Mosquito fauna inhabiting water bodies in the urban environment of Córdoba city, Argentina, following a St. Louis encephalitis outbreak

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ABSTRACT: An understanding of urban aquatic environments as mosquito larval habitats is necessary to prioritize sites for surveillance and control of arbovirus vectors in urban areas. Natural and artificial water bodies at ground level that may be larval mosquito habitats in Córdoba city, Argentina were surveyed. Data on the characteristics of aquatic sites and the presence and abundance of mosquito larvae and pupae were collected in the summer of 2006, coinciding with the first report of human WNV and following an outbreak of St. Louis encephalitis in 2005. Eight species in the genera *Aedes, Culex,* and *Mansonia* were identified. At 64.2% (34 of 53) of the sites, only one species was collected, while 3.8% (2 of 53) had three associated species, the highest richness found per site. *Culex quinquefasciatus* represented over 99% (out of 32,729) of the specimens. It was also the most widely distributed and detected under diverse habitat conditions. Although puddles and semi-permanent pools harbored a greater number of species, drainages and channels may be more relevant as risk factors from an epidemiological point of view because they showed the highest larval densities, mainly of *Cx. quinquefasciatus* (vector of SLE and WNV). Also, higher densities of this species were associated with stormwater runoff and sewage water, thus water management systems should be targeted and closely monitored for mosquito control purposes. ***Journal of Vector Ecology*** **35 (2): 401-409. 2010.**

Keyword Index: Culicidae, larvae, ground water, larval habitats.

INTRODUCTION

Mosquitoes are of medical, veterinary, and economic concern due to their role as vectors of human and other animal diseases. At least 18 arboviruses are known to circulate in Argentina, including St. Louis encephalitis virus (SLEV), West Nile virus (WNV), and dengue. However, the vectors and reservoirs involved in most arboviral diseases are not well known. Field and laboratory evidence indicates that *Cx. quinquefasciatus* is an efficient vector of SLEV in Argentina (Mitchell et al. 1980, 1985) and may transmit WNV as well, as it does in North America (Gleiser et al. 2007).

St. Louis encephalitis virus occurs from Canada to Argentina. In North America it causes outbreaks usually related to climatic factors (for example, see Shaman et al. 2004). In South America the number of documented human infections is usually low. However, recent outbreaks in Argentina in 2005 (Spinsanti et al. 2007) and 2010 (PAHO 2010), and Brazil in 2007 (Mondini et al. 2007) indicate either that SLEV prevalence is underreported or that SLEV is reemerging. West Nile virus (WNV) was first reported in the Americas in 1999 in birds and humans in New York City (Nash et al. 2001), and has spread, reaching Argentina probably in 2005 (Díaz et al. 2008, Petersen and Hayes 2008). The first human cases of West Nile virus in Argentina were reported in 2006 in Córdoba province (in Córdoba city) and in Chaco province.

Many of the WNV- and SLEV-competent vectors in North America, such as *Cx. quinquefasciatus,* do not disperse long distances (not exceeding 2 km range), thus identification of their larval habitats allows for effective targeted control strategies (CDC 2003, Irwin et al. 2008). Dispersal capacity of mosquitoes in Argentina is scarcely known, but it is likely that common species such as *Cx. quinquefasciatus* may have similar characteristics. An understanding of urban aquatic environments as mosquito larval habitats is necessary both for understanding the ecology of the arbovirus vectors and to prioritize sites for surveillance and control in urban areas (Irwin et al. 2008).

The number of investigations of South American mosquito larval habitats has been increasing in recent years. Potential temporary and permanent larval habitats in Argentina have been described in the provinces of Chaco (Stein et al. 2005), Corrientes (Oria and Oscherov 2002), Buenos Aires (Campos et al. 1993, Fischer et al. 2002, Fischer and Schweigmann 2004), and Córdoba (Almirón and Brewer 1996, Gleiser and Pires 2009). In urban environments, most research has focused on containers (Stein et al. 2002), due to the reintroduction and increase in the incidence of dengue (Vezzani and Carbajo 2008). Although *Aedes aegypti* is traditionally considered a container or tree hole mosquito dwelling in clear water, recent reports show it may be able to exploit other environments previously overlooked. In Cuba, immature stages were found in domestic sewage, septic ditches, and storm drainages (Marquetti et al. 2005). In Puerto Rico, not only were larvae and pupa detected in septic tanks, but productivity from these sources was the highest reported (Barrera et al. 2008).

This report presents the results of a survey of urban ground water (or aquatic environments) that may serve as larval mosquito habitats in Córdoba city, Argentina. Data were collected in the summer of 2006, as part of a study on mosquito ecology, to obtain preliminary data on ecological factors related to immature mosquito presence and density in urban aquatic habitats, coinciding with the first report of a human case of WNV, and following an outbreak of St. Louis encephalitis in 2005. Presence, relative abundance, and variation in mosquito diversity were assessed in relation to habitat characteristics.

MATERIALS AND METHODS

Study area

Cordoba is a city of approximately 1,290,000 inhabitants (INDEC 2001). It is located in Cordoba Province, in the center of Argentina (64° 11'W–31° 24'S) at 360 to 480 m above sea level. The climate is temperate, mesothermal, with an average summer temperature of 24° C and an average winter temperature of 11° C. The average annual rainfall ranges between 750 and 800 mm, and rainfalls prevail during October to December and March (Jarsun et al. 2003). The area is included in the Chaco phytogeographic province and has been subjected to intense ecological modifications related to antropic activities (urbanization, industrial development, agriculture, farming, etc.).

Mosquito sampling

Sampling was carried out between January and April 2006 (summer and early fall). The time frame covers the warm and rainy season, when it is more feasible for temporary and semi-permanent pools to occur in the area, as well as when the occurrence of peaks of mosquito abundance are more likely (Gleiser et al. 2000). Samples were collected from 53 natural and artificial water bodies at ground level (pools, ditches, channels, lagoons, water fountains, etc.) in public spaces of the city, mainly in parks, road sides, and margins of water ways. Sampling site selection was based on a combination of random and opportunistic criteria, aiming to cover a variety of conditions characteristic of the city. As a starting point, public sites that may contain bodies of water were located on a map and randomly selected for field confirmation, and sampled if water was detected. When other accessible aquatic sites were detected en route, those were also sampled. In general, 12 to 20 samples were collected from each site with a 600 ml dipper. When no larvae or pupae were detected, additional samples (up to 40) were collected to minimize false negatives. In bodies of water that included microhabitats with evident differences, such as vegetated and bare areas, samples were taken from the different sectors.

Immature stages were counted *in situ* and transported to the lab for further processing. Specimens were killed by immersion in water at 60° C, to avoid shrinking, distortion, and darkening (Rossi and Almirón 2004) and stored in 80% ethanol for taxonomic determination. Third and 4th instar larvae were determined to species level based on morphological characters, while 1st and 2nd instars were determined to genus (Darsie 1985). The abbreviation of generic names is in accordance with the proposal of Reinert (1975). Samples including > 600 specimens were subdivided, such that a minimum of 400 random individuals were identified.

Larval habitat characterization

In each site, the following larval habitat characteristics were recorded (adapted from Zaim 1987, Almirón and Brewer 1996, and Ebsworth et al. 2001):

a) **Nature of water body** (natural or artificial). Artificial bodies were those whose presence and/or characteristics originate from anthropogenic actions (for example, channels, fountains, ditches, drainages, leaking from water pipes) (Irwin et al. 2008). Natural water bodies in general were flooded areas, ponds, and pools that exist due to natural geologic conditions and were not formed by intentional alterations by humans.

b) **Type of water body.** Transient puddle, semi-permanent pool, lagoon, drainage ditch, irrigation channel, pit or opening on concrete ground, fountain.

c) **Permanence.** Transient (holds water intermittently, usually associated with rainfall), semi-permanent (holds water continuously during the rainy season but dries in drier seasons) or permanent. Permanence was assessed from repeated visits and consultation with neighbors to the site.

d) **Plant cover**. Recorded as present or absent.

e) **Vegetation type.** Algae, submerged aquatic plants, floating plants, flooded land plants.

f) **Sunlight exposure**. Sunny, part shade, shade.

g) **Turbidity**. Visually estimated as clear, cloudy/murky, or turbid (when the bottom of the submerged white dipper could not be clearly distinguished).

h) **Water flow rate**. Qualitatively categorized as stagnant, when water movement was not visible; slowly moving, when surface water movement was barely perceptible; fast water, having obvious surface movement with agitation.

i) **Water source.** That most contributed to surface water: rainfall, flooding from river/stream, sewage, pipe water (in fountains and from leaking pipes), stormwater runoff.

j) **Bottom substrates**. Muddy, sandy, rocky/pebbly, or concrete.

k) **Water surface**. Average length x width or diameter.

l) **Average depth.** Estimated from repeated measures taken from the border to the center of the water body. In ditches (and similar linear surfaces) measurements were taken every 3 m.

Data analysis

Larval density (abundance) at each site is reported as the average number of mosquitoes (per species, stages III+IV) per dip (l/d), in real numbers. The following larval density categories were adopted (adapted from Carron et al. 2003): null (larvae were not detected), low (less than 35 l/d), medium (35 to 200 l/d), and high (more than 200 l/d). Correspondence analyses (CA) were carried out to identify relations between habitat characteristics (most of them qualitative) and either presence/absence or larval density categories of a mosquito species. This exploratory analytical data technique is useful to identify systematic relations between categorical variables when there are no (or rather incomplete) *a priori* expectations as to the nature of those relations (Jongman et al. 1995, Quinn and Keough 2002). The main purpose of CA is to summarize the lack of independence between variables in rows and columns of a contingency table as a small number of derived variables known as principal or correspondence analysis axes. The scores for each row and column variable on these axes are used in the ordination plot; points occurring close together on the axis are more similar in their responses, while those at a distance are less similar.

RESULTS

From a total of 53 sites, 79.2% were categorized as artificial (fountains, drainages, channels, pit, or opening on broken concrete ground) and 20.8% as natural (lagoons, ponds, and pools) water surfaces. Of the sampled sites, 47.2% were temporary, while 26.4% were semi-permanent and 26.4% permanent. Eight species of the genera *Aedes, Culex,* and *Mansonia* were identified (Table 1). At 64.2% of the sites, only one species was collected, while 3.8% presented three associated species, and this was the highest richness found per site. Water bodies where more than one species was simultaneously detected were located in the periphery of the city; those with only one species were widespread, while negative sites were located mostly in the center of the city. Overall, more species were collected in natural than in artificial water bodies (Table 2), and there were no evident differences in relation to water permanence. Pools or puddles (both temporary and semi-permanent) were the larval habitats that presented the highest culicid richness. No culicid immatures were detected in pools in the stream/river margins.

Culex quinquefasciatus was the most frequent species, representing over 99% of the specimens. It was also the most widely distributed, and was detected in 56.6% of the sites, in most habitats explored (except lagoons, river/creek margin pools), and under diverse conditions (plant cover, sunlight, depth, etc.). It showed the highest larval density of all species (644 l/d), however larval density presented large variations among sites (s=152.50).

The other species found, in decreasing order, were *Cx. dolosus* Lynch Arribalzaga, *Aedes albifasciatus* (Macquart), *Cx. maxi* Dyar, *Mansonia indubitans* Dyar & Shannon, *Cx. brethesi* Dyar, *Cx. pilosus* (Dyar & Knab), and *Ae. aegypti*. All were detected in densities below 15 l/d. The numbers of specimens, percentage collected of each species, and number of sites where detected, are presented in Table 1. Five sites were excluded from further analysis as only larvae I and II were collected and they could not be determined to species level. *Cx. quinquefasciatus* was the only mosquito species in 90% of the sites where it was found, while in the remaining 10% of the sites it was associated with other *Culex* species.

Considering that most species were found in one and up to seven sites (*Cx. maxi*), further analysis of habitat associations was only assessed for *Cx. quinquefasciatus*. Figure 1 shows associations between the presence/absence of *Cx. quinquefasciatus* larvae and water body permanence (Figure 1a) and sun exposure (Figure 1b). Larval presence was more closely associated with temporary or semi-permanent water bodies than with permanent water bodies. The presence of larvae was associated with shade or partial shade.

Larval density was significantly associated with nature (Figure 2a) and type of water body (Figure 2b), plant cover (Figure 2c), and water source (Figure 2d). Medium and high larval densities were associated with artificial water

Table 1. Mosquito larvae species collected in urban water bodies of Córdoba city, Argentina. Number of specimens collected (percentage of total collections in parenthesis) and number of sites where each species was detected are shown. Same letter in the last column indicates species that were found together in at least one site; a U indicates a species was by itself in at least one site.

Species	**Specimens (%)**	**Sites collected**	**Coexisting**
Ae. aegypti	1 (0.003)	1 (1.89)	U
Cx. brethesi	12 (0.04)	1 (1.89)	A
Cx. dolosus	136 (0.4)	2 (3.77)	U, A
Cx. maxi	65 (0.20)	7 (13.2)	U, B, C
Cx. pilosus	4 (0.01)	1 (1.89)	B
Cx. quinquefasciatus	32,425 (99.07)	30 (56.60)	U, A, C
Ae. albifasciatus	16 (0.05)	3 (5.67)	U, B
Ma. indubitans	70 (0.21)	1 (1.89)	U

Ae. = Aedes; Cx. = Culex; Ma. = Mansonia.

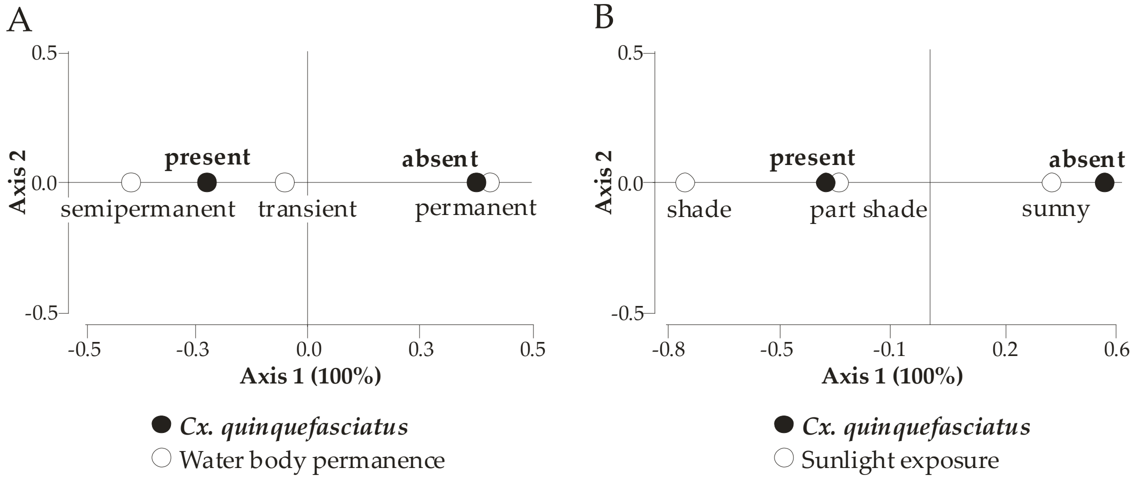

Figure 1. Correspondence analysis (CA) between presence and absence of *Culex quinquefasciatus* in ground water bodies in Córdoba city and a) permanence of water body; b) sunlight exposure. Percentage explained by CA axis is shown in parenthesis.

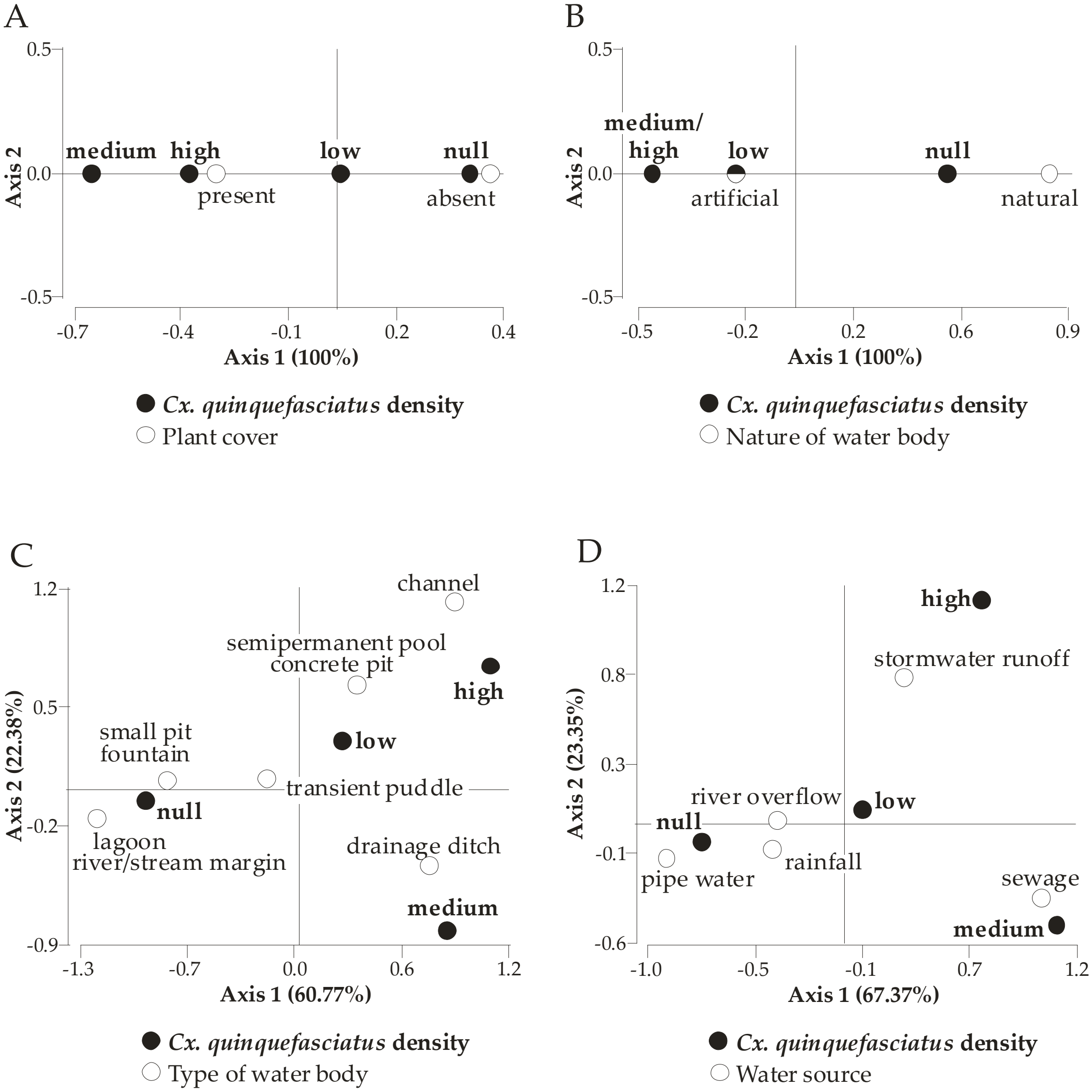

Figure 2. Correspondence analysis (CA) between larval density of *Culex quinquefasciatus* in ground water bodies in Córdoba city, and a) nature of water body; b) type of water; c) plant cover; d) water source. Percentage explained by each CA axis is shown in parentheses.

Table 2. Characteristics of the urban water bodies of Córdoba city, Argentina, where Culicid larvae were found.

		Ae. aegypti	*Cx. brethesi*	*Cx. dolosus*	*Cx. maxi*	*Cx. pilosus*	*Cx. quinq.*	*Ma. indub.*	*Ae. albif.*
Nature of water body	Natural		X	X	X	X	X	X	X
	Artificial	X			X		X		
Type of water body	Channel						X		
	Small pit	X							
	Semipermanent pool		X	X			X		
	Transient puddle				X	X	X		X
	Drainage ditch						X		
	Fountain				X		X		
	Pit on concrete ground						X		
	Lagoon			X				X	
Permanence	Transient	X			X	X	X		X
	Semipermanent		X	X	X		X		
	Permanent			X	X		X	X	
Plant cover	Absent				X		X		
	Present	X	X	X	X	X	X	X	X
Vegetation type	Algae				X		X		X
	Submerged aquatic			X	X	X	X		X
	Floating plants			X			X	X	
	Flooded land plants	X	X	X	X	X	X		X
Sunlight exposure	Sunny	X			X	X	X	X	X
	Part shade		X	X	X		X		
	Shade						X		
Turbidity	Clear			X	X	X	X		X
	Cloudy/murky	X	X	X			X		
	Turbid						X	X	X
Water flow rate	Stagnant	X	X	X	X	X	X	X	X
	Slowly moving						X		
Water source	Rainfall	X			X	X	X	X	X
	River/stream overflow		X	X			X		
	Sewage						X		
	Stormwater runoff			X			X		
	Pipe water				X		X		
Bottom substrate	Muddy		X	X	X	X	X	X	X
	Sandy						X		
	Rocky/pebbly						X		
	Concrete	X			X		X		
Water surface (m^2)	Less than 5	X	X	X			X		
	5 - 15				X		X		
	15 - 50				X	X	X		X
	50 - 100				X		X		
	100 - 600				X		X		X
	More than 600			X			X	X	
Average depth (cm)	Less than 10			X	X		X		X
	10 - 20	X	X	X	X	X	X		X
	20 - 30				X		X		
	30 - 40						X	X	
	More than 40							X	

bodies (Figure 2a) where plants were present (Figure 2c). The first two axes of the correspondence analysis of *Cx. quinquefasciatus* density and larval habitat type explained 83.8% of the lack of independence between the two variables (Figure 2c). Medium and high densities were associated with ditches and channels; absence was associated with lagoons, river/stream margin, and fountains. The first two correspondence axes of water origin explained 90.72% of larval density and indicated that high densities of *Cx. quinquefasciatus* were associated with stormwater runoff; medium densities were associated with sewage water, and absence was related with pipe water, rainfall, and river/stream flooding (Figure 2d).

DISCUSSION

The survey of aquatic habitats in Córdoba city during the warm and rainy seasons detected more species from natural water bodies than from artificial water bodies. Other studies have also reported that natural water bodies were richer in species composition than artificial ones (Almirón and Brewer 1996, Stein et al. 2005). Most culicids have rapid preimaginal development that may allow them to take advantage of temporary aquatic habitats (Fischer and Schweigmann 2004). However, no differences were detected in species richness between water bodies differing in water permanence, as was also reported by Fontanarrosa et al. (2004) in Buenos Aires. On the other hand, in Buenos Aires, Fischer and Schweigmann (2008) found higher species richness in long duration flooding events compared to short duration events. In Resistencia city (Chaco province), *Culex* species were related to permanent water bodies, while *Aedes* and *Psorophora* were associated with transient environments (Stein et al. 2005). Most species detected in the present study develop in a relatively short time, so other factors besides water permanence may be more relevant for the suitability of a site for mosquito breeding, such as nutrient availability, or presence of competitors or predators, etc. (for example, Caillouët et al. 2008).

Puddles and semi-permanent pools together were the habitat types where the highest number of species was collected, while drainages and channels showed the highest larval densities, with *Cx. quinquefasciatus* usually found as the only species. In public fountains, only *Cx. maxi* and *Cx. quinquefasciatus* were detected, both in comparatively low densities; however, it is likely that the ability of these and other species to take advantage of this habitat type may have been underestimated due to cleaning and larviciding activities carried out at the time by the city municipality. It would be interesting to confirm whether other species may dwell in fountains, and if so, to test whether *Cx. quinquefasciatus* is more resistant than the other species to the control measures. On the other hand, although the number of species observed in lagoons was scarce, an opposite pattern was reported in Buenos Aires Province (Fontanarrosa et al. 2004) and Resistencia (Chaco Province) (Stein et al. 2005).

In general, the habitat characteristics of the sites where the different species were found were consistent with what has been documented in the literature. *Cx. quinquefasciatus* was collected from a wide array of habitat types and conditions, although its presence was more associated with artificial water bodies. Other studies in Argentina and abroad indicate that *Cx. quinquefasciatus* takes advantage of a wide range of larval habitats (Oria and Oscherov 2002, Fischer and Schweigmann 2004), including temporary water containers, cisterns, tires, flowerpots, and septic tanks (Almirón and Brewer 1996, Stein et al. 2002, Rossi and Almirón 2004, Hribar 2007, Burke et al. 2010), which were not examined in the present study. The presence and/or density of immature *Cx. quinquefasciatus* is frequently associated with polluted waters with a high content of organic components (Ishii and Sohn 1987, Forattini 2002, Burke et al. 2010). Consistently, correspondence analysis showed an association between medium and high densities of larvae and water bodies from storm runoff and sewage, where organic debris and other pollutants may contribute to its eutrophication. These conditions were mostly observed in ditches and channels in the city. Storm drains were the most productive environment for the species in Corrientes city (Corrientes Province) (Oria and Oscherov 2002) and in Resistencia (Chaco Province) (Stein et al. 2005). As reported by others, *Cx. quinquefasciatus* preferred water bodies not directly exposed to sunlight (Forattini 2002, Almirón and Rossi 2006), and where vegetation was present (Almirón and Brewer 1996). *Culex quinquefasciatus* was not only the most frequent species in terms of the percentage of sites where it was detected, but in most cases it was also the only culicid species at a site. During an SLE outbreak recorded in Córdoba between February and April 2005, *Cx. quinquefasciatus* represented 84% of mosquito adults collected with miniature light traps (Díaz et al. 2006). Another study between October, 2005 and March, 2006 also showed *Cx. quinquefasciatus* to be the most frequent species in adult collections, and that female abundance tended to increase with increasing surface cover (Gleiser and Zalazar 2010). Taken together, these observations reflect the high adaptation of *Cx. quinquefasciatus* to the urban environment. Larval habitats of *Cx. maxi* include puddles, temporary and permanent pools, irrigation channels, ditches, river margins, swimming pools, both shaded or sunny, with clear to turbid water, with or without vegetation, and also some artificial containers (Almirón and Harbach 1996, Oria and Oscherov 2002, Stein et al. 2002, Rossi and Almirón 2004). In the present study, *Cx. maxi* was found both in natural environments (temporary pools and puddles) and in artificial water bodies (fountains), indicating an adaptation of this mosquito to the urban environment. *Culex dolosus* was collected from natural permanent and semi-permanent water bodies, with transparent water and aquatic vegetation. A survey of 42 sites throughout Córdoba province carried out almost two decades ago (Almirón and Brewer 1996), found *Cx. dolosus* both in natural and artificial wet environments, either temporary or permanent, usually with aquatic vegetation with turbid to clear water and also in river margins. In Buenos Aires, Fontanarrosa et al. (2004)

have collected larvae in lagoons with or without vegetation and in temporary pools in parks, with more frequent collections in permanent environments. Our observations are consistent with Campos et al. (1993), who indicate that in urban environments *Cx. dolosus* is associated with clear water based on a considerable decrease of their density in drainages within urban areas of Buenos Aires as pollution increased. However, between April and November (2006-2007, 2007-2008) larvae of this species were collected (although at a low density, up to 2 l/d) in a lagoon to the east of Córdoba city covered by *Pistia stratiotes*, with turbid water and evidently polluted based on the observation of floating trash (Gleiser and Pires 2009).

Aedes albifasciatus was collected from shallow temporary pools, a habitat described as typical by Forattini (2002) and Ludueña Almeida et al. (2002). These authors also report larvae from sites independently of the presence of vegetation and insolation. Fischer et al. (2002), on the other hand, observed a positive association between the presence of *Ae. albifasciatus* larvae and vegetation cover and a negative association with insolation. In the present study, all sites had plant cover and were in the sun. Although in Buenos Aires, *Ae. albifasciatus* breed frequently in temporary rain pools, a low proportion of positive sites (25% of temporary pools sampled) was detected in Córdoba. Considering that sites were not revisited, this may be due to the short development time of *Ae. albifasciatus* and synchronic emergence of adults (Gleiser et al. 2000). According to previous studies, larval abundance is highest immediately after flooding of a site and, during the warm season, decreases after the first week (Ludueña Almeida et al. 2002, Fischer and Schweigmann 2008).

Our results show that mosquito species of sanitary interest breed in urban ground water bodies of Córdoba. Although puddles and semi-permanent pools harbored a higher number of species, drainages and channels may be more relevant as risk factors from an epidemiological point of view because they showed the highest larval densities, mainly of *Cx. quinquefasciatus* (vector of SLE and WNV, as stated above). Also, higher densities of this species were associated with stormwater runoff and sewage water. Water-management systems should be closely monitored for mosquito control purposes. Not only should vector control efforts target these habitats, but drainage systems should be improved to avoid water spill-off and thus reduce availability of larval habitats. Further studies are underway to assess seasonal mosquito productivity of urban ground water bodies.

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