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IMMATURE MOSQUITOES FROM GROUNDWATER HABITATS IN A TEMPERATE WETLAND OF ARGENTINA: ENVIRONMENTAL ASSOCIATIONS AND SEASONAL VARIATION OF COMMUNITY ATTRIBUTES

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ABSTRACT. We studied the seasonal patterns of 3 community attributes (breeding site index, richness, and diversity) of groundwater mosquito immatures and their associations with meso- and microhabitat factors in the Paraná Lower Delta, Argentina, from December 2009 to November 2010. Monthly collections at 4 sites yielded 2,313 mosquito immatures, belonging to 19 species assigned to 6 genera. Immatures developed in a wide range of microenvironmental conditions (water temperature 4.0–30.2°C, pH 5.4–9.1, electrical conductivity 0.02–6.33 mS/cm, and dissolved oxygen 0–235.6%), and their occurrence was significantly associated with lower water temperature and dissolved oxygen values. Most frequently captured species were *Culex dolosus* s.l. (58.6%), *Aedes crinifer* (22.9%), and *Cx. intricatus* (19.0%). Breeding site index was highest in winter (0.76) and lowest in spring (0.54). Species richness and diversity varied significantly among seasons (peaking in summer), land uses, and habitat types.

KEY WORDS Community structure, seasonality, land use, microenvironment, species richness

INTRODUCTION

Studies concerning one or few species are the norm in ecological research, mainly due to the high complexity of mechanisms at higher levels of taxonomic aggregation (Fahrig and Jonsen 1998). However, species-by-species studies cannot be compiled in order to reveal whether general patterns occur at the community level. Mosquitoes have probably been targeted for research more than any other group of insects due to their role as vectors of diseases, apart from being a biting nuisance in many regions (Service 1995). Nonetheless, the ecology of entire mosquito assemblages remains poorly studied (Beketov et al. 2010).

In temperate regions, seasonality plays a major role in the structuring of insect communities (Williams 1996). As regards to mosquitoes, temperature and relative humidity partly determine the onset and length of their life cycle. Some species (e.g., those in the genus *Culex*) overwinter as larvae, therefore immatures can be collected year-round, whereas others deposit drought-resistant eggs in the soil (e.g., *Ochlerotatus* and *Psorophora* spp.) (Bentley and Day 1989). Also, there is plenty of published evidence that mosquito richness and abundance may be determined by physicochemical variables of aquatic habitats in which immatures breed (Berti et al. 2004, Leisnham et al. 2005, Mercer et al. 2005).

Freshwater wetlands have been a neglected area of mosquito-specific research (Dale and Knight 2008). These ecosystems offer abundant, productive, and extremely diverse habitats for insects in general, including pest and disease

vector species (Grillet et al. 2002a, 2002b). Due to their dynamic nature, water level fluctuations represent an important environmental component by which wetland insect communities are organized (Wiggins et al. 1980). Microenvironment variations as a result of fluctuations in water flow and nutrient inputs caused by both natural processes and human activities may be particularly relevant in this context. Increased inputs of dissolved nutrients and bacteria may provide additional resources, thereby altering patterns of mosquito production (Mercer et al. 2005).

The Delta of the Paraná River is the main wetland system in Argentina. Its unique mixing of temperate and tropical elements coupled with its proximity to highly urbanized areas makes it a keystone for both ecological studies and health concern issues. This wetland has recently been the target of mosquito community studies (Loetti et al. 2007; Albicócco et al. 2011; Cardo et al. 2011a, 2011b), after an information gap of almost 50 years (Prosen et al. 1960, García and Casal 1965). Land uses within this region have been shown to support dissimilar groundwater mosquito communities by the differential availability of aquatic habitats (such as temporary pools, irrigation ditches, and canals) (Cardo et al. 2011a). Also, the patterns of occurrence of the 3 most abundant groundwater mosquito species were studied and they all showed a significant seasonal component (Cardo et al. 2011b).

Based on these results, we propose to evaluate the effects of seasonality, land use, and habitat type in structuring the groundwater mosquito community by studying 3 attributes, namely the

breeding site index, the species richness, and the diversity. The specific objectives of this study were to characterize the physicochemistry of mosquito larval habitats and the seasonal patterns of the community attributes within a temperate wetland. With this aim, we performed a stratified sampling in 4 seasons, 4 land uses, and 3 types of larval habitats under the following hypotheses:

H₁: the attributes of the immature mosquito community vary among seasons, showing a peak during the summer.

H₂: the richness and diversity of the immature mosquito community vary among aquatic habitat types (i.e., temporary, semipermanent, and permanent) within a given land use consistently throughout the year.

H₃: a given aquatic habitat type presents the same richness and diversity in different land uses, with an encompassing variation due to seasonality.

MATERIALS AND METHODS

Study area

The Lower Delta of the Paraná River extends over northern Buenos Aires and southern Entre Ríos provinces, covering approximately 2,700 km². It is located at the joint of the Paraná River with the de la Plata estuary (Kandus and Malvárez 2004), and next to Greater Buenos Aires, the 2nd largest megalopolis in South America: 14,451 inhabitants/km² (INDEC 2010). Its islands are formed by the accretion of silts transported and deposited by the Paraná River. Islands are pan shaped, with levees up to 3 m high and a flooded central portion. The region is temperate with mean temperature of 16.7°C (minimum = 6°C; maximum = 30°C) and annual rainfall of 1,073 mm. Its hydrologic regime is affected by lunar tides and wind patterns of the de la Plata estuary (Kandus et al. 1999). Although population density is <1/ha, the delta is a recreational area highly visited during summer and weekends due to its proximity to urban centers.

The main landscape elements in the area (hereafter called land uses) are a mixed result of the geomorphic origin, hydrological regime, and the extent of anthropogenic intervention, and each presents a relatively homogeneous structure, physiognomy, and floristic composition (Kandus et al. 2006). They were detected by satellite imagery and ground proofing, and classified in 4 broad categories, namely peridomestic areas, Salicaceae plantations, secondary forests, and *Scirpus giganteus* Kunth marshes (Baigún et al. 2008). The former two present a high level of anthropogenic intervention, whereas the others are characterized by low human activity. Each land use has a typical location in the islands:

domestic areas in levees, marshes in the inner lowlands, and plantations and secondary forests in the areas in-between (Cardo et al. 2011a).

Sampling design and field work

Sampling sites were selected to obtain replicates of the main larval habitats within the 4 main land uses mentioned recently. Four sites, or islands, were visited monthly from December 2009 to November 2010. Any groundwater habitat, as described by Service (1995), was considered a potential larval habitat and classified in 1 of 3 habitat types based mainly on water stability as follows: temporary (which included pools and flooded ground), semipermanent (irrigation ditches and gutters), and permanent (canals and ponds) (Cardo et al. 2011a).

Three random points were taken monthly in each land use of each site, regardless of its extension, assuming that groundwater habitats are randomly distributed and have equal probability of existing in the same land use of different sites. The habitat of each type nearest to each random point up to a maximum radius of 25 m was sampled for immature mosquitoes. Only habitats larger than 16 m² were included. Samplings were performed, by the same person, in a 4 × 4-m quadrat. Immatures were collected by actively searching for individuals during 12 min with a fine-mesh strainer, a hand net, or a white tray depending mainly on depth, turbidity, and presence of floating vegetation. If there was no groundwater habitat belonging to a certain type within the 25-m radius, nonrandom active search was performed inside each land use in an attempt to complete all types. In each habitat sampled, water physicochemical variables were measured using portable kits: pH, electrical conductivity (Digital Combo meter, Hanna Instruments HI 9813, Padova, Italy), water temperature and dissolved oxygen (Portable Dissolved Oxygen meter, Hanna Instruments HI 9146). All measurements were performed immediately after the mosquito immature sampling within the top 10 cm of water, where mosquito larvae typically spend most of their time (Merritt et al. 1992, Beketov et al. 2010).

All captured larvae were preserved in 70% ethanol; pupae were transported to the laboratory for rearing. Only 3rd and 4th instars and emerged adults were counted and identified with dichotomous keys and specific descriptions (Darsie 1985, Forattini 2002, Rossi et al. 2002). Considering that larval specimens of *Culex dolosus* Lynch Arribálzaga and *Cx. eduardoi* Casal and García have been largely misidentified (Almirón and Brewer 1995, Rossi 2000) and that both taxa may belong to a species complex (Senise and Sallum 2008), all immatures collected were grouped as *Cx. dolosus* s.l.

Data analysis

Groundwater habitats inspected in Salicaceae plantations were excluded from the database due to low sampling size (11% of the total). Therefore, the following analyses refer to the remaining land uses, i.e., peridomestic areas, secondary forests, and *S. giganteus* marshes.

To evaluate how representative of average meteorological conditions of the area is the year under study, monthly mean temperature and cumulative precipitation values for the study period were compared with monthly historic 30-year (1981–2010) average. Both current and historic temperature values were taken from San Fernando Station and precipitation data from Buenos Aires aeroparque station (NOAA Satellite and Information Service 2011).

The 12 sampling surveys were gathered in 4 weather seasons as follows: summer (December–January–February), autumn (March–April–May), winter (June–July–August), and spring (September–October–November). To characterize the seasonality of the mosquito community richness (S , number of species) and diversity (H , Shannon index), monthly and seasonal values of each attribute were calculated (Magurran 2004). Differences of diversity among seasons were tested with Hutcheson t -test (Hutcheson 1970). Rarefaction curves to visualize species accumulation as a function of number of samples were performed. Abundance-based richness estimator $\text{Chao1} \pm \text{SD}$ (Chao 1987) was calculated for each season with EstimateS Win 8.20 (Colwell, Manfield, CT).

The breeding site index ($\text{BSI} = \text{number of groundwater habitats with mosquitoes} / \text{number of groundwater habitats surveyed}$) represents the percentage of occupied habitat and was considered an indicator of relative abundance. Seasonal BSI values for mosquito immatures, and monthly BSI values for the whole community and for the most frequently collected species were calculated. The seasonality of this index was tested in several ways. Seasonal BSI was compared with an independent proportions test followed by Tukey pairwise comparisons. Also, Freedman's test was applied on monthly BSI values to detect departures from a uniform occurrence throughout the year (Abramson 2005). Ratchet circular scan test is based on the maximum number of events in 2 or 3 consecutive months (Wallenstein et al. 1989). Calculations were performed with WINPEPI software (Abramson 2004).

Monthly values of temperature and precipitation and their anomalies respect to the historic mean were associated to monthly S , H , and BSI using Spearman correlations. These were performed both without lag and with a 1-month lag to take into account potential effects of environmental variables on the bionomics of mosquito immatures and adults.

The association between each microenvironmental variable and the occurrence of mosquitoes was analyzed with logistic regressions. The analyses were performed for all species pooled together and then for each of the 3 most frequently collected species. Also, the Kruskal–Wallis test was applied to search for differences among temporary habitats harboring mosquitoes in each of the 3 land uses. Because a significant land use result in the Kruskal–Wallis test could be confounded with a seasonal effect, significant microenvironmental variables in this test were used as response variable in a Generalized Linear Model (GLM) (McCullagh and Nelder 1989) including land use and season as explanatory variables. The `drop1` command was used to evaluate the significance of each explanatory variable when removed from the model.

To evaluate H_2 and H_3 , we performed an analysis combining bootstrap and 2-way ANOVA. Considering 3 main factors, namely season (4 levels), land use (3), and habitat type (3), 5 replicates of each combination (season \times land use \times habitat type) were randomly selected with replacement, and S and H computed for each subset. This was repeated 6 times, resulting in 6 values of S and H for each combination. These values were tested with 2-way ANOVA (main effects plus interaction). Factors were combined in 2 ways: within peridomestic areas (which was the only land use presenting the 3 habitat types), season \times habitat type (H_2), and within temporary habitats (which was the only habitat type present in the 3 land uses), season \times land use (H_3). Three-way ANOVA could not be performed due to the lack of permanent habitats in secondary forests and of semipermanent and permanent habitats in marshes. The entire procedure was repeated 100 times, and confidence intervals for P -values of each main effect and the interaction were calculated. To obtain mean values of S and H for each combination, 5 replicates of each were randomly selected with replacement, and S and H computed for each subset. This was repeated 1,000 times, and assuming a Gaussian distribution of these values within each combination (due to being the mean of a random sample with $N = 1,000$), $S \pm \text{SD}$ and $H \pm \text{SD}$ were calculated. Analyses were performed in R 2.10.1 (R Development Core Team 2009).

RESULTS

Overall, 322 groundwater habitats (169 within a 25-m radius from random points and 153 actively searched) were inspected. All aquatic habitat types acted as breeding sites for mosquitoes. In total, 65.2% (210/322) of the inspected habitats were positive for immature mosquitoes: 63.3% (107/169) randomly selected and 67.3% (103/153) actively searched. Rarefaction analysis

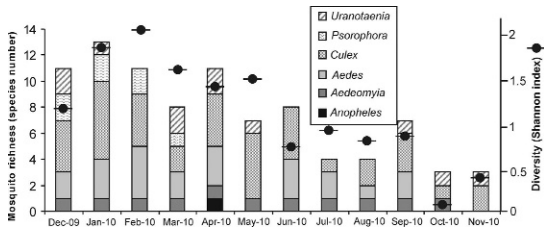


Fig. 1. Monthly mosquito richness (left axis) and diversity (right axis) in groundwater habitats from the Paraná Lower Delta, Argentina. The presence and number of species of each genus is indicated.

showed no significant differences in richness accumulation curves between samples obtained from both sources. Moreover, independent proportion tests showed no differences between total, seasonal, or monthly BSI for both sources. Therefore, all groundwater habitats were pooled for further results.

A total of 2,313 immatures of 19 mosquito species belonging to the genera *Anopheles*, *Aedeomyia*, *Culex*, *Aedes*, *Psorophora*, and *Uranotaenia* were collected and identified. The genus *Culex* ($n = 1,503$) was present year-round and was represented by 8 species mainly dominated by *Cx. dolosus* s.l. and *Cx. intricatus* Brèthes. Species of *Psorophora* were found only during the summer and *Uranotaenia* spp. were absent during the winter (Fig. 1). The most abundant species was *Cx. dolosus* s.l. (48.1% of all immatures collected), followed by *Aedes crinifer* (Theobald) (16.0%) and *Cx. intricatus* (11.8%). These were also the most frequently collected species (BSI = 0.59, 0.23, and 0.19, respectively). Six species (the former three plus *Aedeomyia squamipennis* (Lynch Arribáizaga), *Ae. albifasciatus* (Macquart), and *Psorophora ferox* (Von Humboldt)) accounted for 87.4% of all immatures collected.

Mosquito immatures were found at water temperature values ranging from 4.0–30.2°C, pH 5.4–9.1, electrical conductivity 0.02–6.33 mS/cm, and dissolved oxygen from nondetectable to 235.6%. The occurrence of mosquito immatures was significantly associated with low water temperature and dissolved oxygen values (Table 1). The by-species analysis showed that *Cx. dolosus* s.l. occurred more frequently at lower water temperature and dissolved oxygen values. On the other hand, *Cx. intricatus* was associated with higher water temperature, as well as low values of the other 3 microenvironmental variables. Finally, *Ae. crinifer* presented no significant association to any of the microenvironmental variables considered (Table 1).

As regards the seasonality of the community attributes (H_1), observed richness during the summer (maximum $S = 13$ in January) was

Table 1. Physicochemical parameters of groundwater habitats in the Paraná Lower Delta, Argentina. Median (1st quartile–3rd quartile) values along with t -values of the logistic regression comparing the occurrence of mosquito immatures and most frequently collected species are shown.¹

Mosquitoes	No. samples	Dissolved oxygen (% saturation)		Water temperature (°C)		pH		Electrical conductivity (mS/cm)	
		t	t	t	t	t	t		
Present	210	0 ² (0 ² –9.6)	-3.13**	15.0 (11.4–22.4)	-2.02*	6.8 (6.5–7.2)	-1.24	0.25 (0.18–0.40)	-0.10
Absent	112	3.1 (0 ² –31.9)		17.0 (12.9–22.6)		6.9 (6.6–7.2)		0.26 (0.17–0.40)	
<i>Culex dolosus</i> s.l.									
Present	123	0.8 (0 ² –23.9)	-2.42**	13.6 (9.5–18.5)	-6.26***	6.8 (6.5–7.2)	0.29	0.28 (0.21–0.43)	-1.27
Absent	199	0 ² (0 ² –12.5)		20.0 (14.0–23.1)		6.8 (6.6–7.2)		0.23 (0.17–0.35)	
<i>Aedes crinifer</i>									
Present	48	0 ² (0 ² –11.8)	-1.81	19.5 (11.8–22.8)	0.43	6.8 (6.6–7.3)	0.78	0.29 (0.19–0.59)	1.70
Absent	274	0.8 (0 ² –17.3)		15.4 (11.9–22.3)		6.8 (6.6–7.2)		0.25 (0.18–0.39)	
<i>Cx. intricatus</i>									
Present	40	0 ² (0 ² –0 ²)	-2.46*	21.6 (14.9–23.1)	2.95**	6.6 (6.5–6.8)	-2.40*	0.18 (0.17–0.21)	-3.37***
Absent	282	1.7 (0 ² –18.7)		15.2 (11.6–22.2)		6.8 (6.6–7.2)		0.27 (0.18–0.43)	

¹ * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

² Nondetectable.

Table 2. Characterization of the groundwater immature mosquito community per season in the Paraná Lower Delta, Argentina.¹

Season	No. GWH inspected	BSI (no. of samples/no. of GWH inspected)	Total no. immatures	Shannon diversity index (<i>H</i>)	Evenness	Observed richness	Chao1, mean ± SD
Summer (Dec. 2009–Feb. 2010)	96	0.67 (64/96)	891	2.10	0.74	17	17.5 ± 1.3
Autumn (Mar. 2010–May 2010)	76	0.63 (48/76)	444	1.85	0.72	13	13.0 ± 0.3
Winter (Jun. 2010–Aug 2010)	79	0.76 (60/79)	349	0.84	0.38	9	9.3 ± 0.9
Spring (Sep. 2010–Nov. 2010)	71	0.54 (38/71)	528	0.76	0.37	8	12.5 ± 7.2

¹ GWH, groundwater habitat; BSI, breeding site index.

higher than in autumn, and presented minimum values in winter and spring (minimum $S = 3$ in October and November) (Fig. 1). Chao1 values for each season were very close to the observed values, with the exception of spring, in which the number of samples did not stabilize the estimator (Table 2). The correlation between monthly S and H was 0.65, being the highest diversity observed in February and the lowest value in October (Fig. 1). Pairwise comparisons of the diversity values between seasons performed by Hutcheson test were statistically significant ($P < 0.05$) except for the pair winter–spring. Recorded monthly mean temperature for the study period was very similar to historical records (Fig. 2). Cumulative monthly precipitation, on the other hand, showed an anomalous peak during the summer. Significant positive correlations between temperature/precipitation values and monthly richness/diversity values were found (Table 3).

Mosquito BSI ranged from 0.29 (November) to 0.91 (July); no significant monthly trends or seasonal peaks were found. However, when

months were pooled by seasons, BSI presented a significant variation ($\chi_3 = 8.52, P < 0.05$), the value in winter being significantly higher than the one in spring ($P < 0.05$) (Table 2). A significantly higher BSI for *Cx. dolosus* s.l. was obtained during winter surveys, along with significant results of Freedman’s and Ratchet’s tests, the latter identifying the peak June–August. In concordance, its BSI was negatively correlated with monthly mean temperature with and without lag, while a significant negative correlation with lagged precipitation was also verified (Table 3). On the contrary, *Cx. intrincatus* was more frequently collected during the summer (BSI positively correlated with temperature, Table 3, Freedman’s and Ratchet’s tests significant with peak in January–March), while *Ae. crinifer* showed no seasonal pattern but presented a positive correlation with the anomaly of precipitation (Table 3).

As regards the variation of community attributes among the 3 habitat types within peridomestic areas (H_2), for richness both a season (upper bound confidence interval $P < 0.001$) and a habitat type effect ($P < 0.05$) were verified, along with a significant interaction ($P < 0.001$) between the two; whereas for diversity, only a season effect was encountered ($P < 0.05$) (Fig. 3A).

Concerning the variation of the community attributes of temporary habitats among different land uses (H_3), results matched for richness and diversity; there was a significant land-use effect ($P < 0.001$) in addition to the expected season effect ($P < 0.001$), and no significant interaction (Fig. 3B). In other words, the richness and diversity values presented by temporary habitats varied not only with the season, but also according to the land use in which they were located. In relation to this, temporary habitats presented significant microenvironmental differences among land uses, in particular for electrical conductivity and water temperature (Table 4). For the former, the GLM showed no significant effect of the season and a significant land-use

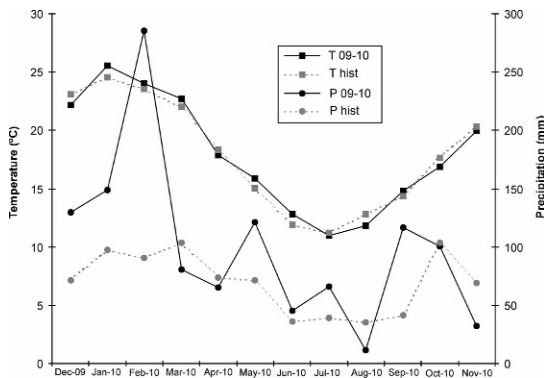


Fig. 2. Monthly mean temperature (°C) and cumulative precipitation (mm) of the year under study (labeled “T 09-10” and “P 09-10”, respectively) compared with 30-year (1981–2010) historical records (“T hist” and “P hist”).

Table 3. Spearman correlation indexes between environmental variables and groundwater mosquito community attributes in the Paraná Lower Delta, Argentina.¹

Environmental variable ²	Richness	Diversity	Breeding site index			
			Mosquitoes	<i>Culex dolosus</i> s.l.	<i>Aedes crinifer</i>	<i>Cx. intricatus</i>
T 2009–10	0.606*	0.601*	−0.242	−0.874**	0.106	0.603*
T 2009–10 with 1-month lag	0.706**	0.755**	0.0981	−0.819**	0.070	0.849**
P 2009–10	0.571*	0.3664	−0.168	−0.441	0.409	0.396
P 2009–10 with 1-month lag	0.521	0.773**	−0.053	−0.560*	0.155	0.528
T anomaly (study period – historic)	0.404	0.476	0.130	−0.280	0.366	0.535
P anomaly (study period – historic)	0.521	0.490	0.046	−0.049	0.697**	0.225

¹ * $P < 0.05$; ** $P < 0.01$.

² T, temperature; P, precipitation.

effect ($P < 0.05$ when removing the variable from the model), peridomestic areas and marshes being equivalent and secondary forests having significantly lower electrical conductivity values. For water temperature, besides the expected effect of season ($P < 0.001$), a significant effect of land use ($P < 0.001$) was verified. In this case,

peridomestic areas and forests were equivalent and marshes presented lower values.

DISCUSSION

The distribution and abundance of mosquitoes in any given location are supposed to be

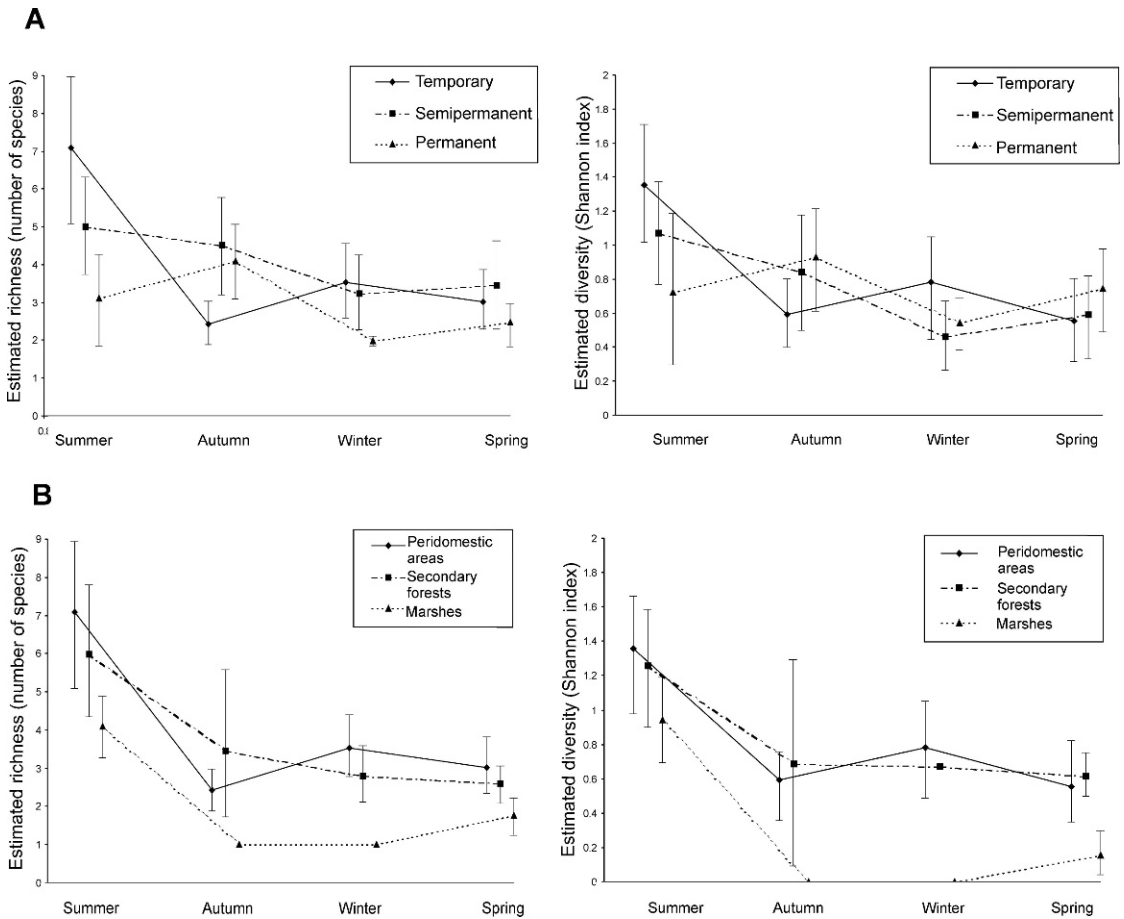


Fig. 3. Bootstrap estimated mosquito richness and diversity (means and SDs) for (A) different habitat types (temporary, semipermanent, and permanent) occurring in peridomestic areas per season; and (B) temporary habitats occurring in 3 land uses (peridomestic areas, secondary forests, and marshes) per season.

Table 4. Physicochemical parameters of temporary groundwater habitats containing mosquito immatures in the Paraná Lower Delta, Argentina. Median (1st quartile–3rd quartile) values along with Kruskal–Wallis statistic comparing habitats in the 3 main land uses in the area are shown.¹

Land use	No. samples	Dissolved oxygen (% saturation)	Water temperature (°C)	pH	Electrical conductivity (mS/cm)
Peridomestic	37	0.8 (0 ² –21.7)	19.1 (13.6–22.8)	6.9 (6.5–7.2)	0.25 (0.18–0.50)
Secondary forest	41	0 ² (0 ² –5.1)	19.8 (13.8–22.8)	6.7 (6.5–7.0)	0.20 (0.17–0.28)
Marsh	28	0 ² (0 ² –11.5)	10.0 (8.8–19.0)	6.8 (6.5–7.5)	0.30 (0.23–0.41)
Kruskal–Wallis statistic		4.004	13.222**	1.058	12.901**

¹ ** $P < 0.01$.

² Nondetectable.

influenced by factors operating across spatial and temporal scales. In the temperate wetland under study, we found significant variations of the community structure breeding in groundwater habitats across seasons, land uses, and aquatic habitat types. Highest mosquito richness and diversity were observed in summer, which was confirmed by significant correlations with higher temperatures. Some species presented a marked seasonality and replaced themselves throughout the year, the reason the percentage of habitats harboring mosquitoes was stable year-round, refuting our initial hypothesis as regards this community attribute. When months were pooled by seasons, BSI was highest in winter, probably reflecting the overwintering of *Culex* spp. larvae. *Culex dolosus* s.l. was collected at low water temperatures, matching its seasonal pattern (higher relative abundance during the winter). The fact of *Cx. intricatus* being collected at significantly higher water temperature values also matched this species' seasonal pattern (higher abundance in summer). In Buenos Aires City, *Cx. dolosus* immatures were previously observed throughout the year, and immature stages were collected at temperatures below 23°C (Fischer and Schweigmann 2004). These results are coincident with other observations on this species in temperate areas of Argentina (Almirón and Brewer 1995, Maciá 1997). Adult sampling with human bait in the study area collected *Ae. crinifer* year-round, with higher numbers in summer and autumn (Loetti et al. 2007). Most frequently captured species matched previous results in the area (Cardo et al. 2011a, 2011b), suggesting that our study period was well representative of previous years.

Studies carried out in the area concluded that the use given to the land drives species richness and composition of groundwater mosquito communities through larval habitat availability. In other words, each land use presents a characteristic availability of habitat types (in terms of identity and relative abundance), and that determines its community structure (Cardo et al. 2011a). In this regard, we verified our 2nd hypothesis, finding a significant habitat type effect in species richness and diversity, along with a significant seasonal effect. However, we rejected

our 3rd hypothesis, implying that the same habitat type did not present the same community attributes in different land uses. We also found significant microenvironmental differences in temporary habitats harboring mosquitoes among land uses, specifically in electrical conductivity and water temperature values. Lowest temperature values were recorded in marshes, in which the lack of canopy may result in higher temperature amplitudes. The occurrence of mosquito immatures was significantly associated with lower water temperature and dissolved oxygen values. The 1st association probably reflects the seasonality of the most abundant species, *Cx. dolosus* s.l., which overall was present in 59% of all groundwater habitats inspected. As regards dissolved oxygen, while some mosquito species are able to survive with low levels, most mosquito predators need higher levels. Perhaps the association of immature mosquito presence with low dissolved oxygen values reflects a release from predation in such groundwater habitats. The 3 most frequently collected species were previously negatively associated with land uses without canopy (marshes and peridomestic areas), suggesting certain dependence on shade or water temperature (Cardo et al. 2011b). Similarly, in a Neotropical wetland in northern Venezuela, Culicidae were negatively associated with water temperature but positively related to conductivity and dissolved oxygen (Grillet et al. 2002b).

Despite comparable sampling effort in the 4 seasons, the breeding site index in spring was considerably lower than in the other seasons; therefore, the number of samples was not sufficient to stabilize the estimated richness index. Even though the total number of immatures identified in spring was higher than in autumn or winter, 2 species were collected in very high abundances, *Cx. dolosus* s.l. (383 of a total of 528 immatures) and *Ae. crinifer* (122/528), and the other 6 species were collected in very low abundances (1–13 immatures). Most of the recorded species were therefore regarded as rare, the reason the index did not stabilize. However, it is rarely cost-effective to record every species in an assemblage, especially in situations with high dominance of few species (Magurran 2004),

which usually characterize mosquito communities (Schäfer and Lundström 2001). Also despite our field effort, the number of samples obtained was insufficient to stabilize the estimators when partitioned by each combination of the 3 factors (season, land use, and habitat type). Although bootstrap estimations did not reflect the expected total richness of each combination, they enabled us to compare the slope of the richness accumulation curve at a fixed sampling effort. Moreover, if we assume that the shape of the curve is identical for each combination, the comparisons performed are still valid for the corresponding plateaus, in terms of which combination has higher total (though unknown) species number.

Mosquitoes are an intrinsic component of most classes of wetlands (Dale and Knight 2008, Schäfer et al. 2008). To control them, it is crucial to understand the bionomics of the target species. This requires the study of the factors affecting immature stages abundance and distribution, which are the main determinants of the fluctuations of the adult populations (Mwangangia et al. 2009). Because of the comparatively small spatial dispersal capacity of mosquito larvae and pupae, the control of immature stages is the principal and most effective tool for mosquito-borne disease management (Killeen et al. 2002). At the scale of the current study, immature mosquito distributions are non-random, and understandable by the relative contributions of season and wetland abiotic and biotic conditions. The current landscape configuration partly determines the composition of the mosquito community. Therefore, changes in the main components of the landscape are expected to modify the entire insect community and the role of surviving and potentially invading mosquitoes as vectors of pathogens (Alfonzo et al. 2005). As Balakrishnan et al. (2011) propose, diversity indexes can be used to monitor mosquito vector species in relation to habitat type and land use, and time-series databases can help to forecast the effects of both natural and human-induced environmental changes in mosquito communities.

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