Journal of Medical Entomology, XX(X), 2017, 1–9
doi: 10.1093/jme/tjx215
Research Article

OXFORD

Population and Community Ecology

Composition of Anopheline (Diptera: Culicidae) Community and Its Seasonal Variation in Three Environments of the City of Puerto Iguazú, Misiones, Argentina

P. G. Ramirez,[1,5] M. Stein,[1,4] E. G. Etchepare,[3,4] and W. R. Almirón[2,4]

[1]Instituto de Medicina Regional, Universidad Nacional del Nordeste, Avenida Las Heras 727, Resistencia, Chaco, Argentina, [2]Centro de Investigaciones Entomológicas, Facultad de Ciencias Exactas Físicas y Naturales, Instituto de Investigaciones Biológicas y Tecnológicas (IIByT-CONICET), Universidad Nacional de Córdoba, Córdoba, Argentina, [3]Facultad de Ciencias Exactas Naturales y Agrimensura, Universidad Nacional del Nordeste, Corrientes, Argentina, [4]CONICET – CCT, Nordeste, Corrientes, Argentina, and [5]Corresponding author, e-mail: patricia.ramirez@conicet.gov.ar

Subject Editor: Douglas Norris

Received 17 May 2017; Editorial decision 3 October 2017

Abstract

In order to extend the knowledge of the composition of the anopheline community and the seasonal variation related to anthropogenic modifications in the city of Puerto Iguazú, adult females were captured between 2009 and 2012. Samples were collected in three environments with different degrees of anthropogenic modification: urban, periurban, and wild. Alpha diversity was evaluated as the 'true' diversity of the species in each environment. Among environments, range-abundance curves were used to compare the composition, abundance, and uniformity of species and cluster analysis was used to analyze the similarities and differences. The temporal distribution was analyzed and the relative abundance of the species captured was correlated with meteorological variables. A total of 4,565 females, belonging to seven species: *Anopheles albitarsis* s.s. (Lynch-Arribálzaga), *Anopheles argyritarsis* (Robineau-Desvoidy), *Anopheles deaneorum* (Rosa-Freitas), *Anopheles fluminensis* (Root), *Anopheles mediopunctatus* (Theobald), *Anopheles strodei* s.l. (Root), and *Anopheles triannulatus* s.l. (Neiva and Pinto) were captured. The wild environment showed higher abundance, diversity, and greater uniformity reflected on the less sharp area of the range–abundance curve. Species richness was the same in the wild and periurban environments. Higher abundances were observed in summer during the months with higher temperatures. Although the wild environment showed greater *Anopheles* abundances and diversity, specific richness and species complementarity were similar among the three environments studied. Thus, the periurban environment would turn into a transition zone of great epidemiological importance due to the introduction of people in this environment, which represents a potential risk of malaria transmission in the area.

Key words: *Anopheles*, community, diversity, ecology, Argentina

The latest cases of malaria in the north-eastern region of Argentina occurred in the city of Puerto Iguazú and date from the year 2008 (WHO 2016). This city constitutes an area of great epidemiological importance. On one hand, it is adjacent to one of the most important tourist areas in the country, Iguazú National Park, which receives thousands of tourists a year from all around the world. On the other hand, it forms the tri-border area called 'Triple Frontera' with the cities of Foz do Iguaçu (Brazil) and Ciudad del Este (Paraguay). In 2014, there were 389.390 cases and 87 deaths reported due to malaria in the Americas, 41% of the deaths occurring in Brazil. As for Paraguay, there have been no autochthonous cases reported since 2012. Of the total cases for the Americas region in 2014, 69% were *Plasmodium vivax* (Grassi and Feletti) infections and 24% were *Plasmodium falciparum* (Welch) infections (PAHO 2017).

There are previous studies on *Anopheles* diversity and seasonal variation in Argentina, including contributions by Hack et al. (1978) and Oscherov et al. (2007) in the province of Corrientes, Campos (1997) in the province of Buenos Aires, Dantur Juri et al. (2003, 2005, 2014) in the north-western region of Argentina, Ramirez et al. (2013) and Stein et al. (2011, 2013, 2016) in the province of Chaco, and Duret (1950, 1951), Bejarano (1959), Rossi (2015), and Ramirez et al. (2016) in the province of Misiones, including the city of Puerto Iguazú. However, it is necessary to deepen the knowledge of the ecological aspects of *Anopheles* present in the region, especially those concerning the diversity changes related to anthropogenic modifications and temporal variation of their populations, which would allow us to identify the months with greater abundance and the fluctuation of populations in relation to climatic variables. It is well known that urban ecosystems can affect mosquito populations by providing breeding sites for immature stages, shelters, and microclimates suitable for surviving winter periods, favoring or limiting host availability. Deforestation and new developments facilitate the proliferation of habitats in artificial containers (Leisnham et al. 2004) that affect immature mosquito populations (Jacob et al. 2003). It has even been concluded that for each 1% increase in forest clearing, there is an 8% increase in the amount of *Anopheles darlingi* (Root), which would be possible since this mosquito may predominate over the others species when they thrive in ponds and wells located in open places and in the sun (Vittor et al. 2006).

Given the importance of this taxonomic group for human health and the need for further ecological studies on this group in the region, the aim of this work is to study the seasonal variation of *Anopheles* female adults in the city of Puerto Iguazú, and to learn about their diversity in three environments with different degrees of anthropogenic disturbance, in order to generate knowledge that contributes to interventions for these mosquito species.

Materials and Methods

Description of Study Area

This work took place in the city of Puerto Iguazú (25°35′50″S; 54°34′43.51″W), in Misiones Province, Argentina. The study area is the Paranaense biogeographic province of the Neotropical region (Morrone 2014). The climate is subtropical with an average temperature of 22ºC, and extreme temperatures of −4.9ºC and 40ºC. Rainfall varies between 1,500 and 2,000 mm annually, with a dry season during June through August and rain during September through May. The dominant vegetation is characterized by a 30 m high tree layer and an understory layer of bamboo, tree ferns, grass, vines, and epiphytes (Cabrera and Willink 1980). The Paranaense rainforest, which used to cover the whole area, was reduced, allowing the insertion of exotic tree species, agriculture, and stockbreeding, as well as urbanization. Currently ~1,490,000 hectares are preserved, representing 58% of the original surface, in various states of degradation and with an average annual deforestation during the period 2004–2010 of 6,700 hectares per year (Milkovic 2012).

Sampling Sites

Samples were collected in three environments with different degrees of anthropogenic modification: a wild environment (nonanthropogenic, with ~90% of forest cover), an environment modified by deforestation (referred to as 'periurban,' ~40% of forest cover), and an environment subject to urbanization (referred to as 'urban,' approximately <10% of forest cover), separated by a distance of 1.7 and 5.7 km, respectively (Fig. 1). In the wild environment (25° 40' 14.03" S 54° 33' 14.70" W), the vegetation is closed. The tree layer is formed by large species, in some cases over 40 m high. The tree species include *Aspidosperma polyneuron* (Müll. Arg.) 'palo rosa,' *Peltophorum dubium* (Spreng.) 'Ivirá-pitá,' *Tabebuia heptaphylla* (Vell.) 'Lapacho negro,' *Patagonula americana* (Linneo) 'Guayaibi,' *Erythrina falcata* (Benth) 'Ceibo,' among others. There are also two palm tree species: *Euterpe edulis* (Mart) 'Palmito' and *Arecastrum romanzoffianum* (Becc) 'Pindó.' Climbing plants are an important element, many of which are woody with thick vines. The ground is covered by a layer of leaves, stems, and branches of different species (Cabrera 1971). The periurban environment was a rural zone of Puerto Iguazú (25°39′57.26″S 54°34′13.89″ W), in an area known as 'Dos mil Hectáreas,' a region that was originally a native forest, but after its partial deforestation many farms (~1,000 families without drinking water or electricity) have been established since 2003. It is an area composed by primary and secondary forest, pigsties, chicken, horses, crops, and houses (farms) and surrounded by natural protected forest reservations and the Parana River (Mastrangelo and Salomon 2010, Fernandez et al. 2015). The urban environment corresponds to family houses in a highly modified area located in the urban area of Puerto Iguazú (25°37′8.76″S 54°35′37.71″W), at a distance of 296 km from the city of Posadas, capital of Misiones province. Puerto Iguazú has 42,849 inhabitants (44.1 inhabitants per hectare). Traps are located in a neighborhood with public transport services, shops, schools, and health centers near and where houses have few small fruit trees and geese and chicken coops.

Capture of Adults

Samples were collected biweekly in the rainy season (September through May) and monthly in the dry season (June through August), between March 2009 and April 2012. This was done using CDC (Center for Disease Control and Prevention) light traps supplemented with 1 kg dry ice blocks. In each of the three environments, two sites separated by a distance of more than 50 m were selected (Service 1993). In each of them, a light trap was placed 1.5 m above ground level remaining active between 6 p.m. and 8 a.m. the next day. Sites where many inmature specimens had been collected in previous samplings were selected, which were located near different types of larval habitats (lagoons, pools, wells, ponds) (Ramirez et al. 2016). The adults collected were identified based on keys by Gorham (1973), Consoli and de Oliveira (1994), and Forattini (2002). The specimens are deposited in the Instituto de Medicina Regional, Universidad Nacional del Nordeste, in the province of Chaco.

Data Analysis

Based on the nonparametric richness estimator ACE (Abundance-based Coverage Estimator) (Chao and Lee 1992), an approximate number of species found in each environment was obtained (Colwell and Coddington 1994), and with the estimated richness value, the percentage of representativeness of the study was determined (Soberón and Llorente 1993). These tests were made using the EstimateS 9.1.0 software (Colwell 2013).The alpha diversity was evaluated as the 'true' diversity of the species (Jost 2006). True diversity was considered based on diversity of order zero (^0D), since its value is completely insensitive to species abundance; therefore, the value obtained is simply equivalent to species richness ($^0D = S$). The second measure is true diversity of order 1 (^1D), where all species are considered in the diversity value, with a weight proportional to their abundance in the community (Hill 1973; Jost 2006, 2007; Tuomisto 2010a,b; Moreno et al. 2011). The true diversity formula with a value of $q = 1$ was estimated using the exponential of the Shannon entropy index ($^1D = \exp(H')$), and the result is shown as the 'number of effective species' (Jost 2006). In order to compare the composition, abundance, and uniformity of species among environments with different anthropogenic interventions, range–abundance curves were used (Feinsinger 2001). For this purpose, the relative abundance of species against their range was illustrated, from the most to the least abundant species. In order to analyze the similarities and differences among the environments regarding the composition

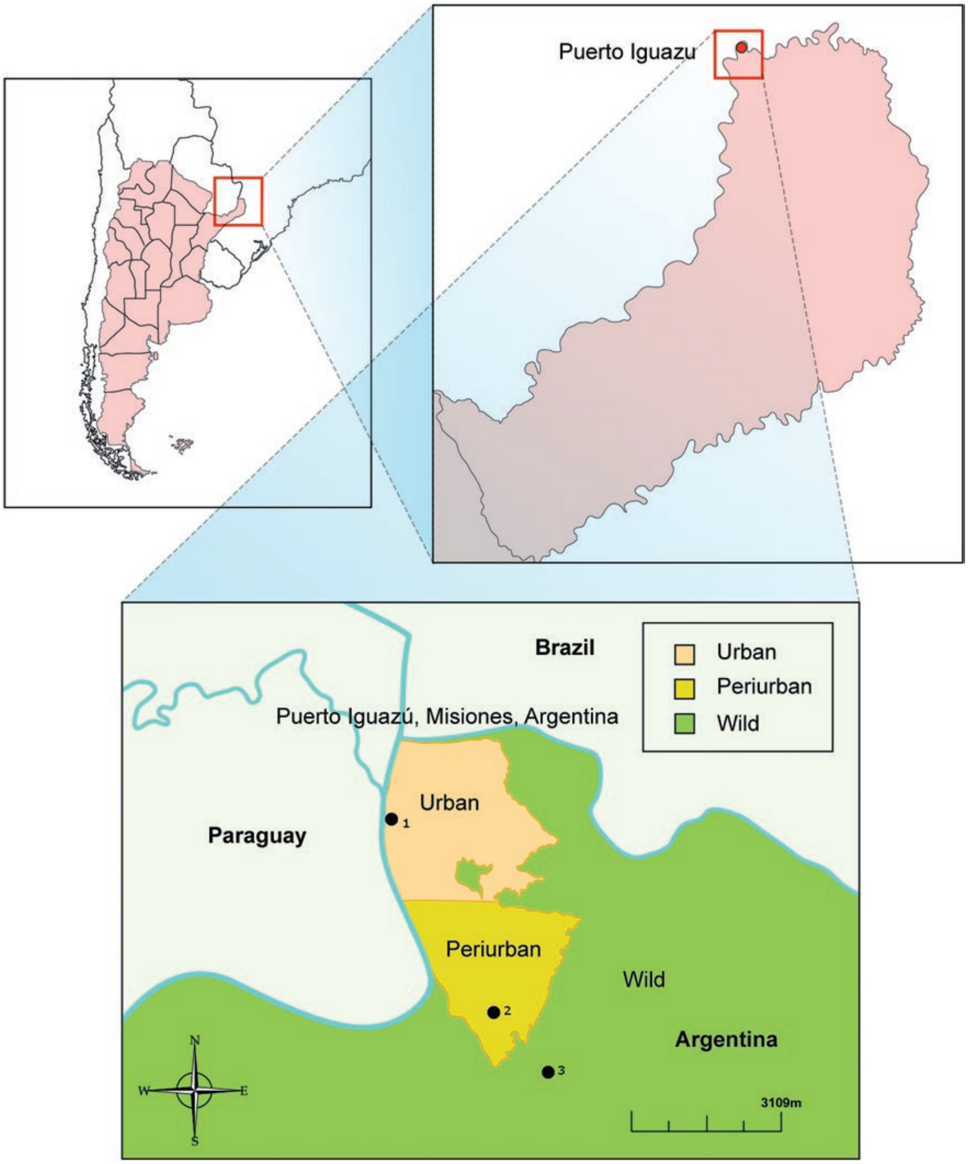

Fig. 1. Location in Argentina. Province of Misiones. Sampling sites: 1. Urban, 2. Periurban, and 3. Wild.

of the existing anopheline fauna, a cluster analysis was made to determine the clustering form in the three environments studied, considering the frequency of all species monthly recorded as variables. The mean Euclidean distance and mean chaining were used (Johnson and Winchern 1998). The temporal distribution of female adult *Anopheles* throughout the study was analyzed. The relative abundance of the species captured in the three environments was correlated with meteorological variables (mean monthly temperature, mean monthly rainfall, and mean monthly relative humidity). Spearman's correlation coefficient was used to analyze the abundance of different *Anopheles* species, with the above-mentioned meteorological variables, using Infostat software version 2012 (Di Rienzo et al. 2012). The meteorological data were obtained from the National Meteorological Service.

Results

A total of 4,565 females of the *Anopheles* genus were captured, of which 1,106 (24%) could only be determined at generic level. The remaining 76% (3,459 specimens) corresponds to seven species; five of them are of the *Nyssorhynchus* subgenus (*An. albitarsis* s.s., *An. argyritarsis, An. deaneorum, An. strodei* s.l., and *An. triannulatus* s.l.) and two are of the *Anopheles* subgenus (*An. fluminensis* and *An. mediopunctatus*). Within the three environments studied, the four most abundant species of the total captured were *An. triannulatus* s.l. (31.71%), *An. strodei* s.l. (29.2%), *An. albitarsis* s.s. (19.31%), and *An. deaneorum* (13.7%). These four species represent 93.92% of the total identified (Table 1).

The richness estimator ACE for the whole study showed a number of species equal to the number found in the field, representing 100% of the expected richness.

Alpha Diversity

The *Anopheles* richness was the same in the wild and periurban environments (seven species), while the lowest number of species was registered in the urban environment (six species). Abundance showed a marked difference, being higher in the wild environment (2,451), followed by the periurban environment (970) and the urban environment (38). True diversity follows a similar trend, with the wild environment showing the greatest diversity (4.3 effective species), followed by the periurban environment (3.2 effective species) and the urban environment (2.7 effective species). This can also be viewed as: the wild environment is 1.34 and 1.6 times more diverse than the periurban and urban environments, respectively. There is 25.60% less diversity in the periurban environment, and 37% in the urban environment, in comparison with the wild environment.

The species estimated for each environment showed that inventories were complete, reporting the same number as the one found in each environment, representing 100% of richness.

The wild environment showed greater uniformity reflected on the less sharp area of the range–abundance curve, in comparison with the urban and periurban environments where only one species prevailed, reflected on the sharper curve (Fig. 2). The more predominant species in the wild environment were *An. triannulatus* s.l. and *An. albitarsis* s.s., with the highest hierarchical positions. However, this position was replaced by *An. strodei* s.l., in the urban and periurban environments, where *An. triannulatus* s.l. and *An. albitarsis* held medium positions. Common and rare species showed changes in the hierarchical order based on the environments; thus, *An. argyritarsis* was among the main common species in the wild and periurban environments, while it was one of the rare species in the urban environment (Fig. 2). Therefore, changes in the structure of the communities were observed.

Beta Diversity

Cluster analysis showed greater similarity between the wild and the periurban environments (Fig. 3). All the *Anopheles* species mentioned earlier were found in both these environments. *Anopheles mediopunctatus* was not found in the urban environment.

Seasonality

Over the 36 mo of study, the greatest abundance was observed in summer, during the months with higher temperatures (Fig. 4). Seasonal patterns were not different between environments so they were analyzed as a whole. Within the three environments studied, in the first warm season *An. albitarsis* s.s., *An. argyritarsis*, and *An. deaneorum* were caught mainly in summer until early autumn, and in the last warm season in summer. *An. fluminensis*, *An. strodei* s.l., and *An. triannulatus* s.l. were captured in all seasons, but the highest abundances correspond to the summer. *An. mediopunctatus* was only caught in summer (Fig. 5).

Table 1. Adult *Anopheles* mosquitoes captured in three different environments using CDC light traps between March 2009 and April 2012 in the city of Puerto Iguazú, Misiones, Argentina

Years		*Anopheles albitarsis*	*Anopheles argyritarsis*	*Anopheles deaneorum*	*Anopheles fluminensis*	*Anopheles mediopunctatus*	*Anopheles strodei*	*Anopheles triannulatus*
	W	0	7	1	5	1	48	6
2009	P	0	1	2	1	1	87	1
	U	0	0	1	1	0	15	0
	W	315	74	118	0	3	76	870
2010	P	44	46	38	0	1	152	100
	U	1	1	1	0	0	7	1
	W	307	27	275	5	0	224	88
2011	P	0	17	37	17	1	382	30
	U	1	0	1	1	0	6	1
	W	0	0	0	0	0	1	0
2012	P	0	0	0	0	0	12	0
	U	0	0	0	0	0	0	0
Total		668	173	474	30	7	1,010	1,097
%		19.31194	5.0014455	13.703382	0.8673027	0.202370627	29.19919	31.714368

W, wild environment; P, periurban environment; U, urban environment.

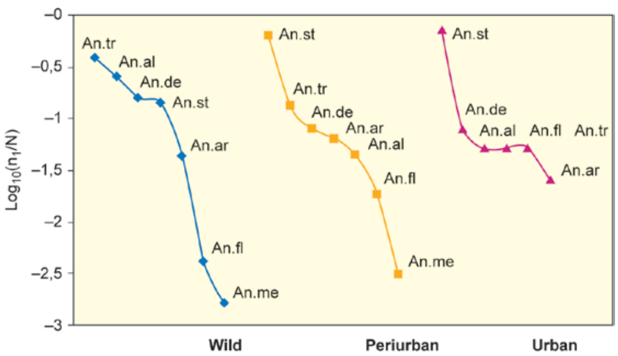

Fig. 2. Range–abundance curves for the *Anopheles* species captured in three environments with different degrees of anthropogenic disturbance in the city of Puerto Iguazú, Misiones, Argentina. Codes for species: *Anopheles albitarsis*: An.al; *An. argyritarsis*: An.ar; *An. deaneorum*: An.de; *An. fluminensis*: An.fl; *An. mediopunctatus*: An.me; *An.stroidei*: An.st; *An. triannulatus*; An.tr.

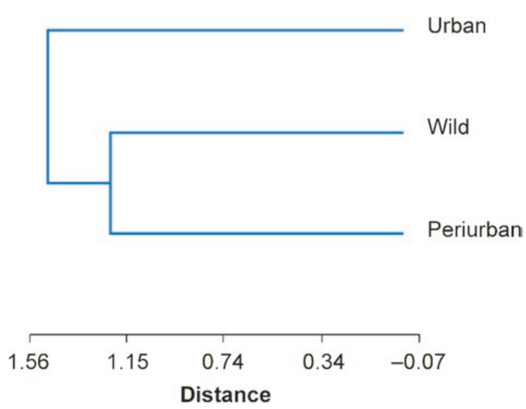

Fig. 3. Phenogram of three operational taxonomic units resulting from cluster analysis. Taxonomic units: wild, periurban, and urban environments of Puerto Iguazú, Misiones, Argentina.

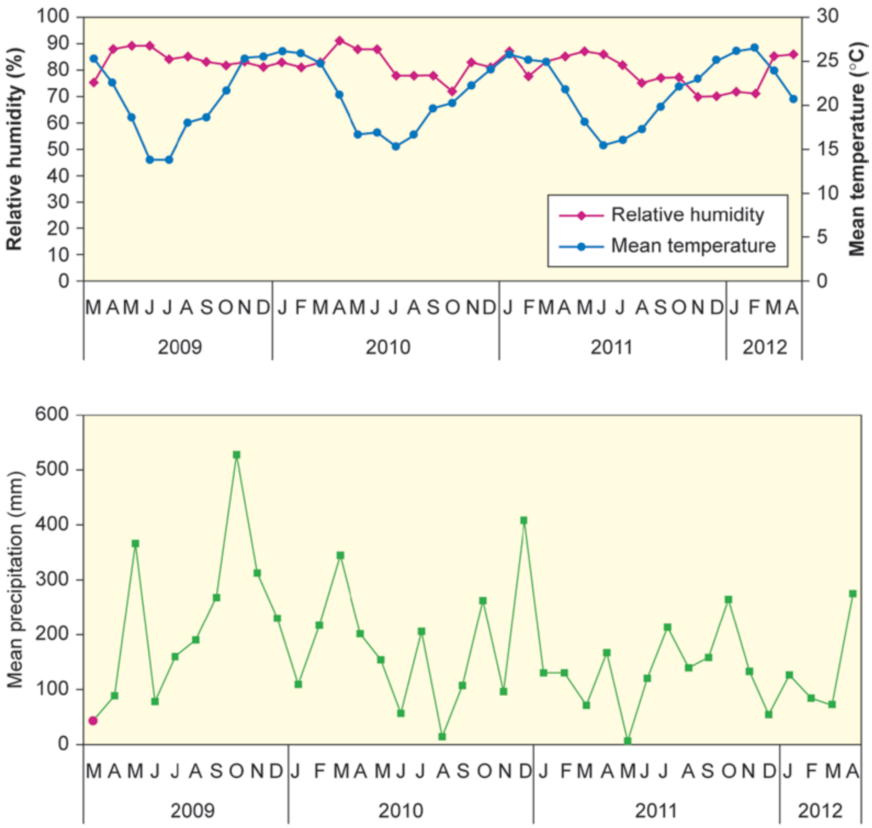

Fig. 4. Monthly variation of relative humidity, mean temperature, and mean precipitation from March 2009 to April 2012 in the city of Puerto Iguazú, Misiones, Argentina.

As for the relationship with meteorological variables, using Spearman's index, *An. albitarsis* s.s., *An. deaneorum*, *An. strodei* s.l., and *An. triannulatus* s.l. showed a positive and significant correlation ($P < 0.05$) for temperature, while *An. mediopunctatus* showed a positive and significant correlation for rainfall (Table 2).

Discussion

In this study, as well as during malaria outbreaks in the province of Misiones from 2006 to 2008, the recognized vector for the northeast region of Argentina, *An. darlingi*, was not found. On the other hand, two of the species found in this study, *An. albitarsis* s.s. and *An. triannulatus* s.l. are recognized malaria vectors in other South American countries (Rubio-Palis and Zimmerman 1997, Olano et al. 2001, Manguin et al. 2008). These species, although in different relative abundances, were found in the three environments studied, two of which—urban and periurban—are frequently visited by man for his activities. During this work, seven of the 23 *Anopheles* species mentioned for the province of Misiones (Rossi 2015) were found. In some cases, such as *Anopheles darlingi*, *An. lutzii*, *An. maculipes*, and *An. parvus*, the records for the city of Puerto Iguazú date back to 1950 and 1960 (Duret 1950, 1951; Bejarano 1959). Some of these species, as well as others cited for the city of Puerto Iguazú, were found mainly in wild areas, some of them near the Iguazú Falls, located at a distance of 20 km of the Puerto Iguazú city.

Malaria transmission anywhere depends on a complex interaction of factors related to the human host, the parasite, the vector, and other environmental, socioeconomic factors and control measures (Marinho e Silva 2012). As for the vector, the main factors determining the transmission of malaria are longevity, population density, susceptibility to plasmodium infection, and blood-feeding (Gillies 1988, Charlwood 1996). Environmental factors such as temperature, rainfall, and air relative humidity also have a significant influence on malaria transmission since they affect the biology of parasites and their vectors, both directly and indirectly.

In general, *Anopheles* abundance increases during the rainy season, which may be related to a greater availability of larval habitats, and decreases during the dry season, when water bodies are reduced in volume and sometimes disappear completely (Berti et al. 1993). In fact, some authors point to precipitation as the main factor controlling the abundance of *Anopheles* because it guarantees the formation or water supply of larval habitats (Forattini 2002, Barbosa and Souto 2011). In this study, the highest abundances were observed in the months where the highest temperatures were recorded, and where the highest rainfall was recorded in the previous months.

Contrary to the observations by Hack et al. (1978) in the province of Corrientes, and by Stein et al. (2016) in the province of Chaco (both provinces belonging to the northeastern region of Argentina, with subtropical climate), who captured *An. albitarsis* s.l. in all four seasons, a limited presence of this species was observed in some months in this study. However, this species was not found in a simultaneous study on immature stages in the same environments (Ramirez et al. 2016). This could be due to the typical larval habitats of this species not being thoroughly sampled in the different collection sites, since *An. albitarsis* is pointed out as a species that can be mainly found in pools, grassland, or artificial lagoons (Lopes and Lozovei 1995,

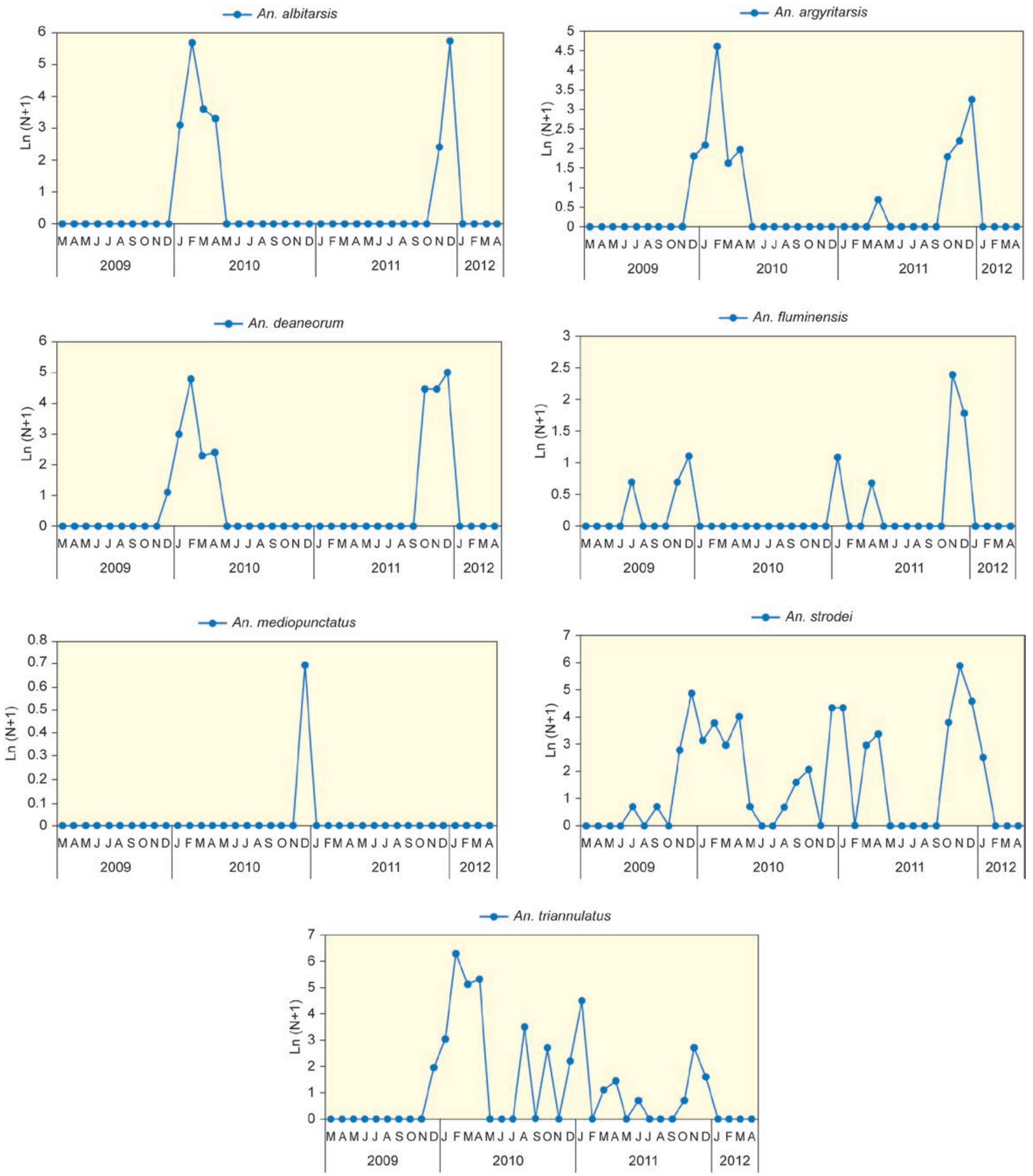

Fig. 5. Monthly *Anopheles* abundance in the wild, periurban, and urban environments from March 2009 to April 2012 in Puerto Iguazú, Misiones, Argentina.

Stein et al. 2011). Moreno et al. (2015) also found a lack of consistency between abundance of adults and larvae of this species in Venezuela, noting that this inconsistency between both populations can be due to different mortality factors.

In this study, *An. triannulatus* s.l. was found in all four seasons, as was observed for the provinces of Corrientes and Chaco (Hack et al. 1978, Stein et al. 2016). Considering all the mosquitoes caught in the three environments, *An. triannulatus* was the most abundant species and is pointed out by several authors for being a ubiquitous species, found in a great variety of environments (Ramirez et al. 2013, Moreno et al. 2015, Stein et al. 2016). Also in this work, *An. argyritarsis* prevailed in summer and fall, unlike what Dantur Juri et al. (2010)

Table 2. Spearman correlation coefficient for anopheline female abundance by species in wild, periurban, and urban environments and temperature, rainfall, and relative humidity recorded by the National Weather Service for the city of Puerto Iguazú, Misiones, Argentina, between March 2009 and April 2012

	Temperature		Rainfall		Relative humidity	
	rs	*P*	rs	*P*	rs	*P*
Anopheles albitarsis	0.32	0.0518*	0.14	0.39	-0.25	0.13
Anopheles argyritarsis	0.24	0.14	0.13	0.43	-0.11	0.49
Anopheles deaneorum	0.48	0.0024*	0.21	0.20	0.23	0.16
Anopheles fluminensis	0.25	0.13	0.09	0.58	-0.17	0.29
Anopheles mediopunctatus	0.23	0.17	0.33	0.0463*	0.10	0.55
Anopheles strodei	0.52	0.0008*	0.15	0.38	-0.22	0.18
Anopheles triannulatus	0.31	0.0541*	0.12	0.47	-0.06	0.71

*Significant ($P < 0.05$).

observed for the province of Tucumán (Argentina), who captured this species mainly in spring and fall. These authors also captured *An. strodei* throughout the year, as was observed in this work.

The socioeconomic factors related to transmission of malaria involve the different forms of space occupation, such as agriculture, road construction, migration, temporal settlements, etc. (Marinho e Silva 2012). The city of Puerto Iguazú is a region that receives thousands of tourists a year. On the other hand, a movement of local human population toward deforestation edge areas has been observed in the last years (Salomón et al. 2016), where there is a part of the secondary Paranaense rainforest that remains, with recent inhabitants in lands used for subsistence agriculture or family production. These inhabitants produce a pattern of deforestation that is irregular in space and dynamic in time, where proximity to the deforestation edge and houses create an area of permanent risk of exposure to vectors (Quintana et al. 2010). Da Silva et al. (2013), who captured *Anopheles* in a preserved area and a modified area in the south-east of Brazil, note that species such as *An. triannulatus* were only captured in the preserved area, while this species was found in environments with and without anthropogenic intervention in this work. However, the abundances found in the wild environment are greater when compared with the other two environments. Stein et al. (2016) also observed this species both in wild and modified environments; and they capture the greatest abundances in an environment with little anthropogenic intervention. In the case of *An. albitarsis* s.s., the results were similar to the ones obtained for the province of Chaco, where the greatest abundances were found in wild environments (Stein et al. 2016).

Although the rainforest (wild environment) showed greater *Anopheles* abundances in populations with a relatively uniform structure, specific richness and species complementarity were similar among the three environments studied, and the periurban environment was in the second place as regards the number of individuals captured. There is growing evidence showing that deforestation creates the ideal conditions for the transmission of pathogens causing diseases transmitted by mosquitoes and other insects, such as malaria, dengue fever, leishmaniasis, and Chagas disease, and it also favors an increased contact between vectors and humans (Bordehore 2001, Guzmán et al. 2001, Riverón Corteguera 2002, Marin-Neto and Rassi 2009, Soler-Tovar et al. 2013). In this study, although the presence of potential malaria vectors was recorded in all three environments, the periurban environment is of great epidemiological importance, since it is a transition zone between the wild and urban environments where the human population is introduced for the realization of diverse activities, thus becoming a potential risk for the transmission of malaria in the area.

Acknowledgments

We are grateful to Fundación Mundo Sano, Secretaría General de Ciencia y Técnica/UNNE, and Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET) for their financial support. M.S., E.G.E., and W.R.A. are members of the Investigator Career of CONICET.

References Cited

Barbosa, L. M. C., and R. N. P. Souto. 2011. Aspectos ecológicos de *Anopheles* (Nyssorhyncus) *darlingi* Root 1926 e *Anopheles* (Nyssorhyncus) *marajoara* Galvão e Damasceno 1942 (Diptera: Culicidae) nos bairros Marabaixo I e Zerão, Macapá, Amapá, Brasil. Biota Amazônia 1: 19–25

Bejarano, J. F. R. 1959. *Anopheles* de la República Argentina y sus relaciones con el Paludismo. Primeras Jorn. Entomoepidemiol. Argent. Primera Parte 2: 305–329.

Berti, J., R. Zimmerman, and J. Amarista. 1993. Spatial and temporal of anopheline larvae in two malarious areas in Sucre State, Venezuela. Mem. Inst. Oswaldo Cruz. 88: 353–362.

Bordehore, C. 2001. Sociología Ambiental. Problemas Ambientales, Problemas Humanos. Grupo Editorial Universitario, España.

Cabrera, A. L. 1971. Fitogeografía de la República Argentina. Bol. Soc. Argent. Bot. 14: 1–42

Cabrera, A. L., and A. Willink.1980. Biogeografía de América Latina: serie de Biología. Secretaría General de la Organización de los Estados Americanos, Washington DC.

Campos, R. E. 1997. Comportamiento estacional de dos especies de *Anopheles* (Diptera: Culicidae) en la provincia de Buenos Aires. Rev. Soc. Entomol. Argent. 56: 31–32.

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

Charlwood, J. D. 1996. Biological variation in *Anopheles darlingi* Root. Mem. Inst. Oswaldo Cruz. 91: 391–398

Colwell, R. K. 2013. Estimates: statistical estimation of species richness and shared species from samples. Version 9. User's Guide and application. http://purl.oclc.org/estimates Accessed 2 May 2017.

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

Consoli, R. A. G. B., and R. L. de Oliveira. 1994. Principais mosquitos de importância sanitaria no Brasil. Fiocruz, Río de Janeiro, Brasil.

Dantur Juri, M. J., M. Zaidenberg, and W. Almirón. 2003. Fluctuación estacional de *Anopheles (*Anopheles*) pseudopunctipennis* (Diptera: Culicidae) en un área palúdica de Salta, Argentina. Entomol. Vect. 10: 457–468.

Dantur Juri, M. J., M. Zaidenberg, and W. Almirón. 2005. Distribución espacial de *Anopheles pseudopunctipennis* en las Yungas de Salta, Argentina. Rev. Saúde Pública. 39: 565–570.

Dantur Juri, M. J., W. R. Almirón, and G. L. Claps. 2010. Population fluctuation of *Anopheles* (Diptera: Culicidae) in forest and forest edge habitats in Tucumán province, Argentina. J. Vector Ecol. 35: 28–34.

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

Da Silva, K. S., I. S. Pinto, G. R. Leite, T. M. das Virgens, C. B. dos Santos, and A. Falqueto. 2013. Ecology of anopheline mosquitoes (Diptera: Culicidae) in the Central Atlantic Forest Biodiversity Corridor Southeastern Brazil. J. Med. Entomol. 50: 24–30.

Di Rienzo, J. A., F. Casanoves, M. G. Balzarini, L. Gonzalez, M. Tablada, and C. W. Robledo. 2012. InfoStat versión 2012. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. http://www.infostat.com.ar Accessed 17 May 2017.

Duret, J. P. 1950. Contribución al conocimiento de la distribución geográfica de los culícidos argentinos (Diptera: Culicidae). Rev. San. Mil. Arg. 49: 363–380.

Duret, J. P. 1951. Contribución al conocimiento de la distribución geográfica de los culícidos argentinos (Diptera: Culicidae). Parte III. Rev. San. Mil. Arg. 50: 211–227.

Feinsinger, P. 2001. Designing field studies for diversity conservation. Island, Washington D.C.

Fernandez, M. S., M. S. Santini, J. I. Diaz, L. Villarquide, E. Lestani, and M. Achinelly. 2015. Parasitism by Tylenchid Nematodes in natural populations of *Pintomyia fischeri* (Diptera: Psychodidae: Phlebotominae) in Argentina. S.M. Trop. Med. J. 1: 1001

Forattini, O. P. 2002. Culicidologia Médica. 2o Volume: Identificação, Biologia, Epidemiologia. Editora da Universidade de São Paulo, São Paulo, Brazil.

Gillies, M. T. 1988. Anopheline mosquitos: vector behaviour and bionomics, pp. 453–485. *In* W. H. Wernsdorfer and I. McGregor (eds.), Malaria. Principles and practice of malariology, vol. 1. Churchill Livingstone, Edinburgh, United Kingdom.

Gorham, J. R., C. J. Stojanovich, and H. G. Scott. 1973. Illustrated key to the anopheline mosquitoes of western South America. Department of Health, Education, and Welfare, Public Health Service, Communicable Disease Center, Washington DC.

Guzmán, M. G., G. Kourí, and J. L. Pelegrino. 2001. Enfermedades virales emergentes. Rev. Cubana Med. Trop. 53: 5–15.

Hack, W. H., G. Torales, M. E. Bar, and E. B. Oscherov. 1978. Observaciones etológicas sobre Culícidos de Corrientes. Rev. Soc. Entomol. Argent. 37: 137–151.

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

Jacob, B. G., J. L. Regens, C. M. Mbogo, A. K. Githeko, J. Keating, C. M. Swalm, J. T. Gunter, J. I. Githure, and J. C. Beier. 2003. Occurrence and distribution of Anopheles (Diptera: Culicidae) larval hábitats on land cover change sites in urban Kisumu and urban Malindi, Kenya. J. Med. Entomol. 40: 777–784.

Johnson, R. A., and D. W. Winchern. 1998. Applied multivariate statistical analysis. 6th ed. Pearson Prentice Hall, NJ.

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.

Leisnham, P. T., P. J. Lester, D. P. Slaney, and P. Weinstein. 2004. Anthropogenic landscape change and vectors in New Zealand. Effects of shade and nutrient levels on mosquito productivity. EcoHealth 1: 306–316.

Lopes, J., and A. L. Lozovei. 1995. Ecologia de mosquitos (Diptera: Culicidae) em criadouros naturais e artificiais de área rural do norte do Estado do Paraná, Brasil. Coletas ao longo do leito de ribeirão. Rev. Saude Publica 29: 183–191.

Manguin, S., P. Carnevale, and J. Mouchet. 2008. Biodiversity of malaria in the world. John Libbey Eurotext Ltd, Montrouge, France.

Marin-Neto, J. A., and A. Rassi Jr. 2009. Actualización sobre la cardiopatía de la enfermedad de Chagas en el primer centenario de su descubrimiento. Rev. Esp. Cardiol. 62: 1211–1216.

Marinho e Silva, M. 2012. Diversidade e ecologia de mosquitos do gênero *Anopheles* (Diptera: Culicidae: Anophelinae) e avaliação do risco de reintrodução de malária no Pantanal de Miranda, Mato Grosso do Sul, Brasil. M.S. dissertation, Oswaldo Cruz Institute, Rio de Janeiro, Brazil.

Mastrangelo, A. and D. Salomon. 2010. Contribution of social anthropology to ecoepidemiological comprehension of an American Tegumentary Leishmaniosis outbreak at '2.000 ha', Iguazú, Argentina. Rev. Argent. Salud Pública 1: 6–13.

Milkovic, M. 2012. Mapa de cobertura forestal de la Provincia de Misiones 2010 mediante el análisis y procesamiento de imágenes satelitales. Informe de consultoría a Fundación Vida Silvestre Argentina, Buenos Aires, Argentina.

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

Moreno, J. E., Y. Rubio-Palis, V. Sánchez, and A. Martínez. 2015. Caracterización de hábitats larvales de anofelinos en el municipio Sifontes del estado Bolívar, Venezuela. Bol. Mal. Salud Amb. 55: 117–131

Morrone, J. J. 2014. Biogeographical regionalisation of the neotropical region. Zootaxa 3782: 1–110.

Olano, V. A., H. L. Brochero, R. Saenz, M. L. Quiñones, and J. Molina. 2001. Mapas preliminares de la distribución de especies de *Anopheles* vectores de malaria en Colombia. Biomedica 21: 402–408.

Oscherov, E. B., M. E. Bar, M. P. Damborsky, and G. Avalos. 2007. Culicidae (Diptera) de la Reserva Provincial Iberá, Corrientes, Argentina. Bol. Mal. Salud Amb. 47: 221–229.

Pan American Health Organization. 2017. Report on the situation of malaria in the Americas, 2014, p. 43. Washington, D.C. http://www.paho.org/hq/index.php?option=com_content&view=article&id=12851&Itemid=42230&lang=en Accessed 9 July 2017.

Quintana, M. G., O. D. Salomón, and M. S. Lizarralde de Grosso. 2010. Distribution of Phlebotominae in primary forest-crop interface, Salta, Argentina. J. Med. Entomol. 47: 1003–10.

Ramirez, P. G., W. R. Almirón, L. Zalazar, and M. Stein.2013. Culicidae (Diptera) en un área rural del Chaco Seco, Argentina. FACENA 29: 53–63.

Ramirez, P. G., M. Stein, E. G. Etchepare, and W. R. Almiron. 2016. Diversity of anopheline mosquitoes (Diptera: Culicidae) and classification based on the characteristics of the habitats where they were collected in Puerto Iguazú, Misiones, Argentina. J. Vector Ecol. 41: 215–223.

Riverón Corteguera, R. L. 2002. Enfermedades emergentes y reemergentes: un reto al siglo XXI. Rev. Cubana Pediatr. 74: 7–22.

Rossi, G. C. 2015. Annotated checklist, distribution, and taxonomic bibliography of the mosquitoes (Insecta: Diptera: Culicidae) of Argentina. Checklist 11: 1–15.

Rubio-Palis, Y., and R. H. Zimmerman. 1997. Ecoregional classification of malaria vectors in the neotropics. J. Med. Entomol. 34: 499–510.

Salomón, O. D., A. V. Mastrángelo, M. S. Santini, D. J. Liotta, and Z. E. Yadón. 2016. La eco-epidemiología retrospectiva como herramienta aplicada a la vigilancia de la leishmaniasis en Misiones, Argentina, 1920–2014. Rev. Panam. Salud Publica. 40: 29–39.

Service, M. W. 1993. Mosquito ecology: field sampling methods. 2nd. Ed. Elsevier Science Publishers. Essex, England.

Soberón, J., and J. Llorente.1993. The use of species accumulation functions for the prediction of species richness. Conserv. Biol. 7: 480–488.

Soler-Tovar, D., P. Hernández-Rodríguez, L. C. Pabón, and A. I. T. Morales. 2013. Pérdida de biodiversidad: un factor determinante en el aumento de enfermedades infecciosas compartidas entre humanos y animales. Biodiversidad Colombia 2: 53–62.

Stein, M., F. Ludueña-Almeida, J. A. Willener, and W. R. Almirón. 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: 400–407.

Stein, M., L. Zalazar, J. A. Willener, F. Ludueña Almeida, and W. R. Almirón. 2013. Culicidae (Diptera) selection of humans, chickens and rabbits in three different environments in the province of Chaco, Argentina. Mem. Inst. Oswaldo Cruz. 108: 563–71

Stein, M., M. Santana, L. M. Galindo, E. Etchepare, J. A. Williner, and W. R. Almirón. 2016. Culicidae (Diptera) community structure, spatial and temporal distribution in three environments of the province of Chaco, Argentina. Acta Tropica 156: 57–67.

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.

Vittor, A. Y., R. H. Gilman, J. Tielsch, G. Glass, T. Shields, W. S. Lozano, V. Pinedo-Cancino, and J. A. Patz. 2006. The effect of deforestation on the human-biting rate of *Anopheles darlingi*, the primary vector of Falciparum malaria in the Peruvian Amazon. Am. J. Trop. Med. Hyg. 74: 3–11.

World Health Organization. 2016. World Malaria Report 2016. WHO Library Cataloguing-in-Publication Data, Ginebra, Suiza. http://www.who.int/malaria/publications/world-malaria-report-2016/report/en/. Accessed 10 May 2017.