

Abiotic factors and population dynamic of *Aedes aegypti* and *Aedes albopictus* in an endemic area of dengue in Brazil

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ABSTRACT

Aedes aegypti and *Aedes albopictus* are exotic species in the Americas with high epidemiological relevance as they are vectors of many pathogens. This study aimed at understanding the population dynamics of *A. aegypti* and *A. albopictus* and the influence of abiotic factors in an endemic area of dengue. The study was conducted in the urban area of Campo Grande, Mato Grosso do Sul, Brazil, over one year. In seven regions of the city, 50 ovitraps were installed in each neighborhood. The development of the larvae was monitored under controlled laboratory conditions until they reached the adult phase. A total of 50,900 eggs of *Aedes* sp. were collected, 26,073 of which reached adulthood: 25,496 (97.8 %) *A. aegypti* and 540 (2.1%) *A. albopictus*. *A. aegypti* was observed in all months during the study. The highest number of *A. albopictus* eggs were collected in June, while in August and September, an absence of this species was noted. Abiotic factors such as temperature, humidity and rainfall were responsible for the observed fluctuations in the mosquito population. The presence of *A. albopictus* in the urban area of the city is concerning because it could become a potential vector for other arboviruses that afflict human populations. The occurrence of these species in Campo Grande reinforces the need for constant entomological and epidemiological surveillance so that informed actions could be taken to decrease potential breeding sites.

KEYWORDS: *Aedes* sp. Entomological study. Infestation. Urban areas.

INTRODUCTION

Aedes aegypti (L.) and *Aedes albopictus* (Skuse) are exotic species to the American continents. They are vectors of several diseases such as yellow fever, chikungunya, zika, and dengue infections¹.

These two species, *A. aegypti* and *A. albopictus* are mainly found in urban and peri-urban areas, respectively, and are closely related to the presence of blood sources and breeding sites.

According to Serpa *et al.*² temperature, humidity, wind and rainfall are determinant factors that control the maintenance of these species and diseases. These climate variables can affect oviposition, viability of eggs, larval development, longevity and adults dispersion³.

In addition to the abiotic factors, Brazil as well as in other American countries, has macrodeterminant factors that help the proliferation of vectors and the transmission of diseases, such as inadequate sanitation conditions, little treatment and selective destination of waste, poor income distribution and low schooling of most population⁴.

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In Campo Grande, the capital of Mato Grosso do Sul (MS) State, 46,632 dengue cases were recorded, with the highest number of deaths, between 2011 and 2013⁵. In 2017, the Brazilian Health Department began to disclose epidemiological data of zika and chikungunya, newly introduced in the country. From 2015 to 2017 45,428 dengue cases, 5,525 zika cases and 555 chikungunya cases⁶ were notified.

Although Campo Grande presents a high prevalence of dengue, there are no studies related to the behavior of these vectors in the urban area. Thus, we studied some aspects of these species population dynamics such as life cycle, female oviposition and density of eggs, as well as abiotic factors like pluviosity, rainfall and temperature that could influence these species in an endemic region of dengue.

MATERIALS AND METHODS

Study area

The municipality of Campo Grande covers 8,092.97 km² and is located approximately in the center of MS State. The city has 74 neighborhoods divided into seven regions: Segredo, Prosa, Bandeira, Anhanduizinho, Lagoa, Imbirussu and Centro. The vegetation of Campo Grande is cerrado (*Lato sensu*), with clean fields of vegetation, cerrado (*Stricto sensu*) and ciliary forests⁷. According to the Köppen Climate Classification System, the climate is tropical savanna, subtype Aw, with two seasons: the dry season between April and September and the rainy season between October and March⁸. The average temperatures during these seasons are 15 and 24 °C, respectively.

Trap locations

A. aegypti and *A. albopictus* eggs were collected monthly between May 2012 and April 2013. Ovitrap were randomly installed in 50 dwellings in each of the following neighborhoods (respective regions are in parentheses): Moreninha (Bandeira), Jardim Santa Emilia (Lagoa), Vila Carvalho (Centro), Jardim do Ze Pereira (Imbirussu), Pioneiros (Anhanduizinho), Vila Nasser (Segredo), and Novos Estados (Prosa). The traps were placed monthly and remained in the dwellings for a week.

The traps in the residences were installed according to standards established by the Technical Department of Entomology, Health Surveillance Secretariat, Ministry of Health. Traps were placed in calm locations, away from people and animals, near breeding sites and potential domestic shelters, such as water tanks, potted plants and tanks, preferably at heights between 0.8 and 1.2 meters⁹.

Monitoring the development of immature forms in the laboratory

The wooden pallets brought from the field were stored in polystyrene boxes until eggs counting. Eggs were placed in plastic boxes with natural water to monitor the development of immature stages, as proposed by Beserra *et al.*¹⁰ Daily larvae were counted and fed with fish food (Alcon Basic®). The pupae were transferred into plastic jars with a fabric across the surface in order to promote air circulation and avoid dispersion of winged animals. Emerged adults were identified based on the taxonomic key according to Consoli and Oliveira¹¹. Immature forms were developed in the laboratory at an average room temperature of 24.3 °C and relative humidity of 62%.

Analysis of abiotic factors

Temperature, humidity, rainfall and wind speed data were obtained from the Campo Grande A702 autonomous weather station maintained by the National Institute of Meteorology (INMET)¹².

Data analysis

The ovitrap positivity index (POI) and egg density index (EDI) were obtained following the criteria established by Gomes¹³.

The association between climate variables and the number of eggs and larvae collected were analyzed using Spearman's correlation. The number of positive ovitraps, mean eggs per trap (MET), male/female ratio and internal/external temperature were analyzed using the Student's *t*-test. The developmental time of immature forms was calculated using descriptive statistics (mean and standard deviation).

RESULTS

From a total of 4,200 placed traps, *Aedes* sp. was observed in 1,529 wooden pallets: 1,475 with *Ae. aegypti*, 9 with *Aedes albopictus* and 45 with both species. The POI and EDI values found were of 36.4 and 32.0, respectively. The remaining pallets were negative for egg counting. The eggs were present in all months of the year, with variations in quantity depending on the trap location and time of the year.

The numbers of positive ovitraps in the dry and rainy seasons were 621 and 908, with 20,298 and 30,602 eggs, respectively. There was no significant difference in positivity between periods ($p > 0,001$) (Table 1).

Table 1 - Average positive ovitrap index (POI), egg density index (EDI), pre-hatching and hatching larvae according to the season (dry or rainy), Campo Grande, MS, May, 2012 to April, 2013.

Season	POI X±SD	EDI X±SD	Period pre-hatching	Larvae hatched X ± SD(%)
Dry	29.6±7.3	28.8± 26.5	1 -6 days	2431.0 ± 29.4 (49.3%)
Rainy	43.2±12.8	34.8 ±15.5	1-5 days	2363.5 ± 21.5 (50.7%)
Total (year)	36.4±19.7	32.0±27.9	1-6 days	2397.2 ± 25.8 (56.5%)

X± SD= standard deviation

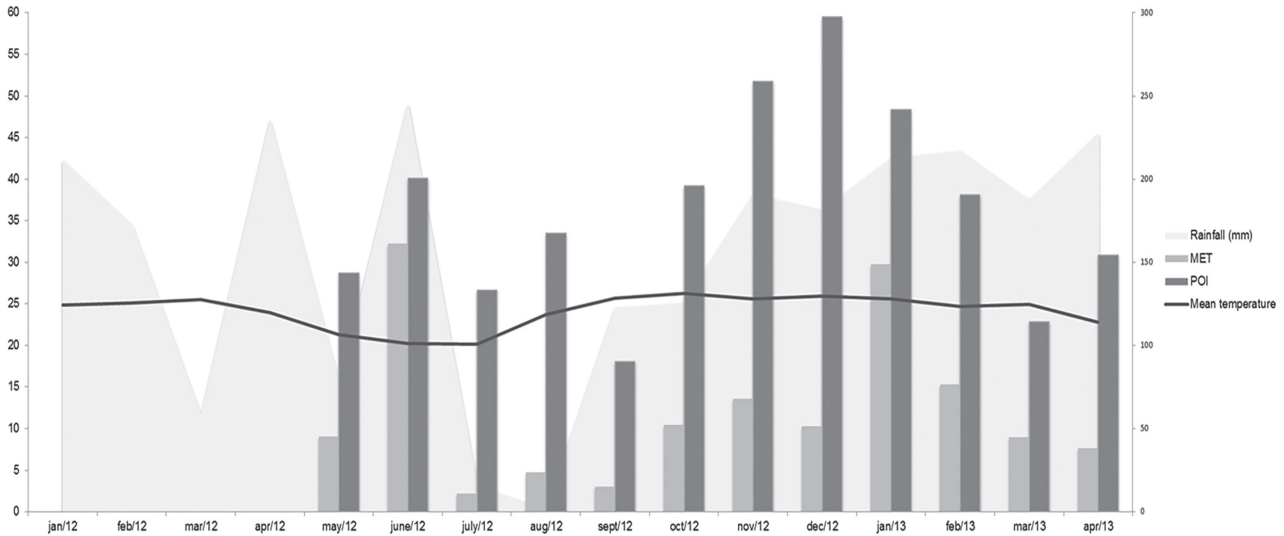


Figure 1 - Monthly distribution of positive ovitrap index (POI), mean egg counts per trap (MET), rainfall and mean temperature, Campo Grande, MS, May/2012 to April/2013.

The mean eggs per trap (MET) was 3,383 for the dry season and 5,100 for the rainy season ($p < 0.001$) (Figure 1).

Climate factors influencing oviposition and larvae hatching were temperature, humidity and rainfall ($p < 0.05$) (Table 2). There were no differences between internal and external laboratory temperature ($t = 0.5396$, $GL = 19.008$, $p = 0.6$) and humidity ($t = -0.7771$, $GL = 17.234$, $p = 0.4$).

Table 2 - Correlation coefficients between the total number of eggs and larvae, and climate variables, Campo Grande, MS, May, 2012 to April, 2013.

Phase	Average temperature (°C)	Relative humidity (%)	Precipitation rainfall (mm)
Eggs	0.17	0.57*	0.70*
Larvae	-0.26*	-	0.71*

* $p < 0.05$

From a total of 50,900 collected eggs, 9,322 larvae hatched during transportation from the field. Therefore, 41,578 eggs were viable for further analysis in the laboratory. The development of immature stages lasted on

average 9.4 days, varying between 5 and 42 days as shown in Table 3, the highest mortality numbers occurred in egg and L2 stages.

A total of 41,578 eggs belonging to both, *A. aegypti* and *A. albopictus* were collected; among them 28,767 completed the larval stage and 26,073 reached adulthood (Table 4). Among those who reached adulthood, 25,496 (97.8%) were identified as *A. aegypti*, 540 (2.0%) as *A. albopictus*, and 37 (0.2%) were not identified due to morphological issues. From the 26,073 specimens, 25,433 were not sexed: (1) 37 specimens could not be identified; (2) it was not possible to sex 603 specimens of *A. aegypti* due to loss of important sexing structures. The overall ratio of males to females was 1.01 for *A. aegypti* and 0.69 for *A. albopictus*. These results are presented in Table 5. There was no statistical difference between the number of males and females.

The presence of *A. aegypti* was observed in every month of the year, whilst that of *A. albopictus* was not observed in August, September and April (Figure 2). Regarding location, the highest numbers of *A. aegypti* and *A. albopictus* were recorded in Pioneiros and Novos Estados. *A. albopictus* was absent in Moreninha.

Table 3 - Duration in days, mean, median and mortality of eggs and *Aedes* sp. immature stages under laboratory conditions, Campo Grande, MS, May, 2012 to April, 2013.

Stages (N)	Amplitude	Mean	Median	Mortality (%)
Eggs (41,578)	-	-	-	30.81
L1 (28,767)	1-7	1.3	1	1.62
L2 (28,300)	1-13	1.6	1	4.34
L3 (27,071)	1-31	3.0	2	1.57
L4 (26,647)	1-39	6.3	4	2.11
Pupa (26,085)	1-38	5.5	4	0.05
Total	5	9.4	8	40.50

Table 4 - Absolute frequency, mean and standard deviation of eggs, larvae and adults according to the neighborhood, Campo Grande, MS, May, 2012 to April, 2013.

Neighborhood	Eggs			Larvae hatched			Adults		
	N	Mean	SD	N	Mean	SD	N	Mean	SD
Moreninha	3,913	6.5	16.8	2,669	4.5	12.9	2,426	4.0	11.9
Jardim Santa Emília	3,170	5.3	14.3	2,172	3.6	11.1	2,010	3.4	10.6
Vila Carvalho	5,598	9.3	24.4	4,287	7.2	20.7	4,047	6.8	20.2
Jardim do Zé Pereira	6,925	11.5	29.5	4,011	6.7	20.3	3,601	6.0	19.0
Pioneiros	12,083	20.1	50.8	9,201	15.3	43.6	8,372	14.0	39.2
Vila Nasser	4,781	8.0	23.4	2,751	4.6	15.2	2,523	4.2	13.9
Novos Estados	5,108	8.5	27.6	3,676	6.1	23.1	3,094	5.2	20.5
Total	41,578	9.8	29.2	28,767	6.9	23.6	26,073	6.2	21.56

N = absolute frequency; X ± SD = Standard deviation.

Table 5 - Number of male and female adults and male/female ratio for *Ae. aegypti* and *Ae. albopictus* in the neighborhood of Campo Grande, MS, May, 2012 to April, 2013 (n=25,433).

Neighborhood	<i>Aedes aegypti</i>	♂	♀	Ratio (male/female)	<i>Aedes albopictus</i>	♂	♀	Ratio (male/female)
Moreninha	2,348	1,166	1,182	0.98	0	0	0	0.00
Jardim Santa Emilia	1,850	995	855	1.16	40	16	24	0.66
Vila Carvalho	4,021	2,042	1,979	1.03	7	4	3	1.33
Jardim do Ze Pereira	3,239	1,560	1,679	0.92	62	36	26	1.38
Pioneiros	8,286	4,148	4,138	1.00	11	7	4	1.75
Vila Nasser	2,327	1,247	1,080	1.15	179	81	98	0.82
Novos Estados	2,822	1,394	1,428	0.97	241	78	163	0.47
Total	24,893	12,552	12,341	1.01	540	222	318	0.69

*p<0.05

DISCUSSION

The utilization of ovitraps allowed us to verify the infestation of *Aedes* sp. According to Vargas¹⁴, this method can identify culicids even at low densities, with the advantage of being inexpensive and effective for vector

surveillance. In this study, the technique was sensitive enough to detect a reduced amount of eggs during the cold and dry months. The ability to detect the presence of *Aedes* sp. in unfavorable climate conditions shows the necessity of continuous surveillance.

The infestation rate of 36.4% and the mean of 32 eggs

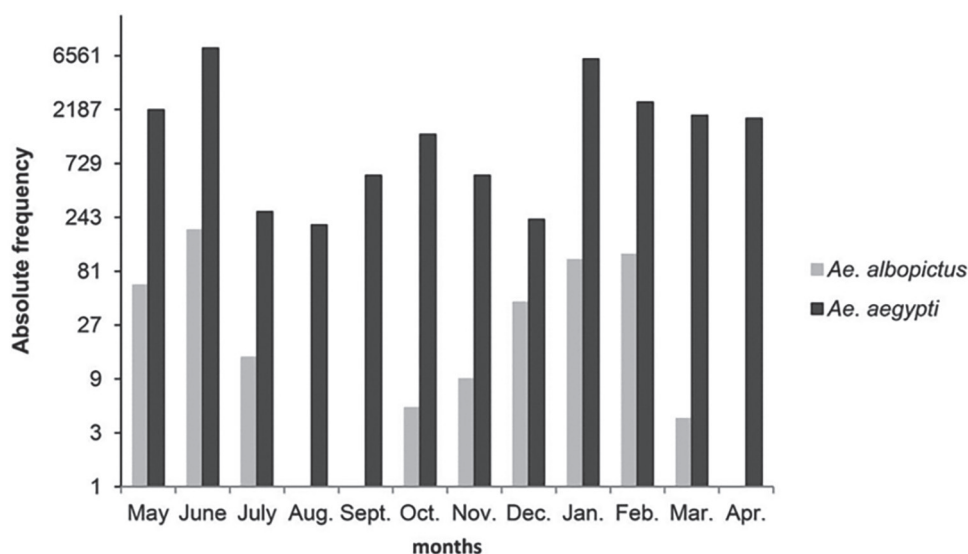


Figure 2 - Monthly distribution of *Aedes aegypti* and *Aedes albopictus* in Campo Grande, MS, May/2012 to April/2013.

per trap allowed an indirect measurement of the abundance of females in the environment¹⁵. Previous studies have reported similar results with respect to POI¹⁶. However, if the trap exposure time is longer, both, POI and EDI are higher, independent of the period of the year¹⁵.

Ribeiro *et al.*¹⁷ stated that abiotic factors, especially rainfall, affects survival, reproduction and causes changes in vectors distribution and density. In the present study, a higher number of positive ovitraps were detected in the rainy season when compared to the dry season, however, the difference was not statistically significant. Regis *et al.*¹⁵ also found a greater abundance of these dipterans during the rainy season. According to Carvalho *et al.*¹⁸ the increase in the density of mosquitoes during this period is associated with large amounts of water deposits and abundant natural breeding sites.

For all sampled neighborhoods, the presence of females in reproductive phase was observed, with variations among collection sites. The greatest positivity of ovitraps and egg density occurred in Pioneiros neighborhood, which is a region of the city with a high population density. Barata *et al.*¹⁹ reported a higher number of females in sites that shared similar characteristics, such as the diversity of artificial breeding sites, suggesting that these places could provide favorable conditions for *A. aegypti* proliferation. Conversely, in the two neighborhoods, Moreninha and Jardim Santa Emilia, which present a high population density, low values of ovitrap positivity were found. This could be explained by the low density of insects or the absence of viable breeding sites inside and around dwellings²⁰. Another relevant fact is that these neighborhoods are old and their residents have lived there for long periods of time so that a greater adherence to vector control measures might have occurred⁷.

In the present study, a greater egg loss and a lower larval yield were observed. During the activities performed in the field, an increase in eggs with hatched larvae was observed in rainy months. On the other hand, high mortality frequencies of dried eggs were detected in periods of low humidity. According to Farnesi *et al.*²¹ the composition of the eggshell and the presence of the serosa cuticle are likely to be responsible for eggs viability and resistance to desiccation. Non-embryonated eggs do not present rapid and uniform development when in contact with water mainly when dried within 24 h of laying and are therefore kept for a few days. This could be an explanation for the change in embryonic development observed in the month of August when humidity was low; this was studied by Shannon and Putnam²², who claimed that moisture is necessary for the development of the first larva inside the egg and once it reaches maturity, becomes sensitive to certain stimuli that trigger the hatching.

The daily monitoring of immature forms in the laboratory have also revealed a significant association with climate variables. The development cycle of larvae and pupae varied from 5 to 42 days, with an average of 9.4 days at 24.3 °C and 62% relative humidity. It was possible to observe fluctuations in the duration of the cycle, mainly in the rainy season. This period is characterized by high temperatures and relative humidity and the development cycles during this period were reduced in time. The period from larva hatching to pupa formation for *A. aegypti* was 5 to 14 days under ideal temperature and food availability²³. An interesting fact occurred in June, in which there was an unusually high amount of precipitation and relative humidity, but low temperatures. Over this period, the larval development period extended up to 42 days. A study

from Ferreira and Yang²⁴ have also demonstrated that the environment temperature and the relative humidity are determinant factors in the development of the mosquito.

The mean development time for stages L4 and pupa were 6.3 and 5.4 days, respectively. These results were higher than those presented by Silva *et al.*²⁵ who obtained averages of between 2.4 and 3.8 days for L4 and between 1.8 and 2.3 days for pupae. These differences in cycle length might be related to the mean laboratory temperature of 24.3 °C in the present study, which was up to four degrees lower than the literature findings.

The effect of temperature on larval development indicates that this variable is indirectly related to the mean duration of larval stage; that is, the increase in temperature reduces the duration of development of all immature stages, which in turn reduces its cycle²⁶. However, Consoli and Oliveira¹¹ claim that the ideal temperature for all mosquito life cycle stages is the one in which the development occurs with the lowest mortality rate.

In addition, several studies have shown that fluctuations in temperature might alter *Anopheles* sp. and *Aedes* sp.²⁷ vector competence. A reduced competence of *A. aegypti* for *Flavivirus* has already been reported in temperatures above 18 °C; however, dengue virus (DENV) transmission increased at lower temperatures²⁸.

An increase in the cycle duration of immatures was observed in ovitraps that were full of eggs. This subject has been addressed by Costa *et al.*²⁹ who hypothesized that stage lengths could be regulated by a high larvae population density. There was a large capture of eggs in June and the hatched larvae were kept in high density possibly influencing the cycle duration. Gama *et al.*³⁰ stated that when larvae are subjected to overpopulation and a shortage of food, their development is compromised and, as a result, mortality increases.

Cohabitation of species was observed in the city of Campo Grande. Forty-five wooden pallets were positive for both, *A. aegypti* and *A. albopictus*, however the first one was the most prevalent. This fact is associated with the capacity of these species to live in urban environments^{31,32}. *A. aegypti* was observed in all months of this study with a high frequency, supporting its predominance in relation to *A. albopictus*. Although *A. aegypti* is resistant to low humidity, a reduction in the frequency of specimens was observed during the dry season; this finding was previously reported by Serpa *et al.*³³ regarding the months of July, August and September. Forattini and Brito³⁴ consider domestic reservoirs as key factors in sustaining *A. aegypti* populations during periods of low rainfall.

The process of urbanization has substantially increased *Ae. albopictus* density, larval development rate and adult

survival time in the cities, and can be frequently found to share breeding sites with *A. aegypti*^{35,36}. Almeida *et al.*³⁷ observed a predominance of *A. albopictus* in relation to *A. aegypti* in the municipality of Dourados, MS. According to Passos *et al.*³⁸ the expansion of *A. albopictus* is causally related to the type of habitat; due to its zoophilic nature, this species develops preferentially in wild environments and places with low population densities. The adoption of new breeding sites could be a consequence of diminished resources and the more these resources are depleted, the higher the probability of intraspecific competition, or rather, population dislocation to seek out habitats that are more viable³⁵. It is believed that the introduction of *A. albopictus* in Campo Grande is recent, since this species has been found in fewer numbers. It is known that this species prefer wild habitats, but the rapid expansion of urban areas has changed natural ecotopes so that *A. albopictus* have moved to anthropophilic areas³⁵.

The greatest density of *A. albopictus* was observed during the months of June and January, while the species was absent during August, September, and April. The suppression of this species during August and September can be explained by intense drought, while the absence in April is most likely due to vector control actions that caused a higher mortality of *A. albopictus* than of *A. aegypti*. The fact that *A. albopictus* was not continuously present indicates that this species is sensitive to abiotic factors³⁹. Forattini *et al.*⁴⁰ found a higher infestation of this species in Ilha Comprida, Sao Paulo (SP), during months of greater rainfall, supporting the idea that *A. albopictus* is sensitive to abiotic factors.

A. albopictus was more prevalent in the Novos Estados neighborhood, which according to the urban planning department of Campo Grande presents a low population density. On the other hand, this species was not found in the Moreninha neighborhood, which is a highly populated region with consolidated urban development and absence of vegetation. Thus, even though *A. albopictus* prefers wild habitats, its recording in residential areas denotes an adaptation to urban environments.

In our study, the sexual ratio of *A. aegypti* was as expected, suggesting the population development stability. The balance between males and females has probably occurred due to the constant temperature (24.3 °C) in most months. However, other studies with *A. aegypti* have shown uneven proportions, with prevalence of males⁴¹. This prevalence was suggested by Craig *et al.*⁴² to be a hereditary trait in certain lineages and single-pair progenies of *A. aegypti*. Under laboratory conditions, Tun-lin *et al.*⁴³ studied the effect of temperature and larval diet on *A. aegypti* sexual ratio. They detected a predominance

of females at a temperature of 30 °C, and homogeneity at temperatures of 15, 20, 25 and 35 °C, suggesting an influence of temperature on the species sexual ratio. Silva and Silva⁴⁴ have also reported a balanced sexual proportion in this species at 28 °C.

In the present study, *A. albopictus* male/female ratio was not significantly different. In a study conducted in Sao Paulo State, Forattini *et al.*⁴⁰ found a greater abundance of females of this species. Yet, Briegel and Timmermann⁴⁵ studying larvae of *A. albopictus* under laboratorial conditions of temperatures between 12 and 32 °C, observed a male/female ratio of 2:1. In Parana State Fantinatti *et al.*⁴⁶ have also described a 1:1 ratio for *A. aegypti* and a higher number of *A. albopictus* males.

A higher ratio of females may increase the number of oviposition and/or affect competition among males. Either way, there might be a significant effect on the composition of the following generations due to a reduced genetic variability. Nevertheless, the similar proportion between males and females observed for Campo Grande may indicate a balance and intraspecific adaptation.

The results found in the present study suggest that an entomologic surveillance program must be conducted continuously, not only during the rainy season⁴⁷. There was a prevalence of *A. aegypti*, which demonstrated a greater ability of this species to colonize residences. The presence of *A. albopictus* in the urban area of the city is concerning because it could become a potential vector of other arboviruses to human populations. The recording of these species in Campo Grande, MS, reinforces the need of constant entomological and epidemiological surveillance to decrease potential breeding sites.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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