Culex mosquitoes in temporary urban rain pools: Seasonal dynamics and relation to environmental variables

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ABSTRACT: The study was conducted in a park of Buenos Aires City, where a total of 89 rain pools were sampled weekly for mosquito immature stages over a one-year period. The aim of the present paper was to investigate the seasonal dynamics of three *Culex* species breeding in temporary rain pools and to analyze the relationships of the presence of these species to pool dimensions, pool age, vegetation, and insolation degree. The three species showed differences in their seasonal patterns, *Culex dolosus* being present during the whole year, *Culex pipiens* mainly in the summer season, and *Culex maxi* almost exclusively during the fall. The variable explaining most of the variation among sampling dates in species composition was weekly mean temperature. A significant positive association was detected among mosquito species, as they were recorded together more frequently than expected by chance. The statistical analyses performed revealed significant positive relationships of all three mosquito species to increasing surface area, whereas no relationship to insolation degree was detected in the studied pools. *Culex pipiens* and *Culex dolosus* showed positive relationships to increasing vegetation cover, whereas the presence of *Culex dolosus* was also related to pool age. ***Journal of Vector Ecology*** **29 (2): 365-373. 2004.**

Keyword Index: Culex, mosquito larvae, environmental variables, urban pools, temporary waters, Argentina.

INTRODUCTION

Mosquitoes are known to be vectors of several diseases, including various types of encephalitis. *Culex* sp. mosquitoes are involved in the primary transmission cycle of St. Louis Encephalitis (SLE). The urban transmission cycle in Argentina may involve *Culex* mosquitoes and several birds such as sparrows or chickens (Sabattini et al. 1998). Although SLE cases are very rare in Argentina, a case was recently reported in Cordoba Province (Spinsanti et al. 2003).

The World Health Organization recommended an emphasis on vector control, including biological control and environmental management (Rejmankova et al. 1991). Knowledge of the relationships between habitats, environmental factors, and occurrence of mosquito larvae is essential for an efficient application of mosquito control methods.

Temporary waters are a major source of breeding habitats for different mosquito species, some of which have proved to be of medical and sanitary importance (Laird 1988). Temporary pools provide an opportunity to study relationships between species presence and/or abundance and several environmental variables under natural conditions. The local abundance of these pools over a relatively small area provides a large number of sampling units differing in size, duration, vegetation cover, and degree of insolation, exposed to the same climatic (temperature and rainfall) regime. Several authors reported relationships between pool duration, pool size, temperature, substrate vegetation, insolation degree, and the presence of different mosquito species in temporary pools (Ward and Blaustein 1994, Nilsson and Svensson 1995, Schneider and Frost 1996, Blaustein et al. 1999).

Among the frequent *Culex* species in Buenos Aires, Argentina, *Culex eduardoi* Casal & García, *Cx. maxi* Dyar, and *Cx. pipiens* Linneus were the most abundant in ephemeral rain pools (Fischer et al. 2000). All three species breed both in natural and artificial habitats (Campos et al. 1993, Almirón and Brewer 1996) and were reported in urban areas of Buenos Aires Province (Campos et al. 1993). *Culex eduardoi* and *Cu. dolosus* are considered to be synonyms (Amirón and Brewer

1995, Rossi, 2000).

Culex dolosus (Lynch Arribálzaga) breeds both in permanent and temporary waters, with aquatic plants, grass, or without vegetation (Almirón and Brewer 1996). In urban areas, this species was mainly associated with clean, non-polluted water (Campos et al. 1993). The immature stages were reported throughout the year in Cordoba (Almirón and Brewer 1995) and Buenos Aires Provinces (Maciá et al. 1997). *Culex pipiens* exploits a great variety of breeding sites, frequently artificial containers and small or medium stagnant water collections. The presence of immatures has been related to shaded environments (Forattini 1965) and to polluted waters with high contents of organic matter (Horsfall 1955, Campos et al. 1993). In Cordoba and Buenos Aires Provinces this species breeds throughout the year (Campos et al. 1993, Almirón and Brewer 1995). *Cx. maxi* breeds in temporary and permanent waters, generally in natural habitats with vegetation, either exposed to sunlight or shaded (Almirón and Harbach 1996). The immature stages of this species have been reported from November to May in Buenos Aires Province (Campos et al. 1993).

The rain pools in the parks of Buenos Aires City are extremely ephemeral, mostly drying out before mosquitoes can complete their development (Fischer et al. 2002). Water permanence is positively related to the amount of water present at a given time, and negatively related to temperature (Fischer[3]). Therefore, the total area covered by water and the mean weekly temperature are the variables that best account for the variability among sampling dates.

The present longitudinal work aims at studying the seasonal pattern of incidence of three *Culex* species and the relationships between microenvironmental variables and their presence in these extreme habitats.

MATERIALS AND METHODS

The study was carried out in a Buenos Aires City park extending over approximately 13 ha. The city is located in a humid temperate climatic region, with an annual mean temperature of 17.6ºC and annual accumulated rainfall exceeding 1000 mm. Saavedra Park (34º33´S - 58º29´W) has an irregular topography that favors the formation of a great number of ephemeral pools (or puddles) after rain. These water bodies range from 0.1 to 600 m² in surface area, 1 to 24 cm in depth, and last from one week to several months depending on the climatic conditions (Fischer et al. 2000). The substrate vegetation of the pools is mainly grass (Gramineae), which is periodically cut, and trees differentially shading the pools. Owing to its location in a densely populated urban area, the park is regularly visited by a great number of people and dogs that regularly perturbate the pools.

All of the 89 pools present in the study area between June 1998 and May 1999 were identified by a code and evaluated weekly. Surface area and maximum depth were determined on every survey. The surface area of each pool was estimated by measuring the area of a rectangle containing the pool, multiplied by the percentage of this rectangle covered by water. Each pool was assigned to one of three insolation categories (shade, half shade, and open sun) and to one of three surface vegetation cover categories (lacking, scarce, and totally covered). In addition, the presence of water in each pool was recorded three times a week from October 1998 through May 1999, in order to assess the age of each pool on every sampling date.

Culex immature stages were sampled weekly. Deeper pools (>5 cm) were dragged along the bottom with a 350 mm hand net (10 x 8 cm) on a single immersion that covered the longest axis of each pool. An 80 ml plastic dipper was used to sample the shallowest pools (<5 cm). The number of dips collected was proportional to the surface area of each pool (from 5 to 160 dips). The samples were fixed *in situ* in 5% formaldehyde and stored in 80% ethanol.

The immature stages of *Culex* were sorted by stage and third and fourth stages were counted and identified to species using the systematic key of Darsie (1985), original descriptions, and new taxonomic criteria for particular groups (Almirón and Harbach 1996). Those larvae identified as *Cx. eduardoi* after Darsie (1985) were assigned to *Cx. dolosus*, owing to the reported impossibility to distinguish between *Cx. dolosus* and *Cx. eduardoi* (Almirón and Brewer 1995, Rossi 2000). Only the information on *Cx. dolosus*, *Cx. maxi,* and *Cx. pipiens* is herein analyzed.

Data analysis

The correlations between weekly total abundance and numbers of colonized pools were analyzed for each mosquito species. Abundance values were transformed to log10 (abundance + 1).

The number of pools shared with other species was divided by the total number of pools where each species was recorded to calculate the percentage of shared pools. The pairwise co-occurrence of mosquito species in the

[3]Fischer, S. 2003. Dinámica estacional de insectos acuáticos en ambientes efímeros urbanos (con énfasis en los culícidos). Ph.D. Thesis, Universidad de Buenos Aires, 113 pp.

pools was analyzed by the Chi^2 test of independence. Only the dates on which both members of a pair were present were included in the analyses.

Seasonal pattern of occurrence

Seasonal cumulative proportions of colonized pools were calculated for each species and compared among seasons by means of tests for independent proportions (Fleiss 1981). The total flooded area and the mean weekly temperature corresponding to each sampling date were used to explore the conditions related to the presence of *Culex pipiens*, *Cx. dolosus,* and *Cx. maxi*. The relationships between weather and flooding conditions and the abundance of all three species were explored by means of multiple regression analyses (Chatterjee et al. 2000). The dependent variable was the abundance (transformed as stated previously) of a species on each sampling date, and the independent variables included were mean weekly temperature, weekly cumulated rainfall, flooded area, and flooded area on the previous sampling date. Totally dry dates were excluded from this analysis.

A canonical correspondence analysis (CCA) was used to summarize the relationships between species composition and temperature, flooding conditions, and season. Transformed species abundance values were used, and season was transformed into dummy variables. Significant effects of each variable on *Culex* mosquito composition were evaluated using forward selection and a Monte Carlo resampling procedure with 999 permutations (ter Braak and Smilauer 1998).

Association to environmental variables

The relationships between environmental variables and the presence of each mosquito species were screened first univariately, in order to enhance the interpretability of results. Weekly records of pools were divided among several categories for every measured variable, according to the following gradients: surface area: 0–1 m^2, 1–10 m^2, 10–100 m^2, >100 m^2; pool depth: 1–5 cm, 6–10 cm, 11–15, >15 cm; vegetation cover: lacking, scarce, totally covered; insolation: shade, half shade, open sun; and pool age: $\leq$1 week, >1–2 weeks, >2–3 weeks, >3 weeks. Differences in proportions among categories of each variable were assessed by means of tests for independent proportions (Fleiss 1981). The associations of the environmental variables and the presence of each mosquito species were then assessed by means of stepwise logistic regression analyses (Chatterjee et al. 2000). The following variables were included in the analysis: surface area, maximum depth, pool age, vegetation cover, and insolation degree. The variables were transformed in order to enhance their normality and the interpretability of the results. Surface areas were logarithmically transformed and depths were transformed to square roots. Pool age was calculated in weeks. Insolation degree was transformed into dummy variables in this analysis. The logistic regression analyses were performed on annual and on seasonal data. Only pool records of dates on which each of the species was present were included both in the univariate and in the logistic regression analyses. Multiple regression and logistic regression analyses were performed with the S-PLUS V.6.02 statistical package.

RESULTS

A total of 1,084 collections were made in 89 different habitats, from where 1,673 third and fourth stage culicids were recorded, 71% *Cx. pipiens*, 25% *Cx. dolosus,* and 4% *Cx. maxi*. Although present on relatively fewer sampling dates as compared to *Cx. dolosus*, the high abundance values attained by *Cx. pipiens* on three occasions accounted for the higher total abundance of this species during the whole study period. *Cx. dolosus* was the most frequently recorded mosquito species (8.4% of the 1,084 weekly samples), followed by *Cx. pipiens* (4.2%) and *Cx. maxi* (2.3%). All three mosquito species showed a positive relationship between the number of colonized pools and the number of collected larvae (Figures 1a, 1b, and 1c). The correlation coefficient showed highest values for *Cx. maxi* ($r = 0.94$, $N = 9$, $p< 0.001$), intermediate for *Cx. dolosus* ($r = 0.82$, $N = 20$, $p< 0.001$), and lowest for *Cx. pipiens* ($r = 0.76$, $N = 12$, $p<0.005$).

The percentage of pools shared with other *Culex* species was lowest for *Cx. dolosus* (35%), intermediate for *Cx. pipiens* (59%), and highest for *Cx. maxi* (68%). The pairwise comparison showed a significant positive association between *Cx. dolosus* and *Cx maxi* ($Chi^2 = 63.36$, df = 1, $p<0.001$), *Cx. dolosus* and *Cx. pipiens* ($Chi^2 = 67.07$, df = 1, $p<0.001$), and between *Cx. pipiens* and *Cx. maxi* ($Chi^2 = 40.69$, df = 1, $p<0.001$).

Seasonal pattern of occurrence

Most pools (one to 68) remained flooded throughout the winter, when no dry dates occurred. During the spring, the highest number of pools recorded was 45, with five dry dates out of 13 surveys. The summer season showed a maximum of 35 pools, and one dry date out of 13 surveys, whereas a range of one to 46 pools was observed during the fall. The distribution of the sampling dates according to temperature and flooded area are shown in Figure 2a. Mean weekly temperatures ranged between 11ºC and 27ºC, while water cover ranged from totally dry (not shown in Figure 2a) up to 2775 m^2.

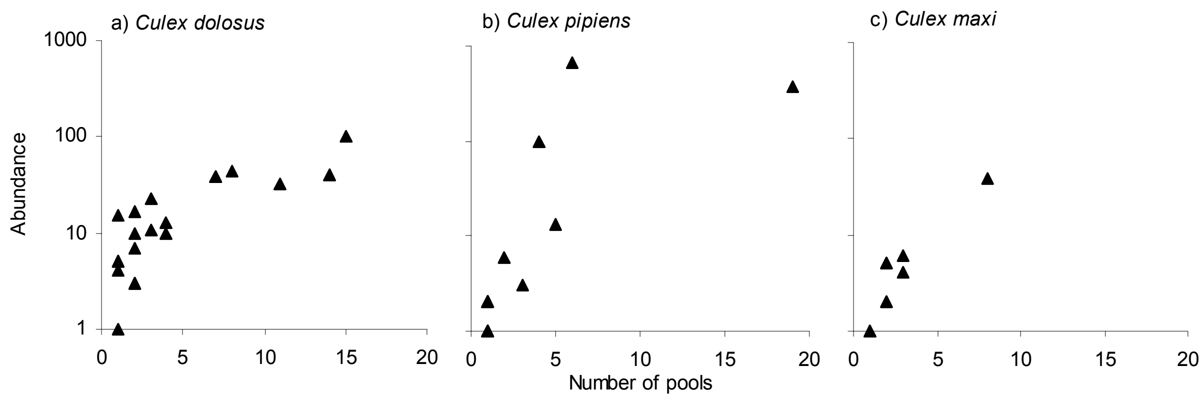

Figure 1. Relationship between the number of colonized pools and the abundance of a) *Culex dolosus*, b) *Culex pipiens,* and c) *Culex maxi*.

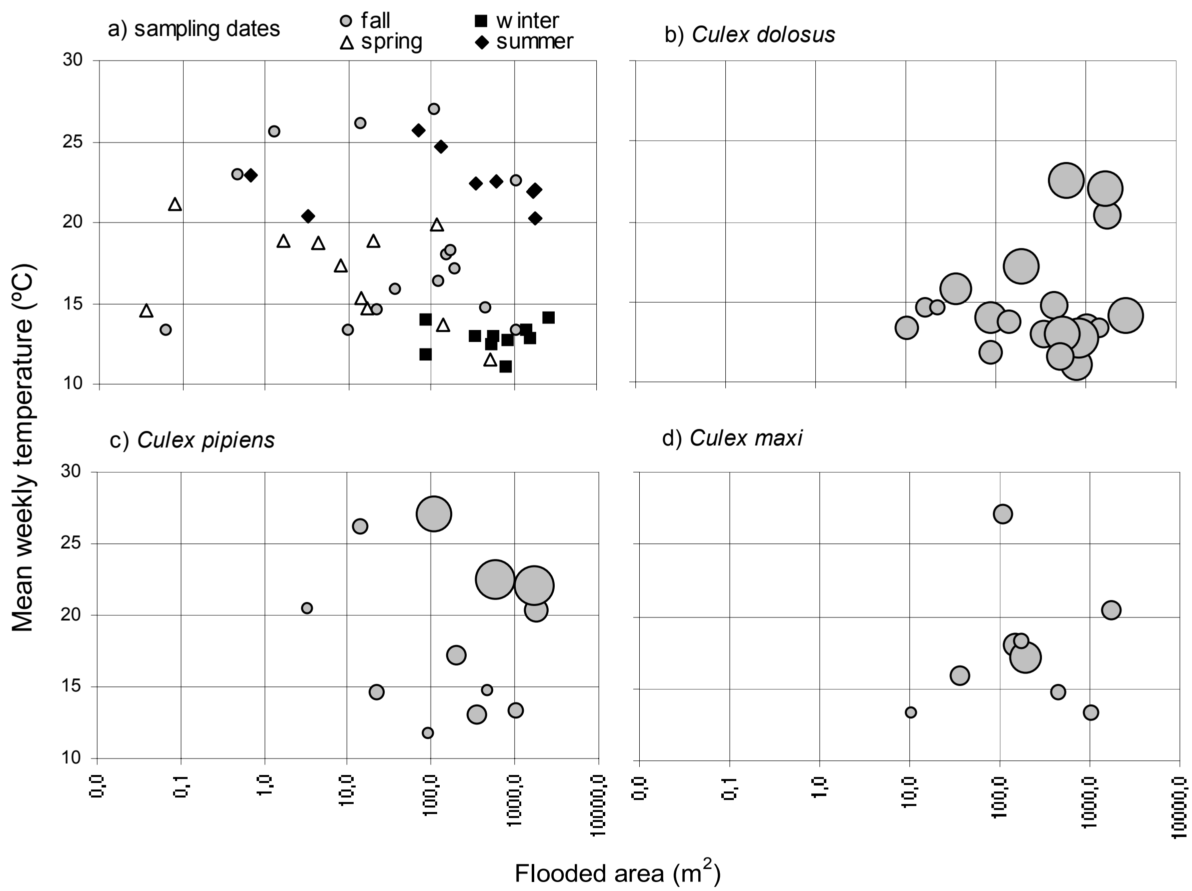

Figure 2. Distribution of temperatures and flooded area on the sampling dates a), and on dates when b) *Culex dolosus*, c) *Culex pipiens,* and d) *Culex maxi* larvae were collected. Circle sizes are related to the number of collected larvae.

Table 1. Multiple regression coefficients of environmental variables (area covered by water, area covered by water during the week before to sampling date, weekly cumulated rainfall, and weekly main temperature) related to *Culex dolosus* and *Culex pipiens* abundance.

	Culex dolosus	*Culex pipiens*
Intercept	-0.386 (p=0.02)	-1.855 (p<0.001)
Flooded area	0.169 (p=0.03)	- (ns)
Flooded area during the week before to sampling date	0.306 (p<0.001)	0.365 (p<0.001)
Weekly cumulative rainfall	- (ns)	- (ns)
Weekly mean temperature	- (ns)	0.082 (p<0.001)

Culex dolosus immatures were observed throughout the whole year (Figure 3a), with proportions of positive pools showing no significant differences among seasons. The immature stages were recorded at temperatures below 23ºC, and on dates having over 10 m^2 of flooded area (Figure 2b). Multiple regression analysis showed a positive relationship between the abundance of *Cx. dolosus* and flooded area on the sampling date and on the previous week ($r^2 = 0.49$, $p<0.001$) (Table 1).

Culex pipiens immatures were mainly observed in the summer (Figure 3b), showing significantly higher positive pools proportions during this season ($p<0.05$). On the other hand, *Cx. pipiens* larvae were recorded for the whole range of temperatures and for almost the whole range of flooded area (Figure 2c). Multiple regression analysis showed a positive association between the abundance of this species and both mean weekly temperature and flooded area on the previous week ($r^2 = 0.42$, $p<0.001$) (Table 1).

Culex maxi reached its highest abundance and incidence values in the fall (Figure 3c), when the proportions of positive pools were significantly higher ($p<0.001$). *Cx. maxi* immatures were collected at temperatures higher than 13ºC, and on dates with more than 10 m^2 of flooded area (Figure 2d). Multiple regression analysis showed no relationship between the abundance of *Cx. maxi* and the analyzed variables. Weekly cumulative rainfall failed to show any relationship to the abundance of all three mosquito species.

The first two axis for the CCA accounted for 57% of the variation in species composition. The significant variables in explaining species composition were temperature ($p<0.03$) and fall season ($p<0.05$), explaining 32% and 18% of the variance respectively. *Cx. dolosus* and *Cx. pipiens* showed opposite trends on the first axis (mainly related to a temperature gradient), the former being more associated with the winter and the latter with the summer season. On the other hand, high proportions of *Cx. maxi* showed a close relationship to the fall season (Figure 4).

Association to environmental variables

The relationships between the presence of all three mosquito species and the analyzed variables for the whole period are shown in Table 2. The proportion of infested pools showed significant differences among categories for each of the analyzed variables, with the exception of pool depth and insolation degree for *Cx. maxi* and pool age for *Cx. pipiens* (Table 2). In general, increasing categories of surface, depth, and age showed higher proportions of pools holding each of the analyzed species. Those pools lacking vegetation were proportionally less colonized, whereas half–shaded pools

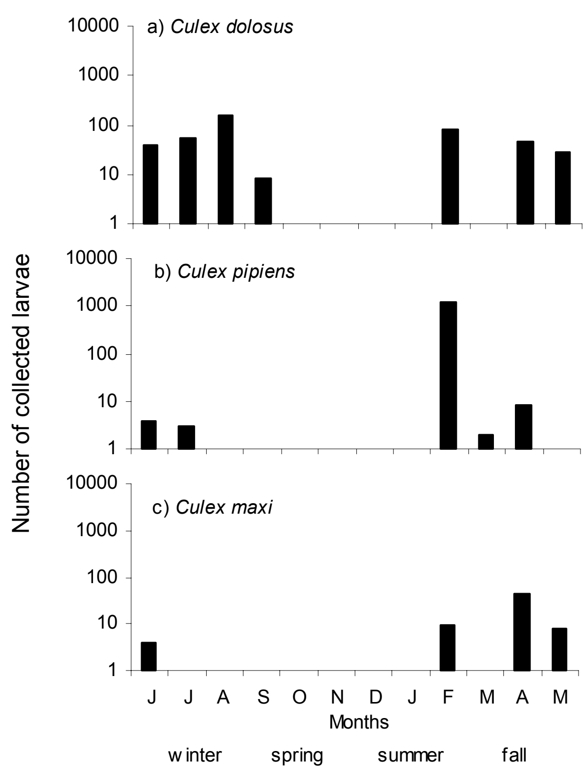

Figure 3. Monthly number of collected larvae of a) *Culex dolosus*, b) *Culex pipiens,* and c) *Culex maxi*.

Table 2. Proportions and number of colonized to total pools for different categories of environmental variables considering the whole study period. P values account for differences among categories of each variable.

	colonized / total	Proportion	p value of differences	colonized / total	Proportion	p value of differences	colonized / total	Proportion	p value of differences
		Culex dolosus			*Culex pipiens*			*Culex maxi*	
Surface area (m^2)									
<1	13/189	0.07		8/102	0.08		4/84	0.05	
1–10	28/263	0.11	p<0.001	12/123	0.10	p<0.001	10/102	0.10	p<0.005
10–100	35/136	0.26		16/61	0.26		8/39	0.21	
>100	16/29	0.55		8/15	0.53		3/8	0.38	
Pool depth (cm)									
1–5	26/336	0.08		13/161	0.08		8/132	0.06	
6–10	40/218	0.18	p<0.001	17/103	0.17	p<0.001	11/72	0.15	ns
11–15	21/52	0.40		9/29	0.31		5/25	0.20	(p>0.05)
>15	5/11	0.45		5/8	0.63		1/4	0.25	
Pool age (weeks)									
≤1	7/175	0.04		7/61	0.11		2/67	0.03	
>1–2	26/203	0.13	p<0.001	21/174	0.12	ns (p>0.05)	12/115	0.10	p<0.005
>2–3	20/65	0.31		7/33	0.21		7/23	0.30	
>3	39/174	0.22		9/33	0.27		4/28	0.14	
Vegetation cover (category)									
0	17/283	0.06		6/134	0.04		4/104	0.04	
0.5	39/189	0.21	p<0.001	23/98	0.23	p<0.001	15/69	0.22	p<0.001
1	36/145	0.25		15/69	0.22		6/60	0.10	
Insolation degree (category)									
0	18/196	0.09		8/96	0.08		5/81	0.06	ns
0.5	35/149	0.23	p<0.005	17/71	0.24	p<0.05	9/59	0.15	(p>0.1)
1	39/272	0.14		19/134	0.14		11/93	0.12	

Table 3. Logistic regression analysis of the presence of *Culex dolosus*, *Culex pipiens* and *Culex maxi* related to environmental variables in rain pools (the statistics reported are odds ratio and confidence interval).

		Surface area	Pool depth	Pool age	Vegetation cover
Culex dolosus	Whole year	3.27 (2.31–4.62)			3.84 (2.11–6.97)
	Winter		2.62 (1.67–4.11)		2.50 (1.11–5.65)
	Summer	3.27 (1.50–1.17)		2.34 (1.01–5.41)	14.26 (3.23-63.16)
	Fall	6.42 (2.72–15.17)		1.36 (1.06–1.76)	
Culex pipiens	Whole year	3.60 (2.19–5.92)			3.96 (1.65–9.52)
	Summer	3.18 (1.72–5.87)			5.60 (1.87–16.79)
Culex maxi	Whole year	3.77 (2.01–7.07)			
	Fall	5.24 (2.29–11.95)			

showed higher proportions of mosquito presence than totally shaded pools or exposed pools.

Logistic regression analysis showed a positive relationship of *Cx. dolosus* to increasing surface area and vegetation cover for the entire year (Table 3). Significant relationships differed among seasons. In the winter season, pool depth and vegetation cover showed a significant positive association; during the fall, surface area and pool age were significantly associated; and during summer, vegetation cover was also important.

The presence of *Culex pipiens* showed a positive relationship to increasing surface area and vegetation cover of the pools (Table 2). Both variables were included in a whole-year model as well as in a model for the summer season considered separately (Table 3). The fall and winter were not analyzed because of the low number of colonized pools.

The presence of *Culex maxi* was positively related to the surface area of the pools, both for the whole period and for the fall season (Table 3). The remaining seasons were not analyzed because of the low number or even absence of colonized pools.

Logistic regression analyses failed to show any significant relationship between insolation degree and the presence of the analyzed mosquito species either for the whole period scale or for individual seasons.

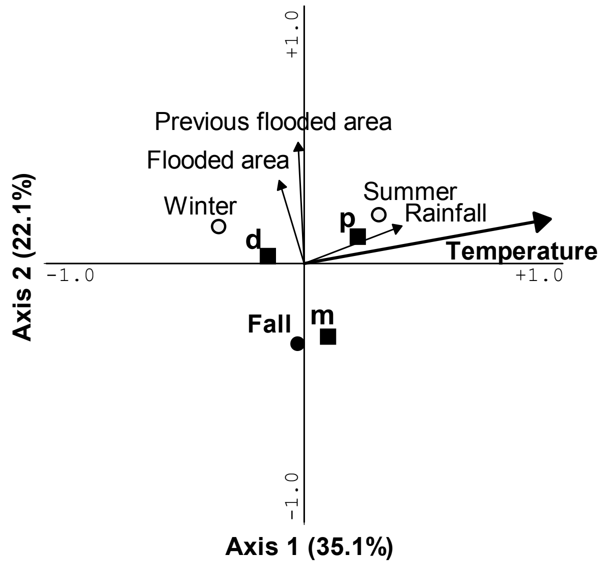

Figure 4. Canonical correspondence ordination diagram for mosquito larvae and temperature, flooding conditions, and season. Thick arrow and full circle indicate significant variables for mosquito ordination. Filled squares indicate the position of mosquito species: d = *Culex dolosus*, p = *Culex pipiens* and m = *Culex maxi*.

DISCUSSION

The differences in cumulative abundance and frequency of detection between *Cx. dolosus* and *Cx. pipiens* contribute to illustrate the different strategies of these species, suggesting a tendency of *Cx. pipiens* to increase explosively as compared to *Cx. dolosus*.

All three mosquito species showed marked differences in their seasonal patterns of abundance. The observed year-round presence of *Cx. dolosus* in Saavedra Park suggests that both the development and the oviposition activity of *Cx. dolosus* are not restricted by the minimum temperatures occurring in Buenos Aires. The results of our research are coincident with other observations on this species in Cordoba and Buenos Aires Provinces (Almirón and Brewer 1995, Maciá et al. 1997). On the other hand, the presence of this species has been reported in Chubut Province in southern Argentina (Schweigmann et al. 2003), supporting the hypothesis of the tolerance of this species to low temperatures.

Previous studies reported the presence of *Cx. pipiens* during the entire year, both in Cordoba and Buenos Aires Provinces (Campos et al. 1993, Almirón and Brewer 1995), indicating that the extreme temperatures of Buenos Aires do not prevent its development. The reported relationship of *Cx. pipiens* to container habitats and to polluted waters (Horsfall 1955, Almirón and Brewer 1996), and its summer prevalence in Saavedra Park suggest that the ephemeral rain pools would not be the main breeding sites for this species, at least at low temperatures.

Culex maxi was the most seasonally restricted of all three analyzed mosquito species. These findings are coincident with previous studies reporting the presence of this species between November and May (Campos et al. 1993), with peak abundance values in April (Maciá et al. 1997). Water permanence in these pools seems to be an important variable for both *Cx. dolosus* and *Cx. pipiens*, as shown by the positive relationships of their abundances and the flooded area during the week before to the sampling date. The positive association of *Cx. pipiens* abundance to increasing temperatures is consistent with the dominance of this species during the summer season. The presence of the three mosquito species was positively related to increasing surface area and/or depth. Pools of greater size may have lesser physicochemical fluctuations than smaller ones. In addition, large pools generally last longer (Schneider and Frost 1996), thus allowing the breeding species to complete their development (Nilsson and Svensson 1995, Wellborn et al. 1996).

The positive relationship between vegetation cover and the presence of both *Cx. dolosus* and *Cx. pipiens* could be related to the role of vegetation as a refuge from potential predators. Although the univariate analysis showed a positive association of *Cx. maxi* to vegetation cover, this was not supported by logistic regression analysis. These contradicting results, together with the previous observations of association of this species to ground vegetation (Almirón and Harbach 1996), suggest the necessity of further studies on the habitat preferences of this species. The degree of insolation did not seem to have a significant relationship to the presence of the studied mosquito species. Nevertheless, this should be tested further, keeping in mind the significantly higher proportions (as shown by the univariate analysis) of half–shaded pools colonized by *Cx. dolosus* and *Cx. pipiens*.

Also, all mosquito species were positively associated to each other, and were recorded together more frequently than expected by chance only. These results are probably related to common habitat requirements, represented by similar associations to individual environmental variables like surface area and vegetation cover.

The main differences among species were related to their seasonal pattern, with *Cx. dolosus* present during the whole year, *Cx. pipiens* mainly in the summer, and *Cx. maxi* in the fall season.

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REFERENCES CITED

Almirón, W. and M. Brewer. 1995. Distribución estacional de Culicidae (Diptera) en áreas periféricas de Córdoba (Argentina). Ecol. Austral. 5: 81-86.

Almirón, W. and M. Brewer. 1996. Classification of immature stage habitats of Culicidae (Diptera) collected in Córdoba, Argentina. Mem. Inst. Oswaldo Cruz 91: 1-9.

Almirón, W. and R.E. Harbach. 1996. Taxonomy and biology of *Culex* (*Culex*) *maxi* Dyar (Diptera: Culicidae) in South America. Mem. Inst. Oswaldo Cruz 91: 579-588.

Blaustein, L., J.E. Garb, D. Shebitz, and E. Nevo. 1999. Microclimate, developmental plasticity and community structure in artificial temporary pools. Hydrobiologia 392: 187-196.

Campos, R.E., A. Maciá, and J.J. García. 1993. Fluctuaciones estacionales de culícidos (Diptera) y sus enemigos naturales en zonas urbanas de los alrededores de La Plata, provincia de Buenos Aires. Neotrópica 39: 55-66.

Chatterjee, S., A.S. Hadi, and B. Price. 2000. *Regression analysis by example.* 3rd edition. John Wiley & Sons, New York, 359 pp.

Darsie, R.F.Jr. 1985. Mosquitoes of Argentina. Part I. Keys for identification of adult females and fourth stage larvae in English and Spanish (Diptera, Culicidae). Mosq. Syst. 17: 153-253.

Fischer, S., M.C. Marinone, M.S. Fontanarrosa, M. Nieves, and N. Schweigmann. 2000. Urban rain pools: seasonal dynamics and entomofauna in a park of Buenos Aires. Hydrobiologia 441: 45-53.

Fischer, S., M.C. Marinone, and N. Schweigmann. 2002. *Ochlerotatus albifasciatus* in rain pools of Buenos Aires: Seasonal dynamics and relation to environmental variables. Mem. Inst. Oswaldo Cruz 97: 767-773.

Fleiss, J.L. 1981. *Statistical methods for rates and proportions*. John Wiley & Sons, New York, 312 pp.

Forattini, O.P. 1965. *Entomología Médica. 2. Culicini: Culex, Aedes* e *Psorophora*. Edit. Univ. São Paulo, 506 pp.

Horsfall, W.R. 1955. *Mosquitoes. Their bionomics and relation to disease*. The Ronald Press Company, New York, 723 pp.

Laird, M. 1988. *The natural history of larval mosquito habitats*. Academic Press, London, 555 pp.

Maciá, A., J.J. García, and R. Campos. 1997. Variación estacional de tres especies de *Culex* (Diptera: Culicidae) y sus parásitos y patógenos en Punta Lara, Provincia de Buenos Aires, Argentina. Rev. Biol. Trop. 44 (3) / 45 (1): 267-275.

Nilsson, A.N. and B.W. Svensson. 1995. Assemblages of dytiscid predators and culicid prey in relation to environmental factors in natural and clear-cut boreal swamp forest pools. Hydrobiologia 308: 183-196.

Rejmankova, E., H.M. Savage, M. Rejmánek, J.I. Arredondo Jimenez, and D.R. Roberts. 1991. Multivariate analysis of relationships between habitats, environmental factors and occurrence of Anopheline mosquito larvae *Anopheles albimanus* and *A. pseudopunctipennis* in Southern Chiapas, Mexico. J. Appl. Ecology 28: 827-841.

Rossi, G.C. 2000. Las especies de mosquitos (Diptera: Culicidae) en la provincia de Buenos Aires, Argentina. Rev. Soc. Entomol. Argent. 59: 141-145.

Sabattini, M.S., G. Avilés, and T.P. Monath. 1998.

Historical, epidemiological and ecological aspects of arboviruses in Argentina: Flaviviridae, Bunyaviridae and Rhabdoviridae. In: A.P.A. Travassos da Rosa, P.F.C. Vasconcelos, and J.F.S. Travassos da Rosa (eds). *An overview of arbovirology in Brazil and neighboring countries*. Belem, Brazil: Instituto Evandro Chagas, pp.113–134.

Schneider, D.W. and T.M. Frost. 1996. Habitat duration and community structure in temporary ponds. J. N. Am. Benthol. Soc. 15: 64-86.

Schweigmann, N., N.E. Burroni, M.G. Freire, M.V. Loetti, M.C. Marinone, and O. Jensen. 2003. Poblaciones de *Ochlerotatus albifasciatus* (Diptera: Culicidae) sostenidas por la actividad humana en un valle patagónico. Publicación especial de la Sociedad Zoológica del Uruguay, Actas de las VII Jornadas de Zoología del Uruguay: p. 150.

Spinsanti, L., A.L. Basquiera, S. Bulacio, V. Somale, S.C.H. Kim, V. Ré, D. Rabbat, A. Zárate, J.C. Zlocowski, C. Quiroga Mayor, M. Contigiani, and S. Palacio. 2003. St. Louis Encephalitis in Argentina: The first case reported in the last seventeen years. Emerg. Infect. Dis. 9: 271-273.

S-PLUS V.6.02 for Windows. 2001. *Guide to statistics*, Vol. 1, Insightful Corporation, Seattle.

ter Braak, C.J.F. and P. Smilauer. 1998. CANOCO reference manual and user's guide to Canoco for Windows: Software for canonical community ordination (version 4). Microcomputer Power (Ithaca, NY), 352 pp.

Ward, D. and L. Blaustein. 1994. The overriding influence of flash floods on species-area curves in ephemeral Negev Desert pools: a consideration of the value of island biogeography theory. J. Biogeogr. 21: 595-603.

Wellborn, G.A., D.K. Skelly, and E.E. Werner. 1996. Mechanisms creating community structure across a freshwater habitat gradient. Annu. Rev. Ecol. Syst. 27: 337-363.