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Bioassay

Species composition and abundance of mosquitoes (Diptera: Culicidae) in a green area surrounded by urbanization in the Neotropical megacity São Paulo, Brazil

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Abstract. The urbanization process can influence mosquito biodiversity and abundance, which in turn might affect public health. Here, we investigated mosquitoes' abundance, diversity and seasonality in an urban green area, located in São Paulo, called Butantan Institute Park. We collected adult mosquitoes for two consecutive years by placing CDC light traps baited with CO_2 in three different sites inside the park covering tree strata (canopy, understory and shrub layer). In total, we identified 27,350 mosquitoes belonging to nine different genera and 20 species, including several epidemiological important mosquito species that occupy the urban, sylvatic, and transitional ecotones. The most abundant species were *Culex quinquefasciatus* (Say, 1823) (Diptera: Culicidae) with 6,892 specimens collected, *Culex nigripalpus* (Theobald, 1901) (Diptera: Culicidae) with 5,878 and *Limatus durhami* (Theobald, 1907) (Diptera: Culicidae) with 4,615. While seasonality did not appear to be a strong factor shaping mosquito diversity and abundance, strata had a pronounced effect on the abundance of mosquitoes. The abundance and diversity (Shannon's index) of mosquitoes was higher at ground level (shrub layer). The high presence of mosquitoes in the park increases the risk of enzootic cycles, re-emergence, or the emergence of new pathogens in the urban environment. For instance, we found one specimen of the genus *Sabethes* (Robineau-Desvoidy, 1827) indicating that monitoring is very important to prevent yellow fever outbreaks in São Paulo city. Finally, several aspects of mosquito biology and dispersion are discussed in more detail.

Keywords: Seasonality, vectors of pathogens, CDC light traps, diseases transmission, public health.

Mosquitoes belong to the family Culicidae and are nematoceran Diptera of cosmopolitan distribution. Both Anophelinae and Culicinae subfamilies draw attention from the public health sector because it includes several species that can act as a vector of pathogens to human and animals all over the world (Consoli & Lourenço-de-Oliveira 1994; Munstermann & Conn 1997).

An important factor influencing the increase of transmission of pathogens by mosquitoes among us is the change in biodiversity due to urbanization (Ezenwa et al. 2006; Swaddle & Calos 2008; Keesing et al. 2010). Urbanization results in a reduction of green areas used as shelter by several species of mosquitoes, forcing them to adapt to the new (semi or completely urban) environment, and consequently a closer coexistence with humans (Taipe-Lagos & Natal 2003), which, therefore, contribute to the spread of infectious-parasitic diseases. Several mosquitoes of medical interest, such as the ones belonging to the genus *Aedes* (Meigen, 1818), *Culex* (Linnaeus, 1758) and *Anopheles* (Meigen, 1818), have already changed their distribution, oviposition sites and feeding habits due to human interference in their environment (Forattini et al. 1989; Laporta et al. 2006; Urbinatti et al. 2001). Two well-known examples are the exotic species *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae) and *Culex quinquefasciatus* (Say, 1823) (Diptera: Culicidae) which are well-adapted to urban areas and commonly found everywhere in tropical countries (Medeiros-Souza et al. 2013), playing an important role as a vector of well-known viruses such as West Nile Virus (WNV), Dengue (DENV), Chikungunya (CHIKV), Zika (ZIKV) and Yellow Fever Virus (YFV) worldwide. Many countries from Asia, Africa, South and Central America have faced continuous outbreaks of these arboviruses every year (Rueda 2008). Nowadays, CHIKV and ZIKV are two arboviruses that deserve attention because they have quickly spread to several countries (Paixão et. al 2018), including Brazil (Ministério da Saúde 2019).

As one of the biggest tropical countries in the world, Brazil has a high diversity and abundance of mosquito species (Foley et al. 2007) where approximately 500 species are found (WRBU 2019). São Paulo is a megacity located in the southeast region of Brazil, where it is possible to find about 65 species of mosquitoes distributed in 14 genera (Medeiros-Sousa et al. 2013). The Butantan Institute Park is one of the oldest and most important tourist spots in São Paulo city.

Mosquito inventories, and monitoring in general, are important for establishing a baseline level of infestation in *area* and to support future management decisions for areas in which mosquito abundance is high. In addition, monitoring can contribute towards the understanding of possible impacts of habitat loss and fragmentation on vector-borne diseases. Thus, the objective of this study was to carry out an adult mosquito survey to investigate the abundance and diversity of mosquitoes (Diptera: Culicidae) in the Butantan Institute park, helping entomologists, parasitologists, epidemiologists, ecologists and public health authorities to set plans to monitor and control mosquito-borne diseases in São Paulo city.

As the Butantan Institute forested area is divided into three major sites ("Horto Florestal", "Trilha da Mata", and "Fazendinha"), we placed 5 CDC light traps randomly in each site. We placed the traps on the branches of the trees and at different strata (canopy, understory, and shrub layer), to increase the chances to sample mosquitoes from different niches. The highest trap was placed at 15.10 m from the ground and the lowest at 1.50 m from the ground. Samplings were performed for five consecutive days and always in the last week of each month, from January 2018 to December 2019. Traps were baited with dry ice CO_2 during the day (from 8:00 to 17:00) and during the night (from 17:00 to 8:00 of the day after) with dry ice CO_2 and light. Prior to identification, all traps were sent to the lab and stored at -4°C.

All mosquitoes sampled were sent to Laboratório de Identificação e Pesquisa em Fauna Sinantrópica da Divisão de Vigilância de Zoonoses (Labfauna/DVZ) for identification by using three taxonomic keys (Lane 1953; Consoli & Lourenço-de-Oliveira 1994; Forattini 2002). The taxonomic classification used in our study followed the one adopted by the Systematic Catalog of Culicidae, developed and maintained by the Walter Reed Biosystematics Unit, Division of Entomology (Walter Reed Army Institute of Research) in (http://www.mosquitocatalog.org/), and by the Mosquito Taxonomic Inventory (https://mosquito-taxonomic-inventory.info). Instructions by Wiley & Liebermann (2011), Vences et al. (2013) and Wilkerson et al. (2015) were also used for the identification of the Aedini Tribe.

Data from 2018 and 2019 were analyzed separately using the software R (R Development Core Team 2016). Differences in the relative abundance of arthropods between months, sites, and strata were analysed using the nonparametric Kruskal-Wallis rank sum test with the *Kruskal.test* function and pairwise comparisons using Wilcoxon rank sum test with the *pairwise.wilcox.test* function in R. To analyse differences in diversity between months, sites, and strata, we used Shannon's diversity index (H') by using the function *ddply* in R.

During two consecutive years of sampling, we collected and identified 27,994 mosquitoes from 20 species belonging to nine different genera. Not all specimens could be identified to the species level and only the genus was identified in those cases (Tab. 1 and Tab. 2). From these 20 species, *Cu. quinquefasciatus* (7,149 specimens), *Culex nigripalpus* (Theobald, 1901) (Diptera: Culicidae) (5,978 specimens), *Li. durhami* (4,766 specimens) and *Aedes scapularis* (Rondani, 1848) (Diptera: Culicidae) (2,315 specimens), were the most abundant species, while other species such as *Sabethes gymnothorax* (Harbach & Petersen, 1992) (Diptera: Culicidae), *Wyeomyia confusa* (Lutz, 1905) (Diptera: Culicidae), *Mansonia wilsoni* (Barreto & Coutinho, 1944) (Diptera: Culicidae), and *Coquilletidia chrysonotum* (Peryassú, 1922) (Diptera: Culicidae), were sampled just once. The high numbers of *Culex* and *Aedes* mosquitoes collected in the park is an important finding since these two genera consist of several species of vectors or potential vectors involved in the transmission of several pathogens. Most of these species sampled reproduce in natural or artificial oviposition sites demonstrating their ability to adapt to new environments and their plasticity in response to urbanization. Nevertheless, we still observed the dominance of anthropophilic mosquitoes over wild mosquito species, indicating that the mosquito fauna of the park is well adapted to the degraded environment over preserved environments.

Except for the species *Ma. wilsoni* and *Sa. gymnothorax*, all these mosquito species have already been identified in surveys carried out in other parks from São Paulo city. For instance, Medeiros-Sousa et al. (2015) identified 57 mosquito species distributed in 13 genera at the Anhanguera Park and Carvalho et al. (2017) identified 27 mosquito species from 7 genera at Santo Dias Park and 36 mosquito species from 8 genera at Shangrilá Park. Both studies sampled immature, using larval dipper, and adult mosquitoes, using manual aspirators, automatic CDC traps and Shannon traps. Hence, comparing these surveys with ours, we have found fewer mosquito species. Because we have only sampled adult mosquitoes using CDC traps, we believe that sampling immature stages might help to identify other mosquito species in the park. In fact, a recent inventory of immature mosquitoes (Diptera: Culicidae) at the Butantan Institute Park has identified some mosquito species that we did not sample in our study, such as *Culex coronator* (Dyar & Knab, 1906), *Aedes serratus* (Theobald, 1901), *Aedes crinifer* (Theobald, 1903), *Culex pleuristriatus* (Theobald, 1903) and *Wyeomyia theobaldi* (Lane & Cerqueira, 1942) (Breviglieri & Lorenz 2017). However, we also identified species that they did not, such as *Aedes fluviatilis* (Lutz, 1904), *Culex chidesteri* (Dyar, 1921), *Culex declarator* (Dyar & Knab, 1906), *Cu. nigripalpus*, *Cu. quinquefasciatus*, *Sa. gymnothorax*, *Co. chrysonotum, Wy. confusa* and *Ma. wilsoni*. Therefore, both studies are complementary, and considering our goal to have the first sampling of adults in the Butantan Institute Park we are confident that we have sampled the most important mosquito species present in the park.

Concerning the abundance of mosquitoes, we collected 14,766 mosquitoes in 2018 (Tab. 1) and 13,228 in 2019 (Tab. 2) with no significant differences in their relative abundance between these years (Kruskal-Wallis chi-squared= 1.6351, df= 1, P= 0.201). For the survey conducted in 2018, the abundance of mosquitoes did not differ between months (Kruskal-Wallis chi-squared= 16.359, df= 11, P= 0.1283, Tab. 1) and sites (Kruskal-Wallis chi-squared = 0.48, df= 2, P= 0.78, Fig. 1A), but it differed between the strata where mosquito traps were placed (Kruskal-Wallis chi-squared= 27.874, df= 2, P= 8.856e-07, Fig. 1B). For the survey conducted in 2019, the abundance of mosquito species collected was different between months (Kruskal-Wallis chi-squared= 81.852, df= 11, P= 6.462e-13, Tab. 2) and strata (Kruskal-Wallis chi-squared= 35.038, df= 2, P= 2.464e-08, Fig. 1B), but not among sites (Kruskal-Wallis chi-squared= 2.78, df= 2, P= 0.24, Fig. 1A). Overall, the abundance of adult mosquitoes in our study was higher than in the studies of Medeiros-Souza et al. (2015) and Carvalho et al. (2017), possibly due to the sampling effort (5 days per month) that was higher in our case.

Mosquito diversity and abundance are known to vary between seasons, mostly due to an increase in temperature and humidity in the summer (Franklin & Whelan 2009; Santiago-Alarcon et al. 2013). Surprisingly, in our study, we did not find this pattern in the abundance of mosquitoes in the year 2018, but we found it in 2019. This difference in abundance highlights the need for more survey in the park, with longer periods and, to keep an eye on significant seasonal changes that might have occurred over the years in São Paulo city. Regarding to the diversity of species, in 2018, the lowest diversity was found in July (H'= 0.43), August (H'= 0.44) and September (H'= 0.72) while December was

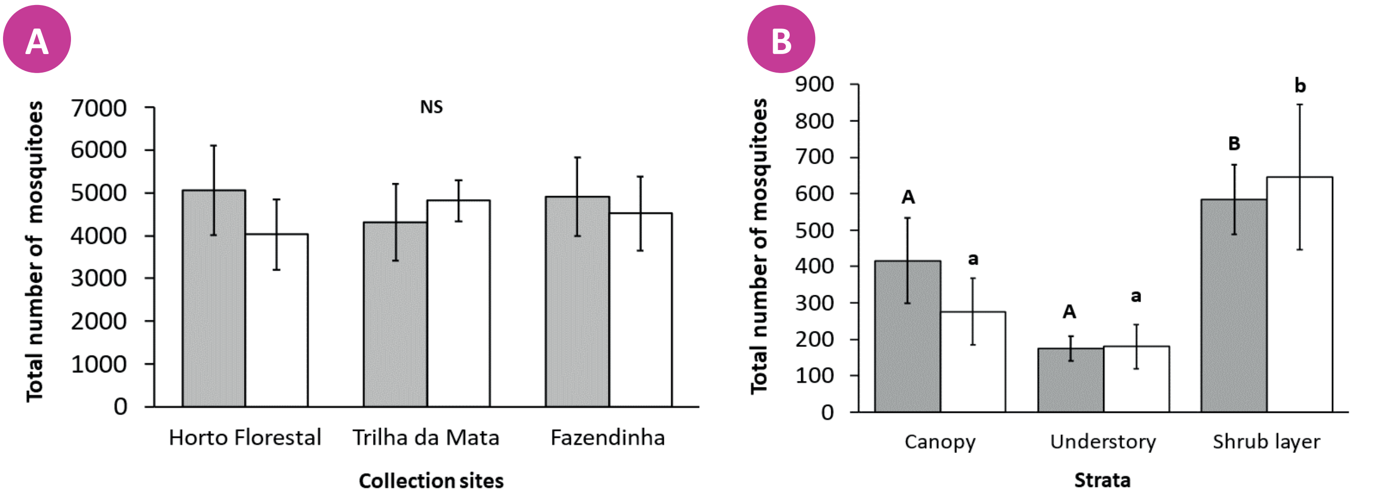

Figure 1. Abundance of mosquitoes collected in 2018 and 2019. Panel (A) shows the abundance of mosquitoes collected in both years in three different locations at the Butantan Institute Park by using CDC mosquito traps. Each trap was placed on the top of a tree and the position of the traps were grouped according to their stratification, into tree strata (canopy, understory and shrub layer). Panel (B) shows the abundance of mosquitoes collected in each strata in 2018 and 2019.

the month with the highest species diversity (H'= 1.46). In 2019, the H' index showed a similar degree of diversity over the months and in all the 3 sites where the mosquitoes were collected. In both years, we observed the highest species diversity in the shrub layer (H= 1.59 in 2018 and H= 1.86 in 2019) than in the other strata.

One specimen of the genus *Sabethes* (Robineau-Desvoidy, 1827) has been sampled in the present study. The natural history of the YFV in the southern Brazilian region, including São Paulo State, is a good and recent example of what may occur if the surveillance is not performed appropriately. Until 2016, this specific pathogen did not occur in urban sites of São Paulo State, but due to an environmental instability, YFV spread from sylvatic to urban sites and infected humans in several cities. The presence of the virus and the high density of the mosquitoes of *Sabethes* spp. and *Haemagogus* spp. migrating between different ecotones was sufficient to spread the diseases in those cities. Therefore, upon detection of these important mosquito species, the government needs to act quickly to retain the spread of the vector and the pathogen before the problem becomes serious.

Finally, because our study was performed in a specific urban park and we did not investigate the presence of pathogens in the sampled mosquitoes, we cannot directly infer or correlate the presence of mosquitoes with the presence of diseases inside or outside the park. Therefore, although our findings have the potential to be used by public health, we should unify efforts to detect the presence of pathogens as

Table 1. Total number of adult mosquitoes collected at the Butantan Institute Park from Jan 2018 to Dec 2018.

Taxon	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Culex quinquefasciatus	0	3	5	653	1041	839	2418	635	267	194	249	335	**6639**
Limatus durhami	638	411	624	417	4	50	3	2	10	59	177	244	**2639**
Culex sp.	151	68	197	495	564	302	344	34	75	76	94	124	**2524**
Culex nigripalpus	105	43	226	431	94	255	202	51	18	90	215	149	**1879**
Aedes albopictus	56	24	89	51	3	6	5	4	3	57	9	23	**330**
Culex chidesteri	23	10	7	34	77	37	58	4	8	7	12	25	**302**
Aedes fluviatilis	38	14	18	28	19	16	11	4	11	13	11	21	**204**
Aedes scapularis	35	4	50	39	2	0	2	2	2	13	20	19	**188**
Aedes aegypti	0	0	4	0	1	0	1	3	1	2	1	1	**14**
Culex section Melanoconion	1	0	1	5	0	1	0	0	0	0	1	0	**9**
Wyeomyia cf. luteoventralis	3	0	5	0	0	0	0	0	0	0	0	1	**9**
Culex dolosus/eduardoi	0	1	0	0	0	4	0	2	0	0	0	0	**7**
Culex group Pleuristriatus	0	0	4	0	0	0	1	0	0	0	0	0	**5**
Aedes sp.	0	0	0	0	1	0	0	0	0	2	0	1	**4**
Psorophora ferox	0	0	0	0	0	0	0	0	0	0	0	3	**3**
Culex declarator	0	0	0	0	0	1	1	0	0	0	0	0	**2**
Coquilletidia chrysonotum	0	0	0	0	0	0	0	0	0	0	1	0	**1**
Mansonia wilsoni	0	0	0	0	1	0	0	0	0	0	0	0	**1**
Wyeomyia sp.	0	0	0	0	0	0	0	0	0	0	1	0	**1**
Total	**1,053**	**578**	**1,230**	**2,153**	**1,808**	**1,511**	**3,046**	**741**	**395**	**514**	**791**	**946**	**14,766**

Table 2. Total number of adult mosquitoes collected at the Butantan Institute Park from Jan 2019 to Dec 2019.

Taxon	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Culex nigripalpus	591	444	1582	507	603	229	51	8	2	1	74	7	**4099**
Culex sp.	360	650	687	376	362	154	123	56	25	77	41	5	**2916**
Limatus durhami	467	517	269	506	182	76	48	7	7	3	6	39	**2127**
Aedes scapularis	198	930	385	428	67	29	3	0	0	0	7	2	**2049**
Aedes albopictus	120	116	148	89	56	29	8	2	5	4	50	15	**642**
Aedes fluviatilis	201	66	173	60	22	22	4	4	3	0	12	9	**576**
Culex quinquefasciatus	97	53	77	157	32	26	3	2	6	3	37	17	**510**
Culex chidesteri	9	3	16	59	38	34	15	1	2	17	3	0	**197**
Aedes aegypti	7	2	8	7	2	2	0	0	0	0	0	1	**29**
Culex dolosus/eduardoi	0	0	2	2	12	6	1	2	3	1	0	0	**29**
Culex sp.	0	0	17	1	0	0	0	0	0	0	0	0	**18**
Psorophora ferox	0	1	7	5	0	0	0	0	4	1	0	0	**18**
Wyeomyia cf. luteoventralis	0	5	0	1	0	0	0	0	0	0	0	0	**6**
Wyeomyia sp.	1	0	0	0	0	0	0	1	1	0	0	0	**3**
Culex declarator	0	0	1	0	1	0	0	0	0	0	0	0	**2**
Culex (Microculex) sp.	1	0	0	0	1	0	0	0	0	0	0	0	**2**
Culex section Melanoconion	1	0	0	0	0	0	0	0	0	0	0	0	**1**
Culex group Imitator	0	0	0	0	1	0	0	0	0	0	0	0	**1**
Sabethes gymnothorax	0	0	0	0	0	0	0	0	0	0	0	1	**1**
Uranotaenia sp.	0	0	0	0	1	0	0	0	0	0	0	0	**1**
Wyeomyia confusa	0	0	0	0	0	0	0	0	0	0	1	0	**1**
Total number of specimens	**2,053**	**2,787**	**3,372**	**2,198**	**1,380**	**607**	**256**	**83**	**58**	**107**	**231**	**96**	**13,228**

well as the number of cases to have a clear epidemiological panorama of certain diseases. Hence, by looking at this mosquito survey and the epidemiological information of some of the diseases found in São Paulo city, the government can develop a better methodology for mosquito control in the city. In fact, surveillance programs and control measures against mosquitoes transmitting ZIKV, DENV, CHIKV and YFV have already been implemented in this city.

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Authors' Contributions

All authors contributed to the study conception and design. Material preparation, data collection was performed by CAM, JCF, FV, LMSA. Taxonomic identification was performed by ABA, CMI, JVAF, RES. Analysis of the data was performed by LMSA. The first draft of the manuscript was written by LMSA and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of Interest Statement

The authors declare that there is no conflict of interests over the ownership of the data presented in this manuscript.

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