

CHAPTER 4

OCCURRENCE OF THE SYLVATIC YELLOW FEVER VECTOR MOSQUITO *HAEMAGOGUS LEUCOCELAENUS* (DIPTERA: CULICIDAE) IN AN ATLANTIC FOREST FRAGMENT OF THE TOURISTIC STATE OF RIO DE JANEIRO, BRAZIL

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ABSTRACT: The yellow fever virus is estimated to cause 30,000 deaths each year worldwide, with the majority of cases and deaths occurring in Africa. The virus is also endemic to Central and South America, including northern and western Brazil. The sylvatic cycle of the virus is related to wild and rural areas, with nonhuman primates as the primary host and wild mosquitoes, specifically from the genera *Haemagogus*, as vectors. The diversity of the mosquito community plays a significant role in the increase of pathogen transmission to humans. In the present study, we detected fluctuation in populations of vector mosquitoes using ovitraps for Culicidae egg collection. The study area is a forest fragment of the Atlantic Forest, one of the most threatened biomes in Brazil. This biome has been suffering significant deforestation due to anthropic activity. Worryingly, the proximity of human populations to forest environments increases the risk of spreading disease from forest fragments to urban areas. Our findings showed that the highest egg abundance occurred in December 2019, with a significant difference ($p = 0.005$) between rainy and dry seasons. Most eggs were collected during the rainy period. Subsequent quantification of

specimens from epidemiologically relevant species hatched from field-collected eggs resulted in 1,131 (86%) *Haemagogus leucocelaenus* (Dyar & Shannon, 1924), 111 (8%) *Aedes terrens* (Walker, 1856), 47 (4%) *Aedes albopictus* (Skuse, 1894), and 21 (2%) *Haemagogus janthinomys* (Dyar, 1921). Finally, we assessed the behavior of different vector species performing oviposition on the same breeding site. The highest correlation coefficient was observed between *Ae. albopictus* and *Ae. terrens* ($\rho = 0.52$) concerning other Culicidae species. Therefore, we believe that Culicidae population surveillance is crucial for disease monitoring since the increase in specimens of a number of vector species influences the emergence of yellow fever cases in nonhuman primates and human populations.

KEYWORDS: *Haemagogus leucocelaenus*, vector, yellow fever, correlation, rainy period

Yellow fever (YF) is an acute viral noncontagious hemorrhagic disease transmitted by infected mosquitoes (WHO 2019). Yellow fever virus (YFV) is estimated to cause 200,000 cases of disease and 30,000 deaths each year (CDC 2018) in the globe. This virus is endemic in tropical areas of Africa and Central and South America (WHO 2019). Specifically, for Brazil, sylvatic YF is endemic in the northern and western areas of the country. YFV has advanced throughout Brazil since its reemergence in the Midwest region in 2014, reaching areas with low vaccination coverage and where vaccination has not been previously recommended (MS 2020a). The sylvatic cycle of the virus is related to wild and rural areas, with monkeys as the primary host and wild mosquitoes, specifically *Haemagogus* spp. Williston, 1896, as vectors (de Abreu et al. 2019). Regarding natural habitats, the Atlantic Forest biome includes a range of forest morphologies harboring a rich and diverse mosquito community that displays considerable spatial variability (Correa et al. 2014). This natural condition is relevant since the risk of pathogen transmission from vectors to humans can significantly increase depending on mosquito community diversity (Keesing et al. 2006). Among this diverse community, the genus *Haemagogus* comprises 28 species widely distributed in the American continent (Marcondes and Alencar 2010). Of these species, *Hg. janthinomys* Dyar, 1921 is known as the primary vector; however, the species *Hg. leucocelaenus* Dyar & Shannon, 1924 is recognized as having a role in maintaining YFV in nature (Vasconcelos et al. 2003). Moreover, *Hg. leucocelaenus* has been detected in the states of Rio de Janeiro and São Paulo, which is alarming since these states represent Brazil's most densely populated regions (Zavartink 1972, IBGE 2010). *Hg. leucocelaenus* and *Hg. janthinomys* were considered the primary vectors in the biggest yellow fever outbreak that occurred in Brazil between 2016 and 2018, and the species *Hg. leucocelaenus* was found naturally infected with YFV in Rio Janeiro State in 2019 (de Abreu et al. 2019). Hence, given the relevance of vector mosquitoes in public health, our study aimed to quantify, compare, and describe the fluctuation of Culicidae populations collected in an Atlantic Forest fragment with circulating YFV in Rio de Janeiro State.

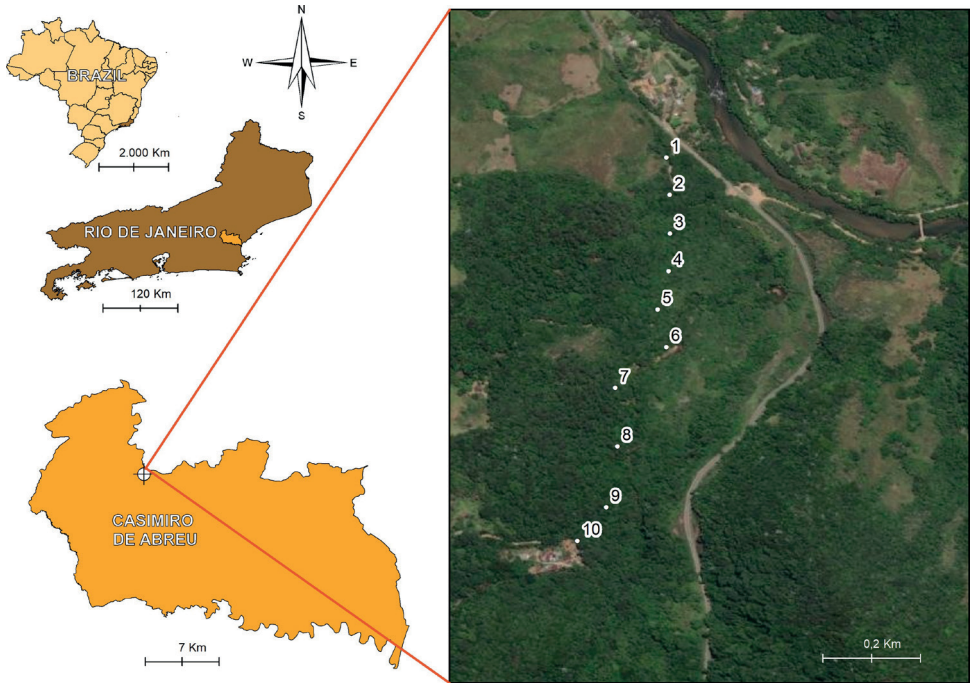


Fig. 1. Sampling sites in the Sana Environmental Protection Area, located in the sixth district of Macaé, Rio de Janeiro, Brazil. $22^{\circ} 25' 44.9''$ S $42^{\circ} 12' 17.5''$ W; $22^{\circ} 25' 47.4''$ S $42^{\circ} 12' 17.3''$ W; $22^{\circ} 25' 49.9''$ S $42^{\circ} 12' 17.3''$ W; $22^{\circ} 25' 52.4''$ S $42^{\circ} 12' 17.3''$ W; $22^{\circ} 25' 55.0''$ S $42^{\circ} 12' 18.1''$ W; $22^{\circ} 25' 57.5''$ S $42^{\circ} 12' 17.5''$ W; $22^{\circ} 26' 00.2''$ S $42^{\circ} 12' 20.9''$ W; $22^{\circ} 26' 04.1''$ S $42^{\circ} 12' 20.7''$ W; $22^{\circ} 26' 08.1''$ S $42^{\circ} 12' 21.5''$ W; $22^{\circ} 26' 10.4''$ S $42^{\circ} 12' 23.4''$ W. Maps were prepared in QGIS 3.14.16 software and edited in Adobe Photoshop CS5 and CorelDraw X5. Reprinted from QGIS 3.14.16, a program under a CC BY license, with permission from Jeronimo Alencar - Fiocruz, original copyright 2021.

MATERIAL AND METHODS

Ethics Statement

The permanent license for collecting, capturing, and transporting the biological material used in this study was granted by the Biodiversity Authorization and Information System (SISBIO)/Chico Mendes Institute for Biodiversity Conservation (ICMBio) under number 34911-1. All research team members were previously vaccinated against YFV.

Study Area

The Figueira Branca Environmental Protection Area (APA) is located near the district of Casimiro de Abreu, in Macaé, Rio de Janeiro State, Brazil. This region is included in the Macaé State Environmental Protection Area, covering an area of 350.37 km² (Fig. 1), and is characterized by a predominant humid mesothermal climate and dense montane and submontane rainforest vegetation (INEA 2014).

Collection and Rearing of Immature Culicids

Culicidae eggs were sampled from September 2019 to April 2021 using 20 ovitraps placed at each collection site in a forest environment at 150 m from each other. The ovitraps were installed in trees at a height of 2.50 m and sequentially numbered. Each ovitrap consisted of a 500 ml lid-less black pot containing four plywood pallets (Eucatex pallets), each measuring 2.5 × 14 cm, fixed vertically inside the trap by clips (Silva et al. 2018). These pallets were examined every two weeks for egg detection and counting. Immediately after arriving in the laboratory, the positive pallets (pallets containing eggs) were immersed in white trays filled with dechlorinated water at $29 \pm 1^\circ\text{C}$ and kept in an acclimatized chamber for hatching. After three days, the pallets were removed from the water and left to air-dry for another three days to quantify the hatched larvae. The immature forms were reared with TetraMint fish food (Tetra, Blacksburg, VA) and monitored daily. After the specimens reached the adult stage, Culicidae identification to genera and species level was conducted. Identifications were made by direct observation of morphological characters under a Zeiss stereo microscope, consulting the respective spp descriptions/diagnoses in dichotomous keys developed by Consoli and Lourenço-de-Oliveira (1994), Forattini (2002), and Marcondes and Alencar (2010). Abbreviations of genera and subgenera names were assigned following Reinert (2009).

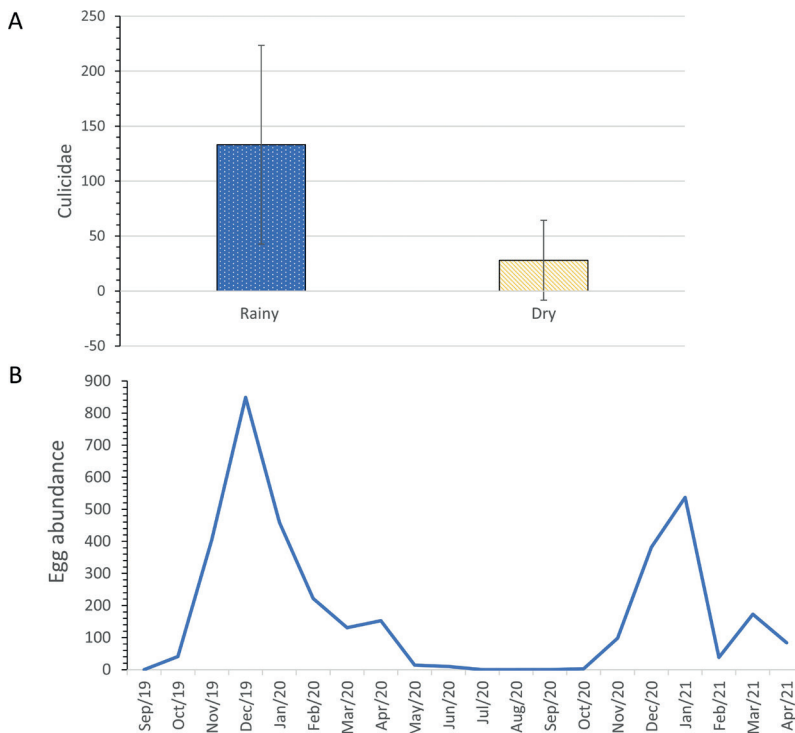


Fig. 2. Statistically significant difference ($p \leq 0.01$) between the number of Culicidae eggs collected during the rainy and dry periods ($p = 0.005$) (A). Egg abundance during the collection period (Sept/19–Apr/21) in the Figueira Branca Environmental Protection Area (APA) (B).

Table 1. Descriptive indices for egg collection and capture in Figueira Branca, Casimiro de Abreu – Rio de Janeiro State, Brazil

Month/year	OPI (%)	EDI (n)	AEI (n)	LPI (%)	LDI (n)	MLI (n)	Total	
							Eggs	Larvae
Sept./19	0	0	0	0	0	0	0	0
Oct./19	0.20	20.50	0.92	0.20	18	1.50	41	36
Nov./19	0.90	45.11	10.35	0.90	36.89	12.30	406	332
Dec./19	0.70	121.29	8.05	0.50	58.20	14.55	849	291
Jan./20	0.90	51.11	9.70	0.90	42.11	12.63	460	379
Feb./20	0.60	37	5.82	0.60	31.33	7.52	222	188
Mar./20	0.20	65.50	4.60	0.20	57.50	4.93	131	115
April/20	0.30	51	3.20	0.30	50.33	5.59	153	151
May/20	0.10	14	0.33	0.20	7	0.63	14	14
June/20	0.10	10	0.17	0.10	10	0.33	10	10
July/20	0	0	0	0	0	0	0	0
Aug./20	0	0	0	0	0	0	0	0
Sept./20	0	0	0	0	0	0	0	0
Oct./20	0.10	3	0.06	0.10	3	0.11	3	3
Nov./20	0.20	49	1.35	0.20	12	0.80	98	24
Dec./20	0.70	54.57	4.95	0.70	38.29	8.93	382	268
Jan./21	0.70	76.71	11.70	0.40	29.25	4.88	537	117
Feb./21	0.20	19	0.66	0.20	8.50	0.59	38	17
Mar./21	0.30	57.67	5.89	0.30	51	6.65	173	153
April/21	0.30	28	1.35	0.40	15	2	84	60

OPI, Ovitrap Positivity Index; EDI, Egg Density Index; AEI, Average Egg Index; LPI, Larvae Positivity Index; LDI, Larvae Density Index; MLI, Mean Larvae Index.

Statistical Analysis

The following indexes were calculated to conduct specific analysis on ovitrap efficiency: 1) Ovitrap Positivity Index (OPI) = (Number of positive traps with eggs/ Number of inspected ovitraps × 100); 2) Egg Density Index (EDI) = (Total eggs in pallets/ Total positive traps); 3) Average Egg Index (AEI) = (Number of eggs collected/Number of ovitraps inspected); 4) Larvae Positivity Index (LPI) = (Number of positive traps with larvae/Number of ovitraps inspected × 100); 5) Larvae Density Index (LDI) = (Total larvae in traps/Total positive traps); and 6) Mean Larvae Index (MLI) = (Number of larvae collected/Number of traps inspected).. The quantity of Culicidae eggs was assessed for dry and rainy seasons, and the Mann–Whitney test was used to analyze the statistical significance of differences in egg abundance between these seasons. A regression analysis was performed between the number of eggs collected and temperature, rainfall, and relative humidity. The environmental variables were obtained from the Climate-Data.org website (Climate-Data. org 2022). In addition, the male-to-female ratio of the

mosquitoes emerging from the collected eggs was calculated. A Kruskal–Wallis test, followed by Dunn’s post hoc test, was used to assess the statistical difference between the number of individuals belonging to each epidemiologically relevant species found in the study area. The correlation between different species found at the same collection sites was assessed by Spearman’s correlation test.

RESULTS

From the 3,601 eggs collected during the entire sampling period, 141 (3.9%) were already hatched on the pallets. Of the remaining 3,460 viable eggs hatched in the laboratory, 1,310 eggs (37.9%) belonged to the Culicidae species of epidemiological relevance *Hg. leucocelaenus* ($n = 1,131$; 86%), *Aedes terreus* Walker, 1856 ($n = 111$; 8%), *Aedes albopictus* Skuse, 1894 ($n = 47$; 4%), and *Hg. janthinomys* ($n = 21$; 2%).

Culicidae Eggs Related to Seasons and Climatic Variables

The number of Culicidae eggs collected showed a highly significant difference ($p \leq 0.01$) between the rainy and dry periods ($p = 0.005$) (Fig. 2A). The month with the highest egg abundance was December 2019, representing 24% of all eggs collected during the study period and thus recording the largest EDI and LDI. After January 2020, there was a decrease in the OPI, which only increased again in December 2020 and January 2021 (70%) (Table 1). Over time, it is possible to notice a decrease in the number of eggs from the beginning to the end of the collection period (Fig. 2B). The decreased culicid population in the studied area during the colder months (July, August, and September 2020) may have influenced the lower number of positive ovitraps since OPI was zero (Table 1).

A regression analysis was conducted between the number of eggs collected and the environmental variables of temperature, rainfall, and relative air humidity to assess whether or not there was a correlation between egg abundance and climatic variables. In this way, the highest coefficient of determination was obtained for the variable rainfall ($r^2 = 0.709$; $p = 0.002$), indicating that this variable explained about 71% of a statistically significant variation ($p \leq 0.05$) in the number of eggs. The coefficient of determination regarding temperature was $r^2 = 0.429$, with $p = 0.040$, a positive and statistically significant correlation ($p \leq 0.05$). In contrast, for the humidity variable, the values were $r^2 = 0.176$ and $p = 0.227$, showing a positive but not statistically significant correlation (Fig. 3).

Epidemiologically Relevant Culicidae Emerged from Eggs Collected with Ovitrap

Ovitrap 3, 9, and 10 showed the highest average and total number of collected eggs (Fig. 4A). Regarding sex ratio, a higher percentage of females ($F = 51\%$) than males ($M = 49\%$) was observed. The percentages for each vector species were as follows: *Hg.*

leucocelaenus ($F = 54\%$, $M = 46\%$), *Hg. janthinomys* ($F = 57\%$, $M = 43\%$), *Ae. albopictus* ($F = 57\%$, $M = 43\%$), *Ae. terrens* ($F = 64\%$, $M = 36\%$). Ovitrap 10 stood out for having collected eggs that produced an equal number of males and females (Fig. 4B). The number of positive ovitraps for each species was *Hg. leucocelaenus* ($n = 9$), *Hg. janthinomys* ($n = 6$), *Ae. albopictus* ($n = 5$), and *Ae. terrens* ($n = 6$).

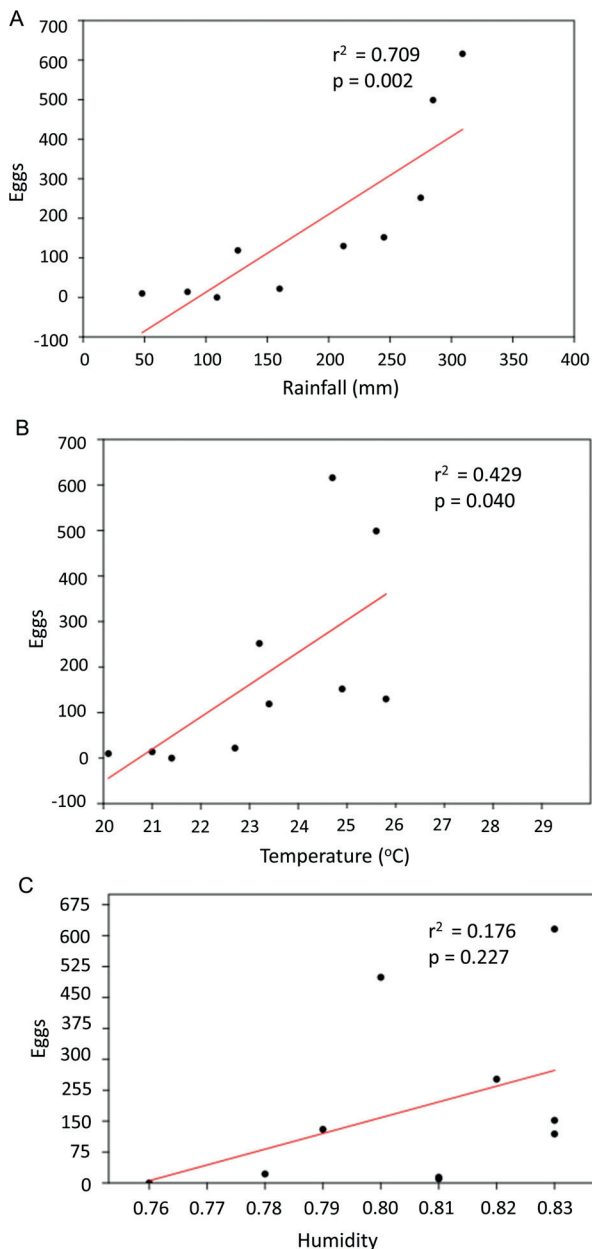


Fig. 3. Regression analysis between the number of eggs collected and environmental variables of temperature (A), rainfall (B), and relative air humidity (C).

Oviposition Correlation Between Vector Species

Several vector species were observed performing oviposition in the same ovitrap. Hence, the data on species frequencies were analyzed to assess correlations between the species found at the same collection site. In this way, we evaluated whether these species were positively or negatively correlated. Although not statistically significant ($p \geq 0.05$), correlations between species were mostly positive. The highest Spearman correlation index was observed between *Ae. albopictus* and *Ae. terrens* ($\rho = 0.52$), with *Ae. terrens* being 8% more abundant than *Ae. albopictus*. A positive correlation was also detected between *Ae. terrens* and *Hg. leucocelaenus* ($\rho = 0.49$), and between *Hg. leucocelaenus* and *Hg. janthinomys* ($\rho = 0.37$) (Fig. 5).

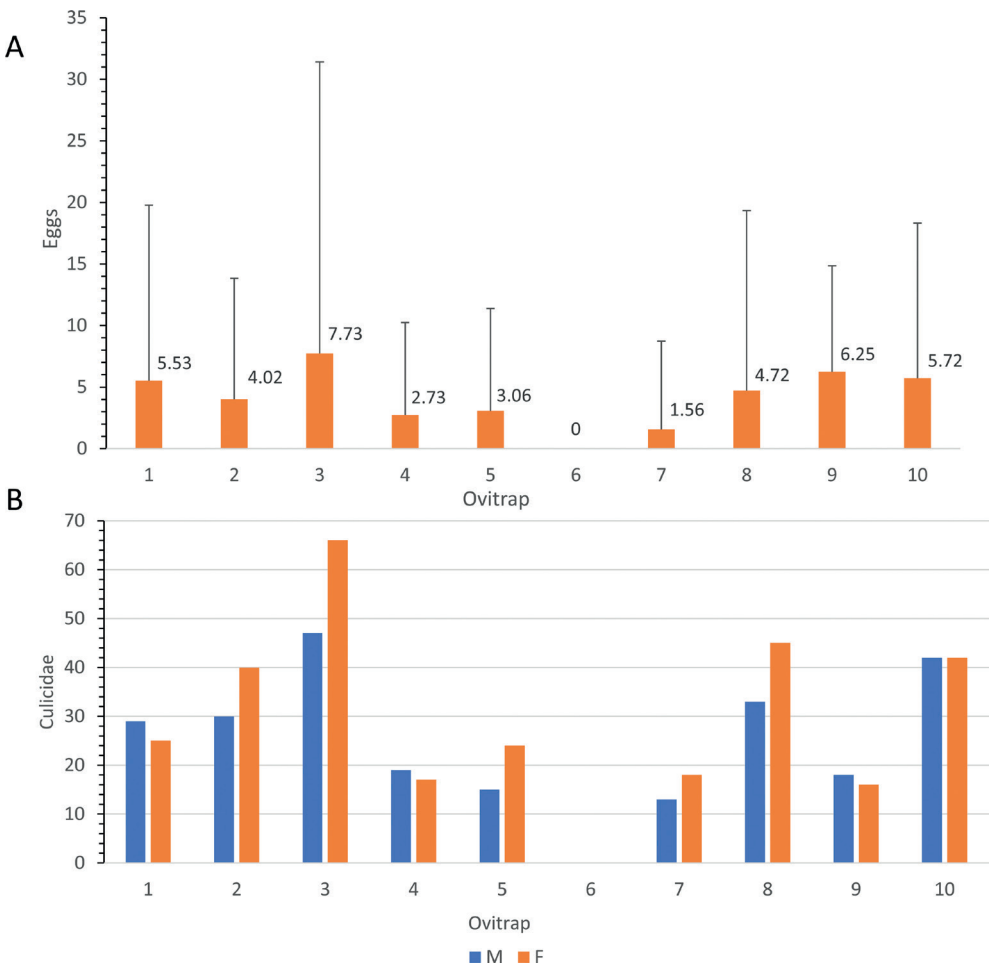


Fig. 4. Mean and standard deviation of Culicidae eggs in the ovitraps (A). Male to female ratio per ovitrap (B).

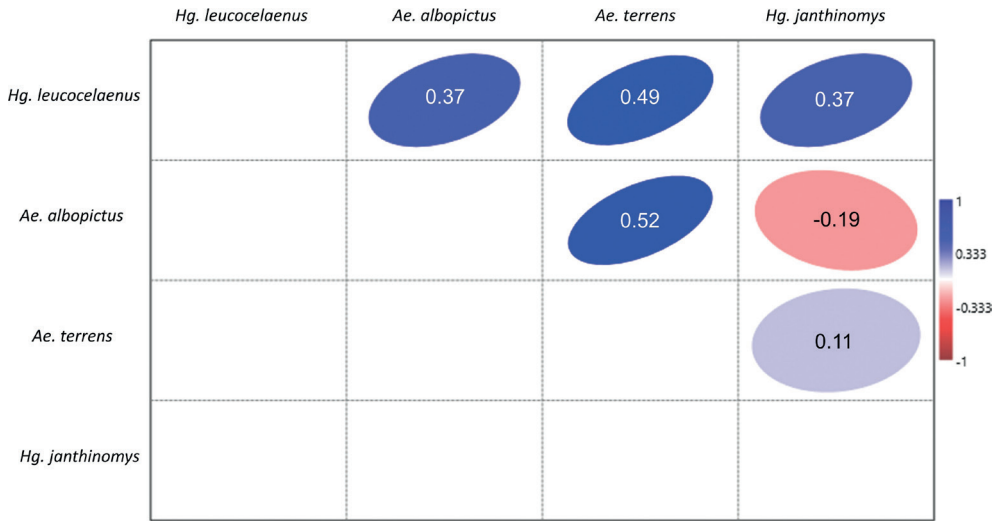


Fig. 5. Spearman correlation index between epidemiologically relevant mosquito species.

DISCUSSION

Mosquitoes of the genera *Haemagogus* inhabit regions of tropical forests, are most active during the hottest hours of the day, and have acrodendrophilic habits. These mosquitoes rarely leave the woods and so are exposed to the meteorological factors of the forest fragments they inhabit (Marcondes and Alencar 2010). Similarly, several species from the genus *Aedes* are also found in preserved areas associated with forest environments and/or rural areas, such as those found in this study (*Ae. albopictus* and *Ae. terrens*) (Lima-Camara et al. 2006, Silva et al. 2021). In addition, it is known that climatic variables, including rainfall, temperature, and humidity, influence Culicidae populations. Hence, these environmental factors can impact the abundance and activity of mosquito vectors, which in turn affect arbovirus transmission (Alencar et al. 2015, 2018; Silva et al. 2018).

In the present study, Culicidae egg abundance had a significantly high correlation with rainfall, a climatic variable that explained 71% of the variation in the number of eggs. Similarly, in a 2016 study by Silva et al., also in the Atlantic Forest of the Rio de Janeiro State, the rainfall variable was significantly correlated with the abundance of mosquito eggs ($p = 0.003561$) (Silva et al. 2018). Interestingly, three years later, this climatic factor continues to have a strong influence on the population of mosquito vectors inhabiting forest fragments within the most threatened Brazilian biome, the Atlantic Forest (Colombo and Joly 2010). A positive and statistically significant correlation was also observed between egg abundance and temperature. The same correlation was observed in a study conducted in Nova Iguaçu, Rio de Janeiro State, for eggs of *Hg. leucocelaenus*, with an increment in the probability of finding more than half positive ovitraps when the mean temperature was higher (Couto-Lima et al. 2020). The peak in the abundance of Culicidae eggs in December 2019 coincides with

the yellow fever seasonal period (from December to May) and the detection of the virus among non-human primates (NHP) (MS 2020b). Females represented 51% of the Culicidae that emerged from the eggs collected in the ovitraps, 2% higher than males, and all vector species identified had a higher number of females than males. More females in culicid populations may increase the risk of pathogen transmission since only female mosquitoes have a hematophagous behavior (Oliveira 1994).

A growing interest in studying the biology and ecology of *Hg. leucocelaenus* has developed due to its role in the sylvatic cycle of YFV and potentially other arboviruses (Cunha et al. 2019). This was the species of medical importance with the highest frequency (81%) found in our study. Similarly, the predominance of this vector was observed in 2015 in the Itatiaia National Park of Poço das Antas Biological Reserve and the Bom Retiro Private Natural Heritage Reserve, both in Rio de Janeiro State (Alencar et al. 2016). The second highest frequency observed was for *Ae. terreus*, a species that has already shown high infection and dissemination rates of Chikungunya virus (CHIKV) under experimental conditions (Lourenço-de-Oliveira and Failloux 2017). *Aedes terreus* is an arboreal species capable of breeding in tree holes and feeding on non-human primates (NHP) near the treetops and humans at ground level (Shannon 1958, Arnell 1973, Schick 1973).

Aedes terreus and *Ae. albopictus* performed oviposition in the same breeding site (ovitraps) and had a strong positive correlation ($\rho = 0.52$) with each other. This behavior shows that besides performing oviposition on a positive breeding site, these Culicidae seemingly prefer breeding sites already colonized by eggs of the same genera and/or species. This behavior was reported by Barbosa and da Silva (2002) for the species *Ae. albopictus*, showing this Culicidae prefers oviposition in sites with immatures of the same species under laboratory conditions (Barbosa and da Silva 2002). *Haemagogus leucocelaenus* also showed a positive correlation with a cogenetic species, *Hg. janthinomys*, ($\rho = 0.37$) and with *Ae. terreus* ($\rho = 0.49$). Coincidentally, Inacio et al. 2020 observed shared breeding sites between *Hg. spegazzinii* Brèthes 1912 and other species, such as *Ae. albopictus*, *Ae. terreus*, *Culex* spp., and *Toxorhynchites theobaldi* (Dyar & Knab, 1906). Thus, *Haemagogus* species seemingly prefer oviposition in breeding sites already colonized by either the same genera or completely different Culicidae species. This behavior could be related to the fact that these Culicidae share the ecological niche, being all sylvatic mosquitoes breeding and feeding near the treetops, and laying their eggs on wet breeding sites close to the surface of the water (Oliveira 1994).

Alarmingly, egg collection in the studied area of the main YFV vectors showed the occurrence of *Hg. leucocelaenus* and *Hg. janthinomys*, the possible CHIKV vector *Ae. terreus*, and *Ae. albopictus*, a known dengue vector in South and East Asia and a secondary DENV vector in the Americas. The presence of these vectors serves as a warning to the human population living around the area of Culicidae egg collections conducted in this study (INEA 2014, Goubert et al. 2016). Moreover, the high number of specimens of primary

arbovirus vectors in some regions of Brazil makes monitoring cases of febrile diseases in their local population and communities living in the surrounding areas imperative.

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AUTHOR CONTRIBUTIONS

Conceptualization: Jeronimo Alencar, Cecilia Ferreira de Mello, Anthony Érico Guimarães. Formal analysis: Shayenne Olsson Freitas Silva. Methodology: Jeronimo Alencar, Cecilia Ferreira de Mello, Paulo José Leite. Supervision: Jeronimo Alencar. Writing – original draft: Shayenne Olsson Freitas Silva, Jeronimo Alencar. Writing – review & editing: Jeronimo Alencar, Anthony Érico Guimarães.

SUPPLEMENTARY DATA

Supplementary data are available at *Journal of Medical Entomology* online.

S1 Table. Data from mosquito collections, carried out in Figueira Branca Environmental Protection Area (APA), municipality of Macaé, State of Rio de Janeiro, Brazil. (XLS)

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