

Structure of *Anopheles* (Diptera: Culicidae) population in areas with different degrees of human settlement: Cantá - Roraima - Brazil

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ABSTRACT

Malaria has still been one of the most important endemic diseases in the Amazonian region. This study presents the impact of human settlements on the structure of *Anopheles* population. Diversity, abundance, richness and distribution of the genus *Anopheles* were observed in two areas with different levels of human settlement in the Cantá city, Roraima State, Northern Brazil. The influence of the dry and rainy seasons on mosquito populations was also observed. Mosquito captures were performed between 6:00 and 10:00 pm during the dry (February and November) and rainy (May and August) seasons at four different sites of each area. Among the 11 species of *Anopheles* identified through the adults' characteristics, *An. albitarsis* s.l. (45.5%) and *An. darlingi* (19.2%) were the most abundant in the more intensively anthropized area while *An. triannulatus* (19.2%) was more common in the less modified area. Other species found were *An. nuneztovari* (10.9%), *An. oswaldoi* (2.0%), *An. evansae* (1.7%), *An. brasiliensis* (0.6%), *An. intermedius* (0.3%), *An. mediopunctatus* (0.5%), *An. periassui* (0.08%) and *An. argyritarsis* (0.04%). The highest mosquitoes' population density was observed in May and the lowest one was observed in February and November. These results demonstrate the existence of a high diversity of anophelines in the study areas, showing that anthropic changes in the environment and climate variability affect both the population density and relative abundance of these vectors.

KEYWORDS: Anophelines, Anthropized, *Anopheles* ecology, Climate variability, Malaria vector.

Estrutura da população de *Anopheles* (Diptera: Culicidae) em áreas com diferentes graus de colonização humana: Cantá – Roraima - Brasil

RESUMO

Malária continua sendo uma das mais importantes doenças endêmicas da região Amazônica. Esse estudo mostra o impacto da colonização humana sobre a estrutura populacional de *Anopheles*. Diversidade, abundância, riqueza e distribuição do gênero *Anopheles* foi observado em duas áreas com diferentes graus de antropização no município do Cantá, Estado de Roraima, Norte do Brasil. A influência das estações seca e chuvosa sobre a população dos mosquitos também foram investigadas. A captura dos mosquitos foi realizada entre 6:00 e 10:00 pm na estação seca (Fevereiro e Novembro) e estação chuvosa (Maio e Agosto) em quatro locais diferentes. Entre as 11 espécies de *Anopheles* identificadas através das características dos adultos, *An. albitarsis* s.l. (45.5%) e *An. darlingi* (19.2%) foram as mais abundantes nas áreas mais intensamente antropizadas, enquanto *An. triannulatus* (19.2%) foi a espécie mais comum na área menos modificada. As outras espécies encontradas foram *An. nuneztovari* (10.9%), *An. oswaldoi* (2.0%), *An. evansae* (1.7%), *An. brasiliensis* (0.6%), *An. intermedius* (0.3%), *An. mediopunctatus* (0.5%), *An. periassui* (0.08%) e *An. argyritarsis* (0.04%). A mais alta densidade populacional de mosquitos foi observada em Maio e as mais baixas em Fevereiro e Novembro. Esses resultados demonstram a existência de uma alta diversidade de anofelinos na área estudada e que mudanças antrópicas no ambiente e variabilidade climática afetam a densidade populacional desses vetores.

PALAVRAS-CHAVE: Anofelinos, Antropização, Ecologia de *Anopheles*, Variabilidade climática, Vetor da malária.

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INTRODUÇÃO

Malaria is still responsible for millions of cases and many deaths every year (Breman *et al*, 2004). Although most cases are reported in Africa, the disease is important in Brazil where 99% of the cases occur in the Amazon basin (Passos & Fialho, 1998; Silva & Oliveira, 2002). Recent surveys have shown a significant increase in the region (MS, 2003), especially in the state of Roraima, which presented a 103% increase in the Annual Parasite Index (IPA) between 1970 and 2000 (FUNASA, 2004). Between 2003-2004, malaria cases have increased by 200% in the city of Cantá, Roraima (SESAU-RR, 2004).

Studies show that 500,000 new cases occur in the Amazon region every year (Boulos, 1990; Silva & Oliveira, 2002). One of the most important factors causing this high number of cases is the intensive human migration to the region. The development of agriculture settlement schemes, uncontrolled migration and non-organized exploitation of natural resources (Oliveira-Ferreira *et al.*, 1992; Chaves & Rodrigues, 2000; Silva-Vasconcelos *et al.*, 2002) has resulted in changes in the ecological niches of anophelines (Boulos, 1990; Guimarães *et al.*, 2004). This has favored the dispersion of vector species to human-made habitats, increasing the transmission of the malaria parasite in the region (Boulos, 1990). The awareness of human settlement and its impact on the ecology of mosquitoes may lead to strategies for controlling the malaria transmission (Barata, 1995; Rebêlo *et al*, 1997; Guimarães *et al*, 2004).

Anopheles (Nynorhynchus) darlingi Root 1926 is considered to be the main vector of malaria in forested areas of Brazil (Deane *et al*, 1988; Oliveira-Ferreira *et al*, 1990; Tadei & Thatcher, 2000; Silva *et al*, 2006), whereas in the coastal region the most important vector is the *Anopheles (Nynorhynchus) aquasalis* Curry 1932 (Silva *et al*, 2006). *Anopheles (Nynorhynchus) albitarsis*, a complex of four species, is described as a secondary vector (Rosa-Freitas *et al.*, 1990; Consoli & Lourenço-de-Oliveira, 1994; Póvoa *et al*, 2006), yet it has been reported as a primary vector in some areas (Arruda *et al*, 1986; Silva-Vasconcelos *et al*, 2002; Póvoa *et al*, 2006).

Although the epidemiological importance of human-induced changes in forest areas is widely acknowledged, few studies have addressed the population dynamics of anophelines in newly anthropized areas. Scarcity of this information is detrimental to designing efficient malaria control measures in those areas. Therefore in this work, the diversity of anophelines was compared in localities with variation levels of anthropic environmental changes. Influences of rainy and dry seasons in the mosquito population were surveyed. Mosquito-human host interactions were also evaluated in both areas.

MATERIAL AND METHODS

STUDY SITES

Mosquito captures were carried out in Cantá city, Roraima State, Northern Brazil (Figure 1), which has 10.826 inhabitants and a territorial area of 7.691 Km² (IBGE, 2006). Cantá's population is concentrated in rural areas and its economic activities are associated with agriculture and livestock. Cantá is 100 m above sea level, presenting an equatorial climate; mostly hot and sub-humid.

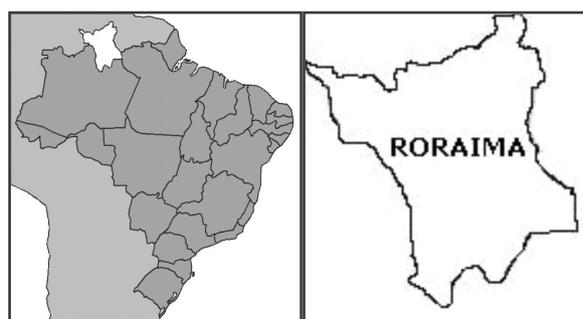


Figure 1 - Location map of the study area; Cantá city, Roraima State, Brazil.

Two Areas (A and B), 14 km distant from each other, were selected for the anophelines' capture. These areas comprise fragments of the tropical rain forest differing from most of region due to their anthropogenic changes.

Area A was located on the city's primary dirt road (N 02°20'00"; W 060°38'07.6"), having characteristics of a farm intensively changed by human activity. It has three houses, a six bedroom dorm-type building, three small man-made lakes,

plantations and livestock (cattle, pigs and fowl). It undergoes an intermittent flow of people, especially on weekends and during the soybean harvest.

Area B is located on the city's secondary dirt road beside a natural lake (vicinal VIII – N 02°15'32.9"; W 060°33'59.1"). Its vegetation is better conserved than area A. It has a small wooden house, a small man-made lake and a barn for cattle.

Mosquito captures were carried out during three consecutive days in both areas (four sites in each one). A gradual stratification was performed in both areas from the main house to the forest, for identifying the human settlement impact on the *Anopheles* population. The border of the forest (site 4) was around 1,500m (Area A) and 1,000m (Area B) from the house. Site 2 was situated at a distance of around 200m from the house and site 3 was between the two former sites. Site 1 was situated around 10m from the house.

In 2004, collections were performed in February and November (dry season) and in May and August (rainy season).

Mosquitoes' captures were carried out from 6:00 to 10:00 pm as they landed on human bait. Previous surveys and the specific literature have shown that this was the time when most of anopheline mosquito bites were reported (Consoli & Lourenço-de-Oliveira, 1994; Guimarães *et al.*, 2000; Forattini, 2002). Eight entomologist technicians from FUNASA-RR worked simultaneously in the two areas (A and B), under the same environmental conditions (temperature; wind; humidity and light).

Specimens were captured by aspiration and kept in individual vials labeled as for the specific site and hour of sampling. Specimens were killed with ether and the taxonomic identification was performed according to the keys of Gorham *et al* (1967), Consoli & Lourenço-de-Oliveira (1994) and Forattini (2002). Specimens belong to the same species were stored in the lab in vials containing silica-gel.

Abiotic factors were hourly measured during the three days of capture. The average maximum and minimum values during this study were: $28 \pm 0.5^\circ\text{C}$ for temperature and $69.7 \pm 2.7\%$ for relative humidity. These values were recorded using a thermohygrometer. Average monthly levels of precipitation were 136 ± 40.7 mm (Relatório de Climatologia Aeronáutico, 2000-2003). Sampling during full moon was avoided, since luminosity affects mosquito behavior (Lourenço-de-Oliveira *et al.*, 1985; Murilo *et al.*, 1988). Samplings were usually performed at new moon or half moon.

The Shannon-Wiener diversity index was used to compare the areas. Where p_i = the proportion of mosquitoes species in the sites of the studied areas.

The Analysis of Variance (ANOVA) was used to compare both areas, and the Tukey HSD was used as Post-hoc test.

RESULTS

Eleven species of anophelines were collected from a total of 2,298 mosquitoes obtained in at all sites between February and November, 2004 (Table 1).

Of this collection, 55.5% were from area A (anthropized), and 44.5% from area B. Eleven species were registered in Area A and eight in Area B. Nevertheless, the daily richness and diversity (H') were significantly higher in Area B compared to A ($F_{(1, 94)} = 4.017$, $p=0.048$ and $F_{(1, 94)}=10.433$, $p=0.002$ respectively).

During the daily richness analysis among sites in both areas, no differences were observed in Area A. However, in Area B, richness was significantly lower at site 1 compared to sites 2 and 3 ($F_{(3, 44)}=7.877$, $p<0.001$) (Figure 2). The mean densities showed different tendencies between areas A and B. A gradual increase in the number of mosquitoes collected was registered from site 1 to 4 in Area B. The opposite was observed in area A, where the density declined towards site 4 (Tabela 1).

Anopheles albitarsis s.l. (45.5%) was the most abundant species, followed by *An. darlingi* (19.2%), *An. triannulatus* (19.2%), and *An. nuneztovari* (10.4%). These species totaled 94% of all captured mosquitoes (Table 1). *An. Albitarsis* s.l. and *An. darlingi* presented higher abundance in area A, $F_{(1, 94)}=4.132$, $p=0.045$ and $F_{(1, 94)}=4.947$, $p=0.029$, respectively. The opposite phenomenon was observed for *An. Triannulatus*, which was four times more frequent in the less modified area, $F_{(1, 94)}=9.103$, $p=0.003$. No density differences were observed among the Areas for other sampled anophelines (Figure 3).

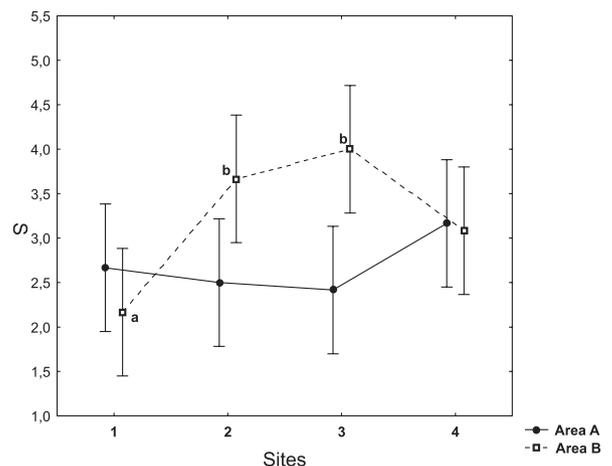


Figure 2 - Anophelines' Richness (S) at the sites of both Areas; A = More anthropized, B= Less anthropized. Cantá city, Roraima State, Brazil. Vertical bars denote 0.95 confidence intervals.

Table 1 - Total number of anophelines captured at the sampling sites, in the areas A and B, Cantá city, Roraima State, Brazil. A= More Anthropized and B= Less Anthropized.

Species	Site 1		Site 2		Site 3		Site 4		Total	%
	A	B	A	B	A	B	A	B		
<i>Anopheles albitarsis</i>	196	28	183	107	324	204	02	01	1045	45.5
<i>An. triannulatus</i>	0	35	05	64	0	46	81	211	442	19.2
<i>An. darlingi</i>	162	31	48	47	64	65	14	10	441	19.2
<i>An. nuneztovari</i>	08	16	20	27	16	37	80	46	250	10.9
<i>An. oswaldoi</i>	0	0	0	02	0	02	19	24	47	2.0
<i>An. evansae</i>	02	0	03	02	01	13	18	0	39	1.7
<i>An. brasiliensis</i>	0	0	08	01	0	05	0	0	14	0.6
<i>An. mediopunctatus</i>	0	0	0	0	0	0	11	0	11	0.5
<i>An. intermedium</i>	0	0	0	0	0	0	02	04	06	0.3
<i>An. periasmui</i>	0	0	01	0	01	0	0	0	02	0.08
<i>An. argyritarsis</i>	0	0	0	0	0	0	1	0	01	0.04
Total	368	110	268	250	406	372	228	296	2298	100

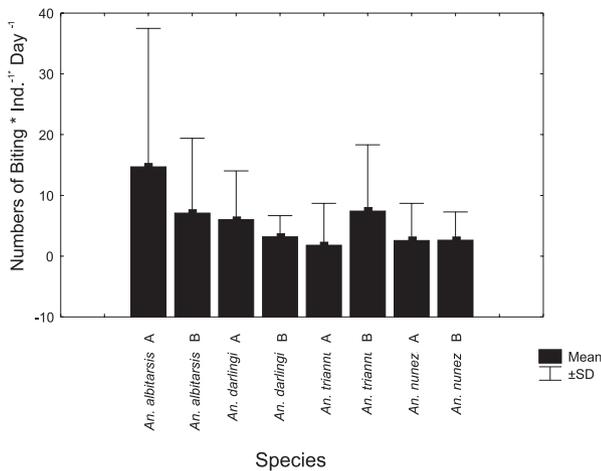


Figure 3 – Dominant anopheline species sampled in each quarter month, from February to November, 2004 in the Areas A and B; Cantá city, Roraima State, Brazil.

An. albitarsis s.l. was significantly more abundant at site 3 and very rare at site 4 of both areas ($F_{(3,92)}=6.599$, $p<0.001$) (Table 1). *An. darlingi*, the primary malaria vector in the Amazon region, was observed at all sites. Higher densities were observed at site 1 and few individuals were captured at site 4 ($F_{(3,92)}=5.980$, $p<0.001$) (Table 1). *An. triannulatus* was found at two sites (2 and 4) in area A and at all four sites in B with significantly higher densities at site 4 than in any other site ($F_{(3,92)}=8.434$, $p<0.001$) (Table 1). *An. Oswaldoi* was captured only at site 4 of Area A, and at 2, 3 and 4 sites of area B, with a preference for site 4 ($F_{(3,92)}=4.636$, $p=0.005$). *An. nuneztovari*

presented significantly higher density at site 4 than in other sites ($F_{(3,92)}=2.953$, $p=0.037$) (Table 1).

February presented a significant lower richness in relation to other months in Area A ($F_{(3,44)}=4.666$, $p=0.006$). The population density of anopheline also varied according to the seasons in area A, with significantly higher density in the rainy season ($F_{(1,46)}=6.955$, $p=0.011$) (Figure 4).

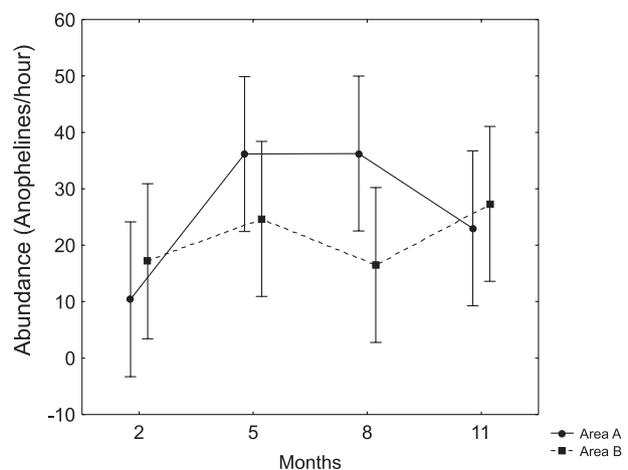


Figure 4 - Anophelines abundance in two areas throughout the months: Cantá city, Roraima State, Brazil. Vertical bars denote 0.95 confidence intervals.

DISCUSSION

The significant lower differences of richness and diversity observed in area A, show that anthropic changes induce a higher abundance of endophilic species of *Anopheles* in the environment. This is reinforced by the dominance of *An. albitarsis* s.l. and *An. darlingi* in the anthropized area. These results are in agreement with those observed by Juri *et al.* (2005) who observed a higher diversity of Anophelinae in preserved areas in Salta Forest, Argentina. Similarly, Montes (2005) observed a higher dominance and lower diversity of Culicidae species in anthropized areas of the Brazilian Atlantic Forests.

The significantly high diversity at site 3 of area B may be related to the concept that moderate impacts of human settlement increase species diversity. This concept is supported by the "intermediate disturbance hypothesis" proposed by Connell (1978) which suggests that species diversity should be highest at moderate levels of disturbance. This fact can be observed in the figure 2 which shows a high abundance at sites 2 and 3 in area B.

The most efficient malaria vector in the Americas, *An. darlingi* (Rozendaal, 1990; Rubio-Pallis, 1995), was the second most common species captured in both areas. It has been considered as the primary vector of this disease in Brazil (Deane, 1986). Silva *et al.* (2006) emphasize the implication of *An. darlingi* as a competent vector. High degree of endophily of *An. darlingi* has been demonstrated in several papers (Forattini, 1987; Roberts *et al.*, 2002; Silva *et al.*, 2006). In this study, this species was present in low densities in the forest (site 4). It was observed almost exclusively at the modified sites, especially around the house (site 1), a fact which confirms its high degree of association with human dwellings (Oliveira-Ferreira *et al.*, 1992; Lourenço-de-Oliveira & Luz, 1996; Souza-Santos, 2002; Guimarães *et al.*, 2004; Silva *et al.*, 2006).

Another important species of anopheline, with regard to malaria epidemiology in the Northern Brazil, is the *An. albitarsis* s.l. It was the most common species collected in this study and presented the highest densities in the most anthropic area, corroborating the results obtained in Boa Vista, Roraima State, by Silva-Vasconcelos *et al.* (2002) and Póvoa *et al.* (2006). Studies carried out by Tubaki *et al.* (2004) also showed an adaptation of *An. albitarsis* s.l. to a newly anthropized area, after a dam construction. The anthropophilic behavior of this species in our study may be observed by the fact that *An. albitarsis* s.l. was hardly found at the forest site in both areas (Table 1). This species is considered the potential malaria vector in several areas of the country (Arruda *et al.*, 1986; Tadei *et al.*, 1988; Klein *et al.*, 1991; Póvoa *et al.*, 2001).

The predominance of *An. albitarsis* s.l. associated with the high degree of infection by *Plasmodium* sp. in Boa Vista,

Roraima, has led several authors to incriminate this species and the *An. darlingi* as primary vectors of malaria transmission in this part of the country (Silva-Vasconcelos *et al.*, 2002; Póvoa *et al.*, 2006; Barros & Honório, 2007). Nevertheless, it is important to consider that the *Plasmodium* infection rate in *An. darlingi* is twice as high when compared to those observed in the *An. albitarsis* s.l. Therefore, simultaneous occurrence of these two species in any given locality may have epidemiological relevance, due to the probability of development malaria outbreaks (Deane, 1986; Teodoro *et al.*, 1994; Tadei & Thatcher, 2000; Póvoa *et al.*, 2006).

In area B, *An. triannulatus* and *An. oswaldoi* colonized different types of sites, although both have shown preference for the forest. Some authors believe that they are mainly associated with wild environments (Lourenço-de-Oliveira *et al.*, 1989; Lourenço-de-Oliveira, 1993; Consoli & Lourenço-de-Oliveira, 1994; Lourenço-de-Oliveira & Luz, 1996). Such observations point to the low degree of anthropophilic activity of those mosquitoes. Similar findings have been reported by Branquinho *et al.* (1993); Oliveira-Pereira & Rebelo (2000); Brown *et al.* (2001) and Póvoa *et al.* (2001). These authors observed low frequency of these species close to and inside human habitations. The absence of *An. triannulatus* in areas with environmental changes may be an indication of its potential as a bioindicator of human impact.

Another species predominant in the forest is *An. nuneztovari* which, however, did not show a clear habitat preference in area A. *An. nuneztovari* presents exophilic habitats (Tadei & Correia, 1982; Olano *et al.*, 1997) and has been observed in low densities close to and inside houses (Souza-Santos, 2002); it was predominantly observed inside the forest (Lourenço-de-Oliveira & Luz, 1996). These results corroborate with those from this investigation, which showed a lower incidence of this anopheline close to houses (site 1).

Rubio-Palis *et al.* (1992) collected 47,704 *An. nuneztovari* in approximately 2 years (74.8% of the sample) inside and outside a house in Venezuela and some were infected with *P. vivax*, detected by the ELISA technique. Moreno *et al.* (2004) reported *An. nuneztovari* (citotype B), for the first time, in southeastern Venezuela. They stress the possibility of this species as a primary transmitter of malaria in the region, since this same citotype is the main vector in Colombia and southeastern Venezuela. In Brazil, *An. Nuneztovari* was found infected with the malaria parasite in the north (Arruda *et al.*, 1986; Póvoa *et al.*, 2001; Silva-Vasconcelos *et al.*, 2002). However, in the urban areas in Roraima, studies have shown low density in the population (Silva-Vasconcelos, 2002; Póvoa *et al.*, 2006) as well as low number of infectious *An. nuneztovari* (Silva-Vasconcelos, 2002).

Although *An. evansae* was collected at all surveyed points, it was very rare at site 1 and presented higher densities at sites 3

and 4, though not significantly different. Low densities of this species were also observed inside houses and in peridomestic areas by Oliveira-Pereira & Rebelo (2000), in the northeastern region of Brazil, and also by Guimarães *et al.* (1997, 2004), in the central-western and southern parts of the country.

Several studies have shown that climatic factors affect the population dynamics of anopheline mosquitoes (Póvoa *et al.*, 2001; Silva-Vasconcelos *et al.*, 2002; Marquetti *et al.*, 1992; Xavier & Rebêlo, 1999; Moreno *et al.*, 2002). The Roraima State presents two climatic seasons: rainy period (May to September) and dry period (October to April) (Chaves & Rodrigues, 2000). In the present study the lowest density of anophelines was observed in February (dry period), which presented a significant lower richness in relation to August and November in Area A. In February, most breeding sites were dry, a fact which may be responsible for the significant decrease in mosquito populations. Silva-Vasconcelos *et al.* (2002) associated the dry season to a decrease in anopheline biting rates. Yet, other studies showed higher mosquito densities at the onset of the dry season (Póvoa *et al.*, 2001) and also an increase in malaria transmission in this period (Charlwood *et al.*, 2000; Chaves & Rodrigues, 2000). Gurgel (2003) in a spatial-temporal analysis of malaria in Roraima suggested that the increase of malaria cases in the beginning of the dry season was related to standing water in several places. At this time, there is also an increase in the flux of people to areas formerly inundated, thus facilitating contact with mosquitoes.

A regular seasonal pattern of mosquito occurrence was not detected in Area B. A similar fact was observed by Guimarães *et al.*, (1997) studying anophelines in Southern Brazil. Their study detected no influence of the rainy season on mosquito densities and observed that breeding sites did not change during the year, regardless of the rainfall regime. The present study observed the same results. This may also be the phenomenon underlying the observed pattern of low population fluctuations of anophelines in area B, where most breeding sites were permanent.

The highest densities of anophelines were recorded during the rainy season, in captures performed from May to August, which corresponds to the period with higher abundance of breeding sites created by rain. Significant higher densities were observed in area A in those months. Zimmerman (1992) reported that the seasonal increase in anophelines is regulated by the abundance of breeding sites being it directly related to the rainfall. Several studies reported the same phenomenon, with highest densities of anophelines being observed during the rainy season (Marquetti *et al.*, 1992; Xavier & Rebêlo, 1999; Moreno *et al.*, 2002, Barros & Honório, 2007).

Human activity favors an increase in temporary breeding sites and has possibly contributed to the increase observed in anopheline populations in area A. The relation between the

breeding of anophelines and the presence of larval habitats created by humans has been reported by other authors. In Africa, Fillinger *et al.* (2004) observed that from 70% of all aquatic habitats found in a man-made area, 67% were colonized by *Anopheles* larvae, especially that of *An. gambiae*.

Data obtained in the present work indicate that ecological changes followed by human occupation in the Brazilian Amazon should be considered. This study showed that changes in the environment affect the population dynamics of *Anopheles*, thus possibly contributing to the increase of malaria transmission. The high degree of sinanthropism of *An. darlingi* and *An. albitarsis* s.l., species, considered as important in the epidemiology of malaria in the region, point to the necessity of monitoring these species in both preserved and modified environments. This will probably generate important information on the control of endemic malaria in the region.

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