Survival of the Immature Stages of the Malaria Vectors Anopheles pseudopunctipennis and Anopheles argyritarsis (Diptera: Culicidae) in Northwestern Argentina

Authors: Guillermina B. Galante, Mirta Santana, Cecilia A. Veggiani Aybar, and María J. Dantur Juri

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SURVIVAL OF THE IMMATURE STAGES OF THE MALARIA VECTORS
ANOPHELES PSEUDOPUNCTIPENNIS AND ANOPHELES ARGYRITARSISS
(DIPTERA: CULICIDAE) IN NORTHWESTERN ARGENTINA

GUILLERMINA B. GALANTE1, MIRTA SANTANA2, CECILIA A. VEGGIANI AYBA2 AND MARIA J. DANTUR JURI1,3,*
1Instituto Superior de Entomología “Dr. Abraham Willink”, Facultad de Ciencias Naturales e Instituto Miguel Lillo, Universidad Nacional de Tucumán, Miguel Lillo 205, CP 4000, San Miguel de Tucumán, Tucumán, Argentina
2Catedra de Bioestadistica, Facultad de Medicina, Universidad Nacional de Tucumán, Lamadrid 875, CP 4000, San Miguel de Tucumán, Tucumán, Argentina
3IAMRA, Universidad Nacional de Chilecito, 9 de Julio 22, CP 5360, Chilecito, La Rioja, Argentina
*Corresponding author; E-mail: juliadantur@yahoo.com.ar

ABSTRACT

In order to optimally time the application of control measures to reduce populations of malaria vectors, program managers need to know precisely when the vulnerable larval stage will be most abundant at each specific breeding site. Therefore at 4 different breeding sites of the malaria vectors, Anopheles pseudopunctipennis Theobald and Anopheles argyritarsis Robineau-Desvoidy in northwestern Argentina, we recorded the calendar dates during spring and summer when different life stages appeared, and in each of these 2 seasons, we measured the duration of each life stage and the probability that it would transition to the subsequent stage or die. Larval samples were collected during the spring and summer of 2008-2009 at 4 localities in northwestern Argentina. These larvae were reared individually in plastic containers in which the volume of water was kept constant, temperature and photoperiod were controlled, and a standard amount of food was provided each day. The data were analyzed by multistate models, a nonparametric model of survival without covariates, a survival model with covariates, a Cox-type survival model with specific co-variates, and models of reduced rank. We collected 1,643 larvae of which 1,404 reached adulthood. Of these 1,119 were An. pseudopunctipennis, and 285 were An. argyritarsis. Both An. pseudopunctipennis and An. argyritarsis were abundant in autumn (55.3% and 66.7%, respectively). Considerably more individuals transitioned from larvae to pupae than from pupae to adults. The probability of an individual remaining in the larval stage for the first 2 days was close to 100% and then decreased. The transition from the larval stage to death was significant in the summer. The breeding site at Rosario de la Frontera exhibited a particularly significant effect on the transition from the larval stage to death, i.e., greatly increased larval mortality. The results obtained in the present study are substantial contributions to the bionomics of An. pseudopunctipennis and An. argyritarsis. According to our results, mosquito source management programs should be focused on the larval stage during the summer season and principally at Rosario de la Frontera River. These actions could substantially reduce the production of the adult vectors and potentially reduce transmission of malaria in northwestern Argentina.

Key Words: immature stages, Anopheles pseudopunctipennis, Anopheles argyritarsis, multistate models, survival, northwestern Argentina

RESUMEN

Para optimizar el tiempo de aplicacion de medidas tendientes a reducir las poblaciones de vectores de la malaria, los directores de programas necesitan saber con precision cuando el estado larval vulnerable sera mas abundante en cada sitio especifico de cria. Por lo tanto, en 4 diferentes sitios de cria de los vectores de la malaria, Anopheles pseudopunctipennis Theobald y Anopheles argyritarsis Robineau - Desvoidy, en el noroeste de Argentina, registramos las fechas durante la primavera y el verano cuando los diferentes estados de vida aparecieron, y en cada una de estas 2 estaciones climaticas, medimos la duracion de cada estado de vida y la probabilidad que habia para la transicion al siguiente estado o para morir. Se recogieron muestras de larvas durante la primavera y el verano de 2008-2009 en 4 localidades en el noroeste de Argentina. Estas larvas fueron criadas individualmente en envases de plastico en la que el volumen de agua se mantuvo constante, la temperatura y el fotoperiodo se controlaron, y una cantidad estandar de la comida se proporciono cada
Malaria remains one of the most important tropical infectious diseases that affect people worldwide. In fact, 219 million cases of malaria and 660,000 deaths were reported in 2010 (Paijmans et al. 2007; World Health Organization 2012). In the Americas, at least 30% of the population of the 21 countries is living at some degree of risk, and approximately 8% of the population is at high risk. Argentina, El Salvador, Mexico, and Paraguay remain in the pre-elimination phase of malaria eradication (World Health Organization 2012).

The number of confirmed cases in the Americas decreased from 1.18 million in 2000 to 490,000 in 2011 (World Health Organization 2012). Brazil and Colombia accounted for 68% of the cases in 2011. Reductions in the number of confirmed cases and in the case incidence rates of more than 75% were recorded in 13 countries (Argentina, Belize, Bolivia, Costa Rica, Ecuador, El Salvador, French Guiana, Guatemala, Honduras, Mexico, Nicaragua, Paraguay, and Suriname) between 2000 and 2011 (World Health Organization 2012).

Anopheles pseudopunctipennis Theobald (Diptera: Culicidae) is the primary mosquito incriminated in malaria transmission in the foothills of South America, including Argentina (Zimmerman 1992). The larval habitats of this species along their wide geographical distribution have been characterized as freshwater stream pools that are partially shaded and contain clean water and filamentous green algae. The few studies that have focused on the biological and ecological aspects of immature forms of An. pseudopunctipennis reported that larvae could also be found in artificial containers (tanks, fountains, rice paddies, and marshy meadows) and that environmental variables, including filamentous green algae, altitude, shallow water, annual rainfall, and water temperature, influence larval abundance (Shannon & Davis 1927; Savage et al. 1948; Rozeboom 1941; Hackett 1945; Downs et al. 1948; Savage et al. 1990; Rejmankova et al. 1991; Fernández-Salas et al. 1994; Berti et al. 1993; Manguin et al. 1996; Grillet 2000; Dantur Juri et al. in press).

Since the beginning of the 20th century, An. pseudopunctipennis was reported as the main vector of malaria in northwestern Argentina, and this pioneering work was based on the characterization of the larval habitats of the species during different seasons (Shannon & Davis 1927). The densities of the immature stages of An. pseudopunctipennis have been shown to fluctuate from the end of the spring season to the autumn season, when malaria cases occur (Dantur Juri et al. in press).

In general, the availability of aquatic habitats depends on precipitation (Koenraadt et al. 2004; Fillinger et al. 2004; Paijmans et al. 2007); however, the mean minimum and maximum air temperatures and the water temperature are also climatic variables that can exert influence on the abundance of An. pseudopunctipennis larvae in northwestern Argentina (Dantur Juri et al. in press).

Rates of larval and pupal mortality are affected by climatological factors, such as temperature (Bayoh & Lindsay 2003; Bayoh & Lindsay 2004; Paijmans et al. 2007). Also precipitation can affect larval population dynamics through the flooding of habitats and the consequent flushing out of larvae (Edillo et al. 2004; Mutuku et al. 2006; Paijmans et al. 2007).

Mortality of the immatures during development can be very high. Several studies have reported that only a small fraction (2-8%) of the hatched larvae survive to eventually become adults, and they attributed this high but variable mortality to the presence or absence of predators, and the presence or absence of predators.
parasites, pathogens (Aniedu et al. 1993; Paa-  
jmans et al. 2007), or cannibalism (Okogun 2005;  
Paaijmans et al. 2007), among other biological  
factors.

The scarcity of larval resources (food/ space)  
results in longer development time and reduc-  
tions in success and size at metamorphosis (Haw- 
ley 1985; Bradshaw & Holzapfel 1992; Renshaw  
et al. 1993; Maciá 2009). In adults, body size, sur-  
vival, fecundity, mating success and flight capac- 
ity are lowered by scarce or inadequate larval re-  
sources (Wada 1965; Steinwascher 1982; Reisen  
et al. 1984; Fisher et al. 1990; Maciá 2009).

To date, no study has quantified the effect of  
season of the year and local habitat on the devel-  
opment times of the different stages, nor the rela-  
tionship between season and local habitat both on  
the duration of each life stage and the probabil-  
ity of transitioning to the subsequent life stage  
in populations of the malaria vectors, Anopheles  
psuedopunctipennis and Anopheles argyritarsis,  
in northwestern Argentina. We sought to meet  
these objectives in this study.

**MATERIALS AND METHODS**

**Study Area**

The subtropical mountainous rainforest called  
“Yungas” extends in northwestern Argentina from  
the Bolivian border (23°S) to the northern area of  
Catamarca province (29°S), and passes through  
Salta, Jujuy, and Tucumán provinces (Brown &  

The mean annual rainfall is 734 mm, and  
ranges from 700 to 1,000 mm. In northwestern  
Argentina the year can be divided into 3 seasons:  
a warm and dry spring (Sep-Dec), a warm and  
rainy summer (Jan-Apr), and a temperate and  
clammy fall-winter season (May-Aug) (Brown &  
Grau 1995).

All of the localities are located on the elevated  
floor of the piedmont of the subtropical moun-  
tainous rainforest, which consists of ecolonal  
areas with the Chaco xerophytic forest (Cabrera  
1976; Brown & Grau 1995). The native vegeta-  
tion—with the exception of the areas near roads  
and stream banks where it becomes less dense—  
is closed arboreal vegetation (Digilio & Legname  
1966; Cabrera 1976; Dantur Juri et al. in press).  
The canopy trees include Blepharocalyx salicifolius  
(H. B. K.) Berg. (Myrtaceae), Enterolobium  
contorsilium (Vell.) Morong, (Fabaceae) and  
Juglans australis Griseb. (Juglandaceae). The  
climber species belong to the Bignoniaceae,  
Ulmaceae, and Amananthaceae. There are also  
vascular epiphytes, such as Bromeliaceae. Tipua-  
na tipu (Benth.) Kuntze, Jacaranda mimosifolia  
D. Don. (Bignoniaceae), Tabebuia avellanedae  
Lor. ex Griseb. (Bignoniaceae), Tecoma stans (L.)  
C. Juss. ex Kunth (Bignoniaceae), and Salix hum-
als and preserved in 70% alcohol in appropriately labelled Eppendorf tubes.

Data Analysis

At first, the total abundance and the percentage of immature forms (larvae and pupae) of An. pseudopunctipennis and An. argyritarsis in relation to the each season and sampling site were calculated. Next, the data were analyzed by multistate models, which are suitable for the analysis of phenomena in which the individuals under study may be at different stages during the observation period. These models allow the estimation of the probability of an individual passing from one stage to another at each instant in time and the effect of certain factors on these probabilities (Meira-Machado et al. 2009). We defined the stages and transitions between stages. In this case, the stages considered were the following: larva (stage 1), pupa (stage 2), adult (stage 3), and death (stage 4). The possible transitions considered were the following: from larva to pupa (T1), from larva to death (T2), from pupa to adult (T3), and from pupa to death (T4) (Fig. 1). The adult and death stages were considered absorbing stages, i.e., the individuals were no longer monitored after reaching these stages.

The number and proportion of individuals who performed each transition were calculated. A non-parametric model of survival without covariates was used to estimate the cumulative risk at each transition over time and the probability of staying at each stage at each instance of time.

A survival model with covariates (semi-parametric model) was then used to estimate the effect of the climatic seasons and the local habitats on overall survival. This model was as follows:

\[ h(t|Z) = H(t) \exp(\beta^T Z) \]

To determine whether the covariates have different effects depending on the transition, a Cox-type survival model was used (Cox 1972; Cox & Hinkley 1974;

Fan and Li 2002; Zeger et al. 2004), which considered specific covariates for each transition depending on the effect of the climatic season and of the local habitat on the transitions. This was modeled as follows:

\[ h_{ik}(t|Z) = h_{ik0}(t) \exp(\gamma_k^TZ), \]

where

\[ h_{ik}(t|Z) \] instantaneous risk at time \( t \) given covariates \( Z \)

\[ h_{ik0} \] instantaneous baseline risk (hazard) at time \( t \)

\[ b = \text{model coefficients.} \]

The data were analyzed using models of reduced rank to estimate a lower number of parameters and thus to obtain more efficient estimators of the effects of each covariate at each transition (Reinsel & Velu 1998; Yuan & Lin 2006). This model was the following:

\[ h_{ik}(t|Z) = h_{ik0}(t) \exp(\gamma_k^TZ), \]

where

\[ k \text{ index of transitions 1, 2, 3, and 4.} \]

In addition, measures the overall effect of each covariate on the risk of each of the transitions and \( \gamma_k \) represents the intensity of the effect on each transition. The \( \alpha Z \) factor is considered to be a prediction score for individuals with covariates \( Z \) and determines the probability that a specific individual experienced an event. The parameter \( \gamma_k \) determines the size of the effect of the prediction score in transition \( k \).

RESULTS

A total of 1,643 larvae was collected of which 1,404 reached the adult stage. The most abundant species was An. pseudopunctipennis with 1,119 individuals, and only 285 of the individuals were identified as An. argyritarsis. The relative abundance of the larvae was high during autumn and spring, which was expected because An. pseudopunctipennis is the most abundant species during these seasons (55.32% and 33.24%, respectively). Anopheles argyritarsis was also equally abundant in the autumn and spring seasons (66.67% and 31.23%, respectively). During the winter, no immature form of either species was collected (Table 1).

The comparison of the abundance of both species by the sampling site (habitat) revealed that An. pseudopunctipennis was the dominant species (35.1% of the population) in El Cadillal and that An. argyritarsis was the most abundant species (51.2%) in Potrero de las Tablas (Table 2).

The number of individuals that made the transitions from larva to pupa (T1) was higher than the number that made the transition from pupa to adult (T2) (Fig. 2). The analysis of the percentage of individuals that made one transition revealed that 83% of the individuals made the transition from larva to pupa (T1) and that 88% of these individuals made the transition from pupa to adult (T3). In addition, a higher proportion of individuals died as larvae (T2, 17%) than as pupae (T4, 12%) (Fig. 3).
The non-parametric model of survival without co-variables was used to determine the accumulated risk for each transition (T1, T2, T3 and T4) at each day. We found that doubling the time (days) increased the cumulative risk, meaning that in the case of the transition from larva to pupa (T1), the risk increases from the first to the second day (from 0 to 0.07). This risk remains relatively unchanged from the second to the fourth day, increases again (from 0.09 to 0.41) from the fourth to the eighth day, significantly increases (from 0.43 to 1.18) from the eighth to the 16th day (1.43 to 2.42), and continues to increase nearly to the end (2.42 to 3.42). In the case of T2, which is the transition from larva to death, the risk was low, i.e., there were few dead larvae. The transition from pupa to adult (T3) exhibited a higher risk than that associated with T1, and T4 (from pupa to death) exhibited a higher risk than that associated with T2 (Table 3).

As shown Fig. 4, the transition from larvae to pupae increased at days 4, 8, 16, and 20, and a similar, albeit higher, behavior was observed for the transition from pupae to adults. In contrast, the transition from larvae to death increased on day 8, but few dead larvae were observed. In addition, the transition from pupae to death doubled within 2 days, increased on day 4, and always exhibited a higher risk than that associated with T2 (Fig. 4).

Using the same model, the probabilities of being alive in the larval, pupal, and adult stages, and death were analyzed (Table 4, Fig. 5). The probability of being in the larval stage was close to 100% until the second day, decreased to 50% by the fourth day, and continued to decrease to 10% by the eighth day. It was found that the probability of being in the pupal stage was close to 0% for the first 3 days, increased on the fourth day to 26%, continued to increase during the next 2 wk to reach 40%, and then began to decrease. The probability of being in the adult stage was low initially, increased to 30% on the eighth day, increased to 67% at 2 wk, and remained high thereafter (Table 4, Fig. 5).

Using the survival model with co-variables, and firstly considering the climatic seasons as a co-variable, it was found that neither the spring nor the summer was significant ($p = 0.78$ and $p = 0.56$, respectively). However, the climatic seasons and the local habitats were considered together as co-variables, the seasons were found to be significant in Rosario de la Frontera River and Potrero de las Tablas River ($p = 0.01$ and $p = 0.02$, respectively).

The Cox survival model with co-variables (jointly considering the climatic seasons and the localities) that are specific to each transition (T1, T2, T3, and T4) showed that the transitions from larvae to death (T2) and from pupae to death (T4) are significant in the summer ($p = 0.003$ and $p = 0.048$, respectively). The transition from larvae to death (T2) decreased during the summer ($p = 0.003$), i.e., a smaller number of larvae died, and the same finding was observed with T4 in relation to the pupal stage (Table 5).

### Table 1. Total number of individual larvae (N) of *Anopheles pseudopunctipennis* and *An. argyritarsis* collected during the four seasons of 2008-2009.

<table>
<thead>
<tr>
<th>Climatic seasons</th>
<th>N</th>
<th>%</th>
<th>N</th>
<th>%</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn</td>
<td>619</td>
<td>55.32</td>
<td>190</td>
<td>66.67</td>
<td>809</td>
<td>57.62</td>
</tr>
<tr>
<td>Spring</td>
<td>372</td>
<td>33.24</td>
<td>89</td>
<td>31.23</td>
<td>461</td>
<td>32.83</td>
</tr>
<tr>
<td>Summer</td>
<td>128</td>
<td>11.44</td>
<td>6</td>
<td>2.11</td>
<td>134</td>
<td>9.54</td>
</tr>
<tr>
<td>Winter</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>1,119</td>
<td>100</td>
<td>285</td>
<td>100</td>
<td>1,404</td>
<td>100</td>
</tr>
</tbody>
</table>

### Table 2. Total number (N) and percentage (%) of larvae of *An. pseudopunctipennis* and *An. argyritarsis* collected at different sampling sites during 2008-2009.

<table>
<thead>
<tr>
<th>Sampling Sites*</th>
<th><em>An. pseudopunctipennis</em></th>
<th><em>An. argyritarsis</em></th>
<th>Total both species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>RF</td>
<td>309</td>
<td>27.6%</td>
<td>30</td>
</tr>
<tr>
<td>VP</td>
<td>128</td>
<td>11.4%</td>
<td>35</td>
</tr>
<tr>
<td>EC</td>
<td>392</td>
<td>35.1%</td>
<td>74</td>
</tr>
<tr>
<td>PT</td>
<td>290</td>
<td>25.9%</td>
<td>146</td>
</tr>
<tr>
<td>Total</td>
<td>1,119</td>
<td>100%</td>
<td>285</td>
</tr>
</tbody>
</table>

*RF, Rosario de la Frontera River in Salta province; and the following in Tucumán province: VP, Vipos River; EC, El Cadillal; and PT, Potrero de las Tablas River.
The analysis of the effect of the local habitats on the transitions to death revealed that, in Rosario de la Frontera River, Potrero de las Tablas River, and Vipos River, there was a significant effect of the habitat on the transition from larvae to death (T2; \( p = 0.050 \), \( p = 0.042 \), and \( p = 0.000 \), respectively). Taking into account the value and sign of the coefficients, the risk of T2 is higher at Vipos River compared with the rest of the localities. Lastly, T4 (transition from pupae to death) was found to be significant only in Vipos River (\( p = 0.020 \); Table 6).

Lastly, the reduced rank model, in which the overall effect of each climatic season and localities (coefficient \( a \)) and the intensity of this effect in each transition (coefficient \( g \)) were estimated separately, showed that the major effects were achieved in the summer and in the Vipos River (\( a = -0.41932767 \) and \( a = 0.84045516 \), respectively). Higher values of the parameter \( g \) corresponded to the transitions from larvae to death (T2) and from pupae to death (T4; \( g = 0.7109526 \) and \( g = 0.8543723 \), respectively). The product of each estimated parameter \( a \) and \( g \) gave the effects of the co-variables on each transition. Thus, the effects of the climatic seasons on T2 and T4 (larvae to death and pupae to death) are higher in the summer than the spring, indicating the decreased risk.

Fig. 2. Number of individuals that underwent a transition from one stage to another (larval, pupal, adult, and death).

Fig. 3. The percentage of individuals that underwent a transition from one the stage to another (larvae, pupae, adult, and death).
of the death of larvae and pupae in the summer (negative sign in the product). In contrast, the effect of the local habitat on T2 and T4 was found to be less in Vipos River compared with El Cadillal, indicating the decreased risk of the death of larvae and pupae in Vipos River compared with El Cadillal (Tables 7 and 8).

**DISCUSSION**

Most of the literature on anopheline mosquitoes has focused on the determination of the presence or absence of adult forms of malaria vectors in the different American countries (Rodriguez & Loyola 1989; Zimmerman 1992; Manguin et al. 1996; Dantur Juri et al. 2003, 2005, 2009, 2010a, 2010b). By focusing on the immature forms and their biology, Forattini (1962) provided detailed descriptions of aspects of anopheline breeding sites. Zimmerman (1992) later reported that most of the research into immature forms referred to the presence and/or absence of larvae and their habitat characteristics. Hoffmann & Samano (1938), Savage et al. (1990), and Fernández-Salas et al. (1994) associated the topography with the annual patterns of local rainfall in the formation of larval habitats of *An. pseudopunctipennis*. Thus, when the rainy season ends, larvae, floating plants, and green algae can be found in the larval habitats; the latter include pools, ponds, and lagoons that developed from the margins of rivers and streams.

Other studies of *An. pseudopunctipennis* were based on the larval habitats (Shannon 1930; Hoffmann 1931; Root & Andrews 1938; Aitken 1945; Levi-Castillo 1945; Rejmankova et al. 1991). In addition, Fernández-Salas et al. (1994) referenced the scarce studies in the ecological and biological aspects of the larvae of *Anopheles* mosquitoes. Therefore, the main objectives of this study were to determine the development time of the different stages, the relationship between the climatic seasons and local habitats on the time at which the specimens remain in each stage of development and on the transitions to the subsequent stages of metamorphosis or death in *Anopheles pseudopunctipennis* and *Anopheles argyritarsis*.

*Anopheles pseudopunctipennis* was collected not only in puddles resulting from the flooding of streams of El Cadillal Dam but also in Mountain Rivers of Rosario de la Frontera, Vipos, and Potrero de las Tablas. As was mentioned by Shannon & Davis (1927), Rozeboom (1941), Hackett (1945), Manguin et al. (1996), and Dantur Juri et

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**Table 3. Accumulated risk for each transition (T1, T2, T3, and T4) over time.**

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Accumulated risk (T1)</th>
<th>Accumulated risk (T2)</th>
<th>Accumulated risk (T3)</th>
<th>Accumulated risk (T4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0.0791</td>
<td>0.10491</td>
<td>0.166667</td>
<td>0.83333333</td>
</tr>
<tr>
<td>3</td>
<td>0.0821</td>
<td>0.10491</td>
<td>0.166667</td>
<td>0.83333333</td>
</tr>
<tr>
<td>3.5</td>
<td>0.094</td>
<td>0.10491</td>
<td>0.166667</td>
<td>0.83333333</td>
</tr>
<tr>
<td>4</td>
<td>0.411</td>
<td>0.13517</td>
<td>0.838200</td>
<td>1.11800487</td>
</tr>
<tr>
<td>7</td>
<td>0.4226</td>
<td>0.13517</td>
<td>0.838200</td>
<td>1.11800487</td>
</tr>
<tr>
<td>7.5</td>
<td>0.4366</td>
<td>0.13517</td>
<td>0.838200</td>
<td>1.11800487</td>
</tr>
<tr>
<td>8</td>
<td>1.1857</td>
<td>0.20651</td>
<td>1.712920</td>
<td>1.2365731</td>
</tr>
<tr>
<td>9</td>
<td>1.1857</td>
<td>0.20651</td>
<td>1.714500</td>
<td>1.23815288</td>
</tr>
<tr>
<td>15</td>
<td>1.2718</td>
<td>0.20651</td>
<td>1.716084</td>
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<td>1.23815288</td>
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<tr>
<td>16</td>
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<td>2.632000</td>
<td>1.32223696</td>
</tr>
<tr>
<td>20</td>
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<td>0.20651</td>
<td>2.632000</td>
<td>1.32223696</td>
</tr>
<tr>
<td>21</td>
<td>3.4298</td>
<td>0.20651</td>
<td>3.623228</td>
<td>1.33100889</td>
</tr>
</tbody>
</table>

The transitions were the following: from larva to pupa (T1), from larva to death (T2), from pupa to adult (T3), and from pupa to death (T4).
**Table 4. Probability of Staying in the Different Stages (Larvae, Pupae, Adult, and Death) Over Time.**

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Larvae</th>
<th>Pupae</th>
<th>Adult</th>
<th>Death</th>
</tr>
</thead>
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**Fig. 5.** Probability of staying in one of the stages (larval, pupal, adult, and death) over time.
al. (in press), An. pseudopunctipennis larvae appear more frequently in bodies of water with oxygen that are generally clean, exposed to the sun, and exhibit the presence of Spirogyra algae. In addition, throughout this investigation, the same characteristics were found in the larval habitats of An. argyritarsis.

Manguin et al. (1996) characterized the larval habitats of An. pseudopunctipennis in the countries of America and provided information related to the larval habitats, including other species of anopheline larvae. Thus, in addition to larvae of An. pseudopunctipennis, larvae of An. aquasalis (at sea level) and An. argyritarsis (in a wide range of distribution) were collected on Grenada Island.

In the present work, as was described by Mühlen et al. (1925) and Manguin et al. (1996), a strong association between the presence of An. pseudopunctipennis and the presence of An. argyritarsis was found because both species were found together, although as different proportions, in the same larval habitats. We observed great seasonal fluctuation in the abundance of anopheline larvae with higher abundance during the autumn and spring seasons and a lower abundance during summer and complete absence during the winter. This finding is consistent with the results reported by Fernández-Salas et al. (1994). These researchers had found that An. pseudopunctipennis exhibited a marked seasonal fluctuation; because the larval habitats were negatively associated with the annual rainfall, and high abundance of larvae was found during the dry season.

In general, the studies conducted by Savage et al. (1990), Rejmankova et al. (1991), Berti et al. (1993), Fernández-Salas et al. (1994), Manguin et al. (1996), and Grillet (2000) focused on ecological aspects of anopheline larvae. In particular, Savage et al. (1990) and Rejmankova et al. (1991) reported on ecological aspects of An. pseudopunctipennis and An. albimanus larvae and the environmental factors that favor their presence; and asserted that the amount of filamentous algae and altitude as determinants of larval abundance. In addition, Fernández-Salas et al. (1994) referred to the possibility of finding larvae of An. pseudopunctipennis in different environments, such as plantations, forests, villages, cities, and swamps. Moreover, although most larval habitats are associated with streams and pools of stream banks, larvae are also found in ditches, ponds, lagoons, and holes in rocks. In addition, larvae have also been found in artificial containers, such as tanks, fountains (Rozeboom 1941), paddy fields, and marshy meadows (Downs et al. 1948). In the present study, An. pseudopunctipennis was the most abundant species in the puddles of El Cadillal Dam, and An. argyritarsis was the most abundant species in Potrero de las Tablas.
Mountain River. Hence, it could be stated that is the current findings are consistent with those previously reported.

In this work, the survival of the different stages was analyzed through multistate models. The number of individuals that made the transition from larva to pupa was higher than the number that made the transition from pupa to adult. We supposed that even though the larval stage was much more subjected to external agents that can affect development, it could survive most challenges. This suggests that the pupal stage is the more delicate and vulnerable.

In addition, the probability of being in the larval stage is close to 100% until the second day after hatching at which point it starts to decrease. This information is vitally important for control programs focused on the breeding sites of the immature stages. Managers of control programs need to know the precise moment at which to attack the larval stage. The results of this study show that the fraction of the population declines after the second day of the development of the first stage larvae. Thus the timing of larvicides must be made early to achieve a great reduction in the larval population, which reduction will be reflected in the subsequent pupal and adults populations. Indirectly, effective reduction of the larval population can influence the potential transmission of malaria.

With respect to the co-variables, which represent the climatic seasons and the local habitats, the transitions from larvae to death and from pupae to death were found to be significant in the summer when the risk of larval death decreased, and the same was observed with the pupae.

The significant transitions of the larvae and pupae during the summer plus decreased mortality during this season demonstrate the important effect of this climatic season on the presence of the larvae that will become pupae and adults. Generally, it is reported that the major abundance of larvae of *Anopheles* occurs during the spring or autumn seasons, when the rainfall has decreased. During the summer, heavy rains increase river flow, which eliminates breeding sites of the immature forms (Dantur Juri et al. in press). Also, Fernández Salas et al. (1994) reported that in Mexico the seasonal rainfall was directly and negatively associated with larval abundance of *An. pseudopunctipennis*. Heavy rainfall generally causes a decrease in the number of immature mosquitoes in both permanent and transitory habitats.

But Dantur Juri et al. (in press) also reported that in several breeding sites called foothill ravines of Aguas Blancas, which are close to Bermejo (Bolivia), the major abundance of *An. pseudopunctipennis* occurred during the spring and summer, i.e., similar to high abundance in breeding sites in this study. Based on this information, efforts should be made to exert a larval control in the breeding sites to avoid the major seasonal abundance of the larvae and pupae and indirectly of the adult malaria vectors.

The analysis of the effect of the local habitats on the transitions revealed that Rosario de la Frontera, Potrero de las Tablas and Vipos Rivers have a significant effect on the transition from larvae to death. Knowledge of the localities where the transition from larvae to death is significant should be a key consideration in deciding when to apply control measures at various breeding sites. These rivers are situated along a national road, where there is an intensive traffic of people throughout the year, and especially during the summer season. Measures to eliminate the larvae forms from these rivers could be an important step to prevent the production of adult vectors that attack travelers in this region.

As was shown in the present investigation, information on the biological and ecological aspects, including the seasonal fluctuations, of the larvae of the known malarial vector *An. pseudopunctipennis* larvae, as well as *An. argyritarsis*, which likely plays a role in the transmission of malaria, will raise awareness of the greater abundance of adult mosquitoes during the epidemiologically important spring season. These results will also raise awareness of the sur-

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PT, Potrero de las Tablas River in Tucumán province; RF, Rosario de la Frontera River in Salta province.

The α parameter measures the overall effect of each covariate on the risk of each of the transitions.

### Table 7. α parameter and its effect on the climatic seasons and the localities.

<table>
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<th>Transitions</th>
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<th>L -&gt; Death (T2)</th>
<th>P -&gt; A (T3)</th>
<th>P -&gt; Death (T4)</th>
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γ represents the intensity of the effect of each covariate on each transition.

**Table 8.**, *γ* parameter and its effects on the transitions (L: Larvae, P: Pupae, A: Adult).
vival time of larvae (48 h) and the relationship between the climatic seasons and localities on the time at which the specimens remain at each stage and the transitions to different stages, i.e., summer was observed to be the season that exerted the most influence on the transitions from larvae to death and from pupae to death, and Vi-pos River was the locality in which both of these transitions was lower, resulting in a reduced risk of larval death in this locality. These results are very important when considering different strategies for the malaria control through the mosquito control in the larval habitats.

REFERENCES CITED


