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Short Communication

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Diversity of Mosquitoes (Diptera: Culicidae) in An Atlantic Forest Urban Park, Salvador, Brazil

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Abstract

We identified mosquito species (Diptera: Culicidae) in an Atlantic Forest fragment located in a large urban park in Salvador, Brazil, one year after a citywide epizootic of yellow fever virus (YFV). Between May 2 and August 2, 2018, adult mosquitoes were collected using the human attraction method, followed by trapping with hand-nets, and CO_2-baited light traps placed at ground level and in the canopy. We collected a total of 11,914 mosquitoes, which belonged to three tribes, five genera, and at least seven species. The most abundant taxa captured by CO_2-baited light traps were *Culex quinquefasciatus* (Say, Diptera: Culicidae) *Limatus* spp. (Diptera: Culicidae), and *Wyeomyia* spp. (Diptera: Culicidae), while by human attraction, *Cx. quinquefasciatus*, *Wyeomyia* spp., and *Aedes albopictus* (Skuse, Diptera: Culicidae) were captured most often. The diversity of mosquitoes by species was greater in the park area with restinga vegetation compared to the area with dense rainforest. Although vectors commonly associated with sylvatic YFV transmission were not captured, we collected several species capable of transmission of other arboviruses. Given the high likelihood of encounters between mosquitoes and human visitors in environments, such as the one studied, periodic entomological surveys to determine the risk of arbovirus transmission in these settings are warranted.

Key words: arbovirus, mosquito, entomological survey, Brazil

In Brazil, dengue (DENV), Zika (ZIKV), and chikungunya (CHIKV) viruses have co-circulated since 2015 (Carvalho et al. 2019). In addition, between late 2016 and mid-2018, Brazil faced the worst human outbreaks and epizootics of sylvatic yellow fever virus (YFV) since the 1940's (Possas et al. 2018). In Salvador, the largest northeastern city of Brazil, an epizootic of YFV affecting 205 non-human primates (NHPs) of the genus *Callithrix* was reported in 2017 (Paploski et al. 2017). Because the city had been affected by simultaneous epidemics of DENV, CHIKV, and ZIKV (Cardoso et al. 2015, Ribeiro et al. 2018, Silva et al. 2019), there were concerns about the potential for establishment of an urban human-mosquito cycle of YFV transmission.

Urban areas adjacent to forests, or containing forest remnants, are considered an ideal setting for resurgence of urban YFV transmission (Possas et al. 2018). However, knowledge about mosquito diversity in forested areas located close to (or within) urban residential areas in Brazil is scarce. The recent outbreaks and epizootics of YFV in Brazil, and the detection of the virus transmission among NHPs living in large urban centers such as Salvador, reinforced the need for inventories of mosquito species in urban forest fragments.

In this study, we performed a comprehensive entomological survey of the Joventino Silva City Park, an urban park that preserves remnants of the Atlantic Forest, and is located near the site where NHPs were found dead during the YFV epizootic in Salvador (Paploski et al. 2017). We aimed to document the mosquito fauna and to investigate the role of the different ecosystems within this forest remnant in harboring mosquitoes of public health importance.

Materials and Methods

Study Area

We performed this survey in Joventino Silva City Park because YFV-infected NHPs were detected in its vicinity in 2017, it receives ~2000 visitors per day, and it borders a densely populated area (Fig. 1). The entire park comprises 72 hectares separated into two forest fragments by walking paths and recreational areas. Its vegetation is characterized by two distinct vegetation types: restinga, a set of plant species associated with quaternary coastal sandy deposits and coastal rocky environments, which may present as herbaceous, sub-shrubby, shrub, and arboreal species; and a dense ombrophylous forest (rainforest), an area with large and medium-sized trees, vines, and epiphytes, present on the Brazilian coast and on the Amazon region (Fig. 1; Carvalho et al. 2010, Campanili and Schäffer 2010). The Park also contains a temporary pond, with higher water level during the rainy season, that can serve as a larval habitat for some mosquito species when the water level reduces during the dry season.

Mosquito Collection and Identification

Mosquito specimens were collected weekly at multiple locations in the park for 10 weeks, between May 2 and August 2, 2018 (the rainy season in Salvador). During this time, the cumulative precipitation was 259.6 mm (INEMET 2018) and the average temperature and humidity were 24.5°C and 76%, respectively. During each capture day, three different locations (~100 meters apart) were sampled, totaling 30 collection points. The captures were performed using human attraction followed by landing catches and Centers for Disease Control (CDC) light traps.

The human landing catch method used to sample diurnal mosquitoes was performed by three researchers at each collection point for one hour between the hours of 09:00 and 16:00. Sampling consisted of using net sweeps to capture adult specimens that landed on the researcher's bodies. The team was vaccinated against YFV and all collectors wore long clothing to avoid inadvertent mosquito bites during the collection process. The Gonçalo Moniz Institute Ethics Committee approved this study (CAAE: 38942620.5.0000.0040).

We also installed two CDC traps at each collection point, one at ground level (60 cm high) and another at the canopy level (~10 m high). The traps were run from 16:00 to 09:00 and were situated approximately 50 m from locations where visitors congregate. To increase attraction of mosquitoes each trap was baited with 250 mg of dry ice as a CO_2 source.

The captured mosquitoes were placed in tubes and transported on dry ice to the entomology facility, at the Oswaldo Cruz

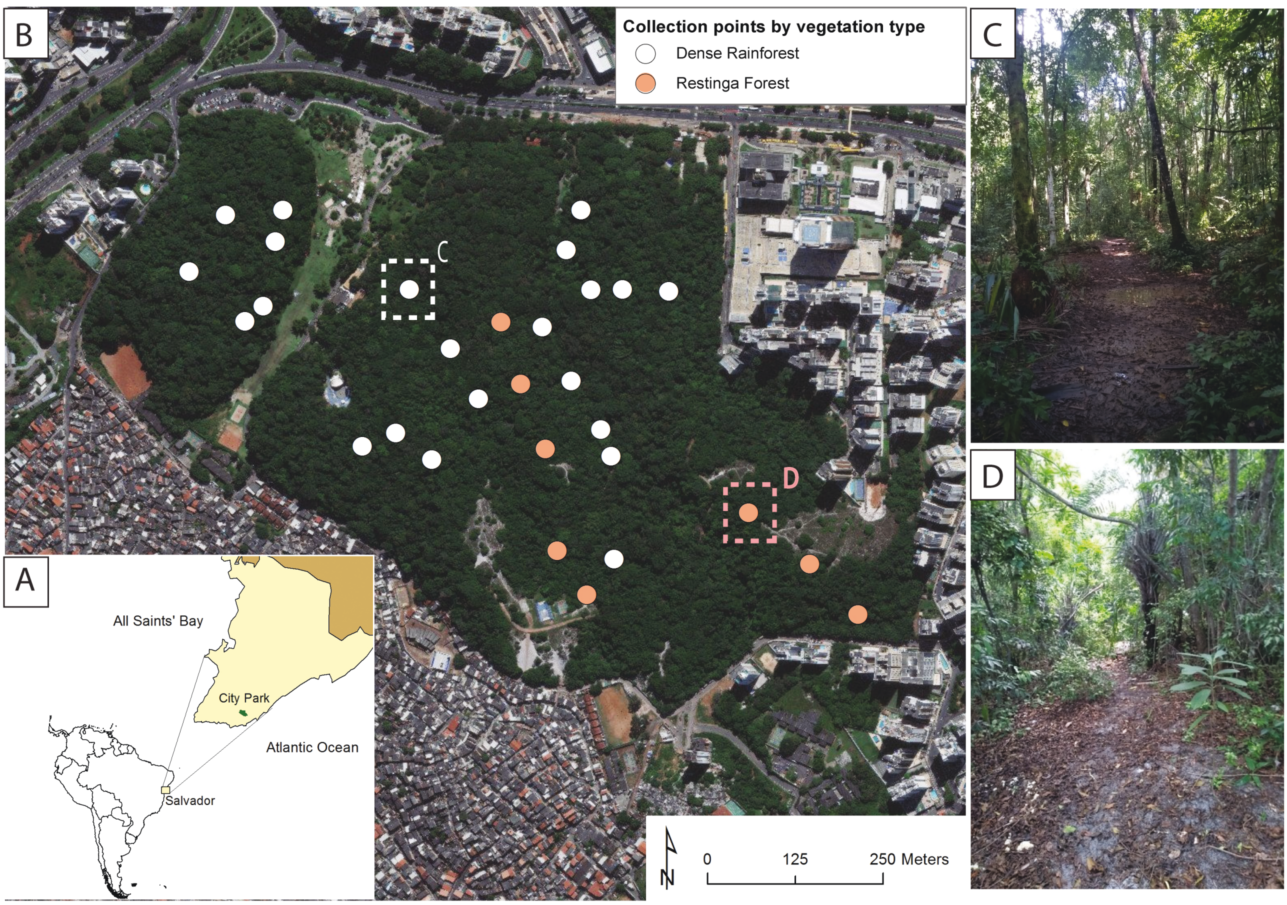

Fig. 1. Spatial distribution of the adult mosquito collection sites in an Atlantic Forest urban park, Salvador, Brazil, May 2 to August 2, 2018. A. Location of Salvador in Brazil and of the park in Salvador. B. Collection points by vegetation type. C. Illustrative photo of a park area characterized by dense rainforest vegetation. D. Illustrative photo of a park area characterized by restinga vegetation.

Foundation (FIOCRUZ), Salvador, Brazil. Two investigators identified all specimens collected to the lowest possible identification level using the taxonomic keys described by Consoli and Oliveira (1994). Because adult mosquitoes of the Sabethini tribe are small, have morphological characteristics that are difficult to separate, and are often damaged during capture and transport, we did not classify them beyond the genus level.

Statistical Analysis

We calculated the frequencies of each species of mosquito collected by capture method and vegetation type, and standardized indices of abundance, such as females per man-hour and females per trap per night. Additionally, two of the multiple available ecological diversity indices, Shannon-Wiener index (H') and Pielou's evenness index (J'), were obtained to compare the diversity of species between the different vegetation types in the park, as well as between the different methods of mosquito collection used (Pielou 1966; Hill 1973; Gorelick 2006). The lower the Shannon-Wiener index, the lower the species diversity. Pielou's evenness index measures uniformity of the distribution among existing species, and ranges between 0 (no evenness) to 1, (complete evenness). Species accumulation and rarefaction curves by collection method were also calculated to evaluate the sampling effort.

Results

A total of 11,914 mosquitoes were collected, of which 887 specimens were captured by human attraction and 11,027 by CO_2-baited CDC traps (6,150 on ground level and 4,877 in the canopy). All were from the subfamily Culicinae, belonging to three tribes (Aedini, Culicini, and Sabethini), five genera (*Aedes*, *Culex*, *Limatus*, *Psorophora*, *Wyeomyia*), and at least seven species (Table 1). The most frequently captured mosquitoes were *Culex quinquefasciatus* (11,038 (92.6%) mosquitoes), *Wyeomyia* spp. (374, 3.1%), and *Limatus* spp. (225, 1.9%) (Table 1). The two most important urban vectors of arboviruses, *Aedes aegypti* (Linnaeus, Diptera: Culicidae) and *Aedes albopictus*, accounted only for a small fraction of mosquitoes with 17 (0.1%) and 166 (1.4%) of each species collected, respectively.

We analyzed the spatial distribution of the density of captured mosquitoes by collection point for the two most frequently captured mosquitoes (*Cx. quinquefasciatus* and *Wyeomyia* spp.) and the two of greatest public health interest (*Ae. aegypti* and *Ae. albopictus*) (Fig. 2). *Cx. quinquefasciatus* and *Wyeomyia* spp. were ubiquitous throughout the park, being captured at all collection points, while *Ae. albopictus* was captured at most (23 out of 30) of the collection points. In contrast, *Ae. aegypti* was captured only at 6 of the 30 collection points, located close to open areas of the park, where movement of people is greater, or in its periphery, near residential areas.

Species accumulation and rarefaction curves showed that the sampling effort using human attraction was adequate, whereas for CDC light traps a greater effort could lead to collection of additional, rarer species (Fig. 3). The CDC light traps on the ground and canopy levels were much more efficient in terms of the number of mosquitoes captured (6,150 and 4,887 specimens, respectively) compared to the human attraction method (887 specimens) (Table 1). However, the species diversity and heterogeneity were lower in CDC traps (Table 1), with >95% of mosquitoes captured comprising a single species (*Cx. quinquefasciatus*), regardless of whether the CDC trap was installed on the ground or in the canopy. The human attraction method captured a greater diversity of mosquito species, including *Cx. quinquefasciatus* (39.2% of the captured specimens), *Wyeomyia* spp. (29.0%), *Ae. albopictus* (16.0%), and *Limatus* spp. (9.2%). Not surprisingly, 16 (94.1%) of the 17 specimens of *Ae. aegypti* and 142 (85.5%) of the 166 specimens of *Ae. albopictus* collected were captured by the human attraction method.

The majority (10,427, 87.5%) of mosquitoes were collected in the dense rainforest area, while 1,487 (12.5%) of specimens were collected in the restinga area (Table 2). In addition, the different vegetation types influenced the diversity and heterogeneity of the species collected (Table 2). While *Cx. quinquefasciatus* accounted for ~90% of all specimens collected both in the dense rainforest and in restinga, *Wyeomyia* spp. and *Ae. albopictus* were relatively less frequent in the dense rainforest area (2.5% and 1%, respectively), compared to the restinga area (7.7% and 3.8%, respectively). Standardized indices of female abundance corroborated that the CDC traps were more productive in capturing *Cx. quinquefasciatus* and the other most abundant species when placed in the dense rainforest compared to

Table 1. Frequency of adult mosquitoes collected in an Atlantic Forest urban park, according to tribe, species, or genus, and capture method; and diversity indices according to capture method, Salvador, Brazil, May 2 to August 2, 2018

Mosquito taxa	No. (%) of adults or index			
	All capture methods	Human attraction	CO_2-baited CDC trap, ground	CO_2-baited CDC trap, canopy
Culicini	11,040 (92.7%)	348 (39.2%)	5,878 (95.6%)	4,814 (98.7%)
Culex quinquefasciatus (Say, Diptera: Culicidae)	11,038 (92.6%)	348 (39.2%)	5,877 (95.6%)	4,813 (98.7%)
Culex spp. (Diptera: Culicidae)	2 (<0.1%)	0 (0%)	1 (<0.1%)	1(<0.1%)
Sabethini	599 (5.0%)	339 (38.2%)	206 (3.4%)	54 (1.1%)
Limatus sp. (Diptera: Culicidae)	225 (1.9%)	82 (9.2%)	116 (1.9%)	27 (0.6%)
Wyeomyia spp. (Diptera: Culicidae)	374 (3.1%)	257 (29.0%)	90 (1.5%)	27 (0.6%)
Aedini	275 (2.3%)	200 (22.6%)	66 (1.0%)	9 (0.2%)
Aedes aegypti (Linnaeus, Diptera: Culicidae)	17 (0.1%)	16 (1.8%)	1 (<0.1%)	0 (0%)
Aedes albopictus (Skuse, Diptera: Culicidae)	166 (1.4%)	142 (16.0%)	22 (0.4%)	2 (<0.1%)
Aedes scapularis (Rondani, Diptera: Culicidae)	89 (0.7%)	39 (4.4%)	43 (0.7%)	7 (0.1%)
Psorophora ferox (Von Humboldt, Diptera: Culicidae)	3 (<0.1%)	3 (0.3%)	0 (0%)	0 (0%)
Total	11,914	887	6,150	4,877
Diversity index				
Shannon-Wiener (H')	0.363	1.468	0.237	0.259
Pielou's evenness (J')	0.174	0.754	0.122	0.142

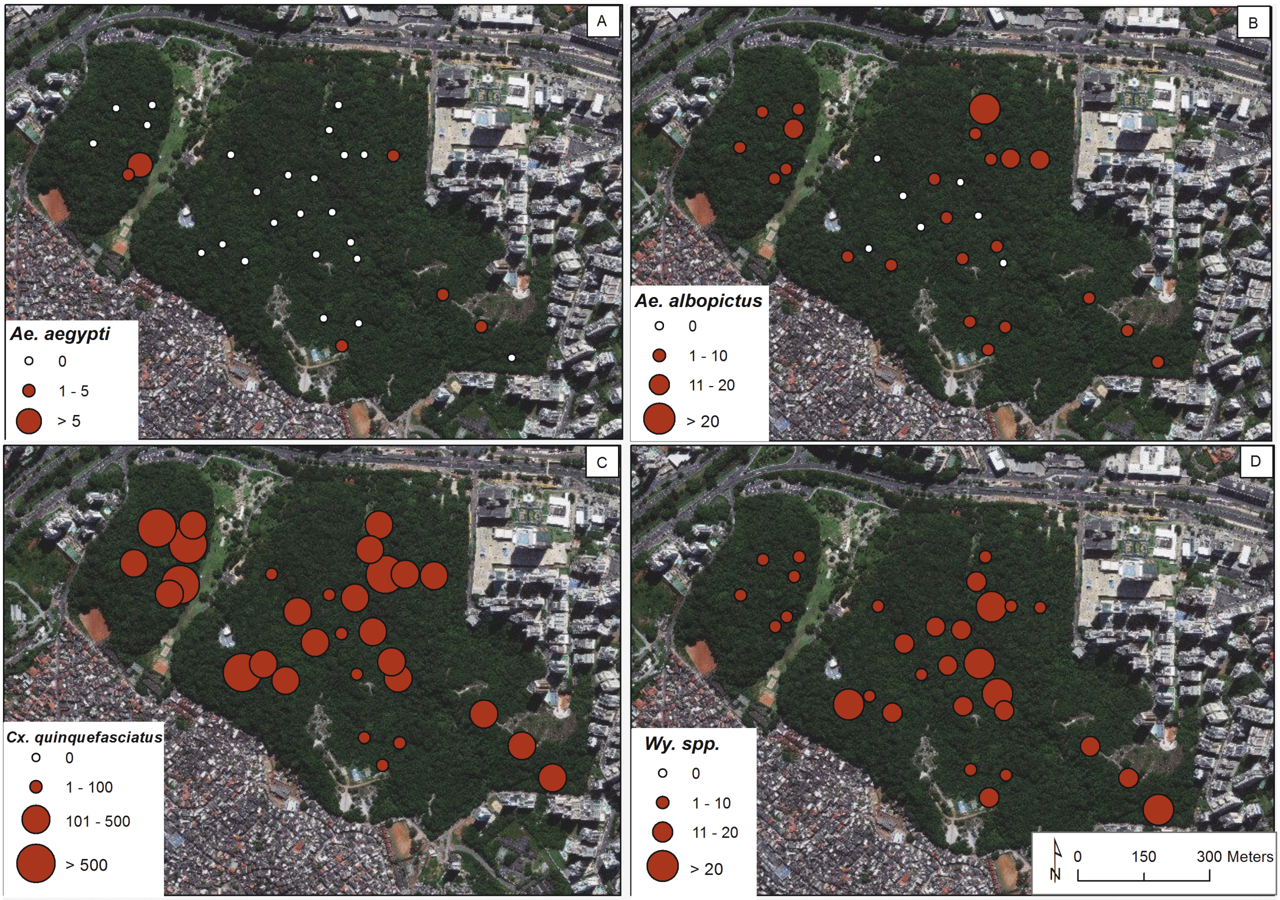

Fig. 2. Spatial distribution of selected species of adult mosquitoes collected in an Atlantic Forest urban park, according to the abundance of captured specimens, Salvador, Brazil, May 2 to August 2, 2018. A. *Aedes aegypti* distribution. B. *Aedes albopictus* distribution. C. *Culex quinquefasciatus* distribution. D. *Wyeomyia* spp. distribution. The legends informing on the abundance of captured specimens are not on the same scales.

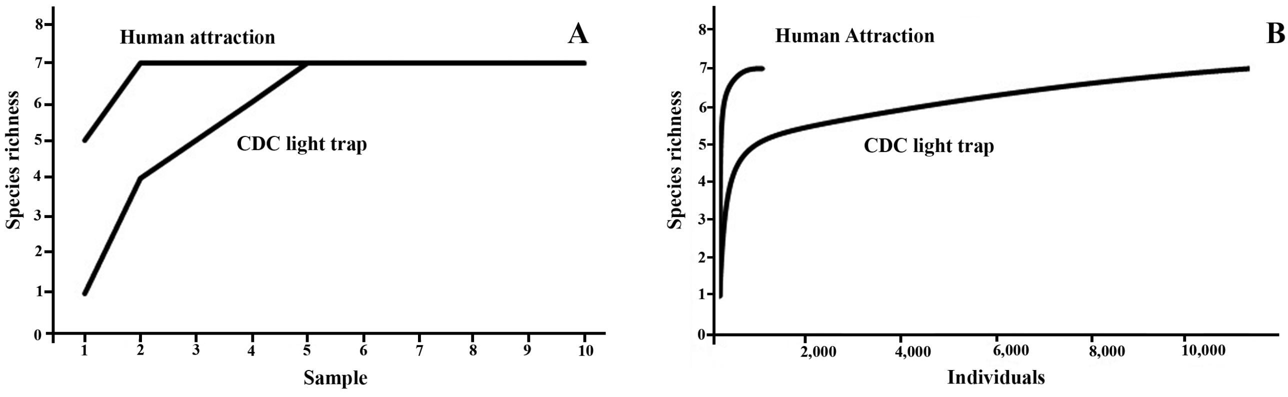

Fig. 3. Species (A) accumulation and (B) rarefaction curves according to the capture method (human attraction and CDC light trap). A. Accumulation curves are shown by sampling effort. B. Rarefaction curves are shown by the number of captured mosquitoes.

restinga, and that the human attraction method was more effective in capturing *Wyeomyia* spp. and *Ae. albopictus* in the restinga area than in the dense rainforest area (Table 2).

Discussion

The seven species of mosquitoes that we identified in this study were also found previously during an entomological survey carried out inside and around the same park and in another preserved Atlantic Forest area in Salvador (Paploski et al. 2017). Together, these findings suggest that mosquito diversity in the urban remnants of the Atlantic Forest in Salvador is low. Neither survey found species of the genera *Haemagogus* (Williston, Diptera: Culicidae) and *Sabethes* (Robineau-Desvoidy, Diptera: Culicidae) which are (to date) the putative vectors implicated in sylvatic YFV transmission. However, *Ae. aegypti* and *Ae. albopictus*, the main vectors for DENV, ZIKV, and CHIKV, were collected, suggesting that urban parks with fragments of Atlantic Forest may offer opportunities for transmission of these arboviruses.

Table 2. Frequency of adult mosquitoes captured by human attraction or CO_2-baited CDC traps and standardized indices of abundance, in an Atlantic Forest urban park, according to species or genus, and vegetation type of the collection point; and diversity indices according to vegetation type, Salvador, Brazil, May 2 to August 2, 2018

Mosquito taxa and diversity	Dense rainforest				Restinga			
	Total number of adult mosquitoes collected (%) or index	Average number of females per man-hour	Average number of females per ground trap per night	Average number of females per canopy trap per night	Total number of adult mosquitoes collected (%) or index	Average number of females per man-hour	Average number of females per ground trap per night	Average number of females per canopy trap per night
Culex quinquefasciatus	9,756 (93.6%)	2.9	247.2	179.1	1,282 (86.2%)	2.0	44.1	101.4
Wyeomyia spp.	259 (2.5%)	2.2	3.9	1.2	115 (7.7%)	4.5	0.5	0.1
Limatus sp.	201 (1.9%)	1.0	4.9	1.1	24 (1.6%)	0.7	1.0	0.0
Aedes albopictus	109 (1%)	0.9	0.9	0.1	57 (3.8%)	1.5	0.3	0.0
Aedes scapularis	88 (0.8%)	0.6	1.9	0.3	1 (<0.1%)	<0.1	0.0	0.0
Aedes aegypti	11 (0.1%)	<0.1	0.0	0.0	6 (0.4%)	<0.1	0.1	0.0
Psorophora ferox	3 (<0.1%)	<0.1	0.0	0.0	0 (0%)	0.0	0.0	0.0
Culex spp.	0 (0%)	0.0	0.0	0.0	2 (0.1%)	0.0	0.1	0.0
Total	10,427	7.7	258.7	181.8	1,487	8.8	46.9	101.5
Diversity index								
Shannon-Wiener (H')	0.327	-	-	-	0.553	-	-	-
Pielou's evenness (J')	0.168	-	-	-	0.284	-	-	-

Overall, *Cx. quinquefasciatus* accounted for 92.6% of all specimens captured. Its high abundance can be partly explained by the multiple larval habitats of this species within the park and its nocturnal habits, coupled with our use of CO_2-baited light traps for mosquito collection (97% of the *Cx. quinquefasciatus* were captured using CDC traps). Although overall numbers were lower, *Cx. quinquefasciatus* comprised the greatest proportion of mosquitoes collected by the human attraction method, indicating that the abundance of *Cx. quinquefasciatus* was not only a function of collection strategy.

We found a greater abundance of mosquitoes in collection points located in areas of dense rainforest than in restinga areas, mainly due to the high numbers of *Cx. quinquefasciatus* collected in CO_2-baited CDC traps in the first setting. However, species such as *Wyeomyia* spp. and *Ae. albopictus* were found relatively more frequently in the restinga area, indicating the importance of this habitat in maintaining a greater diversity and heterogeneity of species in this environment.

In 2017, *Callithrix* spp. monkeys collected dead near the park were found to be positive for YFV. Given that groups of *Callithrix* spp. live in the park, that the park houses both *Ae. aegypti* and *Ae. albopictus*, and that the park is adjacent to densely urbanized areas, a considerable risk exists for the resurgence of urban YFV transmission mediated by *Aedes* mosquitoes. In addition, Salvador has been an epicenter for epidemics of DENV, ZIKV, and CHIKV (Cardoso et al. 2017, Silva et al. 2019, Tauro et al. 2019), and the confluence of people, vectors and animals can provide a favorable environment for these arboviruses to follow the YFV path in re-establishing their primary enzootic cycles (Weaver 2013, Althouse et al. 2016, Terzian et al. 2018, Guth et al., 2020).

Our study was conducted during one season only, which may have influenced the diversity and abundance of species collected. However, the accumulation and rarefaction curves suggest that a greater capture effort using the human attraction method would have a small impact on the diversity of collected species. A greater effort using CO_2-baited CDC light traps could have led to the identification of additional rarer species but would have limited impact on measuring mosquito abundance. Regardless, our survey is one of the few comprehensive entomofauna investigation in a Brazilian urban park containing fragments of the Atlantic Forest. The limited literature on entomofauna in urban forest fragments in Brazil and elsewhere point to the need for additional surveys on the diversity and abundance of hematophagous insects in these areas, given their potential to serve as a link between the sylvatic and urban environments.

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