60.8
Outdoor	198	62.2	4724	61.2	4922	61.6
Total	431	64.3	6509	61.2	6940	61.4

TABLE 4.4
Results of exit-trap dissections used to estimate the pre-gravid rate, Bayama village.

Date	Unfeds		Freshly blood-feds	
	No. diss.	No. null.	No. diss.	No. null.
27/6/91	17	11	50	21
29/6/91	30	17	16	2
4/7/91	36	32	78	14
5/7/91	28	8	45	6
6/7/91	40	23	59	7
Total	151	91(60.3%)	248	50*

* Three of the 50 pre-gravids were parous

combined.

Out of a total of 248 freshly-fed females, obtained from the same exit-traps and maintained for 48 hours at room temperature before dissection, 50 (20.2%) had their ovaries at a stage less than Christophers stage II, and were therefore pre-gravid. This means that they would require another blood-meal to mature their eggs. Although the ovaries of pregravid females had undergone partial development, they were identifiable as parous or nulliparous. The pre-gravid rate when expressed as a proportion of the nulliparous population, was calculated to be 33.3%. Three of the 50 pre-gravid females were parous, thereby exhibiting a condition of gonotrophic discordance.

In July a large number of unfeds were caught in exit-traps and Table 4.5 compares the parous rates of exit-trap and human-bait collections. Again the parous rate of females caught in human-bait collections (59.2%) was significantly higher than the parous rate (48.2%) of females in exit trap collections ($\chi^2=15.479$, $p<0.0001$, $df=1$).

4.3.4. Survival rate of An. gambiae s.s. at Bayama.

Experiments to estimate the survival rates of An. gambiae s.s. were carried out only at Bayama because the numbers of females required for a meaningful analysis of the results were not obtainable in the project villages. Table 4.6 gives the results of the time-series collection experiment performed for the determination of gonotrophic

TABLE 4.5
Parous rates of An. gambiae s.s. caught at human-bait and in exit-trap collections in July 1991, Bayama

Collection method	Number dissected	Number parous	% parous
Exit-trap	438	211	48.2
Human-bait	1152	683	59.3

TABLE 4.6
Outdoor human-bait catches and parous rates of An. gambiae s.s. over 24 consecutive nights at Bayama, from 18/5/91 - 11/6/91.

Time (day)	Total caught	Parous rate	Total parous	Total caught
1	289	0.80	231	
2	254	0.79	201	
3	74	0.77	56	
4	282	0.71	**200**	**289**
5	266	0.59	**157**	**254**
6	136	0.43	**58**	**74**
7	291	0.34	**99**	**282**
8	176	0.18	**32**	**266**
9	261	0.42	**110**	**136**
10	197	0.27	**53**	**291**
11	186	0.24	**45**	**176**
12	324	0.57	**185**	**261**
13	302	0.74	**223**	**197**
14	160	0.82	**131**	**186**
15	242	0.85	**206**	**324**
16	162	0.93	**151**	**302**
17	124	0.93	**115**	**160**
18	112	0.91	**102**	**242**
19	217	0.88	**191**	**162**
20	219	0.73	**160**	**124**
21	212	0.49	**104**	**112**
22	280	0.47	**132**	**217**
23	176	0.47	**83**	**219**
24	171	0.56	**96**	**212**
Lagged total			**2633**	**4487**
Total	5113	0.60	3120	

cycle duration (interval between two blood-meals) and the survival rate per gonotrophic cycle. A total of 5113 females were caught during 24 consecutive outdoor human-bait collections from the 18th of May to the 11th of June 1991. From the total caught and the total parous (parous rate x total caught), a mean parous rate of 61.0% was obtained.

Applying cross-correlation analysis to the time-series describing the abundance of total caught and total parous (Birley & Rajagopalan, 1981; Holmes & Birley, 1987; Mutero & Birley, 1989) the Runs test was first used to test if the population was closed and not affected by emigration or immigration. Using the Minitab command 'Runs' it was shown that daily changes in parous rate was random (P = 0.2108) with 12 observations of daily parous rates above and 12 below the mean parous rate for the 24 observations. This means that the population was closed, with a stationary age distribution. Under these conditions, the mean parous rate is considered an unbiased estimate of the survival rate per gonotrophic cycle (Dye, 1992).

In a closed population with a constant rate of recruitment of nulliparous females, the parous females observed on any given night are produced from the females that existed one gonotrophic cycle before. By lagging one time series of the total density caught (column 2) against the total parous (Column 4),the largest statistically significant cross-correlation coefficient will give the best estimate of the length of the gonotrophic cycle in days (Dye, 1992). By using the Minitab command CCF, a maximum cross-correlation coefficient of 0.234 was observed for day 3 (Figure 4.2), but unfortunately it was not statistically significant. After a modification of the data

Figure 4.2 A profile of peaks in the cross-correlation coefficients obtained by lagging one time series of total female An. gambiae s.s caught against total parous. The profile was obtained using the minitab command **C C F**

Cross-correlation coefficient (CCF)

Day	CCF	-0.6 -0.4 -0.2 0.0 0.2 0.4 0.6 0.8 1.0
		-+----+----+----+----+----+----+----+----+
0	0.689	XXXXXXXXXXXXXXXXXX
1	-0.097	X
2	-0.165	X
3	0.234	XXXXXXX
4	0.059	XX
5	0.021	XX
6	-0.070	X
7	0.083	XXX
8	0.166	XXXXX
9	-0.221	X
10	-0.140	X
11	0.078	XXX
12	-0.053	X
13	-0.044	X
14	0.066	XXX

by the incorporation of weight linear regression and filtered cross-correlation (Holmes & Birley, 1987) the cross-correlation coefficient increased slightly to 0.271, but was still not statistically significant (the weight linear regression and filtered cross-correlation analysis was achieved through the Minitab command Arima 1 0 0). Nevertheless, because of the obvious peak at day three and the presence of other peaks separated by three days, the duration of gonotrophic cycle was considered most likely to be three days. In fact the criterion for detecting statistically significant peaks has been considered by Birley & Mutero (1987) to be too severe.

According to the method of Birley and colleagues already mentioned, in order to calculate the correct value of the survival rate per gonotrophic cycle, it is necessary to take account of the time lag produced by the duration of the gonotrophic cycle. This was achieved by dividing the lagged total parous (column 4, excluding data for the first three days and using values printed in bold in Table 4.6) by the lagged total caught (column 5) and a value of 0.59 was obtained for the survival rate per gonotrophic cycle, which is similar to the mean parous rate (0.61). The mean parous rate was therefore a close estimate of the survival rate per gonotrophic cycle.
The mean parous rates of An. gambiae s.s. in the dry (64.3) and wet (61.2) seasons were therefore considered reasonable estimates of the survival rate per gonotrophic cycle in the respective seasons.

In the project villages, the mean parous rate for An. gambiae s.s. in the wet season was 0.53 (Table 4.1). Whether or not this value was obtained from a closed population of constant age distribution could not be determined.

Attempts in July 1991 to estimate the duration of the gonotrophic cycle and the survival rate of An. gambiae s.s. in Bayama village, using the mark-release and recapture method, were unsuccessful because of the high mortality rate of unfed females marked with fluorescent powders (Table 4.7). A higher proportion of marked females (73.3%) compared to unmarked (6.7%) died after being kept at room temperature for two days. By day six all marked females had died while 66.7% of the unmarked females were still alive. Blood-fed females were not used in this experiment because An. gambiae s.s. could not be bred in large quantities for blood-feeding.

Two sets of mosquitoes were marked and released in July 1991 (Table 4.8). In the first set 660 unfed female An. gambiae s.s. were marked with yellow powder and released, and human-bait catches performed for 11 consecutive days. Marked females were recaptured only up to five days after release. In the second set, marked mosquitoes were caught up to just four days after releasing 572 females marked with red powder. Although the recapture rates in the two sets of marked mosquitoes were high (13.3 and 8.5%) marked females were collected for too short a time for any meaningful data on the duration of the gonotrophic cycle or daily survival rate to be made. All that can be recorded was a slight peak (not statistically significant) in the number of marked females recaptured in both sets on day three after release.

TABLE 4.7
Percentage survival of marked and unmarked female An. gambiae s.s. maintained in polystyrene cups at room temperature from days 0 - 7.

Day	Unmarked			Marked		
	Number alive	Total dead	% survival	Total alive	Number dead	% survival
0	30	0	100.0	30	0	100.0
1	28	2	93.3	14	16	46.7
2	28	2	93.3	8	22	26.7
3	25	5	83.3	5	25	16.7
4	21	9	70.0	5	25	16.7
5	21	9	70.0	2	28	6.7
6	20	10	66.7	0	30	0.0
7	20	10	66.7			

TABLE 4.8
Daily human-bait catches of unfed An. gambiae s.s. after some females had been marked and released, Bayama village

Days after release	**Series 1** (9/7/91) 660 marked females released		**Series 2** (13/7/91) 572 marked females released	
	Number collected	Number marked	Number collected	Number marked
1	935	63	542	36
2	592	10	727	5
3	623	12	522	7
4	607	2	316	1
5	541	1	541	0
6	727	0	252	0
7	522	0	-	-
8	316	0	-	-
9	541	0	-	-
10	252	0	-	-
11	179	0	-	-
Total	5835	88	2900	47

4.3.5 Daily survival rates and vectorial capacities of An. gambiae s.s.

The daily survival rates of An. gambiae s.s. at Bayama was calculated from the mean parous rates using the formula $\sqrt[u]{S}$ where **u** is the duration of the gonotrophic cycle and S is the mean parous rate (Davidson, 1954). In Table 4.9, the daily survival rates were calculated for each month using the gonotrophic cycle duration of three days. The percentage of the population expected to live long enough to become infective (p^n) and the subsequent life expectancy of this population ($p^n/-\log_e p$) was calculated using 10 days as the time from infection (with P. falciparum) to infectivity (Bruce-Chwatt, 1985).

The daily survival rates varied from 0.78 in April to 0.90 in March. In March for example, 35% of the females were expected to survive the extrinsic incubation period of the parasite and these survivors had a further life expectancy of 2.7 days. The life expectancy of An. gambiae s.s. varied from 6.1 days in the wet season to 6.6 days in the dry season (Table 4.10). The calculated vectorial capacities for the dry and wet seasons were 3.0 and 39.3 respectively, and the annual average was 35.4 (formula for calculating vectorial capacity is given under section 4.1) A human blood index of 0.98 calculated from outdoor and spray collections (Chapter 3) was used in the calculation of vectorial capacity. If the duration of the gonotrophic cycle of An. gambiae s.s. in the project villages in the wet season was the same as observed at Bayama (three days), then the estimated life expectancy and vectorial capacity could be calculated as 4.7 and 0.36 respectively.

TABLE 4.9
Components of vectorial capacity[1] calculated from monthly mean parous rates of An. gambiae s.s. at Bayama

Month 1990/91	p	p^n	$\frac{1}{-\log_e p}$	$\frac{p^n}{-\log_e p}$
Nov	0.89	0.31	8.58	2.66
Dec	0.83	0.12	5.37	0.64
Jan	0.86	0.22	6.63	1.46
Feb	0.87	0.25	7.18	1.80
Mar	0.90	0.35	9.49	3.32
Apr	0.78	0.08	4.02	0.32
May	0.83	0.16	5.37	0.86
Jun	0.86	0.22	6.63	1.46
Jul	0.84	0.17	5.74	1.00
Aug	0.82	0.14	5.04	0.70
Sep	0.89	0.31	8.58	2.66
Oct	0.86	0.22	6.63	1.46
Annual	0.85	0.20	6.15	1.23

[1]Duration of gonotrophic cycle = 3 days and intrinsic incubation period for P. falciparum = 10 days.

P = probability of daily survival.
p^n = probability of mosquito surviving to become infective.
$1/-\log_e p$ = vector life expectancy in days.
$p^n/-\log_e p$ = duration of infective life in days.

TABLE 4.10
Vectorial capacities of An. gambiae s.s. at Bayama in the dry and wet season

Season	p	p^n	$\frac{1}{-\log_e p}$	ma	C
Dry	0.86	0.23	6.6	6.0	8.0
Wet	0.85	0.19	6.1	102.7	39.3
Annual	0.85	0.20	6.1	87.9	35.4

4.3.6 Sporozoite rates of An. gambiae s.s and An. funsestus in the project villages

A total of 2356 An. gambiae s.s. and 172 An. funestus from the project villages were tested for P. falciparum sporozoite antigens using the ELISA method. Table 4.11 gives the percentage of females positive (sporozoite rate) in the villages and Table 4.12 gives the sporozoite rates for the dry and wet seasons (all villages combined). There was no significant difference in the sporozoite rates of An. gambiae s.s. in the different villages (χ^2=7.65, p=0.054, df=3). However, the sporozoite rate for the wet season (7.9%) was significantly higher than the sporozoite rate for the dry season (3.4%) (χ^2 = 4.56, p=0.033, df=2). The seasonal difference in sporozoite rate is also clearly shown by the calculated standard errors.

Sporozoite rates of An. funestus were not statistically analyzed at the village or seasonal level because of the low numbers tested. However, the Fisher's exact test showed no significant difference (p>0.05) in the sporozoite rates of this species between the combined high (16.2%) and low altitude (9.6%) villages (Table 4.11).

Table 4.13 gives the seasonal variation in the sporozoite rates of An. gambiae s.s. Sporozoite rates varied from 0.0% in January to 20.0±5.96% in October. Failure to detect positive An. gambiae s.s. in January was probably because only 22 females were tested. A monthly breakdown was not made in the case of An. funestus because of the low numbers tested (172), but sporozoite positives were detected in each month of the year except April (n=28), July (n=0), August (n=1)

TABLE 4.11
ELISA determined P. falciparum sporozoite rates of An. gambiae s.s. and An. funestus in the project villages

Village	Anopheles gambiae			Anopheles funestus		
	Number tested	Number positive	% ±SE	Number tested	Number positive	% ±SE
Nengbema*	578	29	5.0±0.91	13	2	15.4±10.01
N'dyama*	1147	91	7.9±0.80	24	4	16.7±7.61
Mendewa**	298	22	7.4±1.52	114	11	9.6±2.76
Njala Komboya**	333	32	9.6±1.61	21	2	9.5±6.4
Combined Villages	2356	174	7.4±0.54	172	19	11.4±2.42

* Low altitude villages
** High altitude villages

TABLE 4.12
Dry and wet season P. falciparum sporozoite rates (%) of An. gambiae s.s. in the low and high altitude villages

Villages	Dry season			Wet season		
	Number tested	Number positive	% ±SE	Number tested	Number positive	% ±
Low altitude	141	4	2.8±1.39	1548	116	7.5±0.67
High altitude	64	3	4.7±2.65	567	51	8.9±1.20
Combined	205	7	3.4±1.27	2115	167	7.9±0.59

TABLE 4.13
Mean monthly sporozoite rates of An. gambiae s.s. in the combined project villages

Month 1990/91	Number tested	Number positive	% ±SE
February	11	2	18.2±11.63
March	11	1	9.1±8.67
April	54	1	1.9±1.86
May	288	31	10.8±1.83
June	316	17	5.4±1.27
July	802	48	5.0±0.77
August	553	47	8.5±1.19
September	58	9	15.5±4.75
October	45	9	20.0±5.96
November	89	6	6.7±2.65
December	59	3	5.1±2.86
January	22	0	0.0±0.00
Total	2308	174	7.5±0.55

and September (n=2). Although no sporozoite positive An. gambiae s.s. was found in January, one out of six An. funestus tested was positive, indicating that in the project villages, transmission of P. falciparum took place in every month of the year. The difference in sporozoite rates of An. gambiae s.s. caught using the different catching methods (Table 4.14) were not statistically significant (χ^2= 5.100, p=0.277, df=4). Table 4.15 gives the numbers of houses with sporozoite positive females at one time or the other during the period of study. Out of a total of 23 houses sprayed, positive female An. gambiae s.s. or An. funestus were found in 20 of them. Six of the ten houses where light-traps were hung produced positive females. The number of females tested from houses that yielded no positive females varied from 1 to 9. The failure to find positive females in some of these houses was probably due to the few mosquitoes tested. A total of 38 An. hancocki were tested but none were positive.

4.3.7 Sporozoite rates of An. gambiae s.s at Bayama

A total of 7845 An. gambiae s.s. were tested from the Bayama collections (Table 4.16). Samples analyzed by ELISA were collected during all months expect April and October; in April few mosquiotes (18) were collected and dissected because the laboratory had to be closed due to military activity in the study area, and the last ELISA tests were carried out in September because I had to leave for the UK in November. In these two months (April and October), the dissection method alone was used to determine sporozoite rates. A total of 434 An. gambiae s.s. from six months collection were dissected to determine sporozoite rates. From January to

TABLE 4.14
Sporozoite rates (%) of An. gambiae s.s. collected using different catching methods in the project villages.

Catching method	Number tested	Number positive	% ±SE
Exit-trap	159	7	4.4±1.63
Light-trap	216	17	7.9±1.84
Human-bait	146	6	4.1±1.64
Spray	1714	134	7.8±0.65
Outdoor resting	118	10	8.5±2.57
Total	2353	174	7.4±0.54

TABLE 4.15
Number of houses from which sporozoite positive female An. gambiae s.s. were caught in the different project villages.

Village	Spray collections		Light-trap collections	
	Number of houses	Number with positive females	Number of houses	Number with positive females
Nengbema	6	5	-	-
Nyandeyama	6	6	5	5
Mendewa	6	5	5	1
Njala-Komboya	5	4	-	-
Total	23	20	10	6

TABLE 4.16

Mean monthly P. falciparum sporozoite rates (%) of An. gambiae s.s. detemined by dissection and ELISA, Bayama. Standard errors (SE) are provided for the period January to May when sporozoite rates were determined using both methods.

Month 1990/91	Dissection			ELISA		
	Number dissected	Number positive	% ±SE	Number tested	Number positive	% ±SE
Nov	-	-	-	252	17	6.7
Dec	-	-	-	200	14	7.0
Jan	20	3	15.0±7.89	104	7	6.7±2.45
Feb	90	6	6.0±2.50	174	18	10.3±2.31
Mar	62	7	11.2±3.96	189	15	7.9±2.00
Apr	18	2	11.1±7.43	-	-	-
May	50	2	4.0±2.82	2939	97	3.3±0.33
Jun	-	-	-	2519	93	3.7
Jul	-	-	-	992	29	2.9
Aug	-	-	-	388	7	1.8
Sep	-	-	-	88	8	9.0
Oct	194	11	5.7	-	-	-
Total	434	31	7.2	7845	305	3.9

March and during May 1991, sporozoite rates were determined by dissection and by ELISA, and it was found that the mean monthly sporozoite rates determined using the dissection method were statistically similar to rates determined using the ELISA method. At first sight, the sporozoite rates determined by the two methods look different but the overlap in the calculated standard errors clearly shows that they are similar. This finding established that the ELISA method, which is a test for sporozoite antigens, was not overestimating the actual sporozoite rate.

However, to avoid confusion, only sporozoites determined by ELISA were considered in the analysis for seasonal and monthly variations. Sporozoite rates varied from 1.8% in August in the wet season to 10.3% in February in the dry season. Generally, the monthly sporozoite rates were higher in the dry season (December to April) than in the wet season (May to November). The average sporozoite rates for the dry and wet seasons were 8.1 and 3.4% respectively. The annual average was 3.9%.

Out of the 7845 An. gambiae s.s. tested, 7474 were obtained from human-bait catches and 371 from light-trap catches. Most of these (>7000 mosquitoes) were tested during my last visit to The Gambia in September 1991, which lasted only 10 days, so I could not process samples from outdoor resting or exit-trap collections. The light-trap collection tested, only included samples from November 1990 to March 1991 but Table 4.17 gives the mean monthly sporozoite rates for An . gambiae s.s. in the light-trap collections for the dry season (December to March). In November (end of wet season), three (15%) out of the 20 females tested were positive. The dry

season sporozoite rates for the light-trap and human-bait collections were statistically similar ($p>0.05$). Positive females were caught in all six houses at Bayama.

Only 20 An. funestus (all caught in the dry season) were tested, two (10.0%) were found positive.

4.3.8 Visual assessment of sporozoite ELISA results

Out of a total of 7522 An. gambiae s.s. samples tested, 291 and 319 were considered positive by eye and the ELISA plate reader respectively (Table 4.18). The percentage of positive females determined by eye (3.9%) was not significantly different from the percentage (4.2 %) determined by the ELISA reader. After a confirmatory test on all positives, 263 (90.4%) of the visual positives were still considered positive by the ELISA reader (9.6% were false positive), while only 261 (81.8%) of previous reader determined positives were confirmed . The proportion of false positives (18.2%) from the first ELISA reader results were twice as much as from the visual results.

There was no significant difference between the sporozoite rates determined visually (3.9 %) and those determined from the confirmed ELISA reader results (3.5%). However, the sporozoite rate first determined from the unconfirmed ELISA reader results (4.2%) was significantly higher than the rate (3.5%) determined from the confirmed results ($\chi^2=5.826$, $p=0.016$, $df=2$), emphasising the importance of doing a confirmatory test.

TABLE 4.17
Percentage of ELISA positive An. gambiae s.s. in light-trap collections during the dry season.

Month	Number tested	Number positive	% ±SE
December	46	3	6.5±3.63
January	79	3	6.3±2.73
February	102	16	15.7±3.60
March	124	13	10.5±2.75
Dry season*	351	37	10.5±1.64

* In November (wet season), three out of 20 tested were positive.

TABLE 4.18
Number of positives (determined visually and using plate reader) confirmed by a second test using the ELISA reader alone. 7752 An. gambiae s.s. samples were retested. Numbers in parentheses are sporozoite rates.

Confirmatory tests	Visual		ELISA reader	
	Number of positives tested	Number confirmed	Number of positives tested	Number confirmed
1	76	67	75	66
2	74	69	80	68
3	67	62	78	62
3	74	65	86	65
Total	291(3.9%)	263(3.5%)	319(4.2%)	261(3.5%)

4.3.9 Inoculation rates in the project villages

Inoculation rates of An. gambiae and An. funestus calculated using man-biting rates estimated from spray collections are given in Table 4.19. The number of infective bites/man/night varied from 0.038 at Mendewa to 0.097 at Nyandeyama. Inoculation rates for An. funestus varied from 0.002 infective bites/man/night at Nengbema to 0.022 infective bites/man/night at Mendewa. For all villages combined, the inoculation rates for An. gambiae and An. funestus were 0.088 and 0.007 infective bites/man/night respectively. Seasonal variations in combined inoculation rates (all villages) for An. gambiae s.s. are shown in Figure 4.3. Inoculation rates varied from 0.00 to 0.28 infective bites/man/night in January and July respectively, the rates being generally higher during the wet than in the dry season.

Inoculation rates calculated using man-biting rates from human-bait catches are given in Table 4.20 for the wet season because it was only during this period that human-bait catches yielded An. gambiae. At Nyandeyama, where only An. gambiae was caught in the wet season, the mean number of infective bites/man/night for this species was 0.213. At Mendewa, the inoculation rates were 0.088 and 0.012 infective bites/man/night for An. gambiae s.s. and An. funestus respectively.

4.3.10 Inoculation rates of Anopheles gambiae s.s at Bayama

The inoculation rates of An. gambiae s.s. were calculated using the man-biting rates estimated from human-bait collections. Figure 4.4 shows the seasonal variations in

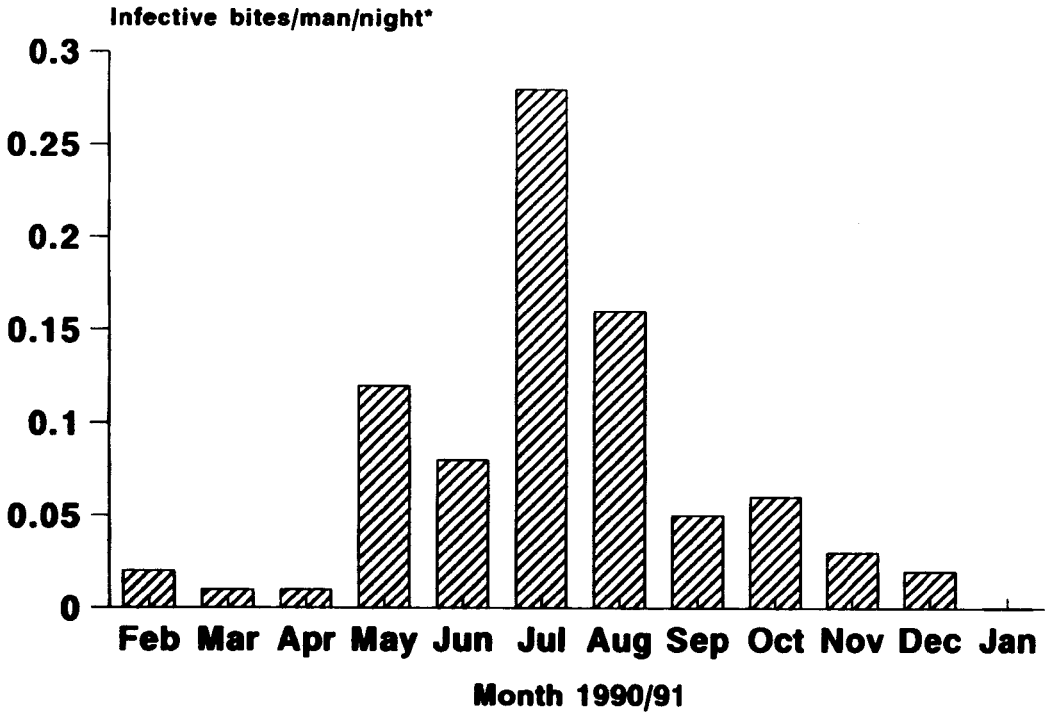
TABLE 4.19
Inoculation rates of An. gambiae s.s. and An. funestus in the project villages. Calculations were done using a human blood index of 1.0 and man-biting rate (**ma**) estimated from spray catches.

Village	An. gambiae	An. funestus	Total
Nengbema	0.057	0.002	0.059
Nyandeyama	0.097	0.003	0.100
Mendewa	0.038	0.022	0.060
Njala-Komboya	0.070	0.003	0.073
Combined villages	0.088	0.007	0.095

TABLE 4.20
Wet season inoculation rates of An. gambiae s.s. and An. funestus in the project villages. Calculations involved man-biting rates from human-bait collections.

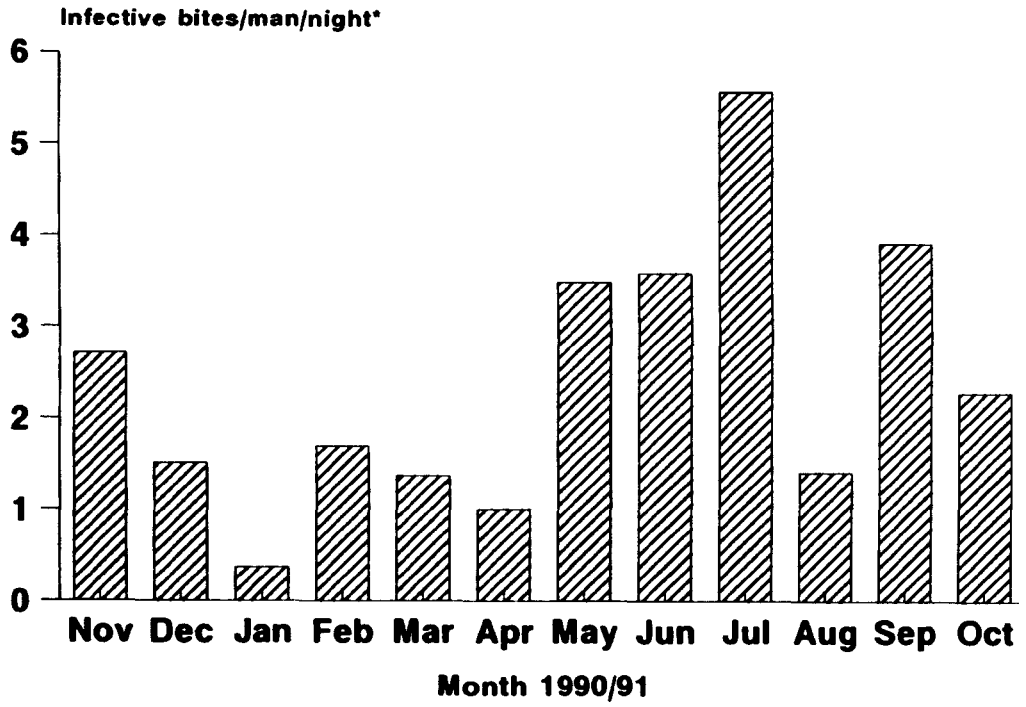
	An. gambiae	An. funestus	Total
Nyandeyama	0.213	0.000	0.213
Mendewa	0.088	0.012	0.010

FIGURE 4.3 Mean monthly inoculation rates of An. gambiae s.s in the project villages



*Man-biting rates were calculated from spray catches

FIGURE 4.4 Mean monthly inoculation rates of An. gambiae s.s at Bayama



Man-biting rates calculated from bait catches

the mean monthly inoculation rates at Bayama. A high estimated number of infective/bites/man (5.58) were received in July, and as in the project villages, the lowest rate was recorded in January (0.37). Mean monthly inoculation rates were higher in the wet season (May - November) than in the dry season (December to April), except for the month of August when the inoculation rate was slightly lower than rates for December and January in the dry season.

4.4 DISCUSSION

Mean parous rates substantially higher than 50.0%, as observed at Bayama, usually indicates that little breeding is taking place in the vicinity of the sampling point, or that the sampling procedure is selectively catching parous females (Birley, 1990). The constant rate of recruitment of nulliparous females at Bayama indicated that outdoor human-bait collections were not selectively sampling any particular age-group, and therefore the mean parous rate was calculated without any bias. Because of this, the mean parous rate (61%) was also a good estimate of the survival rate per gonotrophic cycle (Dye, 1992), which was calculated by the method of Birley and co-workers (Birley & Rajagopalan, 1981; Holmes & Birley, 1987; Mutero & Birley, 1989) to be 59%.

Bayama was located by a large swamp, where rice was transplanted in June, and this should have created an ideal environment for An. gambiae s.l. to breed (Service, 1989; Snow, 1983). However, the recruitment of nulliparous females throughout the whole year did not follow any trend, whereas an upsurge of An. gambiae s.l. would have been expected during rice cultivation if An. gambiae s.l. was breeding in the rice swamp. In fact larvae of An. gambiae s.s. were not found in the swamp during several larval surveys carried out throughout the study period. The few larval breeding sites located along the edges of small streams near the village could not account for the large man-biting-density of An. gambiae s.s. observed in the village. It seems that the majority of the Bayama biting population originated from elsewhere, and this could help explain why the mean parous rates during the wet and dry seasons

were similar, despite there being marked seasonal variations in vector densities.

The mean parous rate of An. gambiae s.s. in the project villages (53.0%) was less than the mean parous rate (61.4%) observed at Bayama possibly because of sampling bias due to the small number of females dissected (148) over the five month period. The mean parous rate of An. gambiae s.s. at Bayama was higher than mean parous rates, 53.0% and 31.0%, observed for An. gambiae s.s. in the savanna zones of The Gambia (Lindsay et al., 1991) and in Burkina Faso (Robert & Carnevale, 1991) respectively. Birley & Mutero (1987) found a mean parous rate of 55.0% for An. gambiae s.s. in Kenya. A small proportion of pre-gravid females were parous (6.0%), thereby exhibiting the phenomenon of gonotrophic discordance (Gillies, 1954). This phenomenon has also been observed in a small proportion (<5%) of An. arabiensis pre-gravids in Kenya (Ijumba et al, 1990).

It is not clear why the parous rate of An. gambiae s.s. in exit-traps was lower than the parous rate of indoor human-bait catches. In Nigeria, Self & Pant (1968) also found that the parous rates of An. gambiae s.l. in exit-traps were lower than in indoor-resting collections. It therefore appears that nulliparous females have a greater tendency towards exophily.

The observation by Mutero & Birley (1987) that the criterion for having a statistically significant peak, in estimating gonotrophic cycle duration using time-series analysis, may be too severe, is supported by the results of this study. Although the largest cross-correlation coefficient (0.234) used for estimating the gonotrophic cycle length

(3 days) was not statistically significant, it was a well pronounced peak, and moreover there followed other smaller peaks indicating an upsurge of parous females at three-day intervals. This criterion was introduced into the model by Holmes & Birley, (1987) because Chatfield (1975) had cautioned that false peaks can occur in cross-correlation analysis. It is not very likely that all the observed peaks at three-day intervals were false peaks. Many studies on An. gambiae s.s. have shown that the length of the gonotrophic cycle is 2-3 days (Gillies & Coetzee, 1987). Peaks at intervals of 2 or 4 days were, however, not observed in this study. In Kenya, a gonotrophic cycle of 3 days, for An. gambiae s.s. in the dry season, was associated with longer flights in search of oviposition sites (Mutero & Birley, 1987), as could well be the case at Bayama. In the present study, the daily survival rates calculated (0.85) using the gonotrophic cycle length of 3 days was similar to what has been calculated for An. gambiae s.s. in Burkina-Faso (0.84) (Brengues & Coz, 1973) and Kenya (0.77 - 0.84) (White, 1972).

Although Reisen & Aslamkhan (1979) working with An. stephesi in Pakistan and Renshaw (1992) working with Aedes cantans Meigen in Britain, found that there was no significant difference between the mortalities of mosquitoes that were dusted with powders and control groups of undusted females, Birley & Chalwood (1989) found that marked unfed An. farauti Laveran in Papua New Guinea had a higher pre-release mortality than blood-fed females. Despite the high mortality rate of marked unfeds compared to unmarked ones in the present study, the recapture rate rates of 13.3 and 8.5% were relatively high. This suggests that An. gambiae s.s. did not disperse far from the village, and fed mostly on people.

The vectorial capacity calculated for An. gambiae s.s. at Bayama (35.4) was much higher than values for An. gambiae s.l. calculated in Nigeria (7.3) by Molineux & Gramiccia, (1980) and Kenya (2.1) by Molineux et al. (1978). At Bayama, survival rates , man-biting densities and human blood indices were relatively high throughout the year. The man-biting rate (**ma**) component of the vectorial capacity was mostly responsible for the high estimated values, and because of this the vectorial capacity for the wet season was five times the value for the dry season. All other components of the vectorial capacity were in fact higher in the dry season. The life expectancy of An. gambiae s.s at Bayama (6.1 days) was similar to the 6.0 calculated for An. gambiae s.l. in Nigeria (Molineux & Gramiccia, 1980) and Kenya (Molineux et el., 1978). The estimated vectorial capacity for the combined project villages during the wet season (0.36) was 10 times the wet season value calculated in an area of irrigated rice cultivation in The Gambia (Lindsay et al., 1991). Anopheles funestus, even though it had a high infection rate (11.4%) and human blood index (0 .99), was not an important vector of malaria in the study area because it was always present in low numbers.

Sporozoite positive An. gambiae s.s. were caught throughout the year, in proportions that were among the highest recorded for countries in subsaharan Africa (Gillies & Coetzee, 1987 and Gillies & de Meillon, 1968), but whether the proportion of sporozoite positive females determined by ELISA is actually a measure of the proportion infective is controversial. Although sporozoite antigens from the oocysts can be avoided by cutting off the abdomen and testing only the head-thorax portion (Nardin, 1982), some of the sporozoites in the thorax may not penetrate the salivary

glands and thus the mosquito may be infected but not infective (Arruda & Cochrane, 1986; Esposito et al., 1986). In Kenya, it was found that 45.4 % of Anopheles vectors containing sporozoite antigens did not contain sporozoites in their salivary glands (Beier et al., 1990). However, in the present study, over a period of four months there was no signicant difference between the mean monthly sporozoite rates determined by ELISA and by dissection. Similarly, in Indonesia, Hoedojo et al. (1987) did not find any significant difference between P. falciparum sporozoite rates determined by the two methods in paired samples of experimentally infected

An. aconitus Doenitz. The proportion of ELISA positives in the present study are considered to represent true sporozoite rates, i.e. the proportion of infective females.

A major problem encountered with the ELISA method is the reliance on a plate reader for the identification of positive results (Beier et al., 1988). The author had to travel twice, from Sierra Leone to The Gambia, to test mosquitoes by ELISA because the small laboratory in Bo did not possess an ELISA reader. Experiments carried out during the second trip showed clearly that there was no significant difference between sporozoite rates determined by visually reading the ELISA plates, and those determined by the ELISA. In fact, the visual reading of the ELISA plates was more reliable than using the ELISA reader because the reader recorded a high proportion of false positives on the periphery of polyvinyl ELISA plates. Collins et al.(1988) also observed that polyvinyl plates produced higher and more variable background readings, especially in the peripheral wells. On one particular 96-well plate, there were nine such 'positives' proven to be false by a confirmatory test. In

fact most parasitologists and immunologists, I have discussed ELISA methods with, avoid using periphery wells as a precaution against false positives. This precaution does not appear to have been transmitted to field entomologists to whom immunoassays are relatively new tools.

The reliability of visually reading sporozoite ELISA plates, which has also been reported from studies in Kenya (Beier & Koros, 1991), is an important observation for entomological field studies in developing countries, because ELISA readers are very expensive and difficult to maintain.

The relatively high sporozoite rates recorded for An. gambiae s.s. (7.4%) in the Bo area of Sierra Leone are similar to those reported in other parts of the country. Wood (1915), working in the Northern Province, found that the sporozoite rate for An. gambiae s.l was 8.8%, while in the Freetown area, Gordon et al. (1932) obtained sporozoite rates of 8.2% for An. gambiae s.l. The sporozoite rate for An. funestus (11.4%) recorded in the study area was similar to the 11.0% obtained in the Northern Province (Wood, 1915) but higher than that observed in the Freetown (4.2%) area by Gordon et al. (1932). None of the 38 An. hancocki tested was positive but Lewis (1956) working in the Northern Province, found one positive female (0.09%) out of 115 dissected.

Sporozoite rates of An. gambiae s.s. and An. funestus were similar in all project villages but they were about twice as high during the wet season, when vector

densities were also larger than during the dry season. Since the human blood index was almost the same in all villages, the man-biting rate was again the most important factor determining transmission intensity. Although sporozoite positive An. gambiae s.s. were not detected in January, most likely because of the of the small number of females tested, it can be safely concluded that the transmission of P. falciparum by An. gambiae s.s. in the project villages was perennial. On the other hand, it appears that An. funestus was capable of transmitting malaria during only the dry season. The role of An. funestus as mainly a dry season vector, was also observed in Freetown by Blacklock & Wilson (1941). Similarly, in Kenya Githeko (1992) found that An. funestus was transmitting malaria mainly during the dry season.

At Bayama, there was also seasonal variation in the sporozoite rates of An. gambiae s.s., but in contrast to what was observed in the project villages, the sporozoite rates were lower in the wet season. There are two possible explanations for the seasonal variation in the sporozoite rates in the different areas. Firstly, in the project villages, An. gambiae s.s. was mainly biting indoors, because there was little or no outdoor human activity after 2100 hr, and because of this the females had ready access to the children (1 - 10 years). Now in Kenya, young children comprise over 70% of the infective P. falciparum reservoir (Githeko, et al., in press) and if this is true, as it likely is, in Sierra Leone, then it would result in higher mosquito infection rates. During the dry season the few biting females were mainly feeding on adults, because of their preference for them over children (Boreman et al., 1978; Bryan & Smalley, 1978; Port et al., 1980), but during the wet season, more children were bitten because of the increase in vector density, so more mosquitoes became infected.

In contrast at Bayama, An. gambiae s.s. was always present in relatively large numbers so everybody in the village, having a total population of less than 50 people, was exposed. But, during the wet season, biting took place outdoors more than indoors and the adult population which remained outdoors till past midnight, became the main source of blood-meals and since they probably constituted a less infective reservoir, the sporozoite rate decreased.

The second possible reason for the seasonal variation in sporozoite rates could be because there was a higher proportion of nulliparous females in the dry season population in the project villages, and this would tend to lower infection rates. However, at Bayama the parous rates in the dry and wet seasons were similar, so parity could not explain the difference in sporozoite rates.

The man-biting rate (**ma**) component of the inoculation rate is difficult to measure accurately and sometimes values obtained need to be adjusted to attain more realistic results (Molineaux & Grammicia, 1980). In an endophilic population, the value of **ma** can be estimated from the number of human-fed indoor resting females and the number of sleepers in the room, so long as all have fed on people. However, most investigators tend to estimate man-biting rates from human-bait catches (Boudin et al., 1991 ,Ijumba et al., 1990; Lindsay et al., 1991; Robert & Carnevale, 1991).

Fortnightly human-bait catches did not yield An. gambiae s.s. mosquitoes during the dry season in Nyandeyama, so during this period inoculation rates could only be calculated using man-biting rates estimated from indoor resting catches. In the wet

season, An. gambiae s.s. inoculation rates, calculated using man-biting rates estimated separately from human-bait and indoor pyrethrum spray collections were similar, namely 0.18 and 0.21 infective bites/man/night, respectively.

At Bayama, the wet season inoculation rates calculated for the same room using man-biting rates estimated from both pyrethrum spray collections and human-bait catches, were 0.07 and 2.31 infective bites/man/night respectively. This difference could be partly explained by the exophilic behaviour of the An. gambiae s.s. population at Bayama, resulting in underestimating man-vector contact from spray collections. However, in a Kenyan village with highly endophilic An. funestus , the calculated inoculation rates from indoor spray collections and human-bait catches were 3.9 and 10.5 infective bites/man/night (Githeko, 1992). It therefore appears that in areas where man-biting rates are high, inoculation rates calculated from human-bait collection tend to be much higher than those calculated from spray collections. Garrett-Jones (annex in Odetoyinbo, 1969) reported that in the Kankiya area of northern Nigeria, eight times more female An. gambiae s.l. were collected on human-bait than the number of blood-fed females per sleeper, obtained from spray collections in the same room. Pull & Grab (1974) working in the Kisumu area of Kenya concluded that human-bait catches overestimated the man-biting rate, and Garrett-Jones & Shidrawi (1969) believed that man-biting rates derived from indoor-resting collections provided a better measure of man-vector contact than human-bait collections.

I am of the opinion that measurements of malaria transmission intensity such as

vectorial capacity and inoculation rates have relatively little absolute value, because of the difficulties encountered in accurately measuring some of the different components. Such measurements are probably more useful when they are used to compare transmission rates in different areas and seasonal trends in the same area, than attempting to try and measure actual transmission intensities.

CHAPTER 5

THE ANOPHELES GAMBIAE SPECIES COMPLEX IN SOUTHERN SIERRA LEONE

5.1 INTRODUCTION

5.1.1 Current status of the Anopheles gambiae complex

The morphological approach to vector species identification revealed two main entomological components in the malaria vectorial system in subSaharan Africa, namely An. funestus and An. gambiae s.l. The An. funestus component has been found to be relatively simple and uniform in most areas of its distribution although several closely related, but morphologically distinct, species are often grouped together as the An. funestus group. However, the An. gambiae component is a complex of at least six morphologically, for the most part, indistinguishable sibling species. More recent studies have revealed further complexities involving chromosomal polymorphism and incipient speciation processes.

The An. gambiae complex consists of two saltwater forms, three freshwater forms and a form that breeds in mineral water pools of hot springs. The distinctive nature of salt-water forms of An. gambiae s.l. was established by studies in Sierra Leone (Muirhead-Thomson, 1945; Ribbands, 1944a,b) in the mid 1940s and later in East

Africa (Muirhead-Thomson, 1951). The evidence for the specific distinctiveness of the saltwater forms was based on behavioral, taxonomic and cross-mating experiments. The genetic basis of the species status of the East African saltwater form, An. merus was demonstrated by Paterson (1962). The West African saltwater form was given the name An. melas.

Until 1963 it was believed that the freshwater An. gambiae was a single species adapted to different ecological conditions. It was Paterson (1963) who provided the direct evidence that what hitherto were known as two 'races'(Davidson and Jackson, 1962; Holstein, 1952) were in fact two sibling species (Species A and B) of the An. gambiae complex. A third sibling species (Species C) was identified from southern Africa by Paterson et al. (1963). The mineral-water breeding species originally documented by Haddow et al. (1947) was recognised as sibling Species D (Davidson & Hunt, 1973; Davidson and White, 1972; Hunt, 1972). To date, only these six members of the An. gambiae species complex have been identified: An.gambiae s.s. (Species A), An. arabiensis (Species B), An. quadriannulatus, (Species C), An. bwambae (Species D), An. melas and An. merus.

Anopheles gambiae s.s. is considered the most efficient malaria vector in Africa because it is highly anthropophagic, endophagic and endophilic (Gillies & Coetzee, 1987; Gillies & De meillon, 1968). It is particularly common in the humid forest areas, but tends to be replaced in the drier areas by An. arabiensis which is more zoophagic, exophagic and exophilic than An. gambiae s.s. Malarial infection rates in An. gambiae s.s. tend to be higher than in An. arabiensis (White, 1974; Molineaux

and Gramiccia, 1980). Anopheles arabiensis is found predominantly in dry zones and the savanna areas. These two species have the widest distribution of all the species of the complex and occur sympatrically over extensive areas. Both An. gambiae and An. arabiensis breed in fresh water habitats, they both colonize transient pools or more permanent sources such as borrow pits and rice fields and the edges of seasonal swamps. However, in many rice-growing areas such as in Kenya, An. arabiensis is the predominant or only species found in the rice fields (Joshi et al., 1975; Service, 1970; Service, 1978b). The two species may show differences in seasonal abundance, and usually exhibit marked differences in their vectorial status (Gillies & Coetzee, 1987).

Anopheles quadriannulatus is a highly zoophagic and exophilic species which has little or no direct role in the transmission of malaria. Its breeding requirements are essentially similar to An. gambiae s.s and An. arabiensis (Gillies & Coetzee, 1987).

Both An. melas and An. merus are salt water breeding species. The former occurs in coastal areas along the West African coast, while An. merus is restricted to East and southern Africa, but in addition to breeding in coastal areas it is often found breeding in inland salt water pools (Gillies & Coetzee, 1987). Anopheles merus is usually exophilic and endophagic and is considered to be a poor malaria vector. In The Gambia An. melas was considered to be of negligible importance in malaria transmission, except when present in large numbers (Bryan,1983; Bryan et al, 1982), but in the Freetown area of Sierra Leone and in Lagos area of Nigeria (Muirhead-Thomson, 1945, 1948), it is of considerable importance as a vector. Anopheles

bwambae is restricted to the forest areas of the Rift Valley, west of Ruwenzori in Uganda. In this area, it can be a local malaria vector among the Bambute pygmies. It breeds in mineral water pools of hot springs.

Identification of the different members of the Anopheles gambiae complex has been achieved by isoenzyme electrophoresis (Miles, 1978, 1979), cuticular hydrocarbon analysis (Phillips et al., 1988, Carlson & Service, 1980) and by DNA probes (Collins et al, 1988; Gale and Crampton, 1987). However , chromosomal identification as pioneered by M. Coluzzi remains the foundation stone of species identification. Identification can be made on the polytene chromosomes of the salivary glands of the 4th-instar larvae and on those in the ovarian nurse cells of half-gravid females. The arrangements of the sequences of chromosome bands is based on paracentric inversions. Some of these inversions occur in the homozygous state (fixed inversions); others, on the contrary, are floating and give a population a polymorphic character for the given inversions. The floating inversions are particularly frequent in An. gambiae s.s. and An. arabiensis. No inversion polymorphism has been found in An. melas, An. quadriannulatus or An. bwambae (Coluzzi et al., 1985).

5.1.2 Chromosomal polymorphism of An. arabiensis and An. gambiae s.s.

Analysis of karyotype frequencies from different localities in West Africa shows that the carriers of the different karyotypic arrangements observed in An. arabiensis constitute a single panmictic unit in Hardy-Weinberg equilibrium. Anopheles

arabiensis is therefore a single genetic population in West Africa. However, behavioral differences have been observed between different karyotypes (Coluzzi et al.,1977, 1979, 1985)

Studies on chromosomal polymorphism of An. gambiae s.s. in Mali have shown that the species can be divided into three genetic populations. In each of these populations: Bamako , Savanna and Mopti , a panmictic unity in Hardy-Weinberg equilibrium is obtained (Touré et al., 1983). Similar observations on the different karyotypic arrangements in An. gambiae s.s. have been made by Coluzzi et al. (1979) in Nigeria; Bryan et al. (1982) in Senegambia and Akogbeto et al. (1987) in Benin.

Chromosome-2 inversion polymorphism in An. gambiae s.s. is highly flexible. It is characterized by marked clinal changes in frequency of karyotypic arrangements which can be correlated with climatic conditions and vegetation zones (Coluzzi et al., 1985). A description of the chromosome structure in An gambiae complex is given by White et al. (1975).

The data available on the distribution of chromosomal arrangements show variations from a situation of normal intergradation to one of complete reproductive isolation. Extending the nomenclature introduced by Touré et al. (1983) for the taxa identified in Mali, various chromosomally characterized, and presumably panmictic, forms have been recognized (Coluzzi et al. 1985; Touré, 1989), namely:-

Forest form

This is monomorphic with chromosome 2 in the standard form. Few inversions (mostly 2**La**, 2**Rb**, 2**Rd**) are observed and only at very low frequency. This form is found in humid forest zones and in the Guinea savannas where it intergrades with various Savanna chromosomal forms.

Savanna form

This name has been used in the general sense to refer to all the chromosomal forms other than those of the forest. In its strict sense, it is characterized by 2**Rb**-2**La** arrangement which is widespread in subsaharan Africa, it intergrades in many areas with the Forest form.

Bissau form

This form is characterized by the arrangement 2**Rd**, its range extends in the coastal rice cultivated areas of The Gambia, south Senegal (Casamance), Guinea Bissau, and Guinea. Studies by Bryan et al. (1982) in The Gambia and neighbouring Senegambia have shown that it partially intergrades with the Savanna form.

Mopti form

This is a chromosomal form typical of the interior delta of the Niger river. It is characterized by the 2**Rbc\u** polymorphism. It is found in the north and south of Mali (Touré, 1989), and in Benin (Akogbeto et al., 1987) but always in zones close to waterways which offer possible breeding sites during the dry season. The Mopti form is found sympatric with Savanna and\or Bamako forms in various localities but

shows partial intergradation only with Savanna or Forest-Savanna intergradation forms.

Bamako form

This is characterized by the **j** inversion which is associated with **cu** and **bcu** in a polymorphism **2Rjcu**. It is found along the length of the Niger river and it's tributaries in southern Mali and northern Guinea. It is sympatric with Savanna and Mopti forms. In the continental zones of Guinea Bissau the inversion is found associated with **d** or **bd** in a **2rjd\jbd** polymorphism (Patrarca et al., 1983), suggesting the existence of a western variant of the Bamako form.

The different chromosomal forms also seem to show differences in their cuticular hydrocarbon components (Phillips et al., 1987). Studies on the savanna forms found in Mali (Toure pers comm., 1990) have shown that there are no major difference in their colonization of transient pools, swamp margins and river edges. In the wet season (July-August) all forms can be found together in the same breeding places. They all contribute to the transmission of malaria.

5.1.3 Anopheles gambiae complex in Sierra Leone

No studies have previously been carried out on the polytene chromosomes of the An. gambiae complex in Sierra Leone. Apart from the early morphological and physiological studies on the salt-water forms of An. gambiae s.l. in Freetown and its environs (Muirhead-Thomson, 1945; Ribbands, 1944a,b), nothing is known about the

distribution of the An. gambiae complex in the country. According to Morgan (1990) who identified specimens from Sierra Leone using the polymerase chain reaction (PCR) technique, An. gambiae s.s. and An. arabiensis are found in the country, but this requires confirmation. The present study involves the first chromosomal identification of the An. gambiae s.l. in Sierra Leone.

5.2 MATERIALS AND METHODS

5.2.1 Chromosome preparations

Freshly blood-fed females caught in the field in the morning were held until 2000 hr when they reached the half-gravid abdominal appearance, which is optimal for chromosomal preparations. Whole females were fixed in modified Carnoys fixative (3:1 ethanol/acetic acid) and stored at 4°C. Half-gravid females caught in spray collections were immediately put into Carnoy's fixative.

Chromosomes were prepared according to the method of Hunt (1973). After at least 24 hr fixing time, the ovaries were dissected out in a small quantity of Carnoy's fixative and placed in a drop of 50% aqueous solution of proprionic acid for 1-2 minutes until the ovaries were swollen to approximately twice their original size. The swollen ovaries were then macerated with a dissecting needle and a small drop of lacto aceto orcein stain was added. The macerated ovaries were agitated in the stain with a dissecting needle to ensure even staining. After about 30 seconds, the stain was then drawn off using filter paper and the tissue washed with several changes of 50% proprionic acid until the macerated tissues had attained a pale pink colour. As much as possible of the proprionic acid was drawn off from the tissues using pieces of filter paper and then a siliconised cover slip placed over them. The preparation was then squashed by tapping with a patella hammer and examined using a phase contrast microscope.

Species were identified in the Bo laboratory by microscopic examination of specific band sequences on chromosome-X. Different paracentric inversion karyotypes were identified in the Malaria Laboratory of the School of Medicine and Pharmacy in Bamako, Mali, with the help of Prof. Y. T. Touré. Chromosomal inversions were scored according to the nomenclature of Coluzzi et al. (1979). Standard arrangements are indicated by + sign following the letter referring to the chromosome section; inverted arrangements are indicated by the letter alone, for example, 2La+/a+ indicates the standard arrangement for inversion **a** on chromosome 2L, while 2La+/a indicates the heterokaryotype for the same inversion.

5.2.2 Statistical analysis

Once inversion and karyotype frequencies had been determined, it was necessary to statistically check for any associations between inversions. When there are no disturbing forces such as selection, mutation or migration that would change gene frequencies over time, and when there is random mating in very large populations, pairs of genes at a locus are known not to be associated. A consequence of this independence is that genotype frequencies are the product of the gene frequencies i.e. they are in Hardy-Weinberg equilibrium, with the genes in Hardy-Weinberg proportions. If the frequencies of the standard (+) and inverted (d) arrangements of the inversion **d** are **p** and **q** respectively, the frequencies of the karyotypes +/+, +/**d** and **d**/**d** in the next generation will be $p^2 : 2pq : q^2$. According to the Hardy-Weinberg equilibrium, in the subsequent generations, this ratio will not change.

Karyotype frequencies were tested for deviation from the Hardy-Weinberg equilibrium using Wright's statistics (Brown, 1970) where $F = (4ac - b2)/[(2a + b)(2c + b)$, a and c being the frequencies of the homozygous classes and b the frequency of the heterozygote. When the absolute value of $|F| > 1.96/\sqrt{N}$ (N = number of specimens) there is a significant departure from the expected values. A positive value of F indicates a deficiency of heterokaryotypes, while a negative value indicates an excess. Wright's F is reliable for samples above 20. However, it is sometimes useful to calculate F even if N is lower than 20 as it may be used to obtain the absolute frequencies of karyotypes by applying the following formulae:

$$a = N[pF + p^2 (1 - F)]$$

$$b = N[2pq (1 - F)]$$

$$c = N[qF + q^2 (1 - F)]$$

where p and q are frequencies of the standard and inverted arrangements, respectively.

5.3 RESULTS

5.3.1 Species identification

Chromosomal examination of the specimens from all the study villages revealed the existence of An. gambiae s.s. only. Specimens were examined from both wet and dry season collections from all villages. Table 5.1 gives the number of samples read, according to collection method from each village. A total of 506 chromosome preparations were made, out of which 366 (72.3%) were readable for species identification.

5.3.2 Chromosomal polymorphism in An. gambiae s.s.

A total of 66 An. gambiae s.s. chromosome preparations from Bayama collections were successfully scored for inversion polymorphism. In the Bayama vector population, An. gambiae s.s. was polymorphic for four inversions on chromosome 2R: **2Rcu, 2Rd, 2Ru, 2La** (Table 5.2). Inversions **2Rcu** and **2Ru** were found in very low frequencies (<6%). Inversion **2Rd** was the commonest (52%), followed by **2La** which was observed only in the heterozygote form in frequencies of less than 10%. The **2Rcu** inversion arrangement was not observed in the exit-trap and outdoor collections probably because of the small sample size (28). The frequencies of inversion **2Rd** in the exit-trap (38.1%) and indoor-resting samples (55.3%) were not statistically different. Using the Fisher's exact test, it was also shown that there was no significant difference ($p > 0.1$) in the frequency of inversion **2Rd** in the indoor

TABLE 5.1
Numbers of chromosomal preparations identified as An. gambiae s.s., the only member of the An. gambiae complex identified from different collections in all villages studied.

Village	Collection method				Total
	Exit-trap	PSC	Light-trap	Outdoor	
Nengbema	-	56	-	-	56
Nyandeyama	31	26	10	14	81
Mendewa	20	16	6	3	45
Njala-Komboya	-	28	-	-	28
Bayama	73	21	21	41	156
Total	124	147	37	58	366

TABLE 5.2
Frequencies (%) of karyotype arrangements in An. gambiae s.s. collected using different methods at Bayama.

		2Rcu system			2Rd system			2Ru system			2La system		
Method	N	+/+	cu/+	cu/cu	+/+	d/+	d/d	+/+	u/+	u/u	+/+	a/+	a/a
Exit-trap	21				61.9	33.3	4.8	95.2	4.8	0.0	90.5	9.5	0.0
Outdoor resting	7				28.6	57.1	14.3						
Indoor resting	38	97.4	2.6	0.0	44.7	44.7	10.5	94.7	2.6	2.6	92.1	7.9	0.0

resting collections when compared to either exit-trap or outdoor resting collections. Only seven specimens were readable from the outdoor samples. Inversion **2La** was also found in frequencies less than 10% in both exit-trap and indoor resting samples. All seven outdoor specimens had inversion **2La** in the standard form.

The observed karyotype frequencies for all inversions were in agreement with the Hardy-Weinberg equilibrium, except in the case of the karyoptype **2Ru** in the indoor collections which had a significant deficiency of heterokaryotypes (Table 5.3). There was an excess of heterokaryotypes in the **2Rd** system in exit-trap collections and a deficiency in the indoor samples. The **2La** system had an excess of heterokaryotypes in both exit trap and indoor samples. The karyotypes in all polymorphic inversions were found to be in agreement with the Hardy-Weinberg expectations. Table 5.4 gives the frequencies of the **2Rd** and **2La** inversions on the different chromosomes. The observed and expected frequencies of the different inversions were the same in all the samples, in agreement with Hardy-Weinberg proportions.

Only five specimens from the project villages were examined for chromosomal polymorphism and all of them showed chromosome **2R** and **2L** in the standard form.

TABLE 5.3
Frequencies (%) of chromosomal inversions observed in preparations from An. gambiae s.s. caught at Bayama village.

			2Rcu		2Rd		2Ru		2La	
Method	N	$1.96/\sqrt{N}$	%	F	%	F	%	F	%	F
Exit	21	0.43			38.1	0.028	4.8	-0.03	9.5	-0.053
Outdoor resting	7	0.75			71.4	0.177				
indoor resting	38	0.32	2.6	-0.018	55.3	-0.005	5.6	0.657	7.9	-0.118

TABLE 5.4
Frequencies (%) of the 2Rd and 2La inversions on the different chromosomes of An. gambiae s.s. from Bayama village

		2Rd system		2La system	
Method	N	+	d	+	a
Exit-trap	42	78.6	21.4	95.2	4.8
Outdoor resting	14	57.1	42.9	100.0	0.0
Indoor resting	76	67.1	32.9	96.1	3.9

5.4. DISCUSSION

Anopheles gambiae s.s. was chromosomally identified for the first time in Sierra Leone. It was the only species identified in the five villages where entomological investigations were carried out. Chromosomal preparations were made from mosquitoes caught in both the dry and the wet seasons, so the failure to identify other species within the An. gambiae complex, especially An. arabiensis, could not be attributed to the well known seasonal variation in the species composition of the complex (Gillies & Coetzee, 1987; Rishikesh et al., 1985). Moreover, specimens were identified from outdoor resting sites such as rice barns which are somewhat similar to granaries in Kenya, and also from pit-shelters, both of which are favoured places for outdoor resting by An. arabiensis in Kenya (Service, 1970; Joshi et al., 1975 and Service et al., 1978).

Although Morgan (1990) identified An. arabiensis from Sierra Leone using the polymerase chain reaction technique, this species has not been reported from neighbouring Liberia or the southern part of Guinea which borders Sierra Leone in the north (Kuhlow & Zielke, 1978; Touré pers comm., 1990), and so the presence of this species in Sierra Leone needs confirmation using chromosomal techniques. Anopheles arabiensis prevails in most Sudan savanna and Sahel savanna localities, but it is also found frequently in forest areas despite its apparent absence from the intervening areas of southern Guinea savanna. Similarly An. gambiae s.s. is widespread in both forest and the Guinea savanna (Colluzi et al., 1979), the main vegetation types in Sierra Leone and neighbouring countries.

Inversion polymorphism is becoming a subject of great interest to entomologists. An inversion is a form of chromosomal mutation, such as an intrachromosomal reversal of a block of genes. For example an hypothetical gene arrangement **abcde** may become **abdce** after rearrangement, with **dc** being the inverted block of the standard **cd** arrangement. The inverted genotype will persist only if it is intrinsically viable and if so, it may or may not be cross fertile with the standard genotype. A successful new inversion is therefore an important evolutionary step of the sort that frequently accompanies dipteran speciation. Specific banding patterns on the polytene chromosomes provide a good guide to karyotype rearrangements between species, and it has been found that changes in banding sequence nearly always accompany speciation (White, 1974). In practice, inversion polymorphism gives selected advantages of restricted genetic variability, while the lack of inversions expose the population to the consequences of unrestricted genetic variability. It has been shown repeatedly, from experimental work on Drosophila, that standard and inverted arrangements function as 'supergenes' that become co-adapted in a stable population. This is presumably through the accumulation of supplementary point mutations, so that the fitness of heterozygotes is maximised (White, 1974). Thus, inversions in a vector population might express specific adaptive values for the environmental conditions in which they originated and alternative arrangements might express ecotypic divergence and epidemiologically important heterogeneties.

The adaptive significance of inversion polymorphism in An. gambiae, its possible origin, and its causal involvement in speciation are of considerable interest to malaria entomologists and epidemiologists. Chromosomal studies on An. gambiae s.s. and

An. arabiensis have revealed a remarkably high level of inversion polymorphism. This characteristic appears to be related to their wide distribution and their association with man and man-made breeding places (Coluzzi et al., 1977, 1979; Bryan et al., 1983; Petrarca et al., 1983 and Touré et al., 1983). Clinal geographical variations in inversion frequencies, closely related to climate and ecological conditions have been observed in An. gambiae s.s. in The Gambia and in the surrounding zones of Senegal (Bryan et al., 1982). The changes in frequencies of the **2La** inversion was interpreted as a cline related to increased aridity, the inversion being more common in the arid north than in the relatively more humid south of the western zone of the study area. It has also been shown in Nigeria (Coluzzi et al., 1979) that the carriers of the **2La** inversion are more prevalent in arid areas than the carriers of the standard chromosome, while the standard arrangement is more common in the humid forest zones (Coluzzi, 1985). In our study area, in the humid forest zones of southern Sierra Leone, 91% of the observed **2La** inversion karyotypes were in the standard form. The few (9%) inverted arrangements of **2La** were in the heterozygote form and the Wright's F statistic indicated an excess of heterokaryotypes. In The Gambia, the inverted arrangement of **2La** was found in frequencies ranging from 23% to 96% and in all the localities the Wright's F statistics indicated an excess of homokaryotypes (Bryan et al., 1982).

In the present study, the **2Rd** inversion arrangements were observed in 52% of the chromosome preparations. According to Coluzzi et al., (1979), this inversion appears to be widespread in humid savanna parts of forest-savanna localities. On the other hand the **2Rb** inversion which is widespread in the savanna areas of The Gambia,

Nigeria and Kenya was very rare in our study area. Detailed, longitudinal chromosomal studies in the Guinea Savanna environment in the Kisumu area of Kenya shows populations of An. gambiae s.s. with inversion frequencies that are consistent with those of the same environment in West Africa, but different from the humid forest environment of West Africa (Petrarca & Beier, 1992).

It has therefore been concluded that the form of An. gambiae s.s. found in the humid rain-forest area of West Africa constitutes a panmictic unit characterised by the chromosome-2 standard arrangement, or with few inversions mostly **2La, 2Rb & 2Rd** (Coluzzi et al., 1985; Touré, 1989). The forest chromosomal form is differentiated from the several savanna forms by having inversion **2La** in the standard form. In the savanna forms **2La** is almost always inverted. In West Africa, the savanna forms are further differentiated by one or more **2R** inversions into Bamako, Savanna, Bissau and Mopti kayrotype arrangements. An apparently unique savanna form, characterised by **2Rb** inversion, is found in East Africa (Coluzzi et al, 1985).

Incipient chromosomal speciation in An. gambiae s.s. is probably a recent phenomenon, which appears to be mostly centred in West Africa, and is possibly related to the late regression of the forest belt and to agricultural development in Africa (Coluzzi et al., 1985). The hypothesis of an ancestral status for the Forest form and incipient speciation following agricultural activity, especially swamp rice cultivation, is quite interesting because in our study area we failed to detect breeding of An. gambiae s.s. in swamps even at times when larvae were common in transient pools. Although some workers have recorded the breeding of An. gambiae s.s in

swamps in Sierra Leone (Blacklock, 1925) others have failed to find larvae in swamps in malaria endemic areas despite long and careful searches (Wood, 1915). In other areas of West Africa where the Savanna form of An. gambiae s.s. is the predominant species larvae are frequently found in swamps . The ancestral status of the Forest form of An. gambiae s.s. is further supported by its somewhat central position in the An. gambiae complex and its involvement in presumably much older speciation processes involving An. quadriannulatus and An. bwambae (Coluzzi et al., 1985). The standard chromosomal arrangement of chromosome-2, which is characteristic of the Forest form, is also the most widespread and the only one which is found to form balanced polymorphism with almost all the inverted arrangements observed in An. gambiae (Coluzzi et al., 1985).

Comparative studies on the bionomics of the different Savanna chromosomal forms of An. gambiae s.s. have shown similarity in biting and resting behaviours and in larval ecology (Coluzzi et al., 1985). The bionomics of the Savanna forms are also similar to those established for An. gambiae s.s. (Gillies & Coetzee, 1987) because most studies on the bionomics of An. gambiae s.s. have been carried out in areas where the Savanna forms predominate (Service, 1963; Molineux and Gramiccia, 1980; White, 1974). However, studies on the bionomics of the Forest form of An. gambiae s.s. in the Bo area have revealed some differences in its biting and resting behaviour, in addition to the fact that it is not commonly found breeding in swamps. Contrary to what has been observed for An. gambiae s.s. in other areas of Africa, the Forest form in Bo was exophilic with a high proportion of blood-feds found resting in outdoor shelters (see chapter 3).

Detailed chromosomal studies in Nigeria (Coluzzi et al., 1979) have shown that the distribution and degree of exophily in An. gambiae s.s. is determined to some extent by chromosomal inversion polymorphism. They found that the standard **2Rd** and **2La** were more common in outdoor than indoor collections. It was concluded that the arrangements which were more frequently found in outdoor collections were those favoured by mosquitoes in humid environments. This is in agreement with the findings from the humid Bo area, where the frequencies of the standard arrangements of **2Rd** and **2La** were 52 and 91%, respectively.

The well-known unimodal biting cycle of An. gambiae s.s., peaking in the second half of the night, was confirmed in this study. However, instead of the biting rate decreasing rapidly after 0500 hr, as has been observed elsewhere (Gillies & Coetzee, 1987), it remained at a high level until catching finished at 0600 hr. In Liberia, where Kuhlow & Zielke (1978) observed biting cycles of An. gambiae s.s. in both the savanna and forest areas, the biting rate in the savanna area where savanna forms are expected, decreased earlier than in the forest area were forest forms are expected. In Burkina Faso the biting activity of the Savanna form (Mopti) peaked at between 2200 and 2300 hr and then dropped off rapidly (Robert & Carnevale, 1992).

Recently, Petrarca & Beier (1992) investigating the possible relationship between chromosomal polymorphism in An. gambiae s.s. and sporozoite rate in the Kisumu area of Kenya showed differences in sporozoite rates associated with the **2La** karyotype. Infection rates for the standard homokaryotypes (+/+) were at least two times higher than the inverted homokaryotype (a/a). The sporozoite rate for the An.

gambiae s.s. population in the Bo area (7.4%), where the frequency of the 2La standard homokaryote was 91%, was higher than that observed in The Gambia (3.5%) and Burkina Faso (5.8%), in areas where the Savanna form is the main vector, and where the frequency of the standard 2La homokaryote is much lower (<25%) (Boudin, et al., 1991; Lindsay et al., 1989).

An important consequence of the exophily exhibited by the Forest form of An. gambiae s.s. in the Bo area is the low man-biting rate estimated from the number of sleepers and the number of blood-fed mosquitoes found resting in a bedroom in the morning. It has been shown in Chapter 3 that the number of blood-feds recorded an exit trap fitted to a bedroom window can greatly out-number those remaining in the room in the morning, indicating that a high number of females leave the room after taking a blood-meal. This is further supported by the high proportion of freshly fed (>50%) females found resting in outdoor shelters in the morning, and could explain why in some humid forest or coastal areas, hyperendemicity of malaria is maintained by relatively low estimated entomological inoculation rates. In southern Nigeria where the prevalence of P. falciparum in the 2-10 year age-group varied between 70 and 80%, the entomological inoculation rate was only 0.005 and 0.03 infective bites/man/night in the dry and wet season, respectively (Bruce-Chwatt, 1952). In this particular area the monthly indoor-resting densities, which yielded the man-biting rates, varied from 0.9 to 19.2 females/room with an annual average of 4.8 females/room (Barber & Olinger, 1932). According to the map of West Africa showing the distribution of chromosome-2 arrangement of An. gambiae s.s. (Coluzzi et al., 1985), the Forest form is the predominant vector in this area. Gordon et al.

(1932) working in a forest village near Freetown, Sierra Leone, noted that 'a comparatively small number of mosquitoes in houses is associated with high malaria infection rates amongst children'. They estimated an indoor-resting density of 3.06 females/room from a survey of 3005 houses.

In the present study, a P. falciparum prevalence of 62% in children 0-7 years old was maintained by an entomological inoculation rate of 0.08 infective bites/man/night. On the other hand, in the savanna area of Burkina Faso where the Savanna form of An. gambiae was the main vector, a similar prevalence (62%) in the same age-group was maintained by an entomological inoculation rate of 0.57 infective bites/man/night (Boudin et al., 1991). The seven fold difference in the inoculation rates was due to the difference in the estimated man-biting rates, because the sporozoite rates for the Forest and Savanna forms in the two areas was 7.4 and 5.8% respectively. Studies in Bayama village have clearly shown that the low man-biting rates estimated from pyrethrum spray collections is due to blood-feds leaving rooms before dawn. Mean monthly indoor-resting densities of up to 270 females/room have been recorded for the Savanna form (Mopti) of An. gambiae s.s. in Burkina Faso (Rossi et al., 1986).

The relationship between chromosomal inversions and resting behaviour in An. gambiae which has been prey to criticism (Curtis & Isherwood, 1985) needs to be carefully re-evaluated because the Forest form of An. gambiae appears to be characterised in its range by low indoor-resting densities which at least in the present study, could be explained by exophily.

CHAPTER 6

HOUSING CHARACTERISTICS, INOCULATION RATES AND PREVALENCE OF MALARIA.

6.1 INTRODUCTION

Proper design of houses can contribute to protection against malaria and other mosquito-borne diseases (Schofield & White, 1984). Eaves, that is the open gaps between the roof and the tops of the outside walls provide important routes through which mosquitoes can gain access to people inside a house (Snow, 1987; White, 1969). The principal African malaria vectors commonly enter and rest in houses in the early part of the evening before biting activity commences (Gillies & De Meillon, 1968).

People living on the outskirts of large villages or towns are most vulnerable to the nightly migration of An. gambiae s.l. which may be breeding in pools, rice fields and swamps some distance away from the houses. The use of bed-nets over beds at night can provide significant protection against the bite of African mosquito vectors, which tend to bite late at night.

The present study evaluated the effect of house design and human activity on vector density and disease prevalence. The value of some entomological indices as measures

of transmission intensity was also evaluated by comparing different indices with infection rates of children in the different villages.

6.2 MATERIALS AND METHODS

6.2.1 Population census and house survey

In December 1989, a population census and house survey was carried out in 15 villages lying along a motorable road to the north of Bo Town. Each house was numbered, and each person was allocated a unique number which showed their village, house and person number. To resolve the problem of some people not remembering when they were born, a National Events diary, which lists important national and local events starting from 1881, was used. By matching adult births to the approximate dates of the events, people were aged to within five years of their birth. All dwelling houses in the villages were inspected by field assistants who recorded on special forms the following information concerning house type and sleeping arrangements:

a) type of wall

b) type of roof

c) number of bedrooms

d) number of bedrooms with ceilings

e) number of beds

d) number of beds with bednets

6.2.2 Clinical survey

Two clinical surveys were carried out by the epidemiological group of the malaria project. A pre-rains clinical survey was conducted in March 1990. In this survey all children aged 0-7 years were selected from eight of the 15 villages in the study area. The post-rains clinical survey was conducted in November/December 1990, when all the 0-7 years-olds were selected from 14 of the 15 villages. In each survey, all children had both a thick and thin blood smear, packed cell volume (PCV) and a blood sample taken from the finger prick. Villages were selected to meet logistical requirements of the epidemiological study.

Parasite counts were made from the thick blood smears (stained with Geimsa) using the method described by Greenwood & Armstrong (1991). The thin smears were used to confirm the diagnosis of the malaria species identified from the thick smears.

Urine specimens were taken to test for the presence of chloroquine (Shenton et al., 1988), and available Under-5's Clinic Cards were examined for the prescription of chloroquine in the previous six months.

6.2.3 Knowledge, attitude and practice (KAP) survey

A KAP questionnnaire was administered to one person in every fourth house throughout the 15 villages. In the first house of every four, the head of the household was interviewed (usually male), and in the other three, a woman of child-bearing age

(15-45 years) was interviewed. Respondents were asked simple questions about malaria and its causes.

Although the author was registered for a PhD in entomology, his responsibilies as a scientist employed in the project, required his participation in all other aspects of the project, including clinical and parasitological studies. All studies described in this chapter were carried out in the same laboratory under the supervision of the epidemiologist (Dr Guy Barnish) and the medical entomologist (the author). On several occasions when Dr. Barnish was away, the author supervised the whole project.

6.2.4 Bayama village

A population census and house survey were conducted separately for Bayama in December 1990 using the same methods used in the project villages. A parasite survey was carried out in November 1991 but KAP questionnaires were not administered and urine samples were not tested for the presence of chloroquine.

6.3 RESULTS

6.3.1 Population census

The population of all 15 project villages was 5913 people, 1619 (27.4%) of whom were children under the age of eight years. The number of people per village varied from 82 to 1165. Table 6.1 gives the population of the different villages.

6.3.2 KAP survey

A total of 210 people responded to the KAP questionnaires. Most (75%) had never had any formal education, and only 2.8% had achieved the Ordinary level of the General Certificate of Education or Higher.

Only 29.5% (62) said that mosquitoes were involved with malaria. Of these 62 respondents, 95.2% said that mosquitoes bite at night; 58.0% said that they bite mainly during the wet season and 31 % said that they bite mainly during the dry season. There was evidence of considerable confusion about the transmission of malaria, one of the more usual ways of catching the disease being thought to be stepping in goat urine.

TABLE 6.1
Results of population censuses and house surveys (wall type) in the project villages.

Village	Number of people	Number of houses	Wall type (%)			
			Mud & Sticks	Mud bricks	Mud & cement	Cement bricks
Bumbe	213	17	76.5	0.0	23.5	0.0
Nengbema*	1059	75	45.3	5.3	46.7	2.7
Nyandeyama*	265	20	100.0	0.0	0.0	0.0
Tondoya	381	31	93.5	0.0	6.5	0.0
Blama I	315	32	78.1	3.1	18.8	0.0
Blama II	134	13	92.3	7.7	0.0	0.0
Ngalu	616	50	40.0	4.0	48.8	8.0
Buma	414	42	35.7	33.3	28.6	2.4
Sami	383	35	51.4	8.6	40.0	0.0
Kunjodoma	156	11	81.8	9.1	9.1	0.0
Kpetema	129	11	54.5	27.3	18.2	0.0
Palima	186	18	66.7	11.1	22.2	0.0
Kpakuma	82	9	77.8	22.2	0.0	0.0
Mendewa*	415	35	40.0	42.9	17.1	0.0
N. Komboya*	1616	108	50.9	13.0	25.0	11.1
TOTAL	5913	507	57.0	12.0	27.2	3.8

* entomological villages.

6.3.3 House survey

All houses (507) in the 15 villages were surveyed. The number of houses per villages varied from nine at Kpakuma to 108 at Njala-Komboya. The two villages with the highest number of houses were Nengbema (75) and Njala-Komboya (108) where the Paramount Chiefs were based. Njala-Komboya, in fact had a secondary school. Table 6.1 shows the variation in the number of houses per village.

Four wall types were identified from the survey: walls built from a network of sticks infilled with mudballs, walls built with mud bricks without a cement plaster or with a cement plaster, and walls built of cement bricks only (Figure 6.1). The relationships between the size of the village (number of houses) and the percentage of houses with walls built of cement bricks ($r=0.84$; $p=<0.0001$; $df=13$) or mud bricks plastered with cement ($r=0.57$; $p=0.027$; $df=13$) were positively correlated, with the correlation coefficients significantly different than zero.

On the other hand, there was a significant negative correlation between the size of the village and the percentage of houses with walls built from just mud and sticks ($r= -0.53$; $p=0.040$; $df=13$). The correlation between the size of the village and the number of houses with walls built with mud bricks was not significantly different than zero.

Out of the 507 houses in the villages, 208 (57.0%) had walls built with mud and sticks, 61 (12.0%) with mud bricks, 138 (27.2%) with mud bricks plastered with

Figure 6.1
Types of houses found in the study villages.

A: Mud and stick house with open eaves and no ceiling.

B: Cement brick house with closed eaves

C: Mud brick house.

D: Mud and stick house with thatch roof.

cement and 19 (3.8%) with cement bricks only.

There were only two types of roofs identified in the villages, thatch and corrugated iron. The percentage of houses with corrugated iron roofing varied from 66.7% in the smallest village (Kpakuma) to 100% in some villages, with the biggest village (Njala-Komboya) having 99.1% of houses with corrugated roofs. Over 90% of house in 12 villages had corrugated iron roofing. Table 6.2 gives the number of houses with the two different roof types in all villages. For all 15 villages, the percentage of houses with corrugated iron and thatch roofing were 94.7 and 5.3% respectively. Almost all the houses in the 15 villages had wide eave gaps.

The total number of bedrooms in all the villages was 2434, indicating that there were approximately 2.4 people per bedroom. Out of the 2434 bedrooms, 856 (35.2%) had a ceiling, some of which were in poor condition. Table 6.3 gives the proportion of rooms with ceilings in the different villages. The percentage of rooms with ceilings was positively correlated with the number of houses in each village (correlation coeffecient, $r = 0.56$, $p < 0.05$).

Out of a total 2820 beds counted in all villages, 165 (5.8%) had bednets. The average number of people per bed was two two, thus only 330 people (5.6%) were sleeping under bednets. Table 6.4 gives the percentage of beds with bednets in the different villages. In three of villages, nobody slept under a bednet. The percentage of beds with bednets in the other villages varied from 1.2 to 9.5%. The percentage of beds with bednets was significantly correlated to the size of the village ($r = 0.68$,

TABLE 6.2
Frequency (%) of roof types in the different villages

Village	Number of houses	Roof type			
		Corrugated iron	%	Thatch	%
Bumbe	17	17.0	100.0	0.0	0.0
Nengbema*	75	73.0	97.3	2.0	2.7
Nyandeyama*	20	18.0	90.0	2.0	10.0
Tondoya	31	30.0	96.8	1.0	3.2
Blama I	32	30.0	93.8	2.0	6.2
Blama II	13	12.0	92.3	1.0	7.7
Ngalu	50	46.0	92.0	4.0	8.0
Buma	42	42.0	100.0	0.0	0.0
Sami	35	34.0	97.9	1.0	2.9
Kunjodoma	11	10.0	90.9	1.0	9.1
Kpetema	11	10.0	90.9	1.0.	9.1
Palima	18	16.0	88.9	2.0	11.1
Kpakuma	9	6.0	66.7	3.0	43.3
Mendewa*	35	29.0	82.9	6.0	17.1
N. Komboya*	108	107.0	99.1	1.0	0.9
Combined villages	507	480.0	94.7	27.0	5.3

*Entomology villages

TABLE 6.3
Percentage of rooms with a ceiling in the different villages

Village	Number of bedrooms	Number with ceiling	% with ceiling
Bumbe	86	24.0	27.9
Nengbema*	386	191.0	49.6
Nyandeyama*	102	42.0	41.2
Tondoya	641	72.0	43.9
Blama I	143	18.0	12.6
Blama II	57	0.0	0.0
Ngalu	241	46.0	92.0
Buma	157	59.0	37.6
Sami	143	46.0	32.2
Kunjodoma	51	6.0	11.8
Kpetema	45	6.0	13.3
Palima	67	21.0	31.3
Kpakuma	31	9.0	29.0
Mendewa*	173	55.0	31.8
N. Komboya*	589	261.0	44.3
TOTAL	2434	856.0	35.2

*Entomology villages

TABLE 6.4
Percentage of beds with bednets in the different villages

Village	Number of beds	Number with bednets	% with bednets
Bumbe	83	0.0	0.0
Nengbema*	469	36.0	7.7
Nyandeyama*	127	7.0	5.5
Tondoya	200	14.0	7.0
Blama I	171	2.0	1.2
Blama II	65	0.0	0.0
Ngalu	282	15.0	5.3
Buma	189	18.0	9.5
Sami	209	15.0	7.2
Kunjodoma	70	2.0	2.9
Kpetema	61	0.0	0.0
Palima	89	2.0	2.2
Kpakuma	38	1.0	2.6
Mendewa*	214	7.0	8.3
N. Komboya*	553	46.0	8.3
TOTAL	2820	165.0	5.8

*Entomology villages

$p<0.005$).

6.3.4 Ceilings and vector density in houses

In Nyandeyama where all houses had their walls constructed from mud and sticks, and all had wide eave gaps, the effect of ceilings on the indoor resting densities of An. gambiae s.s. was investigated. The geometric mean female indoor-resting densities of An. gambiae s.s. in rooms with (64 collections) and rooms without ceiling (72 collections) were 4.46 and 3.15 respectively. A Mann-Whitney rank test showed that there was no significant difference in the indoor resting densities in the two groups of houses ($Z= -1.2$, $p=0.23$).

6.3.5 House design and vector density at Bayama

Bayama was selected for this analysis because mosquitoes were collected from all the five houses normally occupied; the sixth house was used as a guest house. Spray collections were performed in two typical village houses, built from mud and sticks and covered with a thatch roof (Figure 6.1D), and two 'modern' village houses. One of the 'modern' houses was built with mud bricks plastered with cement and the other was built with cement bricks (Figure 6.1B). Both had corrugated iron roofing. The 'modern' houses had rooms with ceilings, no eave gaps and the bedrooms were painted white inside. On the other hand, the typical village houses had wide eave gaps and no ceiling (Figure 6.1A). The geometric mean number of females/room in the typical ($n=28$) and 'modern' houses ($n=31$) were 5.0 and 3.8 females/room, but

a Mann-Whitney test showed that the difference was not significant (Z= -0.7, p=0.4). N is the number of spray collections.

There were 43 people living in Bayama, 17 of whom were children under the age of 11 years. The crude P. falciparum parasite rate was 43.9% and the parasite rates in older children (<11 years) was 56.3%. The 0-10 years olds, rather than the 0-7 years olds examined in the project villages, were considered at Bayama because of the small number of children living there. In each of the five houses in Bayama at least one person was infected with P. falciparum. There were only six bednets in the village, indicating that only 13.9% of the population slept under bednets.

6.3.6 Sporozoite rates of An. gambiae s.s. in the different houses

Table 6.5 gives the sporozoite rates of An. gambiae s.s. collected from seven different houses in Nyandeyama over the entire 12 month period of the study. House to house sporozoite rates varied from 5.8 to 10.1%. However, a chisquare test showed that the difference in sporozoite rates between the different houses was not stastistically different (χ^2=6.7, p=0.3, df=6). The similarity of the sporozoite rates is also indicated from the overlap shown by the standard errors at the 95% confidence level. Nyandeyama was selected for the sporozoite rate analysis because of the large number of female mosquitoes tested. In the other villages, the number of sporozoite positive females per house was usually less than five and this made comparison by chisquare difficult.

TABLE 6.5
Sporozoite rates of An. gambiae s.s. collected from seven different houses at Nyandeyama

House number	Ceiling	Number tested	% positive ±SE[1]
3003	+	87	8.0±2.91
3004	+	76	6.6±2.85
3009*	+	155	7.7±2.14
3010	-	129	10.1±2.65
3011	+	65	10.8±3.85
3012	-	138	12.3±2.80
3014	+	278	5.8±1.40

* Thatch house

[1]Formula for calculating standard errors of sporozoite rates is given in Chapter 4

6.3.7 Prevalence of malaria in the different villages

Table 6.6 gives the pre- and post-rains prevalence of malaria in the eight villages where parasite surveys were carried out. The prevalence of P. falciparum in these eight villages in the pre-rains survey varied from 56.7 % at Buma to 64.3% at Blama II. During the post-rains period, the prevalence of P. falciparum in 14 villages varied from 52.1 to 100%. The pre- and post-rains combined prevalence of P. falciparum in the six villages surveyed, during the pre-rains, was 60.4 and 61.8% respectively. The pre- and post-rains prevalences are statistically compared for the entomology villages (Nengbema, Nyandeyama, Mendewa and Njala-Komboya) in section **6.3.9**. The prevalence of P. falciparum at Bayama in November 1991 (post-rains) was 58.6%.

6.3.8 Chloroquine usage

During the pre- and post-rains surveys, urine specimens from 756 and 839 children (<7 yrs) respectively, were tested for the presence of chloroquine. In the pre-rains survey, 70% were negative, whereas only 42.4% were negative in the post-rains survey.

High levels of chloroquine (1:1000 dilutions) were found in 15.1% of pre-rains survey children, and this had more than doubled to 38.1% during the post-rains survey. In all instances there was very little difference in the proportions between the sexes. The chloroquine analyses were carried out at the MRC Laboratories, Fajara,

TABLE 6.6
Prevalence of P. falciparum in children in the survey villages

Village	Pre-rains Survey		Post-rains survey	
	Total	% positive	Total	% positive
Nemgbema*	242	59.9	199	64.8
Nyandeyama*	61	60.7	64	71.9
Blama II	28	64.3	31	64.5
Ngalu	129	59.7	127	55.9
Buma	67	56.7	44	84.1
Sami	72	61.1	56	80.4
Mendewa*	81	60.5	90	53.3
N. Komboya*	208	61.5	190	52.1
Combined villages	888	60.4	801	61.8
Bumbe			50	87.5
Tondoya			95	75.0
Kunjodoma			49	80.0
Kpetema			31	100.0
Palima			54	53.6
Kpakuma			25	100.0

* Entomology villages

The Gambia.

6.3.9 Man-biting rates, inoculation rates and malaria prevalence.

Some entomological indices associated with prevalence rates and parasite densities of P. falciparum in the four entomology villages during the dry (pre-rains) and wet seasons (post-rains) are given in Table 6.7. The mean man-biting rates of malaria vectors (An.gambiae s.s. and An. funestus combined) in all villages were 0.29 and 1.57 bites/man/night during the dry and wet seasons respectively. The corresponding inoculation rates were 0.013 and 0.127 infective bites/man/night. The mean parasite densities for the children (<8 yrs) in these villages was also lower during the pre-rains survey (10248 parasites/μl blood) compared to the post-rains survey (15408 parasites/μl blood). However, the prevalence rates during the surveys were similar, 60.8 and 59.3% for the pre- and post-rains respectively.

In March, when the pre-rains surveys were conducted, there was no significant differene in the prevalence rates of P. falciparum in all the entomological villages. The prevalence rates varied from 59.9% at Nengbema to 61.5% at Njala-Komboya. However, the entomological inoculation rates varied from 0.002 infective bites/man/night at Nengbema to 0.02 infective bites/man/night at Njala-Komboya, a tenfold difference. The mean parasite density varied from 8235 parasites/μl blood at Nyandeyama to 12922 parasites/μl blood at Njala-Komboya.

In terms of inoculation rates and parasite densities, transmission was more intense in

TABLE 6.7
Dry and wet season entomological inoculation rates compared with pre- and post-rains parasite densities and prevalence in the entomological villages.

A. DRY SEASON (pre-rains)

Village	*Entomological inoculation rate			+Mean parasite densities	Prevalence
	Anopheles gambiae	Anopheles funestus	Total		
Nengbema	0.000	0.002	0.002	9394	59.9
Nyandeyama	0.010	0.002	0.012	8235	60.7
Mendewa	0.000	0.015	0.015	11023	60.5
N. Komboya	0.017	0.003	0.020	12922	61.5
Combined villages	0.007	0.006	0.013	10248	60.8

B. WET SEASON (post-rains)

Village	*Entomological inoculation rate			+Mean parasite densities	Prevalence
	Anopheles gambiae	Anopheles funestus	Total		
Nengbema	0.106	0.000	0.106	12512	64.8
Nyandeyama	0.178	0.005	0.183	12812	71.9
Mendewa	0.056	0.027	0.083	17714	53.3
N. Komboya	0.109	0.003	0.112	21291	52.1
Combined villages	0.119	0.008	0.127	15408	59.3

* Number of infective bites/man/night
+ Number of parasites/μl blood.

the high altitude villages (Mendewa and Njala-Komboya) than in the low altitude villages (Nengbema and Njala-Komboya) during the dry season.

The post rains parasite survey revealed parasite rates that were statistically different in the four entomological villages ($\chi^2 = 12.1$, $p<0.005$, $df=3$). This difference was mainly due to difference in prevalence rates between the high and the low altitude group of villages. There was no significant difference ($p>0.05$) between the prevalence rates for Nengbema (64.%) and Nyandeyama (71.9%), the two low altitude villages. Also the prevalence rates for Mendewa (53.3%) and Njala-komboya (52.1%) were not statistically different. However, the prevalence rates for the combined low altitude villages (66.5%) was significantly higher than the prevalence rates for the combined high altitude villages (52.5%) ($\chi^2=10.5$, $p=0.001$, $df=1$).

The entomological inoculation rates for An. gambiae s.s. and An. funestus, for all villages combined, was 0.119 and 0.008 infective bites/man/night respectively in the wet season and 0.007 and 0.006 infective bites/man/night respectively in the dry season.

6.4 DISCUSSION

Malaria was hyperendemic in the study villages and transmission of the disease was intense and perennial. There was a large seasonal variation in the entomological inoculation rates in the different villages but the prevalence of malaria in children was similar in both the dry and wet season.

There was also a small area variation in the pattern of transmission.

In the high altitude villages, where the mainly grassland vegetation could be described as a forest-savanna mosaic, dry season transmission of malaria was more intense than in the low altitude villages, and An. funestus was the most important vector in this area during this period. It was responsible for about 51% of the transmission. In the low altitude villages, An. funestus was never an important vector, where during both the dry and wet seasons An. gambiae s.s. accounted for over 85% of transmission.

Generally, the wet season entomological inoculation rates were higher than the dry season rates as were the parasite densities in children. During the dry season, parasite densities were higher in the high altitude villages where transmission was more intense, but there was no significant correlation between inoculation rates and parasite densities in the different villages. Because of the high levels of parasite multiplication that occur in the liver and blood of infected people, one would not expect the initial inoculum from a mosquito to greatly influence the outcome of parasitaemia. However, the frequency and size of sporozoite inoculum will have some influence on the time that elapses before a high level of blood parasitaemia is

reached (Greenwood et al., 1991). Extension of this period by only one or two days may give the host an improved chance of developing an immune response that can contain parasite multiplication and thus prevent the development of high parasitaemia. Therefore , although an increase in inoculation rate would tend towards an increase in parasitaemia, the relationship is not a direct and simple one, especially in the case of semi-immune individuals in whom the immune system has already been primed.

Prevalence on the other hand might be expected to be directly correlated with inoculation rate because more people become exposed to infection when the number of infective, biting mosquitoes increases. In the present study, this was not observed. The entomological inoculation rate in the project villages increased tenfold in the wet season, but the prevalence of P. falciparum remained the same. In fact, in Bayama where the wet season inoculation rate was higher than 2 infective/bites/man/night, the prevalence rate during the wet season (56.3%) was lower than that of the entomology project villages (59.3%). In Papua New Guinea, Burkot et al. (1987) found that in a given village the entomological inoculation rates correlated strongly with the prevalence of P. falciparum in children.

A statistically significant difference was observed in the prevalence of malaria between the low and high altitude villages during the wet season; the respective inoculation rates were 0.289 and 0.195 infective bites/man/night. However, in the dry season when the difference between the respective inoculation rates of 0.014 and 0.035 infective bites/man/night was much greater, the prevalence rates were similar. It therefore appears that in the present study area where transmission was very intense

and perennial, parasite prevalence could not be directly related to entomological inoculation rates. The density of asexual parasites was a much better indicator of transmission intensity.

Most children examined (57.6%) during the post-rains survey had chloroquine in their urine samples. The widespeard use of chloroquine might have affected the detection of asexual parasites in the blood. In the two smallest villages, Kpakuma and Kpetema, a prevalence of 100% was recorded, probably because of less chloroquine usage since there were no traders in these villages and drug pedlers who usually travelled during the day found almost everybody had gone to the farm. The small number of children examined in these villages could also have been responsible for the high prevalence observed, but Bayama village, where only 16 children were examined had a prevalence of 56.3%, and in this village chloroquine was readily available because the village was located only 3 km away from Bo, the provincial capital.

In Papua New Guinea where Burkot et al. (1987) found a strong correlation between prevalence and inoculation rates, aminoquinolines were detected by the Dill-Glazko test in only 12% of urine samples. The highest levels of P. falciparum densities (3400 parasites/μl) found by Cattani et al. (1986), working in the same villages as Burkot et al. (1987) , were much lower than densities in the present project villages, which varied from 8235 parasites/μl blood in the dry season to 21291 parasites/μl blood in the wet season. This indicates that transmission was more intense in Sierra Leone than in Papua New Guinea.

Neither the absence of eave gaps nor the presence of ceilings in bedrooms appeared to restrain the entry of mosquitoes into houses contrasting with the findings in The Gambia, that fewer An. gambiae s.l. are found in huts with closed eaves than in those with open eaves, and moreover that the incidence of clinical malaria is lower among children sleeping in huts with closed (Lindsay & Snow, 1988). Studies in Burkina Faso (Majori et al., 1987) and Kenya (Sexton et al., 1990) have shown that mosquito entry into houses, through eave gaps, can be reduced by bridging the gap between the wall and roof by curtains impregnated with insecticide. Although the quality of housing, in terms of number of rooms with ceilings and walls covered with cement, increased with increased size of the villages, the prevalence of malaria was not associated with the size of the villages. The prevalence of malaria in Nyandeyama where all houses were built of mud and sticks and where they had wide eave gaps, was very similar to the prevalence of malaria in Nengbema, where 46.7% of the houses were built with mud bricks covered with cement and usually had closed eave gaps. In The Gambia, malaria prevalence decreased with increasing size of villages partly because bigger villages had better houses (Greenwood, 1989).

In Sri Lanka, Gamage-Mendis et al. (1991) found that poorly constructed houses with mud walls and thatch roofs were prefered resting places for anopheline mosquitoes when compared to houses with plastered brick walls and tiled roofs. Other studies have also shown that poorly constructed houses, with mud walls and thatch roofs, offer dark and cool microenvironments which are more attractive to mosquitoes than 'modern' village houses (Gillies & De Meillon, 1968; Muirhead-Thomson, 1951; Schofield & White, 1984). However, there was no evidence for this at Bayama,

probably because of the exophilic behaviour of the An. gambiae population in the village. In a village with an endophilic population of vectors, unfed mosquitoes entering houses would prefer to rest in a cool microenvironment and wait for their hosts. However, exophilic females would tend to leave the house without resting, irrespective of the microclimate as was the case in Bayama. Very few unfed female An. gambiae s.s. (0.9%) were caught in indoor-resting collection in the present study. In the Kisumu area of Kenya, where indoor-resting population are much higher than in our study area, Githeko (1992) found that 17% of An. arabiensis collected in houses were unfed.

CHAPTER 7

MOSQUITOES OF SOUTHERN SIERRA LEONE

7.1 INTRODUCTION

The mosquito fauna of Freetown and its environs was studied from as early as the first decade of this century, when such eminent men as Ross, Blacklock, Stephens and Christophers investigated the anopheline fauna in connection with the transmission of malaria. Anopheline mosquitoes were well studied in the Freetown area until the closure of the Alfred Lewis-Jones Laboratory, a field station of the Liverpool School of Tropical Medicine, in the early 1940s.

However, few studies were carried out on the mosquitoes of the provinces. Since 1955, when Lewis (1956) conducted an entomological survey of the Tonkolili valley in the Northern Province, nothing has been published on the mosquito fauna of Sierra Leone. Following Lewis's publication many changes in classification and nomenclature had taken place and many of the genera and species recorded have been synonymised or their names changed.

The primary aim of a general mosquito survey in the present study was to provide an updated checklist of mosquitoes of southern Sierra Leone. Species identity, bionomics and medical importance are provided for the study area for the period

December 1989 to November 1991. The observations made in the Southern Province are discussed with reference to the available historical records, and nomenclature is updated.

7.1.1 Historical background to insect collecting in Sierra Leone

The scientific collection of insects is very significant in the history of Sierra Leone; it pre-dates the establishment of the colony in 1787. In 1783, Dr Henry Smeathman, a British naturalist who had been resident in the territory for several years suggested the establishment of Sierra Leone as a settlement for free slaves (Kennan, 1910). Dr Smeathman was known, by the indigenous people, as "fly-catcher" because of his habit of collecting insects. Other fly-catchers in Sierra Leone included Adam Afzelius, a personal student of Linnaeus, who visited Sierra Leone between 1792 and 1794 collecting about 1,600 insects including the first scientific specimen of a tsetse fly subsequently named Glossina longipalpis by Wierdmann in 1830. Mary Kingsley also collected insects when she visited Sierra Leone in 1898.

The "Tumbu fly" maggot was first reared to adult stage in Sierra Leone (Austeen, 1899) where it probably got the common name "Tumbu", a creole word for maggot, Creole being the lingua franca for Sierra Leone.

A review of mosquito studies in Sierra Leone from 1898 to present has been given in Chapter 1. The two most important vectors of malaria in Africa, An. gambiae s.l. and An. funestus were first incriminated as vectors of the disease in Sierra Leone in

1899 (Ross et al., 1900) during the Liverpool School Malaria Expedition. Freetown and its environs continued to be surveyed for many years but little was known about the mosquito fauna of the provinces, until the entomological investigations of Simpson (1913) who travelled extensively in the country between March and November 1912 collecting insects. Several mosquito surveys were carried out in the Northern Province where the Headquarters of the Sierra Leone battalion of the West African Frontier Force was based. The Headquarters was later moved to Daru in the Eastern Province, and Daru is the only town outside the Freetown area and the Northern Province, with a published list of mosquito species (Blacklock, 1925; Evans, 1925). It is interesting to know that Colonel Newstead, who identified a few new mosquito species in Sierra Leone, was one time commanding officer of the Daru Battalion. Almost all medical entomologists who visited Sierra Leone in these early days were members of the military, and were mainly concerned with areas of military interest, which unfortunately, did not include the Southern Province.

The present study provides the first list of the mosquitoes, in Southern Sierra Leone.

7.2 MATERIALS AND METHODS

7.2.1 Collection of immature stages

Plastic bowls and trays were very effective in collecting mosquito larvae in swamps and along the edges of seasonal streams. Soup ladles were used to collect larvae from small pools of water found in roads and playgrounds in the villages. Larvae were transferred from these containers into sample vials using plastic pipettes. Pipettes were also used to collect mosquito larvae from collections of water in domestic containers, and plant receptacles such as the axils of pineapples, bananas and hollow bamboo stems.

Larvae were placed in small glass tubes and a cotton wool plug gently pushed down on top of the larvae to prevent them being damaged during transportation to the laboratory.

7.2.2 Collection of adults

The human-bait, light-trap and exit trap methods described in Chapter 2 were used to collect adult mosquitoes during this survey. Extensive searches were also performed in artificial and man-made outdoor shelters for resting adult mosquitoes. Outdoor resting adults were collected using aspirators and test tubes with the aid of a torches.

7.2.3 Preparation of samples

Pupae and some larvae were individually reared to adults for confirmatory identification and for a genitalia reference collection. Larval skins (pelts) of fourth-instar larvae remaining after they had pupated were mounted on slides and identified taking great care that the various setae were not detached.

Immature stages not reared were preserved in 70% ethanol. Larvae which dried out were covered with 70% acetic acid and kept at a temperature of about 30°C, for two days, after which the acetic acid was replaced with 70% alcohol. Larvae were transferred directly from alcohol and mounted on microscope slides in alcohol-miscible media such as polyvinyl lactophenol (chitomount).

Before placing a cover slip on a dorsally mounted larva, a clean cut was made between abdominal segments VI and VII and the terminal segments turned laterally so as to expose the comb of segment VIII and the pecten of the siphon. Slide preparations were ringed with nail varnish, labelled and stored.

Newly emerged adults were held for 24 hours before killing with ethyl acetate. Each adult was mounted on a stainless steel, headless pin, termed a micropin or minuten. This pin (0.15-0.20mm in diameter and 10mm long) was carefully pushed through the ventral surface of the thorax of the mosquito or through its side, and then into a thin strip (about 10-15mm long, 3-4mm wide and 2mm deep) of polyporus or cork. An entomological stainless steel pin was inserted through the opposite end of this strip.

Old and dry mosquitoes which had to be pinned were first relaxed by placing them, for a few hours or overnight, in a humid atmosphere such as a small sandwich box containing cotton wool soaked in water, taking care the water did not come in direct contact with the adults.

Pinned mosquitoes were stored in airtight entomological cork-lined storage boxes. To prevent attacks by arthropod scavengers and fungi, moth-balls (naphthalene) were firmly glued into the corners of the storage boxes.

7.2.4 Identification keys

Identification of culicine mosquitoes was based on the keys of Hopkins (1952) and Service (1990). Anophelines were identified according to the keys of Gillies and De Meillon (1968) and Gillies and Coetzee (1987). All identifications were checked by Professor M.W. Service of the Liverpool School of Tropical Medicine.

7.2.5 Deposition of specimens

All the collected and identified material was deposited at the Medical Research Council Laboratory, Bo, Sierra Leone, except for one unidentified Anopheles species, which was transferred to the Liverpool School of Tropical Medicine.

7.3 RESULTS AND DISCUSSION

7.3.1 Sites inspected

In addition to Bayama and the project villages, the town of Bo was also surveyed for both larval and adult mosquitoes. Indoor resting, indoor-flying and exiting females were sampled in different types of houses. Outdoor resting sites inspected included rice barns, empty and abandoned houses, overhanging thatch roofs, fences built of oil palm leaves, vegetation around the villages, including buttress roots of large trees, empty domestic containers, eg. buckets, drums, mortars, etc., earth banks, pit shelters and brick piles.

Larval surveys were carried out in swamps, streams and temporary pools including rock pools. Also inspected, were polluted water in cesspits and collections of water in domestic containers, eg. water tanks, bamboo stumps and abandoned vehicles.

7.3.2 Species collected

Table 7.1 lists the 50 species identified during the survey. The collection included seven genera, Anopheles, Aedes, Culex, Uranotaenia, Mansonia, Mimomyia and Eretmapodites. One of the Anopheles species could not be identified using the current keys for Afrotropical mosquitoes (Gillies & De Meillon, 1968; Gillies & Coetzee, 1987). Eleven of the culicines are new records for Sierra Leone.

TABLE 7.1 A list of mosquitoes found in the Bo area during the mosquito survey carried out from December 1989 to November 1991. Species marked with asterisks are new records for Sierra Leone.

Genus Anopheles Meigen

An. barberellus Evans

An. brunnipes (Theobald)

An. coustani Laveran

An. flavicosta Edwards

An. funestus Giles

An. gambiae Giles

An. hancocki Edwards

An. marshalli Theobald

An. obscurus (Grunberg)

An. squamosus Theobald

An. ziemanni Grunberg

Anopheles sp. ident.

Genus Aedes Meigen

Ae. aegypti (Linnaeus)

Ae. africanus (Theobald)

Ae. apicoargenteus (Theobald)

Ae. argenteopunctatus (Theobald)

Ae. luteocephalus (Newstead)

Ae. punctothoracis (Theobald)

TABLE 7.1 continued

Ae. vittatus (Bigot)

Genus Culex Linnaeus

Cx. antennatus (Becker)*

Cx. bitaeniorhynchus group Giles

Cx. cinerellus Edwards

Cx. cinereus Theobald

Cx. duttoni Theobald

Cx. ethiopicus Edwards*

Cx. galliardi Edwards*

Cx. grahami Theobald

Cx. guiarti Blanchard

Cx. horridus Edwards

Cx. insignis (Carter)

Cx. nebulosus Theobald

Cx. perfidiosus Edwards

Cx. perfuscus Edwards*

Cx. poicilipes (Theobald)*

Cx. quinquefasciatus Say

Cx. telesilla De Meillon and Lavoipierre*

Cx. tigripes De Granpré and De Charmoy

Cx. univittatus Theobald*

TABLE 7.1 continued

Genus Eretmapodites Theobald

E. chrysogaster Graham

E. grahami Edwards

E. oidipodeios Graham

E. argyrurus Edwards*

Genus Mansonia Blanchard

Ma. africana (Theobald)

Ma. cristata (Theobald)

Ma. maculipenis (Theobald)*

Ma. uniformis (Theobald)

Genus Mimomyia Theobald

Mi. plumosa (Theobald)

Genus Uranotaenia Lynch Arribalzaga

Ur. alba Theobald*

Ur. balfouri Theobald

Ur. bilineata var. connali Edwards

Ur. nigromaculata Edwards*

Ur. ornata Theobald

7.3.2.1 Distribution, biology and systematics of anophelines occurring in Southern Sierra Leone

The number of Anopheles species (11) identified in the present study was almost half of what has been reported from Sierra Leone. Between 1900 and 1956, 25 Anopheles species were recorded in the country, based on collections made in the Western Area (Blacklock & Evans, 1926; Evans, 1925), Northern Province (Butler, 1915; Gordon, 1929 and Lewis, 1956; Wood, 1915) and the Eastern Province, mainly Daru Town (Blacklock, 1925; Evans, 1925) and collections made during the provincial mosquito surveys that started in 1938 (Anon, 1940; Davey, 1939). In the published literature, Anopheles gambiae, An. funestus and An. brunnipes are the only Anopheles species that had been reported from the Southern Province (Anon, 1940; Butler, 1915; Simpso, 1913).

The list of anophelines of Sierra Leone published by Gillies & De Meillon (1968) does not include An. moucheti Evans and An. maculipalpis Giles which were first recorded in 1940 during the provincial mosquito survey (Anon, 1940).

Most of the Anopheles species not found during the present survey are those that are not commonly found in forested areas, eg. An. pharoensis, An. rhodesiensis and An. domicolus. Anopheles smithii has only been recorded in Mount Aureol in Freetown where it breeds in clear streams with rocky bottoms. It is surprising that An. nili, which appears to be common mosquito in other forested areas in this country, was not found during our survey.

Moreover, because the list of the Anopheles species of Sierra Leone has not been updated for over 40 years, there has been considerable confusion over the existence of certain species reported in the old literature. What was for instance recorded as An. theileri Edward (Blacklock & Evans, 1926) probably refer to An. brohieri because the occurrence of An. theileri in West Africa is doubtful (Gillies and De Meillon, 1968). Anopheles umbrosus Theobald recorded from Daru (Evans, 1925) is now considered a synonym for An. nili Theobald. A larva, resembling An. nili, collected in Daru and identified as An.? nili (Blacklock & Evans, 1926; Gordon, 1929) was later found to be An. somalicus Rivola and Holstein (Gillies & De Meillon, 1968). The original description of An. paludis Theobald from Sierra Leone (Theobald, 1900) was sunk 28 years later as a variant of An. mauritianus Edwards, but it was later, again, given a species status and the original name restored. Anopheles pitchfordi Giles recorded by Wood (1915) in the Northern Province is now considered a synonym for An. marshallii. The
only reference to An. ziemanni occurring in Sierra Leone is recorded as avariant of An. coustani (Davey, 1939).

The confusion over the current status of the anophelines of Sierra Leone is clearly exemplified by the list of Anopheles species in the last review of malaria studies in Sierra Leone by Storey (1972). Storey's list does not include An. maculipalpis, An. moucheti, An. paludis, An. somalicus, An. brohieri and An. ziemanni, all of which were recorded in the country years before his review.

The following species were found during the present survey:

Anopheles barberelus Evans, 1932

Anopheles barberellus was found only in the high altitude villages. Three adults were caught, two resting in pit shelters in a well shaded coffee plantation and one in a light-trap placed inside a house. Immature stages were not found. It was first indentified in Sierra Leone in 1925 (Blacklock & Evans, 1926) and provisionally referred to as An. domiculus Edwards until Evans (1932) described it as a new species. The only other references to An. barberelus as a species in Sierra Leone are Anon (1940), and Lewis (1956). Storey's (1972) list of Anopheles mosquitoes in Sierra Leone does not include this species. It is not important in the transmission of malaria.

Anopheles brunnipes Theobald, 1910

This species was caught only in Bayama, in indoor light-trap collections during the dry season, and was the second most common anopheline in the village. The immature stages which were not observed during this survey, were first described from specimens collected during a mosquito survey of the provinces in Sierra Leone (Davey, 1942). It appears to be wide spread in the provinces because it was caught in the three Provinces during the provincial mosquito survey of 1938.

Anopheles brunnipes has only been observed in Sierra Leone during the dry season and is not important in the transmission of malaria. It has not been recorded from the Freetown area.

Anopheles coustani Laveran, 1900

Larvae of the An. coustani group have been observed in Freetown and in the provinces. One larva, then referred to as An. mauritianus Grandpre (Evans, 1925) was found in a swamp in Daru, in the Eastern Province. In the present study, two larvae were found in swamps.

Few adults were caught, all in indoor light-trap collections, in the project villages and in Bayama , during the dry season. There are, however, records of this species occurring in low numbers in the wet season in both Freetown and the provinces (Anon, 1940; Davey, 1939).

Anopheles coustani was very scarce during this survey and it is not a vector in Sierra Leone. The An. mauritianus, reported to occur in large numbers in some parts of the country (Gordon, 1929; Gordon & Macdonald, 1930) is a synonym for An. paludis Theobald.

Anopheles flavicosta Edwards, 1911

This species was very scarce and was only caught as adults during this survey. It was caught in light-traps placed inside houses, but one was caught outdoors resting in an empty drum. Larvae were not found during this survey but they have been observed in the Northern Province of Sierra Leone (Davey, 1942). This species which is not a vector of malaria in this country, although it may be a minor one elsewhere, has not been recorded in Freetown.

Anopheles funestus Giles, 1900

Anopheles funestus was first incriminated as a malaria vector in Freetown where it was also described (Ross et al., 1900). It has also been reported from all three Provinces but it appears to be more common in the savanna areas of the Northern Province. In the present survey, it was the second most common Anopheles species, in the project villages, after An. gambiae.

During this survey, adults were caught using all the sampling methods applied. Females were caught throughout the year but they were biting humans more during the dry season. Outdoor resting sites included rice barns, mud banks, inside overhanging thatch roofs, pit shelters and palm fences.

This species is also very endophagic , endophilic and anthropophagic, with an HBI of 1.0 in some villages.

Numerous larvae have been observed in clear streams in hills in the Freetown area, but during this survey only two larvae were caught, in swamps. It is a very important vector of malaria in Sierra Leone, especially during the dry season.

Anopheles funestus Giles was the only member of the An. funestus group observed in the present survey.

Anopheles gambiae Giles, 1902

Anopheles gambiae was also first incriminated as a vector of malaria in Freetown (Ross et al., 1900) when it was then referred to as An. costalis Giles. Adults and larvae have been observed in almost all areas surveyed in the country. In the present survey, it was the most common mosquito species and it was caught throughout the year. Females were caught using all sampling methods applied. Outdoor resting sites included those listed for An. funestus . Adults also rested inside empty drums, mortars and buckets. At Bayama, they were also found resting on the buttress roots of large trees.

Despite careful examination of the swamps situated by all the villages, the larvae of this species was found only in temporary pools and the edges of some streams. Blacklock (1925), however, reported An. gambiae breeding in swamps in Daru in the Eastern Province.

Anopheles gambiae s.s. was the only member of the An. gambiae species complex identified chromosomally during the present survey. An. melas breeds in the mangrove swamps along the peninsular of Freetown (Muirhead-Thomson, 1945). According to Morgan (1990), An. arabiensis also occurs in Sierra Leone but he did not state the locality.

Anopheles gambiae s.s. observed in the present survey was very endophagic and anthropophagic but interestingly, it was also highly exophilic.

Anopheles hancocki Edwards, 1929

This species was the third most common mosquito, after An. gambiae and An. funestus, in the village of Mendewa. In the other villages, it was not very common. During the present survey, it was caught in exit trap, light-trap and human bait collections. It was mostly caught during the wet season from May to September. Anopheles hancocki has been reported from Freetown and the Provinces (Davey, 1939). It was the most common Anopheles species in the Tonkolili valley in 1955 (Lewis, 1956). In this valley it was considered a minor vector of malaria because out of 115 females dissected one (0.9%) had sporozoite infected glands. During the present survey, 38 females were tested for P. falciparum sporozoite antigens but none was found positive.

Anopheles marshalli Theobald, 1903

During this survey, this species was collected only in the villages of Mendewa and Nyandeyama. At Mendewa very few adults were caught ,in July and August, in the wet season. Some were caught in light-traps and one was caught resting in a pit shelter in a shaded coffee plantation.

Only one adult was caught in Nyandeyama, in January , in the dry season, resting in a rice barn. This particular female was morphologically, slightly, different from the females caught at Mendewa. It looked rather like An. gibbinsi Evans with vein 3 entirely pale but for a small dark spot at the basal end. However, An. gibbinsi is a highland species of eastern Africa and has never been recorded in Sierra Leone. This An. gibbinsi-like specimen has been deposited at the Liverpool School of Tropical

Medicine, in the custody of Prof. M. W. Service.

Anopheles marshalli has also been recorded from the Koinadugu District (Butler, 1915; Wood, 1915), but it was referred to then as An. pitchfordi Theobald. Anopheles marshalli is of no medical importance in Sierra Leone.

Anopheles obscurus Grunberg, 1905

Between 1912 and 1930, the occurrence of this species in Sierra Leone was recorded as An. umbrosus. Blacklock (1925) found it breeding in Daru, and Wood (1915) captured three females in dwelling houses in Kaballa in the Northern Province. It has also been recorded in Freetown (Anon, 1940) and other parts of the country (Anon, 1940; Davey, 1939). It is not a vector of malaria in Sierra Leone.

In the present survey, it was found breeding in swamps and one adult was caught in a light-trap in Nyandeyama.

Anopheles squamosus Theobald, 1901

This is mainly a dry season mosquito in Sierra Leone. It has been found in very small numbers in both Freetown (Blacklock & Evans, 1926; and the provinces (Davey, 1939; Anon, 1940). It is not medically important in this country.

In the present survey, one adult was caught in a light-trap at Bayama.

Anpoheles ziemanni Grunberg, 1902

Before the present survey, there was only one record of this species in Sierra Leone, made during the provincial mosquito survey of 1938, when it was identified as An. coustani var. ziemanni (Davey, 1939).

Anopheles ziemanni was relatively common during the present survey; it was observed in both the dry and the wet season but it is not a vector of malaria in Sierra Leone. It was caught in human-bait, light-trap and pyrethum spray collections.

7.3.2.2 Distribution and biology of culicines of southern Sierra Leone

A total of 38 different species of culicines were identified during the present survey, 11 of which are new records for Sierra Leone.

Genus Aedes Meigen

Ae. aegypti Linnaeus

During the present survey one adult Ae. aegypti was caught in human-bait collections, at Bayama and a few larvae were found in a domestic water tank at Njala-Komboya.

Aedes aegypti which is a vector of yellow fever has been found to be more common in Freetown than in the provinces (Anon, 1940).

Ae. africanus (Theobald)

This species has only been recorded from the provinces (Anon, 1940; Blacklock, 1925; Butler, 1915).

During the present survey, few adults were caught in light-trap collections.

Ae. argenteopunctatus (Theobald)

Before the present survey there was only one record of this species in Sierra Leone; in the Koinadugu District (Butler, 1915).

In the present survey, it was caught in human-bait and light-trap collections at Bayama and Nyandeyama respectively.

Ae. apicoargentes (Theobald)

Aedes apicoargentes has been found in the Eastern and Northern Provinces (Blacklock, 1925; Wood, 1915).

During my survey larvae were found in collections of water in the hollow stems of bamboo in Njala-Komboya. No adults were found.

Ae. luteocephalus (Newstead)

This species has been found in Freetown and the provinces (Davey, 1939; Anon, 1940). During the present survey it was the second most common mosquito, biting man at Bayama, after An. gambiae.

Ae. punctothoracis (Theobald)

This species which has also been recorded in Freetown and other parts of the provinces (Davey, 1939), was caught, biting man at Bayama, during the present survey.

Ae. vittatus (Bigot)

There are records of Ae. vittatus occurring in Freetown and the provinces (Davey, 1939). It was caught in human-bait collections during the present survey.

Genus Culex Linnaeus

Cx. antennatus (Becker)

This survey provided the first record of Culex antennatus in Sierra Leone. Few adults were caught in light-traps in Bayama in December 1990.

Cx. bitaeniorhynchus group Giles

This species has previously only been recorded in the Koinadugu District, in the Northern Province (Butler, 1915). In the present survey, larvae were found in swamps the Town of Bo.

Cx. cinerellus Edwards

Culex cinerellus was previously only recorded during the 1938 provincial mosquito survey (Anon, 1940). During the present survey, one male was caught in a light-trap placed inside a bedroom.

Cx. cinereus Theobald

This species has been found in Freetown, and it was the most common culicine species recorded during provincial mosquito survey (Davey, 1939; Evans, 1925;).

In the present survey, adults were caught in exit-trap, light-trap and indoor pyrethrum spray collections.

Cx. duttoni Theobald

This species appears to be widespread in the country. It has been caught in Freetown, Eastern and Northern Provinces (Anon, 1940; Evan 1925; Davey, 1939).

During this survey, adults were found resting outdoors on earth banks.

Cx. ethiopicus Edwards

This is the first record of Culex ethiopicus in Sierra Leone.

During my survey one adult was caught in a light-trap, placed in a bedroom in Nyandeyama, in December 1990.

Cx. galliardi Edwards

Culex galliardi has not been previously recorded in Sierra Leone.

In the present survey, adults were found in exit-traps at Mendewa and also resting outdoors on mud banks at Nyandeyama.

Cx. grahami Theobald

This species has only previously been recorded in Freetown (Evans, 1925) and Mabang (Gordon, 1929).

In the present survey, it was caught in a light-trap placed in a bedroom in Nyandeyama.

Cx. guarti Blanchard

This species was recorded during the provincial mosquito survey (Davey, 1939), but no record exists for its occurrence in Freetown.

Larvae were found in swamps ,in Bo town, during the present survey.

Cx. horridus Edwards

Culex horridus has only previously been recorded in Freetown (Gordon et al., 1932). In the present survey, larvae were found, in the hollow stems of bamboo in Njala-Komboya.

Cx. insignis (Carter)

This species has only previously been recorded by Butler (1915) for Koinadugu District. In the present survey it was the most common mosquito resting outdoors on earth banks. It was also caught resting in piles of mud bricks and a few were caught in light-traps.

Cx. nebulosus Theobald

This species has been recorded in all the three provinces in the country and also in Freetown (Blacklock, 1925; Butler, 1914; Davey, 1939; Evans, 1925).

In the present survey, it was found in light-traps and resting in pit shelters.

Cx. perfidiosus Edwards

This species was recorded during the provincial survey of 1938 (Anon, 1940).

In the present survey only larvae were recorded, and these were in very polluted water in the town of Bo.

Cx. perfuscus Edwards

There is no previous record of Culex perfuscus in Sierra Leone. In my survey, it was collected as larvae in very polluted water in a cesspit in Bo.

Cx. poicilipes (Theobald)

The present survey also provides the first record of Cx. poicilipes in Sierra Leone. It was found breeding in swamps and adults were caught in light-trap, human-bait and pyrethrum spray collections.

Cx. quinquefasciatus Say

Surprisingly, the only record of the occurrence of this species in the provinces is that made by Butler (1915) in the Koinadugu District . It was, however, a common species in Freetown in the 1930s (Thomas, 1956).

In the present survey, it was a relatively common mosquito breeding in polluted water. It was caught in light-traps, exit traps and human-bait collections.

Culex quinquefasciatus is a vector of bancroftian filariasis

Cx. telesilla De Meillon and Lavoipierre

There is no previous record of this species in Sierra Leone. In the present survey, larvae were found in heavily polluted water in a cesspit. No adults were found.

Cx. tigripes de Grandpré & de charmoy

Culex tigripes has only previously been recorded in the Tonkolili valley (Lewis, 1956).

During this survey, a few adults were found in an exit trap in Nyandeyama in December 1990.

Cx. univittatus Theobald

This species is also a new record for Sierra Leone. Few females were caught in light-traps placed in bedrooms in Bayama in December 1990.

Genus Eretmapodites Theobald

E. chrysogaster Graham

This species has been recorded from Freetown and the Provinces (Blacklock, 1925; Butler, 1915; Davey, 1939; Evans, 1925).

In the present survey, few adults were caught in light-traps at Nyandeyama.

E. grahami Edwards

Eretmapodites grahami has only previously been recorded from the Northern Province (Lewis, 1956).

In the present survey, it was only observed as larvae found in the hollow stem of bamboo.

E. oidipodeios Graham

This species has been found in Freetown and the provinces (Anon, 1940; Evans, 1925). In the present survey, it was also only observed as larvae in bamboo stem.

E. argyrurus Edwards

I have not seen any reference to the occurrence of Eretmapodites argyrurus in Sierra Leone. In the present survey it was found as larvae in bamboo stem

Genus Mansonia Blanchard

Ma. africana (Theobald)

This species which was relatively common in the present survey was recorded only once (Collett, 1915) prior to 1930. It was found in Freetown in 1932 (Gordon et al., 1932) and during the provincial mosquito survey (Anon, 1940), when it was recorded as Taeniorhynchus africanus .

During the present survey, it was caught in human-bait and light-trap collections throughout the year at Bayama.

Ma. cristata (Theobald)

Mansonia cristata was first recorded in Sierra Leone during the provincial mosquito survey (Anon, 1940). During the present survey, this species was caught in light-traps at Mendewa.

Ma. maculipennis (Theobald)

There is no previous record for Mansonia maculipennis in Sierra Leone.
It was also caught in light-traps in Mendewa during this survey.

Ma. uniformis (Theobald)

Mansonia uniformis has been recorded in the Provinces (Anon, 1940; Butler, 1915; Collett, 1915; Lewis, 1956) but not in Freetown. It was also recorded as Taeniorhynchus uniformis during the provincial survey (Anon, 1940).

In the present survey it was found in light-trap and exit-trap collections throughout the whole year in Bayama.

Genus Mimomyia Theobald

Mi. plumosa (Theobald)

Mimomyia plumosa was found during the provincial survey (Davey,1939), but it was

recorded as a sub genus of Ficalbia. It was found resting on earth banks during the present survey.

Genus Uranotaenia Lynch Arribalzaga

Uranotaenia alba Theobald

Uranotania alba has not been previously recorded in Sierra Leone. In this study, one female was caught resting inside a bedroom in Bo.

Ur. balfouri Theobald

Uranotaenia balfouri has been found in Freetown and parts of the provinces (Davey, 1939 and Evans, 1925).

This species was caught in light-traps in Mendewa during the present survey.

Ur. bilineata var. connali Edwards

The only previous record of this species in Sierra Leone comes from the 1938 provincial mosquito survey (Anon, 1940). During the present survey it was collected in light-traps in Mendewa.

Ur. nigromaculata Edwards

There is no previous record of this species in Sierra Leone. In this survey, it was caught resting outdoors in a shaded pit shelter, in a coffee plantation in Mendewa, and in light-traps at Nyandeyama.

Ur. ornata Theobald

Uranotaenia ornata has only been previously recorded from Freetown (Evans, 1925) and Tonkolili District (Lewis, 1956).

In the present survey it was caught in pyrethrum spray collections in Mendewa.

CHAPTER 8

GENERAL DISCUSSION, SUMMARY AND CONCLUSIONS

8.1 The current malaria situation in southern Sierra Leone

Mosquitoes and their relation to malaria were well studied in Freetown up to the 1950s but very few investigations, concerning the disease and its transmission, were carried out in the provinces. The present study is part of the first attempt at a longitudinal investigation of the epidemiology of malaria in the provinces.

My entomological studies in the Southern Province were carried out in five villages in a high rainfall, forested area. Malaria was found to be hyperendemic in the study area, with transmission taking place throughout the year. The average prevalence of Plasmodium falciparum in both the dry and the wet seasons was about 62% (range 52 - 84%). The prevalence of P. malariae and P. ovale were 12% and 1% repectively. A total of 1105 children , between the ages of 0 and 7 years, were examined for malaria parasites in 14 villages in the wet season, but none was positive for P. vivax. Records of P. vivax in Freetown in the 1920s (Storey, 1972) may have been due to ex-service men who were stationed in the Far East during the First World War.

Preliminary investigations, by Dr G. Barnish into the problem of chloroquine

resistance, albeit based on a small number of samples, indicated that choloquine resistant malaria parasites were present in the area.

Treatment with chloroquine was virtually the only form of malaria control practiced by the community. Very few people used mosquito coils, or aerosols or practiced traditional forms of insecticidal treatment such as burning orange peel in houses. The only observed use of insecticides was against bedbugs. Only 6% of the people used mosquito nets, and the indications were that they were not always used to prevent man-mosquito contact. Possession of bednets appeared to be a status symbol, being more common in the houses of chiefs and well-to-do people.

Anopheles gambiae s.s. was the main vector in the study area responsible for over 90% of malaria transmission. The only other Anopheles species with sporozoite positive glands was An. funestus which was mainly a dry season vector. In one village, Mendewa, An. funestus was more important than An. gambiae s.s. in the transmission of malaria, during the dry season.

Among the 10 other Anopheles species observed during this study, An. brunnipes, An. coustani, An. flavicosta, An. hancocki, An. squamosus and An. ziemanni have been considered secondary or incidental vectors in other parts of Africa (Gillies & De Meillon, 1968). However, the low numbers of these species observed in this study would make them unimportant in malaria transmission, even if some of them were infective. In a sparcely populated area of the Northern Province of Sierra Leone, Lewis (1956) found one out of 115 (0.9%) An. hancocki with an infected gland,

but this could have been with a non-human malaria parasite.

The other Anopheles species observed were An. barberellus, An. marshalli, An. obscurus and one other species (probably new) which could not be identified using the available keys for Afrotropical mosquitoes (Gillies & Coetzee, 1987; Gillies & De Meillon, 1968).

Geographically, the study area was not homogeneous and the pattern of malaria transmission dynamics was different in the two groups of villages which were situated in areas of different landscape and vegetation. One group of villages which was situated at a relatively high altitude was surounded by derived savanna grassland vegetation. In these high altitude villages (300m above sea level), An. funestus was an important vector during the dry season. On the other hand, in the low altitude villages (100m above sea level), in forested areas, An. funestus was not an important vector in either the dry or wet seasons.

Anopheles gambiae s.s. densities were similar in the high and low altitude villages during the dry season, but during the wet season the vector densities in the low altitude villages were significantly larger than in the high altitude villages. The annual average man-biting rates of An. gambiae s.s. and An. funestus, for the combined project villages were 1.1 and 0.1 bites/man/night respectively. Even at these low vector densities, the intensity of transmission was ~~intense~~ high because of the high sporozoite rate (mean 7.4%) and high daily survival rate (0.85) of the main vector, An. gambiae s.s. The mean sporozoite rate for An. funestus was 11.4%.

Unfortunately the daily survival rate of An. funestus could not be determined because few females were caught at any one moment.

The small numbers of vectors caught in the project villages necessitated the identification of another village, Bayama, outside the project area, for ecological studies such, as mark-recapture experiments, which require large numbers of mosquitoes. In Bayama, the prevalence of malaria during the wet season was 58%, similar to the prevalence in the project villages, and An. gambiae s.s. was the only important vector. The mean annual indoor man-biting rate of An. gambiae s.s. in Bayama was 56.8 bites/man/night and was consequently much greater than the annual average for the project villages (1.1 bites/man/night).

It was therefore concluded that even in my relatively small study area, there were village to village variations in the pattern of malaria transmission. This observation is important in the planning of vector control measures.

8.2 The ecology and behaviour of Anopheles gambiae s.s. and Anopheles funestus in southern Sierra Leone

Anopheles gambiae s.s. was very anthropophagic, biting people both indoors and out of doors, from about 1800 - 0600 hr. Although it was endophagic it was surprisingly also highly exophilic, with a relatively small proportion of females remaining indoors to develop their eggs. Outdoor resting sites included rice barns, pit shelters, earth banks, overhanging thatch roofs, buttress roots, fences built with palm leaves and

some domestic containers eg. wooden mortars and buckets.

Anopheles gambiae s.s. also fed on other animals such as goats, sheep, dogs and pigs. Cattle were not observed in or near, the villages during the study period.

Anopheles funestus was also highly anthropophagic but less exophilic than An. gambiae s.s. Blood-feds were found resting outdoors in similar places as used by An. gambiae s.s. Anopheles funestus larvae were found in swamps and along the edges of streams, but surprisingly An. gambiae s.s. was not found breeding in swamps or rice fields. Anophesles funestus larvae were not found in the temporary open pools that were commonly colonised by An. gambiae s.s.

Anopheles gambiae s.s. was the only member of the An. gambiae complex identified, chromosomally, in the area, and all belonged to the Forest form.

8.3. Human behaviour and malaria transmission

The majority of people living in the study area were subsistence farmers and rice farming was their main occupation. Some people went to their farms, with their children, very early in the morning, around 0500 hr, when An. gambiae s.s. was very aggressive. Many people did not return to the villages until after dark, and when the upland rice crop was ripe, during September and August, some people stayed on their farms overnight, because they wanted to be present early in the morning to scare away birds. This practice of bird scaring could be a high risk activity in terms of

malaria transmission. However, the level of exposure to the transmission of malaria on the farms, especially early in the morning and late in the evening, is not known and needs to be investigated. Some people, however, reported being bitten by mosquitoes while on the farm.

House design in the rural community did not deter the entry of mosquitoes. Most houses had large eave gaps and the majority of rooms lacked ceilings. Studies in The Gambia have shown that closed eaves and the presence of a ceiling had a protective effect against malaria infection (Lindsay & Snow, 1988).

8.4 Biotechnology in field entomology

ELISA tests for the identification of mosquito blood-meals were successfully carried out in Bo. Reagents which arrived at the national airport in Freetown some five hours drive from Bo and which were kept overnight in a cool-box before being transported to the Bo laboratory, were still working after 12 months, despite intermittent electricity cuts. An ELISA plate reader was not necessary to identify positive reactions, especially if only freshly blood-fed mosquitoes were used. Weak positive colour changes sometimes occured with samples from semi-gravid female mosquitoes.

An ELISA plate reader has always been recommended in the procedure for sporozoite ELISA tests. However, a double blind, controlled experiment, performed by the author to test the reliability of visually reading sporozoite ELISA plates,

showed that visual assessment was more reliable than plate readers in determining sporozoite rates of An. gambiae s.s.

Both blood-meal and sporozoite ELISA tests have therefore been fully adapted for use in laboratories in the field. However, as Service (1991) emphasised in his review of the use of biotechnology in Anopheles research, there is a greater need for collaboration between molecular biologists and entomologists, especially at the stage of developing 'hightech' tools for use in entomological field research.

8.5 A checklist of mosquitoes of Sierra Leone

Besides Storey's (1972) list of anophelines in Sierra Leone and the list of Anopheles species by country, provided by (Gillies & De Meillon, 1968), there has been no checklist for the mosquito fauna of Sierra Leone. The list of mosquitoes found during the 1938 mosquito survey (Anon, 1940 and Davey, 1939) does not include many of the species found during other surveys. During the present studies, 11 culicine and one anopheline species were identified that have not been previously recorded in Sierra Leone.

Table 8.1 gives an updated checklist of mosquitoes found in Sierra Leone. This checklist is based on findings in the present and other studies carried out between 1900 and 1956 (Anon 1940; Blacklock, 1925; Blacklock & Evans, 1925; Butler, 1914,1915; Christophers & Puri, 1932; Davey, 1939,1941,1942; Evans, 1925, 1926, 1930,1932; Gordon & Macdonald, 1930; Gordon et al., 1932; Simpson, 1913; Wood,

TABLE 8.1 A checklist of the mosquitoes of Sierra Leone. Species marked with asterisks are new records for the country.

Genus Anopheles Meigen

An. barberellus Evans

An. brohieri Edwards

An. brunnipes (Theobald)

An. coustani Laveran

An. domicolus Edwards

An. flavicosta Edwards

An. freetownensis Evans

An. funestus Giles

An. gambiae Giles

An. hancocki Edwards

An. hargreavesi Evans

An. maculipalpis Giles

An. marshalli Theobald

An. melas Theobald

An. moucheti Evans

An. nili (Theobald)

An. obscurus (Grunberg)

An. paludis Theobald

An. pharoensis Theobald

An. rhodesiensis Theobald

An. rufipes (Gough)

TABLE 8.1 continued

An. somalicus Rivola and Holstein

An. smithii Theobald

An. squamosus Theobald

An. ziemanni Grunberg

Anopheles sp. indet.

Genus Aedes Meigen

Ae. aegypti (Linnaeus)

Ae. africanus (Theobald)

Ae. apicoargenteus (Theobald)

Ae. appicoannulatus Edwards

Ae. argenteopunctatus (Theobald)

Ae. argenteoventralis (Theobald)

Ae. cumminsii (Theobald)

Ae. domesticus (Theobald)

Ae. filicis Ingram and De Meillon

Ae. fraseri (Edwards)

Ae. haworthi Edwards

Ae. hopkinsi Edwards

Ae. insolens Edwards

Ae. longipalpis Grünberg

Ae. luteocephalus (Newstead)

Ae. nigricephalus Theobald

TABLE 8.1 continued

Ae. palpalis (Newstead)

Ae. proweri group (Theobald)

Ae. punctothoracis (Theobald)

Ae. scatophagoides (Theobald)

Ae. simpsoni Theobald

Ae. simulans Newstead and Carter

Ae. stokesi Evans

Ae. tarsalis (Newstead)

Ae. vittatus (Bigot)

Genus Culex Linneaus

Cx. albiventris Edwards

Cx. annulioris Theobald

Cx. antennatus (Becker)*

Cx. bitaeniorhynchus group Giles

Cx. cinerellus Edwards

Cx. cinereus Theobald

Cx. consimilis Newstead

Cx. decens Theobald

Cx. duttoni Theobald

Cx. ethiopicus Edwards*

Cx. galliardi Edwards*

Cx. grahami Theobald

TABLE 8.1 continued

Cx. guiarti Blanchard

Cx. horridus Edwards

Cx. inconspicuosus (Theobald)

Cx. ingrami Edwards

Cx. insignis (Carter)

Cx. irritans (Theobald)

Cx. invidiosus Theobald

Cx. kingianus Edwards

Cx. macfiei Edwards

Cx. moucheti Evans

Cx. nebulosus Theobald

Cx. nigricephalus (Theobald)

Cx. perfidiosus Edwards

Cx. perfuscus Edwards*

Cx. philipi Edwards

Cx. poicilipes (Theobald)*

Cx. pruina Theobald

Cx. quinquefasciatus Say

Cx. rima Theobald

Cx. telesilla De Meillon and Lavoipierre*

Cx. thalassius Theobald

Cx. tigripes De Granpré and De Charmoy

Cx. univittatus Theobald*

TABLE 8.1 continued

Cx. weschei Edwards

Cx. wigglesworthi Edwards

Genus Eretmapodites Theobald

E. argyrurus Edwards*

E. chrysogaster Graham

E. dracaenae Edwards

E. grahami Edwards

E. oedipodeios Graham

E. semisimplicipes Edwards

Genus Hodgesia Theobald

H. nigeriae Edwards

Genus Malaya Leicester

Malaya spp

Genus Mansonia Blanchard

Ma. africana (Theobald)

Ma. aurites Theobald

Ma. cristata (Theobald)

Ma. maculipenis (Theobald)*

TABLE 8.1 continued

Ma. metallica Theobald

Ma. uniformis (Theobald)

Genus Mimomyia Theobald

Mi. hispida (Theobald)

Mi. mimomyiaformis (Newstead)

Mi. plumosa (Theobald)

Mi. splendens Theobald

Genus Toxorhynchites Theobald

T. brevipalpis Theobald

T. evansae (Edwards)

T. phytophagus Theobald

Genus Uranotaenia Lynch Arribalzaga

Ur. alba Theobald*

Ur. annulata Theobald

Ur. balfouri Theobald

Ur. bilineata var. connali Edwards

Ur. fusca Theobald

Ur. mashonaensis Theobald

Ur. nigripes (Theobald)

TABLE 8.1 continued

Ur. nigromaculata Edwards*

Ur. ornata Theobald

1915).

8.6 Conclusions and recomendations

Traditional field sampling techniques and 'hightech' tools were successfully used in studying vector biology and malaria transmission in the Southern Province of Sierra Leone.

Anophleles gambiae s.s, the main vector of malaria in the Southern Province was extremely efficient. Hyperendemicity was maintained, even in the dry season, by a vector population density so low that biting was sometimes undetectable by the human-bait sampling technique.

Vector control aimed at reducing vector density, through larval killing or destruction of breeding sites, would be difficult to implement because breeding places are very difficult to locate and often very transient. Similarly, indoor residual spraying of houses to kill resting adults would be largely ineffective because of the exophilic nature of An. gambaie s.s.

Vector control methods such as the use of insecticide-impregnated bednets have received considerable attention for malaria control at community level. Impregnated bednets are very effective in reducing man-mosquito contact and their use in some malarious areas has also substantially reduced the number of infective bites received by a person in (Rozendaal, 1989).

In areas, or during seasons, with low malaria transmission, impregnated bednets have adequately reduced parasite rates, (Sexton et al., 1990), and mortality (Alonso et al., 1991) due to malaria. However, results from studies conducted in highly endemic areas, (Rozendaal, 1989), like ours have shown that a reduction in entomological inoculation rates only caused a reductions in the number of fever cases and parasite densities in positive slides. This is in agreement with our findings in the Bo area, where low entomological inoculation rates in the dry season were associated with low fever rates and parasite densities. In the wet season when the entomological inoculation rates increased substantially there were corressponding increases in fever rates and parasite densities, but not in parasite rates, which remained the same in both the dry and wet seasons. This further supports my suggestion made ealier that parasite rates or prevalence are not good indicators of transmission intensity in highly endemic situations. The fact that impregnated bednet use in highly endemic areas have not always brought about a reduction of prevalence should not, however, be interpreted to mean that they are not effective in reducing the disease. Despite the many years of malaria research, there is still a need to characterise malaria as a disease rather than simply in terms of parasite indices.

Preliminary results from bednet acceptibility studies in the Bo area (Marbiah pers comm., 1992) suggests an overwhelming approval of their use. In a poor rural community like Bo affordebility might pose a problem. However, the use of impregnated screens and curtains, which are sometimes more effective than impregnated bednets in reducing parasite rates (Sexton et al., 1990), could be a cheaper alterntive.

But whatever vector control method is used, planning should be at the village level because it is clear that there can be a small area variations in the pattern of malaria transmission.

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