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VERTICAL AND TEMPORAL DISTRIBUTION OF MEDICALLY IMPORTANT MOSQUITO SPECIES IN AN ATLANTIC FOREST FRAGMENT OF SOUTHEASTERN BRAZIL: ECOLOGICAL INSIGHTS AND PUBLIC HEALTH IMPLICATIONS

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ABSTRACT. Mosquitoes play a significant role in transmitting several major pathogens, with *Haemagogus* and *Aedes* species receiving considerable attention because of their vectorial capacity. The spatial and temporal distributions of mosquito vectors directly influence the maintenance of arbovirus cycles in specific areas. This study presents a spatial-temporal analysis of culicid entomofauna in an Atlantic Forest fragment in the state of Rio de Janeiro, Brazil. Collections were made from March 2020 to February 2024 using installed ovitraps at varying heights (ground level, 2 m, 4 m, and 6 m) in Fazenda Iguapé, located in the municipality of Silva Jardim. Mosquito communities were analyzed by comparing diversity and abundance indices of species per trap and assessing the temporal variation of culicid populations throughout the collection period. The most abundant species, *Haemagogus leucocelaenus* exhibited an increasing oviposition pattern with elevation. *Aedes terrens* showed a positive association with a height of 6 m. *Aedes albopictus* displayed the highest oviposition at a height of 6 m, followed by ground level and 4 m. The highest abundance of medically important mosquitoes at Fazenda Iguapé occurred in September and November 2023.

KEY WORDS *Aedes*, Atlantic Forest, Culicidae, *Haemagogus*, vector

INTRODUCTION

Arthropod-borne viruses, such as those responsible for dengue, yellow fever, chikungunya, and Zika, pose a significant public health threat in tropical and sub-tropical regions of the world (WHO 2024). These diseases can result in a range of symptoms, from mild fever to severe complications, some of which can be fatal. The etiological pathogens of these arboviral diseases are transmitted by arthropods, primarily mosquitoes and ticks (MS 2024). Mosquitoes play a crucial role in transmitting several major pathogens, notably species of *Haemagogus* and *Aedes*, which are vectors for the yellow fever virus (YFV) and dengue virus (DENV), respectively. Additionally, these mosquitoes are also capable of transmitting other arboviruses, including Mayaro virus (MAYV), chikungunya virus (CHIKV), and Zika virus (ZIKV) (Vasconcelos, 2003, Campos et al. 2015, CDC, 2018; Silva et al. 2018, Alencar et al. 2021).

The distribution of mosquito vectors across different locations and periods directly influences the persistence of arboviruses in a given area. Mosquitoes thrive in the presence of water—whether temporary or permanent—and specific environmental conditions, such as light, temperature, and salinity, as well as the presence of plants/their products and microorganisms/their products (Consoli and Lourenço-de-Oliveira, 1994; Silva et al. 2022a). In forest environments, mosquitoes can lay their eggs in a range of breeding sites, from ground level to treetops, depending on their genera or species (Forattini, 2002). *Haemagogus* spp. are primarily sylvatic mosquitoes, characterized by diurnal activity and acrodendrophilic habitats (Arnell 1973, Alencar et al. 2013). In contrast, *Aedes* spp. eggs are commonly found at ground level (Alencar et al. 2016).

The Atlantic Forest biome is one of the richest regions in biodiversity globally, harboring numerous endemic and endangered species. However, it has experienced significant degradation because of deforestation and poor soil conservation practices (SECOM 2024). The remarkable diversity of flora, along with both vertebrate and invertebrate fauna, creates a variety of ecological niches that support the proliferation of culicid species, including those of epidemiological importance (Alencar et al. 2011).

This study analyzed the population density of epidemiologically important species from the genera *Haemagogus* and *Aedes*, as well as their vertical oviposition activity, in an Atlantic Forest fragment in the state of Rio de Janeiro, Brazil.

MATERIALS AND METHODS

Ethics statement

The permanent license for the collection, capture, and transport of zoological material was granted by the Chico Mendes Institute for Biodiversity Conservation (ICMBio) and the System for Authorization

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and Information on Biodiversity (SISBIO) under License N° 84318.

Study area

Sampling was conducted at Fazenda Iguapé, located in the municipality of Silva Jardim, state of Rio de Janeiro, within the Environmental Protection Area (APA) of the Rio São João/Mico-Leão-Dourado Basin (Fig. 1). This region, of significant ecological importance, is situated in the heart of the Atlantic Forest—one of the most biodiverse and threatened biomes on the planet—and holds a strategic position in conservation efforts for native fauna and flora. Fazenda Iguapé spans approximately 500 ha, encompassing native forest ecosystems, fields, and reforested areas, all under environmental protection regimes designed not only for preservation but also for the restoration of degraded ecosystems.

The Rio São João/Mico-Leão-Dourado Basin APA was established primarily to protect the region's natural resources, particularly the watersheds and areas inhabited by the golden lion tamarin (*Leontopithecus rosalia* L.), an endemic and critically endangered species. The golden lion tamarin, a symbol of biodiversity conservation in the state of Rio de Janeiro, depends on the integrity of remaining forest areas for its survival. In this context, Fazenda Iguapé and other properties in the region play a crucial role in preserving the habitat of this primate and many other threatened species (ICMBIO 2020). According to the Köppen classification system, the climate is predominantly AW, with dry winters and humid summers, an average annual temperature of 24.5° C, and an average annual precipitation of 1,200 mm (INPE 2020, Beck 2018).

Ovitrap installation at the collection points

During the sampling period from May 2020 to February 2024, one tree was selected at each collection point—Point 1 (22°30′20.0″S, 42°19′35.9″W) and Point 2 (22°30′27.8″S, 42°19′59.7″W)—for the installation of ovitraps at different heights (ground level, 2 m, 4 m, and 6 m). Four additional ovitraps were installed at a height of 1.5 m, distributed around the tree with a 10-m distance between them. These traps consisted of a black container with a capacity of 500 ml, without a lid (9 cm in high and 12 cm in diameter), resembling a plant pot, and containing four wooden oviposition paddles (2.5 cm × 14 cm) held vertically inside the trap by a clip. Natural water and litter were added to each ovitrap to recreate a micro-ecosystem similar to the surrounding breeding sites.

The traps were carefully installed using a method that involved throwing a rope with a fishing sinker approximately 4 cm in diam. This method allowed precise placement of the rope over the branches of the selected tree. The traps were then hoisted to the desired heights, ensuring effective sampling at various levels.

This installation procedure was repeated at each collection point, with a single tree chosen to represent each sampling unit. By positioning traps at different heights and including additional traps around the trees, the setup offered valuable insights into the variations in mosquito fauna across the tree's vertical structure and its surrounding area.

Rearing of immature culicids

The positive paddles (containing eggs) were transported to the Diptera Laboratory at the Oswaldo Cruz Institute (Fiocruz) in the city of Rio de Janeiro for egg counting. The paddles were then submerged in dechlorinated water in transparent trays placed in a laboratory greenhouse with controlled conditions: a temperature of 28°C ± 1°C, RH of 75–90%, and a 12-h day/night cycle.

After three days, the paddles were removed from the water and left to air-dry for another three days. The water in the trays was checked to quantify the hatched larvae. The immersion and air-drying cycles were repeated until all viable eggs had hatched. The larvae were fed TetraMint fish food (Tetra, Blacksburg, VA, USA) and monitored daily.

The adult mosquitoes reared by this methodology were identified by directly observing their morphological characteristics under a Zeiss stereomicroscope (ZEISS Stemi SV6), following the dichotomous keys developed by Arnell (1973) and Forattini (2002). After species identification, all specimens were deposited in the Entomological Collection of the Oswaldo Cruz Institute.

Statistical analysis

An ANOSIM test was conducted to compare mosquito communities between the two sampling points (Point 1 and Point 2). Principal component analysis (PCA) was used to explore the relationships between heights and the presence of medically important mosquito species: *Aedes albopictus* (Skuse), *Ae. terrens* (Walker); *Haemagogus janthinomys* (Dyar); and *Hg. leucocelaenus* (Dyar and Shannon).

A Kruskal-Wallis test was applied to evaluate differences in species abundance across vertical strata, whereas Bray-Curtis dissimilarity analysis was used to cluster heights based on differences in mosquito species composition.

A logarithmic transformation was applied to minimize species abundance disparities and improve data distribution for statistical analyses. Specifically, a constant value of 1 was added to all abundance data to account for zero values, and the transformed data were calculated as $\log(Y + 1)$, where Y represents the original abundance. This transformation was implemented to stabilize variance and normalize the data.

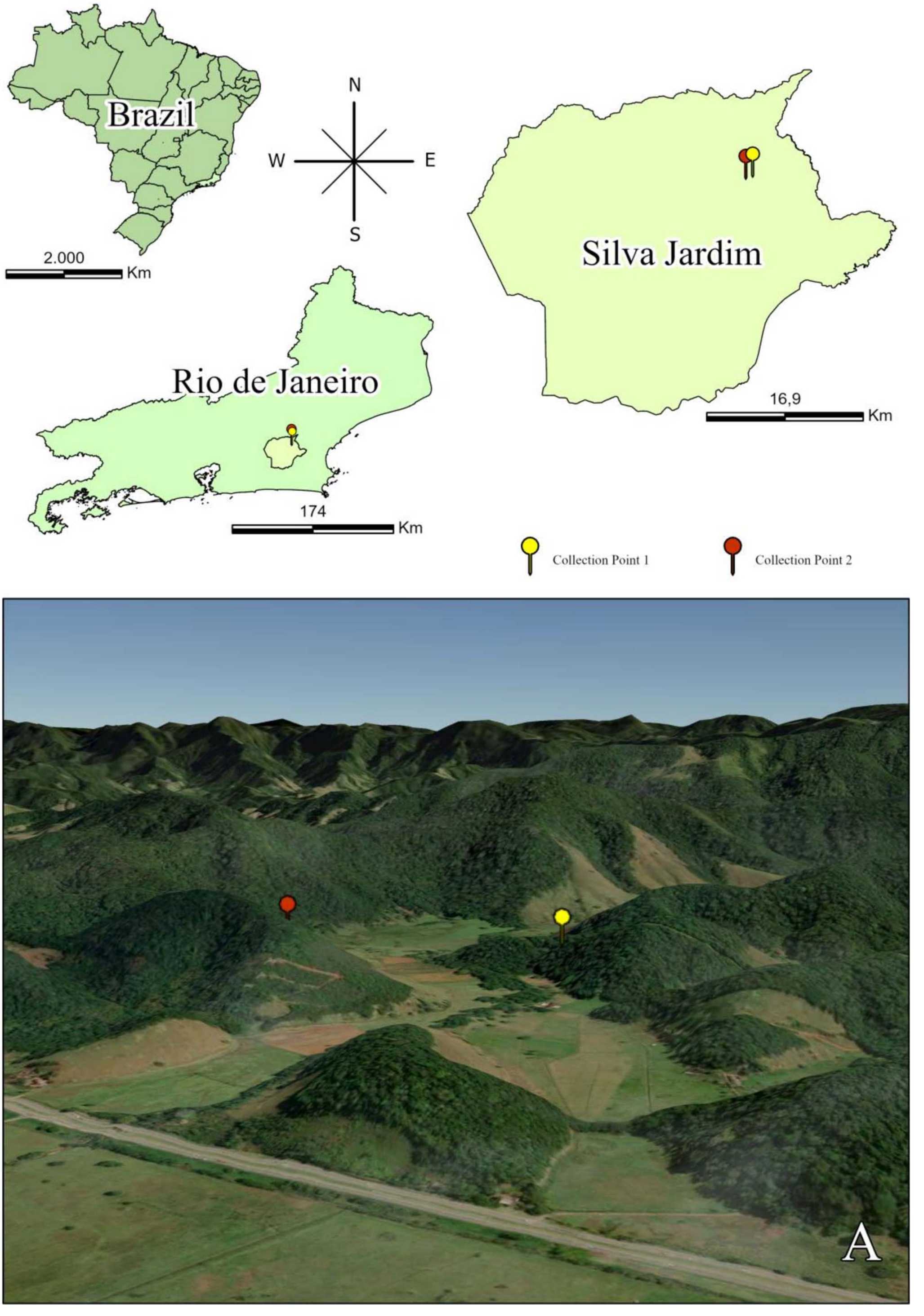

Fig. 1. Sampling location at Fazenda Iguapé, Silva Jardim, Rio de Janeiro, Brazil. Panoramic view of the study area, highlighting the two trap installation points within a homogeneous vegetation zone. Source: Google Earth®, Maxar Tecnologies® satellite image/Pass date: March 2025.

Table 1. Abundance of medically important mosquito species collected at Fazenda Iguapé, Silva Jardim, Rio de Janeiro, Brazil, from March 2020 to February 2024.

Species	Point 1	Point 2	Total
Aedes albopictus	9	80	4,702
Ae. terrens	144	78	72
Haemagogus leucocelaenus	2,176	2,526	89
Hg. janthinomys	30	42	222
Total	2,359	2,726	5,085
Species	4	4	–
Dominance (D)	0.8586	0.8606	–
Shannon Diversity (H)	0.3071	0.3401	–
Equability (J)	0.2215	0.2454	–

RESULTS

Comparison between points 1 and 2

Among the mosquitoes that reached the adult stage and were identified as medically important species, Point 2 recorded a slightly higher abundance (n = 2,726) compared to Point 1 (n = 2,359), representing 54% of the total specimens collected. The most abundant species at both points was *Hg. leucocelaenus*, accounting for 92.2% and 92.7% at Points 1 and 2, respectively. At Point 1, the second most abundant species was *Ae. terrens* (6.1%), whereas at Point 2, *Ae. albopictus* and *Ae. terrens* had nearly identical abundances, each representing 2.9% of the total. The least abundant species at Point 1 was *Ae. albopictus* (0.4%), whereas at Point 2, it was *Hg. janthinomys* (1.5%) (Table 1). Although diversity (H) and evenness (J) indices were slightly higher at Point 2 (H = 0.3401, J = 0.2454), no statistically significant difference was observed between the points ($P = 0.66501$). An ANOSIM test was conducted to compare mosquito communities between the two groups (Point 1 and Point 2), revealing no statistically significant differences between the communities at the two points ($P = 0.3944$).

Vertical stratification of medically important species

At Point 1, *Ae. albopictus* showed the highest oviposition rate at a height of 6 m, followed by the ground level and 4 m. In contrast, at Point 2, this species was not recorded in ovitraps placed at any height. The low sample size of *Ae. albopictus* at 6 m (n = 6) limits the ability to draw strong conclusions about its unusual presence at this height. The rarest and least abundant species, *Hg. janthinomys* showed a preference for 2 m at both points and was also recorded at 4 m and 6 m at Point 2 (Fig. 2A).

The most abundant species, *Hg. leucocelaenus* exhibited an increasing oviposition rate with elevation at Point 1, with the highest abundance recorded in the canopy (6 m, n = 214) and the lowest at ground level (n = 14). This pattern suggests a

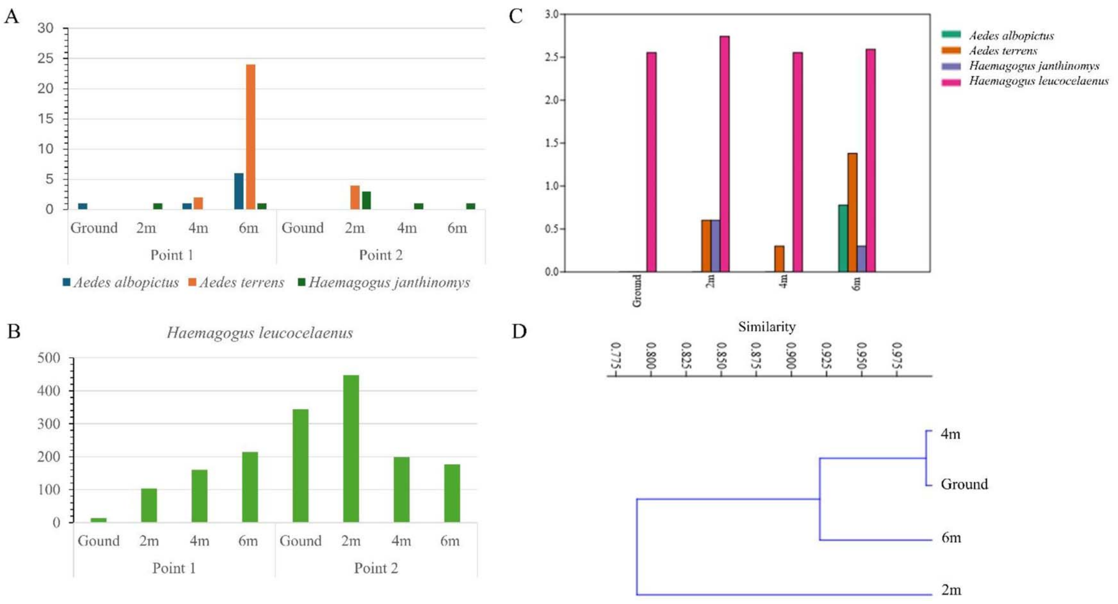

Fig. 2. (A, B) Distribution of medically important mosquito species (*Aedes albopictus*, *Ae. terrens*, *Haemagogus janthinomys*, and *Hg. leucocelaenus*) at different heights (ground, 2m, 4m, and 6m) collected at Fazenda Iguapé, Silva Jardim, Rio de Janeiro, Brazil. (C) Log-transformed abundances (log(Y+1)) of mosquito species across vertical strata, with species color-coded: *Ae. albopictus* (green), *Ae. terrens* (orange), *Hg. janthinomys* (purple), and *Hg. leucocelaenus* (pink). (D) Cluster analysis illustrating the similarity in mosquito species distribution across vertical strata.

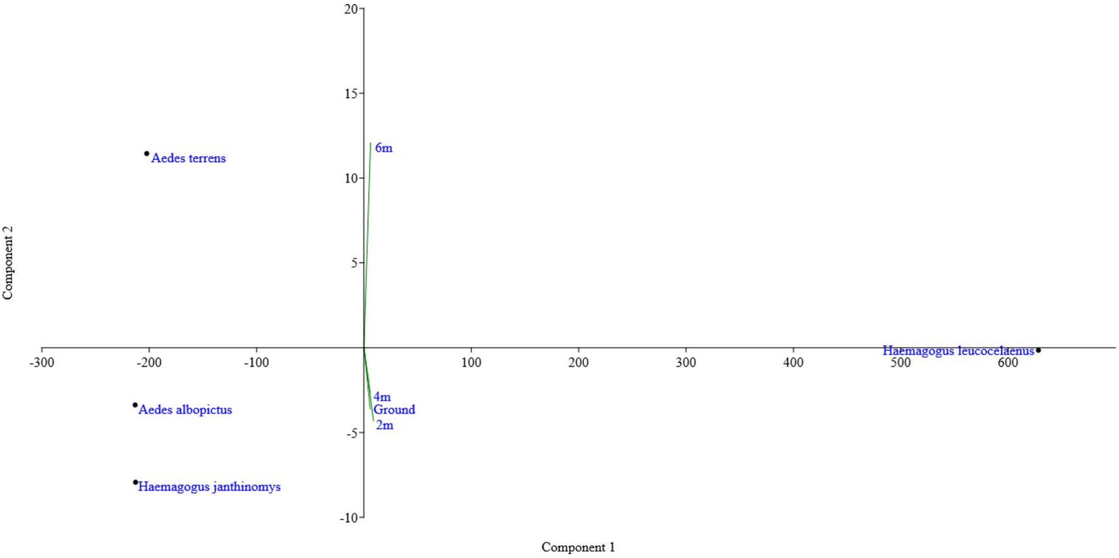

Fig. 3. Principal Component Analysis (PCA) illustrating the relationship between mosquito species and sampling heights at Fazenda Iguapé, Silva Jardim, Rio de Janeiro, Brazil.

gradual preference for greater heights. At Point 2, most eggs from this species were observed at a height of 2 m (n = 448), followed by ground level (n = 344) (Fig. 2B). Due to the significantly higher abundance of *Hg. leucocelaenus*, a logarithmic transformation was applied to reduce the disparity between species abundances and improve data distribution (Fig. 2C).

The Kruskal-Wallis test indicated no statistically significant difference in the median abundances across vertical strata ($P = 0.37$). According to the Bray-Curtis dissimilarity test, the most similar heights were 4 m and ground level, whereas the most distinct height was 2 m, primarily because of the dominance of *Hg. leucocelaenus* (Fig. 2D).

The ordination plot illustrates the distribution of mosquito species in relation to the measured variables. *Aedes terrens* showed a positive association with the height of 6 m, as indicated by its proximity to this variable, suggesting a preference for higher sampling points. In contrast, *Hg. leucocelaenus* was positioned distant from the other species and all analyzed heights, indicating a distinct pattern with no clear association with the measured variables (Fig. 3).

Aedes albopictus and *Hg. janthinomys* were positioned closer to the variables 4 m, 2 m, and ground level, suggesting a potential preference for lower heights or ground-level conditions. However, the short lengths of these vectors indicate that these variables exert a weaker influence on the distribution of these species.

Overall, the results suggest that *Ae. terrens* is associated with higher sampling points, whereas *Ae. albopictus* and *Hg. janthinomys* show a potential relationship with lower heights or ground level. In contrast, *Hg. leucocelaenus* appears to exhibit a distinct distribution that is not strongly influenced by the measured heights.

Comparison between collection years

The mosquito populations at both points exhibited similar seasonal patterns overall, with notable differences at two specific moments. The first occurred in March 2020, when a peak was observed at Point 2 (n = 104), whereas only two individuals were recorded at Point 1. In contrast, Point 1 showed a peak in March 2021 (n = 127), whereas no mosquitoes were recorded at Point 2 during this month and year. Apart from these exceptions, the two collection sites displayed very similar mosquito population fluctuations, with coinciding peaks and troughs throughout the rest of the collection period.

The highest peaks in mosquito populations during the study were observed in November and December 2020, December 2022, January and November 2023, and January and February 2024. When considering all years of collection, the months with the highest abundances were December (n = 653), November (n = 539), September (n = 402), and January (n = 392). The months with the lowest abundances were March and July (n = 0), followed by June (n = 4) and April (n = 7).

The highest abundances of *Hg. leucocelaenus* and *Hg. janthinomys* were recorded in December and November. *Aedes albopictus* was most abundant in January and May, whereas *Ae. terrens* showed higher abundances in January, February, and August (Fig. 4).

DISCUSSION

Haemagogus leucocelaenus was the most abundant species at both collection points in Fazenda Iguapé (municipality of Silva Jardim), which aligns with findings from previous studies in Atlantic Forest

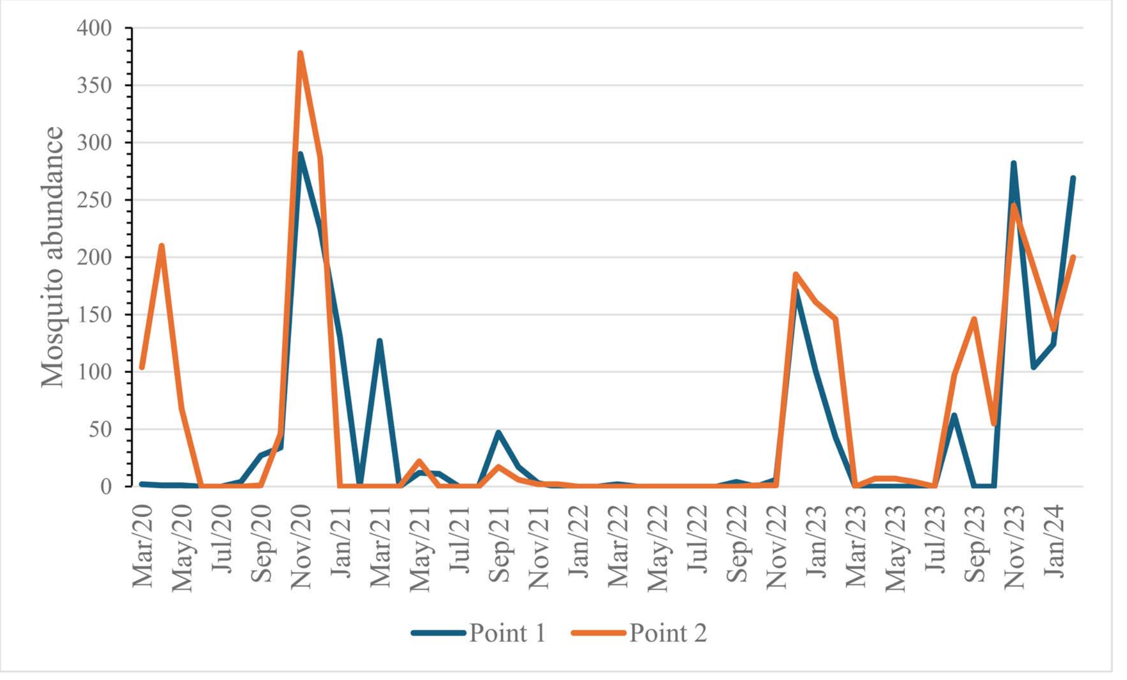

Fig. 4. Fluctuation of medically important mosquito species during the collection period (2020–2024) at Points 1 and 2 in Fazenda Iguapé, Silva Jardim, Rio de Janeiro, Brazil.

fragments in Rio de Janeiro that used similar collection methods (Alencar et al. 2016, Silva et al. 2022b). This species also showed an increase in the number of eggs at higher strata, in agreement with what is extensively reported in the literature about the behavior of this vector (Alencar et al. 2013, Silva et al. 2021, Dias et al. 2023). However, other studies suggest that *Hg. leucocelaenus* is more adaptable, exploring various levels of the forest habitat and moving between the canopy and the ground. This mobility was reflected in the PCA analysis, which did not show a clear preference for any specific height (Tátila-Ferreira et al. 2017; Siches et al. 2021). Nevertheless, the general consensus is that *Haemagogus* spp. are typically associated with acrodendrophilic habitats (Arnell 1973; Marcondes and Alencar, 2010).

Aedes albopictus was more abundant at 6 m, which is unusual since *Aedes* spp. typically oviposit closer to ground level. However, this observation may be explained by the low number of individuals from this species collected (n = 6) (Jin et al. 2023). Similarly, *Ae. terrens* showed a positive association with 6m and was more abundant at this height. This species is considered more related to forest environments and is frequently captured alongside *Haemagogus* spp., which also exhibit acrodendrophilic behavior (Alencar et al. 2013; Silva et al. 2022c).

The highest abundances of *Hg. leucocelaenus* and *Hg. janthinomys* were recorded in December and November, consistent with another study that also observed population peaks during these warmer months, particularly the number of *Hg. leucocelaenus* eggs increased significantly when the mean temperature exceeded 27° C and accumulated precipitation surpassed 100 mm (Couto-Lima et al. 2020). These peaks in mosquito vector populations coincide with yellow fever outbreaks, which have historically occurred in Brazil between December and May (WHO 2019). Since its reemergence in Brazil's Central-West region in 2014, the YFV has progressively spread across the country, especially in areas with low vaccination coverage or where vaccination was not previously recommended. This unprecedented resurgence, including confirmed human cases, hundreds of deaths, and epizootics in non-human primates, prompted the expansion of the Vaccination Recommendation Area (ACRV) to cover the entire country (MS 2020, 2021).

Aedes albopictus was most abundant in January and May. Its importance is tied to its secondary role as a vector for DENV, ZIKV, CHIKV, and YFV (Consoli and Oliveira, 1994; Forattini and Massad, 1998). A particularly high abundance of this vector species was recorded in April 2020 at Point 2 (n= 80). During this month, 876 probable dengue cases were reported in the state of Rio de Janeiro, with a proportion of 47 confirmed cases (SES 2020).

Aedes terrens was the second most abundant species at Point 1, previously reported in a 2011 study in Rio de Janeiro, but with a much lower occurrence (n = 11). In 2022, this species was found in higher numbers (n = 67), and in our study, the numbers increased even further (n = 222). This pattern suggests a potential rise in the population of *Ae. terrens* over the years, likely because of successful environmental adaptation (Alencar et al. 2022, 2011). A high density of this species was observed in May 2020 (Point 2; n = 35), February 2023 (Point 1; n = 22 and Point 2; n = 20), and the highest density in August 2023 (Point 1; n = 54). The species has demonstrated the ability to become orally infected with CHIKV under laboratory conditions, exhibiting high infection and dissemination rates. This finding suggests that *Ae. terrens* could potentially serve as a vector for CHIKV in natural settings, highlighting the importance of monitoring the rise in this mosquito population in Atlantic Forest fragments, particularly in one of the most densely populated areas of Brazil (IBGE 2024; Lourenço-de-Oliveira and Failloux, 2017).

This study underscores the ecological behaviors and public health significance of mosquito vectors in the Atlantic Forest fragments of Rio de Janeiro. The dominance of *Hg. leucocelaenus* and its association with higher forest strata align with its acrodendrophilic behavior. However, some studies suggest habitat plasticity, suggesting this species also occupies lower strata or more disturbed areas under specific conditions. Seasonal peaks in the populations of *Hg. leucocelaenus* and *Hg. janthinomys* during warmer months, driven by climatic factors such as temperature and precipitation, coincide with historical yellow fever outbreaks, reinforcing their role as key vectors in the transmission dynamics of DENV.

The observed increase in *Ae. terrens* populations, coupled with its demonstrated ability to transmit CHIKV under laboratory conditions, highlight the growing epidemiological importance of the species in the region. This trend raises concerns, as *Ae. terrens* may expand its range and contribute to the emergence of new arboviral diseases. Similarly, the detection of *Ae. albopictus* at higher forest strata—though unusual for this species—may reflect behavioral plasticity or local environmental factors, such as microclimate variations, food availability, or human-mediated dispersal. These findings suggest that *Ae. albopictus* is adapting to new ecological niches within the forest ecosystem.

Moreover, the presence of these species in areas near urbanized regions amplifies the potential risk of arbovirus for human populations.

As these vectors adapt to forested environments and interact with urban landscapes, the risk of vector-borne diseases spreading to more densely populated areas escalates.

Our results emphasize the urgent need for continuous surveillance and integrated vector management programs that account for the ecological complexity and behavioral plasticity of these species. A deeper understanding of the ecological roles of these mosquitoes, their distribution patterns, and their potential to transmit diseases across various environments is crucial for developing effective strategies to mitigate the risks posed by emerging arboviruses in regions with significant human-wildlife interactions.

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