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VECTOR BIOLOGY AND MALARIA TRANSMISSION IN WESTERN VENEZUELA

By

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ABSTRACT

The status of all anopheline species reported to occur in western Venezuela is reviewed.

A longitudinal study was conducted in three villages in western Venezuela to assess the malaria risk factors determined by the abundance, parous rate, biting activity, sporozoite rate and human blood index of the various potential vector species in relation to weather and human habits.

The main method of mosquito sampling was on human baits; three other methods tested did not prove to be effective substitutes.

The collections yielded 14 anopheline species, the most abundant being those belonging to the subgenus *Nyssorhynchus*. Because species identification of adult females with available keys proved to be difficult, linked rearings were undertaken.

An. nuneztovari, comprising over 70% of the total anophelines collected, was the most abundant species, followed by *An. triannulatus*, *An. albitarsis s.l.* and *An. oswaldoi*. The anopheline populations showed fluctuations which correlated positively with rainfall and humidity.

The four most abundant species showed different diel patterns of biting. The diel peak for *An. nuneztovari* was close to midnight indoors and outdoors, for *An. triannulatus* between 1900 and 2000 hours outdoors, for *An. albitarsis* mainly before midnight indoors and outdoors and for *An. oswaldoi* outdoors at 1900 hrs, there being an additional smaller peak indoors at midnight. Most of the human population use bed nets, go to bed before 2200 hrs and wake up before 0700 hrs: they are therefore most exposed to the bites of those species that bite early in the night outdoors.

All anopheline species in the study area are exophilic. Some anophelines were collected resting on vegetation around houses between 0600 and 0800 hrs but very few *An. nuneztovari* were found there. The source of blood meals in resting mosquitoes was determined by the ELISA technique. The human blood index for the different species collected showed variations among villages that could not be explained by variation in the ratio of humans to cows in each village.

Over 61,000 anophelines were assayed by ELISA to detect *P. vivax* circumsporozoite protein. The six specimens confirmed as positive belonged to three species: *nuneztovari*, *albitarsis s.l.* and *oswaldoi*. The estimated overall sporozoite rate was 0.0098% (95% confidence limits 0.0036 to 0.0214%). Multiplying this rate by the mean number of bites on the catchers suggests a sporozoite inoculation rate of 10.5 positive bites per person per year.

Recommendations for possible improvements in malaria vector control in this area are made taking into account the endophagic and exophilic behaviour of the incriminated vectors, their diel patterns of biting and some aspects of the behaviour of the human population revealed by questionnaires.

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CHAPTER 1:

GENERAL INTRODUCTION

1.1. MALARIA IN VENEZUELA

Malaria was once the major public health problem in Venezuela, and the main obstacle to the country's social and economic development (Gabaldón, 1959). During the years 1910-1945 endemic and epidemic malaria was prevalent in two-thirds of the country (600,000 km^2) (Fig. 1.1). During epidemic years, in some areas, overall death rates often exceeded 70 per 1,000 population and infant death rates often exceeded 500 per 1,000 births. During these episodes birth rates were reduced and maintained at a low level in more endemic areas. Thus in some years death rates exceeded birth rates and the population decreased over a large area (319,000 km^2) between 1891 and 1920 (Gabladón, 1959). As late as 1941, malaria death rates reached 531 and 1,125 per 100,000 inhabitants in the most afflicted states in central Venezuela. No other disease, even influenza in 1918, caused a higher mortality than did epidemic malaria in Venezuela between 1905-1945 (Gabaldón & Pérez, 1946). Venezuela was the most malarious country in Latin America (Gabaldón, 1959).

In 1936 a programme of malaria control on a national scale was established under the direction of Dr. Arnoldo Gabaldón.

During the early years conventional control measures such as free distribution of quinine and quinacrine, elimination of standing water through drainage and filling operations around towns, use of larvicides (Paris green) and adulticides (mostly pyrethrum) were used with modest results, especially in rural areas (Berti, 1945; Gabaldón & Berti, 1954).

With the availability of DDT in 1945, a nation-wide campaign started and by 1950 the whole endemic area was covered. The malaria death rate per 1,000 population fell from an average of 112.2 in the period 1941 to 1945 to 14.8 in 1948. In 1954 malaria was virtually eradicated from an area of 180,000 km^2. In this region, with 2.4 million

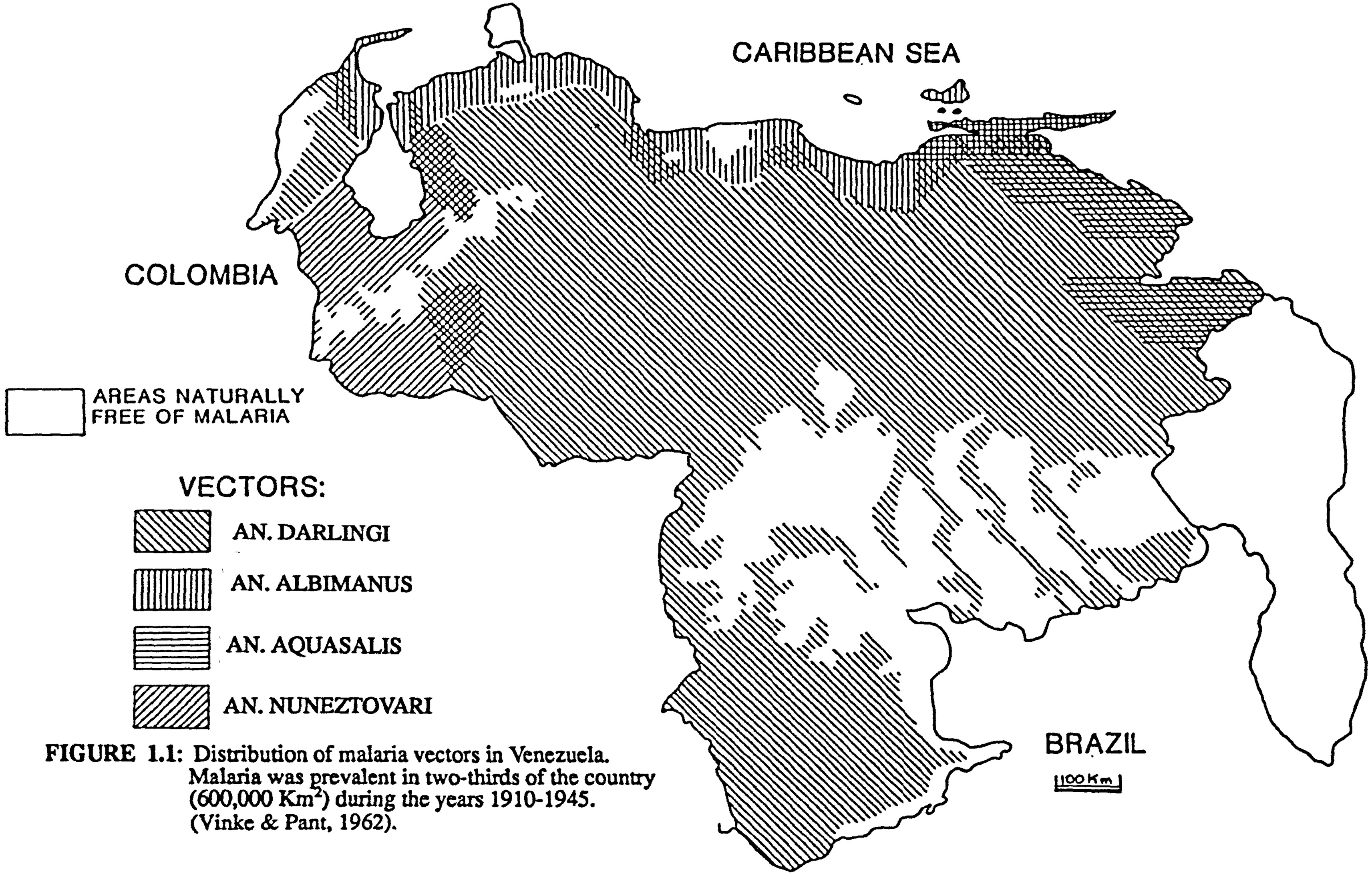


FIGURE 1.1: Distribution of malaria vectors in Venezuela. Malaria was prevalent in two-thirds of the country (600,000 Km^2) during the years 1910-1945. (Vinke & Pant, 1962).

inhabitants, there were only 21 indigenous cases in 1951, 1952 and 1953, 19 of them being in 1951. The area of virtual eradication continued to increase, and in 1959 407,945 km^2 were claimed to be free of malaria (Fig. 1.2). By 1971 such eradication covered 460,054 km^2 with a population of 10 million (Gabaldón, 1983).

During the early years of DDT spraying, results were found to be different in different areas, depending on which species was the main vector. Where *Anopheles albimanus* or *An. darlingi* was present in the coastal and central parts of the country, malaria disappeared rapidly; in contrast, where *An. aquasalis* or *An. nuneztovari* was the exophilic and exophagic vector, malaria decreased slowly (Fig. 1.1). The two types of response to DDT spraying are characteristic of two different kinds of disease: a) "responsive malaria", which decreases rapidly, and b) "refractory malaria" which decreases slowly (Gabaldón & Berti, 1954). Furthermore, Gabaldón (1972) recognized a third type, "inaccessible malaria", characteristic of southern Venezuela where the vector is an exophilic and exophagic form of *An. darlingi* and where the local population mainly comprises amerindians who move from one place to another, staying for a few weeks in a given place according to the opportunities that they find for fishing, hunting etc. In such conditions it is not possible to spray all human dwellings effectively or regularly to survey the human population. Some other inaccessible groups in the area are diamond and gold miners, tonka bean (*Dipterix odorata*) collectors and rubber tappers.

In Venezuela, *P. falciparum* was the commonest malaria species, followed by *P. vivax* and *P. malariae* in that order. However, after the spraying *P. falciparum* and *P. malariae* disappeared more rapidly from areas with reponsive malaria.

Between 1936 and 1945 it was found that malaria incidence had a 5-year cycle coincident with similar cycles in density of the vectors, especially *An. darlingi* (Gabaldón, 1949). The origin of these vector cycles has yet to be explained because they are not related to corresponding fluctuations in yearly rainfall.

During the eradication campaign it was observed that degree of urbanization has an important influence on transmission. Villages with fewest houses have the highest infection rates: the annual incidence of parasitaemia per 1,000 inhabitants is 163.6 when

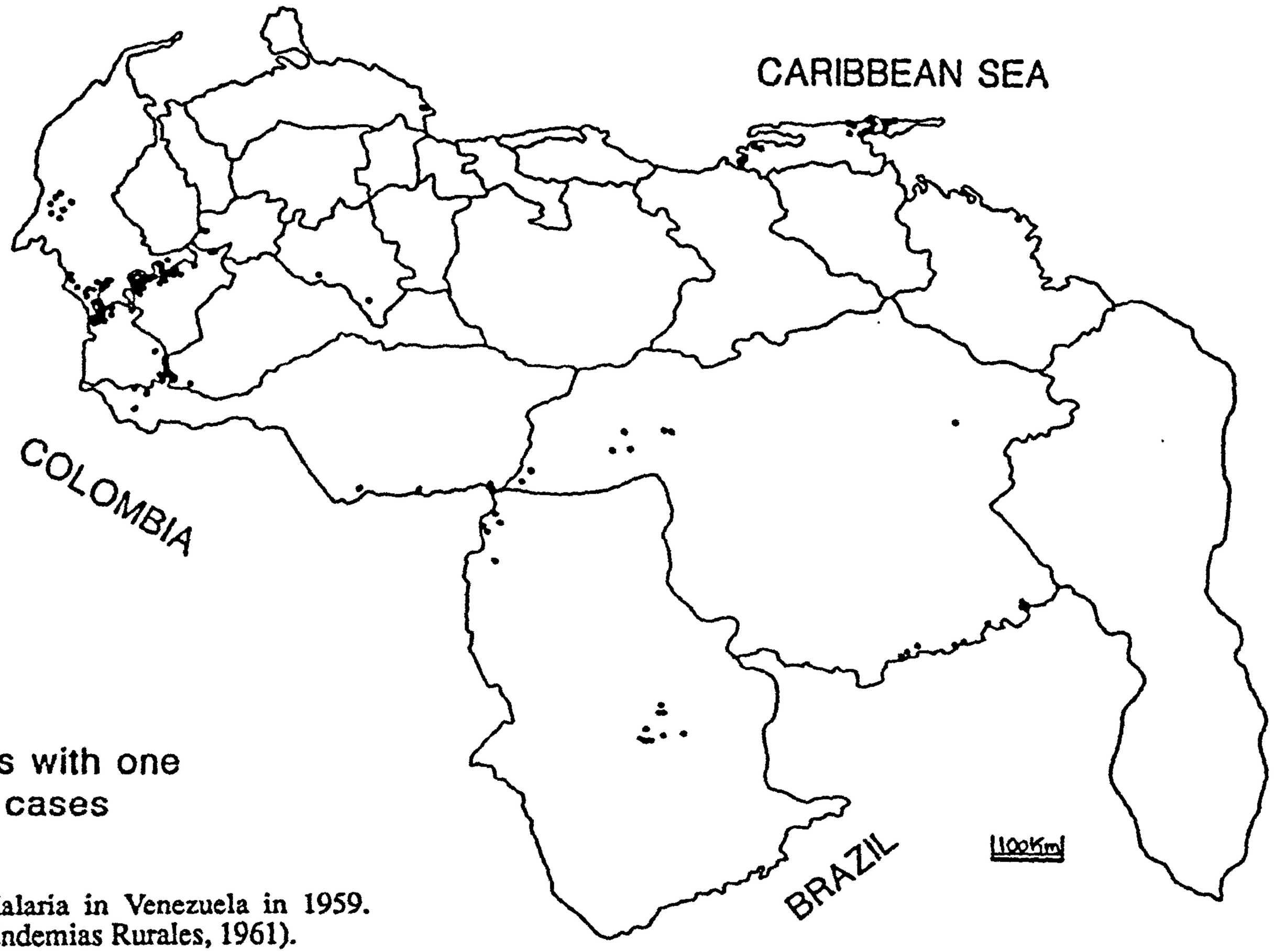


FIGURE 1.2: Malaria in Venezuela in 1959. (Dir. Endemias Rurales, 1961).

the houses number from 1 to 10 and decreases to 41.9 when the houses number over 100 (Gabaldón *et al.*, 1975). This effect has been maintained up to the present time (Table 1.1).

Malaria has persisted for the last 40 years in some localized areas of Venezuela and recently the number of cases has increased and the disease is spreading to areas from which it had been previously eradicated (Table 1.2 & Fig. 1.3). At present, the failure of conventional control measures is attributed mainly to the exophilic habits of the incriminated vectors: *An. nuneztovari* in the west, *An. darlingi* in the south and *An. aquasalis* in the north east (Fig. 1.3).

Persistence of transmission is linked to organizational difficulties and administrative failures, but also to other human factors such as greatly increased mobility of the adult population, which is reflected in the fact that most cases are in adults (Table 1.3). There has also been some resistance of the inhabitants to spraying of houses and some unwillingness to accept advice to take prophylactic antimalarial drugs. Many of the infections are among indigenous tribes, who stay for only a short time in one place and so cannot be reached effectively by anti-malaria squads, especially as the terrain is extremely difficult and the dispersion of the small population over a large area adds greatly to the other difficulties. Furthermore, it has been found that, as in Brazil, certain strains of *falciparum* have become resistant to chloroquine (Gabaldón, 1965).

1.2. MALARIA IN WESTERN VENEZUELA

When "refractory malaria" was recognised in western Venezuela in 1951, the frequency of DDT house spraying was increased from once every 6 months to once every 3 months; this led to a marked decline in the number of cases, which, however, then reached a steady level (Gabaldón *et al.*, 1963). In 1962, 72% of all autochthonous cases in the country occurred in western Venezuela (Gabaldón *et al.*, 1963). Chloroquine was then given to each family, one month's supply at a time, with instructions that prophylactic doses were to be taken each week. However,it was found that the drug was not being taken regularly and in 1959 it was noted that certain cases of *P. falciparum*

Table 1.1: Malaria cases per 1,000 population during 1986 according to the number of houses per village in Venezuela (Dirección de Endemias Rurales, Report Oct. 1989a.)

Number of houses	Number of villages with cases	Cases per 1,000 population
1 - 10	556	108.6
11 - 40	253	36.4
41 - 70	59	29.6
71 - 100	42	23.0
Over 100	70	7.0

Table 1.2: Malaria cases per 1,000 population per year in the three study villages and Venezuela as a whole. (Number of insecticide sprayings in parentheses, * indicates fenitrothion, no asterisk indicates DDT) (Dirección de Endemias Rurales, Records 1979-1989.)

Year	Autochthonous cases in 3 study villages (*P. vivax* only)			All Venezuela (*P.v.* & *P.f.*)
	Caño Lindo	Jabillos	Guaquitas	
1979	224.1(3)	34.5(2)	31.3(2)	0.34
1980	32.6(3)	42.4(3)	0.0(3)	0.28
1981	44.9(3)	23.6(1)	0.0(1)	0.23
1982	41.7(3)	21.0(3)	13.3(2)	0.28
1983	426.0(2)	171.8(3)	80.0(2)	0.51
1984	322.6(2)	22.7(3)	0.0(3)*	0.72
1985	152.5(2)*	25.2(3)*	0.0(3)*	0.82
1986	350.7(3)*	8.9(2)*	153.8(2)*	0.80
1987	127.6(3)*	56.9(3)*	55.1(3)	0.98
1988	77.1(3)*	15.3(3)*	8.7(3)*	2.43
1989	5.8(2)*	6.6(2)*	20.6(2)*	2.25

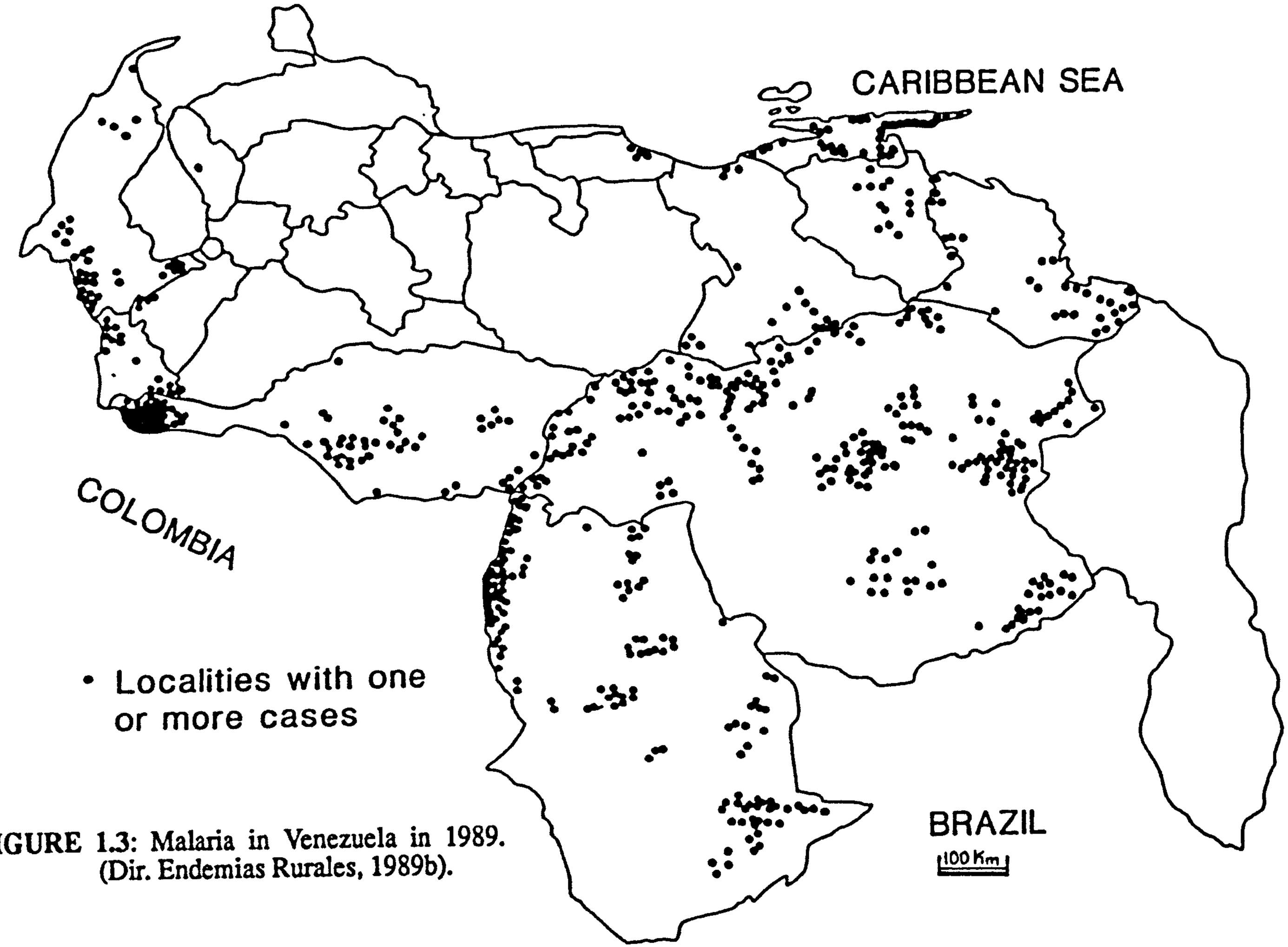


FIGURE 1.3: Malaria in Venezuela in 1989. (Dir. Endemias Rurales, 1989b).

Table 1.3: Distribution of cases by age group during 1986 (Dirección de Endemias Rurales, Report Oct. 1989a.)

Age group (yrs)	Percentage of total cases
Less than 1	0.8
1 to 4	7.7
5 to 9	10.6
10 to 14	11.8
over 15	63.5

Table 1.4: Percentage of cases reported from western Venezuela and the total number of cases in the country. (Dirección de Endemias Rurales, Records 1979-1989.)

Year	Total number of cases in Venezuela	Percentage of cases from western Venezuela
1979	4,722	56.2
1980	3,901	57.4
1981	3,377	60.0
1982	4,269	59.6
1983	8,400	45.2
1984	12,242	35.8
1985	14,305	22.5
1986	14,365	20.1
1987	17,988	15.5
1988	45,662	5.8
1989	43,374	4.2

malaria were not being cured with the usual doses of chloroquine. Pyrimethamine was therefore given to everyone in the area (Gabaldón, 1959). The number of cases fell again but then it was discovered that both *P. falciparum* and *P. vivax* were becoming resistant to pyrimethamine which necessitated a change to amodiaquine (Gabaldón *et al.* 1963). In addition, in view of the exophilic and exophagic activities of *An. nuneztovari*, in 1960 peridomestic insecticides were applied weekly as mists consisting of a 4% solution of lindane in gas-oil, made into an aerosol by powerful pumps (Gabaldón *et al.*, 1963). The fogging was used either early in the morning (0600-0800 hrs) or at dusk (1800-2000 hrs), the latter being more effective. The cost of this fogging was less than giving drugs weekly. Larviciding was impracticable because of the terrain (Gabaldón *et al.*, 1963). Control measures included the radical cure of all infections detected by treatment over a 14-day period with 1,500 mg chloroquine and 210 mg primaquine for adults. Detection was by the taking of blood films from people living within a radius of 5 km of the infected patient; if further cases were found antimalarial treatment was given to all persons suspected of being infected (Gabaldón *et al.*, 1965). This system is still in use.

Transmission was reduced but not stopped. Gabaldón (1981) pointed out that an analysis of the ecological differences between areas of *An. nuneztovari* transmission where malaria was easily eradicated, and those where the disease persisted, suggested that malaria disappeared from areas of open savannah where cattle rearing was the main economic activity, whereas it tended to persist in areas surrounded by bushes, near woods or planted with bananas. He speculated that in pasture land mosquitoes find conditions harsher and die earlier .

These views have been largely supported by observations on the parous rate of *An. nuneztovari* populations carried out by Vincke and Pant (1962). They found that this species was largely exophagic when outside baits were available, and that the proportion of parous females was highest (0.64-0.72) in densely forested areas, and lowest (0.31-0.53) in partly deforested areas.

Based on field observations, Gabaldón (1972) speculated that the exophilic behaviour of *nuneztovari* is not facultative but the result of a genetic change. He noted

that in Venezuela *nuneztovari* was very endophilic before DDT spraying, but was no longer present in recently built, unsprayed houses in villages that had been treated with insecticide for several years. Similar observations were made by García Martín (1955) who worked on the northern slopes of the Andes. Gabaldón (1972) reported that in a small area of the southern slopes of the Andes where houses had been sprayed for 15 years with DDT, but left unsprayed for 5 years, *An. nuneztovari* was still not found inside houses during the day. Thus Gabaldón (1972) considered that intense exophily could be selected by prolonged exposure to insecticide. However, in a larger area sprayed only for 10 years, which remained without insecticide for 8 years, large numbers of *An. nuneztovari* were discovered resting inside dwellings during the day (Gabaldón, 1972).

1.3 VECTOR STUDIES IN WESTERN VENEZUELA

The malaria endemic areas of western Venezuela have been extensively surveyed in the past to monitor the control campaign, but little effort has been made to complete and assemble a coherent picture of the biology, ecology and ethology of the vector(s), to explore ways in which the community could be encouraged to co-operate in the control campaign, to understand transmission dynamics, or to evaluate the feasibility of control measures other than the routinely used ones.

At present, information on entomological factors is scanty: there are some reports on sporozoite index (Pintos & Sabril, 1965; Pintos *et al.*, 1968), susceptibility to *P. falciparum* infection (Scorza *et al.*, 1976) and survival (Scorza *et al.*, 1981), but these are mainly single-point observations where the numbers of mosquitoes collected and dissected were small, and referred only to *An. nuneztovari*. Apparently, no other attempts have been made to quantify or qualify the vectorial capacity of anophelines biting man in western Venezuela.

The knowledge about *nuneztovari* in Venezuela is mostly the result of surveys directed towards monitoring the success of the control campaign and no attempt has been made to organise it coherently. In what follows the available literature is summarised to

give an account of what is known about the biology and ecology of this species.

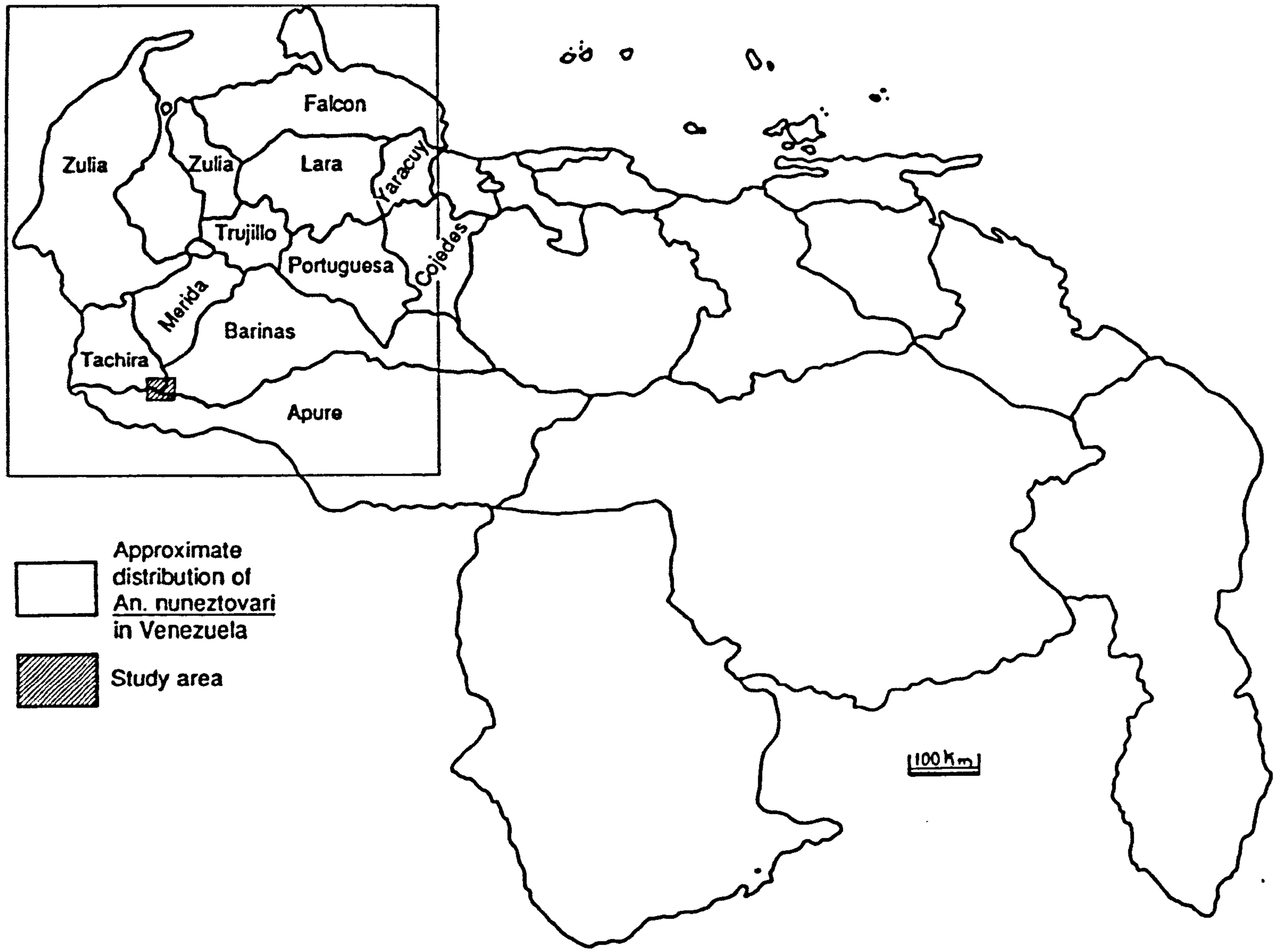
Geographical distribution: In Venezuela, *nuneztovari* occupies both sides of the Andean foothills, encompassing the states of Barinas and Táchira, the extreme west of Apure, and sectors of Mérida, Trujillo, Cojedes, Portuguesa and Zulia (Sutil, 1976) (Fig. 1.4).

Habitat: Scorza and Pintos (1972) found it resting in stands of *Heliconia* sp. (Musaceae) and proposed that the mosquitoes seek sites with low temperature and high humidity to rest, but offered no conclusive support for this hypothesis. There is not enough information to characterise the preferred or typical oviposition site of *nuneztovari.* Scorza *et al.* (1977 a, b, c, d) followed larval fluctuations in two breeding places in Mérida state on the northern slope of the Andes, and Segnini *et al.* (1979), studying physical and physico-chemical features in one such oviposition site found that daily fluctuations were wider than variations in monthly means, which possibly means that critical values and periods may be more important than average conditions in determining what is and what is not a suitable breeding place.

Feeding: The only studies on larval feeding are those mentioned earlier by Scorza *et al.* (1977 a, b, c). The main component of the diet is phytoplanktonic algae; and composition of diet seems to be determined only by size and relative abundance of food organisms.

Scorza (1973) and Scorza *et al.* (1976), using precipitin techniques on blood meals, reported a preference for cattle, but their results could be better understood as indicating a rather opportunistic host selection. On the other hand, Gabaldón (1972) pointed out that *nuneztovari* maintains a preference for human blood of about 80%. There is no published information on host-preference studies designed as such (i.e. comparing host selection when there are equal opportunities for each host type to be chosen).

FIGURE 1.4: Geographical distribution of *Anopheles nuneztovari* in Venezuela (Sutil, 1976) and location of the study area.



Population fluctuations: Scorza *et al.* (1981) followed the fluctuations of larval populations in two larval habitats and variations in abundance of females captured on human baits. Both studies, although performed in different places, showed a tendency for fluctuations to follow variations in rainfall, and adult abundance was positively correlated with cumulative rainfall over the previous 69 days.

There is no record of more extensive studies covering several areas or several places within the area.

There is no information on dispersal or natural enemies.

Vector behaviour: The research so far done on the behaviour of *nuneztovari* has been directed mostly at finding the causes of its exophilic habit. There is also some unpublished information on its biting activity collected by personnel of the Ministry of Health and Social Welfare, but nothing is known about host-finding or mating behaviour or how it identifies resting and oviposition sites.

Regarding the possible explanations of the exophilic behaviour, four major factors have been studied:

a) Scorza and Pintos (1972) studied the possibility that mosquitoes leave houses to seek different environmental conditions. They checked temperature and relative humidity of the resting places found among stands of *Heliconia* sp. and found that ranges were 21.8 to 24.1°C and 80 to 99% for temperature and relative humidity, respectively, but in houses the temperatures were higher and the humidity lower.

Pérez de Valderrama and Scorza (1976) tested three further hypotheses:

b) *nuneztovari* takes smaller meals, and so is able to leave houses earlier, than other species;

c) *nuneztovari* can excrete excess liquid from the blood meal faster than other mosquitoes, so that it can leave house earlier;

d) after a meal, *nuneztovari* is more active than other mosquitoes and this fact facilitates its early departure from houses.

These three hypotheses were tested, comparing *nuneztovari* with *darlingi* and *oswaldoi*, but despite the two authors' claims, no significant differences are apparent in the results.

Despite many years of observations in Venezuela, there is no published information about the diel periodicity of biting activity of *nuneztovari*.

1.4 OBJECTIVES OF STUDY

In order to fill some gaps in understanding the vectors, a small area in western Venezuela was selected to conduct a longitudinal study to assess the entomological malaria risk factors determined by the abundance, parous rate, biting activity and behaviour, sporozoite rate and human blood index (HBI) of the various potential vector species in relation to weather patterns and human habits.

1.5 STUDY AREA

The study villages are located in a problem area, identified by the national malaria control organization, where malaria was fairly important up to 1985 when the decision to conduct the study in that area was made (Table 1.4). This area is on the southern slopes of the Andes near the Venezuelan border with Colombia (approximately 7° 31'N, 71° 41'W) encompassing parts of Barinas, Táchira and Apure states, and covers an area of approximately 760 km^2 (Fig. 1.4). Altitude ranges from 200 to 400 m. This area is characterized by an annual rainfall of 3,000-4,000 mm, a mean temperature of 24°C and 83% relative humidity (Venezuelan Air Force, 1989). The area is classified as wet tropical woodland (Ewell & Madriz, 1968). The human population is about 8,000, and the economy is based on cattle rearing, growing crops (mainly vegetables and plantains), fisheries and forest exploitation. All these activities involve the invasion of previously unoccupied land, deforestation and considerable human movement. Due to the difference in average income between the study region and neighbouring areas of Colombia, a considerable illegal inflow of workers occurs during harvest of the main crops. The region is therefore subjected to profound human interference and continuous ecological

change. Epidemiologically, the area is characterized by high receptivity and vulnerability, that is the number of new cases of malaria that could originate from one imported case and the actual number of imported cases entering the area are high (Bruce-Chwatt, 1985). The malaria incidence per 1,000 population was 3.7 in 1989 and the main parasite is *P. vivax* (98.4%) (Dirección de Endemias Rurales, Records 1989b). The area was regularly sprayed with DDT until 1984-85 when, due to the exophilic habits of the incriminated vector, *An. nuneztovari*, the insecticide was changed to fenitrothion which has a "fumigant" effect. According to an internal report of the Dirección de Endemias Rurales (1985), spraying of 2 gm/m^2 was effective in reducing anopheline populations and parous rates.

Three villages were selected: Jabillos (7°32'10"N,71° 33'44"W), Guaquitas (7°32'6"N, 71°50'10"W) and Caño Lindo de Piscurí (7°33'33"N, 71°51'30"W). Malaria transmission occurs in these villages throughout the year and they have a range of ecological conditions representative of the area. Attempting to carry out a long-term study in existing human dwellings might have created problems with the householders. Therefore, in each village an experimental hut (Fig. 1.5) was built similar to the temporary houses that the people build. It was arranged that routine spraying of insecticide by the Division of Vector Control would not be carried out in the experimental huts in order to avoid interference between the study and the malaria control programme.

FIGURE 1.5: Experimental hut (3 m wide x 5 m long).

CHAPTER 2:

ANOPHELINE SPECIES OF WESTERN VENEZUELA

2.1. REVIEW OF LITERATURE

2.1.1. TAXONOMY

There are 29 anopheline species reported to occur in western Venezuela belonging to 2 genera: *Chagasia* Cruz, 1906 and *Anopheles* Meigen, 1818 (J. Mora, pers. comm.) (Table 2.1). Within the genus *Anopheles* there are 5 subgenera, namely *Anopheles*, *Kerteszia*, *Lophodomyia*, *Nyssorhynchus* and *Stethomyia*. The most abundant species at my study sites were those belonging to the subgenus *Nyssorhynchus* Blanchard, 1902. Of the species now placed in the subgenus *Nyssorhynchus* the first to be named was *Anopheles albimanus*, described by Wiedemann in 1820; a few years later Robineau-Desvoidy 1827 described *An. argyritarsis*. The most distinctive difference between these two species is the presence of a dark band on hindtarsal segment 5 in *albimanus*. For the next 50 years or so these names were applied essentially to all the species in the subgenus depending on the presence or absence of the dark band. Zavortink (1968) stated that the classification of mosquitoes during the early part of this century was in a chaotic state. Linthicum (1988) stressed the fact that the confusion was such that often the same taxa were given different names, descriptions of new taxa were based on heterogeneous material, and closely associated species were not recognised as being related and were placed in different taxa.

The subgenus *Nyssorhynchus* is restricted to the Neotropics (except for *albimanus* which extends into the Nearctic) and contains most of the important malaria vectors of the region. This subgenus has been the subject of recent revision and has in the past been found to be taxonomically complicated (Knight & Stone, 1977; Faran, 1980; Faran & Linthicum, 1981; Linthicum, 1988).

In the past, mosquito identification was based only on morphology of male genitalia and eggs (Hill, 1930; Galvão *et al.*, 1937; Corrêa, 1938; Galvão, 1938a;

Table 2.1: Anophelines reported to occur in western Venezuela (J. Mora, pers. comm.)

Genus	Subgenus	Species
Chagasia		*bathana* (Dyar, 1928)
Anopheles	*Anopheles*	* *apicimacula* Dyar & Knab, 1906
		eiseni Coquillett, 1902
		mattogrossensis Lutz & Neiva, 1911
		* *mediopunctatus* (Theobald, 1903)
		* *neomaculipalpus* Curry, 1931
		* *pseudopunctipennis* Theobald, 1901
		* *punctimacula* Dyar & Knab, 1906
	Kerteszia	*bambusicolus* Komp, 1937
		gonzalezrinconesi Cova García, Pulido & Escalante de Ugueto, 1977
		homunculus Komp, 1937
		lepidotus Zavortink, 1973
		neivai Howard, Dyar & Knab, 1912
		pholidotus Zavortink, 1973
		rollai Cova García, Pulido & Escalante de Ugueto, 1977
	Lophodomyia	*squamifemur* Antunes, 1941
	Nyssorhynchus	* *albitarsis* Arribálzaga, 1878
		* *argyritarsis* Robineau-Desvoidy, 1827
		* *benarrochi* Gabaldón, Cova García & López, 1941
		braziliensis (Chagas, 1907)
		darlingi Root, 1926
		evansae (Brethes, 1926)
		* *nuneztovari* Gabaldón, 1940
		* *oswaldoi* (Peryassú, 1922)
		parvus (Chagas, 1907)
		* *rangeli* Gabaldón, Cova García & López, 1940
		* *strodei* Root, 1926
		* *triannulatus* (Neiva & Pinto, 1922)
	Stethomyia	*kompi* Edwards, 1930

*specimens of these species were collected in the present study

Rozeboom, 1942). According to Faran (1980) anophelines can be reliably identified by chaetotaxy in the fourth-instar larva and by the morphology of the male genitalia. Identification of females is more difficult, especially in closely related species.

Faran (1980) subdivided the subgenus *Nyssorhynchus* into 2 sections, the Albimanus section and the Argyritarsis section. The Albimanus section is distinguished from the Argyritarsis section in adults primarily by the basal dark band on hindtarsal segment 5, and in male genitalia by the variously developed fused ventral claspette.

The revision by Faran (1980), based on the study of 14,792 specimens from different countries recognized 14 species in the Albimanus section. Faran divided the section into 2 groups: the monotypic Albimanus group and the Oswaldoi group. *An. albimanus* is easily differentiated from the Oswaldoi group by several correlated unique features in the adult, male genitalia and larva.

He separated the Oswaldoi group into 2 subgroups: the monotypic Triannulatus subgroup and the Oswaldoi subgroup composed of 12 species, further separated into the Oswaldoi complex and the Strodei complex.

The Oswaldoi complex consists of 9 species: *oswaldoi*, *galvaoi*, *evansae* (= *noroestensis*), *aquasalis*, *ininii*, *anomalophyllus*, *rangeli*, *trinkae* and *nuneztovari*. The Strodei complex contains *strodei*, *rondoni* and *benarrochi*.

Faran and Linthicum (1981) stated that within the Oswaldoi subgroup the external morphology of females is too similar interspecifically and usually too variable intraspecifically to be used alone for identification purposes.

Linthicum (1988) revised the Argyritarsis section of *Anopheles (Nyssorhynchus)* and recognized 8 species, based on the study of 7,659 specimens from different countries. The Argyritarsis section is divided into the Argyritarsis and Albitarsis groups. The Argyritarsis group is separated into 4 distinct subgroups: the Argyritarsis subgroup (comprising 2 species), and the monotypic Darlingi, Lanei and Pictipennis subgroups. The Albitarsis group is separated into 2 distinct subgroups: the Albitarsis subgroup composed of 2 species, *albitarsis* and *marajoara*, and the monotypic Braziliensis subgroup.

Complication in this subgenus arises not because of the above taxonomic scheme, but because almost every species in the taxon has had one or more subspecies or sibling species described for it. Morphological variants abound in the subgenus. For example, *Anopheles albitarsis* was described in 1878 by Lynch-Arribálzaga from Argentina. The great morphological and behavioural variability presented by species in different places has led to the description of many varieties. The first was *An. albitarsis braziliensis* by Root (1926) who, while studying specimens of *An. braziliensis* (Chagas, 1907) obtained in the type locality in Minas Gerais State, Brazil, did not find differences reliable enough to accord *braziliensis* species status. However, Deane *et al.* (1948) reported that Root based his observations on heterogeneous material and moreover had not examined the true *braziliensis* larvae. This led Root to describe *braziliensis* as an *albitarsis* variety and Galvão and Lane (1937) to describe *An. pessoai* (=*braziliensis*) in Lane (1953) as a new species. Also in 1937, Galvão and Lane described the *albitarsis limai* variety based on the finding of an egg morphologically distinct from those figured by Root in 1926. The variety was invalidated when it was verified that in Root's paper the egg described as *albitarsis* was in fact *darlingi* and that no true *albitarsis* eggs were illustrated (Causey *et al.*, 1942).

Galvão and Damasceno (1942) described *An. marajoara* as a species closely related to *albitarsis* among material from Marajó Island, Brazil. They stressed, however, that all taxonomic characters considered were very variable in *albitarsis* and its near relatives. Later Galvão (1944) considered *marajoara* to be synonymous with *albitarsis*.

In 1944 Galvão and Damasceno, based on distinct morphological and behavioural characters, divided the species into two subspecies: a strongly endophilic subspecies which was named *An. albitarsis domesticus* and an exophilic subspecies named *An. albitarsis albitarsis*.

According to Faran and Linthicum (1981), material classified as *An. albitarsis* should be divided into two species: *An. allopha* and *An. albitarsis*, differing by some morphological characters, distinct geographical distribution and vectorial capacity, only

allopha being able to transmit malaria. However, according to Laurenço-de-Oliveira and Deane (1984), none of the known anopheline species conforms with the description by Lutz and Peryassú of *allopha*, which was based on heterogeneous material, and hence should be considered a *nomem nudum*. Later, Linthicum (1988) changed *allopha* to *marajoara*, accepting the former as a *nomem dubium*.

An. albitarsis populations from 18 Brazilian states were studied morphologically by Rios *et al.* (1984). They verified the considerable intrapopulational variability of taxonomically important characters, such as pilosity of the anal lobe of the male genitalia (a character that should differentiate *domesticus* from *marajoara*), and the percentage of black on the 2nd hind tarsomere (supposedly distinguishing *domesticus* from *albitarsis*). Following Root (1926) and Davis (1928), they correlated variation in amount of blackness with latitude, and found it impossible to separate the two species on the basis of criteria used by Galvão and Damasceno (1944). At present the specific name *albitarsis* is given to mosquitoes variable in morphology, karyotype and behaviour, and apparently in the capacity to transmit malaria in different areas, suggesting that *albitarsis* could be a complex of sibling species. It has been regarded either as one of the major Brazilian malaria vectors (Kumm, 1932; Coutinho, 1942 a, b; Schiavi, 1945; Rachou, 1958; Ferreira, 1964) or as a species of minor importance (Freitas, 1942; Deane *et al.*, 1948).

In 1978, genetic studies were initiated in Brazil to characterise electrophoretically various populations of each species in the *Nyssorhynchus* group. To date, the species electrophoretically analysed are: *argyritarsis*, *braziliensis*, *darlingi*, *albitarsis*, *aquasalis*, *triannulatus*, *rangeli*, *oswaldoi*, *evansae* (as *noroestensis)*, *nuneztovari*, *albimanus* and *deaneorum* (Steiner *et al.*, 1982; Rosa-Freitas, 1989).

The main chromosomal studies of the subgenus *Nyssorhynchus* are those of Kitzmiller and his colleagues. To date, chromosomes of 12 of the 22 recognised species have been described (Kitzmiller, 1976, 1977). Chromosomal differences can serve as diagnostic tools to identify individual species because all species show unique banding patterns in the X-chromosome. Kreutzer *et al.* (1976) and Kitzmiller (1977) have

identified three chromosomal types in *An. albitarsis* from Brazil, Colombia and Venezuela respectively. Two of these types are found sympatrically in southern and eastern Brazil. The populations involved evidently do not interbreed, judging by the absence of inversion heterozygotes. The third type is allopatric, being found in Colombia and Venezuela. All three types have been distinguished on the basis of 22 inversions in the X chromosome and the autosomes.

Recent morphological, chromosomal and isoenzyme studies have shown *albitarsis* to be a complex of sibling species (Kreutzer *et al.*, 1976; Steiner *et al.*, 1982; Rosa-Freitas, 1989; Rosa-Freitas *et al.*, 1990).

Early ecological and behavioural observations suggested that *An. nuneztovari* consisted of two distinct forms separated geographically. One of these, found in Brazil, Suriname and Ecuador, bites at sunset, is mainly exophagic, and is considered to be primarily zoophilic (Elliott, 1972). The other, studied in western Venezuela and northern Colombia, bites around midnight, is primarily endophagic, and is a vector of *P. vivax* (Renjifo & de Zulueta, 1952; Elliott, 1972). Cytological studies by Kitzmiller *et al.* (1973) demonstrated the existence of two sibling species of *An. nuneztovari*, one in western Venezuela and northern Colombia and the other in Brazil. These sibling species could be separated by an inversion in the right arm of the X chromosome. Steiner *et al.* (1980) compared isozyme profiles of *An. nuneztovari* from Barinas State (western Venezuela) and from Brokopondo (Suriname). They found high levels of genetic variation in both samples. They suggested that the Est-5 locus may be diagnostic for the two populations. Recently, Conn (1990) studied populations of *An. nuneztovari* from four locations in western Venezuela (three of which were where I did my studies) and found no significant differences in the chromosome banding pattern compared with the populations of *An. nuneztovari* from Barinas State described by Kitzmiller *et al.* (1973). However, Conn found that the frequency of inversion 2La had increased significantly in the 16-year interval since the study of Kitzmiller *et al.* (1973), and considered that this

could be due to one factor or a combination of several such as genetic changes within the 2La inversion, environmental changes or within or between year seasonal variations.

2.1.2. DISTRIBUTION, BIONOMICS AND MEDICAL IMPORTANCE OF ANOPHELINES OCCURRING IN WESTERN VENEZUELA.

CHAGASIA BATHANA (Dyar, 1928).

This species is found from Mexico to Peru. In general the larval habitats are at the shaded margins of water with some current (Forattini, 1962). Immatures (i.e. larvae and/or pupae) were also found in permanent streams and ground pools of clear water (Cova García, 1951). Adults seem to inhabit woodland and to be strictly zoophilic (Forattini, 1962)

ANOPHELES (ANOPHELES) APICIMACULA Dyar & Knab, 1906.

This species is distributed widely in Latin America from Mexico to Bolivia. It also occurs in Trinidad and Tobago (Knight & Stone, 1977).

Immatures are found in shaded ground pools and pools in slowly flowing streams in lowland forest. The adults are zoophilic, feeding on domestic animals; they are rare in houses but common in animal shelters (Gorham *et al.*, 1973). This species has been found in Venezuela at altitudes up to 990 m in temporary or permanent breeding places (Cova García, 1951).

From the medical point of view, this species does not seem significant, except possibly in Mexico where it is a suspected vector of malaria in a small area (Gorham *et al.*, 1973). In Venezuela this species has never been found naturally infected.

ANOPHELES (ANOPHELES) EISENI Coquillett, 1902.

This species is found from Mexico to Bolivia, including some islands of the Antilles such as Trinidad and Tobago, at altitudes from sea level to 1,920 m (Forattini, 1962).

Anduze (1941) found immatures of *eiseni* in Venezuela in tree holes, artificial containers, stream margins and pools with abundant organic matter. Cova García (1951) found them mainly in permanent streams of clear water but in shaded areas. Adults have been found resting in caves and on shaded rocks.

It is a forest species which seems to prefer to feed on wild animals because it has never been found in houses. Deane *et al.* (1948) stated that there is no evidence that this species is a vector of malaria because it does not seem to approach man. Nevertheless, it has been found naturally infected in Colombia where it may play a minor role in malaria transmission (Gorham *et al.*, 1973).

ANOPHELES (ANOPHELES) MATTOGROSSENSIS Lutz & Neiva, 1911.

This species occurs in Brazil, Venezuela, Colombia and Bolivia (Knight & Stone, 1977).

Immatures have been found mainly in ground pools, streams, ponds and lagoons in shaded areas (Cova García, 1951; Deane *et al.*, 1948). Adults were collected inside houses and in traps with animal baits (Cova García, 1951). Deane *et al.* (1948) reported that this was the most abundant species in the upper Amazon. It feeds readily on man although it seems to prefer other animals. Gabaldón (1939) emphasised that *mattogrossensis* is a tenacious biter, attacking man even when near horses. However, it does not seem to be important as a vector of malaria because its distribution in Brazil does not coincide with that of the disease (Deane *et al.*, 1948).

Gabaldón (1939) collected this species in the forests of western Venezuela south of Lake Maracaibo. Due to its scarcity, this species probably plays no role in the epidemiology of malaria in the region where it coexists with *darlingi*. During 1986 and 1987, I collected this species in Guaquitas some 3 km from the experimental hut, whereas it was never collected in or outside the experimental hut. It has never been found naturally infected in my study area in western Venezuela (Dirección de Endemias Rurales, Report 1968).

***ANOPHELES (ANOPHELES) MEDIOPUNCTATUS* (Theobald, 1903).**

An. mediopunctatus occurs from Panama to Argentina (Knight & Stone, 1977).

In Brazil immatures are found in forested areas in clear streams in shade and also in small pools. Adults were collected mainly outdoors on human or animal baits at dusk. Deane *et al.* (1948) stated that it does not seem to be involved in malaria transmission and has never been found infected with *Plasmodium* spp. in nature. Nevertheless, Klein *et al.* (1990) reported that in Rondônia State, Brazil, *mediopunctatus* had the same susceptibility to *P. falciparum* as *darlingi*, whereas it was less susceptible to *P. vivax*. This species has been suspected of being a minor vector in Colombia, where it has been reported naturally infected (Gorham *et al.*, 1973). Its medical importance elsewhere is unknown.

***ANOPHELES (ANOPHELES) NEOMACULIPALPUS* Curry, 1931.**

This species is widely distributed in the Americas from Mexico to Argentina (Knight & Stone, 1977).

Immatures have been found in Venezuela in various breeding places such as ground pools, stream margins, lakes, marshes and ditches. The water may be turbid or clear, temporary or permanent. Adults have been collected mainly in traps and only 1.8% were collected resting inside houses (Cova García, 1951). During the study of Deane *et al.* (1948) only two specimens were collected at dusk on animal bait.

This species has never been found naturally infected with *Plasmodium* spp. in Venezuela (Cova García, 1951; Dirección de Endemias Rurales Internal Report, 1968).

***ANOPHELES (ANOPHELES) PSEUDOPUNCTIPENNIS* Theobald, 1901.**

This species is distributed very widely in the Americas, where it is an important vector in most parts of its range. It extends from central Mexico south to some provinces of Argentina and Chile, and is also present in the Antilles (Gabaldón, 1949). Investigations in Mexico have shown that females enter sprayed houses, bite, and escape rapidly, unharmed by DDT deposits (Martínez Palacios & de Zulueta, 1963; de Zulueta &

Garrett-Jones, 1965; Loyola *et al.*, 1990).

In Venezuela, Hill and Benarroch (1940) never found this species naturally infected with malaria parasites. Gabaldón (1949) found it to be prevalent in the northwestern region of Venezuela, where it appears to be a local vector on both slopes of the Andean foothills. Cova García (1951) never found this species naturally infected but believed that it plays an important role in malaria transmission in some regions of Venezuela where there have been malaria epidemics in which the only anopheline found in houses was *pseudopunctipennis*. On epidemiological grounds, it has also been incriminated as a vector in Colombia (Gast Galvis, 1943) and Ecuador (Levi Castillo, 1945). It has been proved to be a prominent vector in Peru, Bolivia, Chile and Argentina (Gabaldón, 1949).

Andean malaria is practically restricted to the distribution of this species. Among vectors in the western hemisphere it occurs at the highest altitudes. In Peru it was found by Valderrama at 3,200 m in Parco (Villalobos & Valderrama, 1944) and in Bolivia at 2,773 and 2,600 m (Moscoso Carrasco, 1943; Hackett, 1945). It is the only known vector in the Patagonian subregion. Its anthropophilic index is high: 50% according to Davis & Shannon (1928) and even 67.6% according to Vargas (1938); and it occupies human dwellings in large numbers.

***ANOPHELES (ANOPHELES) PUNCTIMACULA* Dyar and Knab, 1906.**

This species is found from Mexico to Argentina (Knight & Stone, 1977).

Immatures have been found in overflowing streams in forested areas (Gorham *et al.*, 1973). Adults of *An. punctimacula* are anthropophilic, but also feed readily on pigs.

An. punctimacula has been found naturally infected with malaria parasites in Panama and Colombia (Rozeboom, 1938; Huffaker *et al.*, 1945; Rey *et al.*, 1945). Simmons (1937) experimentally infected *punctimacula* with *P. falciparum* and *P. vivax* in Panama, and concluded that this species was an important factor in malaria transmission among military forces in the Canal Zone. *An. punctimacula* has been

suspected of being a vector of malaria in Costa Rica (Kumm & Ruiz, 1939), Colombia (Pinzón, 1945, *in* Wilkerson, 1990) and Peru (Villalobos & Valderrama, 1944). In Venezuela, this species have never been associated with malaria transmission (Cova García, 1951; Dirección de Endemias Rurales, Internal Report 1968).

ANOPHELES (KERTESZIA) BAMBUSICOLUS Komp, 1937.

This species has been reported to occur on the eastern slopes of the Andes in Colombia, and in Argentina, Bolivia, Brazil, Ecuador, Guyana, French Guiana, Peru and Venezuela (Zavortink, 1973).

Immatures are found inside unbroken internodes of bamboo (Zavortink, 1973). There are no reports on the adult behaviour or medical importance of this species.

ANOPHELES (KERTESZIA) GONZALEZRINCONESI Cova García, Pulido & Escalante de Ugueto, 1977.

The only information about this species is in the original description by Cova García *et al.* (1977a) which states that the species was collected from aerial bromeliads in Táchira state in western Venezuela at an altitude of 1,500 m.

ANOPHELES (KERTESZIA) HOMUNCULUS Komp, 1937.

This species occurs on the eastern slopes of the Andes in Colombia and Bolivia, and also in Trinidad, southeastern Brazil, Guyana, French Guiana, Peru, Suriname and Venezuela (Zavortink, 1973).

Immatures are found in the leaf axils of epiphytic and terrestrial bromeliads. Adults are attracted to light and females are anthropophilic.

An. homunculus was an important vector of human malaria in small areas of southeastern Brazil and in Trinidad (Forattini, 1962).

***ANOPHELES (KERTESZIA) LEPIDOTUS* Zavortink, 1973.**

According to Zavortink (1973), *An. lepidotus* is found on the eastern slopes of the Andes in Colombia and Bolivia, and possibly also in Brazil, Guyana, French Guiana and Paraguay. It is reported to occur in Venezuela.

Immatures are found in bromeliad leaf axils. Komp (1936) reported that females were common in the jungle where they frequently bit humans during the day and were taken in large numbers on horses in the evening. Recently it has been recorded in Colombia as an exophilic and exophagic mosquito with a biting peak between 1500 and 1800 hours (Quiñones *et al.*, 1984).

An. lepidotus has been incriminated on epidemiological grounds as a vivax malaria vector in Ecuador (Levi Castillo, 1945) and in Central Colombia at altitudes between 1,000 and 1,400 m. (Quiñones *et al.*, 1984).

***ANOPHELES (KERTESZIA) NEIVAI* Howard, Dyar and Knab, 1912.**

This is found from southern Mexico to Ecuador and French Guiana. There are also records from Bolivia, Venezuela, northern Brazil and Peru (Zavortink, 1973).

Immatures are usually found in leaf axils of terrestrial and epiphytic bromeliads and rarely in tree holes. Females commonly bite humans, particularly in the evening (Zavortink, 1973). Astaiza *et al.* (1988) reported that in Chocó, on the Pacific coast of Colombia, *An. neivai* has two biting peaks: one between 0530 and 0630, and a second, higher peak between 1800 and 1900 hrs.

An. neivai is the primary vector of human malaria in the Pacific coastal areas of Colombia (Lee & Sanmartín, 1967; Astaiza *et al.*, 1988). It was the only anopheline found biting man during an outbreak of vivax malaria in 1988 in San Josecito, 60 km west of my study site at an altitude of over 1,000 m (M. Medina, pers. comm.). This species has also been found naturally infected with the virus of yellow fever in Panama (de Rodaniche *et al.*, 1957) and Guaroa virus in Colombia (Lee & Sanmartín, 1967). In Panama it has been found infected with the virus of Venezuelan equine encephalitis, and with Ilheus and Guaroa viruses (Galindo *et al.*, 1966).

***ANOPHELES (KERTESZIA) PHOLIDOTUS* Zavortink, 1973.**

This species is found in the mountains of western Venezuela and western Panama (Zavortink, 1973).

Immatures have been collected in leaf axils of terrestrial and epiphytic bromeliads and females have been captured biting humans in the upper canopy of deep forest (Zavortink, 1973). Its medical importance is unknown.

***ANOPHELES (KERTESZIA) ROLLAI* Cova García, Pulido and Escalante de Ugueto, 1977.**

There is no information on the distribution of this species in the Neotropics, or on its biology or medical importance. The only published information refers to the original description which states that larvae were collected in western Venezuela in terrestrial and epiphytic bromeliads at an altitude of 1,050-1,200 m (Cova García *et al.*, 1976; Cova García *et al.*, 1977b).

***ANOPHELES (LOPHODOMYIA) SQUAMIFEMUR* Antunes, 1937.**

This species occurs in Panama, Colombia, Venezuela, French Guiana and Brazil (Knight & Stone, 1977).

Deane *et al.* (1948) reported that during their studies in Brazil only one female was collected on animal bait at dusk, and that further attempts to collect this species were unsuccessful. It has been collected only on animal baits; hence there is no evidence to incriminate it as a malaria vector (Forattini, 1962).

***ANOPHELES (NYSSORHYNCHUS) ALBITARSIS* Arribálzaga, 1878.**

An. albitarsis occurs in Central and South America from Guatemala to Argentina. In the Antilles it has been reported only from Trinidad (Knight & Stone, 1977).

Immatures have been found in a wide variety of sites such as large ground pools, small stream pools, swampy shores of lakes, turbid marshy depressions in a swamp, small

road puddles and small ponds (Linthicum, 1988). Generally, larval habitats are exposed to full sunlight in areas of secondary growth, such as open savanna or along roads (Linthicum, 1988).

Root (1926) found larvae of *albitarsis* associated with aquatic vegetation such as green algae, water hyacinth, *Ceratophyllum* spp. and *Salvinia* spp. He observed that *albitarsis* preferred for larger bodies of water such as large ponds, marshes, and eddy pools and overflows of rivers. According to Foote and Cook (1959), larvae have been found in Trinidad in rice fields. In large lagoons in the Venezuelan llanos it is very prevalent where floating plants such as *Pistia stratiotes* and *Eichhornia* spp. are present (Gabaldón, 1933).

Immatures have been collected together with those of *darlingi*, *argyritarsis*, *braziliensis*, *rangeli* and *strodei* (Linthicum, 1988).

Adults exhibit behavioural variations. Rozeboom (1937; 1938) reported that *albitarsis* is entirely zoophilic and exophilic in Panama. Similar behaviour was reported by Rozeboom (1942) in Guyana and Trinidad and by Gabaldón (1949) in Venezuela. According to Deane *et al.* (1946), in many parts of Brazil *albitarsis domesticus* can be captured in large numbers in houses by day and night. Rosa-Freitas *et al.* (1990), in a taxonomic and behavioural study carried out in 9 localities of Brazil and the type locality in Argentina, reported a significant decrease of endophily with increase in latitude. The reasons for behaviour differing in different geographic areas are not understood; possibly the difference reflects the habitat in which the species lives or the existance of different sibling species with the same morphology.

The adult female was reported to be able to fly 560 to 1,500 m from its breeding sites (Godoy & Pinto, 1923; Corrêa *et al.*, 1950).

An. albitarsis is not a primary vector of malaria throughout most of its range. It has been experimentally infected and has been found infected with malaria parasites in nature. Klein *et al.* (1990) infected *albitarsis* with *P. vivax* and *P. falciparum* and reported that, although oocysts of both species were often found, sporozoites of *falciparum* were never observed in the salivary glands. Arruda *et al.* (1986) analysed by

ELISA and IRMA over 2,000 specimens of *An. albitarsis* collected in Pará, Brazil for *P. vivax* and *P. falciparum* circumsporozoite protein. They found *P. vivax* sporozoite antigen in *albitarsis* at a higher frequency than that found in *darlingi*. Nevertheless, they failed to detect *P. falciparum* sporozoite antigen. They suggested that either this species is totally refractory to infection with *P. falciparum*, or that the oocysts failed to mature.

An. albitarsis has been found naturally infected in Brazil (Kumm, 1932; Schiavi, 1945; Arruda *et al.*, 1986) and in Colombia (Cadena, 1938). Recently, it has been incriminated as the vector of falciparum malaria together with *darlingi* in São Paulo, Brazil (Andrade *et al.*, 1986). In Venezuela, *albitarsis* has been considered a secondary vector in the north-central states (Gabaldón & Berti, 1954). In 1984, Pintos reported finding one mosquito with five oocysts from Portuguesa State, Venezuela. Nevertheless this species has never been found naturally infected in my study area; nor has it been incriminated as a vector.

***ANOPHELES (NYSSORHYNCHUS) ARGYRITARSIS* Robineau-Desvoidy, 1827.**

An. argyritarsis is widely distributed in the Neotropics. It has been reported from Mexico to Argentina including the Lesser Antilles (Knight & Stone, 1977).

Linthicum (1988) reported that immatures of *argyritarsis* have been found in the following habitats: stagnant ponds, swamps and marshes, drainage ditches, rain puddles and pools, wet meadows, forest springs, streams and pools, plantation and domestic wells, animal tracks, artificial containers such as tins and animal water troughs, rock holes and river margins. These sites were mainly in full sun or partial shade in areas of secondary growth as in plantations, pastures and forest clearings, predominantly at low to intermediate elevations (Linthicum, 1988).

The adults are exophilic and crepuscular (Faran & Linthicum, 1981). *An. argyritarsis* is not considered to be a vector of malaria (Linthicum, 1988). Nevertheless, early reports on the vector status of this species are contradictory. Attempts to infect experimentally *argyritarsis* with various *Plasmodium* species failed (Benarroch, 1931).

Also several authors failed to find wild-caught specimens infected with *Plasmodium* spp. (Stephens, 1921; Benarroch, 1931; Godoy and Pinto, 1923; Earle, 1936). However, other authors have incriminated *argyritarsis* as a malaria vector (Boyd, 1926). Linthicum (1988) pointed out that the apparent contradiction in reports before 1939 regarding the role of *argyritarsis* as a malaria vector is mainly due to the "very poor taxonomic understanding of the Argyritarsis section in the past". It is likely that *darlingi* Root, 1926, an efficient malaria vector, was sometimes misidentified as *argyritarsis*.

ANOPHELES (NYSSORHYNCHUS) BENARROCHI Gabaldón, Cova García & López, 1941.

The distribution of this species is limited primarily to the Orinoco and the eastern side of the Andes including the llanos plateau region of Colombia, parts of the upper Amazon in Brazil and Loreto, Peru (Faran & Linthicum, 1981).

Very little is known of its natural history. Deane *et al.* (1948) and Cerqueira (1961) have found immatures in stagnant ground pools and small streams in full sun or partial shade. It has been collected in association with *triannulatus*, *albitarsis* and *peryassui* (Deane *et al.*,1948).

Females feed primarily on animals and rarely enter houses. Deane *et al.* (1948) in Brazil and Elliott (1972) in Peru reported that *benarrochi* is crepuscular.

It has never been implicated as a vector of malaria (Faran & Linthicum, 1981).

ANOPHELES (NYSSORHYNCHUS) BRAZILIENSIS (Chagas, 1907).

An. braziliensis occurs throughout South America east of the Andes: Colombia, Venezuela, the Guianas, Trinidad, Brazil and Bolivia (Linthicum, 1988).

Immatures have been collected in clear ponds, lakes and pools exposed to full sun or partial shade, mainly in areas of secondary growth such as pastures and clearings in forest (Linthicum, 1988).

Deane *et al.* (1948) reported that in Brazil *An. braziliensis* show two different behaviour patterns: in some places, where there were numerous domestic animals, this

species was exophagic and zoophilic; in other areas it was frequently found in houses and infected with *Plasmodium* spp.

An. braziliensis has been found naturally infected with malaria parasites, and was considered a secondary vector in Brazil by Deane *et al.* (1948). Nevertheless, Arruda *et al.* (1986) examined 178 *braziliensis* from Pará, Brazil where there were *P. vivax* and *P. falciparum* parasites in the human population and failed to detect sporozoites of either species of malaria. However, a sample of only 178 is insufficient to demonstrate its lack of importance as a vector.

***ANOPHELES (NYSSORHYNCHUS) DARLINGI* Root, 1926.**

An. darlingi is widely distributed, occurring from Mexico to Argentina (Linthicum, 1988).

Immatures of *darlingi* have been found in clear streams, ponds and swamps with algae and floating vegetation in partial shade (Root, 1926; Barretto, 1939; Davis and Kumm, 1932; Shannon, 1933). In southern Venezuela, I collected larvae in overflows from the Orinoco river in deep water with floating vegetation.

Deane *et al.* (1946) reported that *darlingi* requires high humidity and rainfall and seems to die out in the dry season. Root (1926), from his studies in Brazil, concluded that *darlingi* was endophilic. Deane and Damasceno (1948) stated that post-feeding resting sites in houses were vertical surfaces within 2 m of the floor. Charlwood and Wilkes (1979), in Mato Grosso, Brazil, Roberts *et al.* (1987) in Amazonas, and Klein and Lima (1990) in Rondônia, Brazil, observed pronounced peaks in biting activity at dawn and dusk.

In Venezuela, the behaviour of *darlingi* is different, with a biting peak between 2200 and 2400 hours (Gabaldón, 1949). Hudson (1984) and Rozendaal (1987) in Suriname, Elliott (1972) in Colombia and Charlwood and Hayes (1978) in Amazonia, Brazil, reported a similar behaviour. However, in French Guiana, Pajot *et al.* (1977) found biting peaks not only at midnight, but also at dusk and dawn. Roberts *et al.* (1987)

found that *darlingi* feeds on humans indoors and outdoors.

All reports in the literature indicate that *darlingi* prefers human hosts to domestic animals.

Although *An. darlingi* was successfully eradicated with DDT spraying from vast areas in northern Venezuela (Gabaldón & Berti, 1954), this species is still a very serious malaria vector throughout most its range, especially in northeastern South America, because of its anthropophilic habits and its high susceptibility to *Plasmodium* spp. (Linthicum, 1988). In Suriname, Rozendaal (1990) found that the geographical distribution of *darlingi* correlates with the occurrence of malaria, and also that it was the only anopheline that occurs throughout the year.

Almost all examinations of *darlingi* in nature have yielded either oocysts of *Plasmodium* on the midgut or sporozoites in the salivary glands (Davis, 1931; Davis & Kumm, 1932; Shannon, 1933; Corrêa & Ramos, 1942a; Corrêa, 1943; Floch & Abonnenc, 1947; Kenney, 1946; Floch, 1954). Davis and Kumm (1932) reported infection rates in Brazil as high as 28.7%, whereas Kenney (1946) reported that 88.8% of the *darlingi* examined during a malaria epidemic in Guyana had oocysts in the midgut. Arruda *et al.* (1986) detected *P. falciparum* sporozoites in 2.7%-4.2% of *An. darlingi* specimens collected in Pará, Brazil and found that 0.9%-1.3% of all specimens tested contained *P. vivax* sporozoites.

In Brazil and Guyana, *An. darlingi* has been found infected with *Wuchereria bancrofti* filariae (Davis, 1931; Giglioli, 1948; Causey *et al.*, 1942).

ANOPHELES (NYSSORHYNCHUS) EVANSAE (Brethes, 1926).

This species is distributed throughout central and southeastern South America. Its northernmost limits are the southern margins of Amazonia and the northeastern states of Brazil. In the west, *evansae* extends to the eastern slopes of the Andes, and south to Argentina (Faran & Linthicum, 1981).

Immatures have been collected in permanent and temporary water in drainage ditches, small ground pools and along stream margins, exposed to the sun or in partial

shade (Faran & Linthicum, 1981).

Various reports suggested that this species is not particularly anthropophilic or endophilic (Deane *et al.*, 1948). The diel pattern of biting is bimodal; featuring a larger peak at dusk and the other at dawn (Faran and Linthicum, 1981). This species does not seem to be an important malaria vector. From their study in northeastern Brazil, Deane *et al.* (1948) concluded that this species was not important in malaria transmission. Forattini (1962) reported that it was possibly a secondary vector.

***ANOPHELES (NYSSORHYNCHUS) NUNEZTOVARI* Gabaldón, 1940.**

This species occurs throughout much of the Amazon basin; it is also found in the Guianas, northern Colombia and Venezuela, and eastern Panama. It is not known how far it extends west in the Amazon basin (Faran, 1980). Recently, Hayes *et al.* (1987) found it in Peru east of the Andes.

Immatures are found in a wide variety of habitats such as open marshy areas, grassy margins of ponds and lakes, small or large permanent or temporary ground pools, animal or wheel tracks, and along stream margins in full sun or partial shade. *An. nuneztovari* is found in clearings within the forest, and in areas of secondary growth (scrub) such as around villages (Faran, 1980). During the present study, immatures were collected together with *oswaldoi* and *albitarsis*.

Elliott (1968) studied adult behaviour of *nuneztovari* in relation to human activity in five localities in Colombia. He found that biting activity was unimodal and that, during months of highest density, the peak was shortly before midnight, indoors and outdoors; in months of low density, however, the peak was about an hour earlier. *An. nuneztovari* collected outdoors in resting places equidistant between animals and houses had a human blood index (HBI) of less than 10% (Elliott, 1972).

Most *nuneztovari* enter houses between 2200 and 2400 hrs. Gabaldón (1972) stated that before the inside walls of houses were sprayed with DDT in Venezuela, *nuneztovari* was very endophilic, remaining in houses and resting on walls and ceiling

after taking a blood meal. Spraying with insecticides, however, selected for "intense exophily". *An. nuneztovari* is still anthropophilic but, immediately after taking a blood meal, females leave houses, thereby avoiding a lethal dose of insecticide that would be received by resting on walls. Gabaldón (1972) stated that, even though it is strongly exophilic, "*An. nuneztovari* in Venezuela, for example, maintains a human blood preference of around 80%, and a man-biting rate of more than 100 during a night indoors". Gabaldón (1972) believes that this intense exophily has been largely responsible for "refractory" malaria in Venezuela.

Panday (1977) and Rozendaal (1987) reported a unimodal distribution of biting activity of *nuneztovari* in Suriname, the peak occurring between 1800 and 1900 hours; meanwhile on the Pacific coast of Colombia, Fajardo & Alzate (1987) found a biting peak outdoors at 2000 hrs and indoors between 2100 and 2200 hrs. Panday (1977) reported a tremendous increase in the numbers of *nuneztovari* in the "hilly and mountainous forest region" in the interior of Suriname. He believed that, to a large extent, this increase was due to the construction of Afobaka dam, resulting in the formation of Brokopondo lake. From daily collections in this area, Panday (1977) concluded that "*An. nuneztovari* was the dominant anthropophilic *Anopheles* species" and implicated it as the primary vector of *P. falciparum*. In laboratory studies on the life cycle of this species, Panday (1977) found that the egg stage lasts one day, the larval stages 7 days and the pupal stage one day (temperature not specified). The first gonotrophic cycle requires 5 days, whereas all subsequent cycles require 4 days. The maximum parous rate found in Suriname was 0.69, the minimum being 0.14 and the mean being 0.34. Panday (1977) also reported that grassy vegetation seems essential for oviposition. Scorza *et al.* (1981), working in western Venezuela, reported that under laboratory conditions at 22°C the development from egg to adult lasted 24 days; in the field they found a parous rate of 0.73.

Unlike *nuneztovari* in Colombia and Venezuela, in Pará, Brazil, females seem to be primarily exophagic. Deane *et al.* (1948) reported that, of the 21,967 females of *nuneztovari* collected, only 411 or 1.9% were captured inside houses. Feeding-preference

studies, comparing a horse and a man as bait, indicated that *nuneztovari* fed on man outdoors. Studies conducted from March 1975 to April 1976 by the US Army Medical Research Unit-Belém in Palestina (100 km SW of Marabá, Pará, Brazil), also indicated that *nuneztovari* is exophilic and most active at sunset, and that it was the dominant anopheline captured in landing and resting collections (Faran and Linthicum, 1981).

Scorza *et al.* (1976) conducted precipitin tests to determine hosts preferences of *nuneztovari* in Santa Barbara, Barinas, Venezuela. Of those that had fed on blood, 75% tested positive for the immune sera used in this study and 25% did not react; of those testing positive 74.2% (289) had fed on cattle, 13% (50) on dogs, 7.4% (29) on humans, 4.5% (19) on chickens, 0.7% (3) on horses, 0.2% (1) on a cat and none on pigs.

An. nuneztovari is a major vector of malaria in western Venezuela and northern Colombia. It was first discovered naturally infected with *Plasmodium* spp. by Rey and Renjifo (1950). Gabaldón and Guerrero (1959) stated that in some areas where *nuneztovari* was transmitting malaria the spleen indices were close to 100%. They also found that in areas distant from the forest, malaria disappeared when the local inhabitants took chloroquine; in districts near forests, however, chloroquine failed to stop transmission. Hamon *et al.* (1970) showed that the importance of *nuneztovari* depends on the amount and density of vegetation around houses, vector density being reduced where peridomestic vegetation has been cleared. In Suriname, Panday (1977) reported that *nuneztovari* may have been the principal vector of *P. falciparum* in recent epidemics and stated that *An. darlingi*, previously thought to be the primary vector of malignant malaria, had not been captured in the epidemic regions. In these same areas *nuneztovari* has been collected in great numbers.

Scorza *et al.* (1976) attempted to infect *nuneztovari* experimentally with *P. falciparum* and *P. vivax* and found this mosquito to be highly susceptible to the former.

An. nuneztovari is responsible for a malaria endemic focus on the Colombian Pacific coast (Fajardo & Alzate, 1987), and it has been found naturally infected in

western Venezuela (Pintos & Sabril, 1965; Pintos *et al.*, 1968), Brazil (Arruda *et al.*, 1986) and in Peru (Hayes *et al.*, 1987).

***ANOPHELES (NYSSORHYNCHUS) OSWALDOI* (Peryassú, 1922).**

An. oswaldoi occurs in Colombia, Venezuela, the Guianas, Brazil, Paraguay, Bolivia, Ecuador, Peru and northern Argentina. Northward, it extends into Panama and Costa Rica. It is also found in Trinidad, but not in Tobago or any other islands of the Antilles (Faran, 1980).

Immatures are usually found in, or on the margins of, tropical forests. The larval habitats are generally in permanent or temporary ground pools that have abundant floating vegetation in shaded areas (Faran & Linthicum, 1981). Immatures have been found in association with *triannulatus*, *rangeli*, *nuneztovari*, *neomaculipalpus* and *punctimacula*. Adults are largely restricted to forest and are exophilic and zoophilic (Rey & Renjifo, 1950; Corrêa & Ramos, 1944). Nevertheless, *oswaldoi* has been found biting humans inside forest, as in the Mojinga Swamp in Panama (Rozeboom, 1941; Curry, 1932) or in forest in French Guiana (Floch & Abonnenc, 1947), or in cacao plantations in Trinidad (Rozeboom, 1942). Deane *et al.* (1948) reported that the peak of biting activity of *oswaldoi* was between 1800 and 1900 hours.

An. oswaldoi has been experimentally infected with *P. vivax* and *P. falciparum* by Rozeboom (1942) in Trinidad, by Fonseca and Fonseca (1942) in the State of São Paulo, Brazil and by Klein *et al.* (1990) in Rondônia State, Brazil. Deane *et al.* (1948) dissected 540 females from the northeast of Brazil and found none infected with *Plasmodium*. Lucena (1940) and Corrêa and Ramos (1942b) reported finding *oswaldoi* var. *metcalfi* naturally infected in Brazil; Faran (1980), however, considered that these investigators were probably dealing with *evansae* or *aquasalis* and not *oswaldoi*. *An. oswaldoi* was reported naturally infected for the first time in Brazil by Arruda *et al.* (1986). They found it positive for *P. vivax* and *P. falciparum* circumsporozoite protein by ELISA. Hayes *et al.* (1987) found it naturally infected in Peru.

ANOPHELES (NYSSORHYNCHUS) PARVUS (Chagas, 1907).

The distribution of this species is uncertain but there are reports from Brazil, Venezuela and Bolivia. Immatures are found in shaded, clean water with little aquatic vegetation, rock holes, rain pools and streams in forested mountains. The adults are zoophilic and rarely found in houses, but will bite man outdoors (Gorham *et al.*, 1973).

It has been suspected to be a secondary malaria vector in Bolivia (Gorham *et al.*, 1973).

ANOPHELES (NYSSORHYNCHUS) RANGELI Gabaldón, Cova García & López, 1940.

An. rangeli occurs in the upper Amazon and Orinoco basins, Colombia, Venezuela, Ecuador and south through eastern Peru and into northern Bolivia (Faran & Linthicum, 1981).

Immatures occur in marshy depressions, temporary ground pools, animal and wheel tracks, semi-permanent ditches, stream margins and lakes. They are usually found in full sun or partial shade and associated with *triannulatus*, *strodei*, *oswaldoi*, *argyritarsis* and *punctimacula* (Faran, 1980).

Bates and de Zulueta (1949) reported that in Colombia the seasonal peak in the populations of *rangeli* occurs in June at the beginning of the rainy season.

The adults are predominantly exophilic (Rey & Ranjifo, 1950; Deane *et al.*, 1948). Elliott (1972) reported that in Peru the peak times of biting by *rangeli* were 1800-2000 and 0400-0600 hours, whereas in Colombia *rangeli* seems to have only one peak of activity outdoors between 1800 and 2000 hours (Quiñones, pers. comm.)

The vectorial capacity of *rangeli* is uncertain. It does not seem to be a vector of malaria, although Forattini (1962) stated that it has been suspected of transmitting malaria in Ecuador. Deane *et al.* (1948) dissected 363 females from Acre, Brazil, and found none infected with *Plasmodium* spp. Rey and Renjifo (1950) did not find *rangeli* naturally infected in the Cúcuta area of Colombia during a malaria epidemic. Nevertheless, Hayes

et al. (1987) found that 0.4% of the salivary glands of *An. rangeli* dissected were positive for sporozoites, and more recently Suárez *et al.* (1990) found *rangeli* positive for *P. vivax* circumsporozoite protein in southern Colombia near the border with Ecuador.

ANOPHELES (NYSSORHYNCHUS) STRODEI **Root, 1926.**

This species is widely distributed throughout Central America and South America east of the Andes. It is not known if it occurs on the Pacific slopes of the Andes. It does not occur on any of the Caribbean islands, including Trinidad and Tobago (Faran & Linthicum, 1981).

Immatures have been found in animal tracks, ponds, lakes, swamps, stream margins, marshy depressions, ditches, seepage areas and rock holes, usually in full sun or partial shade (Faran, 1980). It has been reported to occur at elevations up to 1,600 m (Unti, 1941). Immatures are usually associated with abundant vegetation such as grass, algae and *Utricularia* sp. (Faran, 1980) and have been found with *albitarsis*, *argyritarsis*, *triannulatus* and *rangeli*.

Adult females of *strodei* are exophilic. Deane *et al.* (1948) in Brazil, Kumm *et al.* (1940) in Costa Rica, Rozeboom (1938) in Panama and Quiñones *et al.* (1987) in Colombia occasionally found *strodei* inside houses, but usually it showed a preference for animals and fed outside. The only exception was reported by Corrêa (1938), 95.3% of whose collection of anophelines inside houses consisted of *strodei* in the state of São Paulo, Brazil. In Panama (Curry, 1932; Rozeboom, 1938) and Colombia (Renjifo & de Zulueta, 1952; Bates & de Zulueta, 1949), the peak abundance is during the early part of the dry season. Adults bite most actively around dusk, although they are reported to feed throughout the night (Deane *et al.*, 1948).

An. strodei does not seem to be an important vector of malaria. It has been experimentally infected with *P.vivax* (Galvão & Lane, 1937; Galvão, 1938b; Fonseca & Unti, 1943). Apparently it has only once been found naturally infected with *Plasmodium* spp. in Brazil by Corrêa (1938). Faran (1980) suggested that *strodei* may be a threat to human health only at very high densities.

ANOPHELES (NYSSORHYNCHUS) TRIANNULATUS (Neiva & Pinto, 1922).

An. triannulatus is widely distributed from Nicaragua to Argentina (Faran, 1980).

Immatures are common in permanent ponds, lakes, canals, slow-flowing streams or river margins, ditches and swamps, usually associated with *Pistia* sp., and exposed to full sun or partially shaded (Faran & Linthicum, 1981). In my study site we found it also associated with other aquatic plants such as *Eichhornia*, *Azolla* and *Salvinia*. Immatures have been collected in association with *albimanus*, *oswaldoi*, *nuneztovari*, *rangeli*, *strodei*, *apicimacula*, *neomaculipalpus* and, on one occasion, *aquasalis* (Faran & Linthicum, 1981).

Bates and de Zulueta (1949) found that *An. triannulatus* was more abundant in Colombia during the dry season, whereas it is common in Panama from the end of the dry season until well into the rainy season (Arnett, 1947).

Adult females are primarily exophilic and zoophilic. Floch and Abonnenc (1944) in French Guiana, Deane *et al.* (1948) in Brazil and Rozeboom (1938) in Panama reported that this species was rarely found inside houses. The only contrary finding was by Gabaldón (1949), who reported a large number of *triannulatus* inside a house in the Rio Apure region of Venezuela. Hill (1934), working in the area around Maracay, Venezuela, where malaria epidemics occurred yearly, collected *triannulatus* in stables, houses and dairy farms. He carried out precipitin tests on 262-blood engorged females and found that only 5.3% had fed on man. He concluded that *triannulatus* prefers blood of domestic animals. However, several investigators (Rozeboom, 1935; Deane *et al.*, 1948; Floch and Abonnenc, 1944) have stated that *triannulatus* is a troublesome biter, feeding readily on man outside even during the day although most actively at dusk.

An. triannulatus has been experimentally infected with *P. vivax* and *P. falciparum* by several investigators (Godoy & Pinto, 1923; Rozeboom, 1935; Floch & Abonnenc 1944; Fonseca & Unti, 1943). In comparing the susceptibility of *triannulatus* to *P. vivax* and *P. falciparum* with that of *albimanus*, Rozeboom (1935) found a larger percentage of the *triannulatus* to be refractory to infection.

An. triannulatus has only once been found to have a natural oocyst infection in Venezuela (Gabaldón & Cova García, 1946). Benarroch (1931) incriminated *triannulatus* as a possible vector of malaria at a boys' school near Maracay, Venezuela, on the grounds that it was the most common species present during a malaria epidemic. Hill (1934) stated that at high density "it is probable that this species can act as a malaria transmitter". Recently it has been found naturally infected in Pará, Brazil, at a rate higher than that shown by the well known vector *An. darlingi* (Arruda *et al.*, 1986).

***ANOPHELES (STETHOMYIA) KOMPI* Edwards, 1930.**

An. kompi has been recorded in Panama, Costa Rica, Colombia, Venezuela, Suriname, French Guiana and Brazil (Knight & Stone, 1977).

Immatures were found in permanent or temporary streams and pools with clear or turbid water, with marginal vegetation but without algae (Cova García, 1951). According to Gorham *et al.* (1973) immatures are found in shaded ditches, swamps, streams and ground pools. Adults bite man and domestic animals, but their host preference is unknown. This species is found in forests. Nothing is known of its medical importance.

2.2. TAXONOMIC CRITERIA USED IN PRESENT STUDY

Rozeboom (1942) recognised that many of the characters used to distinguish adult females in the Albimanus section are extremely variable and unreliable for species identification. On the basis of one character alone it is often impossible to identify with any confidence an adult female as belonging to a particular species in this section.

Different authors have different opinions about the most reliable characters for identifying anophelines of the Oswaldoi subgroup. For instance, Pintos *et al.* (1968) considered eggs to be the only reliable means of identifying females of this group, and until the present work all identifications in western Venezuela have been based on eggs. According to Faran and Linthicum (1981) the most reliable characters for species identification are in the male genitalia and the larva. The external morphology of the adult female and pupa, particularly in the case of the Oswaldoi subgroup, is usually rather

variable intraspecifically and rather similar interspecifically. For this reason, the keys for adults and pupae are not always reliable when used by themselves.

Faran (1980), Faran and Linthicum (1981) and Linthicum (1988) considered the following morphological structures to be the most important for differentiating adult females (Fig. 2.1): 1) presence or absence of scales on first abdominal sternum (Fig. 2.2); 2) dark caudo-lateral scale tufts on the abdomen (Fig. 2.3); 3) banding patterns of legs, especially, the dark basal band of hind tarsomere 2 (Fig. 2.4); 4) relative lengths of wing spots, especially those on the costal vein (Fig. 2.5); 5) presence or absence of scales on the anterior and upper mesanepimeron (Fig. 2.6); and 6) scales on palpomeres 4, 5 (Fig. 2.7).

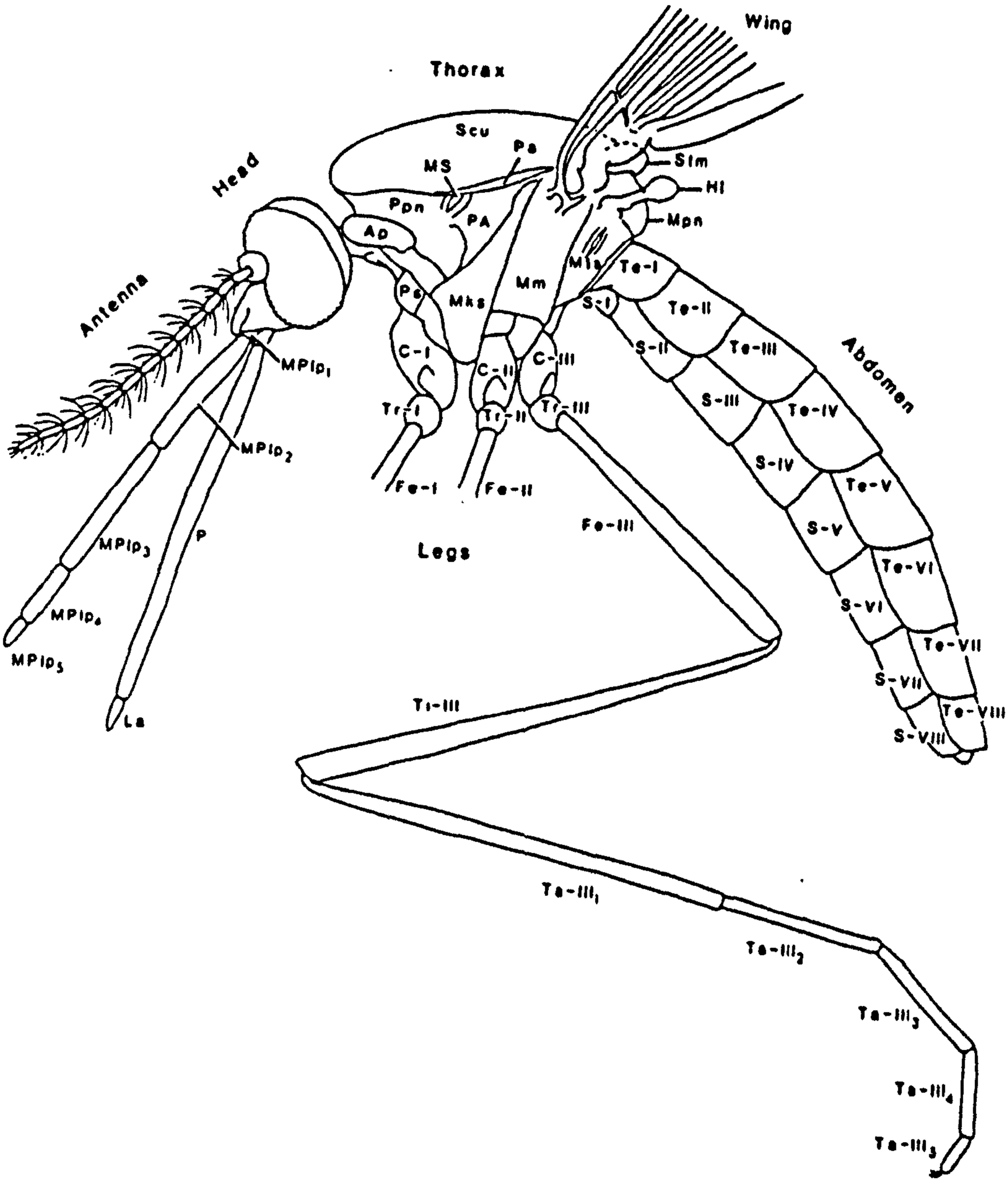
During the present study, species identification of the Oswaldoi subgroup with the available keys proved to be very difficult because the supposedly distinctive taxonomic characters were found to be highly variable, and there were many specimens that could not be identified with the keys. I also found that eggs were highly variable and were unreliable for adult species identification in the field.

As has been emphasised by Belkin *et al.* (1965), it is best to examine more than one specimen. Furthermore, to be certain of an identification, the immatures should be individually reared and slides prepared of their exuviae and of the genitalia of the males to permit the correlation of characters in the different life stages.

In order to determine diagnostic characters in the female adults that would allow us to identify the females in the field, morphometric studies based on associated rearings from field-collected specimens were undertaken by Nereyda Delgado. Larvae were collected at the three villages and reared in the insectary in Maracay at 25 ± 2 °C. Females collected in the field on human baits were blood fed immediately and transported to the insectary in Maracay in order to obtain groups of adult males and females with associated larvae and pupae exuviae derived from individual mothers.

It should be stressed that none of the species of the Oswaldoi subgroup has been colonised and that rearing proved to be very difficult. It was only after 3 years of repeated

FIGURE 2.1: Female anopheline mosquito
lateral view



Abbreviations Fig. 2.1: Ap: antepronotum; C-I: forecoxa; C-II: midcoxa; C-III: hindcoxa; Fe-I: forefemur; Fe-II: midfemur; Hl: halter; La: labellum; Mks: meskatepisternum; Mm: mesepimeron; $MPlp_{1-5}$: maxillary palpus, segments 1-5; Mpn: mesopostnotum; MS: mesothoracic spiracle; Mts: metepisternum; P: proboscis; Pa: paratergite; PA: postspiracular area; Ppn: postpronotum; Ps: proepisternum; S-I-VIII: sterna I-VIII; Scu: scutum; Stm: scutellum; $Ta\text{-}III_{1-5}$: hindtarsomeres 1-5; Te-I-VIII; terga I-VIII; Ti-III: hindtibia; Tr-I: foretrochanter; Tr-II: midtrochanter; Tr-III: hindtrochanter.

From Wilkerson & Strickman (1990).

FIGURE 2.2: Abdomen, ventral view

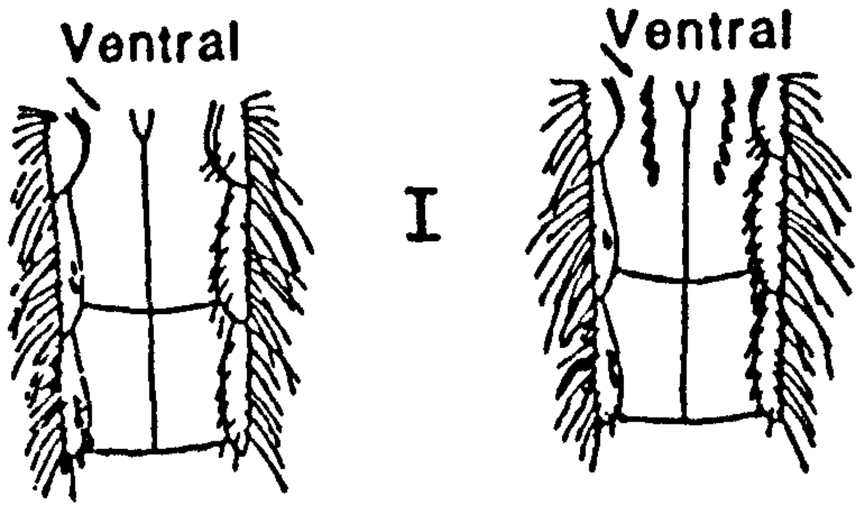


FIGURE 2.3: Abdomen, dorsal view

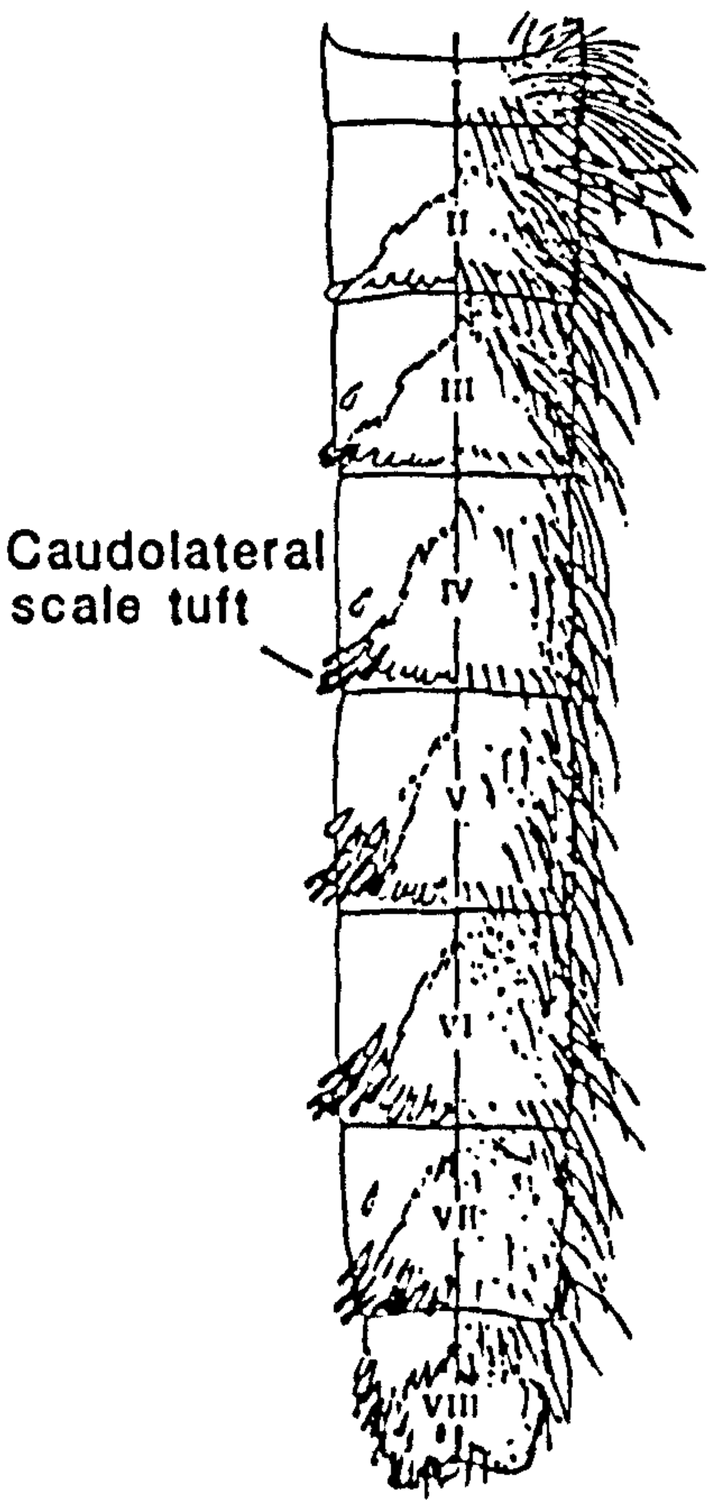
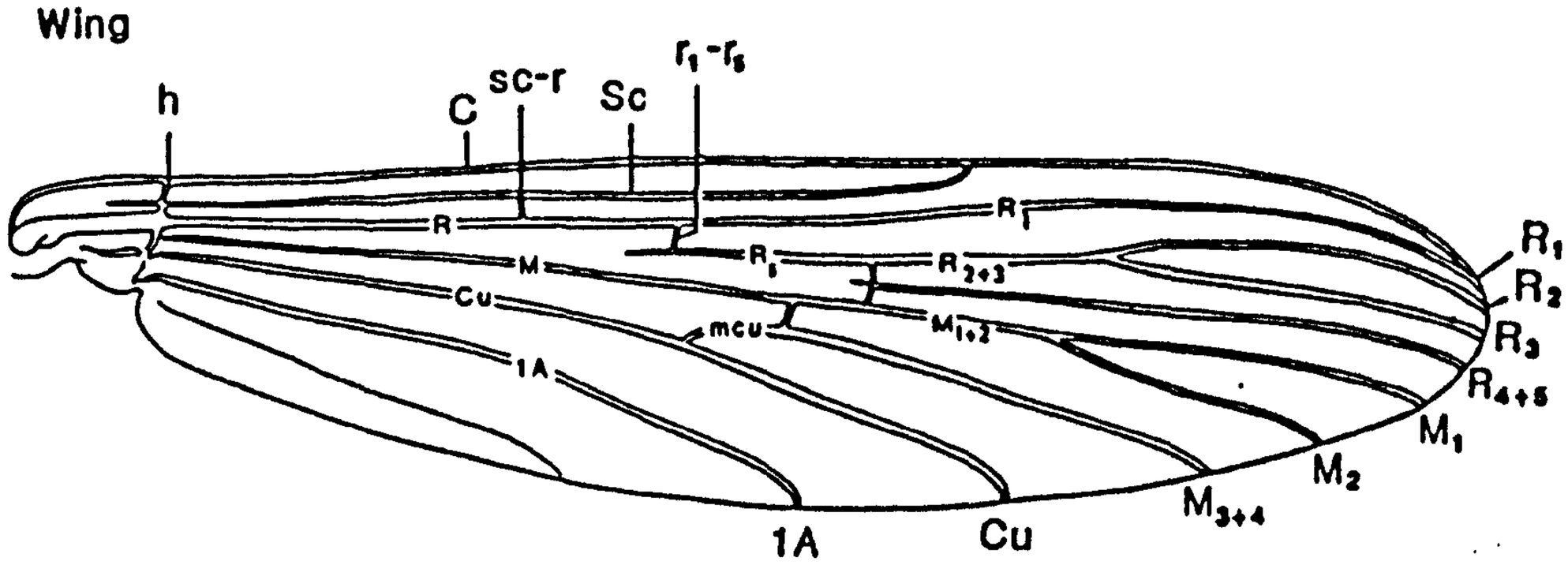


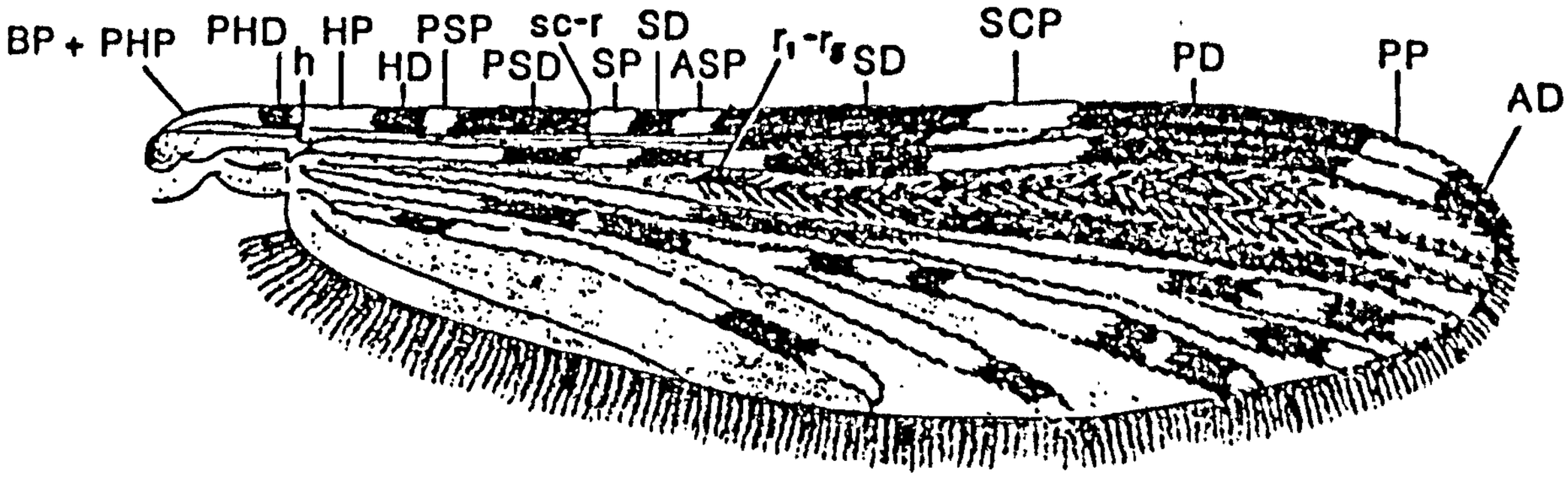
FIGURE 2.4: Hind leg showing banding pattern

From Faran & Linthicum (1981)

FIGURE 2.5: Wing of an *Anopheles* female mosquito



A: Veins and crossveins. C: costa; Cu: cubitus; h: humeral crossvein; M: media; M_1: media-one; M_{1+2}: media-one-plus-two; M_{3+4}: media-three-plus-four; mcu: mediocubital crossvein; R: radius; R_1: radius-one; r_1-r_8: radical crossvein; R_2: radius-two; R_{2+3}: radius-two-plus-three; R_3: radius-three; R_{4+5}: radius-four-plus-five; R_5: radial sector; Sc: subcosta; sc-r: subcostal crossvein; 1A: Anal.

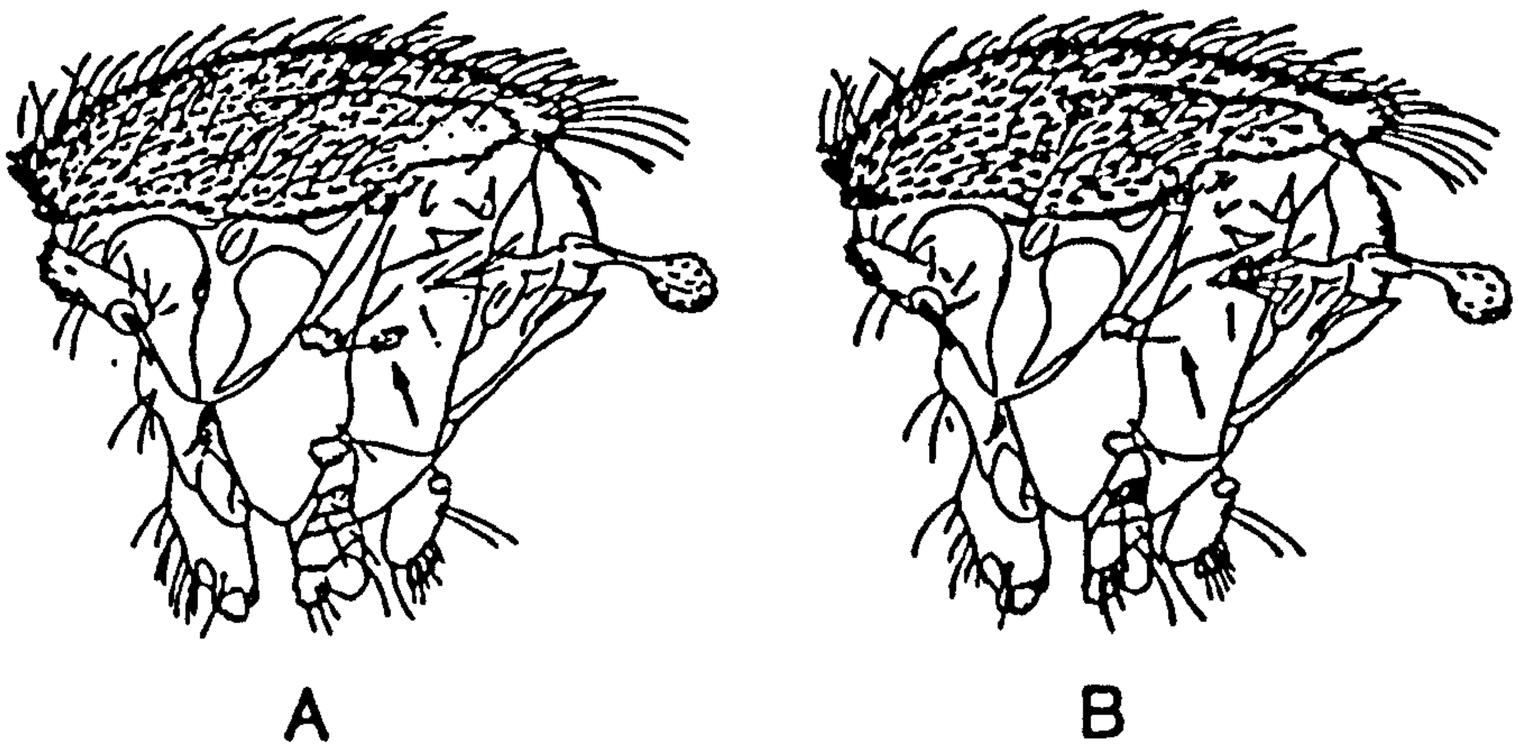


Wing of *Anopheles (Nyssorhynchus) oswaldoi*

B: Wing spots. AD: apical dark spot; ASP: accessory sector pale spot; h: humeral crossvein; HD: humeral dark spot; HP: humeral pale spot; PD: preapical dark spot; PHD: prehumeral dark spot; PHP: prehumeral pale spot; PP: preapical pale spot; PSD: presector dark spot; PSP: presector pale spot; r_1-r_8: radial crossvein; sc-r: subcostal crossvein; SD: sector dark spot; SP: sector pale spot.

From Wilkerson & Strickman (1990).

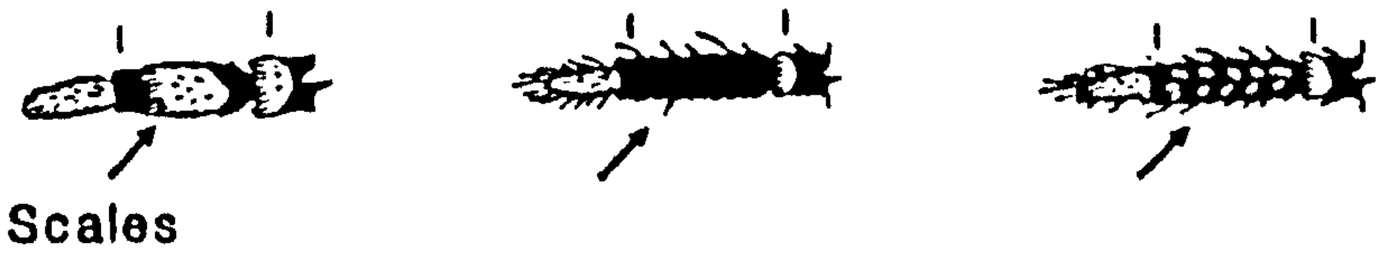
FIGURE 2.6: Thorax, lateral view



A: Anterior mesanepimeron with a conspicuous patch of scales

B: Anterior mesanepimeron without a patch of scales

FIGURE 2.7: Palpomeres 4 and 5



From Faran & Linthicum (1981).

collection and rearing that we were able to obtain progenies in the laboratory.

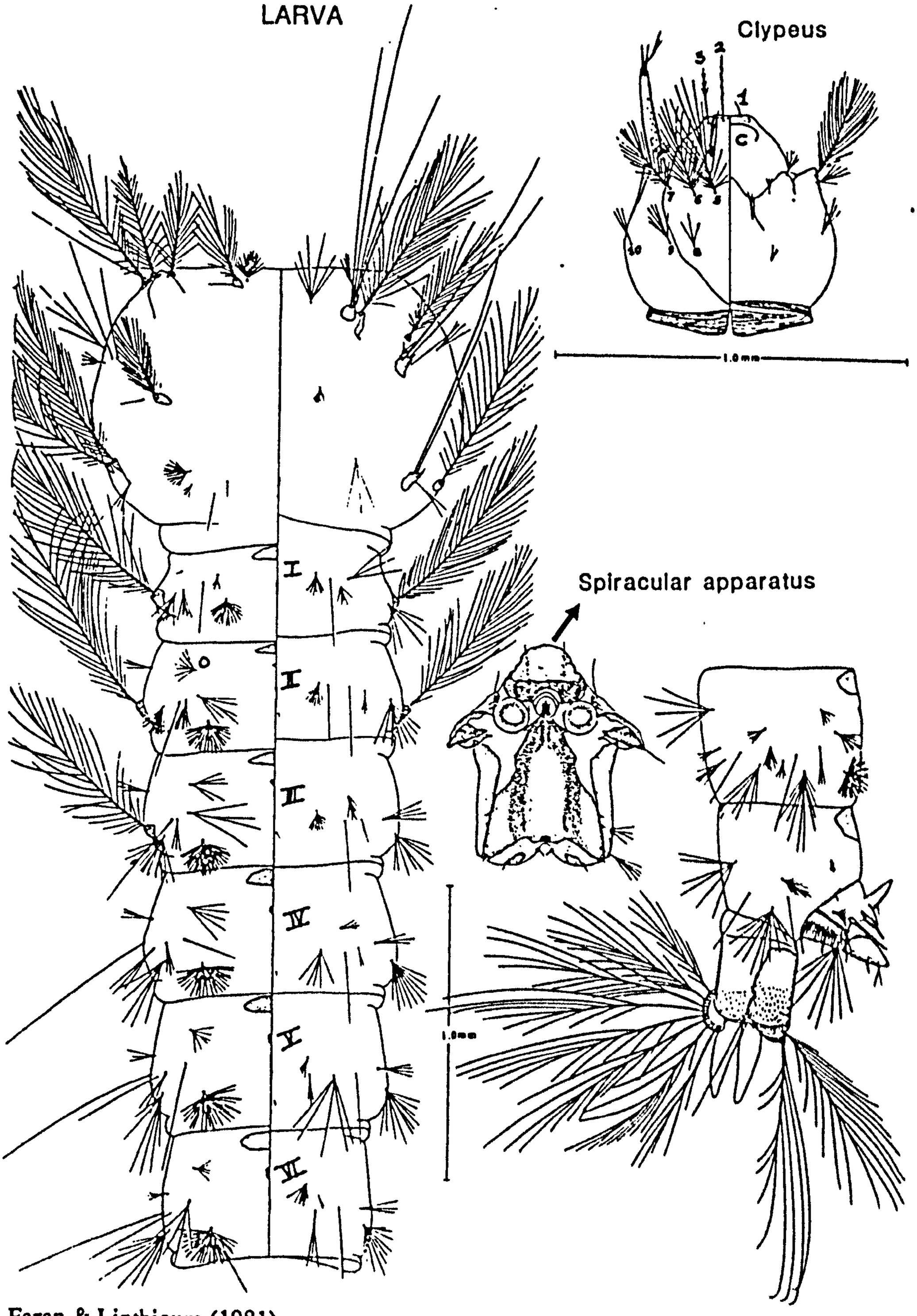
Larval and pupal skins (exuviae) from progenies of field-collected females were preserved in 70% ethanol until mounted on a slide following the method of Lane (1974). Morphometric measurements were carried out by Delgado on the following larval characters: i) length of seta 3-C; ii) distance between seta 3-C and 2-C; iii) distance separating setae 2-C; iv) clypeal index (distance between 2-C and 3-C on one side divided by distance separating setae 2-C); v) Length of seta 4-C divided by length of seta 3-C; vi) length and number of branches of setae 8-C; vii) length of setae 0-II; and viii) length and width of spiracular apparatus (Fig. 2.8). Special attention was paid to the possibility of finding *An. trinkae* Faran 1979, adult females of which could be misidentified as *nuneztovari* (Faran, 1980). On the other hand, examination of field-collected females suggested the presence of a morphological variant of *nuneztovari* that I provisionally called "morphotype 11", and that differed from the majority type in the length of the humeral pale spot on the wing. Hence it was necessary to determine whether we had two species or a single, highly variable one.

Results are shown on Table 2.2. It was found that within progeny from mothers identified as *nuneztovari* there was no significant difference between the mean values of the characters analysed or their frequency distributions (Delgado, pers. comm.). However, when the two groups of progenies (those from *nuneztovari* mothers and those from "morphotype 11") were compared , the clypeal index was significantly different.

Delgado also analysed the male genitalia from progenies from typical *nuneztovari* and "morphotype 11" mothers. The following characters were analysed: i) ventral claspette length divided by the length of sidepiece; ii) width of the apex divided by the length of the claspette; and iii) shape of aedeagus and presence of membranous nonserrated leaflets (Fig. 2.9).

The genitalia analysed from progenies from *nuneztovari* mothers and "morphotype 11" presented characteristics similar to those previously described for *nuneztovari* by Gabaldón (1940) and recently revised by Sutil (1976), Faran (1980) and Savage (1986):

FIGURE 2.8: *An. nuneztovari*



From Faran & Linthicum (1981)

Table 2.2: Larval taxonomic characters measured in progenies of individual mothers categorised as typical *nuneztovari* and "morphotype 11" compared to those studied by Faran (1980) to distinguish between *nuneztovari* and *trinkae*.

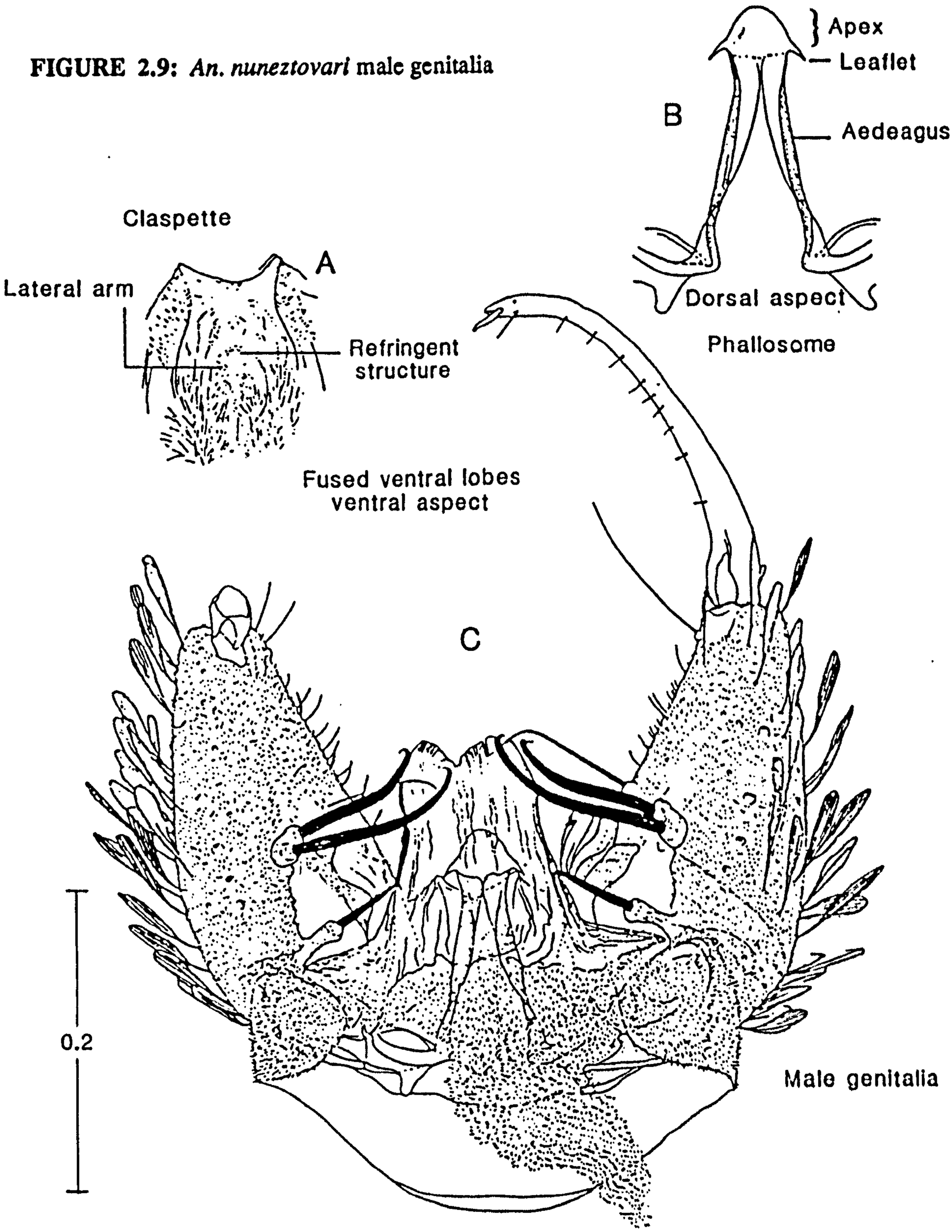
Faran (1980):

Character	*nuneztovari*	*trinkae*
Clypeal index	1.0-1.3	1.25
Length 4-C/3-C	0.3-0.6	0.7-1.0
No. branches 8-C	3-5	2-3
Setae II-0	Conspicuous 5-8 Branches	Inconspicuous 1-3 Branches

Delgado (pers. comm.):

Character	*nuneztovari*			Morphotype 11			
	Mean	S.D.	N	Mean	S.D.	N	Signif. of diff.
Clypeal index	1.535	0.342	68	1.372	0.290	32	$p<0.01$
Length 4-C/3-C	0.472	0.097	68	0.513	0.127	32	n.s.

FIGURE 2.9: *An. nuneztovari* male genitalia



From Savage (1986).

i) length of ventral claspette divided by the length of sidepiece = 0.40-0.50;

ii) width of apex divided by length of claspette = 0.40-0.50;

iii) aedeagus rounded at apex and with small, nonserrated, pointed, basolaterally directed leaflets.

Faran (1980) stated that leaflets may be present or absent in *nuneztovari* but he stated that they were absent in all the specimens that he identified as *trinkae*. Nevertheless, Savage (1986) pointed out that leaflets are always present and are diagnostic for *nuneztovari*. 100% of the specimens analysed by Delgado showed leaflets on the aedeagus, which indicates that none of the specimens was *trinkae*.

In adult females the following morphological characters were measured: length of hind tarsomere 2 (Ta), length of dark band on hind tarsomere 2 (TaD) (Fig. 2.4), length of sector dark (SD), length of subcostal pale (SCP), length of humeral pale (HP) and length of prehumeral dark (PHD) (Fig. 2.5b) (Faran, 1980; Faran & Linthicum, 1981; Wilkerson & Peyton, 1990). These morphological terms and abbreviations follow the usage of Harbach and Knight (1980, 1982) and Wilkerson and Peyton (1990).

Table 2.3 and Figure 2.10 show the mean values of the length of dark band on hind tarsomere 2 divided by the length of hind tarsomere 2 (TaD/Ta), and the frequency distribution of the range of variation of the ratio TaD/Ta for the species of the Oswaldoi subgroup. It was found that the mean values of these parameters for *nuneztovari*, morphotype 11 and *rangeli* are not significantly different while *oswaldoi* showed the smallest ratio and *triannulatus* the largest. This character can be used as diagnostic for these species. In *An. nuneztovari* the range of variation of this character is large, overlapping the ranges of variation of this character in the other species.

Table 2.4 and Figure 2.11 show the mean values of the length of subcostal pale (SCP) spot divided by the length of sector dark (SD), and the frequency distribution of the range of variation of the ratio SCP/SD. In *An. nuneztovari* this character is highly variable but it is significantly larger in *rangeli* and significantly smaller in *triannulatus*.

Table 2.3: Mean values of the length of dark band on hind tarsomere 2 (TaD) divided by the length of hind tarsomere 2 (Ta) of adult females collected in the field.

Species	N	Mean	S.D.	95% Confidence Limits L1	L2
nuneztovari	485	0.270a	0.039	0.266	0.273
Morphotype 11	235	0.267a	0.037	0.263	0.272
rangeli	31	0.250a	0.036	0.237	0.264
oswaldoi	43	0.170b	0.034	0.150	0.180
triannulatus	48	0.391c	0.055	0.375	0.407

Note: Means followed by different letters differ at the p=0.01 level of significance.

FIGURE 2.10: FREQUENCY DISTRIBUTIONS
OF THE RATIO TaD/Ta

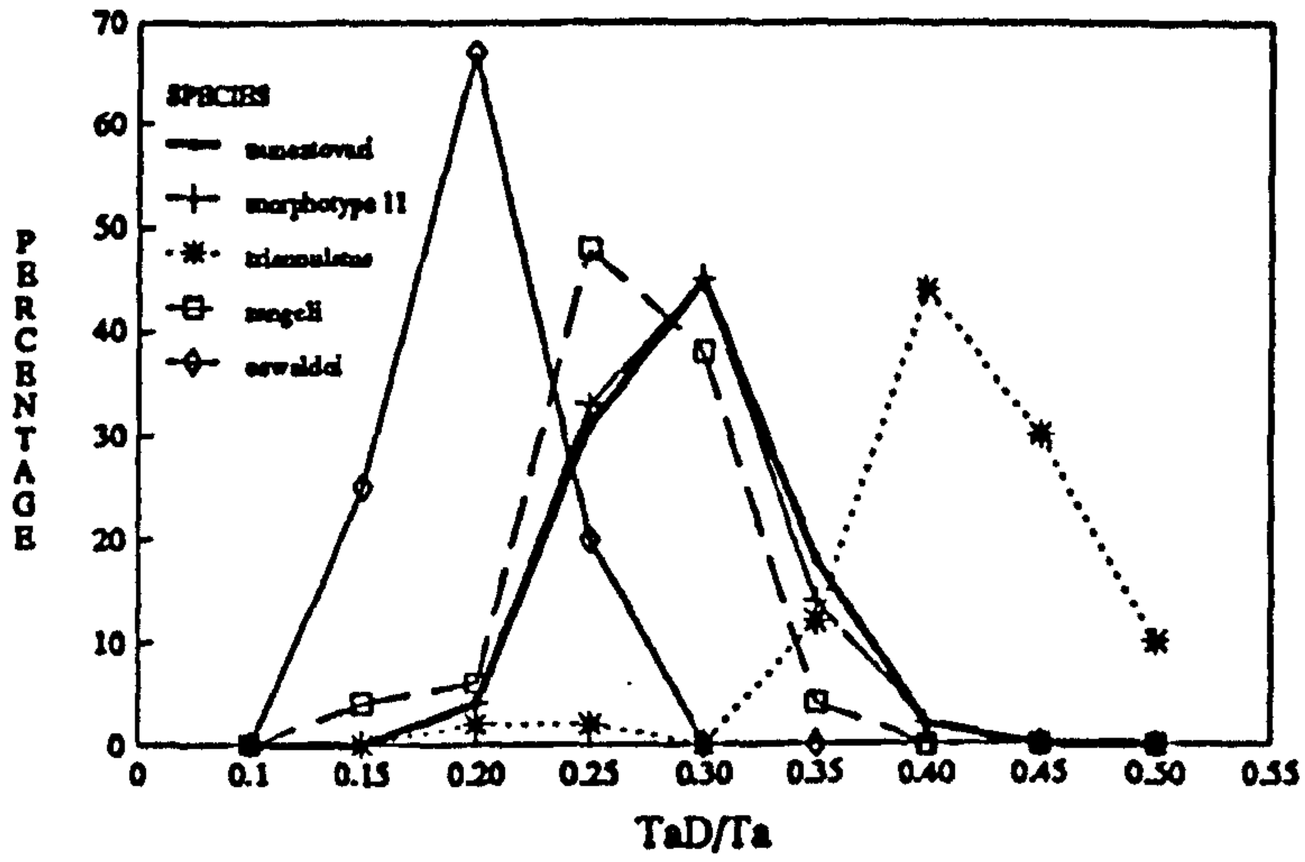


FIGURE 2.11: FREQUENCY DISTRIBUTIONS
OF THE RATIO SCP/SD

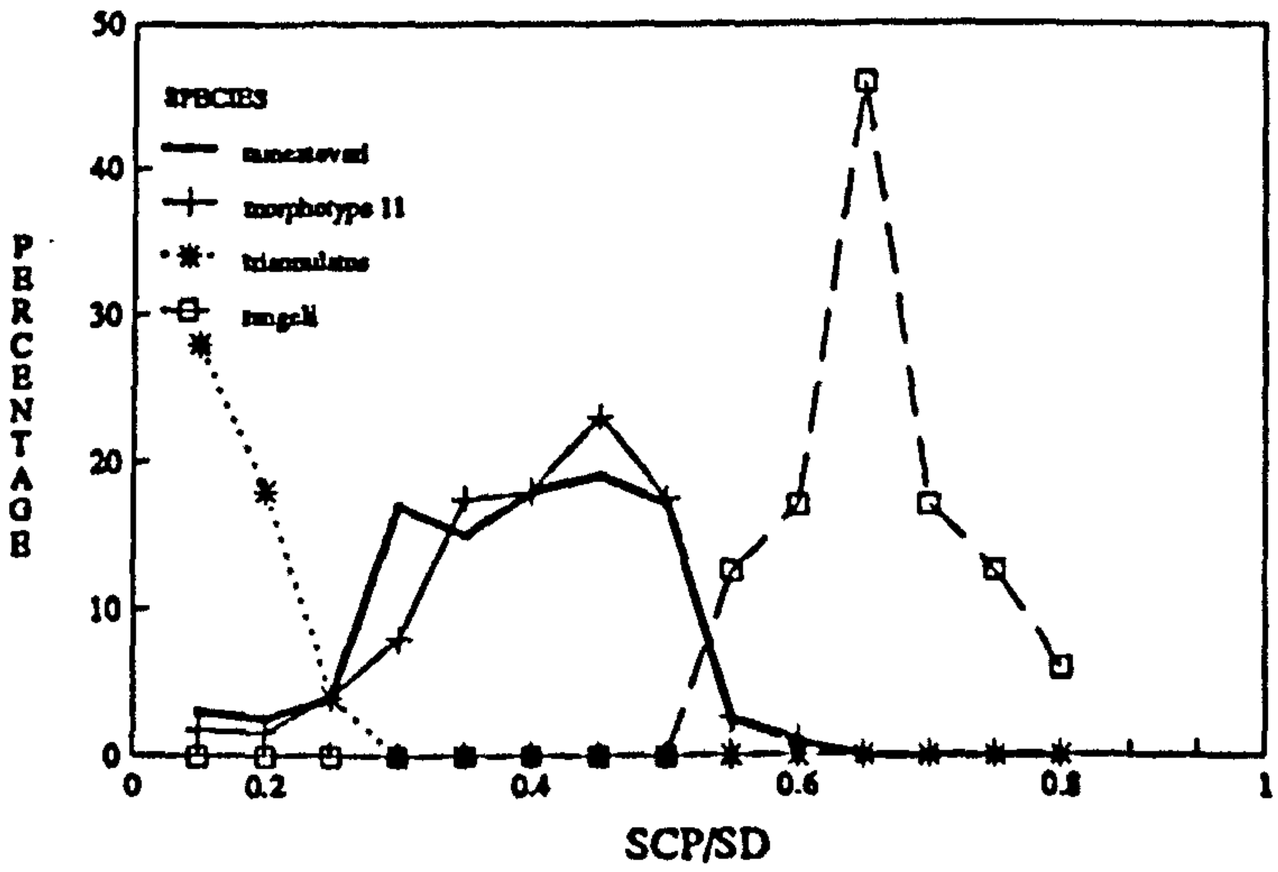


FIGURE 2.12: FREQUENCY DISTRIBUTIONS
OF THE RATIO HP/PHD

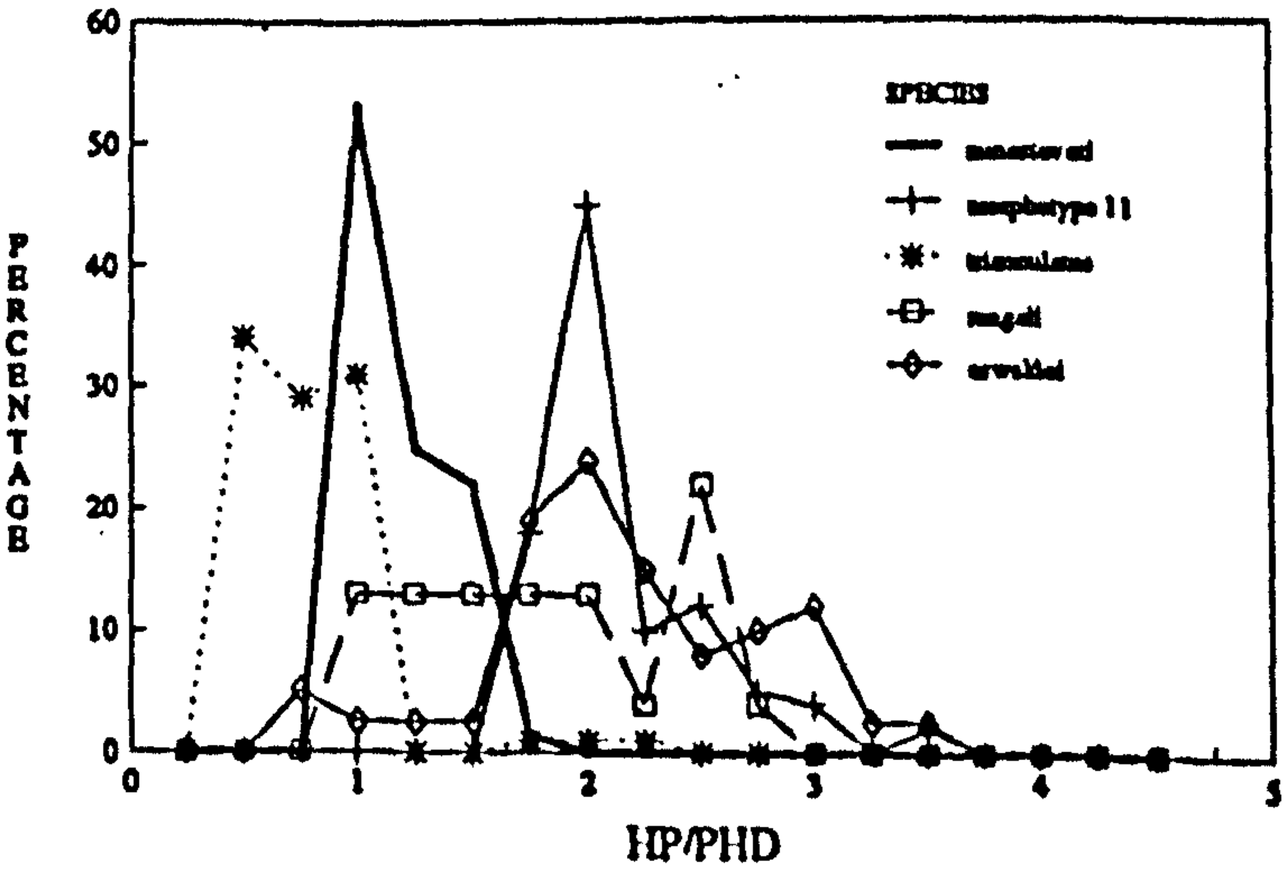


Table 2.4: Mean values of the length of subcostal pale spot (SCP) divided by the length of sector dark (SD) of adult females collected in the field.

Species	N	Mean	S.D.	95% Confidence Limits L1	L2
nuneztovari	485	0.375a	0.159	0.306	0.389
Mophotype 11	235	0.386a	0.095	0.374	0.398
rangeli	31	0.627b	0.107	0.588	0.666
oswaldoi	43	0.255c	0.110	0.220	0.289
triannulatus	48	0.153d	0.034	0.143	0.163

Note: Means followed by different letters differ at the p=0.05 level of significance.

Table 2.5: Mean values of the humeral pale spot (HP) divided by the length of prehumeral dark spot (PHD) of adult females collected in the field.

Species	N	Mean	S.D.	95% Confidence Limits L1	L2
nuneztovari	485	1.093a	0.221	1.070	1.110
Morphotype 11	235	2.149b	0.489	2.087	2.210
rangeli	31	1.910b	0.829	1.606	2.210
oswaldoi	43	2.097b	0.620	1.906	2.288
triannulatus	48	0.750c	0.386	0.638	0.862

Note: Means followed by different letters differ at the p=0.05 level of significance.

Table 2.5 and Figure 2.12 shows the mean values of the length of humeral pale spot (HP) divided by the length of prehumeral dark spot (PHD) and the frequency distribution of the range of variation of the ratio HP/PHD. Comparing the mean value of the ratio for *nuneztovari* and morphotype 11 we found that the ratio was significantly smaller in *nuneztovari* and that there was no overlap of the frequency distributions.

Tables 2.6, 2.7 and 2.8, and Figures 2.13, 2.14 and 2.15 show the comparisons between mothers and progeny of *nuneztovari* and morphotype 11 for the mean ratios of the characters analysed and the frequency distributions of the range of variation of the ratios. The results in Figure 2.15 conclusively show that those specimens considered as morphotype 11 actually belong to the species *nuneztovari*, because we found that typical *nuneztovari* mothers have progenies that include individuals typical of *nuneztovari* as well as "morphotype 11", while typical "morphotype 11" mothers also have progenies that include individuals typical of *nuneztovari* and "morphotype 11". The difference between typical *nuneztovari* and "morphotype 11" shown in Figure 2.12 can be considered as a polymorphism within one species because the distributions do not overlap.

Specimens belonging to the subgenus *Anopheles* were identified using the keys by Cova García and Sutil (1977).

Specimens were examined at 10-60x magnification under an Olympus dissecting microscope with a blue-filtered optical-fibre illuminator. A standard white colour was established as a reference for determining other colours according to the method of Peyton and Ramalingan (1988). This was accomplished by using 60x magnification to position the light source so that a white surface appeared as white as possible. Among the species collected during the study, the whitest structures were hind tarsomeres 2 and 3 in species of the subgenus *Nyssorhynchus*. The colour of the hind tarsomeres was compared to that of the pale spots on the wings (Peyton, pers. comm.; Wilkerson & Strickman, 1990).

A key (see following pages) was developed based on the measurement of the characters specified above in about 1,500 specimens, including wild-caught anophelines

Table 2.6: Comparison between mothers and progeny for the mean ratio TaD/Ta.

		Mean	S.D.	N
nuneztovari	Mothers	0.267	0.037	434
	Progeny	0.263	0.042	91
Morphotype 11	Mothers	0.265	0.032	204
	Progeny	0.278	0.048	52

Table 2.7: Comparison between mothers and progeny for the mean ratio SCP/SD.

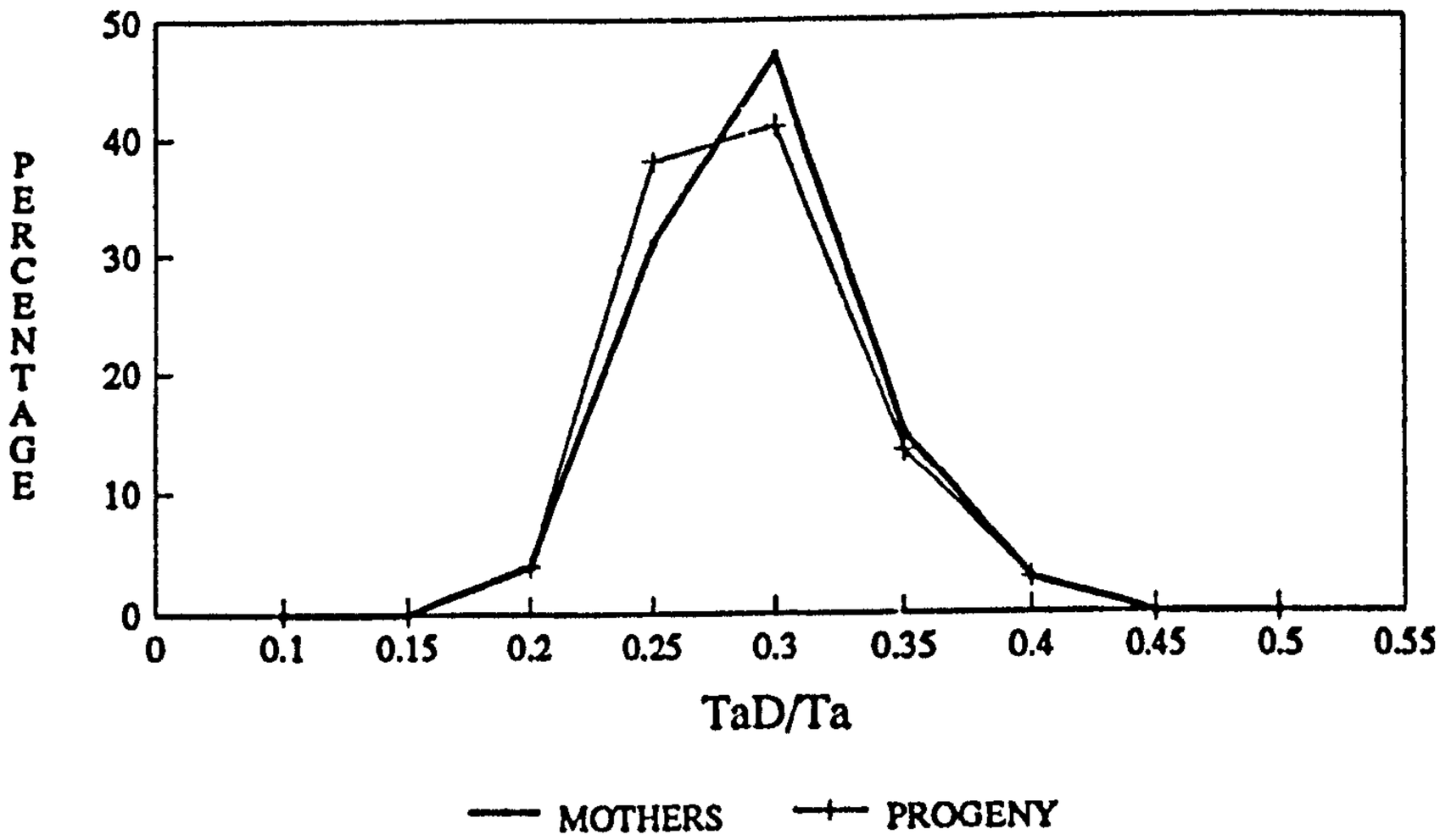
		Mean	S.D.	N
nuneztovari	Mothers	0.355	0.097	434
	Progeny	0.385	0.101	99
Morphotype 11	Mothers	0.377	0.093	204
	Progeny	0.418	0.122	63

Table 2.8: Comparison between mothers and progeny for the mean ratio HP/PHD.

		Mean		S.D.	N
nuneztovari	Mothers	1.050	**	0.242	434
	Progeny	1.526		0.573	99
Morphotype 11	Mothers	2.153	**	0.486	204
	Progeny	1.816		0.662	63

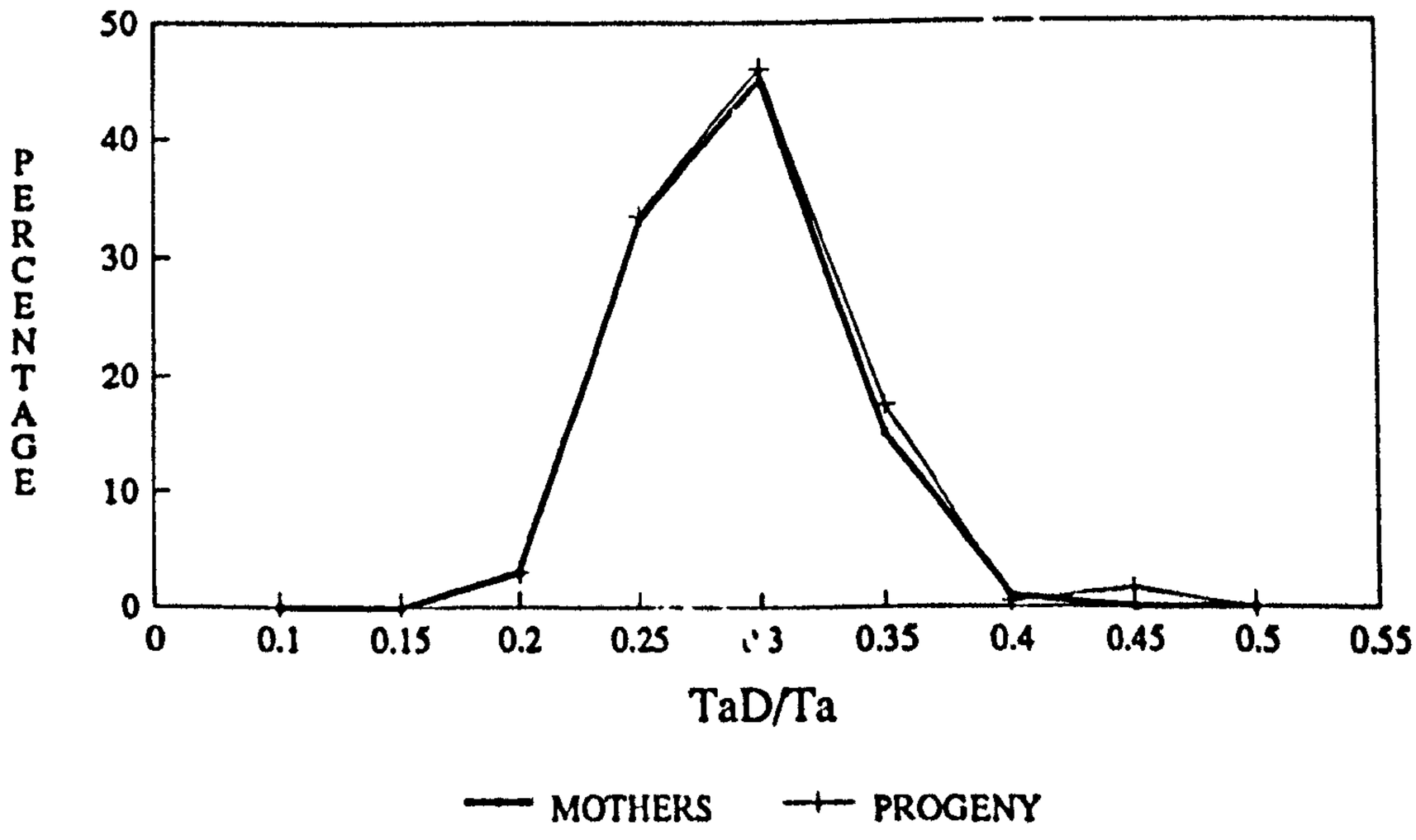
(**) P<0.05

FIGURE 2.13.a: FREQUENCY DISTRIBUTIONS
OF THE RATIO TaD/Ta



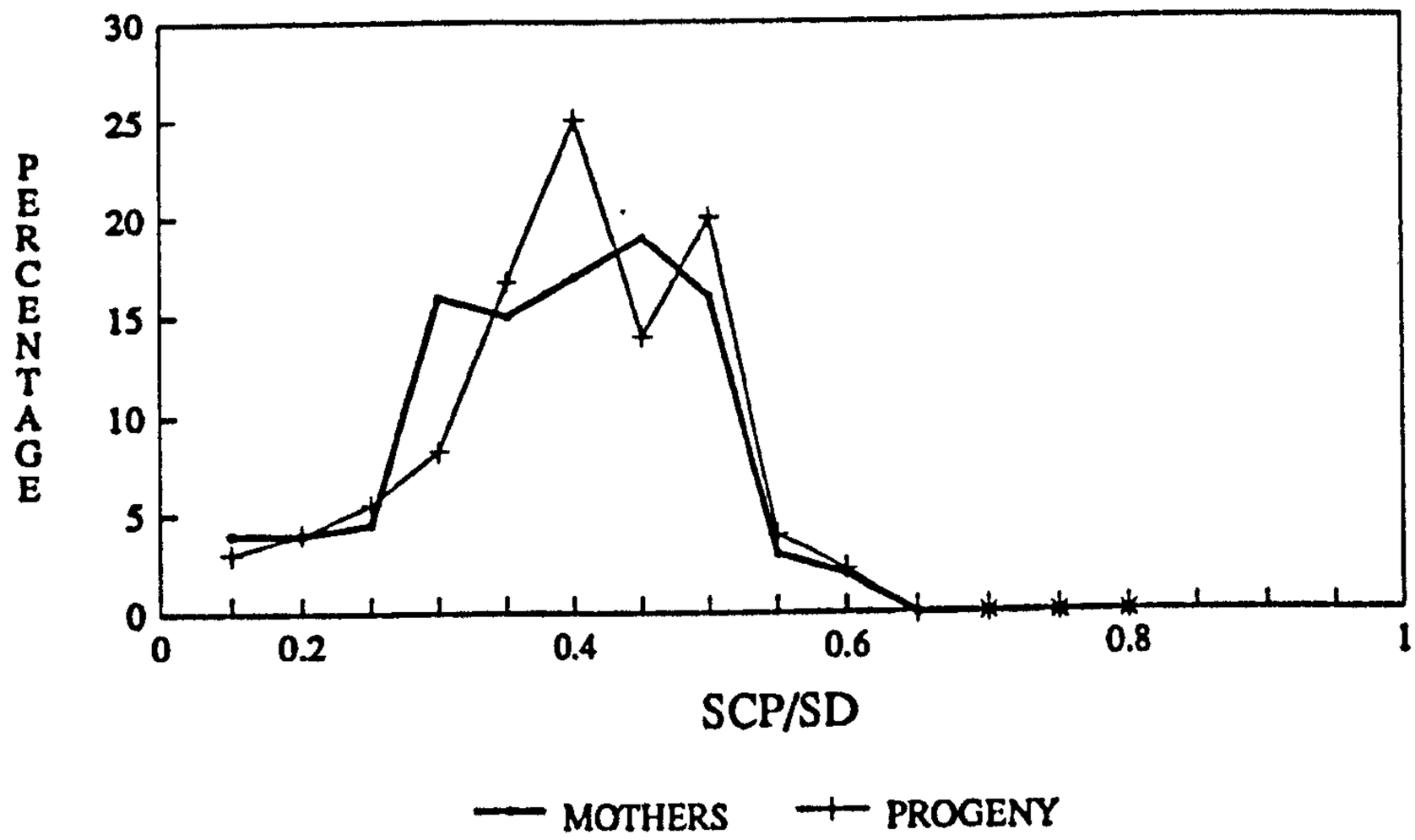
TYPICAL NUNEZTOVARI MOTHERS

FIGURE 2.13.b: FREQUENCY DISTRIBUTIONS
OF THE RATIO TaD/Ta



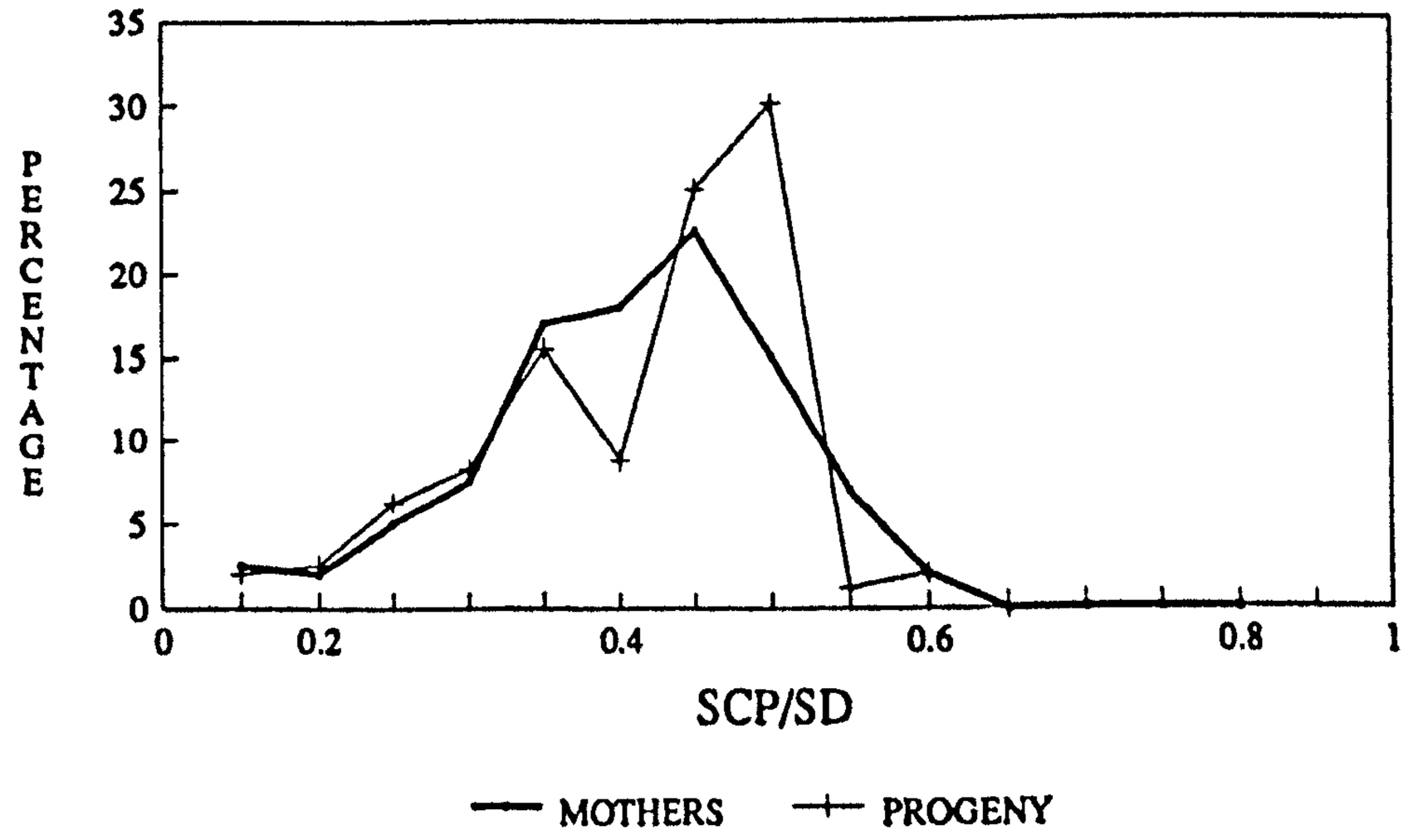
MORPHOTYPE 11 MOTHERS

FIGURE 2.14.a: FREQUENCY DISTRIBUTIONS OF THE RATIO SCP/SD



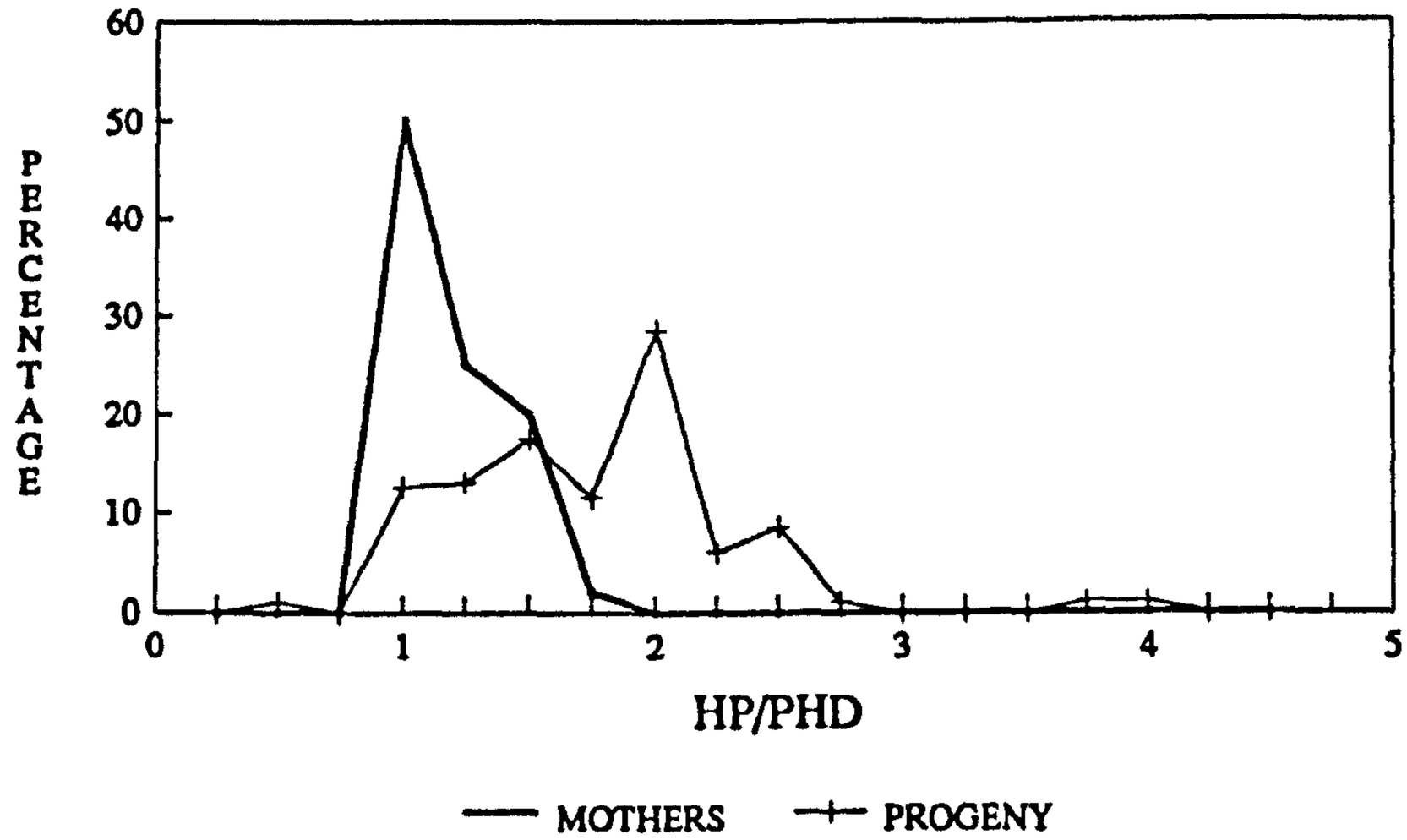
TYPICAL NUNEZTOVARI MOTHERS

FIGURE 2.14.b: FREQUENCY DISTRIBUTIONS OF THE RATIO SCP/SD



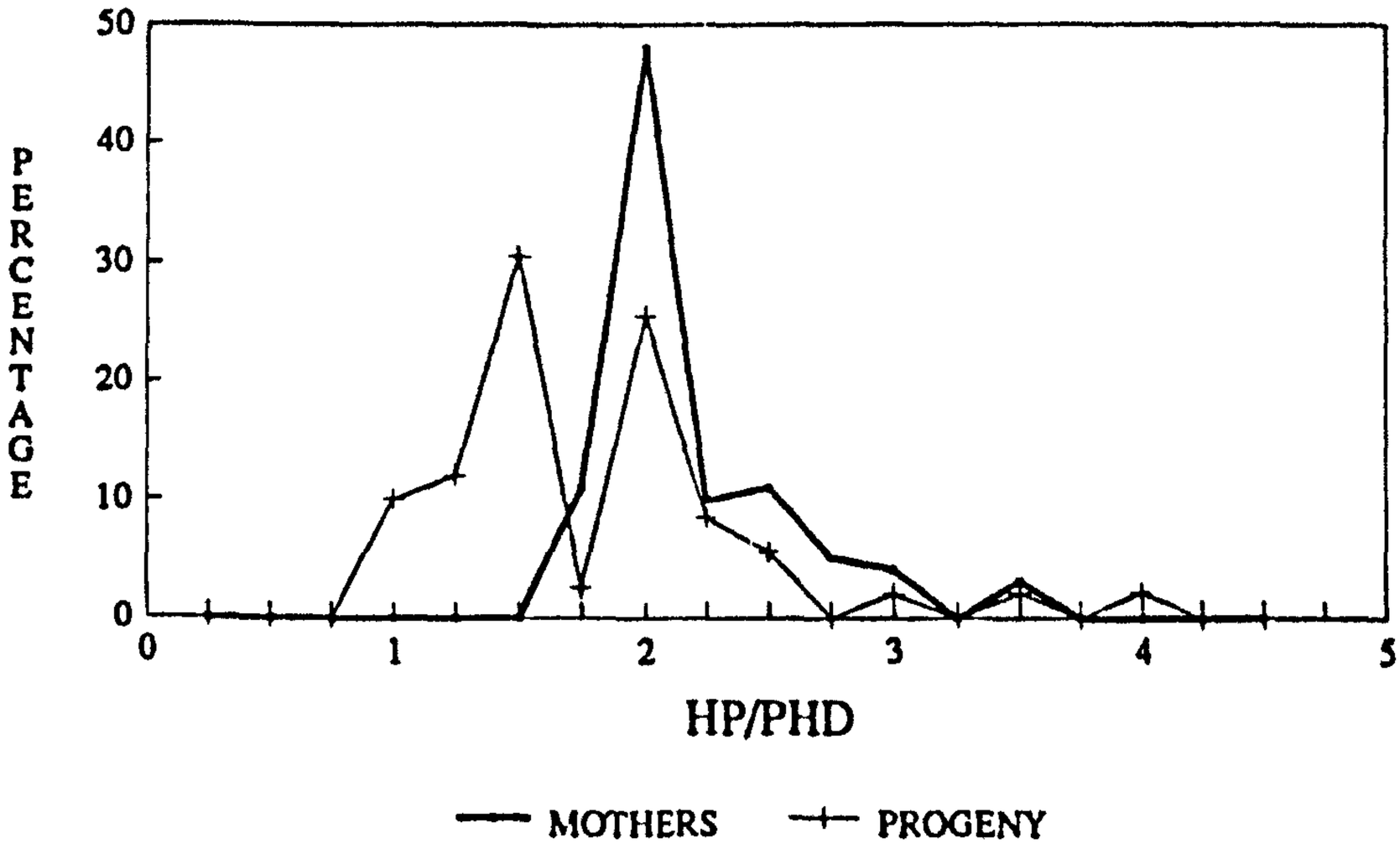
MORPHOTYPE 11 MOTHERS

FIGURE 2.15.a: FREQUENCY DIS'TRIBUTIONS
OF THE RATIO HP/PHD



TYPICAL NUNEZTOVARI MOTHERS

FIGURE 2.15.b: FREQUENCY DISTRIBUTIONS
OF THE RATIO HP/PHD



MORPHOTYPE 11 MOTHERS

and associated reared material. Approximatel y 60% of the specimens belonged to the species *nuneztovari*; the rest were *triannulatus*, *oswaldoi*, *rangeli*, *benarrochi* and *strodei*. Another species commonly collected in the study area was *An. albitarsis s.l.*

Species identification was confirmed by Mr. E. L. Peyton, Smithsonian Institution, Washington, D.C.

ILLUSTRATED KEY TO THE FEMALES OF THE SUBGENUS *NYSSORHYNCHUS* OF *ANOPHELES* COLLECTED IN THE STUDY SITE.

1. Hind tarsomeres 3 and 4 with pale and dark bands or mostly dark (Fig. 2.16) (**Subgenus *Anopheles, Lephodomyia, Kerteszia* and *Stethomyia***)

   Hind tarsomeres 3 and 4 entirely pale (Fig. 2.17)
   Subgenus *Nyssorhynchus* 2

2. Hind tarsomere 5 entirely white (Fig. 2.18)
   Argyritarsis section 3

   Hind tarsomere 5 with a basal dark band (Fig. 2.19)
   Albimanus section 4

3. Dark caudo-lateral scale tufts present on tergum II (Fig. 2.20.a). Hind tarsomere 2 with basal dark band 0.3-0.4 of length of tarsomere 2 ..... ***braziliensis***

   Dark caudo-lateral scale tufts absent on tergum II (Fig. 2.20.b). Hind-tarsomere 2 with basal dark band 0.5-0.7 of length of tarsomere 2 ..... ***albitarsis***

4. Anterior mesanepimeron with a conspicuous patch of light scales (Fig. 2.21.a). Fore tarsomere 4 with a light band in apical 0.4-0.65 of length of tarsomere (Fig. 2.22.a); hind tarsomere 2 with a basal dark band 0.35-0.4 of length of tarsomere (Fig. 2.23). Humeral pale spot on costa 0.7-0.9 of length of prehumeral dark spot (Fig. 2.24) ..... ***triannulatus***

   Anterior mesanepimeron without a patch of light scales (Fig. 2.21.b). Fore tarsomere 4 predominantly dark (Fig. 2.22.b). Basal dark spot on hind tarsomere 2 less than 0.35 of length of tarsomere (Fig. 2.25). Humeral pale spot on costa, 0.7-2.5 times the size of prehumeral dark... 5

5. Basal dark spot on hind tarsomere 2 more than 0.4 of the length of tarsomere ..... ***benarrochi***

   Basal dark spot on hind tarsomere 2 less than 0.4 of the length of the tarsomere 6

6. Basal dark spot on hind tarsomere 2 0.15-0.18 of length of tarsomere (Fig. 2.26). Humeral pale spot 1.9-2.3 times the length of prehumeral dark spot. Subcostal pale spot 0.22-0.29 of the length of the sector dark (Fig. 2.27) ..... ***oswaldoi***

   Basal dark spot on hind tarsomere 2 0.24-0.35 of length of tarsomere (Fig. 2.28), or if less, humeral pale spot less than 0.9 of the length of the prehumeral dark. Subcostal pale spot more than 0.35 of the length of the sector dark 7

7. Subcostal pale spot more than 0.6 of the length of the sector dark. Humeral pale spot more than 1.8 times the length of the prehumeral dark (Fig. 2.29) ..... ***rangeli***

Subcostal pale spot less than 0.5 of the length of the sector dark (Fig. 2.30). Humeral pale spot, 0.7-3.0 times of the length of the prehumeral dark. If length of subcostal pale spot is more than 0.5 of the length of sector dark and humeral pale is less than 1.8 times the length of the prehumeral dark 8

8. Humeral pale spot 0.7-2.5 times the length of the prehumeral dark (Fig. 2.31 a, b, c). Humeral crossvein may or may not touch the apex of the prehumeral dark. Pale spots on wing variable from cream to bright yellow. ..... ***nuneztovari***

Humeral pale spot more than 2.5 times the length of the prehumeral dark. Humeral crossvein does not touch the apex of the prehumeral. Pale spots on wing white ..... ***strodei***

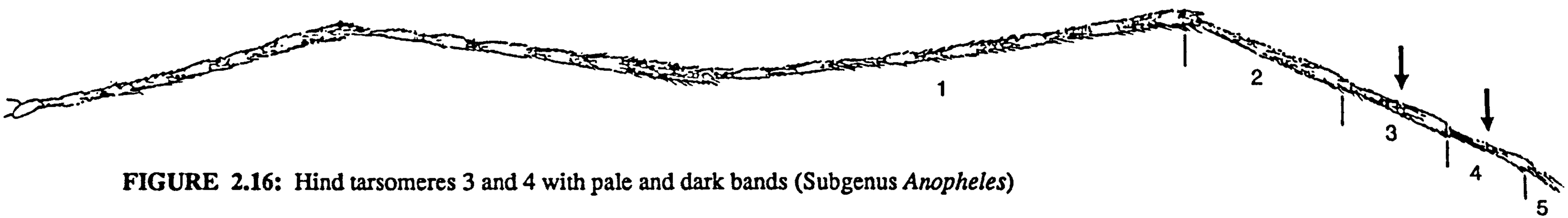


FIGURE 2.16: Hind tarsomeres 3 and 4 with pale and dark bands (Subgenus *Anopheles*)

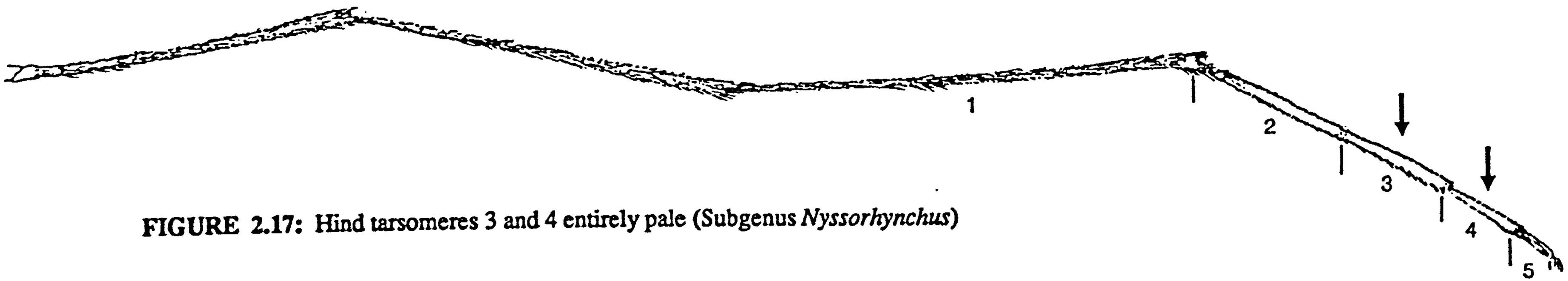


FIGURE 2.17: Hind tarsomeres 3 and 4 entirely pale (Subgenus *Nyssorhynchus*)

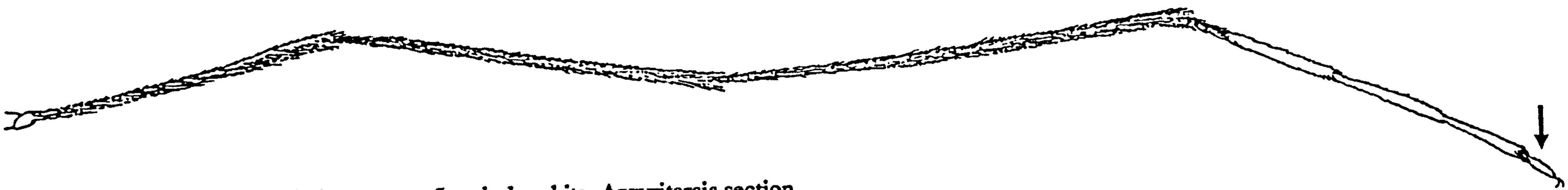


FIGURE 2.18: Hind tarsomere 5 entirely white. Argyritarsis section

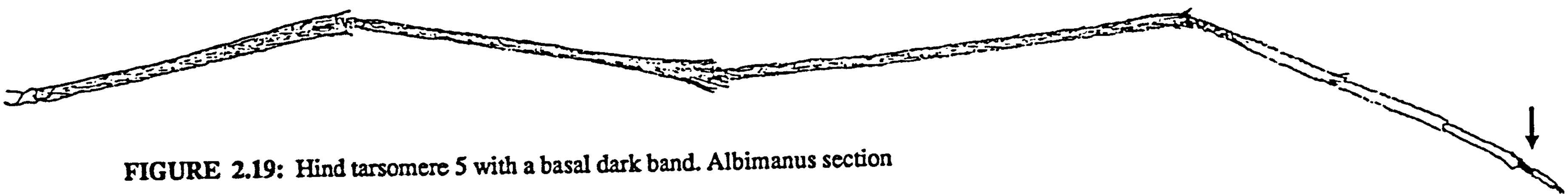


FIGURE 2.19: Hind tarsomere 5 with a basal dark band. Albimanus section

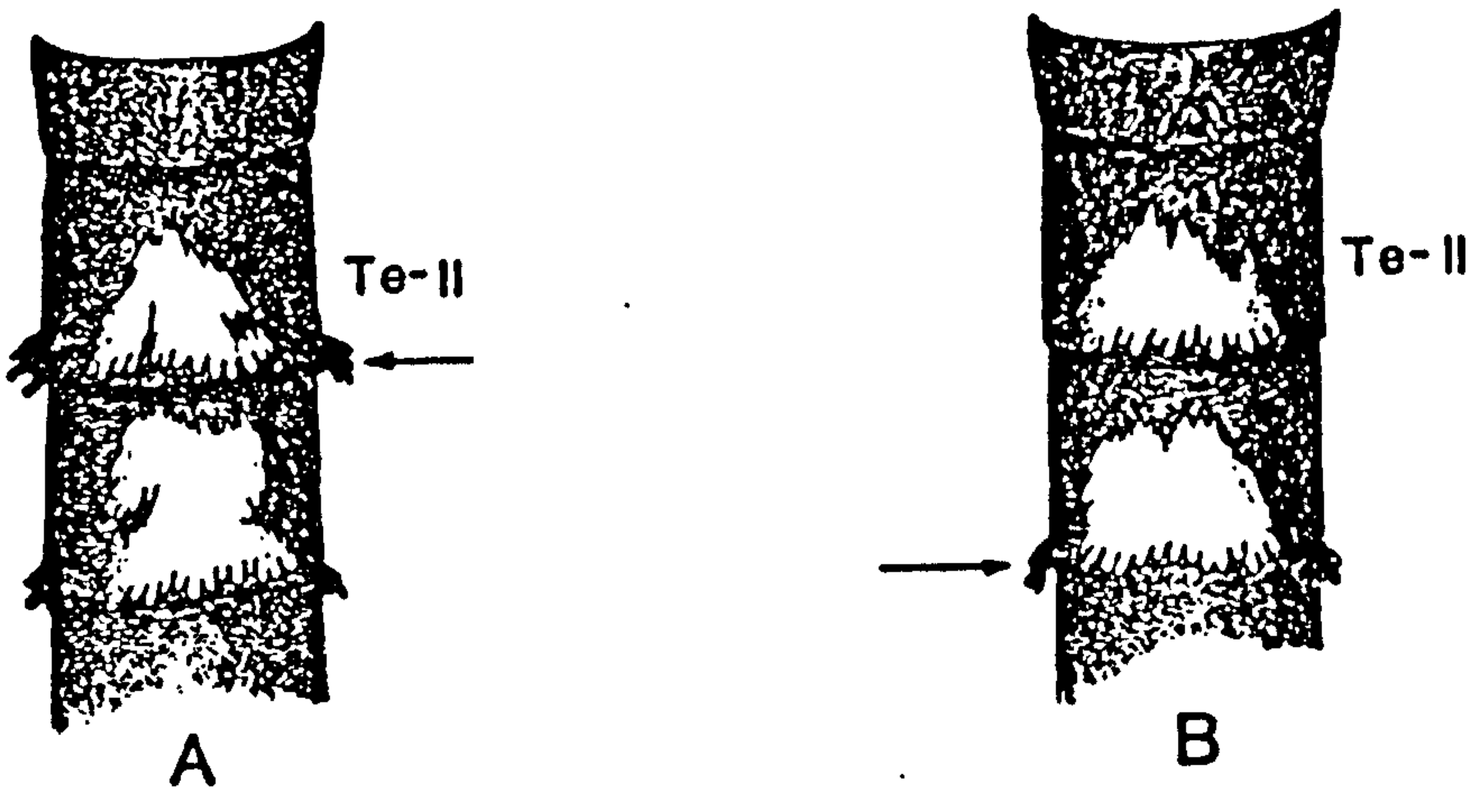


FIGURE 2.20: Abdomen, dorsal view

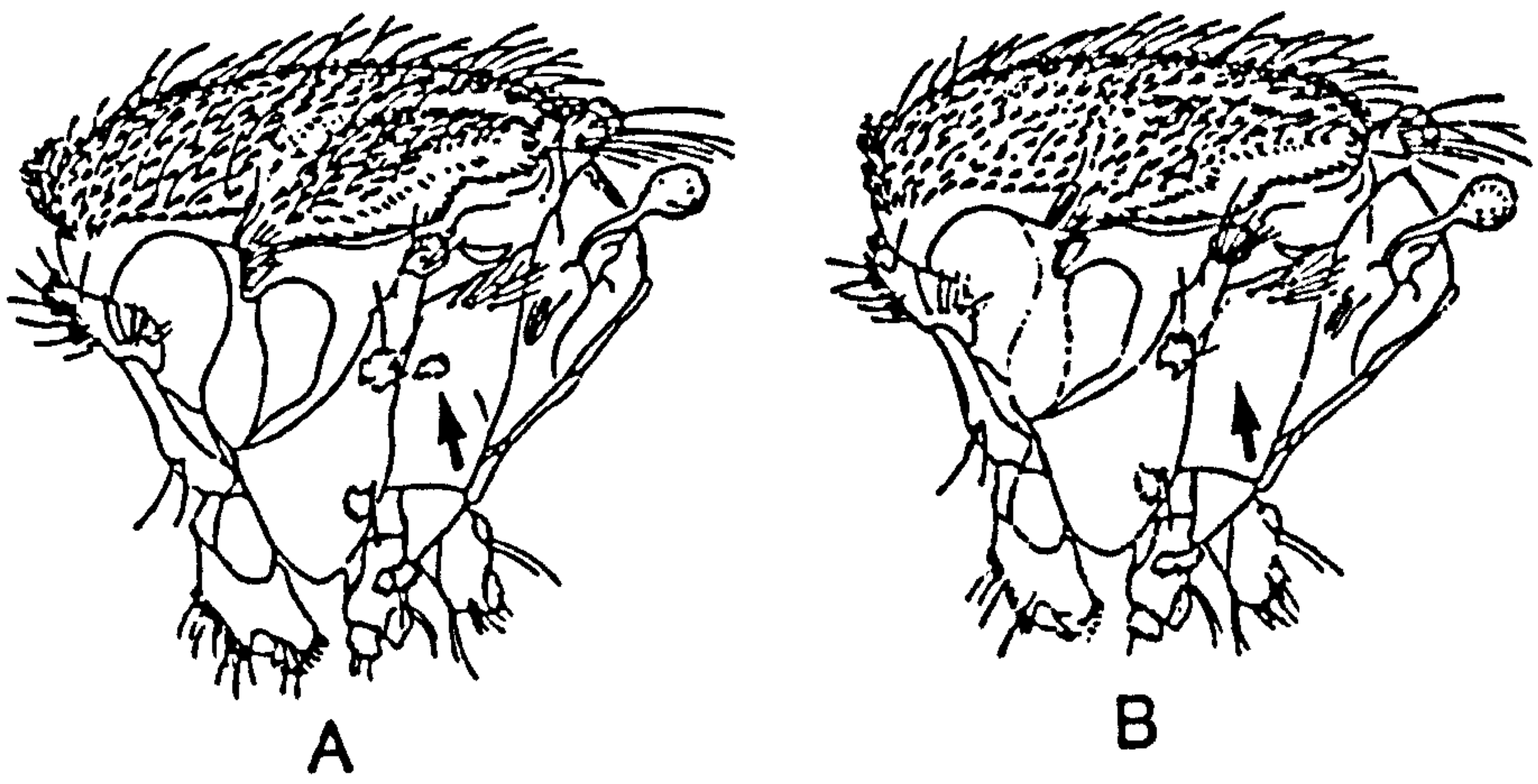


FIGURE 2.21: Thorax, lateral view
Anterior mesanepimeron with a conspicuous patch of scales (A) and without patch of scales (B)

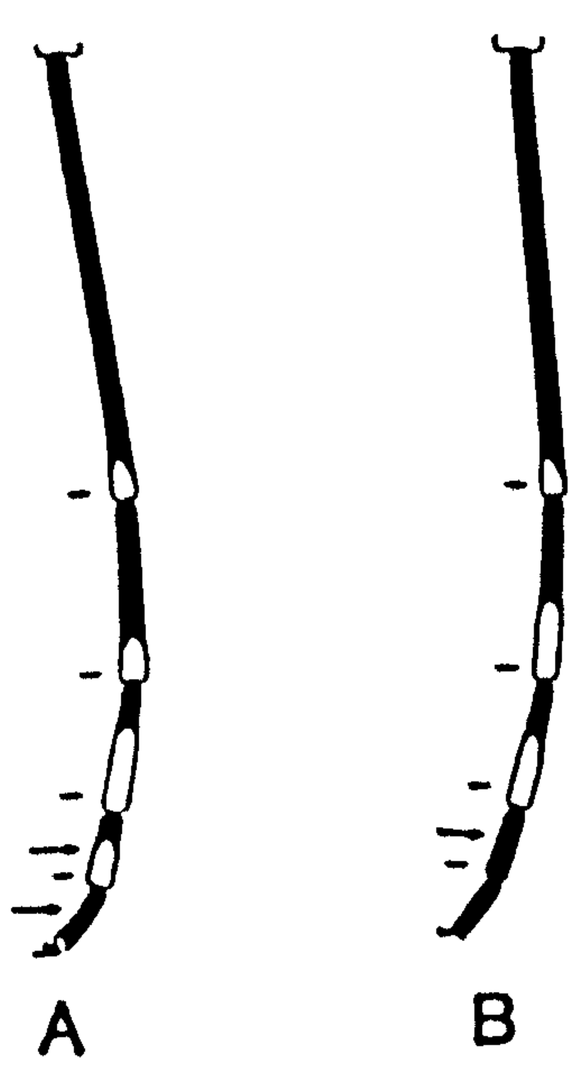


FIGURE 2.22: Fore leg
A: Fore tarsomere 4 with a light band in apical 0.40-0.65 of length of tarsomere
B: Fore tarsomere 4 dark

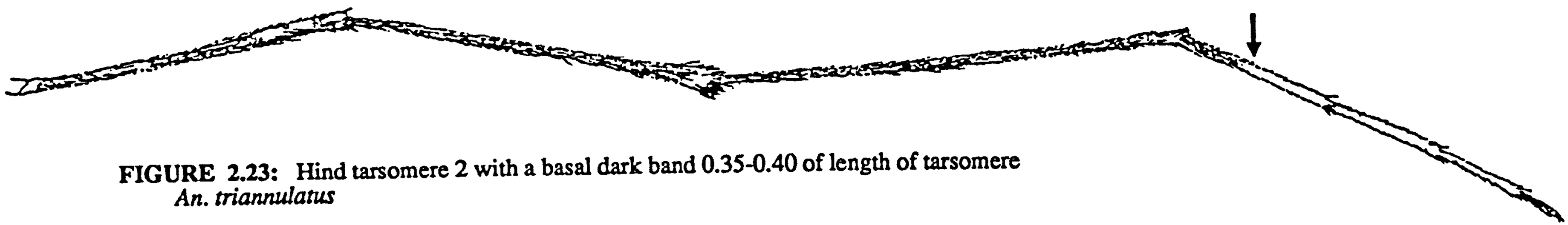


FIGURE 2.23: Hind tarsomere 2 with a basal dark band 0.35-0.40 of length of tarsomere
An. triannulatus

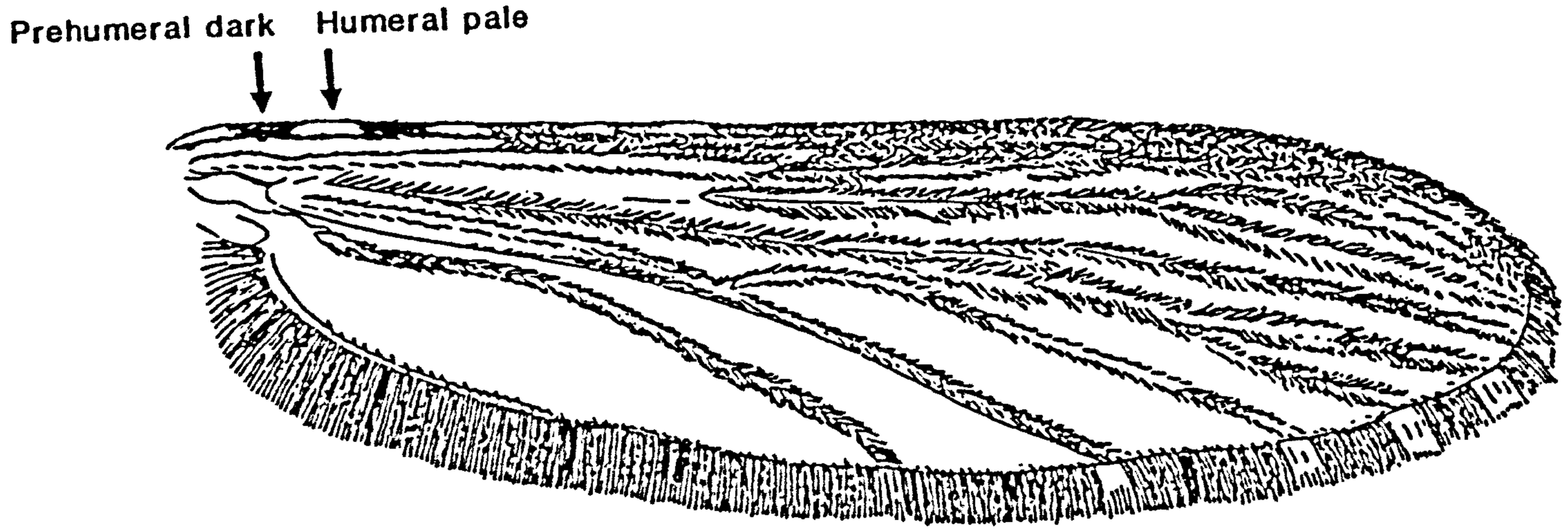


FIGURE 2.24: *An. triannulatus* wing
Humeral pale spot 0.70-0.90 of length of prehumeral

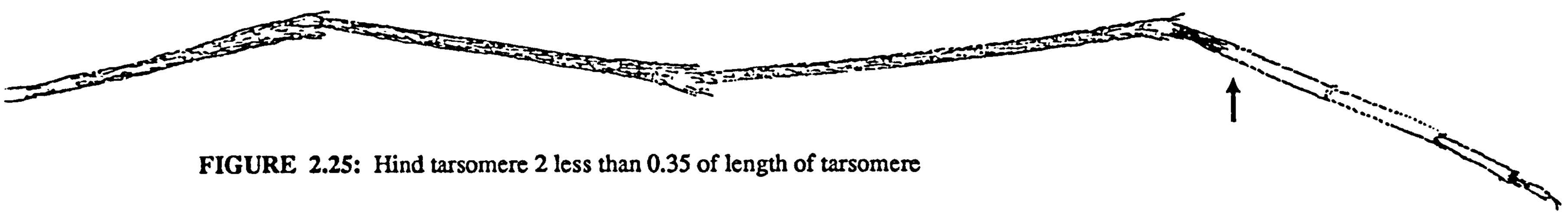


FIGURE 2.25: Hind tarsomere 2 less than 0.35 of length of tarsomere

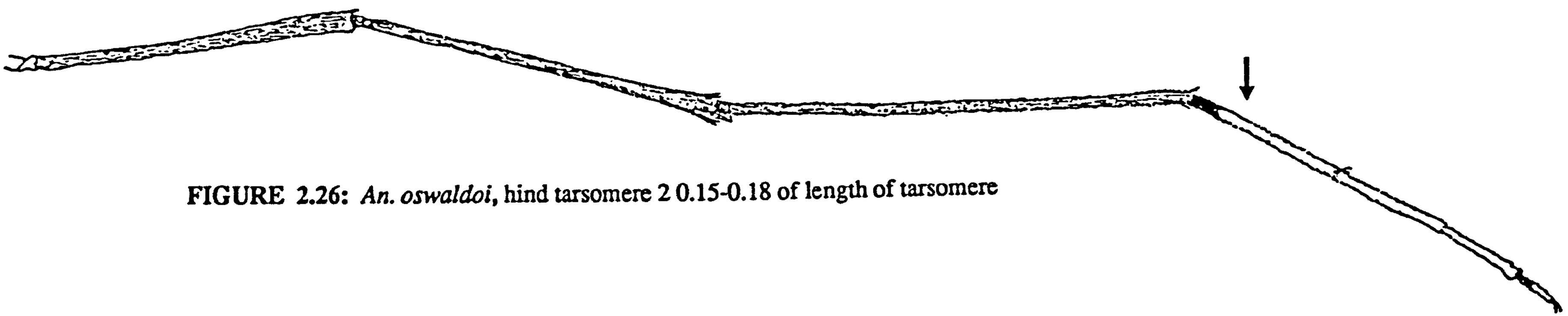


FIGURE 2.26: *An. oswaldoi*, hind tarsomere 2 0.15-0.18 of length of tarsomere

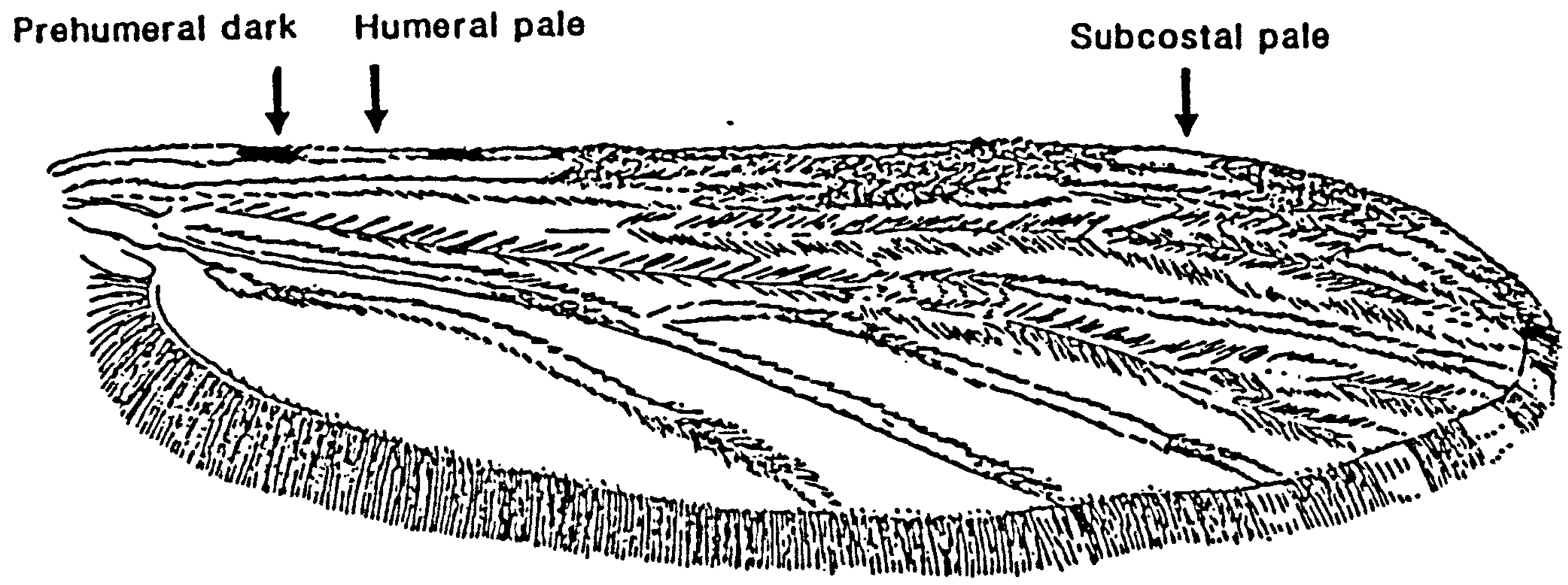


FIGURE 2.27: *An. oswaldoi* wing

FIGURE 2.28: Hind tarsomere 2 variable (hind tarsomere 0.24-0.35 of length of tarsomere)

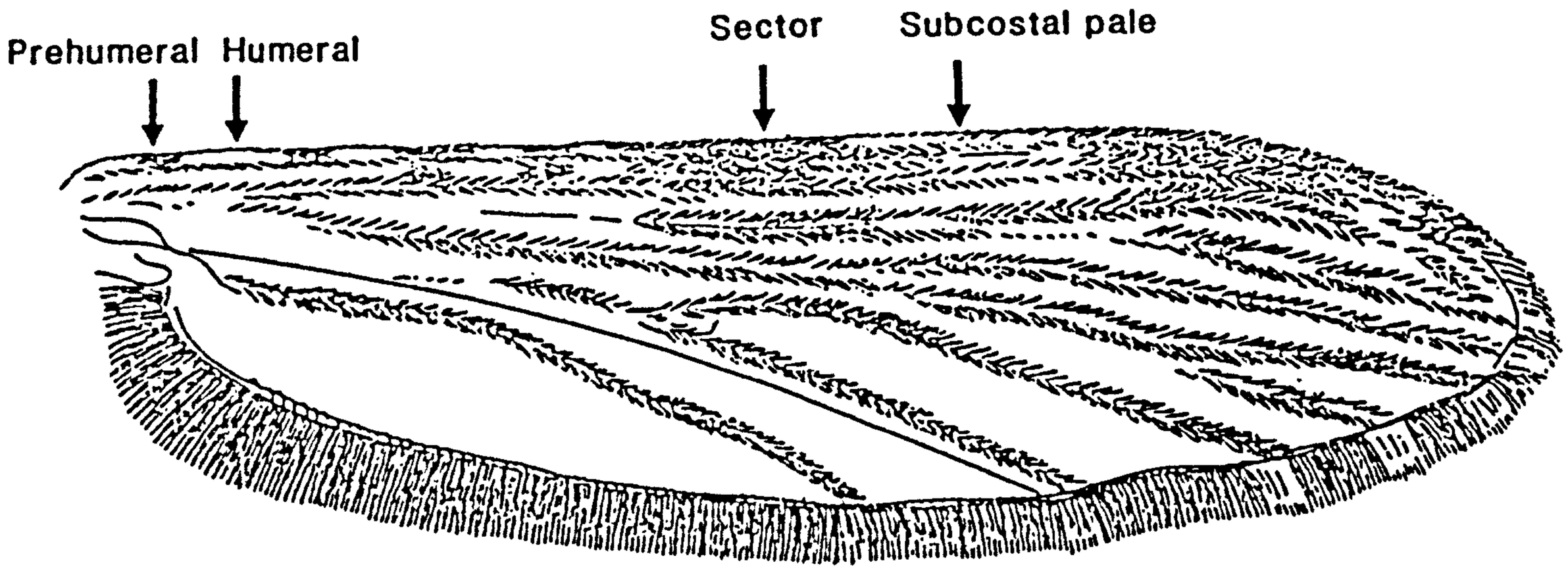


FIGURE 2.29: *An. rangeli*, subcostal pale more than 0.60 of the length of sector dark

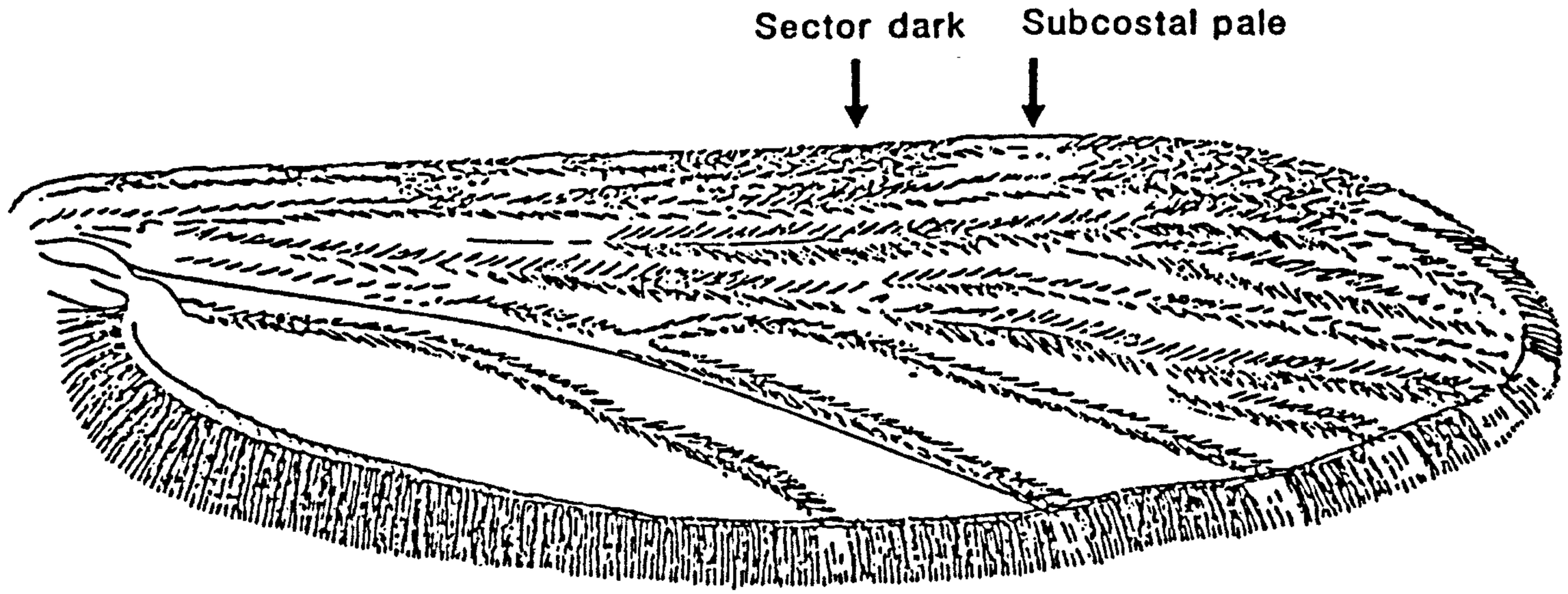
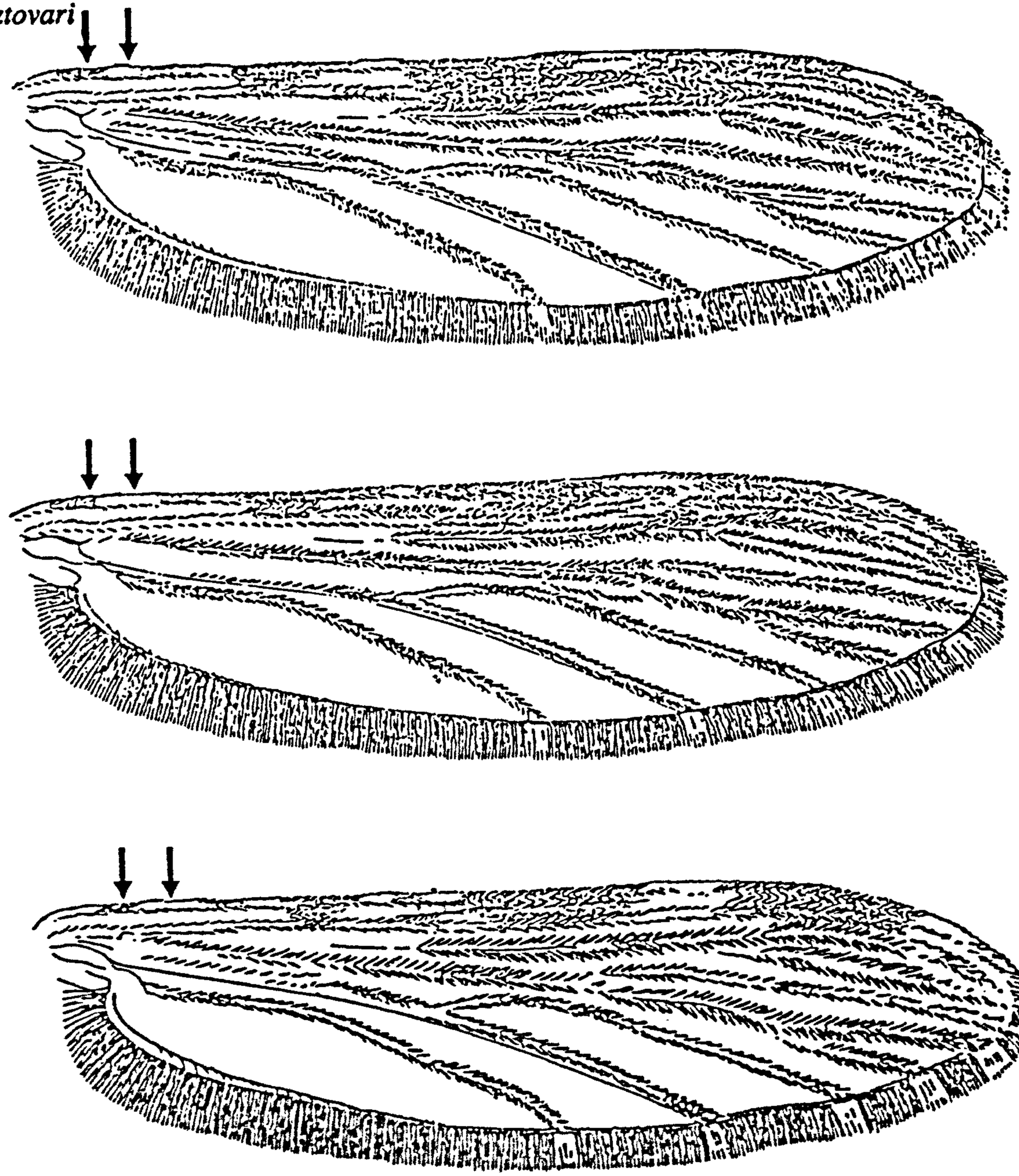


FIGURE 2.30: Subcostal less than 0.50 of the length of the sector dark

FIGURE 2.31 a, b & c: *An. nuneztovari* showing wide range of variation of the humeral pale spot.



CHAPTER 3:

HUMAN-BAIT CATCHES

3.1. INTRODUCTION

Collection of mosquitoes using humans as baits is considered to be the most representative collection method for monitoring the man-biting mosquito population that is the most relevant for the transmission and control of malaria (WHO, 1975; Service, 1976; Molineaux *et al.*, 1988). In addition, Molineaux *et al.* (1988) pointed out that human-bait catches give the "best estimate of the biting cycle".

This method has limitations and some factors have to be considered in order to produce the least biased estimate of the man-biting rate (i.e. the number of bites per person per day). Biases probably arise due to the baits, their locations and time of collections. In normal circumstances and on average, adults are bitten more frequently than children, at least in the few anopheline species in which this has been studied (Carnevale *et al.*, 1976; Bryan & Smalley, 1978). The man-biting rate estimated on adults is thus an overestimate both of the biting rate on children and of the average man-biting rate (Molineaux *et al.*, 1988). Also individual human catchers vary in their attractiveness to mosquitoes and their ability to catch mosquitoes (Shidrawi *et al.*, 1974).

The latter problem was greatly reduced in the human bait catches made during the present study by rotation of the individual team members. Catches were made to determine biting activity, parous rate, seasonal fluctuation and infective rate in the mosquito population.

3.2. MATERIALS AND METHODS

Human-bait catches were carried out for 12 hours between sunset and sunrise (1900-0700 hrs) indoors and out of doors two nights per week per village over a period of 21 months (Jabillos), 17 months (Caño Lindo) and 15 months (Guaquitas). The different sampling periods at the three villages merely reflect difficulties encountered in building

experimental huts and in obtaining funds. Collections were made by a team of 6 catchers and 2 supervisors. Catchers worked in pairs and in shifts of 4 hours, being rotated each day (indoors/out and between shifts). The catchers were seated on stools with their legs below the knees exposed. At any time during a collection there was one catcher out of doors, no less than 3 m from the hut, and one indoors, while the pair for the following shift were inside the hut resting in hammocks and protected by nets.

Before being recruited for the project, catchers read a statement about the aims and possible risks of the catching programme and signed an informed consent. Chloroquine prophylaxis was given every week at a dose of 300 mg.

Mosquitoes were collected with mouth aspirators and placed in paper cups, a new cup being started every hour. The maximum number of mosquitoes placed in a cup was 20. This limit was observed for two reasons: to avoid damage to the mosquitoes which might make identification difficult; and to facilitate estimation of the number of mosquitoes collected in order to decide the proportion to be identified and dissected according to the quota system described below. Cups were kept covered with wet paper towels inside polystyrene boxes. Boxes were sealed with masking tape to prevent ants eating the mosquitoes.

Once in the laboratory, mosquitoes were killed either by freezing if the electricity supply was functional or, if it was not, by exposing them to ethyl acetate or chloroform. Mosquitoes were identified as previously described, counted and a quota of 20 was dissected to determine parity. This procedure was carried out routinely between August 1988 and September 1989. All mosquitoes were stored over silica gel until tested by ELISA for *P. vivax* circumsporozoite protein and host blood-meal identification. The ELISA methods used are described in Chapters 8 and 9.

Parity was determined by the Polovodova technique (Detinova, 1962), i.e. presence or absence of dilatations on the ovariole stalks. This technique was selected, instead of the more common one of examination of ovarian tracheoles because the latter does not permit females in Sella's stages beyond 2 to be diagnosed, i.e. those in which

the ovarian tracheoles are obscured by yolk (Detinova, 1962). However, I found that by the time mosquitoes were taken to the field laboratory in the morning the ovaries were mainly at Sella's stage 3 to 6 which resulted in the stretching of tracheole skeins which can suggest a parous female.

Previous experience has shown that for the Polovodova technique, it is best if mosquitoes are killed by freezing, since the ovaries are then more flexible, making it easier to stretch the ovarioles without breaking them. However, satisfactory diagnosis could also be achieved when mosquitoes were killed with ethyl acetate or chloroform. An illumination system similar to the one described by Gillies and Wilkes (1965) was obtained by using an Olympus dissecting microscope and a ring fluorescent lamp to illuminate the sample uniformly from above. The quota of mosquitoes to be dissected daily was fixed at 20, this being the greatest number of mosquitoes we could dissect carefully and score accurately in a day.

In August 1988, during the wet season, and due to the large numbers of mosquitoes collected (e.g. 3,435 anophelines in one night) it was decided to fix a quota of 500 mosquitoes to be identified per day. The total collection (x) of a given species in a given hour was estimated as:

$$x = y.T/Q$$

where y = no. mosquitoes identified of that species in that hour;

T = the total number of mosquitoes collected;

Q = the quota of mosquitoes which were identified (generally 500).

Climatological data were obtained monthly from the Venezuelan Air Force weather station located approximately 16 km from the study site. It would have been desirable to record variables such a temperature, humidity, wind speed and direction at each capture station but the necessary equipment was not received until towards the end of the field study.

The statistical program SPSS was used for the analysis of data.

3.3. RESULTS

3.3.1. NUMBERS AND SPECIES COLLECTED

A total of 57,956 mosquitoes, representing 12 anopheline species, was collected in all-night catches indoors and out of doors on human baits in the three villages (Table 3.1). The four commonest species were *An. nuneztovari*, *An. triannulatus*, *An. albitarsis s.l.* and *An. oswaldoi*. The most abundant species was *An. nuneztovari*, comprising over 70% of the total anophelines collected in the three sites, and reaching 88% in Caño Lindo. *An. triannulatus*, the second most abundant species in Jabillos and Guaquitas, was rarely collected in Caño Lindo. A small percentage of anophelines (less than 5%) could not be identified due to the loss of wings, legs, scales etc.

3.3.2. SEASONAL FLUCTUATION

Figures 3.1, 3.2 & 3.3 show rainfall and the mean number of bites per month for the four commonest species in the three study sites. The rainy season is between May and December and the dry season is between January and April.

Among the 4 commonest species there was up to a 1,000-fold range between the numbers collected in the dry season and the wet season. For instance, in Guaquitas 2 *An. nuneztovari* were collected in April 1989, whereas in August 3,489 specimens were caught in one night.

In order to normalize the skewed distribution of the numbers of mosquitoes collected, data were transformed to the log (x + 1). An example of the frequency distribution of the untransformed numbers of bites per man per night and the frequency distribution of the transformed data for *An. nuneztovari* is shown in Figure 3.4.a and b. The untransformed data are grossly skewed to the right; the transformed data are more nearly normal but with some skew to the left.

Regression of the log-transformed mean number of bites on rainfall (Tables 3.2 & 3.3) showed a stronger relationship between catches of *An. nuneztovari*, *An. triannulatus* and *An. oswaldoi* in Jabillos and Guaquitas and rainfall during the previous month than with the rainfall in the month in which the catches were made. In Caño Lindo

Table 3.1: Anophelines collected on human baits in western Venezuela.

Species	Jabillos (Feb.88-Oct.89)	Caño Lindo (Jul.88-Oct.89)	Guaquitas (Aug.88-Oct.89)
Total anophelines	16,982	15,451	25,983
	%	%	%
Anopheles (Nyssorhynchus)			
nuneztovari	70.7	88.0	74.9
albitarsis	4.8	2.1	7.7
triannulatus	11.5	0.3	8.0
oswaldoi	3.6	1.5	2.2
strodei	1.6	3.4	1.3
rangeli	1.4	1.7	1.2
benarrochi	0.02	0.1	0.05
Anopheles (Anopheles)			
mediopunctatus	0.0	0.02	0.02
neomaculipalpus	0.5	0.05	0.1
punctimacula	0.01	0.02	0.004
apicimacula	0.0	0.01	0.0
pseudopunctipennis	0.0	0.06	0.04
Unidentifiable	3.2	2.8	5.0

FIGURE 3.1: Human biting catch at Caño Lindo and rainfall

FIGURE 3.1.a: An. nuneztovari

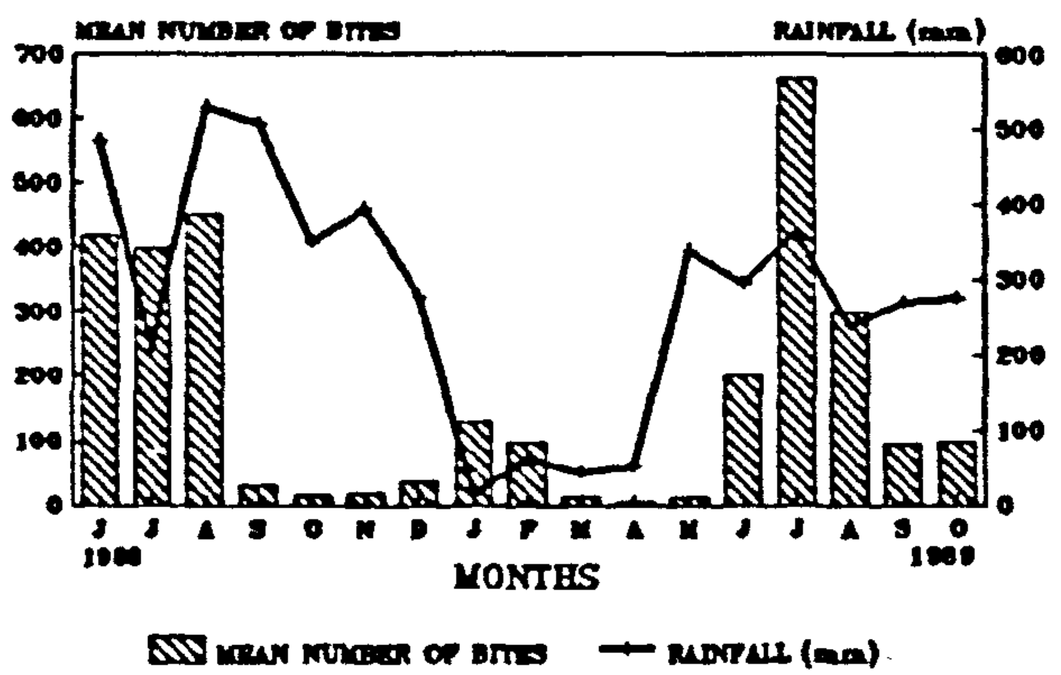


FIGURE 3.1.b: An. triannulatus

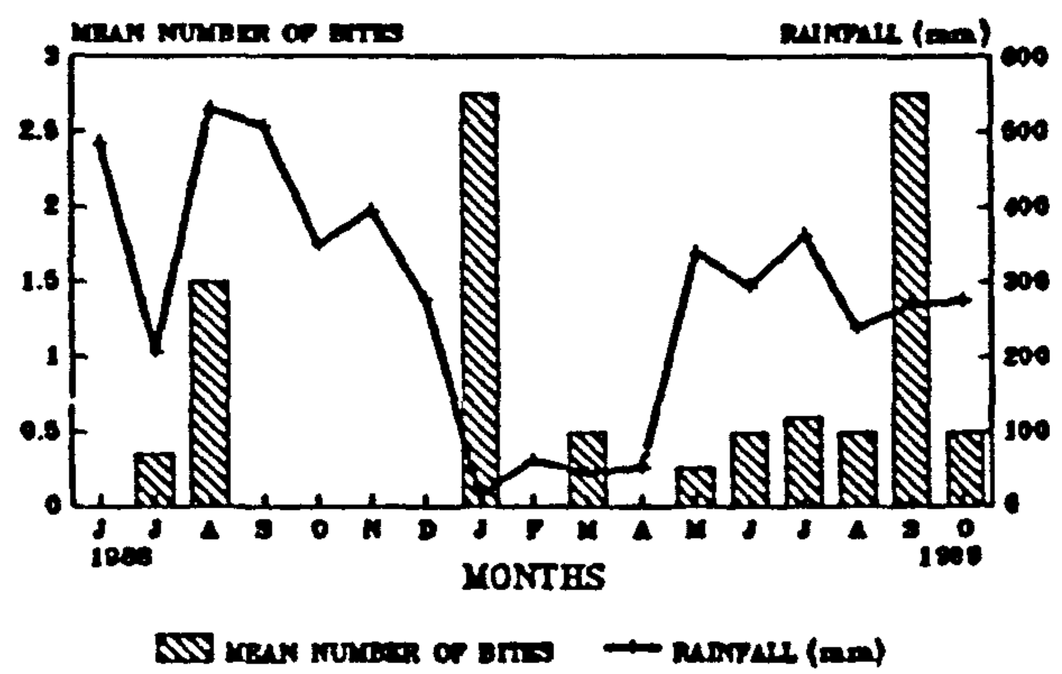


FIGURE 3.1.c: An. albitarsis

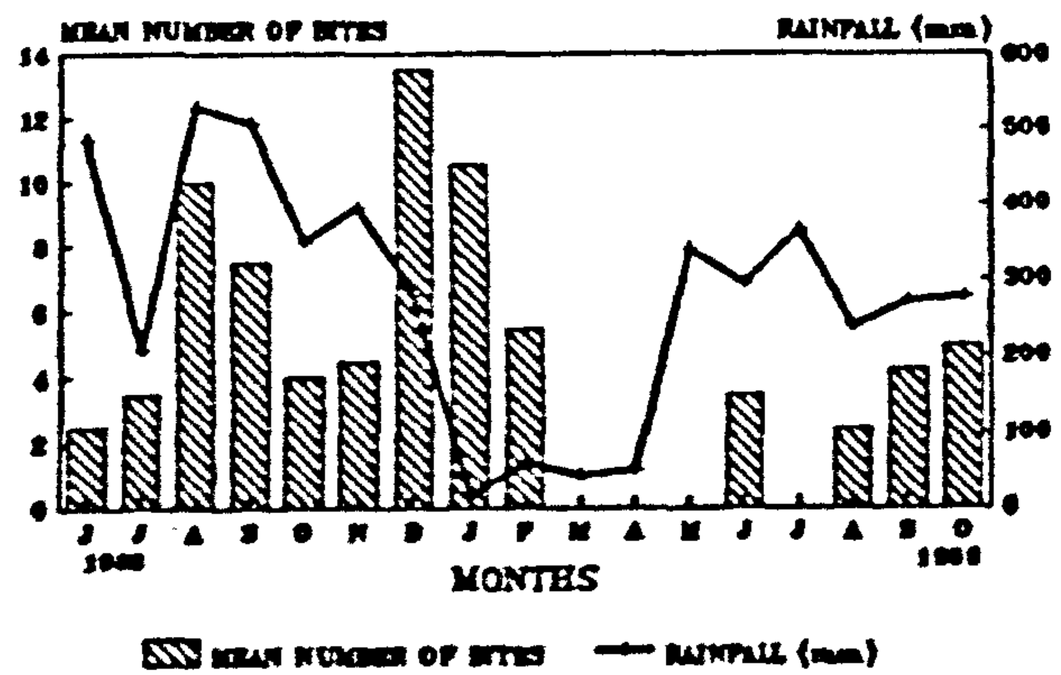


FIGURE 3.1.d: An. oswaldoi

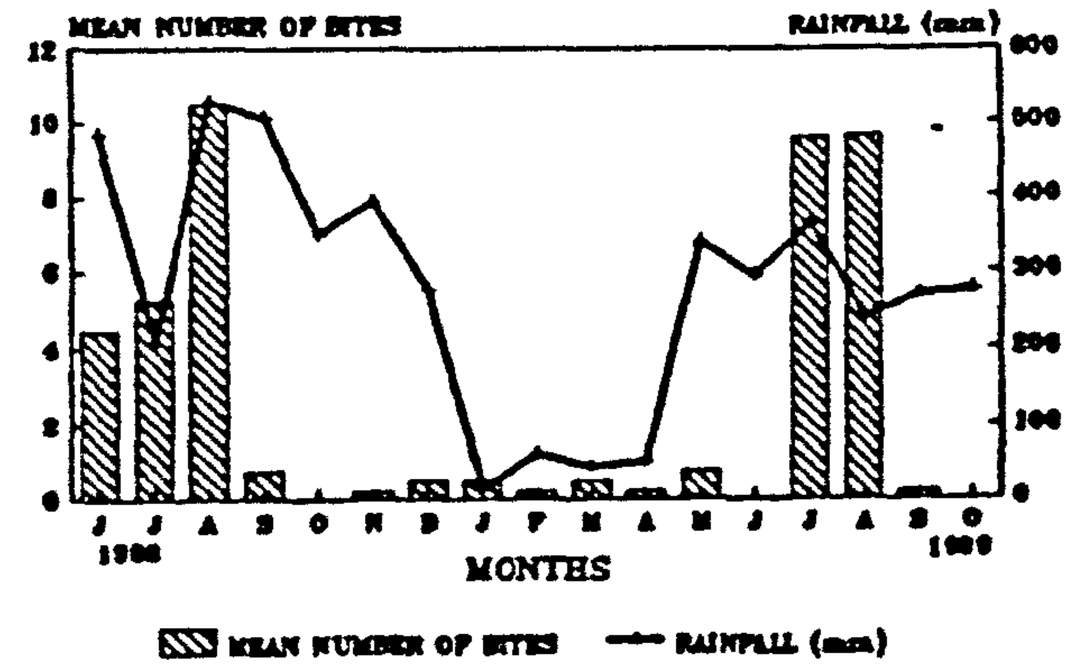


FIGURE 3.2: Human biting catch at Guaquitas and rainfall

FIGURE 3.2.a: An. nuneztovari

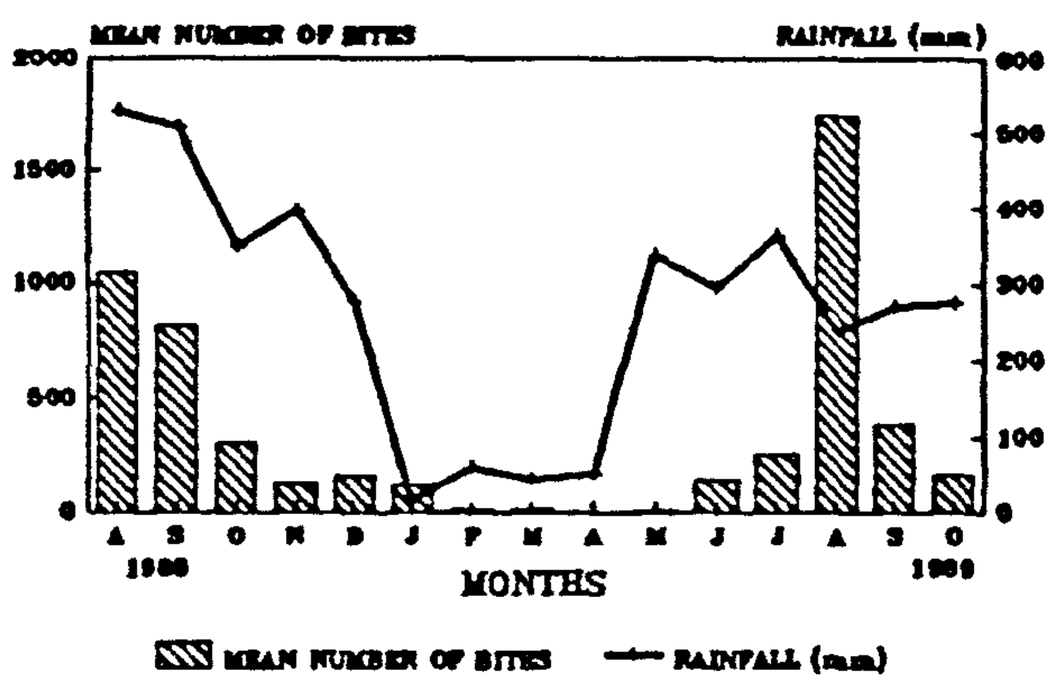


FIGURE 3.2.b: An. triannulatus

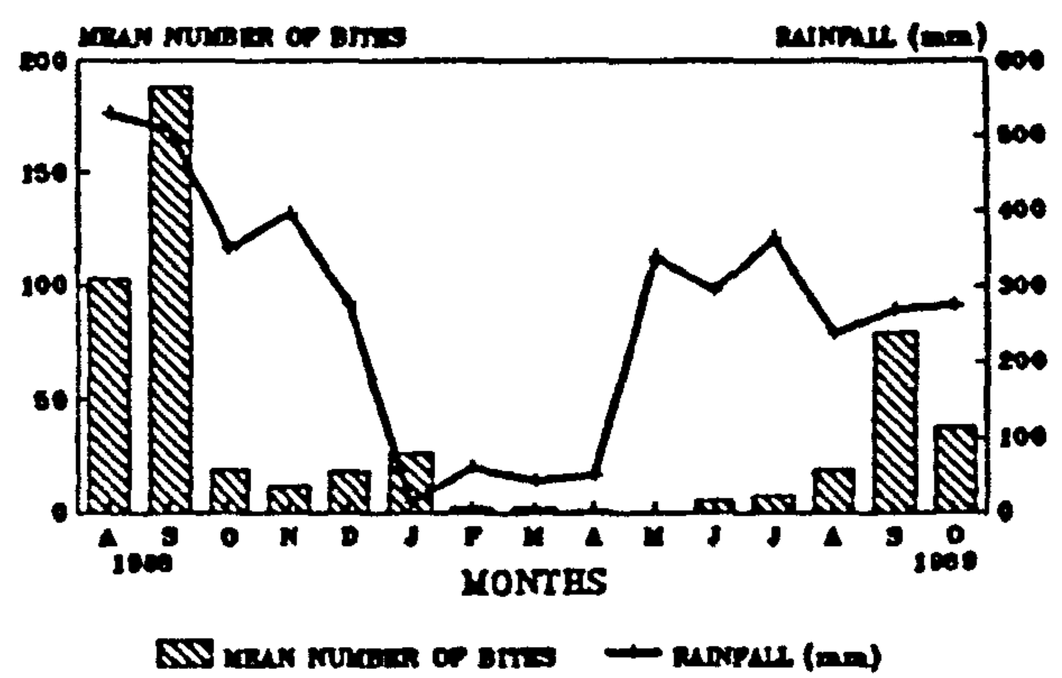


FIGURE 3.2.c: An. albitarsis

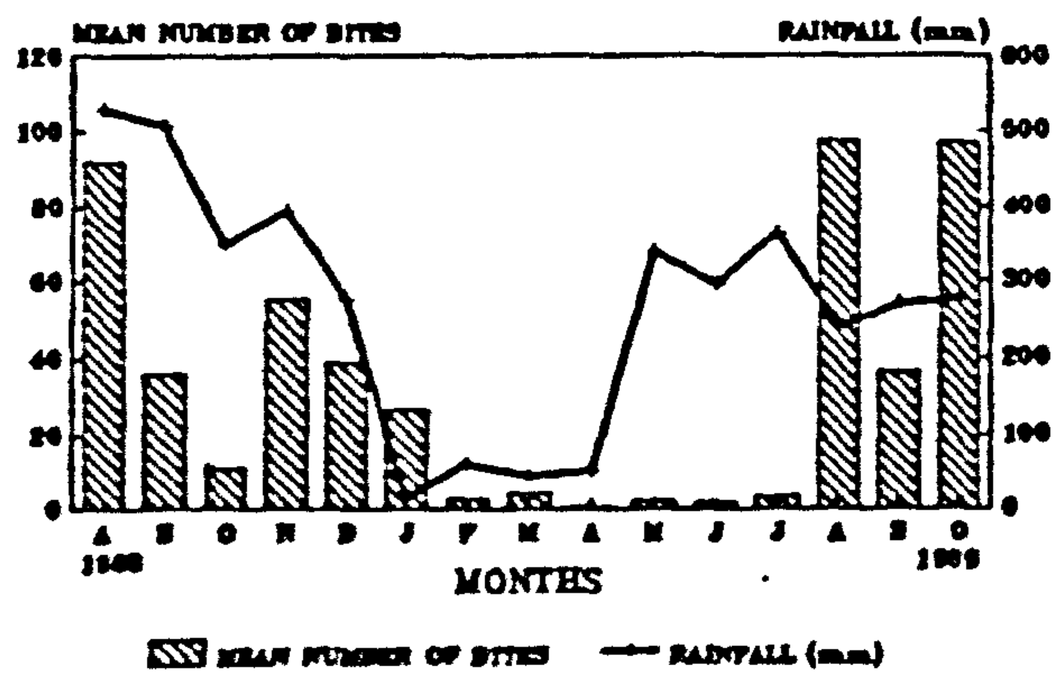


FIGURE 3.2.d: An. oswaldoi

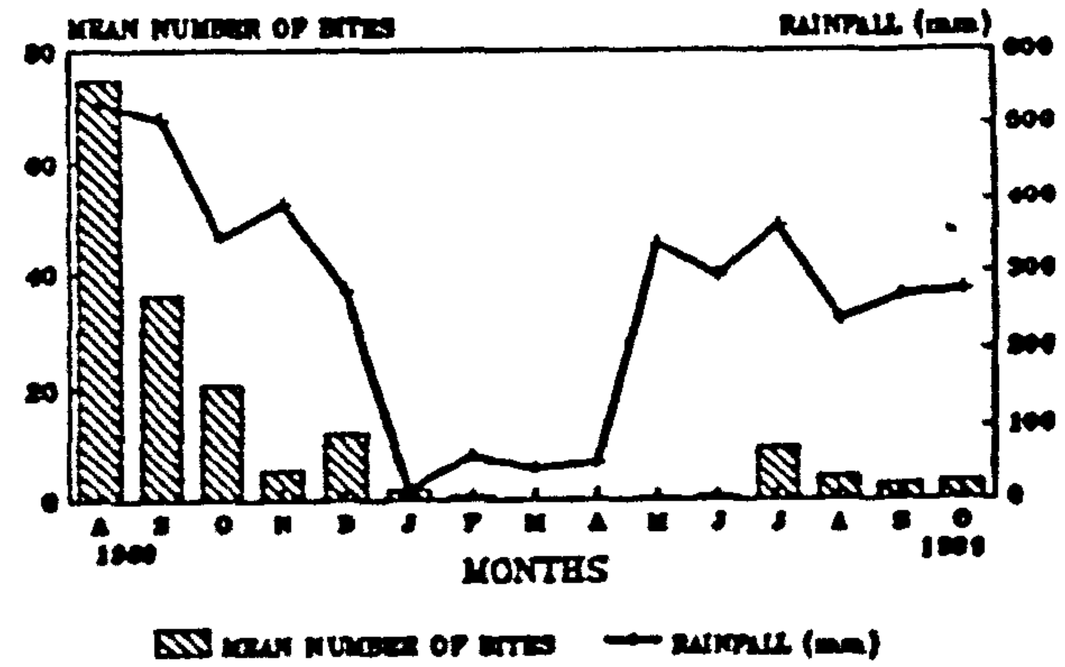


FIGURE 3.3: Human biting catch at Jabillos and rainfall

FIGURE 3.3.a: An. nuneztovari

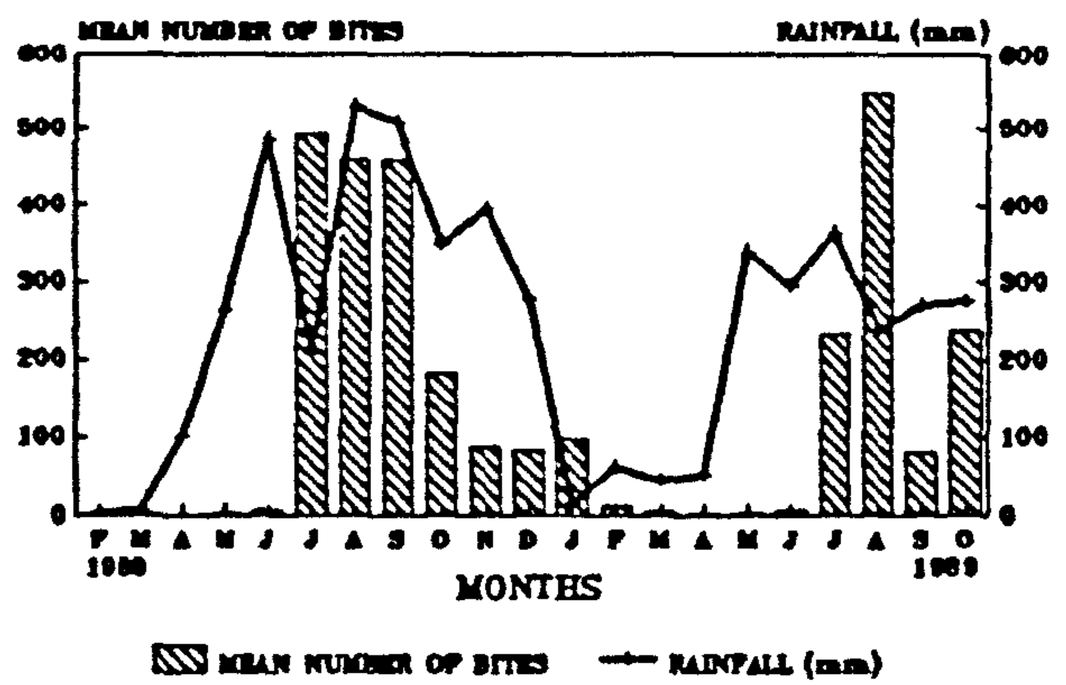


FIGURE 3.3.b: An. triannulatus

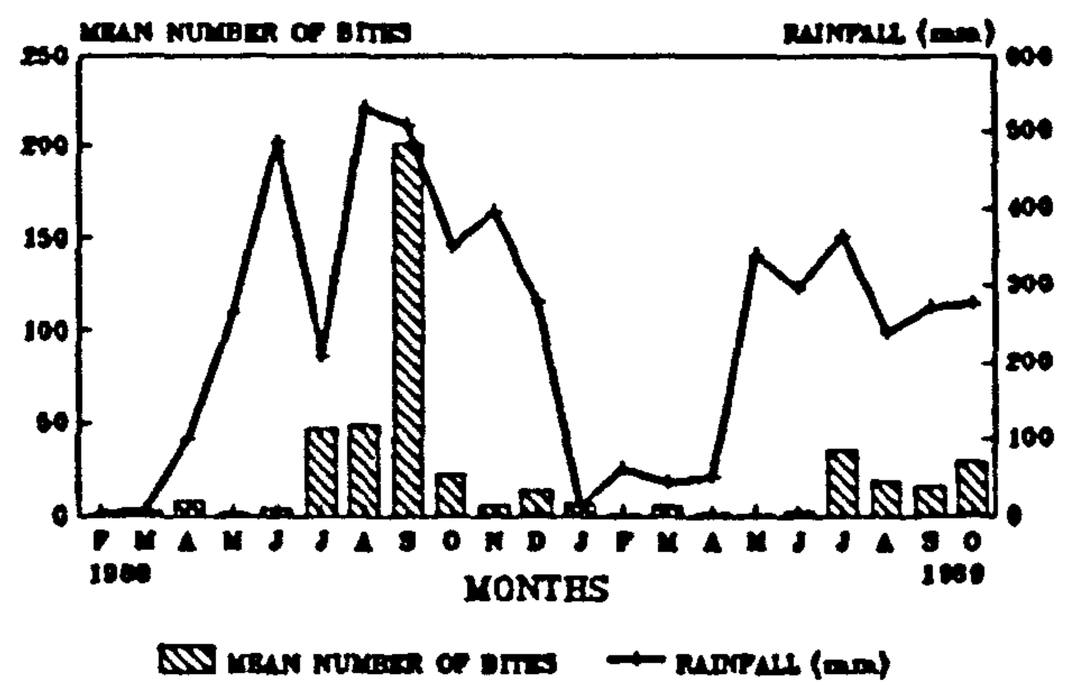


FIGURE 3.3.c: An. albitarsis

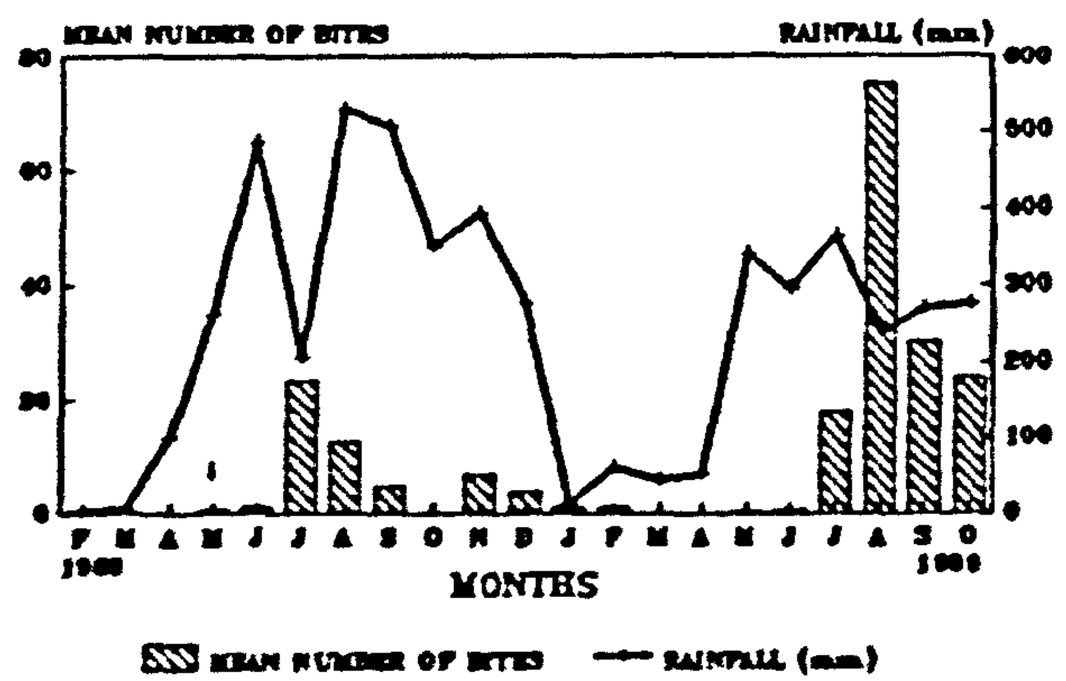


FIGURE 3.3.d: An. oswaldoi

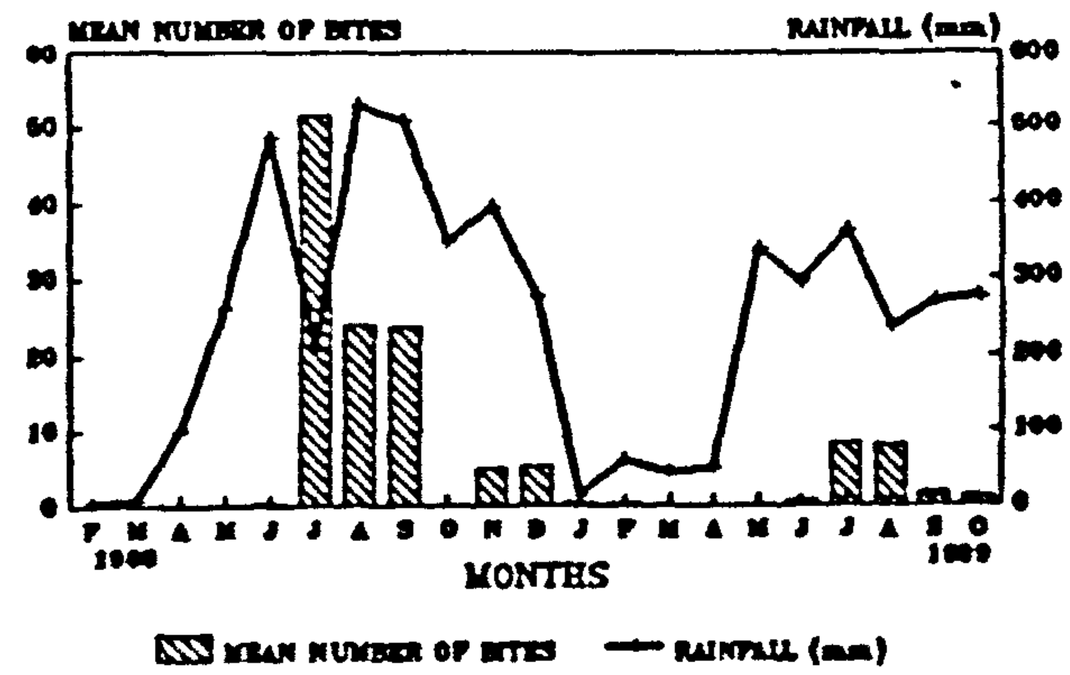
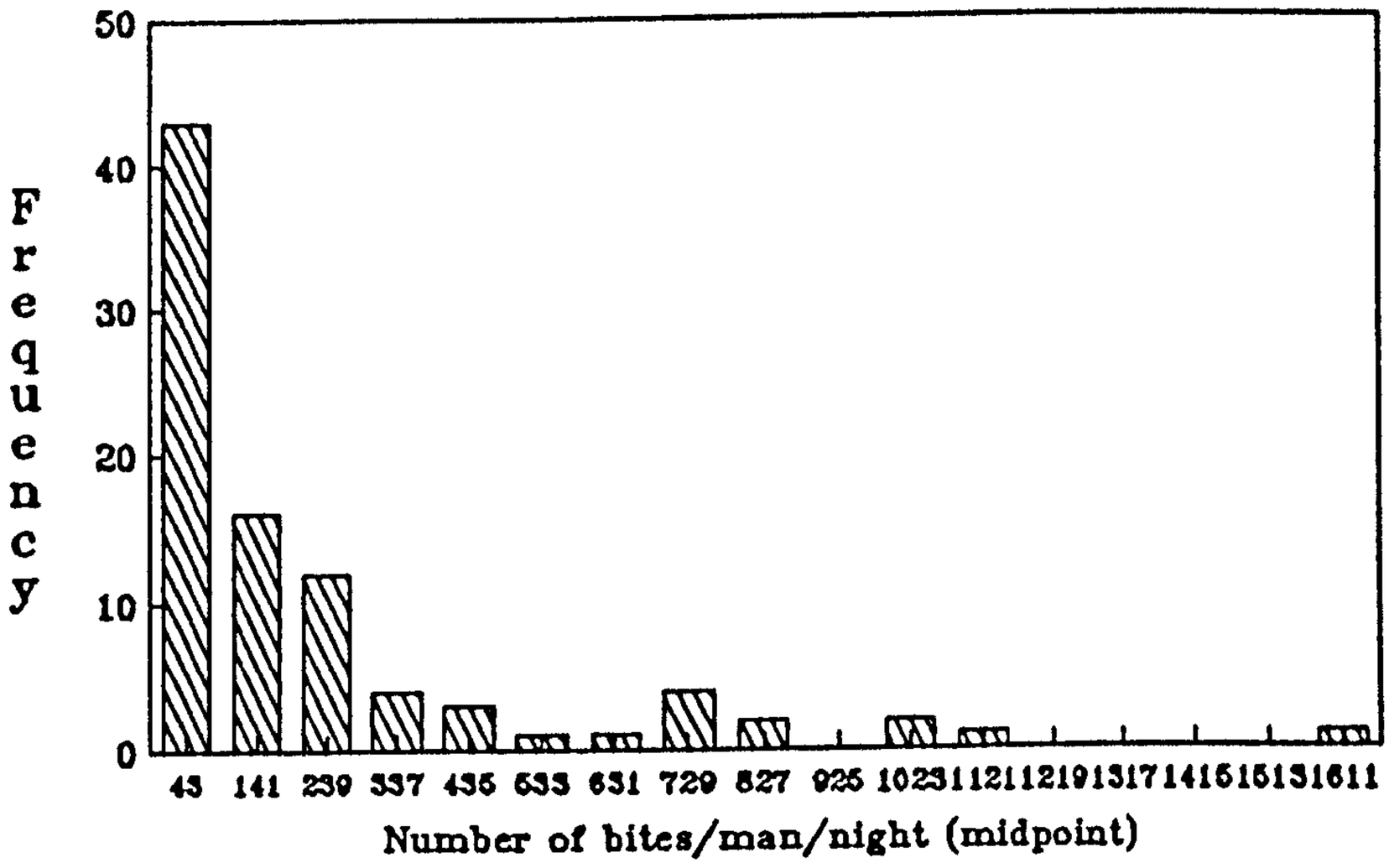
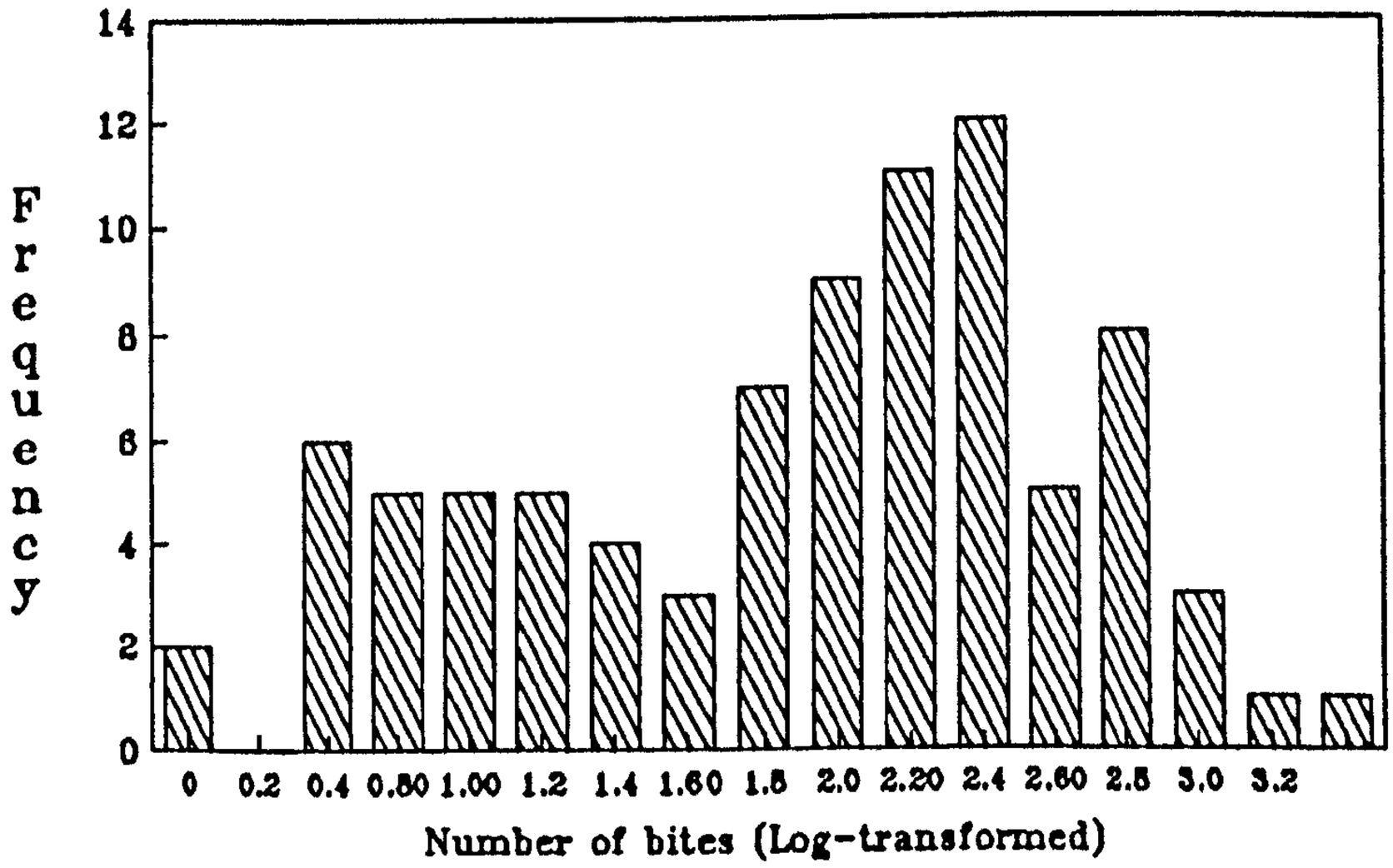


FIGURE 3.4.a: Frequency Distribution of the number of bites per man per night



An. nuneztovari

FIGURE 3.4.b: Frequency Distribution of the log-transformed data



An. nuneztovari

Table 3.2: Regression analysis of the mean number of bites per month on the rainfall in that month for the four most abundant species in the three study villages (data were log-transformed).

Species	Village	Reg Coef.	S.E. of reg.coef.	Signif. of Deviation of reg. coef. from zero t	P
nuneztovari	Jabillos	0.0028	0.0012	2.264	0.035
	Caño Lindo	0.0011	0.001	1.114	0.283
	Guaquitas	0.0034	0.0013	2.621	0.021
albitarsis	Jabillos	0.0014	0.0009	1.552	0.137
	Caño Lindo	0.0005	0.0007	0.815	0.428
	Guaquitas	0.0017	0.001	1.689	0.115
triannulatus	Jabillos	0.0018	0.0007	2.517	0.021
	Caño Lindo	-0.0002	0.0004	-0.626	0.541
	Guaquitas	0.0022	0.0011	2.110	0.055
oswaldoi	Jabillos	0.002	0.0008	2.603	0.017
	Caño Lindo	0.344	0.0007	1.420	0.176
	Guaquitas	0.0031	0.0007	4.232	0.0009

Table 3.3: Regression of the mean number of bites per month on the rainfall in the previous month for the four most abundant species in the three study villages (data were log-transformed).

Species	Village	Reg. Coef.	S.E. of reg.coef.	Signif. of Deviation of reg. coef. from zero t	P
nuneztovari	Jabillos	0.0048	0.0008	6.004	0.0001
	Caño Lindo	0.0009	0.001	0.908	0.378
	Guaquitas	0.0044	0.001	4.361	0.00077
albitarsis	Jabillos	0.0019	0.0008	2.368	0.029
	Caño Lindo	0.0015	0.0006	2.424	0.028
	Guaquitas	0.0044	0.001	4.361	0.00077
triannulatus	Jabillos	0.0026	0.0005	4.908	0.0001
	Caño Lindo	-0.0002	0.0004	-0.451	0.658
	Guaquitas	0.0028	0.0009	3.114	0.008
oswaldoi	Jabillos	0.0026	0.0006	4.034	0.0007
	Caño Lindo	0.0003	0.0007	0.406	0.689
	Guaquitas	0.0028	0.0008	3.466	0.004

there was no significant positive relationship between rainfall and mean number of bites of these species. For *albitarsis s. l.* there was no significant relationship between the mean number of bites and the rainfall in the month of the catches at any of the three sites. However, the relationship with the rainfall during the previous month was significant in all three villages. The differences observed among sites may be due to the differences in the types of larval habitats exploited by each species at each site. Although the investigation of larval habitats was not a major part of the present study, preliminary collections showed that in Caño Lindo immatures mainly were collected in permanent streams whereas in Guaquitas and Jabillos they were in permanent and semi-permanent pools. In Caño Lindo the peak of abundance for *An. nuneztovari* was in July (Fig. 3.1.a) while in Guaquitas (Fig. 3.2.a) and Jabillos (Fig. 3.3.a) the peak was in August. *An. albitarsis* was most abundant during August in Guaquitas and Jabillos but in Caño Lindo its peak was during December. In Jabillos and Guaquitas the peak for *triannulatus* occurred a month later (September) than that for *nuneztovari*. *An. oswaldoi* peaked at the three sites between July and August.

Figure 3.5 shows the percentage relative humidity per month in the study area. During the study the driest month was March 1988 and the most humid was July 1989. In general, the mean humidity only varies from 60-80%. Table 3.4 shows the results of regression of the log-transformed mean number of bites per month on the mean humidity. There was a positive relationship for *nuneztovari* and *albitarsis s.l.* at all the three sites, whereas for *triannulatus* and *oswaldoi* there was a significant relationship only in Jabillos and Guaquitas. Relative humidity is strongly related to rainfall and it is questionable whether it is rainfall (favouring the creation of oviposition sites) or humidity (favouring

FIGURE 3.5: Percentage Relative Humidity

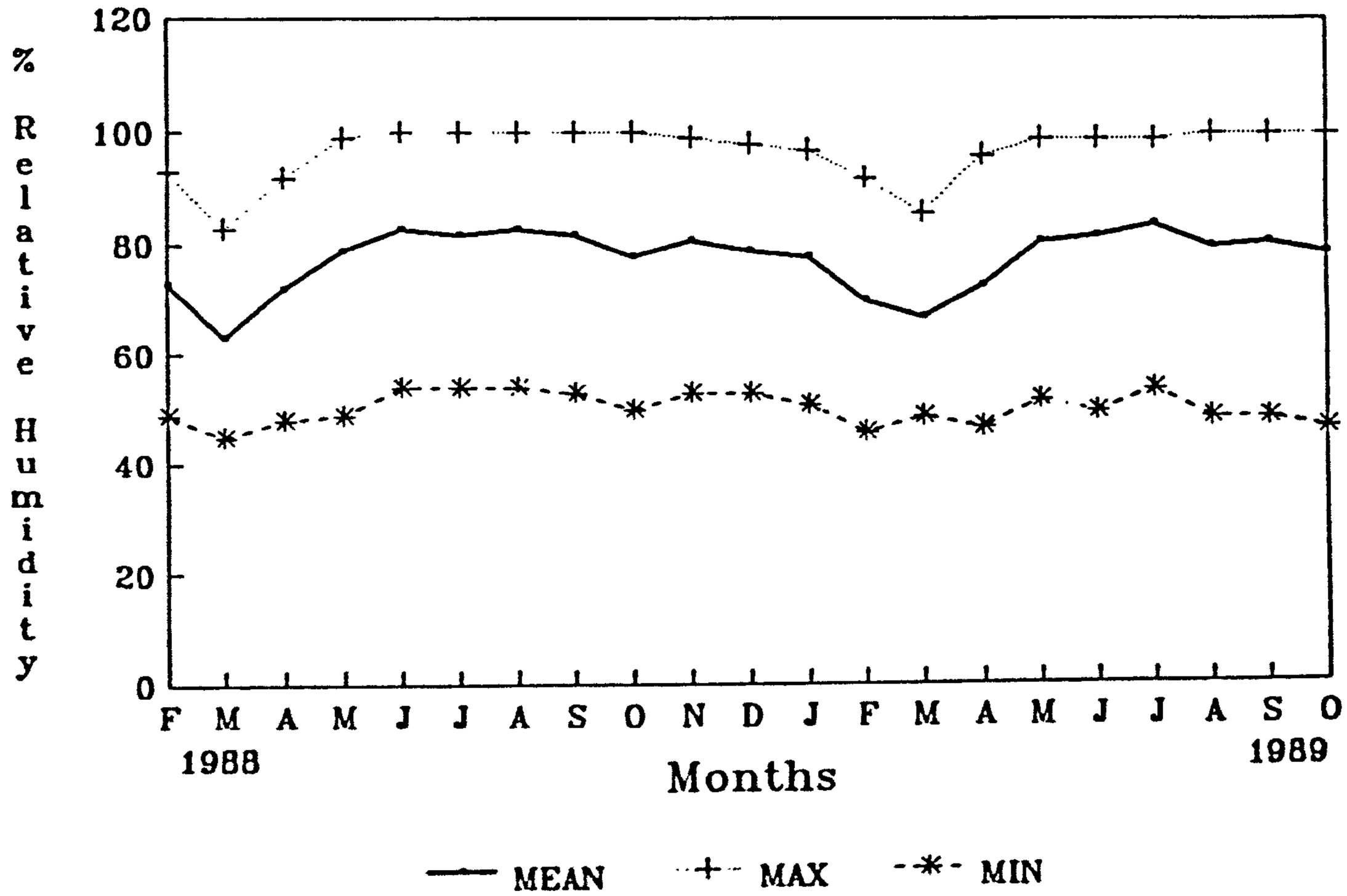


Table 3.4: Regression of the mean number of bites per month on the mean humidity in that month for the four most abundant species in the three study villages (data were log-transformed).

Species	Village	Reg Coef.	S.E. of reg.coef.	Signif. of Deviation of reg. coef. from zero t	P
nuneztovari	Jabillos	6.622	1.232	5.377	0.00003
	Caño Lindo	6.006	1.713	3.507	0.00318
	Guaquitas	11.463	1.304	8.788	0.00001
albitarsis	Jabillos	3.385	1.076	3.146	0.005
	Caño Lindo	4.0343	1.283	3.145	0.006
	Guaquitas	5.9822	1.867	3.203	0.0069
triannulatus	Jabillos	2.505	0.994	2.520	0.021
	Caño Lindo	0.423	0.909	0.465	0.648
	Guaquitas	7.227	1.699	4.253	0.0009
oswaldoi	Jabillos	3.083	1.023	3.014	0.007
	Caño Lindo	2.318	1.582	1.461	0.164
	Guaquitas	6.961	1.537	4.528	0.0005

Table 3.5: Analysis of variance of the monthly mean of the indoor catches of the four commonest species for species, month (August 1988-October 1989) and the three villages (data were log-transformed).

Source of Variation	Sum of Squares	DF	Mean Square	F	p
Main Effects:					
Species	103.366	3	34.455	166.397	<0.0001
Month	66.166	14	4.726	22.824	<0.0001
Site	11.774	2	5.887	28.431	<0.0001
2-way interactions					
Species x Month	20.298	42	0.483	4.652	<0.0001
Species x Site	4.158	6	0.693	6.671	<0.0001
Month x Site	18.469	28	0.660	6.349	<0.0001
Residual	27.428	264	0.104		
Total	251.960	359	0.702		

Table 3.6: Analysis of variance of the monthly mean of the outdoor catches for the four commonest species for species, month (August 1988-October 1989) and the three villages (data were log-transformed).

Source of Variation	Sum of Squares	DF	Mean Square	F	p
Main Effects:					
Species	91.485	3	30.495	290.658	<0.0001
Month	76.044	14	5.432	51.772	<0.0001
Site	21.772	2	10.886	103.757	<0.0001
2-way Interactions:					
Species x Month	19.842	42	0.472	4.503	<0.0001
Species x Site	12.462	6	2.077	19.797	<0.0001
Month x Site	20.043	28	0.716	6.823	<0.0001
Residual	28.537	272	0.105		
Total	269.106	367	0.733		

adult survival) or both factors which is/are the true determining cause. In the case of *nuneztovari* at Caño Lindo a significant relationship was seen with humidity (Table 3.4) but not rainfall. This suggests that humidity may be the more important determining factor.

The significance of differences in abundance of the four commonest species at each village during each month which are apparent in Figures 3.1, 3.2 & 3.3 (a, b, c & d) were corroborated by an analysis of variance. Tables 3.5 and 3.6 show that for the indoor and outdoor monthly mean catches over the full 15-month period for the four commonest species the F values were highly significant ($p<0.0001$) not only between species, sites and months but also in their interactions. The three-way interaction of species x month x site was tested for the indoor and outdoor catches on each night over a six-month period and found to be significant (Tables 3.7 and 3.8). These results suggest that each of the three villages presents different conditions at different times for larval and adult survival.

3.3.3. HOURLY BITING ACTIVITY

Table 3.9a shows the total number and species of anophelines collected indoors and outdoors on human baits. Figure 3.6 (a, b, c, & d) shows the indoor and outdoor biting activity throughout the night for the four commonest species pooled for the three villages in order to give a general picture of the patterns exhibited by each species. Each species has a different diel biting periodicity indoors and outdoors. *An. nuneztovari* peaked in the middle of the night indoors and out. *An. triannulatus* and *oswaldoi* had outdoor peaks soon after dusk, but the latter also had an indoor peak before midnight. *An. albitarsis* bit indoors and outdoors mainly before midnight.

Figure 3.7 shows the ratios of outdoor to indoor biting for the four commonest species in the three villages with confidence limits. For calculating the ratio and confidence limits only nine months of observations were considered because during the dry season numbers collected were very low and there were numerous zero or very low collections which would result in unreliable values for the ratios.

Table 3.7: Analysis of variance of indoor catches on each night of the four commonest species for species, month (June-October 1989) and the three villages (data were log-transformed).

Source of Variation	Sum of Squares	DF	Mean Square	F	p
Main Effects:					
Species	56.474	3	18.825	255.721	<0.0001
Month	8.372	4	2.093	28.433	<0.0001
Site	5.166	2	2.583	35.087	<0.0001
2-way Interactions:					
Species x Month	7.365	12	0.614	8.337	<0.0001
Species x Site	4.354	6	0.726	9.857	<0.0001
Month x Site	4.595	8	0.574	7.802	<0.0001
3-way Interaction:					
Species x Site x Month	4.451	24	0.185	2.519	0.002
Residual	4.417	60	0.074		
Total	95.194	119	0.800		

Table 3.8: Analysis of variance of the outdoor catches on each night of the four commonest species for species, month (June-October 1989) and the three villages (data were log-transformed).

Source of Variation	Sum of Squares	DF	Mean Square	F	p
Main Effects:					
Species	54.231	3	18.077	185.772	<0.0001
Month	8.248	4	2.062	21.190	<0.0001
Site	6.884	2	3.442	35.374	<0.0001
2-way Interactions:					
Species x Month	5.414	12	0.451	4.636	<0.0001
Species x Site	6.831	6	1.139	11.701	<0.0001
Month x Site	3.887	8	0.486	4.993	<0.0001
3-way Interaction:					
Species x Month x Site	4.834	24	0.201	3.618	<0.0001
Residual	3.340	60	0.056		
Total	93.668	119	0.787		

Table 3.9a: Anophelines collected outdoors and indoors on human baits in western Venezuela between August 1988 and October 1989.

Species	Jabillos		Caño Lindo		Guaquitas		
	Out	In	Out	In	Out	In	Total
An. nuneztovari	4,324	5,653	4,505	4,501	11,383	8,084	38,450
An. triannulatus	1,437	204	26	13	1,401	686	3,767
An. albitarsis	447	357	142	139	1,134	760	2,979
An. oswaldoi	242	164	59	76	345	222	1,108
An. strodei	65	65	60	47	219	113	569
An. rangeli	69	80	54	62	192	113	570
An. neomaculipalpus	43	21	5	1	17	10	97
An. benarrochi	3	1	3	4	3	10	24
An. pseudopunctipennis	0	0	5	4	1	0	10
An. punctimacula	2	0	1	2	2	1	8
An. mediopunctatus	0	0	1	1	3	1	6
Total =	6,632	6,545	4,861	4,850	14,700	10,000	47,588

Table 3.9.b: Culicines collected outdoors and indoors on human baits between September and October 1989.

	Jabillos		Caño Lindo		Guaquitas		
	Out	In	Out	In	Out	In	Total
Culicines	781	443	20	41	815	692	2,792
Anophelines	264	321	99	116	1,201	645	2,001

FIGURE 3.6: Biting pattern throughout the night of the four commonest species

FIGURE 3.6.a: An. nuneztovari

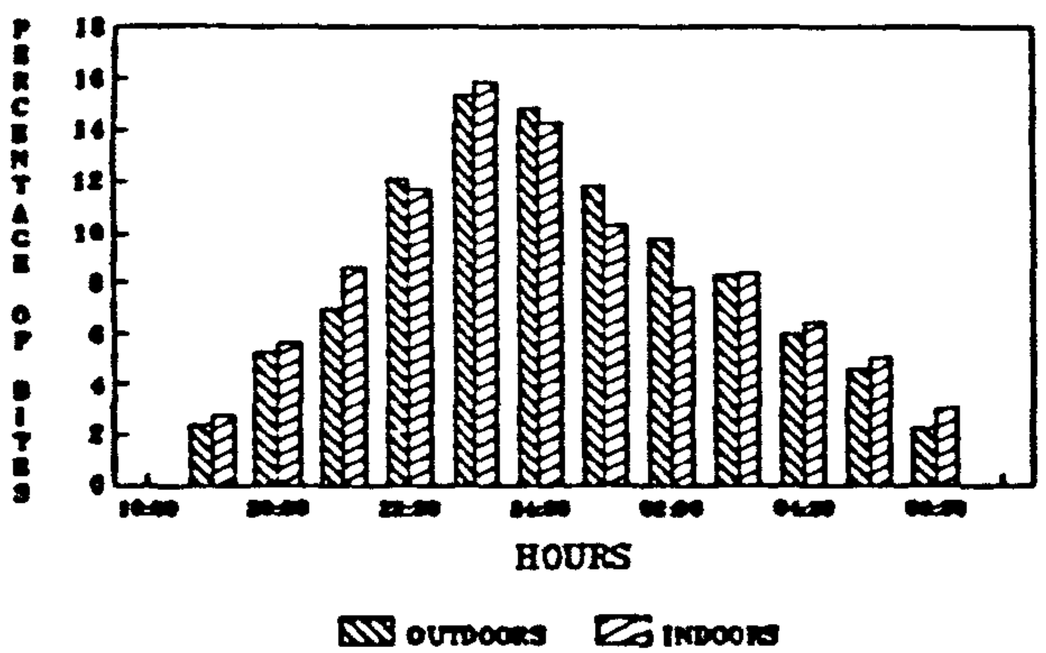


FIGURE 3.6.b: An. triannulatus

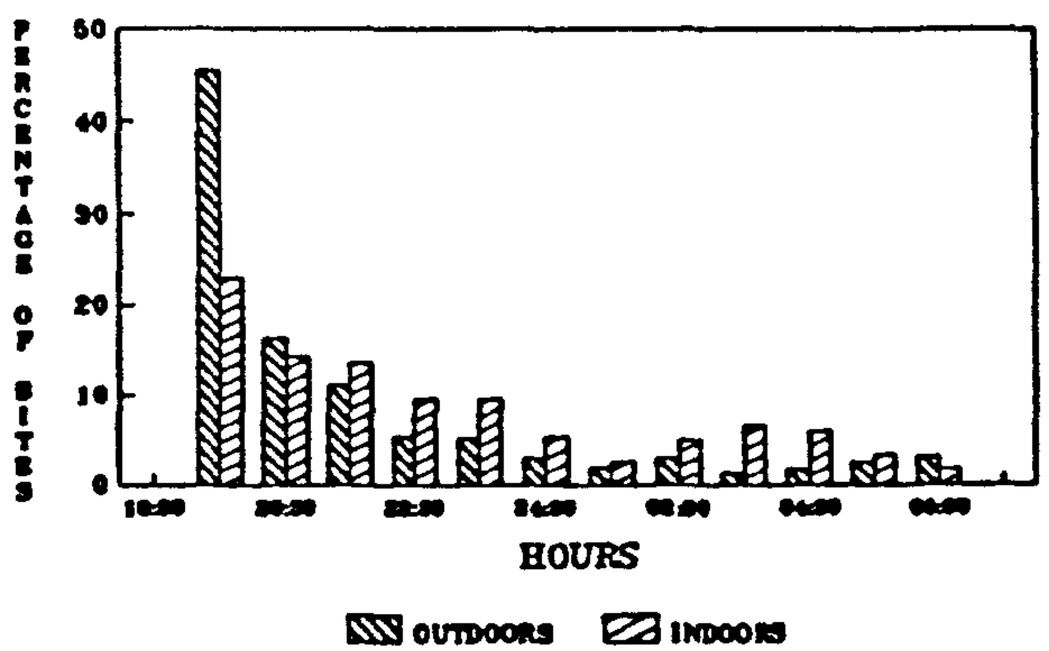


FIGURE 3.6.c: An. albitarsis

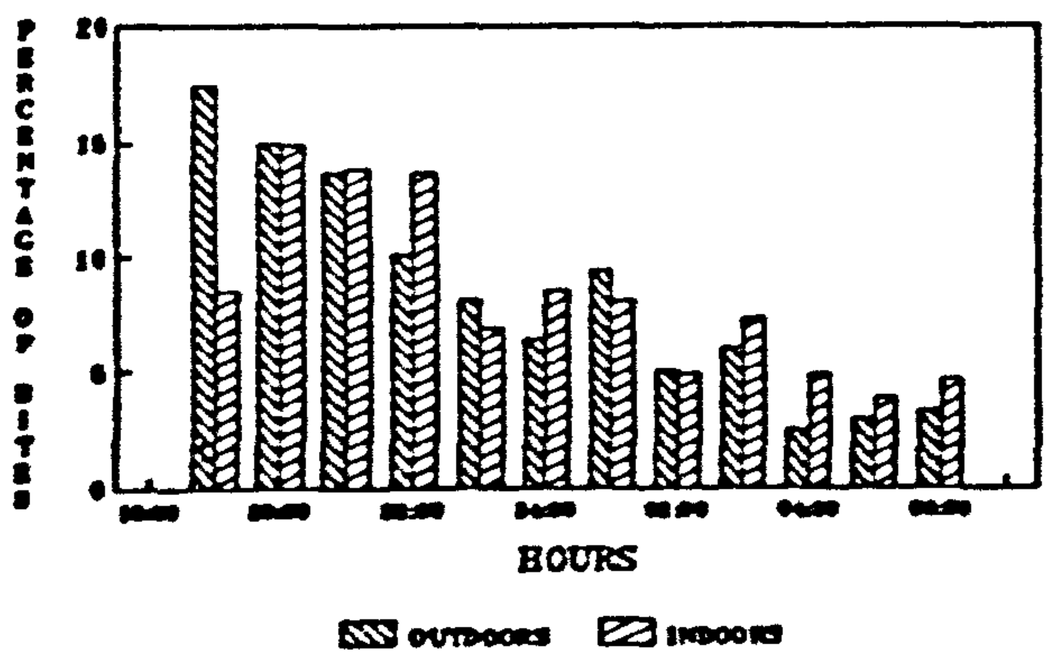
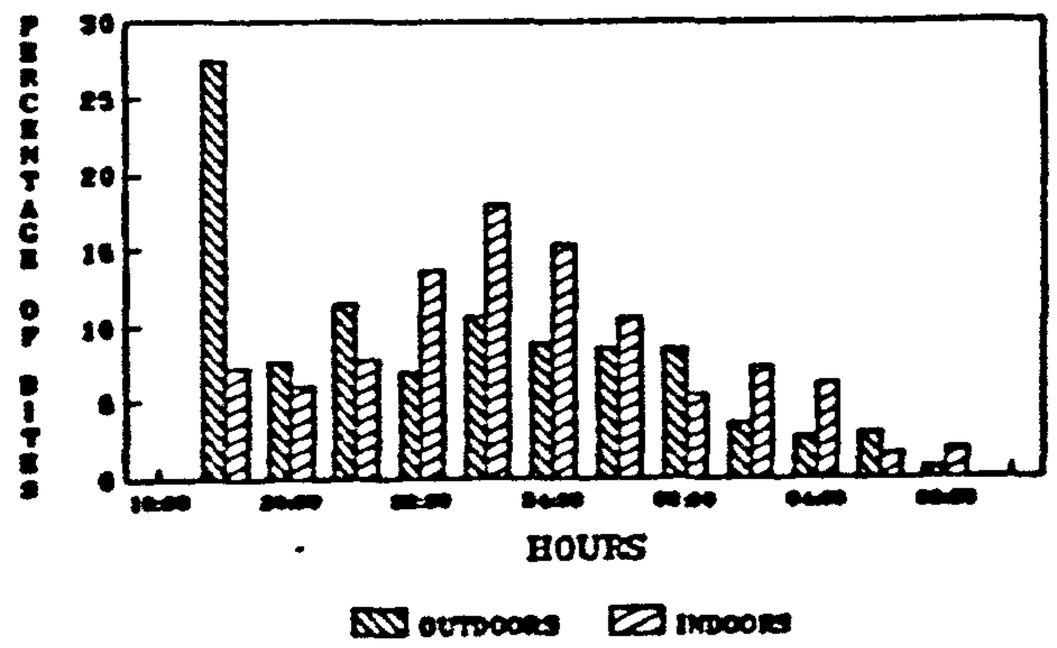


FIGURE 3.6.d: An. oswaldoi



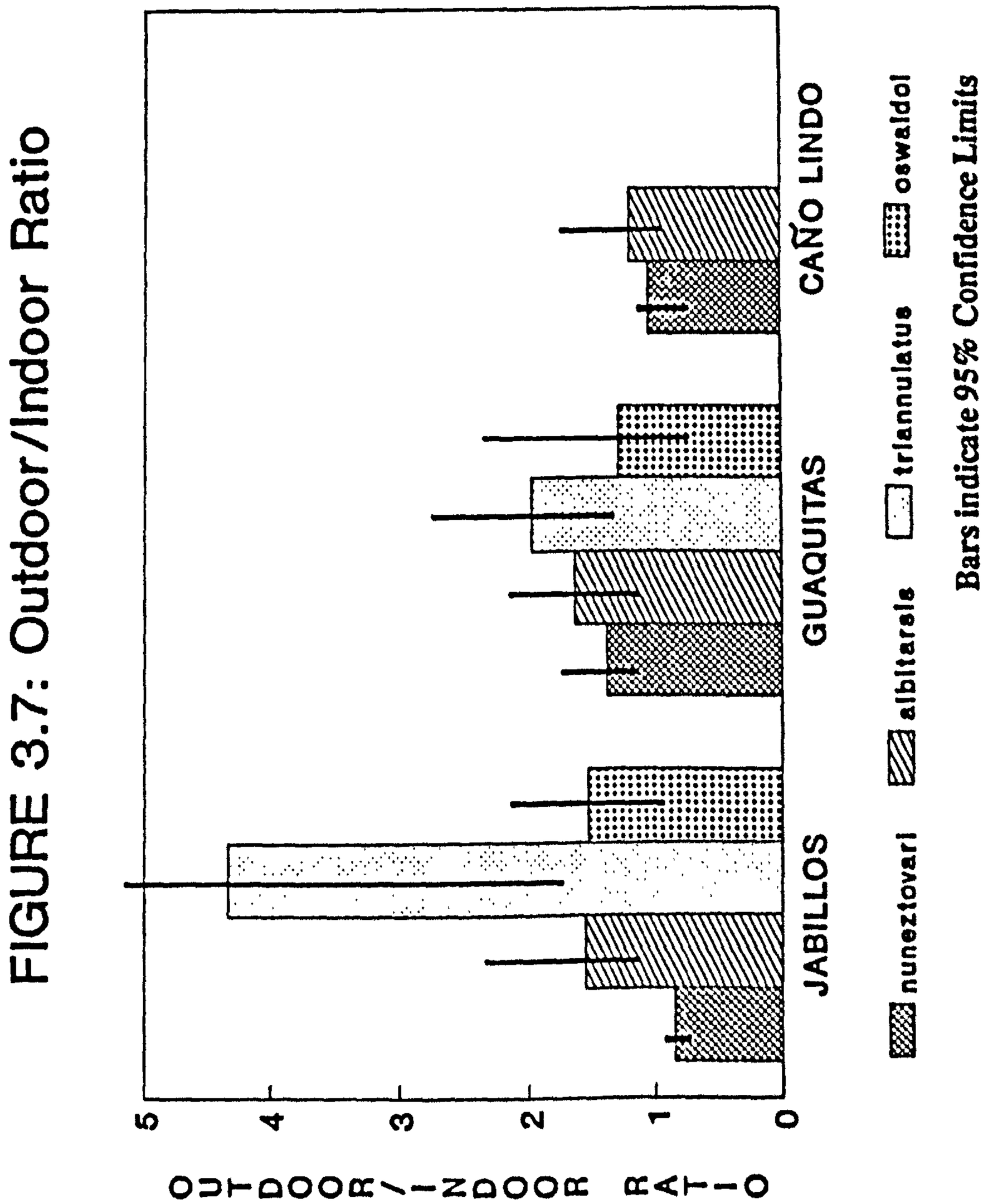


FIGURE 3.7: Outdoor/Indoor Ratio
CAÑO LINDO
GUAQUITAS
JABILLOS
albitarsis
oswaldoi
nuneztovari
triannulatus
Bars indicate 95% Confidence Limits
5
4
3
2
1
0
OUTDOOR/INDOOR RATIO

An. nuneztovari seemed to be more endophagic at Jabillos than Guaquitas. There was no significant difference between the ratios for *albitarsis* and *oswaldoi* among villages while *triannulatus* showed the highest outdoor/indoor ratio, especially in Jabillos.

Analysis of variance performed on the outdoor/indoor ratio for each species by month and village showed that for *An. nuneztovari* (Table 3.10) there was no significant effect of month on the ratio but there was a highly significant effect of the sites, confirming the impression obtained from Figure 3.7; the interaction (month x site) was not significant. For *albitarsis* (Table 3.11) there was no significant effect of months or sites on the ratio, but in this case the interaction (month x site) was significant. For *An. triannulatus* (Table 3.12) the analysis of variance indicated significant effects of months and sites on the ratio, and the interaction (month x site) was also significant. Finally, for *oswaldoi* (Table 3.13) there was no significant effect of site, month or the interaction of both factors on the ratio. When an analysis of variance was performed for the outdoor/indoor ratio of the four commonest species by species, month and site (Guaquitas and Jabillos), there was a significant effect of species and month but not of site. Interactions between species and site, and between month and site were highly significant. Interactions between species and month and species, site and month were close to the border line for significance (Table 3.14).

During September and October 1989, the culicines collected on one night of collection in each village on human baits were counted. Table 3.9.b shows the numbers of culicines collected indoors and outdoors in each village, as well as the numbers of anophelines collected the same night. In general more culicines and anophelines were collected in Guaquitas than in Jabillos or in Caño Lindo.

3.3.4. PAROUS RATE

A total of 1,497 anophelines collected on human baits at the three villages were dissected and parity determined. Of those dissected, 78.5% were *An. nuneztovari* and in general parity in *nuneztovari* did not vary significantly with season (Fig. 3.8.a, b & c),

Table 3.10: Analysis of variance for the outdoor/indoor ratio of the log-transformed data for *An. nuneztovari* between sites and months.

Source of Variation	Sum of Squares	DF	Mean Square	F	p
Main Effects:					
Month	0.191	8	0.024	0.608	0.763
Site	0.810	2	0.405	10.329	<0.0001
2-way Interaction:					
Month x Site	0.826	16	0.052	1.317	0.256
Residual	1.058	27	0.039		
Total	2.885	53	0.054		

Table 3.11: Analysis of variance of the outdoor/indoor biting ratio of the log-transformed data for *An. albitarsis* between sites and months.

Source of Variation	Sum of Squares	DF	Mean Square	F	p
Main Effects:					
Month	1.024	8	0.128	1.572	0.180
Site	0.499	2	0.249	3.061	0.063
2-way Interaction:					
Month x Site	2.941	16	0.184	2.257	0.030
Residual	2.199	27	0.081		
Total	6.664	53	0.126		

Table 3.12: Analysis of variance of the outdoor/indoor biting ratio of the log-transformed data for *An. triannulatus* between sites and months (includes data for small numbers collected at Caño Lindo not shown in Fig. 3.7)

Source of Variation	Sum of Squares	DF	Mean Square	F	p
Main Effects:					
Month	1.890	8	0.236	2.877	0.019
Site	3.178	2	1.589	19.350	<0.0001
2-way Interaction:					
Month x Site	4.618	16	0.289	3.515	0.002
Residual	2.217	27	0.082		
Total	11.902	53	0.225		

Table 3.13: Analysis of variance of the outdoor/indoor biting ratio of the log-transformed data for *An. oswaldoi* between sites and months (includes data for small numbers collected at Caño Lindo not shown in Fig.3.7)

Source of Variation	Sum of Squares	DF	Mean Square	F	p
Main Effects:					
Month	1.533	8	0.192	1.618	0.166
Site	0.197	2	0.098	0.831	0.446
2-way Interaction:					
Month x Site	3.211	16	0.201	1.694	0.110
Residual	3.199	27	0.118		
Total	8.141	53	0.154		

Table 3.14: Analysis of variance of the outdoor/indoor biting ratio for the four commonest species for species, month and villages (Jabillos and Guaquitas) (data were log-transformed).

Source of Variation	Sum of Squares	DF	Mean Square	F	p
Main Effects:					
Species	5.419	3	1.806	19.051	<0.0001
Month	2.540	8	0.318	3.349	0.003
Site	0.159	1	0.159	1.681	0.199
2-way Interaction:					
Species x Month	3.65	24	0.152	1.606	0.064
Species x Site	2.345	3	0.782	8.243	<0.0001
Site x Month	4.593	8	0.574	6.054	<0.0001
3-way Interaction:					
SpeciesxSitexMonth	3.645	24	0.152	1.602	0.065
Residual	6.827	72	0.095		
Total	29.184	143	0.204		

FIG.3.8.a:Parous Rate of An. nuneztovari
CAÑO LINDO

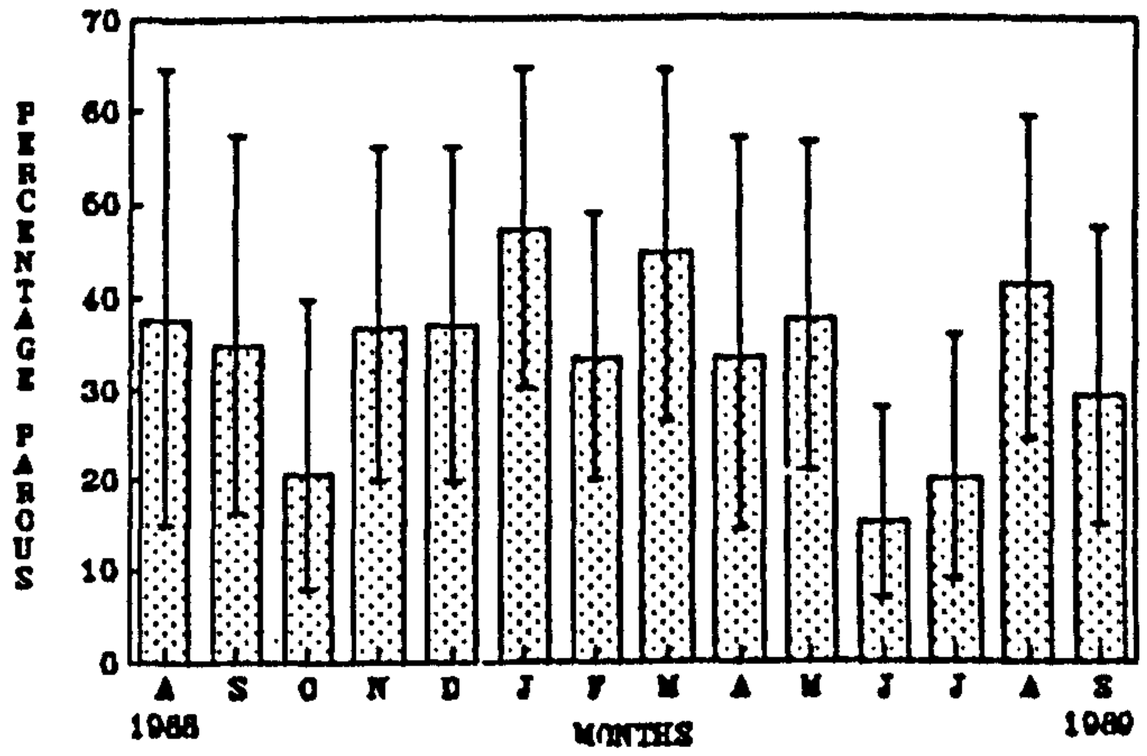


FIG.3.8.b:Parous rate of An. nuneztovari
GUAQUITAS

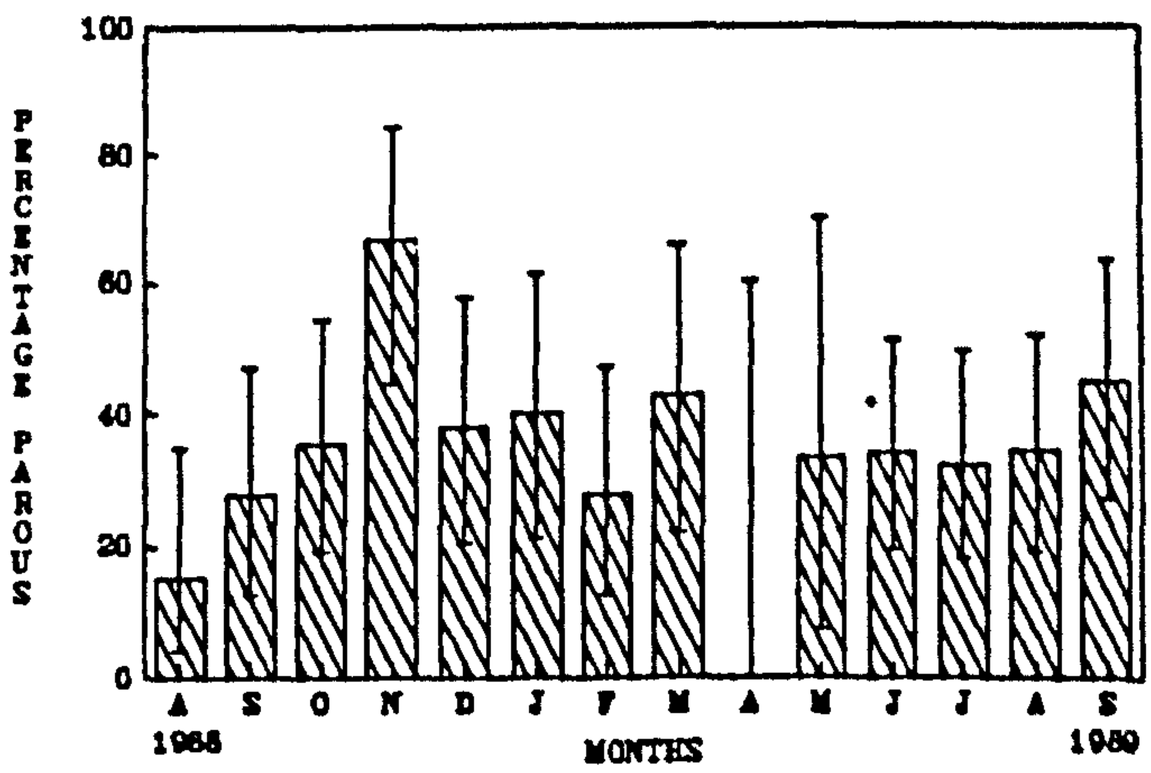
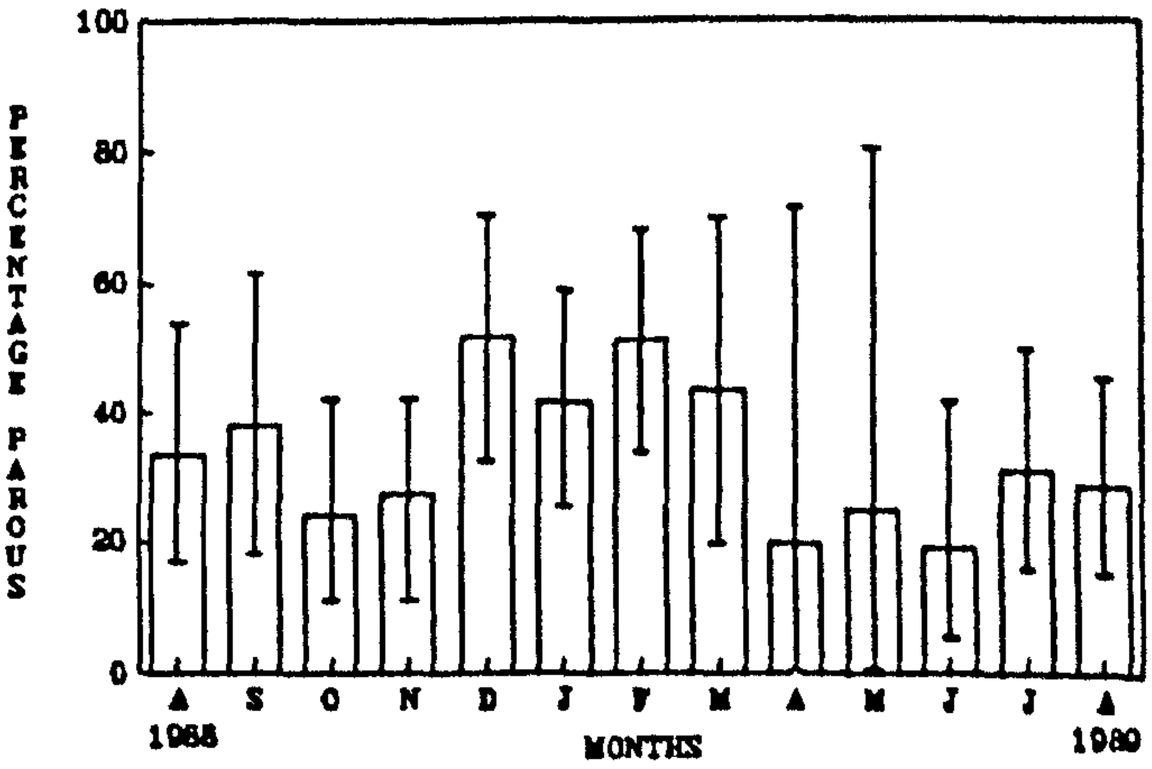


FIG.3.8.c:Parous Rate of An. nuneztovari
JABILLOS



Bars indicate 95% Confidence Limits from Binomial Tables

fluctuating between 20 and 40%. Of 106 *An. albitarsis* dissected, 48% were parous and of 133 *triannulatus* 44% were parous. In general, the parous rate of the species dissected were below 50% which suggests that none would be highly efficient vectors.

3.3.5. EFFECT OF FENITROTHION SPRAYING ON DENSITY AND PAROUS RATE

Fenitrothion apparently has no effect on the *An. nuneztovari* biting densities (Fig. 3.9.a): after spraying an increase was observed in July 1988 and August 1989, but the decreases observed at other times would have been expected at these seasons because of low or declining rainfall, even in the absence of spraying. From the limited sample size dissected, examination of the relationship betweeen fenitrothion spraying and parity of *An. nuneztovari* gives no evidence for a reduction in mean age of the sprayed population (Fig. 3.9.b).

3.4. DISCUSSION

An. nuneztovari is the most abundant anopheline species biting humans in western Venezuela. During its peak of abundance in August a person can receive up to 1,500 bites per night.

Anopheline populations in the study area showed extreme fluctuations which correlated positively with rainfall and humidity, especially for *nuneztovari*. Similar results were reported by Scorza *et al.* (1981) for the *nuneztovari* population on the northern slope of the Andes. However, these results contrast with those reported by Rozendaal (1990) who found that in river valleys in Suriname *nuneztovari* was most abundant during the dry season and almost absent during the rainy season. Rozendaal (1990) found that in that area *nuneztovari* breeds in sunlit rock pools along the river beds. This habitat disappears during the rainy season when the water level in rivers increases.

Highly significant differences in the numbers caught were found between species, site and month and their interactions. This seems to indicate that the main breeding places

Fig.3.9.a: Density of An. nuneztovari in relation to fenitrothion house spraying

(1988-1989)

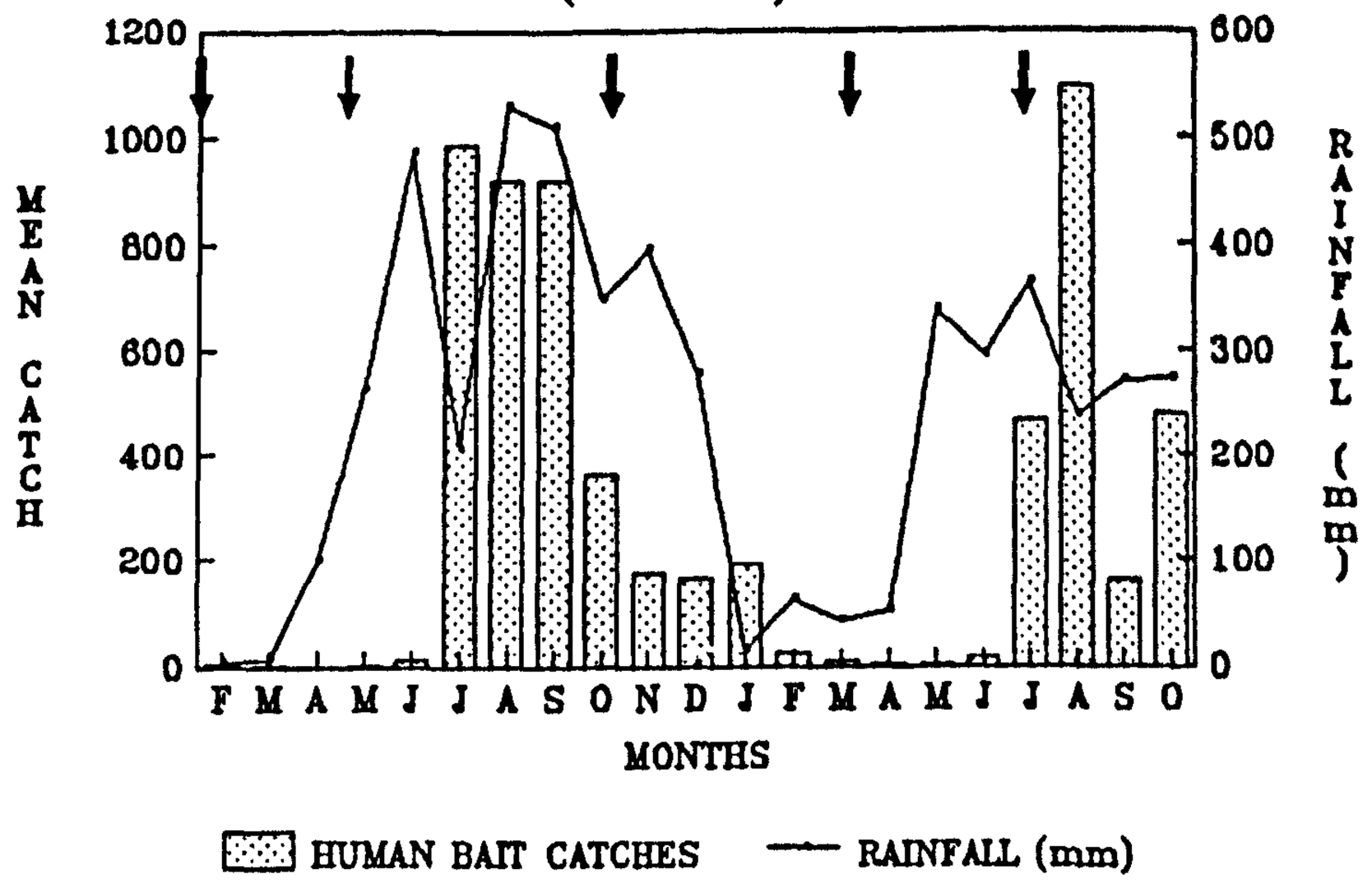
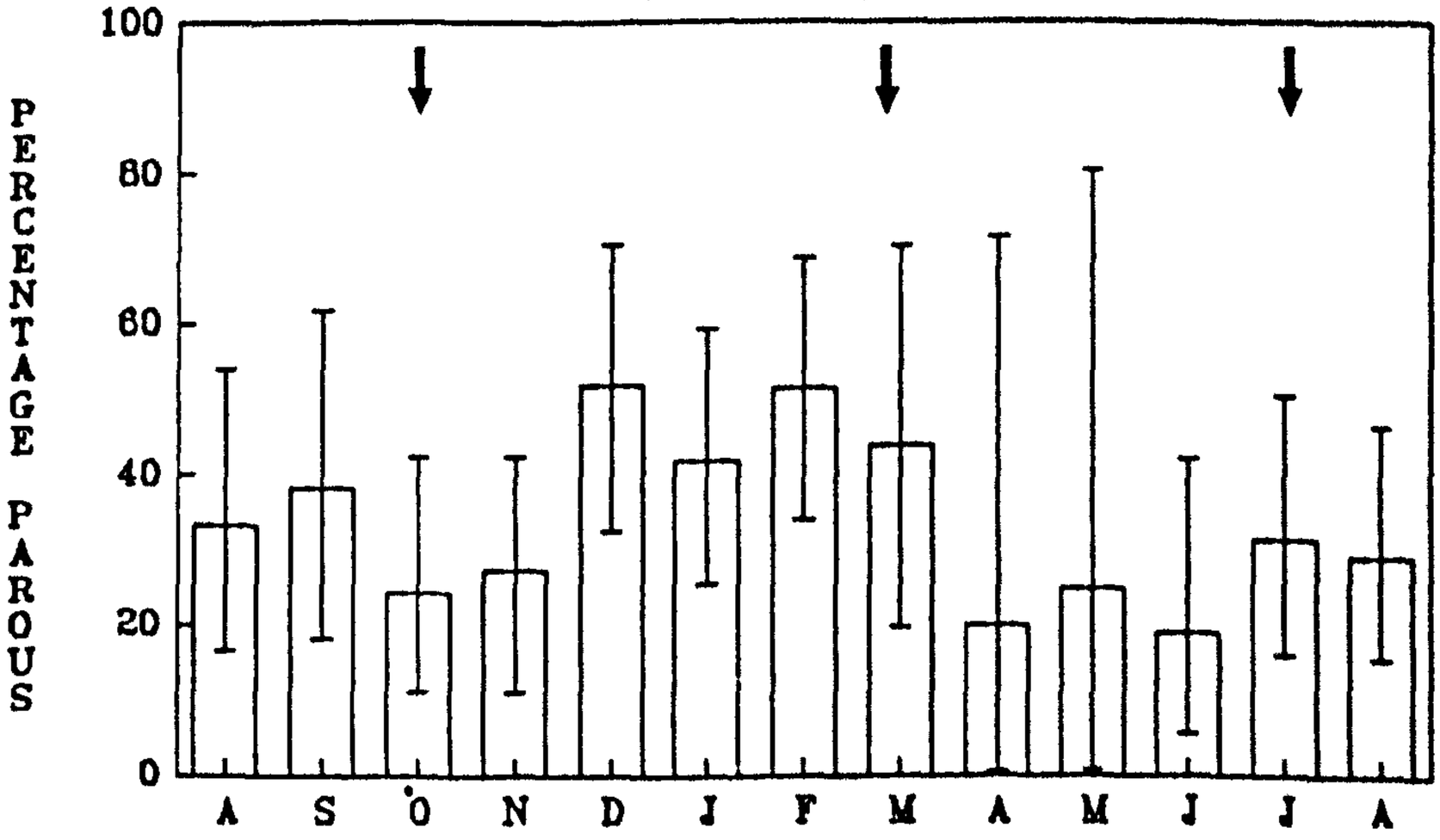


Fig.3.9.b:Parous Rate of An.nuneztovari in relation to fenitrot. house spraying

(1988-1989)



JABILLOS

Arrows indicate the month when the spraying was carried out

exploited by each species at each site and season are different. At present detailed studies on anopheline larval ecology in my study area are being carried out by members of a research team from the Universidad de Los Andes.

Other important parameters to consider are the differences in the amount and type of vegetation in villages that may provide suitable resting places for the mosquitoes. The results reported confirmed the statement in the introduction that there are important differences between the ecological conditions in each village.

An. nuneztovari bites throughout the night and shows a biting peak around midnight. This result agrees with previous reports from Venezuela and Colombia (Vinke & Pant, 1962; Dirección de Endemias Rurales Report, 1967; Elliott, 1967, 1972; Fajardo & Alzate, 1987). The number of outdoor and indoor bites were approximately equal but in Jabillos the mean ratio was found to be less than one, i.e. more bites were recorded indoors. At present any attempt to explain the variation in this ratio would be pure speculation, and detailed studies on the behaviour of *An. nuneztovari* are needed. Regarding the endophagic/exophagic habits of *nuneztovari*, there are contrasting observations in the literature. For instance, Fajardo and Alzate (1987) reported that in Colombia 60% of the total *nuneztovari* were collected inside houses, while Caraballo (1987) reported that in my study area during June 1987, 92.2% of the *nuneztovari* were collected outdoors. On the other hand, Garrón (1986) found that in Guaquitas 54% of the *nuneztovari* were collected outdoors. These results may be due to different behaviour patterns within the same species, to the existence of sympatric sibling species or, more likely, to biases produced in short series of observations by variation between individual catchers or location of collections.

In Suriname and Brazil, *An. nuneztovari* exhibits entirely different behaviour, having a biting peak outdoors immediately after sunset (Elliott, 1972; Panday, 1977; Rozendaal, 1987). Kitzmiller *et al.* (1973) reported that populations from Suriname and Brazil differed from those in Colombia and Venezuela in the polytene chromosome banding pattern, suggesting that *An. nuneztovari* is a complex of at least two sibling species.

The diel biting patterns observed for *triannulatus* and *albitarsis* resemble those reported in other places (Rozeboom, 1935; Deane *et al.*, 1948; Elliott, 1967, 1972). Such is not the case for *oswaldoi*. In fact, Elliott (1967) reported that 55% of the total biting of *An. oswaldoi* was between 2300 and 0100 hours whereas Rozendaal (1987) reported it to be more numerous biting outdoors between 1830 and 2030 hours and Deane *et al.* (1948) reported a biting peak between 1800 and 1900 hours. In my study area the pattern observed combined those mentioned above, i.e. *oswaldoi* showed an early peak out of doors and a smaller peak around midnight indoors. These results seem to indicate that *oswaldoi* may be a complex of at least two sibling species that apparently occur sympatrically in my study area.

During the study, the parous rate of *An. nuneztovari* was low (20-40) and did not vary significantly with season. This seems to indicate that, although there are differences in rainfall and humidity during the year, the environments found by the adults are fairly stable. Vinke and Pant (1962) observed in western Venezuela and northern Colombia that the parous rate of *nuneztovari* was higher in densely forested areas (0.64-0.72) than in partly deforested areas (0.31-0.53). Probably the low parous rate found in the three villages reflects the degree of deforestation in the area.

Since 1945, the study area had been sprayed regularly with DDT, but since 1984-85 fenitrothion has been used instead. The argument used by the National Control Programme for changing insecticide was based on the fact that *nuneztovari* is an exophilic mosquito that did not make sufficient contact with DDT deposits even though it is not physiologically resistant to this insecticide. Fenitrothion has a fumigant effect that lasts about 2 months (Caraballo, 1987) and is considered to be the insecticide of choice to intercept mosquitoes coming for a short time indoors to bite. Trials were conducted between 1984 and 1987 in western Venezuela and, although mosquitoes were collected, reports lack information regarding the effect of fenitrothion on mosquito density and parous rate. The unpublished reports stated that there was a reduction in the number of malaria cases in the area treated with fenitrothion in comparison with the area treated

with DDT. It was also mentioned that the cases occurring in those villages sprayed with fenitrothion were in areas where there were banana plantations and dense forests.

My results seems to indicate that fenitrothion has no effect on mosquito density or its parous rate. Nevertheless, it is important to bear in mind that mosquito collections were made in experimental huts free of insecticide and we do not know whether the insecticide has some effect on anophelines entering sprayed houses, which may be beneficial to the inhabitants.

CHAPTER 4:

CDC LIGHT-TRAP CATCHES

4.1. INTRODUCTION

Light-traps have been widely used for routine sampling of culicine mosquito populations and for the study of culicine vectors of viral diseases, mainly in North America (WHO, 1975; Service, 1976). In Africa, Odetoyinbo (1969) showed that, by placing light-traps inside houses near hosts, large numbers of anophelines were caught and he concluded that light traps were very efficient for sampling anopheline and culicine mosquitoes in The Gambia. Since then, there has been increased interest in using light traps for sampling anophelines by several groups of workers in Africa, with variable degrees of success (Service, 1970; Coz *et al.*, 1971; Carnevale & Le Pont, 1973; Carnevale, 1974; Joshi *et al.*, 1975; Garrett-Jones & Magayuka, 1975, Chandler *et al.*, 1976). Recently, light traps have been also evaluated in South East Asia (Ismail *et al.*, 1982) and they were considered by Hii *et al.* (1986) to be an efficient sampling tool for estimating relative densities of *An. balabacencis* and *An. flavirostris.*

Light traps have been shown in various studies to be a useful supplementary method for entomological evaluation of malaria-control programmes. Particularly encouraging are results reported from Tanzania by Lines *et al.* (1991). These authors, following the method of Garrett-Jones and Magayuka (1975) whereby light traps are set in rooms where people are protected by bednets, found good correlations between the numbers of *An. gambiae* and *Cx. quinquefasciatus* mosquitoes caught, as well as a similar age structure and sporozoite rate in light traps compared to human biting catches.

The situation is distinctly different in Latin America, where the use of light traps for sampling and surveying malaria vectors has not been properly evaluated. The few reports published refer mainly to *An. albimanus.* Pritchard and Pratt (1944) reported that in Puerto Rico more *An. albimanus* were collected in New Jersey light traps than in animal-baited traps. Breeland (1972a) reported that, in El Salvador, New Jersey light-trap

collections were superior to other collecting methods used (human bait, cattle traps, searches in natural resting places) in average numbers of *An. albimanus* caught. He also reported that the method was particularly useful in measuring seasonal fluctuations, in determining the nocturnal activity peak, and in determining what species were present in a given locality. Nevertheless, he found that this method was inadequate for measuring *An. pseudopunctipennis* populations. In field studies in El Salvador, Wilton (1975) evaluated the effectiveness of three different light traps to catch *An. albimanus*: ultraviolet up-draught light traps, New Jersey light traps and CDC light traps. He reported that the ultraviolet up-draught trap was the most effective. More recently, Sexton *et al.* (1986) evaluated light traps to collect *An. albimanus* in Haiti. These authors concluded that the up-draught ultra-violet light trap was a very effective method for collecting *An. albimanus*, being superior to human bait catches and CDC light traps for determination of vector densities.

Suárez and Marinkelle (1980) reported the use of light traps in two regions of Colombia. Traps were hung from tree branches and collected large numbers of *An. triannulatus*, *oswaldoi* and *mattogrossensis*. Nevertheless, traps failed to catch *An. darlingi*, the vector of malaria in those regions (Herrera *et al.*, 1987).

The use of light traps for evaluation of vector-control programmes has several advantages over human bait catches, which have recently been subject to several ethical and practical objections. The use of humans as baits to catch mosquitoes increases the chances of their contracting malaria, and the procedure is labour-intensive, tedious, uncomfortable and expensive in overtime payments. Also, unless the human biting catch team is well motivated and supervised, their results may be unreliable.

In order to evaluate the efficiency of light traps for sampling anopheline populations in western Venezuela, CDC miniature light traps (Sudia & Chamberlain, 1962) were used during the present study.

4.2. MATERIALS AND METHODS

Preliminary trials were conducted using CDC light traps in order to determine whether or not light traps could catch anophelines in western Venezuela. Initially, light-traps were placed in the village of Jabillos on porches near people between 1900 and 2300 hours. Between January and July 1988 traps were placed inside bedrooms, where people were sleeping protected by mosquito nets, and run for 12 hours. The four houses selected were at least 800 m apart. Also, during June 1988 in Caño Lindo, traps were placed in bedrooms where people were sleeping protected by mosquito nets, while human bait catches were carried out in the experimental hut. During August 1988 the method was standardised as follows: for 12 hours a night, CDC light traps operated by a 6-volt rechargeable battery were run simultaneously in the three huts with two human baits per hut sleeping under nets. This procedure was carried out for 2 nights per week, three weeks per month for 15 months. During the night, the light-trap bag was changed every 4 hours and after removal from the trap, the bag was kept wrapped in wet paper towels inside a polystyrene box. This schedule was followed in order to determine whether light-trap catches would reflect changes in mosquito biting activity on humans throughout the night and also to reduce mosquito mortality resulting from many hours exposure to the draught from the trap fan.

In the morning, mosquitoes were killed either by freezing if the electricity supply was functional or, if it was not, with ethyl acetate or chloroform. Mosquitoes were identified under the dissecting microscope and a quota of 20 mosquitoes dissected for determination of parity as previously described in Chapter 3.

4.3. RESULTS

4.3.1. NUMBERS AND SPECIES COLLECTED

Tables 4.1, 4.2, 4.3, and 4.4 show the number of mosquitoes collected of the four commonest anopheline species as well as the total numbers collected during the preliminary trial. Although the number of mosquitoes collected during this period was small, results were encouraging because they showed that anophelines in this part of the

Table 4.1: Light-trap collections on porches in Jabillos between 1900 and 2300 hours during September-November 1987 (wet season).

Numbers of collections	*nuneztovari*	*albitarsis*	*triannulatus*	*oswaldoi*	Total no. collected
11	1	0	1	0	6

Table 4.2: Light-trap collections in 4 bedrooms with nets in Jabillos between 1900 and 0700 hours during January-May 1988 (dry season).

Numbers of collections	*nuneztovari*	*albitarsis*	*triannulatus*	*oswaldoi*	Total no. collected
54	5	18	2	0	29

Table 4.3: Light-trap collections in 4 bedrooms with nets in Jabillos between 1900 and 0700 hours during June-July 1988 (wet season).

Numbers of collections	*nuneztovari*	*albitarsis*	*triannulatus*	*oswaldoi*	Total no. collected
14	23	1	2	0	42

Table 4.4: Light-trap collections in bedrooms with nets in Caño Lindo on three consecutive nights between 1900 and 0700 hrs during June 1988.

House No.	*nuneztovari*	*albitarsis*	*triannulatus*	*oswaldoi*	Total no. collected
7	0	0	0	0	2
8	5	0	0	0	8
62	15	0	0	0	23
7	1	1	0	4	8
8	26	0	0	2	47
62	37	0	0	2	52
7	1	0	1	1	3
62	54	0	0	0	77

country can be caught in light-traps. The results justified embarking on a prolonged study to evaluate the efficiency of the method.

It was apparent that some houses had far more mosquitoes than others. Such was the case in house No.62 in Caño Lindo where relatively large numbers of mosquitoes were collected in June 1988 (Table 4.4). During July 1988, light-traps were used only inside this particular house where on one night 738 anophelines were collected, of which 327 were *An. nuneztovari.*

A total of 7,636 anophelines belonging to nine species was collected during 15 months in the three experimental huts with six nights of collections per site per month (Table 4.5.a). As in human bait catches, the four commonest anopheline species collected in light-traps were *nuneztovari, albitarsis, triannulatus* and *oswaldoi*; but *An. neomaculipalpus* was also frequently collected, especially in Jabillos. Males were rare in light-trap catches. 21.75% of the anophelines collected were unidentifiable. Until towards the end of the study culicine mosquitoes were discarded but, in September and October 1989, 6,235 culicines were counted in 5 night collections in each village (Table 4.5b).

4.3.2. COMPARISON WITH INDOOR BITING CATCHES

Light-traps on six nights per month in each village collected far fewer anophelines (7,661) than did indoor human bait catches (21,395) on two nights per month in each village during the same period. Figure 4.1 shows the regression lines and correlation coefficients of the log-transformed monthly mean catches in each village in light-traps and human bait catches. For the statistical analyses the collections made between February and June 1989 were not considered because of the numerous zero scores in the dry season which would have prevented meaningful ratios being calculated. There were significant correlations between the two methods for the 4 commonest species (r values between 0.58 and 0.81; $p<0.001$). For *triannulatus* the correlation was weakest but it was still significant (Fig. 4.1.b).

Table 4.5.a: Anophelines collected in light traps in Jabillos, Caño Lindo and Guaquitas between August 1988-October 1989. The traps were run on a total of 82 nights (984 hours) in each village.

Species	JAB	CLP	GUA	Total
nuneztovari	1,263	995	2,105	4,363
triannulatus	252	5	469	726
albitarsis	181	31	274	486
oswaldoi	64	20	67	151
neomaculipalpus	49	6	47	102
rangeli	52	7	36	95
strodei	12	3	35	50
benarrochi	0	0	1	1
pseudopunctipennis	0	1	0	1
Not identifiable	475	527	659	1,661
Total	2,348	1,595	3,693	7,636

Table 4.5.b: Culicines caught in light traps on 5 nights in September and October 1989.

	JAB	CLP	GUA	Total
Culicines	2,258	1,252	2,725	6,235

FIGURE 4.1: Numbers of anophelines of the four commonest species caught by light-traps, plotted against the numbers caught by human baits

FIGURE 4.1.a: Light trap/Indoor Biting
An. nuneztovari

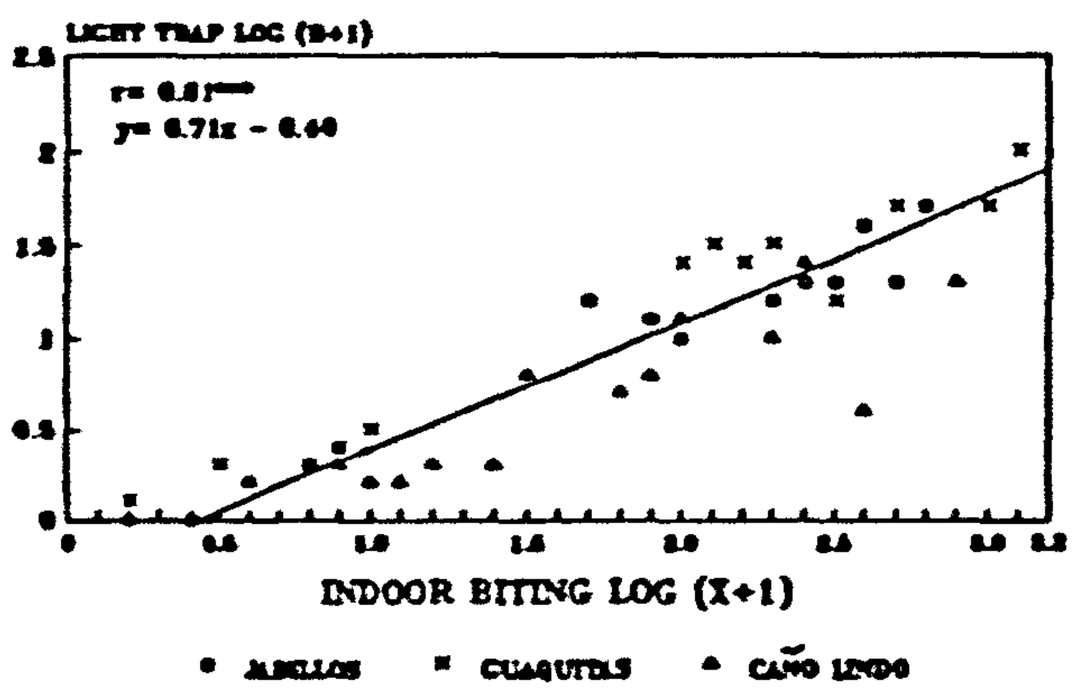


FIGURE 4.1.b: Light Trap/Indoor Biting
An. triannulatus

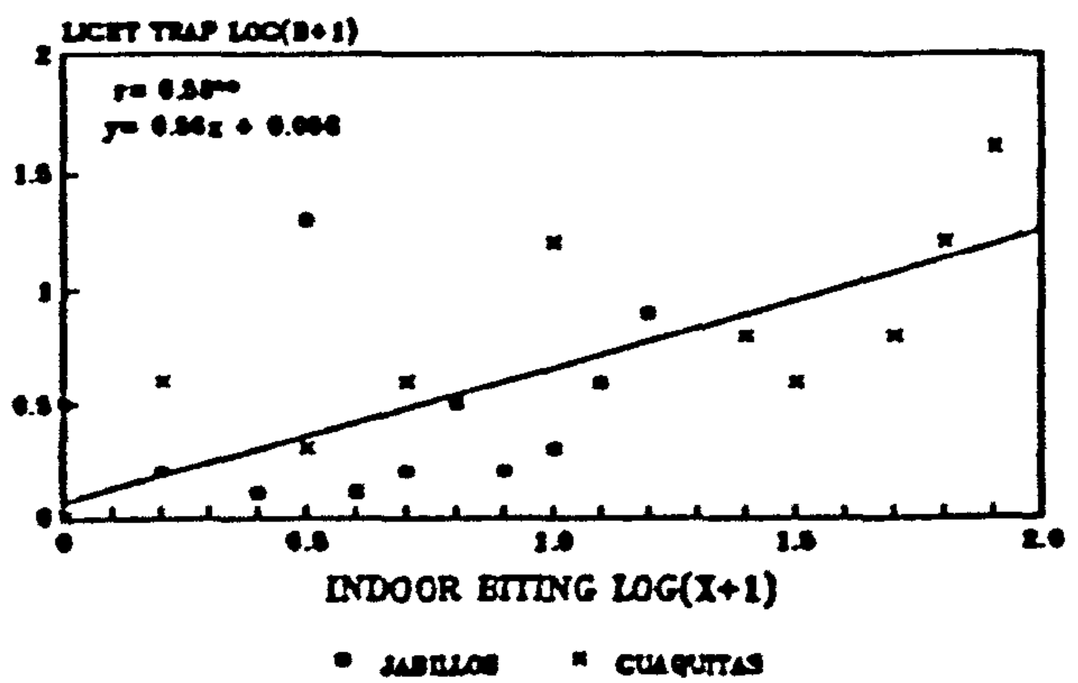


FIGURE 4.1.c: Light trap/Indoor Biting
An. albitarsis

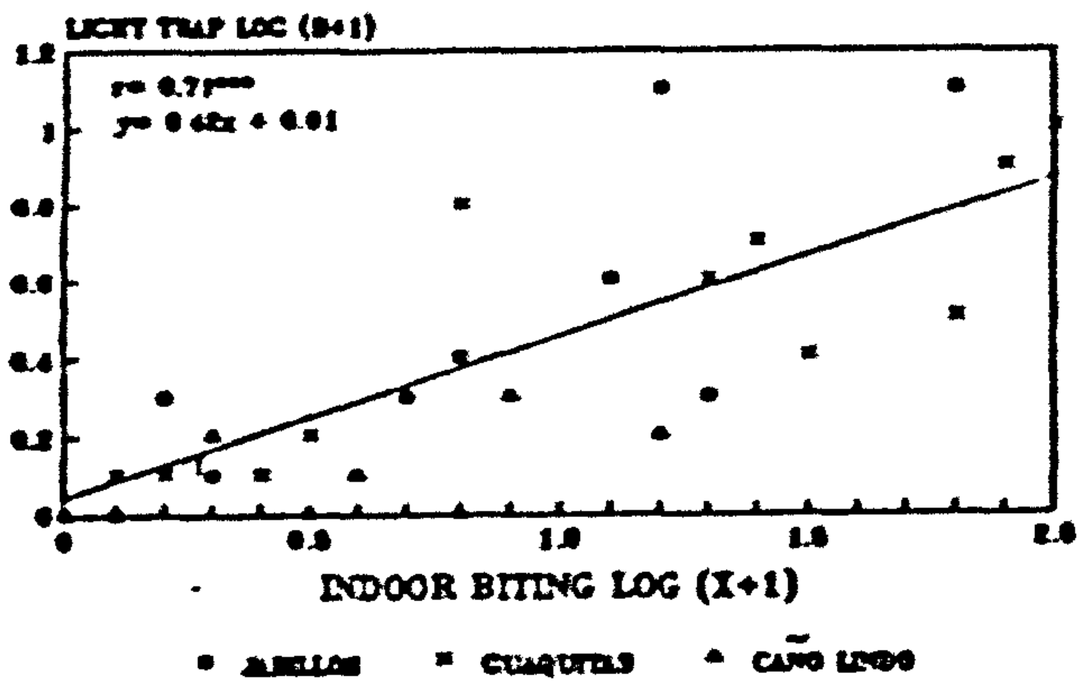
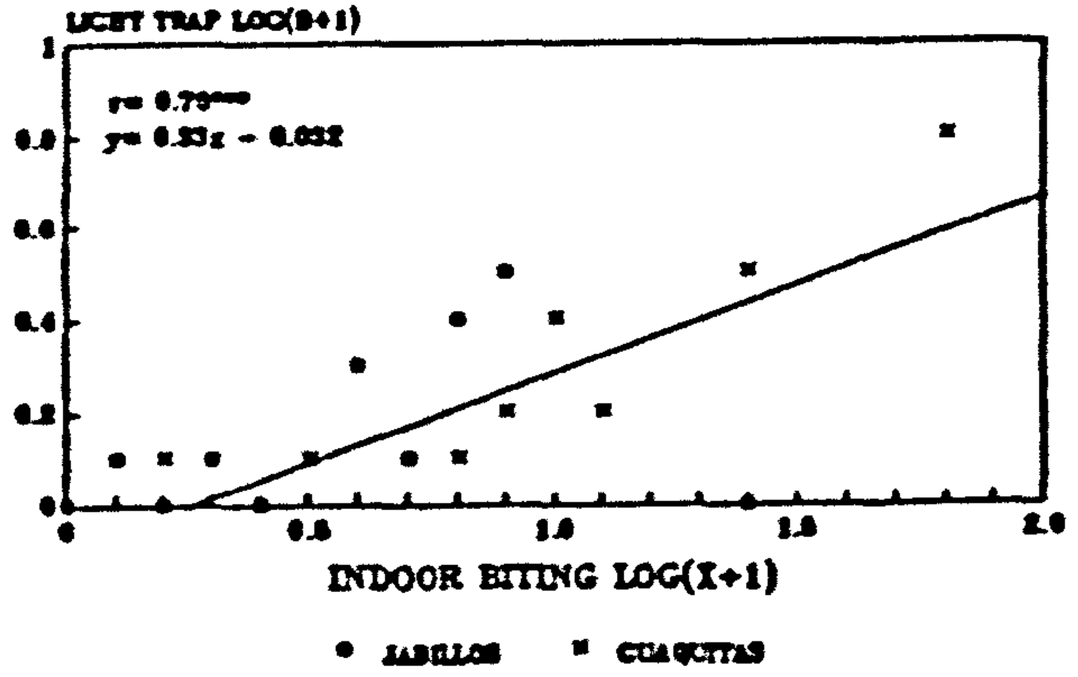


FIGURE 4.1.d: Light Trap/Indoor Biting
An. oswaldoi



In order to determine the efficiency of light-traps compared to indoor biting catches on humans, the monthly mean ratios of light-trap to indoor biting catches were calculated on the log-transformed data for the five most abundant species. The confidence limits were calculated, based on the variance of the log ratios. Figure 4.2 shows the mean ratios for each species, with confidence limits, after back-transformation. Light-traps proved to be particularly inefficient for catching the human biting population of *An. nuneztovari* - the trap only caught 10% as many as the indoor biting catch - but they appeared somewhat more efficient for the human biting population of *albitarsis*, *triannulatus* and *oswaldoi*, and even more efficient for *An. neomaculipalpus*: in Guaquitas the light-trap catch of this species exceeded the human biting catch.

Variation in these ratios between species may be due either to variation in the efficiency of traps for different species or to variations in the human biting tendency of different species after they had entered houses.

To determine if there is a tendency for the light-trap/indoor biting ratio to increase or decrease with increasing mosquito population density, the correlation coefficients of the log-transformed ratios with the log-transformed biting catches were calculated (Table 4.6). There was a tendency in Jabillos for the ratio to increase when the biting populations of *nuneztovari*, *oswaldoi* and *neomaculipalpus* decreased. However, for *triannulatus* and *albitarsis*, the ratio was not significantly dependent on the density of the biting population. In Guaquitas the ratio increased when the populations of *albitarsis* and *oswaldoi* decreased, but the ratio was not dependent on biting population densities for the other species. The same was true at Caño Lindo for *nuneztovari* and *albitarsis*.

To try to clarify this confusing picture, an analysis of variance (Table 4.7) was performed on the light-trap/indoor biting ratios for the five species by month, site and species in two villages (Jabillos and Guaquitas) where good numbers of all the species were caught. This analysis showed that there was a borderline level of significance between species but no significant effect of month or site. Two- and three-way interactions were also not significant. Analysis of variance was also performed for all

FIGURE 4.2: Light Trap as Percentage of Indoor Biting

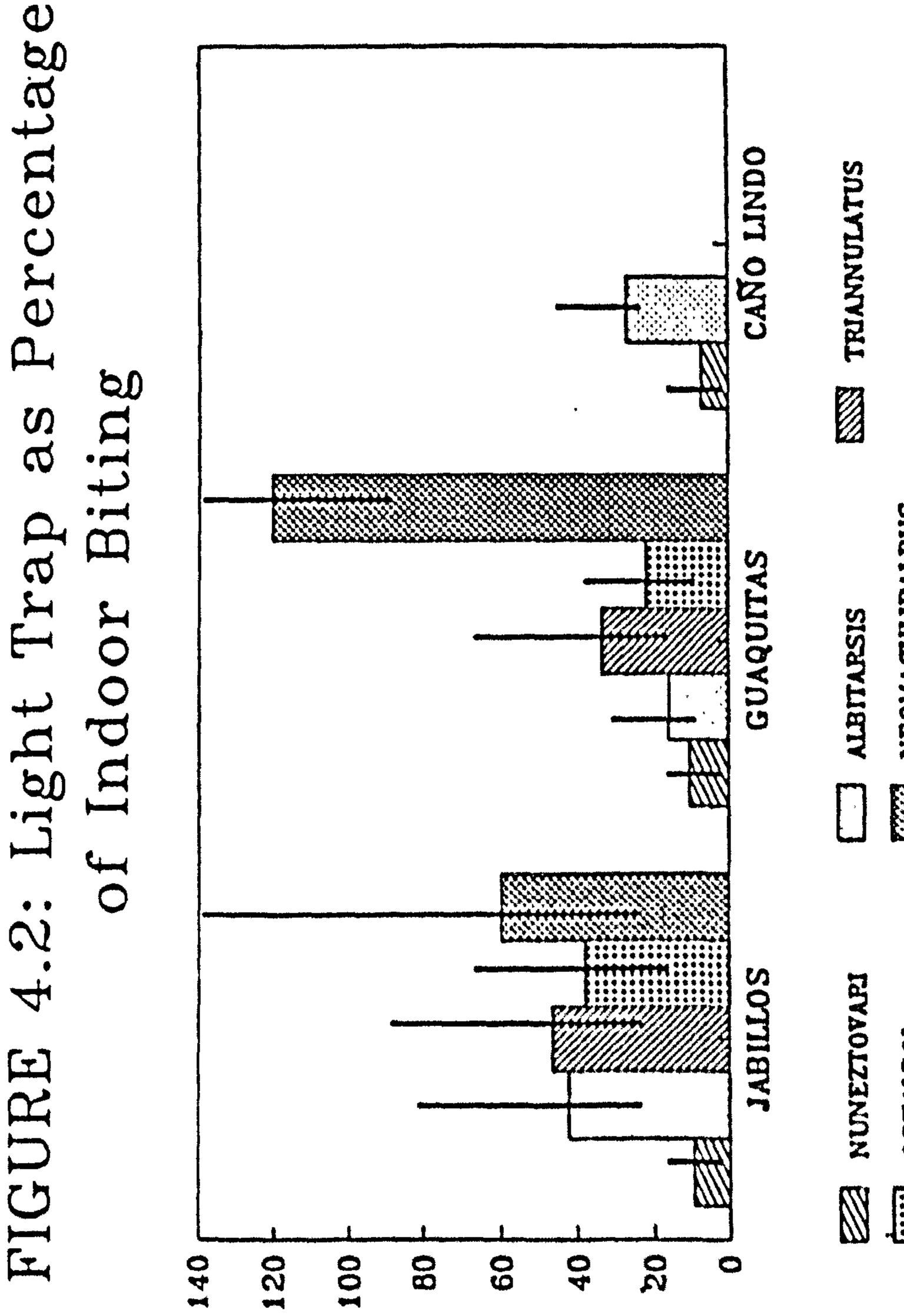


Table 4.6: Correlation coefficients for the biting catch and the log-transformed ratios between light-trap and indoor biting catch (i.e.Log [(LT+1)/(IB+1)] = Log (LT+1) - Log(IB+1)).

Species	Jabillos	Guaquitas	Caño Lindo
nuneztovari	-0.682 p<0.05	-0.254 n.s	-0.488 n.s
albitarsis	-0.578 n.s	-0.643 p<0.05	-0.406 n.s
triannulatus	0.242 n.s	-0.063 n.s	
oswaldoi	-0.837 p<0.01	-0.805 p<0.01	
neomaculipalpus	-0.971 p<0.001	-0.098 n.s	

Table 4.7: Analysis of variance on the log-transformed ratios between the light-trap catches and the indoor human bait catches in two villages (Jabillos and Guaquitas), five species and 9 months.

Source of Variation	Sum of Squares	DF	Mean Square	F	p
Main Effects					
Month	3.402	8	0.425	33.223	0.133
Species	8.469	4	2.117	165.403	0.058
Site	0.571	1	0.571	44.646	0.095
2-way Interaction					
Month x Species	5.638	32	0.176	13.766	0.211
Month x Site	2.436	8	0.304	23.786	0.157
Site x Species	1.969	4	0.492	38.452	0.120
3-way Interactions					
Month x Species x Site	5.192	31	0.167	13.084	0.216
Residual	0.013	1	0.013		
Total	27.671	89	0.311		

Table 4.8: Analysis of variance on the log-transformed ratios between the light-trap catches and the human-bait catches in the three villages of *An. nuneztovari* and *An. albitarsis*

Source of Variation	Sum of Squares	DF	Mean Square	F	p
Main Effects					
Month	1.264	8	0.158	12.348	0.217
Species	3.244	1	3.244	253.451	0.040
Site	0.678	2	0.339	26.482	0.136
2-way Interaction					
Month x Species	1.122	8	0.140	10.958	0.230
Month x Site	1.486	16	0.093	7.254	0.285
Species x Site	0.329	2	0.165	12.864	0.193
3-way Interactions					
Month x Species x Site	1.310	15	0.087	6.822	0.293
Residual	0.013	1	0.013		
Total	9.495	53	0.179		

three sites for *An. nuneztovari* and *An. albitarsis* which were caught in good numbers in all of the sites (Table 4.8). Results showed that there was significant variation between species but no significant effect of month or site; also the interactions were not significant. It is concluded that the indications in Figure 4.2 of variation between species in the light-trap/human biting ratio are correct. However, the suggestions from Table 4.6 and Figure 4.2 of other sources of variation in the ratio are not substantiated by the analysis of variance.

During October 1989, the culicines collected during one night of collection in each village on human baits and in two of the light-trap collections in each village, were counted and the light-trap:indoor biting ratio calculated. Table 4.5.b summarises the data and suggests that the light trap caught culicines much more efficiently than did biting catches (Table 3.9.b). To check this, the ratio was calculated of each light-trap catch and its corresponding human biting catch. Figure 4.3 shows these ratios for each village. The ratios varied widely, especially in Caño Lindo, but always exceeded 1.0, i.e. more culicines were caught in light traps than on human baits. This contrasts with the anopheline data where only for *An. neomaculipalpus* did the ratio ever approach or exceed 1.0.

In order to determine whether the light-trap collections reflected the biting activity pattern throughout the night for *An. nuneztovari*, the proportion of the night's collection which was obtained in each 4-hour interval on human baits and in light traps in each of the three villages was calculated (Table 4.9) and analysis of variance on the arcsine-transformed data was carried out (Tables 4.10 - 4.15). Results are shown only for the first and the last third of the night, i.e. between 1900 and 2300 hrs and 0300 and 0700 hrs. The analysis showed that for the first 4 hours of the night the effect of month and method was not significant, and there were no significant interactions. However, for the third part of the night in Caño Lindo, the effects of month, method and their interaction were highly significant. This follows from the fact, as shown in Table 4.9, that the highest proportion of *nuneztovari* were collected in light traps during the third part of the night, except in November 1988 when no mosquitoes were caught in the four hours before

FIGURE 4.3: CULICINES
Light Trap/Indoor Biting Ratio

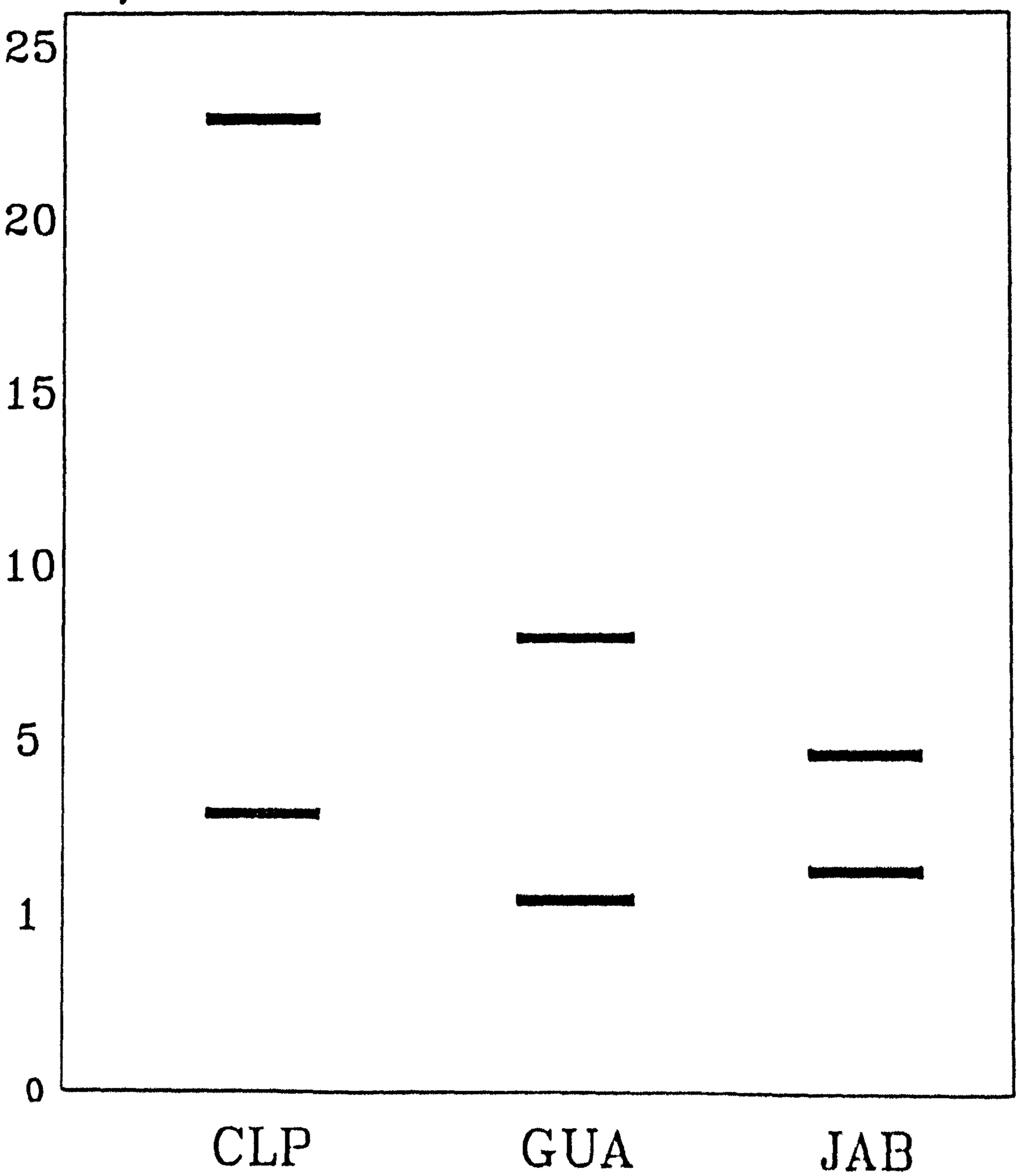


Table 4.9: Mean proportion of *An. nuneztovari* collected in light-traps and on human baits in each 4 hour segment of the night each month in the three villages.

	CAÑO LINDO			GUAQUITAS			JABILLOS		
MONTH	1900 -2300	2300 -0300	0300 -0700	1900 -2300	2300 -0300	0300 -0700	1900 -2300	2300 -0300	0300 -0700
Human-bait									
Sept.'88	0.304	0.522	0.174	0.169	0.526	0.305	0.361	0.582	0.057
Oct.	0.440	0.520	0.040	0.235	0.426	0.139	0.332	0.381	0.287
Nov.	0.512	0.290	0.198	0.286	0.466	0.248	0.361	0.328	0.311
Dec.	0.535	0.310	0.155	0.441	0.350	0.209	0.550	0.372	0.078
Jan.'89	0.283	0.459	0.258	0.392	0.405	0.203	0.232	0.633	0.135
Jul.	0.400	0.356	0.244	0.303	0.442	0.255	0.287	0.420	0.293
Aug.	0.311	0.475	0.214	0.246	0.381	0.373	0.214	0.667	0.119
Sept.				0.222	0.427	0.351	0.513	0.394	0.093
Oct.				0.230	0.301	0.469	0.337	0.426	0.237
Light-trap									
Sept.'88	0.200	0.200	0.400	0.285	0.331	0.384	0.167	0.050	0.783
Oct.	0.000	0.000	1.000	0.526	0.069	0.405	1.000	0.000	0.000
Nov.	0.495	0.495	0.000	0.706	0.228	0.066	0.075	0.262	0.663
Dec.	0.286	0.250	0.464	0.405	0.300	0.295	0.641	0.321	0.038
Jan'89	0.138	0.264	0.598	0.000	0.495	0.495	0.614	0.386	0.000
Jul.	0.125	0.263	0.612	0.050	0.413	0.537	0.250	0.000	0.750
Aug.	0.120	0.446	0.434	0.339	0.521	0.140	0.230	0.600	0.170
Sept.				0.762	0.048	0.190	0.476	0.399	0.125
Oct.				0.434	0.184	0.382	0.676	0.287	0.037

Table 4.10: Analysis of variance of the proportion of the *An. nuneztovari* in Caño Lindo that were collected between 1900 and 2300 hours by method (light-trap and indoor biting) and month (data were arcsine-transformed).

Source of Variation	Sum of Squares	DF	Mean Square	F	p
Main Effects					
Month	0.354	6	0.059	0.375	0.880
Method	0.559	1	0.559	3.553	0.086
2-way Interaction					
Month x Method	0.207	6	0.035	0.219	0.962
Residual	1.731	11	0.157		
Total	2.826	24	0.118		

Table 4.11: Analysis of variance of the proportion of the *An. nuneztovari* in Guaquitas which were collected between 1900 and 2300 hours by method (light-trap and indoor biting) and month (data were arcsine-transformed).

Source of Variation	Sum of Squares	DF	Mean Square	F	p
Main Effects					
Month	1.049	8	0.131	1.395	0.271
Method	0.042	1	0.042	0.444	0.514
2-way Interaction					
Month x Method	0.961	8	0.120	1.278	0.321
Residual	1.504	16	0.094		
Total	3.529	33	0.107		

Table 4.12: Analysis of variance of the proportion of the *An. nuneztovari* in Jabillos which were collected between 1900 and 2300 hours by method (light-trap and indoor biting) and month (data were arcsine-transformed).

Source of Variation	Sum of Squares	DF	Mean Square	F	p
Main Effects					
Month	1.120	8	0.140	1.379	0.274
Method	0.031	1	0.031	0.309	0.586
2-way Interaction					
Month x Method	1.078	8	0.135	1.329	0.295
Residual	1.725	17	0.101		
Total	3.942	34	0.116		

Table 4.13: Analysis of variance of the proportion of the *An. nuneztovari* in Caño Lindo which were collected between 0300 and 0700 hours by month and method (light-trap and indoor biting) (data were arcsine-transformed).

Source of Variation	Sum of Squares	DF	Mean Square	F	p
Main Effects					
Month	0.657	6	0.110	10.562	<0.001
Method	0.639	1	0.639	61.607	<0.0001
2-way Interaction					
Month x Method	1.294	6	0.216	20.794	<0.0001
Residual	0.114	11	0.010		
Total	2.693	24	0.112		

Table 4.14: Analysis of variance of the proportion of the *An. nuneztovari* in Guaquitas which were collected between 0300 and 0700 hours by method (light-trap and indoor biting) and month (data were arcsine-transformed).

Source of Variation	Sum of Squares	DF	Mean Square	F	p
Main Effects					
Month	0.341	8	0.043	0.219	0.982
Method	0.004	1	0.004	0.019	0.892
2-way Interaction					
Month x Method	0.444	8	0.055	0.285	0.961
Residual	3.116	16	0.195		
Total	3.902	33	0.118		

Table 4.15: Analysis of variance of the proportion of the *An. nuneztovari* in Jabillos which were collected between 0300 and 0700 hours by method (light trap and indoor biting) and month (data were arcsine-transformed).

Source of Variation	Sum of Squares	DF	Mean Square	F	p
Main Effects					
Month	2.036	8	0.255	6.000	0.001
Method	0.052	1	0.052	1.216	0.285
2-way Interaction					
Month x Method	1.636	8	0.205	4.822	0.003
Residual	0.721	17	0.042		
Total	4.451	34	0.131		

dawn but they were caught earlier in the night. By contrast in human bait collections the opposite was observed, i.e. in every month the smallest proportion of mosquitoes was collected during the third part of the night. In Guaquitas no significant differences were found for month, method or their interactions during the 4-hour interval between 0300 and 0700 hours (Table 4.14). In Jabillos, there were significant differences in the proportion of *nuneztovari* collected between 0300 and 0700 hours in different months but the difference between methods was not significant; the interaction month x method was significant (Table 4.15).

These results suggest that the use of light-traps to sample *An. nuneztovari* in western Venezuela will not give an exact representation of the biting pattern of this species during the night.

4.3.3. PAROUS RATE

A total of 964 females collected in light-traps was dissected. Of these 73% were *An. nuneztovari*. A larger number of anophelines (1,497) was dissected from human bait collections (see Chapter 3).

The parous rate for *An. nuneztovari, albitarsis* and *triannulatus* are shown in Table 4.16. For each species the significance of the differences observed between these data and corresponding human biting samples were tested by a Mantel-Haenszel chi-squared test (see e.g. Kirkwood, 1988). This statistical method is appropriate since there may well be heterogeneity in the parity between different samples caught by each method. The data were stratified by season (wet and dry) and village, resulting in 9 separate 2x2 contingency tables for each species.

As shown in Table 4.16 the parous rate was significantly higher in the human biting sample than in the light traps for *An. nuneztovari*, but not for *albitarsis* and *triannulatus*. Thus, light-traps would not give an exact representation of the parous rate in the human biting population of *An. nuneztovari* in this area.

Table 4.16: Parity rate in the three commonest species caught by light-traps and human-baits (sample sizes in parentheses). The significance of the differences between the sample caught by the two methods is tested by Mantel-Haenszel chi-squared test stratifying by village and season.

	Human	Light-trap	X^2_{M-H}	p
nuneztovari	34.2% (1,149)	28.9% (702)	5.14	0.02
albitarsis	44.3% (133)	31.2% (93)	3.16	0.08
triannualtus	48.1% (106)	45.3% (106)	0.17	0.919

The parous rate in corresponding catches by the two types of catch are plotted against each other for *nuneztovari*, *albitarsis* and *triannulatus* (Fig. 4.4). In no case was the correlation coefficient significant.

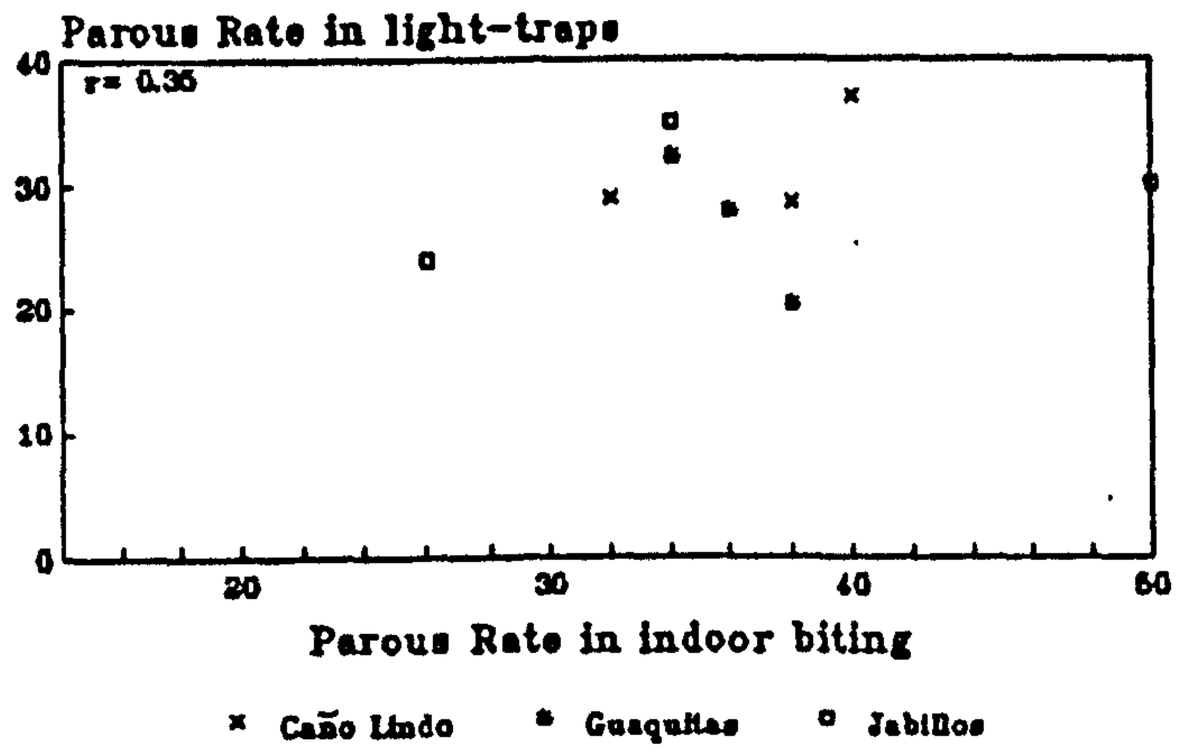
Among the anophelines dissected that were caught in light-traps 18% were blood-fed. This indicates that light-traps not only attract host-seeking anophelines but also some of those already fed either totally or partially.

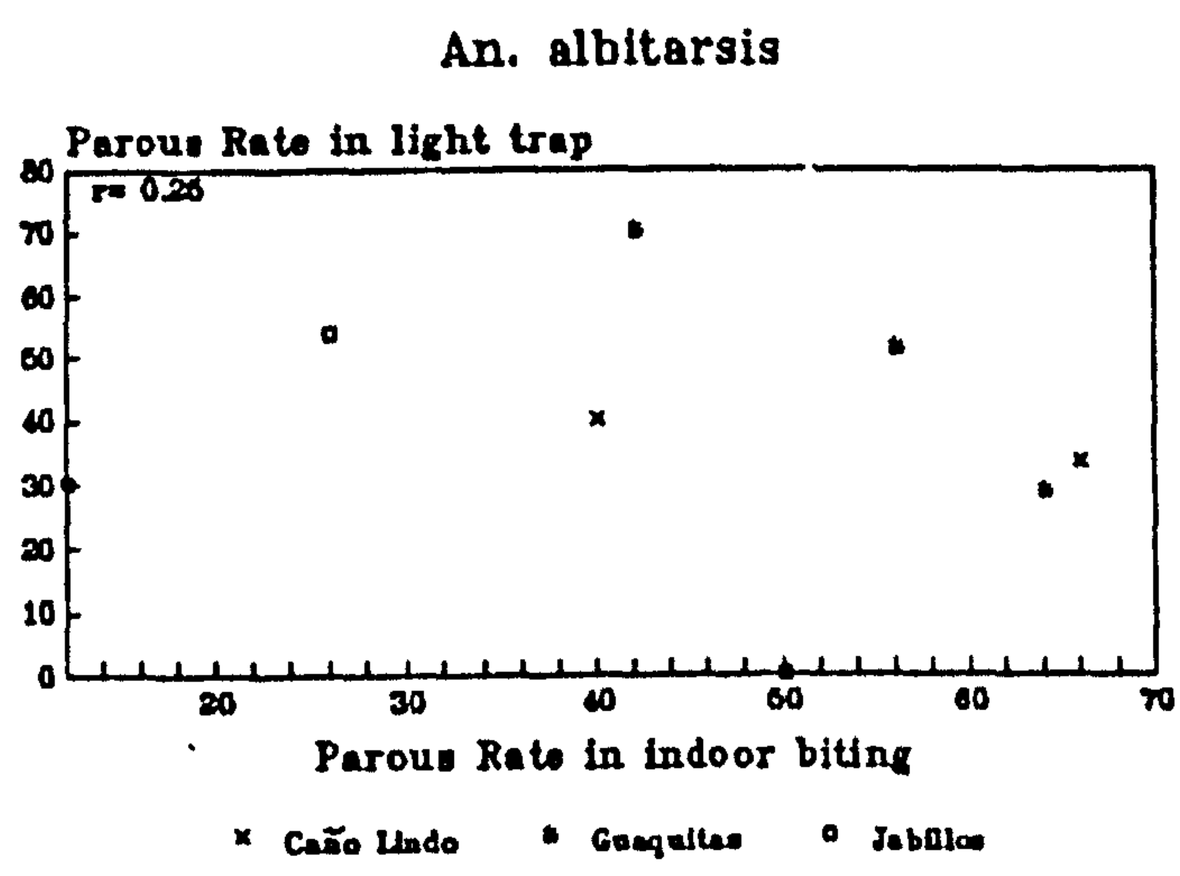
4.4. DISCUSSION

Light-traps have been shown in several studies to be a useful method for entomological evaluation of malaria control programmes. In the present study however, light-traps proved to be relatively inefficient for sampling anophelines, especially *An. nuneztovari*, in that the numbers caught and parous rate were significantly lower than in human bait catches. Similar results were reported for *An. gambiae* by Carnevale and Le Pont (1973) and *An. nili* (Carnevale, 1974). Nevertheless, Lines *et al.* (1991) found a good correlation of the age structure of *An. gambiae* for light-trap compared to human biting catches. Also, in the present study light-traps failed in one village to show the biting pattern of *An. nuneztovari*. Another disadvantage of light-traps for sampling anophelines in this part of the country is that a considerable percentage of mosquitoes were unidentifiable probably due to damage inflicted by the fan.

An. nuneztovari is the most abundant anopheline species in western Venezuela and the incriminated malaria vector (Gabaldón & Guerrero, 1959; Pintos & Sabril, 1965; Pintos *et al.*, 1968). Any method used to sample its population must produce results comparable to the human biting population in which we are interested.

Despite the present conclusions, traps should be further evaluated considering for instance, different types of lights or odour baits, in the search for a satisfactory method of sampling anophelines that would allow one to reduce the use of human baits for routine evaluation of control programmes, especially in areas of southern Venezuela where *P. falciparum* resistant to chloroquine is the main parasite (Dirección de Endemias Rurales, Report 1989).





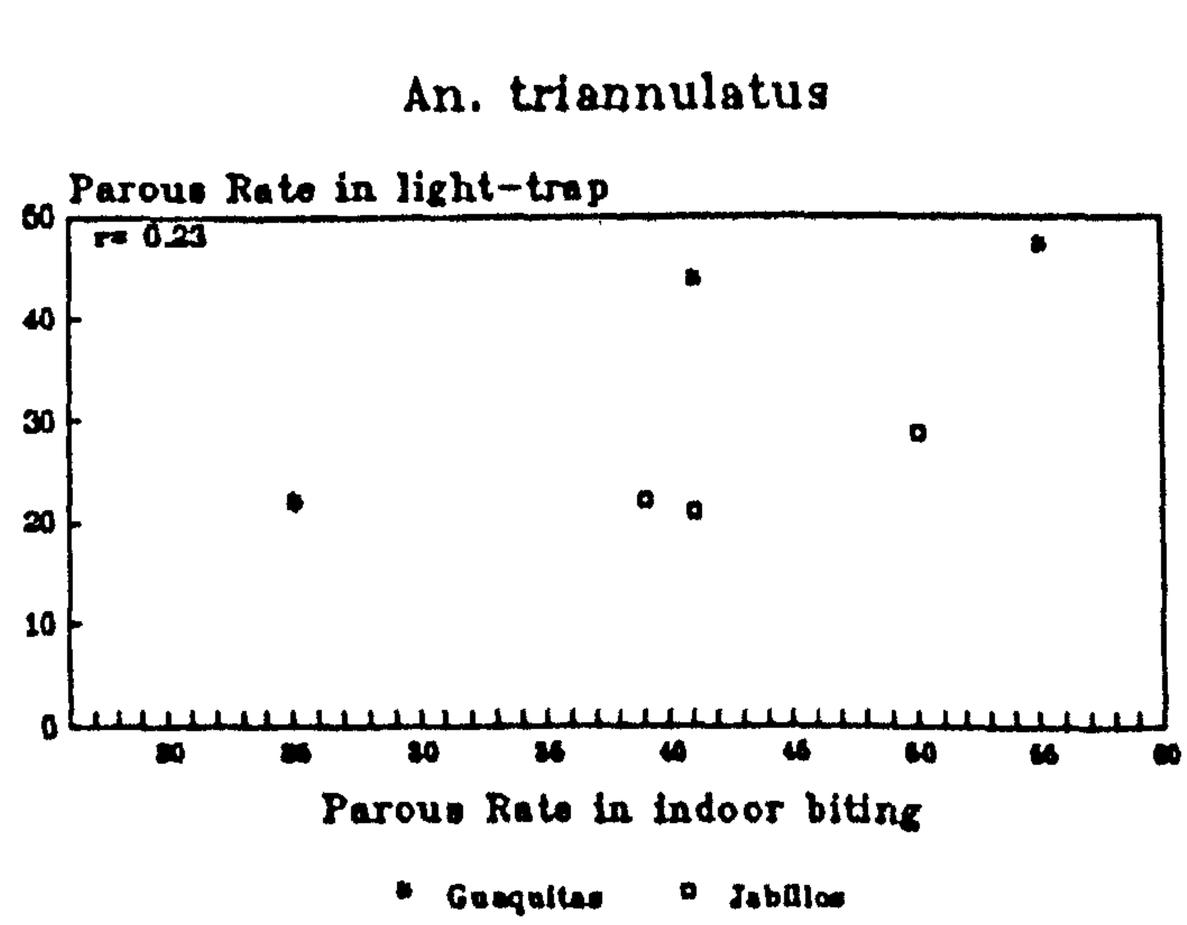


FIGURE 4.4: Relationship of parous rates collections in light-traps and on human baits in different months and villages

Several studies have shown that mosquitoes, and other blood-feeding arthropods, find the host by olfaction. Carbon dioxide, used alone or in conjunction with CDC light-traps, has been widely used to sample mosquito populations (Service, 1976). In general, it is found that the number of mosquitoes collected is higher when carbon dioxide is used together with a light-trap than when a light-trap is operated alone (Newhouse *et al.*, 1966; Carestia & Savage, 1967), and some studies have even demonstrated that catches with CDC light-traps baited with dry ice can closely resemble the human biting collections (Parsons *et al.*, 1974; Slaff *et al.*, 1983). More recently, Takken and Kline (1989) used carbon dioxide and octenol, which are components of ox breath, to study their attractant effect on mosquito populations in Florida. Results showed that both compounds acted synergistically in attracting a greater number of *Aedes taeniorhynchus*, *Anopheles* spp. and *Wyeomyia mitchellii* than either bait used alone. They also reported that the response of *Culex* spp. to octenol was less pronounced. Odour baits dispensed from sachets made of low-density polyethylene were developed for baiting of tsetse traps (Hall *et al.*, 1984). These were used by Yuan (1990) to study the attractant effect of octenol, a mixture of 4-methylphenol:octenol:3-n-propylphenol and acetone to catch *Anopheles gambiae* and *Aedes aegypti* released into a room. He reported that sachets containing octenol and the mixture attracted very few *An. gambiae*, whereas a lower concentration of octenol in paraffin oil attracted similar numbers to those with acetone. Furthermore, acetone and carbon dioxide acted synergistically in attracting significantly greater numbers of *An. gambiae* than either chemical on its own. Nevertheless, the attraction of these chemical odours could not compete with that of a guinea pig.

The use of artificial odours in conjunction with CDC light-traps to sample anophelines offers possibilities that should be evaluated. Nevertheless its implementation for monitoring the malaria control programme in Venezuela, and in general in Latin America, seems unfeasible due to the apparent need to include solid or gaseous carbon dioxide in any effective mixture of odour, and the difficulties of transporting either the

solid or the gas to remote areas. A more realistic approach would be to evaluate light-traps using different types of lights.

In the past 20 years, ultraviolet light traps have been used with some success in mosquito studies. Service (1970) made trials in Nigeria and Kenya with Monks Wood light-traps using white and ultra-violet light. He reported that 3 to 4 times more *An. gambiae*, *An. funestus* and *Cx. pipiens* were caught with the Monks Wood trap than with CDC traps. Nevertheless, the mean number of females caught in the Monks Wood trap using white light and that in the trap with ultra-violet light were not significantly different. Chandler *et al.* (1976) used three different methods to collect anophelines inside houses in Kenya: Monks Wood traps, CDC light-traps and modified CDC traps using ultraviolet light. They found significant differences for the species collected by each trap and considered the ultraviolet/CDC trap to be the most effective for collecting mosquitoes indoors, mainly *An. gambiae* and *An. funestus*. In Haiti, Sexton *et al.* (1986) found that an up-draught ultra-violet light-trap was very effective for catching *An. albimanus*. More recently, B. Jana (1991, pers. comm.) found in a small field trial in north-eastern India, that more *An. minimus* were caught in a modified CDC trap using ultraviolet light than in a standard CDC light trap.

CHAPTER 5:

DOUBLE-NET CATCHES

5.1 INTRODUCTION

Double-nets baited with either humans or animals have been used to collect mosquitoes (WHO, 1975; Service, 1976). The method has the advantage of being an easy and cheap way of collecting mosquitoes attracted to humans and one that greatly reduces the risk that a human bait will contract malaria. The original double-net design by Gater (1935, *in* Service, 1976) consisted of a large net (10 ft long x 7 ft wide x 7 ft high) with two entrances in the longer sides with the bait enclosed in a smaller inner net. This design has been modified by various authors depending on the purpose of the studies: for example it may feature smaller outer nets, nets with one opening or simply raised a few centimetres from the ground (Service, 1976), or nets used in conjunction with an inverted CDC light trap (Charlwood *et al.*, 1986).

5.2. MATERIALS AND METHODS

In order to determine whether double-nets could be used in the study area to sample anophelines attracted to man, 12-hour (1900-0700 hrs) double-net collections were carried out in the experimental huts using as bait a person in a hammock. The bottom of the outer net was raised about 15 centimetres from the ground to allow entry of mosquitoes. Both the inner and outer net were pierced at each end to accommodate ropes that suspended the hammock. Both nets were hung from a string parallel to and above the hammock; holes had to be cut in the outer net for the strings supporting the inner net. The outer net was held out laterally from the inner net with a stick about 1 m long. Searches for specimens trapped between the two nets were made hourly by a second person with a torch and mouth aspirator. Bait and catcher were exchanged every 6 hours.

5.3. RESULTS AND DISCUSSION

A double-net collection conducted outdoors in Jabillos in June 1988 (wet season) for 3 hours failed to catch any mosquitoes whereas 50 mosquitoes were caught in the same period by a human biting catch. In the following month, collections were made indoors for 12 hours in Jabillos and Caño Lindo. In 36 hours of collection only 3 anophelines were collected whereas 1,237 were collected in the contemporary human biting catches.

The double-net method proved to be ineffective for collecting anophelines in western Venezuela, and was abandoned after obtaining these results. Similar results were reported by Hamon (1964), Wilton *et al.* (1985) and Charlwood *et al.* (1986). Akiyama (1973) conducted field trials using human baited traps and human biting catches to assess the density of *An. culicifacies* in a village in Pakistan. Although Akiyama (1973) does not specify whether the men inside the trap were protected by a net, he found that very few mosquitoes, and no *An. culicifacies*, were collected on human baits whereas 2,151 mosquitoes of five species were collected in the man-baited trap. 6.7% of the specimens collected were *An. culicifacies*. Akiyama (1973) concluded that human baited nets were not sampling the man biting population since the collections included males, half-gravid and gravid females and freshly fed females. He also found that all the blood-fed specimens were positive for bovine blood, which means that such mosquitoes had entered the human baited trap to rest inside it and had not been attracted by the human bait. More recently, Wilton *et al.* (1985) reported that far fewer mosquitoes (464) were collected in Colorado, USA in a double-net trap with a human than the number collected inside a trap baited with a horse (2,080) or in light traps (2,532). However, double-nets using as bait a man, a calf or a goat were used successfully in Malaya by Reid (1961) to compare the attraction of mosquitoes to these baits. Apparently this method was also routinely used in Japan to monitor the populations of vectors of Japanese Encephalitis, but it is not clear whether the human baits were protected by nets (Wada *et al.*, 1967, 1970).

CHAPTER 6:

CALF-BAITED TRAP CATCHES

6.1. INTRODUCTION

Animal-baited traps have been used to detect presence and relative abundance of mosquitoes attracted to animals, to evaluate insecticide-spraying campaigns and to collect mosquitoes for other studies, such as susceptibility tests (WHO, 1975). Different methods have been used to collect mosquitoes attracted to animals other than man: stable-traps, tethered animals and nets (WHO, 1975; Service, 1976). Tethered animals such as horses, donkeys and calves have been used outdoors in Latin America to collect anophelines. In Brazil, Davis and Kumm (1932) and Deane *et al.* (1948) reported that *An. albitarsis* and *An. nuneztovari* were mainly collected feeding on animals. Gabaldón (1949) reported that on several different occasions in Venezuela *An. albitarsis* was the most abundant species caught in animal-baited traps. In El Salvador, Lofgren *et al.* (1974) reported that calf-baited traps were unproductive in catching *An. albimanus*. Nevertheless, some years later Lowe and Bailey (1981) designed and used a portable calf-baited trap to collect *An. albimanus* in El Salvador. They concluded that this type of trap was useful for estimating anopheline population densities and for providing live adults for insecticide bioassays. The calf-baited trap was more efficient compared to the standard method used in Central America which entails collecting *An. albimanus* from stables.

Towards the end of the field studies during the present project, a calf-baited trap was used in order to determine whether a calf compared with human baits or light traps would attract the same or different species of anophelines.

6.2. MATERIALS AND METHODS

Between September and October 1989 a calf-baited trap was used in Jabillos between 1900 and 0600 hrs. The trap consisted of a small wooden pen (180 by 120 cm) in which a calf was kept (Fig. 6.1). The pen was covered by a netting roof attached to canvas "walls" which terminated 20 cm above the floor. The trap was set on a roofed *patio* with a cement floor near a cattle-shed. The net was raised about 20 cm from the ground to allow entry of mosquitoes. In the morning, trapped mosquitoes were collected with a large battery-operated aspirator. This aspirator was designed and fabricated primarily to collect resting mosquitoes on vegetation (see Chapter 7) and consisted of a long tube of PVC (14 cm in diameter and 125 cm long), and a small fan operated by a 12-volt motorcycle battery. Later this battery was substituted by two 6-volt rechargeable batteries connected in series which contained gel rather than liquid acid and were therefore more conveniently portable. Mosquitoes were accumulated in the aspirator in removable plastic containers (13.5 cm in diameter and 16 cm long) with lids (Fig. 6.2). After removal from the aspirator containers were placed in polystyrene boxes, covered with wet towels and taken to the field laboratory. Mosquitoes were killed and identified as previously described.

Jabillos was selected for testing the trap for various reasons, such as availability of a calf for an extended period, willingness of the owner to introduce the calf into the pen and lower the net a few minutes before 1900 hours, and security for the calf and net from thieves.

FIGURE 6.1: Calf-baited trap consisting of a small wooden pen

FIGURE 6.2: Aspirator operated by two 6 volt batteries connected in series

6.3. RESULTS AND DISCUSSION

Table 6.1 shows the numbers and species collected from the calf-baited trap during 13 nights at Jabillos. The trap caught all 4 of the main human-biting species, but relatively few *nuneztovari* and many *triannulatus*. A total of 69 anophelines was collected of which 4, or 5.8%, were unidentifiable. During the four human bait catches in September and October 1989, a total of 1,423 anophelines was collected, i.e. a yield per night which was 67x greater than in the calf trap. This may either be due to the fact that anophelines in western Venezuela are less attracted to bovines than to humans, or because the trap, as operated, allowed many mosquitoes to escape. In any further evaluation of this method to sample anophelines the mosquitoes should be collected at 2- or 4-hour intervals in order to reduce the chances of the mosquitoes escaping.

During 48 hours of use of the calf trap, 39 culicines were caught, while only 16 anophelines were caught in the same period. Chapter 3 indicates that the anopheline:culicine ratio on human baits was between 2:1 and 4:1 depending on the village, i.e. the local culicines tended to be more zoophilic than the local anophelines.

Table 6.1: Anophelines in a calf-baited trap in Jabillos in all-night collections in September and October 1989.

Species	Number
nuneztovari	20
albitarsis	13
triannulatus	29
oswaldoi	3
Unidentifiable	4
Total	69

CHAPTER 7:

CATCHES OF RESTING MOSQUITOES

7.1. INTRODUCTION

Mosquitoes seem to spend most of their time resting either inside human dwellings or outdoors in many types of natural or man-made shelters. Collection of resting mosquitoes is the most effective method of obtaining blood-fed specimens for studies of host choice.

Some anopheline species, after taking a blood meal, rest inside houses and are regarded as endophilic species. Such is the case for *An. gambiae* and *An. funestus* in East Africa (Gillies, 1954; Gillies & Smith, 1960; Lines *et al.*, 1986) and *An. minimus* in northeastern India (Muirhead Thomson, 1941). But most anophelines rest exclusively outdoors in natural resting places such as animal burrows, tree trunks, cracks and crevices in the ground and vegetation (Service, 1976). Some species are found resting on man-made sites such as bridges, fences, walls etc. (Service, 1976).

Neotropical anophelines are mainly exophilic, and in general little is known about their natural resting sites. Detailed studies have been conducted in El Salvador by Breeland (1972 a & b) to determine the natural resting places of *An. albimanus* and *An. pseudopunctipennis*. These species were collected during the day resting in rock crevices, tree holes and ground holes (Breeland, 1972 a & b). In the evening, *An. albimanus* has been found in large numbers resting on walls and fences of cattle pens in El Salvador (Breeland, 1974). Less is known about other species.

In 1951, Cova García published a compilation of the bionomic data collected between 1938 and 1945 for 19 anopheline species in different regions of Venezuela. The only information he provides about resting places relates to light conditions. He mentioned that all these species were found in dark, shaded and well-lit places, but gave no further details.

In order to obtain blood-fed specimens to determine the natural host choice of anophelines in the study area, mosquitoes resting on vegetation around houses were collected during the present study.

7.2. MATERIALS AND METHODS

Resting mosquito collections were standardized as follows: indoor resting mosquitoes were searched for inside the experimental huts and collected with a mechanical aspirator (Hausherr's Machine Works, New Jersey, USA) between 0600 and 0610 hours. Outdoors, mosquitoes were collected with a large (14 cm in diameter and 125 cm long) 12-volt battery-operated aspirator (described in Chapter 6) by sweeping vegetation in the villages within an area of radius about 1 km around the experimental huts between 0610 and 0800 hrs on 4 days per month at each village over a period of 14 months. Mosquitoes were trapped inside a large plastic cup (20 cm x 12 cm) which was changed every half hour. Temperature in resting places was recorded by placing a thermometer on or near the ground every half hour. Mosquitoes were kept in a cool environment inside a polystyrene box in order to stop, or at least delay, blood digestion. Mosquitoes were taken to the laboratory, killed either by freezing or with chloroform. Female mosquitoes were identified under the dissecting microscope, counted and kept dry over silica gel for future blood-meal identification by ELISA (Chapter 8). The male anophelines collected were stored over silica gel for future use as negative controls in the ELISA assays.

7.3. RESULTS AND DISCUSSION

7.3.1. NUMBER AND SPECIES COLLECTED

Between August 1988 and September 1989, 2,470 anophelines of 8 species were collected at the three sites (Table 7.1). The number of anophelines unidentifiable represented 13.4% of the total collected. Only three specimens, identified as *An. nuneztovari*, were found in all collections inside huts in the mornings. Before the construction of the experimental huts, searches carried out inside some houses in Jabillos

Table 7.1: Anophelines collected resting outdoors in Jabillos, Caño Lindo and Guaquitas between August 1988 and September 1989.

Species	JAB	CLP	GUA	Total
An. nuneztovari	79	31	686	796
An. albitarsis	41	39	13	93
An. triannulatus	126	1	803	930
An. strodei	1	0	24	25
An. rangeli	7	1	49	57
An. oswaldoi	16	2	154	172
An. neomaculipalpus	47	5	14	66
An. argyritarsis	0	1	0	1
Unidentifiable	21	16	293	330
Total	338	96	2,036	2,470

yielded no anophelines. This result confirmed previous observations that most anophelines (Service, 1976) and certainly those of the subgenus *Nyssorhynchus* (Deane *et al.*, 1948) are exophilic. More *triannulatus* than *nuneztovari* were collected on vegetation around houses. In general very few mosquitoes were collected in Caño Lindo where there is more grass and fewer shrubs around houses than in the other two villages. It is likely that in this village mosquitoes rested in the forest which is patchily distributed and close to houses: in some cases as close as 50 m. Such is the case of house No. 62 where very large numbers of mosquitoes were caught in the light trap (Chapter 4). In Jabillos the most productive resting places were gardens and small plantain plots around houses. In Guaquitas, more resting mosquitoes were collected from shrubs some 30 m from the experimental hut. It is noteworthy that during the study only a few mosquitoes were collected in Guaquitas resting on dense vegetation along the stream.

Temperatures recorded in resting places between 0600 and 0800 hours during the study were remarkably stable, varying only between 22.2 and 24.2 °C, except in December 1988 when 16.5 °C was recorded.

Figures 7.1, 7.2 and 7.3 show the mean number of anophelines collected with the aspirator monthly for the four commonest species in the three villages. In general, fluctuation in the mosquito resting population is related to rainfall, showing a build-up in July, two months after the onset of the rains. This seems to indicate that temperature had no effect on the mosquito resting population. Fluctuation of the mosquito resting population follows a pattern similar to that of the biting population (Chapter 3), except for *An. triannulatus* in Jabillos (Fig. 7.1) which showed a resting peak in January. Very few specimens belonging to other species were collected in January in Jabillos. One may speculate that this was due to chance encounters with concentrations of resting *triannulatus*.

FIGURE 7.1: Mean numbers collected resting on vegetation in Jabillos.

FIGURE 7.1.a: An. nuneztovari

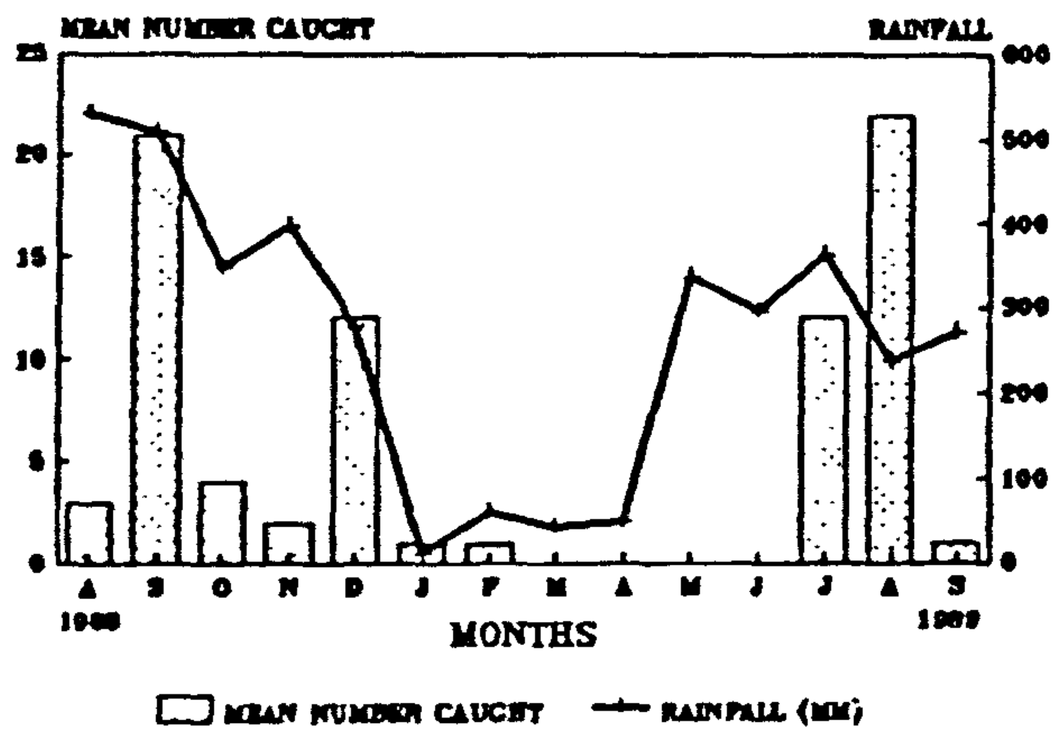


FIGURE 7.1.b: An. triannulatus

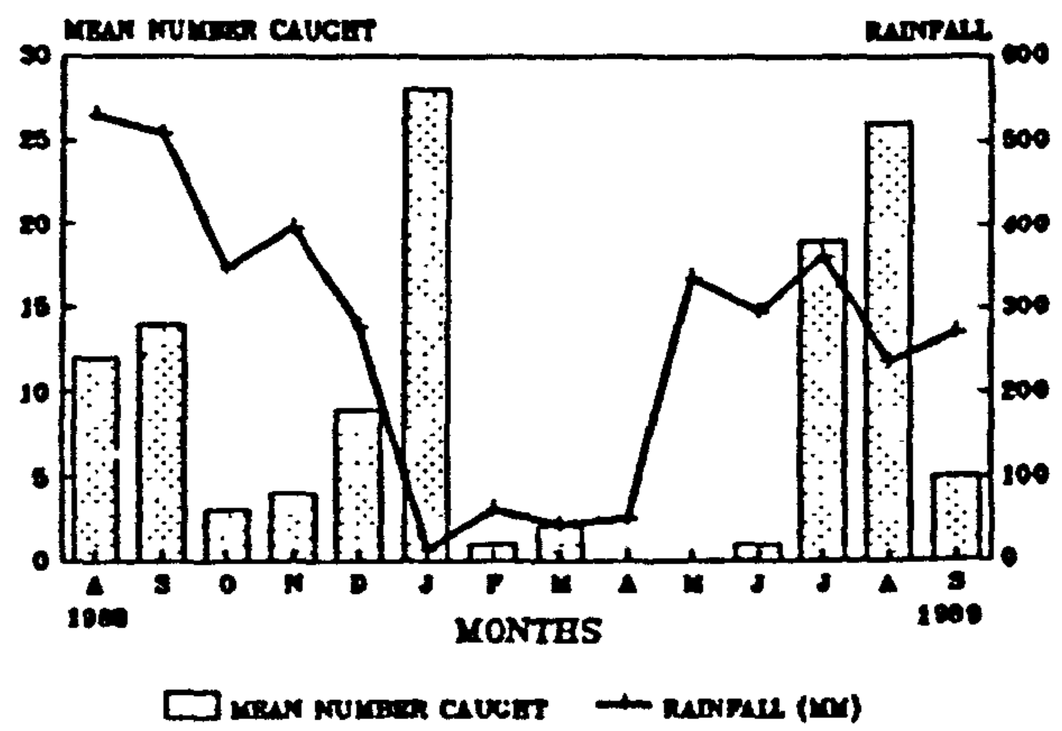


FIGURE 7.1.c: An. albitarsis

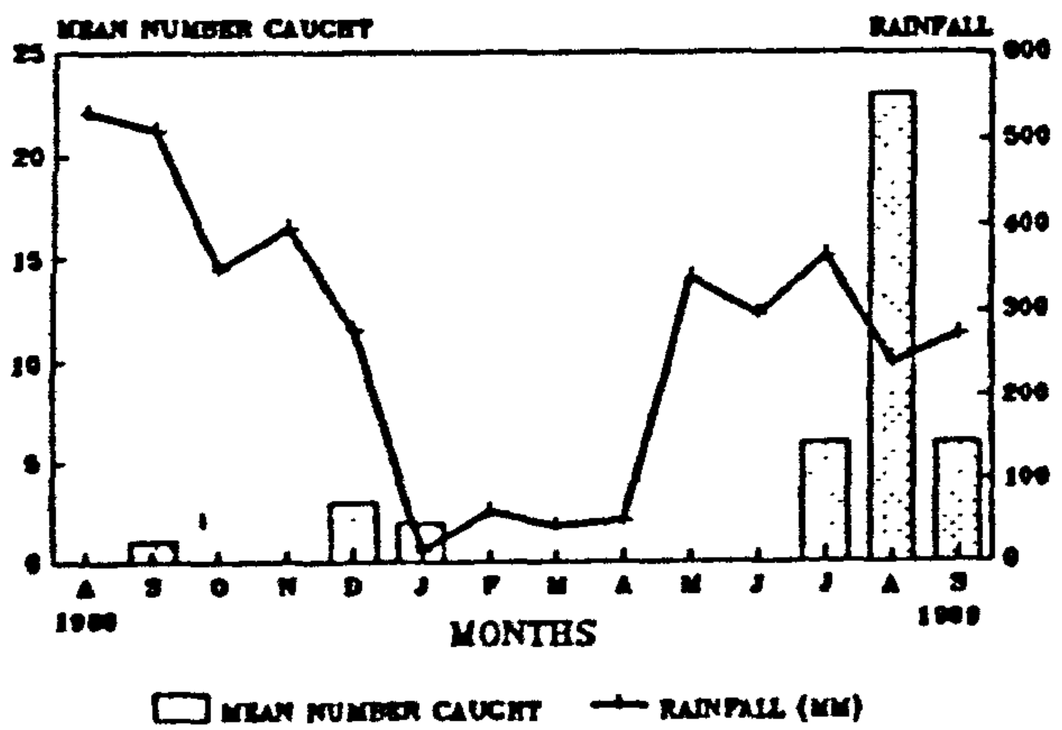


FIGURE 7.1.d: An. oswaldoi

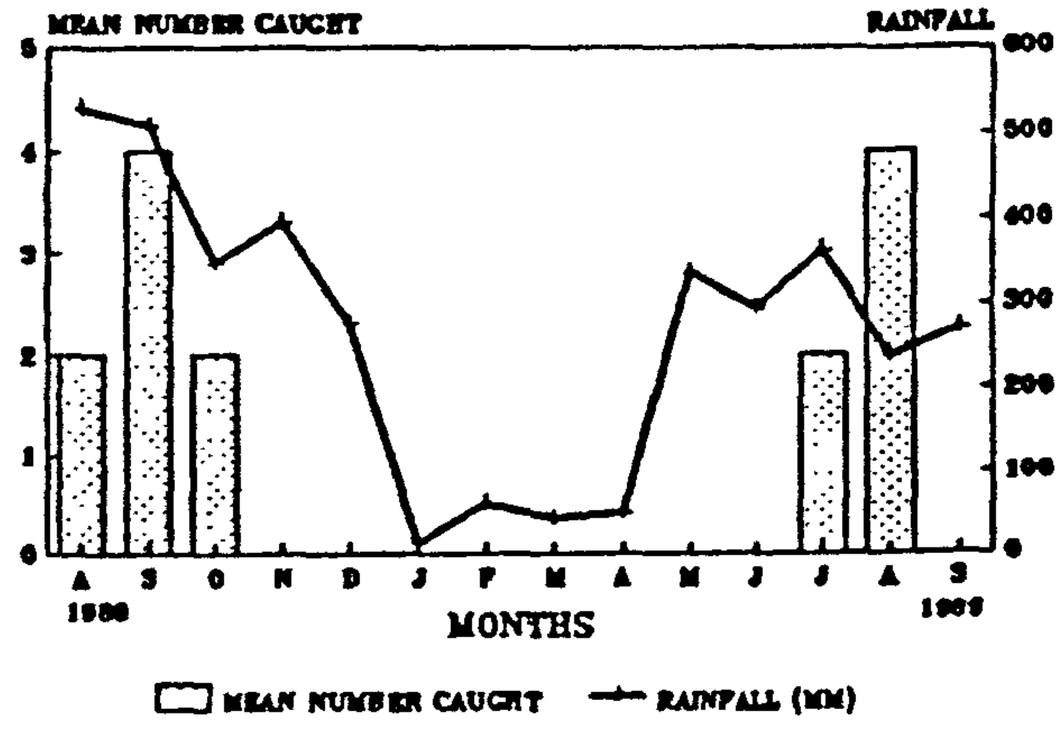


FIGURE 7.2: Mean numbers collected resting on vegetation in Guaquitas.

FIGURE 7.2.a: An. nuneztovari

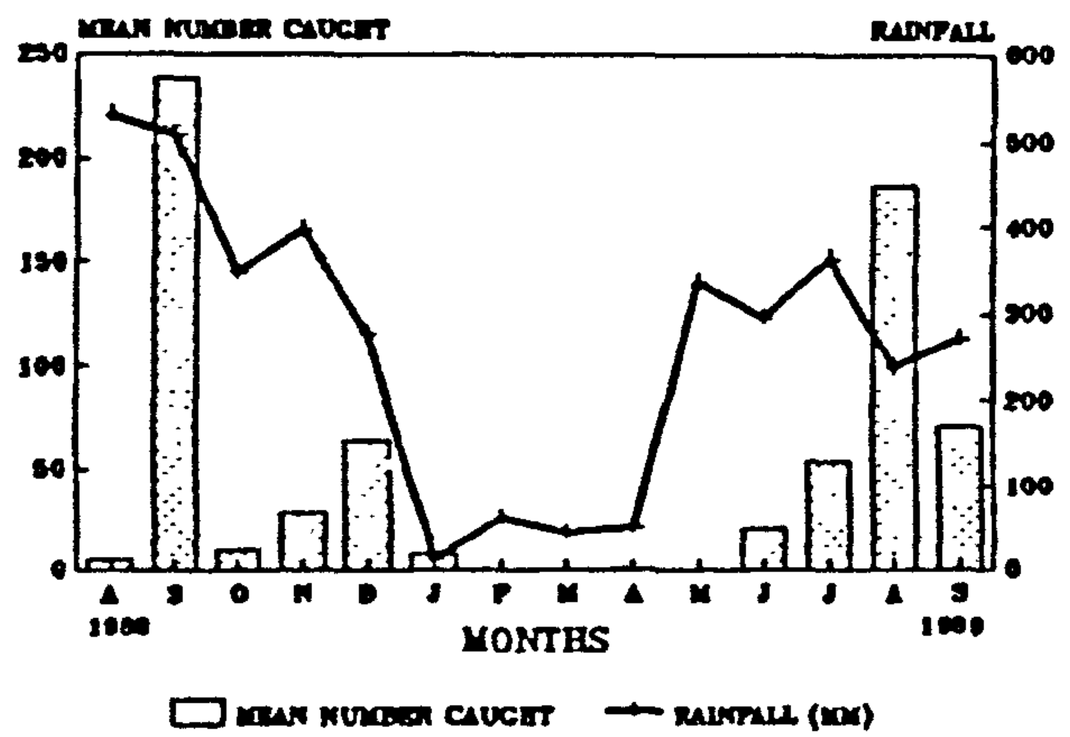


FIGURE 7.2.b: An. triannulatus

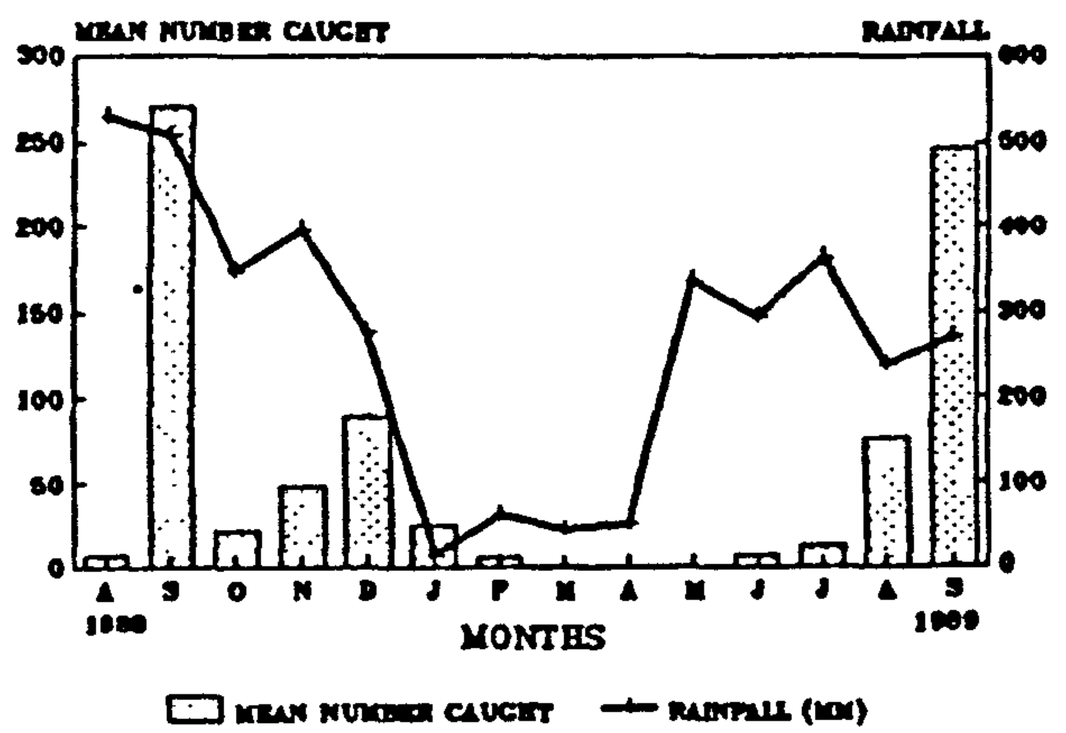


FIGURE 7.2.c: An. albitarsis

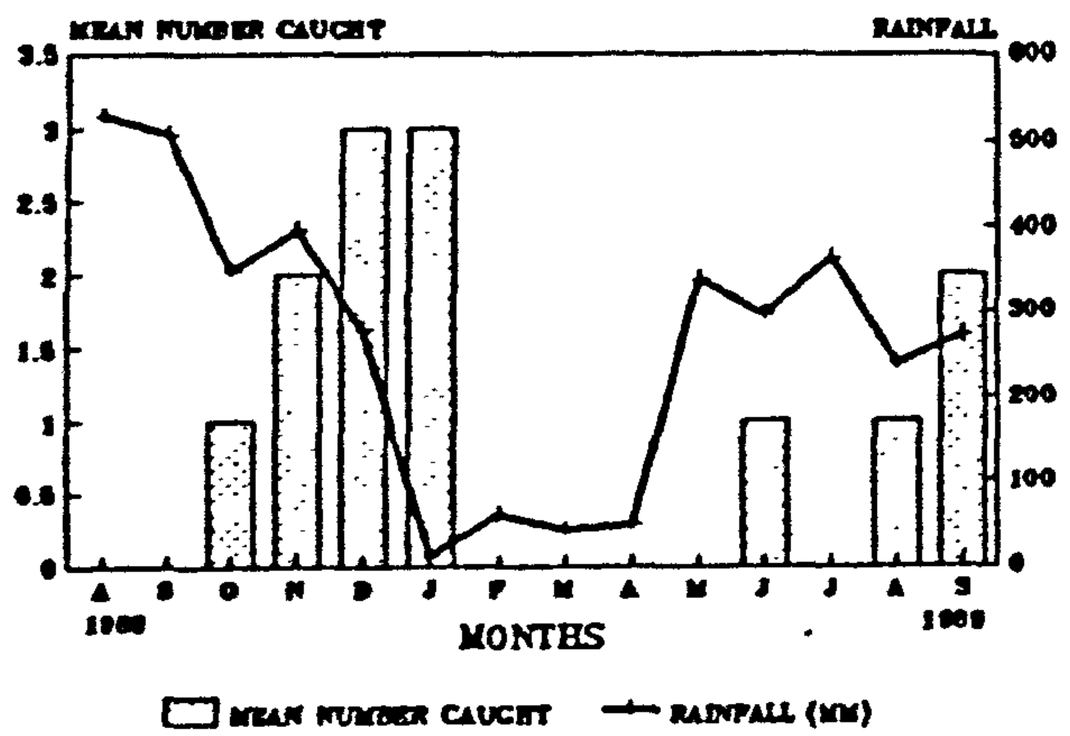


FIGURE 7.2.d: An. oswaldoi

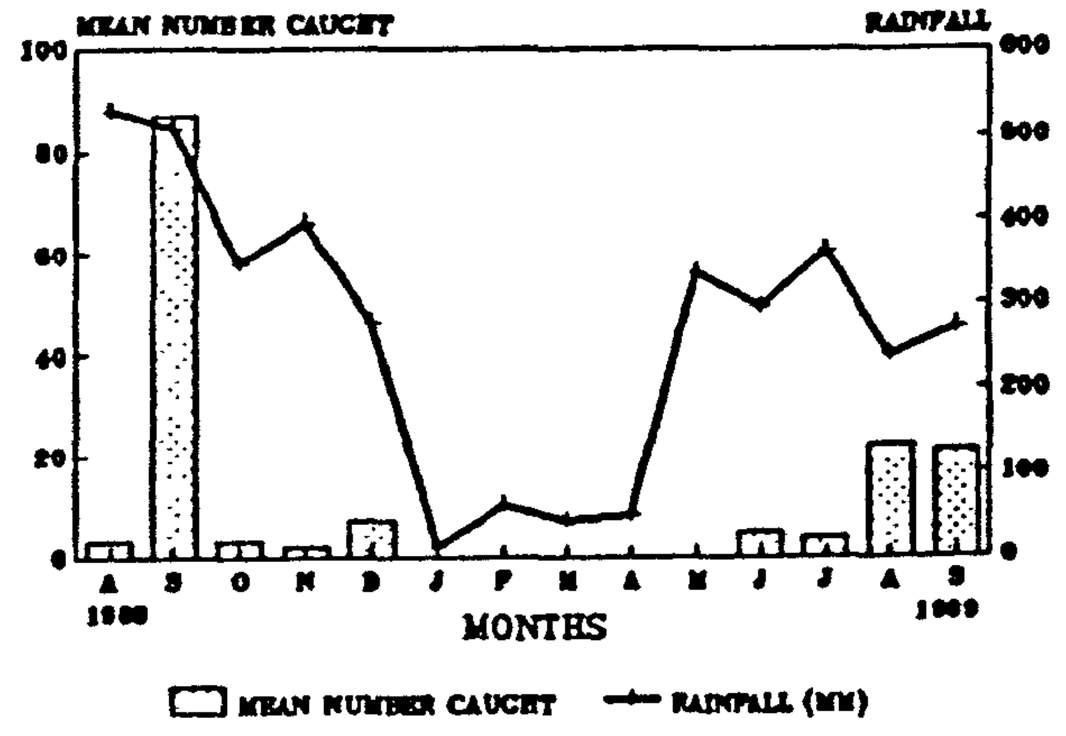


FIGURE 7.3.a: An. nuneztovari

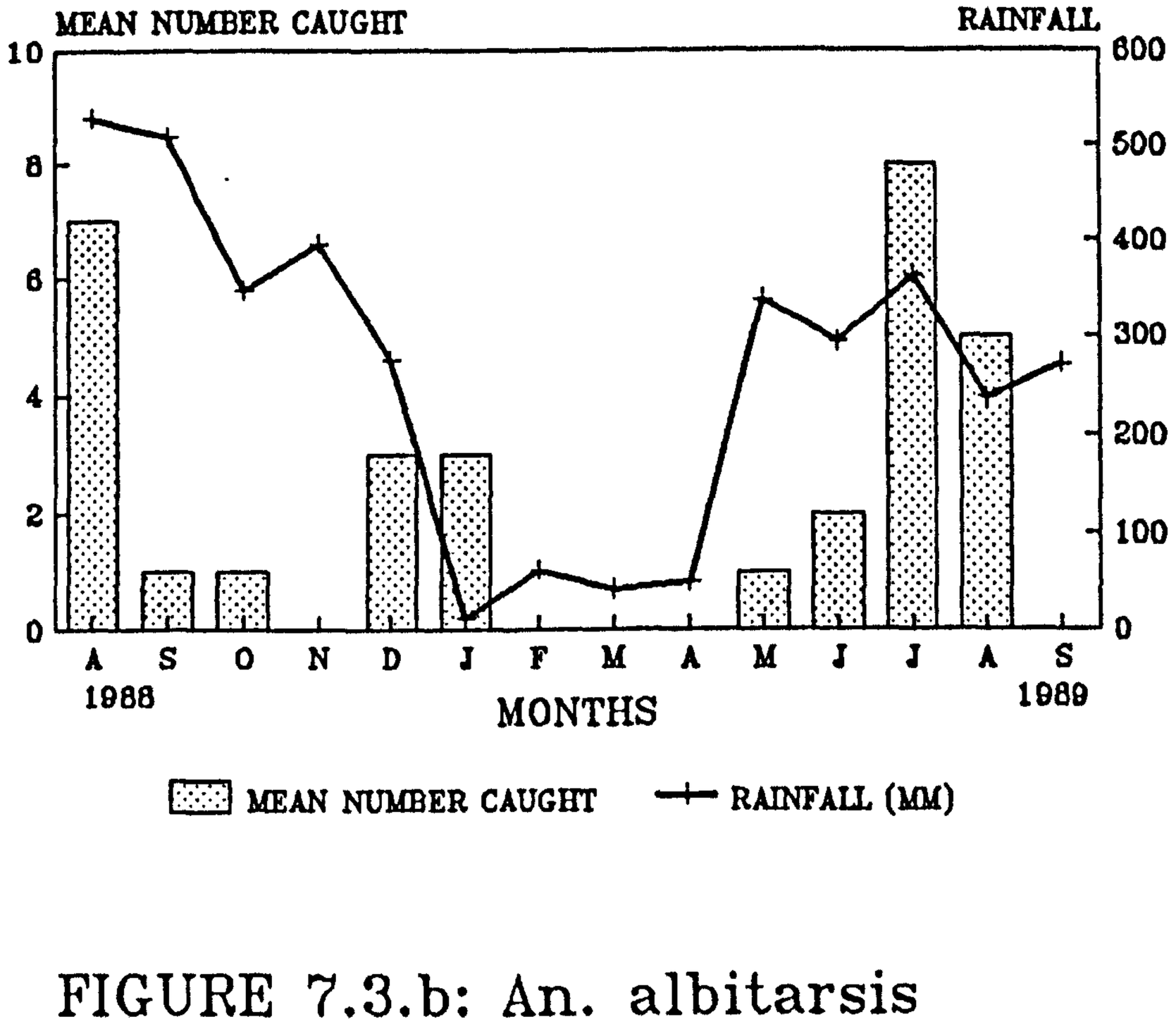


FIGURE 7.3.b: An. albitarsis

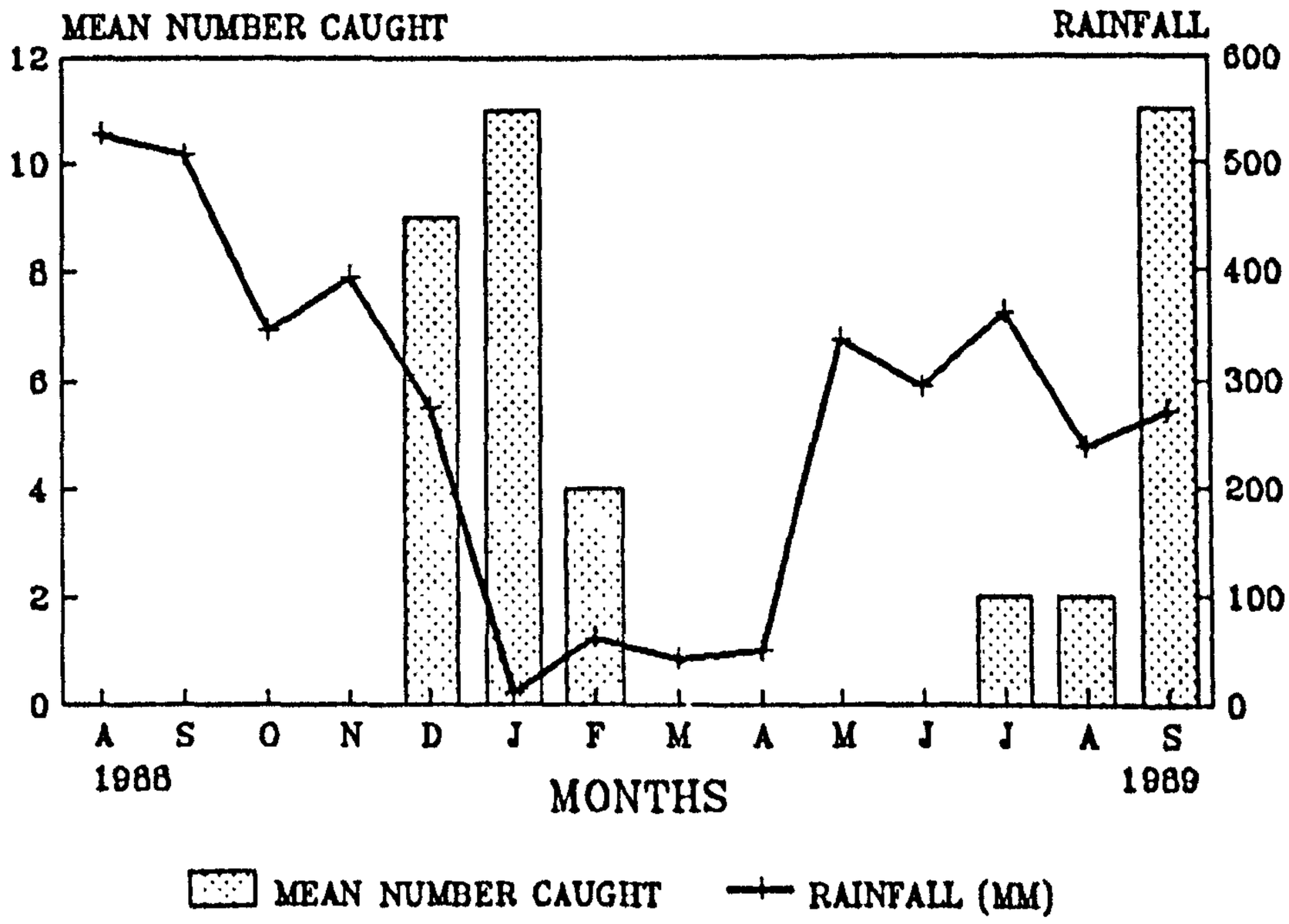


FIGURE 7.3: Mean numbers collected resting on vegetation in Caño Lindo.

7.3.2. CORRELATION WITH INDOOR-BITING CATCHES

The log-transformed monthly mean aspirator catches were plotted against the log-transformed indoor biting catches for the four most abundant species (Fig. 7.4). Collections between February and June were not considered due to the many zero scores. There were significant correlations between the two sampling methods for *An. nuneztovari* and *An. oswaldoi* but no significant correlation was found for *albitarsis* or *triannulatus*.

7.3.3. THE ASPIRATOR:INDOOR-BITING RATIO

In order to determine the relative sampling efficiency of the aspirator in relation to the indoor biting catch, the mean ratio of the log-transformed data was calculated and 95% confidence limits were determined. Ratios are not meaningful when there are zero scores and ratios were therefore calculated only for those months when at least one individual of each species was collected. Figure 7.5 shows that the aspirator was relatively very efficient in collecting *An. triannulatus* in Jabillos and Guaquitas. The method was less efficient for *albitarsis* and *oswaldoi* in Jabillos and Guaquitas, whereas in Caño Lindo more *albitarsis* were collected resting on vegetation than in biting catches indoors. Relatively very few *An. nuneztovari* were collected resting around houses compared with their dominant position among the human biting catches.

The aspirator method of sampling the resting population was in general efficient in collecting the exophilic *triannulatus, albitarsis, oswaldoi* and *neomaculipalpus* but not for *nuneztovari*. These results showed that, except for *nuneztovari*, the anophelines tend to rest on low vegetation around houses. This is particularly true for *triannulatus*, a mosquito biting early that can still be found around houses up to 10 hours after feeding. On the other hand, we failed to find resting places of *nuneztovari* since comparatively few specimens were caught. It would seem that *An. nuneztovari* must rest deep in the forest. Similar behaviour has been reported for *An. dirus* in Thailand. Scanlon and Sandhinand (1965) reported that *An. balabacensis* (= *dirus*) was not found on the inner or

FIGURE 7.4: Relationship of catches with the aspirator and on human baits

FIGURE 7.4.a: An. nuneztovari

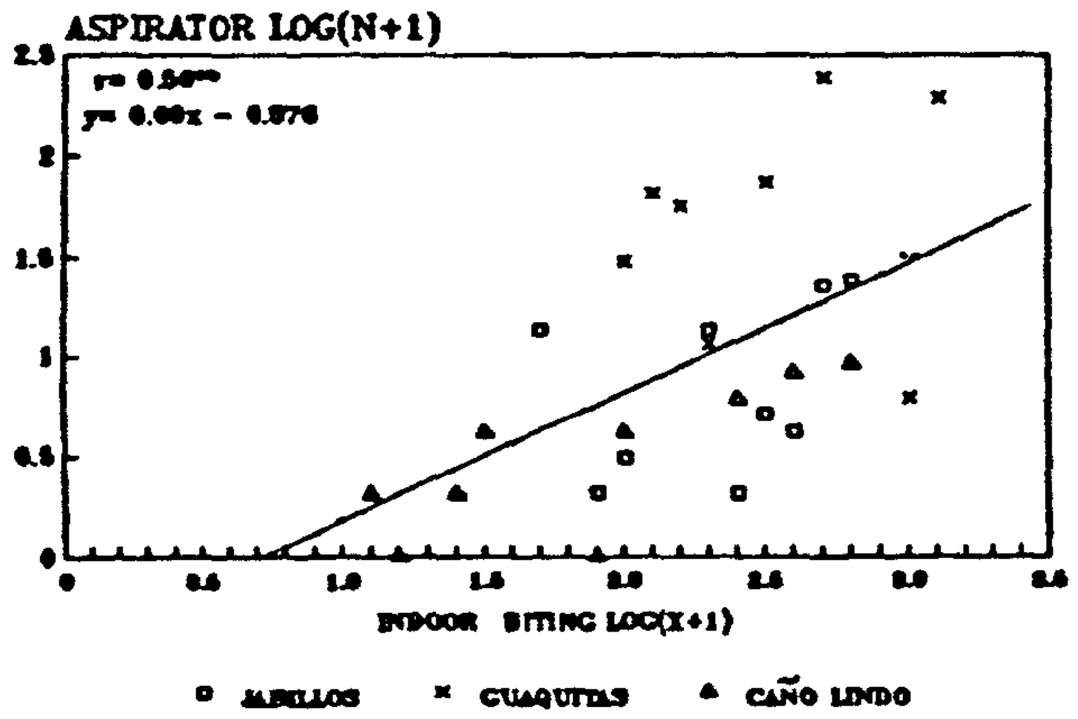


FIGURE 7.4.b: An. triannulatus

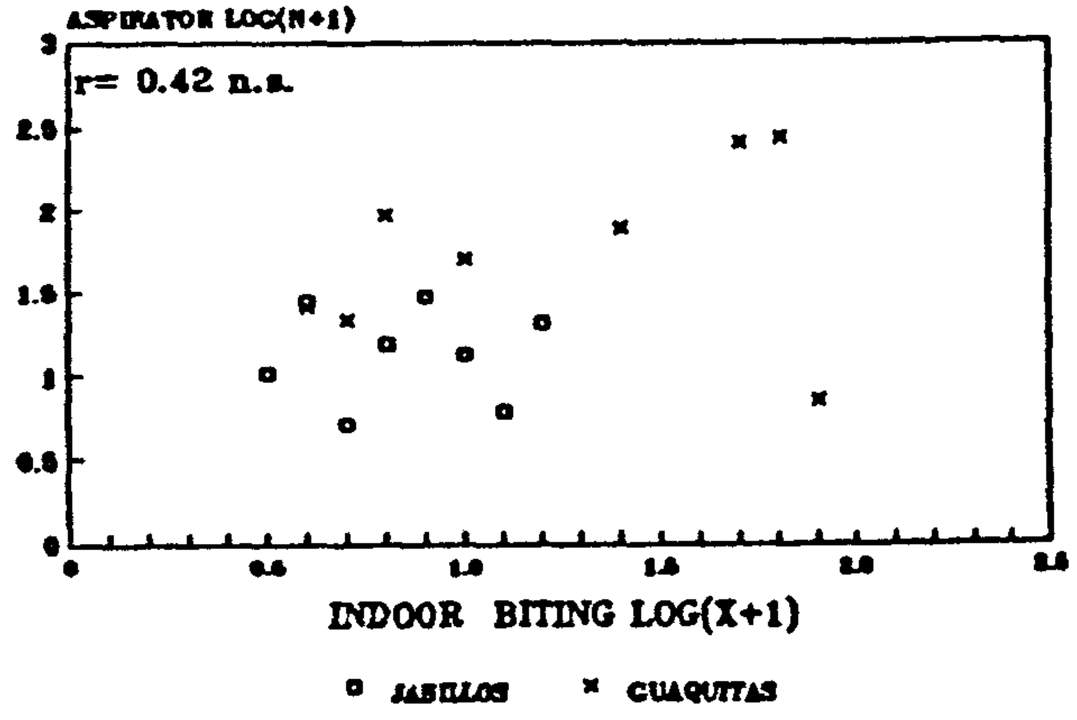


FIGURE 7.4.c: An. albitarsis

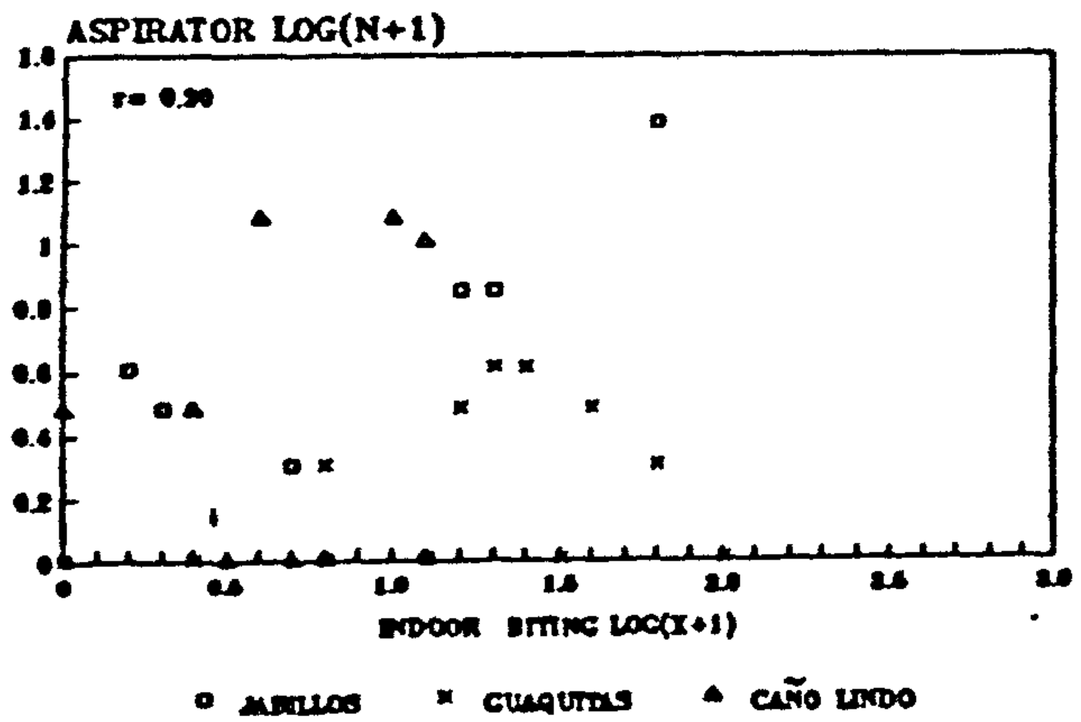


FIGURE 7.4.d: An. oswaldoi

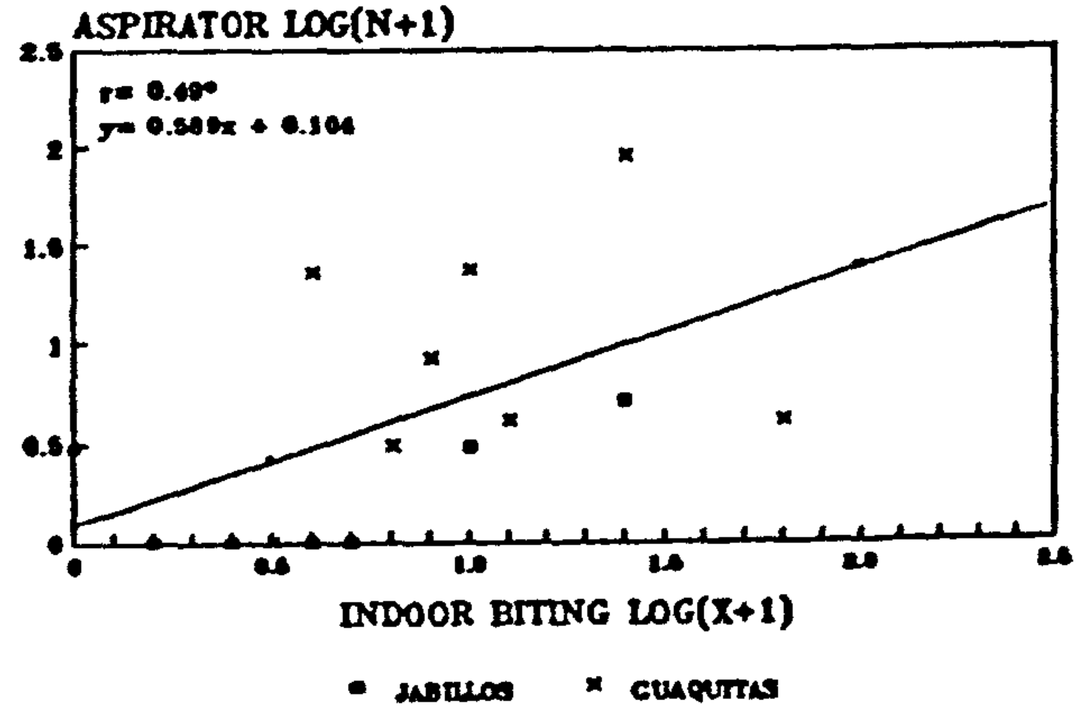
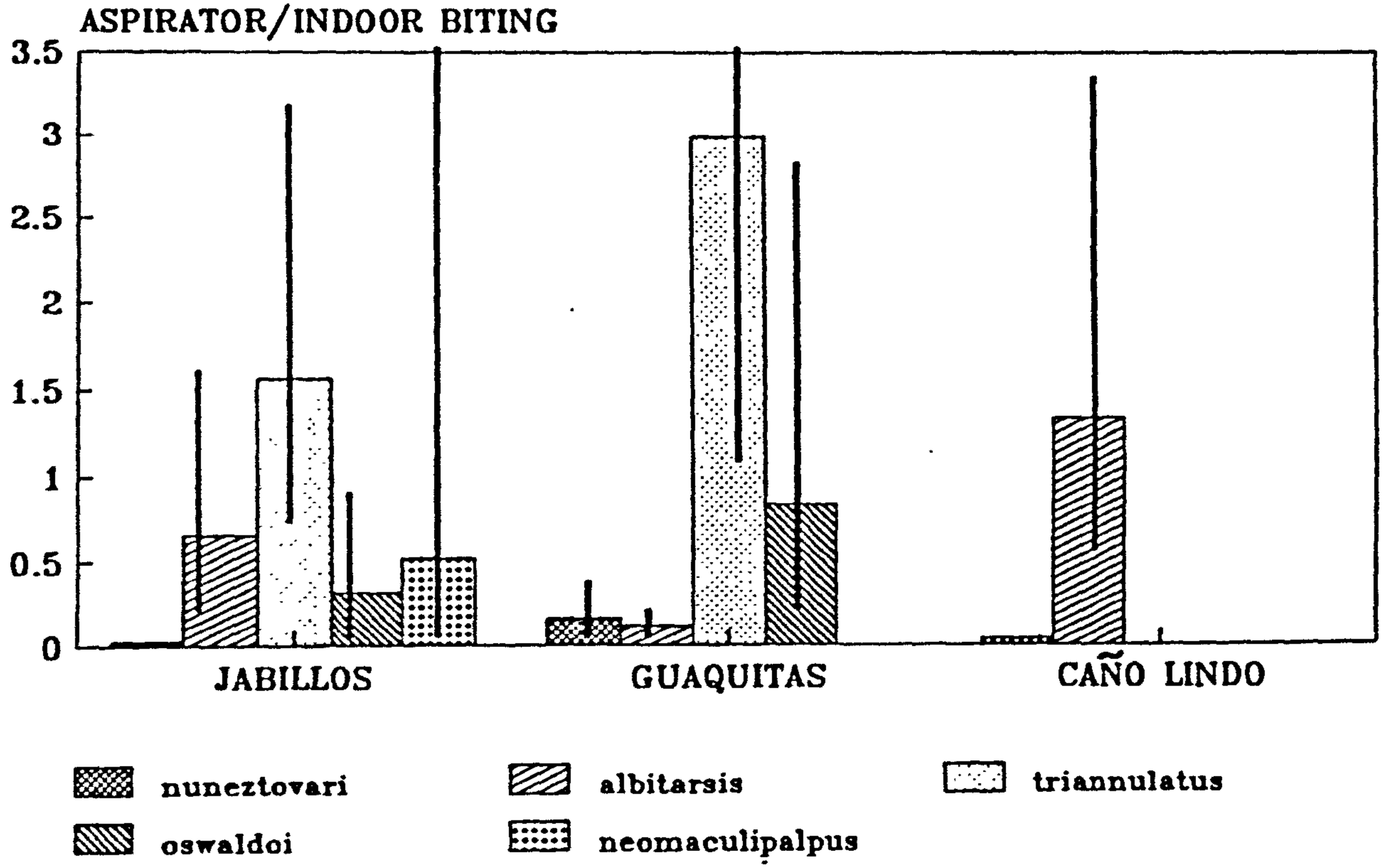


FIGURE 7.5: Aspirator/Indoor Biting Ratios



Note: 95% Confidence limits for *An. nuneztovari* - Jabillos: 0.015-0.06; Caño Lindo: 0.021-0.088

outer walls of their catching station; a small number of females was collected on vegetation near the catching station at 0600 hrs but by 1000 hrs almost all had left the area to rest in the jungle.

After ingesting a blood meal *An. nuneztovari* is apparently able to fly farther than the other species. Pérez de Valderrama and Scorza (1976) found that *nuneztovari* take smaller blood meals than do *darlingi* or *oswaldoi*, and also eliminate more fluid than the other two species. They concluded that this physiological characteristic enables this species to fly out of houses immediately after taking a blood meal.

Collection of resting mosquitoes in the forest is difficult because of the dense vegetation, and dangerous because of poisonous snakes.

CHAPTER 8:

BLOODMEAL IDENTIFICATION

8.1. INTRODUCTION

In ecological and epidemiological studies of arthropod vectors of disease it is essential to identify the source of the blood meals in order to understand the relationships between host and vectors and their role in transmission of disease.

There are several methods for the analysis of blood meals. Tempelis (1975) pointed out that at least four methods have been used to determine the vector host range: visual observation, attraction to bait traps, cytological characteristics of the blood and serology. Of these methods the most extensively used have been the serological.

Various serological methods have been applied to identify blood meals: the precipitin test, agglutination reactions, immunofluorescence and enzyme-linked immunosorbent assays (ELISA). The precipitin test has been widely used but has proven to be not specific enough (Washino & Tempelis, 1983). Agglutination tests also lack specificity between closely related hosts and are less sensitive than the precipitin test (Service *et al.*, 1986). Immunofluorescence techniques require sophisticated equipment and have been evaluated against only a limited number of blood sources (Gentry *et al.*, 1967, *in* Service *et al.*, 1986). The microplate form of the ELISA method described by Voller *et al.* (1974) was modified for the identification of blood meals of *Anopheles* mosquitoes under laboratory conditions by Burkot *et al.* (1981) and Edrissian and Hafizi (1982). At present the ELISA is the most widely used method for blood meal identification and has proved to be more sensitive and specific than other methods (Burkot *et al.*, 1981; Edrissian & Hafizi, 1982; Lombardi & Esposito, 1983; Edrissian *et al.*, 1985; Linthicum *et al.*, 1985; Service *et al.*, 1986; Beier *et al.*, 1988).

Although the techniques for determining the host sources of arthropod blood meals are well established, interpretation of the results can be complex and potentially

misleading. Recent studies have highlighted the problems involving unbiased sampling of blood engorged insects, difficulties of identifying closely related species and multiple feeds, and analysis of data (Boreham, 1975; Tempelis, 1975).

In many reports of feeding patterns little information is given on the numbers and distribution of available hosts, and it is often assumed that blood-meal results simply reflect host preferences, which may well not be true if a very biassed selection of hosts is available (Boreham & Garrett-Jones, 1973).

Data from blood meal analysis are commonly presented as percentages. For anopheline mosquitoes the concept of Human Blood Index was proposed (Garrett-Jones, 1964). The HBI is defined as "the proportion of freshly fed *Anopheles* found to contain human blood". Because of problems in obtaining a representative sample for this index, Garrett-Jones (1964) suggested that the best estimate could be obtained by taking the unweighted mean of samples collected from human dwellings and other habitats. The forage ratio, an index of host selection which considers the relative availability of hosts, is expressed as the percentage of engorged mosquitoes that have fed upon a given vertebrate host, divided by the proportion that the host comprises of the total population of hosts available in the mosquito's habitat (Hayes *et al.*, 1973; Hess *et al.*, 1968). Problems associated with this concept were identified by Edman (1971) as a) neglect of ecological and behavioural differences among hosts and mosquitoes and of host availability and accessibility to the mosquito, and b) difficulty in carrying out a complete numerical census of the animal population.

Kay *et al.* (1979) subsequently introduced the feeding index concept for analysis of host feeding and it was defined as "the observed proportion of feeds on one host with respect to another divided by the expected comparative proportion of feeds on the two hosts, based on factors affecting feeds". These factors include abundance and size of hosts, their temporal and spatial concurrence with the mosquito and its feeding success.

This index is expressed by the following equation:

$$FI = (N/N')/(E/E')$$

where FI= Feeding Index

N = Number of feeds on host 1 N'= Number of feeds on host 2

E and E'= Expected proportion of feeds on hosts 1 and 2 respectively, assuming no preference between any one member of host species 1 or 2, i.e. that the numbers of feeds on the two species would depend only on their relative numbers in the area.

The main advantages of this index were listed as: "it departs from the inference that feeding patterns are attributable to host preference, does not require a full animal census, and assessment of some of the multiple factors influencing feeding patterns".

In order to determine the Human Blood Index and the Feeding Index of anophelines collected resting on vegetation (Chapter 7) during the present study, a direct ELISA was used to identify the source of the blood meals.

8.2. MATERIALS AND METHODS

8.2.1. ELISA: A direct ELISA modified from that described by Beier *et al.* (1988) was used for the identification of blood meals.

Preparation of mosquito sample: Field collected mosquitoes that were kept dry over silica gel for up to 18 months, were prepared individually for testing by trituration in a 1.5 ml microcentrifuge tube to which 50µl 0.01M phosphate buffered saline (PBS-Dulbecco's), pH 7.4, was added. For trituration an electric "GG-Machine" was used, this consisted of an electric drill to which a plastic pestle was attached (R. Wirtz, pers. comm.). Samples were then diluted 1:10 in PBS.

ELISA procedure: Samples were screened for human and bovine serum, the assay being standardized as follows: 100 µl of the sample were added to wells of polyvinyl chloride, U-shaped, 96-well microtitre plates (Dynatech Laboratories, Alexandria, VA, USA), which were covered and incubated at room temperature for 4 hours. Wells on the edge of the plate were not used in order to avoid false positives which

may arise due to an "edge effect" which many ELISA workers have observed (see Chapter 9). Each well was then washed twice with PBS containing 0.5% Tween 20 (PBS-Tw 20). This was followed by the addition of 100 µl host-specific conjugate (anti-host IgG, H & L) diluted 1:500 in 0.5% boiled casein containing 0.025% Tween 20. To the human conjugate a 1:500 dilution of dog serum was added in order to decrease cross-reactivity (Beier *et al.*, 1988). After 1 hour, wells were washed three times with PBS-Tween 20, and 100 µl of ABTS (2,2'-azino-di[3-ethyl benzthiazoline sulfonate]) or TMB (3,3',5,5'-Tetramethylbenzidine) peroxidase substrate (Kirkegaard & Perry) was added to each well. Absorbance at 405 nm (ABTS) or 650 nm (TMB) was determined with an ELISA reader 30 minutes after the addition of substrate. The green positive reactions for peroxidase may also be determined visually. Positive controls on each plate consisted of a 1:10 dilution of the macerate of a field collected anopheline fed either on human or bovine. Negative controls on each plate consisted of 10 field collected male anophelines. Samples were considered positive if the Optical Density values (range 0-3.0) exceeded the mean plus three times the standard deviation of 10 negative controls.

A sample of 100 mosquitoes not reacting to either human or bovine anti-sera were also tested for horse, dog and chicken.

ELISA activity versus bloodmeal digestion: To determine ELISA sensitivity in relation to bloodmeal digestion, 300 *An. nuneztovari* were fed on humans, held at room temperature (25 ± 2°C), and groups of 10-15 mosquitoes were killed by freezing at 0, 4, 8, 12, 16, 20, 24, 36, 40, 44, 48 and 52 hours after feeding. Mosquitoes were held dry over silica gel until tested. Two peroxidase substrate systems were tested:

ABTS (2.2'-azino-di[3-ethyl-benzthiazoline sulfonate (6)]) and TMB (3,3',5,5'-Tetramethylbenzidine).

8.2.2. Questionnaires

In order to determine the most likely hosts of mosquitoes in the study area and to determine the feeding index, a census of the domestic animals in the three villages was carried out by means of questionnaires to householders within a radius of 2 km around

the experimental huts on three different occasions: August 1988 (wet season), February 1989 (dry season) and August 1989 (wet season).

8.3. RESULTS AND DISCUSSION

8.3.1. ASSAY SENSITIVITY

The sensitivity of the ELISA test was determined in relation to blood digestion. Figure 8.1 shows that 24 hrs after blood ingestion 100% of the bloodmeals were identifiable; after 40 hrs the proportion was 50%, when the substrate used was ABTS. Using TMB as substrate 100% of the bloodmeals were identifiable after 40 hrs; after 44 hrs the proportion was 75%. Similar results have been reported recently. Service *et al.* (1986) using a double sandwich ELISA were able to identify blood meals 39-40 hours in three-quarters gravid females held at 24 °C; in a WHO (1987) inter-laboratory trial using various serological tests it was concluded that blood meals were reliably detectable up to 24 hours after feeding; while Beier *et al.* (1988) reported that human blood meals were detectable by direct ELISA up to 32 hours after feeding in mosquitoes kept at 27 ± 2 °C. The sensitivity of the ELISA test has increased in the past few years: earlier workers reported detection times of only 8 hours (temperature not stated) (Edrissian & Hafizi, 1982) or up to 20 hours (temperature not stated) (Lombardi & Esposito, 1983).

In order to check whether there were significant differences for the 10 negative controls on each plate for the two substrates and anti-human and bovine IgG conjugates, an analysis of variance was carried out using the statistical program SPSS (Table 8.1). The results showed that there were significant variations between the conjugates and between substrates. Also the conjugates acted differently with each substrate and a highly significant plate to plate variation was found. The results emphasized the need to test positive and negative controls on each microplate, since inter-plate variations for optical density values of controls can be significant when plates are not read at exactly the same time after the addition of substrate.

FIGURE 8.1: Blood Digestion

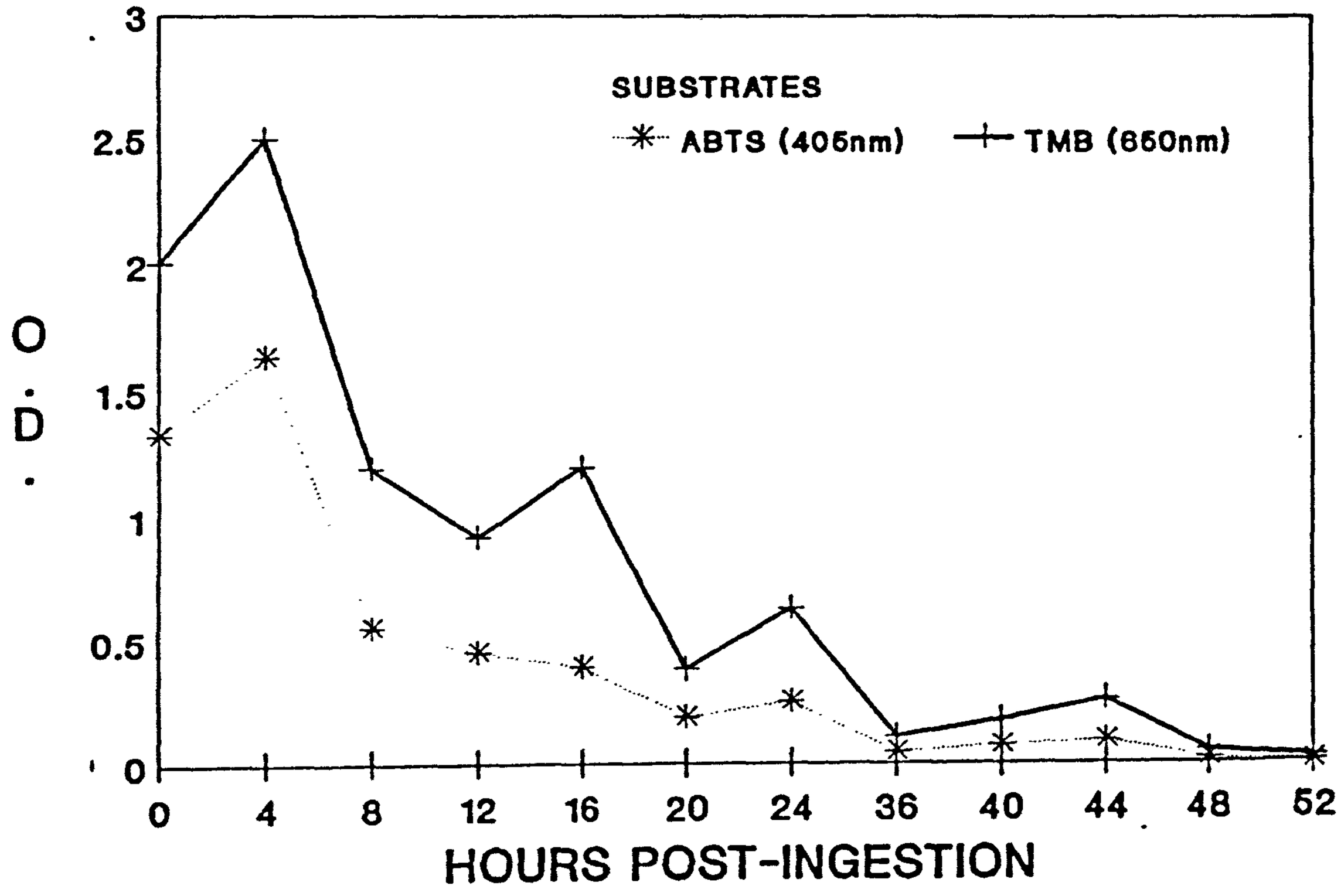


Table 8.1: Analysis of variance of the log-transformed data of the optical densities of the negative controls by serum (human and bovine) and substrate (ABTS and TMB) with subsequent partitioning of the residual variance between plates.

Source of Variation	Sum of Squares	DF	Mean Square	F
Serum	1.128	1	1.128	23.4 ***
Substrate	2.897	1	2.897	60.1 ***
Serum x Substrate	0.648	1	0.648	13.4 ***
Residual	29.692	616	0.048	
Total =	34.485	619		
Plates	24.580	58	0.424	46.3 ***
Residual	5.114	558	0.009	

8.3.2. HUMAN BLOOD INDEX

All anophelines collected resting on vegetation were assayed for blood meal identification regardless of their abdominal condition. Tables 8.2, 8.3, 8.4 and 8.5 show the results of the ELISA for bloodmeal identification of the anophelines collected in the three villages. During September 1989 in Guaquitas and Jabillos culicines also were included. In general, the percentage of blood meals on bovines was higher than on humans in the three villages. A relatively high percentage (up to 12.5%) of mixed blood meals were found. This is a widely observed phenomenon in mosquitoes. For example, Senior-White (1952) reported that 12% of *An. aquasalis* blood meals were mixed; Edman and Downe (1964) found mixed blood meals in up to 61.8% of mosquitoes analysed from Kansas; Burkot *et al.*(1988) in Papua New Guinea, found that 5.2% of the outdoor resting and 4.3% of indoor resting mosquitoes had mixed blood meals. The only contrasting report is that of Tempelis (1970) who found that only 0.1% of the mosquitoes which he analysed had mixed meals.

I found that in all mixed blood meals a stronger positive reaction was recorded for bovine. Also, in the mosquitoes which had only taken one type of blood higher positive absorbance values were generally recorded for bovines than for humans. This may be due to different digestion rates for the two types of blood. Katakumb (1986) using ELISA found that in *An. gambiae* human blood was digested more rapidly than that of bovines. In a review of digestive processes of haematophagous insects, Gooding (1972) mentioned that digestion rates depend on several variables such as the size of the blood meal, temperature (Guelmino, 1951), individual differences within a species (MacKerras & Roberts, 1947), differences between species (Hocking & MacInnes, 1949), physiological age (Detinova, 1962) and blood source (Gooding, 1972). These factors may partially explain the differences in detection time of blood meals after feeding by different authors working under different conditions and with different mosquito species.

Up to 50% of the anophelines assayed were negative for human or bovine IgG. Of these, 400 anophelines, mainly *An. nuneztovari* from Guaquitas were tested for dog, chicken and horse IgG (Table 8.5).

Table 8.2: Results of the ELISA for blood meal identification of anophelines collected in Jabillos. (Percentages in parentheses are the numbers in each host category in relation to the number that could be identified).

Species	No. human	No. bovine	No. mixed	Neither	% ident.	Total tested
nuneztovari	11(33.3%)	19(57.6%)	3(8.3%)	45	42.3	78
albitarsis	3(14.3%)	17(80.9%)	1(4.5%)	17	55.3	38
triannulatus	35(41.7%)	42(50.0%)	7(7.7%)	36	70.0	120
strodei	0	0	0	1	0	1
rangeli	2(40.0%)	3(60.0%)	0	3	62.5	8
oswaldoi	8(57.1%)	6(42.9%)	0	3	82.4	17
neomaculipalpus	13(43.3%)	15(50.0%)	2(6.3%)	18	62.5	48
Unidentifiable	3(27.3%)	8(72.7%)	0	10	52.4	21
Total	75(37.9%)	110(55.6%)	13(6.6%)	133	59.8	331
Culicines	19(9.3%)	164(80.4%)	21(10.3%)	101	66.9	305

Table 8.3: Results of the ELISA for blood meal identification of anophelines collected in Guaquitas (percentages in parentheses are the numbers in each host category in relation to the number that could be identified).

Species	No. human	No. bovine	No. mixed	Neither	% ident.	Total tested
nuneztovari	51(15.8%)	258(80.1%)	13(4.0%)	382	45.7	704
albitarsis	2(25.0%)	5(62.5%)	1(12.5%)	5	61.5	13
triannulatus	128(30.3%)	281(66.6%)	13(3.1%)	393	51.8	815
strodei	4(40.0%)	6(60.0%)	0	14	41.7	24
rangeli	8(30.8%)	18(69.2%)	0	27	49.1	53
oswaldoi	13(23.2%)	43(76.8%)	0	106	34.6	162
neomaculipalpus	2(33.3%)	3(50.0%)	1(16.7%)	7	46.1	13
Unidentifiable	32(17.1%)	149(79.7%)	6(3.2%)	107	63.6	294
Total	240(23.1%)	763(73.6%)	34(3.3%)	1,041	49.9	2,078
Culicines	13(7.1%)	164(89.6%)	6(3.3%)	45	82.5	229

Table 8.4: Results of the ELISA for blood meal identification of anophelines collected in Caño Lindo. (Percentages in parentheses are the numbers in each host category in relation to the numbers that could be identified).

Species	No. human	No. bovine	No. mixed	Neither	% ident.	Total tested
nuneztovari	4(21.1%)	13(68.4%)	2(10.5%)	7	73.1	26
albitarsis	5(15.2%)	28(84.8%)	0	4	82.5	40
triannulatus	0	0	0	1	0	1
oswaldoi	0	0	0	2	0	2
neomaculipalpus	2(50.0%)	2(50.0%)	0	2	66.7	6
argyritarsis	0	0	0	1	0	1
Unidentifiable	4(40.0%)	5(50.0%)	1(10.0%)	2	83.3	12
Total	15(22.7%)	48(72.7%)	3(4.5%)	19	75.0	88

Table 8.5: Mosquitoes which were negative to human and bovine antisera from Guaquitas were tested for dog, chicken and horse (sample size in parentheses).

Species	% Dog	% Chicken	% Horse
nuneztovari	13.0 (115)	1.0 (100)	0 (100)
triannulatus	1.8 (57)	-	-
oswaldoi	0 (23)	-	-
neomaculipalpus	0 (2)	-	-
strodei	0 (3)	-	-
Total	8.0 (200)	1.0 (100)	0 (100)

The Human Blood Index, i.e. the proportion of mosquitoes found to contain human blood (alone or mixed), was calculated for all the species tested (Table 8.6). The highest HBI was observed in *An. oswaldoi* (57.1%) in Jabillos and the smallest in *An. albitarsis* (15.2%) in Caño Lindo. *An. triannulatus* showed a higher HBI than *nuneztovari*. Gabaldón (1972) stated that *An. nuneztovari* "maintains a human blood preference of 80%" but no supporting data were provided. The values of the HBI for *nuneztovari* reported in the present study are higher than those reported by some other authors. Elliott (1972) found that the HBI for *nuneztovari* in Colombia was less than 10% while Scorza *et al.* (1976) reported a HBI of 7.4% in western Venezuela. This marked difference could be due to numerous factors such as numbers and availability of hosts (Boreham & Garrett-Jones, 1973) but also to lack of sensitivity of the precipitin test used by these authors.

In order to determine if the differences in the HBI for the four commonest species between villages were significant, chi-square tests were performed. The results showed that *An. nuneztovari* had a significantly (χ^2=6.5*) higher HBI in Jabillos than in Guaquitas, while the difference was not significant between Jabillos and Caño Lindo (χ^2=0.2) or between Guaquitas and Caño Lindo (χ^2=0.6). There were no significant differences of the HBI of *albitarsis* between the three villages (χ^2=0.01 to 0.6). While *triannulatus* had a significantly higher HBI in Jabillos than in Guaquitas (χ^2=5.7*). Finally, *An. oswaldoi* also had a significantly higher HBI in Jabillos (χ^2=4.6*) than in Guaquitas.

Combining the data for Guaquitas and Jabillos and testing the significance of the difference in the HBI values by means of a Mantel-Haenszel chi-squared test (Kirkwood, 1988), it was found that *An. triannulatus* was more anthropophilic than *nuneztovari* (χ^2_{M-H}=11.76***) and similarly *An. nuneztovari* was more anthropophilic than *An. oswaldoi* (χ^2_{M-H}=12.06***).

Negative *An. nuneztovari* (315) from Guaquitas tested for other sera resulted in only 13% positive to dog. From 100 negative to other sera one responded to chicken and none to horse. It seems unlikely that an appreciable proportion had fed on other hosts and

Table 8.6: Human blood index of anophelines collected resting outdoors in the three villages between August 1988 and September 1989 (in paretheses are the number of blood meals identified to any host species for the mosquito and the village concerned). The data are derived from Tables 8.2, 8.3 and 8.4 with the mixed feeds counted as human feeds.

Species	Caño Lindo	Guaquitas	Jabillos
nuneztovari	28.6 (21)	18.2 (351)	38.9 (36)
albitarsis	15.2 (33)	33.3 (9)	18.2 (22)
triannulatus	0	32.3 (436)	46.2 (91)
strodei	-	40.0 (10)	0
rangeli	-	30.8 (26)	40.0 (5)
oswaldoi	0	23.2 (56)	57.1 (14)
neomaculipalpus	50.0 (4)	42.9 (7)	46.9 (32)

these results suggest that 47.8% of the anophelines collected resting on vegetation and tested by ELISA for blood meal identification had already digested their blood meal, beyond the point at which they could be identified in the assays.

Culicines were less anthropophilic than anophelines (Table 8.3).

8.3.3. FEEDING INDEX

The Feeding Index (Kay *et al.*, 1979) was calculated for the four commonest species in the three villages based on the results of the ELISAs (Tables 8.2, 8.3 & 8.4) and questionnaires to householders (Table 8.7) and the results are shown in Table 8.8. Since mosquitoes assayed for blood meal identifications were collected during a period of 14 months, it was considered more appropriate to calculate the feeding index based on the average numbers of hosts recorded in the questionnaires given on three different occasions. The Index was found to be different in each village for each species, but in some cases the sample sizes were small so some of the observed differences are of doubtful significance. In Guaquitas where there were many cattle, the feeding index for all four species was higher than 1.0, i.e. there was apparently preferential feeding on humans relative to bovines. These results contrast to those in Jabillos where there were fewer cattle and where *nuneztovari*, *albitarsis* and *triannulatus* apparently fed preferentially on bovines. The contrasting results obtained in Jabillos and Guaquitas may be explained if the larger number of cows recorded as belonging to the inhabitants of Guaquitas were not kept so near to where the mosquitoes were collected as in the case of Jabillos. In Caño Lindo, *An. nuneztovari* and *albitarsis* apparently fed preferentially on humans.

The Feeding Index for *An. nuneztovari* in Guaquitas calculated for humans and dogs based on the data on Tables 8.5 and 8.7, was 2.5, which is similar to the feeding index for humans and cattle. These results suggest that *An. nuneztovari* preferentially feeds on humans despite the fact that being a late biting mosquito it might be expected to find dogs more available than humans since dogs are kept outside houses during the night, and generally they sleep in the porches.

Table 8.7: Results of the questionnaires to householders about ownership of animals within 2 km of the experimental huts in the three villages on three different occasions.

Domestic animals	Caño Lindo			Guaquitas			Jabillos		
	Aug.88	Feb.89	Aug.89	Aug.88	Feb.89	Aug.89	Aug.88	Feb.89	Aug.89
Cows	69	296	596	528	414	520	127	327	393
Dogs	35	38	65	31	26	21	82	89	75
Cats	15	13	25	65	11	9	47	51	37
Birds	308	707	942	166	168	158	15,924	16,286	3,610
Pigs	18	114	304	1	6	6	36	50	45
Donkeys	1	0	3	2	2	0	2	1	1
Horses	7	21	16	29	23	28	3	11	13
Mules	0	3	0	0	1	10	3	1	0
Goats	10	6	6	0	2	0	0	7	3
Rabbits	0	0	2	0	0	4	0	0	0
Monkeys	0	0	0	0	0	0	0	0	1

Table 8.8: Numbers of the four commonest species feeding on humans and bovines at the three villages based on the blood meal ELISA results in Tables 8.2-8.4 with mixed feeds counted in both the human and bovine categories. Data are also used on numbers of humans and cattle in each village from averages of the 1988 and 1989 questionnaires (Table 8.7). Feeding Index calculated according to Kay *et al.* (1979)

Species			Guaquitas	Jabillos	Caño Lindo
	Average no. of hosts	E	44	303	114
		E'	487	282	320
nuneztovari	No. of feeds	N	64	14	6
		N'	271	22	15
	Feeding Index		2.6	0.6	1.2
albitarsis	No. of feeds	N	3	4	5
		N'	6	18	28
	Feeding Index		5.5	0.21	2.0
triannulatus	No. of feeds	N	141	42	-
		N'	294	49	-
	Feeding Index		5.3	0.8	-
oswaldoi	No. of feeds	N	13	8	-
		N'	43	6	-
	Feeding Index		3.3	1.2	-

Feeding Index = (N/N')/(E/E')
N = number of feeds on human
E = number of humans
N'= number of feeds on bovine
E'= number of cattle

It is generally considered that feeding patterns on human blood are useful indicators in determining the relative importance of *Anopheles* species as vectors of malaria. These patterns could also be useful in the epidemiological assessment of control activities as a comparative measure of the effect of residual insecticide upon the degree of contact between vector and man (WHO, 1963).

The contrasting results obtained in the present study on host preference based on the HBI and the Feeding Index stressed the differences between villages. Nevertheless, these parameters could be used as indicators to evaluate control measures based on changes in feeding preferences before and after an intervention measure.

CHAPTER 9:

ENTOMOLOGICAL INOCULATION RATE

9.1. INTRODUCTION

The entomological inoculation rate is the number of sporozoite positive bites received by one person in one night (WHO, 1975). It can be represented by the equation: $h = m.a.s.$ where, m is the anopheline density relative to man, a is the number of human blood meals per vector per day and s is the sporozoite rate in the biting population, i.e. the proportion of anophelines with sporozoites in their salivary glands (Macdonald, 1952). Determination of the entomological inoculation rate is generally based on human biting catches and estimation of the sporozoite rate. Such determinations are important for understanding the dynamics of transmission and for planning and evaluating control programmes.

To incriminate a species as a malaria vector one must find sporozoites in the salivary glands of members of the species. The traditional method of determining sporozoite rate requires the dissection of salivary glands of freshly collected mosquitoes. Not only is this method laborious, but the species of *Plasmodium* cannot be determined from the sporozoite morphology. However, this method is still useful in areas where the sporozoite rate is high (e.g. 5-10%), namely some parts of Africa and South East Asia. In areas where the sporozoite rate is low, for example Latin America where the rate is less than 1% (Boyd, 1949), this method is impracticable because of the large numbers of mosquitoes that have to be dissected to obtain a reliable measure of the sporozoite rate.

The production of monoclonal antibodies specific to the circumsporozoite (CS) proteins that cover the external surface of the sporozoite has made possible the development of immunological techniques that can detect and identify by species the sporozoites in mosquitoes (Zavala *et al.*, 1982). Two immunological methods have been developed and tested in the field to detect and quantify sporozoites in field-collected

mosquitoes: the immunoradiometric assay (IRMA) which uses ^{125}I-labelled monoclonal antibodies and the enzyme-linked immunosorbent assay (ELISA) which uses an enzyme-substrate system (Zavala *et al.*, 1982; Collins *et al.*, 1984; Burkot *et al.*, 1984; Wirtz *et al.*, 1985).

For the present study, the method of choice was the ELISA (Wirtz *et al.*, 1985) since it can not only be used to test large numbers of dried mosquitoes, but also because it uses stable reagents.

9.2. MATERIALS AND METHODS

Anophelines collected on human baits were kept dry over silica gel until assayed up to 18 months after collection. The ELISA procedure followed was modified by R. Wirtz (pers. comm.) based on the ELISA method described by Wirtz *et al.* (1987 a, b).

Mosquito preparation: Mosquitoes to be assayed were prepared the day before or on the same day that the ELISA was going to be conducted. The abdomens, wings and legs of females were removed to reduce the risk of detection of CS antigen from parts of the body other than the salivary glands (Wenyon, 1926, *in* Gabaldón & Ulloa, 1978; Robert *et al.*, 1988; Beier & Koros, 1991). Mosquitoes from a given species, village, site, date and hour of collection were analysed separately. When there were numerous individuals belonging to the same one of each of these categories they were analysed in pools of up to 10. When there were few in a category the pools were correspondingly smaller or the mosquitoes were analysed individually. The mosquitoes in a pool were placed in a polypropylene micro centrifuge tube (1.5 ml) and ground in 50 µl of boiled casein buffer (blocking buffer) containing 0.5% of the detergent Nonidet P-40. After grinding, 200 µl of blocking buffer was added to each tube bringing up the total volume to 250 µl.

ELISA: Polyvinyl chloride U-shape microtitre plates were coated with 50 µl of monoclonal antibody against *P. vivax* HD14 (0.025 µg/50 µl PBS), covered and incubated for 30 minutes. Wells were aspirated and filled with 200 µl of blocking buffer.

After 1 hour, wells were aspirated and 50 µl of the sample were added and incubated for 2 hours. After this period wells were aspirated and washed twice with PBS containing 0.5% Tween-20. 50µl of homologous monoclonal antibody conjugated to horseradish peroxidase Pv-HK22-2 (0.05 µg/50 µl) (Kirkegaard and Perry Laboratories) were added. After 1 hour, wells were aspirated and washed 3 times with PBS-Tw20. 100 µl of the substrate ABTS were added to each well. Optical density (range 0-3.00) at 405 nm was determined with an ELISA plate reader 30 minutes after the addition of substrate.

The positive control on each plate consisted of 100 pg of a synthetic *P. vivax* peptide (Wirtz *et al.*, 1987b). Once the assay was standardized and because of the lack of background reactions, I decided to include on each plate only one negative control consisting of a field-collected male anopheline. Samples were considered positive visually by the presence of the characteristic green colour. All samples determined as positive were kept at -70 °C for subsequent confirmatory testing and quantification, where the negative controls consisted of 10 male anophelines. Samples were confirmed positive if absorbance values exceeded the mean plus three times the standard deviation of the 10 negative controls. The positive controls consisted of a serial dilution of the synthetic CS peptide in three replicates (200, 100, 50, 25, 12, 6, 3 pg/well) which allowed the preparation of a calibration curve for estimating the number of sporozoites in each pool of mosquitoes assayed (Wirtz *et al.*, 1987b). Also 23 individual *An. dirus* that had been experimentally infected and confirmed as positive were placed on the same plate.

Monoclonal antibodies, positive controls and experimentally infected *An. dirus* were provided by Dr. Robert Wirtz of the Walter Reed Army Institute of Research, Washington, DC.

9.3. RESULTS

9.3.1. ELISA

A total of 61,068 anophelines collected on human baits in the three villages was assayed. Table 9.1 shows that initially 97 pools of mosquitoes were positive for *P. vivax* CS protein; of these 91 were from pools on the edges of the plates. These 91 pools were retested, generally on the following day but taking care to place them in the centre of the plate. Only three of these pools remained positive, but two of these were found negative on a second repeat. Thus, only one pool from these 91 apparent positives from the edges of the plate was confirmed as positive. Of the 6,797 pools of mosquitoes in the middle of the plates analysed, six were positive and only one of these was negative when retested. One may conclude that there is a very marked "edge effect" which causes 2.5% of mosquitoes to appear as false positives. Negative pools were not retested.

Table 9.2 shows the 6 pools of mosquitoes (equivalent to 6 mosquitoes) that were confirmed as positive (determined visually and spectrophotometrically) for *P. vivax* CS protein: 3 *An. nuneztovari*, 1 *An. oswaldoi*, 1 *An. albitarsis* and 1 species unidentifiable. The overall sporozoite rate was 0.0098% (95% confidence limits 0.0036 to 0.0214%). The rates reported in the present study are lower than those reported in previous studies in other regions of Latin America obtained either by salivary gland dissection or immunoassays. Table 9.3 summarizes previous reports. It is noteworthy that in general the numbers of anophelines dissected have been small, except for *An. evansae* in Colombia where Suárez *et al.* (1990) dissected 3,853 specimens and none was found positive, whereas in 2,192 *rangeli* analysed by ELISA 6.6% were positive.

It is noteworthy that all the O.D. readings were lower when the samples were retested; this is probably due to loss of antigen in the process of freezing and thawing of samples. If so, this fact may account for the finding that the mosquitoes from July, initially found positive but with very low O.D. values, could not be confirmed as positives. Sample 1669-5, which initially had the highest O.D. value, showed a very low value on confirmation, probably due to the thawing of the samples for over 24 hrs when the freezer in our laboratory broke down.

Table 9.1: Results of total pools of mosquitoes analysed, considering a) pools of mosquitoes in the wells on the edge of the plate, and b) pools of mosquitoes in the wells in the middle of the plate (a total of 33 wells were used around the edge of a plate and 60 were used in the middle)

a) Edge wells:

No. Negative	No. +ve initially -ve on repeat	No. +ve initially +ve on 1st repeat -ve on 2nd repeat	No. +ve initially +ve on repeat
3,539	88	2	1

b) Middle wells:

No. Negative	No. +ve initially -ve on repeat	No. +ve initially +ve on repeat
6,791	1	5

Table 9.2: Positive ELISA results.

No.	Date col.	Village	Species	O.D.	-ve control	Cut-off point	Decision
1461-2	Jul.88	CLP	unident.	0.159	-0.055		
				0.002 (*)	0.004	0.013	-ve
1462-13	Jul.88	CLP	*nunez*	0.053 (***)	-0.016		
				0.068 (**)	-0.034	-0.008	
				0.010 (*)	0.004	0.013	-ve
1500-2	Jul.88	CLP	*nunez*	0.065 (**)	-0.016		
				0.010 (***)	-0.034	-0.008	
				0.009 (*)	0.004	0.013	-ve
1669-5	Aug.88	CLP	*nunez*	1.134	-0.021		
				0.018 (*)	0.004	0.013	+ve
2308	Aug.88	JAB	unident.	0.523 (***)	-0.008		
				0.192 (*)	0.004	0.013	+ve
2286	Aug.88	JAB	*albi*	0.438	-0.008		
				0.147 (*)	0.004	0.013	+ve
2212-2	Aug.88	JAB	*nunez*	0.611	-0.008		
				0.467 (*)	0.004	0.013	+ve
2997	Sep.88	JAB	*oswal*	0.190	0.039		
				0.115 (*)	0.004	0.013	+ve
8128	Oct.89	CLP	*nunez*	0.155	0.013		
				0.077	0.009		
				0.052 (*)	0.004	0.013	+ve

(*)Results of final confirmation test with cut-off point= mean (10 male mosq.) + 3 S.D.
(**) 10 neg. mosquitoes on that plate
(***) sample at the edge of the plate

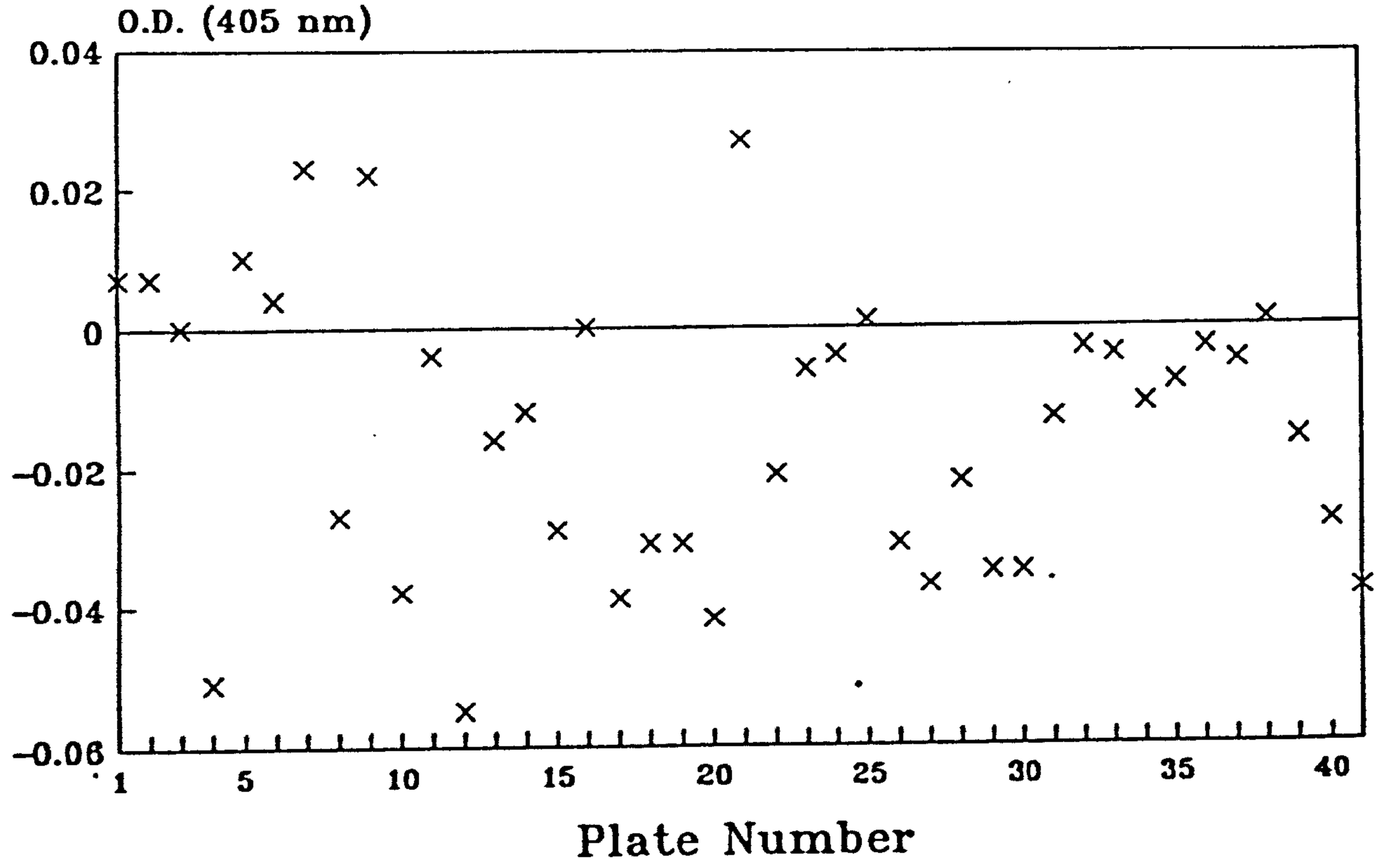
Table 9.3: Sporozoite rates (percentage) in anophelines from other Latin American regions (sample size in parentheses)

Species	Dissection Pf	ELISA Pv		IRMA Pf	Pv	Country	Reference.
nuneztovari	0(405)	-	-	-	-	Brazil	Deane *et al.* (1948)
oswaldoi	0(540)	-	-	-	-		
albimanus	0.61(324)	-	-	-	-	El Salvador	Warren *et al.* (1975)
albitarsis	2.2(186)	0	2.2(227)	0	4.5 (1,858)	Brazil	Arruda *et al.* (1986)
nuneztovari	0.8(254)	0	1.2(135)	0	1.5 (75)		
triannulatus	1.7(119)	0	8.9(433)	0	12.0(593)		
oswaldoi	0(116)	2.3	0.2(442)	-	-		
trinkae	2.3(3,010)	-	-	-	-	Peru	Hayes *et al.* (1987)
nuneztovari	0.5(201)	-	-	-	-		
oswaldoi	0.6(161)	-	-	-	-		
albitarsis	-	-	-	0.36	- (1,110)	Colombia	Herrera *et al.* (1987)
darlingi	-	-	-	0.07	- (2,890)		
darlingi	-	-	-	0.4	0.06(2,298)	Brazil	Deane *et al.* (1988)
triannulatus	-		-	-	0.07(1,160)		
darlingi	-	-	-	0.4	0.1 (9,885)	Brazil	Oliveira-Ferreira *et al.* (1990)
albitarsis	-	-	-	0.6	0.6 (346)		
triannulatus	-	-	-	-	0.5 (931)		
rangeli	0(735)	-	6.6(2,192)			Colombia	Suarez *et al.* (1990)
albitarsis	0(906)	-	0(52)	-	-		
evansae	0(3,853)	-	0(122)	-	-		
vestitipennis	-	-	0.42(3,840)	-	-	Mexico	Loyola *et al.* (1991)

Figures 9.1 and 9.2 show the O.D. values for the individual negative controls on each plate. Figure 9.1 contains those values for the first 41 plates run where the blank contained conjugate plus substrate; because most of the O.D. gave negative readings, I decided to use as blank only substrate, in order to avoid such negative readings. Lower O.D. values for negative controls on plates 76 onwards (Fig. 9.2) may be due because a different batch of plates was used.

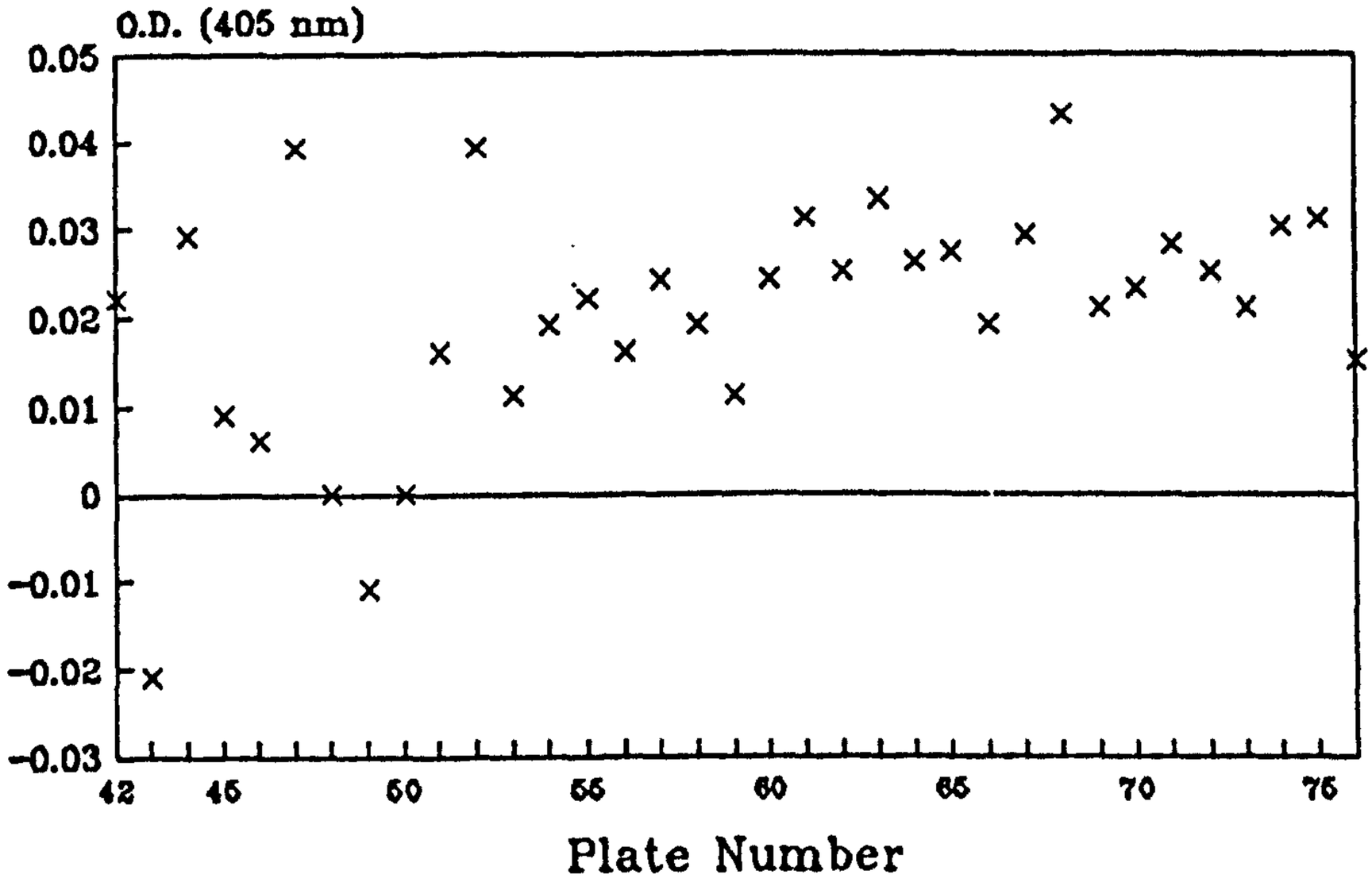
According to Wirtz *et al.* (1987a) it is possible to quantify the number of sporozoites in mosquitoes based on a calibration curve (Fig. 9.3). From the calibration curve and the optical density obtained in the final confirmation test, it is concluded that all positive mosquitoes had less than 50 sporozoites. As previously mentioned, in general the O.D. readings were lower when samples were retested, which may be attributed to loss of antigen activity during freezing and thawing of samples. Nevertheless, it seems that in general the load of *P. vivax* sporozoites in mosquito salivary glands is low. Burkot *et al.* (1987) found that in Papua New Guinea wild-caught anophelines of the *An. punctulatus* complex assayed by ELISA had a geometric mean of 4,000 *P. falciparum* sporozoites per mosquito and only 380 *P. vivax*. The authors, related this to the number of sporozoites produced per oocyst. Baker *et al.* (1987) reported that the levels of CS protein in 50% of the positive anophelines collected on the Thailand-Kampuchea border and tested by ELISA was equivalent to less than 275 sporozoites, and that mosquitoes with *P. falciparum* infections contained more CS protein than those infected with *P. vivax*. Furthermore, Beier *et al.* (1990) reported that 36.8% of the positive salivary glands dissected from *An. gambiae* and *An. funestus* from Kenya contained less than 500 sporozoites, and 26.3% contained less than 100 sporozoites.

FIGURE 9.1: O.D. of individual negative controls on plate nos. 1 to 41, using as blank a well to which was added first conjugate, and then, after washing, substrate.



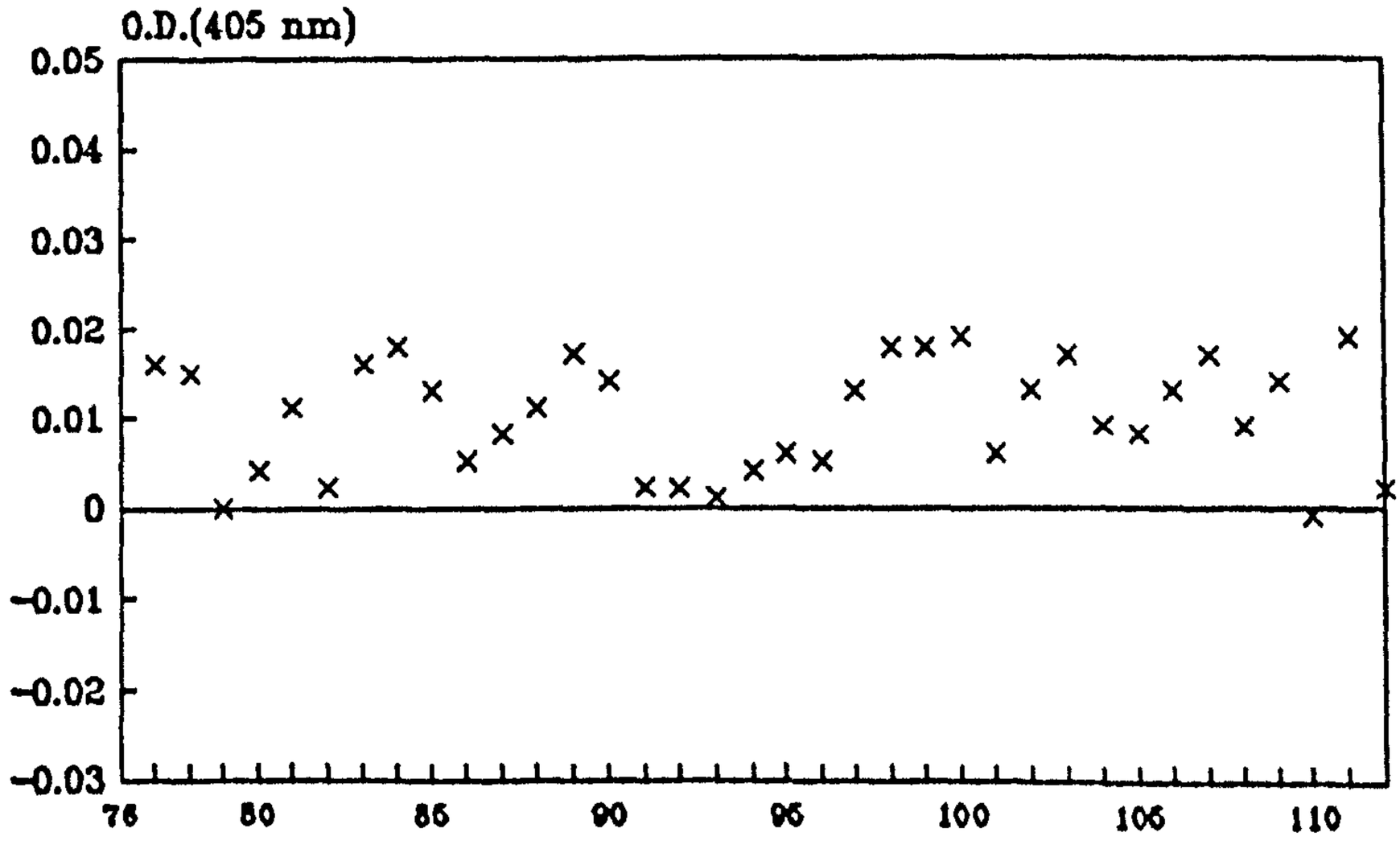
Blank=Conjugate + Substrate

FIGURE 9.2: Sporozoite ELISA
Negative Controls



Blank= Substrate

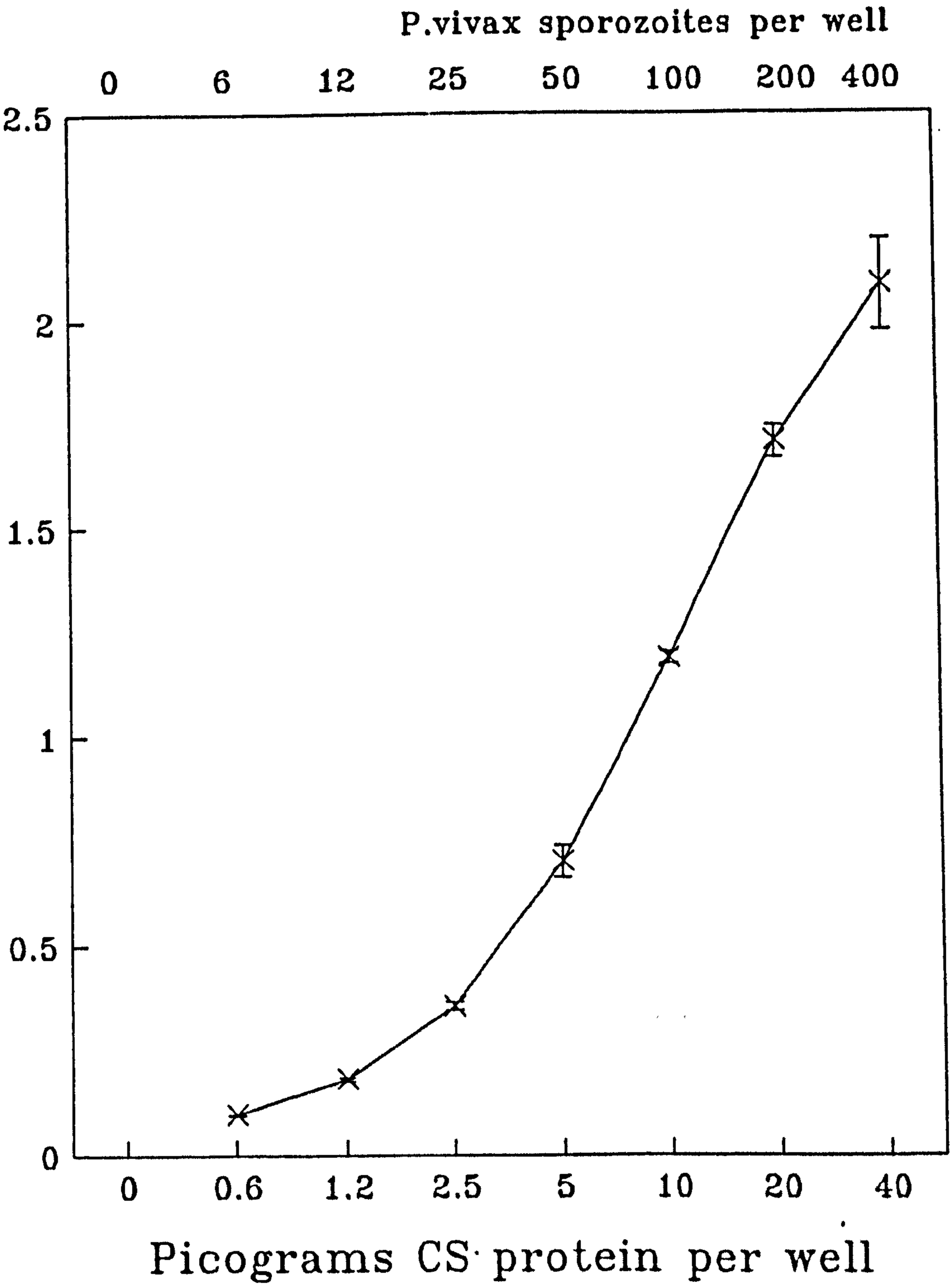
Sporozoite ELISA
Negative Controls



Blank=Substrate

FIGURE 9.2: O.D. of individual negative controls per plate (42 to 112) using as blank a well to which only substrate was added.

FIGURE 9.3: Calibration Curve



9.3.2. Entomological Inoculation Rate

The entomological inoculation rate was calculated for the three species found positive, i.e. *An. nuneztovari*, *An. albitarsis* and *An. oswaldoi* based on the human biting rate (*ma*) obtained from the human biting catches (Chapter 3) and the overall sporozoite rate from the ELISA. Results in Table 9.4 show that in the study area a person may be expected to receive approximately 8.6 sporozoite positive bites per year in Caño Lindo, 15.8 in Guaquitas or 7.1 in Jabillos. The inoculation rate in Guaquitas is about twice the rate in Caño Lindo or Jabillos, which is a reflection of the larger human biting rate. Although *An. triannulatus* were responsible for a larger mean number of bites per person per night (33.6 at Guaquitas) than *albitarsis* and *oswaldoi*, no *triannulatus* were found to be positive for sporozoites. *An. triannulatus* have been found naturally infected with *P. vivax* in Peru (Hayes *et al.*, 1987) and Brazil (Arruda *et al.*, 1986; Deane *et al.*, 1988; Oliveira-Ferreira *et al.*, 1990). The fact that in the present study *triannulatus* was not found to be positive might be due merely to chance and the relatively limited number of mosquitoes tested (4,119), and one cannot discard the possibility that this species could also be involved in transmission.

Although the sporozoite rate estimated is low, it seems to be high enough to maintain malaria transmission in this part of the country (Chapter 1).

Figure 9.4 shows the monthly distribution of malaria cases in the three study villages for 1979-1989, and for 1988 and 1989 separately (Dirección de Endemias Rurales, Records). Positive mosquitoes were collected in Caño Lindo and Jabillos during the rainy season (August and September, 1988; October, 1989) (Table 9.2). I found that there was no correspondence between the presence of human malaria cases and detection of positive mosquitoes, except in Caño Lindo in August 1988. There were positive

Table 9.4: Anopheline species tested by ELISA for vivax-sporozoites and calculation of sporozoite inoculation rate.

Village	Mean no. of bites/person/night				Est. no. of bites/person /year	Number Tested				No. +ve
	nunez.	*albi.*	*osw.*	all 3 spp.		*nunez.*	*albi.*	*osw.*	all 3 spp.	
CLP	232.8	5.2	2.8	240.8	87,892	14,131	416	253	14,800	2
GUA	396.3	31.1	13.9	441.3	161,075	19,108	2,292	872	22,271	0
JAB	180.7	10.3	6.3	197.3	72,015	12,465	897	569	13,913	3
All villages									50,984	5

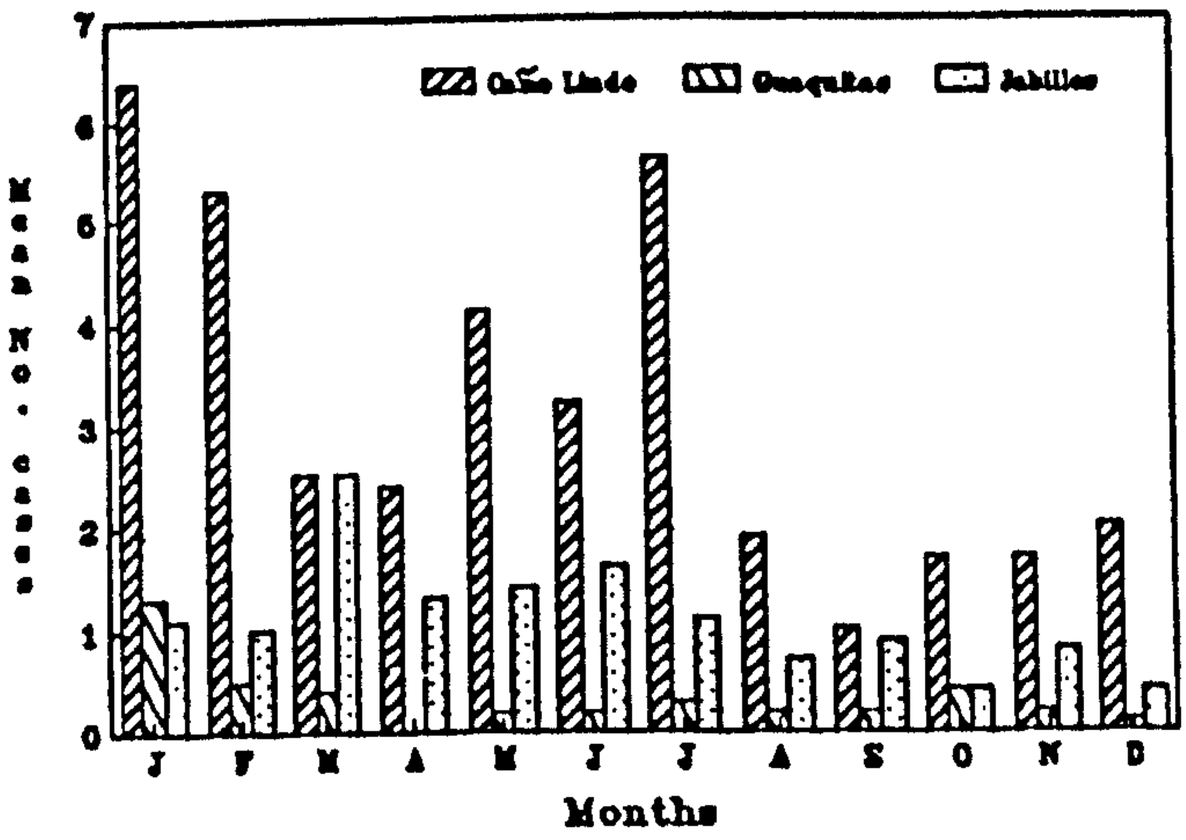
Estimated number of sporozoite positive bites/person/year for each village (using pooled sporozoite rate from all 3 villages and all 3 species):

CLP: (87,892/50,984)x5= 8.6
CLP= Caño Lindo

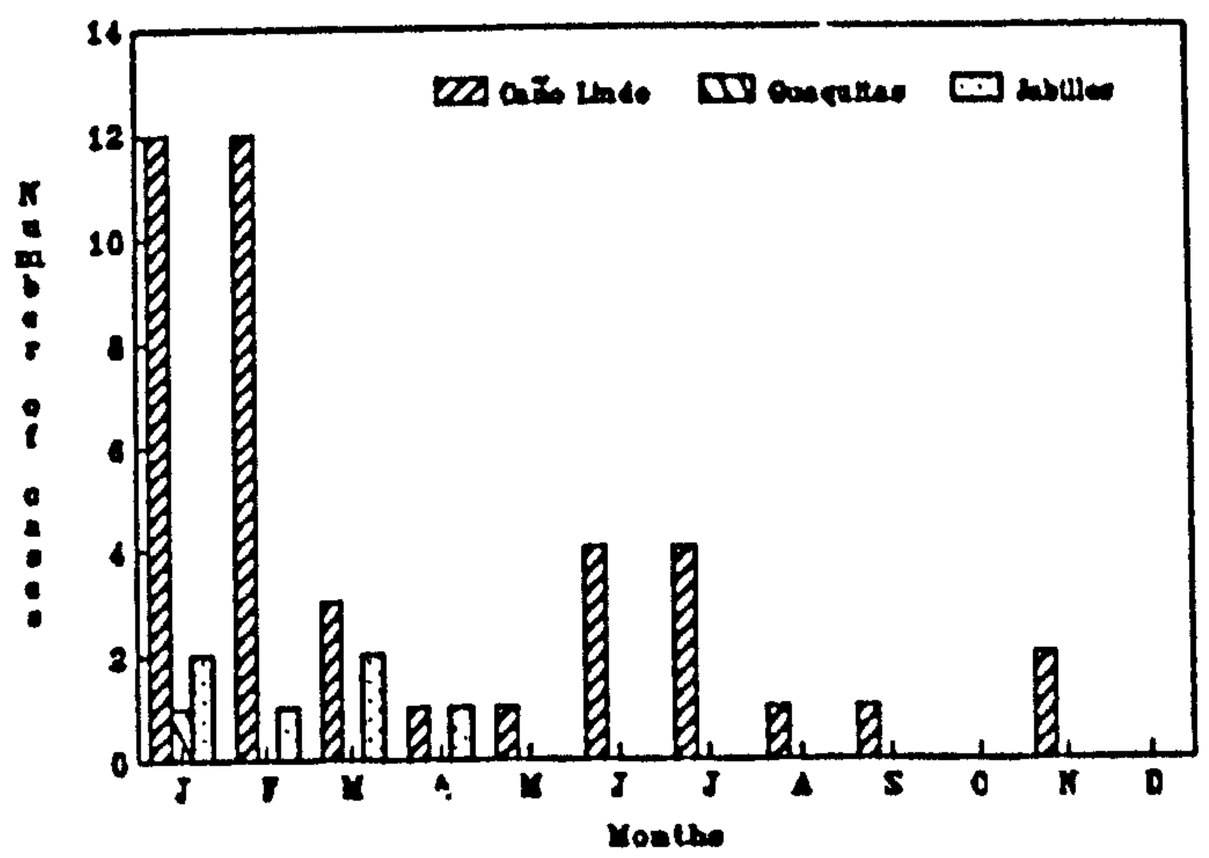
GUA: (161,075/50,984)x5= 15.8
GUA= Guaquitas

JAB: (72,015/50,984)x5= 7.1
JAB= Jabillos

a: Mean number of Cases 1979-1989



b: Number of Cases 1988



c: Number of Cases 1989

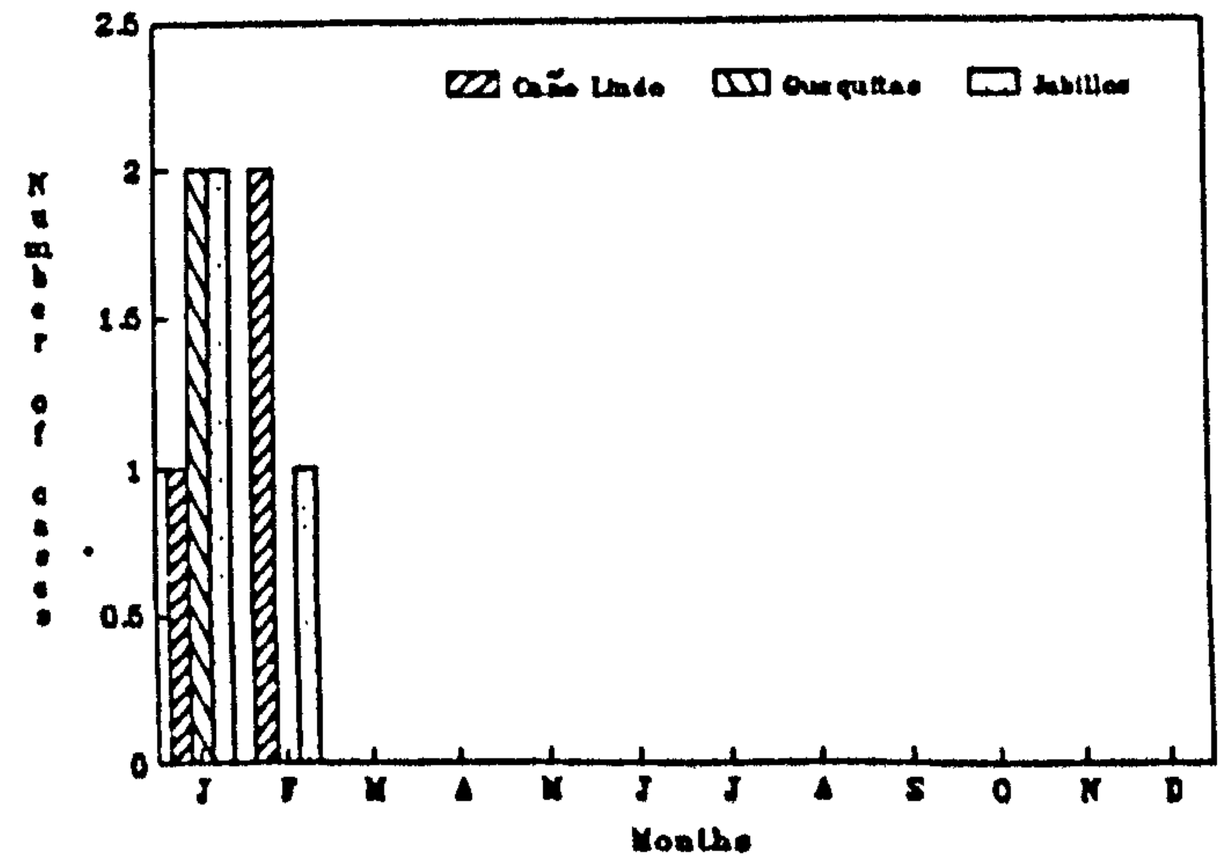


FIGURE 9.4: Number of cases per month in the three villages

anophelines in Jabillos during August and September 1988 while no cases were reported in these months or the following one. These results raise questions about the efficiency of transmission of *P. vivax* and the immune status of the human population in the area.

In an attempt to quantify the transmission efficiency of *P. vivax* in the study area, we can use the data in Table 9.4 where the estimated number of positive bites/person/year was approximately 10.5 averaged over the three villages. On the other hand, Chapter 1 and Fig. 9.4 indicate that in 1988 there were only 0.046 cases/person/year. Dividing 0.046 by 10.5 we come to the conclusion that only 0.44% of the bites containing sporozoites will successfully infect a person. In general, it has been reported that the efficiency of transmission of *P. vivax* is low, probably because of the small load of sporozoites in the mosquitoes' salivary glands (Burkot *et al.*, 1987; Baker *et al.*, 1987). These results contrast sharply with reports from areas where falciparum-malaria is holoendemic. For instance, Pringle and Avery-Jones (1966) reported that in the Ubembe area in Tanzania about two-thirds of the bites from infective mosquitoes gave rise to malaria infections in children.

In order to obtain an idea of the immune status of the population in the study area, 50% of the houses around the experimental huts in the three villages were visited during October 1989 and blood samples taken. Of the 185 samples assayed by ELISA, 84.9% were positive for antibodies against the sporozoites of *P. vivax* and 29% for those against the sporozoite of *P. falciparum*, whereas all blood smears for parasitological analysis were negative (E. Sánchez and E. Vaccari, pers. comm.).

9.4. DISCUSSION

An. nuneztovari, *albitarsis* and *oswaldoi* have been confirmed as vectors of malaria in western Venezuela. Other recent reports have found these species naturally infected in other countries of South America (Table 9.3). It is noteworthy that *An. nuneztovari* and *An. triannulatus* have never been found naturally infected with *P. falciparum* (Arruda *et al.*, 1986; Deane *et al.*, 1988; Oliveira-Ferreira *et al.*, 1990).

The present study sheds some light on the persistence of transmission in western Venezuela despite the efforts to control the vector and the parasite through insecticide house spraying and mass chemotherapy.

The entomological inoculation rate depends on both the sporozoite rate and the man biting rate. Although the sporozoite rate is low, the biting populations are very large (Table 9.4), particularly that of *nuneztovari*, and this factor accounts for the vectorial importance of this species in this part of the country. Nevertheless, it is important to bear in mind that the actual number of bites a member of the public is likely to receive is smaller than that reported in the present study because most people take some precautions against mosquito bites (see Chapter 10) and are therefore much less exposed to mosquito bites than the catchers.

It seems that the low incidence reported in the study area in relation to the entomological inoculation rate, may be due among other factors to the low transmission efficiency of *P. vivax* and/or the presence of antibodies to *P. vivax* in the local population.

During the present study the ELISA technique was the only method used to incriminate the vectors. Dissections were not carried out for the reasons already stressed in the introduction. Recently a debate has centred on the fact that immunological techniques measure sporozoite antigen, which can be widely disseminated throughout the mosquito body (Robert *et al.*, 1988), so that they detect infected mosquitoes but not necessarily infective ones. In fact, Beier *et al.* (1990) reported that the detection of CS protein using ELISA in Afrotropical anophelines overestimated the sporozoite rate because comparative studies showed that 45.4% of the ELISA-positive anophelines did not contain sporozoites in their salivary glands. Similar results have been reported by Esposito *et al.* (1986), Boudin *et al.* (1988) and Magesa *et al.* (1991), although the last of these authors pointed out that the difference they found was not as great as that reported by Beier *et al.* (1990). On the other hand, other authors have reported close agreement between salivary-gland dissections and ELISA-sporozoite-infection rates in field-collected anophelines in different parts of the world (Wirtz *et al.*, 1987b; Collins *et al.*, 1984). In a detailed study, Beier and Koros (1991) showed that on the one hand there is overestimation of the sporozoite rate when ELISA is used but on the other hand, there is underestimation of the sporozoite rate when dissection is used.

Whether dissection by skilled dissectors or carefully controlled ELISA is used for the diagnosis of sporozoites in mosquitoes, the results may be considered to give a measure which can justifiably be used to compare situations or to evaluate control measures. However, in my study area, due to the extremely low sporozoite rate, it would be almost impossible to use the sporozoite rate to evaluate the impact of insecticides because, for example, about 240,000 mosquitoes would be required to detect with

statistical significance a reduction of the observed sporozoite rate to a quarter of its present value; if the adulticide had been successful it would be prohibitively laborious to collect such a large sample of mosquitoes.

CHAPTER 10:

HUMAN BEHAVIOUR

10.1. INTRODUCTION

The frequency of man-biting by mosquitoes depends on the behaviour patterns of both humans and mosquitoes. In order to study people's habits in relation to mosquito behaviour, land use and alternative blood sources for mosquitoes, questionnaires were given to householders in the three villages in August 1988 (wet season), March 1989 (dry season) and August 1989. In October 1989, a more specific questionnaire on people's habits was given in 50% of houses within 2 km around the experimental huts (Appendix 1).

10.2. DESCRIPTION OF THE VILLAGES

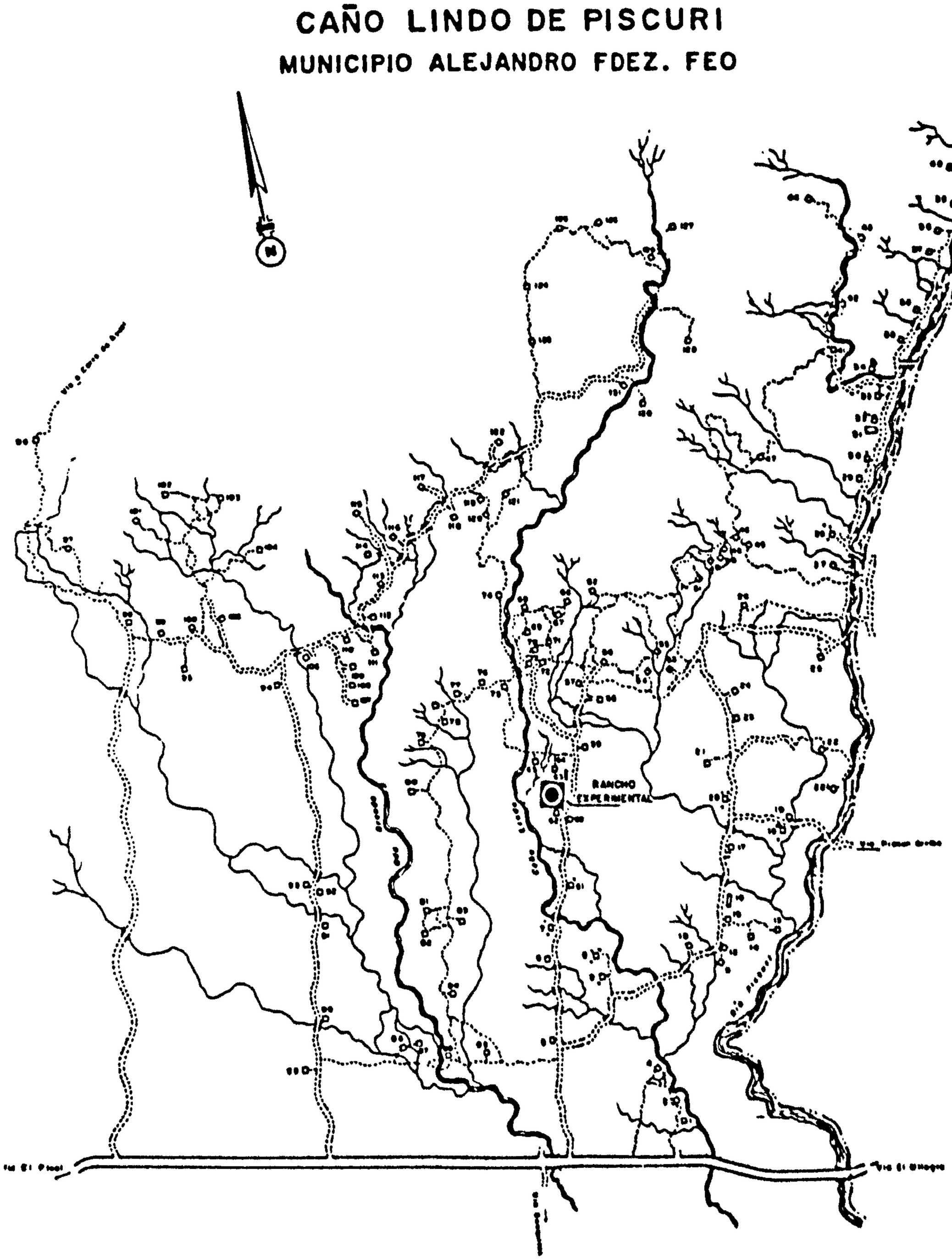
1. Caño Lindo de Piscurí is a fairly recent community resulting from "squatting" in the past 6 years on a *Hacienda*. "Squatting" means migrants taking possession of land that formally belonged to someone else but had been kept unused for several years. The migrants build houses and plant crops; after some years the land can be claimed by the squatters and registered in their names.

This village has no tarred roads, electricity or piped water. The most common type of dwelling is a temporary hut, built of wood with incomplete walls presenting many openings, a thatched or corrugated iron roof and an earth floor.

Figure 10.1 shows the approximate distribution of houses in the village and location of the experimental hut.

2. Guaquitas consists mainly of large farms which in the past were traditionally tobacco plantations. Houses are along an 8-km dirt road (Fig. 10.2) and most are made of brick with corrugated iron roofs; in addition there are temporary structures built by migrant labourers. Mains electricity has been available since July 1988.

FIGURE 10.1: Map of Caño Lindo de Piscurí showing distribution of houses and location of experimental hut.



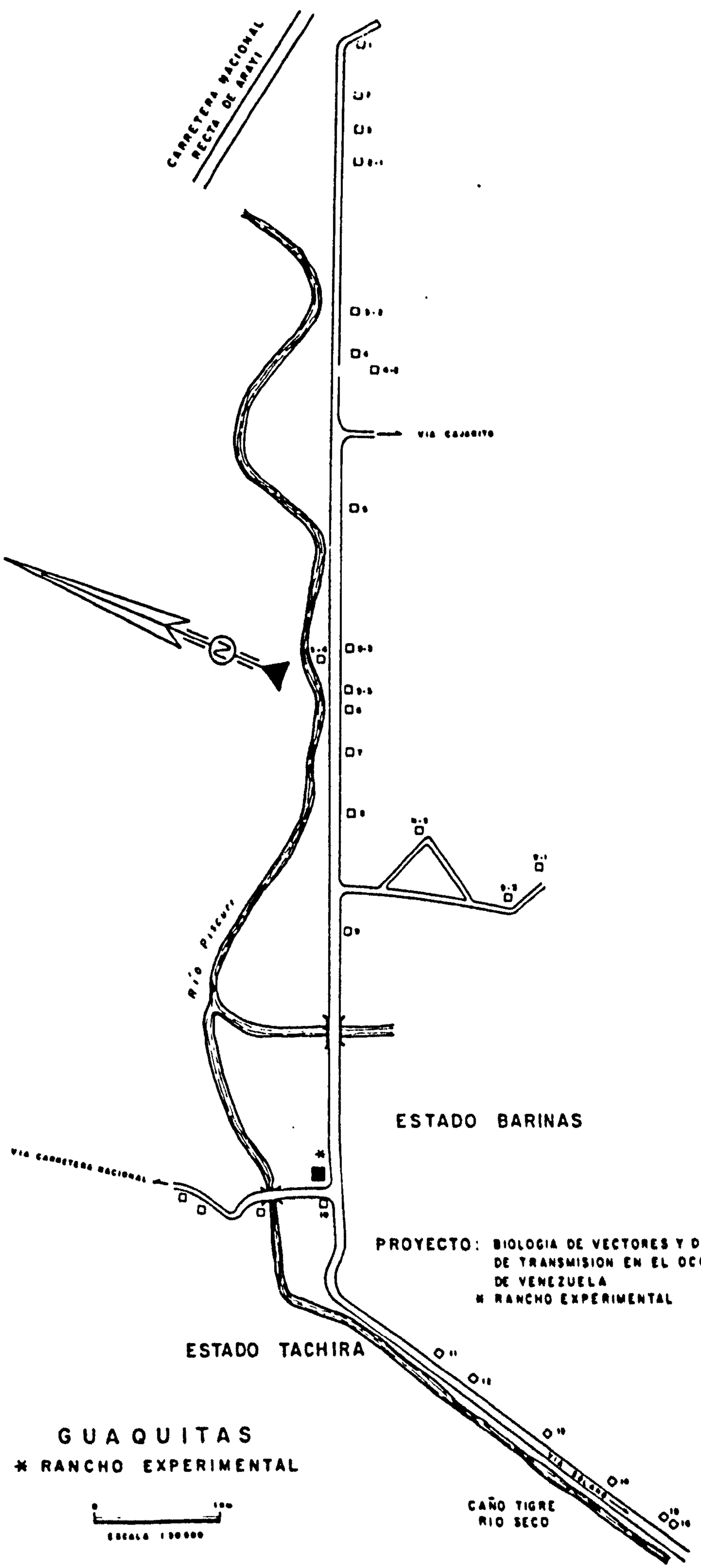


FIGURE 10.2: Map of Guaquitas showing distribution of houses and location of experimental hut.

3. Jabillos is a more stable locality with a tarred road, electricity and piped water, most of the houses having brick walls and corrugated iron roofs. Most houses were built by the Ministry of Health, Division of Rural Endemic Diseases, as part of a national programme to improve housing conditions in rural areas. Houses are distributed along a main road (Fig. 10.3).

10.3. RESULTS AND DISCUSSION

10.3.1. HUMAN BEHAVIOUR

A total of 566 questionnaires covering 3,196 people was given to householders (one questionnaire per house) on 3 different occasions (Appendix 2). A more detailed questionnaire was given to householders in October 1989 in 50% of houses within 2 km around the experimental huts. A total of 42 questionnaires, one per house, covering 263 people was given.

The population age structure varied between the villages: in Jabillos and Caño Lindo over 60% of the population were under 20 years old while in Guaquitas only 27% of the population were under 20 (Table 10.1). Figure 10.4 shows the distribution of the population by age group in the three villages: this suggests some degree of emigration of men in the age groups between 20 and 30 years.

The population was classified into five groups: men and women over 15 years of age; boys and girls from 6 to 15 years; and infants under 5 years.

The numbers of mosquito nets per village and the percentage of people protected by nets varied: in Jabillos and Guaquitas the proportion was higher (84.5% and 93%) than in Caño Lindo (41%) (Table 10.1). This may be due partly to a difference in income; as shown in the Table more people in Caño Lindo reported that they used more traditional mosquito repellents (burning bark). In Jabillos, the oldest and most developed village, 12 people declared that they used electric fans as protection against mosquitoes.

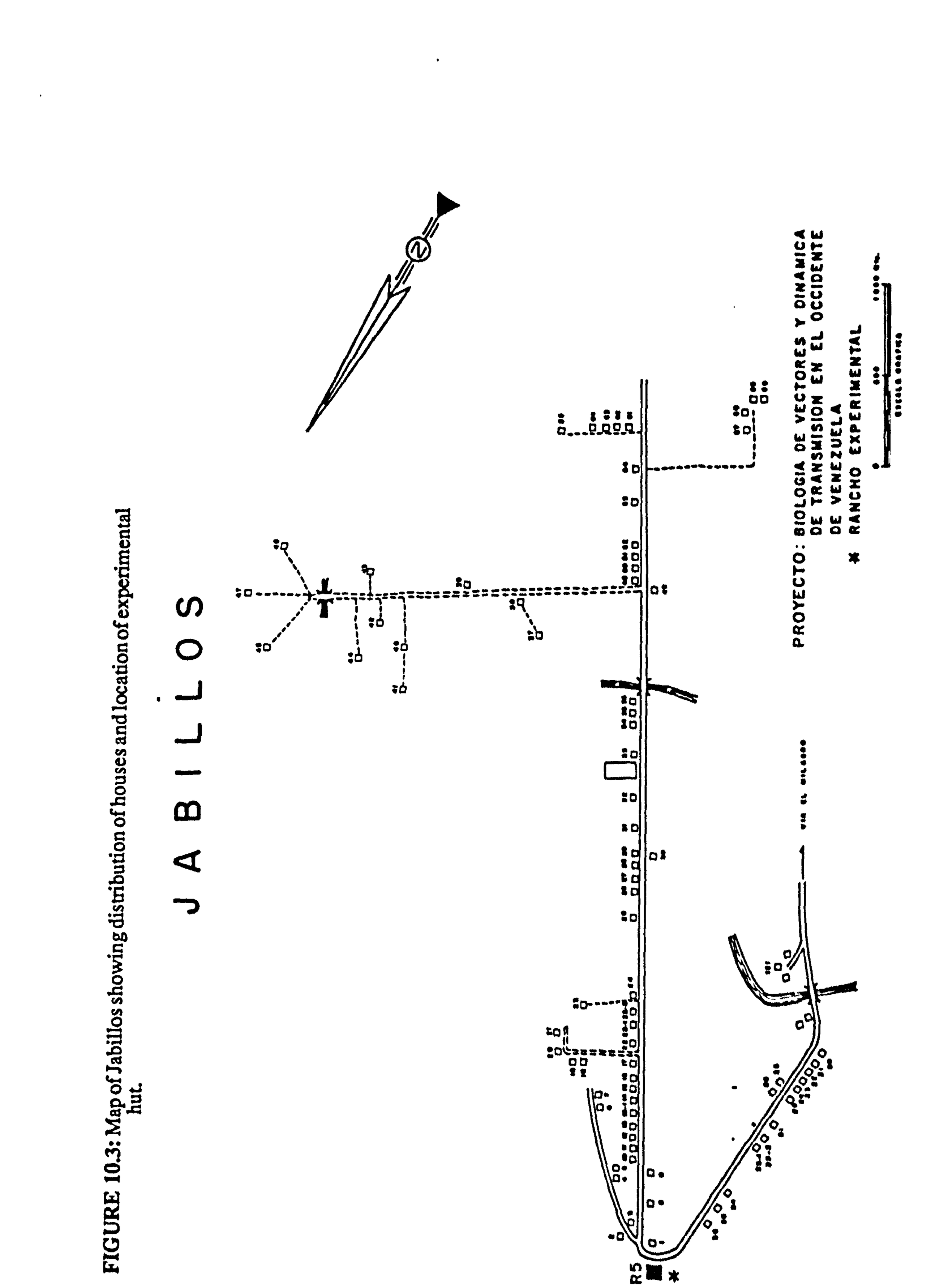


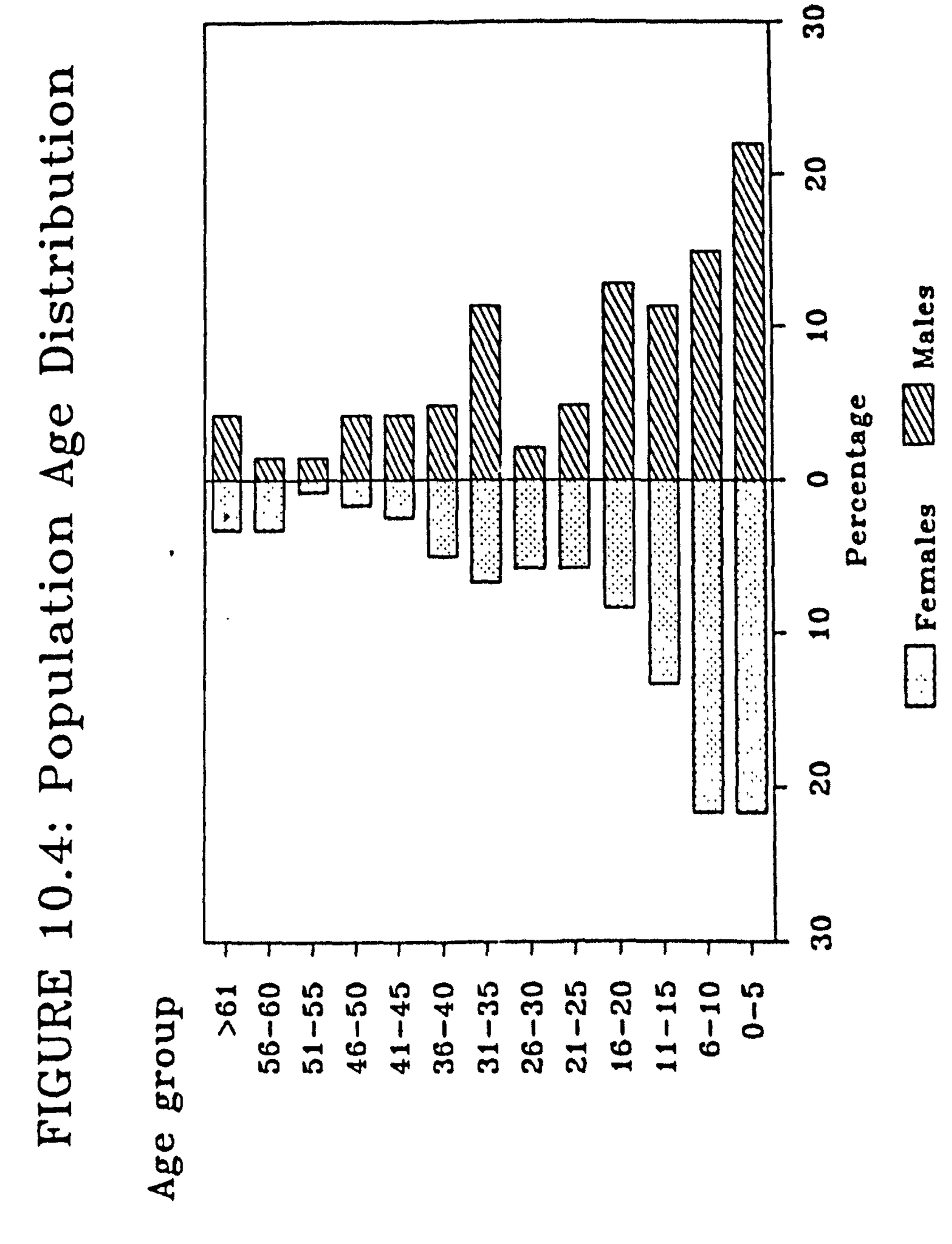
FIGURE 10.3: Map of Jabillos showing distribution of houses and location of experimental hut.

Table 10.1: Summary results of questionnaires carried out in October 1989 in 50% of the houses within 2 km of the experimental huts.

	Village		
	Jabillos	Caño Lindo	Guaquitas
No. houses	24	14	4
No. houses with electricity	13	0	2
No. TV sets	4	0	0
No. inhabitants	155	93	15
No. conventional Catholics	105	58	15
% conventional Catholics	67.7%	62.4%	100%
No. Catholic Revivalists	4	12	0
% of Catholic Revivalists	2.6	12.9	0
No. evangelicals	46	23	0
Percentage evangelicals	29.7	24.7	0
Percentage pop. under 20 yr. old	65	66	27
No. mosquito nets	70	20	12
No. people protected by nets	131	38	14
Percentage protected by nets	84.5	41	93
No. "protected" by fans (*)	12	0	0
No. "protected" by comején[1] (**)	16	20	0
Percentage who go to bed before 2200 hrs.	99	98	100
Percentage who wake up before 0700 hrs.	84	90	100

(*) Run from 2100 to 0500 hrs
(**) Burned from 1900 to 2200 hrs
[1] comején: burned bark

FIGURE 10.4: Population Age Distribution



All but 2 of the 34 mosquito nets inspected were found to be in good condition (Table 10.2).

In Guaquitas most people sleep alone (the population being mainly made up of migrant labourers) and in Caño Lindo and Jabillos most people shared beds (Table 10.3 & Fig. 10.5).

In general people's habits are as follows: they stay outside or near their houses until bedtime; when they go inside their houses they go to sleep immediately. Those people who spent most of the evening inside the house were those who had TV sets; they reported that they go to bed after the *novela* ("soap opera") at 2200 hrs. Generally kitchens and bathrooms are located outside the house, which is why people tend to leave the house as soon as they wake up.

Boys, girls and adults went to bed at about the same time, but infants go to bed earlier and by 2100 hrs most of them are in bed; they also wake up later. Most adults are in bed by 2200 hrs; women tend to go to bed slightly earlier than men (Fig. 10.6 & 10.7).

In an attempt to relate people's habits to mosquito biting activity, one may conclude that the population in the study area is totally exposed outdoors to bites of *An. triannulatus*, *oswaldoi* and *albitarsis* because these species are more active before 2100 hrs (Chapter 3). Of these species, *albitarsis* and *oswaldoi* were found positive for *P. vivax* CS protein (Chapter 9). *An. nuneztovari*, the most numerous species, has its biting peak between 2200 and 0200 hrs, i.e. by the time that almost 100 % of the population is in bed. Therefore, a good method of protection against *nuneztovari* would be the use of mosquito nets, especially if they are impregnated with insecticide. Recent studies in different parts of the world have demonstrated that the widespread use of insecticide-treated bednets results in an overall reduction of anopheline populations, reduction in parous rate, the proportion of mosquitoes that feed on man, the sporozoite inoculation rate and the number of malaria cases. For instance, Charlwood and Graves (1987) in Papua New Guinea reported that the used of permethrin-impregnated nets resulted in a reduction in

Table 10.2: Conditions of mosquito nets observed.

	Caño Lindo	Jabillos	Guaquitas	Percentage
Good	13	15	4	94.0
Medium	1	-	-	2.9
Bad	1	-	-	2.9

Table 10.3: Number of positive answers according to the number of people per bed.

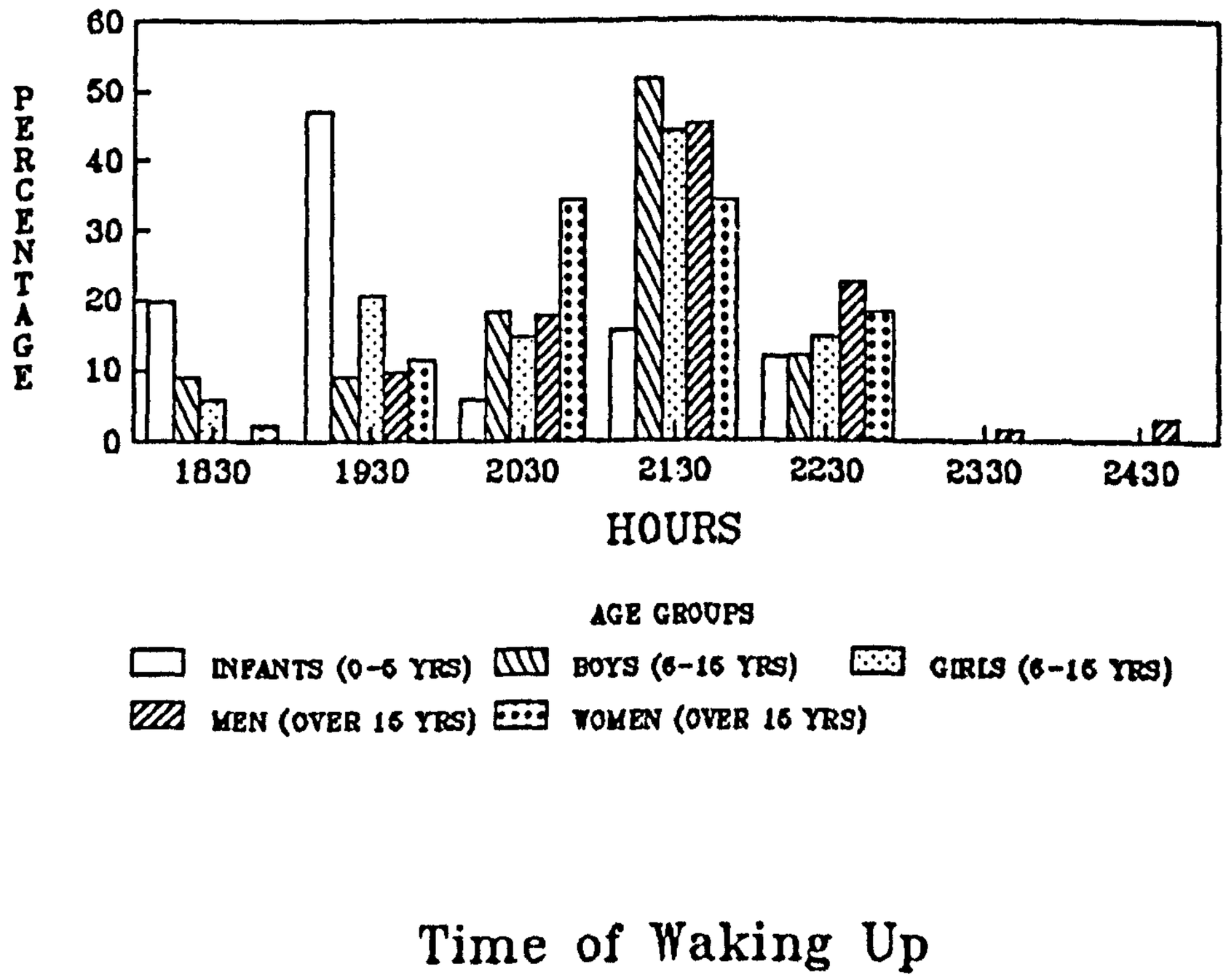
No. per bed	Caño Lindo	Jabillos	Guaquitas	Total	Total No.
1	24	35	11	70 x 1 =	70
2	17	29	2	48 x 2 =	96
3	10	18	0	28 x 3 =	84
4	2	3	0	5 x 4 =	20
5	0	1	0	1 x 5 =	5
				152	270

Mean no./bed= 1.77

FIGURE 10.5: Number of People per bed in the 3 villages

FIGURE 10.6:

Bed Time



Time of Waking Up

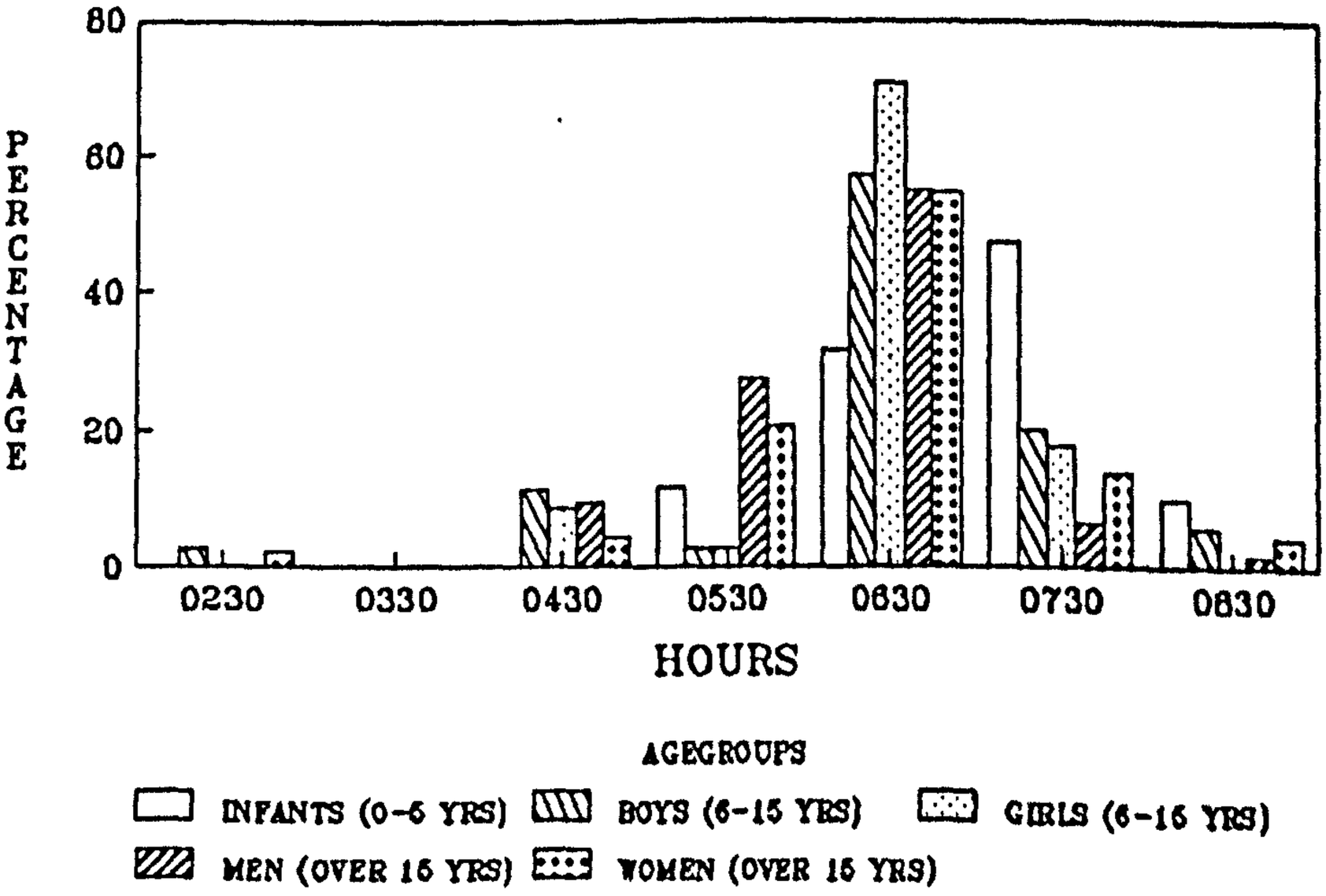
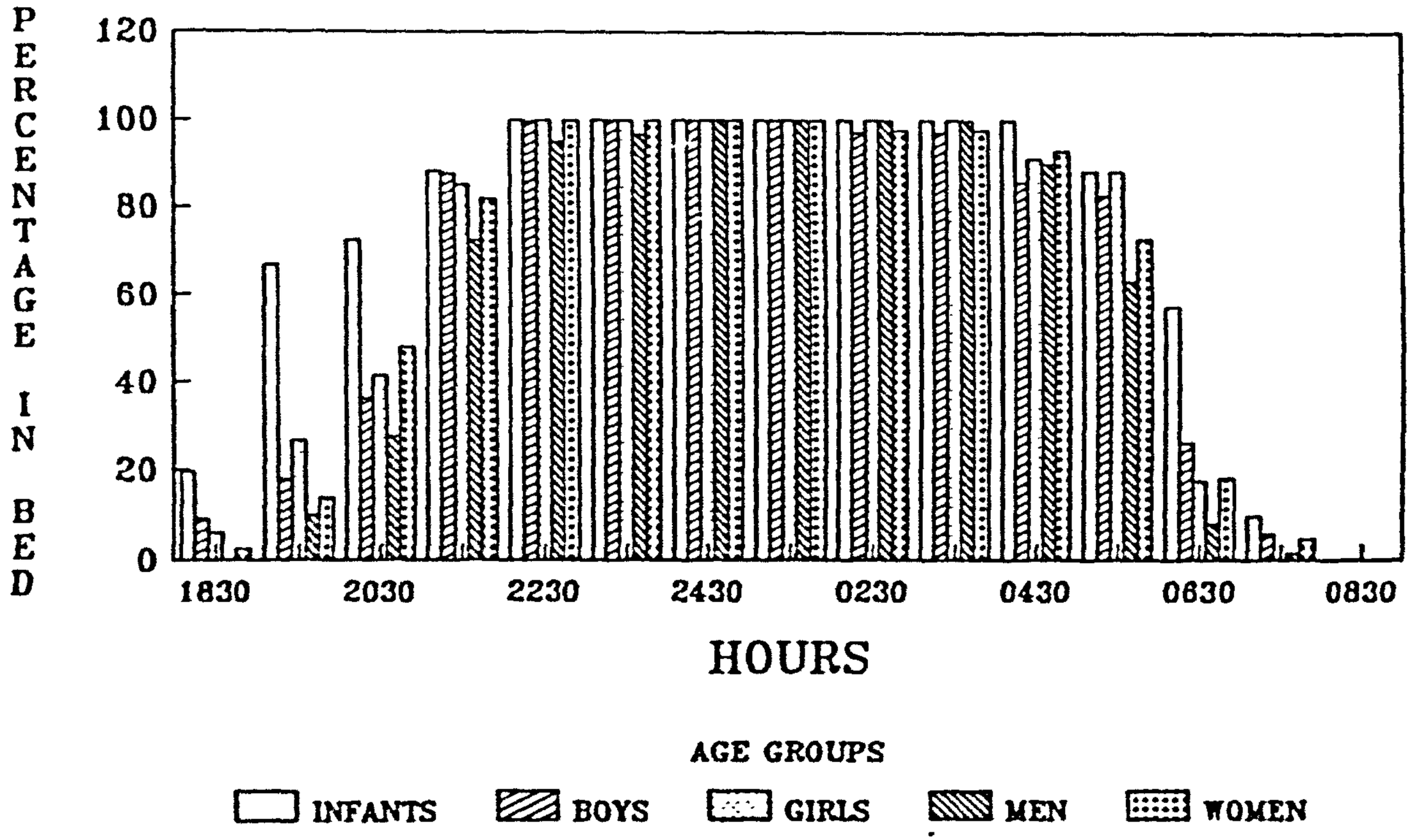


FIGURE 10.6: Bed time and time of waking up by age-group.

FIGURE 10.7:Percentage of People in Bed by Age Group and Sex



populations of *An. farauti* biting humans and resting in houses, and a reduction in the survival, in the proportion of blood-fed mosquitoes and in the human blood index. Carnevale *et al.* (1988) used deltamethrin-impregnated bednets in Burkina Faso and found a marked reduction in the number of *An. funestus* collected on human baits, and a reduction in the parity and sporozoite rate of *An. gambiae* and *An. funestus*. They concluded that the use of bednets resulted in a 90% reduction in the sporozoite inoculation rate. More recently, Magesa *et al.* (1991) have shown in Tanzania that after the introduction of permethrin-impregnated bednets there was a marked reduction in the vector population density and in the survival and sporozoite rate so that there was a reduction of over 90% of the sporozoite inoculation rate into people not under nets in villages where the great majority of people were using impregnated nets.

In the study villages not everybody was protected by nets: only 41% in Caño Lindo but higher percentages in Jabillos (84.5%) and Guaquitas (93%) (Table 10.1).

An interesting finding was that religious affiliation affects exposure to mosquito bites: most conventional catholics go to bed two hours before evangelicals and catholic revivalists (catholics who meet every evening to read the bible and pray) (Fig. 10.8; Table 10.4).

10.3.2. LAND USE

In the study area land use has changed considerably in the past 20 years. According to a sociological study conducted in 1968 (A. Rodríguez, Dirección de Endemias Rurales, Internal Report) crop growing was the main activity (especially tobacco and cotton) and there was little cattle rearing. However the results of questionnaires from 1988 and 1989 (Table 10.5) show that the area devoted to cattle and the number of head is now large and the land area devoted to crops is small.

Comparing the results in Table 10.5 for 1988 and 1989 it is not possible to compare land use between Jabillos and Caño Lindo because the total areas reported on

FIGURE 10.8:Percentage of People in Be
in relation to Religious affiliation

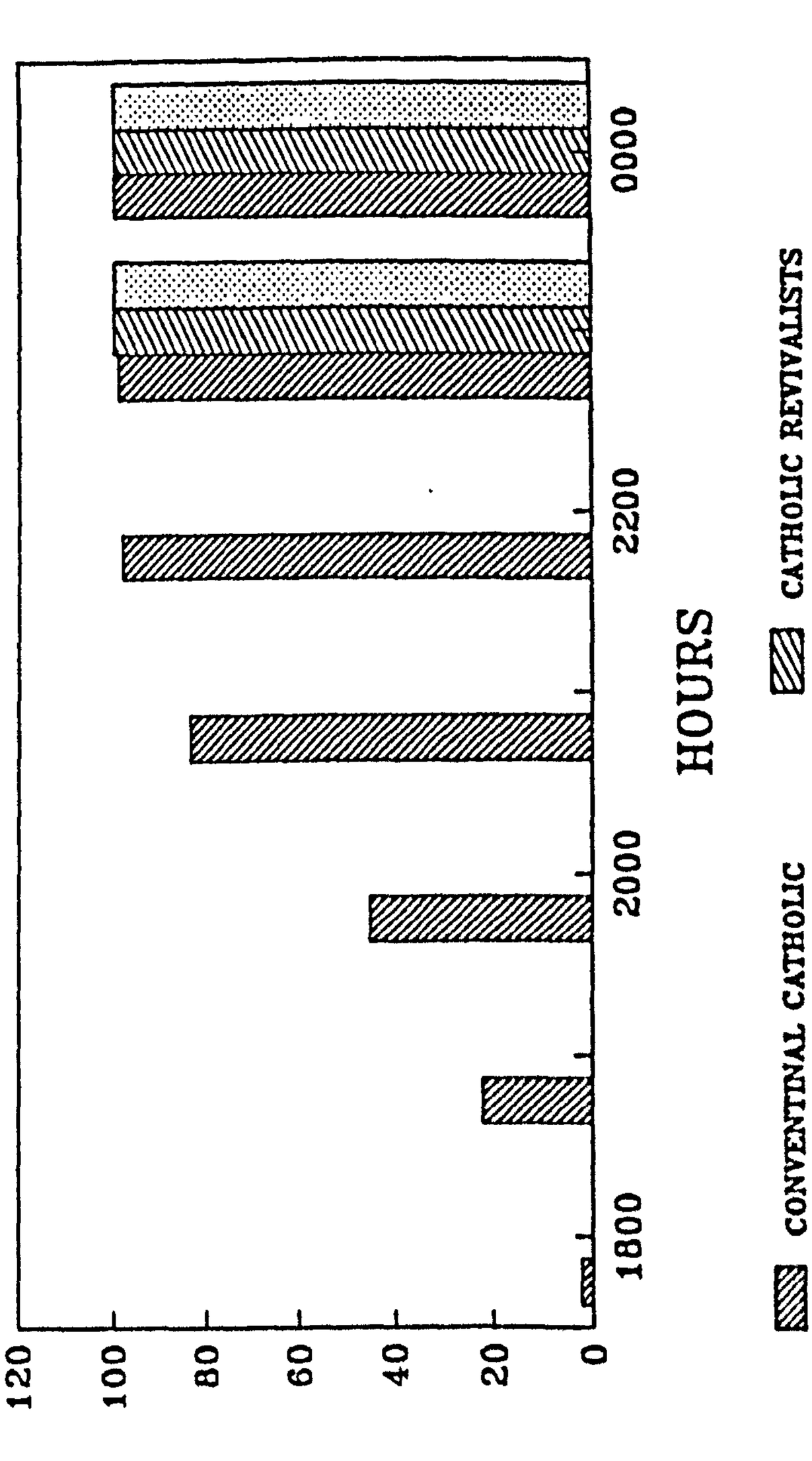


Table 10.4: Percentage of people in bed according to religious affiliation.

	No. of people			Percentage in bed (convent. Catholics)
Bed time	Catholic Revivalists	Evangelicals	Conventional Catholics	
1730-1830	0	0	4	2.3
1830-1930	0	0	34	22.0
1930-2030	0	0	40	45.1
2030-2130	0	0	66	83.3
2130-2230	0	0	26	98.3
2230-2330	11	70	1	98.9
2330-2430	0	0	2	100.0

Table 10.5: Hectares of land used in the three villages according to answers to questionnaires to householders in August 1988 and August 1989. (Number of cattle in parentheses)

	Caño Lindo		Jabillos		Guaquitas	
	1988	1989	1988	1989	1988	1989
Crops	24	46	43	48	118	73
Cattle	82 (69)	471 (596)	109 (127)	210 (393)	603 (528)	390 (520)
Forest (*)	50	194	64	142	162	413
Poultry	0	0	16	1	0	0

(*) Primary & secondary

do not match. However in Guaquitas it seems that between 1988 and 1989 the land area devoted to crop growing was reduced by 60% but there was also a reduction of land reported to be in use for cattle rearing by 64%, although the number of cows reported to be owned in the two years was similar (528-520). In Jabillos and Caño Lindo there was a very marked increase in the number of cows: in Caño Lindo from 69 in 1988 to 596 in 1989 and in Jabillos from 127 to 393.

Regarding the amounts of land reported, it appears that the questionnaire respondents did not have a clear idea of the amount of land they owned. It is noteworthy that in all three villages an increase of forest land (primary & secondary) was reported between 1988 and 1989.

Crops are mainly for local consumption. The most important are bananas and plantains (which are proved resting places for *An. nuneztovari*) and yuca (casava). Tobacco, which was the main crop in the past is now only planted in Guaquitas (Appendix 3).

The census of domestic animals (Appendix 3) showed that cows are by far the most abundant mammals and the most likely source of blood for mosquitoes apart from man.

CHAPTER 11:

CONCLUSIONS AND RECOMMENDATIONS

1. During the present study 14 anopheline species were collected by different sampling methods in western Venezuela. The most abundant species were those belonging to the subgenus *Nyssorhynchus* (Chap. 3, Table 3.1) of which over 75% were species belonging to the Oswaldoi subgroup, namely *nuneztovari*, *oswaldoi*, *strodei*, *rangeli* and *benarrochi*. Identification of adult females of this subgroup required extra care due to the large intraspecific variations and interspecific similarities of the morphological characters used. It was found that in adult females of *An. nuneztovari* the most variable taxonomic characters were the length of humeral pale spot and length of prehumeral dark spot. Based on these characters I initially separated *nuneztovari* into two distinct types "sp.1" (= typical *nuneztovari*) and "sp.11" (= morphotype 11) (Chap. 2, Table 2.5, Fig. 2.12). The results of linked rearings (Chap. 2, Table 2.8, Fig. 2.15) showed that both types occurred in the progeny of individual females. It was concluded that *nuneztovari* and morphotype 11 cannot be separate species but represent a polymorphism within a single species. Any future entomological study in this area should include identification of the female parent and larval skins from linked rearings in order to confirm identification of adult females.

2. The most abundant species were *An. nuneztovari*, *An. triannulatus*, *An. albitarsis* and *An. oswaldoi*. *An. nuneztovari* comprised over 70% of anophelines collected on human baits. *An. triannulatus*, the second most abundant species in Jabillos and Guaquitas, was rarely collected in Caño Lindo (Chap.3, Table 3.1). Highly significant differences in the numbers caught were found between species, site and month and their interactions (Chap.3, Tables 3.5, 3.6, 3.7, 3.8). These results seem to indicate that the larval habitats of each species at each site and season are different and on-going

studies on this question should be encouraged.

3. The efficiency of collections in light traps (Chap. 4, Fig. 4.2), resting on vegetation (Chap. 7, Fig.7.5) and in a double-net (Chap. 5) was much less than that of mosquito collections on human baits. Methods other than collections by human baits were particularly inefficient for the most numerous biting species, *An. nuneztovari*. Also, the parous rate of *An. nuneztovari* was significantly higher in human bait catches than in light traps (Chap. 4, Table 4.16). This means that for monitoring mosquito populations and evaluation of intervention programmes in this part of Venezuela, so far the only reliable method of collecting mosquitoes is by using human baits. However, light traps should be further evaluated using, for example, ultraviolet.

4. The diel biting pattern shown by *An. oswaldoi* (Chap. 3, Fig. 3.6.d) suggests that *oswaldoi* may be behaviourally polymorphic or a complex of at least two sibling species. Integrated taxonomic studies (morphological, biochemical and cytogenetic) are needed in order to elucidate whether *oswaldoi* is a species complex and the implications that this would have for malaria transmission.

5. The parous rate in *An. nuneztovari*, *An. albitarsis* and *An. triannulatus* was below 50% (Chap.3) which suggests that none of these species would be a highly efficient vector. In general, parity in *An. nuneztovari* did not vary significantly with season (Chap.3, Figs. 3.8.a, b & c) which seems to indicate that, although there are differences in rainfall and humidity during the year, environmental conditions in the adult habitat are rather stable.

6. The human blood index showed variations among villages that could not be explained by variation in the ratio of humans to cows in each village (Chap. 8, Tables 8.6 & 8.8). It seems that where the cows are kept is more important than their overall abundance. This factor should be considered when using the human blood index to evaluate control measures.

7. All anophelines in the study area are exophilic and detailed studies on their house entering and leaving behaviour are needed, especially in sprayed houses. Some specimens were collected resting on vegetation around houses between 0600 and 0800 hrs but among them very few *An. nuneztovari* were found (Chap. 7, Table 7.1). This species seems to rest deep in the forest, an inference that should be checked because of its implications for the likely ineffectiveness of peridomestic insecticidal fogging.

8. The study area has been regularly sprayed with insecticides. DDT was used in the area between the 1940's and 1984-85 when there was a change to fenitrothion. Nevertheless, transmission has not been interrupted (Chap. 1, Table 1.2). *An. nuneztovari* is an endophagic and extremely exophilic mosquito and this is presumably why no effects of fenitrothion were observed on mosquito density or on parous rate.

9. Anopheline populations in the study area showed fluctuations that correlated positively with rainfall and humidity (Chap. 3, Fig. 3.1, 3.2, & 3.3, Tables 3.2, 3.3 & 3.4). However, the incidence of vivax malaria in the area (Chap. 9, Fig. 9.4) does not show such obvious seasonal variation which suggests that many of the reported cases are not new infections but relapses.

10. Sporozoites were found in members of three species and the overall sporozoite rate in them, estimated by ELISA on 61,000 specimens, was 0.0098% (95% confidence limits 0.0036 to 0.0214%)(Chap. 9, Table 9.2). Multiplying this rate by the mean number of bites on the catchers indicates a sporozoite inoculation rate of about 10.5 positive bites per person per year (Chap.9, Table 9.4). In 1988 the number of malaria cases per person per year was 0.046 (Chap. 1, Table 1.2) which indicates an efficiency of transmission of 0.44%. This low efficiency of vivax transmission might be related to the small number of sporozoites in the mosquito salivary glands estimated from the optical density of the positive ELISA readings and a calibration curve (Chap. 9, Fig. 9.3).

11. The above calculated figure for the entomological inoculation rate is probably inflated above that experienced by a normal member of the public because,

whereas most people made efforts to protect themselves against mosquitoes (Chap. 10, Table 10.1), the catchers did not. It would be of interest to estimate the actual number of bites received by a person under normal conditions.

12. Entomological evaluation of control measures in western Venezuela should be focused on determining the effect of such measures on mosquito density and parous rate because of the large numbers of mosquitoes which would be required to detect with statistical significance a reduction in the sporozoite rate (Chapter 9).

13. In the past four years malaria has decreased in the study villages while the opposite situation has been observed for the rest of the country (Chap. 1, Table 1.2). As mentioned above, the decline in the study area does not seem to be due to insecticidal house spraying. There may be a natural cycle in malaria transmission, in which case this year or next there may be an increase in the incidence. Another possibility is that deforestation, increased cattle rearing (Chap. 10, Table 10.5) and/or a reduction in human migration from Colombia in the past four years has resulted in a reduction in malaria transmission. This situation should be compared with the present situation in southern Venezuela and the Amazon region of Brazil where human migration into these areas has led to a dramatic increase of malaria transmission (Otero *et al.*, 1986; Cruz Marques, 1987).

14. It is noteworthy that, among the three villages studied, there was more malaria in Caño Lindo (Chap. 1, Table 1.2) but fewer mosquitoes (Chap. 3, Table 3.1). This is probably because the most common type of house in this village offers many openings to mosquitoes and also because only 41% of the population in that village is protected by mosquito nets (Chap. 10, Table 10.1).

15. The four most abundant species showed distinctive biting patterns throughout the night (Chap. 3, Table 3.6): *An. nuneztovari* had a biting peak around midnight indoors and outdoors, *An. triannulatus* had a biting peak outdoors

between 1900 and 2000 hours, *An. albitarsis* bit indoors and outdoors mainly before midnight and *An. oswaldoi* had an early peak (1900 hrs) outdoors and a smaller peak indoors at midnight. When examining the time when people go to bed, based on the results of questionnaires, we found that most people go to bed before 2200 hrs and wake up before 0700 hrs (Chap. 10, Fig. 10.6). However, due to religious affiliation 34% of the population (Chap.10, Table 10.1 & Fig. 10.8) is more exposed to mosquito bites by remaining outdoors up to 2300 hrs.

16. A method of vector control that offers an alternative to traditional house spraying is the pyrethroid impregnation of bednets. Recent studies have shown that the use of such bednets are effective in reducing the vector population density, the survival and the sporozoite rate (Magesa *et al.*, 1991). The encouraging results reported should be considered carefully because the wide use of insecticide-treated nets in the study area may be effective in reducing malaria transmission due to *An. nuneztovari*. Having regard to the reported cost per person of insecticide spraying in Venezuela for 1986 (Dirección de Endemias Rurales, 1989a) and allowing for inflation, it is possible to compare this cost with the cost per person of providing nets to those people found by the questionnaires not to have them (Appendix 2), and impregnating all the nets in the study villages. The estimated present cost per person of spraying is 130 Venezuelan bolivars (= £ 1.30). The corresponding cost of providing impregnated bed nets would be approximately 65.5 Bs. per person, which includes the wholesale price of the nets plus permethrin for impregnation, plus labour (assuming that half the labour is needed for supervising net impregnation compared with house spraying) (Appendix 4). The cost-benefit of introducing impregnated bed nets and the attractiveness of the method to householders observed in other countries and in a small trial in southern Venezuela (Sevilla *et al.*, 1987) and its effectiveness for an exophilic and endophagic mosquito such as *An. dirus* in China (Li Zuzi & Lu Baolin, *in* Curtis *et al.*, 1990) suggests that this measure could be feasible to implement; but further detailed studies are needed.

Nevertheless, this measure may not be effective for controlling those species that bite early and mainly outdoors. Therefore attempts should also be made to motivate the human population to use other protective measures such as repellents on the skin or on clothing.

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APPENDIX 1

Text of questionnaires to householders freely translated from Spanish and an example of a completed form.

The questions were read to each householder either by me or a member of my team who filled in the form in accordance with the householder's answers.

1.1. Text of questionnaire to householders on land use and census of domestic animals within 2 km of the experimental hut (freely translated from Spanish). This questionnaire was carried out on three different occasions: August 1988, February 1989 and August 1989.

MALARIA PROJECT

The objective of the following questionnaire to householders in houses within 2 km of the experimental huts is to determine changes in land use and to carry out a census of domestic animals that could be potential blood sources for mosquitoes.

Please read the questionnaire carefully and follow the instructions. Do not leave any question unanswered.

I- Villages:

1) Caño Lindo
2) Jabillos
3) Abundancia
4) El Milagro
5) Guaquitas
6) Guacas

II- State:

1) Barinas
2) Táchira

III- Date: Day Month Year IV- DDT-#:[1] V- Interviewer:

VI- Name and Surname of householder Sex Age

VII- How many animals do you have?

1) Dogs: _____; 2) Cats: _____; 3) Birds: _____; 4) Pigs: _____;
5) Cows: _____; 6) Donkeys: _____; 7) Horses: _____; 8) Mules: _____;
9) Oxen: _____; 10) Goats: _____; Other: _____.

---

[1]: Since the 1940's, when the DDT-spraying programme started in Venezuela all houses where given a DDT number which is still in practice, especially in rural areas.

VIII- Size of parcel of land (*):

1) Only housing

2) Less than 1,000 m^2

3) Between 1,000 m^2 and 1/2 ha

4) Between 1/2 and 1 ha.

5) Between 1 and 10 ha.

6) Over 10 ha.

(*) Note: 1 ha= 10,000 m^2

IX- Area according to land use:

1) Crop growing: _____

2) Cattle rearing: _____

3) None: _____

X- What type of crops do you have on your land?

There is a list below of different crops(**). It is important to determine the area that each of them occupies. To do so, please write in the corresponding box areas used for them on each householder's land in the ranges defined above.

1) Pasture

2) Banana/plantain

3) Ocumo (*Colocasia*)

4) Yuca (casava)

5) Maize

6) Citrus

7) Vegetables

8) Coffee/Cocoa

9) Tobacco

10) Papaya

11) Sugar cane

12) Fruit trees

13) Ornamentals

(**) Note: 6) Citrus: oranges, mandarins and lemons
7) Vegetables: tomatoes, chilli, green pepper, pumpkin etc.
12) Fruit trees: mangoes, coconut, avocado, etc.

1.2. Example of a completed form on land use and census of domestic animals

PROYECTO MALARIA

El siguiente cuestionario tiene por objeto determinar cambios en el uso de la tierra y realizar un censo de animales que podrían ser fuente de sangre para los mosquitos en las casas ubicadas dentro de un radio de 2 kilómetros alrededor de los ranchos experimentales.

Favor leer cuidadosamente el cuestionario y seguir las instrucciones. No dejar preguntas sin contestar.

1- LOCALIDADES:

1) Caño Lindo	
2) Jabillos	X
3) Abundancia	
4) El Milagro	
5) Guaquitas	
6) Guacas	

2- ESTADO:

1) Barinas	X
2) Táchira	

3- FECHA:

D	M	A
20	8	89

4- DDT-#: 1

5- ENCUESTADOR: YASMIN

6- NOMBRE Y APELLIDO DEL ENCUESTADO

	SEXO	EDAD
Flor González	F	49

7- CUANTOS ANIMALES TIENE?:

1) PERROS: 2; 2) GATOS: 1; 3) AVES: 13;

4) COCHINOS: 1; 5) VACAS: 19; 6) BURROS: 1;

7) CABALLOS: 0; 8) MULAS: 0; 9) BUEYES: 0;

10) OVEJAS: 0; OTROS: 0

6- TAMAÑO DE LA PARCELA(*):

1) Sólo habitación
2) menos de 1000 m^2
3) entre 1000 m y 1/2 há
4) entre 1/2 há y 1 há
5) entre 1 y 10 há — X — 9há
6) Más de 10 há

(*) NOTA: 1 há= 10.000 m^2
1/2 há= 5.000 m^2
1/4 há= 2.500 m^2

7- AREA DE ACUERDO AL USO:

1) AGRICULTURA: 1 há
2) GANADERIA: 3 há
3) SIN USO: —

8- QUE TIPO DE CULTIVO TIENE SEMBRADO AHORA EN SU PARCELA:

A continuación se presenta un listado de tipos de cultivos(*). Es importante precisar la extensión que ocupan, para lo cual debe colocar en la casilla correspondiente a cada cultivo el número correspondiente a los rangos definidos arriba.

1) PASTO — X — 3ha
2) PLATANO/CAMBUR — X
3) OCUMO
4) YUCA
5) MAIZ/SORGO
6) CITRICOS — X
7) HORTALIZAS

8) CAFE y CACAO — X — 1ha
9) TABACO
10) LECHOSA
11) CAÑA DE AZUCAR
12) FRUTALES — X
13) ORNAMENTALES — X

(*) NOTA: 6) CITRICOS= naranjas, mandarinas y limones

7) HORTALIZAS= tomates, ají, pimentón, auyama

12) FRUTALES= mango, zapote, mamones, coco, níspero, guanábana, aguacate, etc.

9- CUANTOS MOSQUITEROS HAY EN LA CASA?: 3

10- A QUE HORA SE ACUESTAN?:

INVIERNO:	1) entre 6 y 7.	
	2) entre 7 y 8	
(Hace ≈ 4 meses) desde que tienen TV se acuestan más tarde	3) entre 8 y 9	X
	4) entre 9 y 10	
	5) entre 10 y 11	
	6) entre 11 y 12	
	7) _____	

VERANO:	1) entre 6 y 7	
	2) entre 7 y 8	X
	3) entre 8 y 9	.
	4) entre 9 y 10	
	5) entre 10 y 11	
	6) entre 11 y 12	
	7) _____	

11- A QUE HORA SE LEVANTAN?:

INVIERNO:	1) entre 4 y 5	
	2) entre 5 y 6	X
	3) entre 6 y 7	
	4) entre 7 y 8	
	5) entre 8 y 9	
	6) _____	

VERANO:	1) entre 4 y 5	
	2) entre 5 y 6	X
	3) entre 6 y 7	
	4) entre 7 y 8	
	5) entre 8 y 9	
	6) _____	

1.3. Text of questionnaire on human habits to 50% of householders within a radius of 2 km around the experimental huts carried out in October 1989 freely translated from Spanish.

Malaria Project

Village :	Date :	DDT - # [1]	Interviewer :	(*) Householder

Mains Electricity (Y/N)	TV (Y/N)	If yes - Indoors or outdoors?	How many mosquito nets do you have ?

No.	Name	Age	Sex	What did you do last night?	When did you come home?	When did you go to bed?	Bed, cot, Hammock?	Do you sleep alone?	did you use a mosquito net last night?	Did you burn Bark last night? /how long?	Did you use a fan last night? /how long?	What time did you wake this morning?	When did you leave the house?

[1]: Since the 1940's, when the DDT-spraying programme started in Venezuela, all houses where given a DDT number which is still in practice, especially in rural areas.

1.4. Example of a completed form on human habits to 50% of householders within a radius of 2 km around the experimental huts.

NOTA: (**) Determinar cuales de las actividades son nocturnas

• Van al culto al Km 21 sábados y domingo. Martes y viernes se reunen en casa de alguno de la Loc.

PROYECTO MALARIA

LOCALIDAD: CLP FECHA: Domingo 15/10/89 SOT-6: 7 ENTREVISTADOR: YASHIN (*)ENTREVISTADO: Carmen Alicia de Becerra

LUZ ELECTRICA: SI / NO X TV: SI / NO X ADENTRO / AFUERA: Cuantos cuartos tiene? 6 Hace toldillos con tapas, 1 un poco descosido / Ninguno con rajas

No.	NOMBRE	Edad	Sexo	Qué hizo anoche?	A qué hora entró en la en la casa?	A qué se acostó?	Duerme en cama, cuna o hamaca	Cuantas duermen en la misma cama	Durmió anoche con mosquitero	Prendió ventilador anoche?	Durante cuantas horas?	Prendió ventilador hoy?	A qué hora se levantó hoy?	A qué hora salió de la casa hoy?	
1	Carmen Alicia	33	F	Jugar con palabra	8:00	9:00	Cama	con 2	No	No	No	—	6:30	6:30	al río
2	Justo	41	M	Juntó de la Iglesia	8:00	"	"		No	"	"		5:30	5:30	
3	Alexander	18	M	"	8:00	"	"	con 9 y 8	No	"	"		8:00	9:00	para el trabajo se para a las 6:a para la Escuela
4	Freddy	17	M	"	8:00	"	"		Si	"	"		8:00	8:00	
5	Gerardo	16	M	"	8:00	"	"		Si	"	"		8:00	8:00	
6	Zaida	14	F	"	8:00	"	"	con 10, 13	No	"	"		6:00	8:00	
7	Gilimar	13	F	"	8:00	"	"		No	"			—	—	
8	Leo Dan	12	M	"	8:00	"	"		No	"			—	—	
9	Richard	10	M	"	8:00	"	"		No	"			7:00	7:00	
10	Yasmina	8	F	"	8:00	"	"		No				7:00	7:00	
11	Alba Ester	3	F	"	8:00	"	"	con 12	No				6:30	7:00	
12	Nelson Ael	2	M	"	8:00	"	"		No				5:00	6:00	
13	Ruth Daniela	—	F	En casa jugando con sus	6:30	6:30	Cama		No				5:00	7:00	
14	Miguel Angel	"	M	"	"	"	Alina	con 15	No				5:00	7:00	
15	Ruben Daniel	"	M	"	"	"	"		No				5:00	7:00	
16															
17															
18															
19															

Asumió Pto. Vivas anoche

Trillizos

APPENDIX 2

Results of questionnaires on human habits to all householders in the three study villages carried out in August 1988, February 1989 and August 1989. An example of a completed form is included.

2.1. RESULTS OF QUESTIONNAIRES ON HUMAN HABITS CARRIED OUT IN AUGUST 1988.

1- VILLAGE	No. HOUSES	POPULATION
CAÑO LINDO (CLP)	99	543
JABILLOS (JAB)	76	425
GUAQUITAS (GUA)	17	102

2- No. OF PEOPLE PER HOUSE, EXPRESSED AS A PERCENTAGE OF THE TOTAL NUMBER OF HOUSES

No. OF PEOPLE	CAÑO LINDO	JABILLOS	GUAQUITAS
1	8.1	6.6	0
2	10.1	5.3	0
3	8.1	15.8	17.6
4	15.2	9.2	11.8
5	12.1	15.8	17.6
6	16.2	9.2	17.6
7	9.1	11.8	11.8
8	9.1	13.2	5.9
9	1.0	5.3	5.9
10	3.0	3.9	5.9
11	4.0	2.6	5.9
12	1.0	0	0
13	1.0	0	0
14	1.0	0	0
15	0	1.3	0
18	1.0	0	0

3- TYPES OF PERSONAL PROTECTION USED.	PERCENTAGE OF POSITIVE ANSWERS		
	CAÑO LINDO	JABILLOS	GUAQUITAS
MOSQUITO NETS	52.5	86.8	82.4
WINDOW SCREENING (*)	-	-	11.8
TRADITIONAL REPELLENTS (*)	-	2.6	-
TOPICAL REPELLENTS (*)	-	-	-
ELECTRIC FAN (*)	-	2.6	5.9
OTHER (*)	-	-	-
NONE	-	10.5	-

(*) Question referred only to mosquito nets but other means were named by some respondents

4- TOTAL No. OF MOSQUITO NETS	135	189	52
No. NETS PER HOUSE	1.4	2.6	3.1
No. NETS PER PERSON	0.25	0.5	0.5

5- PERCENTAGE OF PEOPLE PROTECTED BY MOSQUITO NETS	CAÑO LINDO	JABILLOS	GUAQUITAS
RAINY SEASON	32.5	79.1	79.4
DRY SEASON	29.3	34.8	37.3

6- BED TIME: PERCENTAGE OF POSITIVE ANSWERS

	I- RAINY SEASON			II- DRY SEASON		
HOUR	CLP	JAB	GUA	CLP	JAB	GUA
18:00-19:00	2.0	3.8	0	1.1	2.6	0
19:00-20:00	21.6	8.9	29.4	17.9	0	0
20:00-21:00	31.4	58.2	47.1	31.6	56.3	31.2
21:00-22:00	29.4	25.3	23.5	31.6	31.3	32.5
22:00-23:00	11.8	2.5	0	15.8	12.5	32.5
23:00-24:00	3.9	1.3	0	2.1	1.3	0

7- TIME OF WAKING: PERCENTAGE OF POSITIVE ANSWERS

	I- RAINY SEASON			II- DRY SEASON		
HOUR	CLP	JAB	GUA	CLP	JAB	GUA
3:00-4:00	0	0	0	0	1.3	0
4:00-5:00	0	1.4	0	0	3.9	11.8
5:00-6:00	9.4	14.9	37.5	10.3	28.9	41.2
6:00-7:00	77.1	62.2	56.3	79.4	52.6	47.1
7:00-8:00	11.5	20.3	6.3	10.3	11.8	0
8:00-9:00	2.1	1.4	0	0	1.3	0

2.2. RESULTS OF QUESTIONNAIRES ON HUMAN HABITS CARRIED OUT IN FEBRUARY 1989.

1- VILLAGE	No. HOUSES	POPULATION	% MALES	% FEMALES
CAÑO LINDO (CLP)	95	482	55.8	44.2
JABILLOS (JAB)	68	409	56.2	43.8
GUAQUITAS (GUA)	16	100	71.0	29.0

2- No. OF PEOPLE PER HOUSE, EXPRESSED AS A PERCENTAGE OF THE TOTAL NUMBER OF HOUSES

No. OF PEOPLE	CAÑO LINDO	JABILLOS	GUAQUITAS
1	12.6	5.9	0.0
2	8.4	7.4	12.5
3	8.4	5.9	0.0
4	14.7	11.8	25.0
5	12.6	17.7	12.5
6	15.8	8.8	6.3
7	6.3	10.3	18.8
8	6.3	11.8	0.0
9	5.3	11.8	12.5
10	2.1	4.4	6.3
11	1.1	4.4	0.0
12	1.1	0.0	0.0
13	1.1	0.0	0.0
14	1.1	0.0	0.0
15	0.0	0.0	12.5
19	1.1	0.0	0.0

3- TYPES OF PERSONAL PROTECTION USED-PERCENTAGE OF POSITIVE ANSWERS

PERSONAL PROTECTION	CAÑO LINDO	JABILLOS	GUAQUITAS
MOSQUITO NETS	34.7	94.1	87.5
WINDOW SCREENING	2.1	0.0	18.8
TRADITIONAL REPELLENTS	6.3	32.4	0.0
TOPICAL REPELLENTS	1.1	1.5	0.0
ELECTRIC FAN	1.1	8.8	12.5
OTHER	0.0	1.5	0.0
NONE	59.0	4.4	6.3

4- TOTAL No. OF MOSQUITO NETS	73	180	48
No.NETS PER HOUSE	0.77	2.65	3.0
No.NETS PER PERSON	0.15	0.44	0.48

5- PERCENTAGE OF PEOPLE PROTECTED BY MOSQUITO NETS

	CAÑO LINDO	JABILLOS	GUAQUITAS
RAINY SEASON	24.9	79.2	71.0
DRY SEASON	20.5	73.6	62.0

6- BED TIME: (percentage of positive answers)

HOUR	I- RAINY SEASON: CLP	JAB	GUA	II-DRY SEASON: CLP	JAB	GUA
18:00-19:00	1.1	12.1	0.0	0.0	9.1	0.0
19:00-20:00	19.0	42.4	12.5	16.8	31.8	12.5
20:00-21:00	57.9	29.4	50.0	54.7	24.2	31.3
21:00-22:00	20.0	12.1	25.0	25.3	28.8	18.8
22:00-23:00	2.1	3.0	12.5	3.5	6.1	37.5

7- TIME OF WAKING (percentage of positive answers)

HOUR	I- RAINY SEASON: CLP	JAB	GUA	II- DRY SEASON: CLP	JAB	GUA
2:00-3:00	0.0	0.0	5.9	0.0	0.0	5.9
4:00-5:00	1.1	18.2	5.9	1.1	16.7	5.9
5:00-6:00	61.1	39.4	29.4	59.0	42.4	41.2
6:00-7:00	34.7	36.4	52.9	35.8	36.4	41.2
7:00-8:00	3.2	6.1	0.0	3.2	4.6	5.9

2.3. RESULTS OF QUESTIONNAIRES ON HUMAN HABITS CARRIED OUT IN AUGUST 1989.

1- VILLAGE

VILLAGE	No.HOUSES	POPULATION	% MALES	% FEMALES
CAÑO LINDO	101	548	57.3	42.7
JABILLOS	80	494	57.1	42.9
GUAQUITAS	14	93	71.0	29.0

2- No. OF PEOPLE PER HOUSE, EXPRESSED AS A PERCENTAGE OF THE TOTAL NUMBER OF HOUSES.

No. OF PEOPLE	CAÑO LINDO	JABILLOS	GUAQUITAS
1	4.95	7.5	0.0
2	10.89	2.5	0.0
3	11.88	11.3	0.0
4	12.87	7.5	14.3
5	14.85	16.3	14.3
6	12.87	12.5	21.4
7	10.89	11.3	21.4
8	7.92	12.3	14.3
9	7.92	10.0	0.0
10	0.99	2.5	14.3
11	1.98	2.5	0.0
12	0.99	3.8	0.0
15	0.99	1.3	0.0
17	0.99	0.0	0.0

3- TYPES OF PERSONAL PROTECTION USED: PERCENTAGE OF POSITIVE ANSWERS

PERSONAL PROTECTION	CAÑO LINDO	JABILLOS	GUAQUITAS
MOSQUITO NETS	50.5	90.0	92.9
WINDOW SCREENING	1.9	1.3	14.3
TRADITIONAL REPELLENTS	9.9	28.8	28.6
TOPICAL REPELLENTS	0	5.0	0
ELECTRIC FAN	1.9	13.8	21.4
OTHER	1.9	0	0
NONE	39.6	2.5	0

4-TOTAL No OF

MOSQUITO NETS	129	231	57
No. NETS PER HOUSE	1.3	2.9	4.1
No. NETS PER PERSON	0.3	0.5	1.6

5- PERCENTAGE OF PEOPLE PROTECTED BY NETS

	CAÑO LINDO	JABILLOS	GUAQUITAS
RAINY SEASON	38.7	80.6	84.9
DRY SEASON	32.1	61.7	77.4

6- BED TIME: (percentage of positive answers)

I- RAINY SEASON:	CAÑO LINDO	JABILLOS	GUAQUITAS
a) 18:00-19:00	0.0	7.5	14.3
b) 19:00-20:00	43.4	35.0	50.0
c) 20:00-21:00	41.4	28.8	28.6
d) 21:00-22:00	14.1	18.8	7.1
e) 22:00-23:00	1.0	10.0	0.0

II- DRY SEASON:	CAÑO LINDO	JABILLOS	GUAQUITAS
a) 18:00-19:00	4.1	5.0	0.0
b) 19:00-20:00	39.8	36.3	28.6
c) 20:00-21:00	34.7	25.0	42.9
d) 21:00-22:00	17.3	21.3	28.6
e) 22:00-23:00	4.1	12.5	0.0

7- TIME OF WAKING (percentage of positive asnwers)

I- RAINY SEASON:	CAÑO LINDO	JABILLOS	GUAQUITAS
a) 4:00-5:00	4.0	7.5	0
b) 5:00-6:00	40.0	36.3	42.9
c) 6:00-7:00	45.5	47.5	50.0
d) 7:00-8:00	6.1	6.3	7.1
e) 8:00-9:00	4.0	2.5	0

II- DRY SEASON:	CAÑO LINDO	JABILLOS	GUAQUITAS
a) 4:00-5:00	6.1	7.5	0
b) 5:00-6:00	38.8	38.8	50.0
c) 6:00-7:00	47.9	43.8	42.7
d) 7:00-8:00	6.1	7.5	7.1
e) 8:00-9:00	1.0	2.5	0

2.4. Example of a completed form on human habits

PROYECTO MALARIA

El presente cuestionario tiene por objeto hacer un censo de la población en las localidades de estudio y determinar el tipo de protección personal que la gente emplea contra la picadura de mosquitos.

Favor leer cuidadosamente el cuestionario y seguir las instrucciones.
No dejar ninguna pregunta sin contestar.
Sólo tomar en cuenta las casas ocupadas para el momento de la entrevista.

1- LOCALIDAD (*):

1) Caño Lindo	
2) Jabillos	X
3) Abundancia	
4) El Milagro	
5) Guaquitas	
6) Guacas	

2- ESTADO (*):

1) Barinas	X
2) Táchira	

(*) Colocar una X en la casilla correspondiente.

3- FECHA:

D	M	A
20	8	89

4- DDT-#: 1

5-ENCUESTADOR: YASMIN

6- NOMBRE Y APELLIDO DEL ENCUESTADO (*)

	EDAD	SEXO
Ron González	49	F

7- QUE DEFENSA USA CONTRA LA PLAGA?:
Marcar con una X la casilla correspondiente
Puede marçar más de una respuesta

1) Mosquitero	X
2) Tela metálica	
3) Repelente de ambiente	
4) Repelente sobre la piel	
5) Ventilador	
6) Otro	
7) NO usa	

√

*

8- DIGAME CUANTAS PERSONAS VIVEN AQUI:
Anotar en primer lugar los datos correspondientes al entrevistado(*). Hacer que el entrevistado vaya nombrando a cada persona y luego continuar con las preguntas sobre edad y sexo.
En las casillas correspondientes al uso de mosquitero marcar con una X si la respuesta es afirmativa y con un 0 si es negativa.

	NOMBRE	EDAD	SEXO		MOSQUITERO	
			M	F	INVIERNO	VERANO
1	Flor Gonzalez	49		X	X	0
2	Pablo "	59	X		X	0
3	Maribel "	15		X	X	0
4	Karina "	13		X	X	0
5	Bethy "	11		X	X	0
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						

(*)

APPENDIX 3

Results of questionnaires to all householders within 2 km around the experimental huts carried out in August 1988, February 1989 and August 1989.

3.1. RESULTS OF QUESTIONNAIRES ON LAND USE CARRIED OUT IN AUGUST 1988.

1- VILLAGES	No.HOUSES	POPULATION
CAÑO LINDO (CLP)	13	92
JABILLOS (JAB)	47	242
GUAQUITAS (GUA)	8	52

2- NUMBER AND TYPE OF ANIMALS

SITE	DOG	CAT	BIRD	PIG	COW	DONKEY	HORSE	MULE	GOAT
CLP	35	15	308	18	69	1	7	0	10
JAB	82	47	15,924	36	127	2	3	3	0
GUA	31	65	166	1	528	2	29	0	0

3- SIZE OF PLOTS OF LAND (percentage of positive answers)

SIZE	CAÑO LINDO	JABILLOS	GUAQUITAS
a) House only	0	39.1	0
b) Less than 1000 m^2	0	4.3	0
c) 1000 m^2 to 1/2 Ha	0	13.0	0
d) 1/2 Ha to 1 Ha	15.4	17.4	12.5
e) 1 Ha to 10 Ha	30.8	15.2	12.5
f) More than 10 Ha	53.8	10.7	75.0

4- LAND USE: (Ha)

	CAÑO LINDO	JABILLOS	GUAQUITAS
a) CROP GROWING	24	43.5	118
b) CATTLE REARING	82.5	109	603
c) UNUSED	50	64.5	162
d) POULTRY REARING	0	16	0

5- CROPS GROWN BY EACH FAMILY: PERCENTAGE OF POSITIVE ANSWERS AND AREA USED FOR THEM.

TYPE	CAÑO LINDO	JABILLOS	GUAQUITAS
PASTURE	87.5	19.2	100.0
BANANA/PLANTAIN	81.3	57.5	62.5
OCUMO (*Colocasia*)	12.5	8.5	25.0
YUCA (casava)	47.8	21.3	75.0
MAIZE	12.5	21.3	37.5
CITRUS	56.3	23.4	0
VEGETABLES	31.3	4.3	50.0
COFFEE/COCOA	18.8	23.4	0
TOBACCO	0	0	25.0
PAPAYA	43.8	2.1	12.5
SUGAR CANE	43.8	0	0
FRUIT TREES	81.3	6.4	0
ORNAMENTALS	56.3	2.1	0

3.2. RESULTS OF QUESTIONNAIRES ON LAND USE CARRIED OUT IN FEBRUARY 1989.

1- VILLAGES

	No. HOUSES	POPULATION	% MALES	% FEMALES
CAÑO LINDO (CLP)	18	92	58.7	41.3
JABILLOS (JAB)	56	322	51.9	48.1
GUAQUITAS (GUA)	7	34	76.5	23.5

2- NUMBER AND TYPE OF ANIMALS

VILL.	DOG	CAT	BIRD	PIG	COW	DONKEY	HORSE	MULE	GOATS
CLP	38	13	702	114	296	0	21	3	6
JAB	89	51	16,286	50	327	1	11	1	7
GUA	26	11	168	6	414	2	23	1	2

3- SIZE OF PLOTS OF LAND (percentage of positive answers)

SIZE	CAÑO LINDO	JABILLOS	GUAQUITAS
a) House only	0	37.5	0
b) Less than 1000 m^2	0	16.1	0
c) 1000 m^2 to 1/2 Ha	0	10.7	0
d) 1/2 Ha to 1 Ha	0	10.7	0
e) 1 Ha to 10 Ha	52.9	7.1	16.7
f) More than 10 Ha	47.1	16.1	83.3

4- LAND USE (Ha)	CAÑO LINDO	JABILLOS	GUAQUITAS
a) CROP GROWING	20	29	36
b) CATTLE REARING	221.5	207	574
c) UNUSED	106.5	23	187
d) POULTRY REARING	0	16	0

5- CROPS GROWN BY EACH FAMILY: PERCENTAGE OF POSITIVE ANSWERS

TYPES	CAÑO LINDO	JABILLOS	GUAQUITAS
PASTURE	87.5	25.0	83.3
BANANA/PLANTAIN	81.3	82.1	100.0
OCUMO (*Colocasia*)	12.5	26.8	33.3
YUCA (Casava)	47.8	21.4	83.8
MAIZE	12.5	3.6	0
CITRUS	56.3	53.6	33.3
VEGETABLES	31.3	19.6	33.3
COFFEE/COCOA	18.8	30.4	0
TOBACCO	0	0	33.3
PAPAYA	43.8	39.3	33.3
SUGAR CANE	43.8	12.5	0
FRUIT TREES	81.3	85.7	33.3
ORNAMENTALS	56.3	75.0	0

3.3. RESULTS OF QUESTIONNAIRES ON LAND USE CARRIED OUT IN AUGUST 1989.

1- VILLAGES	No.HOUSES	No.WITH ELECTRICITY	POPULATION	% MALES	% FEMALES
CAÑO LINDO (CLP)	30	2	157	60.5	39.5
JABILLOS (JAB)	57	38	344	52.9	47.1
GUAQUITAS (GUA)	8	4	45	71.1	28.9

2- NUMBER AND TYPE OF ANIMALS

	CAÑO LINDO	JABILLOS	GUAQUITAS
DOG	65	75	21
CAT	25	37	9
BIRD	942	3,610	158
PIGS	304	45	6
COW	596	393	520
DONKEY	3	1	0
HORSE	16	13	28
MULE	0	0	10
GOAT	6	3	0
RABBIT	2	0	4
MONKEY	0	1	0

3- SIZE OF PLOTS OF LAND (percentage of positive answers)

SIZE	CAÑO LINDO	JABILLOS	GUAQUITAS
a) House only	0.0	54.4	12.5
b) Less than 1000 m^2	0.0	7.0	0.0
c) 1000 m^2 to 1/2 Ha	0.0	5.3	0.0
d) 1/2 Ha to 1 Ha	3.3	5.3	0.0
e) 1 Ha to 10 Ha	10.0	10.5	12.5
f) More than 10 Ha	86.7	17.5	75.0

4- LAND USE (Ha)	CAÑO LINDO	JABILLOS	GUAQUITAS
a) CROP GROWING	46	48.5	73
b) CATTLE REARING	471	210	389.5
c) UNUSED	194	141.5	413.5
d) POULTRY REARING	0	1	0

5- CROPS GROWN BY EACH FAMILY: PERCENTAGE OF POSITIVE ANSWERS AND AREA USED FOR THEM (hectares)

	CAÑO LINDO		JABILLOS		GUAQUITAS	
TYPES	% +ve	Area	% +ve	Area	% +ve	Area
PASTURE	83.3	426	28.8	210	87.5	389.5
BANANA/PLANTAIN	80.0	17	87.7	37	62.5	15.5
OCUMO (*Colocasia*)	3.3	2	22.8	0	25.0	4
YUCA (casava)	66.7	12	31.6	3.5	50.0	10
MAIZE	40.0	11	14.0	5	62.5	14
CITRUS	16.7	0.25	47.4	0	12.5	0.5
VEGETABLES	16.7	1	8.8	0	37.5	< 2
COFFEE/COCOA	20.0	2	31.6	2.5	0.0	0
TOBACCO	0.0	0	0.0	0	12.5	20
PAPAYA	10.0	0.5	36.8	0	37.5	6.5
SUGAR CANE	16.7	1	17.5	0.5	25.0	1
FRUIT TREES	80.0	0.5	89.5	0	100.0	< 0.25
ORNAMENTALS	90.0	0	87.7	0	100.0	< 0.25

APPENDIX 4

Comparison between the cost of insecticidal house spraying per person per year in the three study villages and the cost per person of providing the 200 nets needed in the three villages and impregnation with insecticide of all the nets in the three villages.

- COST OF HOUSE SPRAYING/PERSON/YEAR

(Dirección de Endemias Rurales, Report 1989) (*)

Labour and transport	=	93.3 Bs. (**)
Insecticide and consumables	=	36.7 Bs.
Total		130.0 Bs. per person

- IMPREGNATED NETS

Labour and transport (assuming half of that of house spraying)	=	46.7 Bs.
Nets (200 more needed for the population of 1,135, wholesale cost of a net is 300 Bs. and it has about 5 years "life")	=	10.6
Permethrin (15 Bs./net/year, 620 nets for 1,135 people)	=	8.2
Total		65.5 Bs.

(*) An inflation rate of 40% per year was used to estimate the 1991 costs.
(**) £ 1= 100 Bs.