Culicidae (Diptera) community structure, spatial and temporal distribution in three environments of the province of Chaco, Argentina

Marina Stein [a,*], Mirta Santana [b], Liliana María Galindo [b], Eduardo Etchepare [c], Juana Alicia Willener [a], Walter Ricardo Almirón [d]

[a] Laboratorio de Entomología, Instituto de Medicina Regional, Universidad Nacional del Nordeste, Avda. Las Heras 727, Resistencia 3500, Argentina
[b] Cátedra de Bioestadística, Facultad de Medicina, Universidad Nacional de Tucumán, Lamadrid 875, CP 4000 San Miguel de Tucumán, Tucumán, Argentina
[c] Laboratorio de Herpetología, Facultad de Ciencias Exactas y Naturales y Agrimensura, Universidad Nacional del Nordeste, Av. Libertad 5470, Corrientes 3400, Argentina
[d] Instituto de Investigaciones Biológicas y Tecnológicas (IIByT-CONICET), Centro de Investigaciones Entomológicas de Córdoba, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Vélez Sarsfield 1611, Córdoba 5016, Argentina

ARTICLE INFO

ABSTRACT

The aim of this work was to study the composition of the Culicidae community in three environments with different degrees of anthropic disturbance in the province of Chaco. The nonparametric richness estimator ACE was calculated to measure the completeness of the sampling in each environment, and the diversity of each environment (α-diversity) were estimated. The composition, abundance and uniformity of species from the different environments were compared by means of range-species curves. β-diversity was measured as species complementarity, which allowed us to know the degree of dissimilarity among the environments. The synanthropic index was estimated, identifying urban environment (synanthropic) species, eusynanthropic species, and wild species. The influence of climatic factors (mean temperature, relative humidity and rainfall) on the monthly and annual variations of the identified mosquito species was analysed using multilevel Poisson models with over-dispersion. The wild environment showed higher diversity and the semi-urban environment higher species richness. Despite having lower S and abundance, the urban environment showed greater diversity than semi-urban environment, although it also showed lower completeness, which means S could increase. Anthropogenic disturbance can lead to the elimination and/or modification or substitution of habitats, with the subsequent loss of richness in the more sensitive species of Culicidae fauna, although the conditions are also favourable for the settling of opportunistic and exotic species well-adjusted to disturbed environments. Most of the species abundances were positively influenced by the analysed climatic variables. The study area showed a rich Culicidae fauna of public health significance, with a risk of pathogen transmission, suggesting the need of further studies and the febrile and entomological surveillance.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

The study of the composition and diversity of mosquitoes, both in natural and anthropically disturbed areas, is of considerable importance due to their role in pathogen transmission to humans and animals (Forattini, 2002), these may influence, either decreasing disease risk or facilitating the spread of it, for different mechanisms (Alencar et al., 2015). Furthermore, the study of natural environments allows us to know the habitat of those species whose role in transmission cycles remains unknown (Hutchings et al., 2005).

In our country, the records of species from the subtropical climate provinces of the Northeast and Northwest of Argentina, which are characterised by having the greatest Culicidae species richness, are updated by few studies listing the species and, in some cases, the habitats where they are found (Rossi et al., 2006; Visintín et al., 2010; Hoyos et al., 2011; Campos et al., 2011; Dantur Juri et al., 2012; Stein et al., 2012; Rossi and Lestani, 2014; Rossi, 2015). Furthermore, studies analysing species diversity and richness using quantitative estimators are scarce (Ronderos et al., 1991, 1992; Dantur Juri et al., 2005; Ramirez et al., 2014), and no single study has performed a comparative analysis of Culicidae communities from environments with different degrees of anthropization in the

same phytogeographic region. This type of studies would, in some way, allow the identification of changes in an ecologic timeline, data which can be of biologic and epidemiological interest. Also allow measuring the simplification and/or degradation of ecosystems (Badii et al., 2007). The results of this study will reveal the temporal and spatial distribution of the different mosquito species found in a region of epidemiological interest, that will need to be considered when it comes to implementing control measures, identifying periods of greater epidemiological relevance, given the known circulation of different arboviruses (Díaz et al., 2008; Pisano et al., 2013), as well as quantitative estimations of the Culicidae community in 3 types of environments with different degrees of anthropic intervention, in the province of Chaco, located in the subtropical region of Argentina.

2. Material and methods

2.1. Study area

The climate is subtropical with a dry season. It is part of an extensive area, commonly called Parque Chaqueño, where vegetation has a polymorphic appearance and consists of xeric forests, shrubs and herbaceous steppes, savannas, palm groves and grasslands. Phytogeographically, it is divided into two regions: eastern (wet Chaco) and western (dry Chaco) woodland. Both regions are mainly differentiated by rainfall and frost-free period. The eastern woodland, where this study was conducted, is within a flood plain with imperfect or poor drainage. The soil permeability is moderately slow, which allows the formation of flooding areas (Bruniard 1978). The mean annual temperature is 23 °C and the mean rainfall is 1280 mm. In the summer, the mean temperature is 27 °C, with an absolute maximum that can exceed 43 °C. The mean temperature in winter is 15 °C. The rainy season is between November–April, with peaks of rainfall in spring (September–October) and autumn (March–April).

2.2. Study design

In order to determine the seasonal variations of the female adult populations, and to detect the Culicidae species in environments with different degrees of anthropic intervention, samples were collected in 3 types of environments: 1. urban environment—highly modified, located in the urban area of the Resistencia city—(27°30′S 59°W) capital of the province, with 350.000 inhabitants (>1200 inhabitans per hectare); 2. semi-urban environment with little anthropic disturbance—Monte Alto—(27°26′S 58°55′W), with low population density (<600 inhabitants per hectare), without sewage disposal system, public transport services, shops and health centers near; and 3. wild environment, represented by a patch of pristine forest—Kilometer 1031 (27°10′S 58°58′W), 31 km from the city of Resistencia, hereinafter environments 1, 2 and 3, respectively. The collections were made fortnightly, during the period June 2001–October 2003. They were made using a CDC light trap with CO_2 (Service, 1993), supplied through a cylinder with a pressure and a flow controller, which allowed for a continuous gas flow at 0.5 l/min. For each collection and in each site, 1CDC trap was placed 0.5–1.5 m above ground level, remaining active during 16 h for each collection (between 6 p.m. and 10 a.m.). In environment 1, the CDC light trap was located to 1.2 km from the geographical center of the city on the same site where the weather station is found (Fig. 1). In environment 2, the trap was located in a field whose main activity corresponds to animal husbandry, 500 m from the Negro River, nearest to riparian forest, 10 km from the city of Resistencia, which is accessed by the national route N° 16. In environment 3, the trap was located within the forest. Sites where a lot of specimens

were collected on previous sampling and were near to different types of larval habitats (lagoons, river, bromeliads, ditches, pools, artificial containers) within each environment, were selected. The adults captured were taken to the laboratory in the collecting net, killed with ethyl acetate, identified and counted. Identification of collected individuals was based on dichotomous keys (Faran, 1980; Darsie, 1985; Linthicum, 1988; Consoli and Lourenço De Oliveira, 1994). The specimens are deposited in the Instituto de Medicina Regional, Universidad Nacional del Nordeste, in the province of Chaco.

2.3. Data analysis

The nonparametric richness estimator ACE (Abundance-based coverage estimator, Chao and Lee, 1992) was calculated to measure completeness of the mosquito inventory (Colwell and Coddington, 1994). This analysis was performed using the free EstimateS v9.1.0 software (http://purl.oclc.org/estimates; Colwell, 2013).

Alpha diversity was estimated by specific richness (S) and the method proposed by Jost (2006), who introduced the term true diversity:

$$^qD = \left(\sum_{i=1}^{S} p_i^q \right)^{1/(1-q)}$$

where ^qD is the true diversity, p_i is the relative abundance—proportional abundance—of i species, S is the number of species and q is the order of diversity and defines the sensitivity of the index to the species relative abundance (Moreno, 2001; Jost, 2006, 2007; Tuomisto, 2010a,b; García-Morales et al., 2011). The value of the q parameter determines how much the measure of diversity is influenced by common or rare species (Hill, 1973). The result is shown as the "effective number of species" or "real diversity" (Jost, 2006). This way of expressing the diversity of a community complies with the duplication property described by Hill (1973). This allows us to unify the analysis by making the diversity results comparable, as well as to get a direct and simpler interpretation about the difference in diversity between 2 or more communities (Jost, 2007, 2010; Moreno et al., 2011).

In order to compare the composition, abundance and uniformity of species among environments, range-abundance curves were used (Feinsinger, 2001). Relative abundance of species against their range was illustrated, from the most to the least abundant species (Urbina-Cardona et al., 2008).

Also, the beta (β) diversity among environments was estimated by means of the complementarity index or the dissimilarity percentage, as follows:

$$(C_{jk})\, C = \left[\frac{(S_j + S_k) - 2V_{jk}}{(S_j + S_k) - V_{jk}} \right] \times 100$$

where S_j and S_k is the number of species in sites j and k, and V_{jk} is the number of species in common in both sites. This index measures the degree of dissimilarity or similarity in species composition between pairs of biota, (Colwell and Coddington, 1994). When both communities are identical, the result is 0, and when species of both sites are completely different, the result is 1. It was shown as the percentage of species which are complementary among environments.

The synanthropy of the different species was estimated using the Nuorteva Index (Forattini et al., 1993). The relative abundance (%) of species common to the 3 sampling sites was analysed based on the following equation:

$$s = \frac{2a + b - 2c}{2}$$
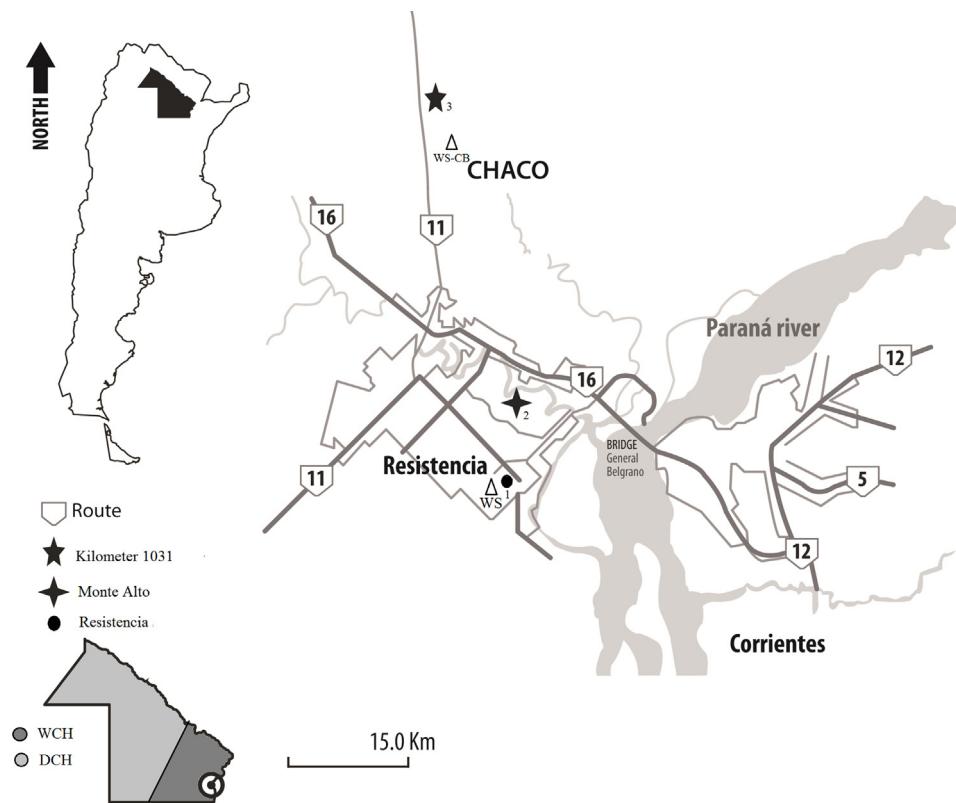
Fig. 1. Location in Argentina. Province of Chaco. Sampling sites: Resistencia (1), Monte Alto (2) and Kilometer 1031 (3). WS: weather station in Resistencia; WS-CB: weather station in Colonia Benitez. Modified Stein et al. (2013).

where s is the synanthropic index, a, b and c represent the percentage of each species in the urban, semi-urban and wild environment, respectively. The resulting values range between −100 and 100, the latter being the maximum synanthropic value.

To determine if the relation between climatic variables (rainfall, mean temperature and relative humidity) and abundance of adults changed monthly or annually, a multilevel model was used. This was a multilevel regression model with Poisson distribution, monthly variable rate, random coefficient or intercept. The software used was HLM6, which uses penalised quasi-likelihood (PQL) estimators (Raudenbush and Bryk, 2002; Bolker et al., 2011).

Two levels were considered for the model, level 1 with units in months, and level 2 with units in years. The incidence rate ratio (IRR), standard error (SE) values and p values were obtained. The IRR enabled the direct observation of the influence percentage of each climatic variable on the mosquito species abundance. To handle the missing data originated during year 2002, the multiple imputation method was used to estimate the missing data with the R software and the mi program (which uses an algorithm known as chained equation approach) (van Buuren and Oudshoorn, 2000; Su et al., 2011).

To assess the reliability of the imputed data, the regression model was applied both to missing and imputed data resulting in a very acceptable level of reliability in all cases, determined by the relative change in IRR values and considering as acceptable changes not exceeding 10%. The meteorological data for the sampling period were obtained from the weather station of the Departamento de Hidráulica, Facultad de Ingeniería, Universidad Nacional del Nordeste, located in the university campus, in the city of Resistencia. For species of the Aedini tribe, *Ochlerotatus* was considered with genus status, as proposed by Reinert (2000).

3. Results

A total of 137,256 specimens, belonging to 82 species from 11 genera, were collected (Table 1). Although a great number of specimens from the *Culex* (*Culex*) were collected, the difficulties to identify females in these species, due to lack of clarity in key distinctive features, only enabled the identification of a great number of specimens to the genus level. Therefore, 6,263 specimens were identified to the species level for this genus and subgenus; 89.27% of the specimens were identified to the subgenus level (*Culex* spp.) (Table 1). On the other hand, a percentage of these (21.74%) could only be identified to the genus level due to damage to their morphological characteristics. The cause of damage, could be the large number of mosquitoes collected in one sampling, which sometimes exceeded 3,000 per CDC trap.

3.1. Diversity and richness at each environment

According to the richness estimator calculated, representation of the sampling in environment 2 was 99% (ACE = 74.5), while in environment 3 it was 89% (ACE = 81.13) and finally, completeness in environment 1 was 86% (ACE = 53.6), showing only in 2 a number of species same to that found in the field. The greatest specific richness and abundance of individuals was registered in environment 2 (S = 74; 73,361 individuals), followed by environment 3 (S = 72; 60,401 individuals) and environment 1 (S = 46; 3,494 individuals) (Table 1). As regards species diversity, the effective number of species was higher in environment 3 (^1D = 13.94 effective species), followed by environment 1 (^1D = 9.34 effective species), the first one being 1.5 times more diverse, while environment 2 (^1D = 9.1 effective species) is 1.53 times less diverse than environment 3, with a 35% reduction in "mosquitoes" diversity in relation to the most diverse environment. Environments 2
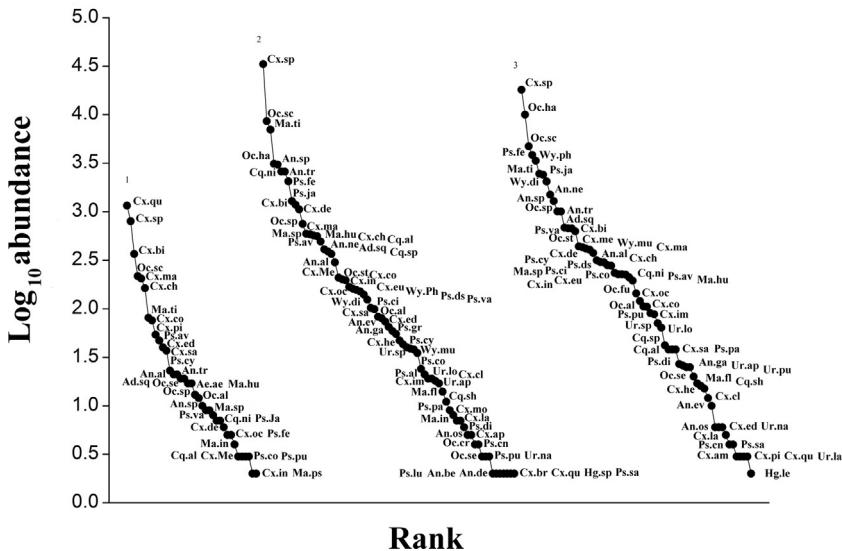
Fig. 2. Range-abundance curves for the species captured in three environments with different degrees of anthropic disturbance in the province of Chaco, Argentina. 1: urban environment; 2: semi-urban environment; 3: wild environment. Species with 0 log (abundance = 1) were not included. (1 = *An.ev*; *Cx.eu*; *Cx.he*; *Cx.la*; *Oc.st*; *Ps.c*; *Ps.ds*; *Ps.Gr* and *Ur.l*; 2 = *Ma.ps*; *Ur.ge y Wy.me*; 3 = *An.ro*; *Ma.in*; *Ma.ps*; *Oc.cr*; *Ps.al*; *Wy.me* and *Wy.wy*).

Codes for species: *Ad. squamipennis*: Ad. sq; *Ae. aegypti*: Ae. ae; *An. albitarsis*: An. al; *An. benarrochi*: An. be; *An. deaneorum*: An. de; *An. evansae*: An. ev; *An. galvaoi*: An. ga; *An. neomaculipalpus*: An. ne; *An. oswaldoi*: An. os; *An.rondoni*: An. ro; *An. triannulatus*: An. tr; *An.* (*Nys.*) spp.: An. sp; *Cq. albicosta*: Cq. al; *Cq. nigricans*: Cq. ni; *Cq. shannoni*: Cq. sh; *Cq.* spp.; Cq. sp; *Cx. ameliae*: Cx. am; *Cx. apicinus*: Cx. ap; *Cx. bidens*: Cx. bi; *Cx. brethesi*: Cx. br; *Cx. chidesteri*: Cx. ch; *Cx. clarki*: Cx. cl; *Cx. coronator*: Cx. co; *Cx. delpontei*: Cx. de; *Cx. eduardoi*: Cx. ed; *Cx. educator*: Cx. eu; *Cx. hepperi*:Cx. he; *Cx. imitator*: Cx. im; *Cx. intrincatus*: Cx. in; *Cx. lahillei*: Cx. la; *Cx. maxi*: Cx. ma; *Cx. mollis*: Cx. mo; *Cx. ocossa*: Cx. oc;*Cx. pilosus*: Cx. pi; *Cx. quinquefasciatus*: Cx. qu; *Cx. saltanensis*: Cx. sa; *Cx.* (*Cux.*) spp.: Cx. sp; *Cx.* (*Mel.*) spp.: Cx. Me; *Hg.spegazzini*: Hg. sp; *Hg. leucocelaenus*: Hg. le; *Ma. flaveola*: Ma. fl; *Ma. humeralis:* Ma. hu; *Ma. indubitans*: Ma. in; *Ma. pseudotitillans*: Ma. ps; *Ma. titillans*: Ma. ti; *Ma.* spp.: Ma. sp; *Oc. albifasciatus*: Oc. al; *Oc. crinifer:* Oc. cr; *Oc. fulvus:* Oc. fu; *Oc.hastatus/oligopistus:* Oc. ha; *Oc. scapularis:* Oc. sc; *Oc. serratus:* Oc. se; *Oc. stigmaticus:* Oc. st; *Oc.* spp.: Oc. sp; *Ps. albigenu/varipes:* Ps. av; *Ps. albipes:* Ps. al; *Ps. ciliata:* Ps. ci; *Ps. cingulata:*Ps. cn; *Ps. confinnis:* Ps. co; *Ps. cyanescens:* Ps. cy; *Ps. dimidiata:* Ps. di; *Ps. discrucians:* Ps. ds; *Ps. ferox:* Ps. fe; *Ps. lutzzi:* Ps. lu; *Ps. pallescens:* Ps. pa; *Ps. paulli:* Ps. pu; *Ps. saeva:* Ps. sa; *Ps. varinervis:* Ps. va; *Ps.* (*Gra.*) spp.: Ps. Gr; *Ps.* (*Jan.*) spp.: Ps. Ja; *Ur. apicalis:* Ur. ap; *Ur. geometrica:* Ur. ge; *Ur. lanei:* Ur. la; *Ur. lowii:* Ur. lo; *Ur. nataliae:* Ur. na; *Ur. pulcherrima:* Ur. pu; *Ur.* spp.: Ur. sp; *Wy. diabolica:* Wy. di; *Wy. melanocephala:* Wy. me; *Wy. muelhensi:* Wy. mu; *Wy.* (*Pho.*) spp.: Wy. Ph; *Wy.* (*Wyo.*) spp.: Wy. Wy.

and 3 showed *Cx.* (*Cux.*) spp. (2: 33,379 individuals, Ln₁₀ = 4.5; 3: 18,051 individuals, Ln₁₀ = 4.3) was at the highest level of the curve (Fig. 2). Environment 3 showed a slightly less pronounced curve, with *An. neomaculipalpus* Curry, *Oc. hastatus/oligopistus* Dyar/Dyar, *Oc. scapularis* (Rondani), *Ps. ferox* (Von Humbolt), *Wy. diabolica* Lane and Forattini and *Ma. titillans* (Walker), at high levels of the curve and a greater number of species in common with environment 2 at intermediate areas of the curve (Fig. 2). With a similar curve *An. triannulatus* (Neiva and Pinto) and *Cq. nigricans* (Coquillet) were dominant in the environment 2 (Fig. 2). Within the rare species range, environments 2 and 3 also shared a great number of species: *An. oswaldoi* (Peryassu), *Cx. quinquefasciatus* Say, *Ma. pseudotitillans* (Theobald), *Ps. saeva* Dyar and Knab, *Ps. cingulata* (Fabricius).

Environment 1 showed a pronounced curve, with *Cx. quinquefasciatus* (1,154 individuals, Ln₁₀ = 3.1) at the highest level (Fig. 2).

As for beta (β) diversity, environment 1 was the one showing higher complementarity, with 42% dissimilarity as compared to environment 2, and 41% as compared to environment 3. Environments 2 and 3, on the other hand, shared a great number of species, indicating low complementarity (20%) or greater similarity between them.

The highest degree of synanthropy was for *Ae. aegypti* (Linneaus) and *Cx. quinquefasciatus* species. Whereas other species such as *Oc. hastatus/oligopistus* or *Oc. stigmaticus* Edwards, showed very low values (Table 1).

3.2. Seasonality and influence of environmental variables on the abundance of mosquito species

Monthly mean temperature, mean rainfall and relative humidity data from the sampling period are shown in Fig. 3. Seasonal patterns were not different between environments so they were analyzed as a whole. Species with abundances exceeding 1% in the different environments studied were analysed. The effects of environmental variables on species abundance were analysed as a single site, since rainfall, relative humidity and mean temperature records from the two weather stations consulted, i.e. Resistencia (27°30′S 59°W) and Colonia Benitez (27°19′25.95″s 58°57′8.36″O) (closer to environment 3) (Fig. 1), correlated positively and significantly ($r = 0.89$; $r = 0.84$; $r = 0.86$, respectively $P < 0.05$). All three climatic variables showed a positive and significant effect of month and year on the abundance of most of the analysed species. Table 2 shows annual mean values for the effect of the different climatic variables on Culicidae abundances.

Culex quinquefasciatus, *Cx. bidens* Dyar, *Cx. chidesteri* Dyar, *Cx. coronator* Dyar and Knab and *Cx. maxi* Dyar, were captured every month of the year and, like most of the species from the same genus, with occasional very marked abundance fluctuations, sometimes reaching very low or zero levels in captures following the peaks of abundance (Fig. 4). In some cases, these reductions occurred the following month, and in other cases they occurred after two or three months. Species from the *Melanoconion* subgenus were more abundant in autumn (Fig. 4) and, unlike species from the *Culex* subgenus, they were scarce in winter. Overall, species from the *Culex* genus were positively influenced by temperature and relative humidity, with the exception of *Cx. chidesteri* and *Cx. maxi* ($P < 0.001$). The IRR showed that a rise of 1 °C resulted in a reduction of abundance of 5% and 3% for these two species, respectively. *Ochlerotatus* and *Psorophora* species were captured mainly in spring–summer–autumn (October–April). As an exception, *Oc. albifasciatus* (Macquart) was mainly captured in winter. *Ochlerotatus hastatus/oligopistus* and *Oc. scapularis* were captured
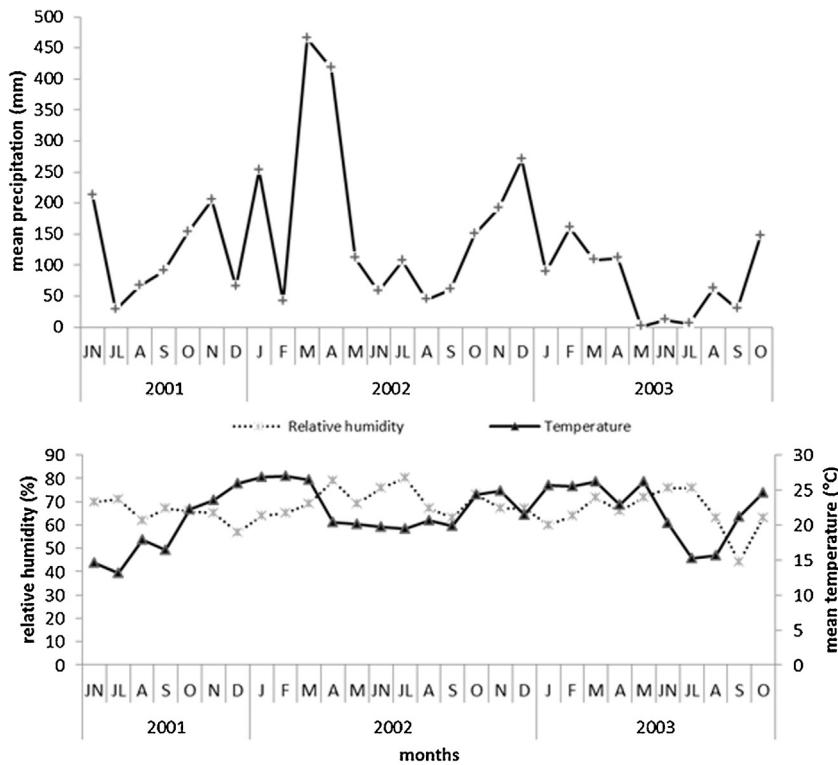
Fig. 3. Monthly variation of mean rainfall (mm), mean temperature and relative humidity from June 2001 to October 2003, in the study area.
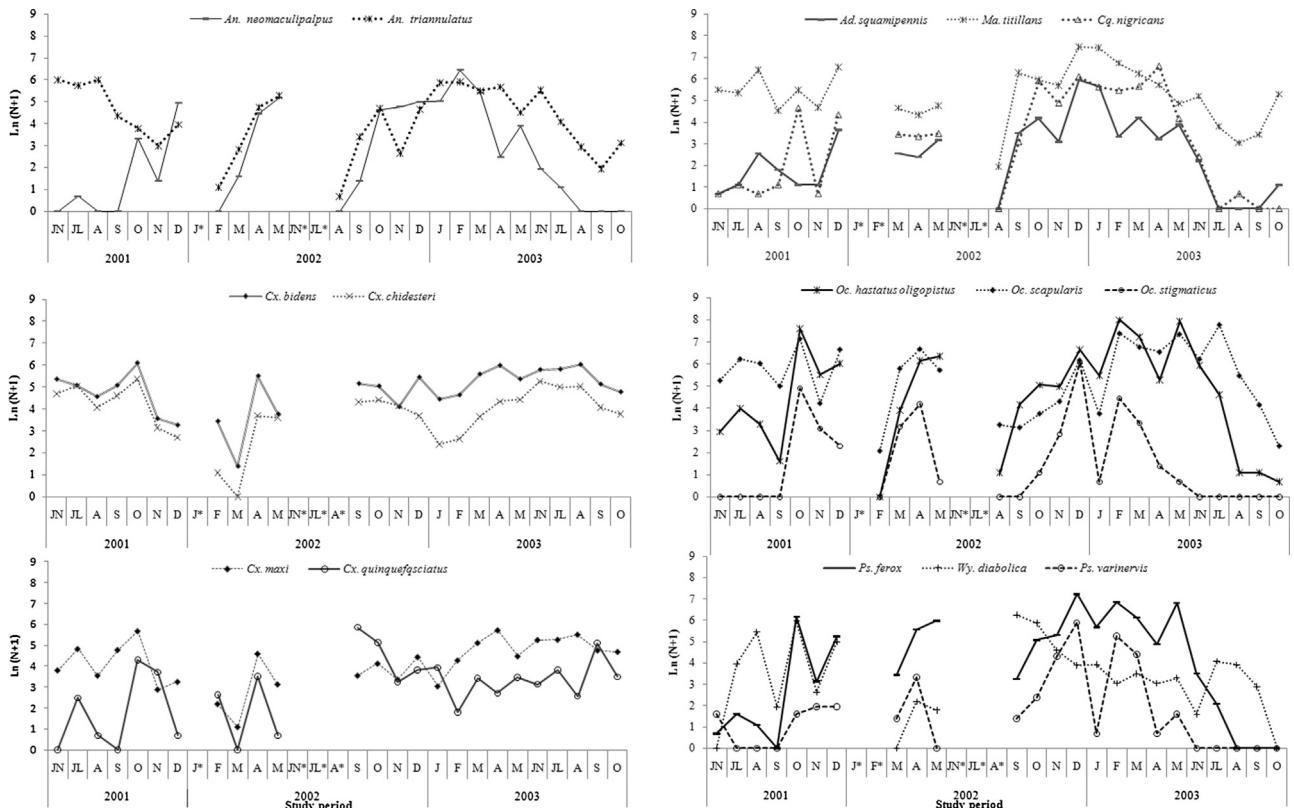
Fig. 4. Monthly mosquito abundance in the urban environment, recorded from June, 2001 to October 2003. *: technical failures of the traps and difficulty accessing the mosquito collection sites did not allow the sampling.

in all four seasons, showing greater abundance in autumn (Fig. 4). Although *Ochlerotatus* species were positively and significantly affected mainly by temperature and relative humidity, *Oc. stigmati-*

cus was negatively affected by the last variable (*P* < 0.001) (Table 2). *Psorophora ferox* showed picks of abundance in spring, summer and autumn, slightly decreasing the captures the next month after

Table 1
Adult mosquitoes captured in three different environments in the province of Chaco, Argentina.

Species	Environments			
	1	2	3	SI
Ad. squamipennis	19	366	685	−45.14
Ae. aegypti	17			100.00
An. albitarsis	21	209	315	−34.77
An. benarrochi		2		1.00
An. deaneorum		2		50.00
An. evansae	1	59	10	29.29
An. galvaoi		47	26	−3.42
An. neomaculipalpus		389	1496	−69.05
An. oswaldoi		5	6	−31.82
An. rondoni			1	−1.00
An. triannulatus	21	2581	1002	8.59
An. (Nys.) spp.	10	3059	1287	5.80
Cq. albicosta	3	493	38	39.61
Cq. nigricans	7	2584	225	38.14
Cq. shannoni		11	16	−38.89
Cq. spp.		300	42	31.58
Cx. ameliae			3	−3.00
Cx. apicinus		5		2.50
Cx. bidens	366	1177	674	−4.68
Cx. brethesi		2		1.00
Cx. chidesteri	163	559	277	10.41
Cx. clarki		18	12	−10.00
Cx. coronator	76	168	105	6.84
Cx. delpontei	6	1053	415	7.76
Cx. eduardoi	40	55	6	−0.57
Cx. educator	1	151	227	−39.71
Cx. hepperi	1	40	15	12.10
Cx. imitator		19	88	−73.61
Cx. intrincatus	2	161	227	−37.05
Cx. lahillei	1	7	5	−23.33
Cx. maxi	205	592	405	2.09
Cx. mollis		8		50.00
Cx. ocossa	5	156	120	−13.17
Cx. pilosus	54		3	89.47
Cx. quinquefasciatus	1154	2	3	99.48
Cx. saltanensis	37	74	38	−10.10
Cx. (Cux.) spp.	797	33279	18051	−0.73
Cx. (Mel.) spp.	3	202	438	−56.94
Hg. spegazzini		2		−25.00
Hg. leucocelaenus			2	−2.00
Ma. flaveola		14	17	−32.26
Ma. humeralis	17	570	195	13.68
Ma. indubitans	4	7	1	54.17
Ma. pseudotitillans	2	1	1	37.50
Ma. titillans	81	7012	2461	11.79
Ma. spp.	9	585	281	2.34
Oc. albifasciatus	12	65	106	−33.61
Oc. crinifer		4	1	20.00
Oc. fulvus		80	144	−46.43
Oc. hastatus/oligopistus	9	3112	10025	−64.35
Oc. scapularis	217	8562	4720	−1.64
Oc. serratus	19	3	20	1.19
Oc. stigmaticus	1	197	628	−63.98
Oc. spp.	13	748	1005	−34.99
Ps. albigenu/varipes	47	409	211	6.07
Ps. albipes		21	1	43.18
Ps. ciliata	1	83	300	−67.06
Ps. cingulata		4	4	−25.00
Ps. confinnis	3	24	234	−83.91
Ps. cyanescens	23	39	375	−76.09
Ps. dimidiata		6	27	−72.73
Ps. discrucians	1	124	302	−55.97
Ps. ferox	5	2054	3848	−47.67
Ps. lutzzi		2		1.00
Ps. pallescens		9	38	−71.28
Ps. paulli	3	3	90	−89.06
Ps. saeva		2	4	−50.00
Ps. varinervis	8	99	677	−79.02
Ps. (Gra.) spp	1	43		51.14
Ps. (Jan.) spp.	7	1286	2400	−47.39
Ur. apicalis		17	25	−39.29
Ur. geometrica		1		0.50
Ur. lanei			3	−100.00
Ur. lowii	1	19	64	−63.69

Table 1 (*Continued*)

Species	Environments			
	1	2	3	SI
Ur. nataliae		3	6	−50.00
Ur. pulcherrima			25	−100.00
Ur. spp.		38	71	−47.71
Wy. diabolica		102	2045	−92.87
Wy. melanocephala		1	1	−25.00
Wy. muelhensi		35	430	−88.71
Wy. (Pho.) spp.		140	3351	−93.98
Wy. (Wyo.) spp.			1	−100.00
Individuals	3494	73361	60401	

Note: 1: Urban environment; 2: semi-urban environment; 3: wild environment; SI: synanthropic index.
Abbreviations: Ad: *Aedeomyia*; An: *Anopheles*; Cq: *Coquillettidia*; Cx: *Culex*; Cux: *Culex*; Gra: *Grabhamia*; Hg: *Haemagogus*; Jan: *Janthinosoma*; Ma: *Mansonia*; Mel: *Melanoconion*; Nys: *Nyssorhynchus*; Oc: *Ochlerotatus*; Ps: *Psorophora*; Ur: *Uranotaenia*; Wy: *Wyeomyia*; Wyo: *Wyeomyia*.

the peaks, while *Ps. albigenu/varipes* (Peryassu)/(Coquillett), *Ps. cyanescens* (Coquillett) and *Ps. varinervis* Edwards reached zero or very low levels of abundance after picks of abundance (Fig. 4). *Psorophora* species were almost absent in winter. These were influenced by all three climatic variables. The IRR showed that *Ps. ferox* abundance increased 1.21 times with a temperature rise of 1° and 2.07 times with an increase of 10 units of rainfall ($P < 0.001$); in the case of *Ps. albigenu/varipes*, abundance increased 32% with a temperature rise of 1 °C (Table 2). *Coquillettidia albicosta* (Peryassu) and *Cq. nigricans* (Coquillett) showed a very similar temporal distribution pattern (Fig. 4). Both species were practically absent in winter months, they were more abundant from October to April. *Mansonia humeralis* Dyar & Knab and *Ma. titillans* Dyar & Shannon were captured every month of the year, the latter being more abundant in summer. *Aedeomyia squamipennis* (Arribalzaga) was more abundant in summer and autumn. The incidence rate ratio (IRR) showed that *Ad. squamipennis* and *Ma. titillans* abundances increased 16% for both species ($P < 0.001$) with a mean temperature rise of 1 °C (Table 2). *Anopheles triannulatus* and *An. albitarsis* (Arribalzaga) showed a similar distribution with peaks of abundance in each season (Fig. 4). Unlike the two previous species, *An. neomaculipalpus* Curry showed peaks of abundance in autumn and summer, and was hardly abundant or practically absent in winter. The abundances of the *Anopheles* species were mainly positively and significantly affected by relative humidity (Table 2).

4. Discussion

The number of species found in this region ($S = 82$), was greater than the number found in the Guapiaçu Ecological Reserve (Brazil) ($S = 48$) (Alencar et al., 2015) which is considered a biodiversity hotspot within the Atlantic Forest, with tropical climate, but smaller than the number found by Ribeiro et al. (2012) ($S = 91$) in the super wet tropical marine climate of the district of Parelheiros, 60 km south of Sao Paulo, Brazil.

The semi-urban environment showed extremely high completeness, suggesting that the number of species is unlikely to grow, even if the sampling efforts were increased. The distribution of the abundances and hierarchical order of the species in the three environments shows that 2 and 3 are structured similarly, with certain unique characteristics, being different to environment 1. Environments 2 and 3 showed higher specific richness with species exclusive and/or specific to the habitat, such as those reared in the axils of Bromeliaceae (*Wyeomyia* spp., *Microculex* spp.), which are more sensitive to human induced changes. Terrestrial bromeliads are very abundant in the underwoodland of the environment 3 and 2, but are absent in urban environment.

Table 2
Relationship of environmental variables to the adult mosquito abundances captured in three different environments in the province of Chaco, Argentina.

Species	Statistical variables	Environmental variables		
		T° mean	RH	PP
Ad. squamipennis	Coef	0.148924	−0.005219	0.001226
	SE	0.01035	0.004165	0.000276
	IRR	1.16	0.99	1.001
	p	<0.001	NS	<0.001
An. albitarsis	Coef	0.028991	0.031322	0.004207
	SE	0.017446	0.009639	0.000438
	IRR	1.03	1.03	1.004
	p	NS	<0.005	<0.001
An. triannulatus	Coef	0.063923	0.034097	0.000456
	SE	0.004339	0.002377	0.000169
	IRR	1.03	1.0005	1.56
	p	<0.001	<0.01	<0.001
An. neomaculipalpus	Coef	0.253246	0.061484	0.000533
	SE	0.007127	0.003502	0.000169
	IRR	1.29	1.06	1.001
	p	<0.001	<0.001	<0.001
Cq. nigricans	Coef	0.187494	0.030264	0.001784
	SE	0.006683	0.002935	0.000186
	IRR	1.21	1.03	1.002
	p	<0.001	<0.001	<0.001
Cx. bidens	Coef	0.009406	0.035319	−0.00072
	SE	0.003807	0.001929	0.000154
	IRR	1.01	1.04	0.999
	p	<0.001	<0.001	<0.001
Cx. chidesteri	Coef	−0.045253	0.051956	−0.00321
	SE	0.006388	0.003754	0.000295
	IRR	0.96	1.05	1.00
	p	<0.001	<0.001	NS
Cx. coronator	Coef	0.074888	0.112728	0.001266
	SE	0.029458	0.020310	0.001201
	IRR	1.07	1.10	1.003
	p	NS	<0.001	NS
Cx. eduardoi	Coef	0.14599	0.17834	0.007063
	SE	0.045025	0.029568	0.001252
	IRR	1.07	1.10	1.003
	p	<0.001	<0.001	<0.001
Cx. delpontei	Coef	0.382603	0.065915	0.001466
	SE	0.012519	0.005376	0.00028
	IRR	1.47	1.07	1.001
	p	<0.001	<0.001	<0.001
Cx. maxi	Coef	−0.020813	0.041260	0.002007
	SE	0.005261	0.002857	0.000249
	IRR	0.98	1.04	1.002
	p	<0.001	<0.001	<0.001
Cx. quinquefasciatus	Coef	0.023201	−0.048849	−0.003371
	SE	0.010108	0.003602	0.000404
	IRR	1.02	0.95	0.997
	p	<0.001	<0.001	<0.001
Ma. titillans	Coef	0.153083	−0.009579	0.001034
	SE	0.003126	0.001359	0.000092
	IRR	1.17	0.99	1.001
	p	<0.001	<0.001	<0.001
Oc. hastatus	Coef	0.297623	0.09764	0.002756
	SE	0.003406	0.001796	0.000093
	IRR	1.35	1.10	1.003
	p	<0.001	<0.001	<0.001
Oc. scapularis	Coef	0.059525	0.087168	0.002131
	SE	0.002344	0.001529	0.000098
	IRR	1.06	1.09	1.002
	p	<0.001	<0.001	<0.001
Oc. stigmaticus	Coef	0.066402	−0.198451	0.019007
	SE	0.024663	0.014167	0.000972
	IRR	1.07	0.82	1.02
	p	<0.05	<0.001	<0.001
Ps. ferox	Coef	0.188194	0.057247	0.001706
	SE	0.004317	0.002176	0.000113
	IRR	1.21	1.06	1.002
	p	<0.001	<0.001	<0.001
Ps. varinervis	Coef	0.790199	−0.274894	0.033217
	SE	0.051898	0.022685	0.002116
	IRR	2.20	0.76	1.03
	p	<0.001	<0.001	<0.001

Table 2 (*Continued*)

Species	Statistical variables	Environmental variables		
		T° mean	RH	PP
Ps. albigenu/varipes	Coef	0.276337	0.202679	0.003435
	SE	0.01734	0.010878	0.000377
	IRR	1.32	1.22	1.003
	p	<0.001	<0.001	<0.001
Wy. diabolica	Coef	0.060918	−0.032085	−0.00233
	SE	0.006622	0.003585	0.000253
	IRR	1.06	0.97	1.00
	p	<0.001	<0.001	<0.001
Wy. muelhensi	Coef	0.024383	−0.014607	−0.006727
	SE	0.014845	0.007326	0.000697
	IRR	1.02	0.99	0.99
	p	NS	NS	<0.001

Note: Abbreviations: Coef: coefficient; SE: standard error; IRR: incidence rate ratio; p: probability value; T° mean: mean temperature; RH: relative humidity; PP: mean monthly precipitation; NS: not significant.

Most of the species from the *Ochlerotatus* and *Psorophora* genera showed synanthropy rates that place them in environments with low or zero anthropic disturbance, as stated by other authors (Hack et al., 1978; Ronderos et al., 1992). These species breed in temporary pools (Stein et al., 2011), the eggs hatch when their habitats are flooded (Forattini, 2002), which can remain longer in less modified environments, deu to higher cover plant, in relation to the environment 1, that was observed in this study. Like other authors, we observed that, *Psorophora* species and some *Ochlerotatus* species *Oc.* stigmaticus after reaching peaks of abundance, decreased significantly until they completely disappeared (Guimaraes and Arlé, 1984; Ronderos et al., 1992; Almirón and Brewer, 1995; Guimaraes et al., 2000b; Méndez et al., 2001). Mean temperatures and rainfall were the two environmental variables that had a more substantial influence on fluctuations in abundance of theses species throughout the year, in coincidence with Alencar et al. (2015). The highest abundances of these species were observed during the rainy and warm season, and they disappear completely in winter.

Ochlerotatus hastatus/oligopistus showed the lowest synanthropy values in this study, so this species does not adjust to human-modified environments, although we consider its biology requires further study. Stein et al. (2011) found this species rearing only in temporary pools, in the study area. Forattini et al. (1995a) found it with frequencies below 1% in agricultural areas of Brazil. On the other hand *Oc. scapularis* showed synanthropy values that suggest a species which breeds well in slightly altered or pristine environments, and can also exist in very disturbed environments (Forattini et al., 1978, 1987, 1993, 1995b; 1997; Lourenço De Oliveira et al., 1985; Guimaraes et al., 1989; Natal et al., 1992; Da Silva and de Menezes, 1996; Tubaki et al., 2004; Alencar et al., 2015). This species was captured every month of the year with similar abundances, which were positively and significantly influenced by the three variables analysed. It is also reported in different investigations (Hack et al., 1978; Lourenço De Oliveira et al., 1985; Forattini et al., 1995b; Guimaraes et al., 2000b). *Ochlerotatus hastatus/oligopistus* showed this behaviour too in the study area. The low abundance of *Oc. albifasciatus* founded in this study, the main vector of west equine encephalitis in Argentina (Avilés et al., 1992), makes us believe that the climatic conditions of the study area is not suitable for reproduction of the species. The species showed greater abundance in winter, in contrast to findings in temperate climates, where shows higher abundance during summer (Ludueña Almeida and Gorla, 1995; Gleiser et al., 1997, 2002; Almirón et al., 2000; Fontanarrosa et al., 2000). Different studies pointed factors favourable for the breeding of the species, such as breeding sites remaining dry for long periods, and for the hatching of eggs (Ludueña Almeida and Gorla, 1995; García and Micieli, 2000;

Fontanarrosa et al., 2000). Almirón et al. (2000) found a significant correlation between the daily relative density of *Oc. albifasciatus* females and the permanence of water in breeding sites. In the study area, rainfall is scarce during early spring, thus developmental success is rather unpredictable for individuals hatched during this period because insufficient water supply and high insolation, in wet season, cause the rapid drying of larval habitats (Gimenez et al., 2015).

Some authors suggest that human interference in the natural environment can lead to the elimination and/or modification or substitution of habitats, with the subsequent loss of richness in the existing Culicidae fauna, although the conditions are also favourable for the settling of exotic or opportunist species well-adjusted to disturbed environments (Consoli and Lourenço De Oliveira, 1994; Dorvillé, 1996; Forattini, 2002).

High abundance of *Cx. quinquefasciatus* in environment 1 is consistent with other findings (Castro Gomes et al., 1987; Castro Gomes and de Forattini, 1990; Ronderos et al., 1991; Campos et al., 1993; Almirón and Brewer, 1995; Lopes et al., 1995; Guimaraes et al., 2000a; Pires and Gleiser, 2010), showing the high adaptation of this species in this one. In coincidence with other studies Stein et al., (2011) collect *Cx. quinquefasciatus* mainly from artificial larval habitats, from ditches with turbid and sewage water with unpleasant odour and solid wastes, in Resistencia city (Oria et al., 2002; Oria and Oscherov 2002; Pires and Gleiser, 2010; Burke et al., 2010). *Aedes aegypti* was an exclusive species of this environment too and its adaptation is well documented in our country (Vezzani and Carbajo, 2008).

Otherwise, *Cx. bidens* and *Cx. chidesteri* are noted for being rarely found in wild environments and for showing a higher level of adjustment to more urban environments (Castro Gomes and de Forattini, 1990). In this study, however, they were very abundant in environments 2 and 3, with synanthropy rates that identify them as eusynanthropic species. These species were collected mainly in ditches and lagoons in the study area respectively (Stein et al., 2011), being more affected by the presence of solid wastes. As found in other studies, *Cx. maxi* and *Cx. bidens* show recurring peaks of abundance without disappearing completely (Almirón and Brewer, 1994, 1995; Maciá et al., 1995; Fisher and Schweigmann, 2004). For *Cx. quinquefasciatus* and *Cx. bidens*, rainfall had a negative and significant effect on abundance. This could be explained by a negative, or washing, effect of rainfall on the larval habitats, like ditches or pools, where these two species were mainly found in the study area (Stein et al., 2011). The same effect was observed for *Wyeomyia* species which abundance can be affected by the washing of their larval micro-habitats in periods of increased rainfall (autumn), being more abundant in months with higher temperatures (spring–summer) and less rainfall. This effect is also observed for some *Anopheles* species in the northwest of Argentina (Dantur Juri et al., 2014). *Culex quinquefasciatus* should be given special attention for its role as vector of the Saint Louis Encephalitis virus during the unprecedented outbreak for South America and Argentina occurring in Córdoba (Spinsanti et al., 2005), by isolating two genotype III strains from *Cx. quinquefasciatus* and *Cx. interfor* (*Cx. bidens* complex) mosquitoes (Díaz et al., 2006; Diaz, 2009).

The niche diversification hypothesis holds that diversity depends on the range of habitats or the degree of specialization of resident species. According to this hypothesis, more stable or less disturbed environments would have higher diversity (Alencar et al., 2015). This would explain what was found in environment 3, with the higher effective number of species. Ronderos et al. (1992), on the other hand, state that even under similar weather conditions, existing vegetation and terrain conditions, the high degree of environmental heterogeneity resulting from anthropic disturbance offers different possibilities of larval habitats, which is eventually favourable for greater diversity (Ribeiro et al., 2012). We consider

that environmental heterogeneity was underestimated in environment 1, thus the lower completeness estimated, however showed, α diversity slightly bigger than environment 2. New studies, with greater sampling effort, should therefore consider this characteristic, as this might allow the possibility to detect other existing species not found in this study. The environment 2 had lower diversity and greater abundance of Culicidae, could be explained in part by the less equity in the distribution of abundances of the dominant species, which are favored when disturbance is neither too rare nor too frequent (Connell, 1978).

In this study, the mean temperature variable was the most closely linked to *Cq. nigricans*, *Ma. titillans*, and *Ad. squamipennis* abundance fluctuations. Higher abundance of these species in summer is consistent with higher temperatures and increased blooming of *Pistia stratiotes* L. in lakes from environments 2 and 3 from where we collected the larvae of two last species (Stein et al., 2011). Contrary to our findings in this study, Alencar et al. (2015) found specimens from the Mansoniini tribe more associated with the environment with higher anthropic disturbance.

Temperature had an important effect on the abundance of *An. triannulatus*, and relative humidity on *An. neomaculipalpus*. *Anopheles triannulatus* is the dominant species of the genus. It is considered a species capable of adjusting to sudden human-induced environmental changes, and is given the role of auxiliary regional vector of malaria in Brazil (Consoli and Lourenço De Oliveira, 1994). This species showed high selection by human bait in the study area (Stein et al., 2013). This species is found mainly in lagoon in the study area which is a more stable habitat (Stein et al., 2011). Given this background, further studies need to be conducted in relation to their *Plasmodium* parasites transmission capabilities, and to have a deeper knowledge of their biology. *Anopheles neomaculipalpus* was mainly found in temporary pools in environment 2 and 3 (Stein et al., 2011), its higher abundance being therefore observed in summer and autumn (associated to rainy and warm season) and hardly represented in winter. The species was described from specimens collected in Panamá, where larvae were taken from cattle footprints exposed to the sunlight in low marshy pastures. Dantur Juri et al. (2011) found the species in bordering the forest, with typical marshy areas where cattle graze.

Hack et al. (1978) and Forattini et al. (1993) capture *An. albitarsis* and *An. triannulatus* mainly in autumn and winter. In this study, they showed peaks of abundance in the four seasons. Unlike the ones registered in tropical forest areas of Brazil (Alencar et al., 2015), low abundances of *An. albitarsis* found in this study suggest that the environments studied are not the most suitable habitats for a high reproduction of the species, which would require an environment with longer and more frequent periods of flooding (Lopes and Lozovei, 1995).

Consistent with other investigations, we found species from the *Melanoconion* subgenus mainly in less anthropic environments (Forattini et al., 1991; Ribeiro et al., 2012). All three climatic variables analysed had a positive and significant effect on abundance of *Cx. delpontei*, the most abundant species from this subgenus. Some *Melanoconion* species play an important role in the maintenance of arboviruses enzootic cycles (Natal, 1981; Mitchell et al., 1985; Forattini et al., 1991).

It should be noted that, in addition to the known vectors of arboviruses in our country (Mitchell et al., 1980; Avilés et al., 1992; Sabattini et al., 1998; Díaz et al., 2008; Vezzani and Carbajo, 2008; Diaz, 2009), a remarkable number of species of epidemiological significance were found in this study. *Ochlerotatus scapularis* is a potential vector of filarias and arboviruses in the Neotropics (Mitchell and Forattini, 1984; Mitchell et al., 1986; Forattini et al., 1995a; Forattini, 2002). *Mansonia titillans*, *Oc. scapularis* and *Ps. ferox* have been found naturally infected with the Venezuelan equine encephalitis virus (VEEV), Rocio virus and UNA (Forattini

et al., 1978, 1987, 1995b, 1997; Lopes et al., 1981; Travassos da Rosa et al., 1998; Méndez et al., 2001; Forattini, 2002). In the United States, the West Nile virus was isolated from *Cx. pipiens* and *Ps. ferox* after the first great outbreak registered in birds, horses and humans (CDC, 2000). Mosquitoes belonging *Wyeomyia* species collected in tropical rain forest have been found infected with several arbovirosis (Motta et al., 2007). Dutary et al. (1989) isolated the Gamboa virus from *Ad. squamipennis* mosquitoes. The subtype Laguna Larga of the Cache-Valley virus was isolated from *Oc. albifasciatus* and a variety of the Maguari subtype from *Ps. varinervis*. Serological studies revealed that this virus is widely spread in our country (Sabattini et al., 1998). Both *Anopheles triannulatus* and *An. albitarsis* play a secondary role in malaria transmission in South America when present in high densities, and *An. neomaculipalpus* is also considered to play a role in the transmission of malaria in some regions of the Americas (Curto et al., 2003; Moreno et al., 2005; Póvoa et al., 2006; Zimmerman et al., 2006). Pisano et al. (2010) found *Oc. hastatus/oligopistus, Cx. coronator, Cx. maxi y Ps. cingulata* capture in the study area, infected with Venezuelan Equine Encephalitis complex viruses. Species of *Anopheles, Aedes, Culex,* and *Uranotaenia* were found infected with uncharacterised viruses from the Bunyaviridae, Coronaviridae, Flaviviridae, and Rhabdoviridae families in the forests of Western Africa (Junglen et al., 2009).

In conclusion, the most conserved environments showed greater richness and abundance of species, share 80% of them. Some of them are absent or less abundant in the urban environment, suggesting a high susceptibility to anthropogenic changes, mainly associated with the availability of larval habitats. The presence of these species could be an indicator of slightly disturbed environments, which can play a role in maintaining arbovirus enzootic cycles. On the other hand the environmental changes caused by humans increase the abundance of opportunistic and exotic species such as *Cx. quinquefasciatus* and *Ae. aegypti* (Guimaraes et al., 2000b) and its consequences on the health of the population. Therefore, the data provided by this research could contribute to potential disease control programmes and they reinforce the need to conduct further studies on the biology of these species since continuous or bigger environmental changes caused by human activity could cause new epidemiological scenarios. It is necessary entomological and control monitoring by health authorities to detect new species possibly involved in pathogen transmission in our country, and whose role has not yet been revealed.

Acknowledgements

References

Alencar, J., de Mello, C.F., Guimarães, A.E., Gil-Santana, H.R., Silva, J.dS., Santos-Mallet, J.R., Gleiser, R.M., 2015. Culicidae community composition and temporal dynamics in Guapiaãu Ecological Reserve, Cachoeiras de Macacu, Rio de Janeiro, Brazil. PLoS One 10 (3), 1–16.

Almirón, W.R., Brewer, M., 1994. Immature stages of mosquitoes (Diptera: Culicidae) collected during the autumn–winter period in Córdoba province, Argentina. Mem. Inst. Oswaldo Cruz 89 (4), 625–628.

Almirón, W.R., Brewer, M., 1995. Distribución estacional de Culicidae (Diptera) en áreas periféricas de Córdoba (Argentina). Ecol. Aust. 5 (1), 81–86.

Almirón, W.R., Ludueña Almeida, F.F., Brewer, M., 2000. Relative abundance and gonotrophic status of *Aedes albifasciatus* (Díptera: Culicidae) during the autumn–winter period in Cordoba Province, Argentina. J. Med. Entomol. 32 (1), 16–20.

Avilés, G., Sabattini, M.S., Mitchell, C.J., 1992. Transmission of western equine encephalomyelitis virus by *Aedes albifasciatus* (Diptera: Culicidae). J. Med. Entomol. 29 (5), 850–853.

Badii, M.H., Landeros, J., Foroughbakhch, R., Abreu, J.L., 2007. Biodiversity, evolution, extintion and sustainability. Int. J. Good Consci. 2 (2), 229–247.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H., White, J.S., 2011. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol. Evol. 24 (3), 127–135.

Bruniard, E.D., 1978. El gran Chaco Argentino: ensayo de interpretación geográfica. Rev. Geogr. 4, 259.

Burke, R., Barrera, R., Lewis, M., Kluchinsky, T., Clanorn, D., 2010. Septic tanks as larval habitats for the mosquitoes *Aedes aegypti* and *Culex quinquefascatus* in Playa-Playita in Puerto Rico. Med. Vet. Entomol. 24, 117–123.

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

Campos, R.E., Spinelli, G., Mogi, M., 2011. Culicidae and ceratopogonidae (Diptera: Nematocera) inhabiting phytotelmata in Iguazú National Park, Misiones province, subtropical Argentina. Rev. Soc. Entomol. Argent. 70 (1–2), 111–118.

Castro Gomes, A., de Forattini, O.P., Natal, D., 1987. Composiçao e atividade de mosquitos Culicidae emprego de armadilla CDC no vale do Ribeira Estado do Sao Paulo, Brasil. Rev. Saúde Públ. 21 (5), 363–370.

Castro Gomes, A., de Forattini, O.P., 1990. Abrigos de mosquitos *Culex* (*Culex*) em zona rural (Díptera: Culicidae). Rev. Saúde Publ. 24 (5), 394–397.

CDC, 2000. Division of vector- borne infectious diseases. Mosquito Species producing WNV positives by year. Available from: http://www.cdc.gov/ ncidod/dvbid/westnile/mosquitospecies.htm.

Chao, A., Lee, S.M., 1992. Estimating the number of classes via sample coverage. J. Am. Stat. Assoc. 87, 210–217.

Colwell, R.K., Coddington, J.A., 1994. Estimating terrestrial biodiversity through extrapolation. Philos. Trans. R. Soc. Lond. Ser. B 345, 101–118.

Colwell, R.K., 2013. Estimates: statistical estimation of species richness and shared species from samples. Version 9. User's Guide and application published at: http://purl.oclc.org/estimates.

Connell, J.H., 1978. Diversity in tropical rain forests and coral reefs. Science 199, 1302–1310.

Consoli, R.A.G.B., Lourenço De Oliveira, R., 1994. Principias Mosquitos De Importancia Sanitária No Brasil. Editora Fiocruz, Rio de Janeiro.

Curto, S.I., Carbajo, A.E., Boffi, R., 2003. Aplicación de sistemas de información geográfica en epidemiología. Caso de estudio: malaria en la Argentina (1902–2000). Available from: epidemiologia.anm.edu.ar/pdf/ publicacioneszcie/2003/Aplicacion_Sistemas_Informacion_Geografica_ epidemiologia_2003.pdf.

Dantur Juri, M.J., Zaidemberg, M., Almirón, W.R., 2005. Distribución espacial de *Anopheles pseudopunctipennis* en las yungas de Salta, Argentina. Rev. Saúde Públ. 39 (4), 565–570.

Dantur Juri, M.J., Stein, M., Rossi, G.C., Navarro, J.C., Zaidenberg, M., Mureb Sallum, M.A., 2012. New records of mosquitoes from northwesternArgentina. J. Am. Control Assoc. 28 (2), 111–113.

Dantur Juri, M.J., Galante, G.B., Zaidenberg, M., Almirón, W.R., Claps, G.L., Santana, M., 2014. Longitudinal study of the species composition and spatio-temporal abundance of *Anopheles* larvae in a malaria risk area in Argentina. Fla. Entomol. 97 (3), 1167–1181.

Darsie, R.F., 1985. Mosquitoes of Argentina. Part I. Keys for identification of adult females and fourth stages larvae in english and spanish (Diptera: Culicidae). Mosq. Syst. 17 (3), 153–253.

Da Silva, A.M., de Menezes, R.M.T., 1996. Encontro de *Aedes scapularis* (Díptera: Culicidae) em criadouro artificial em localidade da região Sul do Brasil. Rev. Saúde Públ. 30 (1), 103–104.

Diaz, A., 2009. Patrones de Actividad y Estacionalidad del Virus St. Louis Encephalitis en Córdoba, Argentina. Facultad de Ciencias Exactas, Físicas y Naturales Universidad Nacional de Córdoba, p. 193.

Díaz, L.A., Re, V., Almirón, W.R., Farias, A., Vázquez, A., Sánchez Seco, M.P., Aguilar, J., Spinsanti, L., Konigheim, B., Morales, M.A., Tenorio, A., Contigiani, M., 2006. Genotype III *Saint Louis* encephalitis virus outbreak, Argentina. Emerg. Infect. Dis. 12 (11), 1752–1753.

Díaz, L.A., Komar, N., Visitin, A., Dantur Juri, M.J., Stein, M., Lobo Allende, R., Spinsanti, L., Konigheim, B., Aguilar, J., Laurito, M., Almirón, W.R., Contigiani, M., 2008. West Nile virus in birds, Argentina. Emerg. Infect. Dis. 14 (4), 689–691.

Dorvillé, L.F.M., 1996. Mosquitoes as bioindicators of forest degradation in southeastern Brazil, a statistical evaluation of published data in the literature. Stud. Neotrop. Fauna Environ. 31, 68–78.

Dutary, B.E., Petersen, J.L., Peralta, P.H., Tesh, R.B., 1989. Transovarial transmission mosquito, *Aedeomyia squamipennis*. Am. J. Trop. Med. Hyg. 40, 108–113.

Faran, M.E., 1980. Mosquito studies (Diptera: Culicidae) XXXIV. A revision of the *Albimanus* section of the subgenus *Nyssorhynchus* of *Anopheles*. Contrib. Am. Entomol. Inst. 15 (7), 1–215.

Feinsinger, P., 2001. Designing Field Studies for Diversity Conservation. Island, Washington D.C, 212 p.

Fisher, S., Schweigmann, N., 2004. Culex mosquitoes in temporary urban rain pools: seasons dynamics and relation to enviromental variables. J. Vector Ecol. 29 (2), 365–373.

Fontanarrosa, M.S., Marinone, M.C., Fischer, S., Orellano, P., Schweigmann, N., 2000. Effects of flooding and temperature on *Aedes albifasciatus* development time and larval density in two rain pools at Buenos Aires University City. Mem. Inst. Oswaldo Cruz 95 (6), 787–793.

Forattini, O.P., 2002. Culicidologia Médica, vol. 2. Editora Universidade de Sao Paulo, Sao Paulo.

Forattini, O.P., Castro Gomes, A., Galato, E.A.B., Robillo, E.X., Iversson, L.B., 1978. Estudos ecológicos sobre mosquitos Culicidae no Sistema da Serra do Mar, Brasil. 2-Observaciones no ambiente domiciliar. Rev. Saúde Públ. 12, 476–496.

Forattini, O.P., Castro Gomes, A., Natal, D., Kakitani, I., 1987. Frequencia domiciliar e endofilia de mosquitos culicidae no vale do ribeira, Sao Paulo, Brasil. Rev. Saúde Públ. 21 (3), 188–192.

Forattini, O.P., de Castro Gomes, A., Kakitani, I., Marucci, D., 1991. Domiciliation of Culex mosquitoes (Melanoconion), in man-made deeply modified environment. Rev. Saúde Públ. 25 (4), 257–266.

Forattini, O.P., Kakitani, I., Massad, E., Marucci, D., 1993. Studies on mosquitoes (Diptera: Culicidae) and anthropic environment 4—survey of resting adults and synanthropic behaviour in southeastern, Brazil. Rev. Saúde Públ. 27 (6), 398–411.

Forattini, O.P., Kakitani, I., Massad, E., Marucci, D., 1995a. Studies on mosquitoes (Díptera: Culicidae) and anthropic enviroment. 3—Survey of adults stages at the rice irrigation system and the emergence of Anopheles albitarsis in south-eastern Brazil. Rev. Saúde Públ. 27 (5), 313–325.

Forattini, O.P., Kakitani, I., Massad, E., Marucci, D., 1995b. Studies on mosquitoes (Díptera: Culicidae) and anthropic enviroment. 9—Synanthropy and epidemiological vector role of Aedes scapularis in south- eastern Brazil. Rev. Saúde Públ. 29 (3), 199–207.

Forattini, O.P., Kakitani, I., Mureb Sallum, M.A., 1997. Encontro de criadouros de Aedes scapularis (Díptera: Culicidae) em recipientes artificiais. Rev. Saúde Públ. 31 (5), 519–522.

García, J.J., Micieli, M.V., 2000. Estacionalidad poblacional de los estados inmaduros de Aedes albifasciatus (Díptera: Culicidae) en la provincia deBuenos Aires, Argentina. Rev. Biol. Trop. 48 (2–3), 623–628.

García-Morales, R., Moreno, C.E., Bello-Gutiérrez, J., 2011. Renovando las medidas para evaluar la diversidad en comunidades ecológicas: el número de especies efectivas de murciélagos en el sureste de Tabasco. Therya 2, 205–215.

Gleiser, R.M., Gorla, D.E., Ludueña Almeida, F.F., 1997. Monitoring the abundance of Aedes (Ochlerotatus) albifasciatus (Maqcuart 1838) (Diptera: Culicidae) to the south of Mar Chiquita lake, centralArgentina, with the aid of remote sensing. Ann. Trop. Med. Parasitol. 91, 917–926.

Gleiser, R.M., Gorla, D.E., Schelotto, G., 2002. Spatial pattern of abundance of the mosquito, Ochlerotatus albifasciatus, in relation to habitat characteristics. Med. Vet. Entomol. 16, 364–371.

Guimaraes, A.E., Arlé, M., 1984. Mosquitos no Parque Nacional da Serra dos Orgaos. Estado do Rio do Janeiro, Brasil. I. Distribucién estacional. Mem. Inst. Oswaldo Cruz 79, 309–323.

Guimaraes, A.E., Motta, M.A., Arlé, M., Machado, R.M., Gonçalves, L.D., 1989. Bionomics of mosquitoes (Diptera: Culicidae) in areas of the Atlantic Forest of Itaguaí municipality, Rio de Janeiro state, Brazil: I intra, peri and extradomiciliary frequency. Mem. Inst. Oswaldo Cruz 84, 243–254.

Guimaraes, A.E., Gentile, C., Lopes, C.M., Sant'Anna, A., Jovita, A.M., 2000a. Ecolog'a de mosquitos (Díptera: Culicidae) em íreas do Parque Nacional da Serra da Bocaina, Brasil. I—Ditribucao por habitat. Rev. Saáde Pábl. 34 (3), 243–250.

Guimaraes, A.E., Pinto de Mello, R., Macedo Lopes, C., Gentile, C., 2000b. Ecology of mosquitoes (Díptera: Culicidae) in áreas of Serra do Mar State Park, State of Sao Paulo, Brazil. I—Monthly frequency and climatic factors. Mem. Inst. Oswaldo Cruz 95 (1), 1–16.

Gimenez, J.O., Fisher, S., Zalazar, L., Stein, M., 2015. Cold aeason mortality under natural conditions and subsequent hatching response of Aedes (Stegomyia) aegypti (Diptera: Culicidae) eggs in a subtropical city of Argentina. J. Med. Entomol. 107, 1–7.

Hack, W.H., Torales, G.J., Bar, M.E., Oscherov, B., 1978. Observaciones etológicas sobre culícidos de Corrientes. Rev. Soc. Entomol. Argent. 37 (1–4), 137–151.

Hill, M.O., 1973. Diversity and evenness: a unifying notation and its consequences. Ecology 54, 427–432.

Hoyos, C., Bangher, D., Jara, G., Stein, M., 2011. New records of mosquito species in the province of Formosa, northeastern region of Argentina. J. Am. Control Assoc. 27 (1), 77–78.

Hutchings, R.S.G., Sallum, M.A.M., Ferreira, R.L.M., Hutchings, R.W., 2005. Mosquitoes of the Jaú National Park and their potential importance in Brazilian Amazonia. Med. Vet. Entomol. 19, 428–441.

Jost, L., 2006. Entropy and diversity. Oikos 113, 363–375.

Jost, L., 2007. Partitioning diversity into independent alpha and beta components. Ecology 88, 2427–2439.

Jost, L., 2010. The relation between evenness and diversity. Diversity 2, 207–232.

Junglen, S., Kurth, A., Kuehl, H., Quan, P.L., Ellerbrok, H., Pauli, G., Nitsche, A., Nunn, C., Rich, S.M., Lipkin, W.I., Briese, T., Leendertz1, F.H., 2009. Examining landscape factors influencing relative distribution of mosquito genera and frequency of virus infection. EcoHealth 6, 239–249.

Linthicum, K., 1988. A revision of the Argyritarsis section of the subgenus Nyssorhynchus of Anopheles (Diptera: Culicidae). Mosq. Syst. 20, 101–271.

Lopes, J., Lozovei, A.L., 1995. Ecología de mosquitos (Diptera: Culicidae) em criadouros naturais e artificillas de área rural do Norte do Estado do Paraná, Brasil. I—Colectas ao longo do leito de ribeirão. Rev. Saúde Públ. 29 (3), 183–191.

Lopes, O.S., Sacchetta, L.A., Francy, D.B., Jakob, W.L., Calisher, C.H., 1981. Emergence of a new arbovirus disease in Brazil III. Isolation of Rocio virus from Psorophora ferox (Humboldt, 1819). Am. J. Epidemiol. 113, 122–125.

Lopes, J., Oliveira, F.J.A., Oliveira, V.D.R.B., 1995. Predominância de Mansonia titillans (Walker, 1848) (Diptera: Culicidae) na área urbana do Município de Londrina—PR, sul do Brasil. Semina-Ci. Biol. Saúde 16 (2), 254–259.

Lourenço De Oliveira, R., Fernandes da Silva, T., Heyden, R., 1985. Algunos aspectos da ecologia dos mosquitos (Díptera: Culicidae) de uma area de planicie (Granjas Calabria), em Jacarepagua, Río de Janeiro. II. Frequencia mensal e no ciclo lunar. Mem. Inst. Oswaldo Cruz 80 (2), 123–133.

Ludueña Almeida, F.F., Gorla, D.E., 1995. The biology of Aedes (Ochlerotatus) albifasciatus Macquart, 1838 (Diptera: Culicidae) in Central Argentina. Mem. Inst. Oswaldo Cruz 90 (4), 463–468.

Maciá, A., García, J.J., Campos, R.E., 1995. Bionomía de Aedes albifasciatus y Ae. crinifer (Diptera Culicidae) y sus enemigos naturales en Punta Lara, Buenos Aires. Neotrópica 41, 43–50.

Méndez, W., Liria, J., Navarro, J.C., García, C.Z., Freier, J.E., Salas, R., Weavwe, S.C., Barrera, R., 2001. Spatial dispersión of adult mosquitoes (Diptera: Culicidae) in a sylvatic focus of Venezuelan Equine Encephalitis virus. J. Med. Entomol. 38 (6), 813–821.

Mitchell, J.C., Monath, T.P., Sabattini, M.S., 1980. Transmission of St. Louis Encephalitis virus from Argentina by mosquitoes of the Culex pipiens (Diptera: Culicidae) complex. J. Med. Entomol. 17 (3), 282–285.

Mitchell, J.C., Forattini, O.P., 1984. Experimental transmission of Rocio encephalitis virus by Aedes scapularis (Diptera, Culicidae) from the epidemic zone in Brazil. J. Med. Entomol. 21, 34–37.

Mitchell, J.C., Monath, T.P., Sabattini, M.S., Cropp, C., Daffner, J., Calisher, C., Christensen, H., 1985. Arbovirus investigations in Argentina. II. Arthropod collections and virus isolations from mosquitoes, 1977–1980. Am. J. Trop. Med. Hyg. 34 (5), 945–955.

Mitchell, C.J., Forattini, O.P., Miller, B.R., 1986. Vector competence experiments with Rocio virus and three mosquito species from the epidemic zone in Brazil. Rev. Saúde Públ. 20, 171–177.

Moreno, C.E., 2001. Métodos Para Medir la Biodiversidad. M & T Manuales & Tesis SEA, Zaragoza.

Moreno, C.E., Barragán, F., Pineda, E., Pavón, N.P., 2011. Reanalizando la diversidad alfa: alternativas para interpretar y comparar información sobre comunidades ecológicas. Rev. Mex. Biodivers. 82, 1249–1261.

Moreno, J.E., Rubio-Palis, Y., Páez, E., Pérez, E., Sánchez, V., Vaccari, E., 2005. Anopheles (Anopheles) neomaculipalpus: a new malaria vector in the Amazon basin? Med. Vet. Entomol. 19 (3), 329–332.

Motta, M.A., Lourenço-de-Oliveira, R., Mureb Sallum, M.A., 2007. Phylogeny of genus Wyeomyia (Diptera: Culicidae) inferred from morphological and allozyme data. Can. Entomol. 139, 591–627.

Natal, D., 1981. Importancia Epidemiológica de Culex Subgénero Melanoconion (Diptera: Culicidae). Dissertacção de Mestrado apresentada àfaculdade de Saúde Pública da Universidade de São Paulo, Departamento de epidemiología, pp. 89.

Natal, D., Soares Barata, J.M., Taipe Lagos, C.B., Moreira Rocha, R., 1992. Nota sobre culicídeos (Diptera: Culicidae) da bacia do rio Purus, Acre, Amazônia (Brasil). Rev. Saúde Públ. 26 (2), 129–131.

Oria, G., Oscherov, E., 2002. Ecología de larvas de Culícidos (Díptera) en desagües pluviales de la ciudad de Corrientes, Argentina. Commun. Cient Tecnol. UNNE http://www.unne.edu.ar/Web/cyt/cyt/2002/06-Biologicas-B-060.pdf.

Oria, G.I., Stein, M., Gorodner, J.O., 2002. Mosquitos, sus criaderos y factores socioculturales de la población en el Nordeste Argentino. Actualizaciones en artropodología sanitaria argentina. Serie Enfermedades transmisibles. Editor. Fundación Mundo Sano 2, 167–172.

Pires, D.A., Gleiser, R.M., 2010. Mosquito fauna inhabiting water bodies in the urban environment of Córdoba city, Argentina, following a St. Louis encephalitis outbreak. J. Vector Ecol. 35 (2), 401–409.

Pisano, M., Belén Oria, G.I., Beskow, G., Aguilar, J., Konigheim, B., Cacace, M.L., Aguirre, L., Stein, M., Contigiani, M.S., 2013. Venezuelan equine Encephalitis viruses (VEEV) in Argentina: serological evidence of human infection. PloS Negl. Trop. D 7 (12), 1–7.

Póvoa, M.M., de Souza, R.T.L., Lacerda, R.N.L., Rosa, E.S., Galiza, D., de Souza, J.R., Wirtz, R.A., Schlichting, C.D., Conn, J.E., 2006. The importance of Anopheles albitarsis E and An. darlingi in human malaria transmission in Boa Vista state of Roraima, Brazil. Mem. Inst. Oswaldo Cruz 101, 163–168.

Raudenbush, S.W., Bryk, A.S., 2002. Hierarchical Linear Models: Applications and Data Analysis Methods, Second Edition. Sage, Newbury Park, CA.

Ramirez, P.G., Almirón, W.R., Zalazar, L., Stein, M., 2014. Culicidae (Diptera) en un órea rural del Chaco Seco, Argentina. FACENA: Rev. Fac. Cienc. Exact. Nat. Agrimens. UNNE 29, 53–63.

Reinert, J.F., 2000. New classification for the composite genus Aedes (Diptera: Culicidae: Aedini), elevation of subgenus Ochlerotatus to generic rank, reclassification of the other subgenera and notes on certain subgenera and species. J. Am. Control Assoc. 16 (3), 175–188.

Ribeiro, A.F., Urbinatti, P.R., Ribeiro de Castro Duarte, A.M., Bicudo de Paula, M., Mendes Pereira, D., Mucci, L.F., Fernandes, A., Silva Homem de Mello, M.H., de Matos Junior, M.O., Correa de Oliveira, R., Natal, D., dos Santos Malafronte, R., 2012. Mosquitoes in degraded and preserved areas of the Atlantic Forest and potential for vector-borne disease risk in the municipality of São Paulo, Brazil. J. Vector Ecol. 37 (2), 316–324.

Ronderos, R.A., Schnack, J.A., Spinelli, G.R., 1991. Species composition and ecology of Culicidae from areas influenced by the Salto Grande Dam Lake. Rev. Bras. Entomol. 35 (1), 17–28.

Ronderos, R.A., Schnack, J.A., Maciá, A., 1992. Composición y variación estacional de una taxocenosis de Culicidae del ecotono subtropical pampásico (Insecta, Diptera). Graellsia 48, 3–8.

Rossi, G.C., 2015. Annotated checklist, distribution, and taxonomic bibliography of the mosquitoes (Insecta: Diptera: Culicidae) of Argentina. Check List. 11 (4), 1712, p.15.

Rossi, G.C., Lestani, E.A., D'Oria, J.M., 2006. Nuevos registros y distribución de mosquitos de la Argentina (Diptera: Culicidae). Rev. Soc. Entomol. Argent. 65 (3–4), 51–56.

Rossi, G.C., Lestani, E.A., 2014. Primeros registros de mosquitos (Diptera: Culicidae) de la provincia de Misiones, Argentina. Rev. Soc. Entomol. Argent. 73 (1–2), 49–53.

Sabattini, M.S., Avilés, G., Monath, T.P., 1998. Historical, epidemiological and ecological aspects of Arboviruses in Argentina: *Flaviviridae*, *Bunyaviridae* and *Rhabdoviridae*. In: Travassos da Rosa, A.P.A., Vasconcelos, P.F.C., Travassos da Rosa, J.F.S. (Eds.), An Overview of Arbovirology in Brazil and Neighbouring Countries. Belem, Para, Brazil, pp. 113–134.

Service, M.W., 1993. Mosquito Ecology: Field Sampling Methods, 2nd. Edition. Elsevier Science Publishers, Essex.

Spinsanti, L., Glatsein, N., Arselan, S., Díaz, L.A., Re, V., Aguilar, J., Farias, A., Morales, M., Almirón, W., Contigiani, M., 2005. Etiología del brote de encefalitis en humanos, detectado en Córdoba (Argentina) en el año 2005. In: Resúmenes de Presentaciones, Jornada de Actualización en Enfermedades de Transmisión vectorial. VIII Simposio Internacional sobre Control Epidemiológico de Vectores, p. 7.

Stein, M., Ludueña Almeida, F., Willener, J.A., Almirón, W.R., 2011. Classification of immature mosquito species according to characteristics of the larval habitat in the subtropical province of Chaco, Argentina. Mem. Inst. Oswaldo Cruz 106 (4), 400–407.

Stein, M., Hoyos, C.B., Oria, G.I., Bangher, D., Weinberg, D., Almirón, W.R., 2012. New records of mosquito species (Diptera: Culicidae) for the provinces of Chaco and Formosa, Argentina. J. Am. Control Assoc. 28 (4), 307–308.

Stein, M., Zalazar, L., Willener, J.A., Ludueña Almeida, F., Almirón, W.R., 2013. Host preference of Culicidae (Diptera) in three different environments in Chaco province, Argentina. Mem. Inst. Oswaldo Cruz 108 (5), 563–571.

Tuomisto, H., 2010a. A consistent terminology for quantifying species diversity? Yes, it does exist. Oecologia 164, 853–860.

Tuomisto, H., 2010b. A diversity of beta diversities: straightening up a concept gone awry. Part 1. Defining beta diversity as a function of alpha and gamma diversity. Ecography 33, 2–22.

Tubaki, R.M., de Menezes, R.M.T., Cardoso Junior, R.P., Bergo, E.S., 2004. Studies on entomological monitoring: mosquito species frecuency in riverine habitats of the Igarapava dam, southern region, Brasil. Rev. Inst. Med. Trop. 46 (4), 223–229.

Travassos da Rosa, J.F.S., Travassos da Rosa, A.P.A., Vasconcelos, P.F.C., Pinheiro, F.P., Rodrigues, S.G., Travasos da Rosa, E.S., Dias, L.B., Cruz, A.C.R., 1998. Arboviruses isolated in the Evandro Chagas Institute, including some described for the first time in the Brazilian Amazon Region, their know hosts and their pathology for man. In: Travassos da Rosa, A.P., Vasconcelos, P.F.C., Travassos da Rosa, J.F.S. (Eds.), An Overview of Arbovirology in Brazil and Neighbouring Countries. Instituto Evandro Chagas, Belém, pp. 19–31.

Urbina-Cardona, J.N., Londoño-Murcia, M.C., García-Ávila, D.G., 2008. Dinámica espacio-temporal en la diversidad de especies de serpientes en cuatro hábitats con diferente grado de alteración antropogénica en el Parque Nacional Natural Isla Gorgona, Pacífico Colombiano. Caldasia 30, 479–493.

van Buuren, S., Oudshoorn, C.G.M., 2000. Multivariate Imputation by Chained Equations: MICE V1.0 User's Manual. TNO Report PG/VGZ/00.038, TNO Preventie en Gezondheid, Leiden. Available from: http://www.stefvanbuuren.nl/publications/MICEV1.0ManualTNO000382000.pdf.

Vezzani, D., Carbajo, A.E., 2008. *Aedes aegypti*, *Aedes albopictus*, and dengue in Argentina: current knowledge and future directions. Mem. Inst. Oswaldo Cruz 103, 66–74.

Visintín, A., Laurito, M., Stein, M., Ramirez, P., Molina, G., Lorenzo, P.R., Almirón, W.R., 2010. Two new mosquito species and six new provincial records inArgentina. J. Am. Control Assoc. 26 (1), 91–94.

Su, Yu-Sung, Gelman, A., Yajima, M., 2011. Multiple imputation with diagnostics (mi) in R: opening windows into the black box. J. Stat. Softw. 45 (2), Available from: http://www.jstatsoft.org/published by the American Statistical Association http://www.amstat.org/.

Zimmerman, R.H., Galardo, A.K., Lounibos, L.P., Arruda, M., Wirtz, R., 2006. Blood meal hosts of *Anopheles* species (Diptera: Culicidae) in a malaria-endemic area of the Brazilian Amazon. J. Med. Entomol. 43, 947–956.