

# Ecological aspects of mosquitoes (Diptera: Culicidae) in the gallery forest of Brasília National Park, Brazil, with an emphasis on potential vectors of yellow fever

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## ABSTRACT

**Introduction:** We analyzed the vertical and monthly distributions of culicid species in the gallery forest of Brasília National Park, with an emphasis on the potential vectors of yellow fever (YF). **Methods:** Between September 2010 and August 2011, mosquitoes were captured on the ground and in the canopy of the forest for five consecutive days per month, from nine to 15 hours. The mosquitoes were examined to verify natural infection with flaviviruses by isolation in *Aedes albopictus* Skuse, 1864 cells followed by indirect immunofluorescence. **Results:** We identified 2,677 culicids distributed in 29 species. Most of the mosquitoes were captured at ground level (69%) during the rainy season (86%). The most abundant species were *Sabethes (Sabethes) albiprivus* Theobald, 1903; *Limatus durhamii* Theobald, 1901; *Haemagogus (Conopostegus) leucocelaenus* Dyar & Shannon, 1924; *Haemagogus (Haemagogus) janthinomys* Dyar, 1921; *Aedes (Ochlerotatus) scapularis* Rondani, 1848; *Psorophora (Janthinosoma) ferox* Von Humboldt, 1819; and *Aedes (Ochlerotatus) serratus* Theobald, 1901. *Haemagogus janthinomys*, *Limatus durhamii*, *Psorophora ferox*, *Aedes scapularis* and *Aedes serratus* showed significant differences ( $p < 0.05$ ) in their habitat use. *Haemagogus janthinomys* was found more often in the canopy, unlike the other species. During the rainy season, the most abundant species were *Sa. albiprivus*, *Haemagogus leucocelaenus* and *Haemagogus janthinomys*. During the dry season, the potential YF vectors exhibited a very low frequency and abundance, except *Aedes scapularis* and *Aedes serratus*. No flavivirus was detected in the 2,677 examined mosquitoes. **Conclusions:** We recommend continued and systematic entomological monitoring in areas vulnerable to the transmission of YF in the Federal District of Brazil.

**Keywords:** Flavivirus. *Haemagogus*. Mosquitoes. Vector ecology.

## INTRODUCTION

Mosquitoes have been widely studied because of their role in the transmission of tropical diseases, such as malaria, dengue and yellow fever<sup>1</sup>. According to Mathers et al.<sup>2</sup>, infectious diseases transmitted by mosquitoes have a high impact on public health and are responsible for approximately 15% of all disability adjusted life years (DALYs) attributed to infectious and parasitic diseases throughout the world.

Yellow fever (YF) is an acute febrile illness that results from infection by an arbovirus of the genus *Flavivirus*<sup>3</sup> that remains

endemic or enzootic in Africa and South and Central America<sup>1,4,5</sup>. In the urban cycle of YF, humans are the only known vertebrate host of the virus, and it is transmitted by the vector *Aedes (Stegomyia) aegypti* Linnaeus, 1762<sup>6</sup>. In contrast, sylvatic YF is primarily associated with nonhuman primates (NHP), and its vectors are female mosquitoes of the genus *Haemagogus* Williston, 1876, *Sabethes* Robineau-Desvoidy 1827 and *Aedes* Meigen 1818 in South and Central America<sup>7,8</sup>.

According to Taui<sup>9</sup>, Brazil is the country with the largest endemic area of sylvatic YF in the world, at approximately 5 million km<sup>2</sup>. Between the years 2000 and 2009, a total of 320 human cases of YF, including 152 deaths, were recorded in 15 states. Between 2007 and 2009, outbreaks of sylvatic YF and epidemics in the NHP population occurred in several Brazilian states, primarily in the extra-Amazonian region<sup>10,11</sup>.

During the last outbreak (2007-2008) in the Federal District (FD), 90 human cases of YF were suspected, 15 of which were confirmed to be sylvatic YF: two were in 2007 (one was autochthonous) (SINAN-net), and 13 were in 2008 (six were autochthonous)<sup>12</sup>. During the same period, the deaths of several

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NHP cases were reported, three of which were attributed to YF<sup>11</sup>. Entomological investigations discovered mosquitoes related to the transmission of this arbovirus, and the YF virus was isolated from *Haemagogus (Haemagogus) janthinomys* Dyar, 1921 and *Haemagogus (Conopostegus) leucocelaenus* Dyar & Shannon, 1924<sup>13</sup>.

The Brasilia National Park (BNP) is a tourist destination that receives approximately 260,000 visitors a year. This preservation area contains populations of NHPs<sup>14</sup> and mosquitoes of the genus *Sabethes* and *Haemagogus*<sup>13,15</sup>. It is noteworthy that during the outbreak of sylvatic YF that occurred between 2007 and 2008 in the FD, multiple NHPs died in the BNP<sup>13,15</sup>. Therefore, it is important to understand the ecological aspects of the mosquito species in the BNP, with an emphasis on the YF potential vectors. Moreover, the detection of flaviviruses in mosquitoes captured in this conservation unit is important information for the surveillance of this arbovirus in the FD.

In this context, the objectives of the present study were the following: I) to analyze the richness and abundance of the mosquitoes captured during different seasons in different strata of the gallery forest of the BNP, with an emphasis on potential YF vectors; and II) to determine the percentage of the captured mosquitoes that are infected with a flavivirus.

## METHODS

### Study area

The FD is located in the Central-West region of Brazil between the parallels 15° 30' and 16° 03' south latitude and the meridians 47° 25' and 48° 12' west longitude, within the Cerrado biome. The average annual temperature ranges from 18°C to 22°C. The average annual rainfall varies between 1,200mm and 1,700mm<sup>16</sup>, but it is unevenly distributed over the year, with a well-defined dry season. The monthly distribution of rainfall accumulated in the FD can be grouped into four quarterly periods. The months between June and August register an average rainfall of zero (the dry period). In April, May and September, there is an average rainfall of 70mm (the intermediate period). Between October and December, an average rainfall of 216mm is observed (the first quarter of rainfall), and between January and March, an average rainfall of 220mm is observed (the second quarter of rainfall)<sup>17</sup>.

The BNP, which is administered by the *Instituto Chico Mendes de Conservação da Biodiversidade* (ICMBio), is an approximately 42,389-hectare environmental conservation area that contains a great diversity of flora and fauna. This park is also a leisure option for residents of the City of Brasilia, and its pools serve as the main attractions. The BNP is situated in the northwestern portion of the FD, approximately 10 km from the city center<sup>14</sup> and just 2km from the *Setor Habitacional Noroeste* (SHN), whose construction began at the end of 2009<sup>18</sup>.

Two capture areas in the gallery forest near the old pool of the BNP were chosen, with a distance of 200m from one another (Area 1 coordinates: latitude 15° 44'13.5"S and longitude 47° 55'37.6"W; Area 2 coordinates: latitude 15° 44 '18.6"S

and longitude 47° 55'41.6"W). The choice of these areas was based on the presence of suitable trees for the construction of platforms. In each area, a platform was built 6m above the ground.

### Mosquito capture

Mosquitoes were captured using entomological netting and manual aspirators<sup>19</sup>. From September 2010 to August 2011, adult mosquitoes were captured each month for five consecutive days from 9a.m. to 3p.m. with a one hour interval between noon and 1p.m., for a total capture effort of 60 days. Entomological capture is a routine activity of the *Diretoria de Vigilância Ambiental em Saúde do Distrito Federal* (DIVAL) and was performed by health agents who were properly immunized and trained for this purpose. Using proper safety procedures and personal protective equipment, two individuals worked simultaneously in each sampling area; one on the ground and another on the platform 6m above the ground.

### Mosquito identification

At the end of the daily activities, the captured specimens were taken to the DIVAL's entomology laboratory and anesthetized by cooling to -6°C. The specimens were transferred to labeled tubes and kept in liquid nitrogen.

The captured mosquitoes were identified with the aid of the dichotomous keys available from Consoli and Lourenço-de-Oliveira<sup>20</sup> and Forattini<sup>8</sup>. The identification occurred on a cold table at -26°C with the aid of a stereoscopic microscope (Olympus Optical CO., Tokyo, Japan). After identification, the mosquitoes were divided into 302 batches according to species and capture area and stored in liquid nitrogen. Subsequently, the mosquitoes were sent to the *Laboratório Central de Saúde Pública* (LACEN) of the FD for flavivirus isolation.

### Virus isolation

The technique used to isolate the flaviviruses from the mosquitoes in LACEN/DF is based on the sensitivity of *Aedes albopictus* Skuse, 1864 cells (clone C6/36) to several flaviviruses<sup>21</sup>. To cultivate these cells, cell culture bottles containing Leibovitz L-15 ® medium were used.

Each batch contained between 1 and 20 specimens of a single mosquito species. Each batch was homogenized in Eppendorf tubes with an antibiotic solution containing penicillin, streptomycin and amphotericin B and then individually inoculated into culture tubes containing C6/36 cells, which were maintained in an incubator at 24°C to 25°C for approximately eight days. Once grown, the inoculated cells were observed daily using an inverted optical microscope to visualize any possible cytopathic effects. Confirmation of viral infection was obtained by indirect immunofluorescence with polyclonal flavivirus antibodies<sup>22</sup>.

### Data analysis

To verify sampling sufficiency, the species accumulation curve was analysed<sup>23,24</sup>. The species constancy during the sampling was assessed by the formula  $C\%=(p/N).100$ , where  $p$ =the number of sampling occasions in which a species was

captured and  $N$ =the total number of captures. The species were then grouped into constancy categories, such as constant ( $C > 50\%$ ), accessory ( $C > 25-50\%$ ) and accidental ( $C < 25\%$ )<sup>25</sup>. To determine the categories of dominance, the classification established by Friebe<sup>26</sup> was determined using the formula  $D\% = (i/t) \cdot 100$ , where  $i$ =the total number of individuals in the species and  $t$ =the total number of individuals captured. Thus, the established categories were eudominant ( $D > 10\%$ ), dominant ( $D > 5-10\%$ ), subdominant ( $D 2-5\%$ ), eventual ( $D 1-2\%$ ) and rare ( $D < 1\%$ ).

To measure the mosquito diversity patterns, the indices of Shannon-Wiener diversity, Shannon-Wiener evenness and Berger Parker dominance were used. Data analysis was performed using the program *Diversidade de Espécies* (DivEs), version 2.0<sup>27</sup>.

The variation in the number of mosquitoes caught between rainfall periods (dry, intermediate, first rainfall quarter and second rainfall quarter) was analyzed using the Kruskal-Wallis test. In this analysis, we considered the daily abundance of seven species captured in the study to be the dependent variable. The daily occurrence of these species on the ground and in the canopy over the 60-day period was compared using chi-squared tests. The tests were performed in the Statistica® program, with significance set at 5%.

## RESULTS

Between September 2010 and August 2011, 2,677 female mosquitoes belonging to the Culicinae ( $n=2,607$ , 97.4%) and Anophelinae ( $n=70$ , 2.6%) subfamilies were captured in the BNP. The Culicinae fauna included specimens of 10 genera and 25 species (**Table 1**). In total, 1,848 (69%) mosquitoes were captured at ground level.

According to the species accumulation curve (**Figure 1**), the overall sampling effort (60 days) was satisfactory to represent the species richness, indicating a trend towards stabilization of the number of accumulated species from the 54<sup>th</sup> day.

The most abundant species were *Sabethes* (*Sabethes*) *albiprivus* Theobald, 1903; *Limatus durhamii* Theobald, 1901; *Haemagogus* (*Conopostegus*) *leucocelaenus* Dyar & Shannon, 1924; *Haemagogus* (*Haemagogus*) *janthinomys* Dyar, 1921; *Aedes* (*Ochlerotatus*) *scapularis* Rondani, 1848; *Psorophora* (*Janthinosoma*) *ferox* Von Humboldt, 1819; and *Aedes* (*Ochlerotatus*) *serratus* Theobald, 1901. Together, these seven species accounted for 86.4% of the total number of mosquitoes captured. The other species were represented by few individuals, and six species were represented by a single specimen (**Figure 2**).

The seven most abundant species were also classified as constant. Five species were classified as accessories, and 17 others were classified as accidental. It was also observed that, in terms of dominance, only three species were eudominant (*Sa. albiprivus*, *Li. durhamii* and *Hg. leucocelaenus*). Another three were dominant (*Hg. janthinomys*, *Ae. scapularis* and *Ps. ferox*), four were subdominant (*Ae. serratus*, *Cq. arribalzae*,

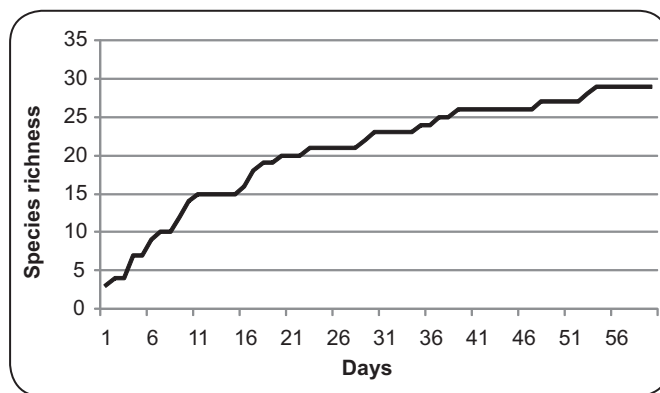


FIGURE 1 - Species richness curve of mosquitoes captured between September 2010 and August 2011 at Brasilia National Park, Federal District, Brazil.

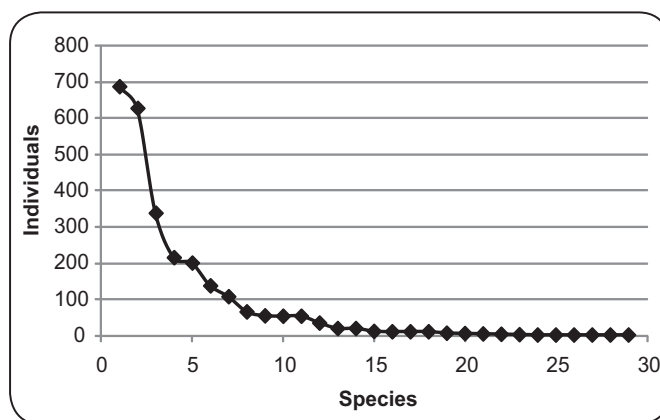


FIGURE 2 - Abundance distribution curve of mosquitoes captured between September 2010 and August 2011 at Brasilia National Park, Federal District, Brazil.

*Anopheles kompi* and *Sa. belisarioi*), two were classified as eventual and all of the others were considered rare (**Table 1**).

With respect to the monthly distribution, the mosquito abundance was higher during the first and second quarter of rainfall. The largest number of mosquitoes at both the canopy and ground levels was captured during the first quarter of rainfall (**Table 2**).

The highest diversity ( $H' = 0.961$ ), evenness ( $J = 0.671$ ) and richness ( $N = 27$ ) of mosquito species were detected at ground level. The Berger-Parker dominance index was similar for the ground ( $D_{bp} = 0.034$ ) and the canopy ( $D_{bp} = 0.035$ ). For the different rainfall classes, we observed a greater richness during the second quarter of rainfall ( $N = 21$ ). The greatest evenness was observed during the intermediate period ( $J = 0.820$ ), and the highest level of dominance was observed during the dry season ( $D_{bp} = 0.147$ ).

The number of captured specimens differed statistically according to rainfall period for *Li. durhamii* (Kruskal-Wallis  $H_{3,60} = 35.2$ ,  $p < 0.01$ ), *Hg. leucocelaenus* (Kruskal-Wallis  $H_{3,60} = 41.1$ ,  $p < 0.01$ ), *Hg. janthinomys* (Kruskal-Wallis  $H_{3,60} = 37.6$ ,  $p < 0.01$ ),

TABLE 1 - Number of mosquitoes captured in the different strata (ground and canopy) and the classifications of dominance and the constancy of species at Brasilia National Park, Federal District, Brazil, from September 2010 to August 2011.

Species	Number of mosquitoes			%	Dom*	Const**
	Ground	Canopy	Total			
<b>Anophelinae</b>						
<i>Anopheles (Nyssorhynchus) argyritarsis</i> Robineau-Desvoidy, 1827	0	1	1	0.03	Rr	Ac
<i>Anopheles (Lophopodomyia) gilesi</i> Peryassu, 1908	9	1	10	0.37	Rr	Ac
<i>Anopheles (Stethomyia) kompi</i> Edwards, 1930	52	2	54	2.01	Sd	A
<i>Anopheles (Nyssorhynchus) parvus</i> Chagas, 1907	5	0	5	0.18	Rr	Ac
<b>Culicinae</b>						
<i>Aedes (Stegomyia) aegypti</i> Linnaeus, 1762	2	1	3	0.11	Rr	Ac
<i>Aedes (Stegomyia) albopictus</i> Skuse, 1864	10	0	10	0.37	Rr	Ac
<i>Aedes (Protomacleaya) argyrothorax</i> Bonne-Wepster & Bonne, 1919	15	4	19	0.70	Rr	Ac
<i>Aedes (Ochlerotatus) fluviatilis</i> Lutz, 1904	1	0	1	0.03	Rr	Ac
<i>Aedes (Ochlerotatus) hortator</i> Dyar & Knab, 1907	12	7	19	0.70	Rr	Ac
<i>Aedes (Ochlerotatus) scapularis</i> Rondani, 1848	178	22	200	7.46	D	C
<i>Aedes (Ochlerotatus) serratus</i> Theobald, 1901	100	7	107	3.99	Sd	C
<i>Aedes (Ochlerotatus) taeniorhynchus</i> Wiedemann, 1821	1	0	1	0.03	Rr	Ac
<i>Coquillettidia (Rhynchoaenia) arribalzagae</i> Theobald, 1903	55	10	65	2.42	Sd	A
<i>Culex (Culex) coronator</i> Dyar & Knab, 1906	32	2	34	1.26	Ev	A
<i>Culex (Culex) quinquefasciatus</i> Say, 1823	10	0	10	0.37	Rr	Ac
<i>Limatus durhamii</i> Theobald, 1901	542	86	628	23.45	E	C
<i>Limatus flavisetosus</i> de Oliveira Castro, 1935	39	14	53	1.97	Ev	A
<i>Mansonia (Mansonia) pseudotitillans</i> Theobald, 1901	6	0	6	0.22	Rr	Ac
<i>Psorophora (Janthinosoma) albipes</i> Theobald, 1907	1	0	1	0.03	Rr	Ac
<i>Psorophora (Janthinosoma) ferox</i> Von Humboldt, 1819	113	24	137	5.11	D	C
<i>Uranotaenia (Uranotaenia) calosomata</i> Dyar & Knab, 1907	2	0	2	0.07	Rr	Ac
<i>Wyeomyia (Dendromyia) melanocephala</i> Dyar & Knab, 1906	0	1	1	0.03	Rr	Ac
<i>Haemagogus (Conopostegus) leucocelaenus</i> Dyar & Shannon, 1924	181	157	338	12.62	E	C
<i>Haemagogus (Haemagogus) janthinomys</i> Dyar, 1921	64	151	215	8.03	D	C
<i>Haemagogus (Haemagogus) tropicalis</i> Cerqueira & Antunes, 1938	1	0	1	0.03	Rr	Ac
<i>Sabethes (Sabethes) albiprivus</i> Theobald, 1903	402	286	688	25.70	E	C
<i>Sabethes (Sabethes) belisarioi</i> Neiva, 1908	10	43	53	1.97	Sd	A
<i>Sabethes (Sabethoides) chloropterus</i> Von Humboldt, 1819	2	2	4	0.14	Rr	Ac
<i>Sabethes (Peytonulus) soperi</i> Lane & Cerqueira, 1942	3	8	11	0.41	Rr	Ac
<b>Total</b>	<b>1,848</b>	<b>829</b>	<b>2,677</b>	<b>100.0</b>		

\*Dom: dominance; \*\*Const: constancy; Rr: rare (D<1%); Ac: accidental (C<25%); D: dominant (D>5-10%); C: constant (C>50%); Sd: subdominant (D>2-5%); A: accessory (C>25-50%); Ev: eventual (D>1-2%); E: eudominant (D>10%).

TABLE 2 - Rainfall classes and number of mosquitoes captured at both the canopy and ground levels in the gallery forest of Brasilia National Park, Federal District, Brazil, from September 2010 to August 2011.

Rainfall classes	Months	Mean precipitation (mm)	Number of mosquitoes		
			canopy	ground	total
Intermediate	April, May, September	70	68	156	224
First rainfall quarter	October, November, December	216	433	958	1,391
Second rainfall quarter	January, February, March	220	314	605	919
Dry	June, July, August	0	14	129	143
Total			829	1,848	2,677

*Sabethis albiprivus* (Kruskal-Wallis  $H_{3,60}=37.4$ ,  $p<0.01$ ), *Ps. ferox* (Kruskal-Wallis  $H_{3,60}=18.6$ ,  $p<0.01$ ) and *Ae. serratus* (Kruskal-Wallis  $H_{3,60}=17.5$ ,  $p<0.01$ ). These species were more abundant during the rainy quarters. During the dry season, the potential YF vectors exhibited very low frequency and abundance, with the exception of *Ae. scapularis* and *Ae. serratus*.

Significant differences were detected between the occurrence of mosquitoes on the ground and in the canopy for the species *Hg. janthinomys* ( $\chi^2=4.9$ ,  $p=0.02$ ), *Li. durhamii* ( $\chi^2=4.0$ ,  $p=0.04$ ), *Ps. ferox* ( $\chi^2=3.9$ ,  $p=0.04$ ), *Ae. scapularis* ( $\chi^2=12.1$ ,  $p<0.01$ ) and *Ae. serratus* ( $\chi^2=24.4$ ,  $p<0.01$ ). Except for *Hg. janthinomys*, these species were more frequent on the ground. With respect to the monthly abundance of these mosquitoes, it was observed that *Hg. janthinomys* was more abundant in the canopy, in contrast to the other species (Figure 3).

None of the 302 batches of mosquitoes (N=2,677) sent to the Virology Laboratory-LACEN/DF for arbovirus isolation tested positive for flaviviruses.

## DISCUSSION

The present study shows that the abundance and species richness of mosquitoes in the gallery forest of the BNP are higher on the ground and during the rainy season. Even species with recognized acrodendrophilic habits, such as *Hg. janthinomys*, were captured on the ground; these results are similar to those of Alencar et al.<sup>28</sup> and Ramirez et al.<sup>29</sup>. These results indicate that precipitation is a crucial factor explaining the richness and abundance of Culicidae fauna in the gallery forests of the Cerrado, as has already been observed in other Brazilian biomes<sup>8,30,31</sup>.

Among the species identified in the BNP, 11 have been previously found to be naturally infected with the YF virus: *Ae. aegypti*, *Ae. scapularis*, *Ae. serratus*, *Ps. albipes*, *Ps. ferox*, *Hg. leucocelaenus*, *Hg. janthinomys*, *Hg. tropicalis*, *Sa. chloropterus*, *Sa. soperi* and *Sa. albiprivus*<sup>13,20,32-36</sup>. Moreover, the natural infection of *Hg. janthinomys* and *Hg. leucocelaenus* by the YF virus has been previously reported in the administrative region of São Sebastião, FD<sup>13</sup>. However, none of the 302 batches of 2,677 mosquitoes examined were

infected with flaviviruses. This may be because of a low rate of infection among primates in the BNP, as this work was conducted during the interepidemic period. Furthermore, the infection of mosquitoes by the YF virus tends to be low, even in areas of YF outbreaks. The results of Cardoso et al.<sup>34</sup> reinforce this hypothesis, showing that *Hg. leucocelaenus* and *Ae. serratus* in YF outbreak areas have minimum infection rates of 3.70 and 1.88, respectively.

Three specimens of *Ae. aegypti* were detected in the gallery forest of the BNP. The occurrence of this species in urban environments has been widely reported<sup>37</sup>; however, the occurrence of *Ae. aegypti* in sylvatic environments is rare. Barbosa et al.<sup>38</sup> and Soares et al.<sup>39</sup> reported the presence of this species in a rural area in the states of Amazonas and Rio de Janeiro. Tauil<sup>6</sup> highlights the difficulty of identifying whether some cases of YF are transmitted by sylvatic or urban vectors. Considering that the sampling areas were located near recreational areas in the BNP, it is possible that the specimens of *Ae. aegypti* captured in the gallery forest were from artificial breeding sites. Future monitoring studies of mosquitoes in gallery forests may better clarify the occurrence of *Ae. aegypti* in sylvatic environments.

Environments that are still considered sylvatic are strongly influenced by the rapid urbanization process, in which population and housing growth generate anthropogenic changes that contribute to the establishment of mosquito species that are better adapted to the human environment<sup>40</sup>. The FD has undergone considerable environmental changes caused by the expansion of its urban area, which can change the behavior of the mosquito species present in the BNP. The occurrence of species that are adapted to urban anthropogenic environments, such as *Ae. aegypti* and *Culex (Culex) quinquefasciatus* Say, 1823, could be attributed to ecological changes that occurred with the construction of the *Setor Habitacional Noroeste*.

*Aedes albopictus* was also found during the survey. The ability of this species to transmit arboviruses has already been demonstrated<sup>41-43</sup>. It is noteworthy that *Ae. albopictus* has been documented as naturally infected with the virus that causes eastern equine encephalitis<sup>44,45</sup> and with dengue virus during an outbreak that occurred in Mexico<sup>46</sup>. Some authors admit the possibility that *Ae. albopictus* may become a vector that

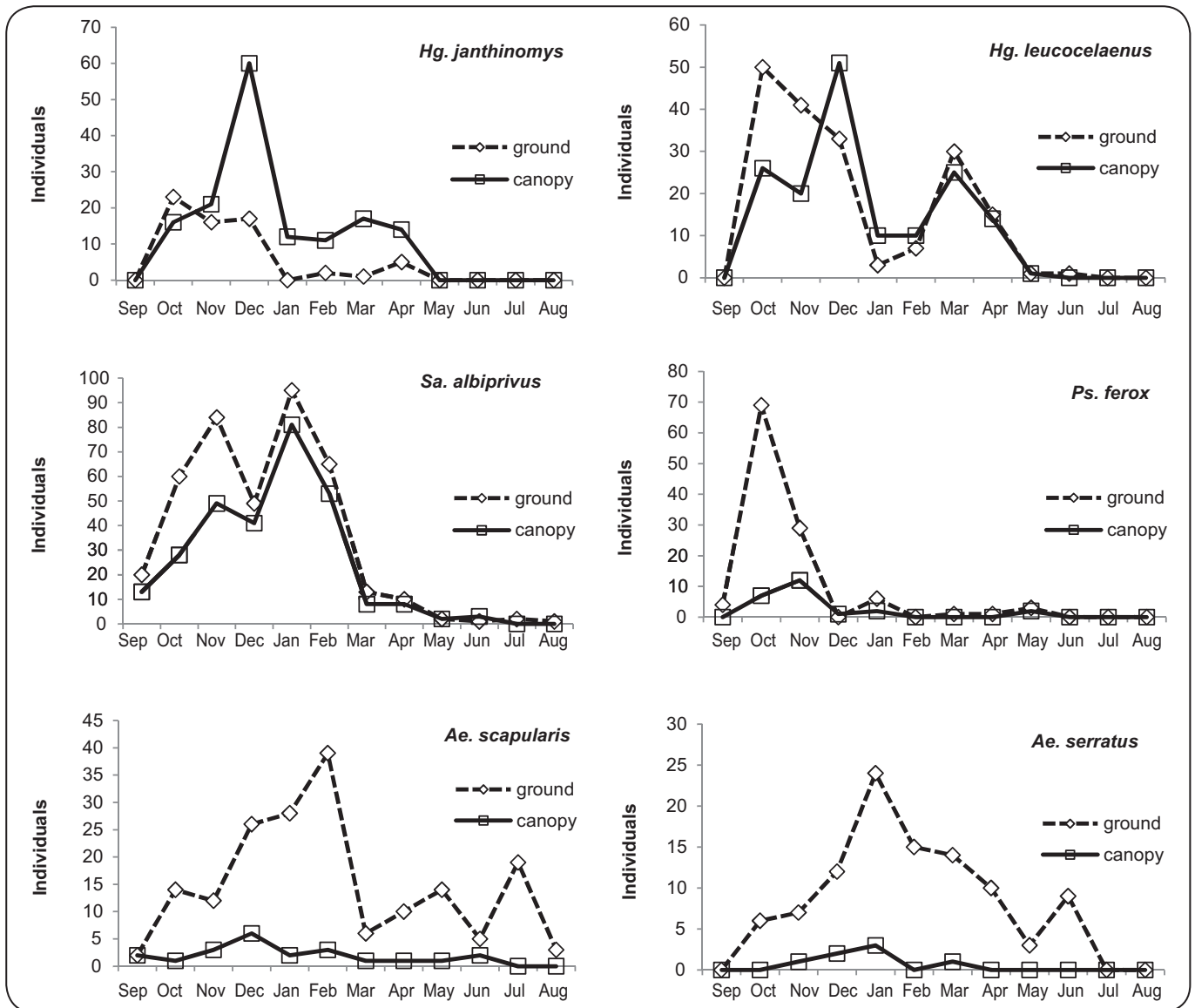


FIGURE 3 - Number of mosquitoes of species that are potential vectors of yellow fever in Brasilia National Park, Federal District, Brazil, on the ground and in the canopy between September 2010 and August 2011. Only the most abundant species (>100 individuals captured) are listed. Hg.: *Haemagogus*; Ae.: *Aedes*; Sa.: *Sabethes*; Ps.: *Psorophora*.

connects sylvatic and urban cycles of YF in Brazil<sup>20,39,47</sup>. Gomes et al.<sup>41</sup> and Albuquerque et al.<sup>48</sup> point to the need to monitor this species in Brazil.

There are few studies related to the vertical distribution of mosquitoes in the Central-West Region of Brazil<sup>49</sup>. In the present study, we detected a higher occurrence of *Hg. janthinomys* in the canopy, reinforcing the known acrodendrophilic behavior of this species<sup>29,50</sup>. Specimens of *Hg. leucocelaenus* were very common on the ground. Forattini et al.<sup>51</sup> in São Paulo and Pinto et al.<sup>50</sup> in Pará also reported the predominance of *Hg. leucocelaenus* on the ground. However, Guimarães et al.<sup>52</sup> showed that 74% of *Hg. leucocelaenus* specimens were captured in the canopy at the National Park of the Serra dos Orgãos, Rio de Janeiro. *Ae. scapularis* and *Ae. serratus* showed significant differences

in habitat use and were more abundant on the ground. The same preference for these species to occur at ground level has also been observed by Fe et al.<sup>53</sup>, Forattini et al.<sup>51</sup>, Guimarães et al.<sup>52</sup> and Julião et al.<sup>30</sup>. Moreover, the highest occurrence of *Ps. ferox* at ground level was also observed by Forattini et al.<sup>51</sup>.

There is evidence that vertical distribution differences between and within species depends upon the type of vegetation cover. Forattini et al.<sup>51</sup> suggested that a vertical distribution difference is most evident in tropical rain forests, where the trees are tall and dense. In less dense forests and in places with long dry seasons, these differences tend to decrease, as observed in the present study.

Other important factors influencing the vertical distribution of mosquitoes are vertical mobility and daily activity. According to Service<sup>54</sup>, mosquitoes can be attracted to a feeding source at a

distance of seven to 30 meters. Forattini et al.<sup>51</sup> and Vasconcelos et al.<sup>55</sup> showed that even species that were predominantly active in the higher levels of the canopy, such as *Hg. janthinomys*, often move to ground level when they detect the presence of a feeding source. Moreover, Guimarães et al.<sup>52</sup> showed differences in the vertical distribution of *An. cruzii* captured at different periods of the day, with the highest number of specimens found in the canopy at night. Therefore, the vertical movement of mosquitoes can weaken the evidence of vertical stratification of species, and it obviously influences the dynamics of pathogen transmission<sup>30</sup>.

The effects of rainfall on the total abundance of mosquitoes captured were evident. There was a strong influence of rainfall on the annual cycle of the species of *Sabethes* and *Haemagogus*, which have eggs that are very resistant to desiccation and sometimes require repeated contact with water to hatch<sup>20</sup>. Similar situations related to these genera have been described in Mato Grosso<sup>31</sup> and Tocantins<sup>56</sup>. Studies in southern Brazil<sup>10</sup> and in Trinidad<sup>57</sup> also indicated a higher occurrence of *Hg. leucocelaenus* during the rainy months. The highest occurrence of *A. scapularis*, *A. serratus* and *Ps. ferox* in the rainy season is also in agreement with the literature<sup>20</sup>.

The present study increases the information available regarding the ecology of important YF virus vectors in Central Brazil. Although seroepidemiological surveys indicate that YF control efforts have achieved good results<sup>58</sup>, entomological monitoring is recommended in the BNP and in other areas that are susceptible to the transmission of the YF virus. We emphasize that *the BNP is located 10km from the center of Brasília and 2km from the Setor Habitacional Noroeste*, where many apartments are being constructed. Furthermore, there are houses located at the edge of a roadway that is very close to the park. Because the flying range of YF vectors can reach 500m<sup>59</sup>, there is the possibility of sylvatic YF transmission to people who reside close to the BNP and to park visitors. This transmission would be more likely during periods of NHP epidemics, when the natural infection rate of mosquitoes tends to be higher<sup>34</sup>. Although no viral isolates were obtained from the specimens captured in this study, studies related to the ongoing natural infection of mosquitoes is of paramount importance as a means of predicting events related to YF and other arboviruses. Such information will contribute to a better understanding of the true role of the vector species that occur within Central Brazil. It is also recommended that the relevant agencies give special attention to the deaths of NHPs and to the vaccination programs against YF for visitors to the BNP.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## REFERENCES

1. Marcondes CB. Entomologia Médica e Veterinária. São Paulo: Atheneu; 2011.
2. Mathers CD, Lopez AD, Murray CJL. The burden of disease and mortality by condition: data, methods and results for 2001. In: Lopez AD, Mathers CD, Ezzati M, Jamison DR, Murray CJL, editors. Global burden of disease and risk factors. New York: Oxford University Press/The World Bank. 2006. p. 45-240.
3. Vasconcelos PFC. Febre amarela: reflexões sobre a doença, as perspectivas para o século XXI e o risco de reurbanização. Rev Bras Epidemiol 2002; 5:244-258.
4. Organização Mundial de Saúde (OMS). Yellow fever: Geographical distribution. [Internet]; [updated 2011 December 1, cited 2011 December 12]. Available at: <http://www.who.int/csr/disease/yellowfev/impact1/en/>.
5. Barrett ADT, Higgs S. Yellow fever: a disease that has yet to be conquered. Ann Rev Entomol 2007; 52:209-229.
6. Tauil PL. Aspectos críticos do controle da febre amarela no Brasil. Rev Saude Publica 2010; 44:555-558.
7. Dégallier N, Travassos-da-Rosa APA, Hervé JP, Travassos-da-Rosa JFS, Vasconcelos PFC, Mangabeira-da-Silva CJ, et al. A comparative study of yellow fever in Africa and South America. Braz J Assoc Advanc Science 1992; 44:143-161.
8. Forattini OP. Culicidologia Médica: identificação, biologia e epidemiologia. Vol. 2. São Paulo: EDUSP; 2002.
9. Tauil PL, Santos JB, Moraes MAP. Febre Amarela. In: Coura JR, editor. Dinâmica das doenças infecciosas e parasitárias. Rio de Janeiro: Ed. Guanabara; 2005. p. 1755-1765
10. Gomes AC, Torres MAN, Paula MB, Fernandes A, Marassá AM, Consales CA, et al. Ecologia de *Haemagogus* e *Sabethes* (Diptera: Culicidae) em áreas epizooticas do vírus da febre amarela, Rio Grande do Sul, Brasil. Epidemiol Serv Saude 2010; 19:101-113.
11. Araújo FAA, Ramos DG, Santos AL, Passos PHO, Elkhoury ANSM, Costa ZGA, et al. Epizootias em primatas não humanos durante reemergência do vírus da febre amarela no Brasil, 2007 a 2009. Epidemiol Serv Saude 2011; 20:527-536.
12. Secretaria de Estado de Saúde do Distrito Federal. Diretoria de Vigilância Epidemiológica. Informativo Epidemiológico (malária, febre amarela, esquistossomose, febre maculosa, DCA e DCJ) Ano 2, nº 2 - Julho de 2012.. [Internet]; [updated 2013 July 1, cited 2013 July 10]. Available at: <http://www.saude.df.gov.br/outros-links/437-informes-epidemiologicos-malaria-febre-amarela-esquistossomose-febre-maculosa-dca-e-dcj.html/>.
13. Obara MT, Monteiro H, Paula MB, Gomes AC, Yoshizawa MAC, Lira-Vieira AR, et al. Infecção natural de *Haemagogus janthinomys* e *Haemagogus leucocelaenus* pelo vírus da Febre Amarela no Distrito Federal, Brasil. Epidemiol Serv Saude 2012; 21:457-463.
14. Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) [Internet]; [updated 2012 January 2, cited 2012 January 10]. Available at: <http://www.icmbio.gov.br/portal/o-que-fazemos/visitaacao/ucs-abertas-a-visitaacao/213-parque-nacional-de-brasil>.
15. Santos JP, Obara MT, Cavalcante KLRJ, Steinke E. Culicídeos encontrados em áreas próximas à piscina velha do Parque Nacional de Brasília, DF. Rev Bras Geogr Med Saude 2008; 3:157-162.
16. Silva JMC, Felfili JM, Nogueira PE, Rezende AV. Análise florística das Matas de Galeria no Distrito Federal. In: Ribeiro JF, editor. Cerrado: Matas de Galeria. Brasília: Embrapa; 1998. p. 53-84.

17. Instituto Nacional de Meteorologia (INMET) [Internet]; [updated 2012 Marh 1, cited 2011 March 26]. Available at: <http://www.inmet.gov.br/html/clima/graficos/plotGraf.php?chklist=2%2C&capita=brasil%2C&peri=99%2C&per6190=99&precipitacao=2&brasil%2C&Enviar=Visualizar/>.
18. Companhia Imobiliária de Brasília (TERRACAP). [Internet]; [updated 2012 Marh 1, cited 2012 March 10]. Available at: <http://www.terracap.df.gov.br/internet/index.php?scoid=35&ctuid=479>.
19. Ministério da Saúde. Secretaria de Vigilância em Saúde. Nota técnica Nº 59/2011. Recomendações de priorização das atividades de investigação entomológica e padronização dos métodos de coleta de amostras para diagnóstico laboratorial de Febre Amarela. [Internet]; [updated 2012 May 1, cited 2012 May 28]. Available at: [http://portal.saude.gov.br/portal/arquivos/pdf/nt\\_59\\_2011\\_in\\_entm\\_fbr\\_amarl\\_corr\\_02\\_01\\_2012.pdf](http://portal.saude.gov.br/portal/arquivos/pdf/nt_59_2011_in_entm_fbr_amarl_corr_02_01_2012.pdf).
20. Consoli RAGB, Lourenço-de-Oliveira R. Principais mosquitos de importância sanitária no Brasil. Rio de Janeiro: Fiocruz; 1994. 228p.
21. Igarashi A. Isolation of a Singh's *Aedes albopictus* cell clone sensitive to dengue and chikungunya viruses. *J Gen Virol* 1978; 40:531-544.
22. Gubler DJ, Kuno G, Sather E, Valez M, Olivre A. Mosquito cell cultures and specific monoclonal antibodies in surveillance for dengue viruses. *Am J Trop Med Hyg* 1984; 33:158-165.
23. Gotelli N, Colwell R. Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecol Lett* 2001; 4:379-391.
24. Cardoso JC, Paula MB, Fernandes A, Santos E, Almeida MAB, Fonseca DF, et al. Ecological aspects of mosquitoes (Diptera: Culicidae) in an Atlantic Forest area on the North coast of Rio Grande do Sul State, Brazil. *J Vector Ecol* 2011; 36:175-186.
25. Silveira Neto S, Nakano O, Barbin D, Nova NAV. Manual de ecologia dos insetos. São Paulo: CERES; 1976.
26. Friebe B. Zur Biologie eines Buchenwaldbodens. Die Käferfauna. *Carolina* 1983; 41:45-80.
27. Rodrigues WC. DivES - Diversidade de espécies. Versão 2.0. Software e Guia do Usuário - 2005. [Internet]; [updated 2012 March 1, cited 2011 March 25]. Available at: <http://www.ebras.vbweb.com.br>.
28. Alencar J, Lorosa ES, Dégallier N, Serra-Freire NM, Pacheco JB, Guimarães AE. Feeding patterns of *Haemagogus janthinomys* (Diptera: Culicidae) in different regions of Brazil. *J Med Entomol* 2005; 42: 981-985.
29. Ramírez P, John E, Yanoviak SP, Lounibos LP. Distribución vertical de *Haemagogus janthinomys* (Dyar) (Diptera: Culicidae) en bosques de la amazonia peruana. *Rev Peru Med Exp Salud Publica* 2007; 24: 40-45.
30. Julião GR, Abad-Franch R, Lourenço-de-Oliveira R, Luz SLB. Measuring mosquito diversity patterns in an Amazonian terra firme rain forest. *J Med Entomol* 2010; 47:121-128.
31. Ribeiro ALM, Miyazaki MS, Zeilhofer P. Spatial and temporal abundance of three sylvatic yellow fever vectors in the influence area of the Manso hydroelectric power plant, Mato Grosso, Brazil. *J Med Entomol* 2012; 49:223-226.
32. Ministerio de Salud y Ambiente Argentina. Instituto Nacional de Enfermedades Virales Humanas. Informe Anual 2009 - 2010. Pergamino: Ministerio de Salud y Ambiente; 2011.
33. Ministério da Saúde. Secretaria de Vigilância em Saúde. Guia de vigilância epidemiológica. Ministério da Saúde. 7<sup>th</sup> ed. Brasília: Ministério da Saúde; 2009.
34. Cardoso JC, Almeida MAB, Santos E, Fonseca DF, Sallum MAM, ? Noll CA, et al. Yellow fever virus in *Haemagogus leucocelaenus* and *Aedes serratus* mosquitoes, southern Brazil, 2008. *Emerg Infect Dis* 2010; 16:1918-1924.
35. Mondet B, Vasconcelos PFC, Travassos-da-Rosa APA, Rodrigues SG, Travassos-da-Rosa JFS, Bicout DJ. Isolation of yellow fever virus from nulliparous *Haemagogus (Haemagogus) janthinomys* in western Amazonian. *Vector Borne Zoonotic Dis* 2002; 2:47-50.
36. Vasconcelos PFC, Sperb AF, Monteiro HAO, Torres MAN, Souza MRS, Vasconcelos HB, et al. Isolations of yellow fever from *Haemagogus leucocelaenus* in Rio Grande do Sul, Brazil in the Southern Cone. *Trans R Soc Trop Med Hyg* 2003; 97:60-62.
37. Braga IA, Valle D. *Aedes aegypti*: histórico do controle no Brasil. *Epidemiol Serv Saude* 2007; 16:113-118.
38. Barbosa MG, Fé NF, Marcião AHR, Silva APT, Monteiro WM, Guerra MVF, et al. Registro de Culicidae de importância epidemiológica na área rural de Manaus, Amazonas. *Rev Soc Bras Med Trop* 2008; 41:658-663.
39. Soares VARC, Rodrigues WC, Cabral MMO. Estudo de áreas e depósitos preferenciais de *Aedes albopictus* (Skuse, 1894) e *Aedes aegypti* (Linnaeus, 1762) no Município de Paracambi - Rio de Janeiro, Brasil. *Entomobrasilia* 2008; 1:63-68.
40. Forattini OP, Kakitani L, Marques GRAA, Brito M. Formas imaturas de anofelíneos em recipientes artificiais. *Rev Saude Publica* 1998; 32:189-191.
41. Gomes AC, Torres MAN, Gutierrez MFC, Lemos FL, Lima MLN, Martins JF, et al. Registro de *Aedes albopictus* em áreas epizooticas de febre amarela nas Regiões Sudeste e Sul do Brasil (Diptera: Culicidae). *Epidemiol Serv Saude* 2008; 17:71-76.
42. Johnson BW, Chambers TV, Crabtree MB, Filippis AMB, Vilarinhos PTR, Resende MC, et al. Vectors competence of Brazilian *Aedes aegypti* and *Aedes albopictus* for Brazilian yellow fever virus isolate. *Trans R Soc Trop Med Hyg* 2003; 96:611-613.
43. Lourenço-de-Oliveira R, Vazeille M, Filippis AMB, Failloux AB. Large genetic differentiation and low variation in vector competence for dengue and yellow fever viruses of *Aedes albopictus* from Brazil, the United States and Cayman Islands. *Am J Trop Med Hyg* 2003; 69:105-114.
44. Gerhardt RR, Gottfried KL, Apperson CL. First isolation of La Crosse virus from naturally infected *Aedes albopictus*. *Emerg Infect Dis* 2001; 7:807-811.
45. Mitchell CJ. Vector competence of North and South American strains of *Aedes albopictus* for certain arboviruses: a review. *J Am Mosq Control Assoc* 1991; 7:446-451.
46. Ibañez-Bernal S, Briseño B, Mutebi JP, Argot E, Rodríguez G, Martínez-Campos C, et al. First record in America of *Aedes albopictus* naturally infected with dengue virus during the 1995 outbreak at Reynosa, Mexico. *Med Vet Entomol* 1997; 11:305-309.
47. Miller BR, Ballinger ME. *Aedes albopictus* mosquitoes introduced into Brazil: vector competence for yellow fever and dengue viruses. *Trans R Soc Trop Med Hyg* 1988; 82:476-477.
48. Albuquerque CMR, Melo-Santos MAV, Bezerra MAS, Barbosa RMR, Silva DF, Silva E. Primeiro registro de *Aedes albopictus* em área da Mata Atlântica, Recife, PE, Brasil. *Rev Saude Pública* 2000; 34:314-315.
49. Pinheiro FP, Travassos da Rosa APA, Moraes MAP. An epidemic of yellow fever in Central Brazil, 1972-1973. II. Ecological studies. *Am J Trop Med Hyg* 1981; 30:204-211.
50. Pinto CS, Confalonieri UEC, Mascarenhas BM. Ecology of *Haemagogus* sp. and *Sabethes* sp. (Diptera: Culicidae) in relation to the microclimates of the Caxiuanã National Forest, Pará, Brazil. *Mem Inst Oswaldo Cruz* 2009; 104:592-598.
51. Forattini OP, Lopes OS, Rabelo EX. Investigações sobre o comportamento de formas adultas de mosquitos silvestres no Estado de São Paulo, Brasil. *Rev Saude Publica* 1968; 2:111-173.
52. Guimarães AE, Arle M, Machado RNM. Mosquitos no Parque Nacional da Serra dos Órgãos, Estado do Rio de Janeiro, Brasil: II. Distribuição vertical. *Mem Inst Oswaldo Cruz* 1985; 80:171-185.
53. Fé NF, Barbosa MG, Fé FAA, Guerra MVF, Alecrim WD. Fauna de Culicidae em municípios da zona rural do Estado do Amazonas, com incidência de febre amarela. *Rev Soc Bras Med Trop* 2003; 36: 343-348.
54. Service MW. Mosquitoes (Culicidae). In: Lane RP, Crosskey RW, editors. *Medical insects and arachnids*. London, United Kingdom: Chapman & Hall 1993. p. 120-240
55. Vasconcelos PFC, Travassos-da-Rosa APA, Rodrigues SG, Travasso-da-Rosa ES, Monteiro HAO, Cruz ACR, et al. Yellow fever in Pará State, Amazon Region of Brazil, 1998-1999: entomologic and epidemiologic findings. *Emerg Infect Dis* 2001; 7:565-569.



56. Silva JS, Pacheco JB, Alencar J, Guimarães AE. Biodiversity and influence of climatic factors on mosquitoes (Diptera: Culicidae) around the Peixe Angical hydroelectric scheme in the state of Tocantins, Brazil. Mem Inst Oswaldo Cruz 2010; 105:155-162.
57. Tikasingh ES, Hull B, Laurent E, Chadee DD. Entomological activities during the yellow fever epidemic in Trinidad, 1978-1980. Bull Soc Vect Ecol 1990; 15:41-47.
58. Machado VW, Vasconcelos PFC, Silva EVP, Santos JB. Serologic assessment of yellow fever immunity in the rural population of a yellow fever-endemic area in Central Brazil. Rev Soc Bras Med Trop 2013; 46:166-171.
59. Reiter P, Amador MA, Anderson RA, Clark GG. Short report: dispersal of *Aedes aegypti* in an urban area after blood feeding as marked by rubidium marked eggs. Am J Trop Med Hyg 1995; 52:177-179.