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Research

Behavior, Chemical Ecology

Monthly abundance and diversity of mosquitoes (Diptera: Culicidae) in an Atlantic Forest area of Rio de Janeiro, Brazil

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Several mosquito species in the Atlantic Forest are yellow fever vectors; therefore, this biome can represent a potential risk to the human population. Studies on mosquitoes from predominantly sylvatic areas produce valuable data for understanding the emergence of new epidemics. In addition, they can elucidate environmental components favoring or hindering biodiversity and species distribution. Our study aimed to evaluate the monthly distribution, composition, diversity, and influence of seasonal periods (dry and rainy) on the mosquito fauna. We used CDC light traps at different levels in a forest area bordering a Conservation Unit of Nova Iguaçu in the state of Rio de Janeiro, Brazil. Specimens were collected from August 2018 to July 2019 by installing traps in sampling sites under different vegetation covers. We detected some species of epidemiological importance in terms of arbovirus transmission. A total of 4,048 specimens representing 20 different species were collected. Among them, *Aedes (Stg.) albopictus Skuse*, 1894 showed recurrent association with the closest level to human residences and *Haemagogus (Con.) leucocelaenus* Dyar and Shannon, 1924 with the most distant levels. Since these mosquitoes are possible vectors of yellow fever, monitoring the area is extremely important. Under the studied conditions, the mosquito populations were directly influenced by dry and rainy periods, posing a risk to the nearby resident population.

Key words: mosquito, vector, diversity

Introduction

Commonly named mosquitoes, several culicids are cosmopolitan nematocerous dipterans that have been related to the transmission of etiological agents of human and other animal diseases (World Health Organization 2014, Bartlow et al. 2019). About 3,619 mosquito species are known, distributed in more than 100 genera (Harbach 2022), or 42 genera, according to the more traditional classification by Wilkerson et al. (Wilkerson et al. 2015).

Studies on the ecological aspects of mosquito fauna have been conducted in Rio de Janeiro; these studies have been mainly motivated by vectors detected in urban environments (Alencar et al. 2011, 2015, 2020; Silva et al. 2017).

Endemic to African and American tropical forests, yellow fever is a mosquito-borne acute infectious disease caused by the yellow fever virus (YFV) from the genus *Flavivirus*, family Flaviviridae. Yellow fever sporadic outbreaks affect humans and non-human primates (NHP). These outbreaks can originate epidemics that drastically affect society, as in some countries in sub-Saharan Africa and Latin America (Mascheretti et al. 2013, Abreu et al. 2019).

Yellow fever is currently transmitted only in Brazilian sylvatic areas, with the last records of urban transmissions reported in the 1940s at Sena Madureira, state of Acre (Vasconcelos 2002). However, the risk of yellow fever re-urbanization must be considered; in 2016/2017, one of the largest outbreaks of wild yellow fever reached states considered non-endemic, including Espírito Santo and Rio de Janeiro, causing deaths in NHP and humans (Cavalcante and Tauil 2017, Silva et al. 2018). The need to monitor re-urbanization risk is evident if competent vector species cohabiting in the same environments are detected. For instance, *Aedes (Stg.) albopictus* Skuse 1894 frequently occurs in peri-urban neighborhoods and/or rural areas, and many studies report it as a possible linking vector between wild and peri-urban/urban environments (Vasconcelos 2002, Alencar et al. 2016, Possas et al. 2018).

Several mosquito species in the Atlantic Forest biome are considered yellow fever vectors, mainly those belonging to the genus *Haemagogus* (primary vectors) and *Sabethes* (secondary vectors) (Couto-Lima et al. 2020). Furthermore, the area where vector species occur represents a risk to the surrounding population and those individuals who enter it without proper care and immunization (Mascheretti et al. 2013).

Studies on mosquitoes from predominantly wild areas are valuable to understand the origin, development, and risk of new epidemics and elucidate environmental factors favoring or harming biodiversity and distribution of culicid species (Guimarães et al. 2000, Alencar et al. 2016). Environmental changes forced by human activities can disrupt mosquito populations by removing controlling conditions of selective pressures (Lyimo and Ferguson 2009).

We aimed to evaluate monthly distribution, composition, diversity, and the influence of seasonal periods (dry and rainy) on the mosquito fauna attracted by CDC light traps at different levels in a forest area bordering a Conservation Unit from Nova Iguaçu in the state of Rio de Janeiro, Brazil.

Methods

Mosquito specimens were collected monthly for one year, from August 2018 to July 2019, in a forest area bordering the Tinguá Biological Reserve (REBIO) of Nova Iguaçu, located 63 km northwest of the city of Rio de Janeiro (22°39′00.9″S/43°31′19.4″W). The study area presents vegetation cover typical of montane and submontane ombrophilous dense forests (Garai and Rizzini 2003), with a humid tropical climate and no defined dry season. The average monthly temperature ranges between 17.3°C and 25.8°C, and relative humidity is between 83% and 85%.

We selected eight sampling sites at an approximate distance of 600 m, paired on four levels, used to guide the installation of traps starting in the area of the residences until the place of most preserved forest, showing differences in vegetation cover, as follows: site A, sloped with several trees and shrubs close to a site with constant human presence (22°39′01″S/43°31′54″W); site B, ombrophilous dense forest fragment between two access roads, close to residences (22°39′01″S/43°31′57″W); site C, area with shrubs and no large trees, approximately 60 m to the right of a main road (22°38′39″S/43°31′31″W); site D, small bamboo grove on the banks of a small creek (reported as frequently used for bird hunting), 50 m to the left of the main road (22°38′36″S/43°31′42″W); site E, alongside an old deactivated road surrounded by new secondary forest with the noticeable presence of several bromeliads on the branches of the bigger trees (22°38′16″S/43°31′28″W); site F, dense forest with a dry creek bed, close to an access road (22°38′19″S/43°31′25″W); site G, area with the densest forest and probably greatest density of native species of all sites (22°38′10″S/43°31′18″W); site H, the most distant location from areas with houses and consequently the most inserted in the dense ombrophilous forest, with a steep slope, close to a large bamboo grove (22°38′03″S/43°31′17″W) (Fig. 1).

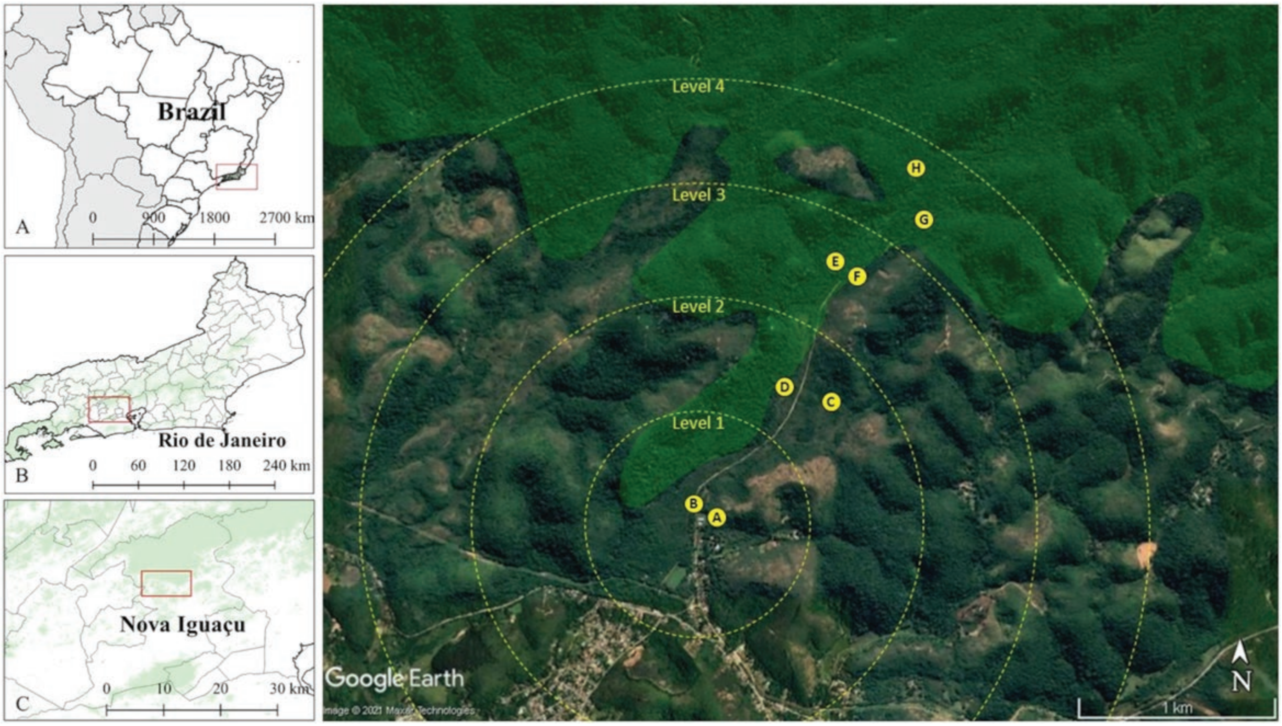

Fig. 1. Study area, (**A**) Brazil; (**B**) state of Rio de Janeiro; (**C**) municipality of Nova Iguaçu; sampling sites (A–H) divided into levels (1–4), with the area of continuous Atlantic Forest highlighted in green.

Table 1. Number of specimens per sampling site (A–H) and level of each species detected and their respective

Species	Sampling sites (A–H)								Total	%
	Level 1		Level 2		Level 3		Level 4			
	A	B	C	D	E	F	G	H		
Aedes (Grg.) fluviatilis (Lutz, 1904)	8	0	5	1	0	0	0	0	14	0.35
Aedes (How.) fulvithorax (Lutz, 1904)	1	2	0	0	1	2	0	0	6	0.15
Aedes (Och.) scapularis (Rondani, 1848)	8	8	1	0	3	0	4	0	24	0.59
Aedes (Och.) serratus (Theobald, 1901)	0	2	2	0	0	0	0	0	4	0.10
Aedes (Pro.) terrens (Walker, 1856)	0	0	7	0	0	0	0	0	7	0.17
Aedes (Stg.) albopictus (Skuse, 1894)	14	45	2	2	1	0	4	0	68	1.68
Aedes sp.	2	0	3	0	3	0	0	0	8	0.20
Culex (Aedinus) sp.	10	7	134	12	0	2	1	0	166	4.10
Culex (Anoedioporpa) sp.	3	29	1	5	105	0	15	1	159	3.93
Culex (Carrollia) sp.	4	0	0	3	0	0	0	1	8	0.20
Culex (Culex) sp.	0	0	82	37	54	0	0	0	173	4.27
Culex (Melanoconion) sp.	3	1	0	3	170	0	0	15	192	4.74
Culex (Microculex) sp.	298	229	728	167	572	89	286	364	2733	67.51
Culex sp.	38	25	19	9	27	15	19	57	209	5.16
Haemagogus (Con.) leucocelaenus (Dyar and Shannon, 1924)	0	5	2	3	3	6	3	4	26	0.64
Haemagogus (Hag.) janthinomys (Dyar, 1921)	0	2	0	0	3	0	0	2	7	0.17
Haemagogus sp.	0	0	0	1	0	0	0	0	1	0.02
Limatus durhamii (Theobald,1901)	12	23	3	9	3	29	12	6	97	2.40
Mansonia sp.	27	3	2	0	0	0	0	1	33	0.82
Psorophora (Gra.) dimidiata (Cerqueira, 1943)	0	14	1	0	0	0	0	0	15	0.37
Psorophora (Gra.) varinervis (Edwards, 1922)	0	2	0	0	0	0	0	0	2	0.05
Psorophora (Jan.) lanei (Shannon and Cerqueira, 1943)	0	0	2	0	0	0	0	0	2	0.05
Psorophora (Pso.) ciliata (Fabricius, 1794)	0	4	0	0	0	0	0	0	4	0.10
Psorophora (Pso.) saeva (Dyar and Knab, 1906)	0	1	2	1	0	0	0	0	4	0.10
Psorophora sp.	0	1	1	0	0	0	0	0	2	0.05
Sabethes (Pey.) undosus (Coquillett, 1906)	0	0	0	0	0	0	2	0	2	0.05
Sabethes (Sab.) albiprivus (Theobald, 1903)	0	0	0	0	0	0	1	1	2	0.05
Sabethes (Sab.) purpureus (Theobald, 1907)	0	0	0	3	0	0	0	4	7	0.17
Sabethes (Sbn.) intermedius (Lutz, 1904)	0	1	0	0	0	1	0	1	3	0.07
Sabethes sp.	1	2	0	1	3	1	0	0	8	0.20
Wyeomyia (Pho.) sp.	1	0	3	0	7	0	0	0	11	0.27
Wyeomyia (Pho.) fuscipes (Edwards, 1922)	3	8	6	1	13	0	2	5	38	0.94
Wyeomyia (Who.) arthrostigma (Lutz, 1905)	0	0	0	0	0	0	0	3	3	0.07
Wyeomyia sp.	1	1	4	1	0	1	1	1	10	0.25
Total per sampling site	434	415	1,010	259	968	146	350	466	4,048	100
Percentage per sampling site (%)	10.72	10.25	24.95	6.40	23.91	3.61	8.65	11.51	100	
Percentage by level (%)	20.97		31.35		27.52		20.16		100	

Table 2. Number of species (S), heterogeneity (H′) and evenness (J′) concerning sampling sites (A–H) and levels (1–4)

Index	Sampling sites (A–H)							
	A	B	C	D	E	F	G	H
Number of species (S):	17	22	21	17	15	9	12	15
Shannon-Weaver (H'):	1.315	1.755	1.049	1.386	1.333	1.208	0.826	0.882
Pielou evenness (J'):	0.464	0.568	0.345	0.489	0.492	0.550	0.332	0.326
	Level 1		Level 2		Level 3		Level 4	
Number of species (S):	26		26		18		19	
Shannon-Weaver (H'):	1.617		1.159		1.428		0.909	
Pielou evenness (J'):	0.496		0.356		0.494		0.309	

The CDC light traps baited with dry ice were used to release CO_2 as an attractant. These traps were installed at a height of 1.80 m (approximate height of a person), where they remained for 24 h at each sampling site (A–H). Other types of traps, such as ovitraps, were not used due to the required operating time and the risk of becoming larval habitats. Reference trees guided the installation of traps in each sampling site, avoiding changes in collection location, totaling eight traps per month. Real-time measurements of temperature and relative humidity were made during the collection using a 'Model RHT 10 – Manufacturer: EXTECH, USA' sensor to obtain data about the microclimate and the temperature and humidity variation of the sites. Regional precipitation measures used in this study are from the National Institute of Meteorology (INMET) database.

The definition of dry (May to October) and rainy (November to April) periods followed Brito et al. (2016) on spatial and temporal patterns of precipitation for the state of Rio de Janeiro.

The specimens of collected mosquitoes were killed by exposure to chloroform and placed in polyethylene flasks standardized according to each sample's characteristics. They were then taken to the laboratory for sorting and identification. Species were identified using dichotomous keys elaborated by Lane (1953). Specific descriptions and diagnoses were also consulted. After species identification, all specimens were deposited in the Entomological Collection of the Oswaldo Cruz Institute, Fiocruz. Abbreviations of genera and subgenera names followed Reinert (2009).

Data Analysis

Shannon-Weaver and Pielou indices were used to analyze heterogeneity and evenness, respectively, in Past v4.12. Principal Component Analysis (PCA) ($\alpha = 0.05$) was conducted in Analyse-it v5.80.2 to simplify the analysis of the data between the sampled species incriminated as vectors of yellow fever with factors like the climatic variables of monthly temperature and precipitation, the transects (levels 1–4), and the seasonal variation of dry and rainy periods.

Results

During the 12 sampling months, we collected 4,048 specimens representing 20 identified species and six genera. Among these samples, we found species of great epidemiological importance in terms of arbovirus transmission. These include *Ae. albopictus*; *Aedes (Grg.) fluviatilis* Lutz, 1904; *Aedes (How.) fulvithorax* Lutz, 1904; *Aedes (Och.) scapularis* Rondani, 1848; *Aedes (Och.) serratus* Theobald, 1901; *Aedes (Pro.) terrens* Walker, 1856; *Haemagogus (Hag.) janthinomys* Dyar, 1921; *Haemagogus (Con.) leucocelaenus* Dyar and Shannon, 1924; *Sabethes (Sab.) albiprivus* Theobald, 1903; *Sabethes (Sbn.) intermedius* Lutz, 1904; *Sabethes (Sab.) purpureus* Theobald, 1907, and *Sabethes (Pey.) undosus* Coquillett, 1906. Among the most abundant taxa is *Culex* spp., with specimens of the subgenus *Microculex* accounting for 67.51% of the total collected. Sampling sites C and E showed the highest abundance of mosquitoes, with 1,010 (24.29%) and 968 (23.91%) specimens, respectively (Table 1).

Despite the high abundance of specimens in sampling sites C and E, the highest diversity was found in sites B (*H*'=1.755) and D (*H*'=1.386). Regarding the transects, sampling sites A and B together corresponded to the highest values of species abundance (*H*'=1.617) among all comparisons. The sampling sites with less homogeneity or evenness indices, i.e., sites G and H, are more inserted in the forest and, consequently, at a greater distance from human residences (Table 2).

Comparative analysis of the number of mosquitoes collected per month detected an increasing trend in the rainy months. However, an evident abnormality occurred in December (10), January (1), and February (67), when the values obtained were far lower than those recorded in March (943) and April (797), which are the other months included in the rainy season (November to April) (Fig. 2).

The region of the study area had unusually dry months in the rainy season during the studied period, as occurred in December (114.4 mm) and January (109.1 mm), contrasting with February (271.7 mm) and March (239.9 mm). In addition, during our study, December displayed an unusual thermal amplitude (±24.8°C), with maximum and minimum averages of 38.8°C and 14°C, respectively (Fig. 3).

When comparing the number of specimens collected monthly with precipitation and temperatures during the sampling period by using a biplot correlation analysis that explains 81.8% of the principal component, we observed the discrepancy related to the unusually dry months of December and January. However, the PCA biplot detected a separate (intermediate) set more associated with temperature than precipitation for other records of rainy months (blue dots) that are distinguishable from the low precipitation and temperature months of the dry period (red squares) (Fig. 4).

Comparative analysis of the species found at the sampling sites throughout the sampling period explained 70.6% of the PCA. Some species showed an expressive correlation, such as *Hg. leucocelaenus*, which was mainly associated with levels 3 and 4, and *Ae. albopictus*, which was associated with levels 1 and 2 (Fig. 5).

In the analysis, the levels appeared in distinct positions in each collection period; these positions can be better understood in Table 3 (PCA loadings).

When conducting a separate examination of the bioplot analysis for periods that differed in precipitation, we found that levels 3 and 4 have similar characteristics and were therefore statistically similar, making it impossible to differentiate the species associated with

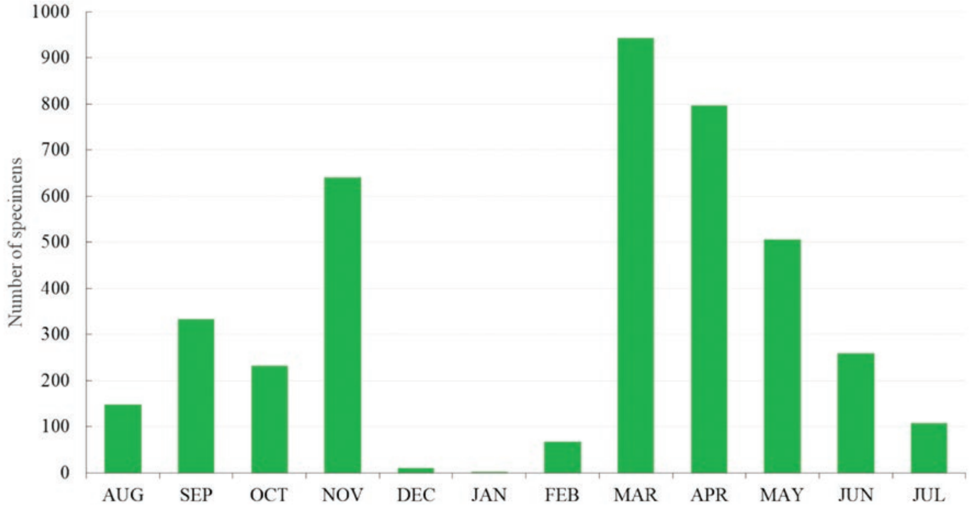

Fig. 2. Number of specimens found per month at all sampling sites in a forest area bordering the Tinguá Biological Reserve (REBIO) of Nova Iguaçu, state of Rio de Janeiro, Brazil.

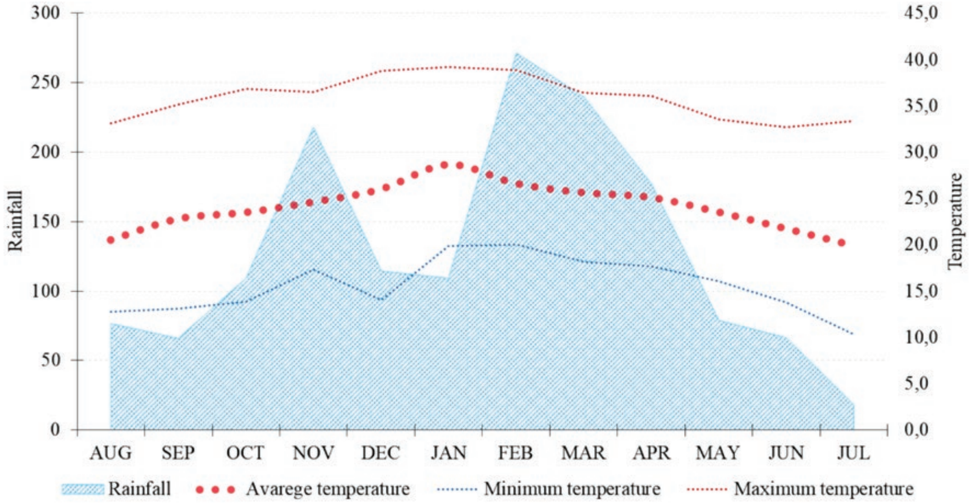

Fig. 3. Average, maximum, and minimum values of precipitation and temperature per month throughout the sampling period.

these two sampling points (3 and 4), as occurred with levels 1 and 2 in this same period. The species repeatedly associated with these two sampling sites was *Hg. leucocelaenus*. In contrast, level 1 differed from the other levels, and again *Ae. albopictus* was more associated with this level (Fig. 6).

Each level stands out during the rainy season, with no species association in the previous analyses. For instance, *Ae. scapularis* is associated with level 4 and *Ae. fluviatilis* with level 2. In addition, *Ae. albopictus* is associated with the dry period of level 1 (Fig. 7).

Some of the species found were not specifically associated with any level for either separate analyses in dry and rainy periods or the entire sampling period. These include *Ae. serratus*, *Sa. albiprivus*, *Sa. intermedius*, and *Sa. undossus*.

Discussion

Gaps in understanding wild arbovirus vectors are evident. Any study in light of entomological indicators must consider the taxonomic status and characteristic habits of vector competence. This assumption follows the logic of no dissociation of entomological research from the evaluation of the vector's adaptive process to modified environments. Hence, we must consider that the human presence, with its essential artifacts, offers favorable conditions to certain mosquito species. Shelter, constant blood-feeding, moderate and continuous climatic factors, and, in some cases, the larval habitat are some of these attractive circumstances. Some *Culex* species have a well-developed ability to adapt to anthropogenically altered environments. In these areas still surrounded by native forests (Forattini et al. 1995, Alencar et al. 2020), *Culex* spp. tend to be easily collected in light traps baited with CO_2 (Carvalho et al. 2017). Although some *Culex* spp. are important vectors of etiological agents causing diseases in humans and/or animals — especially arboviruses in several countries (Dehghan et al. 2016), such as West Nile virus (WNV) in North America and Europe (Nasci et al. 2001, Brugman et al. 2018) — so far there are no reports of wild transmission of the YFV by *Culex* spp. (Evangelista et al. 2013). The massive presence of *Microculex* spp. in expressive numbers on several levels seems to indicate an adaptation of the subgenus, typical of wild habitats.

A survey conducted between September 1994 and April 1995 in the same region using Shannon traps found a variation in

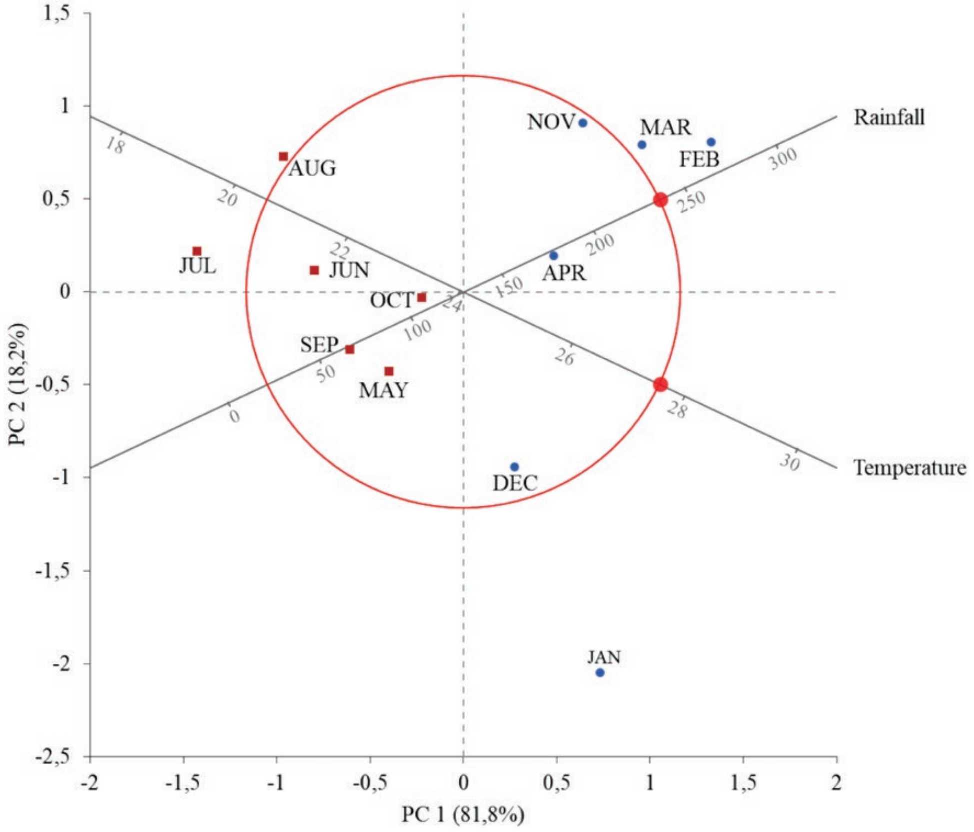

Fig. 4. Biplot graph for the distribution of the number of specimens found per month for precipitation and temperature; red squares represent dry months, and blue dots the rainy months.

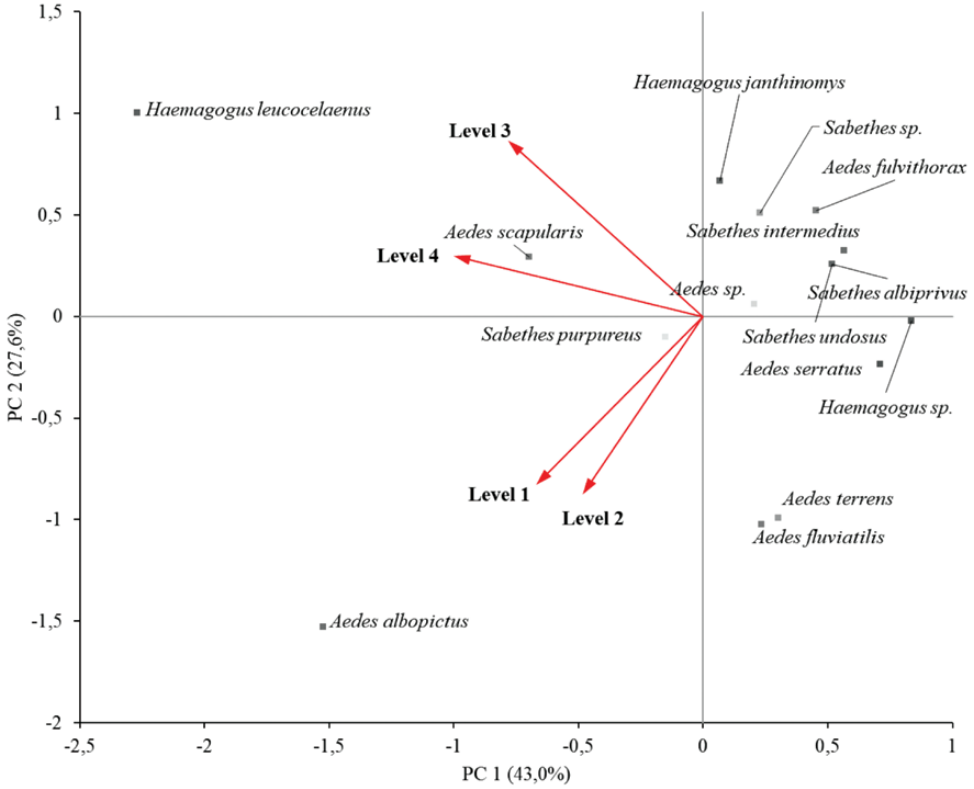

Fig. 5. PCA biplot (70.6%) of the total collection period, correlating the medically important species found with the levels studied.

Table 3. Principal Component (PC) loading variables, per level and collection period.

	PC loadings			
Level	PC 1	PC 2	PC 3	PC 4
Total period				
1	−0.580	−0.575	−0.519	−0.251
2	−0.417	−0.608	0.674	0.038
3	−0.677	0.601	0.243	−0.349
4	−0.866	0.209	−0.167	0.423
Dry period				
1	−0.079	−0.978	0.193	0.008
2	0.561	−0.219	−0.798	−0.038
3	0.873	0.026	0.225	0.431
4	0.855	0.027	0.311	−0.415
Rainfall period				
1	0.752	−0.518	0.282	−0.296
2	0.792	0.433	0.315	0.293
3	−0.540	−0.391	0.736	0.111
4	0.188	−0.883	−0.338	0.268

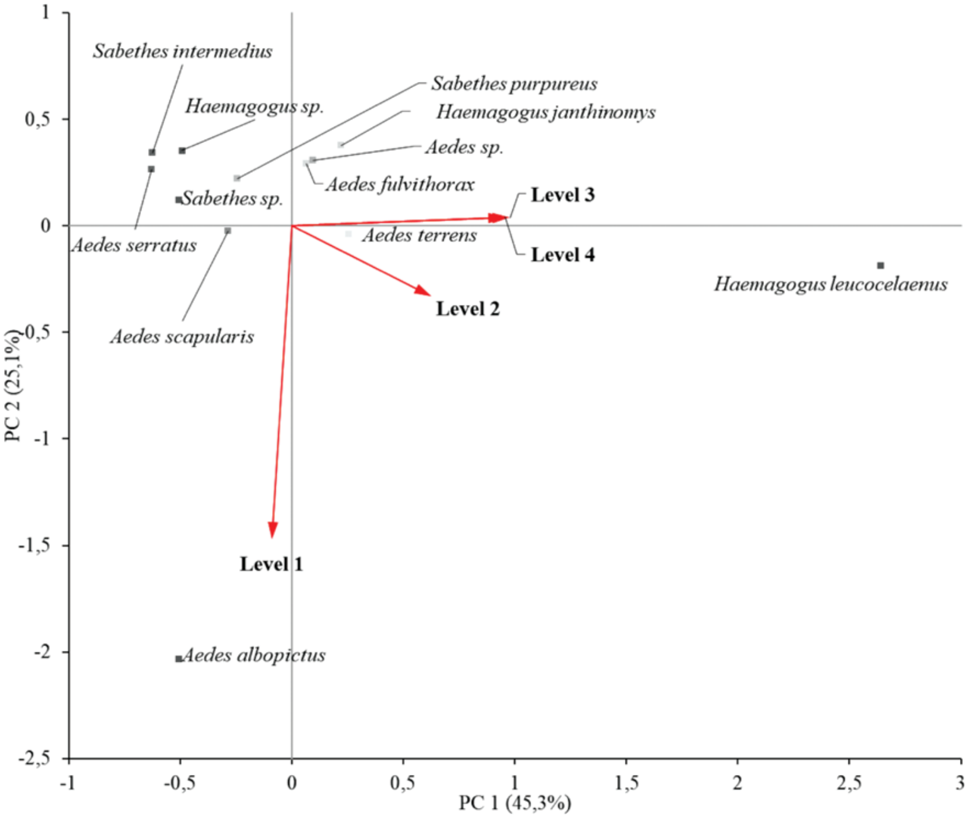

Fig. 6. PCA biplot (70.5%) of the dry collection period (May to October), correlating the medically important species found with the levels studied.

heterogeneity (*H*'= 1.437–2.161) and richness (*J*'= 0.580–0.808) per point (Alencar et al. 2011). In August 2018, a study was conducted at a nearby site, using ovitraps and bamboo traps at different levels, reaching values of heterogeneity of *H*'= 1.251–2.067 and richness of *J*'= 0.503–0.862 (Araujo-Oliveira et al. 2021).

In both studies mentioned above, the diversity was higher than reported in the present study; however, the use of CDC light traps compared to that of other methods can be conflicting, as observed by Alencar et al. (2005), who reported a higher diversity collected using Shannon traps and Hutchings et al. (2018), who found opposite results using CDC light traps. Species commonly considered YFV vectors were found in the present study; they may pose a direct risk to local and neighboring households, as previously observed (Vasconcelos 2002, Abreu et al. 2019). This is mainly the case of *Ae. albopictus* and *Hg. leucocelaenus*, which were the most abundant among these vector species.

We detected *Ae. albopictus* near residences associated with all studied scenarios of dry, rainy, and total sampling periods. Although

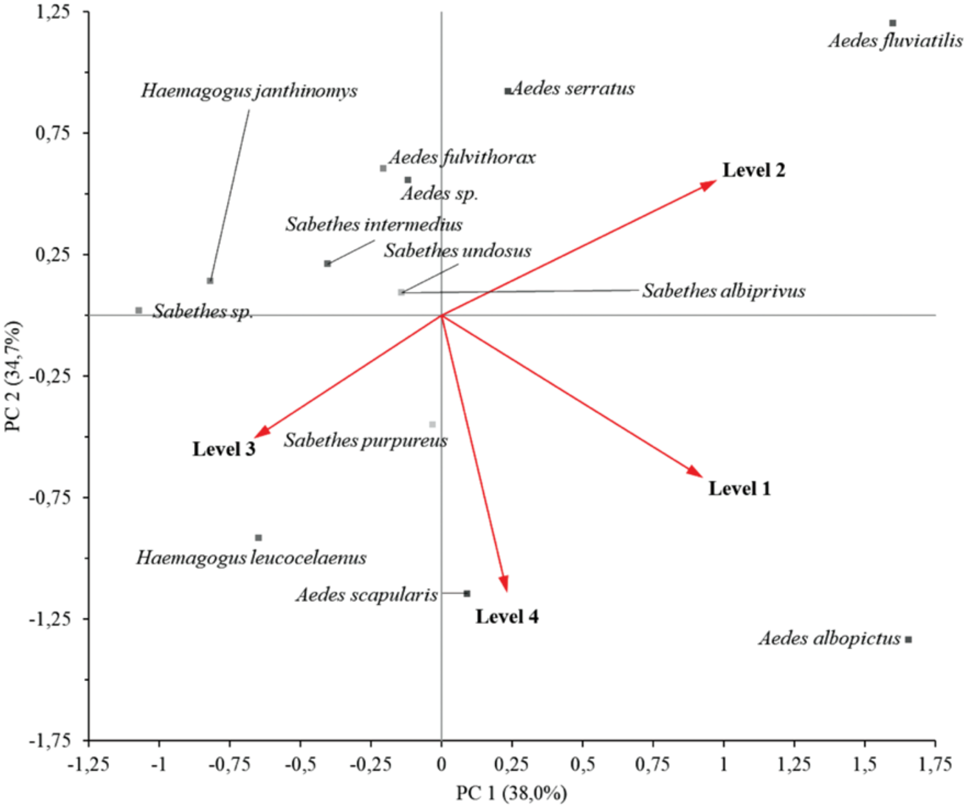

Fig. 7. PCA biplot (72.7%) of the rainy collection period (November to April), correlating the medically important species found with the levels studied.

consistently reported near residences in several studies (Santos 2003, Gomes et al. 2008), the presence of *Hg. leucocelaenus* at site B (level 1) may be an extra factor in the risk of YFV infection of *Ae. albopictus*. Santos (2003) and Gomes et al. (2008) reported the wide dissemination of *Ae. albopictus* throughout Brazilian municipalities, which reinforces the risk of re-urbanization of yellow fever from this vector (Cavalcante and Tauil 2017). *Haemagogus leucocelaenus* has high competence for natural YFV infection and transmission (Abreu et al. 2019, Couto-Lima et al. 2020). Although found at all levels in this study, it was the species most associated with the most distant levels from residences in the whole scenario throughout the sampling period and, therefore, to the forest with less human interference. During the yellow fever outbreak between 2016 and 2019, the months with the highest records of confirmed and suspected cases occurred during the rainy season, with emphasis on the presence of the virus in NHP in Nova Iguaçu (Giovanetti et al. 2020).

We detected *Hg. leucocelaenus* near level 3 during the rainy season. However, since no difference was observed between levels 3 and 4 during the dry period, it is impossible to state whether there was any species displacement during the analyzed period. The high positive correction in these two levels (3 and 4), in contrast to that presented in the rainy period, suggests species tend to migrate to dense forest sites in dry periods and consequently further away from level 1. Although, in the present study, the opposite movement was not observed when analyzing only the rainy period, which may have been influenced by the lack of rainfall in December and January of the collection period. We also observed a more significant association of *Ae. scapularis* at level 4 in the rainy season only. Although Silva and Menezes (1996) define this species as well adapted to anthropogenic environments, we found that *Ae. scapularis* was more related to more preserved forest areas. During the rainy season, there was also an association of *Ae. fluviatilis* with level 2, an area without native or even secondary forest coverage. Caragata et al. (2017) and Cândido et al. (2019) indicated that this species occurs in peri-urban and urban areas with a high risk of transmitting diseases like dengue (Multini et al. 2016, Silva et al. 2017).

Aedes fulvithorax and *Ae. serratus*, potential vectors of the YFV (Ortega-Morales et al. 2019), as well as *Hg. janthinomys* (Abreu et al. 2019), *Sa. albiprivus* (Goenaga et al. 2012), *Sa. intermedius*, and *Sa. undosus* (Cano et al. 2021) did not show a statistically relevant association with the levels at which they were collected, either because they had a small sample size or were equally found at the closest and the most distant levels. However, the lack of association of these species with a specific level suggests that they have a great capacity to disperse in environments and requires in-depth studies.

Conclusions

Under the studied conditions, the mosquito populations were directly influenced by the conditions during the dry and rainy periods; thus, changes may occur at their activity site. It is worth mentioning that a greater diversity of Culicidae at levels farthest from the human residences, consequently inside the more preserved forest, was expected; however, we observed the opposite. Since the mosquito species found in these collections are possible vectors of yellow fever, there is an urgent need to monitor the area. In addition to nearby housing, people's access to leisure and hunting activities is a risk factor for the establishment of the disease in the region. Therefore, more in-depth studies focusing on the ecological interaction between the culicid fauna and vegetation cover are needed in the future to find relationships among the changes that favored the mosquito diversity we found.

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Author Contributions

AAO, WAM, and MTS participated in mosquito collection, data generation, and analysis. AAO and JA also drafted the manuscript. AAO, WAM, and MTS performed mosquito collections and morphological identification. JA and JRSM helped draft the paper by critically reading the original manuscript, were the principal investigators, and participated in its design and coordination. All authors read and approved the final manuscript.

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