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Density and diversity of Culicidae (Diptera), with analysis of viral circulation, in urban public establishments in Northern Paraná, Brazil

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ABSTRACT

Brazil has been suffering a dengue (DENV) epidemic in 2019 and 2022 and records cases of Zika (ZIKV) and chikungunya (CHIKV). Londrina city, Paraná, stands out for the number of cases and deaths from dengue. Therefore, this study aimed to verify the density and diversity of mosquitoes present in urban public establishments of Londrina, to analyze their ecological relationships, and to investigate the presence of the DENV, ZIKV, and CHIKV. Ovitraps were installed in outdoor sites in two public establishments in each region of Londrina, while adult culicids were caught indoors with a Nasci aspirator. A total of 5077 eggs were obtained in 77 ovitraps. The South region had the highest infestation while the East had the lowest (Ovitrap Positivity Index: 38% and 25%; Egg Density Index: 67.83 and 19.03, respectively). From eggs, 704 *Aedes aegypti* females and 71 *Aedes albopictus* females were obtained, while in the aspiration of adults, 99 *A. aegypti* and 11 *Culex quinquefasciatus* were captured, the highest number in the West and the lowest in the North. The DENV-II virus was detected in a pool of 17 *A. aegypti* females in the West region. The results demonstrate high density of pathogen vector species, mainly *A. aegypti*, in and around establishments, alerting to the need for constant local entomological surveillance to prevent viral circulation, mainly in crowded places. The presence of *A. albopictus* around the establishments also alerts to the urbanization of the species and potential participation in the viral circulation, demonstrating the need for constant monitoring.

Introduction

The family Culicidae Meigen, 1818, suborder Nematocera, order Diptera, includes more than 3500 species of insects, popularly known as mosquitoes and designated as culicids. They are distributed in all temperate and tropical regions of the planet, and beyond the Arctic Circle (Forattini, 2002; Harbach, 2022; WRBU, 2022). This family is divided into two subfamilies: Anophelinae and Culicinae, including hematophagous species, which are relevant to public health due to their vectorial capability for several pathogens to humans and domestic and wild animals (Forattini, 2002; Harbach, 2022; WRBU, 2022).

Culicidae have holometabolous development and can have wild, rural, and/or urban habits. Urbanized species have a human-dependent life cycle, benefiting from human actions for reproduction, development, and survival (Consoli and Oliveira, 1994; Forattini, 2002; Rueda, 2008; Dibo et al., 2011; Eiras, 2016).

Culex quinquefasciatus Say, 1823 and *Aedes* (*Stegomyia*) *aegypti* (Linnaeus, 1762) are extremely urban and synanthropic Culicidae (Consoli and Oliveira, 1994; Forattini, 2002; Taipe-Lagos and Natal, 2003; IOC, 2009; Piovezan, 2009; Ministério da Saúde, 2011). Both species are cosmopolitan, but they are distributed mainly in tropical and subtropical regions of the planet, being commonly found throughout the Brazilian territory (Consoli and Oliveira, 1994; Forattini, 2002; Taipe-Lagos and Natal, 2003; IOC, 2009; Ministério da Saúde, 2011; Kraemer et al., 2015).

Culex quinquefasciatus is considered one of the possible vectors participating in the urban cycle of the Oropouche virus in some locations in Brazil, such as the state of Pará (Vasconcelos et al., 1989; Consoli and Oliveira, 1994; Cardoso et al., 2015). In the Northeast region of Brazil, such as in the cities of Recife and Maceió, it is the main vector of bancroftian filariasis, caused by a nematode worm (*Wuchereria bancrofti*) (Consoli and Oliveira, 1994; Forattini, 2002; Brandão et al., 2011; Ministério da Saúde, 2011; Fontes et al., 2012). This species was also found vectoring

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the ZIKV virus in urban areas of Recife, Brazil, in 2016 (Guedes et al., 2017), in addition to having a possible vectorial capacity for this virus, evidenced by studies that detected the presence of Asian and American strains of the ZIKV virus in the salivary glands of mosquitoes of this species, after laboratory infection (Viveiros-Rosa et al., 2020).

However, the most important virus vector in Brazil is *Aedes aegypti*, which has African origin, but has been causing problems for the Brazilian population since the late nineteenth century, when it arrived in Brazil through slave trade ships, in the colonial period (Forattini, 2002; Powell and Tabachnick, 2013). Despite having been eradicated in 1958, *A. aegypti* was reintroduced to Brazil in the 1970s from different geographical locations (Monteiro et al., 2014). Until 1942, urban yellow fever was widely transmitted by *A. aegypti* (Franco, 1969), but currently, only cases of wild yellow fever are registered in Brazil, with no proven relationship with this species (Ministério da Saúde, 2020a).

A. aegypti is the main vector of the four dengue viral serotypes (DENV-1, DENV-2, DENV-3, and DENV-4). These arboviruses are of concern because of their epidemic background in Brazil (Ministério da Saúde, 2020b, 2021; WHO, 2022). *A. aegypti* also transmits the viruses that cause chikungunya (CHIKV) and Zika (ZIKV), which have caused public health concern in the country since 2014 (Nunes et al., 2015; Zanluca et al., 2015; Ministério da Saúde, 2019; WHO, 2022).

Along with *A. aegypti*, *Aedes* (*Stegomyia*) *albopictus* (Skuse, 1894) stands out. Its origin is Asian, where it is described as a DENV vector, and its first record in Brazil is from 1986, in the state of Rio de Janeiro (Forattini, 1986; WHO, 2022). Since then, it has spread rapidly to almost all the Brazilian territory, becoming common in Brazil and increasingly frequent in the urban environment (Silva et al., 2006; Alencar, 2008; Martins et al., 2010; Carvalho et al., 2014; Oliveira and Maleck, 2014; Pancetti et al., 2015; Ayllón et al., 2018; Silva et al., 2018a; Custódio et al., 2019; Rossi da Silva et al., 2021). However, *A. albopictus* prefers places with greater vegetation cover that are close to the population, such as woods and parks (Gomes et al., 2005; Oliveira and Maleck, 2014; Montagner et al., 2018; Silva et al., 2018c; Heinisch et al., 2019; Lutinski et al., 2020).

In the American continent, *A. albopictus* is a potential vector of the same arboviruses carried by *A. aegypti*, having the ability to become infected with more than 20 arboviruses in the laboratory (Moore and Mitchell, 1997; Vega-Rua et al., 2014; Azevedo et al., 2015; Chouin-Carneiro et al., 2016). However, *A. albopictus* has already been found in Brazil carrying DENV (Martins et al., 2012; Medeiros, 2018; Rezende et al., 2020), Zikv (Rezende et al., 2020). These findings demonstrate that this species also has epidemiological importance, therefore requiring more attention from public health agencies.

The detection of the DENV virus and its serotypes in mosquitoes can be used as a virological surveillance strategy, serving as a warning tool for dengue outbreaks, and contributing to the prevention of epidemics, as it makes it possible to verify the circulating serotypes in certain locations, before the disease starts to be transmitted at higher levels, (Chow et al., 1998; Eisen et al., 2009; Bona et al., 2011; Barbosa et al., 2016). Later, given the increased circulation of Zika and chikungunya worldwide, multiplex tests were developed to detect ZIKV and CHIKV viruses, in addition to DENV, simultaneously, following the example of Pabbaraju et al. (2016), complementing the epidemiological studies that aim to analyze viral distribution through vectors.

Brazil has been suffering from a dengue epidemic in 2019 and 2022 and records cases of Zika (ZIKV) and chikungunya (CHIKV) in 2016 (Ministério da Saúde, 2023), with Paraná standing out among the states with the highest number of dengue cases, in addition to Zika and chikungunya (Ministério da Saúde, 2020b). Londrina is one of the municipalities in Paraná with the highest infestation rate, where 3600 cases of dengue were confirmed, with seven deaths in 2019, and two serotypes identified in circulation during this period (DENV-1 and DENV-2), according to the Secretariat of Paraná Health (Paraná, 2020). In 2020, dengue cases increased exorbitantly in the city, characterizing an epidemic outbreak, with more than 20000 cases of the disease being confirmed and 29 deaths, classifying Londrina as the city with the most deaths from dengue in Brazil in 2020 (Paraná, 2020).

Due to the high number of cases and deaths from dengue, Londrina represents a model city for evaluating measures that may be adopted in different regions of the country, in order to help guide vector control actions, contributing to the prevention of arbovirus circulation on a national scale. In association, it is important to know the diversity and distribution of mosquitoes in strategic places in the city. Thus, the current study aimed to verify the density and diversity of Culicidae present in public community facilities – PCF in Londrina, Paraná, Brazil, as well as to analyze their ecological relationships, and to investigate the presence of DENV (and serotypes), ZIKV, and CHIKV in adults of the Culicidae family, to verify vertical and horizontal viral transmission.

Material and methods

Study area

The city of Londrina is located in the northern region of the Paraná state, in southern Brazil (23°08’47” and 23°55’46” S; 50°52’23” and 51°19’11” W). It has 1,652,569 km^2 of land area, and an estimated population of 575,377 inhabitants (IBGE, 2020). According to the Köppen climate classification, Londrina has a humid subtropical climate (Cfa), with hot summers and rainfall in all seasons of the year, with greater abundance in summer (Nitsche et al., 2019; INMET, 2022). Historically the average annual temperature is approximately 21.6 °C, with an annual minimum of around 16.6 °C and annual maximum of around 27.6 °C, while the relative humidity (RH) is in the range from 69 to 76% and the average annual rainfall varies between 1400 and 1800 mm (Nitsche et al., 2019; INMET, 2022).

The samplings were carried out in PCFs located in the urban perimeter, in the five regions of Londrina (North, South, East, West, and Center), as well as in a state school in one of the regions. Two locations were chosen per region, with some exchanges of locations during the collection period, due to temporary changes in the address of the establishments.

The sample collection sites in each region were: South region – PCF 1 (23°22’12.79”S, 51°7’42.48”W), PCF 2 (23°22’26.72”S, 51°8’6.67”W), and PCF 3 (23°20’12.06”S, 51°8’37.30”W); Eastern region – PCF 4 (23°19’22.92”S, 51°6’47.78”W), PCF 5 (23°17’45.60”S, 51°8’15.01”W), and PCF 6 (23°18’40.24”S, 51° 7’54.09”W); North region – PCF 7 (in temporary address) (23°17’27.18”S, 51° 9’30.82”W), PCF 8 (23°15’53.86”S, 51°11’15.37”W), and PCF 9 (23°15’28.56”S, 51°10’7.05”W); Central region – PCF 10 (23°19’40.62”S, 51°9’0.71”W), and PCF 11 (23°18’1.24”S, 51°10’8.91”W); West region – PCF 12 (23°17’39.31”S, 51°10’48.54”W), and PCF 13 (23°18’35.66”S, 51°11’31.72”W) (Fig. 1).

Geographical and meteorological data

The geographic coordinates of each collection site were obtained using a Garmin eTrex® 30 GPS device. Subsequently, they were submitted to the QGIS 3.10.11 ‘A Coruña’ program, for the elaboration of geographic location maps of the study area, with the points from each collection location. Shapefiles provided by the Brazilian Institute of Geography and Statistics - IBGE (2021) and by the City Hall of Londrina (2021) were used.

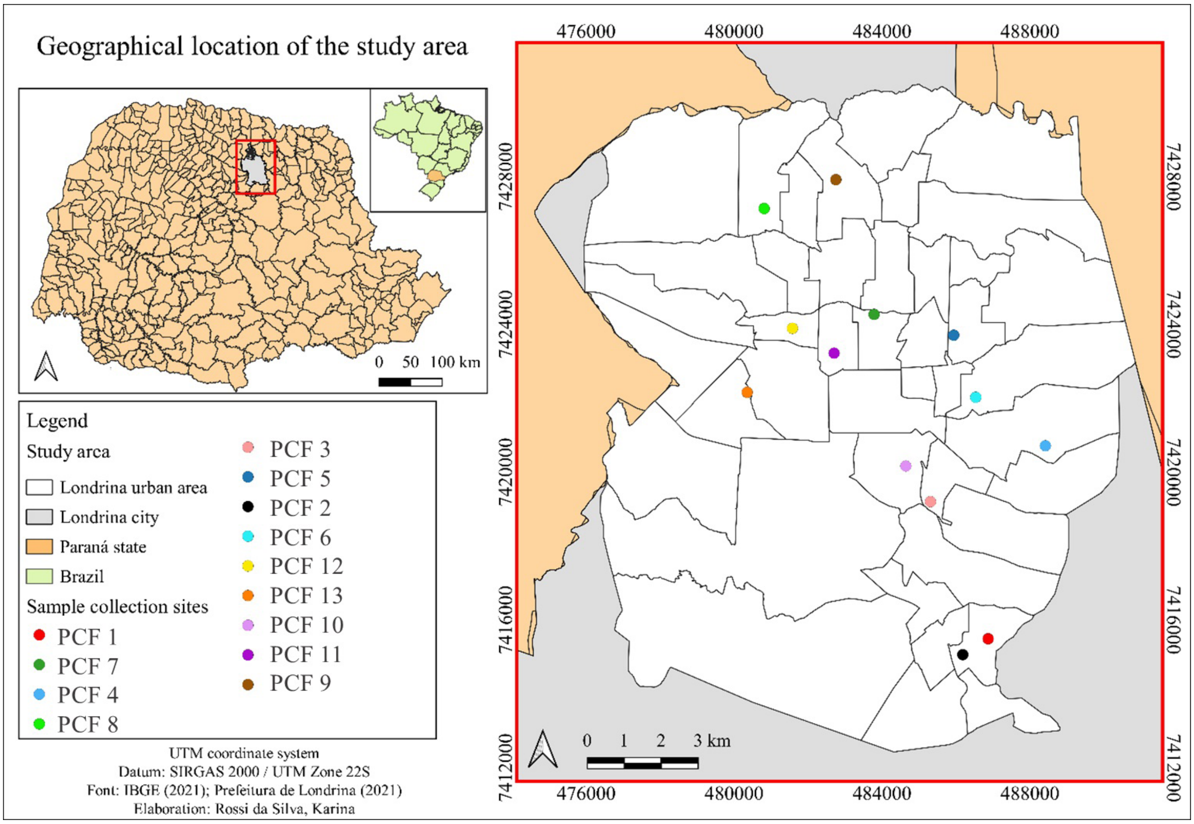

Figure 1 Geographical location of the study area, demonstrating the sample collection sites, carried out between January and September 2019, in the urban area of the municipality of Londrina, Paraná, Brazil.

Temperature and relative humidity values recorded in Londrina were also collected for each collection period, as well as rainfall values recorded in the 14 days prior to each collection period, considering that the average length of the Culicidae mosquito's life cycle is14 days (seven to 20 days) (Forattini, 2002; Eiras, 2016). These data were provided by the Paraná Rural Development Institute – IAPAR-EMATER meteorological station in Londrina (code 23365116), located in the southern region (-23.35454 S and -51.16420 W).

Collection of eggs and adults

Culicidae eggs and adults were collected between January and September 2019. We used a permanent license from SISBIO/IBAMA (23093) and authorization from the Municipal Health Secretary (SMS) of Londrina for collection at the public community facilities. The project was registered and approved by the Dean of Research and Graduate Studies at the State University of Londrina under number 11163, in the category (External development agencies - Resolution 70/2012).

Five samplings were performed in two locations in each region of Londrina, alternately between regions, with an interval of approximately one month between each sampling.

The egg collection was carried out using ovitraps (Fay and Perry, 1965; Fay and Eliason, 1966), which consist of black plastic vessels with a wide mouth (12 cm long × 11 cm in diameter), with a capacity of 750 mL. A total of 200 mL of a solution of deionized water and barm at a concentration of 0.04% (0.4 g L^{-1}) were added to each trap, serving as an attractant for Culicidae females, to increase the chance of the traps being chosen by the females, considering the competition with other breeding sites already existing in the environment. Duratree pallets (14 cm × 3 cm × 3 mm) were also introduced, containing a rough side to facilitate the adherence of the eggs, which remained facing the upper side of the trap.

Five traps were installed in the external areas of each location, with a minimum distance of 10 m each, sheltered from rain and sun and at ground level. Thus, each region received 10 traps in each of the five replicates, totaling 50 traps in each region throughout the sampling period, making a total of 250 traps. The traps were collected after four days in the field, to avoid the possibility of eggs hatching, and sent to the Medical Entomology Laboratory (LEM), State University of Londrina, Paraná.

The collections of adults were performed using a Nasci aspirator (Nasci, 1981) inside each location. The aspirator used in this study consists of a cylindrical aluminum instrument with a propeller, connected to a 12 Volt battery, containing a micro tulle fabric bag tied to the open lower end, where the captured mosquitoes are trapped. The collections lasted two minutes for every 10 m^2 and were carried out only in the morning, between approximately 9:00 and 11:00 h.

Quantification, identification, and storage of collected eggs and mosquitoes

Collected eggs were quantified in the laboratory with the aid of a stereoscopic microscope and, after seven to ten days (period for embryo maturation), they were subjected to hatching in plastic basins (8 cm high × 25 cm in diameter), with the internal edges sanded to facilitate the adherence of emerged mosquitoes, containing water (dechlorinated) up to halfway of the basins, and covered with micro tulle fabric. These basins were kept in a B.O.D. (Biochemical Oxygen Demand) at 25±2 °C.

After hatching, larvae were fed with an autoclaved mixture of wheat germ and barm (1:1) aiming the development of the larvae to adult form.

Emerged females were collected with a Castro catcher and stored in acrylic bottles (7 cm high × 4 cm in diameter) with a malleable opening in the lid and a micro tulle in the base. The bottles remained in B.O.D. at a temperature of 25±2 °C, with cotton soaked with a 12% solution of water and sugar to feed the females, to keep them alive for up to seven days, until accumulation of a sufficient number of females to freeze for the viral search, as explained below.

These bottles were left in a freezer at -20 °C, for 10 min, to reduce the activity of the mosquitoes, to perform their identification and counting, using a stereoscopic microscope and identification keys by Forattini (2002) and WRBU (2022). The mosquitoes were separated by species, location, and collection data, in properly identified 1.5 mL microtubes, containing from one to 50 mosquitoes. These tubes were stored in a freezer at an average temperature of -80 °C, to preserve the nucleic acids in the samples, for viral identification.

The mosquitoes captured with a Nasci aspirator were also stored for 10 minutes in a freezer at -20 °C for further counting, identification, separation, and storage in microtubes in the freezer at -80 °C, in the same way as the females obtained from the eggs, with the only difference being that the collected males were also stored, but separately from the females, for viral research.

Viral analysis

Both the mosquitoes collected with a Nasci aspirator, and the mosquitoes obtained from the eggs collected in the ovitraps were used to carried out viral research at the Laboratory of Morphology and Physiology of Culicidae and Chironomidae (LAMFIC^2) of the Federal University of Paraná (UFPR) – *Campus* Curitiba. The RNA from the mosquito pools containing up to 50 adults was obtained using the QIAamp Viral RNA kit (Qiagen®), according to the manufacturer's instructions. RNA was eluted from the membrane in a final volume of 40 µL, and then its quality and quantity were verified using a NanoDrop® 2000 spectrophotometer.

ZIKV, DENV, and CHIKV arboviruses were identified simultaneously using the multiplex kit XGEN MULTI ZDC (Mobius® - ANVISA register: Nº 80502070063), according to the manufacturer's guidelines; 15 µL of extracted viral RNA was used to analyze the presence of each arbovirus. DENV virus was verified at 520 nm using the FAM fluorophore, while ZIKV and CHIKV viruses were visualized at 550 nm and the fluorophores used were JOE and VIC, respectively. An internal control was used to validate the RNA extraction process, in addition to positive controls for the three arboviruses and a negative control to ensure the success of the analyses, all provided by the kit itself.

The identification of DENV-1, DENV-2, DENV-3, and DENV-4 serotype pools that presented amplification for DENV was performed using the Kit BIOMOL ZDC (Institute of Molecular Biology of Parana – IBMP, ANVISA register: Nº 80780040001) following the producer's instructions. Serotype amplification was confirmed using FAM and VIC fluorophores. Multiplex quantitative polymerase chain reactions (qPCR) were performed on a Rotor-Gene Q (Qiagen®) thermocycler.

Statistical analysis

From the number of eggs collected in the ovitraps, for each sampling region, the Ovitrap Positivity Index - OPI (Nº of positive traps/Nº of examined traps) × 100 was calculated, corresponding to the percentage of positive traps, that is, traps that captured eggs (Gomes, 1998), and the Egg Density Index - EDI (Nº of eggs/Nº of positive traps), corresponding to the average number of eggs per positive trap (Gomes, 1998), in order to verify the infestation rate of *A. aegypti* and *A. albopictus* in each collection site. EDI values from 0 to 20 indicate a satisfactory level of infestation; 21 to 35 indicate an alert situation, and above 35 represents a risk of infestation. These values are based on data obtained by Nascimento et al. (2020), where the LIR*Aa* (Rapid Index Survey for *Aedes aegypti*) technique was compared in the field with ovitraps in the same study area.

Frequency distribution was also calculated for eggs collected in each region of Londrina, considering the positive ovitraps, to analyze the distribution of eggs by region, making it possible to verify the intervals of the most frequent numbers of eggs. Both the mean of eggs and the number of mosquitoes collected with a Nasci aspirator were related to temperature, rainfall, and RH values, recorded during the sampling period, using Spearman's correlation coefficient, since the relationship between the data obtained is not linear. To carry out these analyses, the BioEstat program, version 5.3 (Ayres et al., 2007) was used.

The number of eggs collected and the OPI and EDI, as well as the number of adults of *A. aegypti* captured with a Nasci aspirator in each region, were submitted to the Shapiro-Wilk normality test and subsequently to Analysis of Variance, with ANOVA - 1 factor for normal data or Kruskal-Wallis for non-normal data, in order to verify the statistical difference between samples ($p < 0.05$), and then be submitted to the Tukey test ($p < 0.05$) or Dunn ($p < 0.05$) respectively, to verify which samples differed statistically from each other. The PAST (Paleontological Statistics) version 4.03 program was used to perform these analyses.

Results

Number of eggs collected and infestation level by Aedes aegypti *and* Aedes albopictus

During the entire sampling period, in the five regions of Londrina, a total of 5077 eggs of *Aedes* spp. were collected, in 77 positive ovitraps (30.92%), out of 249 ovitraps analyzed. Of this total, 1768 eggs were collected in the South region (34.82%), 1386 in the North region (27.30%), 844 in the central region (16.62%), 560 in the West region (11.03%), and 519 in the East region (10.22%). However, when comparing the number of eggs collected in each of the five regions, using the Kruskal-Wallis test, no statistically significant differences were found at the 5% significance level ($p = 0.2959$; H = 3.296).

From the distribution of frequencies for eggs obtained in each region, it was found that in the South region there was a higher frequency of traps (53%) containing from one to 50 eggs, in the North region there was a higher frequency of traps (54%) containing from one to 63 eggs, in the central region 26% of the traps had from 18 to 34 eggs, in the West region 57% of the traps had from one to 19 eggs, and in the East region 27% of the traps had from one to 19 and 20 to 38 eggs.

Analyzing the number of eggs collected in each replica of each region, it is observed that in the South region the largest number of eggs was obtained in January, while the smallest number was obtained in July, coinciding with the highest and lowest average temperatures recorded in these periods. In the North region, a greater number of eggs was obtained in March, while in September there was no capture of eggs. In the central region, the month of May had the highest number of eggs, while in the month of August no eggs were collected. In the West region, the largest number of eggs was also obtained in May, while no eggs were collected in September. In the East region, the largest number of eggs was obtained in March, while in July there was no capture of eggs. Therefore, in general, the greatest number of eggs was obtained in summer and autumn, while the least eggs were collected in winter. It was also observed that eggs were obtained from the ovitraps every month.

When analyzing the average values of OPI and EDI obtained in each sampling region, a pattern similar to the number of eggs was observed, where the highest average value of OPI was obtained in the South and Center regions, while the lowest value was observed in the East region. Likewise, the highest average EDI value was also obtained in the South region, while the lowest values were obtained in the West and East regions, respectively (Table 1). However, comparing the OPI and EDI values obtained in each of the five regions, it was found that, statistically, there was no significance in the difference between the OPI values of each region and between the EDI values of each region, at the level of 5% of significance ($p = 0.9332$; $F = 0.2045$ and $p = 0.3876$; $F = 1.084$, respectively) from the ANOVA.

The EDI averages obtained in each region show that the South, North, and Center regions were at risk of infestation by *A. aegypti* and *A. albopictus*, while the West region was in a satisfactory situation, and the East region was in an alert situation (Table 1).

Adult females obtained from eggs collected in ovitraps

A total of 775 females were obtained: 704 of the species *A. aegypti* (90.84%) and 71 of the species *A. albopictus* (9.16%). This total was distributed among the collection regions as follows: 382 in the North region (49.29%), 157 in the central region (20.25%), 96 in the South region (12.39%), 74 in the East region (9.55%), and 66 in the West region (8.52%).

The total number of females can be seen in Table 2, divided between *A. aegypti* and *A. albopictus*, with the percentage of each species obtained in each sampling region. It can be observed that *A. aegypti* was predominant in the regions, except for the East region, where there was a difference of two individuals from *A. albopictus* (Table 2).

Table 1
Average values of Ovitrap Positivity Index (OPI) and Egg Density Index (EDI), obtained in each of the five regions of Londrina, Paraná, from January to September 2019, and estimation of the infestation level of *Aedes aegypti* and *Aedes albopictus* through EDI values.

Region	OPI (%)	EDI	Situation*
South	38	67.83	Risk
North	30	57.31	Risk
Center	38	39.86	Risk
West	28	19.03	Satisfactory
East	25	28.54	Alert

*Nascimento et al. (2020).

Table 2
Total adult females and proportion of *Aedes aegypti* and *Aedes albopictus* obtained from eggs collected in ovitraps in the five regions of Londrina, Paraná, from January to September 2019.

Region	*Aedes aegypti*		*Aedes albopictus*	
	Total of adults	%	Total of adults	%
South	75	78.13	21	21.87
North	379	99.21	3	0.79
Center	154	98.09	3	1.91
West	60	90.91	6	9.09
East	36	48.65	38	51.35
Total	704	90.84	71	9.16

Adult Culicidae captured using a Nasci aspirator

In total, 110 adults Culicidae were captured, 73 males (66.36%) and 37 females (33.64%). Of this total, 99 were *A. aegypti* (90%), being 63 males (63.64%) and 36 females (36.36%), and 11 were *C. quinquefasciatus* (10%), being 10 males (90.90%) and one female (9.10%) (Table 3). The total number of mosquitoes collected with the Nasci aspirator, as well as the percentage in each region, are shown in Table 3.

Note that only *A. aegypti* was collected in all five regions of Londrina. Analyzing the number of *A. aegypti* adults aspirated in each of the five regions of Londrina, it is possible to see that the largest number of mosquitoes was obtained in the West region, while the smallest number was obtained in the North region. The average values of *A. aegypti* obtained in each of the five regions, together with the respective standard deviation values are also shown in Table 3.

Considering the Kruskal-Walli's test, there was a significant difference between the averages of aspirated *A. aegypti* adults, at a 5% significance level ($p = 0.0221$; $H = 8.658$). The Dunn test showed that the Central region differs from the South, North, and East regions, regarding the aspirated mosquito averages. Equally, a difference was observed between the average values obtained in the West and North regions (Table 3).

In relation to the number of *A. aegypti* captured with a Nasci aspirator in each month of the sampling period, April was the only month where *A. aegypti* was not captured. On the other hand, the largest number of adults of this species (both males and females) was captured in May.

Recorded meteorological data and correlation with egg and mosquito data

The average temperature recorded in the collection periods was 21.3 °C, while the minimum temperature was 1.6 °C (July) and the maximum was 36.7 °C (September). The average RH was 73.2% (minimum of 26.2 in September and maximum of 100% in February). Considering the rainfall that occurred in the 14 days before the first collection period, up to 14 days prior to the last collection period, a total of 778.1 mm was recorded, with the lowest rainfall rate recorded in August (0.2 mm), and the highest in February (219.3 mm).

When compared the average of eggs collected in the five regions of Londrina with values of temperature, rainfall, and RH, it was observed a significant correlation with the RH ($p = 0.0048$) in the

Table 3
Total and proportion of adults of *Aedes aegypti* and *Culex quinquefasciatus* captured with a Nasci aspirator in the five regions of Londrina, Paraná, from January to September 2019, average and Standard Deviation from Average for *A. aegypti*, with due differences for the Dunn's test.

Region	*Aedes aegypti* (n)	%	Average ± SDA	*Culex quinquefasciatus* (n)	%
South	5	5.05	0.5±0.40 $^{b, c*}$	1	9.09
North	2	2.02	0.2±0.13 ^{b}	0	0
Center	33	33.33	3.3±1.41 ^{a}	3	27.27
West	55	55.56	5.5±3.61 $^{a, c, d}$	7	63.64
East	4	4.04	0.4±0.27 $^{b, d}$	0	0

Legend: SDA = Standard Deviation from Average. *Different letters in the same column indicate a statistically significant difference ($p < 0.05$) by Dunn's Test.

Table 4
Spearman's correlation between the average of eggs collected in the five regions of Londrina and values of temperature, precipitation, and relative humidity, referring to the period from January to September 2019.

Region	T average		T minimum		T maximum		Precipitation		RH	
	rs	*p-value*	rs	*p-value*	Rs	*p-value*	rs	*p-value*	rs	*p-value*
South	0.7000	0.1881	0.7000	0.1881	0.7000	0.1881	0.7000	0.1881	0.0000	n.s.
North	0.3189	0.5379	0.0580	0.9131	0.7537	0.0835	-0.0580	0.9131	-0.4928	0.3206
Center	0.4000	0.5046	0.8000	0.1040	0.4000	0.5046	0.5000	0.3910	0.8000	0.1040
West	-0.2000	0.7471	-0.2000	0.7471	-0.2000	0.7471	0.2000	0.7471	0.9747	0.0048
East	0.6377	0.1730	0.1479	0.7841	0.4058	0.4246	0.0580	0.9131	0.0290	0.9565

Legend: T = temperature; RH = relative humidity; rs = Spearman's correlation; n.s. = not significant.

Table 5
Spearman's correlation between the number of adults collected in the five regions of Londrina and values of temperature, precipitation, and relative humidity, referring to the period from January to September 2019.

Region	T average		T minimum		T maximum		Precipitation		RH	
	rs	*p-value*	rs	*p-value*	rs	*p-value*	rs	*p-value*	rs	*p-value*
South	0.4472	0.4502	0.4472	0.4502	0.4472	0.4502	0.4472	0.4502	-0.1118	0.8579
North	-0.2070	0.6939	0.0000	n.s.	-0.2070	0.6939	0.8281	0.0418	0.4140	0.4144
Center	-0.5000	0.3910	-0.7000	0.1881	-0.5000	0.3919	0.9000	0.0374	-0.3000	0.6238
West	0.6000	0.2847	0.6000	0.2847	0.6000	0.2847	0.1000	0.8729	0.3591	0.5528
East	0.0000	n.s.	0.4140	0.4144	-0.2070	0.6939	0.6211	0.1881	0.8281	0.0418

Legend: T = temperature; RH = relative humidity; rs = Spearman's correlation; n.s. = not significant.

period of collections carried out in the West region, being positive, with a Spearman's correlation coefficient (rs = 97.47%). As for the other regions, the correlations vary from very weak to strong, for the three meteorological variables, with a negative correlation also with temperature in the West region, as well as with precipitation and with RH in the North region, but without statistical significance ($p > 0.05$) (Table 4).

On the other hand, in relation to the number of mosquitoes aspirated in the five regions of Londrina, there is only a significant correlation with the precipitation recorded in the collection periods in the North ($p = 0.0418$ and rs = 82.81%) and Center ($p = 0.0374$ and rs = 90%), and with the RH recorded in the collection periods in the East region ($p = 0.0418$ and rs = 82.81%), being positive. The other correlations range from very weak to strong, with negative correlations with temperature in the North and Center regions, in addition to a negative correlation with RH in the South and Center regions, but without statistical significance ($p > 0.05$) (Table 5).

Viral analysis

By analyzing the samples of adults captured with a Nasci aspirator, the presence of the DENV-2 virus was detected in one pool of 17 females of *A. aegypti*, among the 24 pools of males and females of the species (36 females and 63 males) and two pools of *C. quinquefasciatus* (1 female and 10 males) analyzed. The sample of females containing virus was collected on May 10, 2019, inside the public community facility located in the West region of Londrina.

As for the samples of females obtained from eggs, none of the three arboviruses analyzed was detected in the 30 pools of *A. aegypti* or seven pools of *A. albopictus* obtained, which totaled 704 and 71 females, respectively.

Discussion

The largest number of eggs of *A. aegypti* and *A. albopictus*, as well as the highest values of OPI and EDI recorded in the South region in this study, are in accordance with the 2019 data reported by the SMS of Londrina, which pointed to the southern region as the most infested in the first, second, and third LIR*Aa* carried out, corresponding to the period from January to August, thus covering the period of collections. Also in agreement, the majority of dengue cases reported in Londrina city in 2019 were concentrated in the South region (Paraná, 2024).

On the other hand, the region indicated by the SMS with the lowest level of infestation in the first LIR*Aa* was the East region, corroborating the present study, which also registered the lowest number of eggs in this region. However, in the second, third, and fourth LIR*Aa*, the place with the least infestation was the West region, matching the lowest mean EDI value obtained in this region in the present study. Although the West region was the second place with the smallest number of captured eggs, there was little difference in relation to the East region.

In relation to the density and diversity of Culicidae obtained from the two collection methods, the greater number of *A. aegypti* in the regions demonstrates that this species predominates in and around public community facilities of Londrina. This result corroborates other studies that observed a high frequency of *A. aegypti* in the urban environment in several locations in Brazil, prevailing in relation to other species (Lima-Camara et al., 2006; Fantinatti et al., 2007; Silva, 2009; Serpa, 2014; Silva, 2016; Medeiros, 2018; Barbosa et al., 2019; Fonseca Júnior et al., 2019; Silva, 2019).

These observations are consistent with the characteristics already attributed to *A. aegypti*, which has a daytime habit and is considered strictly synanthropic and anthropophilic, that is, it is extremely dependent on humans, benefiting from their activities, which provide favorable conditions for reproduction and species survival (Forattini, 2002; Natal, 2002; Lima-Camara et al., 2006; Zara et al., 2016; Wilke et al., 2019).

Although no significant correlation was observed between the number of eggs and adults of *A. aegypti* and temperature, it is valid to consider that the climate of Londrina is favorable to this species, since, according to the literature, the average temperature range, as well as the high rainfall in the municipality and consequent high relative humidity, as evidenced by the meteorological data obtained in the study, are ideal for the proliferation of *A. aegypti*.

According to a recent laboratory study, the optimal temperature range for *A. aegypti* survival is between 15 and 30 °C (Galavíz-Parada et al., 2019). Previous studies by Beserra et al. (2006, 2009), also in the laboratory, concluded that the optimal temperature range for the development of *A. aegypti* is between 21 and 29 °C and between 22 and 32 °C, respectively, while for Marinho et al. (2016) the ideal temperature for the development of this species, verified in the laboratory, is within a range of 22 to 36 °C, being in agreement with the average values registered in the sampling period.

Rainfall also contributes to the proliferation of *A. aegypti*, as it influences the increase in the number of breeding sites available for the development of immature forms of the mosquito (Barrera et al., 2011; Silva et al., 2018b; Custódio et al., 2019). The consequent increase in the relative air humidity has also shown considerable influence on the abundance and survival of *A. aegypti*, with air humidity above 70% required (Canyon et al., 1999; Fouque et al., 2006; Lega et al., 2017). These observations corroborate the statistically significant positive correlations obtained in the current study, with the considerable volume of rainfall recorded and with an average RH above 70%.

Regarding *A. albopictus*, the eggs of this species were obtained in ovitraps installed in the external areas of public community facilities, while no adults were found in the interior, suggesting that this species is more frequent in peridomiciliary areas, using bushes as shelter, in parks, woods, or forest fragments in urban areas, or more distant places, such as plantations adjacent to homes and forests close to urban areas, as verified in several studies (Gomes et al., 2005; Lima-Camara et al., 2006; Silva et al., 2006; Martins et al., 2010; Pedrosa, 2013; Medeiros-Sousa et al., 2015; Heinisch et al., 2019; Silva, 2019; Rossi da Silva et al., 2021). Gomes et al. (2005) also observed a higher frequency of hematophagy of *A. albopictus* in peridomicile compared to intradomicile environments.

However, the capture of *A. albopictus* eggs in ovitraps shows that populations of this species in Londrina have an urbanized habit and usually use the same artificial breeding sites as *A. aegypti* (Lima-Camara et al., 2006; Alencar, 2008; Rossi and Silva, 2009; Rodríguez et al., 2013). In several studies, the presence of *A. aegypti* and *A. albopictus* eggs in ovitraps was observed (Silva, 2009, 2019; Pedrosa, 2013; Oliveira and Maleck, 2014; Serpa, 2014; Silva, 2016; Silva et al., 2018c; Custódio et al., 2019; Heinisch et al., 2019; Dixson et al., 2020; Rossi da Silva et al., 2021), while Honório and Lourenço-de-Oliveira (2001), Silva et al. (2006), Maciel-de-Freitas et al. (2007), Rossi and Silva (2009), Martins et al. (2010), and Ayllón et al., (2018) observed both species sharing several artificial breeding sites.

Regarding *C. quinquefasciatus*, the capture of this species inside public community facilities, even if in small quantities, indicates that, although it has a nocturnal habit to perform hematophagy, it can also be found among the human population during the day, together with *A. aegypti* and *A. albopictus*, possibly using the interior of urban establishments as shelter (Consoli and Oliveira, 1994; Forattini, 2002; Ramos et al., 2019).

However, the absence of *C. quinquefasciatus* eggs in the ovitraps is due to this type of breeding, which is not ideal for this species, as it does not always use the same breeding sites as *A. aegypti* and *A. albopictus*, in addition to demonstrating the specificity of ovitraps and the attractant used to capture *Aedes* spp. This result corroborates studies where only *A. aegypti* and *A. albopictus* eggs were found in ovitraps installed in urban environments (Silva, 2009; Oliveira and Maleck, 2014; Silva, 2016, 2019; Nascimento, 2017; Rossi da Silva et al., 2021). According to Codeço et al. (2015), Silva et al. (2018c), Nascimento et al. (2020), and Rossi da Silva et al. (2021) ovitraps have high specificity and sensitivity for capturing *A. aegypti* eggs and generating infestation indices, showing better performance compared to larval research.

Culex quinquefasciatus prefers to lay eggs in artificial containers containing water rich in organic matter, or in polluted water collections in the ground, in addition to often laying eggs in large places with low-movement or no-movement water, which contains abundant decomposing organic matter and detritus, such as effluent treatment ponds, open sewers, ditches, streams, banks of polluted rivers and lakes, and similar (Consoli and Oliveira, 1994; Forattini, 2002; Morais et al., 2007; Chaves et al., 2009; Borges, 2014). These locations provide ideal conditions for the development of larvae, as they are characterized as excellent food sources, ensuring the reproductive success of the species (Consoli and Oliveira, 1994; Forattini, 2002; Ministério da Saúde, 2011; Wilke et al., 2017).

Considering viral investigation, although there was a high number of infections in Londrina during the entire collection period (more than three thousand registered cases and 7 deaths in 2019), mainly autochthonous cases, as reported by the SMS of Londrina and by Paraná (2019, 2020), the low virus detection in aspirated mosquitoes could be justified by the small number of samples obtained during the sampling period. The low frequency of viral detection in mosquitoes, even in places with vector infestation and the high number of cases of arboviruses in humans, has already been reported in other studies in Brazil (Lourenço-de-Oliveira et al., 2002; Degallier et al., 2003). On the other hand, despite the lower number of confirmed dengue cases in the West region of Londrina, according to the SMS, it is assumed that the possibility of finding viruses in this region was higher due to the large number of mosquitoes captured in a single collection, where the presence of the DENV-2 virus was detected.

The absence of virus in aspirated *C. quinquefasciatus* females can also be explained by the small sample size, but mainly by the low competence and vectorial capacity of this species for the investigated viruses (Viveiros-Rosa et al., 2020). To date, only one study has identified the natural presence of the ZIKV virus in *C. quinquefasciatus* adults collected in abundance in Recife city, where there was a high rate of infestation of the species, due to environmental conditions in the region (Guedes et al., 2017).

The absence of viruses in mosquitoes obtained from the eggs collected in the present study suggests that vertical transmission does not contribute to the high incidence of dengue in Londrina. Grunnill and Boots (2016) demonstrate, based on the review of several studies, that vertical transmission is a rare event, with little relevance to the persistence of the DENV virus in the environment. It is also worth noting that a small sample size was analyzed, obtained from a low number of replicas and in few points in the city. Zeidler et al. (2008) also did not obtain any DENV-positive *A. aegypti* in a larvae pool from samples collected in a neighborhood with a high incidence of dengue, in the city of Boa Vista, Roraima, while Serufo et al. (1993) isolated only two lots of DENV-1 positive larvae among 743 *A. albopictus* larvae collected in the state of Minas Gerais.

According to Paraná (2020), the serotypes circulating in the city, in 2019, were DENV-1 and DENV-2. The simultaneous circulation of these two serotypes, combined with the increase in the population of vectors, is a great risk to the population, as there is a possibility that people who have already been infected by one serotype may catch a secondary infection with another serotype, in a short period of time, or even simultaneously (Martins et al., 2014). This increases the probability of occurrence of new dengue outbreaks and aggravation of the disease, which could lead to Dengue with warning signs and severe dengue, causing organ dysfunction and possible death (Halstead, 2003; Barbosa et al., 2016).

Studies demonstrate singularities for each dengue serotype, regarding the symptoms caused. More severe symptoms were predominant in infections caused by DENV-2 and DENV-3 serotypes, thus indicating that these are the most virulent (Vaughn et al., 2000; Nisalak et al., 2003; Balmaseda et al., 2006; BVS, 2015; Soo et al., 2016; Lovera et al., 2019). Thus, it is evident that one of the most virulent serotypes circulates in Londrina.

Cases of Zika and chikungunya fever were not detected in Londrina in 2019, according to Paraná (2020), which justifies the lack of detection of these viruses in the collected samples. However, the detection of the DENV-2 virus in *A. aegypti* females present in one of the public community facilities, indicates the need to intensify the monitoring and control of vectors in these locations as well as in their surroundings, since they are representative areas of convergence of people. Nonetheless, since the viral research of the present study was carried out on whole mosquitoes, it was not possible to conclude whether the virus was present only in the intestine (recent hematophagy), in the hemolymph (blood in the process of digestion), or in the salivary gland, which would characterize infected females as effective vectors that could transmit the virus.

The results obtained alert to the urgent need to intensify preventive measures and vector control in and around these establishments, to avoid the existence of *Aedes* spp. breeding sites, thus reducing the local infestation of vector mosquitoes and, consequently, the viral circulation. This study also emphasizes the need for constant surveillance of mosquitoes and viruses in nature, not only in *Aedes* spp., but also in other species with vectorial capacity, such as *C. quinquefasciatus.* A more specific viral analysis is also important in these mosquitoes, to identify the presence of viruses in the salivary glands, in order to verify if the mosquito can transmit the virus. These measures make it possible to direct actions to prevent and reduce vectors, thus preventing the occurrence of new epidemics and overloading of the health system and public community facilities.

This work presents a low-cost methodology model for obtaining vector mosquitoes in different locations, demonstrating its effectiveness in vector and viral monitoring. It is important that constant monitoring is performed, allied to the intensification of viral investigation in vectors, to make viral detection more efficient, as this allows for quick actions to be taken if viral circulation is detected at strategic points, such as public community facilities. This methodology can be adopted by all municipalities in Brazil as a form of prevention and primary health care, and it can also be carried out in other places in the urban area that also need surveillance, such as forest fragments.

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Conflicts of interest

The authors declare that there is no competing interest that could inappropriately influence this paper.

Author contribution statement

KRS carried out field collections, conducted the growth of mosquitoes from the collected eggs and identified the species obtained. AMPC and KRS conducted the viral analyzes. KRS and JACZ performed the statistical analyzes. JACZ, MANS, AMPC, LAVB and GFLTVB helped KRS write the manuscript and contributed to its review. All authors read and approved this manuscript.

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