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Artificial container mosquitoes and first record of *Aedes aegypti* in the islands of the Paraná Lower Delta, Argentina

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ABSTRACT

Mosquitoes in artificial containers include *Aedes aegypti* and the *Culex pipiens* complex, both recognized worldwide as vectors of diseases. The goal of this study was to characterize mosquito communities in water-filled artificial containers in the islands of one of the major temperate wetlands in South America, and to assess whether *A. aegypti* is present in the area. Five domestic areas located in the insular Tigre District (Buenos Aires Province, Argentina) were visited monthly between November 2011 and May 2012. A total of 1013 artificial containers (half of them with water) were inspected for mosquito immatures. 3359 specimens corresponding to seven species were collected in 88 containers. *A. aegypti* was recorded for the first time in this wetland, and in all land use categories examined from February to May. Among the remaining six species, only *Culex dolosus* and *C. pipiens* were highly abundant. 88% of the mosquito positive containers were buckets, dustbins and boats, whereas highly available bottles did not act as mosquito breeding habitats; the key breeding container was different for each land use. The Container Index showed differences among land uses, materials, water capacity and volume, and insolation levels. Generalized Linear Mixed Models suggested that the probability of finding mosquitoes was higher for containers located in dumps and recreational areas, made of resin/fiberglass, presenting intermediate water volumes, and not in use if partially or totally shaded but in use for sunlit containers. Recommendations for mosquito prevention and control in the islands are proposed.

Introduction

Mosquitoes are a great epidemiological concern due to their role as vectors of pathogens to humans and animals. From a socio-economic perspective, mosquito transmitted diseases are highly detrimental in terms of human deaths (CDC, 2013) and enormous costs to the sanitary system (WHO, 2013). Among the most world-known mosquitoes are *Aedes aegypti*, the main vector of dengue in America, and the members of the *Culex pipiens* complex, which transmit different arboviruses and filariasis (Becker et al., 2010).

In Argentina, *A. aegypti* was reintroduced in 1986; since then, its geographical distribution has expanded towards the south and the west, and dengue outbreaks have been recorded throughout the country (Vezzani and Carbajo, 2008; MSN, 2009). Also, the recent transmission of Chikungunya virus by *A. aegypti* in neighboring countries constitutes a new threat at the local level (Carbajo and Vezzani, 2015). Regarding *C. pipiens* transmitted diseases, St. Louis encephalitis caused nine casualties in 2005 (Spinsanti et al., 2008) and the transmission of West Nile virus has been demonstrated in horses (Morales et al., 2006). Dengue and St. Louis encephalitis have both expanded towards the south of the continent during the past years, and autochthonous cases of both diseases have been reported in Buenos Aires Province (MSN, 2009; López et al., 2010).

The Lower Delta of the Paraná River stands as one of the most important wetlands in South America. Its islands have recently experienced drastic changes in land use and land cover, developing into a complex mosaic of natural and modified patches. Even though population density is low (<1 inhab/ha), around half of its extension is affected by human activities, mainly Salicaceae plantations and tourism (Kandus and Malvárez, 2004). These are reinforced by its close proximity to Buenos Aires City, the second megalopolis in South America with 14,308 inhab/km^2 (INDEC, 2010). As all wetlands, the Paraná Lower Delta offers a high availability and diversity of aquatic environments, which are optimal for the development of immature stages of mosquitoes (Dale and Knight, 2008). Among them, temporary or permanently flooded depressions in the ground and phytotelmata are abundant in the area and their mosquito communities have been studied during the past years (Albicócco et al., 2011; Cardo et al., 2011a, 2011b, 2012a, 2012b). On the contrary, artificial containers are scarce and

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limited to inhabited dwellings, recreation parks and their immediate surroundings.

Mosquitoes breeding in artificial containers have been widely documented due to their epidemiological importance and their intimate contact with human settlements, and the association with different characteristics of the containers has been studied in detail (e.g., Leisnham et al., 2006; Vezzani and Albicócco, 2009; Rubio et al., 2011). However, no research regarding mosquitoes in artificial containers has ever been performed in the Delta of the Paraná River and, in general, the structure and ecological characteristics of these communities in suburban or rural environments within the region have been poorly studied (e.g., Rubio and Vezzani, 2011). To contribute to the knowledge of mosquito ecology in the region, the main goal of this study was to characterize mosquito communities in water-filled artificial containers in a temperate wetland located near the biggest urban center in Argentina, and to assess if the main dengue vector in South America is present. Specifically, we attempted to: a) describe species composition and their relative abundance; b) determine the presence of *A. aegypti*; c) evaluate the effect of container characteristics and insolation levels on mosquito occurrence; and d) assess the relative importance of different domestic land uses as a source of mosquito infestation.

Materials and methods

Study area

The Paraná Lower Delta is a 2700 km^2 wetland macromosaic which extends over 300 km through the terminal portion of the Paraná River basin. The deposition of sediments by the river determines the constant development of newly formed pan shaped islands (Kandus et al., 2003). These are characterized by temporarily or permanently flooded central areas with herbaceous vegetations, and levees up to 3 m high dominated by forests. The climate is temperate sub-humid, with annual mean temperature and cumulative precipitation values of 16.7 °C and 1073 mm, respectively.

The study was carried out in the first section of islands from Tigre District, Buenos Aires Province, Argentina (34°25′S, 58°34′W). Five sites with a high degree of human activities were selected to guarantee the presence of artificial containers (Fig. 1).

Study design

The five sites were visited monthly from November 2011 to May 2012, being the period of highest abundance of mosquito immatures in artificial containers in Buenos Aires Province throughout the urbanization gradient (Vezzani and Albicócco, 2009; Rubio and Vezzani, 2011; Rubio et al., 2011). Three land uses were distinguished: residential, recreational and dump. Two sites were residential areas composed of 15–20 houses including their front and back yards (each house in the range 35–150 m^2), typically inhabited all-year. The other three sites were 8–16 ha plots used as recreational areas, usually maintained by a manager and visited by tourists mostly during the summer and weekends. In addition, within each island peridomestic areas with dumping grounds were distinguished because they constitute an important and distinct source of artificial containers.

Field and laboratory work

During each visit all artificial containers were inspected, and the type (e.g. bucket, jar, boat) and number of containers with and without water were quantified. For those holding water, the total capacity was categorized as ≤1 L, 1.1–10 L, 10.1–100 L, 100.1–1000 L and >1000 L. The water volume in each container was measured up to 5 L using graduated pipettes and buckets, and estimated for larger volumes. Also, the following qualitative variables were recorded: insolation level (sun, partial shade, total shade), material (plastic, metal, resin/fiberglass, others) and usage (with an apparent function or abandoned). The sampling effort was approximately 4 h/person for each site per month. All the field work was performed by the same person in order to guarantee consistent estimations of quantitative and qualitative variables.

Fig. 1. Location of the 5 sites surveyed (white rings) in the Buenos Aires Lower Delta of the Paraná River, Argentina.

In containers of capacity < 5 L, the content was filtered with a fine mesh strainer and immatures were collected with a pipette. In larger containers, the content was homogenized and the strainer was passed 6–8 times to collect a sample (Silver, 2008). Larvae were preserved in 70% ethanol in the field, while pupae were transported alive to the laboratory and reared until adult emergence.

All larvae 3–4 and adults were morphologically identified to species under stereoscopical microscope following Rossi et al. (2002). As *Culex dolosus* and *C. eduardoi* are considered a species complex (Senise and Sallum, 2008) and their larvae cannot be identified with the currently available keys, they were grouped as *C. dolosus s.l.* Likewise, the members of the *C. pipiens* complex (including *C. pipiens s.s.*, *C. quinquefasciatus* and their hybrids) were not distinguished and grouped as *C. pipiens s.l.*

Data analysis

All the containers inspected were included in a general description of the diversity and availability of artificial containers. However, it was noted that none of the bottles acted as breeding habitats for mosquitoes and 87% of them were present in dumps. Therefore, to avoid confusion among land uses, bottles were excluded from all subsequent analyses.

Univariate

The Container Index (CI), defined as the percentage of containers harboring immatures of mosquitoes among all water-holding containers inspected, was calculated as an estimator of relative abundance (Silver, 2008). The CI values for containers of different characteristics, insolation levels and land uses were compared with a Chi-square test for two or multiple independent proportions according to the number of categories (Fleiss et al., 2003). A posteriori pairwise comparisons were performed by Tukey's procedure (Zar, 1999; Abramson and Gahlinger, 2001) and the Cochran–Armitage test for linear trend was also applied for ordered categories. Statistic comparisons were performed with WINPEPI software (Abramson, 2004).

Multivariate

Generalized Linear Mixed Models were used to analyze mosquito occurrence as a function of the environmental variables considered. The presence of immatures per container was modeled assuming a binomial distribution of errors and applying the logistic function as link. Several random terms (e.g., site, sampling month, land use category and nested combinations) were evaluated on the full set of variables and the model that yielded the lowest Akaike Information Criterion value, as a measure of goodness-of-fit, was selected (Zuur et al., 2009). A quadratic term for the only continuous variable (water volume) and interactions were also tested. Potential collinearity issues were avoided by only allowing terms with variance inflation factors ≤ 4 (Zuur et al., 2010). A manual backward stepwise protocol was performed to remove nonsignificant variables one by one. Once all remaining variables were significant, the levels in a factor that were not significantly different were merged together (Nicholls, 1989). The final model parameters were bootstrapped to discard the effect of very influential observations. To assess the accuracy of the selected model, the agreement Kappa index (K), which indicates the classification improvement of the final model over chance (Fielding and Bell, 1997) and overcomes unequal number of presences and absences (Titus and Mosher, 1984), was calculated. Given that the predicted values are a probability, K was calculated for each 0.01 cut-off point between the complete range of possible values (0–1) and the point that provided the best value of K was reported as the optimal. Modeling was performed in R 3.1.1 (R Core Team, 2014) with lme4 (Bates et al., 2014), car (Fox and Weisberg, 2011) and boot (Canty and Ripley, 2014) packages.

Table 1
Total number of immatures (L3–L4-pupae) collected, number of breeding containers and sites with record for each species breeding in artificial containers in the Paraná Lower Delta.

Species	No. of immatures	No. of breeding containers	No. of sites with record
Culex dolosus s.l.	2387	71	5
C. pipiens s.l.	822	31	5
C. maxi	50	4	2
C. tatoi	28	10	4
C. bidens	3	2	2
C. chidesteri	2	2	1
Aedes aegypti	67	7	3

Results

Out of the 1013 artificial containers inspected, 506 (50.3%) held water and 88 (17.4%) of such harbored immatures of mosquitoes. A total of 3359 specimens (L3–L4-pupae) were collected and identified to seven species belonging to the genera *Aedes* and *Culex* (Table 1).

First record of A. aegypti in the islands

Immatures of *A. aegypti* were recorded from February to May. Its presence was confirmed in the three domestic land use categories defined (residential, recreational and dump) at three of the five sites inspected. In total, seven breeding containers and 67 immatures (63 L3–4 and 4 pupae) were registered. In five of the positive containers, only one immature was found.

Container types

A total of 1006 (99.3%) containers were successfully classified in 14 categories considering their general features (Table 2). Six of them, namely bucket, bottle, dustbin, dish, boat and glass, accounted for 80% of the containers, whereas bag, basin, pot, water tank, car tire, pool and fountain added 12%. The remaining 8% were pooled as “others”, including diverse artificial containers in very low numbers.

Water availability varied substantially among container types, in the range 21.4–88.2% (Table 2). The most abundant water-holding containers were bucket, bottle, dustbin and boat; however, bottles did not act as breeding containers, whereas the highest CIs were recorded for the other three categories (Table 2).

Table 2
Total number of containers, with water and with mosquitoes and Container Index (CI) for each container category.

Container category	No. of containers			CI (%)
	Total	With water (%)	With mosquitoes	
Bucket	232	103 (44.4)	18	17.5
Bottle	186	94 (50.5)	0	0
Dustbin	123	80 (65)	28	35
Dish	112	24 (21.4)	1	4.2
Boat	87	69 (79.3)	32	46.4
Glass	67	18 (26.9)	1	5.6
Bag	39	10 (25.6)	0	0
Basin	32	16 (50)	2	12.5
Pot	13	7 (53.8)	0	0
Water tank	17	15 (88.2)	1	6.7
Car tire	7	2 (28.6)	0	0
Pool	7	5 (71.4)	1	20
Fountain	7	4 (57.1)	0	0
Others	77	59 (76.6)	4	6.8
Total	1006	506 (50.3)	88	

Land use

Container availability varied among land uses; the number of containers in dumps (497) approximately doubled the values in residential (274) and recreational areas (242). However, the percentage of water-holding containers was significantly lower in dumps than in the other land uses ($X^2_{(2)} = 70.46$; $p < 0.0001$; 36.6%, 65% and 60% respectively), rendering similar potential mosquito breeding containers in the three domestic environments.

The availability of the different categories of containers and the most common breeding container (i.e. >50%) differed markedly among land uses. In residential areas, 83% (19/23) of the breeding containers were boats, in recreational areas 53% (19/36) were dustbins and in dumps 54% (14/26) were buckets (Fig. 2). After bottle exclusion, the CI in dumps (CI = 29/100 = 29.0%) was significantly higher than in residential (CI = 23/169 = 13.6%) and recreational areas (CI = 36/143 = 25.2%) ($X^2_{(2)} = 10.76$; $p = 0.005$).

Regarding the total number of mosquitoes, a similar number of immatures was collected at each land use category, ranging between 1037 and 1249 specimens. *C. dolosus* and *C. pipiens*, the two species with the highest CIs, were collected in the three land uses. The former presented high values in the three categories, whereas the latter was abundant only in recreational areas. The total number of species per land use was similar, between 5 and 7.

Material

Water-holding containers were assigned to 11 materials. The most abundant were metal, plastic and resin/fiberglass, followed by ceramic (17), cement (9), glass (5), wood (3), cardboard (2), rubber (2) and foam (1), all of which were grouped as "others". Significant differences in CIs were detected among materials ($X^2_{(3)} = 32.49$; $p < 0.0001$), with higher values in resin/fiberglass (CI = 41%, n = 68) and metal (28%, 111) than in plastic (14%, 194) and others (3%, 39). The highest number of breeding containers and species was recorded in metallic containers, followed by the ones made of plastic and resin/fiberglass. The most abundant species, *C. dolosus*, was collected in the four material categories.

Capacity and volume

The CI increased with the capacity of the container ($X^2_{(4)} = 31.95$; $p < 0.0001$) with a significant positive lineal tendency ($X^2_{(1)} = 29.16$; $p < 0.0001$) (Table 3). However, in pairwise comparisons only the lowest capacity category (≤1 L) presented significant differences with the remaining categories.

In general, species number was highest in containers of 10–1000 L capacity. In particular, *A. aegypti* was collected in small containers whereas *C. dolosus* was present in all the categories (Table 3).

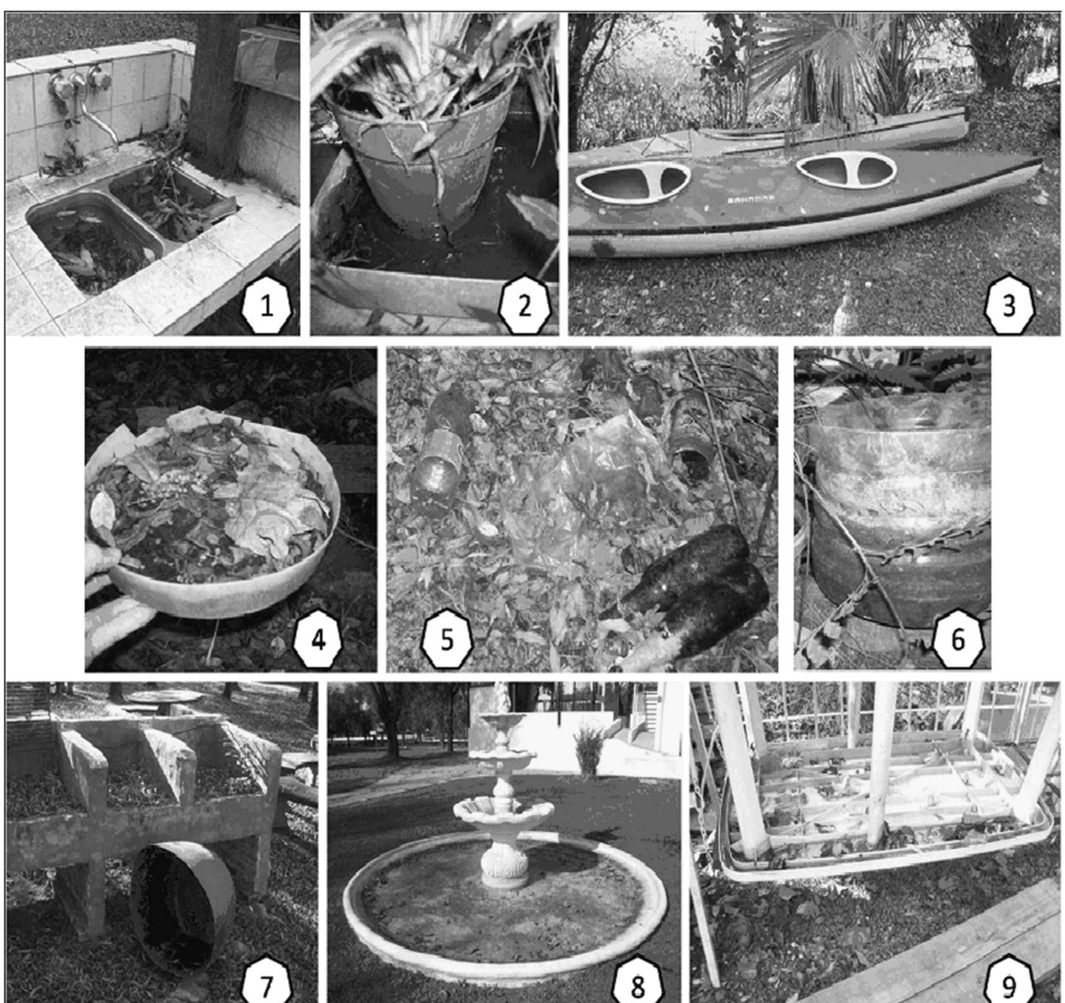

Fig. 2. Artificial containers per land use category: basin, pot and boat (1, 2 and 3 in residential); dish, bottle and bucket (4, 5 and 6 in dumps); and dustbin, fountain and table (7, 8 and 9 in recreational).

Table 3
Species composition, number of breeding containers and Container Index (CI), and total number of immatures collected in containers of different capacities.

Species	Container capacity				
	≤1 L	1.1–10 L	10.1–100 L	100.1–1000 L	>1000 L
Culex dolosus s.l.	X	x	x	x	x
C. pipiens s.l.		x	x	x	
C. maxi			x		x
C tatoi			x	x	
C. bidens				x	x
C. chidesteri				x	x
Aedes aegypti	X	x	x		
No. of species	2	3	5	5	4
No. of breeding containers	3	20	32	27	6
CI (%)	3.2	21.5	24.6	32.9	50
No. of immatures	222	769	1108	1161	99

The water volume of the inspected containers was highly variable, ranging from 2.5 mL to 200 L. More than half (228/412) of the containers had ≤1 L, and less than 5% (19/412) presented >50 L. CI values were minimum for containers holding <1 L or >50 L and maximum for intermediate values, showing a quadratic behavior. The same pattern was observed for the two most abundant species, *C. dolosus* and *C. pipiens*.

Usage

Approximately 2/3 (65.8%) of the containers inspected were not in use. Both the total number of breeding containers (73 vs. 15) and the CIs of the mosquito assemblage (27% vs. 11%) were higher for containers that were not in use ($X^2_{(1)} = 14.67$; $p < 0.0001$). Disused containers also harbored more species (7 spp.) than those in use (4 spp.) during the inspection. When considering the CI per species, only *C. dolosus* showed a significant tendency towards disused containers ($X^2_{(1)} = 19.74$; $p < 0.0001$).

Insolation

Only 7% (29/412) of the water-holding containers inspected were found under a roof and none of them harbored mosquitoes. The number of containers with water and with mosquitoes differed among the insolation levels. The CI for containers in the sun (11%) was significantly lower than for those partially and totally shaded (23 and 29% respectively), with a significant positive lineal trend ($X^2_{(1)} = 7.45$; $p < 0.001$). The number of species collected was similar in containers located in the sun (6 spp.) and under partial shade (7 spp.) but only *A. aegypti*, *C. dolosus*, *C. pipiens*, and *C. tatoi* were collected under total shade.

Multivariate analysis

When considering all explanatory variables simultaneously, the probability of finding immatures of mosquitoes in artificial containers was associated with the land use category, the material, the water volume, the insolation level, whether the container was in use or not and the interaction between the last two variables (Table 4). According to the best model selected (which retained no random term), the probability of harboring mosquitoes was higher in containers (a) located in dumps and recreational areas, (b) made of resin/fiberglass, (c) holding intermediate water volumes, and (d) not in use for partially or totally shaded containers but in use for sunlit containers. In general, such trends were consistent with univariate analyses but an interaction between usage and insolation could be detected. The Kappa value of the model was 0.48 at a cut-off point of 0.42, indicating that the selected model was 48% better than random and that any container with a model fitted probability > 0.42 should be considered positive for immatures of mosquitoes.

Table 4
Coefficient estimates, standard errors (SE), z values and associated probabilities (Pr > |z|) for each term of the selected model for the probability of finding immatures of mosquitoes in artificial containers within the Paraná Lower Delta.

Term	Estimate	SE	z value	Pr > |z|
Intercept	−0.24519	0.22322	−1.098	0.27
Residential	−1.47446	0.40797	−3.614	<0.001
Resin/fiberglass	2.19472	0.43028	5.101	<0.001
Sun	−2.82461	0.79892	−3.536	<0.001
In use	−1.62297	0.46680	−3.477	<0.001
Volume	0.09272	0.01658	5.591	<0.001
$Volume^2$	−0.00126	0.00041	−3.052	<0.01
Sun: in use	3.18672	0.99796	3.193	<0.01

Discussion

Prior to this study, there were no records of *A. aegypti* in the Paraná Lower Delta. Our results suggest that the vector is widely distributed but in very low abundance. This could be due to the fact that the mosquito has recently colonized the area or, alternatively, that environmental conditions are barely suitable for its proliferation due to low human population density and scarce urban development in the region. Even though dengue transmission risk in temperate Argentina is considered moderate (Vezzani and Carbajo, 2008; Bhatt et al., 2013) and no autochthonous transmission of Chikungunya has yet occurred in the territory, it is currently accepted that some factors may worsen the sanitary situation of these transmittable diseases. Among such factors, the expansion of the geographical distribution of *A. aegypti* as a consequence of global climatic change has been highlighted (Vezzani and Carbajo, 2008). In this regard, the availability of water-holding containers in a wetland next to Buenos Aires City is highly relevant for the epidemiological situation of the region. Regarding St. Louis encephalitis, the presence of *C. pipiens* in the islands was previously reported in ground pools (Cardo et al., 2011a, 2012a) and hereby confirmed in artificial containers in close relation with human settlements. Considering the high density of resident and migratory birds and high tourist affluence, the region could maintain the sylvatic cycle and enhance transmission risk to the urban center. Finally, the absence of *Aedes albopictus* in the islands is noteworthy. This vector of important flaviviruses worldwide (Gratz, 2004) was detected in the northeast extreme of Argentina in 1998, where its distribution seems to be restricted and its lack of spreading to the south is really unexpected (Vezzani and Carbajo, 2008).

Mosquito communities

Among over 1000 containers inspected, half of them held water and over 17% of such were infested with immatures of mosquitoes. The species richness was similar to the one recorded in highly urbanized areas from Buenos Aires (5–7 species; Vezzani and Albicócco, 2009; Rubio et al., 2011) and also similar to other mosquito communities in the area, such as phytotelmata (9; Albicócco et al., 2011) and ground pools in domestic areas (7; Cardo et al., 2011a). Besides both vectors mentioned above, the studied community presented other five species of *Culex* that had been previously recorded in other types of aquatic habitats in the islands; all of them in ground pools and *C. pipiens* and *C. dolosus* in tree holes (Albicócco et al., 2011; Cardo et al., 2011a). These findings reveal the differential use of the diversity of aquatic habitats made by each species. In particular, *C. pipiens* and *C. dolosus* could be described as generalists in terms of habitat usage within the wetland. Similar patterns have been observed in other mosquito communities both in urban and rural areas (Martins da Silva, 2002; Calderón-Arguedas et al., 2009). When considering the relative abundance of each species, *C. dolosus* showed higher CI values than the rest. This result contrasts with artificial containers from Buenos Aires City, in which *A. aegypti* and *C. pipiens* were the most abundant (Vezzani and Albicócco, 2009; Rubio et al., 2011). Not coincidentally, *C. dolosus* has also been recorded as the most abundant species in ground pools in the wetland (Cardo et al., 2011a, 2012b).

Container characteristics

Mosquito immature infestation levels differed according to the container material. In urban cemeteries, Vezzani and Schweigmann (2002) showed that for *A. aegypti* the quality of each material as breeding habitat varied according to the level of sun exposure. In shaded areas, the use of each material was proportional to its availability, whereas in sunlit areas plastic containers were highly used and metallic ones were avoided. In the present study, no interaction between the variables insolation and material was detected; this may be due to the fact that metallic containers in the area are much larger than cemetery vases (typically <1 L), therefore heating effects may be lessened.

Regarding capacity, higher CI values for containers in the range 1–1000 L in comparison with ≤1 L could be due to food limitation in small containers (Washburn, 1995). The CI was also higher in containers holding intermediate water volumes, probably because very small volumes represent an unstable aquatic habitat for mosquitoes and large volumes may impose a higher predation pressure by natural enemies (Washburn, 1995). However, we cannot rule out the fact that very large containers may have been undersampled and their true CI underestimated due to habitat complexity (high organic material load and refuge for immatures). *A. aegypti* was found exclusively in small containers in agreement with previous reports elsewhere (Sunahara et al., 2002; Vezzani et al., 2004).

A critical variable affecting habitat quality and CI values was container usage. This variable presents a complex behavior given that some containers are excellent breeding sites for mosquitoes when they are in use (e.g., water tanks) whereas others are optimal when disused (e.g., basins with the drainpipe obtruded). The interpretation of this variable was further complicated by its interaction with the insolation level. In natural areas as the one under study, shade is almost exclusively provided by vegetation, which is a source of food, refuge and detritus for immatures and adults (Vezzani, 2007). In urban areas, the infestation level of *A. aegypti* was higher in containers located in more shady and vegetated environments (Vezzani et al., 2005). Likewise, both for *C. pipiens* and *A. aegypti*, higher CIs were recorded in shaded containers (Vezzani and Albicócco, 2009; Rubio et al., 2011). A similar patter was observed for *C. dolosus* in ground pools of the Lower Delta, due to its positive association with physiognomies with tall vegetation (Cardo et al., 2011b). In our case, sunlit containers may be subjected to higher heating and evaporation processes; because of this, those in use may be more likely to harbor mosquitoes due to human water recharge. On the other hand, shaded containers may be more protected and nurtured by the surrounding vegetation, experience less evaporation and therefore constitute more stable and richer habitats for mosquitoes when not in use.

Key breeding containers were buckets, dustbins and boats; this could be related to their abundance or to the fact that they held water frequently. This is a very important issue because dustbins and boats are not dispensable containers and could act as a reservoir for mosquitoes when other containers are in low abundance or water is scarce (e.g. in times of low tourist affluence or prolonged drought). Besides, the three categories present a large capacity and can hold rain water for several weeks, enabling the completion of the mosquito cycle. Calderón-Arguedas et al. (2009) also reported that, in low urbanized tropical areas, most relevant breeding containers were the non disposable ones.

Domestic land uses

Human activities are unambiguously linked to the proliferation of favorable sites for mosquito development (Matthys et al., 2006). Our findings support the idea that even though wetlands are not characterized by a high density of artificial containers, they provide enough breeding habitats to maintain medically relevant artificial container mosquitoes. Land uses were heterogeneous regarding the proportion of containers with water and with mosquitoes, but similar in species richness and composition. In the Lower Delta, there is evidence that a high degree of human intervention could favor the presence of certain species (e.g., *Culex intrincatus*) in ground pools (Cardo et al., 2011b). However, due to the complexity of the wetland system it is hard to predict the impact of human activities on mosquito presence and abundance.

Finally, the information gathered regarding the availability of water-holding containers per land use could aid in the planning of mosquito control activities. Dumps were characterized by the presence of disused and potentially disposable containers, in opposition to residential and recreational areas which presented non disposable containers. Therefore, recommendations to reduce mosquito densities should be specific for each domestic category. In residential areas, large boats can be covered with a tarpaulin so that they do not accumulate water and smaller boats (kayaks and canoes) can be turned around when not in use. In recreational areas, dustbins should be perforated so that they do not accumulate water, and regularly cleaned so that the holes do not plug. Regarding dumps, alternative proposals to garbage disposal should be promoted. In all cases, providing useful information and encouraging communitarian participation are essential for a successful mosquito control.

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References

Abramson, J.H., 2004. WINPEPI (PEPI-for-Windows) computer programs for epidemiologists. Epidemiol. Perspect. Innov. 1, 6 (Available from www.epi-perspectives.com/content/1/1/6).

Abramson, J.H., Gahlinger, P.M., 2001. Computer Programs for Epidemiologists: PEPI Version 4.0. Sagebrush Press, Salt City.

Albicócco, A.P., Carbajo, A.E., Vezzani, D., 2011. Mosquito community structure in phytotelmata from a South American temperate wetland. J. Vector Ecol. 36, 437–446.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2014. lme4: Linear Mixed-effects Models Using Eigen and S4. R Package Version 1.1-7. Available from http://CRAN.R-project.org/package=lme4.

Becker, N., Petric, D., Zgomba, M., Boase, C., Madon, M., Dahl, C., Kaiser, A., 2010. Mosquitoes and Their Control. Springer.

Bhatt, S., Gething, P.W., Brady, O.J., et al., 2013. The global distribution and burden of dengue. Nature 496, 504–507.

Calderón-Arguedas, O., Troyo, A., Solano, M.E., et al., 2009. Urban mosquito species (Diptera: Culicidae) of dengue endemic communities in the Greater Puntarenas area, Costa Rica. Rev. Biol. Trop. 57, 1223–1234.

Canty, A., Ripley, B., 2014. Bootstrap R (S-Plus) functions. R Package Version 1.3-13.

Carbajo, A.E., Vezzani, D., 2015. Waiting for chikungunya fever in Argentina: spatio-temporal risk maps. Mem. Inst. Oswaldo Cruz 110, 259–262.

Cardo, M.V., Vezzani, D., Carbajo, A.E., 2011a. Community structure of ground-water breeding mosquitoes driven by land use in a temperate wetland of Argentina. Acta Trop. 119, 76–83.

Cardo, M.V., Vezzani, D., Carbajo, A.E., 2011b. Environmental predictors of the occurrence of ground-water mosquito immatures in the Paraná Lower Delta, Argentina. J. Med. Entomol. 48, 991–998.

Cardo, M.V., Vezzani, D., Carbajo, A.E., 2012a. Oviposition strategies of temporary pool mosquitoes in relation to weather, tidal regime, and land use in a temperate wetland. Bull. Entomol. Res. 102, 651–662.

Cardo, M.V., Vezzani, D., Carbajo, A.E., 2012b. Immature mosquitoes from ground-water habitats in a temperate wetland of Argentina: environmental associations and seasonal variation of community attributes. J. Am. Mosq. Control Assoc. 28, 151–159.

CDC, 2013. Centers for Disease Control and Prevention Available from www.cdc.gov.

Dale, P.E.R., Knight, J.M., 2008. Wetlands and mosquitoes: a review. Wetl. Ecol. Manag. 16, 255–276.

Fielding, A.H., Bell, J.F., 1997. A review of the methods for the assessment of prediction errors in conservation presence/absence models. Environ. Conserv. 24, 38–49.

Fleiss, J.L., Levin, B., Paik, M.C., 2003. Statistical Methods for Rates and Proportions. 3rd ed. Wiley & Sons, New Jersey.

Fox, J., Weisberg, S., 2011. An {R} Companion to Applied Regression. Second edition. Sage, Thousand Oaks CA (Available from http://socserv.socsci.mcmaster.ca/jfox/Books/Companion).
Gratz, N.G., 2004. Critical review of the vector status of *Aedes albopictus*. Med. Vet. Entomol. 18, 215–227.
INDEC, 2010. Instituto Nacional de Estadísticas y Censos. Censo nacional de población y viviendas, Secretaría de planeación, Presidencia de la Nación, República Argentina, Buenos Aires. Available from http://www.indec.mecon.ar.
Kandus, P., Malvárez, A.I., 2004. Vegetation patterns and change analysis in the lower Delta Island of the Paraná River (Argentina). Wetlands 24, 620–632.
Kandus, P., Malvárez, A.I., Madanes, N., 2003. Estudio de las comunidades de plantas herbáceas de las islas bonaerenses del Bajo Delta del Río Paraná (Argentina). Darwiniana 41, 1–16.
Leisnham, P.T., Lester, P.J., Slaney, D.P., Weinstein, P., 2006. Relationships between mosquito densities in artificial container habitats, land use and temperature in the Kapiti–Horowhenua region, New Zealand. N. Z. J. Mar. Freshw. Res. 40, 285–297.
López, H., Neira, J., Morales, M.A., Fabbri, C., D'Agostino, M.L., Zitto, T., 2010. Encefalitis por virus San Luis en la ciudad de Buenos Aires durante el brote de dengue 2009. Medicina (Buenos Aires) 70, 247–250.
Martins Da Silva, A., 2002. Imaturos de mosquitos (Diptera, Culicidae) de áreas urbana e rural no norte do estado do Paraná, Brasil. Iheringia Ser. Zool. 92, 31–36.
Matthys, B., N'Goran, E.K., Koné, M., et al., 2006. Urban agricultural land use and characterization of mosquito larval habitats in a medium-sized town of Côte d'Ivoire. J. Vector Ecol. 31, 319–333.
Morales, M.A., Barrandeguy, M., Fabbri, C., et al., 2006. West Nile virus isolation from equines in Argentina, 2006. Emerg. Infect. Dis. 12, 1559–1561.
MSN, 2009. Guía para el equipo de salud. Enfermedades infecciosas: dengue. Ministerio de Salud de la Nación, República Argentina.
Nicholls, A.O., 1989. How to make biological surveys go further with generalised linear models. Biol. Conserv. 50, 51–75.
R. Core Team, 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria (Available from http://www.R-project.org/).
Rossi, G.C., Mariluis, J.C., Schnack, J.A., Spinelli, G.R., 2002. Dípteros vectores (Culicidae y Calliphoridae) de la Provincia de Buenos Aires. Secretaría de Política Ambiental y Universidad de La Plata, Buenos Aires.
Rubio, A., Vezzani, D., 2011. Cubiertas de auto abandonadas como sitios de cría de *Culex eduardoi* (Diptera: Culicidae) en el Parque Provincial Pereyra Iraola, Provincia de Buenos Aires. Rev. Soc. Entomol. Argent. 70, 119–122.
Rubio, A., Cardo, M.V., Vezzani, D., 2011. Tire-breeding mosquitoes of public health importance along an urbanisation gradient in Buenos Aires, Argentina. Mem. Inst. Oswaldo Cruz 106, 678–684.
Senise, L.V., Sallum, M.A., 2008. Redescription of *Culex* (*Culex*) *dolosus* (Lynch Arribálzaga) (Diptera: Culicidae), based on specimens from Pico do Itapeva, Serra da Mantiqueira, São Paulo, Brazil. Zootaxa 1683, 51–62.
Silver, J.B., 2008. Mosquito ecology. Field Sampling Methods. Springer, Dordrecht.
Spinsanti, L., Díaz, L.A., Glatstein, N., et al., 2008. Human outbreak of St. Louis encephalitis detected in Argentina. J. Clin. Virol. 42, 27–33.
Sunahara, T., Ishizaka, K., Mogi, M., 2002. Habitat size: a factor determining the opportunity for encounters between mosquito larvae and aquatic predators. J. Vector Ecol. 27, 8–20.
Titus, K., Mosher, J.A., 1984. Chance-corrected classification for use in discriminant analysis: ecological applications. Am. Midl. Nat. 111, 1–7.
Vezzani, D., 2007. Artificial container-breeding mosquitoes and cemeteries: a perfect match. Tropical Med. Int. Health 12, 299–313.
Vezzani, D., Albicócco, A.P., 2009. The effect of shade on the Container Index and pupal productivity of the mosquitoes *Aedes aegypti* and *Culex pipiens* breeding in artificial-containers. Med. Vet. Entomol. 23, 78–84.
Vezzani, D., Carbajo, A.E., 2008. *Aedes aegypti*, *Aedes albopictus* and dengue in Argentina: current knowledge and future directions. Mem. Inst. Oswaldo Cruz 103, 66–74.
Vezzani, D., Schweigmann, N., 2002. Suitability of containers from different sources as breeding sites of *Aedes aegypti* (L.) in a cemetery of Buenos Aires City, Argentina. Mem. Inst. Oswaldo Cruz 97, 789–792.
Vezzani, D., Velázquez, S.M., Schweigmann, N., 2004. Containers of different capacity as breeding sites of *Aedes aegypti* (Diptera: Culicidae) in the cemeteries of Buenos Aires, Argentina. Entomol. Vectores 11, 305–316.
Vezzani, D., Rubio, A., Velázquez, S.M., Schweigmann, N., Wiegand, T., 2005. Detailed assessment of microhabitat suitability for *Aedes aegypti* (Diptera: Culicidae) in Buenos Aires, Argentina. Acta Trop. 95, 123–131.
Washburn, J.O., 1995. Regulatory factors affecting larval mosquito populations in container and pool habitats: implications for biological control. J. Am. Mosq. Control Assoc. 11, 279–283.
WHO, 2013. World Health Organization. Available from http://www.who.int/en/index.html.
Zar, J.H., 1999. Biostatistical Analysis. Prentice Hall, New Jersey.
Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M., 2009. Mixed Effects Models and Extensions in Ecology with R. Springer, New York.
Zuur, A.F., Ieno, E.N., Elphick, C.S., 2010. A protocol for data exploration to avoid common statistical problems. Methods Ecol. Evol. 1, 3–14.