Tire-breeding mosquitoes of public health importance along an urbanisation gradient in Buenos Aires, Argentina

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Used vehicle tires are a source of mosquito vectors and a means of their introduction and expansion. With the aim of assessing the effects of urbanisation on the main mosquito vectors in temperate Argentina, the infestation levels of Aedes aegypti *(L.) and* Culex pipiens *L. were studied in used tires from highly urbanised cities to low-urbanised small towns in Buenos Aires. Immatures of both species accounted for 96% of the 9,722 individuals collected; the total individuals collected represented seven species. The percentage of water-filled tires containing mosquitoes [container index (CI)] was 33% and the percentage of infested sites [site index (SI)] was 65.2%. These indexes decreased significantly from low to high urbanisation levels for both mosquito species. The relative abundance (RA) of* Ae. aegypti *immatures was slightly higher toward large cities, but showed no difference for* Cx. pipiens*. The CI of shaded tires was significantly higher than the CI of exposed tires for both mosquito species. There was no difference in RA values between shaded and sunlit tires. The CI and the SI were highest during the summer across the urbanisation levels, except for* Cx. pipiens*, which continued to increase during the autumn in small towns. Results related to urbanisation gradient, sunlit exposure and seasonality are discussed.*

Key words: *Aedes aegypti* - *Culex pipiens* - used tires - urbanisation level - artificial containers - mosquito ecology

Mosquitoes use a wide variety of water collections as larval habitats, from ground water bodies of different hydroperiods to natural and man-made containers [see Service (1995) for detailed categories]. Among the latter category, used vehicle tires have some particularities, as reviewed by Yee (2008) that highlight the importance of studying these artificial containers as mosquito habitats. First, many species of medical importance have been found to use discarded tires as breeding habitats. Second, these containers are particularly abundant near human populations. Third, tires are a means of introduction and range expansion for invasive species at country and continental levels. The potential importance of tires as a source of mosquito vectors was noted during the mid-1940s, when war material was returned to the United States (US) containing tires infested with mosquitoes (Reiter & Sprenger 1987). Since then, tires have been targeted for investigations of mosquitoes worldwide, including North America (Yee et al. 2010), Europe (Scholte et al. 2010) and Asia (Higa et al. 2010). Despite the voluminous literature on these man-made containers and their demonstrated relevance to public health, there are no ecological studies focused on water-filled tires as habitats for mosquitoes in Argentina.

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Two tire-breeding mosquito species of cosmopolitan distribution, *Aedes aegypti* (L.) and the complex *Culex pipiens* L., are recognised worldwide as main vectors of pathogens to humans and domestic animals. In Argentina, *Ae. aegypti* was responsible for at least 4,700 cases of dengue fever from 1998-2007 (Vezzani & Carbajo 2008) and approximately 27,000 cases in the 2009 epidemic (Estallo et al. 2011). *Culex pipiens quinquefasciatus* Say was implicated as the vector of St. Louis encephalitis virus (Diaz et al. 2006) and it is a potential vector of West Nile virus, having been detected in horses in Argentina (Morales et al. 2006). Among the mosquito-borne diseases of veterinary concern, the dog heartworm *Dirofilaria immitis* (Leidy) was detected repeatedly in both mosquito species (Vezzani et al. 2011).

Several papers dealing with *Ae. aegypti* and/or *Cx. pipiens* in urban temperate Argentina were published during the last decade. Both species are considered the most abundant species breeding in small artificial containers (García et al. 2002, Vezzani & Albicócco 2009) and in adult collections on private premises (Vezzani et al. 2011). Within the Federal District, the spatial pattern of *Ae. aegypti* oviposition was associated with an urbanisation gradient (Carbajo et al. 2006) and the presence of immatures at the microhabitat scale was related to shaded and vegetated surroundings (Vezzani et al. 2005). However, these studies were restricted to highly urbanised areas and the knowledge of both mosquito vectors in small towns, as well as in used tires, is truly limited in the region. The objective of this paper was to assess the importance of tires as larval habitats and the effects of urbanisation on the main mosquito vectors in temperate Argentina. With these aims, the infestation levels of *Ae. aegypti* and *Cx. pipiens* breeding in used tires were studied in residential areas along an urbanisation gradient in Buenos Aires.

MATERIALS AND METHODS

Study area - Buenos Aires Province is located within the Pampean region; the climate is temperate humid-sub-humid, with annual precipitation ranging from 1,200 mm in the east to 600 mm in the west and an annual mean temperature of 17ºC in the north and 14ºC in the south (Magrin et al. 1997). The original vegetation, mainly consisting of grasses, has been modified by agriculture, farming and human settlements. The study area extends between latitudes 35º26'17.5"S and 34º6'46.82"S and longitudes 59º41'36.66"W and 58º7'32.45"W. This area includes the Aglomerado Gran Buenos Aires (AGBA) (INDEC 2003) and several small towns located up to 100 km from the Federal District, i.e., Buenos Aires. The AGBA covers approximately 3,827 km^2 and has the greatest population density of Argentina (2,995 inhabitants/km^2) (INDEC 2001).

Using a Landsat 5 TM satellite image captured on 22 January 2010, the municipalities of the study area were classified into three urbanisation levels (high, middle and low) by observing the spatial pattern of buildings and green spaces (Fig. 1). A continuous matrix of buildings was assigned to the highest urbanisation level, a mix of buildings and green areas represented the middle values and small well-defined spots of urbanization surrounded by green areas were classified as municipalities of low urbanisation (Table I).

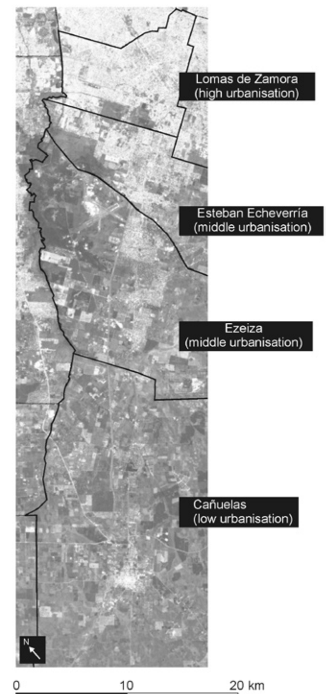

Fig. 1: portion of the Landsat 5 TM satellite image exemplifying the three urbanisation levels defined in the study area.

Data collection - Mosquito surveys were conducted from November 2009 through May 2010. This period includes the maximum abundances of *Ae. aegypti* and *Cx. pipiens* immatures in small artificial containers in the Federal District (Vezzani & Albicócco 2009) and the highest adult collections in neighbouring municipalities (Vezzani et al. 2011). Maintaining a similar sampling effort for each month and urbanisation level (approximately 3 days of 6 working hours each), a total of 279 sites in residential zones within urban areas were visited. Each site consisted of a pile of used tires, with at least

TABLE I

Municipalities of the study area classified according to the urbanisation level[a]

High	Middle	Low
Federal District (14,030)	José C. Paz (5,262)	Campana (250)
Lanús (10,078)	Malvinas Argentinas (5,108)	Luján (134)
La Matanza-East (9,964)	Almirante Brown (4,555)	Zárate (93)
San Fernando (9,811)	Ituzaingó (4,432)	San Vicente (91)
Gral San Martín (7,551)	San Miguel (3,387)	Gral Rodríguez-West (64)
Tres de Febrero (7,473)	Merlo (3,084)	Mercedes (60)
Vicente López (6,947)	La Matanza-West (2,585)	Exaltación de la Cruz (45)
Lomas de Zamora (6,890)	Tigre (2,572)	Cañuelas (44)
Avellaneda (6,200)	Moreno (2,568)	San Antonio de Areco (27)
San Isidro (6,075)	Esteban Echeverría (2,490)	Brandsen (23)
Morón (5,713)	Florencio Varela (2,232)	Lobos (21)
Hurlingham (5,043)	Berazategui (1,703)	San Andrés de Giles (20)
Quilmes (4,647)	Pilar (779)	Marcos Paz-West (20)
	Escobar (758)	Gral. Las Heras (20)
	Ezeiza (676)	San Miguel del Monte (11)
	Pte. Perón (671)	Suipacha (11)
	Gral. Rodríguez-East (517)	Navarro (10)
	Marcos Paz-East (359)	

a: number of inhabitants per km^2 according to Instituto Nacional de Estadísticas y Censos (2010) within brackets.

one tire filled with water. At each site, up to 10 water-filled tires were inspected for mosquito immatures by sweeping the water three times with a fine mesh strainer. Collected larvae were fixed in 70% ethanol and pupae were reared until adult emergence. For each water-filled tire inspected, the sunlit condition (exposed to the sun or under shade) was recorded during the inspection. In addition, we estimated the percentage of water-filled tires per urbanisation level by inspecting 2,813 used tires (with or without water) in 165 sites.

In the laboratory, third and fourth instar larvae and adults were identified using appropriate keys (Darsie 1985, Rossi et al. 2002). Two members of the *Cx. pipiens* complex, *Cx. pipiens* s.s. and *Cx. quinquefasciatus* are sympatric in Buenos Aires (Forattini 2002, Rossi et al. 2002); therefore, these species were not distinguished from one another and are referred to here as *Cx. pipiens*.

Data analysis - To assess mosquito infestation levels, we defined three variables as follows: (i) site index (SI), the percentage of sites with mosquito immatures, (ii) container index (CI), the percentage of water-filled tires with mosquito immatures, and (iii) relative abundance (RA), the median number of immatures collected per infested tire. The SI, CI and RA of *Ae. aegypti* and *Cx. pipiens* were compared among municipalities of high, middle and low urbanisation. Sunlit exposure condition was analysed considering overall data and for each urbanisation level separately.

The SI and CI were compared with the chi-squared test for two or multiple proportions depending on the number of categories involved (e.g., sun-shade, high-middle-low urbanisation) (Fleiss et al. 2003). For the latter, new tests for multiple pairwise proportions were performed using Tukey's procedure to identify groups contributing to significant differences (Zar 1999, Abramson & Gahlinger 2001). The Mann-Whitney *U*-test and the Kruskal-Wallis test were used to compare the RA between two or more categories, respectively (Daniel 1990).

Monthly values of the SI, CI and RA were presented and associations between these variables for each mosquito species were assessed with Spearman's nonparametric correlation coefficient (Daniel 1990). Finally, the SI and CI were compared among urbanisation levels within each season, defined as spring (November and December), summer (January-March) and autumn (April and May).

RESULTS

Considering the entire study period and area, 50.3% of the tires contained water. Out of 2,038 water-filled tires, 673 harboured mosquito immatures (CI = 33%). Among 279 inspected sites with water-filled tires, 182 were positive for mosquito immatures (SI = 65.2%). A total of 9,722 immatures of seven mosquito species were collected and identified (Table II). *Ae. aegypti* and *Cx. pipiens* immatures accounted for 96% of the total and had the highest CIs and SIs. Both species along with *Culex eduardoi* Casal & Garcia were collected from large cities to small localities, whereas the others were found only in middle and/or low urbanisation levels.

The percentage of water-filled tires differed significantly ($X^2_{(2)} = 28.98$, $p < 0.001$) among urbanisation levels, with higher values in small localities (59%) compared to other localities (47%). A well-defined pattern was observed regarding the SI and CI among urbanisation levels for both *Ae. aegypti* and *Cx. pipiens* (Fig. 2). These indexes decreased with increasing urbanisation and in all comparisons, the difference between the extremes of the urbanisation level (i.e., low vs. high) was statistically significant.

TABLE II

Container index (CI), site index (SI) and total number of immatures collected (3rd and 4th instar larvae and pupae) of the mosquito species found in used tires in Buenos Aires Province (Argentina), November 2009-May 2010

Species	CI (%)	SI (%)	Immatures collected (n)	Urbanisation level with record
Aedes aegypti	17.7	47.7	1,995	H, M, L
Culex pipiens	22.7	54.5	7,342	H, M, L
Culex eduardoi	0.9	6.1	123	H, M, L
Culex apicinus	0.5	2.5	136	M, L
Culex acharistus	3 tires	1.1	79	M
Culex tatoi	1 tire	0.4	4	L
Toxorhynchites theobaldi	0.8	3.2	43	M, L

H: high; L: low; M: middle.

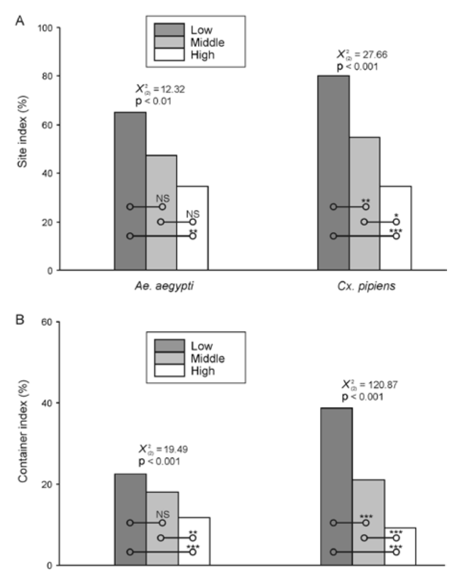

Fig. 2: comparison of site index (A) and container index (B) for *Aedes aegypti* and *Culex pipiens* among urbanisation levels. Results of chi-squared tests for multiple proportions are indicated above bars. Significances of multiple pairwise comparisons by Tukey's procedure are provided between bars. NS: not significant ($p > 0.05$); *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

The number of immatures collected per infested tire (i.e., RA) was significantly higher (U = 50115.5, $p < 0.001$) for *Cx. pipiens* [median = 7, quartile (Q) 1 = 3, Q3 = 19] than for *Ae. aegypti* (median = 3, Q1 = 1, Q3 = 6). The RA of *Ae. aegypti* differed significantly among urbanisation levels ($H_{(2,361)} = 25.46$, $p < 0.001$), with higher values observed for large cities (Fig. 3). However, the estimated RA of *Cx. pipiens* did not differ among categories ($H_{(2,463)} = 3.16$, $p > 0.05$).

Regarding sunlit exposure condition, the CI for shaded tires was significantly higher than the CI for exposed tires for both mosquito species (*Ae. aegypti*: shaded = 24.1%, sunlit = 13.1%, $X^2 = 40.8$, $p < 0.001$; *Cx. pipiens*: shaded = 31.4%, sunlit = 16.4%, $X^2 = 63.7$, $p < 0.001$). A similar trend was observed when both exposure conditions were compared within each urbanisation level, though the observed differences were marginal or not significant in small towns (Fig. 4). Despite this constant trend in the CI, the comparisons of the RA between shaded and sunlit tires were not statistically significant at $p < 0.05$ (Fig. 5).

Fig. 6 describes the monthly variations of the SI, the CI and the RA; both mosquito species were collected throughout the entire study period. *Ae. aegypti* showed a similar temporal pattern for the three variables, with the highest records during summer months. As a consequence, Spearman's correlations between the monthly values of the SI, CI and RA were significant for *Ae. aegypti* ($r_{s\ (RA:CI)} = 0.95$, $p < 0.01$; $r_{s\ (RA:SI)} = 0.86$, $p < 0.05$; $r_{s\ (CI:SI)} = 0.82$, $p < 0.05$). Temporal patterns of *Cx. pipiens* showed some differences among these variables, but all Spearman's correlations were not significant ($p > 0.05$).

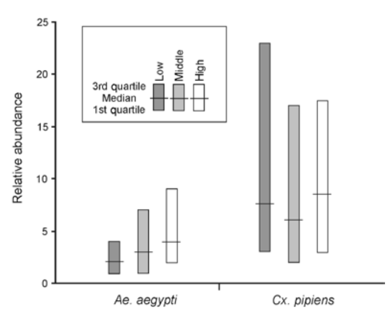

Fig. 3: relative abundance per infested tire for *Aedes aegypti* and *Culex pipiens* in different urbanisation levels.

Finally, the monthly values of the SI and CI were regrouped by season and compared along the urbanisation gradient (Fig. 7). Both mosquito species recorded the highest values of both indexes in the less urbanised localities during spring, summer and autumn. The statistical comparisons differed significantly among urbanisation levels for the SI of *Ae. aegypti* in summer and for the CI of *Ae. aegypti* in spring, whereas the differences were significant for *Cx. pipiens* in every season. A marked difference in the seasonal pattern of *Cx. pipiens* was observed in small towns, where both indexes continued increasing during autumn as opposed to the generalised decreasing trend for both mosquito species in this season.

DISCUSSION

The present study demonstrates that used tires are a considerable source of mosquito vectors in temperate Argentina, with half of the tires containing water and approximately one-third of them harbouring *Ae. aegypti* or *Cx. pipiens* immatures. From a public health viewpoint, it is pertinent to mention that an important dengue outbreak occurred in the study area at the beginning of 2009 (Seijo et al. 2009), and a zoonotic outbreak of West Nile virus was recorded in horses in 2006 (Morales et al. 2006). Therefore, despite frequent advertisements on mosquito prevention, our survey clearly suggests that used tires are not being considered a serious threat in mosquito control activities. Our field data come mainly from used tire shops and piles of discarded tires. In both cases, health authorities have central responsibility in regulating tire commercialisation and in collecting and recycling abandoned tires.

In addition to *Ae. aegypti* and *Cx. pipiens*, five other species were registered, but all in very low abundance. Among them, *Toxorhynchites theobaldi* Dyar & Knab is recognised as a potential biocontrol agent of container-breeding mosquitoes (Collins & Blackwell 2000). In Buenos Aires Province, this is the unique species of the genus, which was recorded only a few times in tree holes (Rossi et al. 2006) and in discarded tires within a forest

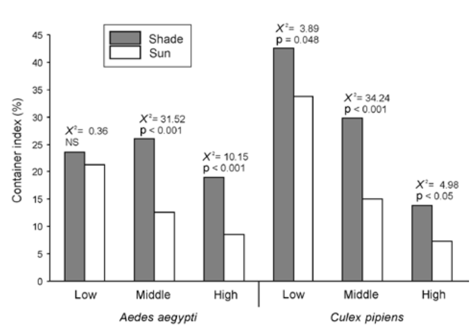

Fig. 4: comparison of container index between shaded and sunlit exposed tires within each urbanisation level. Results of chi-squared tests for two proportions are indicated above bars. NS: not significant.

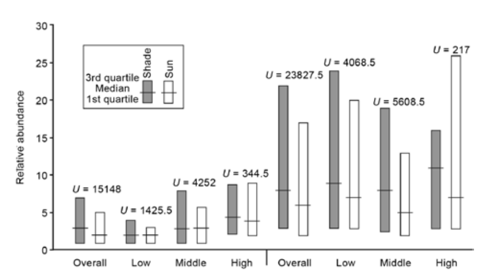

Fig. 5: relative abundance per infested tire for *Aedes aegypti* and *Culex pipiens* in shaded and sunlit conditions within each urbanisation level. All the statistical comparisons were not significant ($p > 0.05$). Mann-Whitney U-tests are indicated above bars.

reserve (Rubio & Vezzani 2011). An interesting finding of our survey is the absence of *Aedes albopictus* (Skuse), which has been invading new areas and expanding its geographic distribution through the worldwide tire trade (e.g., Eritja et al. 2005, Yee 2008). Considering the extension of our study area and sampling size, our data support the idea that *Ae. albopictus* remains restricted to the north of the country (Vezzani & Carbajo 2008).

With respect to the urbanisation level, *Ae. aegypti* and *Cx. pipiens* were the most common tire-breeding mosquito species found along the urbanisation gradient during the three studied seasons. In the Americas, studies from the US and Brazil have documented that *Ae. aegypti* reaches its maximum infestation levels in highly urbanised sites in comparison with rural or forested environments (O'Meara et al. 1995, Braks et al. 2003, Rey et al. 2006). Our survey in temperate Argentina is not consistent with these studies, as our data showed a clear increasing trend of infestation levels toward less urbanised areas (i.e., small towns). However, our survey did not include true rural environments (i.e., agricultural) and further surveys including artificial containers from rural areas are essential to complete the current picture. Another factor that may obscure the comparison is that in the study sites from US and Brazil, *Ae. aegypti* shares larval habitats with *Ae. albopictus*. Interspecific competition between these species has been strongly suggested (e.g., Juliano 1998, Braks et al. 2004) and could be affecting *Ae. aegypti* performance in the less urbanised sites. In other words, the distribution pattern described in temperate Argentina occurs in the absence of a presumably superior larval competitor such as *Ae. albopictus*. Our findings are in accordance with the oviposition pattern of *Ae. aegypti* in Buenos Aires City described by Carbajo et al. (2006); lower infestation levels were found in areas with higher human populations or higher densities of flats.

Sunlit exposure condition was a determinant factor in the CI for both mosquito species; the percentage of infested tires in shaded microhabitats was double that for sun-exposed tires. A similar trend was previously reported in flower vases within the study area (Vezzani & Albicócco 2009) and there is general consensus that there are higher frequencies of containers harbouring *Ae. aegypti* in shaded habitats (e.g., Focks et al. 1981, Kittayapong & Strickman 1993, Vezzani et al. 2005, Barrera et al. 2006). This trend was consistent along the three urbanisation levels, but statistical comparisons suggest that it was less marked within small localities. Considering that large cities register temperatures that are 5-11ºC warmer than their surrounding rural areas due to the effect of the urban heat island (Patz et al. 2005), the differences in habitat suitability between sun-exposed and sun-protected containers would be exacerbated in more urbanised areas. Higher values in shaded habitats could be due to a true oviposition preference or a higher abundance of adults in shaded areas. In addition, current and previous data on the RA of immatures suggest no difference in mortality between shaded and sunlit containers.

Observed seasonal patterns for both species through different urbanisation levels were similar to those previously reported in cemeteries of the Federal District (Vezzani et al. 2004, Vezzani & Albicócco 2009), with the exception of *Cx. pipiens* in small towns, where an increasing trend persisted in autumn. One possible expla-

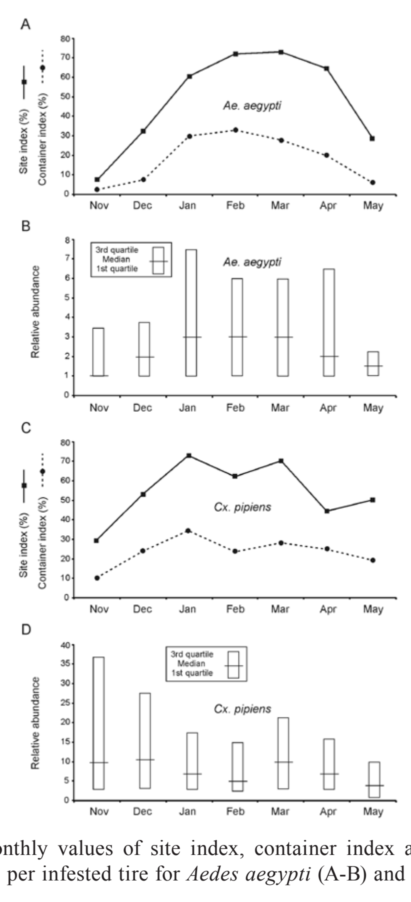

Fig. 6: monthly values of site index, container index and relative abundance per infested tire for *Aedes aegypti* (A-B) and *Culex pipiens* (C-D).

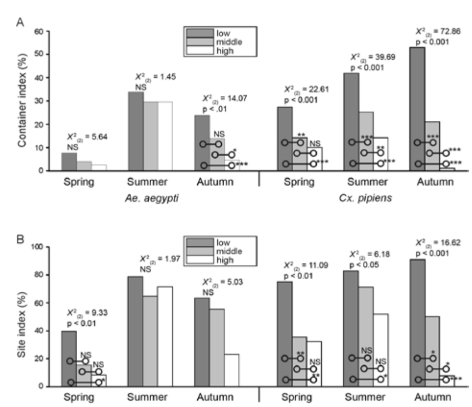

Fig. 7: seasonal comparison of site index and container index of *Aedes aegypti* and *Culex pipiens* among urbanisation levels. Results of chi-squared tests for multiple pairwise proportions are indicated above bars. Significances of multiple pairwise comparisons by Tukey's procedure are provided between bars. NS: not significant ($p > 0.05$); *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

nation is that different forms of the complex show different seasonal patterns and in Buenos Aires Province *Cx. pipiens* s.s. and *Cx. quinquefasciatus* are sympatric (Forattini 2002, Rossi et al. 2002). There are examples compiled by Vinogradova (2000), in which *Cx. pipiens* forms were differentiated according to the urbanisation type; e.g., in Italy, *molestus* (urban) and *pipiens* (rural). Although an overlapping region between the members of the complex was found in Argentina (Almirón et al. 1995), the effect of urbanisation on the geographic distribution of the complex remains unstudied.

Monthly correlations detected among the infestation level for *Ae. aegypti* mean that as its reproductive season progresses, the proportions of infested containers and sites and the abundance of immatures follow a similar pattern. This result could be used as a tool to decide which method for monitoring *Ae. aegypti* provides the best cost-benefit equation. Unfortunately, no significant correlation was found for *Cx. pipiens*. Monthly fluctuations of the indexes described in this study clearly determine that summer is the season with the highest risk of disease transmission by both mosquito vectors. During this period, mosquito control activities focused on tires should be reinforced and considering the above-mentioned pattern of *Cx. pipiens*, these activities should be continued during the autumn at least in small localities.

In summary, our results strongly suggest that in temperate Argentina, tires are more suitable for the proliferation of the main mosquito vectors in small towns than in large cities. The identification of biotic and abiotic factors driving this pattern was beyond the scope of this research and will be the subject of a future set of studies. The results obtained from tires also serve to validate previous investigations performed exclusively within cemeteries of the Federal District, particularly those investigating seasonal patterns and the effects of sunlit exposure. We hope that the present study encourages local health authorities to consider used tires to be a true sanitary problem.

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