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# MOSQUITO COMMUNITIES IN NOVA IGUAÇU NATURAL PARK, RIO DE JANEIRO, BRAZIL

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ABSTRACT. In order to gather information on the culicid fauna of Nova Iguaçu Municipal Park, Rio de Janeiro, Brazil, adult and immature stages were collected with the Centers for Disease Control and Prevention miniature light traps, and dippers and suction tubes, respectively. In all, 828 adult and 990 immature specimens were collected belonging to 12 genera. Among the species collected were Aedes aegypti, Ae. albopictus, Ae. fluviatilis, Ae. scapularis, Haemagogus leucocelaenus, and Psorophora ferox that are considered of potential medical importance. Culicids used a variety of larval habitats and bred under diversion, rock pool, stream, tree hole, and in artificial containers formed by bamboo, bromeliad, ground depression, rock pool, stream, tree hole, and in artificial containers such as abandoned bathtub, car carcass, abandoned sink, plastic cup, waste tire, and water tank. Species richness and diversity increased from lower to higher forest cover and was highest in sites with highest diversity and high number of larval habitats.

KEY WORDS Mosquitoes, immature mosquitoes, adults, light traps, natural park

#### INTRODUCTION

The mosquito faunal inventory of natural environmental areas is of considerable importance in ecological studies. Faunal inventories assess in part the components of animal diversity in a biome or locality, in a determined space and time. They are relevant not only to understand ecosystems, but also are among the pillars upon which the decision-making processes are based in relation to undertakings that will impact the environment (Silveira et al. 2010). Moreover, knowledge of the mosquito fauna is essential to understanding the risk of mosquito-borne diseases, and its utility in vector control planning and/ or decision making.

In Brazil, studies on the ecology of mosquitoes in natural preservation areas are incipient. Since the relevance of knowing the mosquito fauna is mainly associated with their role in pathogen transmission to humans and other vertebrates (Montes 2005), most studies involving mosquitoes are performed as a function of the occurrence of a disease or of the presence of their vectors in risk areas (Guimarães and Arlé 1984). Research on the

diversity of Culicidae in their natural environment may clarify habits still unknown for these vectors (Hutchings et al. 2005).

The knowledge of the biodiversity of mosquitoes in forest ecosystems is of great importance for the implementation of eco-epidemiological analyses on insect vectors of disease. Although research on the ecological aspects of mosquitoes is being carried out in various areas of the Brazilian territory, in Rio de Janeiro State they are always conducted as a function of the presence of vectors in the urban environment (Alencar et al. 2011).

According to Peixoto (1992), even though the Mata Atlântica is considered as one of the regions of highest biodiversity in the world, paradoxically it is among the most highly endangered forest environments, and currently in these biomes <8% remains of its original composition. The unplanned urban growth, increases in agricultural frontiers, energy requirements and related dam building, and deforestation can influence the increases of some vector-borne diseases (Service 1993).

Given the fact that the study area is considered an environmental preservation area and that it is extremely close to the urban border, the objective of the present work was to collect information on the species diversity of mosquitoes, assessing richness, dominance, abundance, and similarity in different environments of Nova Iguaçu Municipal Park, Rio de Janeiro, Brazil.

### MATERIALS AND METHODS

# Description of the study area

Nova Iguaçu Municipal Park is located on the western border of the Mendanha massif, and is included in the natural protection area of

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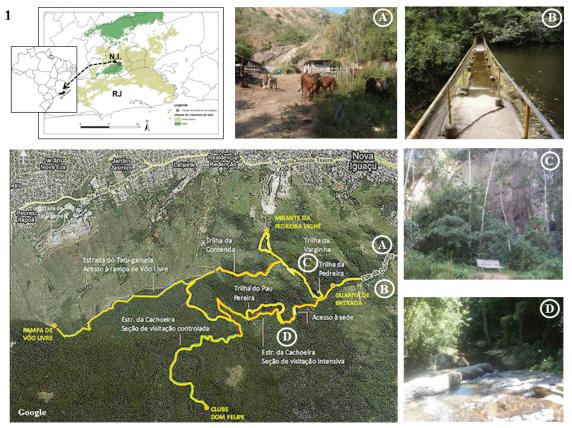


Fig. 1. Location of study area and sampling sites within Nova Iguaçu Natural Park, Rio de Janeiro, Brazil.

Gericinó-Mendanha. It has an area of 11 km<sup>2</sup>, an altitude ranging from 150 m at the entrance of the park to 956 m at the southwestern border, next to Gericinó peak. Mean temperature ranges from 20 to 27°C and annual rainfall is above 1,300 mm. The predominant vegetation is Atlantic Forest, and includes some rare or endangered plant species such as Dalbergia nigra (Vell.) Allem ex Benth, Mezilaurus navalium (Allem.) Taub., Hymenaea courbaril var. altissima L., Virola gardneri Warb., V. oleifera (Schott), Copaifera trapezifolia Hayne, C. lucens Dwyer, Enterollobium glaziovii (Benth.) Mesquita, and Peltogyne angustiflora Ducke, among others. The animal fauna consists of species such as Felis tigrina Thomas, Agouti paca L., Tayssu pecari Link, Bradypus torquatus Illiger, Penelope superciliaris Temminck, Chiroxiphia caudata (Shaw and Nodder), Pyrrhura frontalis (Vieillot), Baillonius bailloni (Vieillot), and Ramphastos vitellinus Lichtenstein. According to the Köppen climate classification based on average temperature and precipitation values, the climate of the region is generically classified as Cw, which is a mild temperate climate with dry winter and hot summer. In the summer, heavy rains occur (December to March) in combination with great heat, not mitigated by winds, and high humidity; while in the winter, the rainfall totals are low.

For our study, 4 sampling sites along a disturbance gradient were established: Site A (corral—22°46′29.0″S, 43°26′51.3″W), 84 above sea level, is located approximately 200 m from the residential area; its forest cover is degraded due to agricultural activity, even in the hilltops, and vegetation is sparse and formed mainly by small trees; Site B (water reservoir— 22°46′47.7″S, 43°27′23.9″W), 98 m above sea level, is located in close proximity to a water reservoir, and riparian vegetation mostly presents medium-sized trees, with no sharp visual impact; Site C (seawall—22°46′47.6″S, 43°27′33.3″W), 193 m above sea level, is localized next to a hillside rock formation, presenting some transient streams and low tree cover; and Site D (headquarters—22°46′59.7″S, 43°27′43.8″W), 158 m above sea level, has a dense cover of medium to large trees and is located next to 2 permanent streams (Fig. 1).

#### Mosquito collections

Mosquitoes were sampled monthly in each site from February 2012 to March 2013. Adults were

collected with automatic Centers for Disease Control and Prevention miniature light traps that were activated from 1800 h to 1000 h once each month. The presence of immature stages was investigated in probable natural developmental sites (ground pools, tree holes, bromeliads, stream, ground depressions, rock pools, internodes of bamboo culms), and artificial sources (car carcass, water tank, plastic cup, tire). Water samples were collected with a dipper (from openwater habitats and large containers) and suction tubes (from tree holes and other phytotelmata) (Silver 2008), from 1000 h to 1600 h 1 day per month per site. Water samples so collected were poured into polyethylene trays, and larvae and pupae were collected with the aid of a soft brush and transported to the laboratory in 250-ml plastic bags (Whirl-Pak Bags®, San Diego, CA). Each sample was identified by site, date, and larval habitat type. In the laboratory larvae were screened and transferred to small individual bowls, where they were maintained in water from the collection site and periodically supplemented with distilled water, and allowed to complete their development. Exuviae and larvae that did not complete their development were fixed in 70% glacial ethanol and mounted on a microscope glass slide with cover slips in Canada balsam for species identification. Pupae were transferred to small containers until their emergence to adults.

The species determination was performed by direct observation of morphological characters under a stereomicroscope, using the dichotomous keys by Lane and Cerqueira (1942), Lane (1953), Faran and Linthicum (1981), Consoli and Lourenço-de-Oliveira (1994), and Forattini (2002). Genera and subgenera were abbreviated according to Reinert (2001) and WRBU (2013). After the specific determination, all specimens were deposited in the Entomological Collection of the Instituto Oswaldo Cruz, Fiocruz, under the title of "Nova Iguaçu Natural Park, Rio de Janeiro, Brasil"

#### Data analysis

For species diversity, the following diversity indices were used: Species richness (S, number of taxa); Dominance (= 1 - Simpson index), a measurement of "unevenness" of the community ranging from 0 when all taxa are equally present to 1 or when one taxon dominates the community completely; and Shannon index (H), which varies from 0 for communities with one single taxon to high values for communities with many taxa, each with few individuals (Hammer et al. 2001, Magurran 2004). Diversity was compared between sites using the "Compare diversity" module of Past software that computes a number of diversity indices for 2 samples, and then compares the diversities using bootstrapping and permuta-

tion as 2 different randomization procedures (Hammer et al. 2001). Species accumulation curves (sample rarefaction using the Mao tau analytical solution [Hammer et al. 2001]) were also used for larvae, adults, and larvae + adults combined, to observe the asymptotic trends of the number of species in the park. The curves were modeled and extrapolated using the Michaelis—Menten equation.

Similarities in species composition between pairs of sampling sites were assessed using Sörensen's (= Dice) similarity index and Bray-Curtis Measure (Magurran 2004). Sörensen's similarity is considered a very effective presence/absence similarity measure, while Bray-Curtis takes into account the relative abundance of the species.

Sörenson's similarity = 
$$2C/(S_A + S_B)$$

where C is the number of species shared by both sites, and  $S_A$  and  $S_B$  are, respectively, the number of species in Site A and Site B.

Bray-Curtis Measure = 
$$2S/(n_A + n_B)$$

where S is the sum of the absolute difference in number of specimens per species from sites A and B, and  $n_A$  is the total number of individuals from Site A and  $n_B$  the total number of specimens from Site B.

Species dominance or relative abundance, was estimated as  $D\% = (i/t) \times 100$ , where i = total number of individuals of a given species and t = total number of individuals collected (of all species).

To evaluate if mosquito diversity was related to larval habitat diversity or availability, indices of larval habitat diversity were correlated with mosquito diversity indices using either linear regressions or Spearman's nonparametric correlations.

#### **RESULTS**

A total of 1,818 mosquitoes were collected during 26 sampling days (24 days in 2012 and 2 days in 2013), comprising 31 species from 12 genera (Tables 1 and 2). More species were collected in their immature stages (29 species belonging to 12 genera) than as adults (16 species from 9 genera). Anopheles, Psorophora, and Toxorhynchites species were only collected in their larval stages. Aedes albopictus (Skuse), Culex declarator Dyar and Knab, Haemagogus leucocelaenus (Dyar and Shannon), Limatus durhamii Theobald, and Ae. scapularis (Rondani) were dominant as larvae and as adults in at least one site. Aedes aegypti (L.) was found only at Site A, where it was dominant (>10%) as adult.

Species accumulation curves were estimated for larvae, adult, and larvae + adult collections, considering total collections from each site as a

Table 1. Total number (%) of mosquito immature (larvae and/or pupae) specimens collected from February 2012 to March 2013, in 4 sites in Nova Iguaçu Natural Park, Rio de Janeiro, and diversity estimates for each site.

	Site A	Site B	Site C	Site D	Total
Species					
Aedes aegypti	3 (0.8)				3 (0.3)
Ae. albopictus	67 (18.4)			44 (13.3)	111 (11.2)
Ae. fluviatilis	55 (15.1)			11 (3.3)	66 (6.7)
Ae. rhyacophilus	13 (3.6)				13 (1.3)
Ae. scapularis	23 (6.3)			45 (13.6)	68 (6.9)
Ae. serratus			6 (2.5)		6 (0.6)
Ae. taeniorhynchus	21 (5.8)				21 (2.1)
Ae. terrens			17 (7.1)	19 (5.7)	36 (3.6)
Anopheles eiseni				20 (6.0)	20 (2.0)
An. tibiamaculatus			7 (2.9)		7 (0.7)
An. evansae		7 (12.5)	3 (1.3)	8 (2.4)	18 (1.8)
Coquilletidia chrysonotum			5 (2.1)		5 (0.5)
Culex (Culex) sp. 1			1 (0.4)	1 (0.3)	2 (0.2)
Culex (Cux.) sp. 2			1 (0.4)		1 (0.1)
Culex (Cux.) sp. 3				1 (0.3)	1 (0.1)
Culex (Microculex) sp.				2 (0.6)	2 (0.2)
Culex (Melanoconion) sp.				1 (0.3)	1 (0.1)
Cx. coronator	56 (15.3)		30 (12.6)	8 (2.4)	94 (9.5)
Cx. declarator	96 (26.3)		52 (21.8)	37 (11.2)	185 (18.7)
Cx. usquatus		49 (87.5)		29 (8.8)	78 (7.9)
Haemagogus leucocelaenus				52 (15.7)	52 (5.3)
Limatus durhamii	24 (6.6)		72 (30.3)		96 (9.7)
Lutzia bigoti	7 (1.9)			16 (4.8)	23 (2.3)
Psorophora cingulata			10 (4.2)		10 (1.0)
Ps. ferox			28 (11.8)	9 (2.7)	37 (3.7)
Runchomyia frontosa				7 (2.1)	7 (0.7)
Toxorhynchites (Lynchiella) sp.			5 (1.8)	3 (0.9)	8 (0.8)
Wyeomyia aphobema			1 (0.4)		1 (0.1)
Wyeomyia sp.				18 (5.4)	18 (1.8)
Index <sup>1</sup>					
Individuals	365 a	56 b	238 с	331 d	
Richness (S)	10 a	2 b	14 ac	19 c	
Dominance	0.16 a	0.78 b	0.18 a	0.10 c	
Shannon H	1.99 a	0.38 b	2.01 a	2.53 c	

<sup>&</sup>lt;sup>1</sup> For each row, sites not sharing a lowercase letter are significantly different (P < 0.05) in that diversity index.

sample (Fig. 2). The curves indicated that, although expected species richness was higher than observed in the current study, the sampling provided an adequate representation of species diversity, since the number of species increased from 3 to 4 samples by 10.5% for adults, 14.7% for larvae, and 12.1% for larvae and adult samples combined. Curve extrapolations using the Michaelis-Menten equation indicated that the species accumulation curve reached an asymptote approximately at 54 species for larvae and larvae + adult curves (at around 35 samples), and at 23 species for adult collections (around 30 samples). These values may overestimate species richness because the sites were selected not randomly but to represent differing environmental conditions within the park.

Overall, the highest total number of mosquito specimens were collected from Sites A and D, while Site B had the lowest collections and also lowest number and diversity of larval habitats (Table 3). Significantly more species were found

as larvae in Sites C and D; Site D was the most diverse (as estimated by Shannon index) and with the lowest species dominance (Table 1). Excluding Site B, larval species richness and diversity significantly increased from Site A to D, while dominance decreased. Site B had the lowest species richness and highest dominance, consistent with a similar pattern observed in larval habitat types (Table 3). Adult mosquito richness and diversity also increased from Sites A to D (excluding Site B); dominance was more similar between sites and fewer significant differences were detected between pairs of sites (Table 2).

Regarding larval habitats, 12 different types were detected (Table 3), including artificial containers, natural water bodies, and phytotelmata. Site B had the lowest diversity of larval habitats (only streams), while other sites had 6 to 8 different types that were relatively evenly represented (dominance ranged from 0.20 to 0.31). Excluding Site B, larval habitat types and diversity significantly decreased from Sites A to

Table 2. Total number (%) of adult specimens collected from February 2012 to March 2013, in 4 sites in Nova Iguaçu Natural Park, Rio de Janeiro, and diversity estimates for each site.

Site A Site B Site C Site D Total

	Site A	Site B	Site C	Site D	Total
Species					
Aedes aegypti	24 (12.6)				24 (2.9)
Ae. albopictus	33 (17.3)	9 (23.7)		62 (12.8)	104 (12.6)
Ae. scapularis	32 (16.8)	11 (28.9)	28 (24.1)	41 (8.5)	112 (13.5)
Ae. serratus			14 (12.1)		14 (1.7)
Ae. terrens				12 (2.5)	12 (1.4)
Coquilletidia chrysonotum			7 (6.0)		7 (0.8)
Culex (Culex) declarator	1 (0.5)		48 (41.4)	12 (2.5)	61 (7.4)
Culex (Cux.) sp.	20 (10.5)		7 (6.0)	17 (3.5)	44 (5.3)
Culex (Microculex) sp.		11 (28.9)		6 (1.2)	17 (2.1)
Haemagogus leucocelaenus	62 (32.5)			199 (41.2)	261 (31.5)
Lutzia bigoti			8 (6.9)	11 (2.3)	19 (2.3)
Limatus durhamii	19 (9.9)	7 (18.4)		45 (9.3)	71 (8.6)
Runchomyia frontosa				43 (8.9)	43 (5.2)
Wyeomyia pilicauda			4 (3.4)	25 (5.2)	29 (3.5)
Wy. aporonoma				8 (1.7)	8 (1.0)
Wy. dyari				2 (0.4)	2 (0.2)
Index <sup>1</sup>					
Individuals	191 a	38 b	116 c	483 d	
Richness (S)	7 a	4 b	7 a	13 c	
Dominance	0.20 a	0.26 ab	0.26 b	0.22 ab	
Shannon H	1.72 a	1.37 b	1.60 ab	1.96 c	

<sup>&</sup>lt;sup>1</sup> For each row, sites not sharing a lowercase letter are significantly different (P < 0.05) in that diversity index.

D, while dominance increased. The proportion of natural larval habitats increased from Site A (34% of samples) to D (99% of samples) in accordance with further distance from urban areas.

Pairs of Sites D–C and A–D were more similar in larval species composition (Table 4, Sorenson's index). The same pattern was observed when comparing larval habitat types between pairs of sites. Moreover, a significant linear relation was detected between shared (similar) larval habitats and shared mosquito species between pairs of sites (each estimated by Sorenson's index;  $r^2 = 0.99$ ; P < 0.01) (Fig. 2); Sites A–B did not share common larval habitat types nor larval species. On the other hand, Sites A–D were more similar when the number of specimens per species were

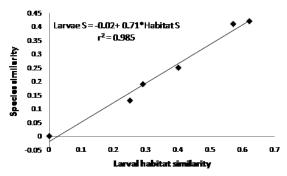


Fig. 2. Relation between larval species similarity and larval habitat similarity of pairs of sites as measured with Sorenson's index of beta diversity.

also considered (Table 4, Bray-Curtis Measure). Sites B-D were more similar in number of common habitats shared (streams, Bray-Curtis Measure). Highest similarity in species composition was also observed between Sites A and D adult data.

When comparing larvae and adult species collected in each site, 59% of the species were collected as larvae and adults in Site A (as indicated by Sorenson's index expressed as percentage), 44% in Site D, 29% in Site C, while there was no coincidence in species collected as larvae and adults in Site B.

# **DISCUSSION**

In all, 31 mosquito species in 12 genera were recorded in Nova Iguaçu Natural Park, including species of reported medical importance such as Ae. albopictus, Hg. leucocelaenus, and Ae. scapularis, that were dominant (>10%) as larvae and/ or as adults. Aedes albopictus is an invasive species originally from Western Pacific and Southeast Asia that is a competent vector of several arboviruses, including dengue viruses (Gratz 2004, Rezza 2012). Haemagogus leucocelaenus is a common epidemiologically important mosquito in Brazil due to its role in the transmission of yellow fever (Kumm and Cerqueira 1961, Vasconcelos et al. 2003, Cardoso et al. 2010), and potential involvement as vector of Ilhéus, Maguari, Tucunduba, and Una viruses (Karabatsos 1985, Hervé et al. 1986). Aedes scapularis is a good vector of the dog heartworm,

Table 3. Total number of larval habitats per type, in each sampling site from February 2012 to March 2013, in Nova Iguaçu Natural Park, Rio de Janeiro, and estimates of their diversity.

Type of larval habitat	Site A	Site B	Site C	Site D
Bamboo culms	_	_	15	_
Bromeliad			1	32
Tree hole	7		30	20
Depression in the ground	93		85	91
Rock pool	21		_	34
Stream	_	34	94	157
Abandoned bathtub	41	_	_	_
Abandoned sink	75	_	39	_
Car carcass	21	_	_	_
Tire	88	_	_	_
Water tank	10	_	_	3
Plastic cup	_	_	19	_
Total habitat <sup>1</sup>	356 a	34 b	283 с	337 d
Habitat types (S)	8 a	1 b	7 ac	6 c
Dominance	0.20 a	1 b	0.24 c	0.31 d
Shannon (H)	1.78 a	0 b	1.59 c	1.37 d

<sup>&</sup>lt;sup>1</sup> For each row, sites not sharing a lowercase letter are significantly different (P < 0.05) in that diversity index.

Dirofilaria immitis Leidy, in Rio de Janeiro State (Labarthe et al. 1998). At least 15 viruses have been isolated from this species—it is a suspected vector of yellow fever and Rocio encephalitis (Forattini et al. 1995, Forattini 2002), and has a tendency to adapt to residential areas (Forattini et al. 1989); therefore, it is potentially very important in the transmission of arbovirus to humans.

Aedes aegypti, the main vector of dengue viruses, was found only at Site A, where it was relatively abundant (12.6%) as adult. Interestingly, this site was the most proximate to urban areas and also to housing linked to the corral. Contrastingly, immature mosquitoes were rare (<1%) even though artificial containers were abundant, including tires often listed as typical Ae. aegypti larval habitat. This suggests that mosquitoes may be breeding closer to the housing area and disperse to trails and corral in search of a blood meal. A recent study in Rio de Janeiro State

found that Ae. aegypti females might disperse as far as 800 m from a release point (Honório et al. 2003); thus, finding the mosquitoes near housing is consistent with observations in other geographic areas linking the species to urban environments. On the other hand, Ae. albopictus, a species that uses similar larval habitats as Ae. aegypti and may exploit both urban and sylvan environments (Hawley 1988), was dominant both as larvae and pupae in most sites.

More species were detected with larval survey compared to adult collections (and correspondingly expected species richness based on species accumulation curves was higher for the former), which might be related to the sampling strategies used, since light traps are not equally attractive to all mosquito species (Silver 2008). Overall, species richness and diversity increased from Site A to D, matching an increase in sylvan habitat. On the other hand, Sites A and D showed the highest

Table 4. Site similarities in larval mosquitoes, larval habitats, and adult mosquitoes detected from February 2012 to March 2013, in 4 sites in Nova Iguaçu Natural Park, Rio de Janeiro, based on Sörensen's similarity index and Bray–Curtis Measure.

Site	Sörensen's similarity index			Bray-Curtis Measure		
	Site A	Site B	Site C	Site A	Site B	Site C
Larval mosquito	es					
Site B	0.00			0.00		
Site C	0.25	0.13		0.39	0.03	
Site D	0.41	0.19	0.42	0.59	0.11	0.23
Larval habitat ty	pes					
Site B	0.00			0.00		
Site C	0.40	0.25		0.21	0.38	
Site D	0.57	0.29	0.62	0.10	0.66	0.35
Adult mosquitoe	S					
Site B	0.55			0.50		
Site C	0.43	0.18		0.42	0.22	
Site D	0.60	0.47	0.50	0.64	0.48	0.28

similarity in species composition, sharing 41% of larvae and 60% of adult species detected (Sörensen's similarity index, Table 4), which could be explained by the shared larval habitat types available at both sites (57% of habitat types). Site B, covered mostly by riparian vegetation, had the lowest collections and also lowest number and diversity of larval habitats.

Interestingly, in Site B species collected with light traps differed from those recorded through larval sampling. Larval habitats found in this site were streams, and the only larval species detected were An. evansae (Brèthes) and Cx. usquatus Dyar, the former reported to breed in marshes and marshy margins of clear freshwater pools, streams, and lakes (Belkin et al. 1968), but also found in eutrophized breeding habitats (Wermelinger et al. 2010). Little is known about the larval habitat characteristics of Cx. usquatus, whose larvae have been collected in old boats, in a fallen tree, in eutrophized puddles, and in water barrels near houses in Suriname (Dyar 1917), but adults have been collected in forest environments in Brazil (Barbosa et al. 2008). Adult mosquitoes included container- and phytotelmata-living Ae. albopictus, Li. durhamii, and Cx. (Microculex) species that probably dispersed from the nearby forest habitat, and the floodwater mosquito Ae. scapularis.

Overall, Sörensen's similarity index and Bray–Curtis Measure were consistent when comparing adult collections between sites, indicating that species in common between sites were also similarly abundant; for example, see pairs of Sites A–B, A–C, B–D (Table 4). Sites C–D shared 42% larval and 50% adults, but the total collections were higher in Site D as was also reflected in a lower Bray–Curtis Measure value), where, for example, 3.5 to 4.3 more adult specimens of *Ae. albopictus*, *Ae. scapularis*, or *Hg. leucocelaenus* were collected.

The finding of potential vectors of arboviral diseases in different environments within the park should be taken into account both for disease surveillance and to highlight the importance of making recommendations to visitors to take personal protection measures against mosquito biting.

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