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LONGITUDINAL STUDY OF THE SPECIES COMPOSITION AND SPATIO-TEMPORAL ABUNDANCE OF *ANOPHELES* LARVAE IN A MALARIA RISK AREA IN ARGENTINA

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Abstract

Species composition and spatio-temporal abundance of *Anopheles* (Diptera: Culicidae) larvae and their relationship with environmental variables were studied in an endemic malarious area of northwestern Argentina, where *Anopheles pseudopunctipennis* is the main vector involved in malaria transmission. From Dec 2001 to Dec 2005, we performed monthly samplings of different aquatic larval habitats, such as puddles, irrigation canals, ponds and pools of Mountain Rivers. To determine the relationship among environmental variables and larval abundance, we used Poisson's regression analysis. We collected 5,079 larvae of which *An. pseudopunctipennis* was the most abundant species followed by *Anopheles argyritarsis* and *Anopheles evansae*. The density of *Anopheles pseudopunctipennis* larvae fluctuated between the end of spring until autumn, when malaria cases occur in the area. Concurrently, the larval densities of the other anophelinae species fluctuated throughout these climatic seasons. Poisson regression revealed that an increase of mean minimum temperature produced an increase in the abundance of *An. pseudopunctipennis* and *An. argyritarsis*. The mean maximum temperature and the water temperature greatly influenced the abundance of *An. pseudopunctipennis*, *An. evansae* and *An. strodei*. Increases of these temperature variables produced increases in abundance of these species. These factors should be taken into consideration when control measures for immature mosquitoes are implemented to reduce the number of larval habitats and the production of larvae, which may ultimately result in the elimination of malaria in this area.

Key Words: larvae, environmental variables, *Anopheles pseudopunctipennis*, *Anopheles argyritarsis*, *Anopheles evansae*, *Anopheles strodei,* malaria, Argentina

Resumen

La composición de las especies y la abundancia espacio-temporal de las larvas de *Anopheles* (Diptera: Culicidae) y su relación con las variables ambientales fueron estudiadas en un área endémica para malaria en el noroeste de Argentina, donde *Anopheles pseudopunctipennis* es el principal vector involucrado en la transmisión de la enfermedad. Entre diciembre de 2001 y diciembre de 2005 se realizaron muestreos mensuales de diferentes hábitats larvales acuáticos tales como charcos, canales de riego, estanques y ríos de montaña. Para determinar la relación entre las variables ambientales y la abundancia larval, se utilizaron análisis de regresión de Poisson. Se recolectaron 5.079 larvas, de las cuales *Anopheles pseudopunctipennis* fue la especie más abundante seguida de *Anopheles argyritarsis* y *Anopheles evansae*. Las larvas de *An. pseudopunctipennis* mostraron una fluctuación desde fines de primavera hasta el otoño, cuando los casos de malaria aparecen en el área. Mientras que las otras especies de larvas de anofelinos fluctuaron a lo largo de las estaciones climáticas. Las regresiones de Poisson revelaron que un aumento de la temperatura media mínima produce un incremento en la abundancia de *An. pseudopunctipennis* y de *An. argyritarsis*. La temperatura media máxima y la temperatura del agua influenciaron en gran medida la abundancia de *An. pseudopunctipennis*, *An. evansae* y *An. strodei*. Un incremento de estas variables produce un aumento en sus abundancias. Estos factores deben tenerse en cuenta al momento de implementar medidas de control sobre las formas inmaduras de estos mosquitos para reducir el número de hábitats larvales y la producción de larvas, lo cual podría al final resultar en la eliminación de la malaria en esta área.

Palabras Clave: larvas, variables ambientales, *Anopheles pseudopunctipennis*, *Anopheles argyritarsis*, *Anopheles evansae*, *Anopheles strodei,* malaria, Argentina

Malaria is an important public health problem in the Americas, and mosquitoes of the genus *Anopheles* (Diptera: Culicidae) are the only vectors implicated in the transmission of the *Plasmodium* parasites, the causative agents of this disease. In North America (Mexico) and Central America, *Anopheles* (*Nyssorhynchus*) *albimanus* and *Anopheles* (*Anopheles*) *pseudopunctipennis* have been reported as the primary *Plasmodium* vectors both in the coastal and foothill regions (Rodríguez & Loyola 1989; Zimmerman 1992). *Anopheles* (*Nyssorhynchus*) *darlingi* is also a *Plasmodium* vector but has a different geographical distribution. In South America, *An. albimanus* and *An. pseudopunctipennis* are the primary malaria vectors on the Pacific coast (Zimmerman 1992). *Anopheles darlingi* is the main vector in the inland regions of Brazil. Also in Brazil, *Anopheles* (*Nyssorhynchus*) *aquasalis* has been reported in the coastal regions, while *Anopheles* (*Kerteszia*) *cruzii* and *Anopheles* (*Kerteszia*) *bellator* are known vectors in southeastern Brazil from the coast into the mountains, and in open places and secondary forests in relatively dry areas along the coast (Zavortink 1973; Zimmerman 1992).

Knowledge of the bioecological aspects of anopheline larvae is limited primarily to the characterization of their habitats. Hoffmann (1929, 1931), Shannon (1930), Hoffmann and Samano (1938), Rozeboom (1941) and Hackett (1945) have reported that larvae are found in freshwater stream pools with typical characteristics, such as the presence of shadows, clean water and filamentous green algae.

Although stream pools were the main larval habitats, immature larvae have also been collected from spring-seepages, ditches, ground pools, lagoons and rock pools. Mosquito larvae have also been found in artificial containers, such as tanks, fountains, rice paddies and marshy meadows (Rozeboom 1941; Downs et al. 1948; Manguin et al. 1996).

According to Savage et al. (1990), Rejmankova et al. (1991, 1993) and Fernández Salas et al. (1994), 3 environmental variables were positively associated with the larval habitats of *An. pseudopunctipennis*, filamentous green algae, altitude and shallow water.

Annual rainfall is the climatic variable associated with *An. pseudopunctipennis* larval abundance, which generally causes a decrease in the number of immature mosquitoes in both permanent and transitory habitats. Fernández Salas et al. (1994) reported that seasonal rainfall was directly and negatively associated with larval abundance. Heavy rains cause river flows to surge and eliminate larval habitats. Therefore, when the wet season is over and rainfall decreases, larval habitats, such as the pools, puddles and ponds that develop along the banks of rivers and streams, can be observed. In these habitats, it is also common to find floating aquatic plants and mats of filamentous algae with immature *An. pseudopunctipennis*.

Manguin et al. (1996) analyzed the larval habitats of *An. pseudopunctipennis* from Mexico to South America considering different biogeographic regions. The larval habitats were found in various environments such as plantations, forest, villages, swamps, and prairies. Larvae were principally collected in clear, shallow stream pools or stream margins with rocky bottoms, but they were also found in spring-seepages, ditches, ground pools, lagoons and rock pools. The exposition to the sun was observed at the majority of the sites as well as the presence of stagnant water; however larvae were also found in flowing water where the presence of green algae seemed to reduce water current velocity. Larvae were also collected in habitats without any visible species of algae. Different vegetation types such as emergent, floating, submersed, or mixtures of these types were also found at the larval habitats. About the pH of the water, larvae were collected not only in acidic and neutral but also in alkaline water. Lastly, *Anopheles albimanus*, *Anopheles aquasalis*, *Anopheles argyritarsis*, *Anopheles darlingi*, *Anopheles hectoris*, *Anopheles punctimacula*, and *Anopheles punctipennis*, were collected in association with *An. pseudopunctipennis*.

These studies already referred to the immature forms of *An. pseudopunctipennis* and were a component of studies regarding the bioecological aspects of *Anopheles* larvae in South America. Indeed, it can be said that there is a scarcity of research in this area (Berti et al. 1993; Grillet 2000).

In Argentina, there are reports on anopheline larval habitats (Shannon & Davis 1927) and cleanup strategies for their elimination (Paterson 1911; Bachmann 1921; Petrocchi 1924); however, these studies are now outdated and insufficient. This is because of the progressive modifications of the environments (Dantur Juri et al. 2010a,b) and the physiographical and climatic factors (Dantur Juri et al. 2010a,b) that may have created new larval habitats.

Based on the existing literature, little is known regarding the composition and effects of environmental factors on the spatio-temporal abundance of *Anopheles* larvae in Argentina. To implement effective malaria control measures through the reduction of malaria vectors, updated information regarding the available aquatic habitats as potential sites to be treated and eliminated is required. Additionally, it is necessary to identify the environmental variables that affect larval production that may have a direct impact on the elimination of malaria in this area.

Consequently, the objectives of this study were to determine the species composition, the spatio-temporal abundance and the effect of environmental variables on *Anopheles* larvae, particularly *An. pseudopunctipennis*, an important malaria vector in Argentina. These data can provide valuable baseline information for the development of future control measures based on larval control strategies in different aquatic habitats in the malarious area of Argentina.

Materials and Methods

Sampling Sites

This study was performed in peri-urban areas of the city of San Ramón de la Nueva Orán (S 23° 08' W 64° 20'), as well as in a Mountain River that flows parallel to a village in El Oculto (S 23° 06' W 64° 30') and in the foothills near the city of Aguas Blancas (S 22° 43' W 64° 22'), Orán department, northwest Argentina. The Bermejo River runs near, Aguas Blancas and San Ramón de la Nueva Orán, whereas the Blanco River flows parallel to El Oculto. Local malaria transmission is known at El Oculto and Aguas Blancas. By contrast, in San Ramón de la Nueva Orán, the importation of cases from the Bolivian border is more common (Boletín Epidemiológico 1997).

The study area is located in the Sierra Baja of Orán, and, thus, in the geological province of the Sub-Andean Sierras. This area is characterized by fast-flowing rivers. During the summer, large volumes of water and the transportation of sediment result in high rates of erosion. This area has high rates of rainfall between Nov and Apr, with peaks in Dec and Mar. The mean annual rainfall is 734 mm with a maximum in Jan (157 mm) and a minimum between Jun and Aug (4 mm). It is possible to recognize 3 seasons, a warm dry season corresponding to the spring (Sep-Dec), a hot wet season corresponding to the summer (Jan-Apr), and a warm-cold and humid season corresponding to the autumn and winter (May-Aug) (Brown & Grau 1993; Brown et al. 2001).

Capture of Immature Mosquito Forms

Mosquito larvae were collected at several habitats situated in the peri-urban areas of the city of San Ramón de la Nueva Orán and in the Mountain River at El Oculto from Dec 2001 to Dec 2005. At the foothills located close to the city of Aguas Blancas, immature sampling was possible during 1 year only, because the heavy rainfall (Jan-Apr) made the road impassable.

Larvae were collected monthly from different habitats of Orán city such as puddles in Tranquera site (S 23° 06' 52.9" W 64° 18'10.3"), an irrigation canal in Don Coca site (S 23° 07' 51.8" W 64° 17' 47.7"), a drainage canal in Recodo site (S 23° 08' 16.6" W 64° 17' 12.2") and a small pond in Matadero site (S 23° 08' 31.5" W 64° 17' 36.5"). In El Oculto, the larval habitat selected was in the Anta Muerta Mountain River (S 23° 07' 1.8" W 64° 29' 49.5") and the samples were taken from its banks, streams and pools.

From Dec 2001 to Dec 2002, the larval habitats sampled in the surroundings of Aguas Blancas city were different ravines on the road along the Arrazayal Mountain River that crosses the mountain and ends in the Bermejo River: Quebrada 1 (S 22° 43' 47.3" W 64° 22' 23.4"), Quebrada 2 (S 22° 43' 19.5" W 64° 22' 53.1"), Quebrada 3 (S 22° 43' 18.4" W 64° 24' 13.4") and Quebrada 4 (S 22° 42' 90" W 64° 24' 3.6").

Larval habitats sampled were previously identified and selected based on the production of the immature anophelinae forms. At all sites, the larvae were collected using standard larval dippers (350 mL) (Service 1976; Fernández Salas et al. 1994). Sampling effort consisted of at least 20 dips per site covering an area up to 5 m^2, and a maximum of 50 dips when habitats covered larger areas. As recommended by Rodríguez et al. (1993), the initial number of larvae collected in the first 5 dips were used to determine the sample size (total number of dips). If the number of larvae collected in the first 5 dips was 0-5 larvae per dip, a maximum of 50 dips was taken; if 5-9, 40 dips was taken, if 10-14, 30 dips was taken; if 15-19, 20 dips was taken; and if 20-25, 10 dips was taken. Independently of the size and the productivity of each larval habitat, samples were taken for up to 1-hr and the larvae were collected along the edge of each body of water whether or not mats of green algae mats were present.

Larvae and pupae were taken to the laboratory for identification. Third and fourth instars were sacrificed and identified by the keys of Faran and Linthicum (1981) and Forattini (2002). As recommended by Berti et al. (1993), when possible, second instars were reared to the third instar for identification. If this was not possible, we assumed that the second instars were part of the same population as the third and fourth instars, and we proportioned them according to the number of each species collected. And, as recommended by Rejmankova et al. (1991), when it was not possible to rear and identify the first instars, these were registered as *Anopheles* spp. Pupae was reared to adults and identified, but if not, they were preserved in alcohol 70%.

Environmental variables were measured at the same time that each sample was taken. Temperature of each body of water was determined using a digital thermo-hygrometer (Springfield Precision Instruments Inc., Wood Ridge, New Jersey, USA), and the pH was determined using reactive strips. Additional environmental data was measured to be carried out further studies based on the characterization of the aquatic larval habitats of *Anopheles* species in Argentina. The type of body of water (natural or artificial), size (length, width and depth), class (spring, stream, river, irrigation canal), exposure to sunlight, water permanence, water movement, turbidity, the color and the odor of the water, kind of substrate, presence, quantity and type of aquatic vegetation, presence of filamentous algae and distance to the nearest human dwelling, were measured. To describe the vegetation, we used the Braun-Blanquet phytosociological method, as recommended by Rejmankova et al. (1991).

From weather stations in San Ramón de la Nueva Orán city (S 23° 07' 60" W 64° 19' 60") and Aguas Blancas city (S 22° 43' 60" W 64° 22' 00") we obtained the monthly measurements of the mean maximum and minimum temperatures and the cumulative monthly rainfall data.

Statistical Analysis

To determine the seasonality of larval densities and whether the relationship between climatic variables and larval density changed annually, a multilevel model for the count data was used. This model was a Poisson regression model with random coefficients. The HLM6 software was used for this model and this software uses Penalized Quasi Likelihood (PQL) estimators (Statacorp 2005). Two levels were considered for the model, level 1 with units in months, and level 2 with units in years. Values of the climatic variables centered on the annual mean ($\bar{X}_{ijk}$) were considered. The following equations defined the models of levels 1 and 2.

Equation for Model of Level One

$$\log(\mu_{ij}) = B_0 + B_1{*}(X_{ij1} - \bar{X}_{ij1}) + B2{*}(X_{ij2} - \bar{X}_{ij2}) + \ldots$$

where

X_{ijk} = value of the k-th climatic variable in month i of year j;

μ_{ij} = is the incident rate or mean density of mosquitoes for month i in the year j, which corresponds to the parameter of a Poisson variable;

B_0 is the intercept of the model for the year j;

B_k is the slope corresponding to the k-th independent variable; and

$\bar{X}_{ijk}$ = annual mean of the k-th climatic variable.

Equation for Model of Level Two

$$B_0 = \alpha + u_0$$
$$B_1 = \beta_1 + u_1$$
$$B_2 = \beta_2 + u_2$$

Given that β, β_1 and β_2 correspond to the fixed effect, u_0, u_1 and u_2 are the random components of the parameters of the model that indicate the variability of the coefficients throughout level 2.

In this case, the better fitting model, using the estimate of the reliability coefficient, was obtained using only the random intercept, such that the level 2 Model (Level-2 Model) was as follows:

$$B_0 = \alpha + u_0$$
$$B_1 = \beta_1$$
$$B_2 = \beta_2$$

It should be noted that the random intercept and also the random slope can be used in this model.

For the sampling sites in Aguas Blancas with only 1 year of larval data, the model used was the same Poisson regression model with a random coefficient but without consideration of the multilevel model.

The following formula was used for the analysis: $1\text{n}\ \lambda = \alpha_0 = \alpha_1 X_1 + \alpha_1, X$

where ln is the Napierian logarithm of the incidence rate or mean density of mosquito larvae, α_0 and α_μ are constants and X is a variable that may or may not be random.

We obtained the incidence rate ratio (*IRR*), standard error (*EE*) values and *P*-values. *IRR* enabled the direct observation of the percentage of influence of each climatic variable on the abundance of the larval species.

Results

Abundance and Temporo-Spatial Distribution of *Anopheles* Species

Throughout the study, a total of 5,079 larvae were collected. Of these, the most abundant species was *An. pseudopunctipennis* (34.36%), followed by *An. argyritarsis* (26.83%), *An. evansae*

(16.15%) and *An. strodei* (15.32%). We also captured *An. triannulatus*, *An. albitarsis* and *An. rangeli* larvae in smaller numbers. Only 2 specimens of the Arribalzagia group of the subgenus *Anopheles* were collected.

When analyzing the seasonal fluctuations of the species across the 4 years of sampling, we observed that *An. pseudopunctipennis* larvae were found in clean bodies of water with a certain amount of water flow, which presumably was related to greater oxygenation, and that these larvae were partially exposed to sunlight. With respect to the fluctuation of the other species, no generalized seasonal pattern could be established for all the sampling sites. In puddles of the Tranquera and in the irrigation canal of Don Coca (Orán city) (Fig. 1), and in the small pond of Matadero (Orán city) and in the Anta Muerta Mountain River (El Oculto) (Fig. 2), we primarily observed a greater abundance of larvae during the spring (dry season). In contrast, in the drainage canal of the Recodo (Orán city), the greatest abundance of larvae was observed at the end of summer and during the autumn (Fig. 3).

Anopheles argyritarsis, *An. evansae* and *An. strodei* were collected in *An. pseudopunctipennis* larval habitats. Seasonal fluctuations of *An. argyritarsis* showed a peak of abundance during the autumn, decreased during the winter without disappearing and increased again during the spring (Fig. 2). In most larval habitats, *An. evansae* showed a fluctuating abundance from the end of summer and during the autumn and winter. The exception was in the puddles of the Tranquera, where larval abundance fluctuated beginning in the autumn, continued towards the end of the winter, and reached its highest peak during the spring (Figs. 1 and 2).

Anopheles strodei was abundant in puddles of the Tranquera; however, when analyzing fluctuations in its abundance, no definite pattern emerged. The highest peaks of *An. strodei* abundance occurred during the autumn and winter but also during the spring and summer months (Figs. 1 and 2).

The fluctuations of anophelinae species in the foothill ravines of Aguas Blancas (Quebrada 1, Quebrada 2, Quebrada 3 and Quebrada 4) were analyzed separately throughout the single year of sampling. For Quebrada 1, *An. pseudopunctipennis* was most abundant during the spring and summer, and the abundance of *An. argyritarsis* increased beginning in autumn and during the winter until the spring (Fig. 4). In Quebrada 2, *An. pseudopunctipennis* behaved differently, with greater peaks in abundance during the summer and autumn that decreased during the winter and spring. The behavior of *An. argyritarsis* was similar to *An. pseudopunctipennis*, and the only difference was that it was most abundant during the winter (Fig. 4). In Quebrada 3, *An. pseudopunctipennis* showed a single peak in the summer unlike *An. argyritarsis*, which was abundant at the end of autumn and during the winter (Fig. 5). Finally, in Quebrada 4, *An. pseudopunctipennis* was abundant in the summer, and *An. argyritarsis* showed increasing abundance from autumn until winter (Fig. 5).

Effect of Environmental Variables on Anopheline Mosquitoes

The effect of environmental variables on larval fluctuation was analyzed separately for each site because each site was considered a different larval habitat, such as puddles, irrigation canals, drainage canals, ponds, streams and pools in the Mountain River and ravines in the foothills.

Influence of Environmental Variables on *An. pseudopunctipennis* and *An. argyritarsis*

The following 2 environmental variables determined the greatest larval abundance of *An. pseudopunctipennis* at Orán city: the mean minimum temperature ($P < 0.007$) and the water temperature ($P < 0.017$). According to the Incidence Rate Ratio (*IRR*), the larval abundance of this species increased by 27% for each rise in mean minimum temperature of one degree centigrade; and larval abundance increased by 19% for each rise in water temperature of one degree centigrade (Table 1).

At El Oculto, the abundance of *An. pseudopunctipennis* was negatively influenced by the cumulative rainfall ($P < 0.001$). The I*RR* showed that the abundance of this species decreased by 1% for each increase in rainfall of 1 mm (Table 2). For the Quebradas of Aguas Blancas, the most significant variables were the mean maximum and minimum temperatures and water temperature ($P < 0.001$, $P < 0.001$, $P < 0.001$; respectively). The *IRR* showed that the species abundance increased by 40% and by 38% when mean minimum and maximum temperature each rose by 1 °C, respectively. Also when the water temperature rose by 1 °C, the larval abundance increased by 32% (Table 2).

The mean minimum temperature was the only significant variable to cause a major increase in the abundance of *An. argyritarsis* in Orán ($P < 0.002$) (Table 1) and Aguas Blancas ($P < 0.015$) (Table 2). The abundance of this species increased by 39% and 17%, when the mean minimum temperature increased by 1 °C at these sites, respectively.

Influence of Environmental Variables on *Anopheles evansae* and *Anopheles strodei*

The environmental variables that exerted the most influence on the fluctuation of *An. evansae* and *An. strodei* were very similar to the above 2 species, i.e., the mean maximum temperature,

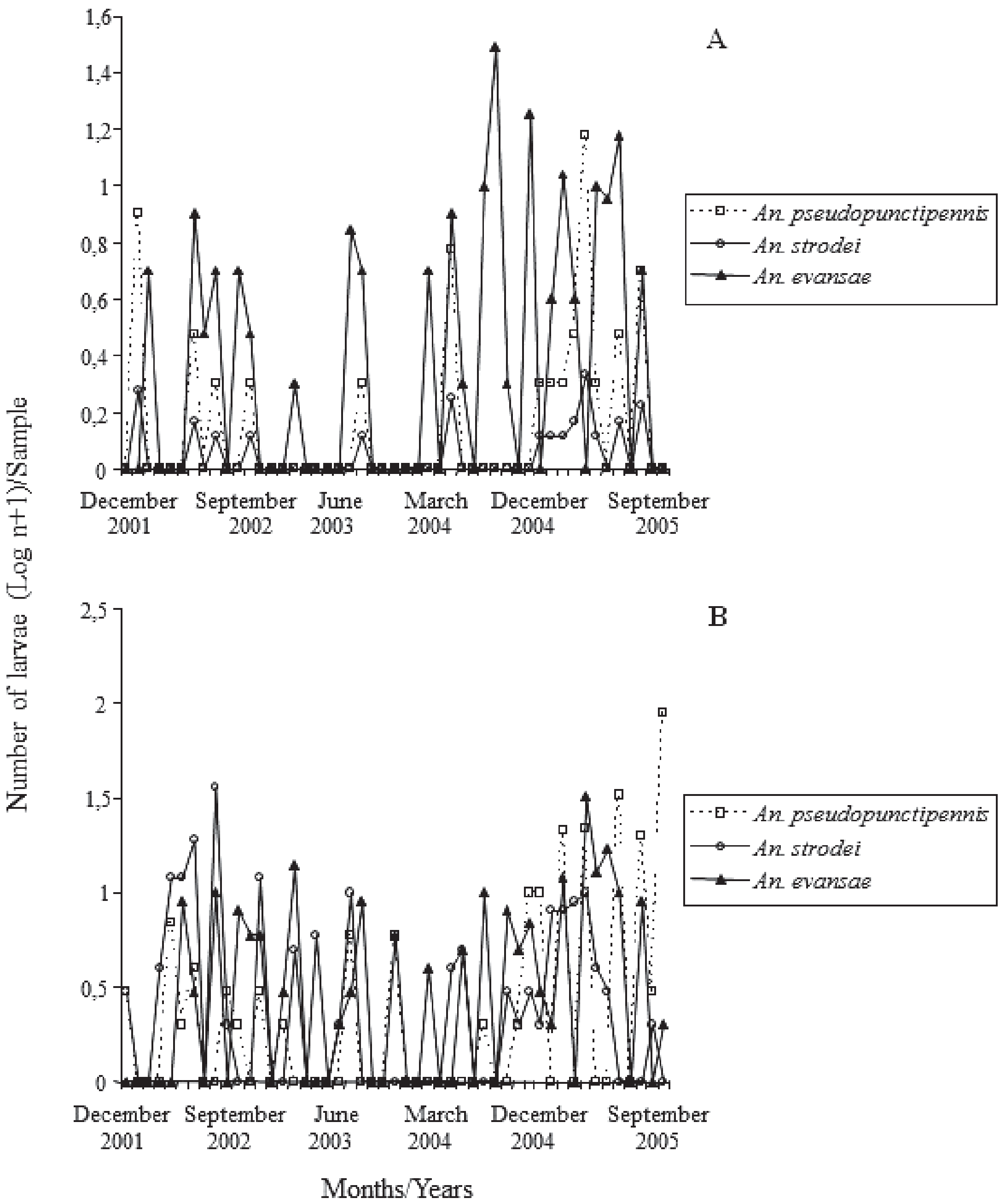

Fig. 1. Seasonal fluctuations of *An. pseudopunctipennis*, *An. strodei* and *An. evansae* in San Ramón de la Nueva Orán, (A) Tranquera and (B) Don Coca from Dec 2001 to Dec 2005.

mean minimum temperature and water temperature.

Water temperature affected the larval population density of *An. evansae* at El Oculto in that it increased larval density by 10% ($P < 0.036$) as the water temperature rose 1 °C (Table 2). The abundance of *An. evansae* increased by 79% when the mean minimum temperature increased by 1 °C ($P < 0.001$). In Aguas Blancas, the mean maximum temperature also increased the abundance of *An. evansae* by 20% when the temperature increased by 1 °C ($P < 0.043$) (Table 2).

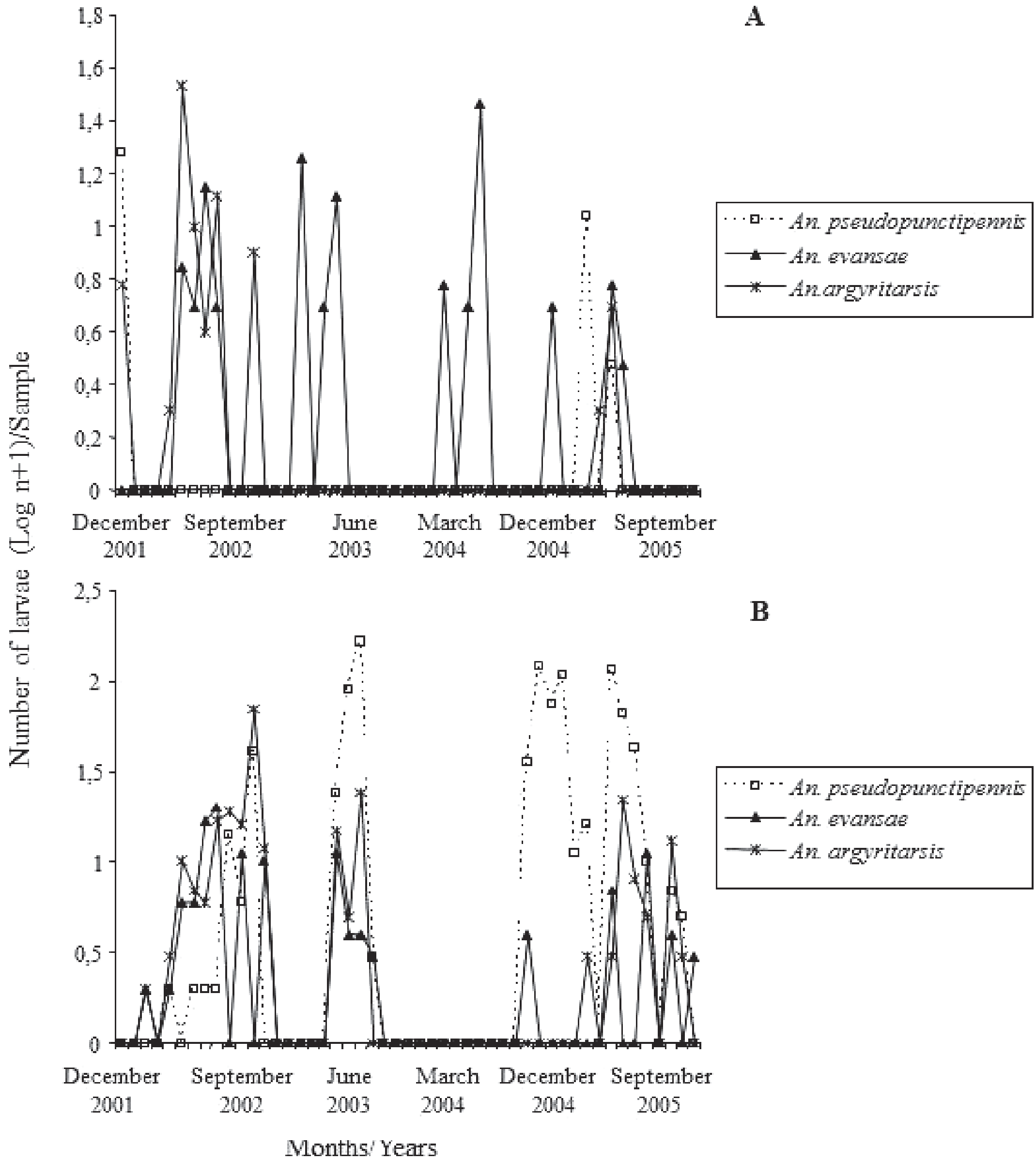

Fig. 2. Seasonal fluctuations of *An. pseudopunctipennis*, *An. evansae* and *An. argyritarsis* in (A) Matadero (San Ramón de la Nueva Orán) and (B) Río Anta Muerta (El Oculto) from Dec 2001 to Dec 2005.

The analyses of *An. strodei* showed that the environmental variables significantly affected this species only in Orán city and El Oculto. The effects of mean maximum temperature, water temperature and mean minimum temperature were all highly significant ($P < 0.004$, $P < 0.001$, $P < 0.001$; respectively). Larval abundance increased by 46%, 25% and 83%, when the mean maximum temperature, water temperature and mean minimum temperature increased by 1 °C, respectively (Tables 1 and 2).

Discussion

In Argentina, studies of the bioecological aspects of immature anopheline mosquitoes (Shannon & Davis 1927) and various sanitation strategies for the treatment and elimination of larval

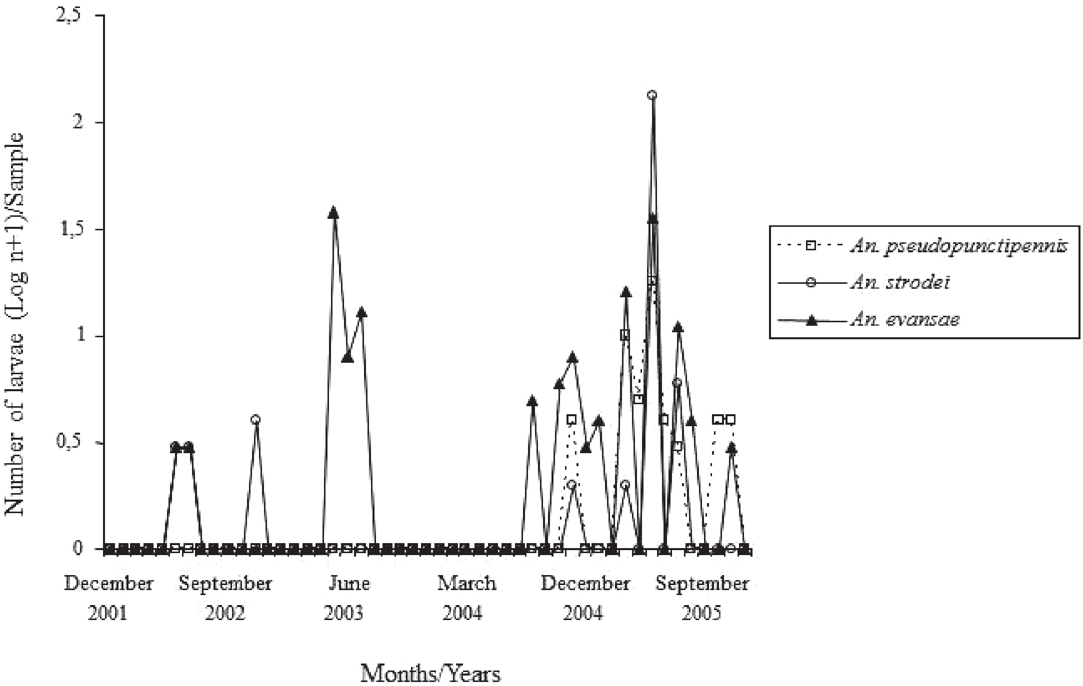

Fig. 3. Seasonal fluctuations of *An. pseudopunctipennis*, *An. strodei* and *An. evansae* in Ricodo, San Ramón de la Nueva Orán, (C) Recodo from Dec 2001 to Dec 2005.

habitats (Paterson 1911; Bachmann 1921; Petrocchi 1924) were performed during the past century. The recommendations from these studies are no longer valid. The results of the present study are important because they provide a baseline for updating the poorly understood bionomics of these mosquitoes in different aquatic environments during different climatic seasons and will enable the objective and rational design of a malaria control program.

Knowledge of which larval habitats are the most productive and/or those in which the malaria vector species are present, as well as the time of year when the habitats have dense populations of immature forms will make it easier to determine a specific control strategy that will indirectly reduce the population of adult mosquitoes.

In the present research, we confirmed that *An. pseudopunctipennis* larvae were mainly collected in clean bodies of water, partially shaded and with the presence of green algae, as was previously reported by Shannon & Davis (1927); Rozeboom (1941); Hackett (1945) and Manguin et al. (1996).

Larvae were found not only in stagnant bodies of water, such as the small pond in Matadero site, and, also, in flowing water as in the Anta Muerta Mountain River and the different ravines of Aguas Blancas. At all of these sites, larvae were found in conjunction with the presence of mats of green algae, as it was previously mentioned by Savage et al. (1990), Rejmankova et al. (1991, 1993); Fernández Salas et al. (1994); Manguin et al. (1996) and Bond et al. (2004). Larvae were collected from within the mats of green algae, where they obtain food and protection against the predators and the water current, as it was reported by Hoffmann & Samano (1938).

The filamentous green algae found in these larval habitats in the present research belonged to the genus of filamentous charophyte green algae, *Spyrogira* (Zygnematales: Zygnemataceae). The abundance of the mats of green algae at each larval habitat varied between high percentages of surface area covered to bodies of water without the presence of the green algae. In the last case, *An. pseudopunctipennis* larvae were found in relation to the presence of emergent, floating or submerged vegetation, as was previously reported by Fernández Salas et al. (1994) and Manguin et al. (1996).

Different larval habitats were found in the proximity of Orán city, i.e., puddles, irrigation canals, drainage canals, and small ponds. The larval habitats were also situated in streams and pools of Mountain River and in ravines of foothills in the subtropical mountainous rainforest in which water flows year round, and not only in dry environments as it was asserted by Manguin et al. (1996).

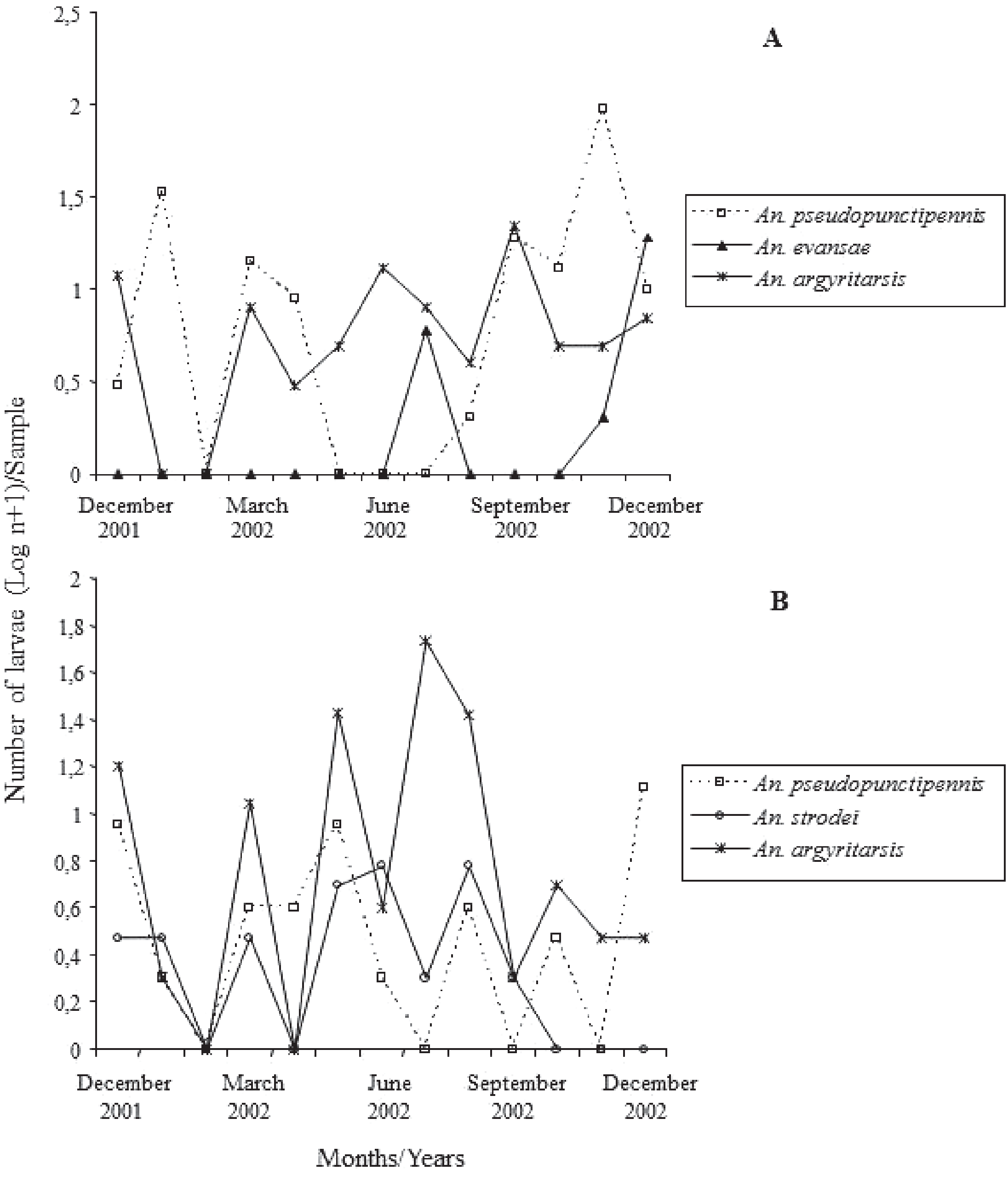

Fig. 4. Seasonal fluctuations of *An. pseudopunctipennis*, *An. evansae*, *An. strodei* and *An. argyritarsis* in Aguas Blancas, (A) Quebrada 1 and (B) Quebrada 2 from Dec 2001 to Dec 2002.

Relationships between larval habitats and proximity to the city, or to the activities developed by humans were observed in our study of the larval habitats located at Orán city. This relationship was cited before by Manguin et al. (1996) in villages, swamps and prairies, and by Rozeboom (1941) in artificial containers like reservoirs, tanks and fountains.

None of the anopheline species showed marked seasonality. Heavy rainfall rates during the summer, low temperatures during winter, and low rainfall rates during the spring did not influence

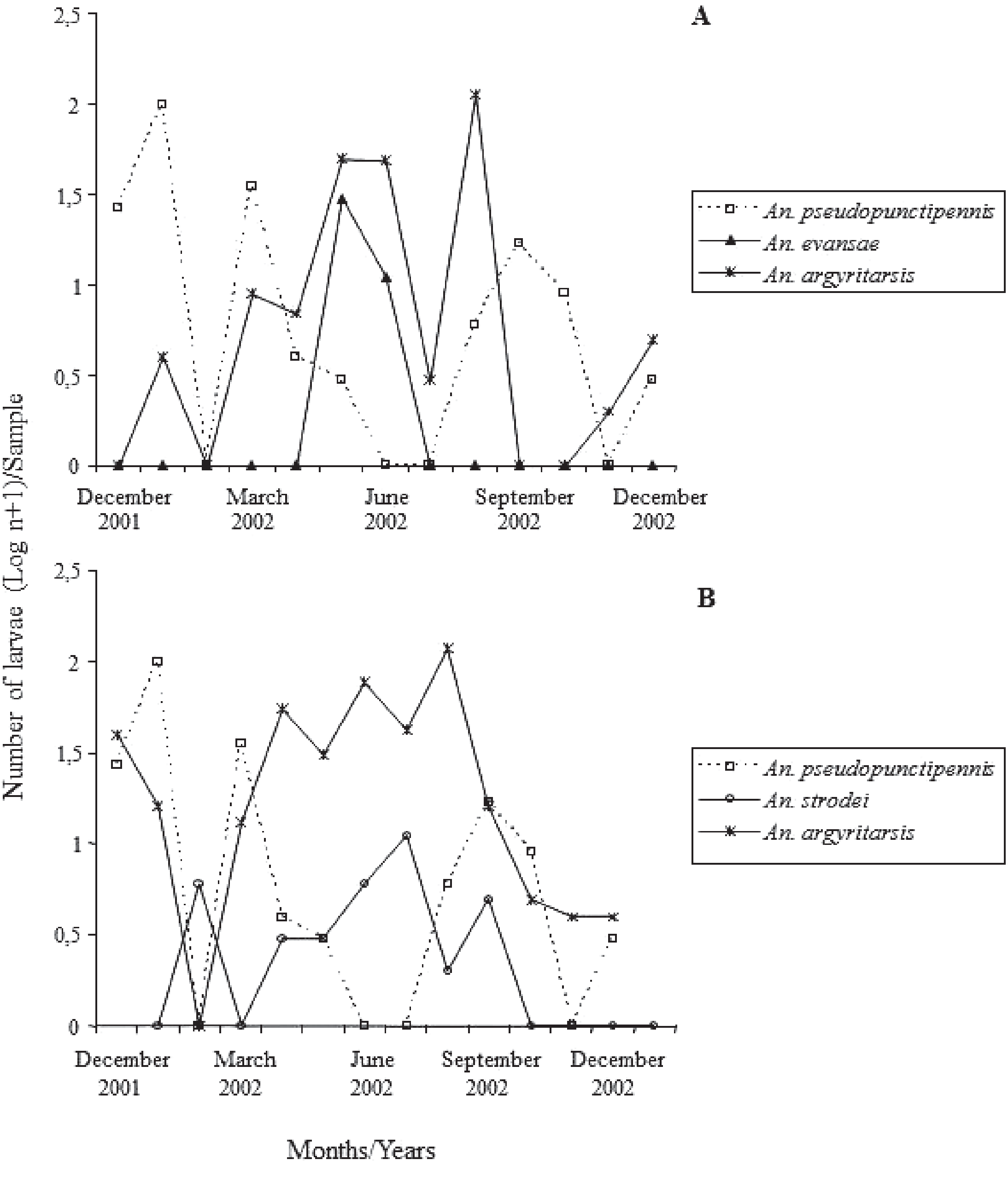

Fig. 5. Seasonal fluctuations of *An. pseudopunctipennis*, *An. evansae*, *An. strodei* and *An. argyritarsis* in Aguas Blancas, (A) Quebrada 3 and (B) Quebrada 4 from Dec 2001 to Dec 2002.

the appearance and abundance of anopheline larvae. This may indicate that larval habitats of these mosquitoes are fairly stable or that the sites may mitigate climatic changes throughout the seasons.

Fernández-Salas et al. (1994) reported a very different situation for southern Mexico in that *Anopheles pseudopunctipennis* larvae showed a marked seasonal fluctuation according to the annual division into dry and wet seasons. In southern Mexico, the larval habitats were negatively associated with annual rainfall; therefore, the greatest abundance of larvae occurred during the dry season (Dec-May). This dry-wet cycle associ-

TABLE 1. RELATIONSHIPS (POISSON REGRESSION COEFICIENTS) OF ENVIRONMENTAL VARIABLES TO THE LARVAL ABUNDANCES OF *AN. PSEUDOPUNCTIPENNIS, AN. ARGYRITARSIS, AN. EVANSAE* AND *AN. STRODEI* CAPTURED IN LA TRANQUERA, DON COCA, RECODO AND MATADERO, SAN RAMÓN DE LA NUEVA ORÁN, NORTHWESTERN ARGENTINA.

Localities-Environmental variables	Species											
	An. pseudopunctipennis			*An. argyritarsis*			*An. evansae*			*An. strodei*		
	Statistical variables											
	IRR	SE	P	IRR	SE	P	IRR	SE	P	IRR	SE	P
Orán City												
Tranquera												
T° water										1.122	0.530	0.015
Don Coca												
T° min med	1.279	0.116	0.007									
T° water										1.258	0.435	0.001
P accumulated			0.994	0.001	0.004							
Recodo												
T° max med										1.466	0.196	0.004
Matadero												
T° water	1.193	0.882	0.017							1.118	0.051	0.015
T° min med				1.391	0.151	0.002						

Abbreviations: *IRR*: Incidence Rate Ratio (Δ abundance/1 C degree); *SE*: Standard Error; *P*: Probability Value; T° water: water temperature; T° max med: mean maximum temperature; T° min med: mean minimum temperature; P accumulated: monthly cumulative precipitation (rainfall).

TABLE 2. RELATIONSHIPS (POISSON REGRESSION COEFICIENTS) OF ENVIRONMENTAL VARIABLES TO THE LARVAL ABUNDANCES OF *AN. PSEUDOPUNCTIPENNIS*, *AN. ARGYRITARSIS*, *AN. EVANSAE* AND *AN. STRODEI* CAPTURED IN THE ANTA MUERTA RIVER (EL OCULTO) AND IN THE FOOTHILL RAVINES: QUEBRADAS 1, 2, 3 AND 4 (AGUAS BLANCAS), NORTHWESTERN ARGENTINA

	Species											
	An. pseudopunctipennis			*An. argyritarsis*			*An. evansae*			*An. strodei*		
	Statistical variables											
Localities-Environmental variables	IRR	SE	P	IRR	SE	P	IRR	SE	P	IRR	SE	P
El Oculto												
Anta Muerta River												
P accumulated	0.993	0.000	0.001				1.009	0.003	0.010			
T° water							1.102	0.051	0.036			
T° min med										1.832	0.343	0.001
Aguas Blancas												
Quebrada 1												
T° max med	1.400	0.054	0.001				1.203	0.110	0.043			
T° min med				1.176	0.078	0.015	1.792	0.321	0.001			
T° water	1.181	0.028	0.001									
Quebrada 2												
T° min med	1.384	0.113	0.001									
Quebrada 3												
T° max med				1.077	0.023	0.001						
T° water	1.329	0.097	0.001									
P accumulated	1.007	0.002	0.005									
Quebrada 4												
T° water	1.129	0.018	0.001									
P accumulated	1.003	0.001	0.001									

Abbreviations: *IRR*: Incidence Rate Ratio (Δ abundance/1 C degree); *SE*: Standard Error; *P*: Probability-Value; ; T° water: water temperature; T° max med: mean maximum temperature; T° min med: mean minimum temperature; P accumulated: monthly cumulative precipitation (rainfall).

ated with the availability of larval habitats depends on rainfall, which varies according to the season, and affects the abundance of the vector; and this has been recorded for other mosquito species and other groups of insects in the tropics (Wolda & Galindo 1981).

The almost constant presence of *An. pseudopunctipennis* during the different climatic seasons including the warm dry season, the hot wet season and the warm-cold and humid season may be related to the fact that when the environmental conditions became favorable, larvae resume their development. Similar situations were reported concerning this species in Oaxaca (Mexico) by Hoffmann & Samano (1938), in Peru by Shannon (1930), in Argentina by Shannon & Davis (1927), and in Ecuador by Levi-Castillo (1945). In addition, Hoffmann (1929) recorded the overwintering of *An. pseudopunctipennis* larvae in springs in Central Mexico.

In relation to the environmental variables, we observed that the cumulative rainfall was one of the most closely related variables influencing the fluctuation of the immature forms of anopheline species. Cumulative rainfall had a negative effect on the larval abundance thorough the years/climatic seasons. Increased rainfall reduced larval densities in the majority of larval habitats, having the same negative effect in the irrigation canals as in the ravines called "Quebradas". This situation was confirmed before by Savage et al. (1990) and Fernández Salas et al. (1994). The last author reported that when heavy rains appeared at the end of the dry season, the pools in the rivers with filamentous algae and the larvae were washed away by the heavy water flow.

For *An. pseudopunctipennis* and *An. argyritarsis*, the cumulative rainfall was negatively significant at the majority of the larval habitats, and we observed the same phenomenon with regard to *An. evansae*. *Anopheles strodei* was the exception because cumulative rainfall was only negatively significant at one larval habitat.

Environmental temperatures (mean minimum and maximum) had important effects on the abundance of the immature. Increases in the mean minimum temperature increased the abundance of *An. pseudopunctipennis* larvae in the irrigation canal of Don Coca at Orán city and in the ravine called Quebrada 2 at Aguas Blancas, whereas in Aguas Blancas, the mean maximum temperature also had positively significant effect on the abundance of this species in the ravine called Quebrada 1. For *An. argyritarsis*, the mean minimum temperature was positively significant in the small pond at Matadero in Orán and at the first ravine called Quebrada 1 in Aguas Blancas. *Anopheles evansae* was positively influenced by the mean minimum and mean maximum temperatures in the ravine called Quebrada 1 at Aguas Blancas; and lastly, *An. strodei* was positively influenced by the mean maximum temperature in the drainage canal of Recodo at Orán city and by mean minimum temperature in the streams and pools of the Mountain River of El Oculto.

Finally, water temperature exerted a positive influence on *An. pseudopucntipennis* larvae, increasing their abundance at the small pond of Matadero at Orán city and at the foothills ravine called Quebrada 3 at Aguas Blancas. This is consistent with Gorham et al. (1973) who reported that *An. pseudopunctipennis* larvae are influenced by water temperature and they are tolerant to its fluctuations. In the Mountain River larval habitat situated at El Oculto, *An. evansae* was positively influenced by water temperature and lastly, *An. strodei* was positively influence at the irrigation canal of Don Coca at Orán city.

The influence of pH on *An. pseudopunctipennis* was not significant. But specimens of this species were found, as reported by Manguin et al. (1996), in acidic, neutral and alkaline water, with pH values ranging from 6.5 to 9.5. In Ecuador, Levi-Castillo (1945) found immature stages in alkaline water with pH values between 7.5-8.5. We observed divergent situations in our study in that this species was found in the irrigation canal of Don Coca with a value of pH of 5.5, and at the Mountain River of El Oculto with a pH values of 8.5.

At the different larval habitats in our study, we observed an association between *An. pseudopunctipennis* and *An. argyritarsis,* the second most abundant larval species collected, and *An. strodei* and *An. evansae*. In Argentina, Mühlens et al. (1925) had confirmed the association between *An. pseudopunctipennis* and *An. argyritarsis* in larval habitats that were close to the dwellings, where the adults bit people. Also, Manguin et al. (1996) confirmed the relationship between this species and other *Anopheles* species in various countries of America. In Grenada, *An. pseudopunctipennis* larvae were collected together with specimens of *An. aquasalis* from bodies of water at sea level, and *An. argyritarsis* was collected from larval habitats with a broad range of altitudes and latitudes. In Belize, *An. pseudopunctipennis* was also collected together with *An. albimanus*, *An. argyritarsis* and *An. darlingi*. In South America, particularly Argentina, larvae of the Argyritarsis section were also collected from all larval habitats of *An. pseudopunctipennis*.

In conclusion, the data obtained from this investigation suggest that larval habitats of *An. pseudopunctipennis* include a variety of bodies of water, but the major production was observed in habitats of the Mountain River and in the ravines called Quebradas. Larvae of this species were found in partially shaded clear water, with the presence of mats of the genus of filamentous charophyte green algae, *Spyrogira*. Considering all the species observed, *An. pseudopunctipennis* is of the greatest epidemiological importance, because it is the most abundant, is found in most larval habitats, and is present during the same period when malaria cases appear. Among the other anopheline species, *An. argyritarsis*, which is a malaria vector (Faran & Linthicum 1981), and *An. strodei* and *An. evansae*, which do not have any roles in malaria transmission (Faran & Linthicum 1981), are most abundant during the autumn. This may be valuable information, because the first species could be related to the late appearance of disease in the area. The mean maximum and minimum temperatures and water temperature appear to be the most significant climatic variables, because they determine the major abundance of the species and favor the appearance of adult mosquitoes and potential malaria cases. Therefore, this study yielded potentially useful information for the subsequent implementation of programs to control the immature forms of the mosquitoes based on the identification of the most productive aquatic environments, which are influenced by ambient air and water temperatures.

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