

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

THE ECOLOGY OF THE ANTHROPOPHILIC ANOPHELES

MOSQUITOES IN THE GHIHE HORTICULTURE

DEVELOPMENT FARM

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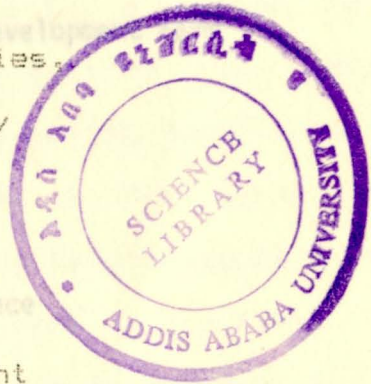
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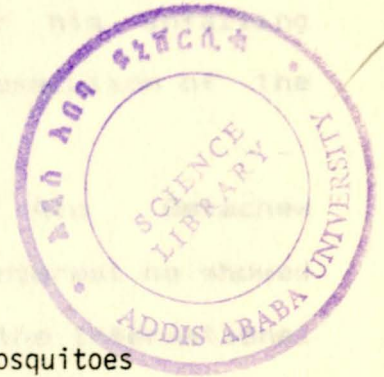


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The Ecology of the Anthropophilic Anopheles Mosquitoes
in the Ghibe Horticulture Development Farm

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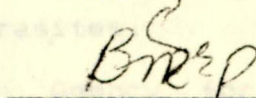
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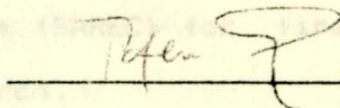
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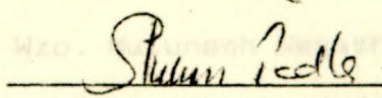
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ABSTRACT

Ecological studies on the anthropophilic *Anopheles* mosquitoes of Ghibe Farm were conducted between April, 1987 and March, 1988 (12 months), and between November, 1988 and February, 1989 (4 months). It was found that malaria is endemic in the

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presumably the most important malaria vector in Ghibe. *An. pharoensis* were mainly exophagous in their feeding behaviour, and may play a secondary role in malaria transmission in the area. The average man-biting rate of *An. gambiae* s.l. (3.0 bites per man per evening) indoors exceeded that of other anopheline species. Outdoor man-biting rate of *An. gambiae* s.l. (2.3) and *An. pharoensis* (2.5) were similar. The other anopheline species were less anthropophilic and more exophagic in their feeding behaviour. 62.7% of *An. gambiae* s.l. and 68.4% *An. pharoensis* caught on human bait were unfed

ABSTRACT

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Ecological studies on the anthropophilic Anopheles mosquitoes of Ghibe Farm were conducted between April, 1987 and March, 1988 (12 months), and between November, 1988 and February, 1989 (4 months). It was found that malaria is endemic in the area. P. falciparum was predominant (21.6% and 46.6%). P. vivax was detected at a very low frequency (1.7%). P. falciparum was more prevalent among children (63.8%) than among adults (36.9%). The anopheline fauna of the area was composed of An.gambiae s.l., An.pharoensis, An.coustani coustani, An.longipalpis, An. pretoriensis, An. natalensis, An. garrhami, An. christyi and An. squamosus. An. gambiae s.l. and An. pharoensis were the most anthropophilic species. An. gambiae s.l. were equally endophagic and exophagic and presumably the most important malaria vector in Ghibe. An. pharoensis were mainly exophagous in their feeding behaviour, and may play a secondary role in malaria transmission in the area. The average man-biting rate of An. gambiae s.l. (3.0 bites per man per evening) indoors exceeded that of other anopheline species. Outdoor man-biting rate of An. gambiae s.l. (2.3) and An. pharoensis (2.5) were similar. The other anopheline species were less anthropophilic and more exophagic in their feeding behaviour. 62.7% of An. gambiae s.l. and 68.4% An. pharoensis caught on human bait were unfed

1. INTRODUCTION

females. Fed females made up 23.8% and 19.7%, respectively. About 25.9% of An. gambiae s.l. and 24% of An. pharoensis dissected were pregravid and nulliparous females. 3-parous and older females constituted about 45.6% and 48.2% of the dissected females, respectively. The oldest females caught were 9-parous. Malaria is a major public health problem in many countries. Attempts to control malaria in most parts of Africa, as in other parts of the world during the last four decades, have met with challenging drawbacks and the disease remains a threat in the foreseeable future (Molineux and Bracciola, 1980; WHO, 1984).

Malaria is known to be a long-standing communicable disease in Ethiopia. At present it is reported as a major health problem in many parts of Ethiopia and its deleterious effect on the economy of the country far surpasses many of the other communicable diseases (Wang, 1966; Gebre Mariam, 1984). In Ethiopia the disease has influenced the settlement and occupational pattern of the population in the past. The gradual movement of the population from the overcrowded highland areas into the fertile and yet malarious lowland regions has been in progress for some time (Schaller, 1978; Gebre Mariam, 1984). Periodic malaria epidemics were common and their effects severe in many parts of the highland regions. Episodes of malaria epidemics at different

1. INTRODUCTION

Malaria is one of the most important vector-borne diseases that prevail in tropical Africa. The impact of the disease as a major public health problem in most parts of the tropics has been long understood. It is one of the worst scourges and a serious obstacle to development in many countries. Attempts to control malaria in most parts of Africa, as in other parts of the world during the last four decades, have met with challenging drawbacks and the disease remains a threat in the foreseeable future (Molineaux and Gramiccia, 1980; WHO, 1984).

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times are well documented (Covel, 1957; Fontaine et al., 1961; Chand, 1965; Wang, 1966; Schaller, 1972). In a summarized presentation on the malaria situation, Gebre Mariam (1984) reported about 75% of the country as either malarious or potentially malarious, and 64% of the Ethiopian population at risk of being infected. Despite the continued control operations dating back to the 1960s when the Malaria Eradication Service was established, the disease has still persisted in many different foci in the different regions of Ethiopia. As in many other countries, altitude, topography and climatological factors mainly determine the prevalence of malaria in Ethiopia (Covel, 1957; Wang, 1966; Schaller, 1972; Gebre Mariam, 1984). The disease is endemic in different foci and the most vulnerable and endemic areas are found in the lowland regions of the country. Malaria transmission seldom occurs above 2000 meters and the disease is unstable in higher altitudes, for instance beyond 1800 m. Climatological factors such as rainfall and temperature at such high altitudes arrest the required population dynamics of vector species and the extrinsic cycle of the malaria parasites in the vector for stability of the disease. However, periodic epidemics occur as a result of exceptional weather, which favour proliferation and longevity of vectors and the extrinsic development of the parasites at altitudes

over 1800m (Wang, 1966; Fontaine et al., 1961).

Variation in the degree of endemicity is also determined by altitude, topography and climatological factors. Meso- and hyperendemic areas are found at lower altitudes with permanent breeding sites. In low-altitude riverine areas with convenient breeding sites, transmission is usually perennial with slight seasonal variations in magnitude. The peak transmission seasons, following the end of the heavy rains in mid-September and around the short-lived showers of April - May, are more or less similar in most parts of the country (Gebre Mariam, 1984).

Anopheles mosquitoes are the only insect vectors of human malaria under natural conditions and are crucially important in the epidemiology of malaria transmission. Pioneer information relating to malaria in Ethiopia goes back to investigations and surveys carried out by Italian workers during the Ethio-Italian war. Both preliminary and detailed studies on the epidemiology of the disease were conducted by Lega et al. (1937), Corradetti (1939, 1940) and Mara (1943, 1950). Melville et al. (1945) also studied the prevalence and transmission of malaria and the vectors involved in different parts of Ethiopia. Giaquinto-Mira (1950) has investigated the distribution and biology of the Anopheles mosquitoes in different parts of Ethiopia. Information on the anopheline fauna was enriched by Covel

(1957) during his investigations on malaria and its vectors in various parts of the country.

Later on, some detailed work on malaria and Anopheles mosquitoes was undertaken in different parts of the country. Fontaine et al. (1961) reported on the 1958 malaria epidemics, which had devastating effects in a number of the highland provinces in the country. Subsequently, Verrone (1962a, 1962b) produced keys for the determination and identification of malaria mosquitoes to clarify the epidemiology of malaria in Ethiopia. O'Connor (1967) also reviewed and recorded the distribution of the Anopheles species of Ethiopia. Krafsur (1970a, 1970b, 1971, 1977) reported detailed studies on the ecology, bionomics, and population dynamics of the Anopheles mosquitoes of Gambela with respect to malaria transmission. Schaller (1972) summarized pertinent information regarding the epidemiology of malaria in Ethiopia.

Notwithstanding the importance of these and similar works, the scarcity of entomological data on vector species in different malaria foci in Ethiopia is strongly felt (Gebre Mariam, 1984). The Ghibe Horticulture Development Farm, where this study was conducted, occupies a part of the Ghibe River valley system. It is one of the many fertile lowland malarious areas which is being turned into a successful agricultural development scheme. In view of

current situations such as famine and resettlement, emphasis is laid upon the expansion of agriculture as one of the ways for development in the country. Malaria is predominantly a rural disease and thus severely hampers agricultural development. It has been long understood that in malarious areas agricultural development schemes could be successful only when the problems of the disease are curtailed or resolved. Entomological studies on the distribution, identity, ecology, behaviour and vectorial status of anopheline mosquitoes, together with a sound knowledge of associated environmental factors of the different malarious areas, would ultimately contribute to the control and possible eradication of malaria from any country, including Ethiopia. It is believed that base-line data obtained from close and effective entomological, parasitological and related studies will facilitate an understanding of the factors which bear upon the persistence or aggravated transmission of malaria in different regions.

The aim of the present project was to gather year-round relevant information on the anthropophilic anopheline mosquitoes of the Ghibe Horticulture Development Farm, and thereby facilitate future control of malaria and its vectors in and around Ghibe.

Here after I will attempt to review the most relevant information on the Anopheles mosquitoes that are widespread throughout different parts of Ethiopia, including that of

and important as vectors of malaria in Ethiopia and the rest of Africa. The large number of the Anopheles species that exist in Ethiopia and elsewhere in Africa have been intentionally left out unless conditions dictated. The following topics have been given special attention in this review.

1.1 Distribution of Anopheles Mosquitoes

Among earlier workers who studied the distribution of Anopheles mosquitoes in Ethiopia, Lega et al. (1937) reported the occurrence of about 14 anopheline species in different parts of Ethiopia. Corradetti (1939, 1940) recorded more than ten anophelines in Wollo province and considered An. gambiae and An. pharoensis as primary and secondary vectors of malaria, respectively. Mara (1943) identified An. d'thali and An. gambiae as the dominant species along the Barca river in Agordat, northern Ethiopia. Mara (1950) also reported about eight anopheline species in Tessenei (North-eastern Ethiopia) and incriminated An. gambiae as the principal vector of malaria, causing epidemics in the area. The other anopheline species including An. pharoensis, An. squamosus, An. coustani, An. d'thali and An. pretoriensis were presumed to play some role in aggravating the epidemic situation. Giaquinto-Mira (1950) described the distribution and breeding habitats of some of the important Anopheles mosquitoes of different parts of Ethiopia, including that of

An. gambiae, An. funestus, An. nili, An. pharocensis, An. longipalpis, An. pretoriensis, An. christyi, An. garnhami and others. Verrone (1962a, 1962b) recognized the existence of about 34 species of Anopheles in Ethiopia. But one of the most comprehensive works on the distribution of Anopheles mosquitoes that of O'Connor (1967). The author reviewed the works of many investigators and recorded the distribution of 34 species and 2 subspecies by province. Lack of evidence of More recent reports indicated that about 42 species of Anopheles mosquitoes have been identified and recorded from Ethiopia (Gebre Mariam, 1984). As for most parts of Africa south of the Sahara, the main vectors of malaria in Ethiopia are the An. gambiae complex and the An. funestus group (Melville et al., 1945; Giaquinto-Mira, 1950; Covel, 1957; Fontaine et al., 1961; Mekuria and Woldetsadik, 1970; Wang, 1966; O'Connor, 1967; Schaller, 1972; Gebre Mariam, 1984). According to these reports, An. gambiae s.l. was the major vector responsible for epidemic outbreaks in some parts of Ethiopia, whereas An. gambiae s.l. and the An. funestus group are the most important vectors responsible for the maintenance of endemic malaria in the country. An. gambiae s.l. is widely distributed throughout Ethiopia (O'Connor, 1967). The recognition of the existence of sibling species of the An. gambiae complex initiated the demarcations of the distribution of these sibling species (Davidson, 1967; White,

1974a). Among the six sibling species in the An. gambiae complex, An. gambiae s.s., An. arabiensis and An. quadriannulatus occur in fresh water habitats; and only An. arabiensis and An. quadriannulatus have been recorded from Ethiopia (White, 1974a; Krafsur, 1977).

An. quadriannulatus has been reported to occur in the highland regions of Ethiopia. So far, this species has not been incriminated as a vector of malaria. Lack of evidence of natural infection in An. quadriannulatus may be ascribed to its low anthropophilic indices, higher degree of exophily and the cool climatic conditions in which this species occurs, which limits extrinsic parasite development (White, 1974a; White et al., 1980). According to these workers cytogenetic methods can be effectively used to distinguish the non-vector An. quadriannulatus from the efficient vector An. arabiensis, both of which occur in Ethiopia. Since An. gambiae s.s. has never been recorded in Ethiopia, malaria transmission by An. gambiae complex as cited by various workers in the past lends credence to An. arabiensis as the principal vector of malaria in the country.

Most of sub-Saharan Africa is infested with a mixture of An. gambiae s.s. and An. arabiensis. But it is also recorded that these species occupy separate geographical areas, as a result of climatic factors (Davidson, 1967; White, 1974a). Observations have indicated that An. arabiensis is widely distributed in a wide range of habitats, usually in semi-permanent water bodies.

associated with more arid habitats. According to White et al. (1972) and White (1974a), An. gambiae s.s. is unknown so far from the Horn of Africa and southern Arabia, where An. arabiensis is common and widespread, probably due to the barriers formed by belts of dry savannah and steppes.

The An. funestus group, a principal vector of malaria second to An. gambiae s.l. in Ethiopia and other parts of Africa, are found in most of the regions in the country. An. funestus have a scattered and relatively localized distributional pattern, probably due to their preference to breed in large and more or less permanent water-bodies, where shade is available (Smith, 1964; O'Connor, 1967).

Vectors of secondary importance in the transmission of malaria in the various endemic foci of Ethiopia include An. nili and An. pharoensis (Krafsur, 1977; Gebre Mariam, 1984). In some restricted localities where the principal vectors are absent or rare, these species were found to be the major vectors. It is also reported as a stream breeder, showing vectors of local importance, both in Ethiopia and elsewhere in Africa (Service, 1968; Wang, 1966, Krafsur, 1970a).

1.2 Breeding Habitats

Anopheles mosquitoes breed in all kinds water-bodies and the breeding habitats are as varied as the altitudinal ranges in which they occur. The most important and widespread malaria vector in Ethiopia, An. arabiensis, breeds in a wide range of habitats, usually in semi-permanent water bodies.

Generally, An. arabiensis has a preference to breed in semi-permanent pools of rainwater or overflow pools, roadside ditches, clogged drainage ditches, small borrow pits, wheel ruts, hoof-prints, natural depressions in the ground, small irrigation canals and man-made cement-lined tunnels (Stevens, 1984; Zahar, 1984). Wang (1966) reported An. gambiae s.l. to breed in habitats such as puddles, shallow ponds, pits, animal footprints, ditches, seepages and along edges of lakes and swamps. O'Connor (1967) also reported An. gambiae s.l. found breeding in small, natural or artificial, sunlit collections of water usually without floating vegetation.

The An. funestus group breeding habitats are characteristically of a more or less permanent nature. Vegetation shade is highly preferred (O'Connor, 1967). Covel (1957) and Krafur (1977) reported An. funestus as the dominant species in swampy areas along the edges of lakes and rivers. It is also reported as a stream breeder, showing strong preference for shade. De Meillon (1971a) reported the An. funestus group to breed in shaded habitats found along perennial streams and rivers. On the whole, clear freshwater is preferred and organic pollution not tolerated.

An. pharoensis has a patchy, more or less similar pattern of distribution to that of the An. funestus group. In Ethiopia this species breeds in shaded permanent water found near swamps, along lake shores, in rice fields and in

irrigation ditches (O'Connor, 1967). De Meillon (1951a, 1951b) reported An. pharoensis as mostly a swamp breeder.

An. nili was reported to be mainly a stream breeder and was frequently found along the edges of streams and large rivers in vegetation or in shade (De Meillon, 1951a, 1951b). Krafsur (1970, 1977) reported that peak density of An. nili correspond nicely with maximum flooding of the Baro river in Gambela. The author recorded the occurrence of about 16 Anopheles species in Gambela and associated the prevalence and density of most species with the flooding of the Baro river. The principal breeding sites of all these species were broad pools and swamps formed by flood water (Krafsur, 1977).

An. coustani coustani, An. Christyi and An. squamosus are mainly swamp breeders in Africa (De Meillon, 1951a, 1951b). According to this author, An. squamosus requires some shade. An. coustani coustani can breed without shade as far as floating vegetation is available. In the case of An. christyi, shade is not essential and larvae are tolerant of organic pollution (De Meillon, 1951b). An. pretoriensis breeds in a wide variety of water-bodies which are not heavily shaded, including seepages.

1.3 Feeding and Resting Habits

Feeding and resting habits of female adult Anopheles

mosquitoes are essential components of the epidemiology of malaria and determine the importance of vector species. Moreover, the effectiveness of indoor residual spraying with insecticides depends, among other factors, on the feeding and resting habits of the vector species involved. An. gambiae s.s. and An. arabiensis are the two most anthropophilic and efficient vector species of the An. gambiae complex (Pant et al., 1981). An. quadriannulatus appears to be entirely zoophilic and exophilic, and probably is not a vector of human malaria (Davidson, 1967; White, 1974a; White et al., 1980). Precipitin test results from several An. quadriannulatus females from Ethiopia indicated a negligible Human Blood Index (HBI) (Smith, 1964; White et al. 1980).

Anopheles gambiae s.s. is more anthropophilic and endophilic than An. arabiensis (Davidson, 1967; Service, 1970; White et al., 1972; White, 1974a; Highton et al., 1979). According to these reports An. arabiensis is more catholic in its feeding and resting behaviour, depending on the availability of hosts and hospitable resting sites.

According to Highton et al. (1979), An. gambiae s.s. was found to be more anthropophilic than An. arabiensis with 92% of the former feeding on man compared to 39% of An. arabiensis. Cattle were the favoured host of An. arabiensis with 59% choosing this host compared to only 5% of An. gambiae s.s.. In the presence of a choice of suitable hosts, the HBI

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of An. gambiae s.s. exceeded that of An. arabiensis (White et al., 1972, Highton et al., 1979). White et al. (1972) have reported HBI values of 91.2% for An. gambiae s.s. and 60.9% for An. arabiensis, whereas the HBI value for An. funestus group was 97.5%. An. arabiensis were more exophilic (2.2 times) than An. gambiae s.s. (Highton et al., 1979). Krafsur (1977) reported that An. gambiae s.l. (presumably An. arabiensis) and An. funestus group females were to a large extent endophagic in their feeding behaviour, whereas An. pharoensis showed a similar choice of feeding indoors and outdoors. An. nili and An. wellcomei showed considerable endophagic behavior in Gambela, but the An. coustani complex were markedly exophagic.

Giaquinto-Mira (1950) reported house frequenting habits both in An. coustani coustani and An. christyi, whereas elsewhere in Africa these species are regarded as more or less exophilic and generally zoophilic (De Meillon, 1951b). An. coustani ziemanni, An. demeilloni, An. implexus, An. rufipes, An. maculipalpis, An. paludis, An. pretoriensis, An. rhodesiensis and An. squamosus are generally considered exophilic species, which do not enter human habitations readily. These species and others are known to occur in Ethiopia. Since they have been found infected with malaria parasites in nature (De Meillon, 1951b), the feeding and resting behaviour of these species have been observed in

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regard to their importance as possible local vectors of malaria.

1.4 Sporozoite Rate and Vectorial Efficiency of Anopheles Mosquitoes

Among the various species of Anopheles mosquitoes described and recorded in Ethiopia, only a few are recognized as important vectors of malaria. The principal vectors are An. arabiensis and the An. funestus group, while An. pharoensis and An. nili play a role as secondary vectors in the transmission of malaria (Gebre Mariam, 1984). Important factors that determine the vector status of a species include longevity of that species, favourable climatic conditions, availability of host, and feeding and resting habits. The combined effect of these factors should ensure the vector to live long enough to allow development and transmission of the malaria parasite to another host.

Vector status of anopheline species can be measured by determining the sporozoite rate in a given population. The sporozoite rate, together with the biting frequency and inoculation rate, give an index of the risk of malaria infection in a given area (Molineaux and Gramiccia, 1980). A number of investigators have recorded sporozoite rates for the main vectors of malaria in Ethiopia and other parts of Africa.

Bruce-Chwatt (1951) reported a mean overall sporozoite rate in the An. gambiae group as 5.89% and a comparable figure of 4.96% in the An. funestus group. De Meillon (1951b) reviewed the results of dissections by various workers and enumerated about 30 Anopheles species and subspecies found naturally infected with malaria parasites. But it was unknown whether the infections were due to human or non-human species of Plasmodium. Vincke (cited by De Meillon, 1951b) reported two infections among 2,116 An. coustani coustani dissected; he suggested a possible short life-span of the female mosquito to explain the minimum possibility of acquiring infection in this species. Giaquinto-Mira (1950) found one specimen of An. coustani ziemanni with salivary gland infection in Harar, but the number of mosquitoes dissected was not specified. De Meillon (1951b) stated that salivary gland infection rates in An. nili ranged from 0.8 to 9.1% in different parts of Africa when the species showed a marked degree of anthropophily and endophily. Davidson and Draper (1953) reported 4.7% of the An. gambiae s.l. and 3.4% of the An. funestus group as naturally infected with sporozoites in their salivary glands. Service (1963) dissected some mosquitoes in Nigeria and found a sporozoite rate of 2.42% in An. gambiae s.l. and 1.65% in An. funestus. White et al., (1972) reported an overall sporozoite rate of 4.23% in An. gambiae s.s., 1.62% in An.

Whittes, 1963). It has been stated in earlier works that the funestus, 0.32% in An. arabiensis and 2.68% in An. gambiae s.l. in Tanzania.

According to O'Connor's (1967) review, attempts to determine the vector status of suspected anophelines in Ethiopia were scarce. Disregarding salivary gland dissections with negative findings, sporozoite rates of 0.03% in Wollo, 0.15% at Marti in the Awash valley, 0.2% in the lake regions in Shoa and Sidamo were reported in An. gambiae s.l. (presumably An. arabiensis). But dissections in An. funestus to determine sporozoite rates, as reported so far, are limited and inconclusive. In Gambela, Krafsur (1977) found that the infection rate in An. gambiae s.l. (most probably An. arabiensis) was 1.87%, in An. funestus 1.23% and that for An. nili was 1.29%. No positive specimens were found in the 155 dissections of An. pharoensis, nor in the 47 dissections of An. wellcomei.

1.5 Age Composition and Population

Structure in Anopheles Mosquitoes

The study of the age composition of populations of Anopheles mosquitoes and other haematophagous Diptera has undergone various modifications through time. The pioneer works of Polovodova and Detinova in the 1950s physiological age determination based on counts of dilations in the ovarioles has lend a strong basis for relatively refined present-day methods used in Anopheles mosquitoes (Gillies and

Wilkes, 1965). It has been stated in earlier works that the method was difficult to apply effectively to the chief vectors of malaria in Africa (An. gambiae Giles and An. funestus Giles) (Detinova, 1962; Giglioli, 1964). Age determination in An. gambiae Giles and An. funestus Giles were therefore restricted to a gross determination of the proportions of nulliparous and parous females in the study population based on the absence or presence of follicular relics, respectively. According to Detinova (1962), this method was recommended to elucidate the population structure of the major Anopheles vectors of malaria in Africa. The same author also stated that mosquitoes of all stages of blood digestion could and should be used for dissections to determine the physiological age of Anopheles populations.

Detinova and Gillies (1964), based on some preliminary studies sponsored by the World Health Organization (WHO), concluded that Polovodova's method could be effectively applied to An. gambiae and An. funestus except that it would be more difficult than in An. maculipennis. Gillies and Wilkes (1965) then carried out a series of dissections for age determination on populations of both An. gambiae and An. funestus in Tanganyika and compared their data with the Calendar age obtained from mark-release-recapture techniques. The authors reported that the reliability of the ovarian aging method, in the case of An. gambiae and An. funestus,

mainly depended on experience and proper handling of the ovaries and ovarioles while dissecting. Nevertheless, the works of Detinova (1962, 1963), Detinova and Gillies (1964), Giglioli (1964) and Gillies and Wilkes (1965) assert that this method of physiological age determination is applicable to major vectors of malaria in Africa. However, the statement that reliability of results depended on experience and expertise should not be overlooked.

Studies on the longevity of An. gambiae s.l. and An. funestus in different parts of Africa indicated that the oldest individuals were those which had completed 13 and 14 gonotrophic cycles (Gillies and Wilkes, 1965). Detinova and Gillies (1964) also reported that the oldest An. gambiae s.l. females were individuals in their seventh gonotrophic cycle and the oldest in An. funestus were females in their tenth gonotrophic cycle. From mark and recapture experiments, Gillies and Wilkes (1965) found that the oldest An. gambiae s.l. female was 34 days old and had laid eggs 10 times. So far, it has been asserted that 3-parous and older females of An. gambiae s.l. and An. funestus are epidemiologically important in the transmission of malaria (Detinova and Gillies, 1964; Gillies and Wilkes, 1965). Studies on the longevity of Anopheles mosquitoes are important to assess the extent to which control measures are effective. The presence of older individuals in a population indicates that probably

control measures are not as effective as they are intended to be (Detinova, 1968). Studies on the age structure of populations of Anopheles mosquitoes have been used to obtain information to estimate the degree of emergence and survival of the vector species in relation to disease transmission.

It must be pointed out here that, apart from the limited volume of information in the literature on malaria vectors in other parts of tropical Africa, there is a scarcity of published information on attempts to determine the age structure of Anopheles mosquitoes in Ethiopia using ovariole dissections. This fact should be appraised in view of the need for such information to elucidate the epidemiology of malaria in any part of Ethiopia. Between November, 1968 and February, 1969. As a result of this, data are now available only for the periods April, 1967 to March, 1968 and November, 1968 to February, 1969.

2.2 Description of the Study Area

The Ghibe Horticulture Development Farm of the Ministry of Agriculture is located in the Ghibe river valley system. The Ghibe river is part of the upper course of the Omo river system in South-western Ethiopia and is also known as the Upper Omo. The study area is situated at about 5 km. to the east of the main Ghibe bridge, which is some 120 km. south-west of Addis Ababa, along the Addis Ababa-Jimma road (Fig.

2. MATERIALS AND METHODS

2.1 Study Periods

Actual sampling activities in Ghibe were preceded by two preliminary field trips in February and March, 1987. These trips were devoted to selection of sampling sites, orientation of field assistants and overall reconnaissance of the study area. Assessment of the malaria problem in the area and the feasibility of a routine year-round study were considered during these trips. Routine monthly field trips to the study area were made between April, 1987 and March, 1988. Technical problems forced the interruption of monthly field trips which were resumed between November, 1988 and February, 1989. As a result of this, data are now available only for the periods April, 1987 to March, 1988 and November, 1988 to February, 1989.

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The Ghibe Horticulture Development Farm of the Ministry of Agriculture is located in the Ghibe river valley system. The Ghibe river is part of the upper course of the Omo river system in South-Western Ethiopia and is also known as the Upper Omo. The study area is situated at about 3 km. to the west of the main Ghibe bridge, which is some 180 km. south-west of Addis Ababa, along the Addis Ababa-Jimma road (Fig. 1).

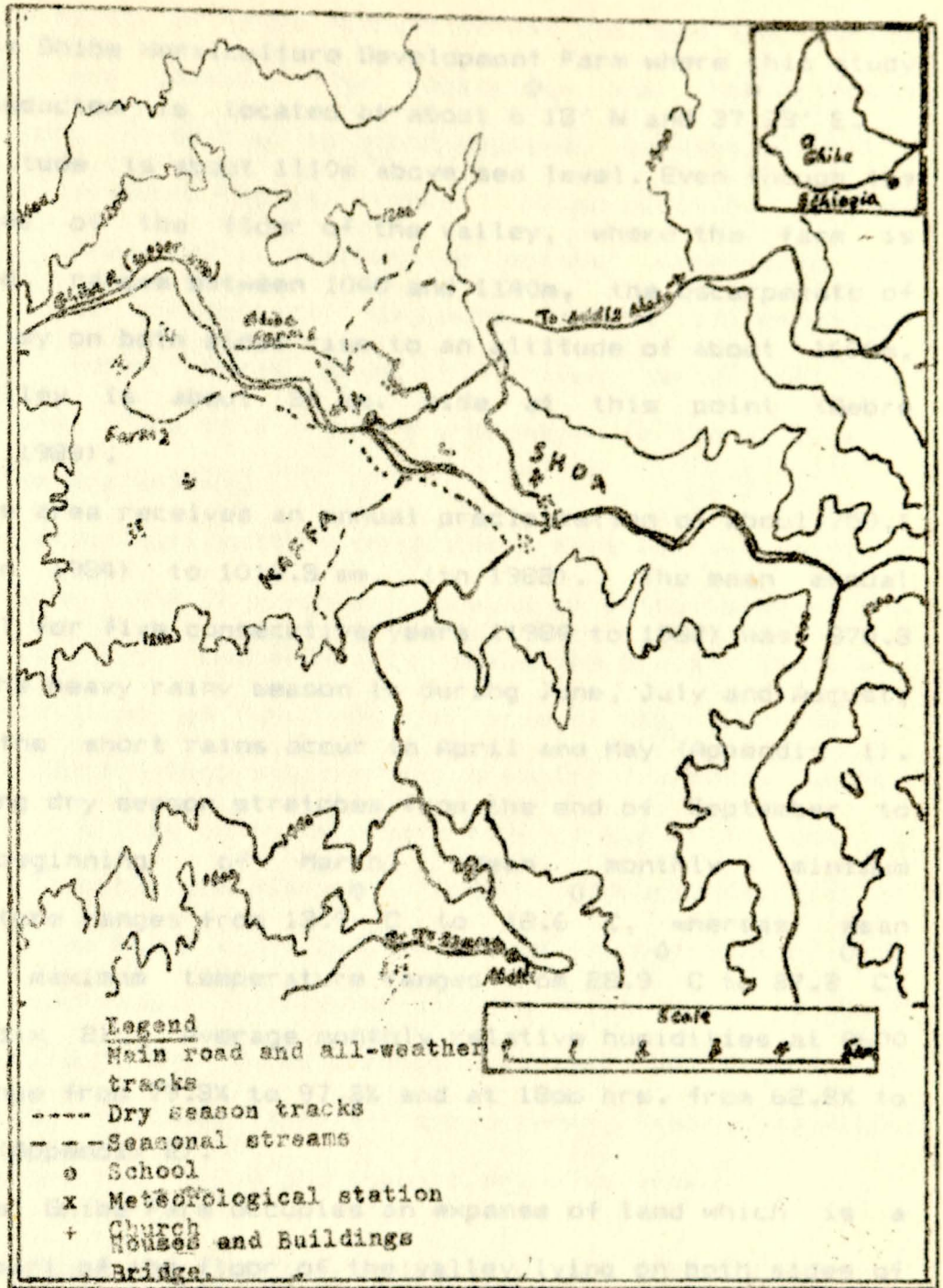


Figure 1 : Map showing Topography of the Ghibe Valley
 (Adopted from Gebre Michael, 1983)

The Ghibe Horticulture Development Farm where this study was conducted is located at about 8° 13' N and 37° 33' E. Its altitude is about 1110m above sea level. Even though the altitude of the floor of the valley, where the farm is situated, ranges between 1040 and 1140m, the escarpments of the valley on both sides rise to an altitude of about 1600m. The valley is about 30 km. wide at this point (Gebre Michael, 1989).

The area receives an annual precipitation of about 759.1 mm. (in 1984) to 1016.3 mm. (in 1988). The mean annual rainfall for five consecutive years (1984 to 1988) was 870.3 mm. The heavy rainy season is during June, July and August; while the short rains occur in April and May (Appendix 1). The long dry season stretches from the end of September to the beginning of March. Mean monthly minimum temperature ranges from 13.9 °C to 18.6 °C, whereas mean monthly maximum temperature ranges from 28.9 °C to 37.3 °C (Appendix 2). Average monthly relative humidities at 0600 hrs. range from 79.3% to 97.3% and at 1800 hrs. from 62.8% to 81.33% (Appendix 2).

The Ghibe Farm occupies an expanse of land which is a large part of the floor of the valley lying on both sides of the river. The Ghibe river, at this point of its course, flows in a west-to-east direction. The type of prevailing vegetation is open grassland savannah. The vegetation

comprises mainly of grass whereas shrubs and woodland species of trees are common. The vegetation and the wildlife composition in addition to other physical parameters, were described by Gebre Michael (1983). Human activity in the area has drastically cleared the natural vegetation for domestic and agricultural purposes. Strips of perennial riverine forest trees along the river banks are but rudimentary. Fire is a frequent phenomenon in the hilly area during the dry season.

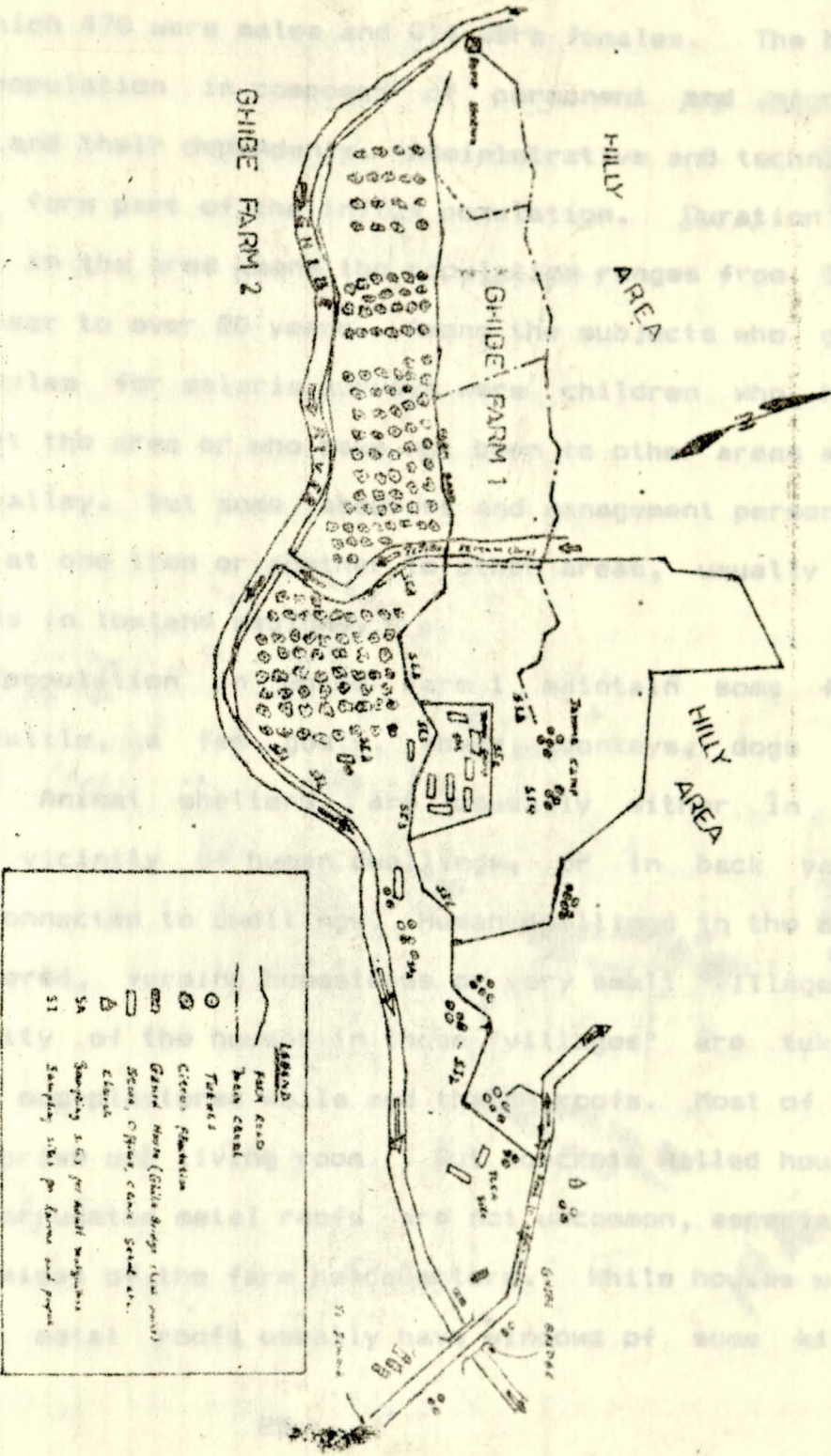
The Ghibe Horticulture Development Farm has two sections (Ghibe Farms 1 and 2) which are located on either side of the river (Fig. 2). Ghibe Farm 1, where the present study was conducted, lies on the northern side of the river and is part of Western-Shoa Administrative Region (formerly Shoa). Ghibe Farm 2 lies on the southern side of the river and is part of Illubabor Administrative Region (formerly Kaffa). At present, the Ghibe farms cultivate some 689 hectares of land in both sections. Cultivated land in Ghibe Farm 1 is about 469 hectares. The farms produce a variety of fruits and vegetables. The most important are oranges, lemons and other citrus products. Maize and peppers are also grown.

2.3 Human and Animal Populations of the Study Area

According to a recent census by the farm management, the human population in Ghibe Farm 1, where the main headquarters



Figure 2 : Sketch Map of the Ghibe Horticulture Development Farm 1.



are is situated, was about 1041. Of these, 535 were males and 506 were females. The human population in Ghibe Farm 2 was 952 of which 478 were males and 474 were females. The bulk of the population is composed of permanent and migrant labourers and their dependents. Administrative and technical personnel form part of the influx population. Duration of residence in the area among the population ranges from less than a year to over 20 years. Among the subjects who gave blood samples for malaria surveys were children who have never left the area or who have not been to other areas away from the valley. But some labourers and management personnel have been at one time or another to other areas, usually to state farms in lowland regions.

The population in Ghibe Farm 1 maintain some four hundred cattle, a few goats, sheep, donkeys, dogs and poultry. Animal shelters are usually either in the immediate vicinity of human dwellings, or in back yards directly connected to dwellings. Human dwellings in the area are scattered, forming homesteads or very small "villages". The majority of the houses in these "villages" are tukuls which have mud-plastered walls and thatch roofs. Most of the tukuls comprise one living room. But concrete walled houses and with corrugated metal roofs are not uncommon, especially in the premises of the farm headquarters. While houses with corrugated metal roofs usually have windows of some kind,

most of the tukuls are constructed without windows. The type of tukul construction, nonetheless, allows entrance of mosquitoes through the eaves to the inside of such houses.

It should be emphasised that house-to-house spraying with DDT by the Division of Control of Malaria and Other Vector-borne Diseases (DCMOVD, formerly Malaria Eradication Service) has been ongoing in the study area for more than five years. Spraying was carried out twice a year, usually in January and June. Spraying was sometimes reinforced by the use of larvicides in places with large temporary collections of water. Field workers from DCMOVD also supply and administer anti-malaria prophylactic drugs at times of severe malaria transmission. The farm clinic under the health assistant treats suspected malaria cases and occasionally provides prophylactic drugs during peak transmission periods. The clinic also refers serious cases to nearby government and missionary hospitals.

One of the most important features of Ghibe during the dry season is the deployment of the river water for irrigation purposes. Water intercepted at a number of sites from the river is run through irrigation canals to the farm. The main pump station is located up-river at a distance of about 1km. from the farm headquarters and the main camp (Fig. 2). In addition to the clinic, the farm runs a school, a motor house and workshops. There is a field station of the

International Livestock Center for Africa (ILCA), which is located immediately by the main Ghibe bridge and on the way to Ghibe Farm 1. Most of the important landmarks in and around the study area are shown in Figure 2.

2.4 Malaria Infection in Humans

At the initial stage, blood samples from about 5% (60 subjects) of the human population in Ghibe Farm 1 were taken in May 1987. Later on, blood samples from about 10% (131 subjects) of the population in Ghibe Farm 1 were taken (in November 1988) to reinforce the first preliminary parasitological findings. Both thick and thin blood smears from individuals of different age groups were stained with standard Giemsa stain. Two sets of both thick and thin blood smears were taken from each person. One set was stained and microscopically examined in the field, and the second pair in the laboratory in Addis Ababa. All thin smears were fixed on the spot with absolute methanol after the smears were taken. All stained slides were microscopically examined for the presence of Plasmodium species. Identified positive slides were taken to DCMOVD for confirmation.

Information on sex, age, occupation and history of movement of the subjects who volunteered to give blood samples were recorded. Positive slides were preserved for reference purposes. Species identification of Plasmodium parasites was effected by reference to Jeffrey and Leach

(1975), Faust et al. (1975) and Nobel and Nobel (1982).

2.5 Survey of the Anopheles Mosquitoes of Ghibe

Anopheles mosquitoes, both mature and immature stages, were sampled using different entomological techniques, as described in the sections below. Adult Anopheles females were sampled on a monthly basis throughout the study period. Immature stages (particularly larvae) of anopheline mosquitoes were collected at different times of the year as conditions allowed. Identification and processing of collected adult and immature stages of mosquitoes were performed using appropriate methods in the field and the laboratory, in Addis Ababa.

2.5.1 Collection and Identification

Larvae and Pupae:

Larvae of Anopheles mosquitoes were collected in different months using a number of sampling techniques recommended by WHO (1963). Choice of sampling techniques largely depended on the type and nature of breeding places at different times. Most of the time anopheline larvae and pupae were sampled using pipettes, rubber bulbs, scoops and ladles. Dip nets with small mesh-sizes were also used. As a result of the semi-permanent and temporary nature of the breeding habitats in the area, sampling sites largely

The field assistant was stationed inside while another depended on where breeding places were available.

Most frequented sampling sites for immature stages are indicated in Figure 2. Collections of larvae and pupae were usually conducted in the morning and in the afternoon, so that collection was possible with minimum discomfort to the collector. Collected pupae were put in cups and left in cages, to allow emergence of adults for species identification.

Collected larvae were preserved in about 70% ethanol and later on processed and mounted on microscope slides as suggested by Lane (1974). Live larvae were also killed in hot water (60 C) and then processed and mounted on slides. Specimens mounted in Canada balsam were allowed to dry and examined later under a compound microscope. Identification to the species level was based on Verrone (1962b). In some cases, live larvae were brought to the laboratory and reared to the adult stage for species identification purposes.

2.5.2 Adult Anopheles Sampling and Identification

Adult anthropophilic Anopheles mosquitoes were collected on a routine monthly basis using human bait. For the 12-months period between April, 1987 and March, 1988, two collection sites (SA1 and SA2) were selected for regular monthly indoor and outdoor collections (Fig. 2). At each

site one field assistant was stationed inside while another was stationed outside, usually from five to ten metres away from the house. Later on, human bait collections at SA2 were interrupted due to difficulties in supervision.

Each collector was provided with a flashlight, a number of test-tubes, cotton wool and plastic beakers, whose open ends were closed with mesh cloth having large holes to enable the transfer of captured mosquitoes from test-tubes to the beakers. Collection normally started at 18:30 hr. and terminated at 24:00 hr. for four consecutive nights per month, unless collection was interrupted by rain, in which case it was postponed to the next evening. Indoor and outdoor collectors exchanged positions at the end of every hour, at which time hourly collections were recorded. Field assistants were supplied with chloroquine tablets and were consistently advised to take them as prophylaxis at the beginning of each monthly trip.

All adult female anopheline mosquitoes collected were identified either the same evening or the next day. Abdominal conditions of freshly caught female mosquitoes were recorded as unfed, fed or gravid. Mosquitoes which died during the night in test-tubes or plastic beakers were identified and pinned. Identification of female Anopheles mosquitoes was made by the keys of Verrone (1962a). Some

identified and pinned specimens were later confirmed at DCMOVD, Ministry of Health.

2.6 Dissection of Adult Anopheles to Determine Age Structure and Natural Infection with Malaria Parasites

Dissections of female Anopheles mosquitoes, freshly killed with chloroform, were conducted in the field by the methods of Furman and Catts (1982), Detinova (1962) and Hodgkin (1950). Dissections were made the same night or the next day.

Mosquitoes were dissected in a drop of physiological saline (0.87% NaCl solution), on a glass slide, under a dissecting microscope. Salivary glands, stomach and ovaries of the dissected mosquitoes were removed and separately examined in fresh drops of physiological saline. Salivary glands were examined under low and high objectives for the presence of sporozoites. Slides under suspicion were fixed with methanol and stained with standard Giemsa stain. Stained slides were later examined with high-dry objectives (x40) or oil immersion objective for the presence of sporozoites.

Stomachs of dissected mosquitoes were transferred to fresh drops of saline, on separate slides, and examined for Plasmodium oocyst both under low (x10) and high power (x40) objectives.

Ovaries of dissected mosquitoes of all stages were transferred to fresh drops of physiological saline, on microscope slides. The ovarioles were then teased with fine dissecting needles and examined for dilations and follicular relics. At least five intact ovarioles were examined before the number of dilations for each mosquito were recorded. Examination and determination of the number of dilations were made according to the recommendations of Detinova (1962, 1968), Giglioli (1963), Detinova and Gillies (1964) and Gillies and Wilkes (1965).

It was reported that malaria was common in Shiba. In the first survey conducted in May 1967, 23.3% of the 60 individuals examined had malaria (Table 1). Most of the positive cases (15/27) were observed among adults of the age group 15 years and above. On the other hand, during the second survey which was conducted in November 1968, 46.6% of the 131 individuals examined were positive for malaria infection (Table 2). The frequency of infection during the second survey was twice as high as that of the first survey.

In both surveys *Plasmodium falciparum* was the most prevalent species in the area. In fact, *P. falciparum* was the only parasite observed during the second survey. *P. vivax* was observed during the first survey at a very low frequency (1.7%). *P. malariae* and *P. ovale* both of which occur in the country were not observed during this study in

3. RESULTS

Parasitological and entomological data obtained from monthly sampling, and also field and laboratory observations, were analysed to give an overall picture of the status of malaria and the possible Anopheles mosquitoes involved in the transmission of the disease in Ghibe during the study period. The greater part of the entomological data were obtained using human bait.

3.1 Prevalence of Plasmodium Species in Man

Microscopic examinations of stained blood smears indicated that malaria was common in Ghibe. In the first survey conducted in May 1987, 23.3% of the 60 individuals examined had malaria (Table 1). Most of the positive cases (85.7%) were observed among adults of the age group 15 years-old and above. On the other hand, during the second survey which was conducted in November 1988, 46.6% of the 131 individuals examined were positive for malaria infection (Table 2). The frequency of infection during the second survey was twice as high as that of the first survey.

In both surveys Plasmodium falciparum was the most prevalent species in the area. In fact, P. falciparum was the only parasite observed during the second survey. P. vivax was observed during the first survey at a very low frequency (1.7%). P. malariae and P. ovale both of which occur in the country were not observed during this study in

Ghibe. The distribution of Plasmodium species among different age groups are also presented in Tables 1 and 2.

TABLE 1 Prevalence of Plasmodium species among the population in Ghibe Farm 1 in May, 1987

Age Group (yrs.)	No. Examined	P.falciparum		P.vivax		P.malariae		P.ovale	
		+ve	%	+ve	%	+ve	%	+ve	%
0 - 4	8	1	12.5	-	-	-	-	-	-
5 - 14	9	1	11.1	-	-	-	-	-	-
15 - 29	21	7	33.3	1	4.8	-	-	-	-
30 - 44	18	3	16.7	-	-	-	-	-	-
45 and above	4	1	25.0	-	-	-	-	-	-
TOTAL	60	13	21.7	1	1.7	-	-	-	-
	60		14(+ve)				23.3%(+ve)		

TABLE 2 Prevalence of Plasmodium species among the population in Ghibe Farm 1 in November, 1988

Age Group (yrs.)	No. Examined	P.falciparum		P.vivax		P.malariae		P.ovale	
		+ve	%	+ve	%	+ve	%	+ve	%
0 - 4	26	16	61.5	-	-	-	-	-	-
5 - 14	21	14	66.7	-	-	-	-	-	-
15 - 29	35	17	48.6	-	-	-	-	-	-
30 - 44	40	12	30.0	-	-	-	-	-	-
45 & above	9	2	22.2	-	-	-	-	-	-
TOTAL	131	61	46.7	-	-	-	-	-	-

3.2 Anopheline Mosquito Fauna of Ghibe

The species composition of Anopheles mosquitoes in the study area were determined based on adult females caught on human bait and on larval specimens collected from different mosquito breeding sites. Pupae were also collected and reared to the adult stage in the field and in the laboratory to confirm identification. From larval and adult specimens collected in the study area, the following anopheline species were identified:

Anopheles gambiae Giles, 1902, Anopheles pharoensis Theobald, 1901, Anopheles coustani coustani Laveran, 1900, Anopheles longipalpis (Theobald, 1903), Anopheles pretoriensis (Theobald, 1903), Anopheles natalensis (Hill and Hayden, 1907), Anopheles garnhami Edwards, 1930, Anopheles christyi (Newstead and Carter, 1911) and Anopheles squamosus Theobald, 1901.

3.2.1 Anopheles larvae collected from different sites and habitats at Ghibe

Anopheles larvae were observed in different breeding sites and through out the study period, during both the wet and dry seasons. In a total of 426 larval identification, the proportions of the nine anopheline species and the comparative preference for shade and sunlight in each breeding site are shown in Table 3. During the dry season small pockets of rain pools and associated collections of

water disappeared. Hence breeding habitats along the river bank and those formed by irrigation activities were sampled. The results of larval surveys during the study period are presented in Table 3.

3.2.2 Adult Anopheles mosquitoes collected from Ghibe

The combined results of adult Anopheles collections, based on indoor and outdoor human bait captures, are shown in Table 4. Human bait collections of adult female mosquitoes were conducted at two sites (SA1 and SA2). An. gambiae s.l. were caught in large numbers (45.3% of the total catches) and An. pharopensis formed 33.8% of the total catches. An. coustani coustani, An. longipalpis and An. pretoriensis were also caught in considerable numbers biting man. On the other hand, An. natalensis, An. garrhami, An. christyi and An. squamosus were collected in relatively low numbers.

Table 4 Species and numbers of adult female Anopheles mosquitoes caught on human bait in Ghibe between April, 1987 and February, 1989

Species collected and identified	Number Examined	Percent Composition of Total
<u>An. gambiae</u> Giles	864	45.3
<u>An. pharopensis</u> Theobald	645	33.8
<u>An. coustani coustani</u> Laveran	152	8.0
<u>An. longipalpis</u> (Theobald)	122	6.4
<u>An. pretoriensis</u> (Theobald)	90	4.7
<u>An. natalensis</u> (Hill & Hayden)	10	0.5
<u>An. garrhami</u> Edwards	7	0.4
<u>An. christyi</u> (Newstead & Carter)	5	0.3
<u>An. squamosus</u> Theobald	12	0.6
TOTAL	1907	100

3.3 Relative Prevalence and Seasonal Abundance of "Anthropophilic" Anopheles Mosquitoes

Monthly sampling of relatively anthropophilic adult female Anopheles species using human bait indicated that An. gambiae s.l. and An. pharoensis were the most abundant man-biting species in Ghibe throughout the year (Table 5) and particularly after the heavy rains (Tables 5 and 6). An. coustani coustani, An. longipalpis and An. pretoriensis were also caught in small numbers throughout the year. An. gambiae s.l., An. christyi and An. squamosus were caught in very small numbers only in some months. Table 5 and 6 show that An. gambiae s.l. and An. pharoensis appeared to be 5 to 6 times more anthropophilic than some of the other species collected on man.

Figure 3 shows the monthly rainfall pattern in Ghibe, as recorded by the National Meteorological Service Agency weather station at Ghibe Farm 1, and the corresponding relative densities of An. gambiae s.l. and An. pharoensis caught on human bait during the study period. It is interesting to note that both An. gambiae s.l. and An. pharoensis show an increase in densities just after the heavy rains (Fig. 3).

TABLE 5

Relative monthly prevalence of Anophele mosquitoes based on human bait collections
at Sites SA1 and SA2 in Ghibe Farm between April, 1987 and March, 1988
(Numbers in brackets are per cent of total monthly catches)

Species	1987									1988			Total
	Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	
<u>Anopheles</u> <u>gambiaes.s.l</u>	38 (48)	59 (56)	62 (54)	60 (57)	62 (48)	84 (47)	78 (46)	67 (42)	50 (35)	41 (38)	38 (45)	45 (44)	684 (46)
<u>Anopheles</u> <u>pharoensis</u>	22 (29)	35 (33)	30 (26)	28 (26)	35 (27)	51 (29)	63 (37)	74 (46)	60 (42)	42 (39)	30 (36)	32 (31)	502 (34)
<u>Anopheles</u> <u>oustani</u> <u>oustani</u>	8	5	16	10	14	7	8	12	10	7	5	9 (8)	111
<u>Anopheles</u> <u>longipalpis</u>	0	5	4	7	11	19	12	8	12	7	3	8	96 (6)
<u>Anopheles</u> <u>pretoriensis</u>	9	0	2	1	4	13	5	0	6	10	8	6	64 (4)
<u>Anopheles</u> <u>natalensis</u>	1	0	0	0	3	0	1	0	2	0	0	0	7 (1)
<u>Anopheles</u> <u>garnhami</u>	0	1	0	0	1	1	0	0	0	0	0	3	6 (1)
<u>Anopheles</u> <u>christyi</u>	0	1	0	0	0	1	1	0	0	0	0	0	3 (1)
<u>Anopheles</u> <u>squamosus</u>	0	1	0	0	0	1	1	0	2	0	0	0	5 (1)
TOTAL	79	106	114	106	130	177	169	161	142	107	84	103	1478

TABLE 6

Relative monthly prevalence of *Anopheles* mosquitoes based on human bait collection in site (SA1) in Ghibe Farm between November 1988 and February, 1989.

(numbers in brackets are per cent of total monthly catches)

Species	1988		1989		Total
	Nov.	Dec.	Jan.	Feb.	
<u>Anopheles gambiae s.l.</u>	65 (44)	48 (44)	32 (41)	35 (37)	180 (42)
<u>Anopheles pharoensis</u>	46 (31)	38 (35)	28 (36)	31 (40)	143 (33)
<u>Anopheles coustani</u> <u>coustani</u>	14	9	10	8	41 (10)
<u>Anopheles longipalpis</u>	11	5	4	6	26 (6)
<u>Anopheles pretoriensis</u>	7	6	3	10	26 (6)
<u>Anopheles natalensis</u>	1	0	0	2	3 ((1)
<u>Anopheles garrhami</u>	1	0	0	0	1 ((1)
<u>Anopheles christyi</u>	2	0	0	0	2 ((1)
<u>Anopheles squamosus</u>	2	2	1	2	7 (2)
TOTAL	149	108	78	94	429

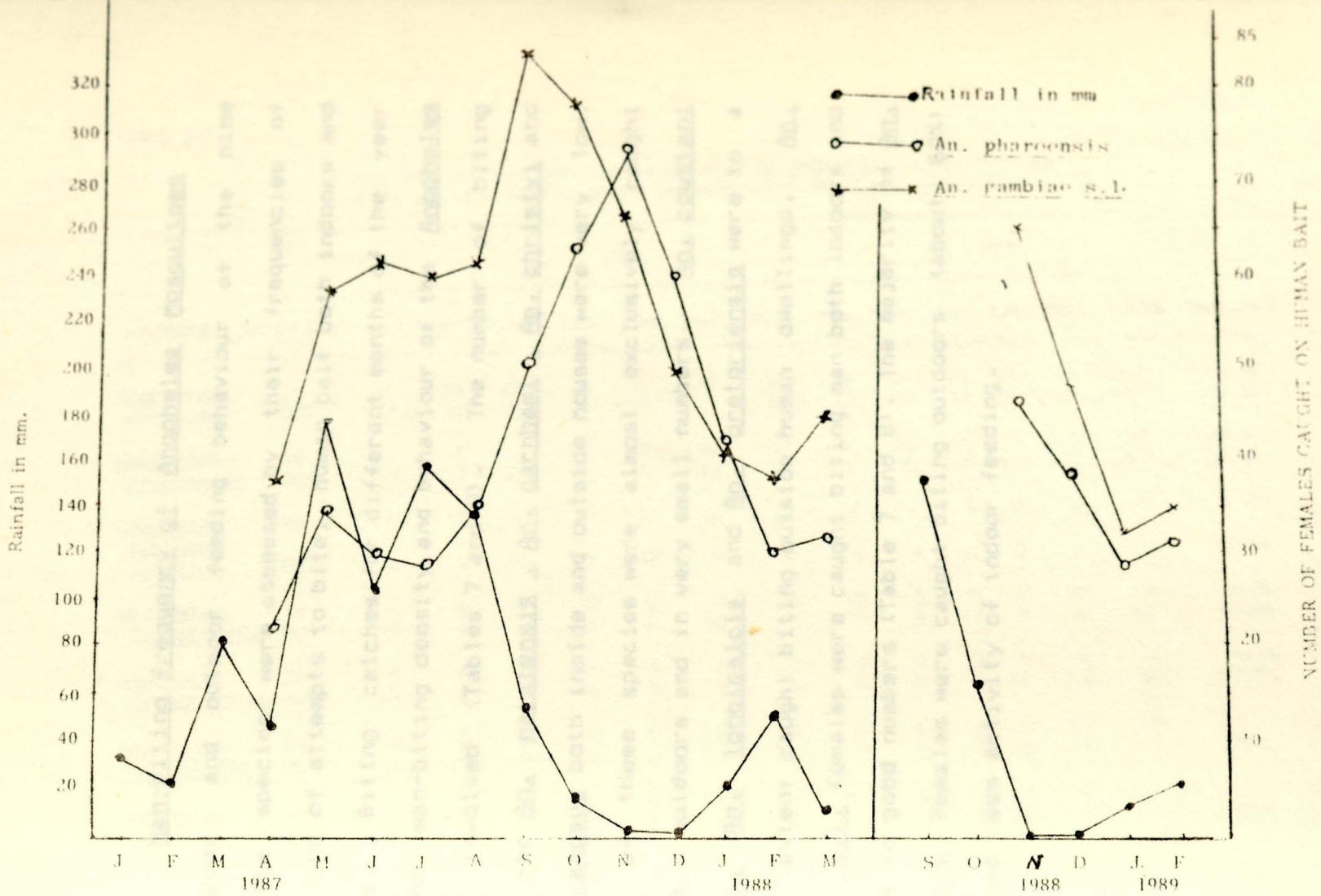


Figure 3 Relative densities of *An. pambiae s.l.* and *An. pharoensis* versus rainfall in mm. in Ghibe Farm.

3.4 Man-biting frequency of Anopheles Mosquitoes

Indoor and outdoor feeding behaviour of the nine anopheline species were assessed by their frequencies of biting, or of attempts to bite, human bait both indoors and outdoors. Biting catches for different months of the year show the man-biting density and behaviour of the Anopheles species involved (Tables 7 and 8). The number of biting catches for An. natalensis, An. gambiae, An. christyi and An. squamosus both inside and outside houses were very low. Females of these species were almost exclusively caught biting man outdoors and in very small numbers. An. coustani, An. longipalpis and An. pretoriensis were to a greater extent caught biting outside human dwellings. An. gambiae s.l. females were caught biting man both indoors and outdoors in good numbers (Table 7 and 8). The majority of An. pharoensis females were caught biting outdoors (about 60%) and showed less activity of indoor feeding.

TABLE 7

Indoor and Outdoor man-biting density of Anopheles mosquitoes at sites SA1 and SA2 in Ghibe between April 1987 and March 1988

SPECIES	LOCATION	1987												1988			Total	Per cent
		Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.					
<u>Anopheles</u> <u>gambiae</u>	IN	11	24	22	26	25	35	27	28	24	12	10	18	262	54.7%			
	OUT	14	16	25	21	17	21	18	24	20	14	15	12	217	45.3%			
<u>Anopheles</u> <u>ghacensis</u>	IN	4	8	3	7	5	13	20	21	16	10	9	8	129	39.6%			
	OUT	9	12	17	10	16	19	24	30	21	15	11	13	197	60.4%			
<u>Anopheles</u> <u>constani</u>	IN	0	0	0	0	0	0	0	1	1	1	0	2	5	6.4%			
	OUT	5	4	10	5	8	4	4	8	9	4	5	7	73	93.6%			
<u>Anopheles</u> <u>longipalpis</u>	IN	0	0	0	0	0	0	0	0	3	0	1	0	4	6.2%			
	OUT	0	3	1	4	6	10	9	6	8	7	2	5	61	93.8%			
<u>Anopheles</u> <u>pretoriensis</u>	IN	0	0	0	0	0	0	0	0	0	0	0	1	1	2.1%			
	OUT	5	0	2	1	3	9	4	0	5	6	8	3	46	97.9%			
<u>Anopheles</u> <u>natalensis</u>	IN	0	0	0	0	0	0	0	0	0	0	0	0	0	-			
	OUT	1	0	0	0	3	0	1	0	2	0	0	0	7	100%			
<u>Anopheles</u> <u>gambani</u>	IN	0	0	0	0	0	0	0	0	0	0	0	0	0	-			
	OUT	0	1	0	0	1	1	0	0	0	0	0	3	6	100%			
<u>Anopheles</u> <u>christyi</u>	IN	0	0	0	0	0	0	0	0	0	0	0	0	0	-			
	OUT	1	0	0	0	0	1	1	0	0	0	0	0	3	100%			
<u>Anopheles</u> <u>scutellaris</u>	IN	0	0	0	0	0	0	0	0	0	0	0	0	0	-			
	OUT	0	1	0	0	0	1	1	0	2	0	0	0	5	100%			

TABLE 8

Indoor and outdoor man-biting density of Anopheles mosquitoes at site SA1 in Ghibe Farm between November, 1988 and February, 1989.

Species	Location		1988		1989		Total	
			Nov.	Dec.	Jan.	Feb.	No.	%
<u>Anopheles gambiae</u>	IN	35	19	9	12	75	52.8	
	OUT	22	20	13	12	67	47.2	
<u>Anopheles pharoensis</u>	IN	15	9	10	8	42	42.4	
	OUT	19	13	11	14	57	57.6	
<u>Anopheles coustani</u>	IN	2	0	1	0	3	10.0	
	OUT	10	7	6	4	27	90.0	
<u>Anopheles longipalpis</u>	IN	2	0	0	0	2	10.0	
	OUT	7	4	4	3	18	90.0	
<u>Anopheles pretoriensis</u>	IN	1	1	0	0	2	10.5	
	OUT	4	3	3	7	17	89.5	
<u>Anopheles natalensis</u>	IN	0	0	0	0	0	-	
	OUT	1	0	0	2	3	100	
<u>Anopheles garrhami</u>	IN	0	0	0	0	0	-	
	OUT	1	0	0	0	1	100	
<u>Anopheles christyi</u>	IN	0	0	0	0	0	-	
	OUT	2	0	0	0	2	100	
<u>Anopheles squamosus</u>	IN	0	0	0	0	0	-	
	OUT	2	2	1	2	6	100	



3.5 Man-vector contact and Man-biting Rates of Anopheles Mosquitoes at Ghibe

The man-vector contact during this study was estimated by the number of bites a person received per evening (between 18:30 and 24:00 hrs). Evening biting catches were recorded for a total of 112 man-evenings both indoors and outdoors. The total indoor and outdoor catches are shown in Table 9. The average man-biting rates for the anopheline species were calculated from indoor and outdoor biting catches during the sampling period. While the mean number of bites a person received indoors from An. coustani coustani, An. longipalpis and An. pretoriensis per evening was less than 0.1 for each of these species, the indoor man-biting rates for An. gambiae s.l. and An. pharoensis were more than 30 and 15 times higher, respectively. The indoor biting rate for An. gambiae s.l. was about twice that of An. pharoensis. The chance of a person receiving bites from An.gambiae s.l. inside houses were higher than that outdoors. The chance of being bitten by An. pharoensis outdoors was greater than indoors. A person might be bitten by An. gambiae s.l. and An. pharoensis outdoors with almost equal chances in the evening.

$$\text{Biting rate of } An. \text{ gambiae s.l. (indoor)} = \frac{\text{Total number of mosquitoes}}{\text{Total number of man-evenings}} = \frac{327}{112} = 2.9$$

TABLE 9

Average man-biting rates of Anopheles mosquitoes at Ghibe Farm between April, 1987 and February, 1989

(* see below for method of calculation)

Species	No. of mosquitoes			Bites per man per evening	
	IN	OUT	TOTAL	INDOOR	OUTDOOR
<u>An. gambiae s.l.</u>	337	284	621	3.0	2.5
<u>An. pharoensis</u>	171	254	425	1.5	2.3
<u>An. coustani</u> <u>coustani</u>	3	100	103	0.1	0.9
<u>An. longipalpis</u>	8	79	87	0.1	0.7
<u>An. pretoriensis</u>	5	63	68	0.0	0.6
<u>An. natalensis</u>	0	10	10	-	0.1
<u>An. garrhami</u>	0	7	7	-	0.1
<u>An. christyi</u>	0	5	5	-	0.0
<u>An. squamosus</u>	0	11	11	-	0.1
TOTAL	529	813	1,342	4.7	7.25

- * Sites SA1 = (12 months + 4 months) x 4 evenings x 1 bait
 = 64 man - evenings (indoors and outdoors each)
 SA2 = 12 months x 4 evenings x 1 bait
 = 48 man - evenings (indoors and outdoors each)

Total = 112 man-evenings indoor (and 112 man-evenings outdoor)

Bite per man per evening = $\frac{\text{Total number of mosquitoes}}{\text{Total number of man-evenings}}$

e.g. Biting rate of An. gambiae s.l. (indoors) = $\frac{337}{112} = 3.0$

3.6 Man-biting Cycles of Anopheles Mosquitoes

The hourly biting activities of Anopheles mosquitoes captured on human bait were recorded to determine the biting pattern of the species involved (Table 10). Anopheline biting activities tended to build up towards the mid-evening and reached a peak around 20:00 hrs. An. gambiae s.l. were vigorous and actively bite for the most part of the evening, with a sharp decline towards mid-night. Peak biting activities were recorded between 19:00 hrs. and 22:00 hrs. Considerable indoor and outdoor biting activities by An. gambiae s.l. were observed around 20:00 hrs. and 19:00 hrs., respectively.

Anopheles pharoensis females indoor peak biting time occurred between 21:00 and 22:00 hrs. Indoor peak biting activity was followed by outdoor peak biting activity. More outside than inside biting by An. pharoensis was observed at all hours of the evening. Biting activities by An. coustani coustani, An. longipalpis and An. pretoriensis occurred throughout the evening but, with lower magnitudes and slight hourly variations. The per cent indoor and outdoor biting activities of An. gambiae s.l. and An. pharoensis are shown in Figure 4.

TABLE 10

Indoor and outdoor hourly man-biting densities of Anopheles mosquitoes in Ghibe Farm between April, 1987 & February, 1989.

Species		Evening hours of biting (beginning at)						Total	
		Location	1830	1900	2000	2100	2200		2300
<u>An.gambiae</u> s.l.	IN		31	73	82	69	59	23	337
	OUT		34	65	56	61	41	27	294
<u>An.pharoensis</u>	IN		13	28	39	42	29	20	171
	OUT		21	40	48	57	61	29	254
<u>An.coustani</u> <u>coustani</u>	IN		1	3	2	1	1	0	8
	OUT		11	18	26	22	17	6	100
<u>An.longipalpis</u>	IN		1	1	4	1	1	0	8
	OUT		8	19	21	14	10	7	79
<u>An.pretoriensis</u>	IN		1	1	2	0	1	0	5
	OUT		4	15	9	20	11	4	63
<u>An.natalensis</u>	IN		0	0	0	0	0	0	0
	OUT		0	3	4	1	2	0	10
<u>An.garrhami</u>	IN		0	0	0	0	0	0	0
	OUT		0	2	3	0	2	0	7
<u>An.christyi</u>	IN		0	0	0	0	0	0	0
	OUT		0	1	4	0	0	0	5
<u>An.squamosus</u>	IN		0	0	0	0	0	0	0
	OUT		0	2	5	2	1	1	11
TOTAL			125	271	303	290	236	117	1342

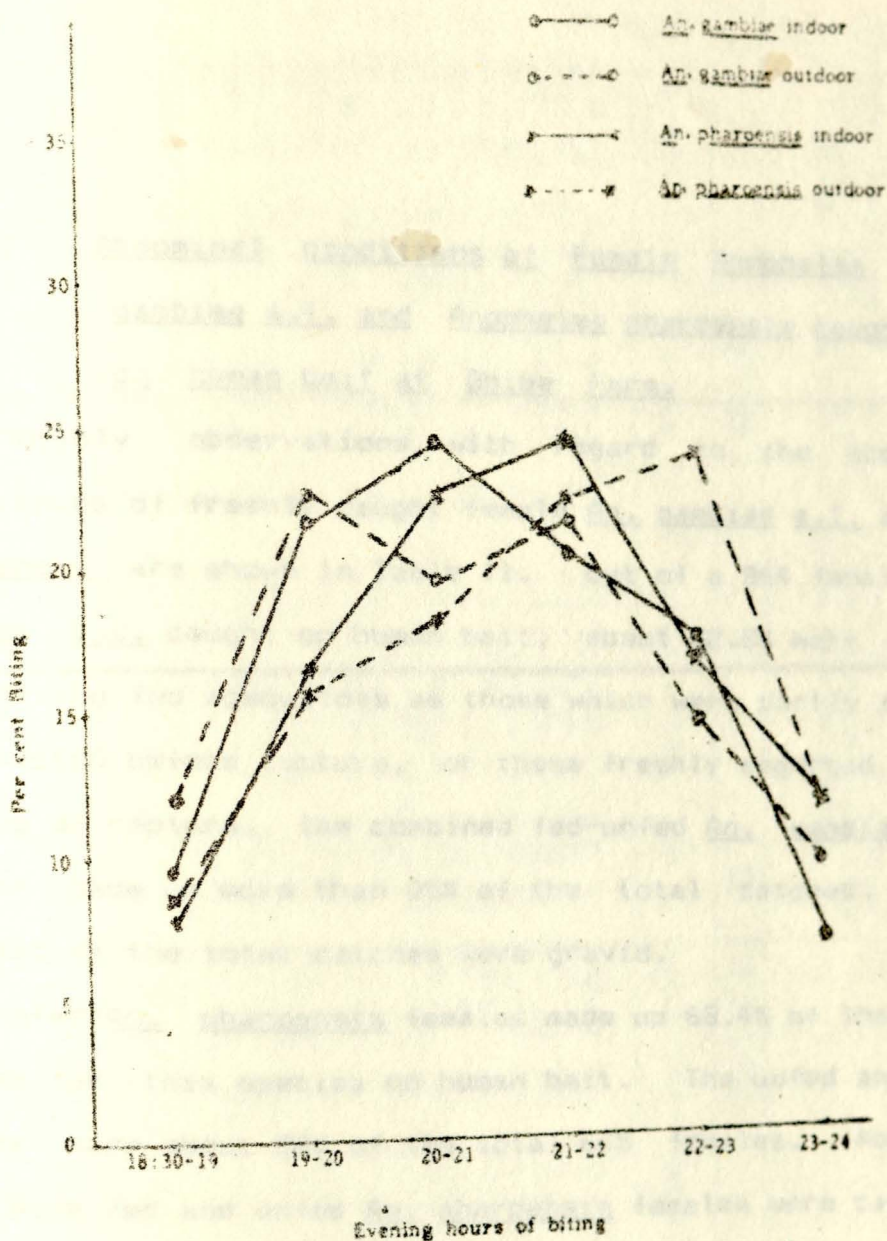


Figure 4 Evening biting cycles of *An. pharoensis* and *An. gambiae* s.l. caught on human bait, indoor and outdoor, in Chibe Farm.

3.7 Abdominal conditions of Female Anopheles
gambiae s.l. and Anopheles pharoensis caught
on human bait at Ghibe Farm.

Monthly observations with regard to the abdominal appearances of freshly caught female An. gambiae s.l. and An. pharoensis are shown in Table 11. Out of a 864 female An. gambiae s.l. caught on human bait, about 62.8% were unfed. Considering fed mosquitoes as those which were partly fed and interrupted before capture, or those freshly engorged on the evening of capture, the combined fed-unfed An. gambiae s.l. females made up more than 85% of the total catches. Less than 15% of the total catches were gravid.

Unfed An. pharoensis females made up 68.4% of the total catches of this species on human bait. The unfed and fed catches were about 86% of the total 645 females. About 8 times more fed and unfed An. pharoensis females were caught while attempting to bite man than did gravid females.

It should be noted that in all the months of sampling, the proportions of unfed females of both An. gambiae s.l. and An. pharoensis by far exceeds that of fed and gravid ones.

TABLE 29

Abdominal conditions of females of two species *Anopheles* mosquitoes collected indoors and outdoors, on human bait, ~~at~~ Ghibe Farm between 1987 and 1989 (Per cent is shown in bracket)

	<i>An. gambiae</i> s.l.					<i>An. pharoensis</i>				
	Unfed	Fed	Gravid	Total	Ratio	Unfed	Fed	Gravid	Total	Ratio
1987 Apr.	26(66.4)	7(18.4)	5(13.2)	38	5:1:1	13(59.1)	6(27.3)	3(13.6)	22	4:2:1
May	33(55.9)	18(30.5)	8(13.6)	59	4:2:1	23(65.7)	8(22.9)	4(11.4)	35	6:2:1
Jun.	26(51.9)	17(27.4)	10(16.1)	62	3.5:2:1	18(60.0)	9(30.0)	3(10.0)	30	6:3:1
Jul.	32(53.3)	19(31.7)	7(11.9)	60	4:2:1	18(64.1)	5(17.9)	5(17.9)	28	4:1:1
Aug.	40(64.5)	15(24.2)	7(11.3)	62	4:2:1	25(71.4)	7(20.0)	3(8.6)	35	8:2:1
Sept.	54(64.3)	20(23.8)	10(11.9)	84	5:2:1	40(78.4)	6(11.8)	5(9.8)	51	8:1:1
Oct.	52(66.7)	18(23.0)	6(7.7)	76	5.5:2:1	48(76.2)	13(31.3)	5(9.5)	63	8:1.5:1
Nov.	42(62.7)	13(19.4)	12(17.9)	67	3.5:1:1	44(59.4)	21(28.4)	12(16.2)	74	5:2:1
Dec.	32(64)	12(24)	6(12)	50	5:2:1	28(56)	15(25)	7(11.7)	44	5:2:1
1988 Jan.	24(58.5)	10(24.4)	7(17.1)	41	3.1:1	30(71.4)	8(19.5)	4(9.5)	42	7:4:2
Feb.	24(65.8)	8(21.0)	5(13.2)	37	5:2:1	22(73.3)	6(20)	2(6.7)	30	11:3:1
Mar.	30(63.7)	11(24.5)	4(8.9)	45	7.5:3:1	19(59.4)	8(25)	5(15.6)	32	7:2:1
1989 Nov.	40(61.5)	17(26.2)	8(12.3)	65	5:2:1	33(71.7)	4(8.7)	9(19.6)	46	14:0.5:1
Dec.	31(64.5)	12(25)	5(10.4)	48	6:2:1	30(78.9)	5(13.2)	3(7.9)	38	10:2:1
1989 Jan.	21(65.6)	7(21.9)	4(12.5)	32	5:2:1	17(57.8)	4(14.3)	5(17.9)	26	4:1:1
Feb.	20(57.1)	8(22.9)	7(20)	35	3:1:1	21(67.7)	6(19.4)	4(12.9)	31	5:1.5:1
TOTAL	453	206	115	864	5:2:1	441	127	77	645	6:2:1
MEAN	0.627	0.238	0.135		5:2:1	0.684	0.197	0.119		6:2:1

3.8 Infection Rates in Anopheles Mosquitoes of Ghibe Farm

On the premise that An. gambiae s.l. and An. pharoensis were the most abundant and anthropophilic species in the area and since I was the only person responsible for all dissections and other observations, examination of a total 1005 Anopheles mosquitoes was considered reasonable for the study period. Results of monthly stomach and salivary gland dissections and examinations of An. gambiae s.l. and An. pharoensis are shown in Table 12. Dissected specimens were both from indoor and outdoor human bait catches.

Out of 568 An. gambiae s.l. females dissected, only one female was found infected with Plasmodium oocyst. Therefore, the relative prevalence of Plasmodium oocysts in An. gambiae s.l. during the study period was 0.2%. Otherwise all salivary gland dissections of An. gambiae s.l. were negative for Plasmodium infection. Out of 417 dissected An. pharoensis, no positive specimens were found. Monthly dissections of An. gambiae s.l. ranged from 24 to 51 females, and that of An. pharoensis ranged from 12 to 36 females with a mean monthly dissection of 37 and 26 females for the two species, respectively.

TABLE 12

Results of Dissections of Anopheles gambiae s.l. and An. pharoensis captured on human bait and examined for Plasmodium infection at Ghibe Fara between April 1987 and February, 1989.

Period	<u>An. gambiae s.l.</u>				<u>An. pharoensis</u>					
	Number dissected	Stomach (oocyst)		Salivary (sporozoite)		Number dissected	Stomach (oocyst)		Salivary (sporozoite)	
		-ve	+ve	-ve	+ve		-ve	+ve	-ve	+ve
1987 Apr.	24	24	-	24	-	12	12	-	12	-
May	36	36	-	36	-	25	25	-	25	-
Jun.	41	41	-	41	-	22	22	-	22	-
Jul.	39	39	-	39	-	19	19	-	19	-
Aug.	32	32	-	32	-	26	26	-	26	-
Sep.	47	47	-	47	-	32	32	-	32	-
Oct.	43	43	-	43	-	30	30	-	30	-
Nov.	39	39	-	39	-	22	22	-	22	-
Dec.	40	40	-	40	-	33	33	-	33	-
1988 Jan.	34	34	-	34	-	27	27	-	27	-
Feb.	30	30	-	30	-	24	24	-	24	-
Mar.	35	35	-	35	-	28	28	-	28	-
Nov.	51	50	1	51	-	36	36	-	36	-
Dec.	39	39	-	39	-	25	25	-	25	-
1989 Jan.	28	28	-	28	-	20	20	-	20	-
Feb.	30	30	-	30	-	26	26	-	26	-
TOTAL	588	587	1	588	-	417	417	-	417	-

3.9 The age composition of populations of *Anopheles gambiae* s.l. and *Anopheles pharoensis* in Ghibe

The age structure of populations of *An. gambiae* s.l. and *An. pharoensis* were determined based on the examination of the number of dilations in the ovarioles of dissected female mosquitoes. In the course of the study, at least 1005 mosquitoes of both species were dissected. As mentioned earlier, mean monthly dissections were 37 for *An. gambiae* s.l. and 26 for *An. pharoensis*.

The age-groups are given in terms of the number of dilations in the ovarioles (gonotrophic cycles completed). Number of dilations (follicular relics) are interpreted as 1-parous, 2-parous, 3-parous, etc. (Tables 13 and 14).

The monthly age composition of *An. gambiae* s.l. are shown in Table 13. Out of 588 *An. gambiae* s.l. dissected, 25.9% were pre-gravid and nulliparous females. 3-parous and older females constituted about 45.6% of the human bait catches that were dissected. The proportions of pre-gravid and nulliparous females were higher than those of any other age group through out the study period.

The age composition of *An. pharoensis* (Table 14) showed that 24% of a total of 417 dissected were pregravid and nulliparous females. 3-parous and older females formed about 48.2% of the females dissected. The proportions of pre-gravid and nulliparous females were higher than those of any

other age group in most of the months during the study period. In both An. gambiae s.l. and An. pharoensis, females older than 8-parous were encountered very occasionally. The oldest females observed in both species during the study period were those which completed nine gonotrophic cycles (8 An. gambiae s.l. and 4 An. pharoensis).

TABLE 13

Physiological age determination based on monthly dissections of Anopheles gambiae s.l. collected on human bait at Ghibe Farm

Period	No. Dissected	% of number of gonotrophic cycles completed									
		0	1	2	3	4	5	6	7	8	9
1987											
Apr.	24	20.8	20.8	8.3	16.7	12.5	8.3	4.2	8.3	-	-
May	36	25.0	11.1	13.9	11.1	11.1	8.3	8.3	5.6	2.8	2.8
Jun.	41	26.8	17.1	14.6	14.6	12.2	7.3	4.8	2.4	-	-
Jul.	39	35.9	15.4	12.8	7.7	10.3	10.3	5.1	2.7	-	-
Aug.	32	21.9	12.5	12.5	15.6	12.5	9.4	9.4	3.1	3.1	-
Sep.	47	24.0	25.5	19.1	4.3	2.1	6.4	2.1	4.3	2.1	-
Oct.	43	30.2	13.9	18.6	9.3	7.0	9.3	4.6	2.3	2.3	2.3
Nov.	39	28.2	23.1	15.4	12.8	5.1	-	7.7	2.7	5.1	2.7
Dec.	40	17.5	12.5	7.5	10.0	15.0	7.5	12.5	10.0	2.5	5.0
1988											
Jan.	34	14.7	11.8	20.6	11.8	11.8	8.8	5.9	5.9	5.9	2.9
Feb.	30	26.7	20.0	10.0	10.0	6.7	10.0	3.3	6.7	3.3	3.3
Mar.	35	28.6	20.0	11.4	5.7	8.6	5.7	8.6	5.7	2.9	2.9
Nov.	51	21.6	9.3	11.8	17.6	13.7	9.8	5.9	5.9	3.9	-
Dec.	39	20.5	10.3	5.1	15.4	12.8	7.7	12.8	7.7	5.1	2.6
1989											
Jan.	28	32.7	7.1	17.9	10.7	10.7	7.1	3.6	10.7	-	-
Feb.	30	26.7	16.7	6.7	6.7	10.0	10.0	10.0	6.7	6.7	-
TOTAL	588	152	91	77	66	59	46	40	32	17	8
%		25.9	15.5	13.1	11.2	10.0	7.8	6.8	5.4	2.9	1.4

TABLE 14

Physiological age determination based on monthly dissections of Anopheles pharoensis collected on human bait at Ghibe Farm

Period	No. dissected	% of numbers of gonotrophic cycles completed									
		0	1	2	3	4	5	6	7	8	9
1987											
Apr.	12	25.0	16.7	8.3	16.7	8.3	8.3	8.3	-	8.3	-
Mar.	25	32.0	16.0	12.0	8.0	8.0	-	8.0	8.0	4.0	4.0
Jun.	22	27.3	9.1	4.5	18.2	9.1	13.6	9.1	9.1	-	-
Jul.	19	21.1	10.5	15.8	10.5	1.1	10.5	5.3	5.3	-	-
Aug.	26	11.5	23.1	11.5	11.5	7.7	19.2	1.5	-	3.8	-
Sep.	32	21.9	12.5	15.6	9.4	9.4	12.5	9.4	3.1	3.1	-
Oct.	30	16.7	13.3	10.0	13.3	3.3	10.0	6.7	10.0	6.7	-
Nov.	32	18.7	21.9	6.2	9.4	9.4	9.4	9.4	9.4	6.2	-
Dec.	33	36.4	18.2	15.1	6.1	9.1	6.1	3.0	3.0	3.0	-
1988											
Jan.	27	22.2	14.8	11.1	11.1	14.8	11.1	7.4	3.7	3.7	-
Feb.	24	20.8	8.3	16.7	8.3	12.5	4.2	12.5	8.3	8.3	-
Mar.	28	14.3	14.3	10.7	14.3	10.7	10.7	7.1	7.1	7.1	3.6
1988											
Nov.	36	33.3	16.7	19.4	8.3	5.6	2.8	5.6	2.8	2.8	2.8
Dec.	25	32.0	20.0	12.0	8.0	8.0	4.0	8.0	4.0	4.0	-
1989											
Jan.	20	35.0	10.0	5.0	15.0	10.0	15.0	5.0	-	5.0	-
Feb.	25	15.4	23.1	11.5	11.5	11.5	3.8	7.7	7.7	3.8	3.8
TOTAL	417	100	66	50	45	43	37	32	22	18	4
%		24.0	15.8	12.0	10.8	10.3	8.9	7.8	5.3	4.3	1.0

4. DISCUSSION AND CONCLUSION

Parasitological surveys conducted during this study for malaria infections in humans indicated that malaria is endemic in Ghibe. P. falciparum was the predominant malaria parasite in the area (Tables 1 and 2). It was observed that P. falciparum was more prevalent among children below 15 years-old (63.8%) than among adults above the age of 14 years-old (36.9%) ($\chi^2 = 8.74$, $P = 0.05$). Moreover it was observed that 61.5% of the younger children between the age of 0 and 4 years old examined during the second parasitological survey had P. falciparum infection. Most of the children in this age group were claimed not to have left the area during their life time. This strongly suggests that endemic malaria transmission existed in the area. The frequent movement of labourers and other farm employees to and from other state farms in malarious areas however, could not be ruled out as a source for the possible introduction of the disease to the Ghibe valley from time to time.

It is evident from Table 1 that P. vivax was detected at a very low frequency during the study. But P. malariae and P. ovale infections were not detected during both surveys in the area.

In earlier surveys for malaria parasites in the country P. falciparum was reported to be the predominant species in many places (Fontaine *et al.*, 1961; Krafsur, 1977; Krafsur

and Armstrong, 1978; Gebre Mariam, 1984). According to their reports, P. vivax was also found to be an important species, even though widely spread at higher altitudes. P. malariae was also commonly reported in Ethiopia. But the frequency of P. ovale infections were obscure and the species was identified in Ethiopia relatively only recently (Armstrong, 1969; Armstrong, 1978; Krafur and Armstrong, 1978).

Fontaine et al. (1961) reported the prevalence of P. falciparum in the country as 60%, with that of P. vivax as 25% and P. malariae as 15%. Krafur (1977) reported a prevalence of about 59% for P. falciparum among children less than 15 years old, in Gambela. Krafur and Armstrong (1978) also reported an average prevalence of P. falciparum as 58% for the same age group and 35% among adults 15 years old and above, in Gambela. The prevalence of P. falciparum observed during this study in Ghibe for the two age groups were comparable to those reported by previous workers in different regions of Ethiopia.

The relatively low frequencies of P. vivax reported by Krafur (1977) and Krafur and Armstrong (1978) in different age groups were similar to that observed in this study. It was suggested that P. vivax thrives better in parts of Ethiopia at elevations between 1000 and 2000m (Armstrong, 1978). As a temperate zone parasite, the apparently very low

frequency of P. vivax in Ghibe may be explained by similar altitudinal factors which favour P. falciparum transmission. The relatively low number of blood smears examined in this study might also possibly explain the detection of very low P. vivax infections and the apparent absence of P. malariae and P. ovale infections at Ghibe.

Another point of interest is that out of the 131 subjects examined during the second survey, 12.2% were P. falciparum gametocyte positives and hence were infective to mosquitoes. It should also be noted that chloroquine tablets were supplied to some of the inhabitants by the farm clinic as a prophylaxis in October and November, 1988. This might have reduced the actual prevalence of malaria infections in the population.

Qualitative observations on collected and identified larval specimens indicated that the nine anopheline species recorded in the area were represented in considerable densities for the most part of the year (Table 3). An. gambiae s.l. were collected from different kinds of breeding habitats, including rock pools and small pockets of water along the river bank, overflow pools and standing water along irrigation canals, and temporary rain pools and roadside ditches formed during the rainy seasons. Most of the breeding places from which An. gambiae s.l. larvae were collected, were generally exposed to sunlight. The capacity

of the freshwater An. gambiae s.l., and particularly that of An. arabiensis, to breed in a wide range of breeding habitats (as observed during this study) was documented by previous investigators (Wang, 1966; O'Connor, 1967; Krafur, 1977; Stevens, 1984; Zahar, 1984). While most observations agree that An. gambiae s.l. breed in sites completely or partially exposed to sunlight, it was also noted that An. gambiae s.l. breeding habitats were devoid of floating vegetation. However, breeding habitats observed along the bank of the perennial Ghibe river, where An. gambiae s.l. larvae were collected, mostly had floating algal vegetation. Roadside ditches and temporary rain pools also had standing grass, which may provide shade at the micro-environment level.

Even though An. pharoensis larvae were collected from most kinds of breeding habitats at Ghibe, considerably larger numbers of larvae of this species were identified from overflow pools associated with irrigation canals, road side ditches with standing water collections, and river side rock pools. These breeding habitats had either standing grass, or suspended detritus, or floating algal vegetation which might provide a shaded or partially shaded micro-environment suitable for this species. O'Connor (1967) described An. pharoensis breeding places as shaded permanent water bodies and irrigation ditches. The relatively higher number of human bait catches at the time of the year irrigation ditches are full and semi-permanent breeding places prevail, at

Ghibe, suggests that this species prefers such kinds of breeding places. De Meillon (1951a, 1951b) reported that An. pharoensis was typically a swamp breeder. But such descriptions may not account for the requirements of mosquito larvae at the micro-environment level.

An. coustani larvae were collected mostly from breeding places along the river bank and from road side ditches and temporary rain pools. Riverside rock pools may provide floating algal vegetation which might favour the species. De Meillon (1951b) reported that An. coustani was a swamp breeder and that shade is not essential if floating vegetation is available.

An. longipalpis larvae were largely collected from rock pools along the river bank, where algae and grass were found. Giaquinto-Mira (1950) reported that the species preferred to breed in clear water riverside pools rich in both vertical and horizontal vegetation and with sufficient shade.

An. pretoriensis larvae were observed commonly in seepage rock pools with clear water, along the river bank. This species was reported to avoid shade and to prefer exposed pools and seepages (De Meillon 1951a, 1951b) in other parts of Africa.

An. natalensis, An. garnhami, An. christyi and An. squamosus were found breeding in most kinds of breeding habitats. Giaquinto-Mira (1950) described An. garnhami and An.

christyi as highland species in Ethiopia, stating that these species might also breed in lowland areas. The author recorded that An. christyi showed a preference for pools and marshes without vegetation and may be found in muddy water. These preferences were also noted by De Meillon (1951b). The breeding places of An. squamosus were mostly small rock pools with floating algal vegetation. The species was reported to prefer some kind of shade and to breed in rice fields and swamps (De Meillon, 1951b).

In general, larval densities of the anopheline species were closely associated with the availability of suitable breeding habitats in different parts of the year.

During the heavy rains season (July to September), the Ghibe river floods and destroys most of the mosquito breeding habitats along its banks. But this seems to be more than compensated by the creation of numerous new breeding sites, such as temporary rainpools and small collections of water in the road side ditches and other depressions on the ground, as a result of local precipitation. As such, mosquito proliferation is favoured during this period. Therefore, the man-biting densities of An. gambiae s.l. and An. pharoensis tend to build up and reach a peak just after the heavy rains (September to November) (Fig.3). After that, the biting densities of these species continued to decrease to a lower level during the dry season. At this time of the year,

mosquito breeding habitats were mainly those formed by the receding river and overflow pools and collections of water associated with irrigation activities in the area. New mosquito breeding sites are not normally formed during the short rains (April-May), unless there is exceptionally heavy rainfall. While rock pools along the perennial Ghibe river bank favoured the occurrence and breeding activities of most of the anopheline species, breeding habitats associated with irrigation canals seemed to favour An. pharoensis.

The changes in the relative densities of An. gambiae s.l. and An. pharoensis (Table 5) observed in some months of the year (e.g. September to December) might be due to the creation of new suitable breeding sites during the rainy season and hence of increased breeding activities by these species. Apparently high relative densities of An. pharoensis biting man corresponded with extensive irrigation activities in the area (October-January). An. gambiae s.l. biting densities seem to decrease at the height of the dry season between February and April (Tables 5 and 6). This may be due to the disappearance of many of the breeding places at this time of the year and probably to effects of house spraying with DDT in January by DCMOVD. On the other hand, An. gambiae s.l. showed an increase in numbers biting during the wet season (June-September) and in some months of the dry season (September-January), probably due to the elastic

breeding activities of the group in many kinds of habitats (Table 5). The effects of house spraying with DDT in June seemed to be curtailed by the creation of numerous breeding sites during the wet season, which favours proliferation of the group.

Anopheles pharoensis man-biting densities showed a decrease in number both during the height of the wet season (June-August), and during the height of the dry season (February-May). The fluctuations in the biting densities observed of both An. gambiae s.l. and An. pharoensis were closely associated with the availability of suitable breeding places in the area. An. gambiae s.l., however, showed least fluctuations due to the capacity of the mosquitoes to breed in both natural and artificial habitats, fully or partially exposed to sunlight (Wang, 1966; Stevens, 1984; Zahar, 1984).

Relative man-biting densities of An. coustani coustani, An. longipalpis and An. pretoriensis did not show a clear cut seasonality, but these species were caught in relatively greater numbers during and after the heavy rains. The relative prevalence of An. longipalpis and An. pretoriensis, however, appeared to decline with the approach of the heavy rain season (Table 5). The scarcity of adults of An. natalensis, An. garrhami, An. christyi and An. squamosus during most of the year may be due to their low population densities in the area, as well as due to the very low degree

of anthropophily (Tables 4-6).

Niether larval nor adult specimens of An. funestus and An. nili were collected in the present study. These species were reported as important vectors of malaria in different regions of Ethiopia (Melville et al., 1945; Covel, 1957; Fontaine, et al., 1961; Wang, 1966; O'Connor, 1967; Krafsur, 1970a, 1977; Gebre Mariam, 1984). The absence of these species in Ghibe is probably due to lack of suitable breeding habitats in the area. Human bait collections, which might be the least biased and probably the most reliable method to study anthropophilous anopheline populations (Service, 1976), should have revealed some An. funestus and An. nili females if these species were present at Ghibe.

Results of human bait collections in this study show that An. gambiae s.l. and An. pharoensis made up the greater proportions of human bait catches (Tables 5-8). It is evident that the two species were the most abundant anthropophilous species in Ghibe. Tables 7 and 8 show that An. gambiae s.l. fed both indoors and outdoors in equal proportions and hence were equally endophagous and exophagous in their feeding behaviour ($T_{0.01} = 1.79, P = 0.044$ (Table 7) and $T_{0.01} = 0.32, P = 0.38$ (Table 8)).

An. pharoensis were mainly exophagous, where the greater proportions of this species were caught biting man outdoors

($T_{0.01} = -2.21, P = 0.019$, and $T_{0.01} = -1.68, P=0.077$, respectively).

Highton et al. (1979) reported An. arabiensis as catholic in its feeding and resting behaviour. Similar observations were reported by White et al. (1972), White (1974a) and White et al. (1980). The higher degree of anthropophily and the tendency for equal endophagic and exophagic feeding behaviour in An. gambiae s.l. (presumably An. arabiensis) observed in the present study are comparable to the findings reported by these workers for An. arabiensis. However Krafur (1977) found An. gambiae s.l. strongly endophagic in Gambela. Krafur (1977) also reported that An. pharoensis in Gambela were equally exophagic and endophagic. This species, however, was mainly exophagous and tended to bite more outside than inside human dwellings at Ghibe in the present study. In other studies in Africa, the species shows inherent variations in its degree of anthropophily and feeding behaviour (De Meillon, 1951b).

Such inconsistent choice of host and feeding behaviour by An. arabiensis and An. pharoensis might be attributed to the availability and location of the host of choice at night. It should also be noted that at Ghibe Farm 1, where man outnumbered other hosts, and where the inhabitants may be found outdoors or indoors in the evening, the feeding behaviour of these species observed in the present study may

differ from some of the previous reports.

Anopheles coustani coustani, An. longipalpis and An. pretoriensis were less anthropophilic and more exophagic at Ghibe. These species were caught biting man in low numbers indoors (about 10% of each) (Table 7 and 8). White (1974b) reported on the occurrence of An. longipalpis in Ethiopia, but little is known about the feeding behaviour and choice of host for this species. Nor is there any evidence of natural infection with Plasmodium species in this species in Ethiopia, or in other parts of Africa (De Meillon, 1951b). An. pretoriensis was not generally considered as an indoor biter in Ethiopia but as high as 21% indoor captures were reported by Giaquinto-Mira (1950). Instances of indoor biting behaviour by An. coustanicoustani were also reported in Ethiopia and elsewhere in Africa (Giaquinto-Mira, 1950; De Meillon, 1951).

The most important anthropophilous species in Ghibe Farm were An. gambiae s.l. and An. pharoensis. These species were reported to be important in the epidemiology of malaria in different regions of Ethiopia and other parts of Africa (Corradetti, 1939, 1940; Mara, 1950; Service, 1963; Wang, 1966; O'Connor, 1967; Krafsur, 197a, 1977; White, 1974a; Russell, 1959; Gebre Mariam, 1964). In view of the accumulated information on vector species, An. gambiae s.l. appears to be the most important vector of malaria at Ghibe.

An. pharoensis is presumably a secondary vector of malaria in this area. This species may be important in aggravating the malaria infection induced by An. gambiae s.l., particularly during the peak transmission season (October-December), and in maintaining the endemic malaria situation. The relatively higher numbers of these species biting man indoors and outdoors, and the malaria incidence observed in this study, may strongly support the implicated and/or asserted role of these species as malaria vectors at Ghibe.

The average man-biting rate indoors of An. gambiae s.l. was 3.0 bites per man per evening, and far exceeds that of other species (Table 9). The value for An. pharoensis average man-biting rate indoors was 1.5, which is about half that of An. gambiae s.l.. Outdoor man-biting rates for both An. gambiae s.l. and An. pharoensis were similar. The combined biting indoors by An. gambiae s.l. and An. pharoensis therefore comprised the overwhelming risk of being bitten by Anopheles species at Ghibe. But the combined average indoor and outdoor man-biting rates of An. gambiae s.l. and An. pharoensis (4.5 and 4.8, respectively) were more or less similar. There was therefore a chance of receiving a similar number of bites per evening indoors and outdoors by these species. Average man biting rates by An. gambiae s.l. and An. pharoensis observed in this study were much higher than those reported by Krafsur (1977) in Gambela. The

average bites per man per evening for the other anophelines inside houses was very low (Table 9).

Mention should be made at this point that the biting cycles of the anopheline mosquitoes over a 12-hours period remains to be investigated. However, the pattern of feeding activities of Anopheles mosquitoes before midnight was remarkable in that there was a decline in biting activities towards midnight (23:00 to 24:00 hrs.) (Table 10). An. gambiae s.l. were characterized by maximum feeding occurring indoors and outdoors between 19:00 and 22:00 hrs. Indoors peak feeding activity was observed between 20:00 and 21:00 hrs. preceded by outdoor peak feeding activity (19:00 to 20:00 hrs). An. pharoensis feeding activities indoors peaked between 21:00 and 22:00 hrs, and preceded outdoor peak feeding activities between 22:00 and 23:00 hrs. (Fig. 4).

Service (1963) reported that maximum feeding by An. gambiae s.l. in Nigeria had a single peak between 03:00 and 04:00 hrs. and commented that this was variably reported by other workers. Krafsur (1977) reported peak An. gambiae s.l. feeding activity to occur indoors between 04:00 and 06:00 hrs. However, this period of the night was not covered in this study and it is difficult to compare with the results of others in this respect. Service (1963) reported that An. pharoensis, in the Guinea savannah zone of Nigeria, showed two peaks, which occurred before and after midnight. But

Krafsur (1977) reported that An. pharoensis were active shortly after dark with most of the biting catches occurring before midnight. In the present study peak biting activities of An. pharoensis were between 21:00 and 23:00 hrs. This observation was somewhat similar to that of Krafsur (1977) in Gambela.

It was observed in this study that the proportion of unfed mosquitoes in human bait catches was always much greater than that of fed and gravid mosquitoes (Table 11). Unfed and fed An. gambiae s.l. and An. pharoensis (>85% in both) were the predominant females in human bait catches and gravid females of these species might have been attracted to man either for the next blood meal, or while looking for a suitable resting site, or for plant sugars.

Monthly dissections of both An. gambiae s.l. and An. pharoensis were not sufficient to provide sporozoite rates for these species at different times of the year (Table 12). Only one female An. gambiae s.l. (0.2%) was positive for Plasmodium oocyst. In the scarcity of positive salivary gland and stomach infections in both An. gambiae s.l. and An. pharoensis during this study, it is difficult to estimate the inoculation rate and the risk of malaria infection by these species.

From this observation, it is difficult to explain the malaria incidence observed in blood samples of the human

population living at Ghibe. O'Connor (1967) reviewed the reports of dissections to determine the vectorial status of Anopheles mosquitoes in different regions of Ethiopia.

Accordingly, reports of sporozoite rates ranged from 0.03% to 0.2% in different places at different times for An. gambiae s.l. This however, did not take into account dissections with negative results. Krafsur (1977) reported an infection rate of 1.87% (156 / 8348) for An. gambiae s.l. in Gambela. In other parts of Africa, sporozoite rates as high as 5.9% were reported for An. gambiae s.l. (Service, 1968). The sporozoite rate of 0.32% in An. arabiensis and 2.68% in An. gambiae s.l. reported by White et al. (1972), and 4.7% in An. gambiae s.l. by Davidson and Draper (1953) fall within the same limits. The mean overall sporozoite rate of 5.89% was somewhat considered as valid by Service (1968).

In the absence of positive salivary gland dissections, as was the case in this study, the foregoing reports of sporozoite rates in An. gambiae s.l. could possibly help to estimate the theoretical number of infective bites a person receives in a given time. This is a function of the biting rate of the vector species (Table 9) and its estimated sporozoite rate. In view of the relatively low number of mosquitoes dissected during this study (Table 12), the sporozoite rate of 1.87% reported by Krafsur (1977) may be assumed as an estimate of the sporozoite rate for An. gambiae s.l. at Ghibe. Based on the foregoing assumption, and the

results of average man-biting rate observed during this study (Table 9), the theoretical infective bites per person per evening at Ghibe by An. gambiae s.l. would be $(2.77 \times 1.87/100) = 0.052$. However, such values may exaggerate the actual infective bites per man per evening, by the mere fact that human baits are intentionally exposed to invite biting mosquitoes, as opposed to the normal condition of avoiding bites.

All dissections of An. pharoensis (417) were negative for sporozoite and oocyst infections. This species was reported to be vector of secondary importance in Ethiopia (Russell, 1959; Gebre Mariam, 1984). Nevertheless most attempts at dissection to determine the sporozoite rate yielded few positive results (O'Connor, 1967; Krafur, 1977). However, some authorities reported a few positive salivary gland infections in Ethiopia and other parts of Africa (O'Connor, 1967; De Meillon, 1951b). Even though this species was caught biting man indoors and outdoors, and were relatively more anthropophilic (second to An. gambiae s.l.) than other anopheline species at Ghibe, there is too little information on the sporozoite rate that could be used as a basis for an estimate. Therefore, the theoretical infective bites a person receives in a given time from An. pharoensis at Ghibe can not be even estimated, as was done for An. gambiae s.l. above.

Since An. coustani coustani and An. pretoriensis were moderately attracted to man, a few examples of these species were dissected during the study but with negative results. In addition to these species, An. christyi and An. squamosus were reported to be infected with Plasmodium sporozoites under natural conditions (De Meillon, 1951b; O'Connor, 1967). But their role in malaria transmission still remains the least known. There is little information in the literature with regard to natural infections in An. longipalpis, An. natalensis and An. garrhami.

Monthly dissections for age determination in An. gambiae s.l. and An. pharoensis during this study were much fewer than those by Detinova and Gillies (1967) and Gillies and Wilkes (1965) in Tanzania. Therefore, it is difficult to determine the true age composition of these species from so short a period of monthly sampling. However, the results of dissections of these species can be used to determine the age at which these mosquitoes were caught biting man.

The proportions of young females (pre-gravid, nulliparous and 1-parous) in both An. gambiae s.l. and An. pharoensis exceeded that of 3-parous and older females in some months of the year (Table 13 and 14). It was also found that nulliparous females made up the greater proportions of these species than any other age group in most parts of the year. There were considerable variations in the proportions

of young females in different months. These monthly irregularities in the proportions of young females may probably be due to variations in the intensities of emergence. Gillies and Wilkes (1965) reported that high proportions of young females corresponded to high adult density and rainfall, thereby associated with an increased rate of new emergences.

The oldest females observed in both An. gambiae s.l. and An. pharoensis were 9-parous. It was repeatedly demonstrated that it took 3 to 5 days to complete the first gonotrophic cycle and 3 days for every subsequent oviposition (Gillies and Wilkes 1965). Therefore the oldest females (9-parous) in the present study would have been 27 to 29 days old. The average age of females which are 3-parous and in their fourth gonotrophic cycle, is about 11 to 13 days. The extrinsic cycle for P. falciparum reported by Davidson and Draper (1953) was 12 to 13 days. Therefore 3-parous females in their mid-fourth gonotrophic cycles would be of sufficient age to develop the parasite to the infective stage. 3-parous females are the youngest age which are dangerous as malaria vectors. It was found that 3-parous and older females of An. gambiae s.l. dissected were 45.6% and that of An. pharoensis were 48.2%.

From mark - release - recapture experiments, Gillies and Wilkes (1965) reported that the oldest recaptured female

was 10-parous and had a calendar age of 34 days. The same authors also reported 14- and 15- parous females under natural conditions. The results of dissections at Ghibe yielded females of An. gambiae s.l. and An. pharoensis as old as 9-parous. From these findings it is difficult to conclude that the average longevity of these species is 27-29 days. The lack of evidence for the existence of older females may probably be due to the increased mortality, due to a higher chance of exposure to residual insecticides in the area. At older ages, the proportions diminish due to mortality. On the other hand, it was found increasingly difficult to count dilations in older specimens, with chains of dilations which are adherent and form clumps. The difficulties which were discussed by senior authorities on ovarian dissection (Detinova, 1962, 1968; Detinova and Gillies, 1964; Gillies and Wilkes, 1965) were well founded and valid.

In the duration of this study, parasitological surveys confirmed that the Ghibe Farm is one of the malaria endemic foci in the country. P. falciparum was the predominant malaria parasite in the area. It was noted that falciparum malaria was significantly more prevalent among children than among adults.

Entomological studies revealed that nine anopheline species occur in and around the Ghibe Farm. Mosquito proliferation in the area was perennial. The most abundant

anthropophilic species were An. gambiae s.l. and An. pharoensis. Adult female densities of these species were closely associated with rainfall, with irrigation activities, and with side pools and rock pools associated with the river.

An. gambiae s.l. and An. pharoensis were presumed to be responsible for the malaria infection observed during the parasitological surveys and at other times. An. gambiae s.l. were equally endophagic and exophagic in their feeding behaviour, whereas An. pharoensis were significantly exophagous in the present study. The indoor and outdoor biting rate of An. gambiae s.l. exceeded that of other anopheline species. The biting rates of An. gambiae s.l. and An. pharoensis observed in this study were much higher than those reported by previous investigators in other regions of Ethiopia. Evening biting catches indicated that most of the indoor and outdoor biting by An. gambiae s.l. occurred between 19:00 and 22:00 hrs. Most of the evening bites by An. pharoensis were observed between 20:00 and 22:00 hrs. indoors and between 21:00 and 23:00 hrs. outdoors. Unfed An. gambiae s.l. and An. pharoensis females made up the greater proportion of the human bait catches.

Dissections for salivary gland infections in both An. gambiae s.l. and An. pharoensis females revealed no positive specimens. Only one An. gambiae s.l. female was found to be positive for Plasmodium oocysts. Monthly dissections for age

determination indicated that there was some variations in the intensities of emergence of both An. gambiae s.l. and An. pharoensis in different months of the year. It was found that the oldest females in these species encountered at Ghibe were 9-parous individuals. 3-parous and older females, which are epidemiologically important in the transmission of malaria, were observed in considerable proportions in different months of the year. Since the average age of the oldest females observed from human bait catches was about 29 days, 2 to 3 monthly sampling and closer monitoring of the pattern of gonotrophic cycles are recommended to determine the true age composition of the populations of these species.

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1984 and February, 1987.

MONTH	Rainfall in mm.					
	1984	1985	1986	1987	1988	1989
January	0.0	12.3	0.0	34.5	22.0	16.5
February	7.6	0.0	31.1	24.2	33.4	23.0
March	10.1	6.9	44.2	34.5	13.9	-
April	8.9	31.2	67.0	46.0	15.8	-
May	133.7	115.2	81.7	129.7	63.7	-
June	144.3	95.2	173.7	104.0	121.1	-
July	212.0	262.9	131.0	156.4	355.1	-
August	133.7	220.3	32.0	133.7	176.6	-
September	31.5	32.1	176.4	35.0	150.1	-
October	10.2	24.2	25.2	13.4	64.4	-
November	12.2	1.3	0.2	7.4	0.0	-
December	4.2	10.3	1.0	3.3	0.0	-
TOTAL	759.1	859.7	860.1	856.1	1016.3	

APPENDIX I

Meteorological Data on Rainfall in Ghibe Horticulture Development Farm between January, 1984 and February, 1989.

MONTH	Rainfall in mm.					
	1984	1985	1986	1987	1988	1989
January	0.0	12.3	0.0	34.5	22.0	14.5
February	7.6	0.0	31.1	24.2	53.4	23.0
March	10.1	6.9	44.2	84.5	13.9	-
April	8.9	51.2	87.0	48.0	15.8	-
May	133.7	115.2	81.7	179.7	43.7	-
June	144.9	95.3	178.7	104.0	121.1	-
July	212.0	268.9	151.0	158.4	355.1	-
August	133.7	222.3	82.0	138.7	176.6	-
September	81.5	52.1	176.4	55.0	150.1	-
October	10.2	24.2	26.8	18.4	64.6	-
November	12.3	1.3	0.2	7.4	0.0	-
December	4.2	10.0	1.0	3.3	0.0	-
TOTAL	759.1	859.7	360.1	856.1	1016.3	

APPENDIX II

Meteorological Data on Temperature and
Relative Humidities in Ghibe Farm (1984 to 1989)

MONTH	Average Monthly Max.-Min. Temp. C		Average Monthly Relative Humidities (%)				
	Max.	Min.	AT 0600	0900	1200	1500	1800
January	36.2	13.8	90.5	72.2	59.8	51.7	65.2
February	36.5	15.7	88.7	76.5	63.2	56.7	63.8
March	37.3	17.0	89.0	76.5	64.0	67.0	62.8
April	36.4	18.6	84.7	77.5	66.8	63.2	69.4
May	34.7	18.5	87.7	82.0	69.6	72.5	77.4
June	31.6	17.0	79.3	85.0	78.0	78.2	76.2
July	28.9	16.4	95.3	91.5	84.4	85.0	81.3
August	29.4	17.9	97.3	92.0	83.2	84.0	81.3
September	31.7	17.9	96.0	89.7	80.8	83.5	80.3
October	35.1	15.6	93.3	82.0	74.6	76.7	79.5
November	36.3	14.5	95.3	72.0	67.0	64.7	73.0
December	36.4	14.7	89.7	69.0	60.8	64.0	68.0

DECLARATION

I, the undersigned, declare that this thesis is my work and that all sources of material used for the thesis have been duly acknowledged.

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Place: ADDIS ABABA UNIVERSITY

Date of submission: JULY, 1989