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Macro- and microenvironmental factors affecting tyre-breeding flies (Insecta: Diptera) in urbanised areas

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Abstract. 1. Urbanisation is often considered a main driver of changes in the structure of insect communities. To assess macro- and microenvironmental factors determining the occurrence and community structure of fly larvae in water-filled artificial containers, a total of 13 848 individuals were collected among 1380 water-filled tyres inspected along an urbanisation gradient in temperate Argentina.

2. According to the best generalized linear mixed model obtained for fly occurrence, the probability of finding an occupied tyre was greater in large tyres with high water volume, containing organic matter and located in shaded conditions under high vegetation cover in backyards. Regarding macroenvironmental factors, fly occurrence decreased as the urbanisation level increased.

3. The ANOVA revealed a negative effect of urbanisation on all variables used to characterize the fly community structure. The number of occupied tyres, the relative abundance of individuals, the species richness, and the Shannon diversity index showed consistent patterns with significantly lower values at high urbanisation level.

4. Community composition also varied along the urbanisation gradient, revealing three major groups of fly species: those found exclusively in highly urbanised areas, those found in the less urbanised sites, and those collected across the entire gradient.

Key words. Artificial containers, Chironomidae, Culicidae, richness, urban ecology.

Introduction

Urbanisation is one of the major human activities impacting biodiversity and a process in continuous growth worldwide. There is a general consensus that increasing urbanisation levels have detrimental effects on invertebrate communities, decreasing their species richness and diversity (Hansen *et al*., 2005; McKinney, 2008). However, human intervention creates highly heterogeneous environments for invertebrates, in which many biotic and abiotic factors could affect different attributes of the community structure, such as the richness and the species composition. These factors may operate at different spatial scales, from the macroenvironment (e.g. land use type) to the microenvironment (e.g. water quality) (McIntyre *et al*., 2001; Paul & Meyer, 2001; Rey *et al*., 2006). To develop an ecological understanding of biological communities functioning within urban systems, man-made and natural components must be integrated in multi-scale analyses.

Vehicle tyres are unavoidable components of domestic environments, from highly urbanised areas to agricultural lands. Given that tyres are built with non-degradable materials, they are a serious hazard worldwide due to the difficulty of disposing of and recycling them. When these containers are improperly disposed, they become aquatic habitats for a broad diversity of fauna, particularly for flies (Insect: Diptera) with aquatic immature stages. Many families from this order have been found in discarded water-filled tyres, including Culicidae, Chironomidae, Psychodidae, Ceratopogonidae, Syrphidae, Corethrellidae, Chaoboridae, and Stratiomyidae (Baumgartner, 1988; Morris & Robinson, 1994; Kling *et al*., 2007). These small urban habitats arouse interest among mosquito researchers as a means of introduction and range expansion of

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these blood-sucking pests and vectors of diseases at the country and continental levels (Yee, 2008). By contrast, other flies found in tyres are considered beneficial. For instance, non-biting culicids (*Toxorhynchites* spp.) are successful biological control agents of mosquitoes (Gordon & Peterson, 1980). Chironomids (Diptera: Chironomidae) are widely used in biomonitoring programmes to obtain information on organic and chemical pollution of aquatic habitats (Morais *et al*., 2010). Other tyre dwellers of unknown bionomics could play a valuable role in urban ecosystem functioning. Consequently, to develop control strategies aimed at suppressing nuisance species from these man-made containers, a comprehensive knowledge of the fly assemblage inhabiting containers and the factors determining its community structure is needed.

Several microenvironmental factors have been proposed to explain the occurrence and abundance of mosquito species in water-filled tyres, from biotic and abiotic conditions of the water to intrinsic characteristics of the tyre. The factors that have attracted most attention are water chemistry (Beier *et al*., 1983), detritus and nutrient concentrations (Kling *et al*., 2007), sunlight exposition (Beier *et al*., 1983; Joy & Sullivan, 2005; Rubio *et al*., 2011), tyre size (Morris & Robinson, 1994; McMahon *et al*., 2008), and relative position and location (Morris & Robinson, 1994). Other studies assessed the association between the mosquito community of tyres and several environmental factors related to detritus, microorganisms and habitat variation (Kling *et al*., 2007; Roiz *et al*., 2007; Yee *et al*., 2010). However, there is a lack of ecological studies linking the entire fly community of tyres with the environmental conditions using a multivariate approach.

So far, the research on macroenvironmental factors associated with tyre inhabitants has also been restricted to culicids of medical or veterinary concern. In general, the occurrence or abundance of mosquitoes was compared among tyres from different settings, e.g. forested versus domestic or peridomestic environments (Joy & Sullivan, 2005; Roiz *et al*., 2007), different land use types (Baumgartner, 1988; Costanzo *et al*., 2005), across urban–rural gradients (Higa *et al*., 2010; Rubio *et al*., 2011) or geographic regions (Joy & Sullivan, 2005; McMahon *et al*., 2008; Higa *et al*., 2010). Most of these studies presume the existence of an association between mosquitoes and the environmental conditions related to the type or intensity of human use. Species-level analyses along urbanisation gradients have suggested, for example, that *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) increase their larval densities towards more urbanised areas, whereas *Culex pipiens* L. is not affected by the urbanisation (Higa *et al*., 2010; Rubio *et al*., 2011). Although the mechanisms behind the variations of certain community attributes may be related to the particular requirements of each species, the complex changes in the community structure cannot be predicted based exclusively on species responses.

In a previous study looking at the entire fly community of other typical urban containers (i.e. flower vases from cemeteries), we found that the percentage of occupied habitat and the abundance of flies peaked at intermediate levels of urbanisation, whereas the species richness and composition were similar throughout the gradient (Rubio *et al*., 2012). It is possible, however, that the observed patterns were strongly influenced by the habitat characteristics of the particular land use type (i.e. cemetery), or by the container type (e.g. Yee *et al*., 2012), in addition to the intensity of the urbanisation in the surroundings. Consequently, here we explore the response patterns of dipteran assemblages in urban environments using a homogeneous container type and the most extended land use type within cities, namely residential and commercial areas. Our objectives were: (i) to identify macro- and microenvironmental factors associated with the presence of flies in water-filled tyres in temperate urban environments; and (ii) to assess the effect of urbanisation on species richness, abundance of individuals, diversity, and species composition of the fly community under study. Under the general hypothesis that human intervention has a detrimental effect on insects, we expect that the occurrence, the abundance, the richness, and the diversity of flies decrease as the urbanisation level increases, also promoting changes in the species composition. In addition, we hypothesize that the microenvironmental factors known to affect the occurrence of mosquitoes in artificial containers also explain the occurrence of flies in general.

Materials and methods

Study area

Buenos Aires Province, Argentina, is located within the Pampean region. Its climate is temperate humid-subhumid with annual precipitation values ranging from 1200 to 600 mm from east to west, and annual mean temperatures of around 14–17 °C (Magrin *et al*., 1997). The original grasslands have been modified by farming, livestock breeding and human settlements. The study area extends between latitudes 35°26′26.53″S and 34°11′11.25″S and longitudes 59°41′36.66″W and 58°5′29.55″W. This area represents a complete urbanisation gradient, from a continuous mosaic of human dwellings around the Federal District, namely Aglomerado Gran Buenos Aires (AGBA), to several small towns located up to 100 km away. The AGBA covers approximately 3827 km^2 and presents the greatest population density of Argentina (2995 inhabitants per km^2) (INDEC, 2001).

Data collection and Diptera identification

Surveys were conducted from November 2009 (spring) to May 2010 (fall), at a monthly sampling effort of 9 days, each of 6 h working. In total, 138 georeferenced sites consisting of used tyre shops or piles of tyres in private premises with at least 10 water-filled tyres were visited (Fig. 1). Each site was visited only once, and we ensured that the entire urbanisation gradient was represented in each monthly sampling. In each site, 10 water-filled tyres were inspected by sweeping the water three times with a fine mesh strainer; the content, including any invertebrates, was slightly mixed before each sweeping to homogenize the water. Collected larvae were fixed in 70% ethanol for further laboratory processing.

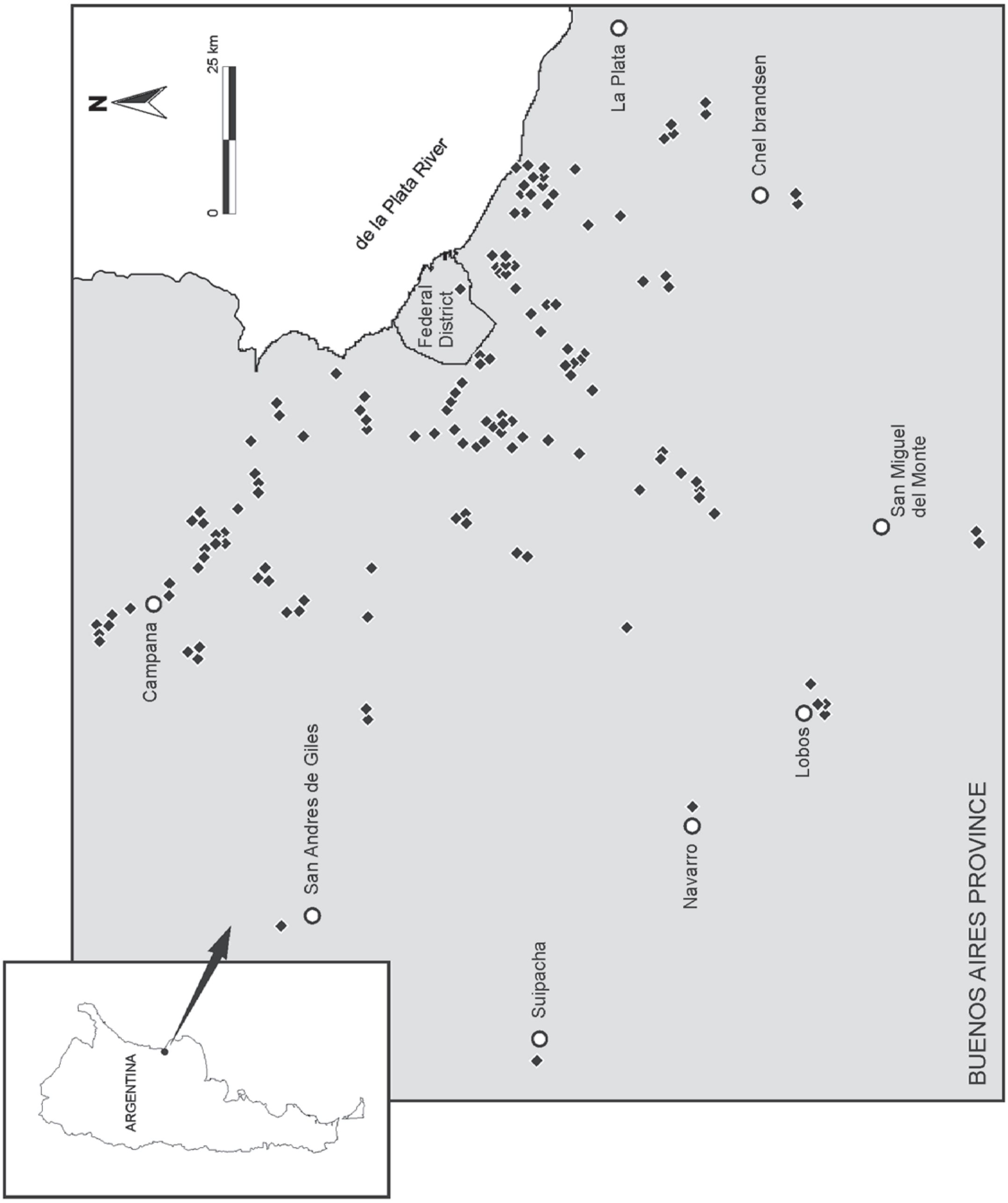

Fig. 1. Geographic location of the 138 sites surveyed in Buenos Aires Province – only peripheral localities are shown.

Tyre features and vegetation cover were measured qualitatively in the field during the surveys. The former were represented by the spatial location regarding the street (LOC), the position regarding the ground (POS), the position within the pile (PIL), the water volume relative to the total capacity (WAT), the size (SIZ), the level of sunlight exposure (SUN) and the presence of organic matter (OMA). Vegetation cover 1 m around each tyre was estimated at different heights representing low (LST), middle (MST) and upper stratums (UST). A full description of the variables and their levels is provided in Table 1.

The urbanisation level surrounding each site was characterized by the percentage of impervious area (PIA). This is one of the primary characteristics of urbanisation and could influence other factors such as urban climate and habitat patterns. An unsupervised classification was performed to identify impervious areas such as paved roads and concrete structures on a 5 TM satellite image captured on 22 January 2010, using ERDAS IMAGINE 8.4 software. The PIA was estimated around each site at three scales (0.5, 1 and 1.5 km radiuses) using GIS-ARCVIEW 3.2 (Table 1).

In the laboratory, larvae of Culicidae were identified to species (Rossi *et al*., 2002), larvae of Chironomidae to genera (Wiederholm, 1983; Epler, 2001) and larvae of other Diptera to family (McAlpine *et al*., 1981). All non-mosquito individuals were then classified to morphospecies.

Data analysis

Analyses were performed considering two sampling units. First, we used individual tyres ($n = 1380$) to identify microenvironmental (tyre features and vegetation cover) and macroenvironmental (urbanisation level) factors determining the occurrence of flies. Then, we considered the sites ($n = 138$) to analyse the effect of the urbanisation level on the structure of the fly community.

Presence/absence model. A multivariate analysis was performed on the presence/absence data of flies in a total of 1380 water-filled tyres surveyed. The spatial dependence among dipteran assemblages of tyres from the same site was taken into consideration in the modelling. For this, we used generalised linear mixed models (GLMMs), which are an extension of generalised linear models (GLMs) that include both random and fixed effects. We attempted to find the best model of fly occurrence per tyre, including the 'site code' as a random factor and the 13 explanatory variables of Table 1 as fixed factors. A binomial distribution of errors was assumed and a logistic function was applied as a link between the fly occurrence and the predictor variables (Bolker *et al*., 2009).

Prior to the GLMM, univariate GLMs were run. For continuous variables, we also included their square (xi^2) and the sum of both ($xi + xi^2$). The inclusion of a variable and its square allows for the modelling of parabolic relationships with maximum or minimum values of the response variable at intermediate values of the explanatory variable. Only significant variables in the univariate analysis were included in the full model, but collinear variables (i.e. those presenting a variance inflation factor > 5) were excluded to avoid redundancy. The goodness-of-fit of the models was evaluated in terms of the Akaike information criterion (AIC) (Akaike, 1974). The model that yielded the lowest AIC was selected (Zuur *et al*., 2009).

The explanatory variables retained in the final model were selected by a stepwise backward procedure. Among the selected variables, all possible two-way interactions were tested. Finally, to simplify the model, the levels in a factor that were not significantly different were merged together (Nicholls, 1989). To assess the classification improvement of the final model over chance (Fielding & Bell, 1997), the higher Kappa index (K) corresponding to the best cut-off point was reported.

All analyses were performed using the open-source software R 2.10.1 (R Development Core Team, 2009) with glmmML and Design packages.

Community structure analysis. Dipteran communities were characterized in each of the 138 sites inspected through the following variables: number of tyres harbouring larvae (i.e. occupied tyres), mean number of larvae collected per occupied tyre (i.e. relative abundance), richness estimated by individual-based rarefaction curves, diversity estimated by the Shannon index, and species composition. For the latter, we first performed a principal component analysis (PCA) using the matrix of species abundances. Then, the component which accounted for the highest variance in the data was used as a new response variable representing species composition of the community. The relationship between species composition and landscape structure can be masked by spatial autocorrelation. In order to discard it, a Mantel test (Legendre & Legendre, 1998) was performed to evaluate the correlation level between the matrix of dissimilarity of the sites based on species composition and the matrix of geographic distance between the sites (Bray–Curtis dissimilarity, 1000 permutations).

The urbanisation level of each site was categorized as low (0–33.3% of impervious area), middle (33.4–66.6%), or high (66.7–100%) for each radius (PIA_{05}, PIA_1 and PIA_{15}). In order to evaluate the categorization at each radius, an ANOVA was used to compare the mean values of PIA among urbanisation levels (Zar, 1999). These tests detected significant differences among urbanisation levels for all PIAs (PIA_{05}: $F = 586.67$, $P < 0.001$; PIA_1: $F = 581.14$, $P < 0.001$; PIA_{15}: $F = 433.64$, $P < 0.001$). Therefore, the selected categorization guaranteed a good representation of the entire urbanisation gradient of the study area for all PIAs (Fig. 2).

To compare the community structure among urbanisation levels, a one-way ANOVA was performed for each dependent variable, i.e. occupied tyres, relative abundance, richness, diversity, and species composition. Relative abundance was square-root-transformed to meet assumptions of normality and homogeneity of variances. Considering that multiple independent analyses of the same data set increase the probability of making a type I error, we used a corrected P-value following the false discovery rate method (Benjamini & Hochberg, 1995). Further pairwise comparisons were

Table 1. Explanatory variables included in the generalized linear mixed model on the occurrence of fly larvae in water-filled tyres.

Variable type	Variable (code)	Levels (codes)	Description
Tyre features	Location (LOC)	Street (str) – front (fro) – backyard (bac)	Spatial location in the site
	Size (SIZ)	< 65 – 65/80 – 80/120 – >120	Total external diameter (cm)
	Position (POS)	Lying (lyi) – inclined (inc) – upright (upr)	Tyre position regarding the ground
	Pile (PIL)	On the ground (1°) – above the first (2°) – above the second (3°) – above the third (4°) – anyone above the fourth (5°)	Tyre position within the pile
	Water volume (WAT)	< ½ (< 1/2) – around ½ (1/2) – > ½ (> 1/2) – full (full)	Water volume relative to the total capacity
	Sunlight (SUN)	Sun (sun) – partial shade (pas) – shade (sha)	Level of sunlight exposure
	Organic matter (OMA)	Yes (yes) – no (no)	Presence of organic matter in the water
Vegetation cover	Low stratum (LST)	Continuous	Percentage of vegetation cover in the low stratum; below 0.5 m
	Middle stratum (MST)	Continuous	Percentage of vegetation cover in the middle stratum; between 0.5 and 1.5 m
	Upper stratum (UST)	Continuous	Percentage of vegetation cover in the upper stratum; above 1.5 m
Urbanisation level	Percentage of impervious area (PIA) for 0.5 km radius (PIA_{05})	Continuous	PIA at 0.5 km radius around the site
	PIA for 1 km radius (PIA_1)	Continuous	PIA at 1 km radius around the site
	PIA for 1.5 km radius (PIA_{15})	Continuous	PIA at 1.5 km radius around the site

performed using the Tukey procedure when necessary (Zar, 1999).

Finally, the proportion of sites with at least one occupied tyre was considered in a separate analysis. This variable was compared among urbanisation levels with the χ^2 test for multiple independent proportions (Fleiss *et al*., 2003) and further pairwise comparisons by the Tukey procedure when appropriate (Zar, 1999).

Results

General findings

A total of 111 (80.4%) sites and 602 (43.6%) water-filled tyres were found harbouring dipteran larvae. The mean number of occupied tyres per site was 4.3 (SE = 0.3) and the mean number of larvae per occupied tyre was 23 (SE = 1.2).

Among the 13 848 larvae collected, a total of 18 species belonging to nine Diptera families were recorded (Table 2). Culicidae and Chironomidae were the richest (six and four species, respectively) and most abundant (72% and 19% of the total, respectively) families. Four species from these families represented 88% of the total individuals captured; these were *C. pipiens*, *A. aegypti*, one species of the genus *Chironomus* and one of the genus *Goeldichironomus*.

Fly occurrence

Univariate GLMs showed that all explanatory variables, except LST, were significantly associated with the occurrence of larvae (Table 3). Three variables regarding tyre and water features (SIZ, WAT and OMA) were the best predictors of fly occurrence, i.e. they yielded the lowest AIC values. The PIAs estimated for different radiuses showed a negative effect of the urbanisation level on fly occurrence.

The multivariate model (AIC_{GLM}, 1375.5) was considerably improved by the inclusion of 'site code' as a random factor [AIC_{GLMM}, 1294; *K* (cut-off point), 0.52 (0.43)]. The best GLMM for fly occurrence included both micro- and macroenvironmental variables (Table 4). The probability of

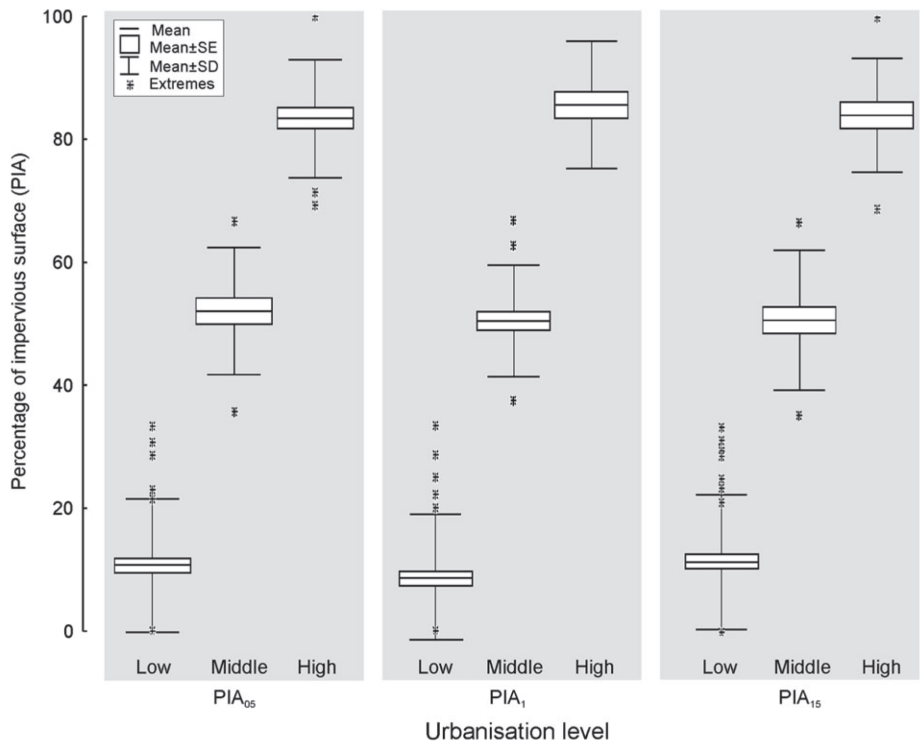

Fig. 2. Box plot comparing the distribution of the percentage of impervious area (PIA) values in each urbanisation level according to PIA_{05}, PIA_1 and PIA_{15}.

finding a tyre harbouring larvae increased with tyre size and water volume, and when the tyre contained organic matter. Also, a location in the backyard, in shaded conditions, and under high vegetation cover of the middle stratum favoured the occurrence of larvae. By contrast, tyres placed above the third position of the pile were less likely to harbour dipterans. Regarding macroenvironmental factors, the model predicted that the probability of fly occurrence decreased as the urbanisation level increased (squared PIA_1). There were no significant interactions between pairs of variables.

Fly community structure

In general, ANOVA analyses revealed a negative effect of urbanisation on all variables used to characterize the fly community of tyres (Table 5). For all PIAs, the number of occupied tyres was significantly lower at the highest urbanisation level. Following a similar pattern, the estimated richness in lowly urbanised sites was approximately 1.5 times the observed in highly urbanised sites, except for PIA_{05}. Regarding the species composition, the first component derived from the PCA accounted for 82.2% of the total variance, and significant differences were detected between the scores of sites corresponding to high and low urbanisation levels. The Mantel test revealed no spatial autocorrelation ($R = -0.04$, $P > 0.05$), meaning that these differences were not due to the distance among sites. The relative abundance and the diversity also recorded the lowest values at highly urbanised sites but these trends were only significant for PIA_{15} and PIA_1, respectively.

Different species presented varying distributions along the gradient, which resulted in characteristic fly assemblages at each urbanisation level (Table 2). Some species were found at the three levels (generalist), whereas others were present only in either high or low urbanisation levels (specialist). As a result, three groups of species were identified: first, species that were found throughout the gradient, represented by *A. aegypti*, *C. pipiens*, *Culex eduardoi* Casal & Garcia and all species of the families Chironomidae and Psychodidae; secondly, species that were collected exclusively in less urbanised sites – these were *Culex apicinus* Philippi, *Toxorhynchites theobaldi* Dyar & Knab and the species of the families Ceratopogonidae, Ephydridae, Muscidae and Syrphidae; and thirdly, species from highly urbanised areas – *Culex acharistus* Root, and the species of Chaoboridae and Sarcophagidae

Finally, the proportion of sites with at least one occupied tyre also showed a marked decreasing trend from low to high urbanisation levels, which was significant only for PIA_{15} (Fig. 3).

Discussion

Urban areas are known to support a rich diversity of insects. Some of them are undesirable or considered pests, whereas others are beneficial or aesthetically pleasing (Frankie *et al*., 2009). Artificial containers filled with water provide aquatic

Table 2. Diptera species composition, total number of individuals collected, and occurrence in each urbanisation level according to the percentage of impervious area (PIA) estimated for radiuses of 0.5 and 1.5 km (PIA_{05} and PIA_{15}, respectively); PIA_1 is not detailed because it is redundant regarding the other PIAs.

		Urbanisation level					
		PIA_{05}			PIA_{15}		
	Number of individuals collected (%)	Low	Middle	High	Low	Middle	High
Family Culicidae							
Aedes aegypti	1467 (10.6)	•	•	•	•	•	•
Culex acharistus	2 (0.01)			•		•	
Culex apicinus	140 (1.0)	•	•		•		
Culex eduardoi	236 (1.7)	•	•	•	•	•	
Culex pipiens	8114 (58.7)	•	•	•	•	•	•
Toxorhynchites theobaldi	21 (0.2)	•	•		•		
Family Ceratopogonidae							
Morphospecies 1	54 (0.4)	•	•	•	•	•	
Family Chaoboridae							
Morphospecies 1	8 (0.1)			•		•	
Family Chironomidae							
Chironomus sp.	1574 (11.4)	•	•	•	•	•	•
Goeldichironomus sp.	952 (6.9)	•	•	•	•	•	•
Limnophyes sp.	36 (0.3)	•	•	•	•	•	•
Monopelopia sp.	17 (0.1)	•	•	•	•	•	•
Family Ephydridae							
Morphospecies 1	52 (0.4)	•	•		•	•	
Family Muscidae							
Morphospecies 1	197 (1.4)	•	•	•	•	•	
Family Psychodidae							
Morphospecies 1	261 (1.9)	•	•	•	•	•	•
Morphospecies 2	672 (4.9)	•	•	•	•	•	•
Family Sarcophagidae							
Morphospecies 1	3 (0.02)			•		•	
Family Syrphidae							
Morphospecies 1	22 (0.2)	•	•	•	•	•	

habitats for 'good' and 'bad' flies in urban areas around the world, and tyres are not the exception. Here, in the greatest megalopolis of temperate South America, over 80% of the tyre piles inspected were infested with fly larvae, suggesting that tyres are a great source of dipterans in urban environments. In support of our general hypothesis, the main findings of the study consistently suggest that the urbanisation process has detrimental effects on the fly community. We also provide evidence that some microenvironmental factors driving mosquito occurrence in artificial containers explain the occurrence of flies in general, supporting our second hypothesis.

Non-culicid species developing in tyres have often been ignored, as most of them are not vectors of human or animal diseases (Kling *et al*., 2007). Among the 18 fly species identified in our survey, 12 belong to the families Chironomidae, Ceratopogonidae, Chaoboridae, Ephydridae, Muscidae, Psychodidae, Sarcophagidae, and Syrphidae. A few fly species within the studied assemblage accounted for 88% of all individuals; a similar pattern was observed for necrophagous dipterans by Hwang and Turner (2005) and for flower vase-breeding flies by Rubio *et al*. (2012).

Identifying the main drivers of the occurrence of dipteran larvae in tyres is the first step towards understanding community functioning. Our final model of fly occurrence identified environmental predictors related to characteristics of the water, the container, its location and close surroundings, and the urbanisation level. Therefore, a multi-scale approach seems indispensable to understand the reasons behind the occurrence of these insects in temporary and artificial aquatic habitats.

Vegetation cover and shade are considered two of the main drivers of species composition and abundance of culicids in water-filled tyres. Possible explanations for higher abundances of some species in shaded tyres, reviewed by Yee (2008), include factors that may increase larval survival, such as greater detritus inputs or lower water temperatures, and factors that may increase oviposition by females, namely higher humidity for resting adults or stronger oviposition cues. In our study, the vegetation of the middle stratum, the presence of organic matter and a shaded condition were associated with a higher probability of fly occurrence. Previous studies showed that the commonest mosquito species from artificial containers in temperate Argentina, i.e. *A. aegypti* and *C. pipiens*, were recorded more frequently in vegetated and/or shaded habitats

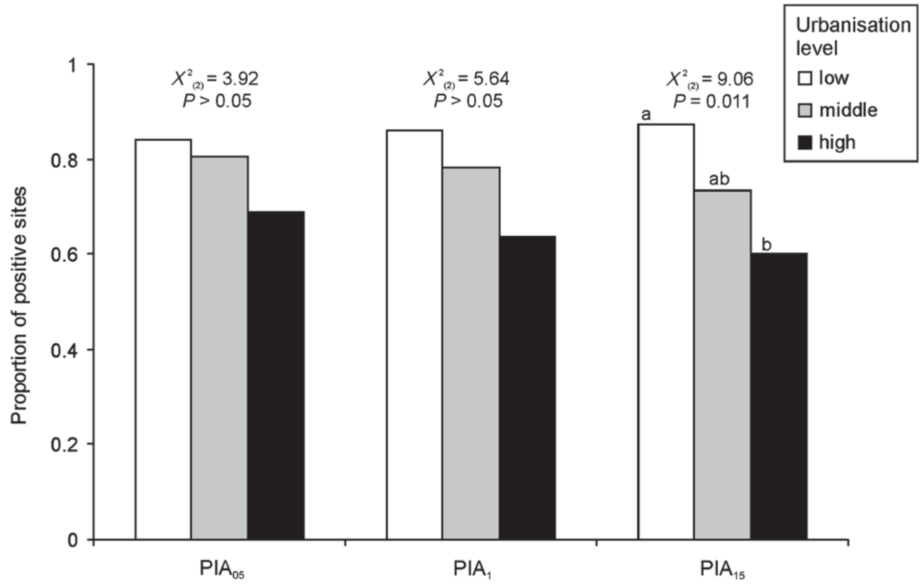

Fig. 3. Multiple comparisons of the proportion of positive sites in each urbanisation level for each estimated percentage of impervious surface (PIA). Different letters indicate significant differences in the pairwise comparisons by the Tukey procedure.

(Vezzani *et al*., 2005; Vezzani & Albicócco, 2009; Rubio *et al*., 2011). Similar to other insect communities, the mechanisms behind the observed patterns may be related to the life history and resource use of each species (e.g. Blair & Launer, 1997).

Primary productivity in tyres is restricted to algae (Beier *et al*., 1983; Kling *et al*., 2007). Given that its nutritional value for mosquito larvae is unknown, the allochthonous input of organic matter is assumed to be important. Some authors have demonstrated the influence of organic matter availability in determining the composition of mosquito assemblages in both tyres (Kling *et al*., 2007) and natural containers, such as tree holes (Srivastava & Lawton, 1998). However, the effect of organic matter is usually difficult to discriminate from that of vegetation and shade, not only for mosquitoes but also for terrestrial insect communities; e.g. ants (Arnan *et al*., 2007). In our study, tyres were shaded not only by vegetation but also by buildings, cars and tyre piles. Therefore, many tyres were located in shaded environments produced by man-made structures and containing organic matter in the absence of vegetation in the surroundings. This may explain why the vegetation cover in the middle stratum, the sunlight condition and the organic matter content were not correlated and could all be included in the final multivariate model.

Other micro-scale factors favouring dipteran occurrence were higher values of tyre size and water volume. Accordingly, McMahon *et al*. (2008) reported that larger tyres were more often infested by mosquitoes; however, Morris and Robinson (1994) recorded the opposite pattern. In both studies, the authors proposed oviposition preferences as the main mechanism explaining the observed pattern. Among the dominant fly species collected in our survey, Harrington *et al*. (2008) found that the presence and abundance of *A. aegypti* eggs increased with the container size, suggesting that bigger containers may provide better resting surfaces for females preparing for oviposition. Similarly, Becker (1995) reported that *Culex quinquefasciatus* larvae were more common in containers of large water volume. In addition, we consider that a greater occurrence of flies could be expected in bigger tyres because high temperature buffering and higher habitat stability during dry periods increase larval survival.

The occurrence of flies was also associated with the spatial location of the tyre, being higher in backyards. Tyres located on the street are more often removed by the government or individual waste collectors for recycling, limiting their colonization by flies. In addition, backyards are usually vegetated, which could enhance tyre suitability in comparison with those not surrounded by vegetation typically found in the front of the sites. Regarding the relative position of the tyre within the pile, our results suggest that the occurrence of flies diminishes at higher positions. A possible explanation for this pattern is that containers closer to the ground remain covered by low vegetation or by other tyres, favouring larger water volume and lower temperatures. Morris and Robinson (1994) also recorded more water-filled tyres closer to the ground but, in contrast to our results, mosquito occurrence was constant throughout the pile.

Previous studies focusing on different arthropod taxa or ecological habitats have produced contrasting results regarding the effect of urbanisation on the community structure, depending on the attribute considered. For example, ground-dwelling invertebrates showed positive, negative or neutral responses of species richness to increasing urbanisation (McIntyre, 2000; Alaruikka *et al*., 2002; Niemelä *et al*., 2002; Ishitani *et al*., 2003; McKinney, 2008). Stream invertebrates recorded lower abundance or diversity as urbanisation increased (Paul & Meyer, 2001; Urban *et al*., 2006). Among flying insects, the abundance and richness of wasps and bees decreased with urbanisation (Zanette *et al*., 2005), the abundance of butterflies

Table 3. Univariate statistics for the explanatory variables used to model the occurrence of fly larvae in water-filled tyres. Generalized linear model parameters and standard errors (B±SE) and Akaike information criterion (AIC) values are reported.

Variable	Level	B ± SE	AIC
Null (∼ 1)			1892.6
LOC	str	-0.57 ± 0.07***	1814.3
	fro	0.42 ± 0.14**	
	bac	1.37 ± 0.16***	
SIZ	< 65	-1.05 ± 0.09***	1711.8
	65/80	1.01 ± 0.18***	
	80/120	1.4 ± 0.13***	
	> 120	2.55 ± 0.29***	
POS	lyi	-0.72 ± 0.09***	1849.2
	inc	0.79 ± 0.12***	
	upr	0.32 ± 0.26	
PIL	1°	$-2.3e^{-3} \pm 6.8e^{-2}$	1857.1
	2°	-0.49 ± 0.17**	
	3°	-0.8 ± 0.29***	
	4°	-0.98 ± 0.25***	
	5°	-0.83 ± 0.21***	
WAT	< ½	-0.84 ± 0.09***	1786.9
	½	0.68 ± 0.13***	
	> ½	1.75 ± 0.2***	
	full	1.23 ± 0.18***	
SUN	sun	-0.61 ± 0.12***	1830.5
	pas	-0.03 ± 0.09	
	sha	0.63 ± 0.18***	
OMA	yes	2.31 ± 0.17***	1621
	no	-2.01 ± 0.15***	
LST		$9.7e^{-4} \pm 1.2e^{-3}$	1894
MST		$4.6e^{-2} \pm 7.9e^{-3}$***	1838.5
UST		$1.1e^{-2} \pm 1.5e^{-3}$***	1840.7
PIA_{05}		$-1.3e^{-2} \pm 2e^{-3}$***	1837.5
PIA_1	†	$-2.1e^{-4} \pm 2.5e^{-5}$***	1804
PIA_{15}	†	$-2.2e^{-4} \pm 2.6e^{-5}$***	1811.4

***$P < 0.001$, **$P < 0.01$.
†Best fit was the squared variable.
Refer to Table 1 for meanings of abbreviations.
Generalized linear model parameters and standard errors (B±SE) and Akaike information criterion (AIC) values are reported.
For continuous variables, only the best fit among the variable, its square, or the sum of both is reported.

decreased but its richness and diversity peaked at moderately disturbed sites (Blair & Launer, 1997), and the richness of hymenopteran parasitoids remained constant throughout the gradient (Fenoglio *et al*., 2009). So, notwithstanding the pattern described in each particular case, the bulk of the literature supports the idea that invertebrates respond to urbanisation in a diverse and complex manner. General findings of our previous (Rubio *et al*., 2012) and current research consistently suggest that the urbanisation level affects several features of the community structure of immature dipterans in artificial container habitats. In small flower vases from cemeteries, the proportion of infested containers and the abundance of individuals peaked at intermediate levels of urbanisation, whereas the species richness and composition were similar along the gradient (Rubio *et al*., 2012). The present study considered the most extended urban land use (i.e. residential/commercial), allowing for a better detection of how the fly community responds to urbanisation, without the influence of the cemetery particularities. We found that all the studied variables reflecting community structure responded negatively to increasing urbanisation.

Table 4. Generalized linear mixed model parameter (B), standard error SE, and Z-value for each variable included in the final model of fly larvae occurrence in water-filled tyres.

Variable code	B	SE	Z-value
Intercept	−2.895	0.237	−12.227***
PIA_1 (squared)	$-8.878e^{-5}$	$3.538e^{-5}$	−2.509*
MST	$3.438e^{-2}$	$9.212e^{-3}$	3.733***
LOC (bac)	0.844	0.224	3.760***
SIZ (65/80)	0.832	0.218	3.815***
SIZ (80/120)	1.263	0.169	7.454***
SIZ (> 120)	1.928	0.334	5.776***
PIL (4°)	−0.739	0.306	−2.412*
WAT (½)	0.519	0.162	3.201**
WAT (> ½ + full)	1.152	0.192	5.999***
SUN (sha)	0.789	0.220	3.589***
OMA (yes)	1.774	0.200	8.867***

***Significant at $P < 0.001$, **$P < 0.01$, *$P < 0.05$.
Refer to Table 1 for meanings of abbreviations.

The detrimental effects of urbanisation on fly species richness from tyres resemble the most general patterns described for many animals and plants, as reviewed by Marzluff (2001) and McKinney (2008). Our results also showed that fly species richness was reduced towards the highest urbanised sites, in agreement with conclusions by McKinney (2002) when assessing available data from different taxa. A possible explanation for this pattern is that highly urbanised areas promote higher extinction rates of native species as a consequence of habitat loss and fragmentation, in addition to the simplification of the vegetation structure, the detrimental effects of the chemical and thermal pollution, and other human disturbances (McIntyre, 2000; McKinney, 2002; McKinney, 2008). Notwithstanding this, the effects of local variables such as the geographic location of the city in its natural ecological matrix and historical and economic factors should not be disregarded (McKinney, 2008).

Species composition differed between the urban core and rural areas, and we identified groups of flies exclusive to the less or the most urbanised sites. These results reinforce the idea that, within a given insect assemblage, the impact of urbanisation is reflected by the community structure independently of the response of each particular species. Similar to our findings, Hwang and Turner (2005) recorded particular fly assemblages in the most urbanised habitats, despite the fact that the diversity showed a decreasing trend towards urban sites.

The relative abundance of larvae per occupied tyre showed the weakest response ($P < 0.05$ for PIA_{15}) in comparison with the general decreasing pattern towards the urban core. However, considering that the intensity of habitat use at the tyre and site scales (number of occupied tyres and sites, respectively) also decreased in highly urbanised areas, the overall abundance of flies is clearly negatively affected by the

Table 5. Results of ANOVA among mean values (±SE) of each response variable characterizing the Diptera community structure in sites located in different urbanisation levels according to the percentage of impervious area (PIA) estimated for different radiuses (0.5, 1 and 1.5 km).

		Urbanisation level						
		Low		Middle		High		
Variable	PIA	n	Mean (SE)	n	Mean (SE)	n	Mean (SE)	F
Occupied tyres	0.5	75	4.95 (0.38) b	31	4.77 (0.59) b	32	2.56 (0.58) a	6.25*
	1	79	5.11 (0.36) b	37	4.19 (0.53) b	22	1.91 (0.69) a	8.53*
	1.5	88	5.36 (0.33) b	30	3.2 (0.57) a	20	1.65 (0.70) a	14.20*
Relative abundance (square root)	0.5	75	3.39 (0.25)	31	3.70 (0.38)	32	2.56 (0.38)	2.51 NS
	1	79	3.55 (0.24)	37	3.07 (0.35)	22	2.59 (0.46)	1.94 NS
	1.5	88	3.64 (0.23) b	30	2.61 (0.39) a	20	2.59 (0.47) a	3.81*
Estimated richness	0.5	55	3.43 (0.13)	23	3.24 (0.14)	15	3.02 (0.26)	1.26 NS
	1	59	3.38 (0.13)b	24	3.45 (0.14) b	10	2.57 (0.22) a	3.94*
	1.5	68	3.38 (0.11)b	16	3.45 (0.20) b	9	2.55 (0.24) a	3.62*
Diversity	0.5	64	0.86 (0.05)	25	0.89 (0.08)	22	0.72 (0.09)	1.22 NS
	1	68	0.86 (0.05) b	29	0.93 (0.07) b	14	0.55 (0.10) a	4.61*
	1.5	77	0.86 (0.05)	22	0.88 (0.08)	12	0.59 (0.11)	2.58 NS
Species composition (PCA scores)	0.5	64	546.95 (0.19) b	25	546.99 (0.22) ab	22	546.07 (0.35) a	3.00*
	1	68	546.95 (0.18) b	29	546.87 (0.27) ab	14	545.84 (0.36) a	3.00*
	1.5	77	546.99 (0.16) b	22	546.52 (0.37) ab	12	545.92 (0.38) a	2.98*

*Significant ($P < 0.05$) after the correction following the false discovery rate method.
NS, not significant; PCA, principal component analysis.
Different letters indicate significant differences in the pairwise comparisons by Tukey procedure.

urbanisation process. The correlation between the proportion of containers harbouring flies and the abundance of larvae has previously been reported for small containers from cemeteries, suggesting that the frequency of occupied containers could serve as an estimation of abundance (Rubio *et al*., 2012).

Regarding the three scales of analysis for the percentage of impervious surface (i.e. $PIA_{05,1,15}$), some differences were observed among the response variables assessed. In particular, the number of occupied tyres, the relative abundance, and the estimated richness were better explained by environmental factors toward the largest spatial scale considered. This pattern was previously described for pollinator communities, in which the environmental context was more important at larger than at smaller scales (Steffan-Dewenter *et al*., 2002). It has been suggested that the relationship between communities and environmental factors can differ with the spatial scale depending on the environmental perception of the species composing the community (Steffan-Dewenter *et al*., 2002; Heatherly *et al*., 2007). Considering that the ecology of many fly species of the studied community is poorly known, the identification of the causes driving the observed differences requires a more comprehensive knowledge of the species involved.

Our findings contribute to the understanding of urban biodiversity patterns. Multi-regional approaches have been considered necessary to find general response patterns of a given community (e.g. ground-beetles) to anthropogenic disturbance, independent of the species identities (Niemelä *et al*., 2002). In this sense, discarded tyres could be used as a study model due to their existence across urban settlements in all regions. Finally, the coexistence of nuisance mosquitoes with other dipteran species gives rise to a well-known dilemma: to control or to conserve? Samways (1996) concluded that it is urbanisation itself, not the act of urban pest control, that is in conflict with biodiversity conservation. However, further studies are needed focusing on the potential loss of urban flies as a consequence of mosquito chemical control. Such an assessment must necessarily include the taxonomic identification of all individuals at the species level, probably the main shortcoming of our research.

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