

Composition, abundance and aspects of temporal variation in the distribution of *Anopheles* species in an area of Eastern Amazonia

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ABSTRACT

Introduction: The diverse and complex environmental conditions of the Amazon Basin favor the breeding and development of *Anopheles* species. This study aimed to describe the composition, abundance and temporal frequency of *Anopheles* species and to correlate these factors with precipitation, temperature and relative humidity. **Methods:** The study was conducted in the District of Coração, State of Amapá, Brazil. Samples were collected monthly during three consecutive nights, from 6:00 PM to 10:00 PM, from December 2010 to November 2011. In addition, four 12-hour collections (i.e., 6:00 PM to 6:00 AM) were performed during this period. **Results:** A total of 1,230 *Anopheles* specimens were collected. In the monthly collections, *Anopheles darlingi* was the predominant species, followed by *An. braziliensis* and *An. albitarsis* s.l., whereas *An. darlingi*, *An. peryassui* and *An. braziliensis* were the most frequent species collected in the 12-hour collections. The greatest number of anophelines was collected in September (the dry season). The highest frequency of anophelines was observed for *An. darlingi* during September, when there were the least rainfalls of the year, along with lower relative humidity and higher temperatures. There was little variation in the abundance of this species in other months, with the exception of slight increases in February, July and August. **Conclusions:** The major malaria vectors, *An. darlingi* and *An. albitarsis* s.l. (likely *An. marajoara*), were the most abundant species collected in the study area. Consequently, prevention and control measures should be taken to prevent malaria outbreaks in the District of Coração.

Keywords: Diversity of species. *Anopheles darlingi*. Malaria vector. Brazilian Amazon.

INTRODUCTION

Entomological studies on the diversity, density, behavioral patterns (particularly anthropophily, zoophily and bite peak) and temporal variations of *Anopheles* species have supported the identification and monitoring of malaria vectors^{1,2}. The capacity of a vector species to transmit malaria involves a combination of factors, including abundance, anthropophily, susceptibility to infection by the malaria parasite, infection rates and female longevity³. Anthropophily, susceptibility and longevity are the major epidemiological factors in malaria transmission, whereas the abundance of anopheline species is the most common entomological measurement used to determine the correlation between vectors and malaria incidence⁴.

In recent years, malaria transmission has decreased in the Brazilian Amazon region, likely because of improved control programs. All states within this region experienced reduced numbers of malaria cases⁵. However, although there was an overall reduction in malaria cases in the State of Amapá, an increase in the number of cases was recorded in some municipalities, such as Macapá, Mazagão, Calçoene and Tartarugalzinho⁵. In addition, the number of malaria cases caused by *Plasmodium falciparum* has increased⁵. This increase is a cause for concern because the main malaria vector, *Anopheles darlingi*, is widely distributed in the state of Amapá^{1,6-9} and has been captured at high densities in some localities^{1,4,10,11}. This framework justifies further studies in this state to improve knowledge of the diversity and behavior of this species, thus facilitating monitoring and vector control efforts. This study aimed to describe the composition, abundance and temporal frequency of *Anopheles* species and to analyze the possible influence of climatic factors on their distribution in a peri-urban area with recent urban developments in the northern Brazilian Amazon region. The data presented here are part of a broader study in which the parameters related to the transmission dynamics (intradomicile and peridomicile, anthropophily, zoophily, parity rate and natural infection rate) of *Anopheles* species were analyzed.

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METHODS

The study area was the District of Coração, located at km 13 Duca Serra Road in the outskirts of Macapá City, State of Amapá, Brazil (0°01'22"N; 51°10'26"W; **Figure 1**). The District of Coração was chosen because, according to the records of the Health Surveillance Secretariat of the State of Amapá,

it is an area of high malaria transmission, and to date, no studies have investigated the diversity and behavior of *Anopheles* species in this area. This district has 1,731 inhabitants. The homes are brick-walled, with most doors and windows protected by screens. The District of Coração belongs to the morphoclimatic Amazon Savanna environment domain, which includes savanna, fields, and islands of non-flooded forest and gallery forest; the majority of its landscape is covered with buriti

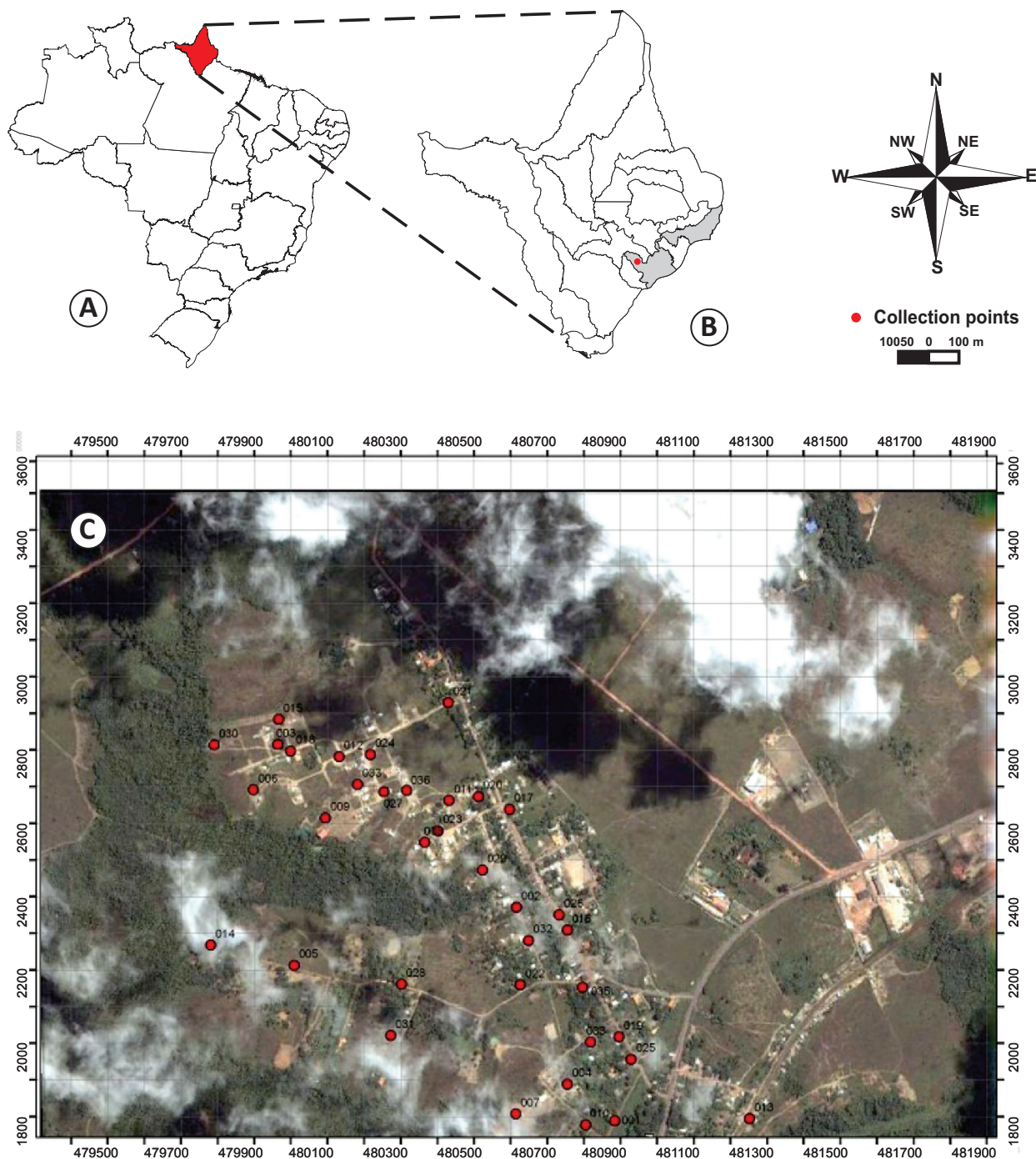


FIGURE 1 - Location of the District of Coração within Brazil. A: In detail, in the State of Amapá. B: Location of the study area in the State of Amapá. C: A satellite image of the studied areas. The collection points are highlighted.

palm trees (*Mauritia flexuosa*)¹². The surrounding wetlands are under the influence of precipitation and the Matapi River. Drainages in the gallery forest connect with the river, which maintains a minimum water level during the dry periods. The climate conditions of the region are typically hot and humid (mean humidity ~85%), with temperatures ranging from 22 to 32°C. The rainy and dry seasons extend from January to July and from August to December, respectively. Climatic data of the monthly accumulated precipitation (mm), mean relative humidity (%) and temperature (°C) were obtained from the National Institute for Space Research/National System for Environmental Data (INPE/SINDA - *Instituto Nacional de Pesquisas Espaciais/Sistema Nacional de Dados Ambientais*).

From December 2010 to November 2011, adult specimens were collected for three consecutive nights each month. Collections were performed at a total of 36 collection sites. The capture duration was four hours between 6:00PM and 10:00PM. The 36 sites were randomly distributed within the urban area of the district, with an average distance of 1,500 meters between each site. Additionally, four uninterrupted 12-hour collections were performed from 6:00 PM to 6:00 AM, two in the rainy season (February and May, 2009) and two in the dry season (August and November, 2009). There were always two collectors for each collection. The mosquitoes were spotted with the aid of a flashlight and captured with a manual-type capturer (Castro) when they attempted to land on the collectors' skin¹³. The entomological investigation and collection methodology adopted in the present study were reviewed and approved by the Research Ethics Committee of the National Institute for Research in the Amazon (INPA - *Instituto Nacional de Pesquisas da Amazônia*, Manaus, Brazil) under process number 036/2010.

After the captures, the mosquitoes were transferred into plastic cups, properly labeled and then transported alive inside tightly closed isothermal boxes¹⁴ to the Arthropoda Laboratory of the Federal University of Amapá (Macapá, Brazil). The *Anopheles* spp. species collected were identified using morphological keys that are specific to the group¹⁵⁻¹⁷, and the genus and subgenus abbreviations used were in accordance with Reinert¹⁸. Because the morphological differences between members of the *An. albitarsis*, *An. nuneztovari* and *An. triannulatus* complexes were not defined in this study, they were referred to as *sensu lato*. However, the species collected belonging to the *An. albitarsis* complex were most likely *An. marajoara*, as previous studies confirmed its presence in this region based on molecular markers¹⁹. Similarly, *Anopheles mediopunctatus* and *An. forattinii*, two cryptic species that cannot be identified by female morphological characters, were identified and separated by a slash in the manuscript.

The frequencies in the figures are expressed as absolute and relative frequencies. The specimens collected during the monthly periods and the four 12-hour periods were analyzed for richness and abundance parameters. For the statistical analyses, the Kruskal-Wallis test and Spearman's correlation analysis were performed using BioEstat software version 5.0²⁰. A significance threshold of $\alpha = 0.05$ was used for all tests.

The specimens obtained in the monthly collections were analyzed for temporal variation. In this analysis, the variables tested were species abundance and abiotic factors, such as precipitation, temperature and relative humidity.

TABLE 1 - Richness and abundance of *Anopheles* species captured in monthly collections in the District of Coração, State of Amapá, Brazil.

Species of <i>Anopheles</i>	Number (Percentage)																									
	Dec/10	Jan/11	Feb/11	Mar/11	Apr/11	May/11	Jun/11	Jul/11	Aug/11	Sep/11	Oct/11	Nov/11	Total (%)													
<i>An. braziliensis</i>	2	0.7	12	3.9	8	2.6	—	—	—	3	1.0	5	1.6	109	35.5	17	5.5	137	44.6	14	4.6	—	—	307	32.7	
<i>An. darlingi</i>	—	—	2	0.5	22	5.8	4	1.1	9	2.4	4	1.1	9	2.4	26	6.9	22	5.8	278	73.7	1	0.3	—	—	377	40.2
<i>An. albitarsis</i> s.l.	1	0.8	21	15.9	34	25.8	8	6.0	18	13.6	3	2.3	2	1.5	10	7.6	1	0.8	32	24.2	2	1.5	—	—	132	14.1
<i>An. mediopunctatus/An. forattinii</i>	—	—	—	—	—	—	—	—	2	100.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	0.2
<i>An. nuneztovari</i> s.l.	—	—	—	—	—	—	—	—	—	—	—	—	1	10.0	—	—	—	—	—	—	—	—	—	—	10	1.1
<i>An. peryassui</i>	—	—	—	—	—	—	—	—	2	4.9	2	4.9	1	2.4	2	4.9	3	7.3	30	73.2	1	2.4	—	—	41	4.4
<i>An. triannulatus</i> s.l.	1	1.4	—	—	2	2.9	—	—	3	4.4	2	2.9	1	1.4	1	1.4	7	10.2	45	65.2	7	10.2	—	—	69	7.3
Total (%)	4	0.4	36	3.8	68	7.3	18	1.9	34	3.6	14	1.5	19	2.0	148	15.8	50	5.3	522	55.7	25	2.7	—	—	938	100.0

An.: *Anopheles*; —: no mosquitos were collected.

RESULTS

A total of 1,230 anophelines were collected and identified as eight species and two subgenera, including five *Nyssorhynchus* and three *Anopheles* species: *Anopheles* (*Nyssorhynchus*) *braziliensis*, *Anopheles* (*Nyssorhynchus*) *darlingi*, *Anopheles* (*Nyssorhynchus*) *albitarsis* s.l., *Anopheles* (*Nyssorhynchus*) *nuneztovari* s.l., *Anopheles* (*Nyssorhynchus*) *triannulatus* s.l., *Anopheles* (*Anopheles*) *intermedius*, *Anopheles* (*Anopheles*) *mediopunctatus*/*Anopheles* (*Anopheles*) *forattinii* and *Anopheles* (*Anopheles*) *perysassui*.

Anopheles darlingi (47.1%) was the predominant species, followed by *An. braziliensis* (27.1%) and *An. albitarsis* s.l. (12.2%). The frequencies of the remaining species (i.e., *An. nuneztovari* s.l., *An. triannulatus* s.l., *An. intermedius*, *An. mediopunctatus*/*An. forattinii* and *An. perysassui*) were < 10%.

In the monthly (four-hour) collections, 938 specimens were captured and distributed into seven species (**Table 1**). The largest number of anophelines was sampled in September (dry season), followed by July (late rainy season). There was a sharp decrease in the anopheline fauna during October, and no anophelines were collected in November.

The species with the highest density (40.2%) during the study was *Anopheles darlingi*, whose greatest frequency was observed in September (73.7%), followed by *An. braziliensis* (32.7%). The latter species was the most abundant in July (35.5%), January (3.9%) and October (4.6%). *Anopheles albitarsis* s.l. was the third most frequent species (14.1%) observed during the collections, with the greatest frequencies in February (25.8%) and September (24.2%). *Anopheles perysassui* and *An. triannulatus* s.l. were not abundant during the study, but they showed population growth in September (73.2% and 65.2%, respectively). *Anopheles mediopunctatus*/*An. forattinii*

and *An. nuneztovari* s.l. had the lowest frequencies (**Table 1**). The Kruskal-Wallis test revealed non-significant differences in the frequencies of the species between the sampling months ($H = 17,700$; $p = 0.088$).

In the four 12-hour collections, a total of 292 specimens were collected and grouped into seven species (**Table 2**). The highest abundances were observed in May (37%) and August (47.3%), and the lowest abundances were observed in February (10.9%) and November (4.8%). *Anopheles darlingi* was the most (69.2%) frequent species, followed by *An. perysassui* (11.6%). Although *An. albitarsis* s.l. was observed at a low (6.2%) frequency, it was present in all four sample periods, as was *An. darlingi*. *Anopheles intermedius* and *An. nuneztovari* s.l. were each only captured during a single period (November and February, respectively) and were found at low (0.3%) frequencies. The Kruskal-Wallis test showed a statistically non-significant difference in the frequencies of *Anopheles* species among the four sampling periods ($H = 2,386$; $p = 0.496$).

During the study period, the heaviest rainfalls occurred in March, April, May and July. Higher temperatures extended from August to November, and the periods of highest relative humidity occurred from January to May. The largest number of specimens was collected in September, during the dry season, when there were the least rainfalls of the year, along with lower relative humidity and higher temperatures. There was no significant correlation between the number of anophelines collected and the tested climatic variables, although the number of anophelines increased with a decrease in precipitation and with an increase in temperature.

Regarding the abundance of *Anopheles* species in this study, the greatest frequency was observed for *An. darlingi* in September (**Figure 2**). In the other months, there was little variation in the abundance of this species, with the exception of slight increases in February, July and August, and there was

TABLE 2 - Richness and abundance of *Anopheles* species distributed between four 12-hour collection periods in the District of Coração, State of Amapá, Brazil.

Species of <i>Anopheles</i>	Number (Percentage)									
	Feb/11		May/11		Aug/11		Nov/11		Total (%)	
<i>Anopheles braziliensis</i>	—	—	1	3.7	20	74.1	6	22.2	27	9.3
<i>Anopheles darlingi</i>	23	11.4	101	50.0	72	35.6	6	3.0	202	69.2
<i>Anopheles intermedius</i>	—	—	—	—	—	—	1	100.0	1	0.3
<i>Anopheles albitarsis</i> s.l.	7	38.9	4	22.2	6	33.3	1	5.6	18	6.2
<i>Anopheles nuneztovari</i> s.l.	1	100.0	—	—	—	—	—	—	1	0.3
<i>Anopheles perysassui</i>	—	—	—	—	34	100.0	—	—	34	11.6
<i>Anopheles triannulatus</i> s.l.	1	11.1	2	22.2	6	66.7	—	—	9	3.1
Total (%)	32	10.9	108	37.0	138	47.3	14	4.8	292	100.0

—: no mosquitos were collected

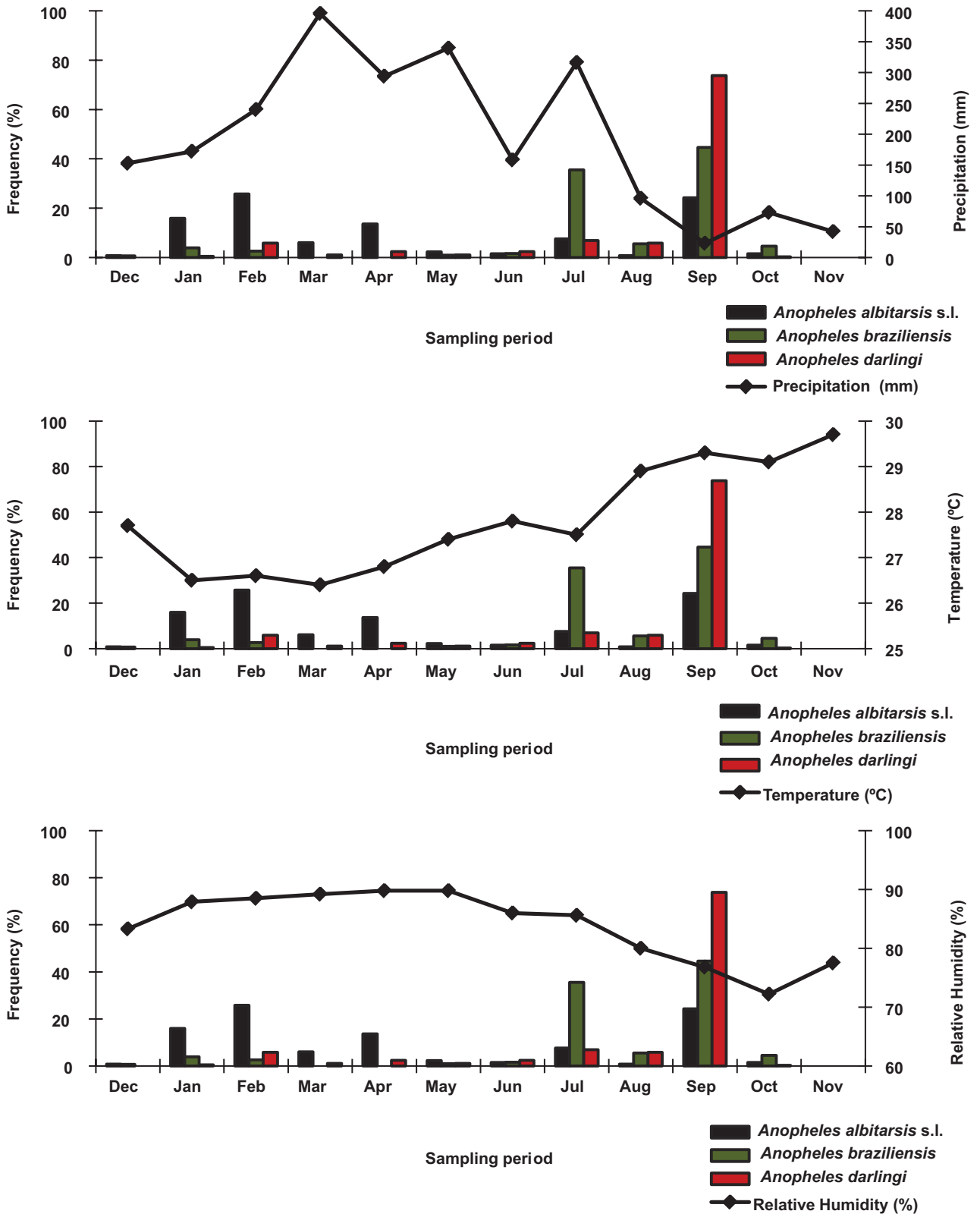


FIGURE 2 - Frequency of *Anopheles albittarsis s.l.*, *Anopheles braziliensis* and *Anopheles darlingi* in relation to the climatic variables, based on the monthly collections performed in the District of Coração, State of Amapá, within Brazil.

a unimodal temporal distribution. As for *An. braziliensis*, there was a pronounced increase in its density in September and July, with a bimodal pattern (temporal distribution) throughout the year. For *An. albitarsis* s.l., higher frequencies were observed in January, February, April and September, with a multimodal temporal distribution. No specimens were captured in November.

Correlation tests were performed for the three most abundant species. There were no statistically significant correlations between rainfall and *An. darlingi* ($r_s = 0.109$; $p = 0.735$), *An. albitarsis* s.l. ($r_s = 0.322$; $p = 0.306$) or *An. braziliensis* ($r_s = -0.345$; $p = 0.271$). In addition, there were no significant correlations regarding temperature.

DISCUSSION

The three predominant species in the study area during the monthly collections were *An. darlingi*, *An. braziliensis* and *An. albitarsis* s.l., whereas the most frequent species during the 12-hour collections were *An. darlingi*, *An. peryassui* and *An. braziliensis*. Among these species, two (*An. darlingi* and *An. albitarsis* s.l.) have been implicated as major malaria vectors in the state of Amapá^{6,10,11,19}. A similar result was obtained by another study²¹ along the Matapí River, an area close to the present study area, where the most abundant species were *An. darlingi* and *An. marajoara*.

Anopheles darlingi is a major malaria vector in the Americas²², where it transmits malaria both within and near homes²³⁻²⁷. Earlier studies implicated this species as being the main malaria vector in Brazil²⁸⁻³⁰. These findings were later corroborated by other reports in the Brazilian Amazon region^{1,24-26,31,32}, as this species is highly anthropophilic and susceptible to infection by *Plasmodium* spp.²⁹. Subsequently, *An. marajoara* was implicated as an important regional and/or local malaria vector in areas of Amapá^{7,19}, including the areas close to the present study^{4,11,21}.

Based on these results, we believe that the District of Coração deserves constant monitoring of *Anopheles* species and epidemiological surveillance, as there is a constant influx of people from malarious areas in the Brazilian Amazon to this district (District of Coração public health care unit: Personal Communication, 2012). These factors may favor the reintroduction and local spread of *Plasmodium* spp., thereby triggering malaria outbreaks. Although the mosquito species that are thought to be malaria vectors were the most abundant in the district, the malaria incidence was not high during this study (District of Coração public health care unit, 2012).

In this study, *An. mediopunctatus*/*An. forattinii* specimens were collected in more distant residences, in the surrounding environment where there is little disturbance by humans and near gallery forest vegetation where the location of water bodies favors the formation of natural breeding sites. We also observed the presence of fish ponds during this study. *Anopheles intermedius* and *An. peryassui* are predominantly zoophilic, preferring the wild or rural environment, and thus far, they have

not been implicated in the transmission of malaria in Brazil. However, these species were reported to be naturally infected with *Plasmodium* spp. in the Brazilian Amazon^{2,7} and may be involved in malaria transmission when present in high densities.

Anopheles nuneztovari s.l. was found to be infected with *Plasmodium falciparum*, *P. vivax* (two variants) and *P. malariae* at sites close to the present study area, together with *An. darlingi* and *An. marajoara*, and it may become an important malaria vector when present at high densities¹¹. *Anopheles braziliensis* was also observed at a high density here; however, its involvement in malaria transmission requires further research.

During the monthly collections, the highest density of anophelines was obtained in September (dry season). Thus, *An. darlingi* had the highest density in September, soon after the rainy season ended, which has been observed in previous studies. Subsequently, a drastic decrease of anophelines was observed in October. We believe that this result was likely because of the intense fires in areas of the District of Coração, which often occur in October because it is the hottest and driest month of the year. In this study, *An. darlingi* and *An. albitarsis* s.l. were the only species captured in all four collection periods (12-hour collections). This result clearly suggests that vector control measures should be performed in the District of Coração to prevent malaria outbreaks and epidemics.

Anopheles darlingi had a unimodal temporal distribution, with the highest density in the dry season (September), whereas *An. albitarsis* s.l. showed two slight peaks (rainy and dry seasons). A similar pattern was observed for these species in the State of Amapá (Brazil) and in Venezuela^{9,33}. In Venezuela³⁴, the highest density of *An. darlingi* was observed when the precipitation level was the lowest, a situation that was also observed in this study and has been reported in other parts of the Brazilian Amazon^{1,9,11,35}. The increased mosquito density was related to the previous rainy period, which maintained a water level that was sufficient for breeding sites; therefore, precipitation is a controlling factor in the density of *Anopheles*⁹. In the humid areas surrounding the District of Coração, there are sources capable of providing a water level that is sufficient to maintain breeding sites even during less rainy periods.

In this study, there was little variation in temperature and humidity throughout the year, and these factors did not significantly affect the *Anopheles* density. Hence, there was no correlation between these variables and the mosquito density. The temperature and humidity varied within the optimal ranges for the vital activities of mosquitoes¹⁷, exerting their influence only at higher intensity variations³⁶. Despite the absence of an established correlation, there is clearly an influence of precipitation on the density of *Anopheles* because it ensures the formation and maintenance of water supplies for breeding sites^{9,21}. Previous studies conducted in Venezuela^{33,37} have also failed to find a significant correlation between these variables and the abundance of anophelines.

In recent years, the Program for Malaria Control in Brazil has focused its strategies on the early diagnosis and immediate treatment of the infected population, as well as the implementation of vector control measures. Here, our findings

revealed that the major vectors were the most abundant species in the District of *Coração*; consequently, this area is at high risk of a malaria outbreak. Therefore, constant prevention measures, particularly in the late rainy season, should be taken to prevent such outbreaks.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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