Composition of *Anopheles* Meigen (Diptera: Culicidae) in a peri-urban area of the Eastern Amazon

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Abstract: Malaria vector mosquitoes belong to the genus *Anopheles* Meigen. The Amazon has been changing due to economic activities such as mining, logging, agriculture, and urbanization, leading to changes in malaria transmission patterns in this region. Therefore, this study aimed to survey *Anopheles* species in a peri-urban area of Macapá, state of Amapá, Eastern Amazon, Brazil. Human landing catches (HLC) and Shannon light traps were used to collect *Anopheles* adults at three points. We screened breeding sites in all accessible water reservoirs within a 2 km radius of each collection point to collect immatures and found two species: *Anopheles* (*Nyssorhynchus*) *albitarsis s.l.* Lynch-Arribalzága (35.7%) and *Anopheles* (*Nyssorhynchus*) *braziliensis* (Chagas) (64.3%). A total of 267 winged specimens of *Anopheles* were collected: *A.* (*N.*) *braziliensis* (62.9%), *Anopheles* (*Anopheles*) *mattogrossensis* Lutz & Neiva (11.6%), *Anopheles* (*Nyssorhynchus*) *triannulatus s.l.* (Neiva & Pinto) (11.6%), *A.* (*N.*) *albitarsis s.l.* Lynch-Arribálzaga (6.7%), *Anopheles* (*Nyssorhynchus*) *darlingi* Root (5.2%), *Anopheles* (*Anopheles*) *peryassui* Dyar & Knab (1.5%), and *Anopheles* (*Nyssorhynchus*) *nuneztovari* Galbadón (0.4%). Considering that Macapá has autochthonous malaria cases, our findings can contribute to developing public health measures in this municipality, therefore helping to protect its habitants.

Keywords: Ecology; Malaria; Medical Entomology; *Plasmodium;* Transmission Dynamics

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Malaria is an infectious disease caused by transmission of *Plasmodium* Marchiafava & Celli (Forattini 2002) protozoans by the bite of female mosquitoes of the genus *Anopheles* Meigen. More than 95% of the malaria cases reported in Brazil are for the Amazon region (Deane 1986). Although some *Anopheles* species are associated with undisturbed forest areas, others are more abundant in anthropized areas due to agriculture and logging, for example (Sallum *et al.* 2020). The Amazon has been suffering from the gradual degradation of its native forest due to these economic activities and others from mining and urban centers, thus changing malaria transmission patterns in the region (Rufalco-Moutinho *et al.* 2016). In this sense, the spatial distribution of the human population and variations in environmental conditions directly affect the occurrence and distribution of vector-borne diseases (Ernst *et al.* 2006).

Anopheles mosquitoes that are malaria vectors belong to the subgenera *Anopheles* (*Anopheles*) Meigen, *Anopheles* (*Cellia*) Theobald, *Anopheles* (*Kerteszia*) Theobald, and *Anopheles* (*Nyssorhynchus*) Blanchard (Tadei *et al.* 1998). *Anopheles* (*Nyssorhynchus*) *darlingi* Root is considered a primary vector of malaria in Brazil (Deane 1986; Baia-da-Silva *et al.* 2019; Carlos *et al.* 2019) and is responsible for the transmission of *Plasmodium falciparum* Welch and *Plasmodium vivax* Stephens (Laporta *et al.* 2015). Other species of *Anopheles* with local relevance (Carlos *et al.* 2019) in the transmission dynamics of *Plasmodium* in the Amazon are *Anopheles* (*Nyssorhynchus*) *albitarsis s.l.* Lynch-Arribalzága (Povoa *et al.* 2006), *Anopheles* (*Nyssorhynchus*) *deaneorum* Rosa-Freitas (Marcondes & Freitas 2013; Gil *et al.* 2015), *Anopheles* (*Nyssorhynchus*) *marajoara* Galvão & Damasceno (Galardo *et al.* 2007, 2009; Galardo 2010), *Anopheles* (*Nyssorhynchus*) *braziliensis* (Chagas), *Anopheles* (*Nyssorhynchus*) *nuneztovari s.l.* Galbadon, *Anopheles* (*Nyssorhynchus*) *oswaldoi s.l.* (Peryassú) , and *Anopheles* (*Nyssorhynchus*) *triannulatus s.l.* (Neiva & Pinto).

Several studies have reported *Anopheles* larvae in breeding sites with polluted water located very close to urban areas and occurring during the rainy and dry seasons, thus showing that these mosquitoes are expanding their niches (Sattler *et al.* 2005; Awolola *et al.* 2007; Mathania *et al.* 2020).

The ecological and behavioral characteristics of the main vectors of *Plasmodium* that cause malaria, along with the environmental conditions of the Amazon, impose significant challenges for adopting large-scale control strategies of these vectors (Ferreira & Castro 2016). Therefore, evaluating the occurrence of *Anopheles* species with the potential to transmit malaria in the Brazilian Amazon is of chief importance for vector monitoring and implementing control strategies (Laporta *et al.* 2015). In this sense, our study aimed to survey the species of *Anopheles* in a peri-urban area of the Eastern Amazon.

MATERIALS AND METHODS

Study Area. The study area is located in the municipality of Macapá, state of Amapá, Brazil, and is characterized by a warm and humid climate, with two well-defined rainy seasons. The season with periods of lowest rainfall extends from August to November, while that with highest rainfall extends from December to July, with 90% of the total annual precipitation (Tavares 2009). Three points of a peri-urban area of Macapá were selected for sampling the anophelines: point 1 (P01; S 0.104663° W -51.117175°), located at an urban construction site; point 2 (P02; S 0.101757° W -51.118915°), corresponding to an area of riparian forest with a small stream and somewhat connected to other forest fragments; and point 3 (P03; S 0.123807° W -51.113129°), located in a small urban nucleus with houses relatively distant from each other (Figure 1).

Sampling of adult *Anopheles*. Three sampling campaigns were carried out in the months of November 2018, March 2020, and June 2020. We collected for three consecutive nights at each sampling point and month. Samplings were performed for 12 uninterrupted hours on the first night (6:00 pm – 6:00 am). The captures lasted four uninterrupted hours on the following two nights, starting at twilight (6:00 pm – 10:00 pm).

Anopheles adults were sampled using human landing catches (HLC) (Brazil 2014) and Shannon light traps (Shannon & Hygiene 1939). We collected the mosquitoes before they fed on blood, using a manual suction catcher (Service 1968). The collected mosquitoes were placed in labeled entomological vials, stating the time and sampling point. In addition, during each capture, we recorded some environmental factors, including temperature, relative air humidity (using a thermo-hygrometer), wind speed (strong, weak, and null), and sky conditions (clean, covert, heavy rain, weak rain).

The collected specimens were placed in a thermal box and sent to the Laboratory of Medical Entomology, Institute of Scientific and Technological Research of Amapá (IEPA). Specimens were morphologically identified using the dichotomous keys of Consoli & Oliveira (1994) and Forattini (2002).

Sampling of immature forms of *Anopheles*. We searched for mosquito breeding sites within a 2 km radius of each sampling point (Brasil 2007). *Anopheles* larvae were collected using entomological scoops with a volume of 350 mL, an opening diameter of 11 cm, and a 1–2 m long handle. We standardized the number of samplings as nine per potential breeding site (Brasil 2007). For breeding sites with a perimeter longer than 100 m, we sampled a maximum of 20 potential breeding sites located 5 m from each other.

The immature specimens collected were placed in 15 mL Falcon tubes with water from their breeding site. Each tube was identified with the collection date and geographical information on the respective breeding site. The specimens were taken to the Laboratory of Medical Entomology (IEPA) and kept in 500 mL plastic pots with water and fish food for insect development until adulthood. After the emergence of adults, the mosquitoes underwent taxonomic identification.

All adult mosquitoes, both directly collected and those that emerged from the sampled larvae, were deposited as vouchers in the Zoological Collection of IEPA and are available for further studies.

Entomological indicators. Vector dynamics of anophelines
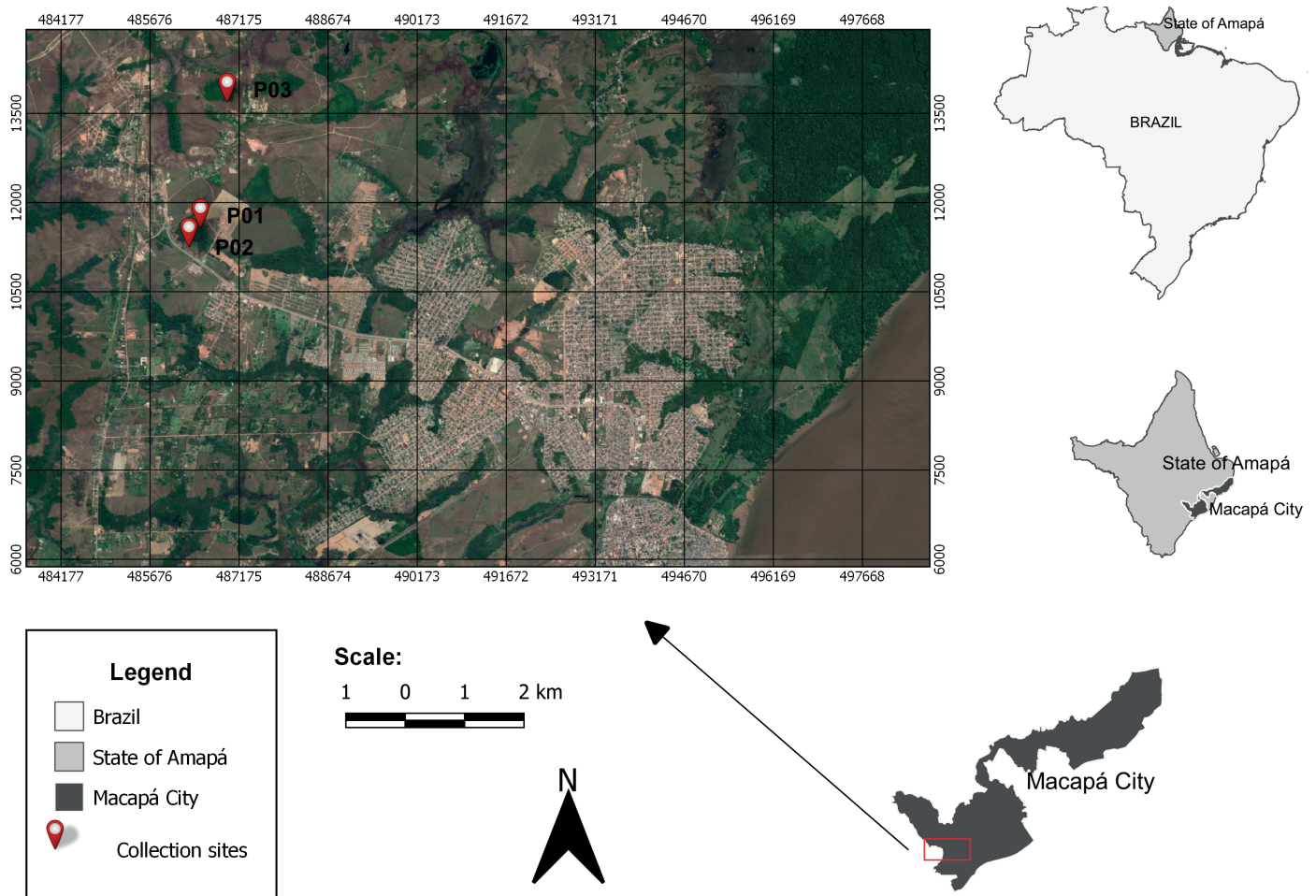

Figure 1. Location of collection points of *Anopheles* in a peri-urban area in the municipality of Macapá, Amapá, Brazil.

was assessed in the study area using two entomological indicators: parity rate and time of feeding activity. Parity or nulliparous (those that have not performed egg-laying) females can provide information about longevity, vector capacity, and reaction to the pressure from insecticides. Parity rate was determined according to Detinova (1962), extracting the ovaries of 50% of the *A.* (*N.*) *darlingi* and *A.* (*N.*) *albitarsis* collected. We studied the time of feeding activity by performing one 12-hour and two 4-hour collections, allowing us to detect the timings of highest activity of females looking for blood supply, and therefore the timings of malaria transmission.

Data Analysis. Data were analyzed in R version 3.6.0. Shapiro–Wilk and Bartlett's tests were used to verify data normality and homogeneity, respectively. Data on richness and abundance between sampled points were compared through the Kruskal–Wallis test complemented by the Nemenyi test. The Mann–Whitney U test was used to compare the means of the numbers of collected adults between the two capture methods. The significance level adopted for all the tests was 5%.

RESULTS

We recorded eight breeding sites when searching for immature forms of *Anopheles*. Sampling point P01 had only one breeding site, P02 had five, and P03 had two (Table 1). However, we collected immature *Anopheles* only in only one breeding site (CRD03) of P02 (78.6%) and one (CRD08) of P03 (21.4%), totaling 11 specimens, identified as *A.* (*N.*) *albitarsis s.l.* (35.7%) and *A.* (*N.*) *braziliensis* (64.3%).

Seven species were identified amongst the 267 collected winged specimens (Table 2). The most abundant species was *A.* (*N.*) *braziliensis* (62.9%), and the least abundant was *A.* (*N.*) *nuneztovari* (0.4%) (Table 02). We collected *Anopheles* (*Anopheles*) *mattogrossensis* Lutz & Neiva, *A.* (*N.*) *nuneztovari*, and *Anopheles peryassui* Dyar & Knab, only in only the third

sampling period, while in the first two sampling periods, we collected the species *A.* (*N.*) *albitarsis s.l.*, *A.* (*N.*) *braziliensis*, *A.* (*N.*) *darlingi*, and *A.* (*N.*) *triannulatus s.l.*

The abundance of anopheline species in the third sample (86.1%), a period corresponding to the months of highest rainfall, was higher than in the first and second samples (6%) and (7.9%), respectively, both corresponding to the months of lowest rainfall. When comparing the mean abundance between the three samplings, the Kruskal-Wallis test showed a significant difference (df=2, *p*-value=6,493e-06), indicating that seasonality contributes to the increase in anopheline abundance in the study area.

The HLC method resulted in a higher abundance of *Anopheles* for the three samplings (81.6%) than the Shannon light traps (18.4%). The Mann–Whitney test showed a significant difference between the mean abundance of individuals collected using these two methods (W=162.5, *p*-value=0.0561).

The density obtained for the 4-hour samplings of *Anopheles* showed that the highest abundance of adults occurred between 7:00 and 8:00 pm for HLC (Figure 2) and between 7:00 and 9:00 pm for the Shannon light traps (Figure 3). Regarding the density of mosquitoes measured with a sampling effort of 12 consecutive hours, we recorded the highest abundance of adults between 7:00 and 8:00 pm. However, we also found density peaks between 3:00 and 5:00 am (Figure 4).

The parity rate was 53.3% for *A.* (*N.*) *albitarsis* and 42.9% for *A.* (*N.*) *darlingi*. This result shows that parity females have already performed oviposition and blood feeding and, therefore, can transmit malaria if infected.

DISCUSSION

The increase in the number of adult individuals, observed from the second sampling campaign and accentuated in

Table 1. Absolute and relative numbers of immature *Anopheles* collected during three samplings in a peri-urban area in the municipality of Macapá, Amapá, Brazil.

Point	Breeding place	Coordinates	A. (N.) albitarsis s.l.	A. (N.) braziliensis	Total	%
P01	CRD01	S 0.106960°; W -51.105689°	0	0	0	0.0
P02	CRD02	S 0.101925°; W -51.118268°	0	0	0	0.0
	CRD03	S 0.102205°; W -51.118323°	3	8	11	78.6
	CRD04	S 0.102124°; W -51.119346°	0	0	0	0.0
	CRD05	S 0.101928°; W -51.119149°	0	0	0	0.0
	CRD06	S 0.103029°; W -51.118978°	0	0	0	0.0
P03	CRD07	S 0.129467°; W -51.113712°	0	0	0	0.0
	CRD08	S 0.131584°; W -51.113825°	2	1	3	21.4
	General Total		5	9	11	100.0

Table 2. Richness and abundance of winged anopheline species collected in three samplings carried out in a peri-urban area in the municipality of Macapá, Amapá, Brazil.

Species	Collection 01			Collection 02			Collection 03			Total	%
	P01	P02	P03	P01	P02	P03	P01	P02	P03		
A. (*N.*) *albitarsis s.l.*	4	0	0	3	1	0	4	3	3	18	6.7
A. (*N.*) *braziliensis*	7	1	0	3	0	14	14	5	124	168	62.9
A. (*N.*) *darlingi*	0	0	1	0	0	0	7	1	5	14	5.2
A. (*N.*) *mattogrossensis*	0	0	0	0	0	0	0	31	0	31	11.6
A. (*N.*) *nuneztovari*	0	0	0	0	0	0	0	1	0	1	0.4
A. peryassui	0	0	0	0	0	0	1	3	0	4	1.5
A. (*N.*) *triannulatus s.l.*	0	3	0	0	0	0	1	26	1	31	11.6
General Total	11	4	1	6	1	14	27	70	133	267	100.0

the third campaign, was probably related to the beginning of the period with highest rainfalls. The rain intensifies from March on, causing a consequent increase in the availability of breeding sites which, in turn, leads to a growth of *Anopheles* populations (Silva-Nunes 2010).
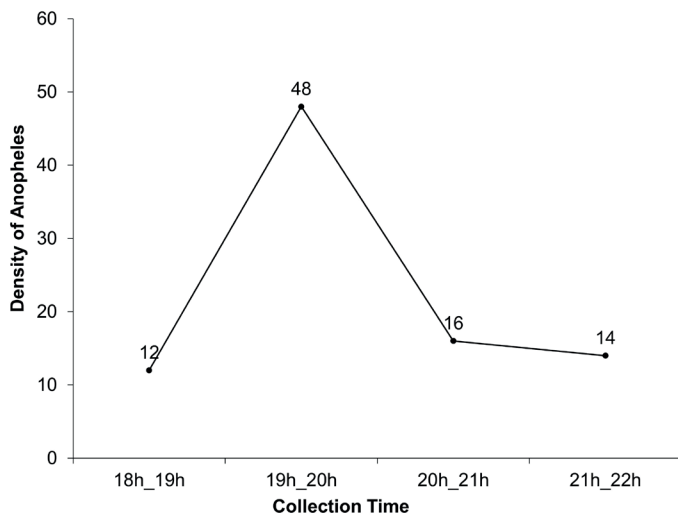

Figure 2. Density of *Anopheles* collected using the HLC method in 4-hour collections in three samplings carried out in a peri-urban area in the municipality of Macapá, Amapá, Brazil.
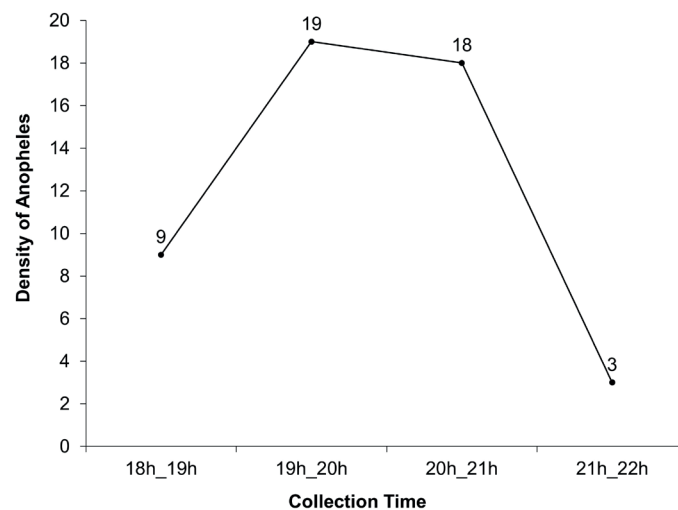

Figure 3. Density of *Anopheles* collected using Shannon light traps in 4-hour collections in three samplings carried out in a peri-urban area in the municipality of Macapá, Amapá, Brazil.
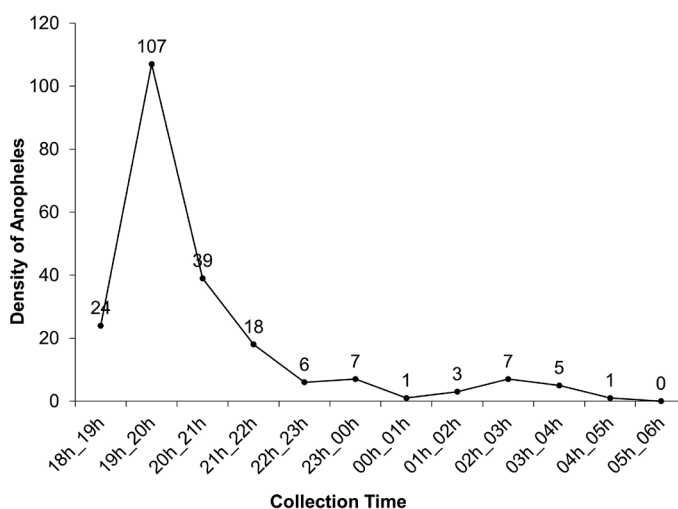

Figure 4. Density of *Anopheles* sampled with the HLC method in 12-hour collections in three samplings carried out in a peri-urban area in the municipality of Macapá, Amapá, Brazil.

The immature forms of *A.* (*N.*) *braziliensis* develop, preferably, in bodies of fresh, constantly replenished clean water, under sunny conditions, and with emerging vegetation (Consoli & Oliveira 1994). The same occurs for *A.* (*N.*) *albitarsis s.l.*, whose immature stages breed in sunny or shaded grasslands (Baia-da-Silva *et al.* 2019). All these characteristics were observed in the breeding sites of the studied area.

The low abundance of *A.* (*N.*) *darlingi*, a primary vector in malaria transmission in Brazil (Laporta *et al.* 2015), may be related to the fact that this species is considered highly anthropophilic, with a very pronounced endophilic behavior (Consoli & Oliveira 1994). Therefore, this species occurred in points where there are dwellings and transit of people, i.e., P01 and P03, unlike P02, characterized by a dense forest cover and a low flow of people.

A. (*N.*) *braziliensis* is a secondary vector in malaria transmission in the Amazon region, while *A.* (*N.*) *albitarsis s.l.* is an important vector in the state of Amapá (Carlos *et al.* 2019), both already reported as having a great abundance of *Plasmodium* (Tadei & Dutary Thatcher 2000; Povoa *et al.* 2001). However, a large number of studies point to *A.* (*N.*) *albitarsis* and *A.* (*N.*) *darlingi* as the *Anopheles* with the highest vector capacities in the Amazon, when in high densities. In addition, *A.* (*N.*) *nuneztovari* is also considered an important vector of *P. vivax* and *P. malariae* in many Amazonian states (Galardo *et al.* 2007).

A. (*N.*) *triannulatus s.l.* was abundantly collected only at point P02. This species, considered a secondary vector in malaria transmission in the Amazon, is found preferably in forest areas (Consoli & Oliveira 1994), as in the present study.

Anophelines usually feed at night and twilight, although this timing of high blood-feeding activity (activity peak) can vary between populations. Therefore, determining the vectors' peak activity has great epidemiological importance since the transmission of the etiological agents of malaria occurs during blood-feeding (Forattini 2002). When vectors' peak activity occurs at twilight, as in the present study, the highest risk of transmission is for people who are awake and those moving mainly in areas outside dwellings. In contrast, when vectors' peak activity occurs at dawn, the highest risk is associated with people who are sleeping. Therefore, knowing the time of vector activity enables the planning of strategies to mitigate the risks of malaria transmission aligned with the behavior of vectors and hosts (humans) and thus allows executing effective actions (Forattini 2002).

Our survey of immature *Anopheles* by larval sampling indicated that *A.* (*N.*) *albitarsis s.l.* and *A.* (*N.*) *braziliensis*, two potential vectors of malaria-causing plasmodia in the Amazon region, successfully colonized breeding sites in the peri-urban area of Macapá. We observed the occurrence of winged specimens of *Anopheles* throughout the year, with a higher incidence in periods of highest rainfall. Considering the occurrence of autochthonous malaria cases in Macapá, our data can contribute to developing public health measures directed to control malaria to protect the human population of this municipality.

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