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SEASONAL DISTRIBUTION AND DIEL BITING PATTERNS OF CULICINE MOSQUITOES IN COSTA MARQUES, RONDÔNIA, BRAZIL

TERRY A. KLEIN*; JOSE B. P. LIMA** & AMAZONIA TODA TANG***

Núcleo de Medicina Tropical e Nutrição, Universidade de Brasília, Brasília, DF, Brasil
U. S. Army Medical Research Unit-Brazil, American Consulate-Rio, APO Miami, FL 34030, U.S.A.

A study of peridomestic man-biting culicines in the Amazon Basin was conducted from January through December, 1987. Fifteen species of mosquitoes from six genera were collected by volunteers in all-night human-bait indoor and outdoor collections at five houses in and near the town of Costa Marques, Rondônia, Brazil. Culex quinquefasciatus *and members of the* Mansonia titillans/indubitans *Group comprised 61 and 33%, respectively, of all culicines collected from human-bait outside houses and 62 and 35%, respectively, of those collected from volunteers inside houses in the town. In rural areas,* Cx. quinquefasciatus *was less abundant and only comprised 2 and 5% of the culicines, respectively, collected inside and outside houses.* Mansonia titillans/indubitans *Group comprised 75% and 86% of the culicines collected inside and outside houses, respectively, from rural residences.* Culex quinquefasciatus *and members of the* Mn. titillans/indubitans *Group were more endophilic than other culicines collected. Nocturnal and seasonal biting rhythms for the more common culicines are described.*

Key words: *Culex* – *Mansonia* – *Psorphora* – *Aedes* – *Aedeomyia* – *Coquillettidia* – mosquito – behavior

Many culicine mosquito populations in the Amazon Basin of Brazil have been implicated or suspected in the transmission of arboviruses and other parasitic diseases affecting human health. Others are peridomestic pest mosquitoes, closely associated with man. Elucidation and control of malaria vectors have been the principal emphasis of the Brazilian Ministry of Health efforts. However, certain culicines and other biting Diptera that transmit a variety of parasites and arboviruses also contribute to health problems in the Amazon Basin. Isolations of arboviruses from culicines of the Amazon Basin emphasize the need for epidemiological studies of this group (Miles, 1960; Causey et al., 1963, 1964; Hoch et al., 1981; Dixon et al., 1981; Calisher et al., 1982; Pannuti et al., 1989).

This study compared the species composition, relative seasonal abundance and nocturnal biting behavior of culicines in and near a frontier town in the Amazon Basin of Brazil.

MATERIALS AND METHODS

Adult culicines from human-bait collections (January to December, 1987) were made in and near the small frontier town of Costa Marques, Rondônia, Brazil. The study sites, collection techniques and meteorological data were the same as described in detail for concurrent studies on anophelines from the same area (Klein & Lima, 1990). The area surrounding Costa Marques is flat and covered with a tropical semi-evergreen seasonal forest (Beard, 1944). Rainfall is seasonal with a dry season from May to September and a wet season from October to April.

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Authors' current addresses:
*Department of Entomology, Division of Communicable Disease and Immunology, Walter Reed Army Institute of Research, Washington, DC 20307-5100, U.S.A.
**US Army Medical Research Unit-Brazil, American Consulate-Rio, APO Miami, FL 34030, U.S.A. or Instituto de Biologia do Exército, Rua Francisco Manuel, 102, 20911-270 Rio de Janeiro, RJ, Brasil.
***Laboratório Central de Entomologia, Divisão de Malária, SUCAM, Ministério da Saúde, Ala A, sala 312, 70058-900 Brasília, DF, Brasil.

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Two rivers and a stream border Costa Marques on three sides. The São Domingos River joins the Guaporé River (Brazil-Bolivia border) on the south. A small temporary stream transects the NW part of Costa Marques and empties into the Guaporé River. During the end of the rainy season, the river systems flood much of the land on three sides of Costa Marques. On the Bolivian side of the Guaporé River, the flood plains cover a large expanse of virgin forest. When the water recedes from these flooded areas, the natural water impoundments form oxbows and temporary and permanent marshes, ponds and pools which serve as highly favorable habitats for immature mosquito development. Natural low lying areas and man-made depressions along the dirt roads and cleared areas form permanent or temporary water impoundments with sparse to abundant vegetation.

Dogs and chickens were the most common peridomestic hosts in the study area. Cattle were common on the periphery of the town and occasionally were observed inside the town. A small dairy farm was located at km 8. These cattle were pastured at km 7 and penned before dusk each night in a corral about 10 m from house 5.

Adult mosquito collections were made at three houses in the town (houses 1, 2 and 3) and at two houses at km 2 and km 7 from the town and along the main road, BR 429 (houses 4 and 5, respectively) (Klein & Lima, 1990). Houses 1, 2 and 4 were constructed of wood and had tin roofs and shuttered windows. The eaves were not screened and the windows were often left open. Houses 3 and 5 were single room houses constructed of scrap lumber. Mosquitoes could gain access through large cracks between the wall boards as well as open doors and windows of these houses.

The study houses were occupied by local nationals and sprayed biannually by the Superintendency for Public Health Campaigns (SUCAM) in January and July of each year. In Costa Marques, 98% of all houses were sprayed with DDT biannually. SUCAM conducted irregular cold fogging with malathion during June and July, 1987 in an effort to reduce malaria transmission.

Nocturnal activity patterns and seasonal distributions of mosquitoes were studied by using systematic human-bait collections by volunteers inside and outside houses 1 and 5 and outside houses 2, 3 and 4 (January to December 1987). Human-bait collections were conducted for 40 min of each hour from 18:00 to 07:00 h by volunteers exposing the lower part of their legs. Mosquitoes were collected with oral aspirators, transferred to screened pint cartons, killed by freezing and identified.

Identifications used in this paper follow Lane (1953). The taxonomy of culicines in Brazil is complicated by recent discoveries of species complexes, and species designations in this paper may change with further systematic studies. Therefore, representatives of wild collected culicines and reared progeny with larval and pupal exuviae were deposited in the Walter Reed Biosystematics Unit at the Smithsonian Institution, Washington, D. C., where our field identifications were confirmed.

RESULTS

In the Costa Marques area, adults of six genera and 15 culicine species were collected on human-bait (Table I). *Culex quinquefasciatus* Say and members of the *Mansonia titillans* (Walker) and *Mansonia indubitans* Dyar and Shannan Group were the most abundant culicines collected on human-bait outside houses (Table I). *Culex quinquefasciatus* and *Mn. titillans/indubitans* accounted for 44% and 46%, respectively, of the culicines collected outside all houses and 36% and 57%, respectively, collected inside house 1. The relative abundance of *Cx. quinquefasciatus* were similar for houses 1, 2 and 3 but in rural areas (houses 4 and 5), adult populations were very low. In town, *Mn. titillans/indubitans* were collected more frequently at houses 1 and 2 near the Guaporé river than at house 3 located near the temporary stream.

Culex quinquefasciatus and *Mn. titillans/indubitans* were the most frequently collected culicines in human-bait collections inside house 1 (Table II). The proportion of most of the culicine species collected indoors was greater at the poorly constructed rural dwelling (house 5) than at house 1. However, numbers of many species collected were insufficient to determine endophilic feeding behavior.

Culex quinquefasciatus adults were frequently collected throughout the year in the town of Costa Marques (Fig. 1). Populations

TABLE I

Culicines collected inside and outside five houses from human-bait during the same period from January through December 1987 in the Costa Marques area. Each number represents the mosquitoes captured during 780 (n) hours of collecting for each collection site

Species	Outside house					Inside house	
	Urban			Rural		Urban	Rural
	1	2	3	4	5	1	5
Aedes							
scapularis	19	6	6	189	222	6	91
serratus	2	2	0	10	3	1	1
fulvus	1	5	0	8	5	0	2
Aedeomyia sp.	0	0	0	1	1	0	0
Coquillettidia							
venezuelensis	42	14	1	35	6	0	0
Culex							
(Melanoconion) sp.	39	118	3	170	64	4	30
quinquefasciatus	1889	2370	2868	158	116	1689	33
corniger	0	1	0	5	0	0	0
spinosus	0	1	0	0	0	0	0
Mansonia							
titillans/indubitans	2469	1288	39	1325	2520	954	1751
amazonensis	12	8	6	15	27	1	13
humeralis	165	154	16	87	132	32	58
Psorophora							
albipes	31	31	0	91	57	16	39
albigenu	24	7	1	19	8	7	2
cingulata	23	4	3	21	6	1	4
ferox	3	1	0	9	0	0	2
Total	4719	4010	2943	2143	2967	2711	2026

TABLE II

Percent of selected culicines biting indoors compared to the total number of each species collected indoors and outdoors at house 1 and house 5 from January through December 1987

Species	House 1		House 5	
	Number inside and outside	Percent indoors	Number inside and outside	Percent indoors
Aedes				
scapularis	25	24	313	29
Coquillettidia				
venezuelensis	42	0	6	0
Culex				
(Melanoconion) sp.	43	9	94	32
quinquefasciatus	3578	47	149	22
Mansonia				
titillans/indubitans	3423	28	4271	41
amazonensis	13	8	40	33
humeralis	197	16	190	31
Psorophora				
albipes	47	34	96	41
albigenu	31	23	8	25
cingulata	24	4	10	40
Total	7423	37	5177	39

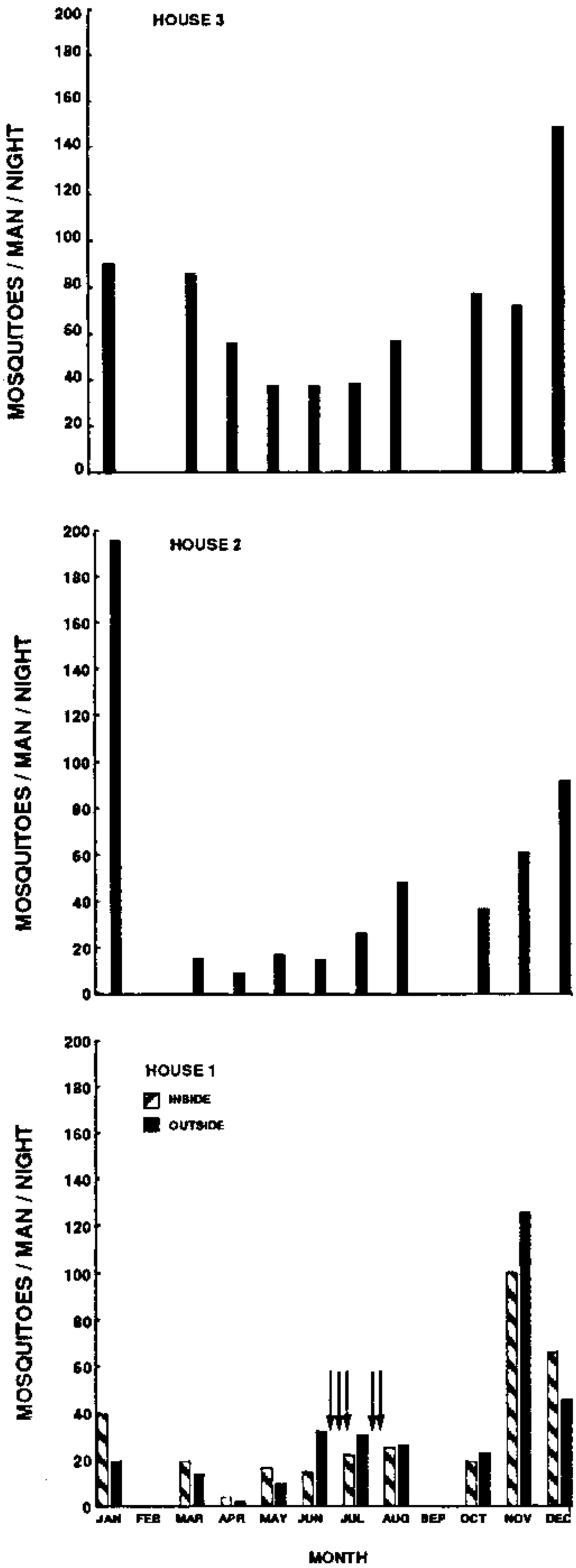

Fig. 1: mean number of *Culex quinquefasciatus* collected per man/night from human-bait inside and outside house 1 and outside houses 2 and 3, Costa Marques, Rondônia, Brazil (January through December 1987). Arrows indicate fogging in the town with malathion.

were more abundant near house 3, where water was improperly drained and raw sewage was discharged on the ground surface.

Mansonia titillans/indubitans Group adults were more frequently collected during the mid-dry and early-wet season (Fig. 2). Even though malathion fogging was applied in the town during June and July, adult *Mansonia* populations decreased more rapidly in the rural area where malathion fogging was not conducted (Fig. 2).

A single peak nocturnal biting activity by *Cx. quinquefasciatus* occurred between 23:00 to 02:00 h (Fig. 3). Blood fed and unfed *Cx. quinquefasciatus* were observed daily resting indoors on DDT sprayed surfaces in human dwellings. The insecticide treatment having no apparent effect on these mosquitoes.

Host-seeking *Mn. titillans/indubitans* Group were active throughout the night with peak biting activities in the crepuscular periods (approximately 18:00-20:00 h and 05:00-06:00 h) (Fig. 4). The bimodal peak biting activity varied throughout the year in association with the timing of sunset and sunrise.

DISCUSSION

More than 40 species of culicines from 12 genera have been collected from the Costa Marques area (Harrison et al., unpublished data). Some of these species are active during the daytime, are collected from forest habitats, or are not normally collected in human-bait and therefore were infrequently or not collected in nocturnal human-bait collections in or near houses. Additionally, *Culicoides* spp., black flies, deer/horse flies and phlebotomine sand flies were seasonally abundant in the area (Wilkerson et al., unpublished data).

Culex quinquefasciatus populations were relatively abundant throughout the year in an urban environment. The greater abundance of *Cx. quinquefasciatus* at house 3 may be due to the lower standards of living and poorer sanitary conditions compared to houses 1 and 2. The relatively low populations in rural areas may reflect its domestic behavior, reduced larval habitats and competition with feral mosquito species (Lourenço-de-Oliveira & Heyden, 1986; Forattini et al., 1987).

Culex quinquefasciatus adults were not susceptible to DDT when tested with the W.H.O. insecticide susceptibility papers (unpublished data) and were frequently observed resting on

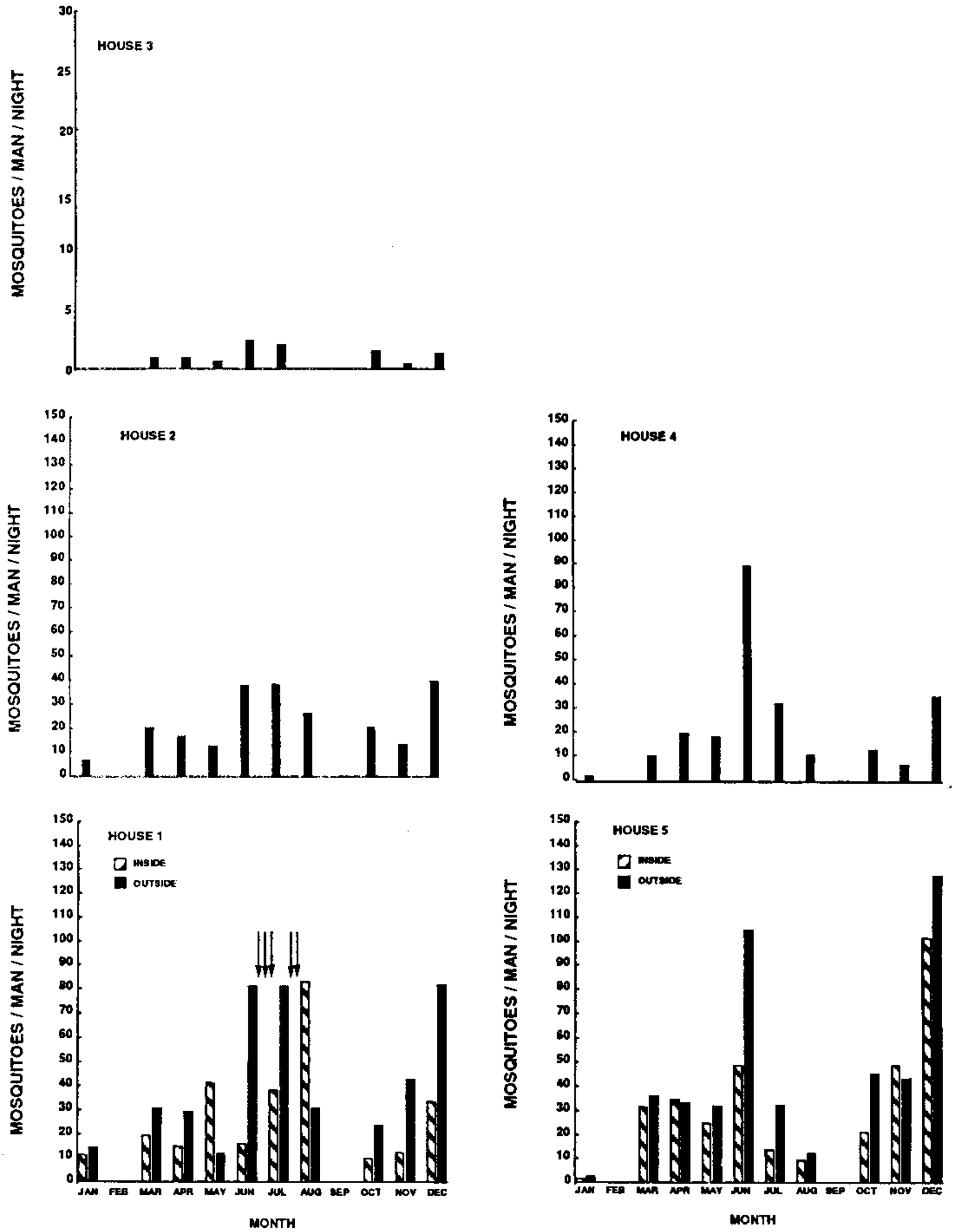

Fig. 2: mean number of *Mansonia titillans/indubitans* collected per man/night from human-bait inside and outside houses 1 and 5 and outside houses 2, 3 and 4, Costa Marques, Rondônia, Brazil (January through December 1987). Arrows indicate fogging in the town with malathion.

DDT treated walls less than 24 h after application in Costa Marques (Klein et al., unpublished data). Household sewage discharged through above ground pipes from some dwellings formed favorable larval habitats. Larval habitat source reduction may provide an effective control measure for this arboviral and filarial vector and pest mosquito.

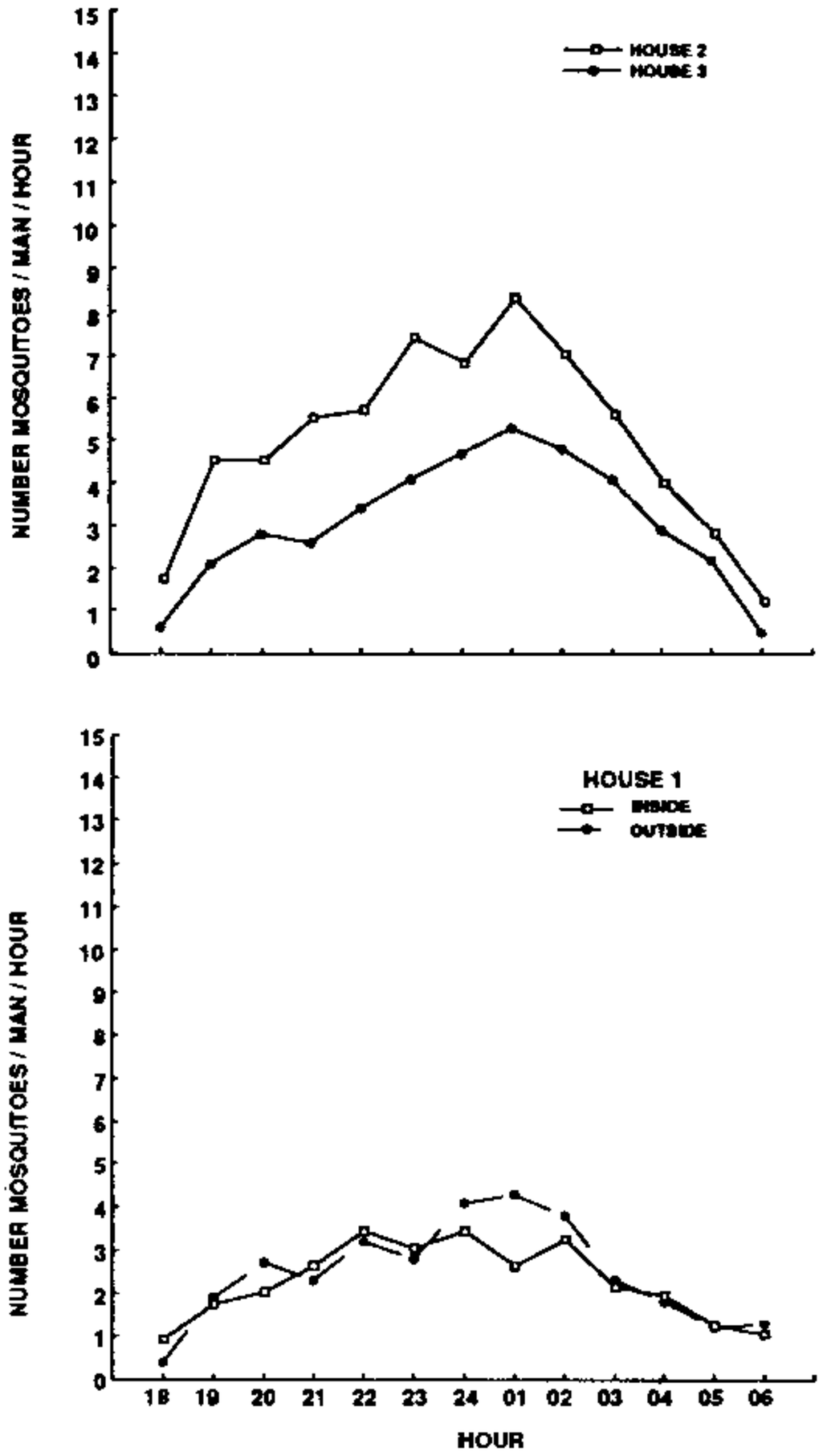

Fig. 3: nocturnal biting behavior of *Culex quinquefasciatus* collected from human-bait inside and outside house 1 and outside houses 2 and 3, Costa Marques, Rondônia, Brazil (January through December 1987).

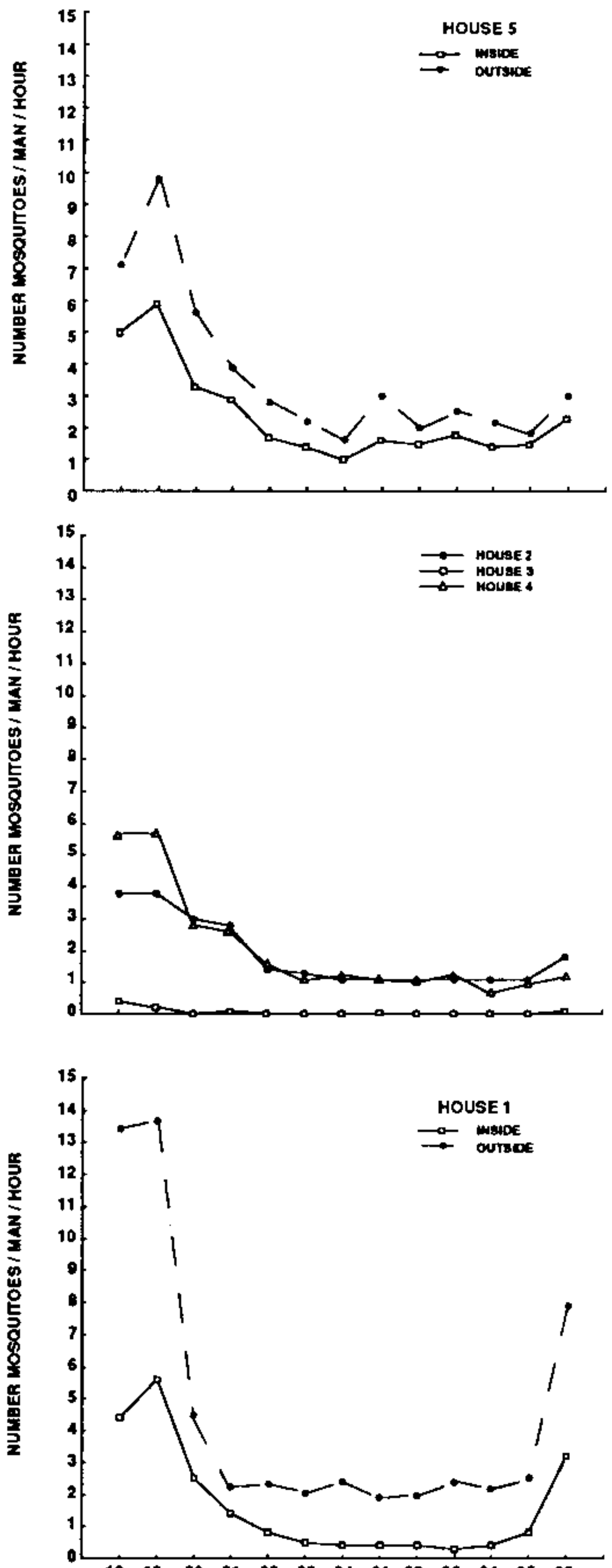

Fig. 4: nocturnal biting behavior of *Mansonia titillans/indubitans* collected from human-bait inside and outside houses 1 and 5 and outside houses 2, 3 and 4, Costa Marques, Rondônia, Brazil (January through December 1987).

Mansonia titillans/indubitans Group are likely a complex (Harrison, personal communication). Population peaks in the early dry season and early wet season could indicate a single bimodal population or may reflect differences within the species complex, i.e., one type may be more common during the wet season and another more abundant in the dry season. Many *Mansonia* spp. are very aggressive biters and have been incriminated in arbovirus transmission (Lourenço-de-Oliveira & Heyden, 1986). In Costa Marques, *Mn. titilans/indubitans* populations are relatively high, adults readily enter houses and are active in the early evening when human hosts are available outdoors, and the two most frequently collected species in Costa Marques are anthropophilic. *Mansonia humeralis* and *Mn. amazonensis* were more frequently collected in bovine-bait collections and much less numerous than *Mn. titilans/indubitans* (unpublished data). It will be very difficult in the Amazon Basin to apply effective source reduction techniques against *Mansonia* species

because of the vast amount of water in the region.

While *Psorophora* species were infrequently collected in nocturnal human-bait collections, they were very abundant and active in forested areas during the daytime. *Psorophora albipes* and *Ps. albigenu* were the predominant species and would follow and attempt to feed on volunteers walking through forest habitats during the daytime. After leaving the forest edge, *Psorophora* spp. would rarely follow and bite (unpublished data). *Psorophora* species have been incriminated in arbovirus transmission, and may be important in sylvatic cycles.

Some culicines, especially *Cx. (Melanoconion)* spp. and *Ae. scapularis,* were infrequently collected near human dwellings in both urban and rural environments. However, human-bait collections near small streams or areas of stagnant pools near the end of the dry season, caught large numbers. Members of the subgenus *Cx. (Melanoconion)* are difficult to speciate without associated larval and/or pupal specimens. *Ae. scapularis* and some *Cx. (Melanoconion)* spp. readily bite man, have a wide range of hosts and have been incriminated in arbovirus transmission (Roberts et al., 1981; Lourenço-de-Oliveira, 1984; Mitchell et al., 1987).

Neither *Cx. quinquefasciatus* nor *Mn. titillans/indubitans* Group adult populations appeared to be reduced by malathion fogging during the mid-dry season (June-July). Application with insecticides should be most effective in the early evening hours when feral mosquitoes are entering the peridomestic environment. However, due to DDT resistance in *Cx. quinquefasciatus* populations, their susceptibility to insecticides and other potential methods of control needs to be evaluated.

More than 60 different arboviruses have been isolated in Brazil (Lopez et al., 1978). While few epidemics have been described, a study of the Amazon Valley demonstrated a large percentage of the local population with antibodies to arboviruses compared to the immigrant populations (Dixon et al., 1981). Additionally, in epidemiological studies by Dixon et al. (1981), it was suggested that arbovirus transmission was largely sylvatic since attack rates were higher for adult males who slept/worked in forested areas. There is no evidence of filariasis transmission in the area, but the prevalence of arbovirus-caused disease is suspected to be relatively high (M. Tada, personal communication).

These data provide baseline information on nocturnal biting culicines and suggest potential risks involved for malaria vaccine trials in the Amazon Basin. Additional research is needed to elucidate the dynamics of culicine transmission of arboviruses in the Amazon Basin.

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REFERENCES

BEARD, J. S., 1944. Climax vegetation in tropical America. *Ecology, 25:* 129-158.

CALISHER, C. H.; KINNEY, R. M.; LOPES, O. S.; TRENT, D. W.; MONATH, T. P. & FRANCY, D. B., 1982. Identification of a new venezuelan equine encephalitis virus from Brazil. *Am. J. Trop. Med. Hyg., 31:* 1260-1272.

CAUSEY, O. R.; CASALS, J.; SHOPE, R. E. & UDOMSAKDI, S., 1963. Aura and Una, two new group A arthropod-borne viruses. *Am. J. Trop. Med. Hyg., 12:* 777-781.

CAUSEY, O. R.; SHOPE, R. E. & THEILER, M., 1964. Isolation of St. Louis encephalitis virus from arthropods in Pará, Brazil. *Am. J. Trop. Med. Hyg., 13:* 449.

DIXON, K. E.; LLEWELLYN, C. H.; TRAVASSOS DA ROSA, A. P. A. & TRAVASSOS DA ROSA, J. F., 1981. A multidisciplinary program of infections disease surveillance along the transamazon highway in Brazil: Epidemiology of arbovirus infections. *Bull. Pan Am. Health Organ., 15:* 1-25.

FORATTINI, O. P.; GOMES, A. C.; NATAL, D.; KAKITANI, I. & MARUCI, D., 1987. Freqüência domiciliária e endofilia de mosquitos Culicidae no Vale do Ribeira, São Paulo, Brasil. *Rev. Saúde Públ., Sào Paulo, 21:* 188-192.

HOCH, A. L.; PETERSON, N. S.; LEDUC, J. W. & PINHEIRO, F. P., 1981. An outbreak of Mayaro virus disease in Beltaerra, Brazil III. Entomological and ecological studies. *Am. J. Trop. Med. Hyg., 30:* 689-698.

KLEIN, T. A. & LIMA, J. B. P., 1990. Seasonal distribution and biting patterns of *Anopheles* mosquitoes in Costa Marques, Rondonia, Brazil. *J. Am. Mosq. Control Assoc., 3:* 700-707.

LANE, J., 1953. *Neotropical Culicidae.* Univ. São Paulo. São Paulo, 2 vol., 1,112 p.

LOPES, O. DE S.; COIMBRA, T. L. M.; SACCHETTA, L. DE A. & CALISHER, C. H., 1978. Emergence of a new arbovirus disease in Brazil I. Isolation and characterization of the etiologic agent, Rocio Virus. *Am. J. Epidemiol., 107:* 444-449.

LOURENÇO-DE-OLIVEIRA, R., 1984. Alguns aspectos da ecologia dos mosquitos (Diptera: Culicidae) de uma área de planície (Granjas Calábria), em Jacarepaguá, Rio de Janeiro. I. Freqüência comparativa das espécies em diferentes ambientes e métodos de coleta. *Mem. Inst. Oswaldo Cruz, Rio de Janeiro, 79:* 479-490.

LOURENÇO-DE-OLIVEIRA, R. & HEYDEN, R., 1986. Alguns aspectos da ecologia dos mosquitos (Diptera: Culicidae) de uma área de planície (Granjas Calábria), em Jacarepaguá, Rio de Janeiro. IV. Preferências alimentares quanto ao hospedeiro e freqüência domiciliar. *Mem. Inst. Oswaldo Cruz, Rio de Janeiro, 81:* 15-27.

MILES, J. A. R., 1960. Epidemiology of arthropod-borne encephalitides. *Bull. WHO, 22:* 339-371.

MITCHELL, C. J.; MONATH, T. P.; SABATTINI, M. S.; CHRISTENSEN, H. A.; DARSIES, R. F. JR; JOKOB, W. L. & DAFFNER, J. F., 1987. Host-feeding patterns of argentine mosquitoes (Diptera: Culicidae) collected during and after an epizootic of Western Equine Encephalitis. *J. Med. Entomol., 24:* 260-267.

PANNUTI, C. L.; IVERSSON, L. B.; DE MENDONÇA, J. S.; TRAVASSOS DA ROSA, A. P. A. & GRANATO, C. F. H., 1989. Relationship between the prevalence of antibodies to arbovirus and hepatitis B virus in the Vale da Ribeira region, Brazil. *Rev. Inst. Med. Trop. Sao Paulo, 31:* 103-109.

ROBERTS, D. R.; HOCH, A. L.; PETERSON, N. E. & PINHEIRO, F. P., 1981. Programa multidisciplinario de vigilancia de las enfermedades infecciosas en zonas colindantes con la carretera transamazonica en Brasil. IV. Estudio entomologico. *Bol. Sanit. Panam., 91:* 379-400.