CHARACTERIZATION OF ANOPHELES PSEUDOPUNCTIPENNIS
LARVAL HABITATS

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ABSTRACT. A survey of Anopheles pseudopunctipennis larval habitats was performed throughout most of
its known geographic range. Eleven key environment variables characterized most larval habitats of this import-
tant vector of malaria in the Americas. Larval habitats occurred mainly in valley and foothill areas which were
often situated in arid regions. Immatures were found primarily during the dry season in sun-exposed freshwater
stream pools with clear, shallow, stagnant water containing abundant filamentous green algae and/or aquatic
vegetation.

INTRODUCTION

Anopheles (Anopheles) pseudopunctipennis Theodore is the most widely distributed anopheline mos-
quitoso in the New World (Rozeboom 1941). It is found from the southern USA (40°N) to the nor-
thern part of Argentina (30°S) along the Andes, with an eastern extension into Venezuela and the Lesser
Antilles. It is the most important malaria vector in the foothills of mountainous areas of Mexico and
Central and South America (Shannon and Davis 1927, Aitken 1945, Rodriguez and Loyola 1989).
These areas correspond to some of the more remote and rugged malaria-endemic areas of North, Cen-
tral, and South America. Anopheles pseudopuncti-
pennis is often the only vector present in areas
above 600 m and transmits malaria up to 2,800 m
in Bolivia (Hackett 1945, Gorham et al. 1973). In
the Americas, An. pseudopunctipennis is consid-
ered a major vector of malaria in 7 of 19 (37%) countries with endemic malaria (Pan American
Health Organization 1994), including Argentina,
Bolivia, Ecuador, Guatemala, Mexico, Nicaragua,
and Peru.
Previous studies have described An. pseudopunc-
tipennis larval habitats from different parts of the
Americas, including Shannon and Davis (1927),
Shannon (1930), Hoffmann (1931), Hoffmann and
Samano (1938), Root and Andrews (1938), Aitken
(1945), and Levi-Castillo (1945). In recent years,
characteristics of An. pseudopunctipennis larval
habitats also have been the subject of studies in
Mexico and Belize (Savage et al. 1990; Rejmanka-
ova et al. 1991, 1993; Fernandez-Salas et al.
1994). However, comparison and characterization
of An. pseudopunctipennis larval habitats from its
entire known geographic range has not been ac-
complished prior to this study.
An extensive investigation of An. pseudopuncti-
pennis along its neotropical distribution was under-
taken in 1991 (Manguin et al. 1995); the charac-
teristics of An. pseudopunctipennis larval habitats
are summarized in this paper.

MATERIALS AND METHODS

Study area: Anopheles pseudopunctipennis was col-
clected along its known geographic range and at
altitudes from sea level up to 2,340 m. Ten coun-
tries (Table 1) were chosen according to critical lo-
cations, such as the type-locality of An. pseudou-
actus pennis in Grenada Island, and areas where 5
subspecies and one variant of this species were de-
scribed (Knight and Stone 1977), and regions pro-
viding a spatial representation of the species’ geo-
graphic distribution. In the Caribbean, An. pseu-
dopunctipennis was sampled on Grenada Island. In
North America, collections were made in the state
of Texas and 2 areas of Mexico, Nuevo Leon
(northeast) and Chiapas (southwest). In Central
America, samples were taken in Belize and in 2
areas of Guatemala located along the drainage pat-
ters of both the Pacific and Atlantic coasts. In
South America, collections were made along the
coastal plain and/or in the Andes in Chile, Colom-
bia, Ecuador, Peru, and Argentina.
Mosquito collections: From 1991 to 1993, larvae
and pupae of An. pseudopunctipennis were collect-
ed mainly along rivers and tributaries in the 10
countries listed above. The longitude and latitude
of each habitat was recorded with a geographic po-
positioning system (Ensign GPS). A standard data
collection form developed by the Walter Reed Bio-
systematics Unit was used to record all the infor-
mation necessary for our study, such as collection
number, state, locality, date, time, type of collec-
tion, type of terrain and environment, and all the
characteristics related to larval habitats. For all
Anopheles-positive habitats, we recorded the type
of larval habitat, water current, depth, shade, veg-
etation (submersed, floating, emergent), and algae

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Table 1. Geographic information on 60 *Anopheles pseudopunctipennis* larval habitats of 4 regions and 10 countries.

<table>
<thead>
<tr>
<th>Region and country</th>
<th>State</th>
<th>Location</th>
<th>No. of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caribbean</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grenada</td>
<td>St. Patrick</td>
<td>Sallee River, Glassy River</td>
<td>Subtotal: 3</td>
</tr>
<tr>
<td>North America</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>USA</td>
<td>Texas</td>
<td>San Antonio: Fort Sam Houston (Area 9)</td>
<td>Subtotal: 2</td>
</tr>
<tr>
<td>Mexico</td>
<td>Nuevo Leon</td>
<td>Monterrey: El Carmen, El Rancho del TEC</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Chiapas</td>
<td>Tapachula, Coatan River: El Plan, El Retiro, La Ceiba</td>
<td>3</td>
</tr>
<tr>
<td>Central America</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guatemala</td>
<td>Escuintla</td>
<td>Escuintla: Guachipilin, Maria Santissima</td>
<td>Subtotal: 22</td>
</tr>
<tr>
<td></td>
<td>Zacapa</td>
<td>Usumatlan: La Palmilla</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>El Progreso</td>
<td>Guastatoya: Barrial, Morazan: Las Pericas</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Baja Verapaz</td>
<td>San Julian: El Patal</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Alta Verapaz</td>
<td>Tactic, Cobán: El Cruce</td>
<td>2</td>
</tr>
<tr>
<td>Belize</td>
<td>Cayo</td>
<td>Caves Branch, Sibun River, Silver Creek</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Cayo</td>
<td>Rio On</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Stann Creek</td>
<td>North Stann Creek</td>
<td>1</td>
</tr>
<tr>
<td>South America</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colombia</td>
<td>Valle</td>
<td>Florida</td>
<td>2</td>
</tr>
<tr>
<td>Ecuador</td>
<td>Imbabura</td>
<td>Salinas</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Pichincha</td>
<td>quito: Tumbaco</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Guayas</td>
<td>Guayaquil: Bacay, El Triunfo</td>
<td>3</td>
</tr>
<tr>
<td>Peru</td>
<td>Lima</td>
<td>Hacienda Villa, Rio Chillon</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Lima</td>
<td>Huachipa, Cieneguilla</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Cuzco</td>
<td>Quillabamba</td>
<td>1</td>
</tr>
<tr>
<td>Chile</td>
<td>Tarapacá</td>
<td>Arica: Rio Lluta, km25, km30, km35, km41, km53</td>
<td>Subtotal: 27</td>
</tr>
<tr>
<td></td>
<td>Tarapacá</td>
<td>Rio Azapa</td>
<td>1</td>
</tr>
<tr>
<td>Argentina</td>
<td>Salta</td>
<td>Puente Polares, Alemania, Santa Barbara</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Tucumán</td>
<td>Rio Tapi, Rio Vipos</td>
<td>2</td>
</tr>
</tbody>
</table>

Results

The following results refer only to *An. pseudopunctipennis* larvae. Data on larval habitats are based on 60 positive collection sites located in 10 different countries (Table 1). The number of collecting sites by region varied from 3 in the Caribbean, where one country was sampled, to 27 in South America, where collections were made in 5 countries.

Types of environment, terrain and larval habitat (Table 2): Of 60 larval collections, 40% were made in relatively dry environments occurring in 7 of 10 sampled countries. In 5 countries, 27% of larval sites were located on agricultural land, such as orange groves, pastures, banana plantations, coffee plantations, and cultivated fields. Environments classified as forests (evergreen, coniferous, and cloud) were encountered in 3 countries. Finally, larval habitats also were found near villages, swampy areas, and prairies.

Larval habitats were encountered in 4 types of terrain. Valleys and foothills were the most common, with a frequency of 52 and 38%, respectively. Larvae also were collected in 4 sites along the coastal plain of Grenada and Peru, and from 2 sites along the Andean plateau of Argentina and Ecuador.

Half of the larval habitats were defined as stream pools, often with rocky bottom, or, in 23% of the cases, as stream margins. However, larvae were collected occasionally in other types of larval habitats, such as spring-seepages, ditches, ground pools, lagoons, and rock pools.

Color and depth of water (Table 3): Water was clear in 54 of the 60 positive larval habitats. Only 4 habitats had colored water, and 2 had turbid water. In one case, the turbidity was caused by con-
Almost half of the sites had an acidic to neutral pH, including 16 habitats with a pH below 7.0. Only one larval site had a pH as low as 4.5. Water conductivity values varied between 45 and 8,350 μS, but the majority of larval sites had freshwater with conductivity values lower than 650 μS.

**Table 1. Extended.**

<table>
<thead>
<tr>
<th>Elevation (m)</th>
<th>Longitude/Latitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>12°12'–11°N/61°37'W</td>
</tr>
<tr>
<td>214</td>
<td>29°25'N/98°30'W</td>
</tr>
<tr>
<td>400</td>
<td>25°29–35°N/100°11–21'W</td>
</tr>
<tr>
<td>400–480</td>
<td>14°47–15°00’N/92°28’W</td>
</tr>
<tr>
<td>40</td>
<td>16°05’N/93°45’W</td>
</tr>
<tr>
<td>250–320</td>
<td>14°15’N/90°47’W</td>
</tr>
<tr>
<td>500</td>
<td>15°00’N/89°30’W</td>
</tr>
<tr>
<td>600</td>
<td>14°50’N/90°00’W</td>
</tr>
<tr>
<td>1,400</td>
<td>15°15’N/90°30’W</td>
</tr>
<tr>
<td>1,500</td>
<td>15°20’N/90°20’W</td>
</tr>
<tr>
<td>60–80</td>
<td>17°06–10°N/88°36–43’W</td>
</tr>
<tr>
<td>480</td>
<td>17°59’N/88°58’W</td>
</tr>
<tr>
<td>80</td>
<td>17°02’N/88°32’W</td>
</tr>
<tr>
<td>1,010</td>
<td>3°20’N/76°12’W</td>
</tr>
<tr>
<td>1,880</td>
<td>0°30’N/78°10’W</td>
</tr>
<tr>
<td>2,340</td>
<td>0°17S/78°32’W</td>
</tr>
<tr>
<td>10</td>
<td>2°16’S/79°20–53’W</td>
</tr>
<tr>
<td>3–100</td>
<td>11°50–12°15’S/76°50–77°00’W</td>
</tr>
<tr>
<td>300–320</td>
<td>12°00–10°S/76°50’W</td>
</tr>
<tr>
<td>988</td>
<td>12°50’S/72°50’W</td>
</tr>
<tr>
<td>200–850</td>
<td>18°20’S/69°30’W</td>
</tr>
<tr>
<td>274</td>
<td>18°20–30’S/69°30–70°00’W</td>
</tr>
<tr>
<td>1,160–1,440</td>
<td>25°00–50’S/65°15’W</td>
</tr>
<tr>
<td>700–800</td>
<td>26°30–40’S/65°20’W</td>
</tr>
</tbody>
</table>

Most larval habitats had shallow water with over 60% being less than 10 cm deep. The association of shallow water with rocky bottom increased the difficulty of dipping for larvae.

**Types of water movement, water body and sun exposure** (Table 3): Stagnant water in larval sites was predominant over moving water in all countries except Grenada where the current was slow in all 3 larval collections. Sixty percent of water bodies were temporary. A total of 88% of the sites were exposed to the sun, including 50% of heavy and 38% of partial presence of sunlight. Only 12% of the sites were fully shaded.

**pH and conductivity** (Table 4): Larvae were collected in water with wide pH values ranging from 4.5 to 8.8. Almost half of the sites had an acidic to neutral pH, with values ranging between 6.02 and 7.0. Only one larval site had a pH as low as 4.5 (Huachipa, Peru). The other half of the sites had an alkaline pH, including 16 habitats with a pH below 8.0 and 9 sites with a pH between 8.0 and 8.8.

Water conductivity values varied between 45 and 8,350 μS, but the majority of larval sites had freshwater with conductivity values lower than 650 μS. Brackish water was found in 10% of larval habitats (conductivity > 5,000 μS).

**Altitude:** Larvae were found from sea level up to 2,340 m, but the collections were mostly made at altitudes below 100 m (36%) and between 100 and 500 m (33%). Above 500 m, larval sites were not always accessible, and the frequency of sampling was reduced to 14% between 500 and 1,000 m and 15% between 1,000 and 2,000 m. Only one larval site, located in the Andes of Ecuador, was sampled above 2,000 m (Table 1).

**Presence and absence of algae and vegetation** (Table 5): In all 10 countries, larvae were highly associated with algae, with a frequency of 93%. Only 4 exceptions occurred, in Belize, Chile and 2 sites in Guatemala where no visible algae were present. In most collections, larvae were associated with mats of either green or filamentous green algae. Different types of green algae such as Cladophora and Enteromorpha were found, but larvae were most commonly associated with the Spirogyra-type, a filamentous green alga.

Most larval sites had emergent, floating, and submerged vegetation. Emergent vegetation was most common. A mixture of these 3 categories of vegetation was also positively associated with the presence of larvae. No larvae were collected in habitats where both algae and vegetation were absent.

**Associated anopheline species:** Of the 60 larval collections of An. pseudopunctipennis, 40% contained larvae of other Anopheles species. In the Caribbean (Grenada), An. pseudopunctipennis larvae were sympatric with 2 Anopheles species (Manguin et al. 1993): Anopheles aquasalis Curry, occurring at sea level, and Anopheles argyritarsis Robineau-Desvoidy, a ubiquitous species in Grenada that was found in a wide range of altitudes (6–480 m). In North America, An. pseudopunctipennis larvae were collected in association with Anopheles punctipennis (Say) larvae in one of the 2 habitats sampled in Texas (USA). In Central America, An. pseudopunctipennis larvae were found in high-altitude habitats (1,400–1,500 m) with Anopheles hectoris Giaquinto-Mira in Guatemala, and in association with 3 species, including Anopheles albimanus Wiedemann, An. argyritarsis, and Anopheles darlingi Root, in Belize at elevations between 60 and 80 m. In South America, larvae of the An. albimanus Section were associated with An. pseudopunctipennis in 2 habitats in Argentina (700 and 1,160 m), one in Ecuador (10 m), and one in Peru (988 m). Larvae of the An. argyritarsis Section were collected with An. pseudopunctipennis in all 5 larval habitats sampled in Argentina (700–1,186 m) and one in Chile (847 m). Finally, larvae of Anopheles punctimacula Dyar and Knab and An. pseudopunctipennis were found in sympathy in one Ecuadorian habitat (10 m).

**DISCUSSION**

Our survey of An. pseudopunctipennis larval habitats throughout its geographic range confirmed contamination with cow feces (Salinas, Ecuador) and in another case by a recent flood (Monterrey, Mexico).
Table 2. Number and frequency of environment, terrain, and larval habitat types and country for positive *Anopheles pseudopunctipennis* larval collections.

<table>
<thead>
<tr>
<th>Environment</th>
<th>Frequency (%)</th>
<th>Country*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dry area</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Scrub</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>2. Desert</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td>24</td>
<td>40</td>
</tr>
<tr>
<td><strong>Plantation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Orange grove</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>2. Pasture</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>3. Banana plantation</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>4. Coffee plantation</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>5. Cultivated field</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td>16</td>
<td>27</td>
</tr>
<tr>
<td><strong>Forest</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Evergreen forest</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>2. Coniferous forest</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>3. Cloud forest</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td>11</td>
<td>19</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Village</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>2. Swamp</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>3. Prairie</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td><strong>Terrain</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Valley</td>
<td>31</td>
<td>52</td>
</tr>
<tr>
<td>2. Foothill</td>
<td>23</td>
<td>38</td>
</tr>
<tr>
<td>3. Coastal plain</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>4. Plateau</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td><strong>Larval habitat</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Stream pool</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>2. Stream margin</td>
<td>14</td>
<td>23</td>
</tr>
<tr>
<td>3. Spring-seepage</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>4. Ditch</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>5. Ground pool</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>6. Lagoon</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>7. Rock pool</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

*AR, Argentina; BHZ, Belize; CH, Chile; COL, Colombia; ECU, Ecuador; GR, Grenada; GT, Guatemala; MX, Mexico; PE, Peru; USA, United States.*

The previous findings that larvae occur most frequently in freshwater stream pools with still, shallow, clean water and abundant filamentous green algae (Hoffmann 1927, 1931; Shannon 1930; Hoffmann and Samano 1938; Rozeboom 1941; Hackett 1945). Recent studies in Mexico and Belize on environmental associations of *An. pseudopunctipennis* larvae identified 3 principal positive variables: filamentous green algae, altitude, and shallow water (Savage et al. 1990; Rejmankova et al. 1991, 1993; Fernandez-Salas et al. 1994). In order to provide a more comprehensive characterization of *An. pseudopunctipennis* larval habitats, the present survey was conducted over most of the known geographic range of the species.

The majority of larval habitats were found in dry environments in valleys or foothills, confirming earlier findings that this species inhabits arid canyons and valleys where the immature forms find ideal conditions for growth in the small, slow-moving streams and side pools of receding rivers containing a rich growth of green algae (Shannon and Davis 1927, Shannon 1930, Aitken 1945). Larvae also are tolerant of water temperature fluctuations and drought (Gorham et al. 1973). Hoffmann (1931) defined *An. pseudopunctipennis* as a xerophile species with a peak abundance during the dry season. Seasonal rainfall has been reported to be negatively associated with larval abundance (Savage et al. 1990, Fernandez-Salas et al. 1994). Heavy rains cause rivers and tributaries to rise suddenly and transform into rapidly flowing waters. As a consequence, river pools containing filamentous algae and larvae are purged. In addition, water which becomes muddy is unsuitable for *An. pseudopunctipennis* larvae (Hoffmann 1931). The increase in numbers of pools in the riverbed was inversely related to rainfall, and it seems likely that with the
**Table 3.** Depth of water, types of water movements, water bodies, and sun exposure for positive *Anopheles pseudopunctipennis* larval collections with country association.

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Depth (d)</strong></td>
<td></td>
</tr>
<tr>
<td>d ≤ 5 cm</td>
<td>26</td>
</tr>
<tr>
<td>5 cm &lt; d ≤ 10 cm</td>
<td>12</td>
</tr>
<tr>
<td>10 cm &lt; d ≤ 50 cm</td>
<td>10</td>
</tr>
<tr>
<td>50 cm &lt; d ≤ 1 m</td>
<td>10</td>
</tr>
<tr>
<td>1 m &lt; d ≤ 2 m</td>
<td>2</td>
</tr>
<tr>
<td>Range: 2 cm–2 m</td>
<td>60</td>
</tr>
<tr>
<td><strong>Water movement</strong></td>
<td></td>
</tr>
<tr>
<td>Stagnant</td>
<td>38</td>
</tr>
<tr>
<td>Slow</td>
<td>21</td>
</tr>
<tr>
<td>Moderate</td>
<td>1</td>
</tr>
<tr>
<td><strong>Water body</strong></td>
<td></td>
</tr>
<tr>
<td>Temporary</td>
<td>36</td>
</tr>
<tr>
<td>Permanent</td>
<td>24</td>
</tr>
<tr>
<td><strong>Sun exposure</strong></td>
<td></td>
</tr>
<tr>
<td>Heavy</td>
<td>30</td>
</tr>
<tr>
<td>Partial</td>
<td>23</td>
</tr>
<tr>
<td>Absent</td>
<td>7</td>
</tr>
</tbody>
</table>

1. AR, Argentina; BHZ, Belize; CH, Chile; COL, Colombia; ECU, Ecuador; GR, Grenada; GT, Guatemala; MX, Mexico; PE, Peru; USA, United States.

Sudden disappearance of preferred oviposition sites, females begin selecting rain pools as alternative sites (Fernandez-Salas et al. 1994).

Larval habitats were found in various environments, such as plantations, forests, villages, swamps, and prairies. While most larval habitats were associated with stream pools and stream margins, larvae were also found in spring-seepages, ditches, ground pools, lagoons, and rock pools (Table 2). Howard et al. (1917) characterized the association of *An. pseudopunctipennis* larvae with clean water. Larvae have also been reported from artificial containers, such as reservoirs, tanks, fountains, well-holes (Rozeboom 1941), rice paddies, and marshy meadows (Downs et al. 1948). Some of these unusual habitats had larvae only during the rainy season when females apparently oviposit in alternative habitats.

During our study, larvae were mainly collected in clear, shallow stream pools with rocky bottoms. A large majority of the sites were exposed to the sun, which is in agreement with earlier findings.

**Table 4.** pH and conductivity of 48 positive *Anopheles pseudopunctipennis* larval collections.

<table>
<thead>
<tr>
<th>Conductivity (C)</th>
<th>Frequency</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pH</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acidic-neutral (4.5 ≤ pH ≤ 7.0)</td>
<td>23</td>
<td>48</td>
</tr>
<tr>
<td>Alkaline (7.0 &lt; pH ≤ 8.8)</td>
<td>25</td>
<td>52</td>
</tr>
<tr>
<td>Range: 4.5–8.8</td>
<td>48</td>
<td>100</td>
</tr>
<tr>
<td><strong>Conductivity (C)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Freshwater (c ≤ 650 μS)</td>
<td>27</td>
<td>56</td>
</tr>
<tr>
<td>Slightly brackish (650 μS &lt; c ≤ 2,000 μS)</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>Moderately brackish (2,000 μS &lt; c ≤ 5,000 μS)</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>Brackish (c &gt; 5,000 μS)</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Range: 45–8,350</td>
<td>48</td>
<td>100</td>
</tr>
</tbody>
</table>

1. AR, Argentina; BHZ, Belize; CH, Chile; COL, Colombia; ECU, Ecuador; GR, Grenada; GT, Guatemala; MX, Mexico; PE, Peru; USA, United States.
### Table 5. Presence/absence on types of algae and vegetation for 60 positive *Anopheles pseudopunctipennis* larval collections.

<table>
<thead>
<tr>
<th></th>
<th>Frequency (%)</th>
<th>Country¹</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Algae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absence</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Presence</td>
<td>56</td>
<td>93</td>
</tr>
<tr>
<td>Type of algae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Green</td>
<td>31</td>
<td>55</td>
</tr>
<tr>
<td>2. Filamentous-green</td>
<td>21</td>
<td>37</td>
</tr>
<tr>
<td>3. Green and red</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>4. Red</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td><strong>Vegetation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absence</td>
<td>14</td>
<td>23</td>
</tr>
<tr>
<td>Presence</td>
<td>46</td>
<td>77</td>
</tr>
<tr>
<td>Type of vegetation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Emergent</td>
<td>21</td>
<td>46</td>
</tr>
<tr>
<td>2. Floating</td>
<td>13</td>
<td>28</td>
</tr>
<tr>
<td>3. Submersed</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>4. Floating and emergent</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>5. Submersed and floating</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>6. Submersed and emergent</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

¹ AR, Argentina; BHZ, Belize; CH, Chile; COL, Colombia; ECU, Ecuador; GR, Grenada; GT, Guatemala; MX, Mexico; PE, Peru; USA, United States.

(Shannon and Davis 1927, Rozeboom 1941, Hackett 1945). The development of algae is dependent on the presence of sunlight and *An. pseudopunctipennis* larvae are significantly (P < 0.0001) associated with the presence of green algae (Rejmankova et al. 1993); therefore, its larval habitats and sunlight are also associated.

Larvae were mostly collected in habitats with stagnant water; however, larvae also were found in flowing water where the presence of green algae seemed to drastically reduce water current velocity within the habitat. Larvae and pupae were often concentrated on top and inside of thick mats of filamentous green algae. Phytoecological relationships between *An. pseudopunctipennis* larvae and green-filamentous algae have been reported throughout the species’ geographic distribution (Shannon and Davis 1927; Root and Andrews 1938; Aitken 1945; Hackett 1945; Savage et al. 1990; Rejmankova et al. 1991, 1993; Fernandez-Salas et al. 1994). Five genera of green algae were significantly associated with larval habitats: the most prevalent was Spirogyra (especially *S. mameara* and *S. mayuscula*), followed by *Oedogonium, Chladorphora, Closterium,* and *Enteromorpha.* The latter alga is known as an indicator of brackish water and was found in abundance in the Sallee River of Grenada Island (Manguin et al. 1993). *Spirogyra* algae form mats that provide not only shelter to *An. pseudopunctipennis* larvae, especially against predators and water current, but also food (Hoffmann and Samano 1938). It is not uncommon to collect larvae with a green color from feeding heavily on green algae. In most cases, abundant algal growth was a key factor for the presence of larvae. Fernandez-Salas et al. (1994) stated that in Mexico (Coatan River, Chiapas), *An. pseudopunctipennis* larval densities were positively associated with the percentage of surface area covered with filamentous algae (P = 0.0001). Range limits for mean numbers of larvae collected were 16.7-53.9 larvae per m² of filamentous algae (Fernandez-Salas et al. 1994). However, during our survey, larvae were also collected in habitats without any visible algae. In different localities, Hoffmann (1927) observed large quantities of larvae between stones and small sand bars of rivers, without the presence of noticeable vegetation, algae, or other aquatic flora. During our survey, larvae were never found in habitats without either algae or aquatic vegetation. Most larval habitats contained various vegetation types—emergent, floating, submersed, or a mixture of these different types. In river pools in southern Mexico, larvae have been positively associated with emergent vegetation such as *Ludwigia octovalvis, Panicum spp., Paspalum spp.,* and *Cyperus spp.* (Fernandez-Salas et al. 1994), whereas in spring seepages, larvae have been associated with *Heteranthera limosa,* a semi-aquatic plant, as well as floating leaves or plants common in ponds, such as a water hyacinth, *Eichhornia crassipes,* and a water lettuce, *Pistia stratiotes.* In Mexico, *Heteranthera reniformis* was associated with *An. pseudopunctipennis* larval habitats along stream margins and floodplain pools during the dry season (Savage et al. 1990). In Grenada, Root and Andrews (1938) observed larvae in floating mats of *Ceratophyllum.* In agreement with Rozeboom (1941), we found that *An. pseudopunctipennis* larvae move onto the upper surface and partially ad-
here to leaves or other floating vegetation. Emergent and floating vegetation, either macrophytes or microphytes, probably have direct or indirect roles in stimulating An. pseudopunctipennis females to select particular oviposition sites (Fernandez-Salas et al. 1994).

Larvae were collected in acidic, neutral, and alkaline water, with pH values ranging from 6.02 to 8.8. Only one site in Peru (Huachipa) had a pH as low as 4.5. In Ecuador, Levi-Castillo (1945) found the water of An. pseudopunctipennis larval sites to be alkaline with a pH ranging from 7.5 to 8.5. We found that most larval habitats contained freshwater with conductivities below 650 \( \mu S \), but larvae were also found in slightly brackish, moderately brackish, and brackish water with a conductivity up to 8,350 \( \mu S \). In the Salle River on Grenada Island, where An. pseudopunctipennis larvae represented 99% of those collected, the river was fed by mineral springs rich in various salts which drastically increased the water conductivity (Manguin et al. 1993). In Peru, some larvae were collected in coastal plain habitats that were fed by seawater infiltration. In the Argentine and Ecuadorian Andes, larvae were found in high-elevation river pools (1,186 and 1,880 m, respectively) that were rich in salts.

During our survey, An. pseudopunctipennis larvae were collected from sea level up to 2,340 m in the Andes. In other studies, larvae were reported at elevations up to 2,300 m in Mexico, 2,800 m in Bolivia, and 3,200 m in Peru (Hoffmann 1931, Aitken 1945, Vargas and Martinez-Palacios 1956, Gorham et al. 1973). Anopheles pseudopunctipennis occurs at near the highest elevation from which malaria is endemic in the Western Hemisphere (Levi-Castillo 1945) and the world (Hackett 1945). However, An. pseudopunctipennis larvae are not restricted to high elevation habitats, but also occur at elevations as low as sea level.

Hackett (1945) stated that several other anopheline species were found to be consistent for the majority of An. pseudopunctipennis larval habitats throughout its geographic distribution. We found that larval habitats of An. pseudopunctipennis mainly occurred in valleys and foothills which were frequently situated in dry environments. Larvae were found in sun-exposed freshwater stream pools containing abundant filamentous green algae and aquatic vegetation, with clear, shallow, and stagnant water. The highest population densities of this species occurred during the dry season. The association of these different variables with An. pseudopunctipennis larval habitats improves our knowledge of the species as well as aids in the development of potential strategies to control this important malaria vector.

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REFERENCES CITED


Vargas, L. and A. Martinez-Palacios. 1956. Anofelinos Mexicanos. Taxonomía y distribución. Secretaría de Salubridad y Asistencia, Mexico, D.F.