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Original Article

Culex quinquefasciatus predominance during integrated mosquito surveillance in an urban area of the Brazilian Amazon

Predominância de *Culex quinquefasciatus* durante a vigilância integrada de mosquitos em uma área urbana da Amazônia brasileira

F. G. F. Rios[a,b]*, C. A. Menezes[a,b], L. R. Silva[a,b], L. H. M. Feitoza[a,b], A. C. A. Meireles[a,c] and G. R. Julião[a,b,d]

[a]Fundação Oswaldo Cruz – Fiocruz Rondônia, Laboratory of Entomology, Porto Velho, RO, Brasil
[b]Universidade Federal de Rondônia – UNIR, Postgraduate Program in Experimental Biology – PGBIOEXP, Porto Velho, RO, Brasil
[c]Fundação Oswaldo Cruz – Fiocruz Rondônia, Postgraduate Program in Biodiversity and Health, Porto Velho, RO, Brasil
[d]Instituto Nacional de Epidemiologia da Amazônia Ocidental – INCT-EpiAmO, Porto Velho, RO, Brasil

Abstract

The presence and establishment of Culicidae in urban areas increase the transmissibility of tropical diseases, since some species can participate as vectors of pathogens. Thus, this study aimed to evaluate the indoor and outdoor abundance of immature and adult populations of Culicidae at the urban area of Porto Velho, Rondônia. Mosquitoes were captured using electric aspirators and ovitraps in September and December 2018 in 27 households spread over nine neighborhoods. A total of 2,342 specimens were collected, distributed among five species, of which *Culex quinquefasciatus* (Say, 1823), *Aedes aegypti* (Linnaeus, 1762) and *Aedes albopictus* (Skuse, 1894) were the most abundant. Considering the sum total obtained by both techniques, more mosquitoes were captured indoors than outdoors. However, the GLM estimates for the ovitrap technique showed that immature *Ae. aegypti*, *Ae. albopictus* and *Cx. quinquefasciatus* were significantly more abundant in the outdoors, on average. The opposite result was observed for electrical aspiration, in which *Ae. aegypti* and *Cx. quinquefasciatus* adults were more abundant indoors. The average number of winged *Ae. albopictus* showed no significant difference between indoors and outdoors. Our findings corroborate the data on the abundance and incidence of these three species in other regions of Brazil, highlighting the need for continuous surveillance due to their importance in disease transmission to humans. We also demonstrated that the ovitrap is a sensitive device to monitor *Cx. quinquefasciatus* larvae and wild species that occasionally frequent urban areas, and thus can be used for surveillance, especially when there are budgetary constraints. Therefore, we emphasize that the combination of techniques, in addition to identifying which species and which stage of development are more frequent inside and outside households, also allows for the implementation of specific and integrated control measures.

Keywords: *Aedes*, *Culex quinquefasciatus*, vector insects, ovitrap, electric aspiration.

Resumo

A presença e o estabelecimento de culicídeos na área urbana aumentam a transmissibilidade de doenças tropicais, pois algumas espécies podem participar como vetores de patógenos. Assim, este estudo teve como objetivo avaliar a abundância de populações imaturas e adultas de espécies de culicídeos em intradomicílio e peridomicílio, em localidades da área urbana de Porto Velho, Rondônia. Os mosquitos foram capturados com o uso de aspiradores elétricos e ovitrampas nos meses de setembro e dezembro de 2018 em 27 residências distribuídas por nove bairros. Coletaram-se 2.342 espécimes, distribuídos em cinco espécies, das quais *Culex quinquefasciatus* (Say, 1823), *Aedes aegypti* (Linnaeus, 1762) e *Aedes albopictus* (Skuse, 1894) foram as mais abundantes. Considerando o somatório obtido por ambas as técnicas, mais mosquitos foram capturados no intradomicílio que no peridomicílio. No entanto, as estimativas de GLM para a técnica de ovitrampa demonstraram que imaturos de *Ae. aegypti*, *Ae. albopictus* e *Cx. quinquefasciatus* foram significativamente mais abundantes em peridomicílio, em média. Resultado inverso foi observado para aspiração elétrica, no qual adultos de *Ae. aegypti* e *Cx. quinquefasciatus* foram mais abundantes em intradomicílio. O número médio de adultos de *Ae. albopictus* não apresentou diferença significativa entre intra e peridomicílio. Nossos achados corroboram os dados de abundância e ocorrência dessas três espécies em outras regiões do Brasil, evidenciando a necessidade de vigilância contínua devido à sua importância na transmissão de doenças aos seres humanos. Nós demonstramos também que a ovitrampa é uma técnica sensível para monitorar larvas de *Cx. quinquefasciatus* e espécies silvestres que ocasionalmente frequentam áreas urbanas, podendo assim, ser empregada para a vigilância, especialmente quando há restrições orçamentárias. Assim, ressaltamos que a

*e-mail: flavia.rios@fiocruz.br
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combinação de técnicas, além de identificar quais espécies e qual fase de desenvolvimento são mais frequentes dentro e fora dos domicílios, permite também estabelecer a implementação de medidas de controle específicas e integradas.

Palavras-chave: *Aedes*, *Culex quinquefasciatus*, insetos vetores, ovitrampa, aspiração elétrica.

1. Introduction

The vast majority of mosquito species are considered to be wild, however, certain species have adapted to the urban settings due to man-made modifications. The changes in the bionomy of these mosquitoes, largely caused by environmental interferences such as deforestation and urbanization, can be exemplified by species of the *Culex*, *Aedes*, and *Anopheles* genera (Taipe-Lagos and Natal, 2003; Burkett-Cadena and Vittor, 2018; Multini et al., 2019).

The mosquitoes *Cx. quinquefasciatus* and *Ae. aegypti* are two species that have benefited from the reduction of urban forest areas, because in addition to reducing the number of predators, they are less dependent on these areas than other species, and can reproduce and proliferate easily in urban areas, both indoors and outdoors (Medeiros-Sousa et al., 2017).

The mosquitoes from the genus *Aedes* can be found all over the world, mainly in tropical and subtropical regions of the planet (Kamal et al., 2018). The species *Ae. aegypti* and *Ae. albopictus* are the most well-known species of this genus and are prominent in the transmission of viruses on all continents, many of which can cause diseases in humans. *Ae. aegypti* is the main vector of the viruses that cause dengue fever (DENV), Zika (ZIKV), chikungunya (CHIKV) and urban yellow fever (YFV). *Ae. albopictus* also has an important epidemiological role in arbovirus transmission, as it is considered a potential or secondary vector of arboviruses (Cecílio et al., 2009; Kotsakiozi et al., 2017; Zanotto and Leite, 2018; Jones et al., 2020).

The genus *Culex* includes the major vector species of West Nile Virus (WNV) which causes West Nile Fever, one of which is *Cx. quinquefasciatus*. This species is important in Brazil, as it is the main vector of *Wuchereria bancrofti*, the etiological agent of lymphatic filariasis (Bhattacharya and Basu, 2016, Xavier et al., 2019). Furthermore, studies by Guedes et al. (2017) showed that the species *Cx. quinquefasciatus*, in addition to being a vector of WNV, may be involved in the transmission of ZIKV in Recife, northeastern Brazil.

Entomological surveillance in urban settings is an important tool for determining integrated control strategies. This tool is generally used for mosquitoes of the genus *Aedes*, but not for other species that are also important for arbovirus transmission. In addition, integrated vector control measures can contribute to the reduction of arbovirus circulation, since the primary form of managing the main urban arboviruses is vector control (Zara et al., 2016).

Thus, an entomological investigation was carried out in the city of Porto Velho, an urban area of the Brazilian Amazon, in the state of Rondônia, where the composition of fauna and the abundance of mosquitoes were assessed using two techniques. Our goal was to measure the abundance of immature and adult populations of Culicidae species among nine neighborhoods of the urban core, in order to characterize the Culicidae fauna and identify which species are circulating inside and outside households, which can be applied to future planning of vector control actions.

2. Materials and Methods

2.1. Ethical aspects

The study was approved by the Ethics Committee in Human Research (CEP FIOCRUZ-IOC #1.420.911 and CEP CEPEM #535.903). In order to carry out entomological collections in the households, all participants provided their written informed consent, and the homeowners had the option to withdraw from participating in the project. In addition, participants were informed about the main objectives, methods, risks, and benefits of the study.

2.2. Study area

Porto Velho city has 67 neighborhoods and is the third largest capital city in the northern region of Brazil, with a metropolitan area of approximately 150 km^2 (Gonçalves et al., 2014). According to the most recent census (2010), the municipality has 428,527 inhabitants, with an estimated population of 548,952 inhabitants in 2021 (IBGE, 2021). The study was carried out during the months of September and December 2018, in nine neighborhoods within the urban core of Porto Velho, in the State of Rondônia. The collections were carried out in 27 households (three per neighborhood) distributed throughout nine neighborhoods: Aponiã, Cohab, Floresta, Nova Esperança, Nova Porto Velho, Ronaldo Aragão, São João Bosco, Tancredo Neves and Triângulo (see Figure 1). The households were chosen on a database from the AETRAPP-WWF-Fiocruz Rondônia Project (https://latinno.net/en/case/3305/) which has information on the collection of *Aedes* spp. eggs through aettraps (https://youtu.be/_Xzw26_hXOA) and ovitraps from 90 households in the urban area of Porto Velho, between September and December 2017. The criterion for choosing these households was the total amount of mosquito eggs collected, selecting for each neighborhood the three households with the greatest number of *Aedes* spp. eggs.

2.3. Sampling techniques

Two techniques were used for mosquito collection, the modified ovitrap (to collect immature insects) and the electric aspirator (to collect adults). The standard ovitrap is a trap intended for the oviposition of mosquitoes, consisting of a black container with a wooden paddle (Reiter et al., 1991). This trap is recommended in the surveillance of *Aedes* spp. mosquitoes, given its high sensitivity and specificity along with its low cost, when compared to other

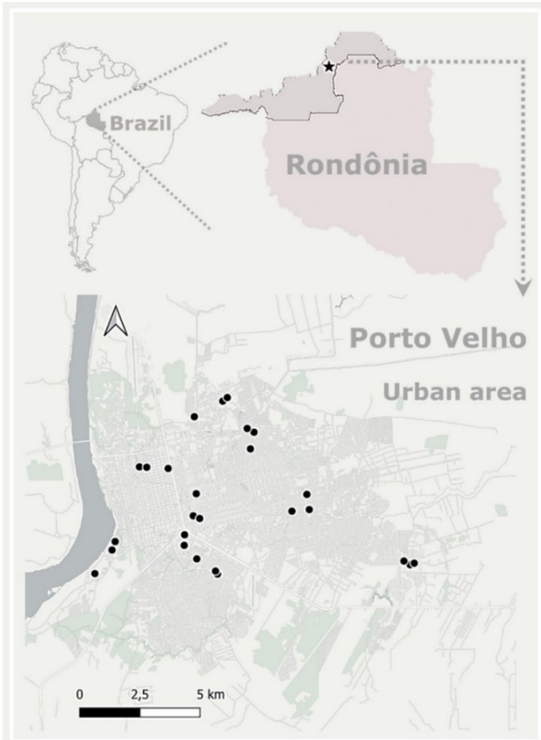

Figure 1. Map of the study area, Porto Velho city in Rondônia State, Brazil, demonstrating the location of each residence of collect (represented by a black circle).

techniques (FIOCRUZ, 2014). Despite being recommended for the acquisition of eggs, in the present study its use was aimed at obtaining larvae, and thus, the paddle was excluded, maintaining water and a hay infusion in the container (100 grams of grass in 20 liters of tap water). 150 mL of hay infusion and 250 mL of running water were placed in each ovitrap, totaling a final volume of 400 mL (Chadee et al., 1993). Adult mosquitoes were captured with a Nasci aspirator (see Figure 2A), consisting of a PVC cylinder covered in aluminum, with a net attached to the inside of the cylinder, where the mosquitoes were captured (Large Electric Aspirator – Horst Traps). The aspirator has a propeller and a motor powered by a 12-volt battery. This apparatus is used to capture both inactive mosquitoes (resting or harbored in natural and artificial shelters) and active mosquitoes (which are in full flight). This aspiration technique can be used to collect diurnal or nocturnal mosquitoes either indoors, outdoors, or extradomiciliary areas (Nasci, 1981; Forattini, 2002). For transportation purposes, the net intended for the interior of the vacuum was replaced by a plastic container with a screen (see Figure 2B and 2C). The container was introduced into the nozzle of the aspirator (see Figure 2D), and after the capture session the container was capped with the aspirator still in operation (see Figure 2E), and the mosquitoes were trapped inside it.

The collected specimens were taken to Entomology Laboratory I at Fiocruz Rondônia. The immature specimens captured in the ovitrap remained in the insectary, with controlled temperature and humidity conditions, until they

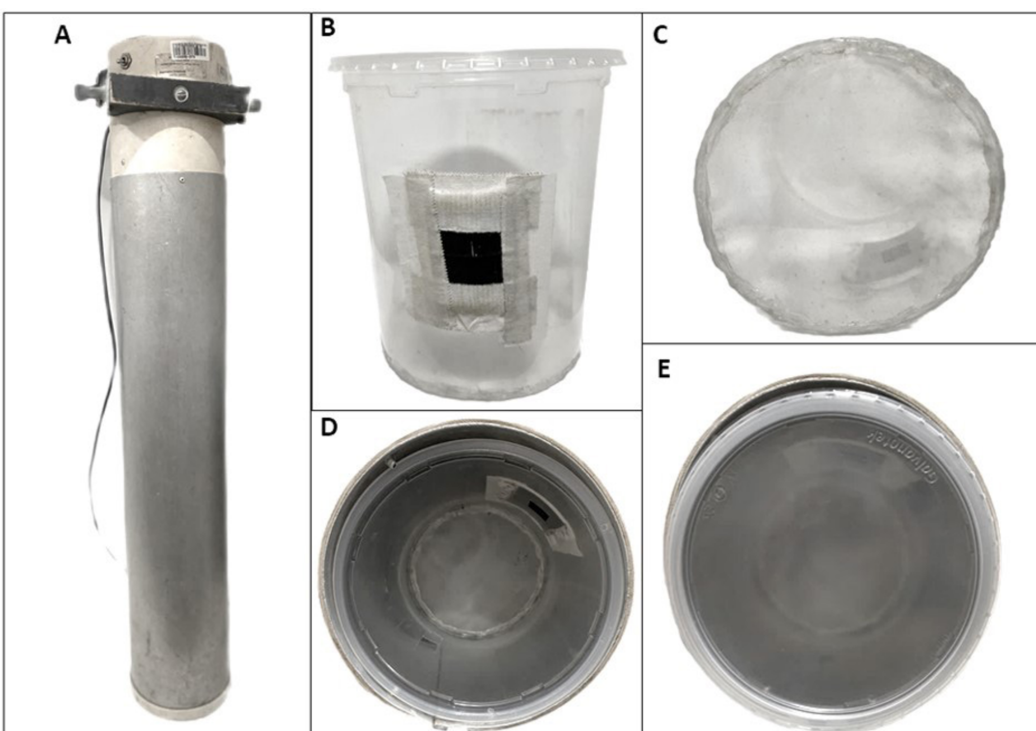

Figure 2. Nasci Aspirator (A) and a plastic pot (B) with a screen (C) adapted for electric vacuuming. Open pot (D) and pot with a lid (E) in the nozzle of the aspirator.

reached the winged phase of development. The ovitraps that did not contain larvae at the time of removal received TetraMin fish food to stimulate the hatching of eggs that might have been deposited in the water or on the wall of the container and were not visualized, these were kept in the laboratory for three days (Imam et al., 2014). All mosquitoes were killed at -20°C for taxonomic identification of the species, using the keys proposed by Consoli and Lourenço de Oliveira (1994) and the atlas prepared by Segura and Castro (2007).

2.4. Sample design

Mosquito collections were carried out in 27 households during the day, with 27 electrical aspirations indoors and 27 electrical aspirations outside the home, totaling 54 electrical aspirations. Each aspiration lasted 15 minutes. Twenty-seven ovitraps were also installed indoors and 27 in outdoors (54 collections with ovitraps), which remained in the residence for 7 days. Two collection events were carried out (September and December), thus totaling 108 collections by electrical aspiration and 108 collections by ovitrap, and each event was carried out over a period of two weeks: in the first week, ovitraps and electrical aspirations were performed. In the second week, the ovitraps were removed.

2.5. Data analysis

Comparison of species abundance between household habitats (indoor and outdoor), for the two sampling techniques (ovitrap and electrical aspiration) was performed using generalized linear models (GLM) with Poisson error distribution corrected for over- or under-dispersion (correction for standard errors using a Quasi-GLM model), and log-binding function, indicated for count values (Crawley, 2007). This error distribution adequacy was compared with other distributions using the hp package, with a visual diagnosis of the fit of the models, verifying whether or not the residuals were dispersed within the simulated envelopes (Moral et al., 2017). The packages *sjPlot*, *sjmisc* and *sjlabelled* were used to generate the model estimation results. The estimates were automatically transformed (exponential) and in addition to the average number of mosquitoes at the baseline level of the variable (indoor), an estimate of relative risk in percentage values was provided, which indicated greater or lesser risk than the other level of the variable (outdoor). Mosquito collection data were tabulated in Microsoft Excel and analyzed on the free platform R (R Core Team, 2022).

3. Results

A total of 2,342 mosquitoes were obtained, distributed among five species, of which *Cx. quinquefasciatus*, *Ae. aegypti* and *Ae. albopictus* were the most abundant. Of this total number, 1,202 were females and 1,140 males (as shown in Table 1). Sixty percent of the mosquitoes (n=1,412) were sampled indoors while 930 individuals were obtained outside households.

Table 1. Number of mosquito individuals by species and sex, collected in September and December 2018 in nine neighborhoods of the city of Porto Velho, Rondônia, Brazilian Amazon.

Species	Female	Male	Total
Aedes aegypti	218	169	387
Aedes albopictus	83	30	113
Culex quinquefasciatus	869	916	1,785
Limatus durhamii	30	21	51
Toxorhynchites haemorrhoidalis	2	4	6
Total	**1,202**	**1,140**	**2,342**

The species *Cx. quinquefasciatus* was the most abundant in both habitats, followed by the species *Ae. aegypti*, which showed little difference between indoors and outdoors. *Ae. albopictus* was the third most abundant and presented the highest number of mosquito individuals in the outdoor habitats (see Figure 3A).

The number of mosquitoes was slightly higher in captures by electrical aspiration compared to ovitrap sampling (56.66% x 43.34%). *Cx. quinquefasciatus* was the most abundant species when considering both techniques (aspiration and ovitrap). *Ae. aegypti* and *Ae. albopictus* mosquitoes were more numerous in ovitrap sampling. There was no capture of individuals from the species *Limatus durhamii* (Theobaldi, 1901) or *Toxorhynchites haemorrhoidalis* (Fabricius, 1787) using the electric aspiration technique, whereas they were captured in the ovitrap (see Figure 3B). *Limatus durhamii* were stored at -80°C for further studies. The specimens of the species *Toxorhynchites haemorrhoidalis* were assembled and deposited in the Entomological Collection of Fiocruz Rondônia (COLRO).

The estimates of the generalized linear model for the ovitrap devices, which take into account the sampling effort by household habitat, showed that the species *Ae. aegypti*, *Ae. albopictus* and *Cx. quinquefasciatus* were significantly more abundant in the outdoor habitat, with 58%, 0.09% and 0.04% more mosquitoes when compared to the average number of mosquitoes captured inside the home, respectively (as shown in Table 2).

The GLM estimates for the electric aspiration technique also point to statistical differences between habitats, in relation to the average number of mosquitoes. The species *Ae. aegypti* and *Cx. quinquefasciatus* were more abundant indoors: there were 36% and 48% fewer mosquitoes outdoors in relation to the average number of mosquitoes sampled indoors, respectively. The average number of *Ae. albopictus* specimens showed no significant difference between the two habitats (as shown in Table 3).

4. Discussion

One of the limitations in controlling mosquito vectors, such as *Ae. aegypti*, is the scarcity of specific techniques and equipment for monitoring their adult forms. Thus, several

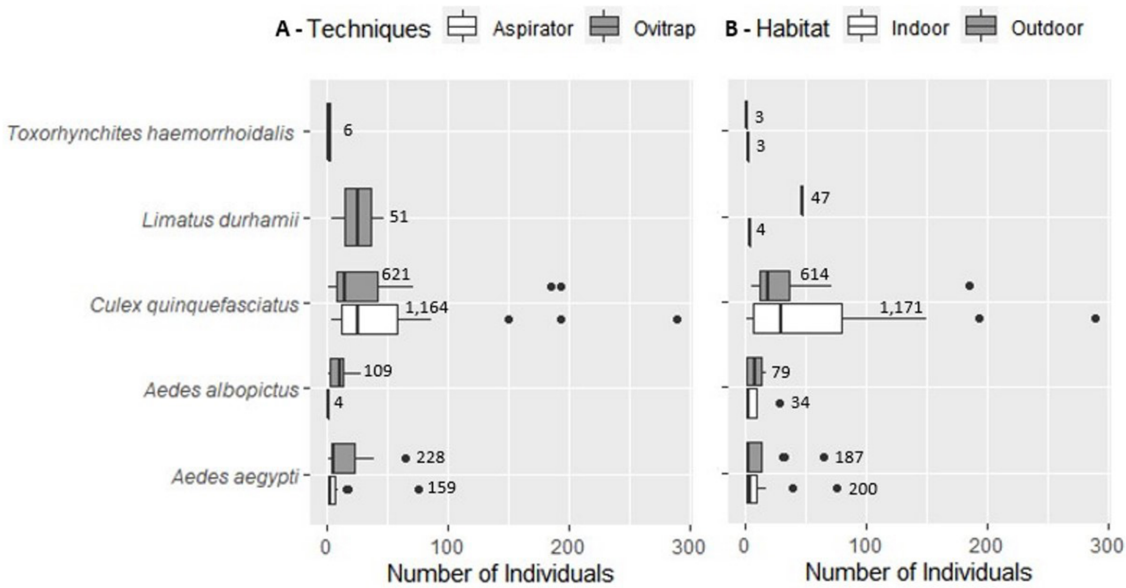

Figure 3. Number of mosquitoes by species using two sampling techniques (A) collected indoors and outdoors (B) in nine neighborhoods from the city of Porto Velho, Rondônia, Brazilian Amazon.

Table 2. Relative risk estimates for the number of mosquitoes sampled indoors and outdoors, through ovitraps in nine neighborhoods from the city of Porto Velho, Rondônia, Brazilian Amazon.

Species	Habitat	Relative risk		
		Estimates	IC	p*
Ae. aegypti	Indoor	3.30	1.72-5.63	**<0.001**
	Outdoors	1.58	0.81-3.31	0.202
Ae. albopictus	Indoor	3.67	1.56-7.13	**0.002**
	Outdoors	1.09	0.46-2.87	0.851
Cx. quinquefasciatus	Indoor	13.53	8.78-19.74	**<0.001**
	Outdoors	1.04	0.61-1.77	0.899

IC: confidence interval. *Bolded p-values indicate significant differences between predictor levels.

Table 3. Relative risk estimates for the number of mosquitoes sampled indoors and outdoors, through electric aspiration in nine neighborhoods from the city Porto Velho, Rondônia, Brazilian Amazon.

Species	Habitat	Relative risk		
		Estimate	IC	p*
Ae. aegypti	Indoor	3.62	2.61-4.87	**<0.001**
	Outdoors	0.36	0.15-0.75	**0.014**
Ae. albopictus	Indoor	1.00	1.00-1.00	0.553
	Outdoors	1.00	1.00-1.00	1.000
Cx. quinquefasciatus	Indoor	9.05	7.34-11.01	**<0.001**
	Outdoors	0.48	0.31-0.74	**0.001**

IC: confidence interval. *Bolded p-values indicate significant differences between predictor levels.

alternative techniques have been used for entomological surveillance, such as electric aspirators, aimed at estimating the adult populations. However, the use of this apparatus, as well as the installation of ovitraps, is limited to the consent of the residents for collection inside their homes, as well as to the training and quantity of people needed to perform the techniques properly in order to have a reliable sampling that is truly representative of mosquito populations at a site. The ovitrap is a method that differs from electric aspiration because it is more economical and because it is passive, that is, it does not require a technical operator (Barrera, 2016).

Entomological indicators have been proposed for monitoring vector mosquitoes that may vary depending on the mosquito's stage in its life cycle, available sites for larval development and collection methods (Gomes, 1998). Ovitraps and electric aspiration generate a variety of indicators, such as larval/egg indices and the number of adult mosquitoes per household, respectively. In addition, the use of georeferenced technological systems associated with ovitraps has been proven efficient for monitoring the presence and density of Culicidae. These systems and indicators can guide vector control procedures. However, the abundance of mosquitoes may not sufficiently demonstrate the risk of infection in humans due to the complexity of the transmission chain that may involve several variables, such as susceptibility to human and vector infection (Regis et al., 2013; Cromwell et al., 2017).

Despite the ovitrap being a technique considered specific for the surveillance of mosquitoes of the genus *Aedes*, our study demonstrated that this device is able to monitor larvae of *Cx. quinquefasciatus*, a species often found to be naturally infected with WNV, ZIKV and the Mayaro virus (Bhattacharya and Basu, 2016; Serra et al., 2016; Guedes et al., 2017). In addition, we demonstrate that this technique can also be used for surveillance based on the eggs/larvae of sylvatic species that may breed in urban areas. Thus, despite the need for a combination of techniques (ovitrap and electric aspiration) for a better sampling of Culicidae and for vector control, the ovitrap has proven to be an important technique for the larval monitoring of species, especially when there are budget constraints (FIOCRUZ, 2014).

The mosquito with the highest abundance was observed to be *Cx. quinquefasciatus*, followed by the species *Ae. aegypti* and *Ae. albopictus*, constituting one of the main results analyzed and expected from this study, due to the fact that the first two species are domiciled, a very endophilic and highly anthropophilic behavior (Consoli and Lourenço de Oliveira, 1994; Wilke et al., 2019).

In the last Rapid Survey of the *Aedes aegypti* Infestation Index (LIRAa) carried out in October and November 2021, the municipality of Porto Velho was classified in an alert situation for mosquito infestation, with garbage (plastic containers and cans), scraps, debris and water storage deposits in low places being the predominant breeding sites (AGEVISA, 2021). As in Porto Velho, solid waste without proper disposal has been recognized worldwide as facilitating the transmission of diseases in urban areas, not only since it provides a favorable habitat for immature *Aedes*, but also because solid waste is directly or indirectly associated with the incidence of other diseases vectors, such as triatomines and sandflies, as well as urban zoonoses (Krystosik et al., 2020).

The hegemony of adults of the species *Cx. quinquefasciatus* and *Ae. aegypti* has been recorded in several Brazilian municipalities, with captures carried out with an electric aspirator, resting box and hand-held net (Barata et al., 2007; Serra et al., 2016). Despite the fact that the species *Ae. albopictus* is increasingly common in urban areas of Brazilian states, occupying mainly artificial breeding sites from human activity, in addition to natural breeding sites (Carvalho et al., 2014), the fortuitous incidence of this species in the urban area of Porto Velho can be explained by its behavior and habit, since it has a preference for places with greater vegetation cover (exophilic) (Lima-Camara et al., 2006), as can also be observed in another Amazonian capital city, Manaus (Ríos-Velásquez et al., 2007). As opposed to the synanthropic *Cx. quinquefasciatus* and *Ae. aegypti*, periurban forested sites have been demonstrated as preferred habitat for some species (Forattini, 1986), and this seems to explain the low incidence of *Ae. albopictus* in most of the capture sites in Porto Velho, which were located in neighborhood with little and/or no vegetation.

Our findings corroborate the data obtained in southeastern Brazil, where adults of the species *Cx. quinquefasciatus* and *Ae. aegypti* were prevalent within households (Barata et al., 2007). However, there is no consensus regarding the preference of adults of these species for any of these habitats. *Cx. quinquefasciatus* adults were predominant in outdoors in the southern region of the country (Ramos et al., 2019).

In our study, there was little difference between indoors and outdoors regarding the abundance of *Ae. aegypti*, a result also observed in captures using human attractants (Gomes et al., 2005). On the other hand, *Ae. albopictus* females were more abundant in the collections performed by outdoor electrical aspiration, compared to the indoor habitats, a fact also verified with the captures using human attractants performed by Gomes et al. (2005). In addition, the sexual ratio of *Ae. aegypti* in an area of endemic dengue fever transmission seems to be concordant in both indoor and outdoor habitats. Males and females were more abundant indoors, accounting for 82.4% and 87.3%, respectively, through collections with Nasci aspirators (Barata et al., 2001).

Cx. quinquefasciatus was the most abundant species captured through electrical aspiration; however, *Ae. aegypti* and *Ae. albopictus* represented the highest number of specimens in the ovitrap. It is concluded that integrating techniques in different environments and habitats is necessary for vector monitoring, allowing researchers to obtain a greater number of individuals and to characterize some aspects of the biology of Culicidae, in addition to the target species. In this sense, the ovitrap proved to be useful in detecting the presence of typically wild species, even if at a low frequency, and their sporadic incidence in households in urban areas; in addition, our study confirms the potential for oviposition of these species indoors. *Limatus durhamii* mosquitoes, a wild species, were obtained in a single neighborhood (Triângulo), which is the most distant of the 9 neighborhoods from the urban area, located in the most peripheral portion of the city (with greater vegetation cover), close to the Madeira River. *Toxorhynchites haemorrhoidalis*, another wild species, though not hematophagous, was also found less frequently. Some studies have also demonstrated the presence of species *Li. durhamii* and *Toxorhynchites* sp. in urban areas, associated with artificial breeding sites (Lopes et al., 1993; Calderón-Arguedas et al., 2009; Montagner et al., 2018). The recent finding of Zika virus in *Li. durhamii* in a wild area of Brazil (Barrio-Nuevo et al., 2020) reinforces the need for more research and surveillance actions for this species.

All species were sampled using ovitraps in both habitats. However, more specimens of each species were obtained in the outdoors, suggesting that there was a preference for laying eggs in external areas adjacent to the households. This behavior may also suggest that females enter households to feed and rest and leave to lay their eggs in the outdoor habitats, where more eggs were found in the ovitraps (Dibo et al., 2005).

The preference for laying eggs in outdoors may be due to the greater quantity and variety of natural reservoirs (bamboo internodes, tree holes, bromeliads) and/or artificial resources (tires, cans, glass, bottle shards, dishes) existing outside the households that can serve as oviposition sites (Consoli and Lourenço de Oliveira, 1994).

Despite the sample coverage being satisfactory on a spatial scale, involving nine neighborhoods in the urban area of an Amazonian city, the collection period (two months) was a limiting aspect of this study, as it restricts the conclusions regarding the list of species in both outdoor and indoor habitats, since the results may be subject to seasonal influences (Calderón-Arguedas et al., 2009; Codeço et al., 2015; Rodrigues et al., 2015). Despite this, our study corroborates data on the abundance and incidence of the species *Cx. quinquefasciatus*, *Ae. aegypti* and *Ae. albopictus* in an urban area (Serra et al., 2016). Due to their importance in the transmission of diseases to humans, these species should be monitored more frequently, since, in general, there are few studies addressing the Culicidae fauna in urban cores in the Brazilian Amazon (Ríos-Velásquez et al., 2007; Saraiva et al., 2020). Furthermore, the use of a strategy for the combination of techniques makes it possible to identify which species and which stage of development are most frequent inside and outside households and establishes the implementation of specific (immature/adult) and integrated (species) vector control measures.

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References

AGÊNCIA ESTADUAL DE VIGILÂNCIA EM SAÚDE DE RONDÔNIA – AGEVISA, 2021 [viewed 9 February 2022]. *Semana Epidemiológica 50/2021* [online]. Available from: https://rondonia.ro.gov.br/publicacao/boletim-dengue-semana-50-2021/

BARATA, E.A., COSTA, A.I.P., CHIARAVALLOTI-NETO, F., GLASSER, C.M., BARATA, J.M.S. and NATAL, D., 2001. *Aedes aegypti* (L.) population in an endemic area of dengue in the Southeast Brazil. *Revista de Saude Publica*, vol. 35, no. 3, pp. 237-242. http://dx.doi.org/10.1590/S0034-89102001000300004. PMid:11486145.

BARATA, E.A.M.D.F., CHIARAVALLOTI-NETO, F., DIBO, M.R., MACORIS, M.D.L.G., BARBOSA, A.A.C., NATAL, D., BARATA, J.M.S. and ANDRIGUETTI, M.T.M., 2007. Capture of culicids in urban areas: evaluation of the resting box method. *Revista de Saude Publica*, vol. 41, no. 3, pp. 375-382. http://dx.doi.org/10.1590/S0034-89102007000300008. PMid:17515990.

BARRERA, R., 2016. Recommendations for the surveillance of *Aedes aegypti. Biomédica*, vol. 36, no. 3, pp. 454-462. http://dx.doi.org/10.7705/biomedica.v36i3.2892. PMid:27869394.

BARRIO-NUEVO, K.M., CUNHA, M.S., LUCHS, A., FERNANDES, A., ROCCO, I.M., MUCCI, L.F., SOUZA, R.P., MEDEIROS-SOUSA, A.P., CERETTI-JUNIOR, W. and MARRELLI, M.T., 2020. Detection of Zika and dengue viruses in wild-caught mosquitoes collected during field surveillance in an environmental protection area in São Paulo, Brazil. *PLoS One*, vol. 15, no. 10, e0227239. http://dx.doi.org/10.1371/journal.pone.0227239. PMid:33064724.

BHATTACHARYA, S. and BASU, P., 2016. The southern house mosquito, *Culex quinquefasciatus*: profile of a smart vector. *Journal of Entomology and Zoology Studies*, vol. 4, no. 2, pp. 73-81.

BURKETT-CADENA, N.D. and VITTOR, A.Y., 2018. Deforestation and vector-borne disease: forest conversion favors important mosquito vectors of human pathogens. *Basic and Applied Ecology*, vol. 26, pp. 101-110. http://dx.doi.org/10.1016/j.baae.2017.09.012. PMid:34290566.

CALDERÓN-ARGUEDAS, O., TROYO, A., SOLANO, M.E., AVENDAÑO, A. and BEIER, J.C., 2009. Urban mosquito species (Diptera: Culicidae) of dengue endemic communities in the Greater Puntarenas area, Costa Rica. *Revista de Biología Tropical*, vol. 57, no. 4, pp. 1223-1234. PMid:20073347.

CARVALHO, R.G., LOURENCO-DE-OLIVEIRA, R. and BRAGA, I.A., 2014. Updating the geographical distribution and frequency of *Aedes albopictus* in Brazil with remarks regarding its range in the Americas. *Memorias do Instituto Oswaldo Cruz*, vol. 109, no. 6, pp. 787-796. http://dx.doi.org/10.1590/0074-0276140304. PMid:25317707.

CECÍLIO, A.B., CAMPANELLI, E.S., SOUZA, K.P.R., FIGUEIREDO, L.B. and RESENDE, M., 2009. 2015. Natural vertical transmission by *Stegomyia albopicta* as dengue vector in Brazil. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 69, no. 1, pp. 123-127. http://dx.doi.org/10.1590/S1519-69842009000100015. PMid:19347154.

CHADEE, D.D., LAKHAN, A.N.S.O.N., RAMDATH, W.R. and PERSAD, R.C., 1993. Oviposition response of *Aedes aegypti* mosquitoes to different concentrations of hay infusion in Trinidad, West Indies. *Journal of the American Mosquito Control Association*, vol. 9, no. 3, pp. 346-348. PMid:8245947.

CODEÇO, C.T., LIMA, A.W., ARAÚJO, S.C., LIMA, J.B.P., MACIEL-DE-FREITAS, R., HONÓRIO, N.A., GALARDO, A.K.R., BRAGA, I.A., COELHO, G.E. and VALLE, D., 2015. Surveillance of *Aedes aegypti*: comparison of house index with four alternative traps. *PLoS Neglected Tropical Diseases*, vol. 9, no. 2, e0003475. http://dx.doi.org/10.1371/journal.pntd.0003475. PMid:25668559.

CONSOLI, R.A.G.B. and LOURENÇO DE OLIVEIRA, R., 1994. *Principais mosquitos de importância sanitária no Brasil.* Rio de Janeiro: Editora Fiocruz, 225 p. http://dx.doi.org/10.7476/9788575412909.

CRAWLEY, M.J., 2007. *The R book*. Chichester: John Wiley & Sons, 942 p.. http://dx.doi.org/10.1002/9780470515075.

CROMWELL, E.A., STODDARD, S.T., BARKER, C.M., VAN RIE, A., MESSER, W.B., MESHNICK, S.R., MORRISON, A.C. and SCOTT, T.W., 2017. The relationship between entomological indicators of *Aedes aegypti* abundance and dengue virus infection. *PLoS Neglected Tropical Diseases*, vol. 11, no. 3, e0005429. http://dx.doi.org/10.1371/journal.pntd.0005429. PMid:28333938.

DIBO, M.R., CHIARAVALLOTI-NETO, F., BATTIGAGLIA, M., MONDINI, A., FAVARO, E.A., BARBOSA, A.A. and GLASSER, C.M., 2005. Identification of the best ovitrap installation sites for gravid *Aedes* (Stegomyia) *aegypti* in residences in Mirassol, state of São Paulo, Brazil. *Memorias do Instituto Oswaldo Cruz*, vol. 100, no. 4, pp. 339-343. http://dx.doi.org/10.1590/S0074-02762005000400001. PMid:16113880.

FORATTINI, O.P. 2002. *Culicidologia médica: identificação, biologia e epidemiologia*. São Paulo: EDUSP, vol. 2, 864 p.

FORATTINI, O.P., 1986. *Aedes* (*Stegomyia*) *albopictus* (Skuse) identification in Brazil. *Revista de Saúde Pública*, vol. 20, no. 3, pp. 244-245. http://dx.doi.org/10.1590/S0034-89101986000300009. PMid:3809982.

FUNDAÇÃO OSWALDO CRUZ – FIOCRUZ, 2014 [viewed 20 May 2022]. *Nota técnica n.º 3/2014/IOC-FIOCRUZ/Diretoria. Avaliação de armadilhas para a vigilância entomológica de Aedes aegypti com vistas à elaboração de novos índices de infestação* [online]. Available from: http://www.fiocruz.br/ioc/media/nota_tecnica_ioc_3.pdf

GOMES, A.C., 1998. Medidas dos níveis de infestação urbana para *Aedes (stegomyia) aegypti e Aedes (stegomyia) albopictus* em Programa de Vigilância Entomológica. *Informe Epidemiológico do Sus*, vol. 7, no. 3, pp. 49-57. http://dx.doi.org/10.5123/S0104-16731998000300006.

GOMES, A.D.C., SOUZA, J.M., BERGAMASCHI, D.P., SANTOS, J.L., ANDRADE, V.R., LEITE, O.F. and LIMA, V.L., 2005. Anthropophilic activity of *Aedes aegypti* and of *Aedes albopictus* in area under control and surveillance. *Revista de Saude Publica*, vol. 39, no. 2, pp. 206-210. http://dx.doi.org/10.1590/S0034-89102005000200010. PMid:15895139.

GONÇALVES, K.D.S., SIQUEIRA, A.S.P., CASTRO, H.A.D. and HACON, S.D.S., 2014. Indicator of socio-environmental vulnerability in the Western Amazon. The case of the city of Porto Velho, State of Rondônia, Brazil. *Ciencia & Saude Coletiva*, vol. 19, no. 9, pp. 3809-3818. http://dx.doi.org/10.1590/1413-81232014199.14272013. PMid:25184586.

GUEDES, D.R., PAIVA, M.H., DONATO, M.M., BARBOSA, P.P., KROKOVSKY, L., ROCHA, S.W.D.S., SARAIVA, K., CRESPO, M.M., REZENDE, T.M., WALLAU, G.L., BARBOSA, R.M., OLIVEIRA, C.M., MELO-SANTOS, M.A., PENA, L., CORDEIRO, M.T., FRANCA, R.F.O., OLIVEIRA, A.L., PEIXOTO, C.A., LEAL, W.S. and AYRES, C.F., 2017. Zika vírus replication in the mosquito *Culex quinquefasciatus* in Brazil. *Emerging Microbes & Infections*, vol. 6, no. 8, e69. http://dx.doi.org/10.1038/emi.2017.59. PMid:28790458.

IMAM, H., ZARNIGAR., SOFI, G. and SEIKH, A., 2014. The basic rules and methods of mosquito rearing (*Aedes aegypti*). *Tropical Parasitology*, vol. 4, no. 1, pp. 53-55. http://dx.doi.org/10.4103/2229-5070.129167. PMid:24754030.

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA – IBGE [online], 2021 [viewed 18 February 2022]. Available from: https://cidades.ibge.gov.br/brasil/ro/porto-velho/panorama

JONES, R., KULKARNI, M.A., DAVIDSON, T.M. and TALBOT, B., 2020. Arbovirus vectors of epidemiological concern in the Americas: A scoping review of entomological studies on Zika, dengue and chikungunya virus vectors. *PLoS One*, vol. 15, no. 2, pp. 1-17. http://dx.doi.org/10.1371/journal.pone.0220753. PMid:32027652.

KAMAL, M., KENAWY, M.A., RADY, M.H., KHALED, A.S. and SAMY, A.M., 2018. Mapping the global potential distributions of two arboviral vectors *Aedes aegypti* and *Ae. albopictus* under changing climate. *PLoS One*, vol. 13, no. 12, pp. 1. http://dx.doi.org/10.1371/journal.pone.0210122. PMid:30596764.

KOTSAKIOZI, P., GLORIA-SORIA, A., CACCONE, A., EVANS, B., SCHAMA, R., MARTINS, A.J. and POWELL, J.R., 2017. Tracking the return of *Aedes aegypti* to Brazil, the major vector of the dengue, chikungunya and Zika viruses. *PLoS Neglected Tropical Diseases*, vol. 11, no. 7, e0005653. http://dx.doi.org/10.1371/journal.pntd.0005653. PMid:28742801.

KRYSTOSIK, A., NJOROGE, G., ODHIAMBO, L., FORSYTH, J.E., MUTUKU, F. and LABEAUD, A.D., 2020. Solid wastes provide breeding sites, burrows, and food for biological disease vectors, and urban zoonotic reservoirs: A call to action for solutions-based research. *Frontiers in Public Health*, vol. 7, pp. 405. http://dx.doi.org/10.3389/fpubh.2019.00405. PMid:32010659.

LIMA-CAMARA, T.N.D., HONÓRIO, N.A. and LOURENÇO-DE-OLIVEIRA, R., 2006. Frequency and spatial distribution of *Aedes aegypti* and *Aedes albopictus* (Diptera, Culicidae) in Rio de Janeiro, Brazil. *Cadernos de Saude Publica*, vol. 22, no. 10, pp. 2079-2084. http://dx.doi.org/10.1590/S0102-311X2006001000013. PMid:16951879.

LOPES, J., SILVA, M.A., BORSATO, A.M., OLIVEIRA, V.D. and OLIVEIRA, F.J.D.A., 1993. An ecological study of the mosquito *Aedes (Stegomyia) aegypti* L. and associated culicifauna in an urban area of southern Brazil. *Revista de Saude Publica*, vol. 27, no. 5, pp. 326-333. http://dx.doi.org/10.1590/S0034-89101993000500002. PMid:8209165.

MEDEIROS-SOUSA, A.R., FERNANDES, A., CERETTI-JUNIOR, W., WILKE, A.B.B. and MARRELLI, M.T., 2017. Mosquitoes in urban green spaces: using an island biogeographic approach identify to drivers of species richness and composition. *Scientific Reports*, vol. 7, no. 1, pp. 17826. http://dx.doi.org/10.1038/s41598-017-18208-x. PMid:29259304.

MONTAGNER, F.R.G., SILVA, O.S.D. and JAHNKE, S.M., 2018. Mosquito species occurrence in association with landscape composition in green urban areas. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 78, no. 2, pp. 233-239. http://dx.doi.org/10.1590/1519-6984.04416. PMid:28793030.

MORAL, R.A., HINDE, J. and DEMÉTRIO, C.G.B., 2017. Half-normal plots and over dispersed models in R: the hnp package. *Journal of Statistical Software*, vol. 81, no. 10, pp. 1-23. http://dx.doi.org/10.18637/jss.v081.i10.

MULTINI, L.C., WILKE, A.B.B. and MARRELLI, M.T., 2019. Urbanization as a driver for temporal wing-shape variation in *Anopheles cruzii* (Diptera: culicidae). *Acta Tropica*, vol. 190, pp. 30-36. http://dx.doi.org/10.1016/j.actatropica.2018.10.009. PMid:30359567.

NASCI, R.S.A., 1981. Lightweight battery-powered aspirator for collecting resting mosquitoes in the field. *Mosquito News*, vol. 41, no. 4, pp. 808-811.

R CORE TEAM, 2022. *R: a language and environment for statistical computing.* Vienna: R Foundation for Statistical Computing. Available from: https://www.R-project.org/

RAMOS, C.J.R., BELLATO, V., DE SOUZA, A.P., SARTOR, A.A., MOURA, A.B., CENTENARO, F. and MILETTI, L.C., 2019. Mosquitoes fauna (Diptera: Culicidae) in the domiciles and the peripheral environment in the city of Lages, SC. *Revista Brasileira de Higiene e Sanidade Animal*, vol. 13, no. 3, pp. 387-400. http://dx.doi.org/10.5935/1981-2965.20190029.

REGIS, L.N., ACIOLI, R.V., SILVEIRA JÚNIOR, J.C., MELO-SANTOS, M.A.V., SOUZA, W.V., RIBEIRO, C.M.N., DA SILVA, J.C.S., MONTEIRO, A.M.V., OLIVEIRA, C.M.F., BARBOSA, R.M.R., BRAGA, C., RODRIGUES, M.A.B., SILVA, M.G.N.M., RIBEIRO JÚNIOR, P.J., BONAT, W.H., MEDEIROS, L.C.C., CARVALHO, M.S. and FURTADO, A.F., 2013. Sustained reduction of the dengue vector population resulting from an integrated control strategy applied in two Brazilian cities. *PLoS One*, vol. 8, no. 7, e67682. http://dx.doi.org/10.1371/journal.pone.0067682. PMid:23844059.

REITER, P., AMADOR, M.A. and COLON, N., 1991. Enhancement of the CDC ovitrap with hay infusions for daily monitoring of *Aedes aegypti* populations. *Journal of the American Mosquito Control Association*, vol. 7, no. 1, pp. 52-55. PMid:2045808.

RÍOS-VELÁSQUEZ, C.M., CODEÇO, C.T., HONÓRIO, N.A., SABROZA, P.S., MORESCO, M., CUNHA, I.C., LEVINO, A., TOLEDO, L.M. and LUZ, S.L., 2007. Distribution of dengue vectors in neighborhoods with different urbanization types of Manaus, state of Amazonas, Brazil. *Memorias do Instituto Oswaldo Cruz*, vol. 102, no. 5, pp. 617-623. http://dx.doi.org/10.1590/S0074-02762007005000076. PMid:17710307.

RODRIGUES, M.M., MARQUES, G.R.A.M., SERPA, L.L.N., DE BRITO ARDUINO, M., VOLTOLINI, J.C., BARBOSA, G.L., ANDRADE, V.R. and DE LIMA, V.L.C., 2015. Density of *Aedes aegypti* and *Aedes albopictus* and its association with number of residents and meteorological variables in the home environment of dengue endemic area, São Paulo, Brazil. *Parasites & Vectors*, vol. 8, no. 1, pp. 115. http://dx.doi.org/10.1186/s13071-015-0703-y. PMid:25890384.

SARAIVA, J.F., MAITRA, A. and SOUTO, R.N.P., 2020. Diversity and abundance of mosquitoes (Diptera, Culicidae) in a fragment of Amazon Cerrado in Macapá, State of Amapá, Brazil. *EntomoBrasilis*, vol. 13, pp. 1-8. http://dx.doi.org/10.12741/ebrasilis.v13.e901.

SEGURA, M.D.N.D.O. and CASTRO, F.C., 2007. *Atlas de culicídeos na Amazônia brasileira: características especificas de insetos hematófagos da família Culicidae.* Belém: Instituto Evandro Chagas, 67 p.

SERRA, O.P., CARDOSO, B.F., RIBEIRO, A.L.M., SANTOS, F.A.L.D. and SLHESSARENKO, R.D., 2016. Mayaro virus and dengue virus 1 and 4 natural infection in culicids from Cuiabá, state of Mato Grosso, Brazil. *Memorias do Instituto Oswaldo Cruz*, vol. 111, no. 1, pp. 20-29. http://dx.doi.org/10.1590/0074-02760150270. PMid:26784852.

TAIPE-LAGOS, C.B. and NATAL, D., 2003. Culicidae mosquito abundance in a preserved metropolitan area and its epidemiological implications. *Revista de Saude Publica*, vol. 37, no. 3, pp. 275-279. http://dx.doi.org/10.1590/S0034-89102003000300002. PMid:12792675.

WILKE, A.B., CHASE, C., VASQUEZ, C., CARVAJAL, A., MEDINA, J., PETRIE, W.D. and BEIER, J.C., 2019. Urbanization creates diverse aquatic habitats for immature mosquitoes in urban areas. *Scientific Reports*, vol. 9, no. 1, pp. 15335. http://dx.doi.org/10.1038/s41598-019-51787-5. PMid:31653914.

XAVIER, A., OLIVEIRA, H., AGUIAR-SANTOS, A., BARBOSA JÚNIOR, W., SILVA, E., BRAGA, C., BONFIM, C. and MEDEIROS, Z., 2019. Assessment of transmission in areas of uncertain endemicity for lymphatic filariasis in Brazil. *PLoS Neglected Tropical Diseases*, vol. 13, no. 11, e0007836. http://dx.doi.org/10.1371/journal.pntd.0007836. PMid:31765388.

ZANOTTO, P.M.D.A. and LEITE, L.C.D.C., 2018. The challenges imposed by Dengue, Zika, and Chikungunya to Brazil. *Frontiers in Immunology*, vol. 9, pp. 1964. http://dx.doi.org/10.3389/fimmu.2018.01964. PMid:30210503.

ZARA, A.L.D.S.A., SANTOS, S.M.D., FERNANDES-OLIVEIRA, E.S., CARVALHO, R.G. and COELHO, G.E., 2016. *Aedes aegypti* control strategies: a review. *Epidemiologia e Serviços de Saúde: Revista do Sistema Único de Saúde do Brasil*, vol. 25, no. 2, pp. 391-404. http://dx.doi.org/10.5123/S1679-49742016000200017. PMid:27869956.