Biting Indices, Host-seeking Activity and Natural Infection Rates of Anopheline Species in Boa Vista, Roraima, Brazil from 1996 to 1998

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The epidemiology of the transmission of malaria parasites varies ecologically. To observe some entomological aspects of the malaria transmission in an urban environment, a longitudinal survey of anopheline fauna was performed in Boa Vista, Roraima, Brazil. A total of 7,263 anophelines was collected in human bait at 13 de Setembro and Carañã districts: Anopheles albitarsis sensu lato *(82.8%),* An. darlingi *(10.3%),* An. braziliensis *(5.5%),* An. peryassui *(0.9%) and* An. nuneztovari *(0.5%). Nightly 12 h collections showed that* An. albitarsis *was actively biting throughout the night with peak activities at sunset and at midnight.* An. darlingi *bit during all night and did not demonstrate a defined biting peak. Highest biting indices, entomological inoculation rates and malaria cases were observed seasonally during the rainy season (April-November). Hourly collections showed host seek activity for all mosquitoes peaked during the first hour after sunset.* An. darlingi *showed the highest plasmodial malaria infection rate followed by* An. albitarsis, An. braziliensis *and* An. nuneztovari *(8.5%, 4.6%, 3% and 2.6%, respectively).* An. albitarsis *was the most frequently collected anopheline, presented the highest biting index and it was the second most frequently collected infected species infected with malaria parasites.* An. albitarsis *and* An. darlingi *respectively, are the primary vectors of malaria throughout Boa Vista.*

Key words: *Anopheles* - biting index - infection rates - malaria - Roraima - Brazil

Malaria continues to be the most important endemic disease in Brazil. Approximately 500,000 cases, primarily in the Amazon Basin (99%), have been reported annually during the last decade (Fundação Nacional de Saúde-FNS/ Funasa, Ministry of Health). The majority of cases are reported from the states of Pará and Amazonas (Funasa 2000). However, nowhere does malaria have a greater impact on health than in Roraima, as shown by the record annual parasitic indices, (API) (number of cases/ 1,000) reported for the last five years (150.9 in 1995, 143.3, 1996; 104.9, 1997; 81.6, 1998; 135.8, 1999, FNS 1996, Funasa 2000). For the period January-April 2000, a total of 14,973 cases were reported, approximately 40% of those registered in 1999 (Funasa 2000). Routine inquiries by health authorities provided evidence for the origin of many of the malaria cases (FNS 1996). These investigations showed that people living in small agricultural settlements (37.8%) and small villages (14.6%) contributed >50% of the reported malaria cases. This is an underestimate of the total malaria in the area since may people acquire medication from pharmacies (self-treatment) or private doctors and are therefore not reported. During 1996, malaria cases among native Indians represented 16.5% of the total, whereas people living in periurban areas accounted for <15% (FNS 1996). Approximately 50% of the mortality (*causa mortis)* among native Indian populations were accredited to falciparum malaria (FNS 1996).

Vector control is still one of the most successful ways of controlling malaria (WHO 1991). The identification of entomological variables in different malaria areas, such as the prevalent species, density, seasonal variation, larval habitats and infection rates, may assist in the development of effective malaria control programs. Those programs should take into account the way and time to interfere in the transmission cycle to increase effectiveness.

Our objectives were to identify anopheline species, their peak of biting activity, biting indices and natural infection rates and, to collect meteorological data aiming to understand the malaria ecology in the area. Longitudinal studies were conducted in two urban districts in Boa Vista, Roraima, where malaria rates were the highest during 1995: 13 de Setembro (May 1996 to April 1998) and Carañã (June 1997 to April 1998) (FNS 1996). This is the first longitudinal study on malaria vectors performed in the State of Roraima.

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MATERIALS AND METHODS

Study site - To observe malaria transmission in a highly endemic urban area, anopheline human-bait collections were conducted at Boa Vista (02°49'11"N, 60°40'24"W), State of Roraima, Brazil, from May 1996 through April 1998. The study was performed in the districts of 13 de Setembro (May 1996 to April 1998) and Caranã (June 1997 to April 1998). These two districts are situated along river margins and had the highest number of reported malaria rates for Boa Vista (from 1970 to 1996, FNS 1997).

Roraima is the northernmost state of Brazil (Fig. 1). Roraima has 225,116 km^2 with a population of 254,499 (IBGE 1996). Roraima's rural population density is <1/ km^2, while the population density of its capital, Boa Vista, is approximately 27/km^2 (154,000 people or 62% of the state population, IBGE 1996, FNS 1998). Approximately 85% of Roraima is forested, with the remaining 15% covered by savanna (Silva 1993) (Fig. 1). The climate is tropical humid with mean temperatures 27.8ºC (range 27.3 to 28.9ºC, 10 years) and mean rainfall 429 mm (range 391mm to 451mm, 4 years) with low annual variation (NASA 2001).

Savanna is the predominant ecological environment in Boa Vista. The climate presents two distinct seasons; a rainy season between April and November with high rainfall indices during the months of June and July (Schmidt 1942). The Branco River crosses the city in the northwestern direction (Fig. 2). During the driest months (December-March) the Branco River margins are intensely exploited for brick manufacturing. All the manufacturing processes are manual and locally made, from clay extraction to molding, burning and sales. This makes a floating population of around 3,000 people that to move to the Branco River margins, some with their families living in huts without walls at 13 de Setembro. The daily excavation of clay produces shallow pits (2-3 m depth, ~3 m diameter) where water accumulates. Even though NaOH (used in brick manufacturing), is washed into those pits, we decided to check whether they could be providing breeding sites for anophelines.

Fig. 1: Brazil, expanded area, Roraima. Light gray, savana; dark gray, equatorial forest with predominance of *várzeas* and *igapós* (permanent and semi-permanent flooded areas); white equatorial forest with predominance of non-flooded areas (IBGE 1992)

The district of 13 de Setembro, with 12,000 inhabitants, is situated southeastern of Boa Vista (Fig. 2). Besides Branco River, another smaller stream, the Pricumã *Igarapé*, borders the district together with dense marginal vegetation. This dense vegetation in combination with pools and slow moving waters provide suitable habitats for many anopheline mosquitoes. The district of 13 de Setembro accounts for the highest number of reported malaria cases in Boa Vista (1970 to 1996, FNS 1997).

The district of Caranã, with 10,800 inhabitants, is located in western Boa Vista at the margin of a large permanent stream, the Cauamé *Igarapé* (IBGE 1996) (Fig. 2). While no longer active, there was evidence of clay exploitation for brick manufacturing along the margins of the Cauamé. From this exploitation, shallow pools that could provide suitable habitats for mosquitoes were formed. Caranã district had the second highest number of reported malaria cases in Boa Vista (FNS 1997).

Selected entomological variables investigated were seasonal and hourly biting indices and natural infection rate for the various anopheline species. The suitability of the brick pools for anopheline habitats were also checked in 13 de Setembro. Meteorological and reported human malaria cases data were provided by local authorities. Rainfall indices, temperature and humidity averages were those from the Air Force Meteorological Station in Boa Vista. Malaria cases data was obtained from Funasa-RR.

Adult collection - At 13 de Setembro, adult human-bait collections were performed for 7 to 10 consecutive days/month from May 1996 through April 1997 and 1 to 5 consecutive days/month from May 1997 through May 1998. Human-bait collections were performed at dusk (18:00-20:00 h ± 30 min, sunset time variation throughout the year) in a house located ~100 m from the right margin of the Branco River and ~50 m from the Pricumã *Igarapé*, in a neighborhood called Olaria. Collections were done on the veranda of a house with screened windows and doors. People living in this house (a family of 17 members) were previously affected by many malaria episodes. They normally interrupted their outdoors activities by 18:00 h for dinner and TV, a common behavior for the whole neighborhood.

A 12 h collection, from 18:00 to 06:00 h, was performed in 13 de Setembro for one of the ten days of the consecutive collection period, from May 1996-April 1997, to determine biting activity peaks.

In Caranã, adult collections were conducted for one to five consecutive days/month (June 1997-April 1998). Human-bait collections also were performed at dusk (18:00-20:00 h) on the veranda of a wood-constructed house located approximately 50 m from the Cauamé *Igarapé*.

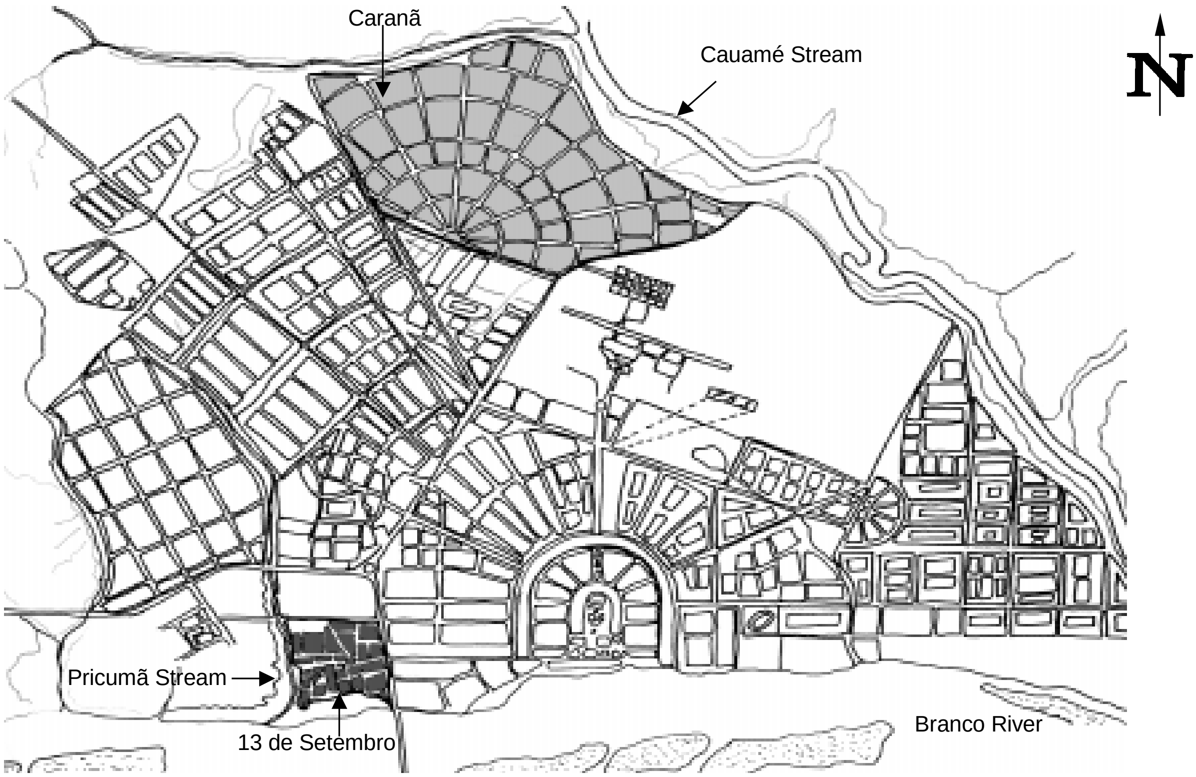

Fig. 2: municipality of Boa Vista, Roraima, with the districts of 13 de Setembro and Caranã, Branco and Cauamé rivers and Pricumã *Igarapé*.

Anopheline species were identified using taxonomic keys (Consoli & Lourenço-de-Oliveira 1994). Data were recorded for the number of specimens collected by species, collection time and date, and number of collectors. Biting indices were calculated for *Anopheles albitarsis sensu lato* (hereafter only *An. albitarsis), An. darlingi, An. braziliensis, An. nuneztovari* and *An. peryassui* for the first hour after sunset (18:00-19:00 h) and for *An. albitarsis* and *An. darlingi* for the second hour (19:00-20:00 h). The indices were obtained by dividing the number of mosquitoes collected daily by the number of collectors and the hours of collection daily. Monthly means (based on the 5-10 collections/month) for the 1st, 2nd and for the 2 h period were also calculated. Mosquitoes were kept in silica-gel for natural malaria infection determination.

Excavation pits as anopheline habitats - Immature collections in the brick shallow pits were performed with 400 ml metallic dippers ladles in the early morning from 06:00-06:30 h in water flooded excavations ~2 km along the Branco River margin, at 13 de Setembro. Investigations were performed five days per month during the same period as the adult mosquito collections, whenever the Branco River water level permitted. Larvae and pupae were either placed in 70% alcohol or reared in the laboratory to 4th instars or to adults, respectively, for identification. Water temperature and pH were recorded during collections and means calculated. Species identification were as previously described for adults.

Determination of anopheline infection rates - The assays were performed at the Laboratório de Entomologia da Malária, Serviço de Parasitologia, Instituto Evandro Chagas, Belém, Pará. Specimens had their identification reconfirmed based on the same taxonomic key (Consoli & Lourenço-de-Oliveira 1994). Only the head and prothorax of the specimens were used. *An. darlingi, An. nuneztovari* and *An. peryassui* were individually assayed. For *An. albitarsis sensu lato* and *An. braziliensis* 5 specimens from the same collection date were pooled per tube.

Methodology used ELISA techniques essentially the same described in Wirtz et al. (1987a,b).

Lyophilized monoclonals antibodies for *Plasmodium falciparum, P. vivax* VK210, *P.vivax* VK247 and *P. malariae* and, positive controls used were kindly provided by Dr Pamela Patterson, National Center for Infectious Diseases, Centers for Disease Control and Prevention-CDC, produced by Kirkegaard & Perry Laboratories.

Statistics - Results were analyzed by Pearson's correlation coefficient, Student's t test and, standard error of the correlation coefficient (Rodrigues 1993). Pearson's correlation coefficient (r) was used to evaluate the degree of dependence between variables. Student's t test (t) identified significant levels of dependence between variables to determine whether one had an effect on other variables or were related by chance. Significant levels were designated as twice correlation coefficient difference from the correlation coefficient r.

TABLE I

Number (Nr) and biting indexes (BI) for *Anopheles albitarsis*, *An. darlingi*, *An.braziliensis* and *An. nuneztovari* for the first and second hours and overall mean (mean ± standard deviation). Collections were made at sunset (18:00-20:00 h ± 30 min) between May 1996 and April 1998 at 13 de Setembro district, Boa Vista, Roraima, Brazil

Year	Month	Days	Nr collectors Mean	Nr. *An. albitarsis*	BI *albitarsis* 1st h	BI *albitarsis* 2nd h	BI *albitarsis* Mean	Nr. *An. darlingi*	BI *darlingi* 1st h	BI *darlingi* 2nd h	BI *darlingi* Mean	Nr. *An. braziliensis*	BI *braziliensis* Average	Nr. *An. nuneztovari*	BI *nuneztovari* Average	Subtotal
1996	May	8	3.3	363	4 ±5.6	0.6 ± 1.7	2.3 ± 9	26	0.8 ± 1.2	0.4 ± 0.5	0.6 ± 0.8	39	0.8 ± 0.6	4	0.1 ± 0.2	432
	June	10	3.6	319	5.1 ± 2.1	4.2 ± 2.2	4.7 ± 1.7	138	1.8 ± 1.6	2.3 ± 3.8	2.1 ± 2.6	0	0	8	0.1 ± 0.2	465
	July	10	2.9	179	4.5 ± 2	1.5 ± 0.9	3 ± 1.4	82	1.7 ± 1.7	0.8 ± 0.8	1.3 ± 0.8	35	0.4 ± 0.6	4	0.15 ± 0.3	300
	August	8	2.9	179	6.2 ± 4.3	1.6 ± 1.2	3.9 ± 2.1	64	1.5 ± 0.7	1.5 ± 1.1	1.5 ± 1.3	31	0.3 ± 0.6	4	0.1 ± 0.2	278
	September	7	2.6	668	27.7 ± 24.4	8.5 ± 6.3	18.1 ± 14	193	7.6 ± 12	4.9 ± 7.7	6.3 ± 20.3	66	1.7 ± 1	5	0.2 ± 0.1	932
	October	9	2.6	380	13.3 ± 11.4	3.1 ± 2.7	8.2 ± 2.7	16	0.4 ± 0.6	0.5 ± 0.5	0.5 ± 0.3	27	0.5 ± 0.4	3	0.1 ± 0.1	426
	November	9	3.1	204	3.2 ± 3.3	0.8 ± 1.1	2 ± 2.6	16	0.6 ± 0.5	0.4 ± 0.7	0.5 ± 0.3	27	0.4 ± 0.4	0	0	247
	December	10	2.2	25	0.8 ± 0.9	0.2 ± 0.4	0.5 ± 0.5	5	0.1 ± 0.2	0.3 ± 0.4	0.2 ± 0.2	13	0.3 ± 0.3	0	0	43
1997	January	10	2.6	678	19.1 ± 14.5	6.1 ± 7.5	12.6 ± 8.4	6	0.4 ± 0.5	0.04 ± 0.1	0.2 ± 0.4	24	0.5 ± 0.2	6	0.04 ± 0.1	714
	February	10	2.8	828	21.2 ± 11.8	12.5 ± 11	16.9 ± 11.1	0	0	0	0	2	0.03 ± 0.06	3	0.1 ± 0.2	833
	March	8	3.3	60	1.8 ± 1.7	0.3 ± 0.4	1.1 ± 0.9	1	0	0.03 ± 0.1	0.02 ± 0.04	5	0.08 ± 0.1	1	0.03 ± 0.1	67
	April	10	2.5	7	0.2 ± 0.3	0.1 ± 0.3	0.2 ± 0.2	1	0.03 ± 0.1	0	0.02 ± 0.1	0	0	0	0	8
Subtotal		109		3,890				548				269		38		4,745
1997	May	10	2.4	15	0.5 ± 0.4	0.2 ± 0.2	0.4 ± 0.5	2	0	0.1 ± 0.1	0.03 ± 0.1	0	0	0	0	17
	June	5	2.6	33	2.3 ± 1.3	0.3 ± 0.3	1.3 ± 0.6	0	0	0	0	0	0	0	0	33
	July	5	1.2	67	9.3 ± 10.7	4 ± 5.6	6.7 ± 0.1	4	0.4 ± 0.6	0.2 ± 0.5	0.3 ± 0.3	5	0.4 ± 0.6	0	0	76
	August	5	3.2	593	26.4 ± 24.7	12 ± 12.3	19.2 ± 18	3	0.2 ± 0.2	0	0.08 ± 0.1	3	0.07 ± 0.2	0	0	599
	September	5	3.2	208	4.5 ± 1.1	1.8 ± 0.6	3.4 ± 0.8	0	0	0	0	0	0	0	0	208
	October	5	2.8	89	5 ± 1.1	1.8 ± 0.6	3.4 ± 0.8	6	0.4 ± 0.3	0.1 ± 0.2	0.2 ± 0.2	0	0	0	0	95
	November	5	2.2	68	5 ± 0.8	1.2 ± 1	3.1 ± 0.8	6	0.4 ± 0.3	0.1 ± 0.2	0.3 ± 0.2	0	0	0	0	74
	December	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1998	January	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	February	5	1	4	0.4 ± 0.9	0.4 ± 0.6	0.4 ± 0.4	0	0	0	0	0	0	0	0	4
	March	5	1	3	0.4 ± 0.6	0.2 ± 0.5	0.3 ± 0.3	0	0	0	0	0	0	0	0	3
	April	1	2	15	4	3.5	3.75	0	0	0	0	0	0	0	0	15
Subtotal		51		1,095				21				8		0		1,124
Total		160		4,985				569				277		38		5,869

Rainy season is April to November; dry season is December to March. Collections were not performed in December 1997 and January 1998; from May 1996 to April 1997, in one day, a 12 h collection (18:00-06:00 h) was performed; Nr. *An.albitarsis s.l.*, *darlingi*, *braziliensis* and *nuneztovari* is the total number of mosquitoes collected in 2 h.

TABLE II

Number (Nr) and biting indexes (BI) for specimens of *Anopheles albitarsis*, *An. darlingi*, *An.braziliensis* and *An. peryassui* for the first and second hours and overall mean (mean ± standard deviation). Collections were made at sunset (18:00-20:00 h ± 30 min) between June 1997 and April 1998 at Caranã district, Boa Vista, Roraima, Brazil

Year	Month	Days	Nr *An. albitarsis*	BI *albitarsis* 1st h	BI *albitarsis* 2nd h	BI *albitarsis* Mean	Nr *An. darlingi*	BI *darlingi* 1st h	BI *darlingi* 2nd h	BI *darlingi* Average	Nr *An. braziliensis*	BI *braziliensis* Average	Nb *An. peryassuy*	BI *peryassuy* Average	Total
1997	June	5	0	0	0	0	0	0	0	0	0	0	0	0	
	July	5	313	12.3 ± 24.6	8.6 ± 6.8	10.5 ± 14.7	24	0.6 ± 0.8	1 ± 0.8	0.8 ± 0.7	97	3.2 ± 3.1	34	2.1 ± 1.6	
	August	5	55	5.5 ± 8.3	-	2.8 ± 4.1	6	0.6 ± 1.1	-	0.3 ± 0.5	12	0.6 ± 0.8	23	1.2 ± 1.8	
	September	5	23	2.3 ± 3.4	-	1.2 ± 1.7	6	0.6 ± 1.1	-	0.3 ± 0.5	7	0.4 ± 0.6	7	0.4 ± 0.6	
	October	5	0	0	0	0	0	0	0	0	0	0	0	0	
	November	5	7	0.5 ± 1.2	0.2 ± 0.3	0.4 ± 0.6	1	0.1 ± 0.2	-	0.1 ± 0.1	0	0.3 ± 0.5	0	0	
	December	-	-	-	-	-	-	-	-	-	-	-	-	-	
1998	January	-	-	-	-	-	-	-	-	-	-	-	-	-	
	February	5	-	-	-	-	-	-	-	-	0	0	0	0	
	March	5	-	-	-	-	-	-	-	-	0	0	0	0	
	April	1	68	2.8 ± 3.2	4 ± 3.2	3.4 ± 3.2	-	-	-	-	2	0.1 ± 0.1	0	0	
Total	46	466				503	37				124		64		691

Rainy season is April to November; dry season is December to March. Collections were not performed in December 1997 and January 1998; f rom May 1996 to April 1997, in one day, a 12 h collection (18:00-06:00 h) was performed. Nr *An. albitarsis s.l.*, *darlingi*, *braziliensis* and *peryassui* is the total number of mosquitoes collected in 2 h. Mean number of collectors was 2.2.

RESULTS

Adult collections - From May 1996 to April 1998, 7,263 specimens were identified to five species: *An. albitarsis* (82.8%), *An. darlingi* (10.3%), *An. braziliensis* (5.5%), *An. peryassui* (0.9%), and *An. nuneztovari* (0.5%).

The number of collectors/night ranged from 1-6, 2.6 mean, during the study period in 13 de Setembro. In Caranã, the number of collectors/night ranged from 2-3 (mean 2.2). Total man-hours of collection was 1,194 for 13 de Setembro and 110 for Caranã.

Mosquito biting indices (BI) were highest seasonally during the first hour after sunset (18:00-19:00 h) during the rainy season (April-November) at 13 de Setembro district (Tables I, II). At both 13 de Setembro and Caranã districts, *An. albitarsis* BI, while variable seasonally, were higher than for other anophelines species (as high as 27.7 in September 1996). *An. nuneztovari* specimens were collected only at 13 de Setembro with BI of = 0.2 (May-October 1996 and January-March 1997). *An.peryassui* was collected only at Caranã. *An. darlingi*, *An. braziliensis*, *An. nuneztovari* and *An. peryassui* were infrequently collected throughout the study at both districts.

To determine host-seeking activity, monthly 12 h collections (18:00-06:00 h) were performed at 13 de Setembro from May 1996 through April 1997. A total of 1,334 specimens were collected, *An. albitarsis* (76%), *An. darlingi* (21.2%), *An. braziliensis* (2.1%) and *An. nuneztovari* (0.7%) (Fig. 3).

An. albitarsis, the most abundant species, was found throughout the night with peaks at sunset (18:00-20:00 h) and during the middle of the night (23:00-02:00 h). These peaks were observed in May and August through November 1996. *An. darlingi* was found biting throughout the night (May, July, August and November 1996). A biting peak was observed for *An. darlingi* only in September 1996, when most specimens were collected at sunset. *An. braziliensis* and *An. nuneztovari* were present in small numbers, always at sunset.

Immature collections - At 13 de Setembro, a total of 342 immature anopheline specimens were collected during 80 days of collections from irregular excavations along the north margin of Branco River associated with manual brick manufacturing. Species collected were 209 *An. albitarsis*, 17 *An. nuneztovari*, 17 *An. darlingi* and 10 *An. oswaldoi*. *An. oswaldoi* was found only in this type of collection. Water was turbid, mixed with clay, with temperatures and pH ranging from 22.7-27.6°C and 6-6.5 mean, respectively, during the time of collection, from May 1996 through January 1997 (Table III).

Mosquito natural infection rates - A total of 7,198 specimens were analyzed by ELISA for malaria parasites. Malaria infections were found in 383 anophelines, 31 being mixed infections (MI). *An. darlingi* had the highest natural infection rate (IR) (8.5%, excluding MI) followed by *An. albitarsis* (4.6%), *An. braziliensis* (3%) and *An. nuneztovari* (2.6%). *An. albitarsis* was positive for all plasmodia investigated. *An. peryassui* was not found infected. Only one *An. darlingi* specimen was found infected in Caranã (Table IV). IR were calculated monthly as the number of infected specimens among the total analyzed per species (Table V).

P. vivax was the most prevailing plasmodium infecting anophelines (72.3%, both strains) followed by *P. falciparum* (26.1%) and *P. malariae* (1.6%).

Among the 27 specimens of *An. albitarsis* with MI, 14 were found with *P. vivax* VK210 and *P. falciparum*, 10 with *P. vivax* VK 210 and *P.vivax* VK 247 and 3 with *P. vivax* VK 247 and *P. falciparum*. The 4 specimens of *An. darlingi* with MI, 2 had *P. vivax* VK210 and *P. vivax* VK 247 , 1 with *P. vivax* VK 210 and *P. falciparum* and 1 with *P. vivax* VK 247 and *P. falciparum*. The other species showed no MI.

Entomological inoculation rates - Entomological inoculation rates (EIR) were calculated monthly as the product of BI and IR (Shiff et al. 1995) (Table V). EIR varied seasonally. *An. albitarsis* displayed the highest monthly EIR followed by *An. darlingi* and *An. braziliensis*.

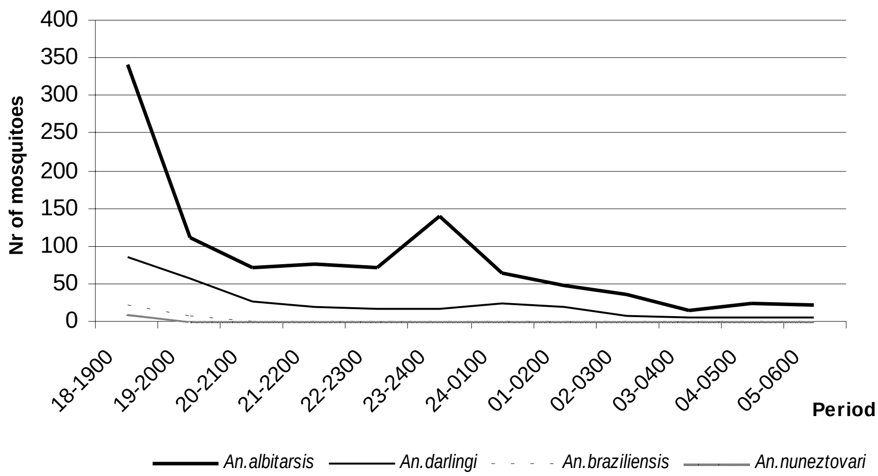

Fig. 3: peak of host seeking activity as expressed by the mean number of mosquito specimens collected per hour during 12 h collections (18:00-06:00 h) for the period May 1996 to April 1998 in the 13 de Setembro district, Boa Vista, Roraima.

TABLE III

Number of immature anophelines specimens (Nr) collected in excavations at the Branco River margin during May 1996 through April 1998, 13 de Setembro district, Boa Vista, Roraima, Brazil

Year	Month	Days [a]	Rainfall (mm)	Temperature (°C)	Water temperature (°C)	pH	Nr *An. albitarsis*	Nr *An. darlingi*	Nr *An. nuneztovari*	Nr *An. oswaldoi*	Subtotal
1996	May	5	474.2	28.2	22.7	6.5	4	0	5	0	
	June	5	541.2	27.1	-	-	2	4	3	0	
	July	5	331.6	27.1	25.6	6	28	0	45	1	
	August [b]	0	208.2	28.2	27.5	6	0	0	0	0	
	September	5	109.6	29.5	27.5	6	1	1	0	0	
	October	5	12	30.2	27.6	6	7	0	0	0	
	November	5	32.8	29.6	26	6	48	5	18	4	
	December	5	9.4	29.8	26.1	5	55	6	28	2	1997
	January	5	113.5	29.5	25.3	5	19	1	6	3	
	February	5	129.7	28.3	25	6	0	0	0	0	
	March	5	3.8	29.9	-	-	0	0	0	0	
	April	5	52.6	29.6	-	-	0	0	0	0	
Subtotal		55					164	17	105	10	296
1997	May	5	203.9	28.5	-	-	0	0	0	0	
	June	5	209.4	28.7	-	-	1	0	1	0	
	July	5	217.4	28	-	-	0	0	0	0	
	August	5	144.8	29.2	-	-	0	0	0	0	
	September [c]	5	3.1	30.8	-	-	44	0	0	0	
Subtotal		25					45		1		46
Total (%)		80					209 (61)	17 (5)	106 (31)	10 (3)	342

Rainy season is April to November. Dry season is December to March; *a*: 2 collectors, 30 min collections; *b*: collections were negative in August 1996 because margins were flooded and, *c*: were negative from October 1997 to April 1998 because of drought; - not determined

TABLE IV

Plasmodium vivax (VK210, VK247 and both-Σ*Pv*), *P. falciparum* (*Pf*) and *P. malariae* (*Pm*), as determined by ELISA, in *Anopheles albitarsis, An. darlingi* and *An. braziliensis* between May 1996 and April 1998 in two districts of Boa Vista, Roraima, Brazil

Year	Month	*An. albitarsis*						*An. darlingi*						*An. braziliensis*						Subtotal			Total
		VK 210	VK 247	Σ*Pv*	*Pf*	*Pm*	Σ	VK 210	VK 247	Σ*Pv*	*Pf*	*Pm*	Σ	VK 210	VK 247	Σ*Pv*	*Pf*	*Pm*	Σ	*Pv*	*Pf*	*Pm*	*Pv+Pf+Pm*
1996	May	4	7	11	0	0	11	0	0	0	0	0	0	0	2	2	0	0	2	13	0	0	13
	June	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	July	0	3	3	0	0	3	19	2	21	8	0	29	0	0	0	0	0	0	24	8	0	32
	August	6	15	21	1	0	22	10	4	14	4	0	18	0	2	2	0	0	2	37	5	0	42
	September	32	2	34	19	0	53	1	1	2	3	0	5	0	2	2	6	0	8	39[a]	28	0	67
	October	4	3	7	7	0	14	1	0	1	2	0	3	0	0	0	0	0	0	8	9	0	17
	November	0	0	0	6	0	6	0	0	0	2	0	2	0	0	0	0	0	0	0	8	0	8
	December	0	0	0	1	0	1	0	3	3	2	0	5	0	0	0	0	0	0	3	3	0	6
1997	January	14	12	26	14	0	40	0	0	0	0	0	0	0	0	0	0	0	0	26	14	0	40
	February	61	56	117	16	0	133	0	0	0	0	0	0	0	0	0	0	0	0	117	16	0	133
	March	0	3	3	0	0	3	1	0	1	0	0	1	0	0	0	0	0	0	4	0	0	4
	April	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	May	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	June	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	July	0	1	1	1	0	2	0	1	1	1	0	2	0	0	0	0	0	0	2	2	0	4
	August	0	3	3	7	6	16	0	0	0	0	0	0	0	0	0	0	0	0	3	7	6	16
	September	0	0	0	0	0	0	1[b]	0	1[b]	0	0	1[b]	0	0	0	0	0	0	1[b]	0	0	1[b]
	October	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	November	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	December	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1998	January	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	February	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	March	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	April	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mixed infections							27						4						0				31
Subtotal							277/5987 (4.6%)						62/730 (8.5%)						12/396 (3%)				352
Total		121	105	226	72	6	304	33	11	44	22	-	66	0	6	6	6	-	12	277[a]	100	6	383[b]

Σ: sum of infections*;* ΣS*Pv:* sum of both *P.vivax* strains*; a*: includes 1 *An. nuneztovari* with *P. vivax* (1/ 38, 2.6%)*; b*: 1 *An. darlingi* infected with *P. vivax* from Caranã; mixed infections: 27 specimens of *An. albitarsis*, 14 with *P. vivax* VK210 and *P. falciparum,* 10 with *P. vivax* VK 210 and VK 247 and 3 with *P. vivax* VK 247 and *P. falciparum;* 4 specimens of *An. darlingi*, 2 with *P. vivax* VK210 and VK 247, 1 with *P. vivax* VK 210 and *P. falciparum* and 1 with *P. vivax* VK 247 and *P. falciparum*

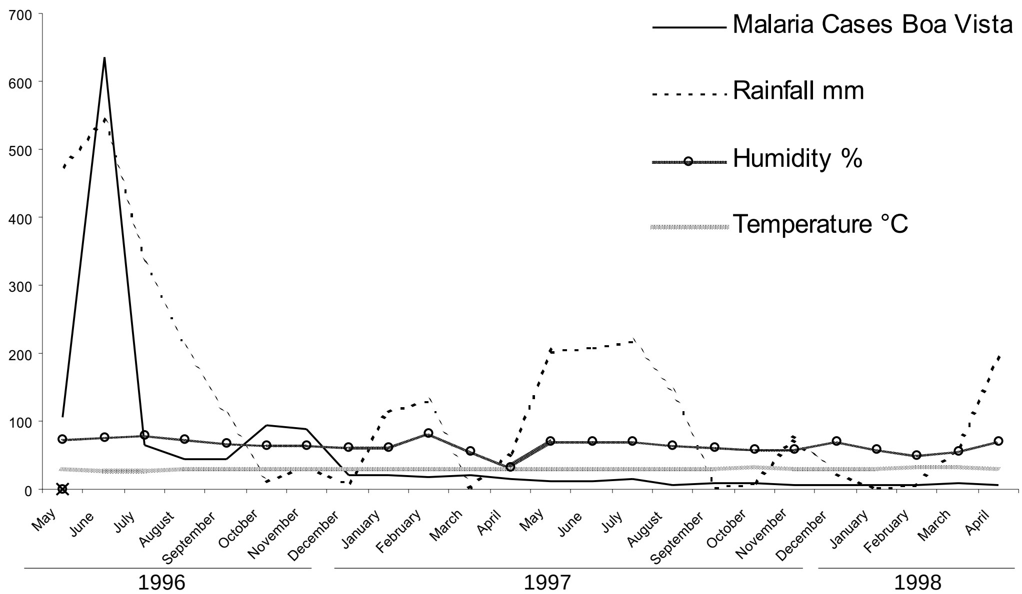

Fig. 4: malaria cases, rainfall, humidity and temperature indices for the period May 1996 through April 1998 in Boa Vista, Roraima

Meteorological variables, mosquitoes and malaria cases correlation - Mean monthly BI for *An. albitarsis, An. darlingi, An. braziliensis* were plotted against IR and EIR, rainfall, temperature, humidity and reported malaria cases for both 13 de Setembro and Caranã districts and, Boa Vista. *P. malariae* data for human malaria cases were not routinely diagnosed by health authorities.

Results showed that there was no significant correlation between meteorological variables tested (temperature, humidity and rainfall) and BI for 13 de Setembro ($r < 0.4 \pm 0.21$, $p > 0.05$) or Caranã ($r < 0.6 \pm 0.3$, $p > 0.05$).

There was no significant correlation between meteorological data (temperature, humidity and rainfall), IR and reported malaria cases for 13 de Setembro ($r < 0.4 \pm 0.21$, $p > 0.05$) and Caranã ($r < 0.6 \pm 0.3$, $p > 0.05$). However, there was a positive significant correlation between rainfall and malaria cases for all Boa Vista ($r < 0.65 \pm 0.21$, $p < 0.01$).

DISCUSSION

A 2-year longitudinal entomological survey was conducted at two highly malaria endemic districts (13 de Setembro and Caranã districts, Fig. 2), of Boa Vista, Roraima, Brazil.

An. albitarsis, An. darlingi, An. braziliensis, An. peryassui and *An. nuneztovari* were collected at human bait. Morphological differences among most members of the albitarsis complex (Rosa-Freitas et al. 1990) were not defined. Random amplified polymorphic DNA (RAPD, Wilkerson et al. 1995) was not performed, therefore *An. albitarsis* refers to *An. albitarsis sensu lato. An.albitarsis* was the most common anopheline found throughout the period. Low numbers of *An. darlingi* were collected. In Boa Vista, the predominant savanna vegetation may account for this, since *An. darlingi* is often associated with low lying vegetation along forested river margins (Forattini 1962, Consoli & Lourenço-de-Oliveira 1994, Rubio-Palis & Zimmerman 1997).

Collections for the immature forms showed that the excavations made along the Branco River margins at 13 de Setembro for brick manufacturing contributed to adult anopheline populations. *An. nuneztovari* was the second most frequently collected species along the river margin (31%), but was least frequently collected at human-bait (0.6%). In this area, *An. nuneztovari* may be highly zoophilic and comparisons between bovine and human bait collections should be performed to confirm this. *An. oswaldoi* was found only in its immature forms and not in human-bait collections. In some areas *An. oswaldoi* is highly anthropophilic, while in this site, it appears to prefer other animals. *An. braziliensis* and *An. peryassui* were collected only as adults and were not found in excavation pits as immatures.

BI and malaria reported cases showed no monthly correlation. Considering that the pre-patent period for *P. vivax* (the most prevalent species in Boa Vista) is 10-20 days (mean ~12 days at 24ºC, Garnham 1966) and considering that mosquito (infected) bites can increase in one month that would be registered (as disease) in the following month, statistical correlations of BI and IR means of a given month and malaria cases means of the next month were performed. Again, these variables showed no correlation perhaps due to small sample size an/or populations becoming infected elsewhere where BI and EIC were not measured.

BI varied considerably seasonally, hourly and by species. Among the selected environmental and biological parameters, there were significant differences for the rainfall and malaria indices (0.65, $p < 0.01$) and, *An. albitarsis* infections and malaria indices (0.62, $p < 0.01$ for *P. vivax* and 0.47, $p < 0.05$ for *P. falciparum)*.

Host attraction activity demonstrates that while anophelines bite predominantly in the early evening (mainly at the first hour after sunset), small numbers continue to bite throughout the evening. Therefore malaria

TABLE V

Malaria human cases for *Plasmodium vivax* (*vivax*), *P. falciparum* (*falc.*) and mixed infections (*vivax + falc.*), number of collected (Nr) and infected (NI) anopheline specimens, infected rates (EIR), mean monthly biting indices (BI), and entomological inoculation rates (TR) for *Anopheles albitarsis s.l.*, *An. darlingi* and *An. braziliensis* between May 1996 and April 1998 in two districts of Boa Vista, Roraima, Brazil

		Human malaria reported cases																								
		Boa Vista				13 de Setembro			Caranã			*An. albitarsis*					*An. darlingi*					*An. braziliensis*				
Year	Month	*vivax*	*falc.*	*vivax + falc.*	Total	*vivax*	*falc.*	Total	*vivax*	*falc.*	Total	Nb	NI	IR	BI	EIR	Nb	NI	IR	BI	EIR	Nb	NI	IR	BI	EIR
1996	May	695	352	8	1055	-	-	25	-	-	10	363	11	0.03	7.3	0.21	26	0	0	0.6	0	39	2	0.05	0.8	0.04
	June	425	201	2	628	-	-	34	-	-	11	319	0	0	5.1	0	138	0	0	2.6	0	0	0	0	0	0
	July	439	211	8	658	-	-	8	-	-	9	179	3	0.02	2.1	0.04	82	29	0.35	1.3	0.5	35	0	0	0.4	0
	August	180	47	7	236	-	-	9	-	-	4	179	22	0.12	3.5	0.42	64	18	0.28	1.5	0.42	31	2	0.06	0.3	0.02
	September	174	63	0	237	-	-	9	-	-	9	668	53	0.08	18.1	1.45	193	5	0.03	6.2	0.19	66	8	0.12	1.7	0.2
	October	276	104	3	383	-	-	17	-	-	20	380	14	0.04	8.2	0.33	16	3	0.19	0.5	0.1	27	0	0	0.5	0
	November	247	103	3	353	-	-	12	-	-	15	204	6	0.03	3.5	0.11	16	2	0.13	0.5	0.07	27	0	0	0.4	0
	December	340	182	2	524	-	-	42	-	-	20	25	1	0.04	0.5	0.02	5	5	1	0.2	0.2	13	0	0	0.3	0
1997	January	395	197	3	595	37	4	41	25	10	35	678	40	0.06	12.6	0.76	6	0	0	0.2	0	24	0	0	0.5	0
	February	336	192	1	529	12	14	46	16	6	22	828	133	0.16	16.9	2.7	0	0	0	0	0	2	0	0	0.03	0
	March	314	137	4	455	14	2	17	18	0	18	60	3	0.05	1.1	0.06	1	1	1	0.02	0.02	5	0	0	0.08	0
	April	318	125	4	447	11	2	11	16	1	22	7	0	0	0.2	0	1	0	0	0.02	0	0	0	0	0	0
	May	247	105	2	354	8	1	9	11	1	12	15	0	0	0.4	0	2	0	0	0.03	0	0	0	0	0	0
	June	266	93	3	362	12	1	13	11	1	12	33	0	0	1.3	0	0	0	0	0	0	0	0	0	0	0
	July	163	57	1	221	13	5	18	5	1	6	67	2	0.03	6.7	0.2	4	2	0.5	0.3	0.15	5	0	0	0.4	0
	August	85	21	3	109	8	2	9	1	0	2	593	16	0.03	19.2	0.6	3	0	0	0.1	0	3	0	0	0.07	0
	September	81	15	1	97	7	0	8	6	0	6	208	0	0	6.5	0	0	0 (1[a])	0	0	0	0	0	0	0	0
	October	77	17	2	96	6	4	10	6	0	7	89	0	0	3.4	0	6	0	0	0.2	0	0	0	0	0	0
	November	59	16	0	75	2	2	4	2	0	2	68	0	0	3.1	0	6	0	0	0.3	0	0	0	0	0	0
	December	64	17	1	82	3	1	4	5	0	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1998	January	60	16	1	77	1	2	3	3	0	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	February	69	14	1	84	3	1	4	1	0	1	4	0	0	0.4	0	0	0	0	0	0	0	0	0	0	0
	March	89	13	2	104	5	0	5	5	0	5	3	0	0	0.3	0	0	0	0	0	0	0	0	0	0	0
	April	61	6	1	68	0	1	-	0	0	-	15	0	0	3.8	0	0	0	0	0	0	0	0	0	0	0
	Total				12678			358			158	4985	304				569	66				277	12			

Rainy season is April to November; September to December 1997, insecticide was spatially sprayed in Boa Vista; collections were not performed in December 1997 and January 1998; malaria cases as expressed by the number of positive slides FNS-RR; *a*: 1 *An. darlingi* found infected in Caranã; - data not available transmission may occur throughout the night, even though it is more likely to occur at sunset. The concomitant BI decrease for the period September-December 1996 and malaria cases observed could be due to a successful Malathion ultra-low volume pesticide fumigation program, aimed at *Aedes aegypti* reduction, due to a dengue outbreak in September 1996. Large decreases in rainfall indices could, on the other hand, account for the decrease of BI observed (Table V).

Rainfall indices appeared to affect the malaria rates. This has also been observed in Costa Marques, Rondônia, where increases in rainfall indices positively affected mosquito population numbers (Klein & Lima 1990). During the long period of drought that extended from September 1997 to February 1998 in Boa Vista, there was a large decline in BI (< 6.5 thereafter for *An. albitarsis* and < 0.3 for *An. darlingi*) and an associated low number of malaria cases. Between September 1997 and April 1998, the lowest rainfall indices in 20 years were observed (IBGE 1999). As a result, the water flow of the Branco and Cauamé rivers nearly halted. Large areas of vegetation were consumed by natural fires around Boa Vista, as well as other areas throughout the state. Not only mosquito immature populations might have been affected by the lack of suitable breeding habitats, adult populations might also have been reduced due to resting site reduction and the dry and smoky atmosphere prevailing in Boa Vista.

In contrast, during July and August 1997 rainfall indices were very high, 217.4 mm and 144.8 mm, respectively. During that period, large areas of the city were flooded. During the extreme water levels, there was a corresponding decrease of malaria (135 to 63 cases, respectively) followed by an increase in the BI for *An. albitarsis* (6.7 to 19.2, July-August 1997) and a decrease in the *An. darlingi* BI (0.3 to 0.1, July-August 1997).

Biting indices for *An. darlingi* were generally low, suggesting a low participation of this species in the malaria transmission. However, even though low numbers of *An. darlingi* specimens were collected, it had the highest combined infection rate (8.5%). Deane (1986) stated that even though populations of *An. darlingi* were low, they significantly contribute to transmission due to its high susceptibility to *Plasmodium* parasites. Minimal biting indices could be enough for keeping the transmission cycle. In fact, the high IR observed in the period of July-December 1996, accounted for a steady EIR even with low BI (Table V). The second highest natural infection rate was observed for *An. albitarsis* (4.6%). Even though presenting the second highest IR, *An. albitarsis* was the most frequently collected found species (82.8%) and had the highest BI and EIR (Table V). *An. albitarsis* should be considered the primary malaria vector in Boa Vista due to its high density, high infection and potential transmission rates.

An. albitarsis s.l. has been recently assigned as a vector of primary importance in malaria transmission in Pará with IR of 4.5% (Arruda et al. 1986), Amapá with 1.6% IR (Segura 1998) and 0.8% IR (Póvoa et al. 2001) and, Rondônia, 0.5% (Oliveira-Ferreira et al. 1990).

Malaria control is largely based on vector control. Usually, the moment to apply vector control measures is dictated by an outbreak of malaria cases and not before to prevent the outbreak. Nevertheless, as our results indicate, mosquito populations could have been decreased when malaria becomes "visible" through the diagnosis of cases. This would decrease the effectiveness of insecticide-based vector control methods. Therefore, vector-based malaria control programs should rely on mosquito data, such as natural infection and biting rates rather than reported malaria cases.

Even though the importance of an integrated and decentralized approach to the malaria control program has been recognized in the last few years, little malaria control has been achieved as shown by the increasing numbers of malaria cases in Brazil and approximately 500 thousand cases have been reported annually over the last years.

Even with occurrences of torrential rain (in the period May-July 1996), drought (September-October 1997 and January-February 1998), insecticide fumigation application (September-December 1996), low mosquito BI for the two most frequent species, malaria cases did not subside in Boa Vista.

Statistical results indicated that malaria cases are not linked to mosquito BI, even though mosquitoes were found infected in the two most malarigeneous districts of Boa Vista. This might demonstrate that imported malaria cases from other municipalities may play a putative role on the maintenance of the endemicity in Boa Vista, that deserve to be better investigated. Roraima has localities of high API (as for the municipality of Mucajaí in 1999 with an API of 600, Funasa 2000). People transit intensively from Boa Vista towards those localities, and vice-versa. Farmers and weekenders lead this transit. This intense flow of people can be a large contributor for the malaria maintenance in the capital and in the rest of the state.

The correct appreciation of the variables that compose the malaria panorama in a given area is critical. These variables can play distinct roles in different endemic areas. Important components of the control effort have been appointed in the past. They were related to better housing, that decrease the man vector contact and increase residual insecticide activity, the determination of the case origins, society engagement and, early diagnosis and treatment of the malaria cases (Botelho et al. 1986, Sawyer 1986, Motta 1992, Barata 1995, Marques 1995, Voorham 1997). We suggest that measures that could decrease people flow could play a putative role in malaria control and should be considered in the infrastructure of any government settlement programs. Among those measures, the availability of health care in the interior, farming supplies and equipments as well as produce cooperatives. To those variables related to vector biology, population fluctuation and natural infection of malaria vector species should be inserted in the routine of the entomological control teams. Entomological studies should include detailed taxonomy of vectors, monthly BI and natural infection determination to better assess the correct intervention time for larvicide and adulticide application. Routinely assessed mosquito BI and natural infection rates should allow health authorities to draw a threshold level. Larvicide and adulticide control measures should be undertaken every time this threshold level is reached. Success on vector control can be achieved when intervention times coincide with that of a low-high borderline mosquito populations. Then larval and adult control might prevent mosquito populations to increase. On the other hand, lack of correlation between reported malaria cases and biting indices may indicate that malaria is acquired elsewhere. In Roraima, human population daily flow movements from the interior to the capital Boa Vista and vice-versa could be of great importance to the malaria persistence. Cooperation teams working on entomology, protozoology, human ecology and, developmental infrastructure for a better quality of life will provide the tools to control malaria in the Amazon.

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