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DISTRIBUTION OF *HAEMAGOGUS* AND *SABETHES* SPECIES IN RELATION TO FOREST COVER AND CLIMATIC FACTORS IN THE CHAPADA DOS GUIMARÃES NATIONAL PARK, STATE OF MATO GROSSO, BRAZIL

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ABSTRACT. Members of the genera *Haemagogus* and *Sabethes* are the most important biological vectors of the wild yellow fever virus (WYF) in the forested areas of the Americas. The ecologies of *Haemagogus janthinomys*, *Hg. leucocelaenus*, *Sabethes chloropterus*, and *Sa. glaucodaemon* were studied in a forest of the Chapada dos Guimarães National Park, state of Mato Grosso, Brazil, to evaluate the influence of climatic factors (temperature and relative humidity [RH]) on their abundance. We also examined the association of climate with landscape structure on species distribution patterns throughout the seasons of the year. Multiple stepwise regressions showed that RH was most likely to influence the density of mosquito populations. A multidimensional scaling (MDS) was used to evaluate the effects of forest cover on the composition of mosquito populations at different radii (100-, 250-, and 1,000-m-radius buffer areas). The MDS provided 2 dimensions with values that indicated a higher similarity in the composition of culicid populations between sites 1 and 3, while site 2 was separate from the others in the ordination space. Site 2 had a much higher forest cover ratio at 100-m radius compared with sites 1 and 3. We found a possible relationship between the forest cover and the composition of the mosquito populations only in the 100-m radius. These results enabled us to infer that RH directly favored the activity of mosquito populations and that the forest cover located closest to the sampling site may influence the species composition. Since mosquito abundance was higher in the sites with lower local forest cover, forest fragmentation may be a key factor on the presence of WYF vector.

KEY WORDS Climate factors, Culicidae, *Haemagogus*, landscape ecology, *Sabethes*

INTRODUCTION

There is currently an ongoing outbreak of sylvatic yellow fever in Brazil. The outbreak probably started at the end of 2016, when the 1st case was reported from the state of Minas Gerais, but has since spread to the states of Espirito Santo, São Paulo, and Rio de Janeiro. According to a World Health Organization (2017) report, as of April 2017, the yellow fever virus transmission (epizootics and human cases) continues to expand towards the Atlantic coast of Brazil in areas not previously deemed to be at risk for yellow fever transmission.

Members of the genera *Haemagogus* Williston and *Sabethes* Robineau-Desvoidy are the most important biological vectors of the wild yellow fever virus (WYF) in the forested areas of the Americas (Arnell 1973). *Haemagogus* and *Sabethes* are diurnal sylvan mosquitoes that are active in the treetops, with some species showing a tendency to favor domestic habitats (Marcondes and Alencar 2010). Yet, the ecology of these mosquitoes, which may influence their role in virus transmission, is still not very well known.

Changes in climate and land cover–land use are some general factors that may explain recent emergences (or reemergences) of infectious diseases caused by mosquito-borne pathogens (Anyamba et al. 2014), for example by affecting conditions for pathogen and vector survival and proliferation (Parham et al. 2015, Zhong et al. 2016) and altering contact between vectors and reservoir hosts (Wilcox and Ellis 2006). The main climatic factors influencing biocoenosis and, therefore, the living organisms involved in the disease transmission process, are temperature, relative air humidity, and precipitation (Rouquayrol 1994).

Diseases such as yellow fever and dengue, among others, may reemerge due to deforestation and its unintended effects (Wilcox and Ellis 2006). A

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progressive increase in the number of cases of WYF in epizootic or transition areas has been found due to large-scale deforestation over the last 30 years (Pessanha 2009).

The joint use of biological landscape structure and occurrence of the main vector species of disease-causing pathogens can contribute to a better understanding of the ecology of species, including those in the family Culicidae. The advent of technology based on techniques such as remote sensing, photo interpretation, map scanning, Global Positioning System (GPS), and Geographic Information System opened up the possibility of monitoring landscapes, which are closely related to land use and cover.

This study aimed to analyze the influence of climatic factors (temperature and RH), and their association with the landscape structure, on the distributions of *Haemagogus* and *Sabethes* populations. These mosquitoes are effective or potential vectors of WYF in areas of the Chapada dos Guimarães National Park, state of Mato Grosso, Brazil.

MATERIALS AND METHODS

Three sampling sites were established in the Chapada dos Guimarães National Park, state of Mato Grosso, Brazil (Fig. 1). Sites were selected that are frequented by park visitors, representing typical forest covers with increasing degree of anthropization. Site 1 was located at 15°24′19.8″S and 55°50′07.6″W, altitude of 590 m, near the main tourist attraction of the park, the "Véu de Noiva" waterfall in the "Valley Forest" area. Site 2 was located at 15°24′26.8″S and 55°49′21.8″W, altitude of 601 m, near the "Namorados" waterfall (formed by the Coxipozinho River and surrounded by dense gallery forest). Site 3 was located at 15°25′46.5″S and 55°50′05.4″W, altitude of 645 m, covered by gallery forest. Geographical coordinates were obtained using a Garmin (Chicago, IL) GPS Map 60CS GPS.

Insects were collected every 2 months, from June 2001 to December 2002, during the day (1000–1200 h and 1400–1600 h). Each sampling period consisted of 6 consecutive days of collecting in preselected areas. Members of the Diptera Laboratory Team (Oswaldo Cruz Institute [OCI]) captured mosquitoes that were attracted to the oral suction tubes (Castro capturer) and/or those resting in the surrounding vegetation in each sampling period. All field samples were collected according to the biosafety standards established by OCI, with all possible risks minimized by routine measures of preventive vaccination against yellow fever. Temperature and RH were measured every hour during sampling. Maximum and minimum values were assessed by Oregon Scientific (Portland, OR) thermo-hygrometers. The thermo-hygrometers were fixed at the capture site, at a height of 1 m from the ground. All climate data measured during the catches were transcribed into field data sheets, with the description of the location and date of sampling.

Species were identified by direct observation of morphological characteristics under a stereomicroscope (Zeiss®, Jena, Germany), using dichotomous keys prepared by Arnell (1973) and Forattini (2002). Abbreviations of generic and subgeneric names follow those proposed by Reinert (2001). All specimens were incorporated into the Entomological Collection of OCI after identifying the species.

Generalized linear and mixed models were used to assess differences in species richness and abundance per species between sites (Infostat software [Di Rienzo et al. 2014]). Dependent variables were richness and abundances, which were assumed to be Poisson log distributed. Fixed effects were sites; random effects were sampling month–year. The threshold for assessing significant differences was set at $P < 0.05$.

Meteorological variables (temperature and RH, recorded during mosquito collections) were compared between sites with Kruskal–Wallis nonparametric analysis. Total number of mosquitoes collected were compared between months also using Kruskal–Wallis nonparametric analysis. Multiple regressions (Stepwise in InfoStat software) assessed the unique contribution of a meteorological variable (temperature, RH) during the mosquito collections on the dependent variable (richness or abundance), once the contributions of the other variables are taken into account (Streiner 2013). Abundance data (total or per species) were previously transformed to $\ln(n + 1)$. The criterion for retaining variables was $P < 0.15$.

In addition, the influence of forest cover on the composition of mosquito populations was analyzed at different radii. The radii used were based on buffer areas with radii of 1,000, 250, and 100 m around the sampling sites, within which the proportion of forest cover was calculated (Fig. 1). The forest cover ratios were estimated manually using ArcGIS 10 software (Environmental Systems Research Institute, Redlands, CA). A Landsat 7 satellite image (from 2002) downloaded from the United States Geological Survey platform "LandsatLook" (http://landsatlook.usgs.gov/viewer.html) was used to delineate the forest cover polygons. The image resolution was 30 m for bands 1–7, and 15 m for band 8. Bands were fused, resulting in a final image of 15-m resolution. The composition of the mosquito communities was assessed from multidimensional scaling (MDS). The MDS is a method to measure the similarity between data sets, which in this study refers to the composition of the mosquito populations (data sets) in each sampling unit (Jongman et al. 1995, Borg and Groenen 2005).

A graphic of the MDS outcome and the proportion of plant cover within each area of different radii (1,000, 250, and 100 m), was constructed to infer about the effect of forest cover on the composition of mosquito populations. The IBM (Chicago, IL) SPSS Statistics 22 software was used to build the MDS.

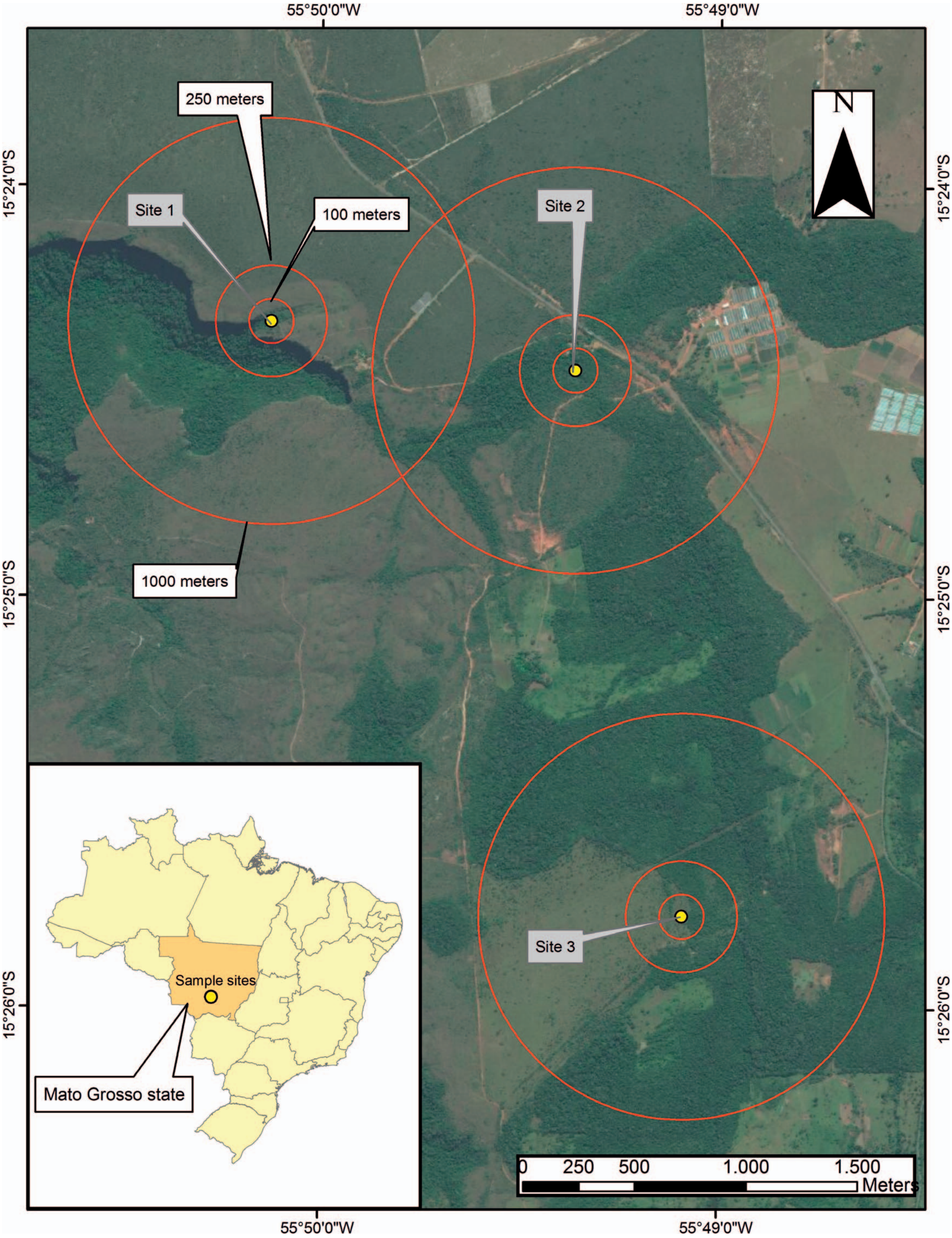

Fig. 1. Study sites and their corresponding area radii, Chapada dos Guimarães National Park, Mato Grosso, Brazil.

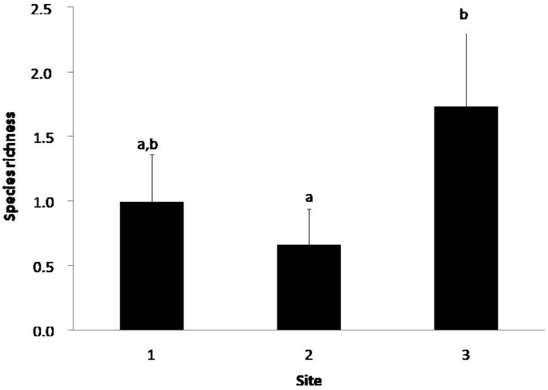

Fig. 2. Observed species richness (adjusted mean + standard error from generalized linear and mixed models [GLMM]), Chapada dos Guimarães National Park, Mato Grosso, Brazil.

RESULTS

In all, 2,630 mosquito specimens were collected in 3 different capture sites, covering 2 genera and 4 species, two of which occurred at a frequency ≥10%. The species collected were *Haemagogus janthinomys* Dyar (1,800 specimens), *Hg. leucocelaenus* (Dyar and Shannon) (140 specimens), *Sabethes chloropterus* (von Humboldt) (470 specimens), and *Sa. glaucodaemon* (Dyar and Shannon) (220 specimens).

Species richness differed between sites ($P = 0.04$). On average, site 3 was significantly richer than site 2 (Fig. 2), which may be due both to higher overall mosquito abundances per species on site 3 and to *Hg. leucocelaenus* not having been found at site 2 (Table 1).

The monthly frequencies of species (Fig. 3), species richness (Fig. 2), and climate data records (temperature and RH; Fig. 4) at the sampling sites were analyzed. Total mosquito abundance significantly differed between months ($H = 17.5$; $P = 0.03$); higher mosquito numbers were collected in December 2001, April and February 2002, and lowest records (no mosquitoes) were from June 2001 and 2002.

No significant differences were detected between sites in RH ($H = 5.37$; $P = 0.72$) or temperature ($H = 6.42$; $P = 0.59$). Species richness was positively related to RH ($-0.97 + 0.03 \times \mathrm{RH}$; $P = 0.03$), while temperature was not retained in the model; however, the model only explained 16% of the variation in the data. A similar pattern was detected regarding total mosquito abundance ($P = 0.02$), where RH explained 17% of the data variation ($-1.28 + 0.05 \times \mathrm{RH}$) and temperature did not alter the model. Correlations between mosquito abundances per species and climatic variables were mostly weak and nonsignificant. Only the abundance of *Hg. janthinomys* was significantly related to RH ($P = 0.01$), which was the only variable retained in the model ($-2.98 + 0.06 \times \mathrm{RH}$) and explained 20% of the variation in the data.

The forest cover ratios at the radii of 1,000-, 250-, and 100-m buffer areas are shown in Table 2. The sampling site that exhibited the highest forest cover varied, depending on the buffer area examined. Site 3 had the highest forest cover ratio for a 1,000-m radius; however, site 2 had the highest forest cover ratio for the 250- and 100-m radii. Site 1 always had the lowest forest cover ratio, while for site 2, forest cover ratio was inversely proportional to the radius. The pattern observed for site 3 was the opposite of site 2, as forest cover ratio was directly proportional to the radius.

As shown in Fig. 5, the MDS provided 2 dimensions (2 coordinates) with values that indicated a higher similarity in the composition of the mosquito populations between sites 1 and 3, while site 2 was located separate from the other two in ordination space. The greatest similarity between sites 1 and 3, and dissimilarity between these sites and site 2, was mainly related to the 1st dimension of the MDS (eigenvalue: site 1 = –0.386, site 2 = +0.792, site 3 = –0.405).

We found a possible relationship between the forest cover and the composition of the mosquito populations only in the 100-m radius (Fig. 6).

DISCUSSION

Since the 1980s, the number of cases of yellow fever in African and American continents has increased with increasing urbanization (Vasconcelos 2003). Species of the genera *Haemagogus* and *Sabethes* are epidemiologically important as sylvan vectors of the yellow fever virus. Therefore, knowledge about the bioecology of these genera is

Table 1. Mosquito abundance per site during June 2001 and December 2002, in Chapada dos Guimarães National Park, Mato Grosso, Brazil.

Species	Site[1,2] 1	2	3
Haemagogus janthinomys	1.37 ± 2.25 a	0.11 ± 0.18 b	3.36 ± 5.50 c
Hg. leucocelaenus	0.45 ± 0.50 a	0.00 ± 0.00 a	0.60 ± 0.67 a
Sabethes glaucodaemon	1.45 ± 0.78 a	3.39 ± 1.76 b	5.81 ± 2.98 c
Sa. chloropterus	1.34 ± 1.71 a	0.63 ± 0.81 b	1.74 ± 2.21 c

[1] Data shown are adjusted mean ± standard error.

[2] For each species, sites not sharing a letter (a–c) differ significantly ($P < 0.05$).

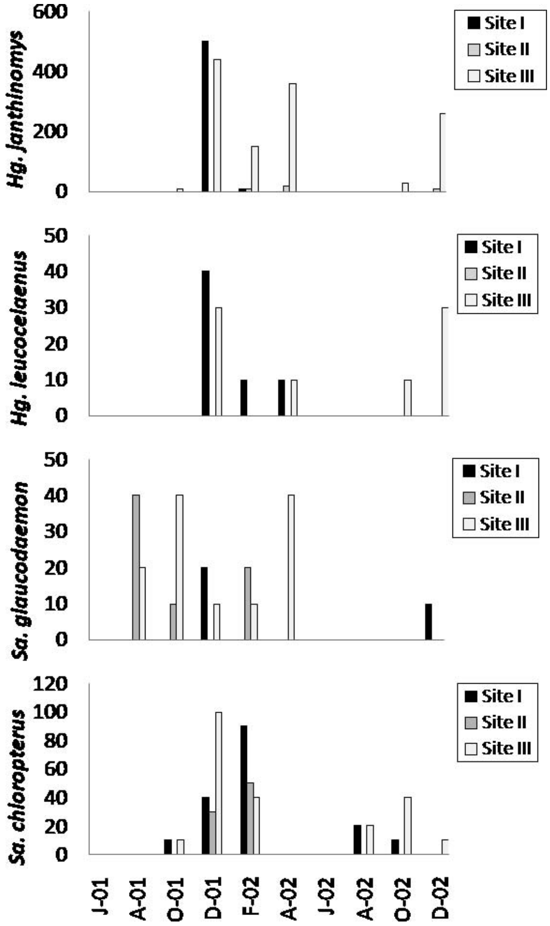

Fig. 3. Seasonal variation in mosquito abundance per site during June 2001 and December 2002.

relevant to better assess the risk of disease transmission. Even though collections were carried out at ground level (understory), *Hg. janthinomys* was the species most frequently collected. Species of *Haemagogus* have strong acrodendrophilic habits, and *Hg. janthinomys*, considered arboreal, has been mainly found in the canopy of rain forest or deciduous forest (Trapido and Galindo 1957, Ramírez et al. 2007, Tubaki et al. 2010), and at ≥16 m above the ground (Pinto et al. 2009). Still, Alencar et al. (2005), based on blood meal patterns from different regions of Brazil, concluded that the feeding habits of *Hg. janthinomys* are eclectic and females move from tree crowns to the ground based on host availability. *Haemagogus leucocelaenus*, on the other hand, although frequently searches for blood meals at the canopy, is also active at ground level (Pinto et al. 2009) and lays its eggs at a broad range of heights (Alencar et al. 2016).

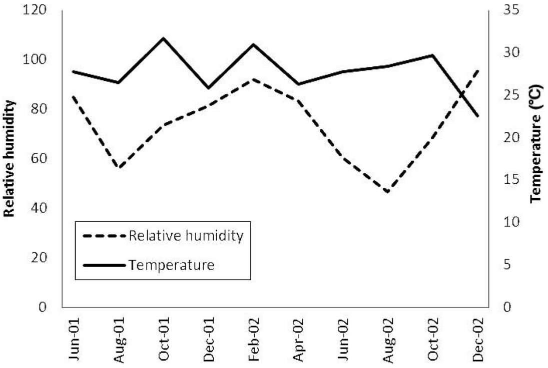

Fig. 4. Seasonal temperature and relative humidity variation throughout mosquito sampling during June 2001 and December 2002.

Some regions of Brazil alternate between tropical and temperate climates (Nimer 1979); we may consider that the geographic regions in this study have prevailing tropical characteristics. We observed that temperature did not have a significant effect on the number of specimens or species collected. Since temperature varied within a range that is suitable for mosquito activity (average 27.6°C, range 21.5–32.7°C), these results were expected. Relative humidity, on the other hand, positively influenced total mosquito abundance and species richness at Chapada dos Guimarães National Park. Guimarães et al. (2000) reported a positive effect of RH on 3 species, *Culex nigripalpus* Theobald, *Aedes serratus* (Theobald), and *Wyeomyia pilicauda* (Root)), and this effect was associated with an increase in the number of specimens captured. Therefore, the hot and wet months, with higher rainfall rates, contributed to the increase in diversity and density of the mosquito species captured. Still, contributions of RH to species richness or abundances were moderate in the present study. Weak or no significant correlations between mosquito diversity and climate variables were observed in a tropical Atlantic Forest area within Guapiaçu Ecological Reserve (Rio de Janeiro) (Alencar et al. 2015), suggesting that factors other than climate may have more influence on temporal variations in the mosquito community.

Relative humidity favored *Hg. janthinomys* populations, supporting observations made by Alencar et al. (2008, 2010). Alencar et al. (2008) reported that *Hg. janthinomys* was collected when the RH values were >70% and the mean temperature was high (~24–30°), conditions most commonly found in the Amazon and afferent gallery forests. The authors highlighted that RH also directly favored the activity of the *Hg. leucocelaenus* populations (Alencar et al. 2008). Contrastingly, other studies, for example from the Peruvian Amazon, did not show significant correlations between *Hg. janthinomys* abundance and RH or temperature (Ramírez et al. 2007).

Guimarães and Arlé (1984) reported the occurrence of *Hg. leucocelaenus* in every month of the year, except June and November, which differs from the observations in this study, where *Hg. leucoce-*

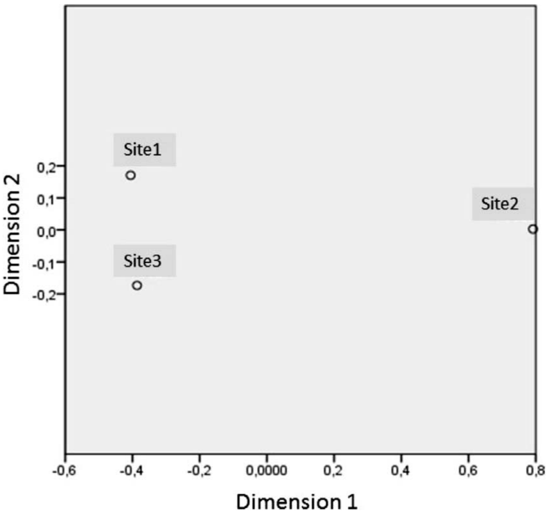

Fig. 5. Nonmetric multidimensional scaling of 3 sites in the Chapada dos Guimarães National Park, Brazil.

laenus specimens were collected from December to April. *Haemogogus janthinomys* was also mostly absent in June and August, similar to observations in gallery forest in Sao Paulo State, Brazil (Tubaki et al. 2010).

Table 2. Forest cover ratios (percent) per study site during June 2001 and December 2002, in Chapada dos Guimarães National Park, Mato Grosso, Brazil.[1]

	Forest cover ratio (%)		
Site	1,000 m	250 m	100 m
Site 1	26.05	38.53	35.25
Site 2	32.56	66.61	99.92
Site 3	63.98	51.73	35.50

[1] 1,000 m = plant cover ratio for the area with a radius of 1,000 m; 250 m = plant cover ratio for the area with a radius of 250 m; 100 m = plant cover ratio for the area with a radius of 100 m.

Multidimensional scaling demonstrated, in 2 dimensions, a similarity in species composition between sites 1 and 3, and a dissimilarity between these 2 sites and site 2. These results also reflect similarities in the total richness and abundance data for the respective sites, where site 2 had the lowest species richness (3 species) and abundance (190 specimens), whereas sites 1 and 3 had the same species richness (4 species), and total abundances greater than site 2 (site 1: 770; site 2: 1,670). The graphic relation between the MDS axes and the forest cover ratios within the different circumferences (buffer areas) pointed to a relationship between the 1st coordinate/dimension (highest dissimilarity between areas) and the 100-m radius area (smallest radius analyzed). Therefore, we may infer that the similarity in species composition between sites 1 and 3 is a consequence of the lower forest cover at the 100-m-radius scale, since both sites had a similar forest cover ratio. Although we did not detect significant differences in temperature or RH between sites, the effects of microclimatic conditions related to forest cover might influence mosquito communities. We need more sample sites to make an assertive conclusion about that. A possible relationship was found only for the 100-m scale, the smallest in this

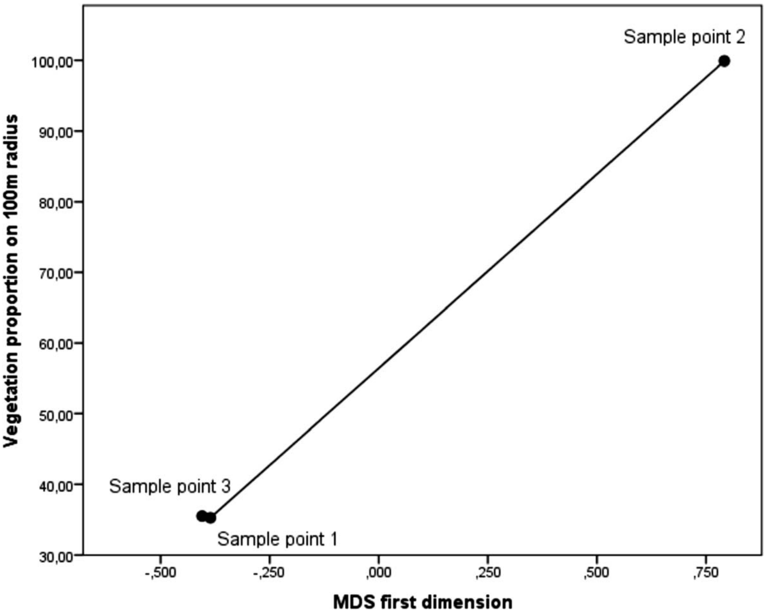

Fig. 6. Graphic showing the relationship between the forest cover and the composition of the Culicidae populations in the 100-m radius during June 2001 and December 2002.

study. Therefore, the vegetation located close to the site may be an important factor for the populations collected in a given site in the region. Nevertheless, the presence of forest cover at the 250- and 1,000-m radius had no graphic relation on the composition of the mosquito populations studied.

Because the number of sites and conditions was limited, further studies on a larger spatial sample are needed to confirm this hypothesis. Interestingly, Schäfer et al. (2006) found contrasting results in Swedish wetlands considering 5 spatial radii ranging from 100 to 3,000 m, where forest cover affected mosquito populations only at the large radius, suggesting the scale at which landscape characteristics have an effect on culicid communities, depending on the biome.

In conclusion, we note that the influence of RH directly favored the activity of mosquito populations and forest cover was a limiting factor for the species studied. Since mosquito abundance was higher in the sites with lower local forest cover, the influence of forest fragmentation in the presence of wild yellow fever vector need to be investigated.

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