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SCIENTIFIC NOTE

LONG-TERM TRENDS IN MOSQUITO VECTOR POPULATIONS AND THEIR IMPACT ON YELLOW FEVER OUTBREAKS IN ATLANTIC FOREST FRAGMENTS OF RIO DE JANEIRO, BRAZIL (2016–2021)

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ABSTRACT. Among all living beings, mosquitoes account for the highest number of human fatalities. Our study aimed to determine mosquito egg abundance fluctuation from 2015 to 2020, in order to observe which years had the highest mosquito vector densities and whether they coincided with yellow fever virus outbreaks in both human and nonhuman primates. The study area included Atlantic Forest fragments in the state of Rio de Janeiro. Studies from the Diptera Laboratory at FIOCRUZ were selected and compared along a timeline period of the field collections. The highest peak in egg abundance from the analyzed studies was observed from 2016 to 2017 and from 2015 to 2016. The lowest egg abundance was during the collection periods from 2018 to 2019 and 2019 to 2020. The species with the highest abundance throughout all the periods of the studies analyzed was *Haemagogus leucocelaenus,* representing 87% of all epidemiological species identified. The species with the lowest abundance was *Hg. Janthinomys,* representing only 1%. Monitoring the population of mosquitoes is imperative for disease surveillance, as the rise in specimens of various vector species directly impacts the occurrence of yellow fever cases in both nonhuman primates and human populations.

KEY WORDS Egg abundance, *Haemagogus leucocelaenus*, outbreak, vector density, yellow fever

Yellow Fever (YF) is an arboviral disease of significant importance in the American continent. It is caused by yellow fever virus (YFV) belonging to the genus *Flavivirus*, family Flaviviridae, with its origin in Africa and likely spreading to the Americas and the Caribbean during the seventeenth to nineteenth centuries, through maritime transport driven by the slave trade (Bryan et al. 2004). The first recorded outbreak of YF in the Americas occurred in 1647 in the Yucatan Peninsula of Mexico (Franco 1969). The disease has a long history and has been extensively studied, as it has been responsible for major epidemics in the Americas and Africa since the 17th century (Benchimol 1999). Molecular biology studies have made it possible to establish phylogenetic relationships between the strains circulating on both continents, confirming the African origin of the virus (Mutebi and Barrett 2002).

Remarkably, yellow fever virus (YFV) circulation has not been documented in the Atlantic Forest in Brazil since the 1930s, despite it being the biome where sylvatic transmission was initially identified (Laemmert and Kumm 1950). Between the mid-twentieth century and 1999, YFV expansion from the Amazon reached the Cerrado biome. After 2000, YFV expansion waves reached the southern pampa biome and progressed eastward across the Cerrado. Until 2016, the stretch of the Brazilian coast from Piauí to Rio Grande do Sul was considered free of YFV, leading to an absence of vaccination recommendations. However, a significant outbreak occurred in the central-western region of the country between 2014 and 2015. In late 2016, YFV spilled from Cerrado into a transition zone between this biome and the Atlantic Forest in the state of Minas Gerais, rapidly spreading across the southeast. This outbreak quickly extended into the most populous section of the Atlantic Forest (southeast), where the virus had not circulated for nearly 80 years (de Abreu et al. 2019).

Brazil has been heavily impacted by the disease, both in terms of public health and economics, and its elimination from urban areas at the beginning of the 20th century was a significant achievement. However, in the 1920s, it was suspected that transmission had occurred in the forest areas of Colombia, and this was subsequently confirmed in the Canaã valley, in the Brazilian state of Espírito Santo, demonstrating that the virus had adapted to wild mosquitoes (Soper et al. 1933).

Mosquito species belonging to the genera *Haemagogus* Williston and *Sabethes* Robineau-Desvoidy are

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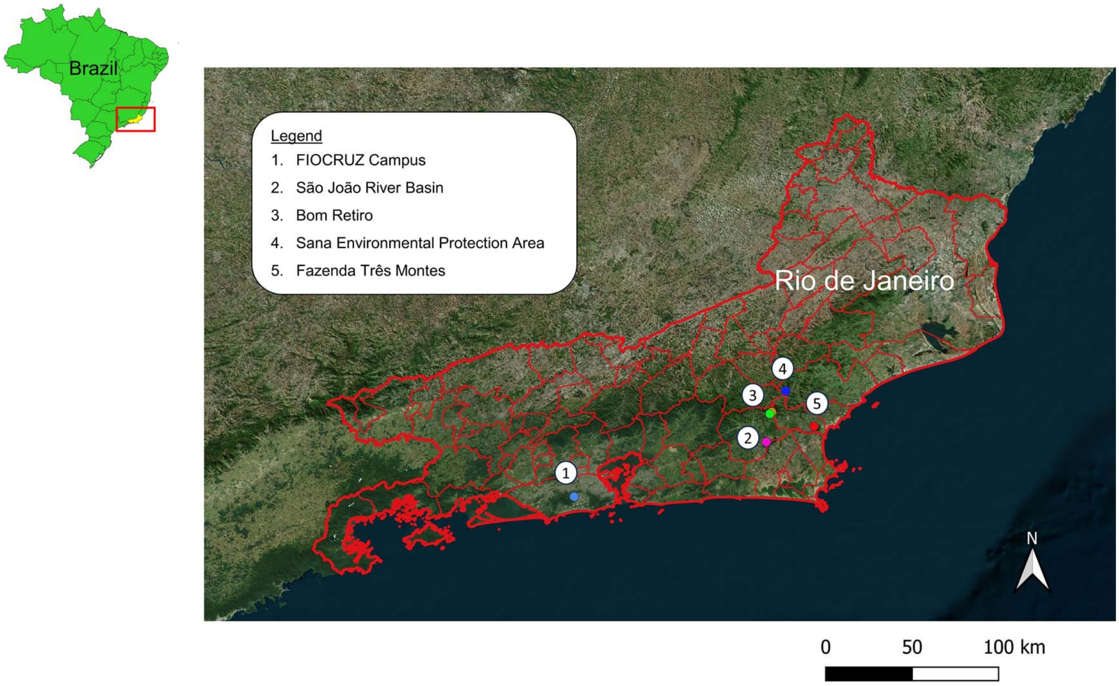

Fig. 1. Map of immature collection areas in Atlantic Forest Biome, within the state of Rio de Janeiro, Brazil. The map was generated using QGIS software, version 3.22.16.

of epidemiological importance because of their efficiency in becoming infected with and transmitting the wild yellow fever virus (YFV). These vectors are responsible for maintaining the natural cycle of YFV in forested areas of the Americas. *Haemagogus* contains 28 species, of which nine are found in Brazil (Marcondes and Alencar 2010). These are essentially wild, diurnal, acrodendrophilous mosquitoes, which mainly inhabit dense forests and usually feed on the blood of arboreal animals, mainly nonhuman primates (NHP). These perform blood meals at specific times of the day, usually around noon (Alencar et al. 2016).

Immature forms of *Haemagogus* spp. usually develop in tree hollows, cut bamboo, and bromeliads (Marcondes and Alencar 2010). Eggs of these mosquitoes are deposited on moist substrates and become drought-resistant. When the embryo receives negative stimuli from the environment, a drastic reaction occurs, reducing its metabolism and blocking larval hatching. This increases the probability that the embryo will survive (Perez and Noriega 2013). Understanding the hatching response is therefore essential for developing mosquito control strategies. The present study aimed to assess the number of eggs from vector mosquitoes from 2015 to 2020 and evaluate whether the seasonal distribution coincides with YF outbreaks in humans and nonhuman primates in Atlantic Forest fragments in the state of Rio de Janeiro.

The data that compose the present study were collected through the period of 2015 to 2020 in different Atlantic Forest fragments in the state of Rio de Janeiro, with a focus on YFV vectors (Fig. 1). The Atlantic Forest vegetation is composed of dense submontane rainforests, a specific type of tropical forest. Some areas within the Atlantic Forest exhibit moderate to advanced stages of regeneration. This ecosystem comprises evergreen foliage, canopies up to 50 m high, emerging trees up to 40 m high, and dense shrubby vegetation with ferns, shrubs, and bromeliads (MMA 2008).

The data from the period of 2015 to 2016 were sampled in the Mata Atlântica FIOCRUZ Campus (CFMA) (22°56′00.0″S, 43°25′00.0″W), located in the municipality of Rio de Janeiro, and in the RPPN (a private natural heritage reserve) of Bom Retiro (22°27′19.4″S, 42°18′09.5″W); (22°27′14.1′′S; 42° 17′34.9′′W), located in the Casimiro de Abreu municipality, Rio de Janeiro State. The data from 2016 to 2018 were from eggs sampled at the Sana Environmental Protection Area (SPA) (22°20′ 01.3″S, 42°12′ 24.0″W), located in an area of secondary Atlantic Forest in the municipality of Macaé, 189 km from FIOCRUZ Campus CFMA, and 39.5 km from the Private natural heritage reserve of Bom Retiro in Casimiro de Abreu municipality. The samples from 2018 to 2019 were collected at the São João River Basin Environmental Protection Area (EPA) (22°37′10.7′′S, 42°18′59.5′′W), in Silva Jardim, 150 km from Mata Atlântica FIOCRUZ Campus (CFMA), 56.6 km from Private natural heritage reserve of Bom Retiro in the city of Casimiro de Abreu, and 66.4 km from Sana Environmental

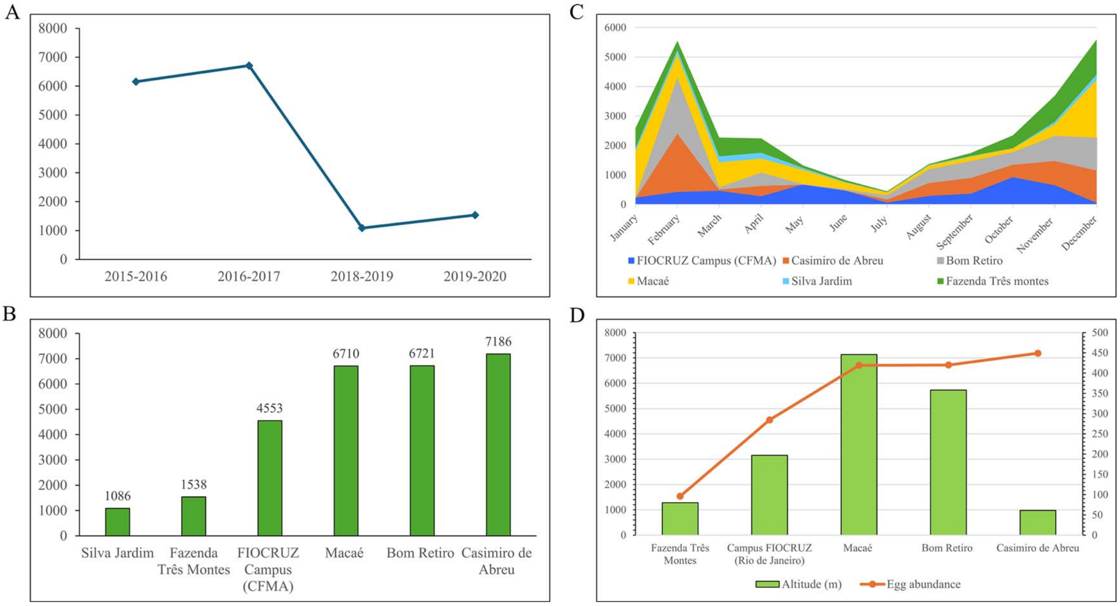

Fig. 2. Egg abundancy per study period from 2015 to 2020 (A), per study area (B), per month (C), and per altitude (D) in fragments of the Atlantic Forest in the state of Rio de Janeiro, Brazil.

Protection Area (SPA). Samples for 2018 to 2020 were collected at Fazenda Três Montes (22°31′50.8″S, 42° 02′56.3″W) and Morro Grande Private Natural Heritage Reserve (22°32′29.6″S, 42°00′49.0″W), both in the city of Casimiro de Abreu.

The total number of culicid eggs collected for each time was plotted on a graph in ascending chronological order to observe the fluctuation in the culicid population during the 6 years of observation. The quantitative values of the total number of eggs collected in each study were plotted on a line graph over a specified time interval of one year. A bar graph was used to illustrate the abundance of mosquito eggs per collection area. To compare the number of eggs collected per field visit (monthly) between studies and evaluate the study areas, a normality test was performed. The data did not follow the normal distribution. Therefore, the Kruskal-Wallis test was used for nonparametric data, followed by Dunn's post hoc to observe between which studies and/or areas there was a statistically significant difference. Regression analysis was performed between the number of eggs collected and the altitude of the collection areas of the studies, after excluding an outlier identified through Cook's distances calculations. All recorded data were analyzed using software PAST version 4.09 (Hammer et al. 2001).

At the FIOCRUZ Campus (CFMA) *Hg. leucocelaenus*, a known YFV vector, was reported. The study conducted in this area only focused on this specific species, thus no other vector species were registered. In the fragments at the municipality of Carimiro de Abreu, important vector species were present: *Hg. leucocelaenus* (Dyar and Shannon), *Hg. janthinomys* Dyar, *Aedes albopictus* (Skuse), *Ae. aegypti* (L.), *Ae. terrens* (Walker). The most abundant was *Hg. leucocelaenus* [n = 992 in Bom Retiro Private Natural Reserve (RPPNBR)] and (n = 1,525 in Fazenda Três Montes and Morro Grande). The second most abundant vector species was *Ae. albopictus* (n = 103 in RPPNBR) and (n = 1,100 in Fazenda Três Montes and Morro Grande). The fragment Sana Environmental Protection Area (SPA), located in the municipality of Macaé, showed a higher abundance of *Hg. leucocelaenus* (n = 10,946), followed by *Ae. terrens* (n = 172). Considering the fragment São João River Basin Environmental Protection Area (EPA) in Silva Jardim municipality, the most abundant species found was also *Hg. leucocelaenus* (n = 1,525), with a very high abundance of *Ae. albopictus* (n = 1,100). This was the only fragment in which another species (*Ae. albopictus*) was found with an abundance almost as high as *Hg. leucocelaenus.*

The highest peak in egg abundance from the studies analyzed comprehending the period from 2015 to 2020 was observed from 2016 to 2017 (n = 6,710) and from 2015 to 2016 (n = 6,153). The lowest egg abundancy was during the collection periods from 2018 to 2019 (n = 1,086) and 2019-2020 (n = 1,538) (Fig. 2A). The areas with the most elevated culicid egg abundance were Casimiro de Abreu (n = 7,186), Bom Retiro (n = 6,721) and Macaé (n = 6,710). The lowest egg abundance was observed in Silva Jardim (n = 1,086), Fazenda Três Montes (n = 1,538), and FIOCRUZ Campus (CFMA) (n = 4553). Silva Jardim was the location that showed statistical difference with all the other locations analyzed: FIOCRUZ Campus

(CFMA) (Kruskal-Wallis test, $P = 0.004$), Casimiro de Abreu (Kruskal-Wallis test, $P = 0.013$), Bom Retiro (Kruskal-Wallis test, $P = 0.010$), Macaé (Kruskal-Wallis test, $P = 0.001$) (Fig. 2B). Regardless of the year of collection, the months of February, November and December showed very distinct and high peaks in the graph for the majority of the collection areas in the studies analyzed. The pattern of mosquito egg abundance was highly similar among the collection areas. July was the month with the lowest egg abundance (Fig. 2C).

The number of eggs collected in different Atlantic Forest fragments had a positive and significant statistical correlation to the altitude ($r = 0.96$, P-value $= 0.0053$) (Fig. 2D).

Research on mosquito biodiversity in the Atlantic Forest enhances our comprehension of their population distribution patterns. It sheds light on potential alterations in mosquito behavior to adapt to diverse environmental conditions due to the impacts of anthropic activity within this biome (Alencar et al. 2021). In 2000, Guimarães and collaborators (Guimarães et al. 2000) noted a rise in the occurrence of epidemics associated with human-induced environmental impacts. These impacts can cause changes in the behavior of vectors and pathogens' transmission patterns. *Haemagogus* species are found in tropical forest areas, exhibit peak activity during the hottest hours of the day, and demonstrate acrodendrophilic tendencies (Silva et al. 2022).

Most of the eggs collected in this period from the studies analyzed, considering only the medically important mosquito species and genera, were from *Hg. leucocelaenus*. This mosquito species, along with *Hg janthinomys* were considered the primary vectors in the major YF outbreak in Brazil from 2016–2018 (de Abreu et al. 2019). The elevated number of mosquito eggs with a high abundance of eggs from *Hg. leucocelaenus* is alarming as it was observed that YFV transmission persisted in the same Atlantic Forest area for at least three consecutive transmission seasons without the need for new introductions (de Abreu et al. 2019).

The highest peak in mosquito egg abundance was observed in the studies from 2016 to 2017, which coincides with one of the biggest outbreaks in the history of YF in forest areas of Brazil, with 28 confirmed YF human cases (9 deaths) in the state of Rio de Janeiro (32.1% lethality) (SES 2018). The second outbreak happened from 2017 to 2018 (MS 2020). The year 2017 marks the reemergence of the yellow fever virus (YFV) in non-endemic Southeastern Brazilian states (Mir et al. 2017). In Brazil, from December 1, 2016, to February 2, 2017, 901 cases of YF were reported, including 143 deaths (54 confirmed, 3 discarded, and 86 under investigation), with an overall lethality rate of 16%, with 36% among confirmed cases and 12% among suspected cases (OMS 2017). At the beginning of January 2017, the Brazilian Ministry of Health informed the World Health Organization of a rising number of officially verified instances of YF in the state of Minas Gerais, located in Southeastern Brazil (Rossetto et al. 2017).

The collection areas analyzed showed a very similar pattern of egg abundance throughout the months, regardless of the collection year. This is probably because all of them were in the Atlantic Forest biome, state of Rio de Janeiro. It is interesting to note how consistently the egg abundance rose in hot rainy months and decreased in drier and colder months of the year. This observation has also been noted and reported extensively throughout research involving mosquito populations and climatic variables, resulting in a seasonal pattern (Hamlet et al. 2021). The influence of climate on mosquito abundance indicates that predictive models based on climate variables can provide important information on mosquito population dynamics and etiological agents transmitted by them (Wilke et al. 2017).

Regarding altitude, considering the studies analyzed, the highest number of eggs were observed at 61m ($n = 7186$), 358m ($n = 6721$) and 446m ($n = 6710$). In the Brazilian city of Belo Horizonte, capital of Minas Gerais State, *Ae. albopictus* was collected at an altitude of 858 m above sea level; in the studies reviewed, this species has been collected in elevations of 28 m, 61 m and 358 m (Bezerra 2016). Elevations with less than 500m have moderate to heavy mosquito populations, in contrast, mountain areas higher than 500m have low populations. Regions at high altitudes are practically free of dengue due to low temperatures and oxygen depletion, the vector is not usually found above 1,000 meters of altitude (Kaul and Rastogi 1997). This variable may act as a limiting factor in the distribution of *Aedes* spp. mosquitoes.

As for *Haemagogus* species, according to Alencar et al. (2009), the analyses examining the relationship between altitude and abundance indicate little to no correlation observed for both *Hg. capricornii* Lutz and *Hg. janthinomys* (Alencar et al. 2009). In contrast, in the studies included in this revision, altitude was shown to positively correlate with mosquito eggs, including eggs from *Haemagogus* spp. This might be because the areas of studies ranged from 28 m to 446 m, thus not exceeding 500m. In Brazil, *Hg. capricornii* and *Hg. janthinomys* are not only restricted to lowland regions; they can also be found in areas at altitudes greater than 800 m above sea level (Alencar et al. 2009).

The Atlantic Forest fragment with the highest number of eggs collected was in Casimiro de Abreu, this fragment also demonstrated elevated percentages of *Hg. leucocelaenus* (83%), when compared to other vector species. This species is considered one of the main YFV vectors and has already been found infected in Brazil (de Abreu et al. 2019). In 2017, YF cases were detected in Casimiro de Abreu, Rio de Janeiro state, resulting in one fatality (Silva et al. 2022). This highlights the importance of epidemiological surveillance in forest areas, detecting and quantifying mosquito vector populations. Early detection of these mosquito species can help identify higher-risk areas for YFV transmission, making these a priority for YF vaccination both among humans and nonhuman primates.

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REFERENCES CITED

Alencar J, de Mello CF, Rodríguez-Planes L, dos Santos Silva J, Gil-Santana HR, Bastos AQ, Albuquerque HG, Rodrigues WC, Guimarães AÉ. 2021. Ecosystem diversity of mosquitoes (Diptera: Culicidae) in a remnant of Atlantic Forest, Rio de Janeiro state, Brazil. *Austral Entomol* 60:244–256.

Alencar J, dos Santos Silva J, Maués Serra-Freire N, Érico Guimarães A. 2009. dispersion and ecological plasticity patterns of *Haemagogus capricornii* and *H. janthinomys* (Diptera: Culicidae) populations in different regions of Brazil1. *Entomological News* 12:53–60.

Alencar J, Mello CF, de Barbosa LS, Gil-Santana HR, Maia DA, Marcondes CB, Silva JS. 2016. Diversity of yellow fever mosquito vectors in the Atlantic Forest of Rio de Janeiro, Brazil. *Revista Da Sociedade Brasileira de Medicina Tropical* 49:351–356.

Benchimol, JL. 1999. *Da etiologia ao modo de transmissão*. Rio de Janeiro: Editora FIOCRUZ/Editora UFRJ.

Bezerra, JMT, Araújo RGP, Melo FF, Gonçalves CM, Chaves BA, Silva LD, Pimenta PFP. 2016. *Aedes (Stegomyia) albopictus*' dynamics influenced by spatiotemporal characteristics in a Brazilian dengue-endemic risk city. *Acta Tropica* 164:431–437.

Bryan CS, Moss SW, Kahn RJ. 2004. Yellow fever in the Americas. *Infectious Disease Clinics of North America* 18:275–292.

de Abreu FVS, Ribeiro IP, Ferreira-de-Brito A, Santos AAC, de Miranda RM, Bonelly I S, Neves MSAS, Bersot MI, Santos TP, Gomes MQ, da Silva JL, Romano APM, Carvalho RG, Said RFC, Ribeiro MS, Laperrière RC, Fonseca EOL, Falqueto A, Paupy, Lourenço-de-Oliveira R. 2019. *Haemagogus leucocelaenus* and *Haemagogus janthinomys* are the primary vectors in the major yellow fever outbreak in Brazil, 2016–2018. *Emer Microbes Infec* 8:218–231.

Franco O. História da Frebre Amarela no Brasil 1969. Ministério da Saúde. *Dep Nac Endem Rurais* 1:1–8.

Guimarães AÉ, De Mello RP, Lopes CM, Gentile C. 2000. Ecology of mosquitoes (Diptera: Culicidae) in areas of Serra do Mar State Park, state of São Paulo, Brazil. I - monthly frequency and climatic factors. *Memorias Do Instituto Oswaldo Cruz* 95:1–16.

Hamlet A, Ramos DG, Gaythorpe KAM, Romano APM, Garske T, Ferguson NM. 2021. Seasonality of agricultural exposure as an important predictor of seasonal yellow fever spillover in Brazil. *Nature Communications* 12:3647.

Hammer Ø, Harper DAT, Ryan PD. 2001. Paleontological Statistics Software Package for Education and Data Analysis Past: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4:1–9.

Kaul SM, Rastogi RM. 1997. Prevalence of Aedes aegypti and Aedes albopictus-Vectors of Dengue and DHF in India. In: *Dengue Bulletin*. 85p. New Delhi: Indian Council of Medical Research.

Laemmert HW, Kumm HW. 1950. the geographical distribution of immunity to yellow fever among the primates of Brazil. *The American Journal of Tropical Medicine and Hygiene* 30:733–748.

Marcondes CB, Alencar J. 2010. Revisão de mosquitos Haemagogus Williston (Diptera: Culicidae) do Brasil. *Revista Biomedica* 21:221–238.

Mir D, Delatorre E, Bonaldo M, Lourenço-de-Oliveira R, Vicente AC, Bello G. 2017. Phylodynamics of Yellow Fever virus in the Americas: new insights into the origin of the 2017 Brazilian outbreak. *Scientific Reports* 7:7385.

MMA. 2008. Plano De Manejo Da Área de Proteção Ambiental da Bacia do Rio São João/Micoleão-Dourado. Ministério do Meio Ambiente. 325 p. https://www.gov.br/icmbio/pt-br/assuntos/biodiversidade/unidade-de-conservacao/unidades-de-biomas/mata-atlantica/lista-de-ucs/apa-da-bacia-do-rio-sao-joao-mico-leao-dourado/arquivos/apa_bacia_rio_sao_joao.pdf

MS [Ministério da Saúde]. 2020. Situação epidemiológica da febre amarela no monitoramento 2019/2020. In *Boletim Epidemiológico.* 19 p. Available from Ministério da Saúde Secretaria de Vigilância em Saúde, SRTVN Quadra 701, Via W5 – Lote D, Edifício PO700, 7° floor, Brasília, Distrito Federal, Brazil.

Mutebi JP, Barrett ADT. 2002. The epidemiology of yellow fever in Africa. *Microbes and Infection* 4:1459–1468.

OMS [Organización Panamericana de la Salud]. 2017. *Actualización Epidemiológica Fiebre amarilla* [Internet]. Avaliable from Organización Panamericana de la Salud. https://www.paho.org/es/documentos/2-febrero-2017-fiebre-amarilla-actualizacion-epidemiologica-1

Perez MH, Noriega FG. 2013. *Aedes aegypti* pharate 1^{st} instar quiescence: A case for anticipatory reproductive plasticity. *J Insect Physiol* 59:318–324.

Rossetto EV, Angerami RN, Luna EJA. 2017. What to expect from the 2017 yellow fever outbreak in Brazil? *Revista Do Instituto de Medicina Tropical de São Paulo* 59:17–20.

SES [Secretaria de Estado de Saúde]. 2018. Informe epidemiológico 001/2018 [internet]. Available from Centro de Informações Estratégicas de Vigilância em Saúde/Unidade de Resposta Rápida – CIEVS/URR, Rio de Janeiro. Available from: http://www.riocomsaude.rj.gov.br/Publico/MostrarArquivo.aspx?C=%2Bp77vqe5mOs%3D

Silva SOF, de Mello CF, Érico Guimarães A, José Leite P, Alencar J. 2022. 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. *J Med Entomol* 59:1891–1897.

Soper FL, Penna H, Cardoso E, Serafim J, Frobisher M, Pinheiro J. 1933. Yellow fever without *Aedes aegypti.* Study of a rural epidemic in the valle do chanaan, Espirito Santo, Brazil, 1932. *Am J Epidemiol* 18:555–587.

Wilke ABB, Medeiros-Sousa AR, Ceretti-Junior W, Marrelli MT. 2017. Mosquito populations dynamics associated with climate variations. *Acta Tropica* 166:343–350.