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Effects of seasonality on the oviposition activity of potential vector mosquitoes (Diptera: Culicidae) from the São João River Basin Environmental Protection Area of the state of Rio de Janeiro, Brazil

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Abstract
The Atlantic Forest is home to several arboviruses potentially pathogenic to humans. Therefore, it is crucial to assess the effects of seasonality on mosquito populations circulating in this domain. We evaluated the influence of seasonal variation on the oviposition activity of epidemiologically important mosquito populations in an Environmental Protection Area in Rio de Janeiro, Brazil. Mosquito eggs were collected using ovitraps for 1 year. During the sampling period, 1,086 eggs were collected. Of these, 39 (3.6%) did not hatch, and 1,047 (96.4%) reached the adult stage. *Aedes albopictus* (44.8%), *Ae. terrens* (6.4%), and *Haemagogus leucocelaenus* (48.8%) eggs and adults were identified. The changes in this community over the seasons were also analyzed. Season influence on the collections was significant. The highest numbers of collected eggs were collected in the summer and autumn, with *Hg. leucocelaenus* dominant in the summer and *Ae. albopictus* in the autumn. These two seasons were more similar to each other in terms of the composition of the collected mosquito community, forming a separate cluster from winter and spring groups. Summer, autumn, and winter presented values of Dominance (D), Shannon Diversity (H), and Evenness (J) closer to each other than spring. Climatic factors recorded throughout the collection period were not associated with egg abundance, except for temperature, which was positively correlated with *Ae. albopictus* presence. Finally, seasonality seemed to influence the oviposition activity of the three species recorded. Summer and autumn were the most critical seasons due to *Ae. albopictus* and *Hg. leucocelaenus* circulation. These findings should be considered in prophylaxis and implementation of entomological control strategies in the study area.

Keywords: *Haemagogus leucocelaenus*, *Aedes terrens*, *Aedes albopictus*, *seasonality*

Introduction

The Atlantic Forest has experienced severe human activity impacts associated with population densification of the Brazilian coast throughout history. Currently, the most extensive and preserved remnants of the Atlantic Forest are located within Conservation Units (Grelle et al. 2021). These preserved areas serve as refuges for fauna, including mosquito species (Evangelista et al. 2021).

Many studies have been focused on mosquitoes due to their epidemiological importance, associated with the circulation of various arboviruses in wild environments. Much of the research focuses on geographic distribution and the association of climatic and environmental aspects that directly influence the population dynamics of several Culicidae species (Asigau et al. 2017; Freire et al. 2021). The effect of seasonality on mosquito populations in the Atlantic Forest, as well as the dynamics of arboviruses trans-

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mitted by them, is an important public health issue (Possas et al. 2018).

There are approximately 3,600 species of mosquitoes described worldwide (Wilkerson et al. 2021), 530 of which have been reported in the Brazilian territory (Hutchings et al. 2020). The vast majority of these species are from the Atlantic Forest. Among these species, those belonging to the genera *Aedes* and *Haemagogus* are notable for carrying and transmitting yellow fever, Zika, and other arboviruses (Vanlandingham et al. 2015; Lourenço-de-Oliveira & Failloux 2017; Alencar et al. 2021).

Aedes albopictus (Skuse 1895) is an invasive species that has been adapted successfully even in degraded Atlantic Forest fragments, with the remarkable ability to move between the forest edge and urban environments (Santos et al. 2018). They are mosquitoes with diurnal behavior mainly concentrated in two activity peaks comprising morning and afternoon (Forattini 2002). *Aedes terrens* (Walker 1856) is very eclectic in the type of habitat and has been found in preserved forests to small forest fragments inserted in the urban environment (Stahlhöfer et al. 2021). This species is diurnal, and oviposits and feeds preferentially on the treetops (Guimarães et al. 1985). *Haemagogus leucocelaenus* (Dyar & Shannon 1924) is considered the most common species of *Haemagogus* in Brazil, as its broad phenotypic plasticity allows it to inhabit degraded wild environments (Marcondes & Alencar 2010). This culicid species also has acrodendrophilic habits (Pinto et al. 2009).

The use of ovitraps to collect mosquito eggs, especially those of *Ae. albopictus, Ae. terrens* and *Hg. leucocelaenus* has been shown to be effective in egg collection, when installed in wild environments (Maia et al. 2020). This collection methodology even proves to be important in studies on the detection of arboviruses in these mosquito species, as it allows eggs to be collected in nature and transported to the laboratory, where adults emerging from this material can be quickly identified and killed, which facilitates the preservation of viral genetic material (Alencar et al. 2021).

Therefore, understanding the biological responses and population dynamics of vector species such as *Ae. albopictus, Ae. terrens* and *Hg. leucocelaenus* to seasonal variations implies a better assessment of arbovirus transmission patterns. This study aimed to evaluate the influence of seasonal variation on the oviposition activity of epidemiologically important mosquito populations in the Environmental Protection Area of the São João River Basin, allowing us to establish which climatic seasons are more prone to the proliferation of these culicids in an area of the Atlantic Forest.

Materials and methods

Ethics statement

The collection, capture, and transport of zoological materials were authorized by Chico Mendes Institute for Biodiversity Conservation (ICMBio), Ministry of the Environment of Brazil, under license No. 44333-1. All members responsible for the collections received vaccination against yellow fever virus and were aware of the potential risks in the study area.

Study area

The study was conducted within the São João River Basin Environmental Protection Area (EPA), in Silva Jardim, state of Rio de Janeiro, Brazil. The EPA of the São João River Basin is a conservation unit with 150,374.61 hectares of Atlantic Forest. This conservation unit is located at Km 214 of BR 101 Highway in the city of Silva Jardim, Rio de Janeiro, Brazil (ICMBIO 2020). The São João River is the main watercourse of the region, significantly contributing to the water supply of the surrounding regions through the Juturnaíba Reservoir of Araruama, state of Rio de Janeiro. The vegetation is characterized by dense ombrophilous forests, also known as tropical rainforests. This ecosystem comprises evergreen foliage, canopies up to 50 m high, emerging trees up to 40 m tall, and dense shrub vegetation composed of ferns, bushes, and bromeliads (MMA 2008).

Collections and laboratory procedures

Between July 2018 and June 2019, monthly collections of mosquito eggs were carried out using ovitraps. Eighteen ovitraps were distributed using convenience sampling, a non-probabilistic sampling technique (Kenkel 1989), where traps were positioned at a minimum distance of 40 m from each other. Each ovitrap was placed at a height of 2.50 m affixed to trees located along a walking trail near the edge of the forest fragment used by visitors.

Eighteen ovitraps were installed at random intervals, with one trap placed at a height of 2.50 m per tree, between July 2018 and June 2019 (Figure 1 and Table I).

Ovitraps were used as a standard tool for the collection of Culicidae eggs. These traps consist of

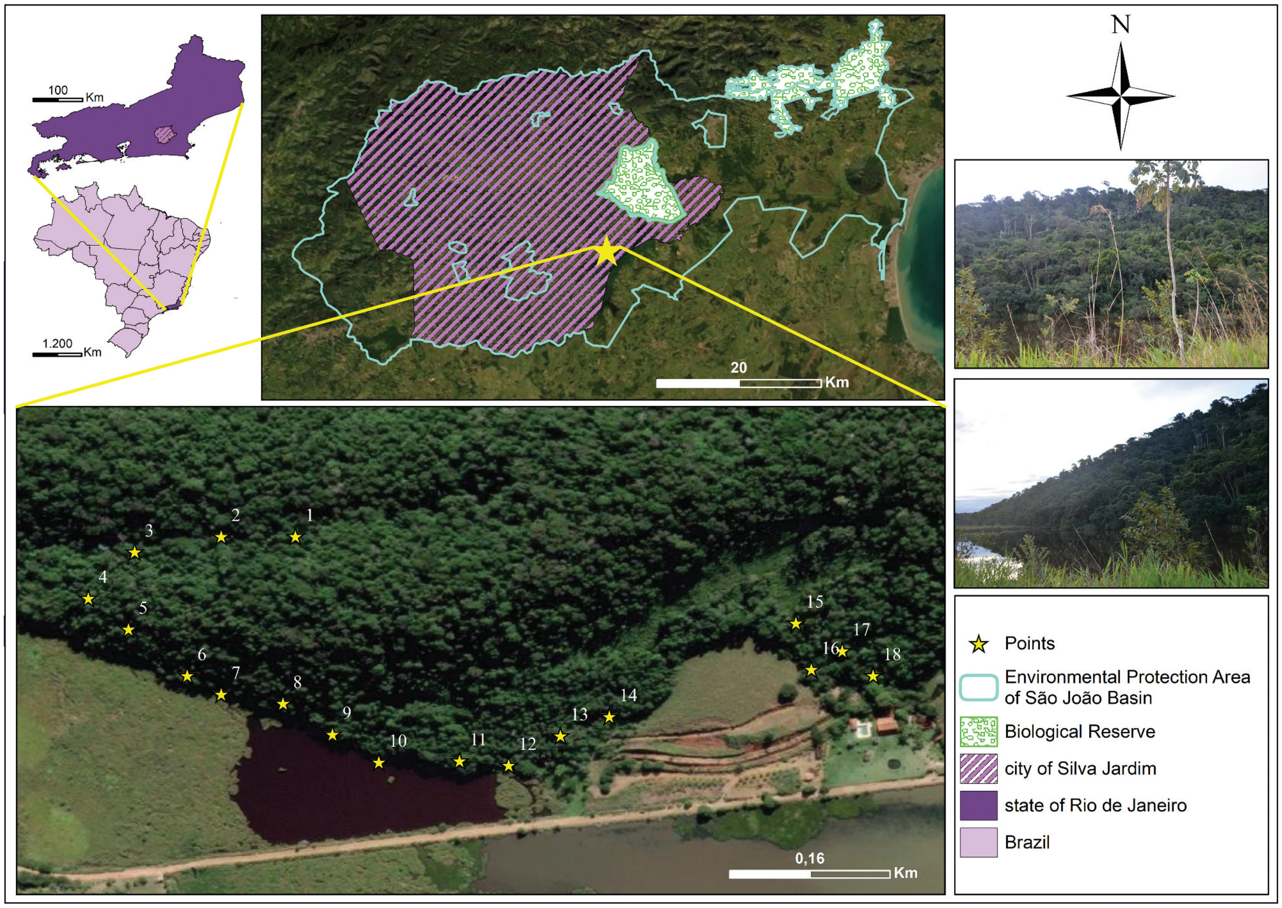

Figure 1. Location of the eighteen sampling points in the Environmental Protection Area of São João Basin, Biological Reserve of the city of Silva Jardim, state of Rio de Janeiro, Brazil. Georeferenced positions for the ovitraps: Point 1. 22°37′10.7″S, 42°18′59.5″W; Point 2. 22°37′10.7″S, 42°19′01.9″W; Point 3. 22°37′11.2″S, 42°19′04.7″W; Point 4. 22°37′12.7″S, 42°19′06.2″W; Point 5. 22°37′13.7″S, 42°19′04.9″W; Point 6. 22°37′15.2″S, 42°19′03.0″W; Point 7. 22°37′15.8″S, 42°19′01.9″W; Point 8. 22 °37′16.1″S, 42°18′59.9″W; Point 9. 22°37′17.1″S, 42°18′58.3″W; Point 10. 22°37′18.0”S, 42°18′56.8″W; Point 11. 22°37′18.0″S, 42°18′54.0″W; Point 12. 22°37′18.1”S, 42°18′53.8″W; Point 13. 22°37′18.1″S, 42°18’ 52.6″W; Point 14. 22°37′13.8”S, 42°18′43.2″W; Point 15.22°37′13.5”S, 42°18′43.3″W; Point 16.22°37′15.0″S, 42°18′42.8″W; Point 17. 22°37′14.4″S, 42°18′41.8″W; Point 18. 22°37′15.2”S, 42°18′40.8″W.

a 500 mL black-colored unlidded container resembling a plant vase. Each ovitrap contained four wooden oviposition paddles (2.5 × 14 cm), vertically held by a clip inside the trap. A total of 300 mL of natural water and approximately 100 g of leaf litter were added to each ovitrap in an effort to recreate a microecosystem resembling the natural environment. The ovitraps were inspected for the presence of eggs, and their aquatic content changed every 10 days throughout the collection period.

Once collected from the ovitraps, the paddles were packed in a polyethylene box and sent to the Diptera Laboratory of the Oswaldo Cruz Institute (FIOCRUZ). Paddles containing eggs were considered positive; these were sorted in the laboratory and subjected to egg counting. Then, these paddles were immersed in transparent trays containing dechlorinated water. Subsequently, the eggs were placed in a laboratory greenhouse under a controlled experimental environment and regulated thermoperiod (28°C ± 1°C, 75–90% relative air humidity, and a 12 h light/12 h dark cycle). These conditions allowed us to keep the specimens alive and perform species identification on adult forms after hatching, according to the methodology described by Alencar et al. (2013).

Species identification was carried out by directly observing morphological characters under a stereomicroscope and using dichotomous keys developed by Arnell (1973) and Forattini (2002). Abbreviations of genus and subgenus names were based on Reinert’s proposal (2009). After species identification, all specimens were listed in the FIOCRUZ Entomological Collection.

Table I. Abundance, relative frequency, dominance, diversity, and evenness in different seasons of mosquito species collected between July 2018 and June 2019 in the EPA of the São João River Basin, Rio de Janeiro, Brazil.

Species	Summer *N* (%)	Autumn *N* (%)	Winter *N* (%)	Spring *N* (%)
Ae. albopictus	175 (40.4)	259 (55.6)	18 (40.9)	17 (16.3)
Ae. terrens	9 (2.1)	13 (2.8)	1 (2.3%)	44 (42.3)
Hg. leucocelaenus	249 (57.5)	194 (41.6)	25 (56.8)	43 (41.4)
Total	433 (100.0)	466 (100.0)	44 (100.0)	104 (100.0)
Dominance (D)	0.49	0.48	0.49	0.38
Shannon diversity (H')	0.76	0.79	0.77	1.03
Evenness (J)	0.70	0.72	0.70	0.93

Data analysis

We used the Chi-square test to analyze the effect of seasonal variation on the abundance of mosquito species using the statistical software GraphPad Prism 8.01 for Windows. To analyze the effect of seasonal variation on the mosquito community, the Bray–Curtis similarity index was calculated, and values for Dominance (D), Shannon Diversity (H), and Pielou's evenness (J) were obtained using the PAST 4.03 statistical software (Hammer et al. 2001). This software was also used to generate a canonical correspondence analysis (CCA), which was applied to verify the effect of the evaluated climatic variables (mean temperature and accumulated rainfall) on the sampled mosquito species. The level of significance used in the statistical tests was $p < 0.05$.

Mean temperatures and accumulated rainfall during the study period were obtained from the Weather Prevision Center and Climate Studies (CPTEC) of the National Institute for Space Research (INPE 2020).

Results

During the sampling period, 1,086 eggs were collected in the São João River Basin area. Of these, 39 (3.6%) did not hatch, and 1,047 (96.4%) evolved to the adult stage, corresponding to the following species: *Ae. albopictus* (Skuse, 1895) (469–44.8%), *Ae. terrens* (Walker, 1856) (67–6.4%) and *Hg. leucocelaenus* (Dyar & Shannon, 1924) (511–48.8%).

The three species of mosquitoes were present in the collections in all seasons. *Haemagogus leucocelaenus* was the most abundant species in the summer (57.5%) and winter (56.8%). In contrast, *Ae. terrens* was the most abundant species in the spring (42.3%) and *Ae. albopictus* was the most abundant in the autumn (55.6%) (Table I). The influence of seasonality on the collections was significant by Chi-square analysis ($\chi 2$ = 222,9; d.f. = 6; $p < 0.0001$).

The highest number of eggs was obtained in the collections carried out in the autumn (466–44.5%) and summer (433–41.4%), followed by spring (104–9.9%) and winter (44–4.2%), respectively. Summer, autumn, and winter presented D, H, and J values closer to each other than when compared to spring (Table I). The Bray-Curtis index indicated a high similarity between the composition and abundance of mosquito species collected in the autumn and summer (85%), whereas the cluster formed by species collected in the spring and winter had a similarity of approximately 60% (Figure 2).

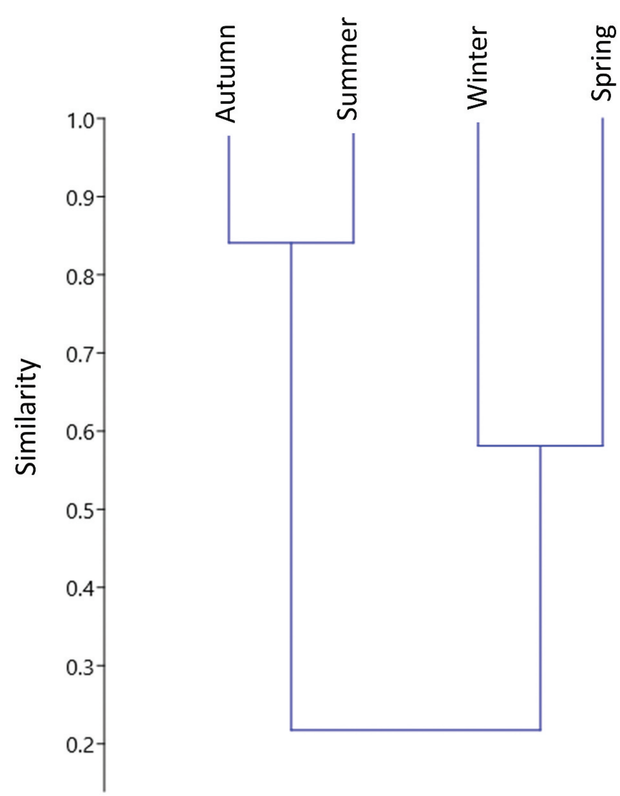

Figure 2. Cluster analysis (Bray-Curtis index) of mosquito samples collected in different seasons between July 2018 and June 2019 in the EPA of the São João River Basin, Rio de Janeiro, Brazil.

The CCA indicated significant positive correlations between *Ae. albopictus* with temperature (°C) ($r = 0.59$; $p < 0.05$), but not with precipitation (mm) ($p > 0.05$). The other species did not show a positive correlation with temperature or rainfall ($p > 0.05$) (Figure 3).

Discussion

The three species of mosquitoes recorded in this study had already been captured by ovitraps in a wild environment, including in Atlantic Forest areas of the state of Rio de Janeiro (Alencar et al. 2016). When litter is added to its aquatic content, these traps simulate the conditions of breeding sites formed by tree holes (Alencar et al. 2004). Immature forms of *Ae. terrens* and *Hg. leucocelaenus* are commonly found in tree holes (Mangudo et al. 2018). *Aedes albopictus* has less frequent records of occurrence in these breeding sites than the other two, as it is a species whose occurrence in a wild environment is limited to areas most impacted by human activity (Pancetti et al. 2015).

Our results showed a greater abundance of *Hg. leucocelaenus* eggs, a species dominant in the summer and autumn. Studies involving the installation of ovitraps in fragments of the Atlantic Forest in Rio de Janeiro usually indicate dominance of this species (Alencar et al. 2016) often in all seasons of the year (Silva et al. 2018a). *Aedes albopictus* was the second most abundant species identified, dominant in the autumn. Silva et al. (2018b) observed that *Ae. albopictus* was also the second most abundant species collected from ovitraps installed in forest areas. In a study using ovitraps to monitor *Ae. albopictus* in an urban area of the state of São Paulo, this species was present during the summer and autumn, with higher population numbers registered in April (Serpa et al. 2006). Autumn and summer seem to offer climatic conditions that favor the proliferation of *Ae. albopictus* and *Hg. leucocelaenus*. *Aedes terrens* was the least abundant species, whose presence was more prevalent in the spring and nearly absent in the winter. In a study developed by Silva et al. (2021), *Ae. terrens* was also less frequent in ovitraps installed in a forest area of Rio de Janeiro than *Hg. leucocelaenus* and *Ae. albopictus*. Campos (2016) observed in natural breeding sites formed by bamboo stumps that *Ae. terrens* only occurred in spring, being absent in other seasons.

The greater absolute abundance of eggs, as well as greater similarity in the composition of species recorded in the autumn and summer (Table I and Figure 2), indicates that these seasons possibly offer climatic conditions that favor the proliferation of mosquitoes, especially of *Ae. albopictus* and *Hg. leucocelaenus*. Although several studies suggest that the structure and complexity of the environment have an important effect on the diversity and composition of mosquito species (Catenacci et al. 2018),

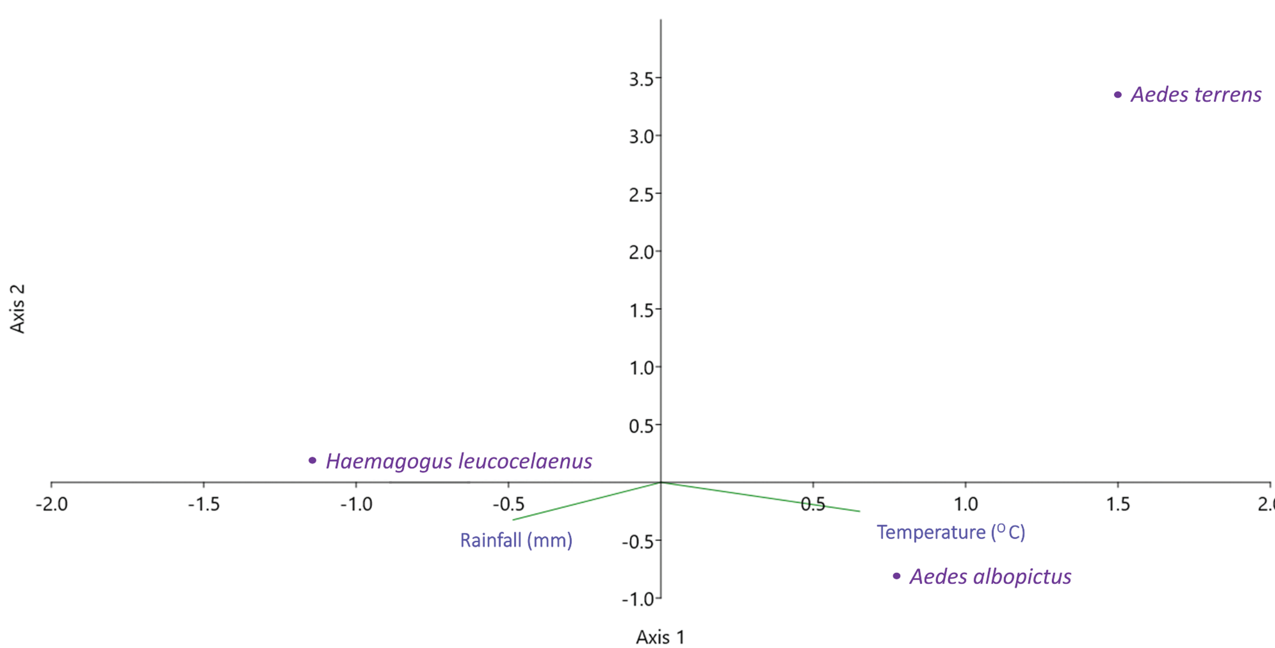

Figure 3. Ordination diagram of mosquito species and climatic variables recorded between July 2018 and June 2019 in the EPA of the São João River Basin, Rio de Janeiro, Brazil, from canonical correspondence analysis (CCA).

variations in climatic conditions are also an important element (Wilke et al. 2017). As reported in other studies, the increase in rainfall and temperature in the summer allows an increase in mosquito populations due to the formation of new breeding sites and the intensification of the activity of these insects (Chadee & Tikasingh 1991; Muller & Marcondes 2007; Medeiros-Sousa et al. 2015). In the present observations and as previously reported, autumn proved to be very similar to summer, which can be explained by the delay in climate change influenced by seasonality that affects mosquito communities. Probably, the weather conditions generated in the summer were still influencing the mosquito records carried out throughout the autumn (Couto-Lima et al. 2020). Couto-Lima observed a significant effect of temperature and rainfall on the oviposition of sylvatic mosquitoes after four weeks. The same pattern was observed for *Ae. aegypti* in an urban environment, where the egg density was affected by the variables temperature and rainfall after three and 4 weeks of their records (Honório et al. 2009), indicating that, possibly, climatic variables recorded in a season can influence the abundance of mosquito eggs in the following season.

The abundances of *Hg. leucocelaenus, Ae. albopictus* and *Ae. terrens* showed similar values in the spring when compared to other seasons. This implied higher values of H and J and lower values of D. The high abundance of *Hg. leucocelaenus* in summer, of *Ae. albopictus* in the autumn and the reduced abundance in the winter influenced the results with lower values of H and J, and higher values of D at these stations (Table I). In contrast to the present study, Guedes and Navarro-Silva (2014) collected adult mosquitoes in an area of Atlantic Forest in the state of Paraná and observed lower values of evenness and diversity and greater dominance during the spring. This pattern was also observed by Cardo et al. (2012), who studied immature forms of mosquitoes in different breeding sites in a wild area of Argentina.

In this study, no significant correlation was found between the abundance of eggs of the three species and climatic factors, except for *Ae. albopictus*, which showed a positive correlation with temperature (Figure 2). This result corroborates the study by Silva et al. (2021) conducted in a region near the São João River EPA using ovitraps and showing a positive correlation between *Ae. albopictus* egg abundance and temperature. In the present study, water was initially added to the ovitraps, and these were revised every 10 days when the paddles were changed; if necessary, more water was added during inspections. By these means, eventually, in drier and hotter periods, the traps continued to have water available for female mosquitoes' oviposition. This methodology may have influenced the lack of a significant correlation between rainfall and egg abundance.

In conclusion, seasonality seemed to influence the oviposition activity of the three species recorded. Summer and autumn can be considered the most critical seasons for *Ae. albopictus* and *Hg. leucocelaenus*, which should be considered in any prophylaxis and entomological control strategies implemented in the São João River Basin Environmental Protection Area.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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