

Seasonal and Environmental Variables Related to the Abundance of Immature Mosquitoes in Rain Pools of a Peri-Urban Park of Buenos Aires (Argentina)

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Abstract

The aim of the present study was to investigate the variables associated with the composition of mosquito species in rain pools of 'Bosque de Ezeiza', a large peri-urban forested park of Buenos Aires city, Argentina. A total of 12 rain pools were sampled biweekly over a 1-yr period in search of mosquito immature stages. Mosquito immature stages were present in all the occasions in which water bodies were recorded. A total of 14 species of five genera were identified, with the highest abundances observed in autumn and spring. The total abundance varied among dates according to previous temperature, precipitation, and flooding conditions. Only one species, *Psorophora cyanescens* (Coquillett) (Diptera: Culicidae), was associated with extreme conditions (high temperature and prolonged period of drought). Besides drought periods, two main variables were related to species composition: temperature, which accounted for seasonal changes in species composition, and flooding history, which accounted for the predominance of floodwater mosquito species on recently flooded dates and for that of stagnant water species on dates with a longer permanence of water. Regarding the pools, the most important variables accounting for species composition were the insolation level and the variability in the flooded area, with floodwater mosquitoes associated with pools with high variability in the flooded area. Interestingly, *Culex (Mel.) pilosus* (Dyar and Knab) (Diptera: Culicidae) showed temporal and spatial dynamics more similar to floodwater species than to stagnant water species in the rain pools studied.

Key words: immature mosquito, environmental variable, seasonal dynamics, floodwater mosquito, stagnant water mosquito

Temporary pools constitute important larval habitats for mosquitoes and contribute significantly to the production of adults, because they are geographically widespread, can be locally abundant (Williams 2006), and may contain high abundances of mosquito immature stages (Chase and Knight 2003). These aquatic environments exhibit a recurrent drought phase of variable duration (Williams 1997), and, due to their small size and important variations in water volume, they are subject to drastic fluctuations in their chemical and physical conditions, including their total drying (Williams and Felmate 1992). The water balance of these environments depends on the weather conditions and the characteristics of the rain pool: the water supply is determined by the magnitude of the rainfall, while the water loss is influenced by evaporation, evapotranspiration, and drainage. Therefore, water loss is closely related to variables such as vegetation cover, degree of insolation, temperature, and dimensions of the rain pool (Brooks and Hayashi 2002). In addition, the reduction of the water level is associated with fluctuations of other variables such as temperature and pH (Williams 1997).

Mosquitoes that inhabit temporary pools can be classified into two groups, floodwater mosquitoes and stagnant water mosquitoes, depending on whether their eggs are resistant to desiccation or not (Becker et al. 2010). Females of the former group (e.g., *Aedes* Meigen (Diptera: Culicidae) and *Psorophora* Robineau-Desvoidy (Diptera: Culicidae)) deposit their eggs on moist substrates that are periodically flooded, and synchronized hatching of these drought-resistant eggs may occur when the substrate is covered by water. In contrast, females of the latter group (e.g., *Anopheles* Meigen (Diptera: Culicidae), *Culex* L. (Diptera: Culicidae) and *Uranotaenia* Lynch Arribálzaga (Diptera: Culicidae)) lay their eggs on the surface of water, and hatching occurs immediately after embryonic development is completed. For the laying of eggs, the species of this group depend on the detection of aquatic environments once formed, and thus take opportunistic advantage of the temporary pools when they are available (Clements 1992). The final selection of the oviposition site depends on different factors, including the age, nutritional

value, and permanence of water of the temporary pool, and may also vary according to meteorological variables (Day 2016).

In urban and suburban areas, large green spaces frequently harbor high numbers of temporary pools, in places where the topography favors the accumulation of water after rains (Williams and Felmate 1992). Thus, these parks may produce high abundances (Irwin et al. 2008, Pires and Gleiser 2010) and richness of adult mosquitoes (Medeiros Souza et al. 2015), affecting the health and well-being of the people who visit them or live in nearby areas (Lyytimäki and Sipilä 2009).

Within the Metropolitan Area of Buenos Aires, Argentina, previous studies have reported a total of 26 species in temporary aquatic habitats, 10 of which correspond to floodwater and 16 to stagnant water mosquitoes (Fontanarrosa et al. 2009; Cardo et al. 2011, 2012; Urcola and Fischer 2015). Some of the mosquito species recorded in the present study are epidemiologically important both for humans and animals: *Aedes albifasciatus* (Macquart) (Diptera: Culicidae), *Aedes scapularis* (Rondani) (Diptera: Culicidae), *Culex maxi* Dyar (Diptera: Culicidae), *Culex pipiens* Linnaeus (Diptera: Culicidae), and *Psorophora ferox* (Von Humboldt) (Diptera: Culicidae) are related to the transmission of viruses that affect humans and domestic animals (CDC 2000, Forattini 2002, Pisano et al. 2010). In particular, *Ae. albifasciatus* is the most problematic species, due to its high abundance and to the fact that it is the main vector of the western equine encephalitis virus and potentially of other arboviruses in Argentina (Mitchell et al. 1987, Aviles et al. 1992).

In the Metropolitan Area of Buenos Aires, the evidence suggests that, in the urban area (i.e., Buenos Aires city), the abundance of adult floodwater mosquitoes sometimes increases drastically, transported by the wind from the nearby Bosque de Ezeiza (Bejarán et al. 2008). The Bosque de Ezeiza is a green wooded area extending over 450 ha at the transition between the urban and rural landscape. This large recreational park receives about 80,000 visitors on weekends. The irregular relief of the land favors the formation of several temporary pools after rainfall events (Urcola and Fischer 2015). However, in spite of its importance as a source of adult mosquitoes to the city, no studies on the community of mosquitoes that develop in this peri-urban park have yet been performed.

In contrast, in urban parks of Buenos Aires city, exhaustive studies on the mosquitoes developing in temporary pools have been performed, and, together, have reported a total of 14 species (six floodwater and eight stagnant water species) in the temporary pools studied (Fischer et al. 2000, Fontanarrosa et al. 2009, Quiroga et al. 2013). The seasonal variations in these sites have been associated mainly with temperature, with some species as *Cx. pipiens*, *Cx. maxi*, *Psorophora cyanoescens* (Coquillett) (Diptera: Culicidae), and *Ps. ferox* present mainly during the warm season, and others such as *Culex dolosus* (Lynch Arribáizaga) (Diptera: Culicidae) also recorded during the cold season (Fischer and Schweigmann 2004, Fontanarrosa et al. 2009, Quiroga et al. 2013). In addition, the presence of other species is relatively independent of the temperature or season (Fontanarrosa et al. 2000, Fischer et al. 2002, Fontanarrosa et al. 2009, Quiroga et al. 2013) and mainly associated with the pattern of rainfall, e.g., *Ae. albifasciatus*, which initiates development immediately after the filling of the pools (Fischer and Schweigmann 2008). In these urban pools, the species richness of immature mosquitoes is affected by the permanence of the aquatic habitats, with more species present in pools with longer water permanence (Fischer and Schweigmann 2008). In addition, the composition of the immature mosquito community may change in relation to the water permanence, with some species of the genus *Culex* associated with pools

of longer permanence (filled by the constant leaking of water from broken pipes), and floodwater mosquitoes such as *Ae. albifasciatus* associated with the more ephemeral pools (filled by rain) (Quiroga et al. 2013). Other variables such as insolation and ground vegetation have also been shown to be important for mosquito immature stages, since pools with intermediate insolation and intermediate or high ground vegetation are colonized more frequently by *Ae. albifasciatus* (Fischer et al. 2002), *Cx. pipiens*, and *Cx. dolosus* (Fischer and Schweigmann 2004).

Based on the above, the aim of the present study was to analyze the seasonal variations of the community of culicid immature stages in temporary rain pools in the Bosque de Ezeiza, and their association with the environmental variables of these water bodies.

Materials and Methods

Study Area

The Bosque de Ezeiza is located at the southern edge of the Metropolitan Area of Buenos Aires (Fig. 1). Most of the area of the Bosque de Ezeiza is covered by planted *Eucalyptus* sp. L'Her (Myrtales: Myrtaceae), which differentially shade the ground where temporary pools are formed after rainfall events. The climate is temperate humid, with seasonally varying temperatures (winter mean: 10°C, summer mean: 23°C). The mean annual rainfall is 1,021 mm, and rainfall events are recorded throughout the year, being highest in the warm season (between November and April) and lowest in the cold season (between June and September) (Servicio Meteorológico Nacional 2018).

Data Collection

Twelve temporary pools were surveyed every 2 wk from October 2013 to September 2014. All the pools studied were located within an area of 90,000 m², meaning a distance of less than 350 m from one pool to the other (Fig. 1C), which is considered below the average dispersal measured for adult mosquitoes (Guerra et al. 2014). On each sampling date, the surface area covered by water was assessed for each pool by multiplying the maximum length, width, and the proportion of water of the resulting rectangle occupied by water. In addition, the degree of insolation was estimated by eye on a scale between 0 and 1, with 0 for pools located completely in the shade, 1 for those completely exposed to sunshine, and intermediate values according to the proportion of the pool area exposed to sunlight during the sampling day. Samples of mosquito immature stages were taken with a hand net (10 × 12 cm, mesh size 350 µm). The sampling effort was approximately proportional to the surface covered by water (i.e., the number of 1-m net hauls was equivalent to 4 times the log₁₀ (surface area + 1)). Samples were fixed in situ in 80% alcohol. The mosquito immature stages were sorted by stage and the third and fourth stages were counted and identified to species level by using the appropriate systematic keys (Darsie 1985).

Meteorological data (daily precipitation and hourly temperature) during the sampling period for the nearby meteorological station in Ezeiza (5 kilometers away from the study site) were obtained from the National Meteorological Service of Argentina.

Data Analyses

Temporal Variation

Cumulative rainfall was calculated based on the daily records of rainfall, both for each season and for the complete study period. The mean daily, seasonal and annual temperatures were calculated based on hourly temperature data. In addition, the following variables

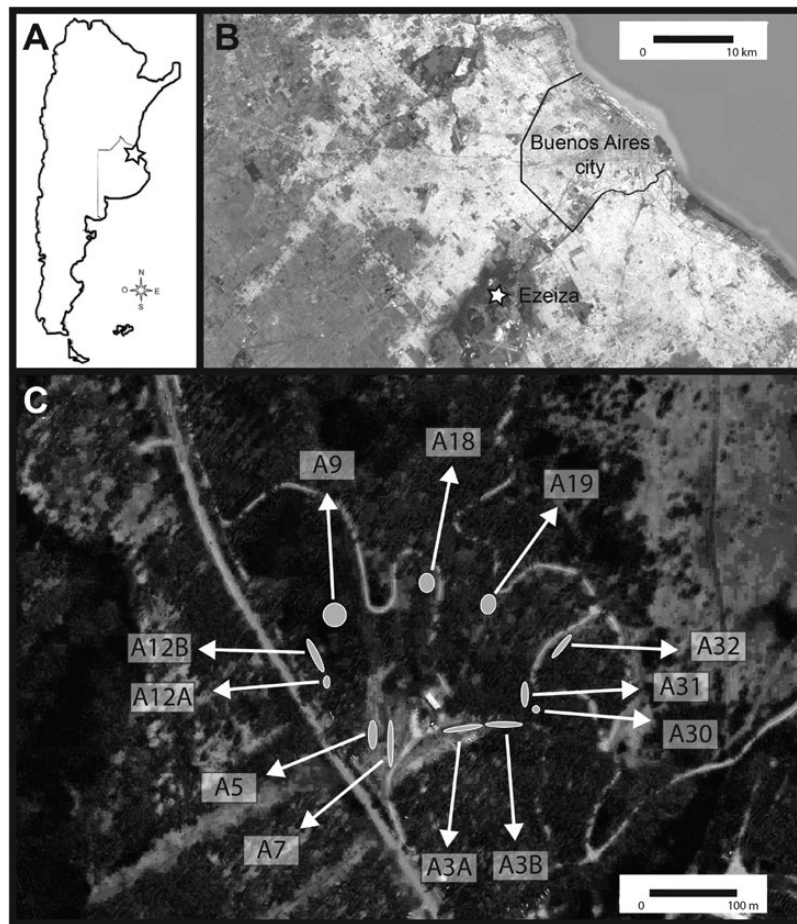


Fig. 1. Location of the Bosque de Ezeiza, Buenos Aires, Argentina. (A) Map of Argentina; (B) Location of the study site (represented with a star) in the Metropolitan Area of Buenos Aires; (C) Spatial arrangement of the studied pools (numbers and letters represent the code names of each pool).

were calculated for each sampling date: total flooded area (St, sum of the surfaces covered by water of the pools analyzed, square root transformed), previous temperature (T15, average temperature during the 15 d previous to each sampling), previous precipitation (P15, the cumulative rainfall during the 15 d previous to each sampling), change in flooded area (Fch, differences in St between consecutive dates, with negative values meaning a decrease in St and positive values meaning an increase in St). The total number of floodwater and stagnant water mosquitoes was calculated for each pool and date by multiplying the number of collected individuals by the flooded area, divided by the number of net hauls in the corresponding pool. Total abundances were summed over all pools for each sampling date. The temporal variability of the abundances of both groups in relation to St was analyzed by a graph. The relationships of total abundance of floodwater mosquitoes and total abundance of stagnant water mosquitoes (both variables $\log(n + 1)$ transformed) with environmental variables were analyzed with Generalized Linear Models, using the R software, Version 3.2.3 (R Core Team 2015), accessed through a user-friendly interface in Infostat software. The Poisson distribution with the log link function was used, and models were fitted and parameters were estimated using the Maximum Likelihood method (Pinheiro and Bates 2000). The variables initially included in the models were: St, Fch, T15, P15, and the T15 \times P15 interaction. The best model was selected using the Akaike information criterion, and the models were then validated by residual analysis, and by inspecting the autocorrelation function and the partial autocorrelation

function of the residuals to detect any remaining temporal structure (Zuur et al. 2007). To identify the most abundant and frequent mosquitoes of the Bosque de Ezeiza, we calculated the relative abundance (mosquitoes collected of one species / total mosquitoes collected \times 100) and occurrence (number of visits in which the species was present / number of visits to the park \times 100) of each species. To analyze the presence of each species throughout the study period, we calculated the monthly number of mosquitoes for each species.

To analyze the temporal variation in the community of immature mosquitoes, we performed a correspondence analysis (CA) of the number of individuals collected of each species per sampling date ($\log(n + 1)$ transformed) (Zuur et al. 2007). The relationship of the two principal components of the analysis with St, Fch, T15, and P15 was analyzed with a correlation analysis.

Distribution Among Aquatic Environments and Association With Environmental Variables

The mean surface area (Sm, sum of the surface area of the pool at each visit divided the number of visits), mean insolation (Im, average insolation of the pool in all visits when the pool contained water), an index of water permanence (PI, number of visits that the pool contained water divided by the total number of visits), and the coefficient of variation of the flooded area (CVs, standard deviation of the surface of the pool divided by Sm) were calculated for each pool. In addition, the cumulative numbers of stagnant water mosquitoes and floodwater mosquitoes were calculated for each pool. The

relationships of the cumulative abundance of each group with the mean surface area, mean insolation, the coefficient of variation and the permanence index for each pool were analyzed with Spearman rank correlations.

To analyze the spatial variation of mosquito species, we used a CA of the total number of individuals collected of each species transformed to $\log(n + 1)$ for each pool. The relationship of the first two components with the variables Sm, Im, PI, and CVs was analyzed with a correlation analysis.

Results

Fourteen mosquito species belonging to five genera were identified during the study period. Specimens of *Anopheles* and *Uranotaenia* were identified only to the genus level. A total of 4,282 third and fourth stage culicids were collected (Table 1), 36% of which corresponded to *Ae. albifasciatus*, 20% to *Cx. tatoi* Casal and García (Diptera: Culicidae), 16% to *Culex pilosus* (Dyar and Knab) (Diptera: Culicidae), 9% to *Culex eduardoi* Casal and García (Diptera: Culicidae), 6% to *Cx. pipiens* and 6% to *Aedes crinifer* (Theobald) (Diptera: Culicidae). The remaining species had a pooled relative abundance of less than 2%. *Culex eduardoi* was the most frequently recorded mosquito species (79.2% of the sampling dates on which the park had water), followed by *Ae. albifasciatus* (62.5%), *Cx. pipiens* (45.8%), *Ae. crinifer* (41.7%), *Cx. tatoi* (33.3%), *Anopheles* sp. (33.3%), *Cx. maxi* and *Ps. cyanescens* (29.2%), *Cx. dolosus* and *Cx. pilosus* (25.0%), and *Uranotaenia* sp. (16.7%). The rest of the species presented a frequency of detection lower than 10%.

Temporal Variation

During the study period, 1,481 mm of accumulated rainfall were recorded, spread over 101 d, during which it rained from 0.1 to 71.8 mm. The accumulated rainfall was highest in summer (December–February, 541 mm); lowest in autumn (March–May, 253 mm) and winter (June–August, 233 mm), and intermediate in spring (September–November, 408 mm). The mean annual temperature was 17.7°C. The mean temperature was 20.8°C in spring, 23.1°C in summer, 14.9°C in autumn, and 11.7°C in winter. The

maximum values of flooded area by date were 1185.7 m² (in spring) and 1181.9 m² (in summer), whereas the mean flooded area in these seasons (331 m² in spring and 411 m² in summer) was lower than in autumn (526 m²) and winter (633 m²). The pools were found dry only on three sampling dates (Fig. 2B).

Mosquito immature stages were present on all the sampling dates on which the water bodies were recorded, with the highest abundances observed in autumn and spring. Stagnant water mosquitoes were the more abundant group and were collected on all sampling dates when at least one pool was recorded, except for three dates (7 November 2014, 10 January 2014, and 23 January 2014). On these three opportunities, the flooded area was larger than in the previous visit (null for the first two dates and 12.5 m² for the third date). Floodwater mosquitoes were recorded on all sampling dates in which at least one pool was recorded, except on six dates (17 October 2013, 14 November 2013, 20 February 2014, 1 May 2014, 7 August 2014, and 21 August 2014). On all these dates, there was a reduction of the flooded area as compared to the previous sampling date (Fig. 2).

The variables included in the model explained 38% of the variability in the log-transformed abundance of floodwater mosquitoes. T15, P15, and Fch were positively related, whereas the T15 × P15 interaction was negatively related to the abundance of floodwater mosquitoes (Table 2). For stagnant water mosquitoes, the selected model explained 60% of the variability in the log-transformed abundance. Fch was negatively related, whereas St was positively related to the abundance of stagnant water mosquitoes (Table 2).

The first two axes of the CA accounted for 50.5% of the temporal variability of the species composition. Only two dates (10 January 2014 and 24 January 2014), which were characterized by highest temperatures in the study period and a previous drought period (the flooded area was null in the two previous visits), separated from the rest on the first axis (29.4%). The floodwater species *Ps. cyanescens* was associated with these dates.

To analyze in detail the variability in the composition of the remaining species, the analysis was repeated, eliminating these two dates (10 January 2014 and 24 January 2014). After doing this, the first two axes of the CA accounted for 50.9% of the temporal variability in the species composition. The first axis of the CA showed a positive correlation with the mean temperature ($r = 0.75$, $P < 0.001$),

Table 1. Mean surface area and monthly and total number of third and fourth instar larvae collected in the Bosque de Ezeiza, of Buenos Aires province (Argentina)

Flooded area (m ²)	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Total
	714.1	1472.5	-	49.4	1782.3	1046.7	974.9	1544.2	1532.8	1545.7	613.0	1214.1	12489.7
Species													
<i>Ae. albifasciatus</i> (Macquart)	7	144	-	-	-	208	461	133	7	4	-	584	1,548
<i>Ae. crinifer</i> (Theobald)	-	15	-	-	-	41	119	34	8	13	-	6	236
<i>Ae. scapularis</i> (Rondani)	-	-	-	-	-	-	-	1	-	-	-	-	1
<i>Anopheles</i> sp. Meigen	-	-	-	-	-	1	38	19	-	2	1	-	61
<i>Cx. dolosus</i> (Lynch Arribálzaga)	-	-	-	-	16	12	15	-	2	-	-	-	45
<i>Cx. eduardoi</i> Casal and García	3	4	-	-	20	8	30	12	95	140	38	26	376
<i>Cx. pilosus</i> (Dyar and Knab)	-	-	-	-	44	546	115	-	-	-	-	-	705
<i>Cx. pipiens</i> L.	1	6	-	-	93	98	37	3	-	4	-	-	242
<i>Cx. maxi</i> Dyar	-	3	-	-	12	15	39	-	1	-	-	-	70
<i>Cx. tatoi</i> Casal and García	-	3	-	-	96	22	749	1	-	-	-	1	872
<i>Ps. ciliata</i> (F.)	-	-	-	-	-	11	-	-	-	-	-	-	11
<i>Ps. cyanescens</i> (Coquillett)	-	4	-	34	1	23	1	-	-	-	-	-	63
<i>Ps. ferox</i> (Von Humboldt)	-	1	-	-	-	2	-	-	-	-	-	-	3
<i>Uranotaenia</i> sp. Lynch Arribálzaga	-	-	-	-	-	15	32	2	-	-	-	-	49

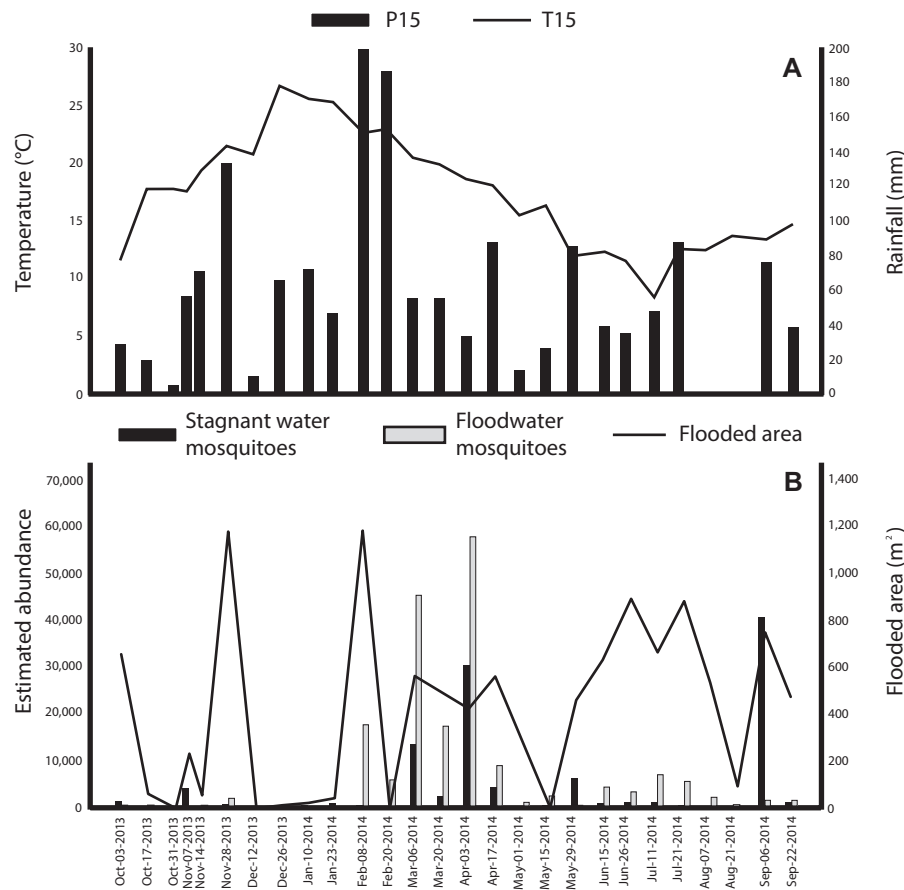


Fig. 2. Variations in relevant variables along the study period in the Bosque de Ezeiza, Buenos Aires, Argentina. (A) temperature (T15) and cumulative rainfall (P15) during the 15 d previous to each sampling date; (B) degree of flooding and estimated numbers of immature stagnant water mosquitoes and floodwater mosquitoes on each sampling date.

Table 2. Parameter estimates for the models describing the relationships of abundances of floodwater mosquitoes and stagnant water mosquitoes with meteorological and environmental variables in the Bosque de Ezeiza, of Buenos Aires province (Argentina)

Floodwater mosquitoes ^a				
Variables	Estimate	SE	z value	Pr(> z)
(Intercept)	-1.49	1.1	-1.35	0.177
P15	0.05	0.02	2.74	0.006
T15	0.12	0.06	1.98	0.048
Fch	0.03	0.01	2.52	0.012
P15 × T15	-2.50E-03	9.10E-04	-2.8	0.005
Stagnant water mosquitoes ^b				
Variables	Estimate	SE	z value	Pr(> z)
(Intercept)	-0.77	0.58	-1.33	0.184
Fch	-0.03	0.01	-2.36	0.018
St	0.08	0.02	3.42	0.001

^a*Ae. albifasciatus*, *Ae. crinifer*, *Ae. scapularis*, *Ps. ciliata*, *Ps. cyanescens*, and *Ps. ferox*.

^b*Anopheles* sp., *Cx. dolosus*, *Cx. eduardoi*, *Cx. pilosus*, *Cx. pipiens*, *Cx. maxi*, *Cx. tatoi*, and *Uranotaenia* sp.

and, to a lesser extent, with the accumulated precipitation ($r = 0.49$, $P < 0.05$). *Aedes albifasciatus*, *Ae. crinifer*, *Ae. scapularis*, and *Cx. eduardoi* were associated with lower temperatures, whereas *Cx.*

dolosus, *Cx. maxi*, *Cx. pilosus*, *Cx. pipiens*, *Cx. tatoi*, *Psorophora ciliata* (F.) (Diptera: Culicidae), and *Uranotaenia* sp. were associated with high temperatures. The second axis of the CA showed a marginally significant relationship with Fch ($r = 0.41$, $P = 0.056$), with recently flooded dates located at positive values and not recently flooded dates located at negative values of this axis. No correlation with the remaining variables analyzed was detected. All floodwater species were plotted together with *Cx. pilosus* at positive values, and separated from stagnant water mosquito species, which were all plotted at negative values on the second axis (Fig. 3A–B).

Distribution Among Aquatic Environments and Association With Environmental Variables

Immature mosquitoes were collected from all the pools studied, although the abundance of floodwater and stagnant water mosquitoes varied in the different types of pools. A positive correlation of the abundance of floodwater mosquitoes with the coefficient of variation was detected ($r_s = 0.74$, $P < 0.01$), while the remaining variables showed no relation to the abundance of this group. A marginal positive correlation of the abundance of stagnant water mosquitoes with the mean surface area ($r_s = 57$, $P = 0.051$), and no relation to the remaining variables was detected. Among stagnant water mosquitoes, *Culex* species were collected in pools with different levels of insolation, whereas *Anopheles* and *Uranotaenia* were restricted to pools with high and intermediate sun exposure. Among floodwater mosquitoes, all species were recorded mainly in pools with low or

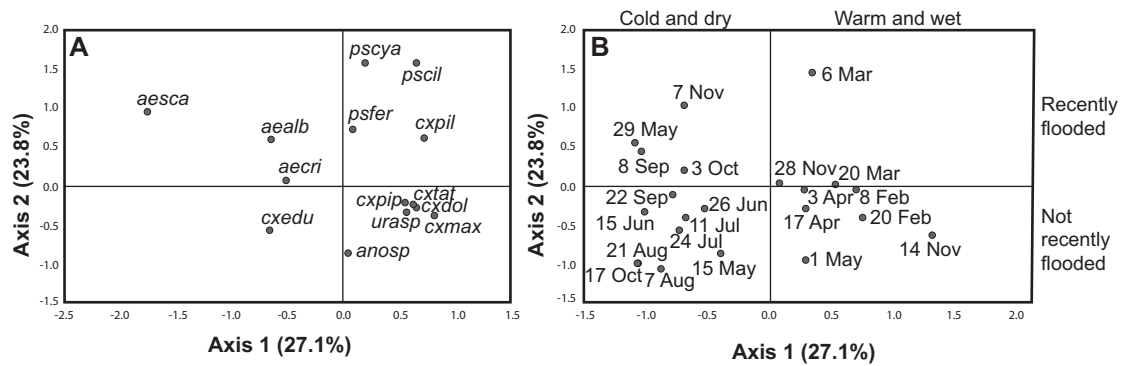


Fig. 3. Correspondence analysis ordination diagram for seasonal variation of mosquitoes, including all sampling dates (except January 10 and 24). (A) Mosquito species. (B) Sampling dates. Codes: *Anopheles* sp.: anosp; *Ae. albifasciatus*: aealb; *Ae. crinifer*: aecri; *Ae. scapularis*: aesca; *Cx. dolosus*: cxdol; *Cx. eduardoi*: cxedu; *Cx. pilosus*: cxpil; *Cx. pipiens*: cxpip; *Cx. maxi*: cxmax; *Cx. tatoi*: cxtat; *Ps. ciliata*: pscil; *Ps. cyanescens*: pscya; *Ps. ferox*: psfer; *Uranotaenia* sp.: urasp.

Table 3. Relevant environmental variables, proportion of floodwater mosquitoes and number of third and fourth instar larvae of each species collected in each pool during the study period in the Bosque de Ezeiza, of Buenos Aires province (Argentina)

	A3A	A5	A7	A12A	A12B	A9	A18	A3B	A19	A32	A30	A31	
Variables													
Mean flooded area (m ²)	1115.8	2481.7	999.9	147.0	1239.7	2145.5	1479.3	835.9	986.5	472.9	371.2	214.5	
Mean insolation	1.00	1.00	1.00	0.83	0.81	0.79	0.60	0.35	0.23	0.15	0.07	0.02	
Coefficient of variation of flooded area	0.44	0.56	0.53	0.55	0.57	0.69	0.62	0.51	0.74	1.04	0.61	0.58	
Proportion of floodwater mosquitoes	0.23	9.24	55.25	5.56	17.65	63.19	31.70	24.77	60.87	73.32	70.76	43.89	
Species													
<i>Ae. albifasciatus</i> (Macquart)	2	2	90	1	1	500	77	58	201	404	148	64	Total 1,548
<i>Ae. crinifer</i> (Theoblad)	-	-	-	-	-	10	29	20	73	45	45	14	236
<i>Ae. scapularis</i> (Rondani)	-	-	-	-	-	-	-	-	-	1	-	-	1
<i>Anopheles</i> sp. Meigen	14	21	11	5	2	-	3	4	-	-	1	-	61
<i>Cx. dolosus</i> (Lynch Arribálzaga)	17	5	-	-	4	-	-	11	4	2	2	-	45
<i>Cx. eduardoi</i> Casal and Garcia	18	21	44	7	16	26	24	22	60	97	29	12	376
<i>Cx. pilosus</i> (Dyar and Knab)	11	33	1	1	0	264	167	122	81	16	6	3	705
<i>Cx. pipiens</i> L.	70	11	13	-	5	12	8	40	13	14	29	27	242
<i>Cx. maxi</i> Dyar	6	4	6	-	-	1	2	18	5	10	17	1	70
<i>Cx. tatoi</i> Casal and Garcia	724	2	5	4	1	-	21	10	15	28	4	58	872
<i>Ps. ciliata</i> (F.)	-	-	-	-	-	3	1	1	2	2	2	-	11
<i>Ps. cyanescens</i> (Coquillett)	-	9	10	-	5	6	2	3	4	7	17	-	63
<i>Ps. ferox</i> (Von Humboldt)	-	-	-	-	-	-	1	-	-	-	1	1	3
<i>Uranotaenia</i> sp. Lynch Arribálzaga	1	11	1	-	-	-	12	22	2	-	-	-	49

intermediate intensity of insolation, except for *Ps. cyanescens*, which was also found in pools with high insolation (Table 3).

The first two axes of the CA accounted for 55.8% of the variability in species composition among pools. The first axis of the analysis showed a negative correlation with the mean insolation ($r = -0.705$, $P < 0.02$), and a positive one with the coefficient of variation ($r = 0.59$, $P < 0.05$), whereas the second axis showed a negative correlation with the mean surface area ($r = -0.75$, $P < 0.01$). *Culex pilosus* and all floodwater mosquito species (except *Ps. cyanescens*) were associated with pools with lower mean insolation and higher coefficient of variation, whereas the opposite trend was observed for the remaining species. The species most associated with pools of greater Sm were *Cx. pilosus*, *Ps. ciliata*, *Ps. cyanescens*, and *Uranotaenia* sp., whereas *Ae. scapularis*, *Cx. tatoi*, and *Ps. ferox* were associated with smaller pools (Fig. 4A and B).

Discussion

The results of the present study show that the temporary pools of the Bosque de Ezeiza produced mosquitoes throughout the year and

in all the pools studied. The species composition was similar to that observed in rain pools from urban areas within the same region (Fischer et al. 2000, Fontanarrosa et al. 2009, Quiroga et al. 2013), although the richness recorded in the Bosque de Ezeiza was higher than that reported individually by each of these studies. These differences are due to the presence for example of *Ps. ciliata*, a rare species in the urban environment (Campos et al. 2004), and *Cx. pilosus*, a species recently cited for the first time in the region (Urcola and Fischer 2015).

The presence of mosquitoes on all sampling dates with water and the relatively high proportion of advanced immature stages of stagnant water mosquitoes suggest that the pools studied have a longer duration than the urban rain pools previously studied, which mostly dry out before floodwater mosquitoes could complete their development (Fischer et al. 2002), or before stagnant water mosquitoes colonize them (Fischer and Schweigmann 2008, Quiroga et al. 2013). However, it has to be noted that the record of rainfall during the study period was 45% higher than the historical average (Servicio Meteorológico Nacional 2018), which may have also favored the

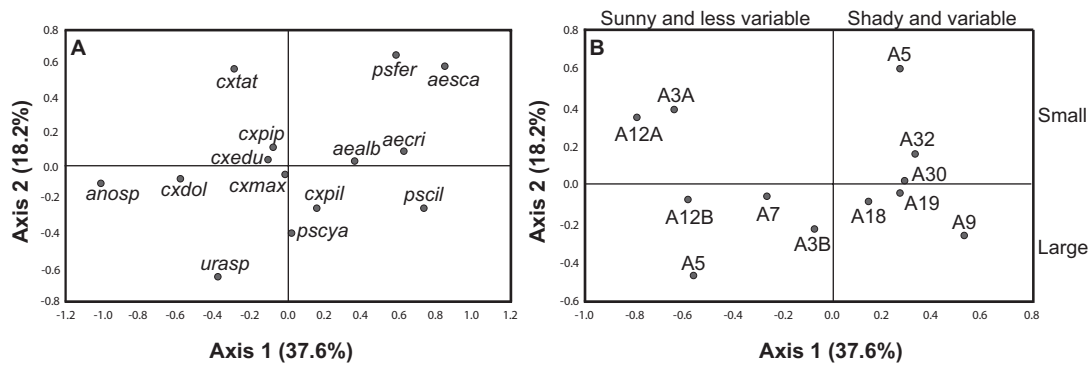


Fig. 4. Correspondence analysis ordination diagram for spatial variation of mosquitoes. (A) Mosquito species. (B) Temporary pools. Codes: *Anopheles* sp.: anosp; *Ae. albifasciatus*: aealb; *Ae. crinifer*: aecri; *Ae. scapularis*: aesca; *Cx. dolosus*: cxdol; *Cx. eduardoi*: cxedu; *Cx. pilosus*: cxpil; *Cx. pipiens*: cxpip; *Cx. maxi*: cxmax; *Cx. tatoi*: cxtat; *Ps. ciliata*: pscil; *Ps. cyanescens*: pscyca; *Ps. ferox*: psfer; *Uranotaenia* sp.: urasp.

permanence of the pools and the sustained mosquito production. This was particularly evident in autumn and winter, when lower temperatures prevented the pools from rapidly drying out.

Under these conditions, the temporal dynamics of floodwater mosquitoes was predicted by meteorological and flooding variables. Both the association of floodwater mosquitoes with recently flooded sites and the association of stagnant water mosquitoes with large not recently flooded sites are coincident with that expected according to the oviposition strategy of each group. The eggs of floodwater mosquitoes laid on moist soil may hatch immediately after flooding, allowing the detection of third and fourth larval instars within a few days. In contrast, even stagnant water mosquitoes that prefer laying eggs on 2- to 3-day-old recently flooded sites (Beehler and Mulla 1995, Day 2016) are detected later because of the time elapsed from flooding to egg laying, the time to complete embryonic development, and the time to reach the third and fourth larval instars. This has been observed in temporary pools in Buenos Aires city, where peak abundances of floodwater mosquitoes were detected within the first week, while *Culex* larvae were recorded mainly 2–3 wk after flooding (Fischer and Schweigmann 2008). Furthermore, the association of stagnant water mosquito abundance with dates with larger pool areas and with pools of larger size might be explained by the increased possibility of visual detection of these pools (Day 2016).

In the present study, the temperature, rainfall, and flooding dynamics were the most important variables explaining the temporal variability in species composition. Only one species, *Ps. cyanescens*, was associated with extreme conditions of high temperature and recent flooding after a prolonged period of drought. This species is characterized by its unusually short larval period, between 3 and 4 d (Schwardt 1939), and similarly to our results, in North America, the population increases after heavy summer rains (Carpenter and LaCasse 1955).

Besides these extreme conditions, the variability in species composition is given by seasonal changes in temperature and short-term flooding and precipitation variability.

Regarding thermal seasonality, the association of *Ae. albifasciatus* and *Ae. crinifer* with colder temperatures and the association of *Ps. ciliata*, *Cx. maxi*, and *Cx. tatoi* with warm temperatures are consistent with previous studies in the region (Campos et al. 1993, Campos et al. 1995, Fischer et al. 2000, Fontanarrosa et al. 2009, Quiroga et al. 2013). Furthermore, the presence of *Ps. cyanescens* and *Ps. ferox* in the warm season, the records of *Anopheles* sp. in autumn and winter, and the presence of *Cx. pipiens* throughout the year are also coincident with previous observations for these species in the region (Campos et al. 1993, Campos et al. 1995, Fischer

et al. 2000, Quiroga et al. 2013). Finally, the association of *Cx. dolosus* with the warm season in the Bosque de Ezeiza contrasts with previous studies reporting the presence of immature stages of this species in the region throughout the year (Maciá et al. 1997, Fischer et al. 2004).

Besides thermal seasonality, the flooding history of the pools was the second source of temporal variability in species composition. The association of *Cx. pilosus* with floodwater mosquitoes (*Aedes* and *Psorophora*) and not with stagnant water mosquitoes (*Anopheles*, *Culex*, and *Uranotaenia*) supports the hypothesis that this species has a life strategy more similar to that of floodwater mosquitoes, which might relate to a certain drought resistance of the eggs, as suggested by several authors (King et al. 1944, Galindo et al. 1951, King 1960, Castro Gomes et al. 1998, Urcola and Fischer 2015).

The association of *Ae. albifasciatus* with habitats with lower insolation in the Bosque de Ezeiza agrees with previous observations in the city of Buenos Aires (Fischer et al. 2002), where a greater association of larvae of this species with intermediate and low insolation was observed (mainly in summer), and disagrees with other works where the species was associated with environments exposed to sunshine (Maciá et al. 1995, Fontanarrosa et al. 2000). The fact that *Ae. albifasciatus* larvae are associated with different levels of insolation depending on the season and site suggests a certain plasticity that allows this species to avoid temporary adverse environmental conditions. For example, in temperate regions, in summer, this species is present in the shaded pools (to avoid the premature desiccation of the pool or extremely high temperatures), whereas in winter, the species prefers pools with greater insolation (to avoid the coldest conditions) (Fischer et al. 2002). The association of *Ae. crinifer* with partial and completely shaded pools is consistent with other studies, in which this species was found in areas of dense forest (Cardo et al. 2011, Cardo et al. 2013).

The identification of factors that affect the temporal and spatial variation of mosquito vectors is valuable information to develop models that predict the risk of exposure of humans and animals, and to design mosquito control strategies. This study shows associations between the abundance of mosquito species and environmental variables such as the permanence of the water, the dimensions of the aquatic environment, its exposure to the sun, and climatic variables such as the temperature and the precipitations of the previous days. The Bosque de Ezeiza has a strategic location as a source of adult mosquitoes to the surrounding urban areas, and future studies should assess the frequency of high mosquito abundances dispersing to these urban areas, including Buenos Aires city.

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