



## ECOSYSTEMS

# Updated *Anopheles* mosquitos abundance and distribution in north-eastern malaria-free area of Argentina

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**Abstract:** Malaria is the most important parasitic disease worldwide. In 2019, more than 679,441 cases of malaria were reported in the American region. During this study, Argentina was in malaria pre-elimination autochthonous transmission phase with the aim of being declared as malaria-free country. The aim of this work was to assess the influence of remote sensing spectral indices (NDVI, NDWI) and climatic variables (temperature, relative humidity and precipitation) on the distribution and abundance of *Anopheles* mosquitoes, in four localities with different degrees of anthropogenic disturbance and with previous malaria cases records located in a historical malarious area in northeastern of Argentina. Between June 2012 and July 2014, mosquitoes were collected. We collected 535 *Anopheles* adult mosquitoes. *Anopheles strodei* s.l. was the most abundant species. The greatest richness, diversity and abundance of species were registered in wild and semi-urban environments. The abundance of *Anopheles* presented a negative association with relative humidity and mean temperature, but positive with mean maximum temperature. The most important variables determining *Anopheles* total abundance and distribution were NDWI Index and distance to vegetation. The abundance of *An. strodei* s.l., was positive associated with water areas whereas the NDVI Index was negatively associated.

**Key words:** *Anopheles*, malarious risk area, subtropical region, landcover.

## INTRODUCTION

According to the World Health Organization (WHO), malaria is the most important parasitic disease worldwide, causing 409,000 deaths in 2019 (WHO 2020). The Pan American Health Organization (PAHO) has included malaria in the list of neglected diseases, a group of infectious diseases that mainly affects the poorest populations and with limited access to health services; especially those who live in remote rural areas and slums (PAHO 2020).

This disease caused by protozoa of the *Plasmodium* genus is transmitted to humans through the bite of infected female mosquitoes

belonging to the *Anopheles* genus (WHO 2020). *Plasmodium falciparum* (Welch) and *P. vivax* (Grassi & Feletti) are the most frequent species that parasitize human, the latter being responsible for 74.1% of malaria cases in 2017 in the Region of the Americas (PAHO 2020), although infections acquired by *P. falciparum* can progress to severe illness, and lead to death if no treated (Idro et al. 2010, CDC 2019).

In 2019, more than 679,441 cases of malaria were reported in the American region (PAHO 2020). Although it continues to be a serious public health problem in endemic areas, information on incidence, morbidity, mortality, distribution of parasite species and fatal cases is still

scarce. In Argentina the malaria area comprised 349,051 km<sup>2</sup> in the 1950' decade, encompassing mainly the north-western geographical region (with marked endemicity) and north-eastern geographical region (characterized by outbreaks) of the country. These geographical regions of Argentina are territorial divisions, defined by geographic and historical-social characteristics (INDEC 2023). These ones corresponding to the provinces of Salta, Jujuy, Tucumán, Santiago del Estero, Catamarca, La Rioja (north-western), Formosa, Chaco, Misiones, Corrientes (north-eastern) and small areas in San Juan, San Luis and Córdoba provinces (Curto et al. 2003). By the end of the 1980s, there were outbreaks in the provinces of Salta (11,725 km<sup>2</sup>) and Jujuy (3,249 km<sup>2</sup>), that is, that only 4% of the traditional malaria area registered cases. This situation changed, with several outbreaks in the northwestern Argentina (NWA) with the worst during 1996 with more than 2,000 cases as well as some sporadic cases in the northeastern Argentina (NEA) (González Cappa 1991, Cuba Cuba et al. 2012). In 2006 and 2007 significant outbreaks occurred in Misiones province (NEA), the last one in 2008, with only 19 cases, in Puerto Iguazú city, located on the triple border Brazil/Paraguay/Argentina (WHO 2016). Between 2015 and 2016, only nine imported malaria cases were reported corresponding to travellers from endemic malaria countries (Ministerio de Salud de la Nación 2017). Years later, in May 2019, Argentina received the certification as a malaria-free country (WHO 2019).

Regarding the *Anopheles* species involved in malaria transmission in Argentina, *An. pseudopunctipennis* (Theobald) is the vector in the geographical region of NWA and *An. darlingi* (Root) in the geographical region of NEA, out of a total of 31 species cited for the country (Lifshitz et al. 1946, Rossi 2015). From the rest of the *Anopheles* species recorded in the NEA, it is

known that some of them, such as *An. albitarsis* s.l. (Lynch Arribáizaga), *An. punctimacula* (Dyar and Knab) and *An. triannulatus* s.l. (Neiva and Pinto) are secondary vectors of malaria in other South America countries (Rubio-Palis & Zimmerman 1997, Olano et al. 2001, Manguin et al. 2008). Although, in Argentina is not known if they have a role in transmission of malaria. *Anopheles albitarsis* s.l. has also been involved in malaria transmission in the NEA in the 1940s (Lifshitz et al. 1946).

Temperature, precipitation and relative humidity have a significant role affecting vector abundance species, their survival, geographic spread, as well as transmission dynamics (Gage et al. 2008, Sáez Sáez et al. 2007, Rocklöv & Dubrow 2020).

Although climate affects vector dynamics, land cover such as distribution of larval habitats, and the type of vegetation cover, also determines available vector habitats, and therefore influences on the abundance and distribution of the species (Linthicum et al. 1987, Patz et al. 2000, López Vélez & Molina Moreno 2005). In addition, human impacts on the environment with clear-cut forestry, dam construction, urbanization, with habitat loss, as well as fragmentation, can also affect the diversity, spatial and temporal patterns of vector populations, favoring the creation of new and more artificial larval habitats and allowing survival of winter periods (Jacob et al. 2003, Leishnam et al. 2004). In fact, entire ecosystem habitats could be modified, wild and urban, in which vectors may thrive or fail (Rocklöv & Dubrow 2020).

Remote sensing data have allowed characterizing key environmental variables to understand their influence on the spatial and temporal patterns of disease transmission risk (Lourenço et al. 2011). Ceccato et al. (2005) in their study of remote sensing applications for

malaria highlights that temperature, humidity, surface water, climate seasonality as well as type of vegetation influence the abundance of vectors. Vegetation type or land use may influence mosquito abundance by affecting the presence of animal or human hosts and thus the availability of blood meals (Ceccato et al. 2005). In addition, vegetation around larval habitats may also determine the abundance associated with these sites by providing resting sites, supplies for adult mosquito feeding, and protection from climatic conditions. Several studies have investigated how environmental changes affect the abundance of vector mosquitoes and the occurrence of malaria, analyzing the landscape coverage and spectral indices such as the Normalized Difference Vegetation Index (NDVI) and the Normalized Difference Water Index (NDWI) (Lourenço et al. 2011, Obsomer et al. 2013, Djamouko-Djonkam et al. 2019). Spatiotemporal changes of the NDVI has shown positive correlation with incidence rates of mosquito vectors and epidemic outbreaks (Tourré et al. 2008). Studies of malaria incidence in Africa and Asia have shown their association with NDVI (Liu & Chen 2006, Gaudart et al. 2009). In the malarious area of northwestern Argentina, Dantur Juri et al. (2015) found that NDVI and Land Surface Temperature were directly related to the increases in abundances of *Anopheles* species.

Due to the human health impact of *Anopheles* mosquito vectors and the historical malarious transmission scenarios in Argentina, our aim was to assess the influence of NDVI, NDWI, and meteorological variables on the distribution and abundance of *Anopheles* mosquitoes, in localities with different degrees of anthropogenic disturbance with recorded malaria cases, in northeastern Argentina.

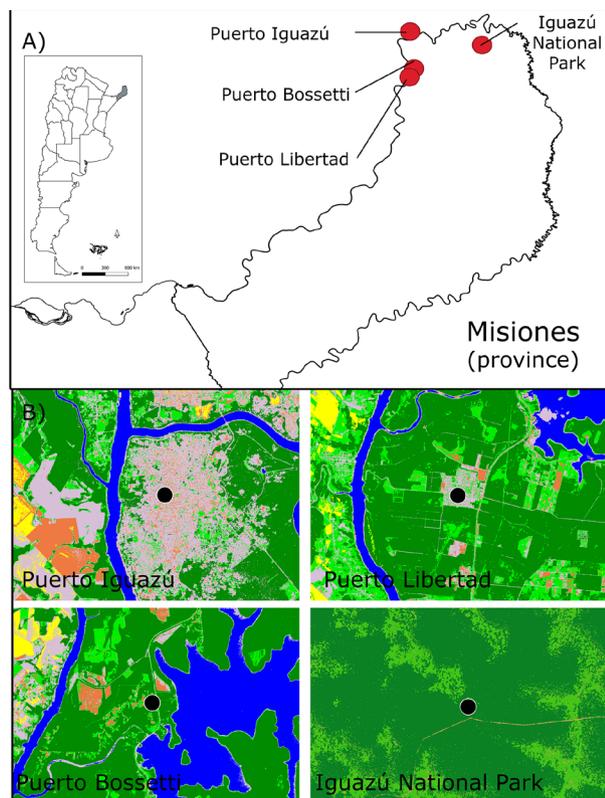
## MATERIALS AND METHODS

### Study area

Misiones province is in the northeast of Argentina and belongs to the Paranaense biogeographic province of the Neotropical region (Morrone 2014). The climate is subtropical with an average annual temperature of 22 °C, and extreme values of -4.9 °C and 40 °C. The rains vary between 1500 and 2000 mm annually, with a no marked dry season during winter (June to August) and rainfall in summer (September to May). The dominant vegetation is characterized by having a tree layer that reaches 30 meters in height and an undergrowth made up of bamboos, tree ferns, herbs, lianas, and epiphytic plants (Cabrera & Willink 1980). The Paraná jungle, which originally covered the entire study area was considerably reduced, for the implantation of exotic tree species, agriculture, and livestock, as well as urbanization (Bertolini & Gil 1999). Currently, approximately 1,490,000 hectares are conserved, which represents 58% of the original surface, in different states of degradation and with an average annual deforestation during the 2004-2010 period of 6,700 hectares per year (Milkovic 2012).

### Adult mosquito capture sites

For the capture of mosquitoes, four localities with previous malaria case records were selected (Fig. 1): Puerto Iguazú (25°36'39" S; 54°34'49" W), Puerto Libertad (25°55'17" S; 54°35'04" W), Puerto Bossetti (25°51'47.23" S; 54°33'22.86" W) and Iguazú National Park (25°41'09" S; 54°18'43" W). Puerto Libertad city represents urban environments and shows 6,694 inhabitants with a density of 9.1. This city has 83.4% of homes with a water supply connection and 1.72% sewer. The 21.6% of homes have unmet basic needs (INDEC, 2010). Puerto Iguazú city has 42,849 inhabitants, and a population density of 44.1. Water supply is available by pipe for 70% of the population



**Figure 1. a) Location in Argentina of Misiones province and sampling sites. b) Satellite images of sampling sites.**

and 22.16% have sewers. The 21.08% of the population has unmet basic needs. For locating the CDC light traps, we select a peri-urban environment within the city. This site is partially deforested area, with the presence of patches of primary and secondary forest, with the activity of raising domestic animals (pigs, goats, horses, chickens), surrounded by a protected nature reserve. Five km from Puerto Libertad is Puerto Bossetti composed by a few homesteads, which represents a semi-urban environment located next to the Urugua-í dam, where the regional flora was subjected to forest extractions. The wild environment was represented by a patch of original jungle belonging to the Iguazú National Park, located on the side of provincial route 101, 17 km approximately from the intersection

of national route 12 and provincial route 101, next to the Ibicuy stream, on the way to the Cabure-i city, in Misiones province. In it, the type of jungle that develops in the area has the same characteristics of the Neotropical jungle described above, (Bertolini & Gil 1999). Between June 2012 and July 2014, the mosquitoes capture was carried out monthly, using CDC light traps supplemented with dry ice. Due to logistical problems, sampling was not performed in several months (July and October 2012; February, October, and December 2013; January, February, and June 2014), 17 months were effectively sampled. Two light traps were placed at each selected site, one night per month, 1.5 meters above ground level and separated one from each other for a distance not less than 50m. All traps remained active between 6 PM and 8 AM of the next day. The collected adult mosquitoes were cold euthanized and determined using the keys of Gorham et al. (1973), Faran & Linthicum (1981), Linthicum (1988), Consoli & 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.

Monthly measurements of meteorological variables were obtained from the Iguazú AERO Weather Station (25.73° S; 54.47° W): mean temperature, minimum temperature, minimum mean temperature, maximum temperature, maximum mean temperature (°C), relative humidity (%) and accumulated precipitation (mm).

### Satellite environmental characterization

#### **Normalized Difference Vegetation Index and the Normalized Difference Water Index**

To analyze the relationship between the abundance of *Anopheles* mosquitoes and each site (Puerto Iguazú, Puerto Libertad,

Puerto Bossetti and Iguazú National Park), a multispectral (green, red, near infrared and middle infrared) Satellite Pour l'Observation de la Terre-5 (SPOT-5) image with high spatial resolution (10 m pixel) were used (ESA Copernicus Services Coordinated Interface 2000).

On each satellite image vegetation indices were calculated, Normalized Difference Vegetation Index (NDVI) and Normalized Difference Water Index (NDWI). Normalized Difference Vegetation Index reflects the contrast of vegetation reflectivity between the spectral regions of Red (R) and Near Infrared (NIR) reflectance (Eq.1). This is commonly used to measure vegetation cover, and as a proxy for suitable conditions of mosquito development, since values close to +1 are associated to areas with vigorous vegetation, and values close to zero are related to bare soil (Chuvienco Salinero 2002, Pettorelli et al. 2005, Amri et al. 2011). On the other hand, NDWI computed using the near infrared (NIR) and the short-wave infrared (SWIR) reflectance (Eq.2), which makes it sensitive to changes in liquid water content and in spongy mesophyll of vegetation canopies taking values between -1 and +1 (Gao 1996, McFeeters 1996, Ceccato et al. 2001). According to Gao (1996), NDWI is a good indicator for vegetation liquid water content therefore it has been used in several mosquitoes' studies known as the water index because relating NDWI as an indirect measure for precipitation and soil humidity (Estallo et al. 2012).

$$NDVI = (NIR-R) / (NIR+R) \quad (Eq.1)$$

$$NDWI = (NIR-SWIR) / (NIR + SWIR) \quad (Eq.2)$$

### Land cover classification

Supervised classification (maximum likelihood) was performed using the ENVI 5.3 Software (2013) to determine landscape coverage (Supplementary Material - Table SI). Six land

cover classes were selected: water (rivers, streams, lakes, and artificial containers), bare soil (soil without any vegetation cover), farmland (agricultural crop), urban areas (buildings, streets and roads), low vegetation (herbs and grasses) and high vegetation (trees and shrubs usually with a closed canopy). To be able to discriminate more efficiently the landscape coverage during the classification process, the NDVI and the NDWI were added as two more bands with the satellite spectral bands (Amri et al. 2011).

Accuracy of the classification was measured by selecting an equivalent number of pixels in each land cover class, using historical images of Google Earth (<https://earth.google.com/web>) to know their "true" type of coverage (Qian et al. 2015). It was estimated the Kappa's coefficient and the confusion matrix which shows the accuracy of the classification (Chuvienco Salinero 2002).

Around each sampling site, circular buffer areas of 3km were generated, considering the greater *Anopheles* flight range (Verdonschot & Besse-Lototskaya 2014). Therefore, from each buffer area average values of NDVI and NDWI were obtained as well as percentages of each landscape coverage.

In addition, the distance from each sampling site to the closest point of water, vegetation (both low vegetation and high vegetation) and urban areas was calculated using Google Earth.

### Data analysis

#### *Anopheles mosquito community*

To assess the completeness of the data (Moreno et al. 2011), the non-parametric richness estimator ACE was calculated (Abundance-based Coverage Estimator) (Chao & Lee 1992). This analysis was performed with Estimate S open access software version v8.2.0 (Colwell 2009).

To measure alpha diversity, were quantified species (specific richness-S) and individuals (abundance-Ab), by locality and by type of environment. The true diversity measure was used (Jost 2006, Moreno et al. 2011). For this study the exponent used was  $q=1$ , where all species are included with a weight exactly proportional to their abundance in the community. This measurement is expressed in units called “effective number of species” (Hill 1973, Jost 2006). Range-abundance curves were used to compare the composition, abundance, and uniformity of species between environments (Feinsinger 2001). The curve was plotted according to the logarithm ( $\text{Log}_{10}$ ) of the proportion ( $n_i / N$ ) of each species, ordering the results in decreasing order.

To analyzed beta diversity, that is, the degree of replacement and the joint occurrence of species between the localities, the number of shared species was compared, and multivariate clustering techniques (cluster analysis) were applied with the data transformed to the fourth root, prior to the application of the Bray-Curtis similarity index “B” (Bray & Curtis 1957, Somerfield 2008).

### **Environmental variables and abundance of *Anopheles* mosquitoes**

Generalized Linear Mixed Models (GLMM) were developed for two response variables, total abundance of *Anopheles* mosquitoes and *An. strodei* s.l. Root (most abundant mosquito species in our captures) to identify the association with the satellite environmental characterization and meteorological variables.

In the analysis of satellite environmental characterization and abundance of *Anopheles* mosquitoes, we evaluated the association between the mentioned response variables and the satellite data: NDVI, NDWI and land cover classes. “Sites” were incorporated as a random

effect to include the spatial dependency. While in the temporal analysis, the response variables used were the monthly abundance of total *Anopheles* mosquitoes and the monthly abundance of *An. strodei* s.l. These were obtained from the sum of the data collected at all the sites. To incorporate temporal dependence, “Years” were included as a random effect.

In first place, data exploration was implemented following the protocol described in Zuur et al. (2010). Therefore, we decided to use GLMM with a negative binomial distribution and logarithmic link function, since the data presented overdispersion. Correlation analysis among explanatory variables were performed to avoid multicollinearity in the models, not incorporating explanatory variables in the same models with Spearman’s correlation coefficients ( $r$ ) greater than 0.7 (Table SII). In addition, correlations among meteorological variables and the response variables were investigated at different time lags. Time lags between one and two months were used considering the mosquito biology (Walker et al. 2013). To include in the models, Spearman’s correlation coefficient was used to determine which time lags of each meteorological variable were best correlated with the abundance of *Anopheles* (Table I).

The explanatory variables were standardized, and univariate models were developed to choose the most important variables and developed multivariable models with “glmmTMB” package (Brooks et al. 2017). Variables whose p-value were less than 0.05 were considered significant and added at the model. Due to high correlation between some explanatory variables, we decided to model each one of the high correlated explained variables separately, and finally compare the selected models.

Initially, univariate models were performed to choose the most important explanatory

variables to start building the models. Then, a manual forward stepwise procedure was used by adding the other explanatory variables. Starting with the univariate GLMMs and following the Akaike's Information Criteria (AICc) for low sample sizes and Akaike weights (Zuur et al. 2009) explanatory variables were added by the "model.sel" function of the "MuMin" package (Barton 2009). The variables that were significant were in turn used as starting points in the different branches of the modeling. The multicollinearity between variables of the final models was evaluated using the variance inflation factor (or VIF) and in addition, overdispersion and the normality of the residual distribution were checked (Zuur et al. 2009). The "qqnorm", "shapiro.test" functions and the "DHARMA" package (Florian 2021) was used to validate the final models selected by plotting residuals versus fitted values. All the statistical analyzes were developed in the free software R, version 3.5.3 (R Core Team 2018).

## RESULTS

### Mosquitoes collected

Between June 2012 and July 2014, a total of 535 *Anopheles* mosquitoes corresponding to 11 species were collected (Table II). Numerous female specimens could not be clearly determined due to the lack of diagnostic characters for which they were named as spp. for the calculation of the total abundances. *Anopheles strodei* s.l. was the most abundant species with 59.60% of the identified specimens followed in order of abundance by *An. triannulatus* s.l. (11.17%), *An. fluminnensis* Root (10.64%), *An. argyritarsis* Robineau-Desvoidy (8.51%), *An. albitarsis* s.l. (3.46%) (excluding *An. deaneorum*), *An. benarrochi* s.l. Gabaldón, Cova García y López, *An. deaneorum* Rosa-Freitas and *An. punctimacula* Dyar y Knab with 1.60% each. The rest of the species were collected in percentages less than 1%. *Anopheles* (*Nys.*) spp. and *An.* (*Ano.*) spp. corresponds to species not identifiable due to lack of morphologic

**Table I. GLMM parameter estimates for the selected explanatory variables in the total *Anopheles* mosquito abundance model and the *Anopheles strodei* s.l. abundance model.**

Response variable	Model	Parameter	Estimate	Std. Error	z-value	p-value
Total <i>Anopheles</i> abundance	GLMM8	Intercept	1.8984	0.2179	8.711	< 2e-16
		NDWI	-0.5432	0.1794	-3.028	0.0025 *
		Dist_veg	-0.3365	0.1453	-2.316	0.0206 *
	GLMM9	Intercept	1.9057	0.2166	8.799	< 2e-16
		NDWI	-0.7613	0.2166	-3.515	0.0004 *
		Urban	-0.4002	0.1758	-2.276	0.0228 *
<i>Anopheles strodei</i> s.l. abundance	GLMM10	Intercept	0.4336	0.3097	1.400	0.1615
		Water	0.0367	0.0111	3.311	0.001 *
	GLMM13	Intercept	0.8352	0.2484	3.362	0.0008
		NDVI	-0.5522	0.1843	-2.996	0.0027 *

An asterisk indicates significant p-value (<0.05).

characters. *Anopheles argyritarsis* (Robineau - Desvoidy), *An. strodei* s.l. Root and *An. triannulatus* s.l. were captured in the four study locations, *An. albitarsis* s.l., *An. deaneorum* and *An. fluminensis* Root in three, *An. benarrochi* s.l. and *An. mediopunctatus* s.l. Lutz in two and the rest in only one locality (Table II).

### Anopheles mosquito community

The richness estimator reflected a number of species slightly higher to that found in the field, representing 97% of the expected richness (ACE = 11.34).

Regarding localities, the greatest number of species and their abundance was registered in

Puerto Bossetti (S = 11; Ab = 163). While Iguazú National Park followed in a species number (S = 9; Ab = 78), Puerto Iguazú ranks second in terms of their abundances (S = 6; Ab = 96). Lastly, in the town of Puerto Libertad the lowest abundance and specific richness were found (S = 5; Ab = 21) (Table II).

Regarding true diversity, Iguazú National Park is the one that presented the highest number of effective species ( ${}^1D = 5.4$ ), being 1.5 times more diverse in *Anopheles* species than Puerto Iguazú ( ${}^1D = 3.6$ ) and 1.6 times more than Puerto Bossetti ( ${}^1D = 3.4$ ). At last, doubling in

**Table II. Abundance, richness and diversity of *Anopheles* mosquitoes collected in four sites in Misiones province between June 2012 and July 2014.**

Species	Sites collection				Total
	Puerto Bossetti	Puerto Iguazú	Iguazú National Park	Puerto Libertad	
<i>An. albitarsis</i> s.l.*	4	6	3	0	13
<i>An. argyritarsis</i>	8	13	10	1	32
<i>An. benarrochi</i> s.l.	4	2	0	0	6
<i>An. deaneorum</i>	1	4	1	0	6
<i>An. evansae</i>	11	5	8	1	25
<i>An. fluminensis</i>	12	0	24	3	39
<i>An. lutzii</i>	1	0	1	0	2
<i>An. malefactor</i>	1	0	0	0	1
<i>An. mediopunctatus</i> s.l.	2	0	2	0	4
<i>An. evandroi</i>	1	0	0	0	1
<i>An. punctimacula</i>	2	0	3	1	6
<i>An. strodei</i> s.l.	100	48	18	15	181
<i>An. triannulatus</i> s.l.	16	17	8	1	42
An. ( <i>Anopheles</i> ) spp**.	9	2	11	0	22
An. ( <i>Nyssorynchus</i> ) spp**.	41	85	22	7	155
TOTAL	213	182	111	29	535
S	11	6	9	5	
${}^1D$		3.6	5.4	2.6	

S: richness;  ${}^1D$ : true diversity;\*\*not identifiable at specific level, \*excluding *An. deaneorum*.

diversity (2.1) to Puerto Libertad ( ${}^1D = 2.6$ ), which lost 51% of diversity (Table II).

Regarding the environments, the least modified environments ( $S = 11$ ;  $Ab = 241$ ;  ${}^1D = 4.4$ ) exceeded in all values (richness, abundance, and diversity) to the anthropogenic environment ( $S = 8$ ;  $Ab = 117$ ;  ${}^1D = 3.9$ ), in the first environment the diversity of *Anopheles* is 1.1 times greater than in the second. In other words, the least modified environment represents 11% more diversity.

Analyzing the structure of the community in the two localities with the highest number and abundance of species, we find that the Iguazú National Park presents the greater equitable distribution of its taxa, expressing itself in a curve without a steep slope. There are no marked dominances, being *An. strodei s.l.* the one that occupied the highest hierarchical position, forming a group in this sector of the curve with *An. fluminensis* as subdominant species. While in Puerto Bossetti, this range change and there is less equality, in this case, the dominance of *An. strodei s.l.* is notably higher compared to the previous locality, and *An. fluminensis* occupy a position that corresponds to the common species within the slope being displaced by *An. triannulatus s.l.* (Fig. 2).

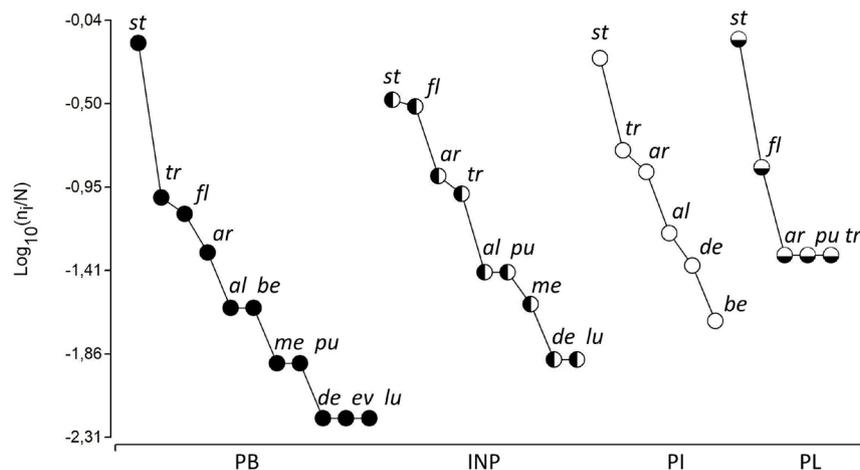
Regarding the localities with greater anthropogenic modifications, communities with

a single species prevalence can be observed (*An. strodei s.l.*) with steep slope and less equitativity. Although both cases exhibit curves with steep slopes, the number of rare species in Puerto Libertad stands out (*An. argyritarsis*, *An. punctimacula* and *An. triannulatus s.l.*) that occupy intermediate ranges in the rest of the environments.

### Beta diversity

Puerto Bossetti recorded the only exclusive species (*An. evandroi*) while in the rest of the localities no such finds were found. The highest degree of similarity by locality was found between Puerto Bossetti and the Iguazú National Park ( $B = 0.86$ ), sharing 82% of the *Anopheles* species found in both sites. While Puerto Iguazú and Puerto Bossetti ( $B = 0.72$ ) shared 55% of the species; Puerto Iguazú and Iguazú National Park ( $B = 0.65$ ) shared 56% of the species recorded in both locations. The lowest similarity was recorded in the localities of Puerto Iguazú and Puerto Libertad ( $B = 0.46$ ), reflecting a higher value of complementary, differing in 63% of the species.

With the grouping analysis, a single dendrogram was observed, with Puerto Bossetti and the Iguazú National Park forming a consolidated group. In more separated



**Figure 2.** Range-abundance curves for the species captured in four sites in the province of Misiones, Argentina. Puerto Bossetti (PB); Iguazú National Park (INP); Puerto Iguazú (PI); Puerto Libertad (PL). Codes for the species. *An. albitarsis s.l.*: al; *An. argyritarsis*: ar; *An. benarrochi s.l.*: be; *An. evandroi*: ev; *An. deaneorum*: de; *An. fluminensis*: fl; *An. lutzii*: lu; *An. mediopunctatus s.l.*: me; *An. punctimacula*: pu; *An. strodei s.l.*: st; *An. triannulatus s.l.*: tr.

nodes are the localities that present greater anthropogenic modifications, Puerto Iguazú and Puerto Libertad (Fig. 3).

Regarding the environments, 27% of the species (*An. lutzii*, *An. mediopunctatus s.l.*, and *An. evandroi*), appeared exclusively in the least modified environments, however, they presented a relatively high similarity ( $B = 0.8$ ) sharing eight species (73%) of the total recorded in this work.

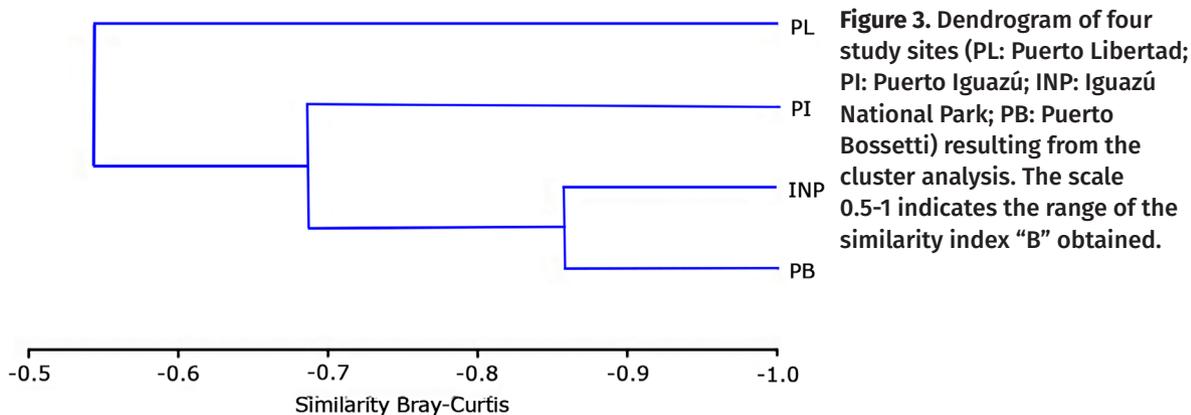
The abundance distribution patterns, and the hierarchical order of the species compared between the slightly modified environments (INP and PB) with those more altered (PI and PL) showed differences, observing a less steep and more equitable slope in the first case (Fig. 4).

While in both environments *An. strodei s.l.* represented the dominant, *An. fluminensis* followed in the least modified. However, in environment with greater alterations is replaced by *An. triannulatus s.l.* in the hierarchical order. Regarding the range that the common species occupy in the curve, the slightly modified environments presented a smoother slope in this sector. In the case of *An. punctimacula*, presents an abrupt change, going from being a species that occupies the rank within the common ones in little modified environments, being found at the end of the slope, which is considered rare in those more altered.

### Environmental characterization

According to SPOT-5 image's classification (Fig. 1), six land classes were identified and classified (water, urban, high vegetation represented by trees, low vegetation represented by herbaceous plants, farmland and bare soil). Puerto Iguazú, Puerto Libertad and Puerto Bossetti presented all six land cover classes, while in the image corresponding to Iguazú National Park no water nor urban areas were identified. The measurement of accuracy in the land cover maps obtained showed excellent agreement between the classification results and the groups of verification areas (Kappa index  $>0.84$ , total accuracy  $>87.5\%$ ).

The environmental classification for the mosquito sampling sites (urban, semi-urban and wild environments) agree with the results obtained in the land cover maps of the supervised classifications. Puerto Libertad presented 7.79% of urban areas and 74.83% of high vegetation (corresponding to commercial plantations), Puerto Iguazú presented 46.71% of urban area and 25.48% of high vegetation, Puerto Bossetti 4.96% of urban area and 46.81% of high vegetation, and 33.55% of water areas, while Iguazú National Park presented 63.59% of high vegetation and 35.59% of low vegetation.



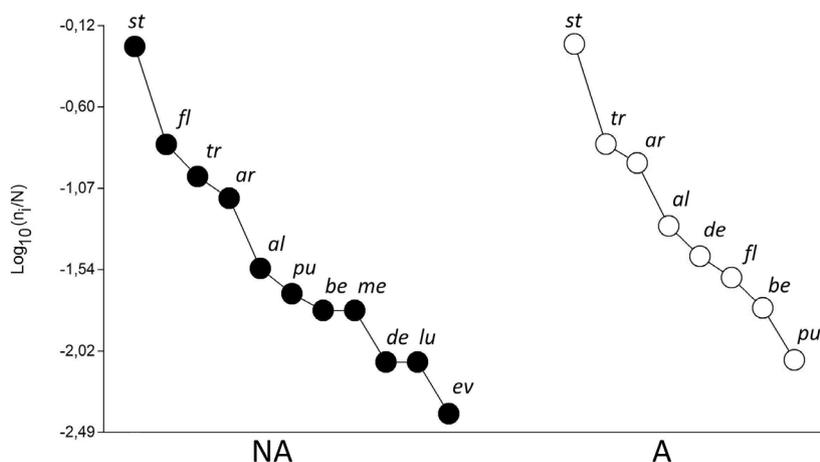
## Models development

For the total abundance of *Anopheles* mosquitoes, the best fit models include NDWI and distance to vegetation (GLMM8), as well as NDWI and urban areas (GLMM9) (Table SIII). Based on this model, a negative association is observed between NDWI, distance of vegetation, urban areas, and the total abundance of *Anopheles* mosquito.

While for *Anopheles strodei* s.l., the more parsimony and best fit models were the univariates with water areas (GLMM10) and with NDVI (GLMM13) (Table SIV). Based on these models, *An. strodei* s.l. abundance is positively related to the presence of water, but negatively related to the NDVI (Table II).

## Seasonality

To identify seasonal patterns, we estimate the mean number, monthly collected in all sites for each *Anopheles* species (total number in 4 sites/8 traps). Over the effective 17 months of *Anopheles* collected, the greatest abundance of *Anopheles* mosquitoes was observed in spring, with mean temperatures (20.9°C to 24.9°C) lower to those of summer (24.8°C to 26.4°C) (Fig. 5). This pattern of abundance was similar for most of the species captured in the four climatic seasons.



**Figure 4.** Range-abundance curves for the species captured in two environments with different degrees of anthropogenic disturbance (NA: natural environment; A: artificial modified environment) in the province of Misiones, Argentina. Codes for the species. *An. albitarsis* s.l.: al; *An. argyritarsis*: ar; *An. benarrochi* s.l.: be; *An. evansae*: ev; *An. deaneorum*: de; *An. fluminensis*: fl; *An. lutzii*: lu; *An. mediopunctatus* s.l.: me; *An. evandroi*: ev; *An. punctimacula*: pu; *An. strodei* s.l.: st; *An. triannulatus* s.l.: tr.

*Anopheles albitarsis* s.l., *An. fluminensis* and *An. strodei* s.l. were collected in all climatic seasons. *An. argyritarsis*, *An. benarrochi* s.l., *An. deaneorum* and *An. punctimacula* were caught in three seasons (autumn, winter, and spring). During winter, *An. punctimacula* was not captured, although a specimen was captured in summer (Fig. 6).

All the rest of the species were scarce capture during one or two climatic seasons. *Anopheles evansae* and *An. triannulatus* s.l. were captured in spring and autumn, *An. lutzii* was captured in winter and autumn, *An. mediopunctatus* s.l. in spring and winter, *An. evandroi* was captured in autumn and *An. malefactor* in spring.

## Models development

For the total abundance of *Anopheles* mosquitoes, the best models were GLMM1 and GLMM8, which include the variables relative humidity, mean temperature and mean maximum temperature respectively (Table SV).

Moreover, for *An. strodei* s.l. abundance, the more parsimony and best fit models were the univariates that include the variable relative humidity (Table SVI).

The abundance of *Anopheles* presented a negative association with relative humidity and mean temperature, but positive with mean maximum temperature. In himself, *An. strodei* s.l.

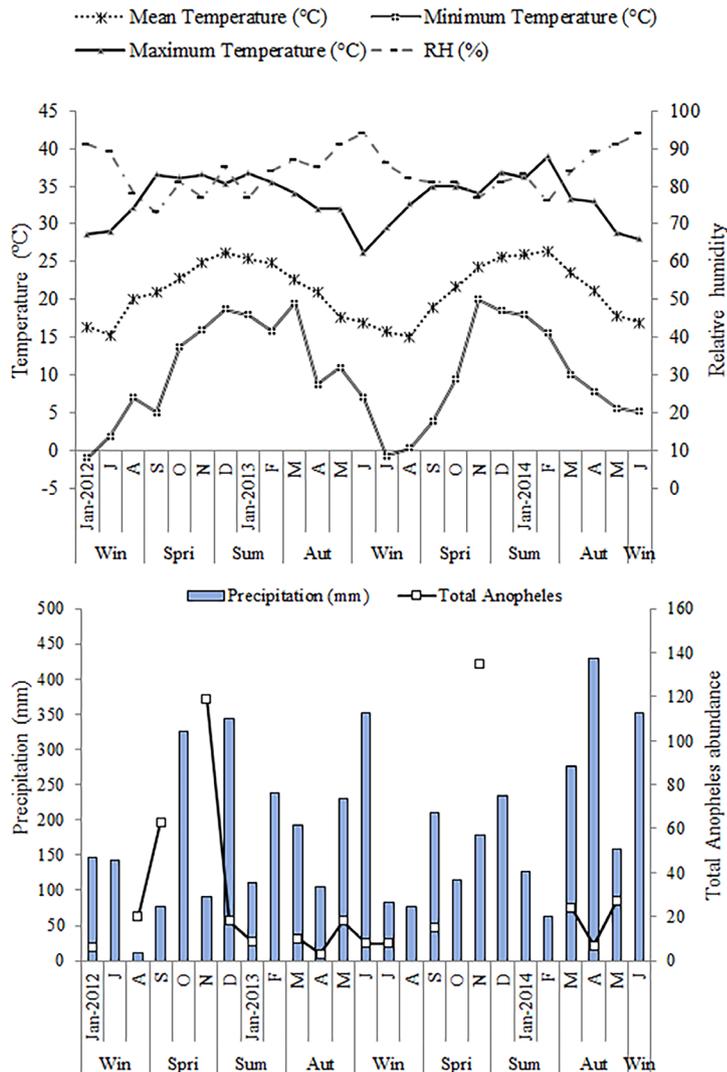
presented a negative association with relative humidity (Table III).

**DISCUSSION**

The historical northeastern malarious area of Argentina has been extensively modified, so to evaluate the changes of landscape coverage from the use of high spatial resolution satellite products and the influences of meteorological variables and their relationship with the abundance of *Anopheles* mosquitoes in the study area, constitute in data of interest to be analyzed. In this study, 11 of the 29 known

recorded *Anopheles* mosquito species for Misiones province (Rossi 2015) were collected. Although the low collected abundance of specimens, which we attribute to possible technical failures during the captures, the results in relation to the community structure are similar to those obtained in the area for a previous study (Ramirez et al. 2017). In the present study a good approximation to the knowledge of the local fauna was observed (97%).

It is noteworthy that, *An. darling*, which is the main malaria vector in the northeastern Argentina, was no detected during the two sampling years of this study, in agreement with

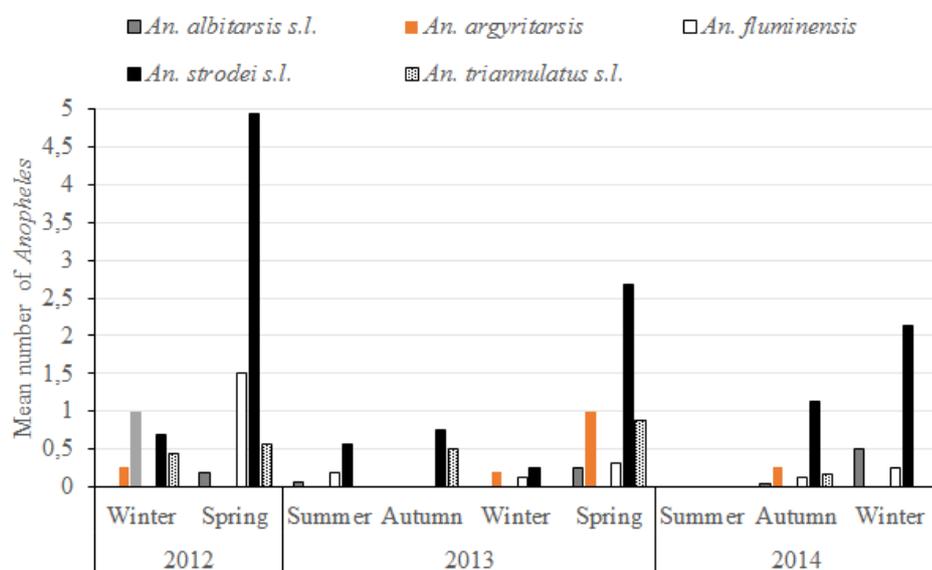


**Figure 5.** Monthly variation of total mosquito abundance, accumulated rainfall (mm), mean, minimum, maximum temperature, and relative humidity from June 2012 to July 2014, in the study area.

the previous research of our group during the years immediately preceding the present study in Puerto Iguazú (Ramirez et al. 2016, 2017). These situations left us the question about *An. darlingi* environmental determinants in our subtropical study area, related fundamentally to the larval habitats' characteristic of these species as well as the consequences of anthropogenic climate change leading to climatic process changes that affected mainly temperatures and precipitation patterns (Paaijmans et al. 2009). On several Brazilian amazon areas is suggested that deforestation could be one of the main causes for *An. darlingi* absence as consequence of open clusters with exposed habitats inappropriate for vector development, favoring the replacement of the main vector of malaria by a secondary vector (Tadei & Dutary-Thatcher 2000, Conn et al. 2002). Also a recent research made by Laporta et al. (2015) in Brazil showed that climate changes could affect *An. darlingi* geographic distribution and as a consequences of that its role in malaria transmission may decrease in future, and some species of the Albitarsis complex (between them *An. deaneorum*) could adopt a more significant role in South America. According to this study, *An. darlingi* is more dependent on

high precipitation levels than Albitarsis complex species, which could affect the quality of larval habitats so a potential distribution expansion in some species of the Albitarsis complex, and the reduction of the geographical distribution of *An. darlingi* may occur. Secondly, Shannon & Del Ponte (1928) stated that malaria transmission in northeastern Argentina at the beginning of the 20th century was linked to migrations along the Paraná River. Therefore, we suggest that for that times as postulated by Del Ponte (1940), *An. darlingi* presence may be due to flourishing of rice cultivation and therefore the outbreaks of malaria in this area. Currently 65% of the Misiones territory is highly modified as a result of forestry agribusiness, and cultivation of yerba mate, tea and tobacco (IPEC 2012), completely changing the landscape that was conducive to the reproduction of *An. darlingi* in the last century.

In regarding *An. punctimacula*, *An. triannulatus s.l.*, and *An. albitarsis s.l.*, the present study expanding knowledge about their geographic distribution range in areas with historical records of malaria cases (Duret 1950, Lifshitz et al. 1946, Castro et al. 1959, Bejarano 1959, Ramirez et al. 2016). It is data of interest



**Figure 6.** Mean number of *An. albitarsis s.l.*, *An. argyritarsis*, *An. fluminensis*, *An. strodei s.l.* and *An. triannulatus s.l.* recorded from June 2012 to June 2014. \*: white spaces: technical failures of the traps and difficulty accessing the mosquito collection sites did not allow the sampling.

considering that they are secondary vectors of malaria in other South American countries and the lack of main malaria vector records since 50' in Puerto Iguazú city (Duret 1950, 1951, Bejarano 1959, Rubio-Palis & Zimmerman 1997, Olano et al. 2001, Manguin et al. 2008, Ministerio de Salud y Desarrollo Social 2018).

In the present study *An. strodei s.l.* was the most abundant species, in consistency with Ramirez et al. (2018) who found it highly represented in urban, semi-urban, as well as wild environments. Dantur Juri et al. (2010) report *An. strodei s.l.* mostly in the forest in the Yungas, northwestern Argentina (Tucumán province, Argentina) but also it was the most abundant *Anopheles* species in forest edge in the same study.

Most of the species were collected in all environments studied, indicating their ability to colonize a wide variety of environments, being some of them found, in both natural and artificial larval habitats (Stein et al. 2011, Ramirez et al. 2016, 2018, da Silva et al. 2013, Djamouko-Djonkam et al. 2019).

The less modified environments (Puerto Bossetti and Iguazú National Park) present the higher diversity and richness species in coincidence with other studies (Stein et al. 2016, Ramirez et al. 2018).

On the other hand, Puerto Iguazú (urban environment) records species as the second most abundant site, also, was less diverse, which is explained by the more hierarchical and dominant occupation of a single species (*An. strodei s.l.*). Human impacts on the peri-urban environment of Puerto Iguazú, with clear-cut forestry, that generates fragmentation and environmental heterogeneity, adds new niches and larval habitats, which can be colonized by the malaria vectors (Mattah et al. 2017). This situation affected diversity of mosquito populations, facilitating the proliferation of artificial habitats for immature mosquitoes due to urbanization influence (Jacob et al. 2003, Leisnham et al. 2004, Ramirez et al. 2017) and therefore a higher abundance of opportunistic *Anopheles* species in this site consequently (Consoli & De Oliveira 1994, Dorvillé 1996, Forattini 2002).

Likewise, we identified species more susceptible to environmental changes such as *An. fluminensis*, *An. punctimacula* and *An. benarrochi s.l.*, which were located to the end of the curve in the most disturbed environment.

Several studies showing that distributions of mosquito species are related to land cover such as wetlands availability, type of surrounding vegetation and presence of agricultural crops (Stefani et al. 2013, Altamiranda-Saavedra et

**Table III. GLMM estimate parameters for the selected explanatory variables in the *Anopheles* mosquitoes total abundance model and the *Anopheles strodei s.l.* abundance model.**

Response variable	Model	Parameter	Estimate	Std. Error	z-value	p-value
Total <i>Anopheles</i> abundance	GLMM1	Intercept	3.2281	0.1949	16.566	< 2e-16
		RH	-0.6328	0.1882	-3.362	<0.01*
	GLMM8	Intercept	3.1582	0.2407	13.120	< 2e-16
		Tm	-0.7112	0.2968	-2.396	0.016*
<i>Anopheles strodei s.l.</i> abundance	GLMM9	Tmaxm	0.5085	0.2351	2.163	0.03*
		Intercept	1.4391	0.3135	4.590	4.44e-06
	GLMM9	RH	-1.2565	0.3331	-3.772	<0.01*

An asterisk indicates significant p-value (<0.05).

al. 2017). Resolution satellite products have been used to describe and predict spatial and temporal changes in the abundance of vector mosquitoes and malaria transmission (Adimi et al. 2010, Machault et al. 2011). The low number of specimens found in Puerto Libertad draws our attention, being an environment represented with high percentages of high vegetation according to SPOT-5 image's classification. However, the high vegetation was represented by pine plantations for commercial purposes surrounding the city, which is not a favorable environment for the proliferation of characteristic habitats of *Anopheles* mosquitoes. On the other hand, we think that the CDC light trap could not be very efficient when the abundances of *Anopheles* are not high (Mbogo et al. 1993).

We found that a high abundance of *Anopheles* mosquitoes was negatively associated with NDWI, distance of vegetation and urban areas. Various studies showed that *Anopheles* mosquitoes occupy areas where NDWI values were low, such as bare soils and low forest cover ecosystems (Stefani et al. 2013, Altamiranda-Saavedra et al. 2017).

In Venezuela (Rubio-Palis et al. 2013) and Peru (Vittor et al. 2006), find higher abundances of *Anopheles* associated to agricultural areas, characterized by early secondary vegetation. In Brazil, in altered areas by constructions of hydroelectric dams (Tadei & Dutary Thatcher 2000) found a greater number of *Anopheles* species in places where the percentage of natural vegetation was low, in agreement with our study. In Puerto Bossetti (semi-urban environment), located next to the Urugua-í dam, where the regional flora was subjected to forest extractions, we founded lower diversity but greater abundance of Culicidae than wild environment.

The NDWI is known to be strongly related to the plant water content, consequently, high

NDWI values would indicate abundant rainfall and high relative humidity. In Argentina, Dantur Juri et al. (2009) reported that rainfall favors the presence of the malaria vectors in northwestern of the country. We find a negative association between the abundance of *Anopheles* mosquitoes and the relative humidity without time lag. Dantur Juri et al. (2010) showed that in subtropical mountainous rainforests in northwestern Argentina, the relative humidity with a 15-day delay is the major determinant of the abundance of *Anopheles* mosquitoes.

Additionally, our results showed a negative association of *Anopheles* mosquitoes with two months-time lag medium temperature, but a positive relation of them with maximum temperature without time lags. Temperature increases result in rapid hatching eggs, shorter larval development, adequate conditions for reaching adult stage and increased mosquito abundance (Sáez Sáez et al. 2007, Munga et al. 2009). Our results showed high mosquito abundance during spring and the beginning of summer, similar to observed by Ramirez et al. (2018) who found higher *An. strodei* s.l. abundance during summer, and as well as *An. albitarsis* s.l., *An. argyritarsis*, and *An. deaneorum* abundance positively related to temperature. Dantur Juri et al. (2015) analyzed the effect of day Land Surface Temperature (LST) on the *Anopheles* abundances in northwestern of Argentina, detecting negative association. They mentioned that higher day LST also has been related to a lower relative humidity, proving a less appropriate environment for mosquito survival. In relation to different findings, it is necessary to analyze multiple factors, which would be interconnected, to allow us to understand the changes on the *Anopheles* densities.

Our results confirm the opportunism behavior of *An. strodei* s.l., being abundant in all study sites, occupying the most hierarchical

position, showing its ability for colonize and breed in modified environments, being more abundant in semi-urban sites, in accordance with other studies (Meneguzzi et al. 2009, da Silva et al. 2013, Ramirez et al. 2018). Our models showed that the water and NDVI are highlighted explanatory variables of the abundance of *An. strodei* s.l. NDVI values were negative associated with the abundance of this species. Rezende et al. (2009) found *An. strodei* s.l. frequency is increasing near houses and decreasing inside the forest and, where there is a reduction in the number of sources of domestic and wild blood, due to environmental modifications. At high population densities, *An. strodei* s.l. is considered a secondary vector in areas of malaria transmission (Linthicum 1988, Consoli & de Oliveira 1994). De Carvalho et al. (2014) collected *An. strodei* s.l. feeding on humans and dogs in municipal parks in São Paulo, Brazil, added to it was found infected with either *P. falciparum* or *P. vivax* in malaria endemic areas, all data of interest for considering its potential role as malaria vector. Its major abundance also is positively associated to the presence of water, which could be associated with the availability of different larval habitats (Stefani et al. 2013).

It is well based the association between infectious disease emergence and land-use changes (Patz et al. 2004). In the Amazon basin, Vittor et al. (2009) concluded that deforestation and associated ecologic alterations increase malaria risk. Part of our study area showed a considerable degree of deforestation, with the consequent transformation of natural forests into family settlements with development of needed activities such as raising pigs, chicken, and horses, growing exotic forests for wood used, and the environment transformation with commercial purposes and a high percent of poor houses (Mastrangelo & Salomon 2010). Some studies affirm that vector capacity increases by

77% in the deforested areas than in the forested ones of the same altitude (Afrane et al. 2006). In western Kenya, deforestation for agricultural purposes, environment with dramatic changes in land use and cover, increases the availability of *Anopheles* mosquito habitats, their abundances, and the risk of malaria (Munga et al. 2009).

Considering that, *An. strodei* was the most abundant species in the study area and knowing it represents a complex with seven possible species (Bourke et al. 2013), new studies are necessary to know the effective identification in the study area.

The abundance of *Anopheles* mosquitoes was associated to different environment and climatic variables in the study area and which together explain their seasonal and spatial changes, data that results in interest for possible appearance of malaria cases.

Currently, Argentina is a malaria-free country, with no record of the main vector in this study area, but with other potential vectors, so it is needed multidisciplinary and transversal works for prevention and vector management over time. To prevent malaria reintroduction in this area, more studies that allow identifying the effects of environmental variables on the abundances and presence of different species of *Anopheles*, which shares an extensive border, commercial links, and population movements with Bolivia and Brazil, countries that register autochthonous cases are needed.

### Acknowledgments

This work was partially funded by Dirección Nacional de Control de Vectores, Ministerio de Salud de la Nación. We thank the technicians of the Ministerio de Salud de la Nación (National Health Ministry) for their technical support in the collecting specimens. We also want to thank Dr. Débora N. Bangher for her technical support to edit some images. The authors have no conflict of interest to declare. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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## SUPPLEMENTARY MATERIAL

### Table SI-SVI.

#### How to cite

STEIN M, MARTIN ME, RAMÍREZ PG, ETCHEPARE EG, ORIA GI, ROSSI GC, KURUC J & ESTALLO EL. 2023. Updated *Anopheles* mosquitoes abundance and distribution in north-eastern malaria-free area of Argentina. *An Acad Bras Cienc* 95: e20220956. DOI 10.1590/0001-3765202320220956.

*Manuscript received on November 5, 2022; accepted for publication on April 5, 2023*

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Conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the manuscript, and final approval of the version to be submitted: MS; Acquisition of data, analysis and interpretation of data, drafting the manuscript, and final approval of the version to be submitted: ELE, MEM, PGR, GIO and EGE. Acquisition of data and final approval of the version to be submitted: JK; revising it critically for important intellectual content, and final approval of the version to be submitted: GCR (Adviser from CEPAVE). MS and ELE are members of CONICET.

