CHAPTER 15

EVALUATION OF MULTIPLE IMMERSION EFFECTS ON EGGS FROM *HAEMAGOGUS LEUCOCELAENUS*, *HAEMAGOGUS JANTHINOMYS*, AND *AEDES ALBOPICTUS* (DIPTERA: CULICIDAE) UNDER EXPERIMENTAL CONDITIONS

Acceptance date: 01/03/2024

Shayenne Olsson Freitas Silva
Diptera Laboratory, Oswaldo Cruz Institute (Fiocruz), Rio de Janeiro, Brazi
Postgraduate Program in Tropical medicine, Oswaldo Cruz Institute (Fiocruz), Rio de Janeiro, Brazil

Cecilia Ferreira de Mello
Diptera Laboratory, Oswaldo Cruz Institute (Fiocruz), Rio de Janeiro, Brazi
Postgraduate Program in Animal Biology, Institute of Biology, Federal Rural University of Rio de Janeiro, Rio de Janeiro, Brazil

Raquel M. Gleiser
Universidad Nacional de Córdoba - CONICET. IMBIV, Centro de Relevamiento y Evaluación de Recursos Agrícolas y Naturales (CREAN), Córdoba, Argentina
UNC, Facultad de Ciencias Exactas, Físicas y Naturales, Córdoba, Argentina

Alexandre A. Oliveira
Diptera Laboratory, Oswaldo Cruz Institute (Fiocruz), Rio de Janeiro, Brazi
Interdisciplinary Entomological Surveillance Laboratory in Diptera and Hemiptera, Oswaldo Cruz Institute (Fiocruz), Rio de Janeiro, Brazil

Daniele de Aguiar Maia
Diptera Laboratory, Oswaldo Cruz Institute (Fiocruz), Rio de Janeiro, Brazi
Postgraduate Program in Animal Biology, Institute of Biology, Federal Rural University of Rio de Janeiro, Rio de Janeiro, Brazil

Jeronimo Alencar
Diptera Laboratory, Oswaldo Cruz Institute (Fiocruz), Rio de Janeiro, Brazi

ABSTRACT: Studies on the bioecology of *Haemagogus leucocelaenus* Dyar and Shannon 1924, *Haemagogus janthinomys* Dyar 1921, *Aedes albopictus* Skuse 1895 (Diptera: Culicidae) mosquitos are extremely important from an epidemiologic point of view, as they are known to be vectors of many important pathogens and, therefore, act as the main factor responsible for the maintenance of several zoonoses natural cycles. The present work aimed to elucidate their seasonal egg-hatching rate using the immersion method. Ovitraps were used to collect mosquito eggs from an Atlantic Forest fragment, in the State of Rio de Janeiro, Brazil, from November 2015 to November 2016. After collection, the eggs

were immersed 40 times to assess their hatching rate and evaluate the number of immersions resulting in the highest hatchability during the study period. Differences in the proportion of hatched eggs between species and seasons (spring, summer, fall, winter) and in the numbers of immersions in which eggs hatched were assessed using odds ratios. *Hg. leucocelaenus* was the species with the highest number of eggs hatching in all sampling periods, followed by *Ae. albopictus.* Most *Ae. albopictus* eggs hatched on first immersion regardless of season. Both the numbers of eggs and the number of immersion in which the *Haemagogus* eggs hatched showed high variability within seasons. In spring, the proportion of eggs that hatched on the first compared to further immersions was similar, while in fall and winter a higher percentage (over 94%) of *Hg. leucocelaenus* eggs hatched on the first immersion; the opposite pattern was observed in the summer. These results differ from previous observations linking increased hatching to warmer months.The number of immersions in which *Hg. leucocelaenus* eggs hatched varied between seasons, however differences were not statistically significant. These results evidence the need for further studies to elucidate factors that influence hatching patterns.
KEYWORDS: Culicidae, *Haemagogus leucocelaenus*, *Hg. janthinomys*, *Aedes albopictus*, egg, hatching rate

The highly diverse genus *Haemagogus* Williston 1896 (Diptera: Culicidae) comprises 28 species. From an epidemiologic point of view, they are extremely important as vectors of the yellow fever virus, being responsible for the maintenance of the natural cycle of this zoonosis (Marcondes and Alencar 2010). The species of this genus prefer to lay eggs in tree holes, although they can be found colonizing bamboo internodes, fruit peels, and puddles formed in stones and tires (Arnell 1973, Chadee 1983).

Among the species that have been identified as potential transmitters of the sylvatic yellow fever (SYF) virus, *Haemagogus janthinomys* Dyar 1921 (Diptera: Culicidae) has been highlighted as its main vector in the American continent. This mosquito also vectors other viruses such as Mayaro (Vasconcelos et al. 1998), Ilhéus (de Rodaniche and Galindo 1961), Tacaiuma (Contigiani and Diaz 2009), and Jurona and Mucambo (Hervé et al. 1986); its geographic distribution corresponds to endemic areas of this disease (Vasconcelos 2003). This species was incriminated as a vector for Mayaro virus in Belterra, Para State (Hoch 1981). Azevedo et al. (2009) reported that this arbovirus occurs in epidemic outbreaks in many areas of Amazonia, causing symptoms similar to those triggered by the dengue fever.

Haemagogus leucocelaenus Dyar and Shannon 1924 (Diptera: Culicidae), the most common species in Brazil, was found naturally infected with some arboviruses, and is considered the primary vector of SYF in the south-eastern region of the country (Vasconcelos 2010). Its geographic distribution extends from Trinidad to southern Brazil and northern Argentina (Arnell 1973). According to laboratory studies (Waddell 1949), *Hg. leucocelaenus* is a more efficient vector when compared to *Aedes aegypti* Linnaeus 1762 (Diptera: Culicidae).

Aedes albopictus Skuse 1894 (Diptera: Culicidae) is a competent vector for at least 22 arboviruses including dengue fever virus and yellow fever virus (Lourenço-de-Oliveira et al. 2003, Gratz 2004). Besides its importance as potential vector for different arboviruses, this species has a higher capacity of adaptation to different environments, acting as a link between forest areas where yellow fever circulates and urban agglomerations (Gratz 2004).

Currently, there is an outbreak of SYF in Brazil. The outbreak probably began by the end of 2016, when the first case was reported in the state of Minas Gerais and rapidly spread to the states of Espírito Santo, São Paulo, and Rio de Janeiro. In agreement with the report of the World Health Organization, as of April 2017, the transmission of the yellow fever virus (epizootic and human cases) continues to expand to the Atlantic coast of Brazil in areas previously considered without risk for yellow fever transmission (World Health Organization 2017).

Haemagogus and *Aedes* (Diptera: Culicidae) develop in temporary habitats such as phytotelmata, and lay drought-resistant dormant eggs on damp substrates. Egg dormancy (either diapause or quiescence) is considered to be a reproductive strategy of multivoltine mosquitoes that allows them long-term survival under unfavorable environmental conditions for hatching (Vinogradova 2007). Partial hatch of viable eggs is known as installment hatching (Gillett 1955). A previous exploratory study in the area of Simplício Hydroelectric Complex (AHES), state of Minas Gerais, Brazil, showed that most *Ae. albopictus* eggs hatch upon first immersion, while *Haemagogus* showed a varied instalment hatching response (Alencar et al. 2014). Knowledge about the installment hatching response is important for understanding mosquito population dynamics and developing mosquito control strategies (Vinogradova 2007).

The present study aimed to evaluate the effects of multiple immersions of *Hg. leucocelaenus*, *Hg. janthinomys*, and *Ae. albopictus* eggs in a tropical climate area and to assess the highest hatchability rate during the study period. Based on the previous observations, it was expected that a higher percentage of the eggs would hatch upon the first and/or second immersion during the rainy months, while the number of immersion required for eggs to hatch should be higher for eggs collected during the dry winter season.

METHODS

The eggs of *Hg. leucocelaenus*, *Hg. janthinomys*, and *Ae. albopictus* were collected using ovitraps, from November 2015 to November 2016. The present study was carried out in the Bom Retiro Private Reserve of Natural Heritage (Reserva Particular do Patrimônio Natural Bom Retiro, RPPNBR), located in south-eastern Brazil (22°27′14.1″S; 42°17′34.9″W), approximately 140 km from the city of Rio de Janeiro. The main landcover of the region is a typical Atlantic Forest vegetation, with dense ombrophilous sub-mountain forests in moderate and advanced stages of regeneration. The region of RPPN Fazenda Bom Retiro, located in the hydrographic basin of São João River, is situated in the intertropical zone (at low latitudes) and highly influenced by the Atlantic Ocean. Thus, its climate is predominantly of humid tropical type (Takizawa 1995). The average temperature is 26.8°C, with a relative humidity of 56% and 1,200 mm precipitation (National Institute of Meteorology (INMET) 2017). Higher rainfall levels are recorded from October to March.

Samples were collected in the RPPNBR at five sampling sites (1 – 22°27′ 19.4″S; 42°18′ 09.5″ W; 2 – 22°27′ 15.4″ S; 42°18′ 02.4″ W; 3 – 22°27′ 19.5′ S 42°18′ 01.5″ W; 4 – 22°27′ 14.1″ S; 42°17′ 34.9″ W; 5 – 22°27′ 19.4″ S 42°18′ 09.5″ W). Geographical coordinates of the sampling sites were obtained using a Garmin GPSMAP 60CS (Garmin International, Inc., Olathe, KA).

At each sampling site, samples were collected with three ovitraps. The ovitraps were made of black plastic containers, with a capacity of 1 liter and a cylindrical shape, containing four wooden paddles (2.5 × 14 cm). The traps were placed at a height that varied between 2 and 10 m above soil level. The details on the use and manufacturing of the ovitraps can be found in the studies by Silver (Silver 2008) and Alencar (Alencar et al. 2013). The paddles in traps were examined every 2 wk to detect and quantify the eggs, the eggs' age were not considered as an additional variable. Immediately after arriving in the laboratory the positive paddles were immersed in white trays filled with dechlorinated water at 29 ± 1°C, these trays were kept in acclimatized chamber for hatching. After 3 d, the paddles were removed from the water and left to air dry for another 3 d to quantify the hatched larvae. Immature forms were reared as described by Alencar (2008). To evaluate the influence of multiple immersions on egg hatching in the biological cycle, immersion was repeated 40 times with 3 d intervals.

Immature forms that died before completing development to adult stage were fixed in ethanol 80% and identified to the lowest taxonomic level (genus or species). Both immature (larvae or pupae) and adult specimens were identified through direct observation of morphological characters under stereoscopic microscope,and transmitting light microscope using the dichotomous keys proposed by Arnell (1973) and Forattini (2002). The abbreviations of generic and subgeneric names follow the proposal by Reinert (2001).

Differences between seasons categorized as spring (October– December), summer (January–March), fall (April–June), and winter (July–September) in the proportion of *Hg. leucocelaenus* eggs that hatched or in the number of immersions in which eggs hatched were assessed with odds ratios (R Studio). Statistical analyses were only carried out on *Hg. leucocelaenus* data because the number of eggs of *Hg. janthinomys* was very low for meaningful statistical inferences and most *Ae. albopictus* eggs hatched on first immersion regardless of season.

Ethical Considerations

All research was performed in accordance with scientific license number 34911 provided by SISBIO/IBAMA (Authorization and Information System on Biodiversity/ Brazilian Institute of Environment and Renewable Natural Resources) for the capture of culicids through- out the Brazilian national territory. All members of the collection team were vaccinated against the yellow fever virus and were aware of the possible risks they could encounter in the study area.

RESULTS

During the sampling period, 6,721 eggs were collected. Of these, 1,928 eggs (28.7%) were already hatched on the paddles. Of 4,793 eggs that did not hatch, 3,833 eggs (57.0%) hatched after being immersed in the laboratory. These hatched eggs belonged to three species: *Hg. leucocelaenus*, 1,100 specimens (28.7%); *Hg. janthinomys*, 62 specimens (1.6%); *Ae. albopictus*, 327 specimens (8.5%). In total, 1489 (38.8%) specimens reached the adult stage in the labora- tory and 2344 (61.2%) did not.

Table 1 shows a summary of the number of eggs and number of eggs hatched per species and season. Overall, higher numbers of eggs were collected (hatched) during the spring and summer. *Haemagogus janthinomys* was the species with the lowest frequency of eggs hatched during sampling, amounting to a total of 57 hatched eggs. Only two eggs hatched during the fall and winter compared to 25 during the spring (2015 + 2016) and 30 during the summer. Most eggs hatched within the first immersion, except for a batch of 30 collected in February (summer), which showed installment hatching with eggs hatching up to the 37 immersion. Because the number of eggs collected most seasons was very low, these data were excluded from further statistical analyses. *Hg. leucocelaenus* was the species with the highest number of eggs that hatched in all sampling periods, followed by *Ae. albopictus*. The numbers of eggs that hatched of both species showed high variability within seasons. Most *Ae. albopictus* eggs hatched on first immersion regardless of season, being 2 the maximum number of immersions required for eggs to hatch.

Hg. leucocelaenus showed significant effects of season on the proportion of eggs that hatched on first immersion. The proportion of eggs that hatched on the first compared to further immersions was similar, and thus spring was the reference value for odds ratio analysis. In fall and winter, a higher percentage (over 94%) of *Hg. leucocelaenus* eggs hatched on the first immersion compared to spring; in contrast, a higher proportion of eggs hatched after first immersion during the summer (Table 2).

The number of immersions in which *Hg. leucocelaenus* eggs hatched varied between seasons. However, differences were not considered statistically significant based on odds ratios analysis (Table 3).

DISCUSSION

The knowledge on *Hg. leucocelaenus, Hg. janthinomys,* and *Ae. albopictus* hatching rate presented herein contributes with impor- tant data on the biology of these species, which will aid the ento- mological surveillance of areas with confirmed SYF cases in Rio de

Janeiro's Atlantic Fores. For example, *Hg. leucocelaenus* showed installment hatching up to the 37th immersion; this delayed hatching may have several consequences on the mosquito populations such as the promotion of species coexistence, the alteration of microevolutionary dynamics, and the migration of alleles from the past (Evans and Dennehy 2005, Juliano 2009), which may influence pesticide resistance or vector capacity.

In their comparative study on the effects of multiple immersions of Aedini eggs, Alencar et al. (2014) observed that most eggs of *Aedes* and *Ochlerotatus* Reinert 2000 (Diptera: Culicidae) hatched during the first immersion, in contrast, the hatching of the eggs in *Haemagogus* varied according to the incubation period (instalment hatching) and required multiple immersions in order to hatch. Consistently, most *Ae. albopictus* hatched during the first immersion regardless of collection season, while *Haemagogus* hatching pattern was more variable.

Hovanitz (1946) observed hatching of *Hg. janthinomys* eggs right after one immersion. According to Clements and Kerkut (1963), in some Aedini, such as *Haemagogus*, *Aedes*, and *Psorophora* (Fabricius, 1794) (Diptera: Culicidae), the eggs hatch to first-instar larvae as soon as the environmental conditions are favorable. Similar results were found in the present analysis, in which the hatching of *Hg. leucocelaenus*, *Ae. albopictus*, and *Hg. janthinomys* eggs were usually higher during the first immersions.

Egg diapause is a long and stable pause during the incubation, even when the environmental conditions are favorable (Mullen and Durden 2009). Quiescence, however, is induced by unfavorable environmental conditions, and it is interrupted after the exposure to appropriate hatching stimuli such as floods. For *Ae. albopictus* there was no evidence of egg diapause or quiescence because almost all eggs hatched on first immersion regardless of season. In contrast, the proportion of eggs of *Hg. leucocelaenus* that hatched on first immersion increased in fall and winter, the seasons when rainfall is lower. Exposure to warmer temperatures before and after immersion tends to benefit the hatching (Campos and Sy 2006), however, the lowest percentage of egg hatch on first immersion was recorded in the summer. It could be speculated that delayed hatching in the seasons with higher rain could be a strategy to reduce mortality due to flushing from the larval habitat.

Regardless of season, *Haemagogus* hatching response varied between batches, from most eggs hatching on first immersion to hatchings up to the 37 immersion, as observed on a previous study on the effect of multiple immersions in different species of mosquito in Minas Gerais, Brazil (Alencar et al. 2014) and in Linhares Municipality, in the Brazilian south-eastern State of Espírito Santo (Alencar et al. 2008). This could be explained by the fact that egg diapause is expressed at different intensities, not only in eggs exposed to different conditions, but also in those exposed to the same condi- tions and laid by the same female.

Table 1. Number of eggs that hatched and number of immersions in which eggs hatched per species and season

Species	Season	Eggs	Mean immersion	Weighted immersion[b]	Maximum Immersion
Ae. albopictus	Spring	19.1 (1–65)	1.3 (1–2)	1.1 (1–1.5)	2
	Summer	5 (3–7)	1 (1–1)	1 (1–1)	1
	Fall	46.3 (3–117)	1 (1–1)	1 (1–1)	1
	Winter	19 (12–26)	1 (1–1)	1 (1–1)	1
Hg. janthinomys	Spring	4.2 (1–16)	1.2 (1–2)	3.2 (1–7)	8
	Summer	30[a]	11	9.3	37
	Fall	1[a]	1	1	1
	Winter	1[a]	1	2	1
Hg. leucocelaenus	Spring	60.6 (2–179)	3.5 (1–9)	1.8 (1–4.2)	12
	Summer	39.5 (2–124)	5 (1–12)	7.1 (3.2–14)	22
	Fall	28 (19–37)	2 (1–3)	1.1 (1–1.3)	7
	Winter	11.7 (1–25)	1.3 (1–2)	1.1 (1–1.2)	2

Values in third to fifth columns represent average and minimum-maximum range (in parenthesis). Maximum immersion shows the highest immersion event where hatchings were recorded on a given season.

[a]Only one batch each.

[b]Weighted per number of eggs.

Table 2. Percentage of *Hg. leucocelaenus* eggs that hatched per season on first immersion and following (later) immersions

Season	First immersion	Later immersions	Total	Odds ratio	95% CI
Spring	53.1 (32.6)	46.9 (28.7)	61.1		
Summer	30.4 (7.5)	69.6 (17.2)	24.6	0.4	0.3, 0.6[a]
Fall	94.6 (8.7)	5.4 (0.5)	8.7	15.6	4.8, 50.9[a]
Winter	94.3 (5.5)	5.7 (0.3)	5.5	14.6	3.4, 61.7[a]

Data shown are percentage of eggs that hatched on first or later immersions per season, and in parenthesis the relative frequencies of eggs hatching in rela- tion to total eggs hatched (expressed as percentages). Spring was the reference value for odds ratio analysis.

[a]Significant differences.

Table 3. Average number of immersions in which *Hg. leucocelaenus* eggs hatched per season (40 immersions tested; in parenthesis minimum and maximum number of immersions in which eggs hatched per season)

Season	Number of immersions	Percent per season	Odds ratio	95% CI
Spring	3.5 (1–9)	61.1		
Summer	5 (1–12)	24.6	1.5	0.8, 2.6
Fall	2 (1–3)	8.7	0.5	0.2, 1.5
Winter	1.3 (1–2)	5.5	0.4	0.1, 1.0

Spring was the reference value for odds ratio analysis.

Ferreira et al. (2017) reported that despite an abrupt decrease in egg hatching of *Hg. leucocelaenus* after the 8th immersion, egg viability was observed until the 12th immersion. However, in the present study, a different egg-hatching pattern was seen in *Hg. leucocelaenus*, with egg viability observed all the way until the 21st immersion.

Our results indicate differences between species in their hatch- ing schedule that are consistent with Alencar et al. (2014)`s findings. Installment hatching would prevent the loss of all descendants of a parent to any single drought. A previous study in the Atlantic Forest of Rio de Janeiro State on the vertical oviposition activity of mosquitoes showed that while *Ae. albopictus* laid most eggs near the ground, *Hg. leucocelaenus* oviposit at a broad range of heights and more frequently in ovitraps set at 5 m or higher (Alencar et al. 2016), likely more prone to dry, which might explain a higher hatch delay in the later species.

The evidence for active SYF viral transmission in the area where eggs were collected merit further studies on factors that influence hatching patterns, which will contribute to better understanding their population dynamics and eventually their ecoepidemiology of the viral diseases they transmit.

ACKNOWLEDGMENTS

This work was supported by the Foundation for Research Support of the State of Rio de Janeiro (FAPERJ; grant numbers 26/010.001630/2014, E-26/202.819/2015). Thanks also to Dr. David Gorla (National University of Cordoba, Argentina), for this helpful collaboration in software R.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES CITED

Alencar J, H. M. de Almeida, C. B. Marcondes, and A. É. Guimarães. 2008. Effect of multiple immersions on eggs and development of immature forms of *Haemagogus janthinomys* from south-eastern Brazil (Diptera: Culicidae). Entomol News. 119: 139–244.

Alencar, J., F. Morone, C. F. De Mello, N. Dégallier, P. S. Lucio, N. M. de Serra-Freire, and A. E. Guimarães. 2013. Flight height preference for ovi- position of mosquito (Diptera: Culicidae) vectors of sylvatic yellow fever virus near the hydroelectric reservoir of Simplício, Minas Gerais, Brazil. J. Med. Entomol. 50: 791–795.

Alencar, J., R. M. Gleiser, F. Morone, C. F. Mello, J. D. O. S. S. Silva, N. M. Serra-Freire, and A. É. Guimarães. 2014. A comparative study of the effect of multiple immersions on Aedini (Diptera: Culicidae) mosquito eggs with emphasis on sylvan vectors of yellow fever virus. Mem. Inst. Oswaldo Cruz. 109: 114–117.

Alencar, J., C. F. de Mello, H. R. Gil-Santana, A. É. Guimarães, S. A. de Almeida, and R. M. Gleiser. 2016. Vertical oviposition activity of mos- quitoes in the Atlantic Forest of Brazil with emphasis on the sylvan vector, *Haemagogus leucocelaenus* (Diptera: Culicidae). J. Vector Ecol. 41: 18–26.

Arnell, J. H. 1973. Mosquito studies (Diptera, Culicidae) XXXII. A revision of the genus *Haemagogus*. Contrib Am Entomol Soc. 10: 1–174.

Azevedo R. S. S., E. V. P. Silva, V. L. Carvalho, S. G. Rodrigues, J. P. N. Neto, A. O. Monteiro, V. S. Peixoto, O. Jannifer, Chiang, M. R. T. Nunes et al. 2009. Mayaro fever virus, Brazilian Amazon. Emerg Infect Dis. 15: 1830–1832.

Campos, R. E., and V. E. Sy. 2006. Variation in the hatching response of *Ochlerotatus albifasciatus* egg batches (Diptera: Culicidae) in temperate Argentina. Mem. Inst. Oswaldo Cruz. 101: 47–53.

Chadee, D. D. 1983. Rock hole breeding *Haemagogus* mosquitoes on Monos Island, Trinidad, West Indies. Mosq News. 43: 236–237.

Clements, A. N., and G. A. Kerkut. 1963. The physiology of mosquitoes. Pergamon Press, London, England.

Contigiani M. S., and L. A. Diaz. 2009. Togaviroses: encefalites e outras doen- ças. *In* C. B. Marcondes. Doenças transmitidas e causadas por artrópodes. Editora Atheneu, São Paulo.

de Rodaniche E., and P. Galindo. 1961. Isolation of the virus of Ilheus enceph- alitis from mosquitoes captured in Panama. Am J Trop Med Hyg. 10: 393–394.

Evans, M. E. K., and J. J. Dennehy. 2005. Germ banking: bet-hedging and variable release from egg and seed dormancy. Quarterly Rev Biol. 80: 431–451.

Ferreira, A. T., D. A. Maia, F. V. S. Abreau, W. C. Rodrigues, and J. Alencar. 2017. Oviposition behavior of *Haemagogus leucocelaenus* (Diptera: Culicidae), a vector of wild yellow fever in Brazil. Rev Inst Med Trop São Paulo. 1-6. Article ID 59e60. doi:10.1590/S1678-9946201759060

Forattini O. P. 2002. Culicidologia Médica. vol 2: Identificação, biologia, epi- demiologia. Editora da Universidade de São Paulo, São Paulo, Brasil.

Gillett, J. D. 1955. Variation in the hatching-response of *Aedes* eggs (Diptera: Culicidae). Bull Entomol Res. 46: 241–254.

Gratz, N. G. 2004. Critical review of the vector status of *Aedes albopictus*. Med. Vet. Entomol. 18: 215–227.

Hervé, J. P., N. Dégallier, P. A. Amélia, T. Rosa, P. F. Pinheiro, and C. G. S. Filho. 1986. Arboviroses: aspectos ecológicos. *In* Instituto evandro chagas: 50 anos. Instituto Evandro Chagas, Belém, Brasil.

Hoch, A. L., N. E. Peterson, J. W. LeDuc, and F. P. Pinheiro. 1981. An outbreak of Mayaro virus disease in Belterra, Brazil. III. Entomological and eco- logical studies. Am. J. Trop. Med. Hyg. 30: 689–698.

Hovanitz, W. 1946. Comparisons of mating behavior, growth rate, and fac- tors influencing egg-hatching in South American Haemogogus mosquitoes. Physiol. Zool. 19: 35–53.

Juliano, S. A. 2009. Species interactions among larval mosquitoes: context dependence across habitat gradients. Annu. Rev. Entomol. 54: 37–56.

Lourenço-de-Oliveira R., M. Vazeille, A.M. Bispo De Filippis, and A.B. Failloux. 2003. Large genetic differentiation and low variation in vector competence for dengue and yellow fever viruses of *Aedes albopictus* from Brazil, the United States, and the Cayman Islands. Am Jour Trop Med & Hyg. 69: 105–114.

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

Mullen, G. R., and L. A. Durden. 2009. Medical and veterinary entomology, 2nd ed. Academic Press, Amsterdam, Netherlands.

National Institute of Meteorology (INMET). 2017. Estação Meteorológica de Observação de Superfície Automática. (http://www.inmet.gov.br/portal/ index.php?r=estacoes/estacoesautomaticas) (accessed 14 September 2017).

Reinert J. F. 2001. Revised list of abbreviations for genera and subgenera of Culicidae (Diptera) and notes on generic and subgeneric changes. J Am Mosq Control Assoc. 17: 51–55.

Silver, J. B. 2008. Mosquito ecology: field sampling methods, 3rd ed. Springer, NY. **Takizawa, F. H. 1995.** Levantamento pedológico e zoneamento ambiental da Reserva Biológica de Poço das Antas. Universidade de São Paulo, Piracicaba, Brasil.

Vasconcelos, P. F. C. 2010. Yellow fever in Brazil: thoughts and hypoth- eses on the emergence in previously free areas. Rev Saude Publ. 44: 1144–1149.

Vasconcelos P. F. C., A. P. A. Travassos da Rosa, F. P. Pinheiro, R. E. Shope, J. F. S. Travassos da Rosa, S. G. Rodrigues, N. Dégallier, and E. S. Travassos da Rosa. 1998. Arboviruses pathogenic for man in Brazil: an overview of arbovirology in Brazil and neighboring countries. Instituto Evandro Chagas, Belém, Brasil.

Vasconcelos, P. F., A. F. Sperb, H. A. Monteiro, M. A. Torres, M. R. Sousa, H. B. Vasconcelos, L. B. Mardini, and S. G. Rodrigues. 2003. Isolations of yellow fever virus from *Haemagogus leucocelaenus* in Rio Grande do Sul State, Brazil. Trans. R. Soc. Trop. Med. Hyg. 97: 60–62.

Vinogradova, E. B. 2007. Diapause in aquatic insects, with emphasis on mos- quitoes, pp. 83–113. *In* V. R., B. Alekseev de Stasio, J. J. Gilbert (eds.), Diapause in aquatic invertebrates, theory and human use, Series: mon- ographiae Biologicae 84, Springer-Verlag, New York.

Waddell, M. B. 1949. Comparative efficacy of certain South American Aëdes and Haemagogus mosquitoes as laboratory vectors of yellow fever. Am. J. Trop. Med. Hyg. 29: 567–575.

World Health Organization. 2017. Yellow fever – Brazil. http://www.who.int/ csr/don/04-april-2017-yellow-fever-brazil/en/ (accessed 10 June 2017).