



Influence of pH, light, food concentration and temperature in *Aedes aegypti* Linnaeus (Diptera: Culicidae) larval development

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EntomoBrasilis 15: e999 (2022)

Abstract. *Aedes aegypti* Linnaeus mosquito is a vector of several viruses that cause diseases of medical and veterinary importance. Dengue, yellow fever, Zika and Chikungunya viruses are more important arboviruses transmit by mosquitoes. *A. aegypti* life cycle goes through 4 stages of development and the time for development from egg to adult mosquito depends on a series of biotic and abiotic factors such as temperature, food availability and population density, studied in different species of insects. In this work we studied the effects of different food concentrations, temperatures variation, pH gradient and luminosity on the development of *A. aegypti* larvae. The eggs were collected in the city of Tangará da Serra/MT and larvae in the L1 stage were used for the tests. The results showed that all the factors studied interfered in the larval development. The increase in food concentration and temperature accelerated the development of larvae to pupae. The acidic pH (pH = 4) proved to be unsuitable for the development of larvae (100% lethality), with the ideal pH for the development of larval stages being equal to pH = 6. Although in all light variations (dark, light and photoperiod 10L/ 14D) there was complete development of the larvae, the photoperiod test proved to be more adequate. This study helps to better understand the success, dispersion and adaptation of the *A. aegypti* mosquito in different regions under different environmental conditions.

Keywords: arboviruses; biotic factors; dengue; metamorphosis; mosquito.

Edited by:

Jeronimo Augusto Fonseca Alencar

Article History:

Received: 01.iii.2022

First Answer: 29.v.2022

Accepted: 19.vii.2022

Published: 17.x.2022

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Funding agencies:

↳ Fundação de Amparo a Pesquisa do Estado de Mato Grosso - FAPEMAT



doi: [10.12741/ebrazilis.v15.e999](https://doi.org/10.12741/ebrazilis.v15.e999)

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Arboviruses (viruses transmitted by arthropod invertebrates) transmitted by mosquitoes of the genus *Aedes* are one of the biggest public health problems in the world. The distribution of mosquitoes of the genus *Aedes* is mainly located in the tropical and subtropical regions of the globe, and in Brazil there is a differentiation in the distribution of the two main species of this genus, *Aedes aegypti* Linnaeus and *Aedes albopictus* (Skuse) (KRAEMER *et al.* 2015). The North and Northeast region of Brazil concentrates the *A. aegypti* mosquito and in the Southern region we found higher population density of *A. albopictus* (EBI & NEALON 2016).

Only females have the hematophagous habit necessary for their reproduction and egg development (FORATTINI 2002; NATAL 2002; CARDOSO *et al.* 2010). These insects have important adaptations related to hematophagy, such as: anesthetic substances, anticoagulants and dilator vessels present in the saliva of the animal that act at the time of blood repast (RIBEIRO & FRANCISCHETTI 2003). Such molecules aid for a better blood supply, reducing the time of contact with the host, thus ensuring the survival of the insect (CIPRANDI *et al.* 2003; CALVO *et al.* 2009).

The relatively short life cycle and the large number of eggs that females can oviposit throughout their life aid in the reproduction of this insect. Another important factor to be considered is the ability of eggs of mosquitoes of the genus *Aedes* to be resistant to desiccation, thus increasing the dispersal capacity of this mosquito (FARNESI *et al.* 2017). The life cycle of *A. aegypti* is partially aquatic, in which females prefer to oviposit on moist surfaces (air-water interface), in places of preferably clean and stop water with little organic matter that are dark or low luminosity (NELSON *et al.* 1986). In addition, they undergo complete metamorphosis passing through four larval stages (L1, L2, L3, L4), pupa stage and the adult stage. The total time between the egg to winged insect phase varies depending on environmental conditions and food availability, being between 7 and 10 days. Several studies are found in the literature on the development of mosquitoes under different environmental conditions, mainly related to temperature, rainfall, population density and different food sources (ALTO & JULIANO 2001a, 2001b; ARAÚJO *et al.* 2015; COURET *et al.* 2014; CHRISTIANSEN-JUCHT *et al.* 2015). The lack of adequate food for mosquito development and high temperatures lead to mosquitoes of smaller sizes, which interfere with their physiology. The correlation between mosquito size and reproductive capacity, longevity and vector capacity were studied by different authors (GAMA *et al.* 2005; STEINWASCHER 2018). Other factors such as luminosity, pH levels and feed availability were better studied in flies (*e.g.*, *Drosophilidae*) and there is a study deficit related to the genus *Aedes*, especially regarding the parameter for the establishment of laboratory rearing (VILLANUEVA *et al.* 2016).

In order to complement the knowledge about the *A. aegypti* mosquito, this study evaluated the influence of different parameters (food availability, temperature, luminosity and pH) that may cause physiological changes, thus affecting its development. This helps in understanding the dispersion and adaptation in which the *A. aegypti* mosquito is suffering under different biotic and abiotic conditions.

MATERIAL AND METHODS

Eggs were collected by ovitramp method (FAY & PERRY 1965) in Tangará da Serra municipality (State of Mato Grosso, Brazil) in different neighborhoods. After seven days the palettes were removed and observed under stereomicroscope to verify their positivity (presence of eggs) and eggs count. The palettes were placed in a transparent plastic container with 500 mL of distilled water for the hatching of the eggs. After 24 h, the larvae in stage L1 were used to perform the experiments described below.

Ethics. The collection and transport of the target specimens was authorized through the Biodiversity Information and Authorization System (SISBIO) - number 68486.

Experimental. All experiments were carried out using L1 larvae (as described above), under the same temperature conditions (except for the temperature variation experiment), in transparent plastic pots with 500 mL of distilled water. The larvae were fed with Alcon Colours fish feed® crushed and the addition of food was performed when necessary (depending on the test - see below). The experiments were observed daily, with the annotation of the development of larval stages until the metamorphosis of the pupa and/or the death of the individual.

Experiment 1: Larvae development in different food concentrations. In this experiment, three plastic pots were used with 20 larvae in stage L1. This experiment was carried out in duplicate, obtaining a total of 40 larvae for each condition analyzed. The pots were kept in the same temperature and luminosity conditions. In pot 1 - 2 mg of food was added, in pot 2 - 4 mg of food was used and finally in pot 3 - 8 mg of food were added.

Experiment 2: Development of larvae at different temperatures. For this experiment the pots were kept in BOD at the different temperatures analyzed. This experiment was carried out in duplicate, obtaining a total of 40 larvae for each condition analyzed. Pot 1 was kept at a constant temperature of 18 °C (low), pot 2 at constant temperature of 25 °C (ideal) and pot 3 at constant temperature of 31 °C (high). The larvae were fed with 4 mg of fish feed and, whenever necessary, the same concentration was added in all pots.

Experiment 3: Development of larvae at different pH values. The three containers were kept in different pH ranges: pot 1 - pH = 3.0 (acid), pot 2 - pH = 6.0 (control) and pot 3 - pH = 8.0 (alkaline). The water pH was adjusted using hydrochloric acid 1 M or sodium hydroxide 1 M. The pHs were measured and adjusted every day as aid of pH indicator tapes. This experiment was carried out in duplicate, obtaining a total of 40 larvae for each condition analyzed.

Experiment 4: Development of larvae in different conditions of luminosity. For luminosity control, the pots were kept in B.O.D and at a controlled temperature of 25°C. The experiment was carried out in duplicate with 20 L1 larvae. Pot 1 was left with light access throughout the experiment, pot 2 was used the photoperiod with control of 14 hours of dark (D) and 10 hours light (L) and, pot 3 was kept without light throughout the analyzed time. Food replacement was

performed every two days or when necessary, with 4 mg of fish feed.

Statistics. The values obtained were plotted in a spreadsheet in the Microsoft® Excel program, and later the data were analyzed in the R Statistical System (R Core Team 2015), where survival was analyzed using a nonparametric distribution with Weibull test to verify the significance of the results.

RESULTS AND DISCUSSION

Several studies carried out on different insects and, particularly in mosquitoes, show that some factors interfere in the development, slowing, accelerating and even causing the death of some individuals. One of the main parameters affecting larval development and food availability. Figure 1 shows a direct relationship between the amount of food available and larval development until its pupa metamorphosis. At the concentration of 8 mg of available food (gray line) all pupae were obtained on the eighth day after experimental initiation. At the concentration of 2 mg of food it was not possible to observe the metamorphosis of all larvae until the end of the experimental period (red line). The curve most consistent with larval development for *A. aegypti* described in nature was observed using 4 mg of available food (dashed line).

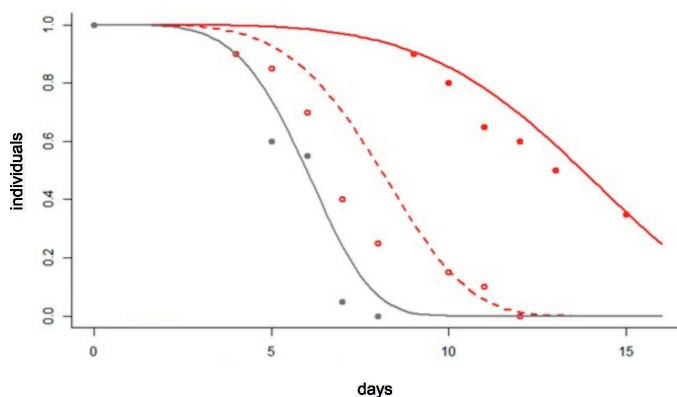


Figure 1. Food availability influence offered to *Aedes aegypti* larvae. Red line: 2 mg - food; Dashed line: 4 mg - food and Gray line: 8 mg - food.

Result obtained by us in the survival test for food concentration corroborates with another works utilizing another mosquitoes (GIMNIG et al. 2002; YEE et al. 2004; YOSHIOKA et al. 2012; STEINWASCHER 2018). Studies conducted in *A. albopictus* (YOSHIOKA et al. 2012) and *Anopheles gambiae* Giles (YOSHIOKA et al. 2012) showed that the lower amount of food and the increase in larvae density slow the development of larvae.

Another important factor that modifies the speed in the development of larvae is temperature. The results obtained for the temperature tests (Figure 2) showed that there was statistical difference between the parameters analyzed ($P=2.816 \times 10^{-19}$). The graph shows a higher delay in larva metamorphosis to pupa at lower temperature (19 °C - red line) compared to the other temperatures analyzed (25 °C - dashed line/ 30 °C - gray line). Studies conducted in the laboratory using temperature gradient showed that the larval and pupal period is lower the higher the temperature (BAR-ZEEV 1958; CALADO & SILVA 2002; COURET et al. 2014). Our results are in accordance with the described by these authors (Figure 2), except in the highest temperatures studied. BAR-ZEEV (1958) in his work with *A. aegypti* analyzed the mean development time in each larval stage (L1-L4) and in pupae in 8 temperature conditions (between 16 °C to 38 °C). He noted that the greatest differences in the development of juvenile stages up to the adult insect occurred between 16-28 °C. At the highest temperatures (30 - 38 °C) the average

time was closer. A study conducted in *A. albopictus* showed no significant difference in the development time of larvae and pupae at three different temperatures (25 °C, 30 °C and 35 °C) (MONTEIRO *et al.* 2007).

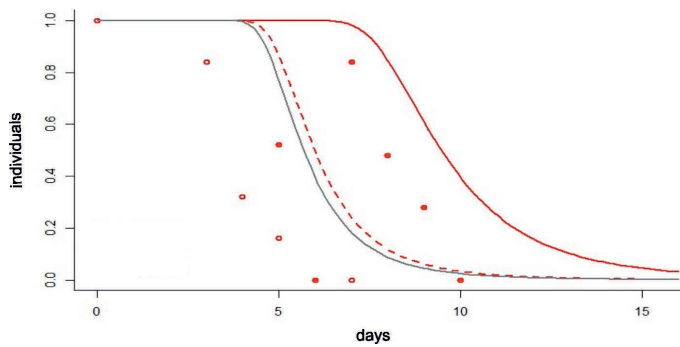


Figure 2. Temperature influence on the development of *Aedes aegypti* larvae. Red line: 19 °C; Dashed line: 25 °C and Gray line: 30 °C.

The pH values in aqueous environment may vary due to the influence of several factors, such as temperature, carbon dioxide concentration, dissolved organic matter, etc. Water pH also interferes with the proliferation of microorganisms that can be used as a feeding source for mosquito larvae. All larvae that were submitted to the most acidic pH (pH = 3) died after 24 h. The other data obtained at pH 6 and pH 8 are expressed in Figure 3, which showed a statistical difference ($P=1.417 \times 10^{-5}$) between them. We can observe (Figure 3) that in pot 2 (pH = 6 - red line) presented faster development when compared to pot 3 (pH = 8 - dashed line).

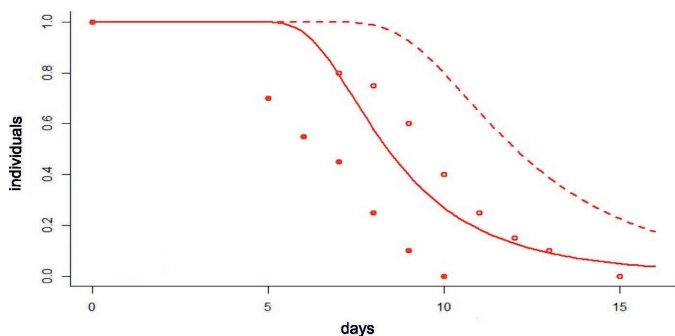


Figure 3. pH influence on the development of *Aedes aegypti* larvae. Red line: pH: 6.0; Dashed line: pH: 8.0.

Our results corroborate studies conducted on larvae of mosquitoes of the genus *Aedes* (PARADISE & DUNSON 1997; CLARK *et al.* 2004; LOPEZ *et al.* 2011). It has been described that more acidic pH (equal to 4) interferes in the development of mosquito larvae, significantly increasing their mortality (PARADISE & DUNSON 1997; LOPEZ *et al.* 2011; CLARK *et al.* 2004) observed that larvae of *A. aegypti* present great tolerance in pH variations, but at pH equal to 3 or 12, obtained 100% mortality. DANTAS (2011) the pH of water directly interferes in the development of the mosquito affecting from its weight to its survival, and acidic pHs cause death or harmful physiological effects on the development of insects, due to favoring the proliferation of bacteria harmful to *A. aegypti*. CLARK *et al.* (2004) also observed that larvae of *A. aegypti* in an environment with neutral pH undergo metamorphosis for pupa faster than in a basic environment, with no statistically significant difference in the total development time in acidic or basic environments.

The exposure of insect larvae to luminosity can affect their development in several ways causing their delay (diapause) (WAY *et al.* 1949; SAUNDERS 2014), modifying its longevity (CHOCOROSQUI & PANIZZI 2003), altering reproduction through the development of ovarian follicles (SANBURG & LARSEN 1973; REZNIK & VAGHINA 2011), influencing the flight capacity (ASLAM *et al.* 1994), among others. Analysis performed for

the influence of luminosity (Figure 4) showed a statistically significant difference between the development of pot 2 with photoperiod ($P=0.0282152$), when compared to pot 1 (light) and pot 3 (dark). Interestingly, pots 1 and 3 showed no statistically significant difference. Our results showed that the larvae of *A. aegypti* tolerate large differences in the photoperiod, ending larval development until pupa in all trials performed. CONSOLI & OLIVEIRA (1994) state that some mosquito species do not end their development in the absence of luminosity, although most mosquito species studied can develop in complete darkness.

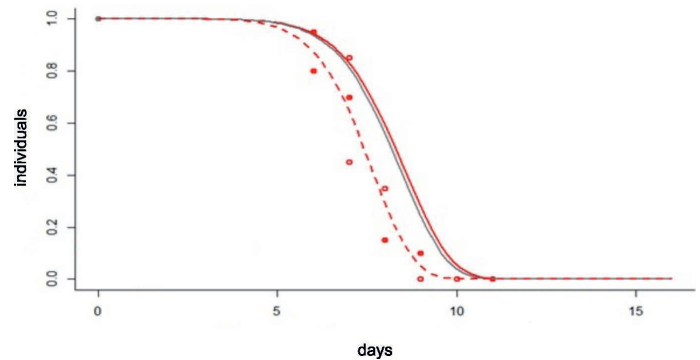


Figure 4. Photoperiod influence on the development of *Aedes aegypti* larvae. Red line: dark (D); Dashed line: photoperiod (14 h D/ 10 h L) and Gray line: light (L).

The influence of photoperiod on mosquitoes is better studied in *Culex quinquefasciatus* (SANBURG & LARSEN 1973; UKUBUIWE *et al.* 2017; UKUBUIWE *et al.* 2018) and in *A. albopictus* (YEE *et al.* 2012; COSTANZO *et al.* 2016). In *A. aegypti* there were no statistically significant changes for survival, fecundity and body size (COSTANZO *et al.* 2016), however, the photoperiod interferes with the hematophagous habit of females and may increase their vector capacity (COSTANZO *et al.* 2015). GARZÓN *et al.* (2021) showed that larvae of *A. aegypti* present slower development when exposed to shorter luminosity time (8 h - light) compared to those exposed to more time with light (16 h - light). Our results also showed a delay in development when the larvae were submitted to total darkness in relation to the one with a photoperiod of 10 h light and 14 h dark. Although we have not found studies that have submitted larvae of *A. aegypti* to extreme conditions as we performed (larval development totally in the dark or in clarity), larvae of *Culex quinquefasciatus* Say (UKUBUIWE *et al.* 2017) submitted to these conditions showed differences in their development, a result that differs from the data obtained by us. This is probably due to different species mosquitoes which inhabit different biological niches and have different biological aspects.

Our study showed that all parameters analyzed interfered in the development of *A. aegypti* larvae. The amount of food and temperature have already been described in several other classes of insects as one of the most important factors for the development of arthropods. Luminosity and pH were poor studied in mosquitoes. The results obtained in our work showed that the larvae of *A. aegypti* develop independent of luminosity and that the medium with acid pH significantly slows the development of mosquitoes and can be used as one of the ways to eliminate larvae in artificial breeding sites, found mainly in urban environment. Alkaline pH also interferes with development by slowing it down compared to control.

ACKNOWLEDGEMENTS

The authors thank the FAPEMAT (Fundação de Amparo a Pesquisa do Estado de Mato Grosso) for funding this work and the UNEMAT (Universidade do Estado de Mato Grosso Carlos Alberto Reyes Maldonado) for all support.

REFERENCES

- Alto, BW & SA Juliano, 2001a. Precipitation and Temperature Effects on Populations of *Aedes albopictus* (Diptera Culicidae): implications for range expansion. *Journal of Medical Entomology*, 38: 646-656. DOI: <https://doi.org/10.1603/0022-2585-38.5.646>
- Alto, BW & SA Juliano, 2001b. Temperature Effects on the Dynamics of *Aedes albopictus* (Diptera, Culicidae) Populations in the Laboratory. *Journal of Medical Entomology*, 38: 548-556. DOI: <https://doi.org/10.1603/0022-2585-38.4.548>
- Araujo, RV, MR Albertini, AL Costa-da-Silva, L Suesdek, NC Franceschi, NM Bastos, Katz G, VA Cardoso, BC Castro, ML Capurro & VL Allegro, 2015. São Paulo urban heat islands have a higher incidence of dengue than other urban areas. *Brazilian Journal Infection Disease*, 19: 146-55. DOI: <https://doi.org/10.1016/j.bjid.2014.10.004>
- Aslam, M, DW Hagstrum & BA Dover, 1994. The Effect of Photoperiod on the Flight Activity and Biology of *Rhyzopertha dominica* (Coleoptera: Bostrichidae). *Journal of Kansas Entomological Society*, 67: 107-115.
- Bar-Zeev, M, 1958. The Effect of Temperature on the Growth Rate and Survival of the Immature Stages of *Aedes aegypti* (L.). *Bulletin of Entomological Research*, 49: 157-163. DOI: <https://doi.org/10.1017/S0007485300053499>
- Calado, DC & MAN Silva, 2002. Avaliação da influência da temperatura sobre o desenvolvimento de *Aedes albopictus*. *Revista de Saúde Pública*, 36: 173-179. DOI: <https://doi.org/10.1590/S0034-89102002000200009>
- Calvo, E, VM Pham, O Marinotti, JF Andersen & JMC Ribeiro, 2009. The salivary gland transcriptome of the neotropical malaria vector *Anopheles darlingi* reveals accelerated evolution of genes relevant to hematophagy. *BMC Genomics*, 10: 57-83. DOI: <https://doi.org/10.1186/1471-2164-10-57>
- Cardoso, AF, RL Cres, AS Moura, F de Almeida & AT Bijovsky, 2010. *Culex quinquefasciatus vitellogenesis*: morphological and biochemical aspects. *Memórias do Instituto Oswaldo Cruz*, 105: 254-262. DOI: <https://doi.org/10.1590/S0074-02762010000300003>
- Chocorosqui, VR & AR Panizzi, 2003. Photoperiod influence on the biology and phenological characteristics of *Dichelops melacanthus* (Dallas, 1851) (Heteroptera: Pentatomidae). *Brazilian Journal of Biology*, 63: 655-664. DOI: <https://doi.org/10.1590/S1519-69842003000400012>
- Christiansen-Jucht, CD, PE Parham, Saddler, A, JC Koella & MG Basáñez, 2015. Larval and adult environmental temperatures influence the adult reproductive traits of *Anopheles gambiae* s.s.. *Parasites Vectors*, 8: 456. DOI: <https://doi.org/10.1186/s13071-015-1053-5>
- Ciprandi, A, F Horn & C Termignoni, 2003. Saliva de animais hematófagos: fonte de novos anticoagulantes. *Revista Brasileira de Hematologia e Hemoterapia*, 25: 250-262. DOI: <https://doi.org/10.1590/S1516-84842003000400012>
- Clark, TM, JF Benjamin & SK Remold, 2004. pH tolerances and regulatory abilities of freshwater and euryhaline Aedine mosquito larvae. *Journal of Experimental Biology*, 207: 2297-2304. DOI: <https://doi.org/10.1242/jeb.01021>
- Consoli, RAGB & RL Oliveira, 1994. Principais mosquitos de importância sanitária no Brasil. *Fiocruz*, Rio de Janeiro, Brazil.
- Costanzo, KS, RA Dahan & D Radwan, 2016. Effects of photoperiod on population performance and sexually dimorphic responses in two major arbovirus mosquito vectors, *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). *International Journal of Tropical Insect Science*, 36: 177-187. DOI: <https://doi.org/10.1017/S1742758416000163>
- Costanzo, KSS, KJ Schelble & M Keenan, 2015. The effect of photoperiod on life history and blood-feeding activity in *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). *Journal of Vector Ecology*, 40: 164-71. DOI: <https://doi.org/10.1111/jvec.12146>
- Couret, J, E Dotson & MQ Benedict, 2014. Temperature, larval diet, and density effects on development rate and survival of *Aedes aegypti* (Diptera, Culicidae). *PLoS One*, 9: e87468. DOI: <https://doi.org/10.1371/journal.pone.0087468>
- Dantas, ES, 2011. Avaliação da influência de algumas características do criadouro e da água na frequência de formas imaturas e no tamanho e peso de adultos do mosquito *Aedes aegypti* (Diptera: Culicidae) no Rio de Janeiro. *Dissertação (Mestrado em Medicina Tropical) - Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro*.
- Ebi, KL & J Nealon, 2016. Dengue in a changing climate. *Environmental Research*, 151: 115-123. DOI: <https://doi.org/10.1016/j.envres.2016.07.026>
- Farnesi, LC, HCM Vargas, D Valle & GL Rezende, 2017. Darker eggs of mosquitoes resist more to dry conditions: Melanin enhances serosal cuticle contribution in egg resistance to desiccation in *Aedes*, *Anopheles* and *Culex* vectors. *PLoS Neglected Tropical Diseases*, 11: e0006063. DOI: <https://doi.org/10.1371/journal.pntd.0006063>
- Fay, RW & AS Perry, 1965. Laboratory studies of ovipositional preferences of *Aedes aegypti*. *Mosquito News*, 25: 276-281.
- Forattini, OP, 2002. *Culicidologia Médica. Identificação, Biologia, Epidemiologia*. Vol. 2. São Paulo, Editora Universidade de São Paulo.
- Gama, RA, KC Alves, RF Martins, AE Eiras & MC Resende, 2005. Efeito da densidade larval no tamanho de adultos de *Aedes aegypti* criados em condições de laboratório. *Revista da Sociedade Brasileira de Medicina Tropical*, 38: 64-66. DOI: <https://doi.org/10.1590/S0037-86822005000100014>
- Garzón, MJ, L Maffey, A Lizuain, D Soto, PC Diaz, M Leporace, OD Salomón & NJ Schweigmann, 2021. Temperature and photoperiod effects on dormancy status and life cycle parameters in *Aedes albopictus* and *Aedes aegypti* from subtropical Argentina. *Medical and Veterinary Entomology*, 35: 97-105. DOI: <https://doi.org/10.1111/mve.12474>
- Gimnig, JE, M Ombok, S Otieno, MG Kaufman, JM Vulule & ED Walker, 2002. Density-dependent development of *Anopheles gambiae* (Diptera: Culicidae) larvae in artificial habitats. *Journal of Medical Entomology*, 39:162-72. DOI: <https://doi.org/10.1603/0022-2585-39.1.162>
- Kraemer, MU, ME Sinka, KA Duda, AQ Mylne, FM Shearer, CM Barker, CG Moore, RG Carvalho, GE Coelho, W Van Bortel, G Hendrickx, F Schaffner, IR Elyazar, HJ Teng, OJ Brady, JP Messina, DM Pigott, TW Scott, DL Smith, GR Wint, N Golding & SI Hay, 2015. The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. *Elife*, 4: e08347. DOI: <https://doi.org/10.7554/eLife.08347>
- Lopez, LCS, EGB Silva, MG Beltrão, 2011. Effect of tank bromeliad micro-environment on *Aedes aegypti* larval mortality. *Hydrobiologia*, 665: 257-261. DOI: <https://doi.org/10.1007/s10750-011-0605-8>
- Monteiro, LC, JR Souza, CM Albuquerque, 2007. Eclosion rate, development and survivorship of *Aedes albopictus* (Skuse) (Diptera: Culicidae) under different water temperatures. *Neotropical Entomology*, 36: 966-971. DOI: <https://doi.org/10.1590/s1519-566x2007000600021>
- Natal, D, 2002. *Bioecologia do Aedes aegypti*. *Biológico*, 64: 205-207.
- Nelson, JM, 1986. *Aedes aegypti*: Biologia y Ecologia. *Organizacion Panamericana de la Salud*. Washington, DC.
- Paradise, CJ & WA Dunson, 1997. Effects of pH and sulfate on insects and protozoans inhabiting treeholes. *Archives of Environmental Contamination and Toxicology*, 33:182-187. DOI: <https://doi.org/10.1007/s002449900240>
- R Core Team, 2015. R: A language and environment for statistical computing. R Foundation for Statistical

- Computing, Vienna. Available in: <<https://www.R-project.org>>. Access: 10.vi.2015.
- Ribeiro, JM & IM Francischetti, 2003. Role of arthropod saliva in blood feeding: sialome and post-sialome perspectives. Annual Review Entomology, 48: 73-88. DOI: <https://doi.org/10.1146/annurev.ento.48.060402.102812>
- Reznik, S & N Vaghina, 2011. Photoperiodic control of development and reproduction in *Harmonia axyridis* (Coleoptera: Coccinellidae). European Journal of Entomology, 108: 385-390. DOI: <https://doi.org/10.14411/eje.2011.048>
- Sanburg, LL & JR Larsen, 1973. Effect of photoperiod and temperature on ovarian development in *Culex pipiens pipiens*. Journal of Insect Physiology, 19: 1173-1190. DOI: [https://doi.org/10.1016/0022-1910\(73\)90202-3](https://doi.org/10.1016/0022-1910(73)90202-3)
- Saunders, DS, 2014. Insect photoperiodism: Effects of temperature on the induction of insect diapause and diverse roles for the circadian system in the photoperiodic response. Entomological Science, 17: 25-40. DOI: <https://doi.org/10.1111/ens.12059>
- Steinwascher, K, 2018. Competition among *Aedes aegypti* larvae. PLoS One, 13: e0202455. DOI: <https://doi.org/10.1371/journal.pone.0202455>
- Ukubuiwe, AC, IK Olayemi, MO Odeyemi, IM Salihu, Al Jibrin, CC Ukubuiwe & RY Yunusa, 2017. Influence of photoperiod on larval growth indices and energy budget for metamorphosis in *Culex quinquefasciatus* mosquito (Diptera: Culicidae); its implication in integrated vector management. Biotechnology Society of Nigeria 2017 Book of Proceedings.
- Ukubuiwe, AC, IK Olayemi, ICJ Omalu, FO Arimoro, BM Baba & CC Ukubuiwe, 2018. Effects of Varying Photoperiodic Regimens on Critical Biological Fitness Traits of *Culex quinquefasciatus* (Diptera: Culicidae) Mosquito Vector. International Journal of Insect Science, 10: 1179543318767915. DOI: <https://doi.org/10.1177/1179543318767915>
- Villanueva, OK, G Ponce, B Lopez, SM Gutierrez, IP Rodriguez, G Reyes, KJ Saavedra, WC Black, J Garcia, B Beaty, L Eisen & AE Flores, 2016. Effect of photoperiod on permethrin resistance in *Aedes aegypti*. Journal of The American Mosquito Control Association, 32: 308-314. DOI: <https://doi.org/10.2987/16-6577.1>
- Way, MJ, B Hopkins & PM Smith, 1949. Photoperiodism and Diapause in Insects. Nature 164: 615. DOI: <https://doi.org/10.1038/164615a0>
- Yee, DA, B Kesavaraju & AS Juliano, 2004. Interspecific differences in feeding behavior and survival under food-limited conditions for larval *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). Annals of the Entomological Society of America, 97 (4): 720-728. DOI: [https://doi.org/10.1603/0013-8746\(2004\)097\[0720:idifba\]2.0.co;2](https://doi.org/10.1603/0013-8746(2004)097[0720:idifba]2.0.co;2)
- Yee, DA, SA Juliano & SM Vamosi, 2012. Seasonal photoperiods alter developmental time and mass of an invasive mosquito, *Aedes albopictus* (Diptera: Culicidae), across its north-south range in the United States. Journal of Medical Entomology, 49: 825-832. DOI: <https://doi.org/10.1603/me11132>
- Yoshioka, M, J Couret, F Kim, J McMillan, TR Burkot, EM Dotson, U Kitron & GM Vazquez-Prokopec, 2012. Diet and density dependent competition affect larval performance and oviposition site selection in the mosquito species *Aedes albopictus* (Diptera: Culicidae). Parasites Vectors, 5: 225. DOI: <https://doi.org/10.1186/1756-3305-5-225>

