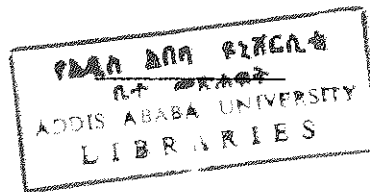


A STUDY ON THE ANTHROPOPHILIC BLACKFLIES (DIPTERA: SIMULIIDAE)
IN GILGEL GHIIBE RIVER AREA (SW ETHIOPIA) WITH
REFERENCE TO THE TRANSMISSION OF ONCHOCERCIASIS

A Thesis
Presented to the
School of Graduate Studies
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of Master of Science in Biology*

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ABSTRACT

Studies on the anthropophilic blackflies in the Gilgel Ghibe area with reference to the transmission of onchocerciasis were conducted between April 1994 and March 1995.

The results of this study showed that onchocerciasis was of low endemicity in the area. Parasitologically positive skin snips were recorded for 17.1% of the persons examined. The prevalence of infection in males (19%) was higher than in females (14.9%). The geometric mean number of microfilariae per skin snip was 11.14, and was higher in males than in females. Clinical signs and symptoms were observed in 26.3% of the population examined- itching (18.4%), pigmentary changes (3.5%), itching with pigmentary changes (3.07%) and itching with papular rash (1.3%).

The collection of biting flies at four selected sites revealed Simulium damnosum complex to be the predominant anthropophilic species in the locality followed by S. ethiopiense which appeared in very small numbers.. Dissections of 6,483 S. damnosum complex showed an average parous rate of 74.7%. The parous rate was observed to vary between the four sites. Parous rates of 87.8%, 62.4%, 56.9% and 41.4% were recorded at sites 1, 2, 3 and 4, respectively, suggesting a differential dispersal of parous and nulliparous flies. Of the total 4,844 parous flies dissected, 1.8% were harbouring L3 larvae morphologically indistinguishable from O. volvulus. The average number of L3 O. volvulus per fly was 5.1. The infection rate at the different sites was, 2.32% (Site 1), 0.75% (Site 2), 0.97% (Site 3) and 2.98% (Site 4).

The annual biting rate (ABR), the annual infective biting rate (AIBR) and the annual transmission potential (ATP) was, 24,928.75, 328.7 and 1,669.5 respectively.

Of the total 5 specimens of S. ethiopiense 60% (3) were parous and none of these were infected. The species plays little or no role in the transmission of onchocerciasis in the Gilgel Ghibe area.

1. INTRODUCTION

Human onchocerciasis, or river blindness, is the disease resulting from infection with the parasitic filarial worm, Onchocerca volvulus, transmitted from person to person by the bites of blackflies, belonging to the genus Simulium, which breeds in fast flowing streams and rivers (Duke, 1990a). The species of Simulium that serve as intermediate hosts and transmit the filarial worm to man at the time they take a blood meal obtain their infection from infected human beings in whose skin the microfilariae are migrating (Beaver *et al.*, 1984).

The disease is endemic in intertropical regions, having been found in most African countries, in Saudi Arabia and North Yemen, and in six Latin American countries (Brazil, Colombia, Ecuador, Guatemala, Mexico, and Venezuela) (WHO, 1976).

Although the disease does not directly result in mortality, it causes extreme itching and incapacitating eye lesions and may be the precipitating factor in reduced social productivity and mass abandonment of agriculturally fertile regions where the population is concentrated (WHO, 1987).

It is extremely difficult to make accurate estimates of the numbers of persons infected with O. volvulus in any country because the disease is one of remote areas and relatively few reliable prevalence survey results are

available. The most recent global figures are those in the third report of the WHO Expert Committee on onchocerciasis (WHO, 1987): the estimated global total of persons living in endemic areas, and thus exposed to the risk of infection, was 85.6 million. Of them, 17.8 million were infected, 336,400 were blind, and a similar number were thought to be suffering severe visual impairment from ocular onchocerciasis. Africa (and the Yemen) accounted for 17.7 million infected and 335,000 blind. The remainder being from the six Latin American countries.

There is a wide spectrum of clinical manifestation associated with onchocerciasis and there are marked geographical variations. In Africa, the worst endemic areas of onchocerciasis occur in the western part of the continent, being more serious in the savanna than in the forest areas. This is due to the existence of forest and savanna strains of *O. volvulus* (Duke *et al.*, 1966) and the Savanna strain being more pathogenic and invasive (Duke and Anderson, 1972). In the worst afflicted villages in the savanna zone, up to 15% of the whole population and more than 40% of the male populations of working age may be blind from onchocerciasis (Duke, 1990a). Communities harassed by onchocerciasis to this extent cease to be economically viable after a few years. Their inhabitants move to a less dangerous area, deserting the fertile land near the rivers in an exchange for a less productive land further away from the *Simulium* breeding sites.

1.1 The Parasite

The filarial aetiology and the skin lesions associated with onchocerciasis were first recognized by John O'Neill in 1875 when he found microfilariae in the skin of patients in West Africa. Later in 1893 the German Parasitologist Leuckart described the adult worms for the first time from subcutaneous nodules sent to him by a medical missionary working in Ghana. However, it was several years before it was realized that the skin lesions and microfilariae described by O'Neill were related to the adult worms described by Leuckart (Nelson, 1991).

Onchocerca volvulus is predominantly a parasite of man maintained in nature by inter-human transmission. The only report of the parasite in an animal under natural conditions is by Van den Berghe et al. (1964) when he found an adult worm from a gorilla.

The adult worms of O. volvulus are commonly located in fibrous but well vascularized nodules in the subcutaneous tissue, although at times they are so deeply situated that they cannot be easily palpated (WHO, 1987). The living worms are white, opalescent, and transparent, with distinct transverse striations of the cuticle. The male worms are relatively small and mobile, the females are long and thin (Beaver et al., 1984).

Adult O. volvulus may live for 15 years and are capable of producing many millions of living embryos, known as microfilariae for at least (nine) to (ten) years

(Roberts et al., 1967; Duke, 1981). The biological function of these microfilariae is to invade the skin and await ingestion by a suitable female Simulium vector when it comes to take a blood-meal from the human host. The anatomical distribution and concentration of these microfilariae in the hosts skin is often related to the site of the bite of the vector (Shelley, 1994).

1.2 The Disease

Chronic infection with O. volvulus, causes dermatologic and ocular complications including blindness. These disease manifestations are thought to be evoked by the host's immune reaction to the parasite (Yarzabal, 1985).

It is the microfilariae that are responsible for most of the disease processes in human onchocerciasis. These microfilariae are occasionally found in peripheral blood and in the urine and sputum, but they are typically found in the skin, eyes, and lymph nodes (WHO, 1976).

There may be 50 - 200 million microfilariae present in a heavily infected O. volvulus carrier (Duke, 1990a), and only a very small proportion of these are ingested by feeding Simulium. The majority of microfilariae are never ingested and they eventually die in the tissues causing scarring and disease (WHO, 1976).

The clinical picture of onchocerciasis includes nodules, a wide variety of skin changes, ocular lesions, lymphatic and systemic manifestations.

1.2.1 Nodules

The adult worms of O. volvulus live either singly or, more often, as coiled entangled masses in deep fascia and subcutaneous tissue. They become encapsulated by fibrous tissue to form discrete onchocercal nodules (onchocercomata) of 0.5 cm to 10 cm or more in diameter in the deep dermis and subcutaneous tissue (Beaver et al., 1984; WHO, 1987).

The distribution of these nodules on the body varies in different geographical regions. Nodules that are visible and palpable from the surface are found mainly over bony prominence, while many others are deep and impalpable (WHO, 1987). Nodules may be used in the assessment of onchocerciasis in an area. Nodules on the head may be very dangerous since they produce abundant microfilariae near the eye. Otherwise, they are usually painless and cause the patient little or no inconvenience (WHO, 1976).

1.2.2 Dermal Onchocerciasis

The skin manifestations are common and due to their social implications may be of major concern to the patient. Their manifestations vary according to the microfilarial density in the skin, immune response of the host and duration of infection.

The lesions due to O. volvulus infection result from inflammatory reactions around damaged or disintegrating microfilariae and are characterized by Pruritus, edema, thickening and wrinkling, atrophy and altered pigmentation

of the skin. When the depigmentation is spotted, it is known as "leopard skin" (Domonkos, 1983).

The lesions may be divided into two main groups, the early and late skin lesions. The early skin lesions are the distressing, itching urtico-papular rashes often confined to one anatomical part of the body seen in recently and relatively lightly infected persons while the late skin lesions develop as a result of prolonged heavy microfilariae infection of the skin (Nelson, 1991). Here after years of chronic infection of the skin, Pigmentary changes, fibrosis and atrophy will be produced.

Among the various types of skin changes observed in infected individuals leopard skin is one. It is initiated by a phase of variable duration characterized by a progressive loss of pigment (Browne, 1960). Rapid assessment of its prevalence in a village population may provide a preliminary estimation of the prevalence of onchocerciasis (WHO, 1987).

Based on the observation that the leopard skin developed on his skin where blackflies bit him remote from the shoulder where his microfilariae are concentrated, Fugslang (1983) Observed the distribution of leopard skin coinciding more with the favoured sites of Simulium bites and concluded that the bites of blackflies, rather than onchocercal dermatitis are the causes of his leopard skin. However, Connor and Palmieri (1985) noticed some patients with onchocerciasis developing leopard skin at sites not

readily exposed to biting simuliids, suggesting that not all depigmentation is explained by repeated Simulium bites. These observations indicate that the causes of leopard skin could be attributed either to the presence of microfilariae in the skin or to the bites of blackflies.

1.2.3 Ocular Onchocerciasis

The most disastrous public health and socio-economic result of onchocerciasis is the damage microfilariae do in the delicate ocular tissue which leads to severe ocular disease and eventually to "river blindness". The ocular lesions of onchocerciasis are caused by living or dead microfilariae and these organisms have been demonstrated in all tissues of the eye (Thylefors, 1978). Lesions of the eye responsible for blindness due to onchocerciasis usually build up over a long period (WHO 1976). In early and light infection with O. volvulus a few microfilariae may invade the cornea producing characteristic "fluffy" or "snowflake" opacities which clear up spontaneously to be succeeded by others. These opacities disappear rapidly following microfilaricidal treatment (Duke, 1990a). However, heavier and more long-standing invasion of the eye by microfilariae can lead to any of the severe and potentially blinding lesions in the anterior and posterior segment of the eye (WHO, 1987). These lesions are in the cornea, the iris and ciliary body, the retina and choroid, and the optic nerve.

Among the several lesions, sclerosing keratitis, the

result of massive invasion of the cornea by microfilariae giving rise to permanent corneal damage, is the predominant cause of onchocercal blindness in the Sudan-savanna area of Africa (Thylefors, 1978a).

1.2.4. Lymphatic Lesions

In heavy microfilarial invasion of the skin, some of the microfilariae may get into the lymph nodes draining the area and produce a lymphadenitis with accompanying fibrosis. The inguino-femoral nodes may enlarge grossly in hanging pockets of skin (known as "hanging groin") predisposing to herniae and sometimes accompanied by elephantiasis of the scrotum (Duke, 1990a).

1.2.5. Systemic Onchocerciasis

In heavily infected patients, microfilariae of O. Volvulus have been found in the urine, blood and cerebrospinal fluid. Eventhough, the systemic effects of onchocerciasis are not fully understood, cases of dwarfism and epilepsy have been found associated with the disease (WHO, 1976). Recently, Gilbert (1991) observed patients with positive skin snips in Malawi weighing significantly less than persons with negative skin snips, suggesting that onchocerciasis should no longer be considered a disease affecting the eye and skin only but an infection which produces systemic effects as well.

1.3 Host Immune Response

The majority of persons resident in endemic onchocercal areas do not present clinical manifestations of

the disease, even when parasitologic examinations of the skin reveal the presence of microfilariae (Anderson et al., 1974). Similarly infections in hyper-endemic areas are acquired early in life, but the density of microfilariae in the skin does not rise indefinitely. Often it reaches a plateau and some balance seems to be reached even during apparently high levels of continuing exposure (WHO, 1976). Ngu and Blackett (1976) suggest that this may be the consequence of the immune response on the part of the host.

Eventhough information obtained from various studies confirms the existence of different immune response patterns to O. volvulus infection, the present understanding of the immune response to O. volvulus infection in man is quite limited. It is still not known accurately what part cellular mechanisms and antibodies play in the pathogenesis of onchocerciasis.

It is generally assumed that in onchocerciasis, the immunologic fight begins at the moment the infective larvae penetrate the skin, having been introduced by the vector. These larvae possess inherent surface antigenic determinants and are also likely to carry immunogens of simuliid origin. It is possible that such antigens stimulate the synthesis of diverse types of antibodies (Yarzabal, 1985). It has also been shown by Ngu and Blackett (1976) that infection with O. volvulus stimulates the synthesis of antibodies directed against antigenic determinants of the different stages found in the human

host (L3 larvae, microfilariae, and adults). These antibodies belong fundamentally to the IgG, IgM, and IgE classes of immunoglobulins. In many people with onchocerciasis, particularly, the IgE levels are raised to extremely high levels, and a greater proportion of IgE antibody is directed against parasite antigen (WHO, 1987).

Likewise, Green et al. (1981) have demonstrated that eosinophils and neutrophils from normal individuals have the capacity to kill microfilariae of O. volvulus in vitro. Similar results were also obtained in experiments made with fresh microfilariae and with cells from infected individuals. They have also observed that the addition of fresh serum increases the lethal capacity of the polymorphonuclear cells, especially the eosinophils. According to WHO (1987), the presence of degranulating eosinophils around dying microfilaria suggests that eosinophils may be a major effector cells in antibody-dependent killing of microfilariae. Eventhough they lack in vivo evidence of this resistance, these experiments indicate that there are humoral and cellular mechanisms that assure the host of some resistance against onchocercal infection.

1.4 Diagnostic Methods in Onchocerciasis

1.4.1 Parasitological

Parasitological methods are the most frequently used techniques to assess the prevalence and severity of onchocerciasis in an area. These methods could be employed

both for the detection of the adult worms as well as the microfilariae.

a) Nodules

The adult worms of O. volvulus live in fibrous but well-vascularized nodules. The nodules are found mainly over bony prominences (Duke, 1990a). The detection of these nodules could be used for the assessment of onchocerciasis.

The detection of nodules, however, is of limited use for establishing the diagnosis of onchocerciasis since some people, especially those who are lightly infected, do not have detectable nodules and some nodules are deep and impalpable so that they cannot be detected. Besides this, the presence of nodules in patients living in areas where transmission has been interrupted for several years is less conclusive since these nodules may contain only dead worms (WHO, 1987).

b) Skin Snips

The detection of microfilariae in a bloodless skin snip is by far the most widely used diagnostic method for the diagnosis of onchocerciasis (Taylor et al., 1989).

Skin snips can be taken with various instruments, including simple razor blades, sharp scissors and the various types of scleral punches. Until recently, scleral punches were most commonly used because they are less painful and threatening to the patient than a razor blade and, in practised hands, may give more uniform biopsies

(WHO, 1987). However, with the advent of HIV, it has become less popular in prevalence surveys. Instead, a razor blade per person which shall be disposed after use is much safer and cheaper.

In prevalence surveys, atleast two skin snips from each person should be taken. The snips should be taken from the sites most likely to be heavily infected, which vary depending on the geographical strain of the parasite. According to WHO (1976) in Africa the preferred site is below the iliac crest, whereas, in Mexico the preferred site is behind the shoulder and in Yemen the highest concentration of microfilariae occurs around the ankle.

The snips are usually examined for living microfilariae either in water or saline. Earlier the use of distilled water for 30 minutes was recommended as a standard method for field studies (Buck, 1974). However, Sowa and Sowa (1975) got better results in saline water. Schulz-Key (1978) from a comparative study of the emergence of microfilariae showed that only 20% of the total number were released after 30 minutes in distilled water, whereas, 50% emerged at this stage in isotonic saline. After 24 hours incubation in isotonic saline, 80% were found.

The skin snip test is a quantitative one and microfilariae are usually quantified against the weight of the skin snip from which the number of microfilariae per milligram of skin can be calculated, and expressed proportional to the weight of the snip.

The disadvantage of skin snip method is that it often fails to detect early and light infections. However, the sensitivity is improved by taking additional snips. The more skin snips that are taken, the more accurate the diagnosis. Taylor *et al.* (1989) has found that the overall sensitivity of 6 snips (i.e. 1 from each shoulder, hip, and calf) per person was estimated to be 91.6%. When the microfilariae density was ≥ 3.5 mff/mg, skin, there were no false negatives. However, at lower microfilariae densities, the sensitivity declined markedly.

During skin snip examination most workers teased or unteased skin snips. To determine the efficiency of these methods, Tada *et al.* (1973) after taking skin snip from patients, teased some into small pieces and others roughly into two pieces and thus left intact. After 22 hours of incubation they observed high microfilarial density in the intact skin snips and lowest in the teased snips.

The use of collagenase to digest the skin enables to assess the total number of microfilariae in biopsies. And the number of false negative skin snips can be reduced as near to zero as possible (Schulz-Key, 1978). However, when compared to incubation in saline for 24 hours, this use of collagenase increased the sensitivity of this test significantly only in patients with very low parasite loads (WHO, 1987).

1.4.2 The Mazzotti Test

Diethylcarbamazine (DEC) which has proved to be of great value for the control of lymphatic filariasis caused by W. bancrofti, cannot kill adult O. volvulus. However, it produces severe pruritic reaction as a result of the death of microfilariae in the skin. The recognition of this reaction by Mazzotti, 1948 (cited in Nelson, 1991) forms the basis of a reliable diagnostic test, the so-called Mazzotti test, which can be used to supplement the bloodless skin snip method.

The Mazzotti test is a useful aid to the diagnosis of O. volvulus infections in whom parasitological evidence of infection cannot be detected. It is usually used in persons in whom the disease is suspected on clinical grounds but the parasite cannot be found. The test consists of giving a small dose of diethyl carbamazine (usually 50 mg for an adult), and observing the development of the clinical reaction in the skin, which results from the destruction of microfilariae. The reactions include increased itching and papular rash. These reactions in the skin may begin as early as 15 minutes after the administration of DEC, or it may be delayed up to 24 hours. Usually the test should be read first, if possible, after about 3 hours, and again at 24 hours (WHO, 1976).

Eventhough this test is a useful aid in the diagnosis of onchocerciasis when parasitological evidence of infection is a failure, the following disadvantages have

been noted (Oomen, 1969a ; WHO, 1976).

1) Some individuals produce no clinically detectable skin reaction to DEC, despite the presence of microfilariae in the skin, i.e. false negative results.

2) In Africa, patients harbouring microfilariae of Dipetalonema Streptocera in their skins may react to doses of DEC higher than 50 mg.

3) Severe reactions such as shock, collapse, loss of consciousness and even death may result.

1.4.3 Immunodiagnosis

To circumvent some of the problems associated with the classical methods of diagnosing onchocerciasis such as skin snip and Mazzoti test, the potentials of immunological tests have been investigated. However, most of these have been hampered by poor specificity caused by immunological cross-reactivity between O. volvulus and sympatric parasites (Chandrashekar et al., 1991). According to Lobos et al (1990) the specificity of antibody detection systems can be improved by the use of low molecular weight fraction.

Using low molecular weight parasite antigens Lobos et al. (1990) have identified and characterized a complementary DNA fragment coding for an O. volvulus antigen (OV-16) which is highly immunogenic early in infection and is also O. volvulus specific. When purified OV-16 was used in ELISA to analyze the antibody response of 41 onchocerciasis patients, they were able to detect OV-16

specific antibodies in 90% of the patients with onchocerciasis. Based on this observation, the Authors claim that OV-16 can be exploited to identify the presence of O. volvulus parasites even in the early prepatent period of infection when microfilariae in the skin or adults in nodules cannot be detected. Likewise, Ogurinde et al., (1993) have recently identified & characterized two recombinant Onchocerca volvulus antigens, OC 3.6 and OC 9.3, which appeared to be promising reagents for use in the diagnosis of onchocerciasis on the basis of preliminary results. While, 95% of serum specimens from patients with onchocerciasis were reactive with OC 3.6; the reactivity with OC 9.3 was 81% suggesting that these might be useful for detecting prepatent infections in humans.

1.4.3.1 Skin Test

The use of O. volvulus microfilarial antigens has made cutaneous tests useful for seroepidemiological survey. Using the crude extract of microfilariae, Hashiguchi et al., (1979) found positive results in 85-86% patients with onchocerciasis and false positive in 1-13% of subjects without onchocerciasis.

1.5 The Simuliid Vectors of Onchocerciasis

The simuliidae, commonly known as blackflies, constitute the family Simuliidae (Diptera, Nematocera). Not all of them are black, some Neotropical species are

even predominantly yellow or orange in color. They are stout bodied, small (mostly 2 -6 mm long) flies with high arched mesothorax and short broad and transparent wings. The mouth parts of the female are adapted for blood-sucking but male flies do not bite (Crosskey, 1973).

Blackflies are cosmopolitan in distribution being found almost anywhere if there are suitable rivers and streams for the developmental stages. The outlets of ponds and lakes are also suitable habitats for filter feeding larvae of Simulium (Davies et al., 1978). Generally blackflies are absent from areas devoid of running water such as polar regions and deserts (Crosskey, 1973).

The eggs of blackflies when first laid are pale and often whitish but darken to a brown or black color. They are about 0.1 mm long, and are more or less triangular in shape. Usually some 150 - 800 eggs are laid in sticky masses or strings on a level with, or just below the water line on submerged objects (Service, 1986).

The larvae which hatch from the eggs are always found in free water, the almost universal requirement being moving usually fast-moving water. The larval life is generally completed in about 10 days to a little to over two weeks in the tropics whilst in the temperate zones the period of development of the larva lasts about three to four weeks. Temperature and seasonal influences have a profound effect on the larvae. Pupation takes place in the larval habitat. The pupal stage lasts two or three days to

a week but it may be prolonged with a lowering of temperature (Crosskey, 1973; Gordon and Lavoipierre, 1962).

Blackflies bite by the day and in the open, although Simulium ochraceum s.l. (Dalmat, 1955) and S. amazonicum, and S. pertinax (Shelley, 1994) will bite in small numbers in houses and at night fall when biting populations are high.

Several of the anthropophilic species select particular feeding sites on the human body (Gordon and Lavoipierre, 1962). For instance females of S. damaosum of Africa tends to concentrate its attack on the lower extremities (Duke and Beesly, 1958) whilst the Central America species, S. ochraceum, bites on the upper part of the body (Shelley, 1988).

Blackflies affect man and his domestic animals both by their bites and as intermediate hosts of parasites (Dalmat, 1955). A few blackfly species cannot bite, but the great majority of them bite and suck the blood of warm-blooded vertebrates. Only about 10% (150 of the 1500 or so species) bite man and his domestic animals (Crosskey, 1973). As a consequence of this feeding habit simuliids are able to transmit several pathogenic organisms.

In both tropical and non-tropical areas of the world simuliids can cause a very serious biting problem, since their bites can be painful. Although the severity of the reaction to bites differs in different individuals, localized swelling and inflammation frequently occurs,

accompanied by intense irritation lasting for several days or even weeks (Service, 1986).

Simuliids in addition, to being notorious for their blood sucking and consequent toxic effects, transmit protozoan and nematode parasites to domestic and wild birds as well as to mammals (Freeden, 1977). They may even play some role as vectors of encephalitis viruses which they may propagate among birds and transmit to humans (Anderson et al., 1961). More importantly, the simuliidae are notable for the transmission of filarial parasites to man, his domestic animals and birds.

It is now known that nine filarial species are transmitted by simuliids. These include Dirofilaria ursi in bears, Mansonella ozzardi in man, Onchocerca cervipedis and O. trasicola in deer, O. dukei, O. lienalis, and O. ochengi in cattle, O. volvulus in man, and Splendofilaria fallisensis in ducks (Shelley, 1994). Other filarial species undoubtedly have simuliids as vectors because unidentified filariae are often found in flies, especially in onchocerciasis areas in Africa (Duke, 1967; Nelson and Pester, 1962; Voelker and Garms, 1977). These unidentified species have been assigned to the genus Agamofilaria (Voelker and Garms, 1977).

Eventhough Simuliids are known to transmit several pathogenic parasites, their medical importance lies in their ability to transmit the two human filariae,

Mansonella ozzardi and Onchocerca volvulus. Mansonella ozzardi is restricted to tropical America, where it is also transmitted by species of Culicoides (Ceratopogonidae) (Shelley et al., 1980). Onchocerca volvulus is found in Africa, Latin America, and Southern Arabia (WHO, 1976).

Of the two filarial species of medical importance O. volvulus, the causative agent of human onchocerciasis is seriously pathogenic (Nelson, 1970). Blackflies are the only vectors of human onchocerciasis. Their habit of tearing and rasping the skin to rupture the blood capillaries when obtaining a blood meal makes them particularly suited for the ingestion of microfilariae of O. volvulus (Gordon and Lavoipierre, 1962, Service, 1986).

In Africa, human onchocerciasis is transmitted by members of Simulium damnosum complex and species of the S. neavei group (WHO, 1976). Simulium damnosum s.l. is the most widely distributed and the most important vector in the whole continent, members of the S. neavei group transmit the disease only in East and Central Africa (Raybould and White, 1979).

In Latin America, larger number of vector species are involved. Simulium ochraceum, S. metallicum, S. gonzalezi, S. callidum, S. veracruzianum, and S. haematopotum are anthropophilic and have been incriminated in the transmission of the parasite in Central America (Shelley, 1988).

Because of the relative homogeneity of the family, requiring the use of characters from more than one stage of life cycle, the taxonomy of simuliidae generally depends upon a combination of larval, pupal and adult characteristics (Raybould and White, 1979).

Simulium damnosum which was formerly regarded as a single but variable species by freeman and de Meillon (1952) is now known to be a complex of forms which cannot be distinguished morphologically except by the banding patterns of the larval chromosomes (WHO, 1976). This identification to cytospecies level is of crucial importance to fully understand transmission cycles and their subsequent relevance to control programmes, because different members of the complex show differences in aspects of their biology with regard to vectorial capacity, biting behaviour, and habitat preference (Shelly, 1994).

Since the discovery that, S. damnosum is a complex of sibling species, a considerable number of distinct cytotaxonomic categories have been recognized by Dunbar (1966, 1969) and others within the conventional morphological concept of "S. damnosum". Only some of these categories have man-biting females. Simulium damnosum of Africa is now known to consist of over 40 members. Of these, twelve are vectors of O. volvulus in Africa and one in the Arabian Peninsula (WHO, 1987). Nine cytospecies are now known from larval chromosomes from West Africa and these are divided into four sub-complexes: damnosum

containing S. damnosum s.s., S. sirbanum, and S. dieguerense, Squamosum containing S. squamosum and S. yahense, Sanctipauli containing S. sanctipauli, S. soubrense, and S. soubrense B, and "Kibwezi" containing S. menegnse (Post et al., 1991 cited in Shelley, 1994).

Regarding members of the complex from east Africa, recently Procunier and Muro (1993) revealed the presence of eight distinct taxa from central and northern Tanzania. These cytoypes are Nyamagasani form, Nkusi form, Sanje form, Kiswani form, Kibwezi form - Kibwezi A1 and Kibwezi A2, Ketaketa form - Ketaketa C1 and Ketaketa C2. Otherwise, the vernacularly named East African cytota remain inadequately described. From the West African cytospecies, S. damnosum s.s. and S. siribanum, extend east to the Nile System (WHO, 1987). In Ethiopia, a preliminary work has so far revealed the presence of at least three forms (Raybould and White, 1979; WHO, 1987).

The Simulium neavei group of East Africa on the other hand, includes all simuliids in which the larvae and pupae live in phoretic association on river crabs of the genus Potomonautes (Raybould and White, 1979). The structural uniformity of members of this group and the lack of any cytological studies, makes identification of potential vector species in the group difficult. However, at least four species are recognized within the group (WHO, 1987). These are S. ethiopiense, S. neavei s.s., S. nyalalandicum, and S. woodi. Their distribution in Africa is from

Ethiopia southward to Malawi through the Zaire river basin to Cameroon and Liberia. No vector species extends into West Africa. Recently Procunier and Muro (1994) presented detailed larval salivary gland polytene chromosome maps for two taxa designated S. neavei Amani form A and S. neavei Amani form B, which are probably synonymous with the previous described morphospecies, S. nyasalandicum and S. woodi respectively. However, extensive cytological work on the S. neavei group as a whole is still in need.

Work on vector sibling species in Latin America is at a more preliminary stage. Of the eleven O. volvulus vector species present, larval cytotaxonomy has established the presence of species complexes in the following. These include three cytotypes in S. ochraceum (Hirai et al., 1994), eleven in S. metallicum (Conn et al., 1989 cited in Hirai et al., 1994), four in S. exiguum, two in S. gonzalezi and two in S. oyapockense (Shelley, 1994).

1.6 Development of O. volvulus in the vector and its transmission to man

The first description of the development of O. volvulus in Simulium was that of Blacklock (1926) (cited by Nelson, 1991). The position of O. volvulus microfilariae in the skin is an evolutionary adaptation for transmission by the local vector (Nelson, 1970). They are spread in the surface of the skin in the anatomical region most likely to be bitten, and at the right depths where they can be easily

ingested by the short proboscis of the vector (Kershaw et al., 1954).

Microfilariae ingested with the blood during infective feed pass down the alimentary canal to the midgut, where digestive enzymes as well as the chitinous peritrophic membrane are secreted. In S. damnosum, immediately after feeding, a peritrophic membrane begins to form around the blood-meal in the dilated posterior portion of the midgut. This membrane provides an impenetrable barrier to O. volvulus microfilariae, and those trapped within it die before it finally disintegrates (Duke and Lewis, 1964)

The peritrophic membrane forms initially within 30 minutes of feeding, and it has been shown that it severely restricts migration of microfilariae to the hemocoel (Duke and Lewis, 1964). Successful penetration of the stomach by microfilariae therefore, has to occur soon after their entry into the gut. Laurence (1966) has shown that microfilariae leave the blood-meal rapidly after engorgement, 40 - 50% of them leaving the stomach within the first 60 minutes, the first micro-filariae appearing in the thorax after 20 minutes.

Having penetrated the stomach wall and entered the insects hemocoel the microfilariae migrate to the flight muscles in the thorax where they penetrate the muscle cells. Development through the L1 and L2 to the L3 stage then occurs if the parasite has been ingested by the

appropriate vector (Nelson, 1991). The infective larvae then appear in the proboscis as early as the sixth day after the original ingestion of the microfilariae. Temperature and humidity are important determinants for the successful development of the organisms in the vector (Nelson and Pester, 1962).

Transmission occurs when infected flies take a blood-meal. Little is known of the exact mechanism by which the infective stage (L3) pass from the mouth parts of the fly into the skin of man, but very probably they penetrate through the wound made when the fly bites, and those which fail to do so perish on the skin surface (Duke, 1973). Very little is known again, about the subsequent development of O. volvulus in man, but it is assumed that the parasites migrate through the subcutaneous tissue, undergoing two further moults before they reach the adult stage, six to twelve months after infection (Nelson, 1991).

1.7 The Existence of Non-human derived Filarial L3 Larvae in Simuliid Vectors

Since no simuliid species is totally anthropophilic, sympatric human and animal filariae can be found in the same vector species. There is a variety of evidence from the finding of non-human derived filarial larvae in S. neavei group (Nelson and Pester, 1962) and in S. damnosum s.l. (Duke, 1967; Garms and Voelker, 1969; Gebre-Michael, 1983; Gebre-Michael and Gemetchu, in press) showing their zoophilic behaviour to varying degrees. This can

cause overestimates of vector competence in epidemiological and control programmes for O. volvulus. For example, the cattle parasite, Onchocerca ochengi which is widely distributed in Africa have been found in S. damnosum, the vector of the human parasite, O. volvulus. The L3 larvae of both filariae are morphologically indistinguishable (Trees, 1992). Other filariae of animal origin which are quite distinct from O. volvulus or bovine Onchocerca spp. are also known in simuliid vectors and have been assigned to the genus Agamofilariae (Voelker and Garms, 1977).

The taxonomy and identification of filariae are still far from satisfactory because of their parasitic habit and hence relative inaccessibility in their definitive and intermediate hosts. Since the earlier work in Africa, morphometric analysis have been used to distinguish O. volvulus from other filaria species found sympatrically in S. damnosum (Duke, 1967; Nelson and Pester, 1962; Voelker and Garms, 1977; McCall et al., 1992). Besides the morphological, non-morphological methods, such as biochemical, histo-chemical and immuno-chemical methods have been developed to distinguish both species and strains of filariae with varying degrees of success. More recent work on DNA sequences has provided a precise method of identification for species as well as intraspecific strains. Probes for separating O. volvulus from O. gibsoni, O. ochengi, O. gutturosa and O. cervicalis as well as savanna from forest strains of O. volvulus have now

been developed (Shelley, 1994).

Despite the development of the non-morphological methods, microscopic examination of vectors using morphological characters for species, parasite number, and life stage determination is the standard field technique still in use. Morphometric examination in general has the quality of simplicity and applicability in any field study. However, it suffers the disadvantage of not being definitive in the case of individual L3_s, and of being unable to identify L3_s of other possibly occurring but, as yet, undescribed Onchocerca species (Trees, 1992). This is why currently considerable effort has been directed to molecular methods of identification.

1.8 Onchocerciasis Control

The Onchocerciasis Control Programme (OCP) of the World Health Organization in the Sudano-Guinean savanna zone of West Africa has been operational since 1974. Its objectives are: (1) to put an end to onchocerciasis as a disease of public health and socioeconomic importance and (2) to ensure that there will be no recrudescence of the disease thereafter (WHO, 1976). The strategy at first has been to interrupt transmission through larviciding alone, however, since the beginning of 1988, a second weapon, this time chemotherapeutic, has become available for control of onchocerciasis (Duke, 1990b).

When the programme started in 1974, it focussed on African Sudano-Guinean savanna belt where blinding

onchocerciasis is of sufficient public health importance and hinderance to socio-economic development. At first, the programme was centred in Burkina Faso and parts of the six surrounding countries (Benin, Cote d'Ivoire, Ghana, Mali, Niger and Togo) in the Volta River Basin areas (WHO, 1976). Later, it was extended into four other countries: Guinea, Guinea-Bissau, Senegal and Sierra Leone (WHO, 1987).

The insecticide selected for OCP was a biodegradable insecticide tempehos (Abate) (WHO, 1976). Later, the constant threat of insecticide resistance has necessitated the use of four newly developed back up insecticides on a rotational basis (Duke, 1990a).

The results of the OCP over the first ten years were remarkably successful. After ten years of control transmission was virtually interrupted over nearly 90% of the original area. Progression of eye lesions in all but a few of the worst cases was halted. The incidence of new infections among three million children born in the programme area during its first ten years of operation was reckoned to be almost zero. The concentration of microfilariae in the skins and eyes of persons who were already infected at the outset of the OCP had fallen steadily (Remme et al., 1986).

Parasitological surveys carried out 14 years later revealed that the community microfilarial load (CMFL) was close to zero in all villages, and prevalence of infection declined at an accelerated rate particularly in those

villages that had the highest level of endemicity during the precontrol period (Remme *et al.*, 1990).

The incidence of onchocerciasis infection in children born since the start of the vector control is one of the indicators used in the epidemiological evaluation of the OCP. The results of 14 years Simulium control in the OCP area suggests that larviciding had achieved a 99% reduction in the incidence of infection in children (DeSole and Remme, 1991). The results therefore, leave no doubt that onchocerciasis has been eliminated as a problem of public health importance from the central OCP area. After 14 years of vector control, the level of onchocerciasis has fallen to such a low level that consideration is being given to end larviciding without the risk of recrudescence (DeSole and Remme, 1991).

Since the beginning of 1988, the Merck, Sharp and Dohme (MSD) compound Ivermectin has been brought up to registration in France, for use in human onchocerciasis under the name of Mectizan. Many studies indicate that Ivermectin is effective against Onchocerca volvulus as a single dose, non-toxic, quick acting microfilaricide as well as micro-filarial suppressant of some three - twelve months duration. It radically changes onchocerciasis prevalence and intensity (Duke, 1990b). However, according to Whitworth (1992) the use of Ivermectin alone cannot be expected to achieve adequate degree of transmission control. There are many problems - for example, not

everyone in endemic areas will be treated, not all microfilariae are removed from the skin, some remaining available for uptake by the vector. Ivermectin does not kill blackflies, nor does it prevent filarial development to the infective (L3) stage, and infected blackflies may invade from untreated areas (Whitworth, 1992). Furthermore, as a means of control for onchocerciasis, ivermectin needs to be regularly administered over a period of not less than five years, usually at an annual frequency (Taylor and Green, 1989). Therefore, in the control of onchocerciasis both the control of Simulium through the use of larvicides as well as the treatment of patients using the chemotherapeutic agent, ivermectin, should be carried out.

1.9 Onchocerciasis and Simuliidae in Ethiopia

The existence of human onchocerciasis and simuliid vectors in the Keffa region of Ethiopia has been demonstrated for the first time in 1939 by Giaquinto Mira (cited in Oomen, 1969a). Since then, extensive epidemiological surveys conducted by several workers (Oomen, 1967a, b; Oomen 1969 a, b; Iwamoto et al., 1973; Lester and Tsega, 1974; DeSole and Walton, 1976; Mengesha and Jembere, 1977; Taticheff et al., 1987; Gundersen et al., 1988 ; Yeneneh et al., 1989 ; Zein 1986, 1990, 1993) have confirmed the presence of the disease, with varying degrees of endemicity, in the south-western, western and north-western parts of the country. The infection being

particularly associated with coffee growing areas in the south-west (WHO, 1987) and with cotton and oil seed farming areas in the north-west (Zein, 1986). The coffee growing areas in the southwest are thought to provide suitable ecological conditions for the vector as well as for the parasite in the dense human populations associated with coffee (DeSole and Kloos, 1976). Prevalence of the disease in Ethiopia ranges from a high of 82-84% in western and southwestern endemic zones (Gunderson *et al.*, 1988; Workineh *et al.*, 1993) through 56% in the south-west (Iwamoto *et al.*, 1973) to a low of 19.5% in the north-west (Zein, 1986). Therefore, based on WHO's (1966) classification of endemicity levels, there are hyperendemic, mesoendemic and hyperendemic areas in the country. In general, prevalence of the disease is greater in the lowlands.

Earlier Oomen (1969a) estimated the number of microfilariae carriers in the endemic regions of Keffa, Wollega and Illubabor (area of 132,000 km²) to be about half a million. Later, WHO (1987) estimated the number of people at risk in the onchocerciasis endemic areas to be about 7.3 million out of which 1.38 million are infected with *O. volvulus*. Recently, Zein (1993) estimated about 10 million of the Ethiopian population to be at risk from the disease (in the endemic area covering about 200,000 km²).

The clinical picture of onchocerciasis in Ethiopia has been described as mild, involving a wide variety of skin

changes (Oomen, 1969 a, b; Iwamoto et al., 1973; Woodruff et al., 1977; Zein, 1986). It is believed that onchocerciasis does not cause blindness in Ethiopia, the main causes of blindness in the country being trachoma, cataract, glaucoma and blindness of childhood (Budden, 1981; cited in Zein, 1988). However, since information on blindness due to onchocerciasis in the country is inadequate, the findings of moving microfilariae in the anterior chamber of the eyes (Gundersen et al., 1988) and Onchocercomata on the head (Zein, 1986) needs further and extensive investigation since these features of onchocerciasis eventually lead to blindness.

Work on Ethiopian simuliidae carried out by (Van Someren, 1944; Grenier and Ovazza, 1956; Oomen 1969a; Tanaka et al., 1973; Raybould and White, 1979; Mebrahtu et al., 1980) suggests that Simulium flies, both anthropophilic and non-anthropophilic, have wide distribution in all regions of Ethiopia, extending beyond the known endemic area of the western part of the country. Of these, at least seven species show anthropophilic tendencies, of which S. damnosum s.l. and S. ethiopiense (a member of the S. neavei group) are regarded as the vectors of onchocerciasis in the country (Raybould and White, 1979; WHO, 1976, 1987). A detailed checklist of the simuliidae of Ethiopia is given by Mebrahtu et al. (1980).

Simulium damnosum s.l. is widely distributed throughout the endemic and non-endemic areas of the country

and is regarded as the most important vector

(Grenier and Ovazza, 1956; Oomen, 1969a; Raybould and White, 1979 ; Mebrahtu et al.,1980). This has been confirmed by more detailed studies in some selected foci in south-west Ethiopia (Gebre-Michael, 1983; Gebre-Michael and Gemetchu, in press).

According to WHO (1987) three chromosomal taxa of the complex are recorded in the country. These are the "Kisiwani", "Kulfo" and "Jimma" forms. The "Jimma" form is the only member of the S. damnosum known from the south-west (Kaffa region) and might be the vector (Raybould and White, 1979).

On the other hand, S. ethiopiense (a member of the S. neavei group) which has a limited distribution in the smaller rivers of south-western midlands and highlands and often sympatric with S. damnosum s.l. has long been suspected of playing a secondary role. S. ethiopiense shows a strong anthropophilic behaviour but its density is often remain quite low at all seasons. As a result, it has long been suspected of playing a secondary role in onchocerciasis transmission in Ethiopia (Grenier and Ovazza, 1956; Oomen, 1969 a,b; TenEyck, 1973; White, 1977; Raybould and White, 1979). The few attempts to isolate infective larvae resembling O. volvulus from S. ethiopiense in Gilgel Ghibe and Gojeb river valleys have so far been unsuccessful (White, 1977; Gebre-Michael and Gemetchu, in press). However, its vector potentiality have only been

shown by experimental infection on patients (Schmidt, M.L., Pers. Comm. to White, G.B., 1977).

1.10 Objectives of the study

The present study in the Gilgel Ghibe river area was carried out with the following objectives.

1. To determine the prevalence and intensity of human onchocerciasis in the study area.
2. To determine the annual transmission potential of onchocerciasis (ATP) and its association with prevalence of the disease in the area.
3. To investigate further on the relative importance of S. damnosum complex and S. ethiopiense, and thereby determine the role of the latter in the transmission of O. volvulus in the study area.

2. MATERIALS AND METHODS

2.1. The Study Area and Selection of Study Sites

Gilgel Ghibe river (1710 m a.s.l.), where the study is conducted is located at a distance of 285 km along the Addis Ababa - Jimma main road in the south-western part of Ethiopia (Fig.1).

Together with the Gojeb and the Gibbie Ennaraya the Gilgel Ghibe river forms the right bank tributary of the Omo/Gibbie river system which flows into Lake Turkana on the Ethio-Kenyan border in the south. The Gilgel Ghibe arising in the high rain fall region of western Ethiopia, flows throughout the year and forms broad valleys which contrast with the deep gorges of the northern plateaus (Wolde-Mariam, 1972).

The area around the Gilgel Ghibe river bridge where the study is undertaken is a flat plain with sparse vegetation. According to Gebre-Michael (Pers. Comm.) ,the natural vegetation at the river side was richer some 10-12 years ago, but is now greatly disturbed by increasing human activity. The outstanding vegetation now found along the river bank include, Acacia tortilis, Acacia seyal, Croton macrostachys, Ourqus gurgus, Albizia spp, together with other shrubs and grasses.

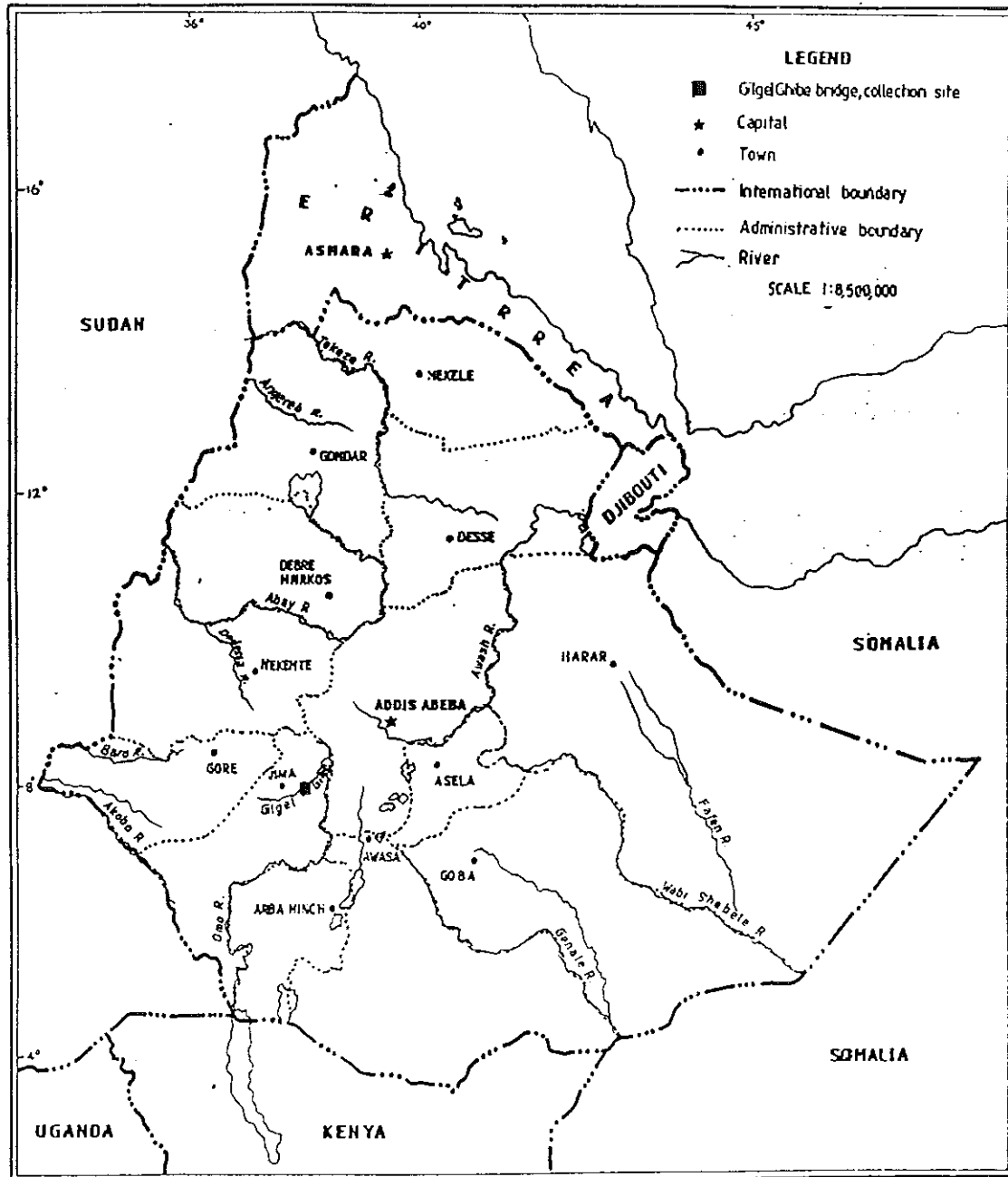


Fig. 1. MAP SHOWING THE STUDY AREA

Hippopotamus (Hippopotamus amphibus), Colobus monkey (Colobus polykomos), Warthog (Phacoerus aethiopicus) are the most commonly observed wild animals in the area.

Few farms are found scattered along the river bank. There is a saw mill factory in a small village of 400 - 500 inhabitants (Personal communication with Ato Dinka, Chairman of the Kebele Assosation) at a distance of 300 - 400 metres from the river bank. The majority of adult males of working age in the village are labourers in the facotry. The remainder are farmers and small merchants. The females are usually housewives and children are either students or look after cattle or farm.

2.2. Diagnosis for Human Onchocerciasis

For the parasitological and clinical examination, the purpose of the study was explained to the chairman of the kebele and to few influential persons well ahead of time. As a result, the study was conducted on subjects who understood the purpose of the study and voluntarily reported for examination. The aim at first was to examine the whole population but only those willing were examined. These accounted for about 46-57% of the total population.

2.2.1. Clinical Examination

A brief medical history was obtained from each participant and recorded on a questionnaire form. The questionnaire contained the name, sex, age group,

occupation, birth place and duration of residence in the village (See Appendix). Each participant was specifically asked if he/she experienced any itching and if so, to what degree and for what length of time.

In the clinical examination, each individual was visually examined for skin lesions as well as palpated for Onchocerca subcutaneous nodules.

2.2.2. Parasitological Examination

(i) Collection of Specimen

The iliac crest was the site of skin-snipping (WHO, 1987) and samples were obtained using a sterile needle and razor blade which were discarded after use on each person. From each person two skin snips, each 2 - 3 mm in diameter were obtained from the pelvic girdle. The snips could not be weighed since the appropriate balance was not available.

(ii) Examination of Specimens

The two skin snips were placed in two separate wells of a microtitration plate containing 100 micro litre of normal saline. When all wells were full, the plate was covered with adhesive plaster to reduce evaporation or spillage and was kept for 24 hours at room temperature. After 24 hours, the saline was pipetted onto slide and was examined under a compound microscope with the X10 objective. When microfilariae were detected, they were counted using a talley counter and were expressed as the

number of microfilariae per skin snip (mf/ss) for each person.

2.3. Entomological Studies

2.3.1. Simulium Sampling

In the present study, Simulium population sampling was carried on human baits using aspirators and/or test tubes. Four catching sites of biting simuliids located at 0 to 400 mts away from the river banks were used throughout this investigation (Fig 2). Site one was located on the river bank, and site two and site three at distances of 150 meters and 300 meters, respectively, away from the river bank towards the village; all three were on one side of the river. Site four was located at a distance of 400 meters away from the bank of the river was on the other side. All the catching sites were under shade of trees.

Twelve hours (from 0600h to 1800h) collection of flies attacking human baits for one day a month at each site was conducted on four consecutive days every month for a total period of 12 months starting from April 1994 to March 1995. A pair of catchers/baits worked at each site.

The catchers/baits worked while seated together on low chairs or 'burchumas', with their trousers and shirt-sleeves rolled well up above knees and elbows. For this purpose it was initially intended to use infected boys but skin positives could not be found in the few local boys examined. Therefore apparently healthy individuals were

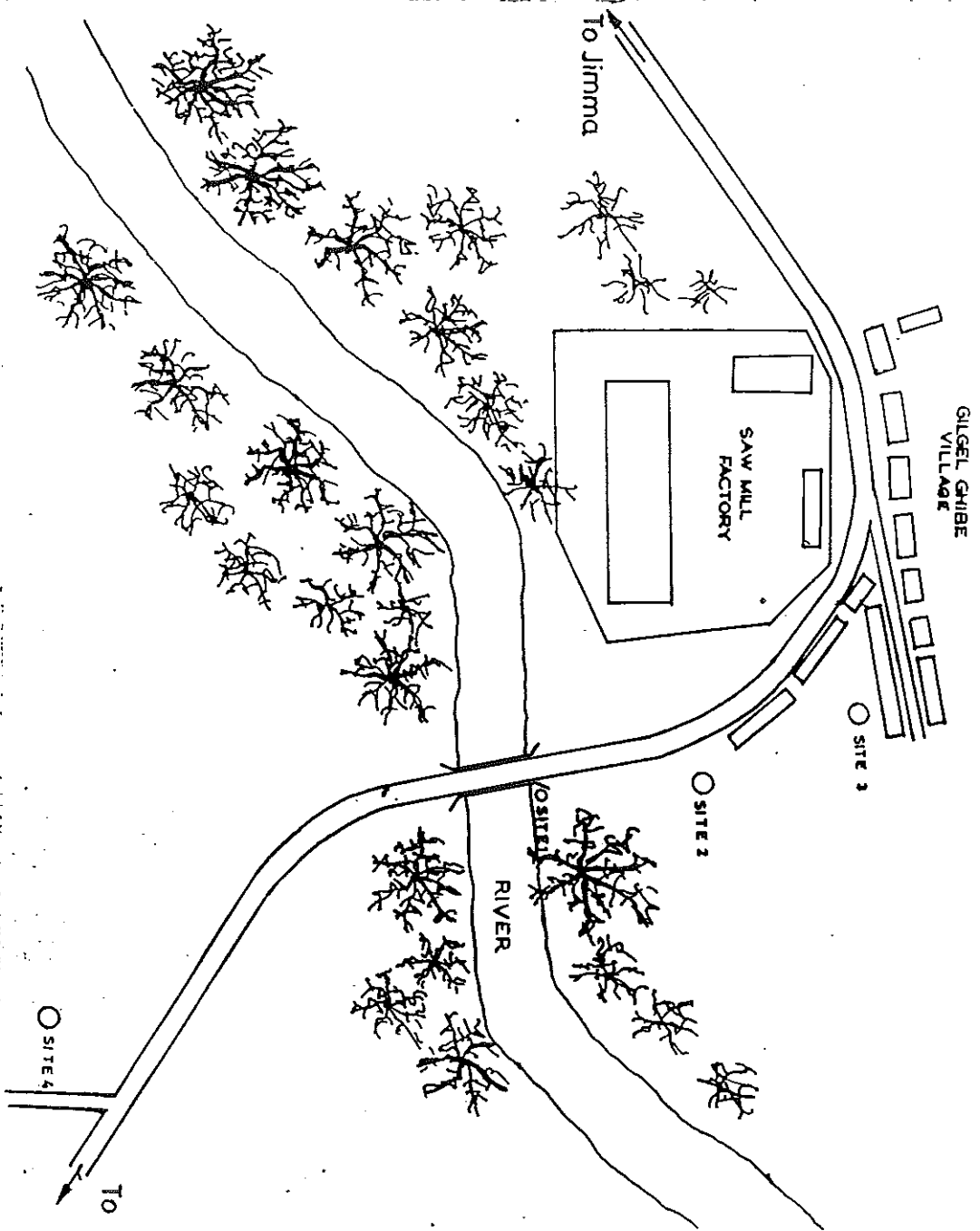


Fig 2. Location of Biting Collection Sites

used as baits with appropriate precautions. For safety reasons, flies were collected immediately after landing and well before they commenced feeding on a bait/catcher. To minimize boredom, the baits were replaced every six hours at each site.

2.3.2. Identification and Parity Determination

At the end of every 30 minutes or an hour, flies were transferred to test tubes plugged with cotton wool for identification and dissection for age determination. Flies were first killed with chloroform vapour and then transferred to a petridish with damp cotton wool. The identification of the flies was then done under a binocular dissecting microscope using a key provided by Crosskey (1973). Then, the hourly batch of flies were immediately dissected to determine parity.

Dissection for parity was performed on a drop of physiological saline on a glass slide under a stereoscopic microscope. Using fine entomological needles, the abdomen of the fly was severed near the last two or three posterior tergites and the contents of the abdomen drawn out until the ovaries and the hind gut associated with Malpighian tubules were observed. Parous and nulliparous flies were distinguished using the criteria recommended by WHO (1976). The indicators used for this are the state of the ovaries, Malpighian tubules, and fat body.

After the hourly catches were dissected and divided

into nulliparous and parous, the parous flies were preserved in 80% alcohol, in vials, for later staining and dissection for filarial parasites. Nullipars were discarded in the field.

2.3.3. Filarial Infection Rate Determination in Simulium

In the Laboratory in Addis Ababa, the alcohol preserved parous flies were stained in Mayer's acid Haemalum according to the method recommended by WHO (1966). Flies were first washed in descending dilutions of ethanol (i.e. 70%, 50%, 30% and 15%) and finally in water for about 40 minutes in each case. Afterwards, the flies were stained for three days in Mayer's acid Haemalum and then differentiated in water for 3 days. Finally, the flies were transferred to glycerol to await dissection.

To examine for Onchocerca volvulus or any other filarial parasite, the head, thorax and abdomen were separately dissected in separate drops of glycerol on a slide, using fine entomological needles. The filariae isolated from each region were mounted in glycerol on slides and their number recorded.

2.3.4. Filariae Identification

The identification of filarial larvae was entirely made by morphological examination and measurements from camera lucida drawings of the infective larvae (third stage) found in the head, thorax and abdomen. This was

based on the dimensions recorded for Onchocerca volvulus by several investigators. Important distinguishing characters were total length, oesophageal/intestinal ratio and the anal ratio (Nelson and Pester, 1962; Duke, 1967; Voelker and Garms, 1977; McCall et al., 1992). All infective larvae with dimensions falling in the ranges of O. volvulus given by the above authors were identified as "morphologically indistinguishable from O. volvulus".

2.3.5. Collection of Crabs

To look for larvae and pupae of Simulium ethiopiense (a member of the S. neavei group) collection of crabs was performed, during the study period.

Perforated cans (8X8cms) suspended from a branch hanging on the river and positioned under rocks (Fig.3) were used for the collection of fresh water crabs. The cans were baited with fresh meat and fish.

The crabs collected after being marked with the name of the catching place and the date of capture were placed in Jars for later identification.

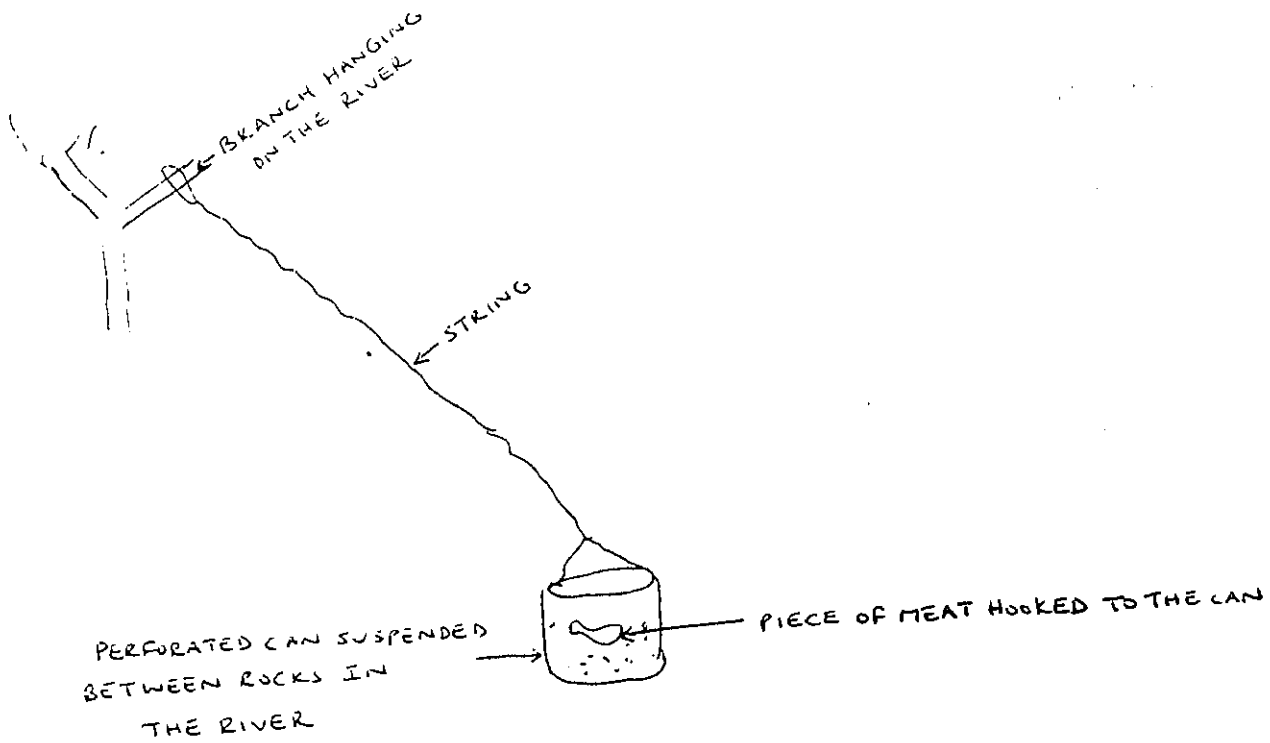


Fig3. Sketch of the trap used for the collection of crabs.

3. RESULTS

3.1 Prevalence of Human Onchocerciasis in Gilgel Ghibe Area

The age and sex distribution of the 228 people examined for onchocerciasis in this study in the Gilgel Ghibe area is presented in Table 1. Of the total 228 people examined, males constituted 53.1% (121/228) while females form 46.9% (107/228). Most of the individuals examined are from the age group 1-14 and relatively few from the age group 50 and above. Microscopic examination of skin snips from the iliac crests showed the existence of O. volvulus infections among the inhabitants of Gilgel Ghibe village. Table 2 shows the parasitological results. The prevalence of infection in males was 19% (23/121) and was higher than in the females, 15% (16/107), but this difference was not significant ($X^2 = 0.40357$; $P > 0.5$). The overall prevalence rate of onchocerciasis in the 228 persons examined in Gilgel Ghibe area was 17.1% (39/228).

Generally, a higher rate of infection was observed in males of all age groups than in females of a corresponding age group. There was a steep rise in prevalence with age in both sexes until about age 29 and then tends to stabilize at older age (Table 2).

The age and sex specific infection rate shows that a maximum infection rate is attained at age 15-29 by both

Table 1 Number of persons in the study grouped by age

Age group Years	Males		Females		Total No. %
	No. examined	% Total	No. examined	% Total	
1-14	48	21.1	36	15.8	84(36.8)
15-29	25	11.0	38	16.7	63(27.6)
30-49	40	17.5	22	9.6	62(27.2)
> =50	8	3.5	11	4.8	19(8.3)
Total	121	53.1	107	46.9	228(100)

Table 2. Age and sex standardized prevalence of *Q.volvulus* in Gilgel Ghibe area.

Age group Years	Males		Females		Total	
	No. exam	No. Pos(%)	No. exam	No.Pos(%)	No exam	No.Pos(%)
1-14	48	2(4.2)	36	1(2.8)	84	3(3.6)
15-29	25	9(36)	38	9(23.7)	63	18(28.6)
30-49	40	10(25)	22	4(18.2)	62	14(22.6)
> =50	8	2(25)	11	2(18.2)	19	4(21.1)
Total	121	23(19)	107	16(15)	228	39(17.1)

males and females and the least infection was observed in the age group 1-14 in both sexes. While 46.2% of the positive cases are found in the age group 15-29, only 7.7% are infected from the age group 1-14.

Out of the total 39 positive cases 74.4% or 29 individuals had microfilariae in biopsies from both the right and left iliac crest, while 25.6% or 10 individuals had microfilariae from either the right or left iliac crest.

3.1.1 Intensity of microfilarial burden in skin snips

The parasite load in the village expressed as the geometric mean of microfilariae per skin snip is presented in Table 3. The community microfilarial load was 11.14 mf/skin snip. The microfilarial count ranged between 1 and 132 per skin snip. The mean microfilarial load was higher in males (13.8 mf/ss) than in females (7.94 mf/ss), although this was not stastically significant ($t=0.088$, d.f 66; $p>0.05$).

The mean microfilarial load in males was observed to increase with age up to the age of 15-29 and then decreased. In females the mean microfilarial load continued to rise with age up to the age of 15-29 and then decreased. However, the greatest difference occurred in females aged 50 and above, where the load rose again. Of all the age groups in both males and females the load in females of age group of 50 and above was the highest.

3.1.2 Effect of duration of residence on onchocerciasis infection

Table 4 shows the infection rate by duration of residence. A higher rate of onchocercal infection 77% (30/39) was found in individuals resident in the village for more than 5 years than those with a residence period of less than 5 years 23% (9/39). Of those that have resided for more than 5 years, 70% (21/30) were males and 30% (9/30) were females. In individuals with a residence period of less than 5 years 22.2% (2/9) are males and 77.8% (7/9) are females.

3.1.3 Clinical manifestations

In the present investigation, signs and symptoms suggestive of onchocerciasis were observed in 26.3% (60/228) of the study population examined clinically. Of these, 36.7% (22/60) were positive parasitologically. In other words, of the 39 individuals with positive skin snip, only 22 (56.4%) had typical signs and symptoms suggestive of onchocerciasis. However, microfilariae with no clinical sign of onchocerciasis were found in 17 (43.6%) of the positive cases. The frequency of clinical signs and symptoms of onchocerciasis in the study population by age and sex is presented in Table 5.

Itching was the most common complaint being observed in 18.4% (42/228) of the people examined. Pigmentary changes, itching with pigmentary changes and itching with papular rash was observed in 3.5%, 3.07% and 1.3% of the

Table 3 Geometric Mean and range of microfilariae density per skin snip

Age group Years	Males			Females			Total		
	Pos.	G.mean	(range)	Pos.	G.mean	(range)	Pos.	G.mean	(range)
1-14	2	15.85	(4-37)	1	1	-	3	6.9	(1-37)
15-29	9	16.98	(2-68)	9	10.72	(2-67)	18	13.49	(2-68)
30-49	10	1.22	(1-86)	4	2.89	(1-13)	14	7.94	(1-86)
>=50	2	14.8	(6-61)	2	21.38	(4-132)	4	17.78	(4-132)
Total	23	13.8	(1-86)	16	7.94	(1-132)	390	11.14	(1-132)

Table 4 Positivity by duration of residence in the village

Age group (years)	Duration 1-5 years		Duration >5 years	
	Males (%)	Females (%)	Males (%)	Females (%)
1-14	-	1(2.6)	2(5.1)	-
15-29	2(5.1)	5(12.8)	7(17.9)	4(10.2)
30-49	-	-	10(25.6)	4(10.2)
>=50	-	1(2.6)	2(5.1)	1(2.6)
Total	2(5.1)	7(17.9)	21(53.8)	9(23.2)

Table 5. The frequency of clinical signs and symptoms by age and sex

Age group (years)	Itching		Pigmentary changes		papular rash		Nodules		Itching and Pigmentary changes		Itching and papular rash	
	M	F	M	F	M	F	M	F	M	F	M	F
1-14	5(2)	1(0)	-	-	-	-	-	-	-	-	-	-
15-29	6(2)	8(2)	2(0)	1(0)	-	-	-	-	2(2)	-	-	2(2)
30-49	14(1)	5(4)	3(1)	-	-	-	-	-	4(3)	-	1(1)	-
≥50	3(0)	-	1(1)	1(0)	-	-	-	-	-	1(1)	-	-
Total	28(5)	14(6)	6(2)	2(0)	-	-	-	-	6(5)	1(1)	1(1)	2(2)

() = Numbers in parenthesis show the No. of skin snip positives

people examined, respectively. Of the 42 individuals complaining of itching, 11 (26.2%) had O. volvulus. Two (25%) of the 8 patients with pigmentary changes, Six (85.7%) of the 7 patients with itching and pigmentary changes, and all 3 patients with itching and papular rash were positive for O. volvulus. Patients with nodules were not found.

3.2 Entomological Findings in Gilgel Ghibe area

3.2.1 Anthropophilic blackfly species and their relative abundance.

Results of the twelve months long (April 1994 - March 1995) collection of adult female blackflies on human baits at 4 sites in Gilgel Ghibe river area show the presence of two anthropophilic blackfly species, Simulium damnosum s:l and S. ethiopiense, the overall catch being 6488 blackflies (Table 6). S. damnosum s:l was the most common and important species, comprising 99.92% of the total catch. S. ethiopiense was caught in very small numbers, and accounted for only 0.08% of the total biting catch. It appeared only during the rainy season and all came from site 1.

3.2.2 Biting densities, seasonal trends and the biting rhythm

The biting density of S. damnosum s.l flies caught at all the sites is presented in Table 7. The total number of flies caught, the number of flies per man per day and flies

Table 6 Black fly species collected on human baits in the Gilgel Ghibe river area and the monthly Meteorological data (April 1994 - March 1995)

Year	Month	Max Temp	Min Temp.	Mean Temp.	Rain fall (mm)	No. <i>S.damnosum</i> (%)	No. <i>S.ethiopiens</i> (%)	TOTAL
1994	Apr	27.6	14.3	21.0	160.0	50(100)	-	50
	May	26.4	14.1	20.3	113.2	206(100)	-	206
	Jun	24.4	14.4	19.4	257.4	778(99.87)	1(0.13)	779
	Jul	23.6	14.4	19.0	190.6	2460(99.96)	1(0.04)	2461
	Aug	23.3	14.5	18.9	303.1	2190(99.86)	3(0.14)	2193
	Sep	24.6	13.5	19.1	131.7	408(100)	-	408
	Oct	27.3	12.0	19.7	7.0	56(100)	-	56
	Nov	27.7	11.4	19.6	13.5	28(28)	-	28
	Dec	28.5	8.5	18.5	0.0	22(100)	-	22
	1995	Jan	29.2	10.2	19.7	9.4	59(100)	-
Feb		29.6	14.0	21.8	34.4	93(100)	-	93
Mar		(-)	(-)	(-)	(-)	133(100)	-	133
					6483(99.92)	5(0.08)	6488.00	

(-) Data not available

Table 7. The biting density of *Simulium damnosum* s.l at different sites in the Gilgel Ghibe river area (April 1994 - March 1995)

Year	Month	Site 1			Site 2			Site 3			Site 4			Total for all sites		
		Tot. no. flies	Flies/ man/ day	Flies/ man/ hour	Tot. no. flies	Flies/ man/ day	Flies/ man/ hour	Tot. no. flies	Flies/ man/ day	Flies/ man/ hour	Tot. no. flies	Flies/ man/ day	Flies/ man/ hour	Tot. no. flies	Flies/ man/ day	Flies/ man/ hour
1994	Apr.	43	21.5	1.8	7	3.5	0.29	0	0	0	0	0	0	50	6.25	0.52
	May	166	83	6.9	34	17	1.42	5	2.5	0.20	1	0.5	0.042	206	25.75	2.15
	Jun.	344	172	14.3	333	166.5	13.88	94	47	3.92	7	3.5	0.29	778	97.25	8.10
	Jul.	1169	584.5	48.7	738	369	30.75	451	225.5	18.8	102	51	4.25	2460	307.5	25.63
	Aug.	1117	558.5	46.5	714	357	29.75	320	160	13.33	39	19.5	1.63	2190	273.75	22.81
	Sep.	283	141.5	11.8	78	39	3.25	34	17	1.42	13	6.5	0.55	408	51	4.25
	Oct.	47	23.5	1.96	9	4.5	0.38	0	0	0	0	0	0	56	7	0.58
	Nov.	28	14	1.17	0	0	0	0	0	0	0	0	0	28	3.5	0.29
	Dec.	22	11	0.92	0	0	0	0	0	0	0	0	0	22	2.75	0.23
1995	Jan.	46	23	1.92	13	6.5	0.54	0	0	0	0	0	0	59	7.38	0.62
	Feb.	83	41.5	3.46	5	2.5	0.20	5	2.5	0.20	0	0	0	93	11.63	0.97
	Mar.	129	64.5	5.38	4	2	0.17	0	0	0	0	0	0	133	16.63	1.39
	Total	3477	1738.5		1935	967.5		909	454.5		162	81		6483	810.4	

per man per hour is given in Table 7. No such data are presented for S. ethiopiense since the numbers were extremely low.

It may be seen from Table 7 that the biting density of S. damnosum s.l flies vary between the four sites. With more flies concentrating on the river bank, the biting density was observed to decline as the distance from the river bank increases. While biting flies were caught throughout the year at Site 1, they were less abundant and sometimes totally absent, particularly during the dry season from sites 2, 3 and 4. Thus highest biting densities of S. damnosum s.l flies was observed at the river bank (Site 1) and the lowest at Site 4.

The highest biting density at site one was during the middle of the rainy season in July, when 584.5 flies/man/day (or 48.7 flies/man/hour) were recorded, and the lowest, 11 flies/man/day (or 0.9 flies/man/hour) was recorded in December during the dry season. During the wet season (April-September) the fly density ranged between 21.5 flies/man/day to 584.5 flies/man/day (or 1.79 flies/man/hour to 48.7 flies/man/hour). During the drier months between October and March, the biting density ranged between 11 flies/man/day to 64.5 flies/man/day (or 0.9 flies/man/hour to 5.4 flies/man/hour).

Similar trends of biting density were also observed at sites 2, 3, and 4 where greater numbers of S. damnosum s.l bit during the rainy season and relatively few during the

dry season, although densities at these sites were much lower than at Site 1. In comparison with the other sites the least biting density for S. damnosum was recorded at site 4 where flies were caught only during the rainy season.

Generally at all the sites the biting density of S. damnosum s.l flies was observed to exhibit a distinct seasonal pattern. The seasonal fluctuation as percentage of all flies caught at each site is depicted in Figs. 4 to 8. It can be seen that highest biting densities at each site occurred during the rainy season and the lowest in the dry months. The peak biting densities in the wet months correlated positively with rainfall ($r=0.74$) but not with temperature ($r= - 0.43$).

The diurnal biting cycle of S. damnosum at each site over the year is presented in Figs. 9 to 12. The biting cycle at site 1 (Fig. 9) was essentially bimodal showing two peaks, one in the morning from 800 hour-1200 hour and the other in the afternoon from 1600 hour-1800 hour. The biting activity was suppressed between noon and the early part of the afternoon. The pattern during the rainy season is somewhat different where the flies bit in quite large numbers throughout the day with a smaller peak in the afternoon.

The biting activity of S. damnosum s.l. at Sites 2, 3 and 4 was basically similar to Site 1, with two peaks, one in the morning the other in the afternoon (Figs. 10 - 12).

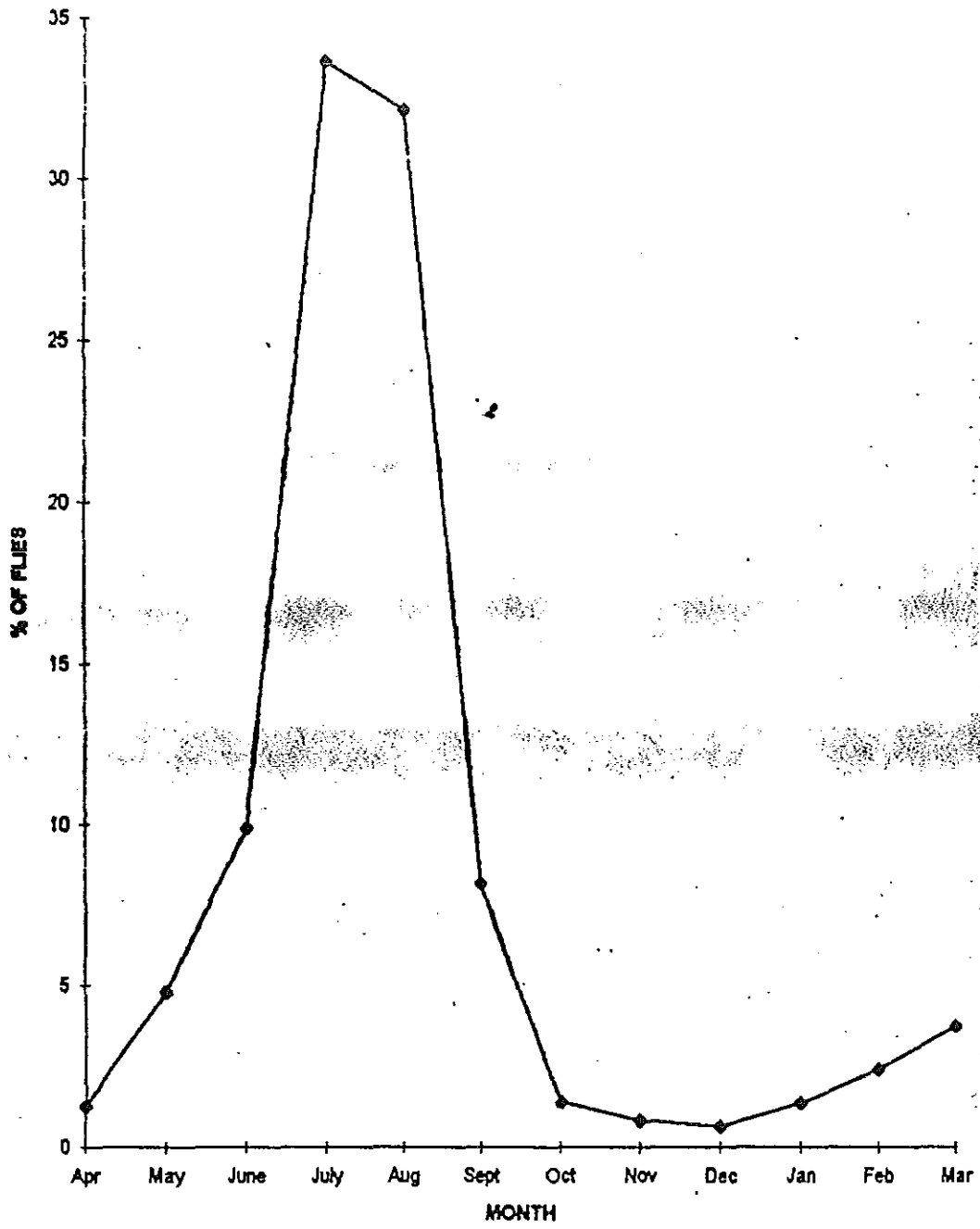


Fig. 4 Seasonal fluctuation in the population density of *S. damnosum* at Site 1 (April, 1994 to March 1995)

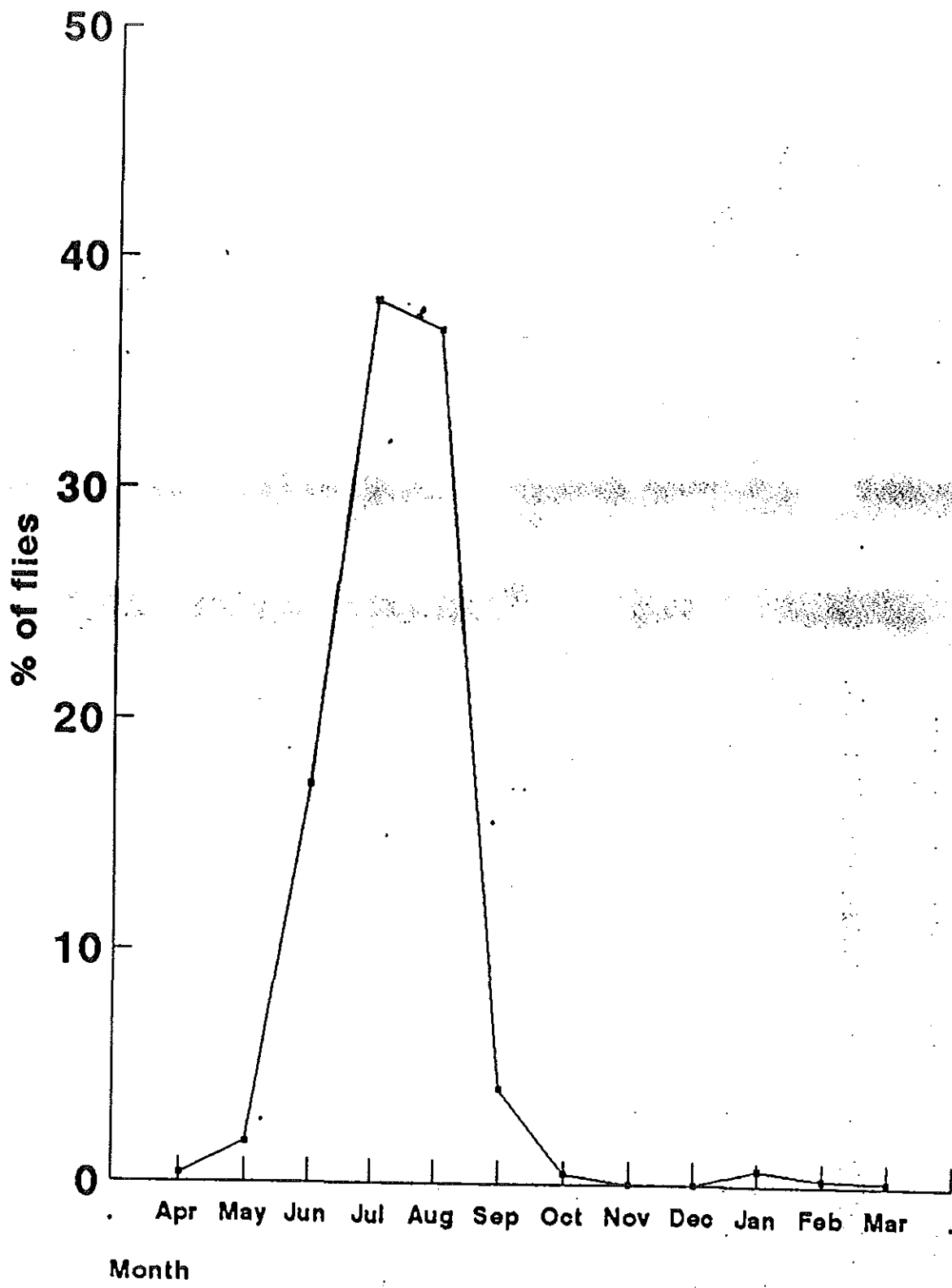


Fig. 5 Seasonal fluctuation in the population density of *S. damnosum* at Site 2 (April, 1994 to March 1995)

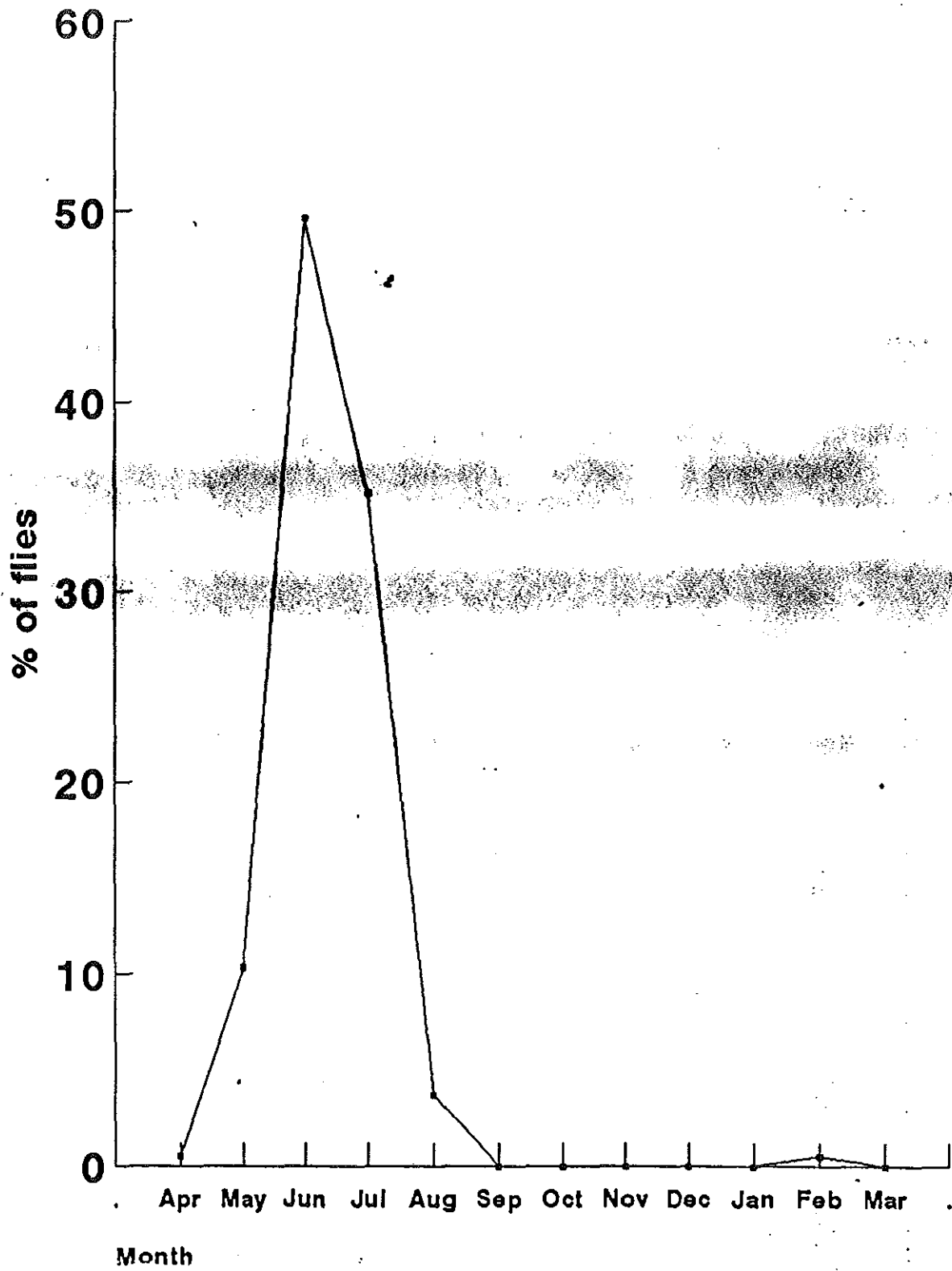


Fig. 6 Seasonal fluctuation in the population density of *S. damnosum* at Site 3 (April, 1994 to March 1995)

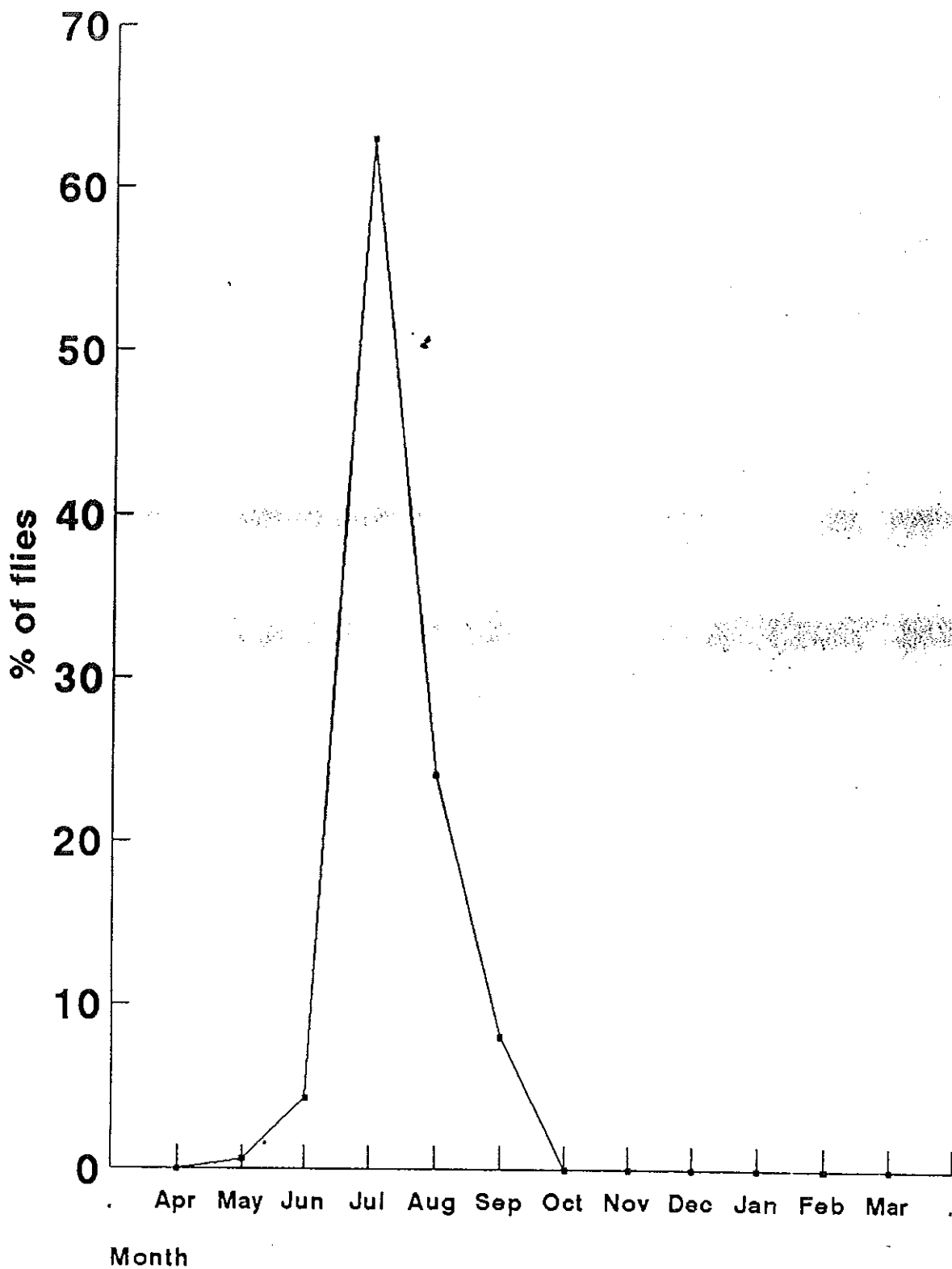


Fig. 7 Seasonal fluctuation in the population density of *S. damnosum* at Site 4 (April, 1994 to March 1995)

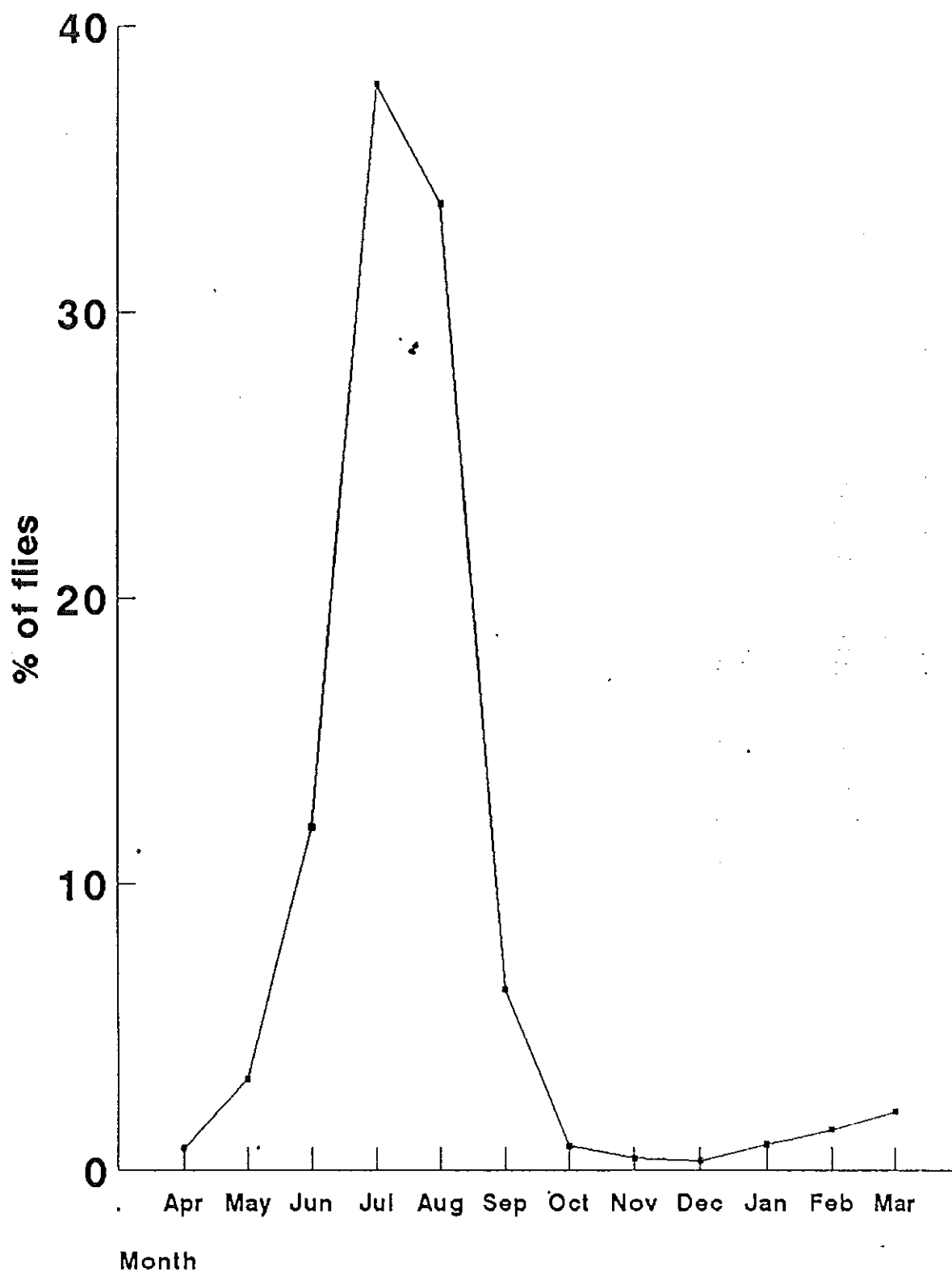


Fig. 8 Seasonal fluctuation in the population density of *S. damnosum* all Sites (April, 1994 to March 1995)

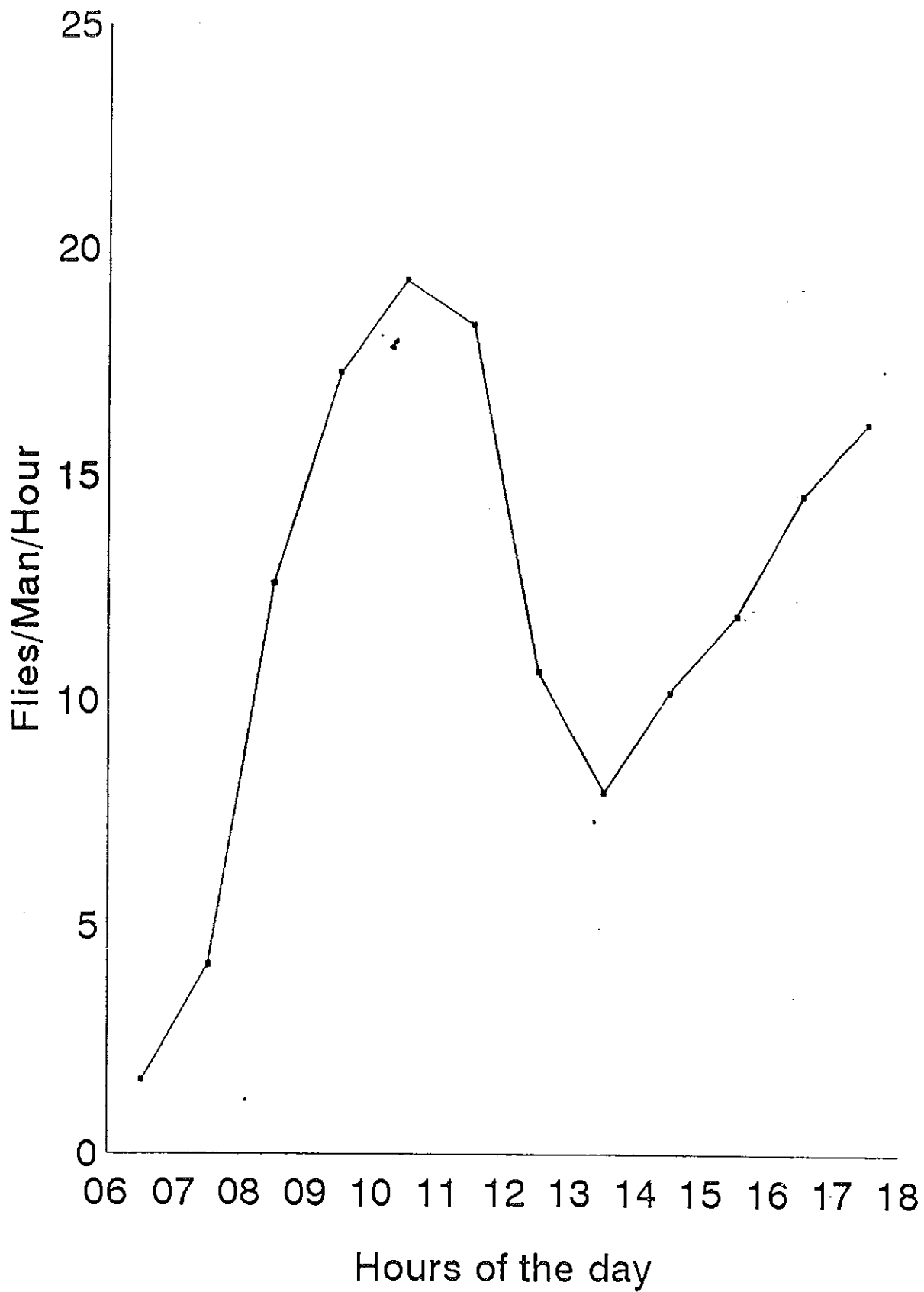


Fig. 9 The diurnal biting cycle of *S. damnosum* at Site 1 (April, 1994 to March 1995)

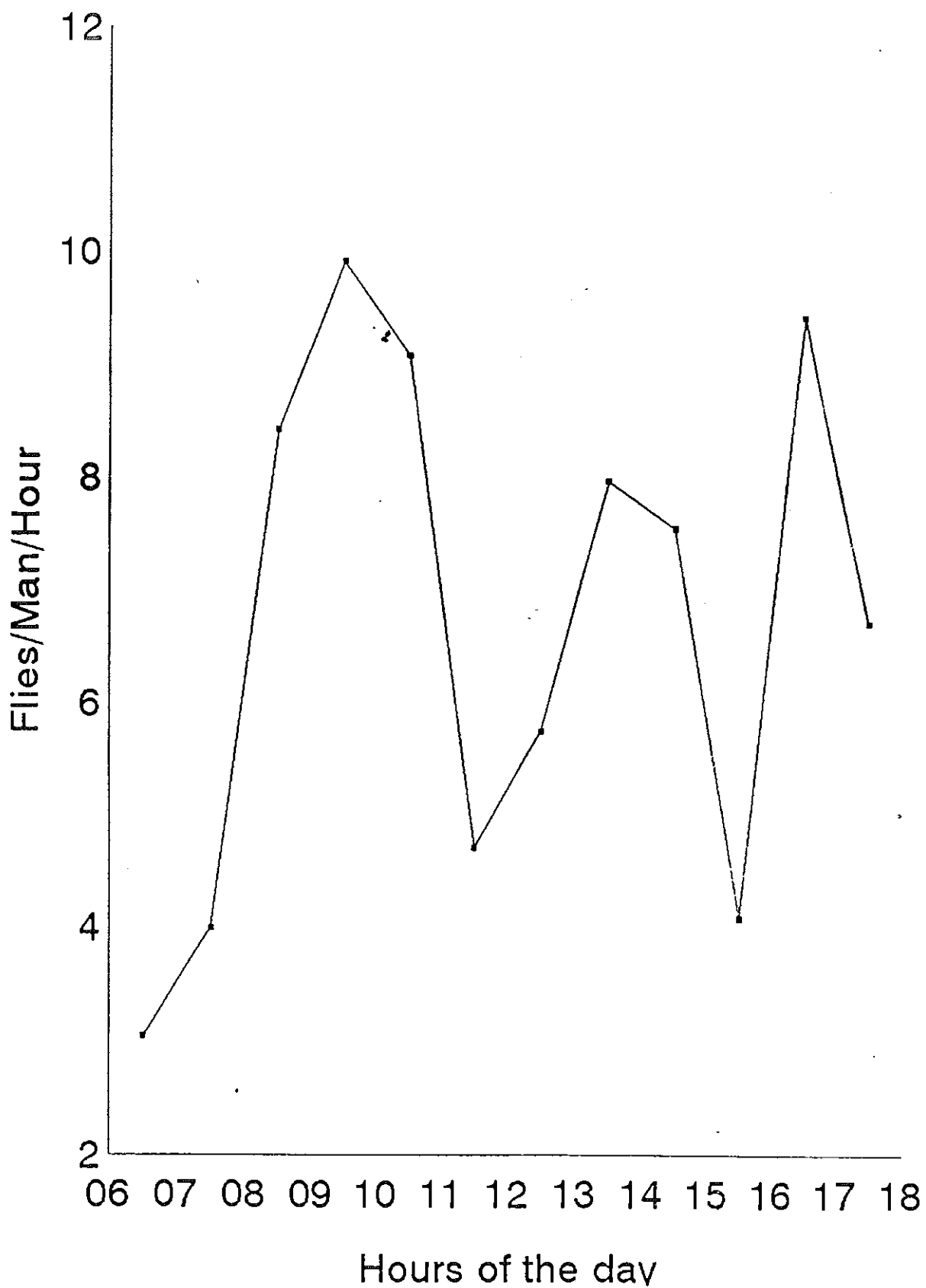


Fig. 10 The diurnal biting cycle of *S. damnosum* at Site 2 (April, 1994 to March 1995)

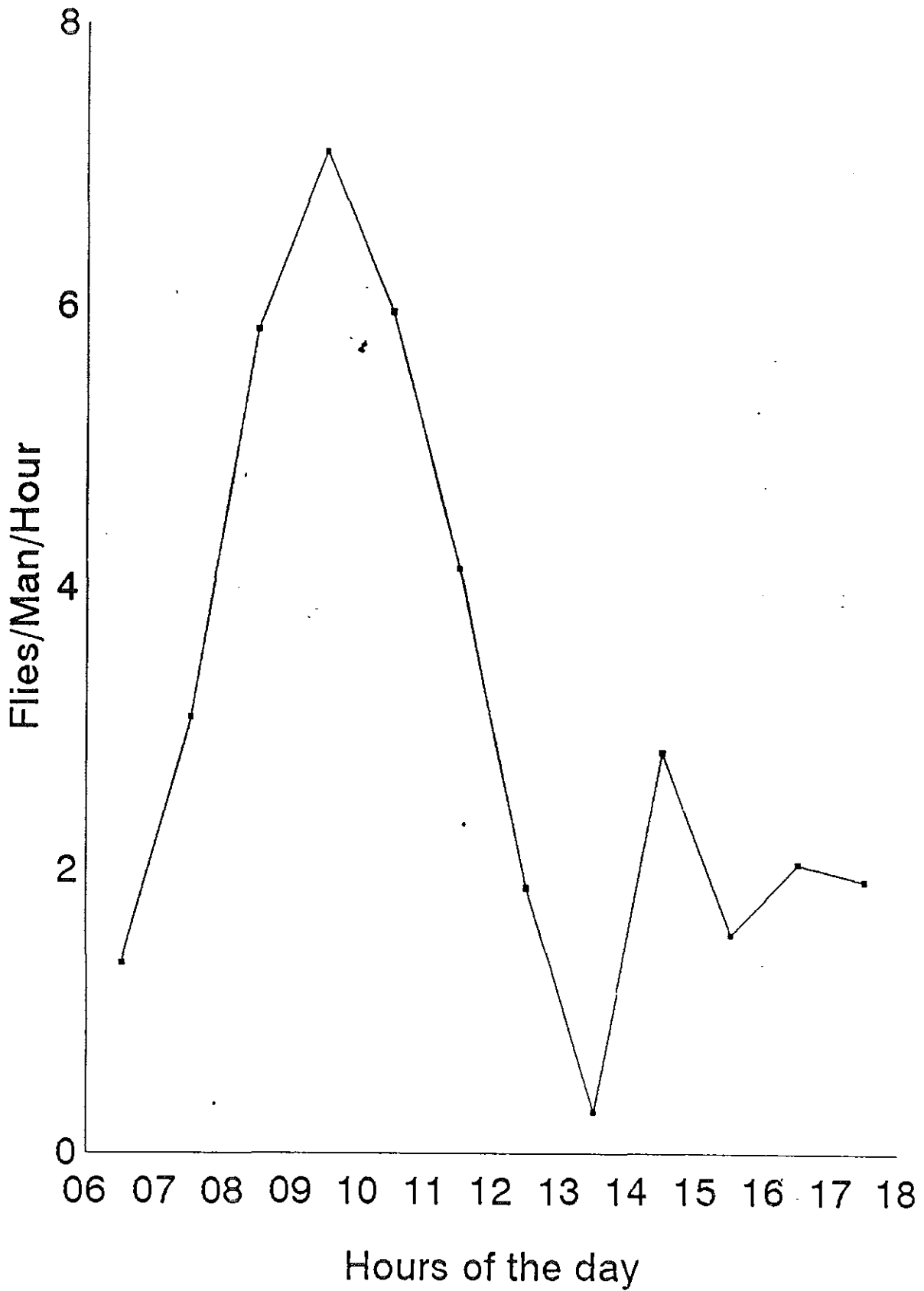


Fig. 11 The diurnal biting cycle of *S. damnosum* at Site 3 (April, 1994 to March 1995)

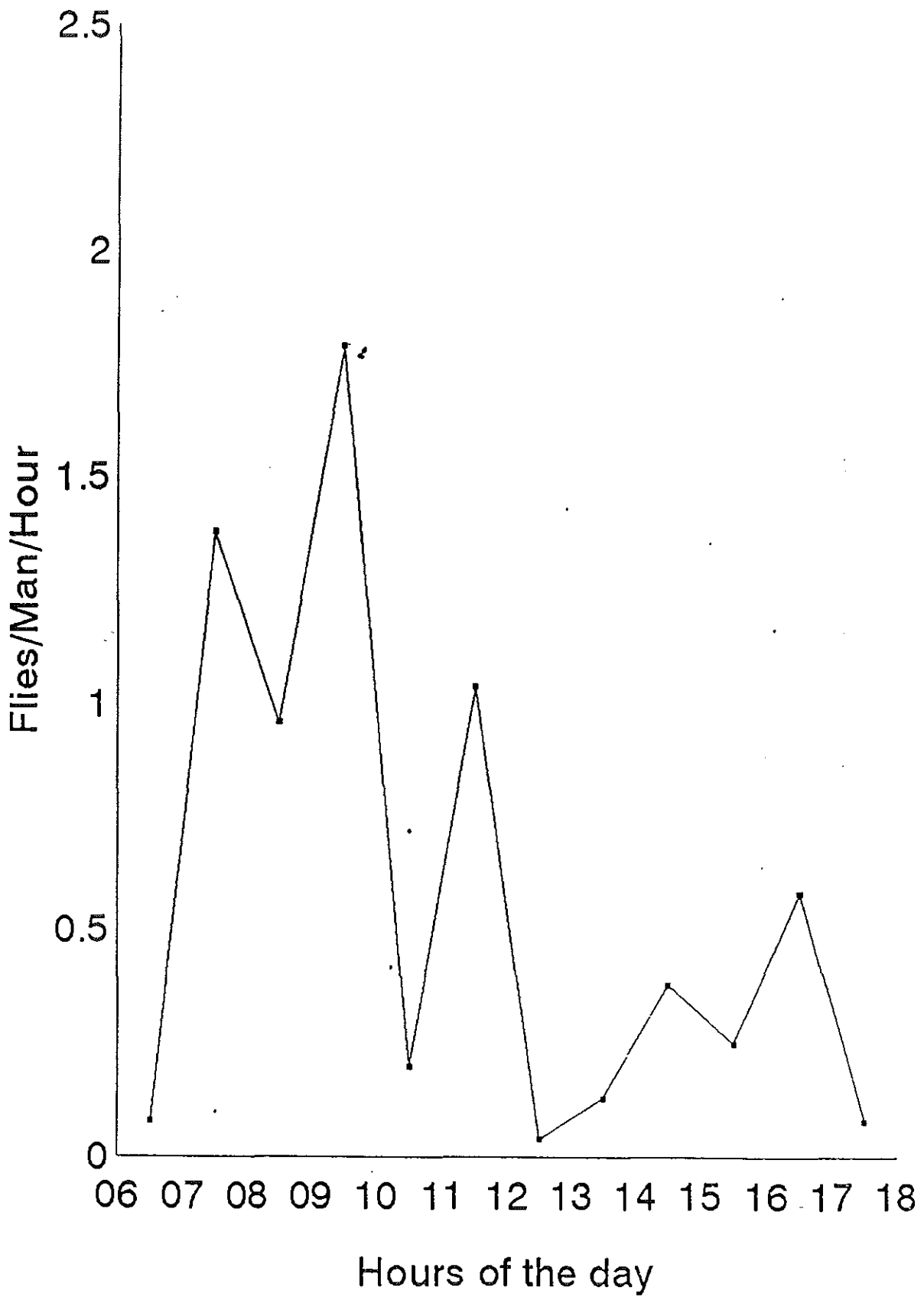


Fig. 12 The diurnal biting cycle of *S. damnosum* at Site 4 (April, 1994 to March 1995)

3.2.3 Parous rates of Simulium flies dissected.

a) Simulium damnosum.

The parous rate of S.damnsum flies at different sites in the Gilgel Ghibe river area is given in Table 8.

Of the total 6,483 S. damnosum s.l collected and dissected for age determination during the study period, the overall parous rate was 74.7% (4,844) while the remaining 25.3% (1,639) flies were nulliparous. The parous rate ranged from 70.6% in July to 90.2% in March, with significant seasonal difference ($X^2 = 14.26$; $P < 0.001$). The parous rate was observed to vary between the different sites and there was a decrease in parous rates as the distance away from the river bank increases.

The monthly parous rate at Site 1 ranged from 76.5% in May to 92.47% in August with an average rate of 87.7% but no seasonality in parous rates was observed ($X^2 = 0.0365$, $P > 0.5$). The proportion of parous flies caught at Site 1 was found to be significantly higher than all other sites: Site 2 (62.4%: $X^2 = 476.6$, $p < 0.001$); Site 3 (56.9%: $X^2 = 330.43$, $P < 0.001$) and Site 4 (41.4%: $X^2 = 273.13$, $P < 0.001$). The least parous rate was observed at Site 4 which was the furthest site from the river bank. In other words, the proportion of nulliparous flies were greater at the sites away from the river bank.

b) Simulium ethiopiense

Of the total 5 specimens of S. ethiopiense caught and dissected throughtout the study period, a parous rate of 60% Was observed (Table 9).

Table 8. Parous rate of *S. damnosum* flies at the different sites in the Gilgel Ghibe river area (April 1994 - March 1995)

Year	Month	Site 1		Site 2		Site 3		Site 4		Total		
		Flies col. & dissected	No. Parous (%)	Flies col. & dissected	No. Parous (%)	Flies col. & dissected	No. Parous (%)	Flies col. & dissected	No. Parous (%)	Flies col. & dissected	No. Parous (%)	
1994	Apr	13	34(26.1)	7	2(28.6)	0	0	0	0	50	36(72.0)	
	May	160	127(79.4)	34	18(52.9)	5	2(40.0)	1	0	200	117(58.5)	
	Jun	344	310(90.1)	333	163(48.9)	94	49(52.1)	7	3(42.9)	778	525(67.5)	
	Jul	1169	991(84.7)	738	465(63.0)	451	237(52.5)	102	43(42.2)	2460	1737(70.7)	
	Aug	1117	1033(92.5)	714	488(68.3)	320	204(63.8)	39	14(35.9)	2190	1739(79.4)	
	Sep	283	24(8.5)	78	55(70.5)	34	24(70.6)	13	7(53.8)	408	331(81.1)	
	Oct	4	3(75.0)	9	5(55.6)	0	0	0	0	56	41(73.2)	
	Nov	28	22(78.6)	0	0	0	0	0	0	28	22(78.6)	
	Dec	22	19(86.4)	0	0	0	0	0	0	22	19(86.4)	
	1995	Jan	46	40(87)	13	7(53.8)	0	0	0	0	59	47(79.7)
		Feb	83	36(43.3)	5	3(60.0)	5	1(20.0)	0	0	93	34(36.6)
		Mar	129	11(8.5)	1	2(50.0)	0	0	0	0	133	120(90.2)
	Total	3477	3079(88.5)	1935	1208(62.4)	909	517(56.9)	162	67(41.4)	6483	4841(74.7)	

Table 9 Parous rate of *S. ethiopiense* in the Gilgel Ghibe river area

Year	Month	No. of <i>S. ethiopiense</i> caught & dissected	Nulliparous <i>S. ethiopiense</i> No. (%)	Parous <i>S. ethiopiense</i> No. (%)
1994	Apr.	-	-	-
	May.	-	-	-
	Jun.	1	1(100)	-
	Jul.	1	-	1(100)
	Aug.	3	1(33.3)	2(66.7)
	Sept.	-	-	-
1995	Oct.	-	-	-
	Jan.	-	-	-
	Feb.	-	-	-
	Mar.	-	-	-
	October	-	-	-
	December	-	-	-
	January	-	-	-
Total		5	2(40%)	3(60%)

4.2.4 Filarial infection rate in Simulium damnosum

Filarial dissection results of S. damnosum at each site is given in Tables 10-14. Out of the total 3052 parous flies dissected and examined for filarial at Site 1, 226 (7.4%) flies were infected with both developing (L1 and L2) larvae (Figs. 13 a and b) and infective (L3) larvae (Fig.14) (Table 10). Of these, 155 (5.1%) flies harboured developing (L1 and L2) larvae and 71 (2.32%) flies harboured infective larvae resembling Onchocerca volvulus (L3). The percentage of parous flies with developing larvae ranged from 2.2% in August to 15% in January, and of parous flies with infective (L3) O. volvulus like larvae from 0.5% in August to 26.5% in April. The average number of (L3) O. volvulus larvae/fly was 5.1. Infection rates with L3 larvae in the dry season was significantly higher ($X^2 = 82.25$, $p < 0.001$) than in the wet season but not significant difference in infection with young (L1 and L2) larvae ($X^2 = 3.076$, $p > 0.05$).

The dissection and examination of 1208 parous flies at site 2 revealed 69 (5.7%) of the flies to be infected both with developing (L1 and L2) larvae and L3 O. volvulus larvae (Table 11). Of these, 60 (4.9%) were infected with developing larvae and 9 (0.7%) with L3 O. volvulus larvae. The monthly infection rate with developing larvae ranged from 1.2% in August to 14.3% in January and the rate with L3 O. volvulus larvae ranged from 0.2% in August to 14.3% in January. The average number of L3 O. volvulus larvae/fly

Table 10 Filarial dissection results of *S. damnosum* at site 1 (April 1994 -March 1995)

	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Tot.
Flies dissected	43	166	344	1169	1117	283	47	28	22	46	83	129	3477
No. Parasitic (%)	34 (79.1)	127 (76.5)	310 (90.1)	992 (84.8)	1033 (92.4)	248 (87.6)	39 (82.9)	22 (78.6)	19 (86.4)	40 (86.9)	70 (84.3)	118 (91.5)	3052 (87.8)
No. (%) Parasitic flies with dev. (L1 & L2) larvae	4 (11.8)	6 (4.7)	16 (5.2)	84 (8.5)	23 (2.2)	0	4 (10.3)	3 (13.6)	1 (5.3)	6 (15.0)	3 (4.3)	5 (4.2)	155 (5.1)
No. (%) Parasitic flies with L3 <i>Q. volvulus</i> larvae	9 (26.5)	8 (6.3)	3 (0.96)	16 (1.6)	5 (0.5)	0	2 (5.1)	3 (13.6)	2 (10.5)	2 (5.0)	17 (24.3)	4 (3.4)	71 (2.32)
Tot. No. of L3	31	39	10	70	12	0	15	24	23	25	91	20	360
<i>Q. volvulus</i> larvae Average No. of L3	3.4	4.9	5	4.4	2.4	0	7.5	8	11.5	12.5	5.2	5	5.1

Table 11 Filarial dissection results of S.damnosum at site 2 (April 1994 -March 1995)

	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Tot.
Flies dissected	7	34	33	738	714	78	9	-	-	13	5	4	1935
No. Parous (%)	2 (28.6)	18 (52.9)	163 (48.9)	465 (63)	488 (68.3)	55 (70.5)	5 (55.5)	-	-	7 (53.8)	3 (60.0)	2 (50)	1208 (62.4)
No. (%) Par. flies with dev. (L1 &L2) larvae	-	-	9 (5.5)	44 (9.5)	6 (1.2)	-	-	-	-	1 (14.3)	-	-	60 (4.9)
No. (%) Par. flies with L3 <u>Q. volvulus</u> larvae	-	2 (11.1)	1 (0.6)	4 (0.9)	1 (0.2)	-	-	-	-	1 (14.3)	-	-	9 (0.7)
Tot. No. of L3 <u>Q. volvulus</u> larvae	-	6	1	10	3	-	-	-	-	23	-	-	43
Average No. of L3 <u>Q. volvulus</u> larvae	-	3	1	2.5	3	-	-	-	-	23	-	-	4.77



(a)



(b)

Fig13. (a) Developing larvae (L1) isolated from S.damnsum
(b) Developing larvae (L2) isolated from S.damnsum



Fig4. Infective stage (L3) larvae of O.volvulus
isolated from S.damnosum

was 4.77. Significant seasonal difference was observed in infection rates with L3 Q. volvulus larvae ($X^2 = 8.24$, $p < 0.005$) but not with with developing larvae ($X^2 = 0.05320$, $p > 0.5$).

Out of the total 517 parous flies examined for filariae at Site 3, 18 (3.48%) were infected with both developing and L3 Q. volvulus larvae (Table 12). The number and percentage of parous flies with developing and L3 Q. volvulus larvae was, 13(2.5%) and 5(0.97%) respectively. The monthly infection rate with developing (L1 and L2) larvae ranged from 2% in June to 2.9% in August, and the monthly infection rate with (L3) Q. volvulus larvae ranged from 0.5% in August to 1.7% in July. The average number of (L3) Q. volvulus larvae per fly was 1.8.

Filarial dissection results of S. damnosum at site 4 is given in Table 13. Of the total 67 parous flies dissected and examined for filariae at this site, 6 (8.95%) harboured both developing larvae and L3 Q. volvulus larvae. Flies with developing larvae were 4 (5.9%) and with L3 Q. volvulus larvae were 2 (2.98%). The average number of L3 Q. volvulus larvae/fly was 11.

The results from all the sites show that the proportion of parous flies infected with developing and L3 larvae at Site 1, was generally greater than the proportion of parous flies infected with the larvae at sites 2, 3 and 4. There was a general decreasing pattern in infection rates at sites further away from the river bank.

Table 12 Filarial dissection results of *S. damnosum* at site 3 (April 1994 -March 1995)

	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Tot.
Flies dissected	-	5	94	451	320	34	-	-	-	-	5	-	909
No. Parous (%)	-	2	49	237	204	24	-	-	-	-	1	-	517
	-	(40)	(52.1)	(52.5)	(63.8)	(70.6)	-	-	-	-	(20)	-	(56.9)
No. (%) Par. flies with dev. (L1 &L2) larvae	-	-	1	6	6	-	-	-	-	-	-	-	13
	-	-	(2.0)	(2.5)	(2.9)	-	-	-	-	-	-	-	(2.5)
No. (%) Par. flies with L3 <i>Q. volvulus</i> larvae	-	-	-	4	1	-	-	-	-	-	-	-	5
	-	-	-	(1.7)	(0.5)	-	-	-	-	-	-	-	(0.97)
Tot. No. of L3 <i>Q. volvulus</i> larvae	-	-	-	7	2	-	-	-	-	-	-	-	9
Average No. of L3 <i>Q. volvulus</i> larvae	-	-	-	1.75	2	-	-	-	-	-	-	-	1.8

Table 13 Filarial dissection results of *S. damnosum* at site 4 (April 1994 -March 1995)

	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Tot.
Flies dissected	-	1	7	102	39	13	-	-	-	-	-	-	162
No. Parasitic (%)	-	-	3 (42.9)	43 (42.2)	14 (35.9)	7 (53.8)	-	-	-	-	-	-	67 (41.4)
No. (%) Parasitic flies with dev. (L1 &L2) larvae	-	-	-	3 (6.9)	1 (7.1)	-	-	-	-	-	-	-	4 (5.9)
No. (%) Parasitic flies with L3 <i>O. volvulus</i> larvae	-	-	-	2 (4.6)	-	-	-	-	-	-	-	-	2 (2.98)
Tot. No. of L3 <i>O. volvulus</i> larvae	-	-	-	22	-	-	-	-	-	-	-	-	22
Average No. of L3 <i>O. volvulus</i> larvae	-	-	-	11	-	-	-	-	-	-	-	-	11

The overall results of dissection carried out at all the sites in Gilgel Ghibe area is summarized in Table 14. Out of the total 4,844 parous S. damnosum flies dissected and examined for filariae at Gilgel Ghibe, 319 (6.58%) were infected with both developing and infective larvae. Developing larvae were isolated from 232 (4.74%) of the parous flies. The number of these larvae ranged from 1 to 35 per fly. Infective (L3) O. volvulus larvae were found in 87 (1.8%) of the parous flies dissected. They were found in the head, thorax and abdomen. However 76.7% of the infective (L3) O. volvulus larvae were isolated from the head region alone. The average number of (L3) O. volvulus larvae per fly was 5.1. The infection rate measured by the percentage of parous flies harbouring developing (L1 and L2) larvae per month ranged from 2.1% in August to 14.9% in January, and those of parous flies (L3) O. volvulus larvae ranged from 0.4% in August to 22.9% in February. The monthly infection rate was observed to show a seasonal trend. Infection rates with infective (L3) O. volvulus larvae during the dry season was significantly higher than the wet season ($X^2 = 119.42$, $p > 0.001$). Similar trends were also observed in the infection rate with young (L1 and L2) larvae ($X^2 = 4.083$, $p > 0.05$).

No infection was observed in the small number of parous S. ethiopiense flies (n = 3) dissected.

Table 14 Filarial dissection results of *S. damnosum* . Combined results of all four sites (April 1994 -March 1995)

	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Tot.
Flies dissected	50	206	778	2460	2190	408	56	28	22	59	93	133	6483
No. Parasit. (%)	36 (72)	147 (71.4)	569 (73.1)	1737 (70.6)	1739 (79.4)	334 (81.8)	44 (78.6)	22 (78.6)	19 (86.4)	47 (79.7)	74 (79.6)	120 (90.2)	4844 (74.7)
No. (%) Parasit. flies with dev. (L1 &L2) larvae	4 (11.1)	6 (4.1)	26 (4.6)	137 (7.9)	36 (2.1)	0	4 (9.1)	3 (13.6)	1 (5.3)	7 (14.9)	3 (4.1)	5 (4.2)	232 (4.8)
No. (%) Parasit. flies with L3 <i>Q. volvulus</i> larvae	9 (25)	40 (6.8)	4 (0.7)	26 (4.6)	7 (0.4)	0	2 (4.5)	3 (13.6)	2 (10.5)	3 (6.4)	17 (22.9)	4 (3.3)	87 (1.8)
Tot. No. of L3 <i>Q. volvulus</i> larvae	31	45	19	109	17	0	15	24	23	48	91	20	442
Average No. of L3 <i>Q. volvulus</i> larvae	3.4	4.5		3.92	2.4	0	7.5	8	11.5	16	5.35	5	5.08

3.2.5 Onchocerciasis transmission by S. damnosum

The monthly biting rate (MBR), the monthly infective biting rate (MIBR) and the monthly transmission potential (MTP) are indices used for expressing the transmission of onchocerciasis in an area. These values for Gilgel Ghibe area were calculated based on the following formula (WHO, 1987).

$$\text{MBR} = \frac{\text{No. of flies caught} \times \text{No. of days in the month}}{\text{No. of catching days}}$$

$$\text{MIBR} = \frac{\text{MBR} \times \text{No. of flies with infective O. volvulus larvae}}{\text{No. of flies dissected}}$$

$$\text{MTP} = \frac{\text{MIBR} \times \text{No. of infective O. volvulus larvae isolated}}{\text{No. of flies with infective larvae}}$$

The annual biting rate (ABR) is the sum of the 12 monthly biting rates for the year, the annual infective biting rate (AIBR) is the sum of the 12 monthly infective biting rates, and the annual transmission potential (ATP) is the sum of 12 monthly transmission potentials. The values of these transmission indices estimated for the Gilgel Ghibe area are presented in Table 15.

The annual biting rate (ABR) was 24928.75 flies, with the MBR ranging from 85.25 flies in December to 9,532.5 flies in July. The MBR was observed to show a seasonal trend, with more adult Simulium damnosum s.l flies biting in the wet season than in the dry season.

The annual infective biting rate (AIBR) was 328.7

Table 15 The monthly and annual biting rate and transmission potential of *S.damnosum* s.l in Gilgel Ghibe river area (made from all the sites)
(April 1994 - March 1995)

Year	Month	No. of man days	Monthly biting rate (MBR)	Monthly infective biting rate (MIBR)	Monthly transmission potential (MTP)	
1994	Apr	8	187.5	33.75	116.25	
	May	8	798.25	38.75	174.4	
	Jun	8	2917.5	15.00	71.25	
	Jul	8	9532.5	100.75	422.4	
	Aug	8	8486.25	27.1	65.9	
	Sep	8	1530	0	0	
	Oct	8	217	7.75	58.1	
	Nov	8	105	11.25	90	
	Dec	8	85.25	7.75	89.1	
	1995	Jan	8	228.6	11.6	186.1
		Feb	8	325.5	59.5	318.5
		Mar	8	515.4	15.5	77.5
Total			24928.75 (ABR)	328.70 (AIBR)	1669.5 (ATP)	

infective flies/year. The MIBR ranged from 0 in September to 100.75 infective flies in July. In comparison with the dry months, the highest MIBR was during the rainy season.

The monthly transmission potential (MTP) ranged from 0 in September to 422.4 in July. The MTP shows the transmission of Q. volvulus in the Gilgel Gibe area to be perennial, with two peaks of transmission; one in July (422.4 L3 Q. volvulus /man/month), and the other in February during the dry season (318.5 L3 Q. volvulus/man/month) could be transmitted. The annual transmission potential (ATP) was 1669.5 infective larvae/man/year.

3.2.6 Collection of crabs

A total of five crabs were collected during the study period and were caught during the dry season. These were probably Potomonantes (Lobopotomanantes) antheus which were earlier collected by White (1977) from the same locality. In spite of the close search using a hand lens no larvae and pupae of Simulium were found attached to the crabs.

4. DISCUSSION

The present parasitological investigation for Onchocerca volvulus infections among the inhabitants of Gilgel Ghibe village indicated the endemicity of the disease in the area. Based on the presence of positive skin snips an overall prevalence of 17.1% was found. During his 1967 survey in Asendabo, a village not far from the present study area, Oomen (1969a) found 16% of the males over 15 years of age to be positive for O. volvulus by skin snip examination and suggested that onchocerciasis is of low endemicity in this area. The prevalence rate of onchocerciasis shown by the present study is low (17.1%) and Gilgel Ghibe can be regarded as hypoendemic according to WHO's (1966) standard levels of endemicity. A similar low level of endemicity was also reported in north-western Ethiopia (Zein, 1986). Other studies carried out elsewhere in the southwestern and western parts of the country have reported onchocerciasis as hyper, hypoendemic or mesoendemic (Taticheff et al., 1987, 1993; Gundersen et al., 1988; Yeneneh et al., 1989).

The prevalence of infection in males (19%), though not statistically significant ($p < 0.5$), was found to be higher than in females (14.9%) suggesting males to be at greater risk of infection. This observation is consistent with the findings of other investigators in Ethiopia (Oomen, 1969a, b; Taticheff et al., 1987; Yeneneh et al., 1989) and

elsewhere (McMahon et al., 1988; Porter et al., 1988). The higher rate of infection observed in males may be due to the sexual division of labour. Males are usually involved in activities like farming and fishing which bring them closer to the Simulium breeding sites. Contrary to this females work a great deal of their time in their house and don't frequent the river bank, thus they are probably less exposed to the Simulium bites than males. Moreover, the dressing habits of most females with long skirts which may prevent the Simulium bites could also explain the low rate of infection.

In this investigation higher rate of infection was observed in individuals of age group 15-29 than others. Moreover, the infection rate (26.5%) in people of working age was higher than the prevalence of 16.9% in the remaining age groups. Individuals of this age were observed to frequently visit the river bank for fishing, washing, swimming, or other activities. They prefer the early morning or late afternoon part of the day for these purposes. This indicates that individuals of this age are therefore at highest risk of acquiring infection. The observed diurnal biting cycle of the Simulium species in the area shows the biting activity of the vector to be the highest during this part of the day. It is therefore more probable that this may predispose them to the bites of Simulium. The observation that the prevalence of infection increases with age suggests that repeated exposure through

years is required for onchocerciasis transmission.

Taylor et al (1989) suggested that the sensitivity of skin snip could vary with the number of skin snips taken, the more skin snips that are taken, the more accurate the diagnosis. Of the 39 positive cases, microfilariae were detected in 74.4% in biopsies from the right and left iliac crest while 25.6% were detected in biopsies taken from either the right or left iliac crest. In other words, 25.6% of the positive cases would have been missed if biopsies were to be taken from either the right or left iliac crest.

The mean microfilarial load per positive skin snip which is a measure of parasite burden, was calculated to be 11.14 per snip for the community. In the Blue Nile Valley of Western Ethiopia, a prevalence rate of 38% and a mean microfilarial count of 12 per snip was recorded (Gunderson et al., 1988). Workneh et al. (1993) recorded a mean microfilarial count of 34 per snip among labourers in Teppi Coffee plantation Project (southwestern Ethiopia) where the prevalence rate of onchocerciasis was 82.7%.

The mean parasite load was higher in males (13.8 mf/ss) than in females (7.9 mf/ss) and increased with age up to ages 15-29 and declined thereafter with the exception of one individual. Other investigators also reported higher mean microfilarial count in males than females and an increase in microfilarial count with age (Budden, 1963, McMahon et al., 1988, Gunderson et al., 1988; Moyou Somo et al., 1993). It has been speculated that hormonal factors

which lead to the relatively high resistance of females may be responsible for the observed differences between males and females (WHO, 1976).

Differences in the prevalence of infection related to the duration of residence period in the village was also noted. The highest rate of infection, 77% of the positive cases, was in individuals with a residence period of more than five years while 23% of the infected individuals have a residence period of less than 5 years. This also suggests that onchocerciasis infection requires repeated exposure to Simulium bites which needs a prolonged residence period in the endemic area. However, if the individuals are in the prepatent stage of infection, there is the probability of microfilariae being not detected in skin snips.

The prevalence of clinical manifestation in the present study, 26.3% was found to be higher than the prevalence of positive skin snips, indicating the presence of signs and symptoms suggestive of onchocerciasis in individuals with negative biopsy. Similar observations have also been noted by others in the country and this was explained partly by the non specific nature of the symptoms (Oomen, 1969 a; Yeneneh et al., 1989). The most frequent of the clinical signs was itching. Of the 39 positive cases, clinical findings suggestive of onchocerciasis were observed in only 22 (56.4%) while the remaining 17 (43.6%) of the positive cases are clinically negative. The clinical manifestations of onchocerciasis are known to depend on

numerous factors, the host immune response, bioclimatic zone, and duration of exposure (WHO, 1987). Therefore this may be the reason for the positive cases with no clinical sign of onchocerciasis. In fact many of the signs and symptoms develop over the years and this individuals may have acquired the infection relatively recently. Moreover, onchocerciasis in Ethiopia has been described as mild (Oomen 1969 a,b; DeSole and Walton, 1976; Mengesha and Tiruneh, 1977).

According to WHO (1991), communities with prevalence rate of 40% and above should be targeted for mass treatment with Ivermectin. Both the intensity and prevalence of the disease in Gilgel Ghibe were shown to be low and therefore could not at present be a priority area for mass treatment with Ivermectin.

The collection of black flies on human baits, during the study period revealed the existence of two anthropophilic species, Simulium damnosum complex and S.ethiopiense, in the Gilgel Ghibe river area (Table 6). Previous investigations have also reported these species from the area (White, 1977; Mebrahtu et al., 1980; Gebre-Michael and Gemetchu, in press). Of the two anthropophilic species, S.damnsum complex was observed attacking man throughout the year, and was caught in quite large numbers. Of the total 6,488 Simulium flies caught, 6483 were S. damnosum and only 5 were S. ethiopiense, and these were caught during the rainy season. Therefore based on the

relative abundance of the flies, *S. damnosum* complex was found out to be the most common and important species in the area. Previous investigators (White, 1977; Gebre-Michael and Gemetechu, in press) have also recorded the two species but *S. damnosum* was the predominant species. During his survey in the rainy season of August, White (1977), recorded a biting rate of about 43 *S. ethiopiense*/man/day. Later in 1984, Gebre-Michael and Gemetechu (in press) recorded a biting rate of 4 *S. ethiopiense* flies per man per day for the same month and annual biting rate of 1343.5 flies. During the present study a biting rate of 1.5 flies/man/day in August and annual biting rate of 38.5 was observed. These observations indicate the biting density of *S. ethiopiense* has always been minimal in the area and declining over the years. The absence of immature stages of *S. ethiopiense* on the few crabs (n = 5) collected in the present study might reinforce the declining pattern of *S. ethiopiense* over the years. The reasons for the decline in *S. ethiopiense* in Gilgel Ghibe area are not immediately clear, but could be due to ecological changes in the area. The *S. neavei* group as a whole are known to thrive best in heavily-forested riverine environments (McCrae, 1969) and seem to be sensitive to changes in the vegetation cover. In fact, deforestation was the sole means used to eradicate the vector (*S. neavei*) and onchocercias from some foci in Kenya (McMahon et al., 1958; Therefore, the declining biting density of *S. ethiopiense* around Gilgel Ghibe might be due

to the continued clearance of the riverine forest in the area. S.ethiopiense flies have been suspected of playing a secondary role in the transmission of onchocerciasis in the south western part of Ethiopia (White, 1977; Raybould and White, 1979; Mebrahtu et al., 1980). However, the present study and a previous one (Gebre-Michael and Gemetechu, in press) show that any role played by S. ethiopiense can be discounted at least in the Gilgel Ghibe area. However, this does not rule out the possibility of its role as a secondary vector in other localities (TenEyck, 1973; Mebrahtu et al., 1980).

Population peak of S. damnosum (Table 6, and Figures 4-8) was observed to fluctuate seasonally. Biting densities upto 307.5 flies/man/day or 25.63 flies/man hour was recorded in the month of July during the rainy season in contrast to the relatively few flies caught during the dry season. The existence of high fly numbers during the rainy season has been observed by several investigators in Ethiopia or elsewhere (e.g. Gebre-Michael, 1983; Crosskey, 1955) and might be due to favourable ecological factors (e.g. rain; $r = 0.74$) for the immature and adult fly population. However, this might not always be true and highest fly densities could be collected during the dry season in some foci in Togo (Cheke et al., 1992).

The abundance of S. damnosum complex (Table 7) was observed to vary between the different sites. Of the total 6,483 S. damnosum flies collected, the numbers caught at

sites 1, 2, 3 and 4, were 3477, 1935, 909 and 162 respectively. Highest biting density S. damnosum flies was recorded at site 1, and the lowest was at site 4, located at about 400 meters away from the river bank. This observation indicates villages located further away from rivers and streams are at less risk of being bitten by flies and consequently of onchocercal infection.

The diurnal biting cycle of S. damnosum flies (Figures 9-12) shows the flies tend to bite in large numbers in particular hours of the day. Two major peaks were apparent at all the sites, one in the morning, and another in the afternoon suggesting highest man-fly contact during these hours of the day. The bimodal biting rhythm of S. damnosum observed in the present study is similar to previous observations in the same locality or elsewhere (White 1977; Kaneko *et al.*, 1973; and Gebre-Michael 1983). This is assumed to be due to favourable meteorological conditions like temperature and relative humidity. The range of favourable temperature for maximum fly (S. damnosum) biting activity was 21.4°C to 27.6°C in Ghibe valley (Gebre-Michael, 1983) and 22°C-26°C in Tanzania (Hauserman, 1969). Increased light intensity has been reported to inhibit biting by S. damnosum (Kaneko *et al.*, 1973). The annual parous rate of 75.4% (for all the sites combined) recorded in the present study was lower than the previous record of 91.5% and 84% in Gilgel Ghibe and Gojeb rivers, respectively, from flies collected at the river banks

(Gebre-Michael and Gemetechu, in press). The lower parous rate was due to the location of fly catching sites where three of the 4 sites were located at various distances away from the river bank and the proportion of parous flies in these sites was lower (Tables 8). The overall parous rate was however, higher than the rate, 64.7% recorded from river bank sites in the Ghibe river valley (Gebre-Michael 1983). The observed decreasing pattern in parous rates away from the river bank in the present study is typical of the savanna situation in West Africa where differential dispersal of parous and nulliparous flies were reported (Le Berre, 1966; Duke, 1975). In these areas it was noted that nulliparous flies dispersed mainly inland away from their riverine breeding sites and parous flies concentrated more at the river bank. No such differences in dispersal pattern between parous and nulliparous flies has been observed in the rainforest zone of West Africa. The observed low parous rates combined with low biting density at sites away from the river bank in the present study and elsewhere in savanna areas in West Africa implies that villages further away from the river bank are less exposed to onchocerciasis transmission.

The results from the dissection of S. damnosum complex flies for filarial larvae at Gilgel Ghibe (Table 14) shows 1.8% of the parous flies dissected to be infected with L3 O. volvulus larvae. This was found to be higher than the 1.3% recorded at the same locality by Gebre-Michael and

Gemetchu (in press), but lower than the 3.1% in Ghibe valley (Gebre-Michael, 1983), 3.4% in the Mahenge mountains in Tanzania (Houserman, 1969) and 4.38% in Togo (Cheke et al. 1992). The filarial infection results from the different sites show the infection rate to vary between the different sites with decreasing tendency towards sites away from the river bank. This suggests the highest risk of infection could be presented to persons whose activity brings them closer to the river, whereas activities further away from the bank of the river presented little risk of infection. Duke (1975) observed a similar trend in a savanna area of Cameroon and concluded that a given biting density of S. damnosum on the river bank to be much more dangerous than a similar biting density inland.

The proportion of parous flies acquiring infection has been observed to exhibit a seasonal trend in that the proportion of parous infected during the dry season was higher than the proportion during the rainy season (Table 14). This might give a wrong impression that the transmission of O. volvulus is intense in the area during the dry season. However, the monthly transmission potential (Table 15) which takes account of the biting rate, infective biting rate and quantitative filarial data shows the transmission of O. volvulus to occur through out the year with the highest transmission occurring in July (846.3 larvae/man/month) and the lowest in October (117.8 larvae/man/month).

The overall annual transmission potential (ATP) for the Gilgel Ghibe area was estimated to be 1669.5 larvae/man/year which is paradoxically associated with a low prevalence rate of the disease, 17%. This transmission potential is much lower than the ATP level of 3,567 recorded in the Ghibe river valley by Gebre-Michael (1983) where the prevalence was not determined. In savanna areas in west Africa ATP levels between 500 - 18000 have been associated with high prevalence (70- 100%) of disease and high rates of blindness (Duke et al., 1975; Thylefors et.al., 1978b). In forest villages in West Africa, prevalence rates of 30%- 93% were associated with ATP levels of 897 to 87846 (Duke et al., 1972; Moyou Somo et al., 1993). The discordance between low prevalence of the disease and high ATP level in the present study is not clear. But this may be due to the existence of animal filariae, morphologically indistinguishable from O. volvulus which have been shown to exist in simuliids (Nelson and Pester 1962, Garms and Voelker, 1969, Duke, 1967).

Earlier, an even higher ATP level of 5,478 was estimated for the same locality by Gebre-Michael and Gemetchu (in press). However, this was due to the collection of flies at the river bank resulting in high parasitic rates, higher infection rates and consequently an exaggerated ATP. Since no individual stays for 12 hours a day at the river bank or at any other fixed spot, ATP level

estimated from flies collected at different sites with different distances inland from the river bank would give a better picture of the transmission of onchocerciasis of a given area.

5. CONCLUSION AND RECOMMENDATION

1. The prevalence of onchocerciasis (17.1%) in the Gilgel Ghibe area, suggests that this is not a priority area for control of onchocerciasis through mass administration of ivermectin at present. However; this may need re-evaluation in the future.
2. Simulium ethiopiense was suspected of playing a secondary role in the transmission of onchocerciasis in the country; however, the present study showed this not to be the case at least in the Gilgel Ghibe area. To establish the vectorial importance of this species in the areas beyond the present study area further investigations are required.
3. To establish the status of the vectors of onchocerciasis and the disease in Ethiopia, extensive entomological and parasitological surveys should be carried out in the country.
4. Effort should be given for the development of DNA probes for field use in entomological study of onchocerciasis in Ethiopia and elsewhere.

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ONCHOCERCIASIS SURVEY FORMI. Questionnaire

Name _____ Sex _____

Age group 1-4; 5-9; 10-14; 15-19; 20-24; 25-29; 30-39;
40-49; >50

Occupation _____ Tribe _____

Birth place _____ Duration of residence _____
in the village

Previous residential areas _____ years lived _____

How often do you go to the river bank? (always, usually,
sometimes) _____For what purpose? (Bathing, swimming, fishing, wash
cloths, fetch water, other (specify) _____Have you had itching? (Yes, No, Don't know). If yes,
since when _____Do you have nodules? (Yes, No, Don't know). If yes,
since when _____

Site of nodules _____ Number _____

_____Do you have skin rashes? (Yes, No, Don't know). If yes,
since when _____

Do you have eye/visual problems? (Yes, No, Don't know)

Remark _____
