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ADULT ABUNDANCE, BITING BEHAVIOR AND PARITY OF ANOPHELES AQUASALIS, CURRY 1932 IN TWO MALARIOUS AREAS OF SUCRE STATE, VENEZUELA

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The principal vector of malaria in eastern Venezuela, Anopheles aquasalis, *is exophagic and exophilic. Control using indoor insecticide house sprays has failed to lower the number of malaria cases. Therefore, studies were initiated in two villages of the eastern coastal state of Sucre to better understand this vector's biology and develop a more integrated control program.* An. aquasalis *was found to have a crepuscular biting behavior with a major peak at dusk and a minor peak at dawn. Mosquitos were collected more outdoors than indoors. Forty-seven percent of the biting took place before people went to bed (22:30 hr) and 69% of the mosquitos biting during this time period bite outdoors. Outdoor biting could be the reason why indoor spraying alone did not lower malaria cases. Seasonal abundance was greater in the rainy season compared to the dry season. Seasonal parous rates were high (78.3%-100%) and similar indoors and outdoors and between dry and wet season in Santa Fé. In Guayana, the seasonal parity was lower (34.6%-42.2%) than Santa Fé with indoor parity slightly higher than outdoors. Malaria cases were higher in Santa Fé, but adult mosquito density was much lower than in Guayana. This difference could have been due to higher parity in Santa Fé compared to Guayana. The greater distance to the nearest breeding site and presence of alternative hosts in Guayana can not be discounted as factors which contributed to the difference in malaria transmission between locations. We concluded that knowledge on seasonal occurrence, biting activity, resting behavior and breeding site location can be used to design a new control strategy for this vector.*

Key words: *Anopheles aquasalis* – Venezuela – malaria – abundance – activity – parity

Anopheles aquasalis is considered the principal coastal vector of malaria from eastern Venezuela to southern Brazil (Fleming, 1986). Its sporozoite rate is low in comparison with other vectors of malaria (Senior-White, 1951; Rachou, 1958) and therefore high densities are assumed necessary to maintain transmission (Deane et al., 1948; Senior-White, 1952). Adult control of malaria vectors is done by the use of indoor house sprays, which assumes that the vector rests inside houses long enough to obtain a lethal dose of the insecticide.

In Brazil (Deane et al., 1948), and in the Caribbean (Earle, 1936; Senior-White, 1952) *An. aquasalis* rests inside houses. But in Venezuela, it seldom rests inside houses (Cova Garcia, 1964; pers. obs.). Therefore, indoor house sprays have proven inadequate in control of this vector.

When vivax malaria reappeared in Sucre State, Venezuela, in 1982, house spraying failed to contain the disease. Malaria cases actually rose from 2 in 1982 to more than 6,000 in 1990. Biological studies were urgently needed to design an integrated control program for *An. aquasalis.*

In this paper we present the first results of these studies on *An. aquasalis.* We examine seasonal abundance, biting activity and parity by site and season.

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MATERIALS AND METHODS

Two locations were selected for study in the eastern Venezuelan State of Sucre, in the village of Santa Fé (Lat 10° 17'N, Long 64° 24'W) and the other on the peninsula of Paria in the village of Guayana (Lat 10° 35'N, Long 63° 57'W) (Fig. 1). Santa Fé is located in one of a series of coastal valleys along the Cordillera de la Costa Oriental mountain range. The area is dominated by littoral vegetation consisting of coastal mangrove, xerophytic and halophytic vegetation, and coconut plantations. Cultivation consists of tropical root crops, bananas, maize and fruits. Livestock was virtually nonexistent at the time of our study. Two rivers come down a pair of valleys and empty into the ocean at Santa Fé. The riparian vegetation is a deciduous dry forest. Average yearly rainfall in this area is 760 mm and is seasonal, beginning in late May and abruptly ending in late November or early December.

Guayana is located in a dry tropical forest, but due to the large number of streams running off the Cordillera the area is abundant in riparian vegetation. The littoral zone is approximately 5-10 km from Guayana and is similar to Santa Fé, but is much more extensive with a seasonally wet Juncaceae marsh (> 40,000 ha) next to the coastal mangroves. Cultivation practices are similar to Santa Fé, but in this area there is considerably more livestock present. Average yearly rainfall in Guayana is 1040 mm and is seasonal beginning in late May and ending in December.

The annual parasitic indices for malaria (per 1000 inhabitants, API) in Santa Fé for 1989 and 1990 were 105.4 and 266.3, respectively. In Guayana, the API's for 1989 and 1990 were 41.0 and 60.2, respectively (DER, 1989, 1990).

Monthly all-night collections were made indoors and outdoors within 5 m of a preselected house at each location. One person collected at each position. The two houses were sprayed with Fenitrothion® on a routine basis before the study began, but during the study they were excluded from the spray program. Experiments started three months after the last spray cycle. Captures began at 18:30 hr and ended at 07:30 hr. Collections were made using handheld aspirators and placed in paraffin lined paper cups hourly. Collectors rotated positions every hour and individuals did not collect mosquitos for more than four consecutive hours each night. Mosquitos were brought to the field laboratory, identified to species using the key of Cova Garcia & Sutil (1977). Those without presence of blood or eggs were dissected for parity determination using the traqueole method of Detinova (1962).

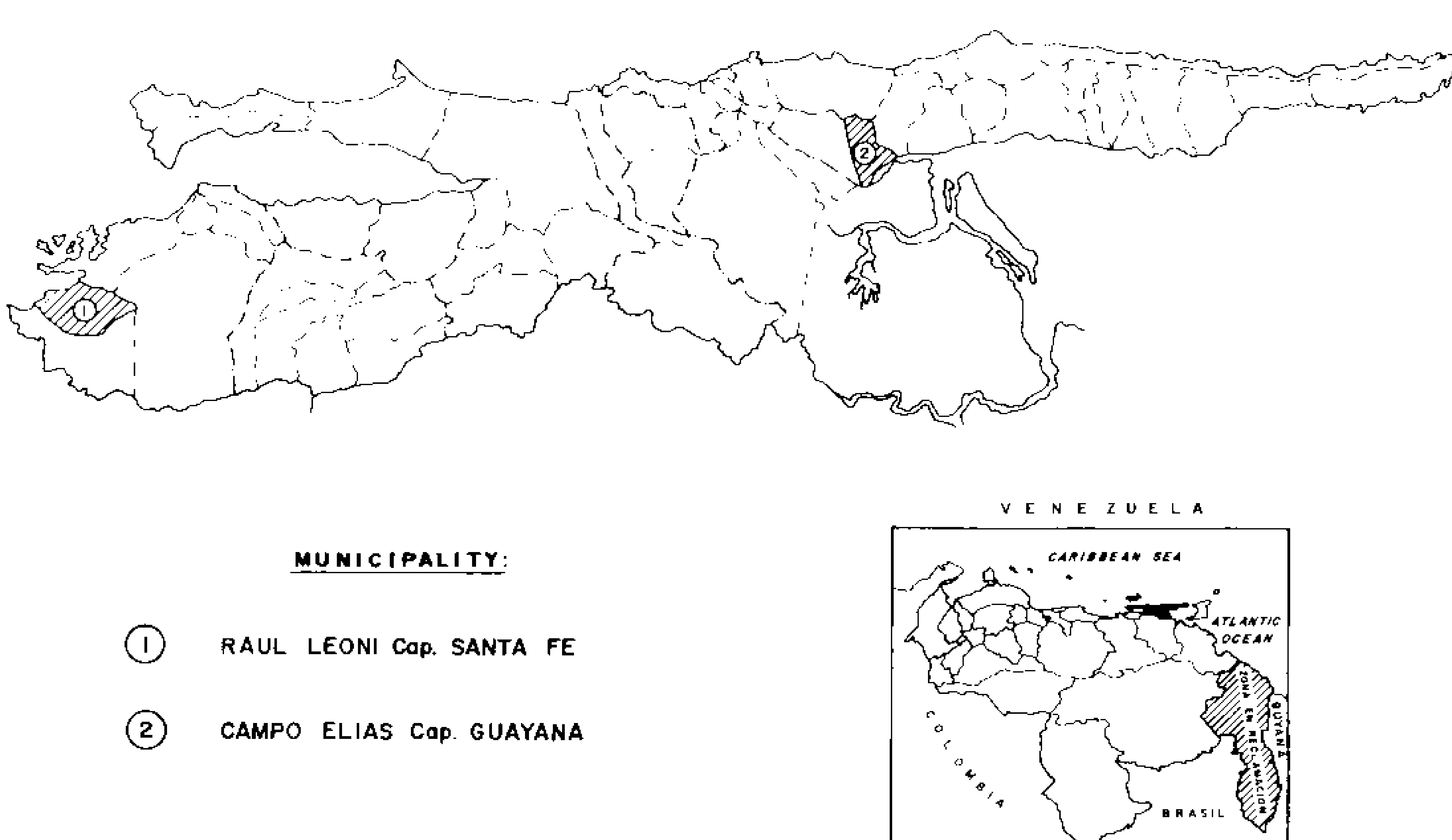

Fig. 1: study locations in Sucre State, Venezuela.

Rainfall data were taken from the nearest meteorological station; for Santa Fé, 40 km away in Barcelona, and for Guayana, 30 km away in Irapa.

Data analysis on parity was done using chi-square, 2x2 contingency tables (df=1) with Yates' correction for continuity (Snedecor & Cochran, 1978). No analysis of seasonal trends was attempted due to low number collected in Santa Fé and only one person collected inside and outside monthly at one house at each location.

RESULTS

Anopheles aquasalis was collected in much greater numbers in Guayana than in Santa Fé (Table I). The only other anopheline collected was *An. oswaldoi* in Santa Fé (N = 2). Mosquitos were also collected in greater numbers outside houses than inside; 62.9% of the mosquitos were collected outside in Guayana and 79.0% collected outside in Santa Fé.

Adult mosquito abundance in Santa Fé increased soon after the first rains and decreased rapidly at the beginning of the dry season (Fig. 2). In Guayana there were more mosquito collected in the wet season, but a more gradual decrease in adult abundance was observed as the dry season began (Fig. 3).

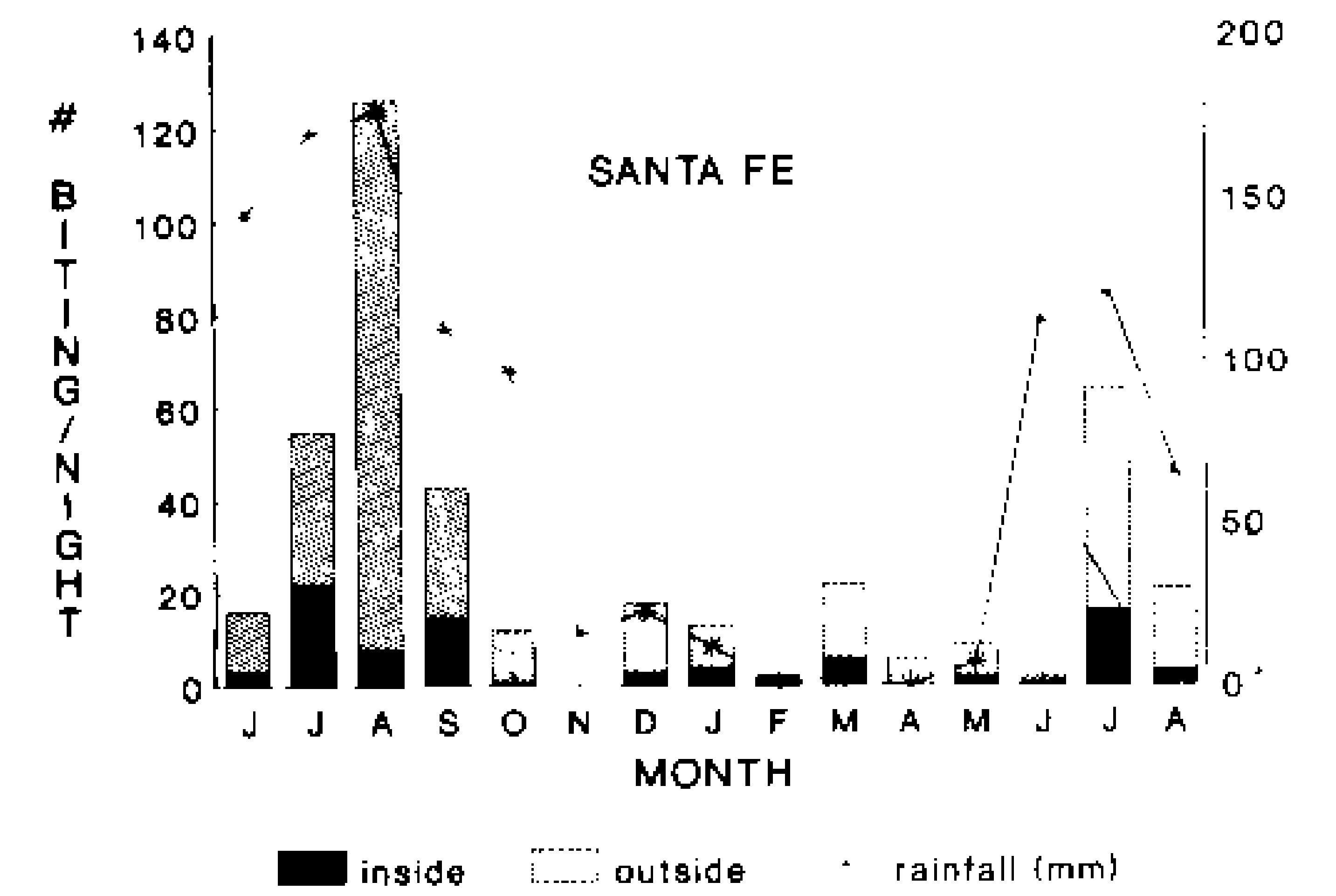

Fig. 2: number of *Anopheles aquasalis* biting man per night inside and outside houses in Santa Fé, Venezuela from June 1988 to August 1989.

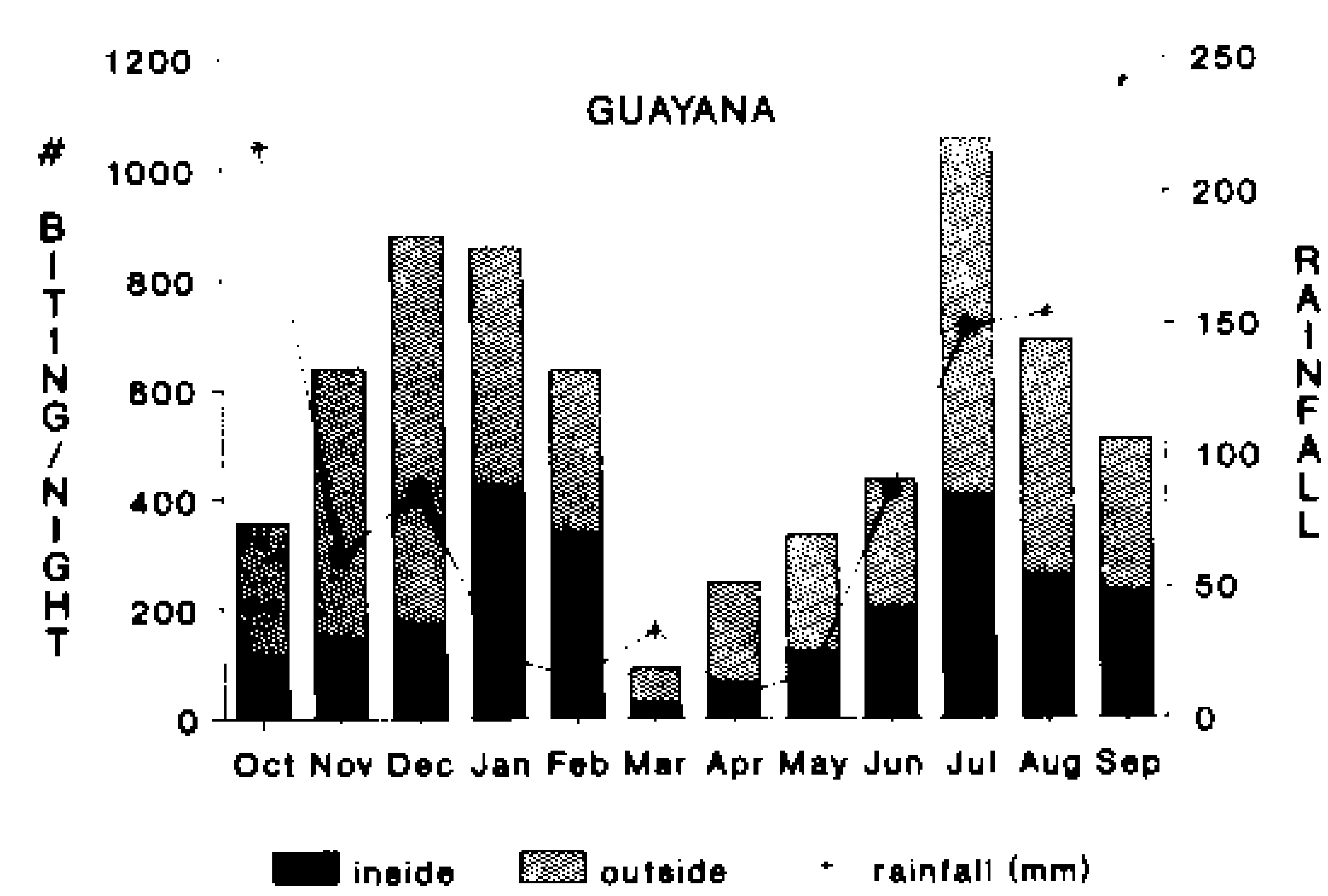

Fig. 3: number of *Anopheles aquasalis* biting man per night inside and outside houses in Guayana, Venezuela from October 1988 to September 1989.

TABLE I

Monthly biting counts of *Anopheles aquasalis* in Sucre, Venezuela, from June 1988 to September 1989

Month	Santa Fé Indoors	Santa Fé Outdoors	Santa Fé Total	Guayana Indoors	Guayana Outdoors	Guayana Total
June	3	13	16	–[a]	–	–
July	22	33	55	–	–	–
August	8	118	126	–	–	–
September	15	28	43	–	–	–
October	1	11	12	110	248	358
November	0	0	0	144	497	641
December	3	15	18	173	709	882
January	4	9	13	424	436	860
February	2	0	2	339	298	637
March	6	16	22	28	64	92
April	0	6	6	65	183	248
May	2	7	9	120	215	335
June	1	1	2	201	235	436
July	16	48	64	405	649	1054
August	3	18	21	260	431	691
September	–	–	–	231	278	509
Total	86	323	409	2500	4243	6743

a: – all night collections not taken.

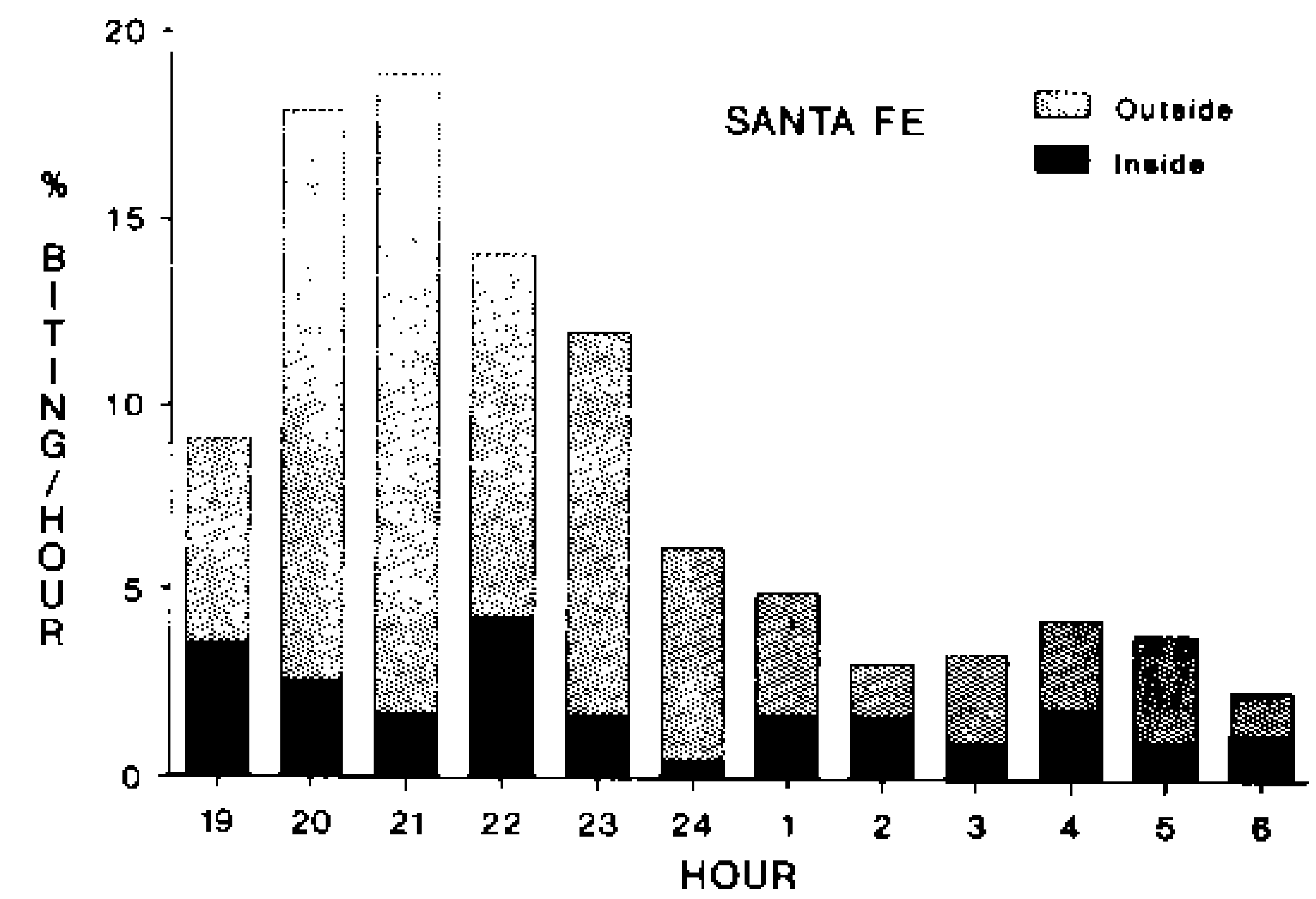

Fig. 4: percentage of *Anopheles aquasalis* biting man per hour inside and outside houses in Santa Fé, Venezuela from June 1988 to August 1989.

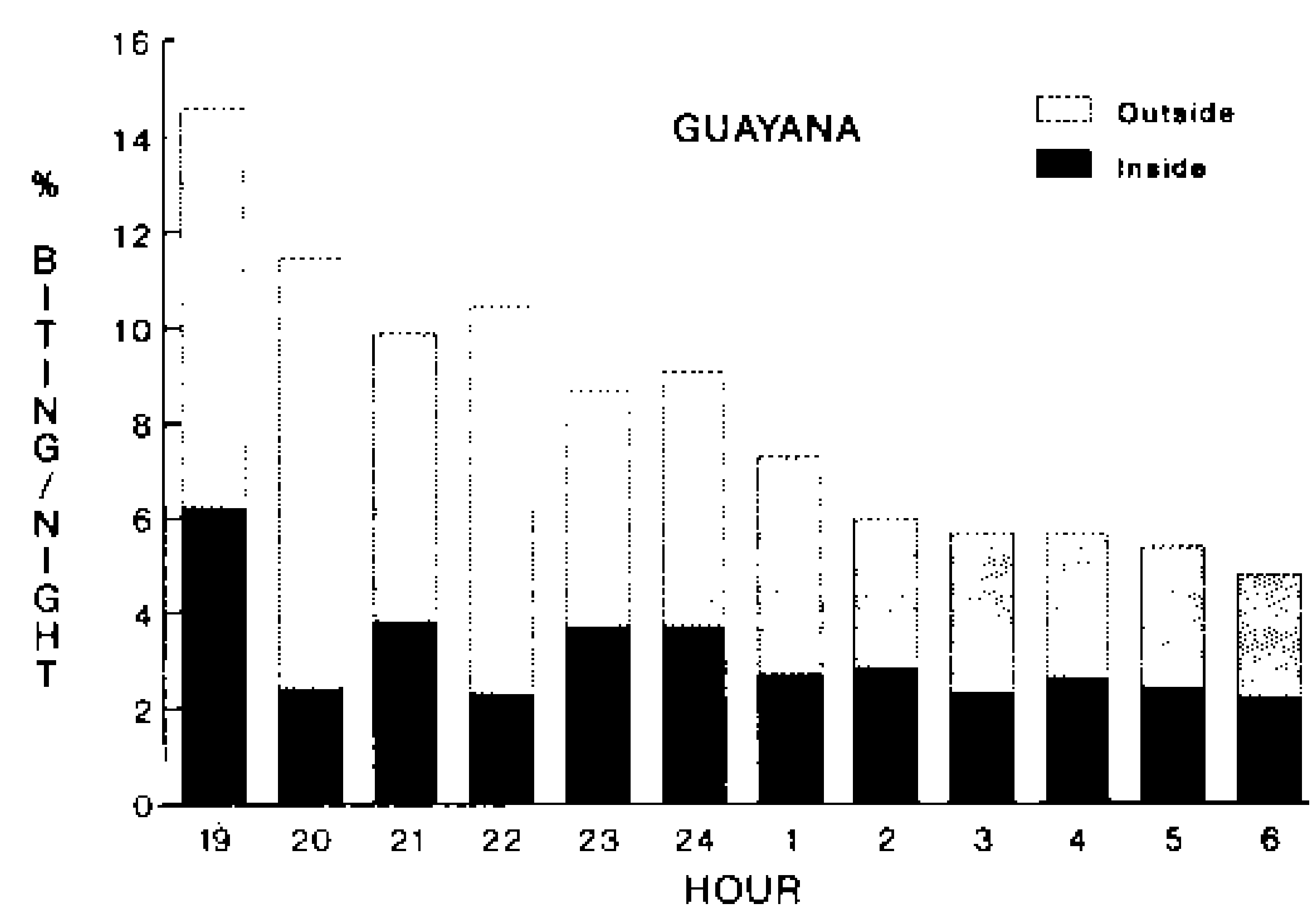

Fig. 5: percentage of *Anopheles aquasalis* biting man per hour inside and outside houses in Guayana, Venezuela from October 1988 to September 1989.

Biting activity began approximately 30 min after sunset and peaked in Santa Fé between 20:00 hr and 21:00 hr and in Guayana 19:00 hr (Figs 4, 5). Biting activity gradually decreased throughout the night at both locations. Two *An. aquasalis* were collected biting during the day while we were surveying breeding sites.

Parous rates were calculated by season (June-December, wet season; January-May, dry season) and parity comparisons were made at each location (Table II). No significant difference in parity was observed in Santa Fé when indoor captures were compared by season ($\chi^2 = 0.233$, $P > 0.10$). The same was true for outdoor collections ($\chi^2 = 0.014$, $P > 0.10$). Parity comparisons between indoor and outdoor captures by season showed no significant differences, also ($\chi^2 = 3.34$, $P > 0.05$, rainy season; $\chi^2 = 0.10$, $P > 0.10$, dry season). In Guayana, parity was not significantly different for indoor captures by season ($\chi^2 = 0.124$, $P > 0.01$), but outdoor parity was significantly higher in the wet season compared to the dry season ($\chi^2 = 26.0$, $P < 0.005$). Parity comparisons between indoor and outdoor captures within season showed no significant difference during the wet season ($\chi^2 = 0.026$, $P > 0.01$), but parity was significantly higher inside houses verses outside houses during the dry season ($\chi^2 = 4.03$, $P < 0.05$), in Guayana.

TABLE II

Seasonal parous rates of *Anopheles aquasalis* in Sucre, Venezuela[a]

Rainy season					
Santa Fé Indoors	Santa Fé Outdoors	Total	Guayana Indoors	Guayana Outdoors	Total
100% (20)[b]	80% (70)	84.4% (90)	42.2% (204)	41.2% (711)[c]	41.7% (915)
Dry season					
Santa Fé Indoors	Santa Fé Outdoors	Total	Guayana Indoors	Guayana Outdoors	Total
87.5% (8)	78.3% (23)	80.6% (31)	40.3% (402)[a]	34.6% (547)[ab]	37.1% (949)

a: only months when collections were made at both sites were used.
b: percent parous (number dissected).
c: parous rates followed by the same letter are significantly different according to Chi Square analysis (a, $x^2 = 26.0$, $P < 0.05$; b, $x^2 = 4.03$, $P < 0.05$).

Because there was more than a 10-fold difference in number of mosquitos dissected between locations (Santa Fé, N = 121; Guayana, N = 1864) we did not make statistical comparisons between locations. Previous experience showed that low numbers dissected can lead to possible misinterpretation of comparison results. Nevertheless, the parity of *An. aquasalis* in Santa Fé was much higher than in Guayana (Table II) both in the dry and wet seasons. The difference in parity between the two locations was also seen in previous and subsequent studies (unpub. data).

Monthly and hourly parous rates were only calculated for Guayana because of low numbers dissected in Santa Fé. Monthly parous rates ranged from a low of 25.0% in November 1988 to a high of 59.8% in September 1989 inside. Outside parity ranged from a low of 18.8% in November 1988 to a high of 51.8% in July 1989 (Fig. 6). The mean parous rates were 43.7% inside and 39.2% outside. No noticeable change was seen in parity between dry and wet seasons. In fact, the trend after the rains in late May was toward an increase in parity, not a decrease as expected due to the emergence of more nulliparous females.

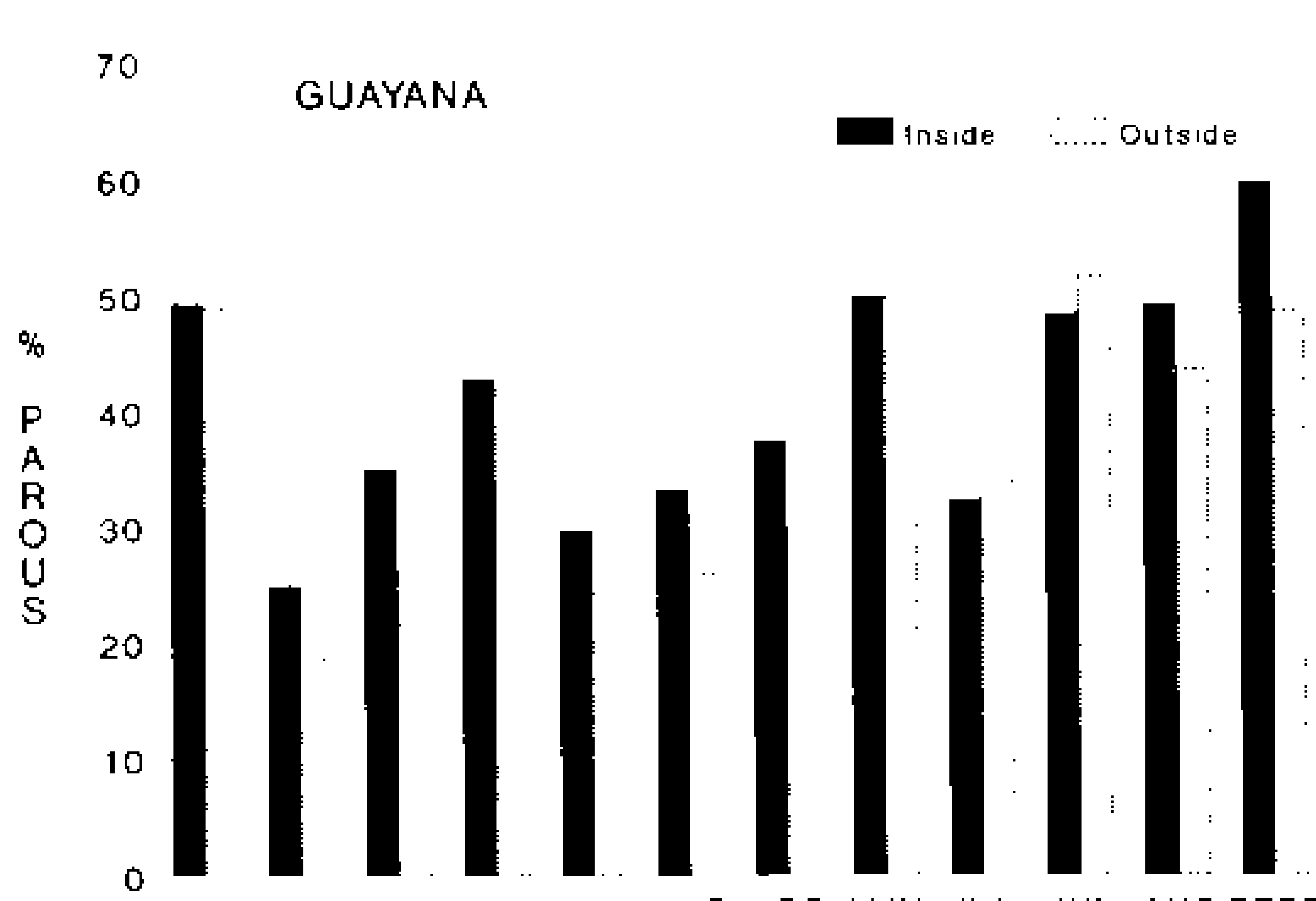

Fig. 6: monthly parous rates of *Anopheles aquasalis* collected inside and outside houses in Guayana, Venezuela from October 1988 to September 1989.

Hourly parous rates in Guayana ranged from a low of 29.8% at 04:00 hr to a high of 55.3% at 23.00 hr inside. Outside parity ranged from a low of 30.6% at 04:00 hr to 49.0% at 01:00 hr (Fig. 7). Throughout the night the hourly parity rate was equal or higher in the females collected indoors.

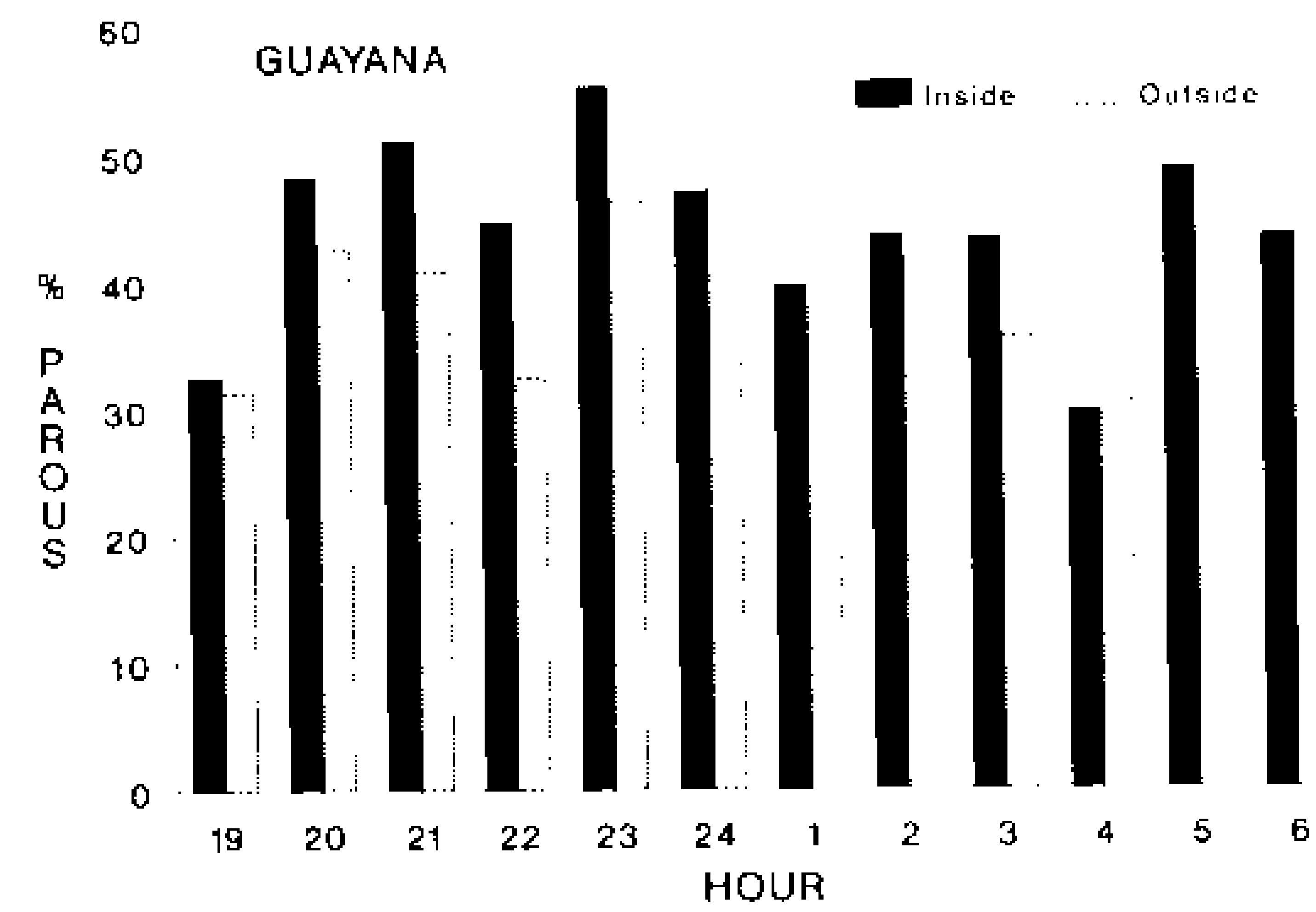

Fig. 7: hourly parous rates of *Anopheles aquasalis* collected inside and outside houses in Guayana, Venezuela.

Anopheles aquasalis was not found resting inside houses, but was observed resting on vegetation around the houses just before biting activity began. They continued to rest outside houses throughout the night and engorged mosquitos were seen resting on bushes around the houses within a half hour after biting activity began. By daybreak no more mosquitos were observed resting on the bushes.

DISCUSSION

Our results on seasonal abundance and biting behavior of *An. aquasalis* in Sucre, Venezuela are similar to those from other studies in the Americas (Earle, 1936; Ayroza Galvão et al., 1942; Lucena, 1946; Deane et al., 1948; Giglioli, 1948; Senior-White 1951, 1952; Silvain & Pajot, 1981; Zimmerman, 1992). Our study confirmed that *An. aquasalis* abundance was influenced by rainfall and increased at the beginning of the rainy season. But, contrary to our study where the biting activity peaked between 19:00 hr and 21:00 hr depending on the location, Deane et al. (1948) found *An. aquasalis* biting activity to peak after midnight inside houses and Lucena (1946) after 22:00 hr.

The greater abundance of *An. aquasalis* in Guayana as compared to Santa Fé most likely was due to the presence of more extensive breeding sites and the persistence of these breeding sites into the dry season (Berti et al., 1993). In Santa Fé the major breeding sites were several temporary ponds and a 400-500 ha mixed species mangrove. In Guayana the major breeding sites were at least 5 km away. Two important sites were a 40,000 ha semipermanent saltmarsh and an extensive coastal mangrove. The presence of water in the saltmarsh in Guayana for several months at the beginning of the dry season may also be why adult collections did not decrease as rapidly in the dry season in Guayana compared to Santa Fé (Figs 2, 3).

Rachou et al. (1950), in Ceará State, Brazil, collected 70.8% of the *An. aquasalis* inside houses. We collected means of 62.9% and 79.0% of the *An. aquasalis* outside houses in Guayana and Santa Fé, respectively. It was suggested by Deane et al. (1948) that the difference in biting behavior in Ceará State and similar areas was due to extremely dry weather. The lack of other suitable resting sites may also affect resting site selection (Giglioli, 1948). The only suggestion of a behavioral change that we saw related to dry weather was a slightly higher parous rate inside houses compared to outside houses (Table II).

Seasonal parity was slightly higher in the rainy season than in the dry season. This is contrary to what would be expected if one considers that the increase in abundance of *An. aquasalis* after the rains is due to emergence of nulliparous females. This would presumably lower the parous rates for a certain period of time. The lowering of parity may have been masked by an increase in longevity due to more favorable climatic conditions during the rainy season.

Parous rates for *An. aquasalis* in Santa Fé were much higher than in Guayana, but both were higher than recorded by Panday (1975) in Surinam. He found a mean parous rate of 15% with a high of 46% from July 27-August 10. He concluded that in his area of study *An. aquasalis* would not be considered an important vector.

Forty-seven percent of the biting activity of *An. aquasalis* occurred before most people went to bed (22:30 hr) and 69% of the mosquitos biting during this time period bite outdoors. Therefore, a significant proportion of the human population was exposed to the vector outside houses at peak biting hours. This, coupled with our observation that *An. aquasalis* did not rest on walls inside houses, suggests to us that indoor house spraying has minimum lethal effect on this exophilic vector. Outdoor transmission was confirmed in a subsequent study when 3 collectors contracted vivax malaria over a 1.5 year period, while only collecting *An. aquasalis* in Santa Fé outside houses from 18:30 hr to 21:30 hr. In a concurrent study, *An. aquasalis* collected biting outside in Santa Fé and Guayana were found positive for *P. vivax* sporozoites by the ELISA method (Caceres, 1993). In Santa Fé, outdoors, 1.7% (30/1739) of the mosquito pools tested were positive; indoors no pools were positive (0/115). In Guayana, outdoors, 0.7% (52/7853) of the mosquito pools were found positive; indoors 0.5% (23/4371) of the pools were positive.

Contrary to previous studies (Deane et al., 1948; Senior-White, 1952), our study indicates that adult longevity is a key entomological indicator of malaria transmission by *An. aquasalis,* not adult density. In our study we collected much lower numbers of *An. aquasalis* in Santa Fé compared to Guayana, but, there were many more cases of malaria in Santa Fé. This contradiction may be due to differences in parity between locations. Theoretically the most sensitive variable in the vectorial capacity model is vector life expectancy, which regulates the latent period of the parasite and therefore the transmission rate (Dye, 1986). If we use parity differences between sites (Table II) as a relative estimate of longevity, then malaria transmission would be higher in Santa Fé compared to Guayana, all other factors being equal.

Other factors can influence malaria transmission, including the presence of alternative hosts and location of breeding sites. Unfortunately, we did not calculate human blood indices in our study, but we knew that no livestock were present in Santa Fé, except an occasional burro or pig. However, in Guayana, there were cattle herds positioned between the major breeding sites and the village as well as a few cattle and burros present in the village.

In addition, our larval study showed that the breeding sites in Santa Fé were within meters of the houses and that the majority of the breeding sites in the vicinity of Guayana were at least 5 km away (Berti et al., 1993). Host preference and distance between human settlements and breeding sites cannot be discounted as impacting malaria transmission in the area of our study.

Results of this study suggest that a new control approach is needed where *An. aquasalis* is the primary vector in Venezuela. Alternatives should take advantage of the seasonal abundance of the vector, its time and place of activity, the fact that it rests on vegetation around the houses before and after biting, and the distance between human settlements and major breeding sites. An example of such an approach could include the use of spatial sprays on vegetation around houses where *An. aquasalis* rests, at peak biting activity, coupled with larval control of key breeding sites ranked by larval abundance and closeness to human settlements. This example would also take into account seasonal adult abundance and the number of malaria cases over seasons.

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