Journal of the American Mosquito Control Association, 39(2):000–000, 2023

FAUNA AND VIROLOGICAL INVESTIGATION OF MOSQUITOES IN URBAN PARKS IN SÃO PAULO, BRAZIL

MARTA R. HEINISCH,[1] ANTÔNIO R. MEDEIROS-SOUSA,[2] PÂMELA S. ANDRADE,[1] PAULO R. URBINATTI,[1] ROSA M. M. S. ALMEIDA[1] AND TAMARA N. LIMA-CAMARA[1,2]

ABSTRACT. The mosquito fauna in urban parks in the city of São Paulo, Brazil, was investigated and compared for richness and diversity, and the abundance of each species was associated with climatic variables. Simultaneously, a virological investigation was performed to test the presence of *Flavivirus* and *Alphavirus*. Aspirations of adult mosquitoes were conducted in 3 urban parks for 3 consecutive weeks of each season between October 2018 and January 2020. A total of 2,388 mosquitoes were identified, with *Culex quinquefasciatus*, *Cx. nigripalpus*, and *Aedes aegypti* being the most abundant species. Mosquito assemblages showed similar richness and diversity, showing variability in individual results. Temperatures and *Ae. aegypti* abundance correlated significantly in one of the parks investigated herein. Urban parks represent areas of shelter and refuge for both anthropophilic and opportunistic species, such as *Cx. quinquefasciatus* and *Ae. aegypti*, as well as species that still need moderately preserved environments to develop.

KEY WORDS Diversity, green areas, mosquitoes, richness, seasonality

INTRODUCTION

Mosquitoes are important vectors of infectious agents of human diseases. Multiple environmental factors affect the mosquito behavior and pathogen transmission as well as interaction with vertebrate hosts (Brugueras et al. 2020). Climatic factors such as temperature and rainfall are associated with the spatial, temporal, and seasonal distribution of species, influencing all stages of its developmental cycle and population dynamics (Tabachnick 2013).

Landscape is also an important environmental factor in vector distribution, affecting mainly species abundance and dispersal and, consequently, influencing the interaction between mosquitoes and vertebrate hosts (Lambin et al. 2010). Landscape modification is closely related to urban sprawl, which directs environmental changes globally because of the demographic growth of human population (Gubler 2011). The urbanization process has modified natural environments to suit human needs, altering the structure, function, and dynamics of ecological ecosystems (Shochat et al. 2006). Consequently, the species community composition is in general modified, with a decrease in species richness as more generalist species, adapted to urban environments, increase in abundance (Chaves et al. 2011).

Urban landscapes are a formation of heterogeneous mosaics, interspersing various types of land cover and land use, between built areas and highly fragmented vegetation, mainly represented by parks (Breuste et al. 2008). Urban parks contribute to the preservation of native flora and fauna and provide essential ecosystem services to cities besides offering natural spaces for nature contemplation, leisure, sports practice, and culture to the urban population (Limnios and Furlan 2013).

Parks within the urban fabric may harbor several species of mosquitoes, including those belonging to the *Aedes* and *Culex* genera (Medeiros-Sousa et al. 2013a). These urban green areas may offer a variable availability of breeding sites, favoring the maintenance of populations of these mosquitoes (Ceretti-Júnior et al. 2014, Medeiros-Sousa et al. 2015), as well as vertebrate hosts for bloodfeeding (Carvalho et al. 2014), ensuring the survival and proliferation of anthropophilic and opportunistic mosquitoes vis-à-vis their interactions with climatic factors (Wilke et al. 2017, Heinisch et al. 2019). Thus, given the need to prioritize more effective strategies for the monitoring and control of these vectors in urban areas, it is essential to keep gathering information on the diversity, distribution, and seasonality of mosquito species in urban parks.

The objective of this work was to investigate the mosquito fauna in urban parks of the city of São Paulo, Brazil, comparing the species diversity of these areas and associating their abundance with climatic variables. Simultaneously, a virological investigation was performed on the collected mosquitoes for the presence of *Flavivirus* and *Alphavirus* (these data are included in the supplemental data).

MATERIALS AND METHODS

Study area

The city of São Paulo has more than 12 million inhabitants in a land area of 1,521.110 km^2 (IBGE 2021). The climate is classified as humid subtropical,

[1] Laboratory of Entomology in Public Health, School of Public Health, University of São Paulo, Avenida Dr. Arnaldo, 715, Cerqueira César, São Paulo, SP, 01246-904, Brazil.

[2] Epidemiology Department, School of Public Health, University of São Paulo, Avenida Dr. Arnaldo, 715, Cerqueira César, São Paulo, SP, 01246-904, Brazil.

Fig. 1. Map of the city of São Paulo, highlighting the location of the investigated urban parks. On the left: city of São Paulo in dark gray, highlighting the east zone in light gray. The locations of the parks are identified in red circles: CM, Chico Mendes; LJ, Lajeado; and PQ, Piqueri. On the right: each frame shows the area of parks (green polygons) inserted in the urban fabric (gray lines). This map was created using QGIS v2.18.9 (http://www.qgis.org).

marked by hot and rainy summers, and dry winters with mild temperatures (Alvares et al. 2013). Part of the Atlantic Forest biome is still preserved in fragments of secondary natural vegetation in Conservation Units, Environmental Protection Areas, and urban municipal parks (SVMA/PMSP 2014). For this study, 3 urban parks (Ecological Chico Mendes: 23°30′25.7″S, 46°25′39.2″W; Lajeado – Izaura Pereira de Souza Franzolin: 23°32′18.3″S, 46°24′18.6″W; and Piqueri: 23°31.36.6″S, 46°34.41.5″), in eastern São Paulo, were selected for our study (Fig. 1). Their selection was not only based on the distance between them, since mosquito collection by aspiration had to be performed on the same day, but also because this region of São Paulo has a history of autochthonous human cases of dengue fever during the large 2014–15 epidemic (SUS/PMSP 2016).

Mosquito collection, identification, and viral testing

Adult mosquito collections were made with 12-V battery-operated electric aspirators, corresponding to the model described by Nasci (1981). This method was selected to sample mosquitoes sheltering in vegetation and those in full flight searching for vertebrate hosts (Silver 2008). Aspirations were conducted for 3 consecutive weeks of each season between October 2018 and January 2020, from spring 2018 to summer 2020. Once every week, in the daytime, aspirations were conducted in 4 fixed internal areas of the park, for approximately 15 min each, totaling about 1 h of aspiration per park, avoiding the edge areas of the park sampled. During the study period, the sampling effort totaled 54 h of aspiration, divided into 18 h per park (3 h of aspiration per sampling season). The sampling sufficiency of the study period was estimated in the construction of rarefaction and extrapolation curves for each park.

Aspirated specimens were placed in entomological cages and transported alive to the Entomology Laboratory of the School of Public Health, University of São Paulo, where the cages were kept at –20°C. Mosquitoes were identified on a cold table at –10°C, following the dichotomous keys of Consoli

Table 1. Distribution of adult mosquitoes collected in Chico Mendes, Lajeado, and Piqueri parks, São Paulo, SP, Brazil, between September 2018 and January 2020.

	Chico Mendes		Lajeado		Piqueri		
Taxon	Female	Male	Female	Male	Female	Male	Total
Aedes aegypti	36	36	26	31	37	78	244
Ae. albopictus	2	1	2	8	17	3	33
Ae. fluviatilis	3	3	16	5	10	23	60
Ae. scapularis	56	5	16	2	20	3	102
Ae. serratus	0	0	3	0	1	1	5
Aedes spp.	12	0	4	3	1	2	22
Culex spp.	68	23	39	62	10	24	226
Cx. bidens	1	0	0	1	1	0	3
Cx. chidesteri	11	10	54	109	0	2	186
Cx. coronator	9	0	24	18	6	11	68
Cx. declarator	3	0	0	0	1	0	4
Cx. dolosus/eduardoi	0	1	3	0	0	0	4
Cx. bigoti	0	1	0	0	0	0	1
Cx. nigripalpus	94	11	106	12	24	1	248
Cx. quinquefasciatus	106	126	227	622	35	66	1,182
Subtotal	401	217	520	873	163	214	2,388
Total specimens collected	618		1,393		377		2,388
Taxonomic units (S)	14		13		12		15
Taxa identified at species level	12		11		10		13

and Lourenço-de-Oliveira (1994) and Forattini (2002). Abbreviations of the genera and subgenera of mosquito species followed Reinert (2001). All identified mosquitoes, separated by sex, collection site, and season, were grouped into pools of up to 10 specimens (engorged females were individually packed in isopropyl alcohol), stored at –80°C for subsequent virological investigation following the protocols described by Patel et al. (2013) and Giry et al. (2017).

Statistical analysis

The comparison of richness and diversity among the different study sites was analyzed by means of rarefaction (interpolation) and prediction (extrapolation) curves. The indices were calculated, and the curves constructed using the models proposed by Chao et al. (2014), based on Hill numbers (Hill 1973). This method enabled obtaining interpolated and extrapolated comparisons of richness and diversity among the 3 parks investigated. The diversity indices obtained are equivalent to the exponential of Shannon's Entropy Index (EXP H′) and to the Inverse of Simpson's Index (1/D). The 95% confidence intervals for the estimates were calculated by the Bootstrap method based on 100 replications. Rarefaction and extrapolation curves were constructed based on individuals. All analyses were performed in the R computing environment (R Foundation for Statistical Computing 2019) with the aid of the iNEXT package (Hsieh et al. 2016).

Climatic variables data were obtained from the meteorological station Mirante Campo de Marte in the National Institute of Meteorology (INMET 2021). For these analyses, we applied the Spearman's correlation (Rho) considering the total abundance and the most abundant species, observing the relationship between these abundances with the average maximum and minimum temperatures and average and total accumulated rainfall, all these accumulating for the 30 days preceding the collections, and added to the 22 days of collections.

RESULTS

A total of 2,388 mosquito specimens were collected—1,084 females and 1,304 males—distributed into 15 taxonomic units and identified to species level in 13 taxa. All mosquitoes collected belonged to the subfamily Culicinae, tribes Aedini and Culinici, comprising 2 genera: *Aedes* (19.51%; $n = 466$) and *Culex* (80.49%; $n = 1{,}922$). The most abundant species was *Culex* (*Cux.*) *quinquefasciatus* Say ($n = 1{,}182$), followed by *Cx.* (*Cux.*) *nigripalpus* Theobald ($n = 248$), *Aedes* (*Stg.*) *aegypti* (L.) ($n = 244$), and *Cx.* (*Cux.*) *chidesteri* Dyar and Knab ($n = 186$). All species were ubiquitous in the 3 parks. Other *Aedes* species were less abundant, such as *Ae.* (*Och.*) *scapularis* Rondani ($n = 102$), *Ae.* (*Stg.*) *albopictus* (Skuse) ($n = 33$), *Ae.* (*Och.*) *fluviatilis* Lutz ($n = 60$), and *Ae.* (*Och.*) *serratus* Theobald ($n = 5$) (Table 1).

In Chico Mendes park, 619 mosquitoes were collected, with *Cx. quinquefasciatus* and *Cx. nigripalpus* being the most abundant species ($n = 232$ and $n = 105$, respectively), followed by *Ae. aegypti* ($n = 72$) and *Ae. scapularis* ($n = 61$). In Lajeado park, 1,393 mosquitoes were collected, with the most abundant species being *Cx. quinquefasciatus* ($n = 849$), *Cx. nigripalpus* ($n = 118$), *Cx. chidesteri* ($n = 163$), and *Ae. aegypti* ($n = 57$). In Piqueri park, we collected 377 mosquitoes, with *Ae. aegypti* ($n = 115$)

Table 2. Asymptotic diversity estimates (est.) along with related statistics.

Park	Diversity index	Observed	Estimator	Lower est.	Upper est.
Chico Mendes	Species richness	14	16.995	14.353	39.392
	Shannon diversity	6.117	6.203	6.117	6.683
	Simpson diversity	4.615	4.642	4.615	5.077
Lajeado	Species richness	13	13	13	14.65
	Shannon diversity	4.062	4.08	4.062	4.344
	Simpson diversity	2.496	2.499	2.496	2.667
Piqueri	Species richness	12	13.995	12.18	34.075
	Shannon diversity	6.675	6.799	6.675	7.401
	Simpson diversity	5.145	5.202	5.145	5.773

and *Cx. quinquefasciatus* (n = 101) the most abundant species (Table 1).

General descriptive analyses of Culicidae faunal diversity by park are presented in Table 2. The columns indicate: estimated diversity index, observed richness, asymptotic estimator-based richness, and the lower and upper limits for estimated richness at the 95% confidence interval (Lower est. and Upper est.).

Species richness—be it observed or estimated—showed little variation among parks: up to 2 species between the parks with the highest and lowest richness—Chico Mendes (S_{obs} = 14 species) and Piqueri (S_{obs} = 12 species), respectively. Lajeado park showed an intermediate species richness (S_{obs} = 13 species).

Diversity indices were high for all parks, especially in Piqueri and Chico Mendes. Although the former exhibited the lowest species richness, its diversity indices were the highest (EXP H′ = 6.675; 1/D = 5.145). In the latter park, where the highest species richness was observed, diversity indices were lower (EXP H′ = 6.177; 1/D = 4.615). On the other hand, Lajeado park, with an intermediate species richness value (S = 13 species), showed the lowest indices among the sampled sites (EXP H′ = 4.062; 1/D = 2.496). Figure 2 shows the comparison among the 3 study areas based on diversity estimates. For the Shannon (Fig. 2A) and Simpson (Fig. 2B) indices, estimates are presented for up to 100 randomly obtained individuals. For species richness (Fig. 2C), an extrapolated estimate of up to 1,600 individuals per park is presented. The shaded areas around the curve represent the error associated with the estimate (95% CI). Diversity estimates were similar to the diversity indices for each park. Piqueri and Chico Mendes parks show similar diversity with overlapping confidence intervals (Fig. 2A, 2B), whereas, comparatively, Lajeado park shows lower diversity (Fig. 2A, 2B).

The rarefaction and extrapolation curves of the species were reasonably asymptotic at the end of the collection period for the Chico Mendes and Piqueri parks and approached the asymptote for the Lajeado (Fig. 2C). Mosquito diversity between parks was also compared as a function of the season of collection (spring 2018; summer, autumn, winter, and spring 2019; and summer 2020). For this analysis, the observed values and the confidence interval for the Exponential of Shannon's index and the Inverse Simpson's index were obtained (Fig. 3). Numerical results are shown in the supplementary materials (Supplementary Table 1).

Concerning the observed species richness between seasons, winter 2019 showed the lowest richness in all 3 parks, with a similar number between Lajeado and Piqueri (S = 3 species), and Chico Mendes exhibiting 1 more species (S = 4 species). Autumn had the highest species richness in all parks, especially in Lajeado (S = 12 species), followed by Chico Mendes (S = 11 species), and Piqueri (S = 10 species). Nonetheless, diversity was highly variable between seasons, particularly in Lajeado park which, during summers, showed the lowest diversity among parks in 2019 (EXP H′ = 1.223; 1/D = 1.317) and 2020 (EXP H′ = 7.053; 1/D = 5.03).

The relationship between climatic variables and mosquito abundance in each park was verified using Spearman's correlation analyses (Rho), considering the total mosquito abundance, and the abundance of the most abundant species for the genera identified in this study—*Ae. aegypti* and *Cx. quinquefasciatus*—with the average minimum and maximum temperatures and the average and accumulated total rainfall, all these accumulated for the 30 days preceding the collections and added to the 22 days of collections (Supplementary Table 2). Only the abundance of *Ae. aegypti* in Chico Mendes park showed a statistically significant correlation (at the 0.05 confidence level) with maximum and minimum temperature values, with identical values for both variables (Rho = 0.943; P = 0.017). The variations in abundance of *Ae. aegypti*, *Cx. quinquefasciatus*, and total number of mosquitoes collected, as well as the variations in maximum and minimum temperatures calculated for each sampling season, are shown in Fig. 4.

Aedes aegypti was more abundant during higher temperature periods, such as the summer of 2019, when the average maximum and minimum temperature values were the highest among all sampling seasons: 31°C and 20°C, respectively (Fig. 4A); a total of 88 *Ae. aegypti* specimens were captured in this season. In comparison, less than half that number of *Ae. aegypti* mosquitoes were collected in the summer of 2020 (n = 40), when the average temperatures were slightly lower (29°C and 19.2°C,

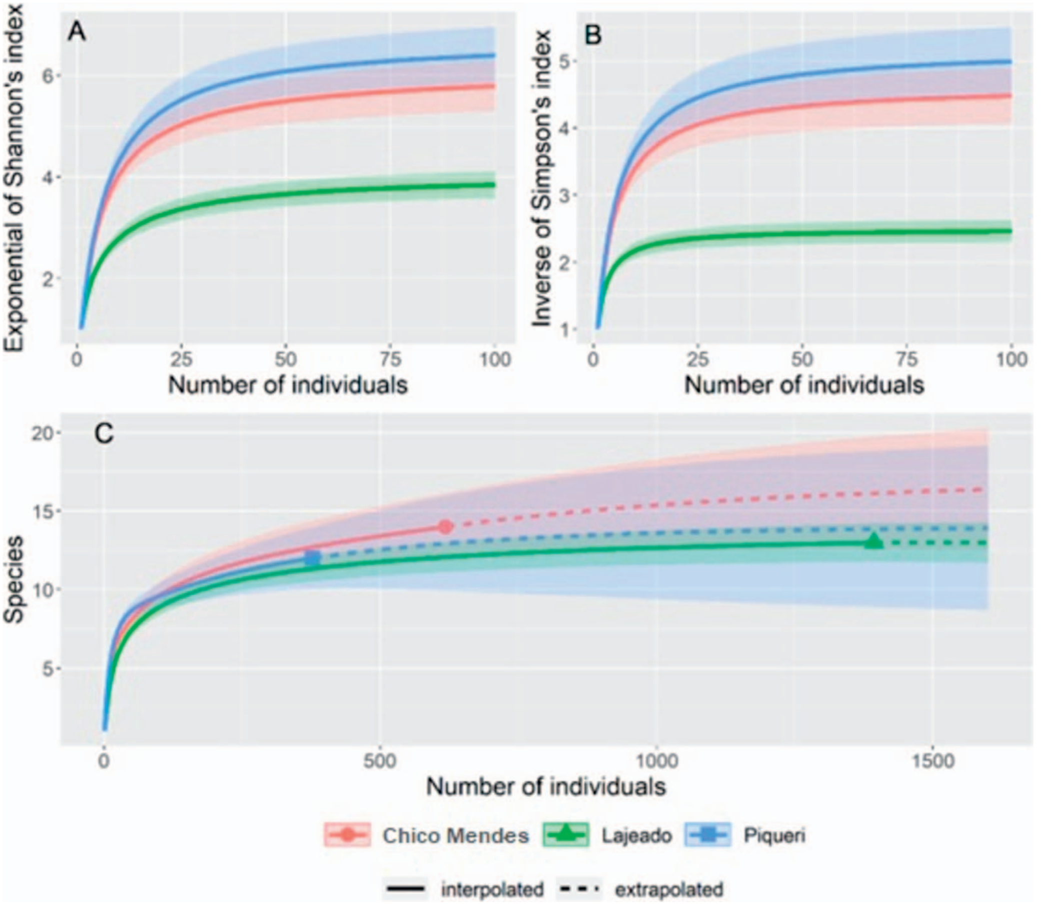

Fig. 2. Interpolated and extrapolated comparisons of richness and diversity of the 3 urban parks investigated. In the upper part, (A) is Exponential of Shannon's index and (B) is Inverse of Simpson's index. In the lower part, (C) is species accumulation curve. Solid lines represent interpolated values, dashed lines represent extrapolated values, and shaded areas represent the 95% confidence interval.

respectively). In the autumn of 2019, *Ae. aegypti* abundance remained high only in Piqueri park ($n = 39$) (Fig. 4A, blue bar); the average maximum and minimum recorded temperatures were 26.4°C and 18.1°C, respectively. In the spring of 2018 and 2019, average maximum and minimum temperatures were also similar from year to year: 26°C and 17°C; 28°C and 18.1°C, respectively. However, in 2019, the abundance of *Ae. aegypti* was higher compared with 2018 ($n = 36$ and $n = 16$, respectively) (Fig. 4A).

Regarding *Cx. quinquefasciatus,* the average maximum and minimum temperatures did not show significant correlation in the analyses, but we observed that its abundance showed great seasonal variability among the different parks (Fig. 4B). The highest representativity of this species was in Lajeado, mainly during spring 2019 ($n = 354$) (Fig. 4B, yellow bar).

For the total mosquito abundance, variability among parks was high throughout the same season (Fig. 4C). Overall, mosquito abundance was higher in autumn 2019, a season with milder temperatures than summers. Mosquitos were also abundant during spring 2019 and summer 2019, especially at Lajeado ($n = 354$ and $n = 380$, respectively) (Fig. 4C, yellow bar). At Piqueri, the autumn and summer of 2019 showed the highest abundances ($n = 113$ and $n = 111$, respectively) (Fig. 4C, blue bar). At Chico Mendes park, in addition to autumn 2019, spring 2018 was also an abundant season ($n = 246$ and $n = 129$, respectively) (Fig. 4C, green bar). Winter was the season with the lowest mosquito abundances for all sampled parks, when average maximum and minimum temperatures reached 23.3°C and 13°C, respectively (Fig. 4C). The virological investigation did not detect the presence of *Flavivirus* or *Alphavirus*.

DISCUSSION

The mosquito fauna in 3 urban parks in eastern São Paulo was investigated from spring 2018 to summer 2020, highlighting the mosquito richness and

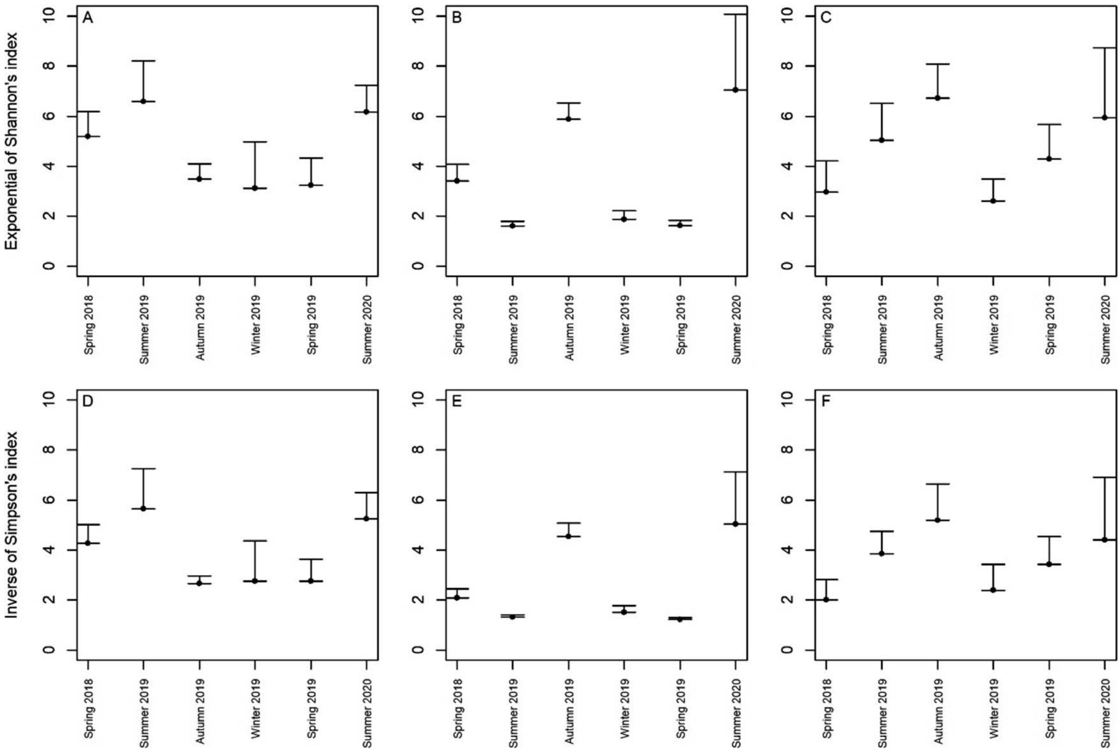

Fig. 3. Seasonal diversity of mosquitoes in the 3 urban parks investigated during the study period. In the upper part, values are obtained with the Exponential of Shannon's index for each park: (A) Chico Mendes, (B) Lajeado, and (C) Piqueri. In the lower part, the values obtained with the Inverse of Simpson's index for each park: (D) Chico Mendes, (E) Lajeado, and (F) Piqueri.

diversity in these areas, as well as relating species abundance with climate variables. The results show the presence of epidemiologically important vector mosquitoes therein, with little variation in richness and diversity in mosquito assemblages, albeit with high seasonal variability.

The aspiration collection method used in the study allowed us to collect a gamut of adult mosquitoes during both resting and active periods, but it may also have caused us to attain lower richness values compared with previous studies. The species rarefaction and accumulation curves show that our sampling effort was probably sufficient to quantify diversity, but the species richness observed in our study was lower than in other studies conducted in Chico Mendes and Piqueri parks (Paula et al. 2015, Wilke et al. 2017). This is possibly due to the different culicid collection techniques used in those studies, yielding a higher species richness. However, for Lajeado park, our study obtained the best richness and diversity estimates of the mosquito fauna so far, since data about this urban park are only preliminary (Medeiros-Sousa et al. 2013a).

The predominance of the *Culex* genus observed in this study is also evidenced by other authors, such as Medeiros-Sousa et al. (2013b), who conducted preliminary research in Chico Mendes, Lajeado, and Piqueri parks, and Paula et al. (2015), who collected in Chico Mendes park. *Culex quinquefasciatus* was the most abundant in both our study and as well as other studies conducted in São Paulo urban parks (Medeiros-Sousa et al. 2013a, 2013b). Similarly, in Piqueri park, *Cx. quinquefasciatus* was the main *Culex* species, and the 2nd most abundant among those identified therein (Ceretti-Junior et al. 2016, Wilke et al. 2017).

Culex nigripalpus was the 2nd most abundant species, including in Chico Mendes park, where few specimens had been sampled previously (Paula et al. 2015). On the contrary, few *Cx. nigripalpus* specimens were collected in Piqueri park as compared with previous studies (Wilke et al. 2017).

Regarding the *Aedes* genus, the results mainly show *Ae. aegypti* and *Ae. scapularis* as the most abundant species, in contrast to the low numbers of *Ae. albopictus*, usually associated with areas with higher vegetation-cover levels (Lima-Camara et al. 2006, Heinisch et al. 2019). In Piqueri park, for example, where we collected more *Ae. aegypti* specimens, Wilke et al. (2017) reported a greater abundance of *Ae. albopictus* compared to the other aedines. Similarly, the abundance of immatures collected in bamboo internodes and bromeliads in Piqueri park was higher for *Ae. albopictus* than for

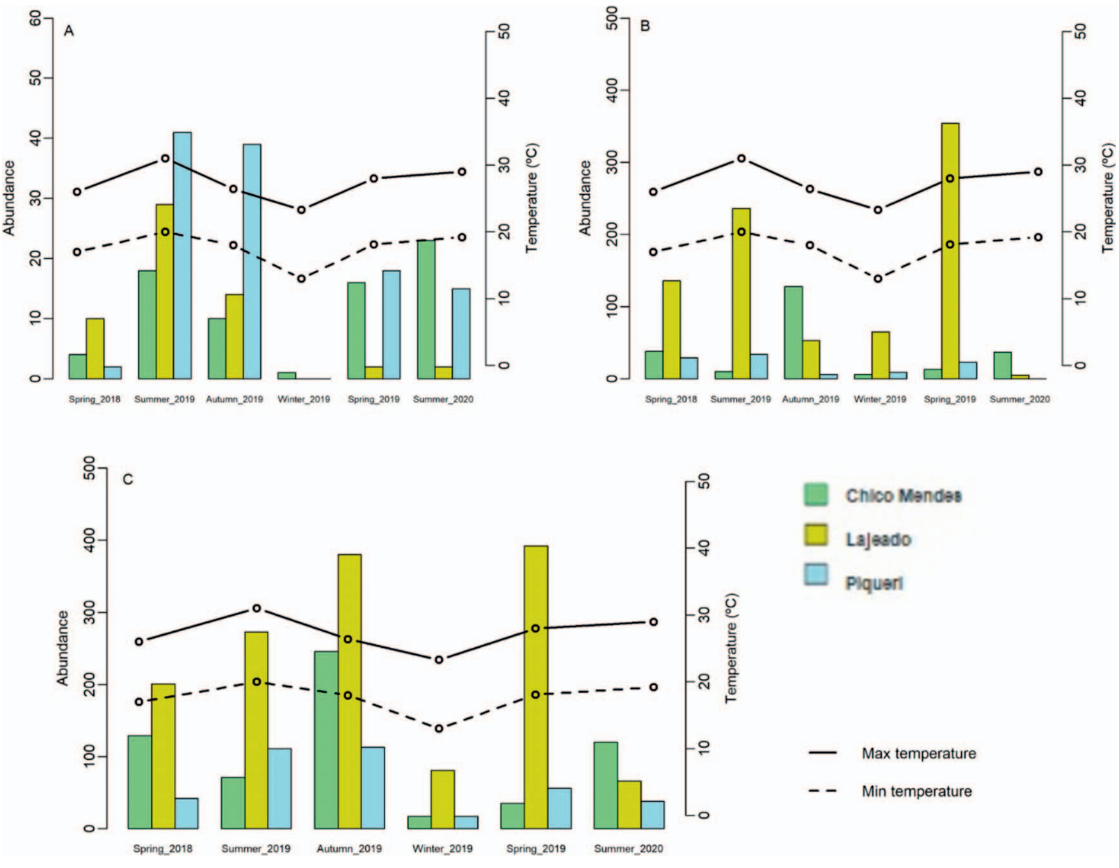

Fig. 4. Variation of species abundance was more abundant with the average maximum and minimum temperatures by season. In the upper part, (A) is abundance of *Aedes aegypti* and (B) is abundance of *Culex quinquefasciatus*. In the lower part, (C) is total abundance of species collected.

Ae. aegypti (Ceretti-Júnior et al. 2014, 2016). However, Paula et al. (2015) described *Ae. scapularis* as the most abundant species of the genus in Chico Mendes park.

As mentioned earlier, the differences in species abundance between this study and the existing literature may stem from the use of different collection techniques, since only entomological aspirations were used in this study. Nevertheless, the results add valuable information to previous research on mosquito fauna in urban parks, suggesting that green areas in urban networks are favorable environments for the permanence of species that still need moderately preserved environments to complete their developmental cycle, such as *Cx. nigripalpus* and *Ae. scapularis*, as well as for species more adapted to urban environments, such as *Cx. quinquefasciatus* and *Ae. aegypti*.

Besides the recognized involvement of *Ae. aegypti* and *Cx. quinquefasciatus* in the transmission of human pathogens (Brasil 2009, Diaz-Badillo et al. 2011, Cardoso et al. 2015, Lima-Camara 2016), mosquitoes in urban settings constitute a nuisance factor, as female specimens are highly anthropophilic (Forattini 2002). The permanence and abundance of these vectors in urban green areas reinforce the generalist and opportunistic behavior of these mosquitoes in finding breeding and sheltering habitats in urban settings.

Other species, such as *Cx. nigripalpus* and *Ae. scapularis*, are also considered vectors or suspect vectors in the transmission of some arboviruses (Mitchell and Forattini 1984, Day and Stark 2000, Pauvolid-Correa et al. 2013). Both species are versatile, adapted to natural environments with anthropogenic modifications, and eclectic with respect to hemophagy (Forattini 2002). Therefore, they require attention since they may also feed on a wide variety of vertebrates that circulate in urban parks in addition to humans (Laporta et al. 2008, Carvalho et al. 2014).

In this study, the diversity in the mosquito assemblages of the parks was reported. The species richness was similar, with little differentiation in diversity indices and estimates, but still suggesting that the mosquito assemblages from Chico Mendes and Piqueri parks were relatively more heterogeneous compared to the Lajeado assemblage, which appeared more homogeneous.

These results can be related to the Equilibrium Theory of Island Biogeography (ETIB) developed by MacArthur and Wilson (1967), which can explain the diversity patterns of mosquito assemblages in urban parks of São Paulo (Medeiros-Sousa et al. 2017). Urban parks are green area fragments with different area sizes and degrees of isolation, separated by the urban matrix that constitutes an inhospitable environment for most species and, therefore, form island-like habitats (Faeth et al. 2012, Fattorini 2016). The richness and composition in the mosquito assemblage in urban parks follow ETIB patterns, i.e., higher species richness in larger than in smaller parks, which consequently show greater similarity in species composition (Medeiros-Sousa et al. 2017). Thus, the fact that the parks analyzed herein have relatively smaller areas compared to others in São Paulo and a high degree of isolation within the urban network, would explain the relatively low and similar richness that we observed in these 3 sites, which also have similar mosquito assemblages, corroborating Medeiros-Sousa et al. (2017).

The summer of 2020 was the season with the best diversity indices in relation to the others for all parks, while autumn presented more species richness and high abundance, and winter was predominantly the season with the lowest mosquito richness and diversity in all 3 parks. Rainy seasons are associated with greater abundance of mosquitoes (Vieira et al. 2020, Balthazar et al. 2021); however, dry season, such as autumn, may have greater species richness (Abella-Medrano et al. 2015, Mayi et al. 2020), and these differences may be more associated with the availability of breeding sites within the areas of each park, mainly for the permanence of some species of the genus *Culex*, more abundant in this study.

In this study, mean and cumulative rainfall values did not show a statistically significant correlation. Even though it is one of the climatic factors that affects the quantity and quality of breeding sites (Jemal and Al-Thukair 2018), the correlation is not always significant (David et al. 2012, Heinisch et al. 2019) since, besides availability, the physico-chemical characteristics of the breeding sites and other biotic factors are important for the development of immatures and definition of the mosquito assemblage (Medeiros-Sousa et al. 2020). Maximum and minimum temperatures were significantly correlated with the abundance of *Ae. aegypti* only in Chico Mendes park. This correlation may stem from the higher frequency and abundance of the species in this park exclusively in the warmer seasons in contrast to the permanent frequency between seasons in Piqueri park, except for winter, and the low frequency throughout the entire period in Lajeado park.

Indeed, temperatures seem to affect *Ae. aegypti* populations in urban parks within São Paulo (Wilke et al. 2017, Heinisch et al. 2019). *Aedes aegypti* oviposition was analyzed as a function of climatic variables in Piqueri park; similar to these results, egg abundance did not show a significant association with rainfall, but was strongly associated with temperature, evidencing that the increase in minimum temperatures also conditions the increase in *Ae. aegypti* oviposition rates (Heinisch et al. 2019). Wilke et al. (2017) reported positive correlations between temperature and *Ae. aegypti and Cx. quinquefasciatus* abundance in Piqueri and Previdência parks, but the correlation with rainfall was different between species and parks. The observations of the present study suggest that the different responses of abundance to climate variations may be influenced by a species-dependent relationship regarding the habitats found in each park, determining species abundance, including the presence and resilience of more generalist species better adapted to urban environments, such as *Ae. aegypti* and *Cx. quinquefasciatus*.

Finally, our virological investigation results although negative, do not rule out the possibility of arbovirus circulation in urban parks, albeit at low levels (Ferreira-de-Lima et al. 2020).

Entomological and virological investigations in urban parks are essential, since they are not traditionally part of municipal control and surveillance programs. Studies that include other methodologies and analyses can provide additional knowledge on the dynamics of mosquito assemblages in urban green areas and assist in the development and targeting of strategies to control species implicated in arbovirus transmission as or those acting as bridge vectors that may have the potential to transmit pathogens.

SUPPLEMENTAL MATERIAL

Supplementary Table 1. Observed values and the confidence interval for the Exponential of the Shannon's Index and for the Inverse of the Simpson's Index in the Chico Mendes, Lajeado, and Piqueri parks.

Supplementary Table 2. Analysis of the Spearman (Rho) correlation for the total abundance of mosquitoes and the most abundant collected in the parks in relation to mean minimum and maximum temperatures and precipitation.

Supplementary Table 3. Total mosquito abundance, and the abundance of the most abundant species for the genera identified in this study—*Aedes aegypti* and *Culex quinquefasciatus*—with the average minimum and maximum temperatures and average and accumulated total rainfall.

Supplementary Table 4. Mosquitoes submitted to virological tests for the detection of *Flavivirus* and *Alphavirus*.

ACKNOWLEDGMENTS

We thank the Departamento de Parques e Áreas Verdes (DEPAVE 3) da Secretaria do Meio Ambiente da Prefeitura Municipal de São Paulo Municipal and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior–Brasil (CAPES) for partial funding—Finance Code 001. We also thank the administrations of the Ecological Chico Mendes, Lajeado – Izaura Pereira de Souza Franzolin, and Piqueri parks and who contributed to the field and laboratory work.

REFERENCES CITED

Abella-Medrano CA, Ibáñez-Bernal S, MacGregor-Fors I, Santiago-Alarcon D. 2015. Spatiotemporal variation of mosquito diversity (Diptera: Culicidae) at places with different land-use types within a neotropical montane cloud forest matrix. *Parasit Vectors* 8:487.

Alvares CA, Stape JL, Sentelhas PC, de Moraes Gonçalves JL, Sparovek G. 2013. Köppen's climate classification map for Brazil. *Meteorol Z* 22:711–728.

Balthazar TD, Maia DA, Oliveira AA, Marques WA, Bastos AQ, Vilela ML, Mallet JRS. 2021. Entomological surveillance of mosquitoes (Diptera: Culicidae), vectors of arboviruses, in an ecotourism park in Cachoeiras de Macacu, state of Rio de Janeiro-RJ, Brazil. *PLoS ONE* 16:e0261244. https://doi.org/10.1371/journal.pone.0261244

Brasil. 2009. *Guia de vigilância epidemiológica e eliminação da filariose linfática* [Internet]. Brasília, Brazil: Ministério da Saúde (Série A. Normas e Manuais Técnicos) [accessed October 26, 2022]. Available from: https://bvsms.saude.gov.br/bvs/publicacoes/guia_vigilancia_filariose_linfatica.pdf.

Breuste J, Niemelä J, Snep RPH. 2008. Applying landscape ecological principles in urban environments. *Landsc Ecol* 23:1139–1142.

Brugueras S, Fernández-Martínez B, Martínez-de la Puente J, Figuerola J, Porro TM, Rius C, Larrauri A, Gómez-Barroso D. 2020. Environmental drivers, climate change and emergent diseases transmitted by mosquitoes and their vectors in southern Europe: a systematic review. *Environ Res* 191:110038.

Cardoso BF, Serra OP, Heinen LB da S, Zuchi N, Souza VC de, Naveca FG, Santos MAM dos, Slhessarenko RD. 2015. Detection of Oropouche virus segment S in patients and in *Culex quinquefasciatus* in the state of Mato Grosso, Brazil. *Mem Inst Oswaldo Cruz* 110:745–754.

Carvalho GC, dos Santos Malafronte R, Miti Izumisawa C, Souza Teixeira R, Natal L, Marrelli MT. 2014. Blood meal sources of mosquitoes captured in municipal parks in São Paulo, Brazil. *J Vector Ecol* 39:146–152.

Ceretti-Júnior W, Medeiros-Sousa AR, Multini LC, Urbinatti PR, Vendrami DP, Natal D, Marques S, Fernandes A, Ogata H, Marrelli MT. 2014. Immature mosquitoes in bamboo internodes in municipal parks, city of São Paulo, Brazil. *J Am Mosq Control Assoc* 30:268–274.

Ceretti-Junior W, de Oliveira Christe R, Rizzo M, Strobel RC, de Matos Junior MO, de Mello MHSH, Fernandes A, Medeiros-Sousa AR, de Carvalho GC, Marrelli MT. 2016. Species composition and ecological aspects of immature mosquitoes (Diptera: Culicidae) in bromeliads in urban parks in the city of São Paulo, Brazil. *J Arthropod Borne Dis* 10:102–112.

Chao A, Gotelli NJ, Hsieh TC, Sander EL, Ma KH, Colwell RK, Ellison AM. 2014. Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. *Ecol Monogr* 84:45–67.

Chaves LF, Hamer GL, Walker ED, Brown WM, Ruiz MO, Kitron UD. 2011. Climatic variability and landscape heterogeneity impact urban mosquito diversity and vector abundance and infection. *Ecosphere* 2:art70.

Consoli RAGB, Lourenço-de-Oliveira R. 1994. *Principais mosquitos de importância sanitária no Brasil* [Internet]. Rio de Janeiro, Brazil: Editora FIOCRUZ [accessed October 26, 2022]. Available from: https://static.scielo.org/scielobooks/th/pdf/consoli-9788575412909.pdf.

David MR, Ribeiro GS, Freitas RM de. 2012. Bionomics of *Culex quinquefasciatus* within urban areas of Rio de Janeiro, Southeastern Brazil. *Rev Saude Publica* 46(5):858–865.

Day JF, Stark LM. 2000. Frequency of Saint Louis Encephalitis virus in humans from Florida, USA: 1990–1999. *J Med Entomol* 37:626–633.

Diaz-Badillo A, Bolling BG, Perez-Ramirez G, Moore CG, Martinez-Munoz JP, Padilla Viveros AA, Camacho-Nuez M, Diaz-Perez A, Beaty BJ, de Lourdes Munoz M. 2011. The distribution of potential West Nile virus vectors, *Culex pipiens pipiens* and *Culex pipiens quinquefasciatus* (Diptera: Culicidae), in Mexico City. *Parasit Vectors* 4:70.

Faeth SH, Saari S, Bang C. 2012. Urban biodiversity: patterns, processes and implications for conservation. *eLS* [Internet]. John Wiley & Sons, Ltd: Chichester [accessed October 26, 2022]. Available from: https://onlinelibrary.wiley.com/doi/10.1002/9780470015902.a0023572.

Fattorini S. 2016. Insects and the city: what island biogeography tells us about insect conservation in urban areas. *Web Ecol* 16:41–45.

Ferreira-de-Lima VH, Andrade P dos S, Thomazelli LM, Marrelli MT, Urbinatti PR, Almeida RMM de S, Lima-Camara TN. 2020. Silent circulation of dengue virus in *Aedes albopictus* (Diptera: Culicidae) resulting from natural vertical transmission. *Sci Rep* 10:3855.

Forattini OP. 2002. *Culicidologia médica*. 1st edition. Volume 2. São Paulo, Brazil: EDUSP. 864 p.

Giry C, Roquebert B, Li-Pat-Yuen G, Gasque P, Jaffar-Bandjee M-C. 2017. Improved detection of genus-specific *Alphavirus* using a generic TaqMan® assay. *BMC Microbiol* 17:164.

Gubler DJ. 2011. Dengue, urbanization and globalization: the unholy trinity of the 21st century. *Trop Med Health* 39(Suppl):S3–S11.

Heinisch MRS, Diaz-Quijano FA, Chiaravalloti-Neto F, Pancetti FGM, Rocha RC, Andrade PS, Urbinatti PR, de Almeida RMMS, Lima-Camara TN. 2019. Seasonal and spatial distribution of *Aedes aegypti* and *Aedes albopictus* in a municipal urban park in São Paulo, SP, Brazil. *Acta Trop* 189:104–113.

Hill MO. 1973. Diversity and evenness: a unifying notation and it is consequences. *Ecology* 54:427–432.

Hsieh TC, Ma KH, Chao A. 2016. iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods Ecol Evol* 7:1451–1456.

IBGE [Instituto Brasileiro de Geografia e Estatistica]. 2021. *Panorama, população no último censo 2010* [Internet]. Rio de Janeiro, Brazil: Instituto Brasileiro de Geografia e Estatistica [accessed July 21, 2020]. Available from: https://cidades.ibge.gov.br/brasil/sp/sao-paulo/panorama.

INMET [Instituto Nacional de Metereologia]. 2021. *Banco de dados meteorológicos do Instituto Nacional de*

Metereologia [Internet]. Brasilia, Brazil: Instituto Nacional de Metereologia [accessed July 21, 2020]. Available from: https://bdmep.inmet.gov.br/.

Jemal Y, Al-Thukair AA. 2018. Combining GIS application and climatic factors for mosquito control in Eastern Province, Saudi Arabia. *Saudi J Biol Sci* 25:1593–1602.

Lambin EF, Tran A, Vanwambeke SO, Linard C, Soti V. 2010. Pathogenic landscapes: interactions between land, people, disease vectors, and their animal hosts. *Int J Health Geogr* 9:54.

Laporta GZ, Crivelaro TB, Vicentin EC, Amaro P, Branquinho MS, Sallum MAM. 2008. *Culex nigripalpus* (Theobald) (Diptera, Culicidae) feeding habit at the Parque Ecológico do Tietê, São Paulo, Brazil. *Rev Bras Entomol* 52:663–668.

Lima-Camara TN. 2016. Emerging arboviruses and public health challenges in Brazil. *Rev Saude Publica* 50:36.

Lima-Camara TN de, Honório NA, Lourenço-de-Oliveira R. 2006. Freqüência e distribuição espacial de *Aedes aegypti* e *Aedes albopictus* (Diptera, Culicidae) no Rio de Janeiro, Brasil. *Cad Saude Publica* 22:2079–2084.

Limnios G, Furlan SA. 2013. Parques urbanos no município de São Paulo - SP (Brasil): espacialização e demanda social. *Rev LabVerde* 6:173–189. https://doi.org/10.11606/issn.2179-2275.v0i6p173-189.

MacArthur RH, Wilson EO. 1967. *The theory of island biogeography*. Princeton, NJ: Princeton Univ. Press.

Mayi MPA, Bamou R, Djiappi-Tchamen B, Fontaine A, Jeffries CL, Walker T, Antonio-Nkondjio C, Cornel AJ, Tchuinkam T. 2020. Habitat and seasonality affect mosquito community composition in the West Region of Cameroon. *Insects* 11:312.

Medeiros-Sousa AR, Ceretti W, Urbinatti PR, De Carvalho GC, De Paula MB, Fernandes A, Matos MO, Orico LD, Araujo AB, Nardi MS, Marrelli MT. 2013a. Mosquito fauna in municipal parks of São Paulo city, Brazil: a preliminary survey. *J Am Mosq Control Assoc* 29:275–279.

Medeiros-Sousa AR, Ceretti-Júnior W, de Carvalho GC, Nardi MS, Araujo AB, Vendrami DP, Marrelli MT. 2015. Diversity and abundance of mosquitoes (Diptera:Culicidae) in an urban park: larval habitats and temporal variation. *Acta Trop* 150:200–209.

Medeiros-Sousa AR, Ceretti-Junior W, Urbinatti PR, Natal D, Carvalho GC de, Paula MB de, Fernandes A, Mello MHSH de, Oliveira RC de, Orico LD, Maurelli MT. 2013b. Biodiversidade de mosquitos (Diptera: Culicidae) nos parques da cidade de São Paulo I. *Biota Neotrop* 13:317–321.

Medeiros-Sousa AR, Fernandes A, Ceretti-Junior W, Wilke ABB, Marrelli MT. 2017. Mosquitoes in urban green spaces: using an island biogeographic approach to identify drivers of species richness and composition. *Sci Rep* 7:17826.

Medeiros-Sousa AR, de Oliveira-Christe R, Camargo AA, Scinachi CA, Milani GM, Urbinatti PR, Natal D, Ceretti-Junior W, Marrelli MT. 2020. Influence of water's physical and chemical parameters on mosquito (Diptera: Culicidae) assemblages in larval habitats in urban parks of São Paulo, Brazil. *Acta Trop* 205:105394.

Mitchell CJ, Forattini OP. 1984. Experimental transmission of Rocio encephalitis virus by *Aedes scapularis* (Diptera: Culicidae) from the epidemic zone in Brazil. *J Med Entomol* 21:34–37.

Nasci RS. 1981. A lightweight battery-powered aspirator for collecting resting mosquitoes in the field. *Mosq News* 41:808–811.

Patel P, Landt O, Kaiser M, Faye O, Koppe T, Lass U, Sall AA, Niedrig M. 2013. Development of one-step quantitative reverse transcription PCR for the rapid detection of *Flaviviruses*. *Virol J* 10:58.

Paula MB de, Fernandes A, Medeiros-Sousa AR, Ceretti-Júnior W, Christe R, Stroebel RC, Pedrosa L, Almeida RMM de S, Carvalho GC de, Pereira UD, Jacintho MCO, Natal D, Marelli MT. 2015. Mosquito (Diptera: Culicidae) fauna in parks in greater São Paulo, Brazil. *Biota Neotrop* 15:e20140026. https://doi.org/10.1590/1676-0611-BN-2014-0026

Pauvolid-Correa A, Kenney JL, Couto-Lima D, Campos ZMS, Schatzmayr HG, Nogueira RMR, Brault AC, Komar N. 2013. Correction: Ilheus virus isolation in the Pantanal, West-Central Brazil. *PLoS Negl Trop Dis* 7:e2318. https://doi.org/10.1371/annotation/13ca0354-e5eb-42bd-b99c-606fe873df2c

Reinert JE. 2001. Revised list of abbreviations for genera and subgenera of Culicidae (Diptera) and notes on generic and subgeneric changes. *J Am Mosq Control Assoc* 17:51–55.

R Foundation for Statistical Computing. 2019. *R: a language and environment for statistical computing* [Internet]. Vienna, Austria. R Development Core Team [accessed October 8, 2019]. Available from: https://www.r-project.org.

Shochat E, Warren P, Faeth S, Mcintyre N, Hope D. 2006. From patterns to emerging processes in mechanistic urban ecology. *Trends Ecol Evol* 21:186–191.

Silver JB. 2008. *Mosquito ecology: field sampling methods*. 3rd edition. New York, NY: Springer Science and Business Media.

SUS/PMSP [Sistema Único de Saúde da Prefeitura Municipal de São Paulo]. 2016. *Coordenação de Vigilância em Saúde Plano de Contingência Municipal da Dengue: 2015/2016* [Internet]. São Paulo, Brazil: Sistema Único de Saúde da Prefeitura Municipal de São Paulo [accessed August 25, 2022]. Available from: https://www.prefeitura.sp.gov.br/cidade/secretarias/upload/chamadas/plano_contigencia_ final_1462895236.pdf.

SVMA/PMSP [Secretaria do Verde e Meio Ambiente da Prefeitura Municipal de São Paulo]. 2014. *Guia dos parques municipais de São Paulo* [Internet]. 4th edition. São Paulo, Brazil: Secretaria do Verde e Meio Ambiente da Prefeitura Municipal de São Paulo [accessed July 21, 2020]. Available from: https://www.prefeitura.sp.gov.br/cidade/secretarias/upload/meio_ambiente/arquivos/guia-parques-municipais.pdf.

Tabachnick W. 2013. Nature, nurture and evolution of intra-species variation in mosquito arbovirus transmission competence. *Int J Environ Res Public Health* 10:249–277.

Vieira CJ da SP, Thies SF, da Silva DJF, Kubiszeski JR, Barreto ES, Monteiro HA de O, Mondini A, São Bernardo CS, Bronzoni RV de M. 2020. Ecological aspects of potential arbovirus vectors (Diptera: Culicidae) in an urban landscape of Southern Amazon, Brazil. *Acta Trop* 202:105276.

Wilke ABB, Medeiros-Sousa AR, Ceretti-Junior W, Marrelli MT. 2017. Mosquito populations dynamics associated with climate variations. *Acta Trop* 166:343–350.