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Factors Associated with Distribution of *Anopheles aquasalis* and *Anopheles oswaldoi* (Diptera: Culicidae) in a Malarious Area, Northeastern Venezuela

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ABSTRACT Spatial and temporal abundance patterns of anopheline larvae and their relationships with wetland conditions were studied in an endemic malaria area in northeastern Venezuela, where *Anopheles aquasalis* Curry is the main vector. Larvae were sampled over a 2-yr period in 7 wetland types (brackish and freshwater herbaceous swamps, mangrove swamps, freshwater ponds, clear-cut marsh forests, small irrigation canals, and swamp forests), covering 3 environmental gradients (salinity, aquatic vegetation, and habitat permanence). Twelve variables were quantified to describe each habitat. Two species of anophelines were collected. *An. aquasalis* was the species with the widest distribution, and its highest abundance was in the seasonal brackish mangrove habitat during the rainy season. *An. oswaldoi* Peryassu was rarely encountered, but was mainly associated with the dry season and with the permanent freshwater wetlands (such as ponds). Principal components and correlation analyses revealed that the physicochemical (salinity, dissolved oxygen) variables of the wetland were associated most strongly with the spatial distribution of both species. Variations in salinity were strongly associated with the abundance of *An. aquasalis.* Both the occurrence and abundance of *An. oswaldoi* were most closely correlated with dissolved oxygen. Changes in seasonal abundance of both species were associated with rainfall. The relevance of these results to vector control in northern Venezuela is discussed.

KEY WORDS *Anopheles aquasalis*, *Anopheles oswaldoi*, spatial and temporal distribution, larval habitats, environmental factors, malaria

MOSQUITOES (DIPTERA: CULICIDAE) are often dominant members of wetland ecosystems where the larvae play an important trophic role as filter-feeders (Wallace and Merritt 1980). Assuming that the species distributions are nonrandom and predictable based on habitat conditions, the characterization and interpretation of distribution and abundance patterns of immatures can provide insight about how mosquito communities are structured. Physical factors, such as habitat permanence or degree of spatial heterogeneity, and biotic interactions, such as predation, are known to influence wetland mosquito species assemblages (Batzer and Wissinger 1996). These generalizations are based on studies in temperate ecosystems and it is uncertain whether they apply to Neotropical mosquito populations because few comparative data exist (e.g., Rejmankova et al. 1991, 1993).

Knowledge of mosquito distribution patterns is critical when considering the management of vector species. In Mexico and Belize, for example, the determination of spatial and temporal distribution patterns of anopheline species has provided insights into the dynamics of malaria transmission, and has resulted in efficient monitoring of vector breeding sites (Pope et al. 1994).

Most studies on preadult anopheline ecology in the Americas have been more descriptive than comprehensive (see review in Zimmerman 1992). However, recent work on *An. albimanus* Wiedemann and *An. pseudopunctipennis* Theobald in Mexico and Belize have demonstrated a strong association between larval distribution and the distribution of some habitat factors: cyanobacterial mats and filamentous algae (Rejmankova et al. 1991, 1993).

Anopheles aquasalis Curry is considered the main coastal *Plasmodium vivax* malaria vector from northeastern Venezuela (Berti et al. 1993b) to southern Brazil (Fleming 1986). Larvae of *An. aquasalis* have been collected in brackish (Senior-White 1951, Berti et al. 1993a) and fresh water habitats (Ayroza Galvao et al. 1942, Berti et al. 1993a); in habitats with (Senior-White 1951) and without, aquatic vegetation (Silvain and Pajot 1981); and in permanent and seasonal wetlands (Berti et al. 1993a). Although these studies have been mainly descriptive, with few attempts to relate preadult distribution with any of those environmental factors, they suggest that *An. aquasalis* is adapted to a

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Table 1. Wetland descriptions in northeastern Venezuela

Habitat (code)	Water salinity	Dominant aquatic vegetation species	Habitat permanence
Mangroves (M)	Mesosaline	Basin mangrove: *Avicenia germinans*	Seasonal
	Polysaline	Coastal mangrove: *Rhizophora* sp., *Laguncularia racemosa*	Seasonal
Brackish herbaceous swamps (Bhs)	Oligosaline	*Eleocharis mutata* (emergent)	Permanent
	Mesosaline	*Eleocharis mutata* (emergent)	Seasonal
Freshwater herbaceous swamps (Fhs)	Freshwater	*Typha dominguensis* (emergent)	Permanent
		Cyperus articulatus (emergent)	Seasonal
Ponds (P)	Freshwater	*Cyperus* sp. (emergent)	Temporal
		Typha dominguensis (emergent)	Seasonal
		Ludwigia affinis (emergent)	Permanent
		Lemna sp., *Nymphaea* sp. (floating)	
Clear-cut marsh forests (Ccmf)	Freshwater	*Colocasia* sp. (emergent)	Temporal
		Lemna sp., *Pistia* sp. (floating)	
Canals (C)	Freshwater	No aquatic vegetation	Temporal
Swamp forests (Sf)	Freshwater	No aquatic vegetation	Temporal

broad range of ecological conditions, and that separate studies for each particular geographic area must be made to understand and explain species distribution.

The study reported here was conducted to determine the spatial and temporal distribution of anopheline species in relation to breeding site conditions in a diversity of wetlands in a malaria endemic area in northeastern Venezuela. Gradients of 3 environmental parameters were chosen for this purpose. The parameters were water salinity, aquatic vegetation, and annual habitat permanence. These gradients can be interpreted as measures of adversity (Southwood 1977), structural heterogeneity (Orr and Resh 1989), and frequency of habitat disturbance (Schneider and Frost 1996), respectively. They are considered critical axes along which the wetland mosquito communities should be organized (Batzer and Wissinger 1996).

The information gathered in this study should contribute to the understanding of species assemblage patterns of Neotropical mosquitoes and will help form the basis for future integrated control programs for malaria vector control in northeastern Venezuela. This study is part of a multidisciplinary effort (biosystematic, ecological, and vector control) aimed at the control of *An. aquasalis* larvae with the use of biocontrol agents (see Delgado et al. 1998, Grillet et al. 1998).

The specific objectives of the study were to characterize physicochemical, structural, and biotic factors in the larval mosquito habitat; describe spatial and temporal patterns of mosquito abundance in relation to selected environmental parameters; and identify the factors that influence the abundance and distribution of mosquito species in the habitat.

Materials and Methods

Study Site. The study was carried out in the southern coastal lowland areas of the Paria Peninsula (10° 17′ N, 63° 57′ W), 0–10 km from the littoral zone, in Sucre State, northeastern Venezuela. Annual mean temperature is 27–28°C and total annual rainfall is 1,200–1,700 mm, with a rainy season from May to November and a dry season from December to April. The vegetation is dominated by deciduous forests, a relatively undisturbed area of coastal mangroves, and herbaceous and woody swamps (Huber and Alarcón 1988). Aquatic habitats were classified using 3 parameters: water salinity, the dominant species of aquatic vegetation (>60% of cover vegetation), and habitat permanence. Categories of salinity for wetlands were freshwater, oligosaline, mesosaline, and polysaline and refer to saline contents of <0.5, 0.5–5, 5.1–18, and >18 ppt, respectively. Habitat permanence was determined as the number of months per year the habitat contained any standing water. Permanent wetlands are wet all year. Seasonal wetlands are dry for 3 or 4 mo each year. Temporal wetlands are dry for 6–8 mo each year. The first 14 mo of the sampling period in this study provided the basis for defining habitat permanence. Within the categories for habitat permanence, the term marsh applies to wetland having marked seasonal fluctuations in moisture conditions ranging from water-logged, in the rainy season, to dry in at least the upper layers of the soil in the dry season (Fanshawe 1952). When the term swamp is used, it means that the soil is rarely dry; the land may be inundated year-round, or for varying periods, and that it has a high water table (Fanshawe 1952).

Habitat Types. Seven wetlands were chosen as a representative and contrasting subset of natural wetlands prevailing in this region. These differed in size, shape, and substratum type and were termed brackish and freshwater herbaceous swamps, mangrove swamps, freshwater ponds, clear-cut marsh forests, small irrigation canals, and swamp forests. A brief description of each habitat type is given in Table 1.

Temporal Sampling Design. To determine the temporal (within year) distribution of *Anopheles* species, a sampling was performed at 10 sites, 2 of each habitat type (mangrove, brackish and freshwater herbaceous swamp, pond, and clear-cut marsh forest), at ≈3-wk intervals over a 14-mo period (July 1993–August 1994).

Spatial Sampling Design. To determine spatial (among habitats) distribution of *Anopheles* species, a sampling was carried out at 28 rainy season (corre-

sponding 4 mangrove, 6 brackish herbaceous swamp, 3 freshwater herbaceous swamp, 4 pond, 3 clear-cut marsh forest, 4 canals, and 4 swamp forest habitats) and 16 dry season sites (corresponding 1 mangrove, 3 brackish herbaceous swamp, 5 freshwater herbaceous swamp, 2 ponds, 4 canals, and 1 swamp forest habitats). Accessibility and extensive time needed to make the sampling limited the number of sampled sites in each habitat type. Each site was visited once at each occasion (October 1994, corresponding rainy season, and February 1995, corresponding dry season), although many of the sites previously surveyed in the rainy season were dry during the dry season. The temporal sampling provided the basis for selecting these 2 mo in which the highest (October) and lowest (February) larval abundance of *An. aquasalis* (the dominant anopheline species of the study area) was observed.

Mosquito Sampling. Larvae were sampled with a long-handled dipper (1.5 liter), and the number of larvae per dip was calculated from 30 such samples from each collection site. Dipper samples were made in a transect at 1.5-m intervals, 2–3 m from the shore. Samples were always taken by the same individuals. During each sampling, the habitat was visited at the same morning (0700–1100 hours) or afternoon (1500–1800 hours) hours, every month (temporal sampling) or season (spatial sampling). Third- and 4th-instar larvae and pupae of anophelines were picked from the dipper sample, transported to the laboratory, and reared to the adult stage; the associated larval and pupal exuviae were used for species identification using the characters described by Cova García and Sutil (1977) and by Faran and Linthicum (1981). Water from each sample was sieved through a 0.5-mm mesh screen, and the samples were preserved in 80% ethanol (20 ml volume) to enable identification of all anopheline larvae in each sample.

Environmental Variables. At each occasion a site was visited; 12 variables were measured previously in the environment. The 12 variables were as follows: (1) habitat permanence (permanent, seasonal, or temporal), (2) type of dominant aquatic vegetation (emergent, floating, or submerged), (3) total cover of dominant aquatic vegetation (mean from 6 replicates; 0.5 by 0.5-m quadrats; Braun-Blanquet method, Mueller-Dumbois and Ellenberg 1974), (4) mean height of emergent dominant vegetation above the water surface (6 replicates), (5) chlorophyll *a* content (Wetzel and Likens 1991), (6) mean water depth (6 replicates), (7) temperature, (8) pH, (9) salinity, (10) alkalinity, (11) dissolved oxygen, and (12) dissolved carbon dioxide. Portable field meters were used for pH (model 5996–70, Cole Palmer, (Chestertown, MD)), and for salinity and water temperature (model 33, YSI). Chemical Kits (Standard LaMotte, LaMotte Chemical, (Vernon Hills, IL)) were used for dissolved oxygen, dissolved carbon dioxide, and alkalinity. Spot measures were made for variables 7–12 at the depth of mosquito sampling (up to 17–20 cm below the surface) in 1 site chosen randomly in the wetland. Phytoplankton density was estimated by measuring chlorophyll *a* concentration at each collection site from a 500-ml sample of water taken 30 cm below the surface in a plastic bottle, with the sample concentrated to 2 Whatman (0.45 μm) GF/F filters (Whatman, Hillsboro, OR) and stored at 4°C in the dark. Chlorophyll *a* was extracted from each filter sample using 90% methanol (for freshwater samples) or acetone (for saline water samples). Extractions were performed in the dark for 24-h. Spectrophotometric readings were made at 665 and 750 nm, before and after acidification of each sample (with HCl), to correct for chlorophyll degradation products in the sample (Wetzel and Likens 1991).

Data Analyses. The $\log_{10}(x + 1)$ or $\log_{10}$ transformation (larval abundance, height of dominant emergent vegetation, chlorophyll *a* content, water depth, salinity, alkalinity, dissolved oxygen, and dissolved carbon dioxide) and angular transformation (total cover of dominant aquatic vegetation) were made before the statistical analysis (Zar 1999). The data from each temporal and spatial sampling period were analyzed separately. For the temporal analysis, data for the 2 sites within each of the 5 wetland types were combined. Similarly, in the dry or rainy season spatial sampling, the samples of each wetland type were combined. We used a multivariate approach, principal components analysis, to elucidate environmental gradients in the studied wetlands (ter Braak 1987). Principal component analysis was carried out to reduce and order the environmental data into a smaller number of statistically independent principal components. Each principal component is a linear combination of the original habitat variables, with each successive principal component accounting for a smaller percent of the variation in the original data set. A preliminary examination of the habitat data suggested that a linear description of relationships among habitat variables was reasonable. Interpretations of each principal component was based on Pearson correlation analysis between scores of derived environmental components and the original habitat data (Ludwig and Reynolds 1988). Similarly, the association between abundance of *Anopheles* species and scores of derived environmental components was determined by correlation analysis, because a linear description of species–habitat relationship was suggested (ter Braak 1987). Spearman nonparametric correlation analysis was used to correlate rainfall with larval density in each habitat. A Student *t*-test was used to compare the mean density of different anopheline species within each sampling period. All statistical tests were considered significant at $P < 0.05$. The analysis were performed using the Multivariate Statistical Package (MVSP 1998) and the Statistica for Windows Package (Statsoft 1995).

Results

Habitat Characterization. Overall, mangrove swamps were the most brackish wetlands, with high dissolved carbon dioxide and phytoplankton content, and with fewer aquatic macrophytes (Tables 2 and 3). Ponds were the habitats with the warmest and most oxygenated waters, with great values of phytoplank-

Table 2. Environmental variables (mean ± SE) used to characterize the wetlands, during the temporal sampling

Variables	Habitat types				
	Bhs (n = 26)	Fhs (n = 26)	Ccmf (n = 13)	P (n = 20)	M (n = 20)
Depth, cm	28.4 ± 1.7	40.3 ± 3.3	18.4 ± 1.3	24.6 ± 2.0	19 ± 1.7
Vegetation cover, %	62.4 ± 5.4	81.1 ± 3.7	52 ± 9.8	60 ± 6.0	39 ± 7.6
Vegetation ht, cm	67.6 ± 5.2	239 ± 12.2	95 ± 5.2	119 ± 15.0	11 ± 3.6
Temp, °C	28.1 ± 0.4	28.6 ± 0.3	28 ± 0.7	30 ± 0.6	27 ± 0.8
pH	6.7 ± 0.2	7.1 ± 0.2	7.0 ± 0.1	7.1 ± 0.1	6.0 ± 0.4
Oxygen, ppm	1.4 ± 0.1	1.8 ± 0.2	1.2 ± 0.2	3.6 ± 0.5	0.9 ± 0.2
Salinity, o/oo	0.7 ± 0.1	0.3 ± 0.1	0	0	15.0 ± 2.0
Alkalinity, ppm	240 ± 30	179 ± 8.8	227 ± 18.5	113 ± 14.5	139 ± 25
CO_2, ppm	41.5 ± 4.2	32.9 ± 3.2	53 ± 8.0	22.5 ± 2.6	106 ± 33
Chlorophyll, μg/L	28.7 ± 5.7	32.0 ± 7.0	23 ± 13.0	61.0 ± 13.7	60 ± 16

Bhs, brackish herbaceous swamps; Fhs, freshwater herbaceous swamps; Ccmf, clear-cut marsh forests; P, ponds; and M, mangroves. n, Total number of sampling occasions in each habitat type (2 wetlands for each habitat type were sampled).

ton, and low content of dissolved carbon dioxide and alkalinity. Freshwater herbaceous swamps were deeper and with larger emergent vegetation than other habitats. Canals were the wetlands with the lowest amount of phytoplankton, and with high values of oxygen and pH (Tables 2 and 3).

Three principal components (PC) explained 59.2, 60.2, and 69.6% of the total variance based on the environmental variables measured during the 3 sampling periods, respectively (Table 4). The 1st principal component was a linear combination with high loads on the chemical variables such as salinity and carbon dioxide, describing a strong salinity gradient that separated the polysaline and mesosaline habitats (mangroves and brackish herbaceous swamps sites laying on the right or positive side of PC1) from the rest of freshwater wetlands (sites laying on the left or negative side PC1). Additionally, the 1st axis separated mangrove from other habitats based on the absence of macrophytes and low depth. PC2 was a linear combination with high loads on water alkalinity, emergent aquatic vegetation and oxygen, describing mainly an alkalinity gradient during the temporal and rainy season spatial sampling, and a weaker heterogeneity gradient (or aquatic vegetation gradient) during the dry season spatial sampling. In the alkalinity gradient, sites with high PC2 scores (on the positive side of PC2) were sites of high alkalinity but without aquatic vegetation (canal habitats). In the heterogeneity gradient, sites with high PC2 scores were sites with emergent aquatic vegetation but little oxygenated (freshwater herbaceous swamps). Finally, PC3 axis separated sites with low dissolved oxygen (the clear-cut marsh forest sites ordenated on the positive side of PC3) from sites with high dissolved oxygen (pond habitat sites ordenated on the negative side of PC3),

Table 3. Environmental variables (mean ± SE) used to characterize the wetlands, during the spatial sampling

Variables	Habitat type						
	BHS	Fhs	Ccmf [a]	*P*	M [b]	C	Sf [b]
	(n = 6) (n = 3)	(n = 3) (n = 5)	(n = 3)	(n = 4) (n = 2)	(n = 4)	(n = 4) (n = 4)	(n = 4)
Depth, cm	20 ± 5.0	34 ± 4.1	15 ± 5.2	28 ± 5.2	20 ± 6.6	24 ± 2.8	13 ± 0.9
	16 ± 1.4	29 ± 6	—	26 ± 11	—	27 ± 4.9	—
Vegetation cover, %	69 ± 11	60 ± 12	65 ± 18	46 ± 95	42 ± 18	23 ± 18	43 ± 8.9
	20 ± 7.7	51 ± 20	—	62 ± 23	—	36 ± 7.0	—
Vegetation ht, cm	71 ± 10	208 ± 27	91 ± 19	99 ± 18	27 ± 13	77 ± 15	0
	63 ± 5.9	216 ± 35	—	110 ± 56	—	36 ± 17	—
Temp, °C	28 ± 0.5	28 ± 0.9	22 ± 7.7	29 ± 1.3	27 ± 0.5	28 ± 0.3	26 ± 0.4
	26 ± 0.1	27 ± 0.1	—	31 ± 0.2	—	29 ± 0.0	—
pH	6.0 ± 0.3	7.0 ± 0.1	6.8 ± 0.0	6.5 ± 0.1	6.8 ± 0.1	6.9 ± 0.2	6.9 ± 0.2
	7.3 ± 0.1	7.7 ± 0.1	—	7.3 ± 0.3	—	8.2 ± 0.1	—
Oxygen, ppm	0.8 ± 0.3	1.9 ± 1.3	0.8 ± 0.2	3.9 ± 0.8	0.9 ± 0.4	2.6 ± 1.0	2.6 ± 1.1
	0	1.6 ± 1.2	—	6.7 ± 1.1	—	5.9 ± 0.5	—
Salinity, o/oo	4.0 ± 2.3	0	0	0	13 ± 4.1	0	0
	1.7 ± 0.3	0.6 ± 0.3	—	0	—	0	—
Alkalinity, ppm	121 ± 28.4	176 ± 14.4	200 ± 58	70 ± 29	70 ± 29	231 ± 29	165 ± 51
	482 ± 11	234 ± 118	—	109 ± 79	—	202 ± 26	—
CO_2, ppm	118 ± 30	27 ± 9.9	93.3 ± 3.3	24 ± 5.8	24 ± 5.8	54 ± 25.5	31 ± 51
	70 ± 15	41 ± 6.4	—	18 ± 3.5	—	15 ± 1.0	—
Chlorophyll, μg/L	42 ± 12	37 ± 24	32 ± 5.5	30 ± 2.3	30 ± 2.3	7 ± 0.3	67 ± 16
	30 ± 11.5	21 ± 9.9	—	44 ± 6.2	—	5.3 ± 3.6	—

Bhs, brackish herbaceous swamps; Fhs, freshwater herbaceous swamps; Ccmf, clear-cut marsh forests; P, ponds; M, mangroves; C, canals; and Sf, swamp forests. Values (n, number of sampled habitats) from rainy (top) and dry (bottom) season spatial sampling.

[a] Wetland dried out.

[b] Only one each of the M and Sf habitats were sampled in the dry season.

Table 4. Principal component analysis of habitat variables and interpretation of derived principal components (PC), based on Pearson correlation analyses between wetlands variables and derived principal components, during the temporal and the spatial sampling

Variables	Temporal sampling[a]			Rainy season[b]			Dry season[c]		
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
Depth, cm	−0.55*	−0.15	0.23	−0.53*	−0.21	0.35	−0.64*	0.23	0.55*
Vegetation type	−0.85*	−0.22	0.02	−0.28	−0.80*	−0.35	0.12	0.89*	0.22
Vegetation Height, cm	−0.68*	−0.28	−0.06	−0.48*	−0.44*	−0.19	0.02	0.64*	−0.01
Oxygen, ppm	−0.46*	−0.01	−0.64*	−0.49*	0.01	−0.61*	−0.70*	−0.61*	0.13
Salinity, o/oo	0.90*	0.04	−0.22	0.84*	0.05	0.19	0.90*	−0.14	−0.09
Alkalinity, ppm	−0.28	0.75*	0.21	−0.03	0.74*	−0.33	0.24	0.26	0.58*
CO_2, ppm	0.68*	−0.44*	0.10	0.58*	−0.26	0.49	0.89*	0.32	0.01
% variance explained	36.4	13.11	9.7	26.5	19.4	14.3	34.4	19.5	15.7

*, $P < 0.05$. The + and − signs indicate the direction of change with increasing PC scores. Note: Some variables were omitted in the table by any significant association with each PC.

[a] $n = 98$ (number of sampled habitats + number of sampled months in each habitat type).

[b] $n = 28$.

[c] $n = 16$ (number of sampled habitats).

during the temporal and rainy season spatial sampling (Table 4). In the dry season, the canal habitats were separated from the rest of the habitas based on their deeper and more alkaline waters.

Richness, Abundance, and Associated Habitat Factors. Two species of anophelines were collected during the study period: *An. aquasalis* Curry and *An. oswaldoi* Peryassu (Table 5). Larvae were routinely found near emergent structures such as mangrove roots, emergent aquatic vegetation, and floating leaves and debris. *An. aquasalis* was the most plentiful species in each of the 3 sampling periods ($t = 6.7$, df = 212; $t = 7.1$, df = 54; and $t = 2.7$, df = 30; $P < 0.05$; respectively) with mean larval abundance ($10.6 \pm 4.3, 7.0 \pm 3.1, 12 \pm 1.7$; temporal, rainy, and dry season spatial sampling, respectively) highest in the mangrove habitat (primarily, *Avicennia*), followed by the brackish herbaceous swamp habitat (primarily, the mesohaline, 7.5 ± 0.9) during the rainy season. However, *An. aquasalis* was observed in all habitats except clear-cut marsh forest. *An. oswaldoi* was most abundant in ponds, but most frequently collected in the clear-cut marsh forests and canals during the rainy and dry season, respectively (Table 5). No larvae were found in the mangroves and brackish herbaceous swamps.

Results of the correlation analysis between anopheline larval density and habitat factors (or principal components) showed a significant and positive association between *An. aquasalis* abundance and PC1 (implying a strong association with the positive side of the PC1, Table 6). Larvae were most frequently collected in saline habitats, such as the mangrove wetlands. In contrast, the abundance of *An. oswaldoi* was significant and negatively correlated with PC3 and PC1 (meaning an association with the negative side of each component) during the temporal sampling. Larvae of *An. oswaldoi* were associated with oxygenated freshwater habitats, such as ponds (Table 6).

The temporal abundance of each species varied according to the habitat permanence of each wetland, but overlapped little. *An. aquasalis* was most abundant during the rainy season and at the beginning of the dry season (Fig. 1a), whereas *An. oswaldoi* was most abundant at the end of the dry season ($r_s = -0.82$, $n = 14$, $P < 0.05$; ponds; Fig. 1b).

Table 5. Mean density (larvae per dip) ± SE of *An. aquasalis* and *An. oswaldoi* over sampled wetlands during the temporal and the spatial sampling

Species	Habitat types						
	M	Bhs	C[a]	Fhs	*P*	Sf[a]	Ccmf[b]
	($n = 600$) ($n = 120$) ($n = 30$)	($n = 780$) ($n = 180$) ($n = 90$)	 ($n = 120$) ($n = 120$)	($n = 780$) ($n = 90$) ($n = 150$)	($n = 600$) ($n = 150$) ($n = 60$)	 ($n = 120$) ($n = 30$)	($n = 390$) ($n = 90$)
An. aquasalis	5.5 ± 2.4	2.2 ± 0.6	—	0.8 ± 0.1	0.4 ± 0.1	—	0
	6.6 ± 1.4	5.0 ± 1.2	2.6 ± 0.2	1.1 ± 0.2	1.6 ± 0.6	0.7 ± 0.5	0
	12 ± 0.0	1.4 ± 0.4	2.9 ± 1.4	0.8 ± 0.4	3.0 ± 3.0	1.6 ± 0.0	—
An. oswaldoi	0	0	—	0.1 ± 0.02	0.2 ± 0.02	—	0.05 ± 0.01
	0	0	0.3 ± 0.3	0.1 ± 0.1	0	0	0.5 ± 0.5
	0	0	1.8 ± 1.8	0.1 ± 0.1	1.0 ± 1.0	0.9 ± 0.0	—

M, mangroves; Bhs, brackish herbaceous swamps; C, canals; Fhs, freshwater herbaceous swamps; P, ponds; Sf, swamp forests; and Ccmf, clear-cut marsh forests. Values from temporal (top; *n*, total number of sampling occasions in each habitat type × 30 dips), rainy (middle; *n*, number of sampled habitats × 30 dips), and dry (bottom; *n*, number of sampled habitats × 30 dips) season spatial sampling.

[a] Wetlands not sampled during the temporal sampling.

[b] Wetland dried out completely during the dry season.

Table 6. Results of Pearson correlation analyses between species abundances and derived principal components (PC) during each sampling occasion

Species	Temporal sampling			Rainy season			Dry season		
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
An. aquasalis	0.26*	0.12	0.00	0.51**	−0.11	0.19	0.36	−0.46	0.03
An. oswaldoi	−0.25*	−0.09	−0.33*	−0.17	0.06	−0.07	−0.42	−0.31	0.05

*, $P < 0.05$, $n = 98$; **, $P < 0.05$, $n = 28$. The + and − signs indicate the direction of change with increasing principal component scores.

Discussion

The importance of studying spatial and temporal variations in the distribution and abundance of wetland mosquitoes across environmental gradients was demonstrated in this study when some mosquito species–environment relationships in northeastern Venezuela were elucidated.

Anopheles aquasalis was the most common, abundant, and widely distributed species in this study, whereas *An. oswaldoi* was rare. Spatial abundance of both species varied in association with water physicochemistry, mainly the salinity. *An. aquasalis* was found mostly in the seasonal saline wetlands, such as basin mangrove swamps of *Avicennia.* Nevertheless, salinity seems not to be the only environmental variable associated with *An. aquasalis* occurrence, because this species was also collected in freshwater permanent wetlands during the dry season. Spatial distribution of *An. aquasalis* is in agreement with the previous studies done in the Americas (e.g., Senior-White 1951, Silvain and Pajot 1981, Berti et al. 1993a); however, such studies did not differentiate between occurrence and abundance. *An. oswaldoi* was most abundant and frequent in the freshwater permanent oxygenated vegetated wetlands, such as ponds or in nonvegetated habitats such as canals. Previous descriptive studies of this species only refer to its association with freshwater habitats (Faran and Linthicum 1981).

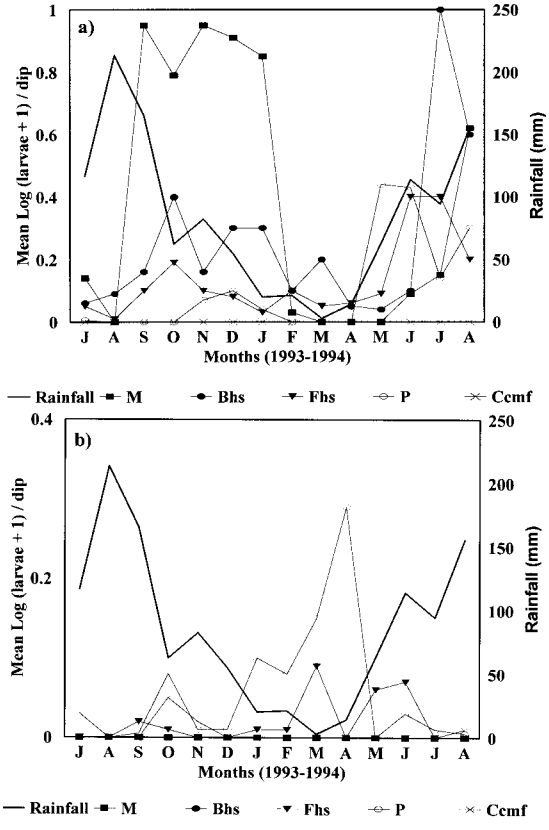

Fig. 1. Rainfall and relative abundance of *An. aquasalis* (a) and *An. oswaldoi* (b) in the studied wetlands in northeastern Venezuela, during the temporal sampling.

The mechanism by which salinity affects the *An. aquasalis* abundance and occurrence is yet unknown, although at the physiological level, it is likely to involve the ionic balance of the larvae. Salinity can determine directly species occurrence through physiological tolerances, and *An. aquasalis* appears to tolerate a wide salinity range (Berti et al. 1993; current study). Physiological processes can determine the upper limits of salinity tolerance for the aquatic organisms; lower limits, if any, may be attributed to biotic interaction (Ward 1992). In addition, salinity appears to affect indirectly the abundance and occurrence of some aquatic faunal through its general negative effect on the macrophytes and other aquatic organisms (Ward 1992). Consequently, the few species adapted to tolerate high salinities can occur in enormous numbers (Batzer and Wissinger 1996). The positive association between dissolved oxygen levels and *An. oswaldoi* abundance is surprising because most anopheline species respire primarily at the water surface, and the oxygen has not been usually considered as a relevant habitat factor to the anopheline preadult distribution. Nevertheless, some anopheline species are affected in water that contains little oxygen in a yet unexplained way (Unti 1943).

Oviposition site selection has been recognized as a critical factor for the occurrence and survival of mosquito species (Fleming 1986). We suggest that *An. aquasalis* does not select specific oviposition habitats, but is able to use a variety of wetlands. This ability probably involve a high levels of adult dispersion, a short life cycle, and a tolerance to high salinity conditions. In addition, because mosquito abundance may be influenced mainly by biotic interactions (Schneider and Frost 1996), opportunistic species such as *An. aquasalis* may be poor competitors or may develop few antipredator defenses; thus, they are less abundant in those heterogeneous habitats with high diversity of

aquatic vegetation where they could be negatively affected by biotic interactions. The low species richness and abundance of predators in the saline environments, such as mangrove swamps, could explain the high abundance of *An. aquasalis* in such wetlands. We found the richness and abundance of aquatic predators in this study to increase in relation to a decreasing salinity and increased vegetation in the habitat (M.E.G., unpublished data).

Anopheles aquasalis showed a wider temporal occurrence than *An. oswaldoi.* Seasonal changes in the larval population of both species were attributed to rainfall patterns; and the temporal distribution of both species appears strongly related to the hydrologic regimes (drying/flooding) of each wetland. Studies on the temporal dynamics of anopheline breeding site (species colonization and habitat permanency) in this region deserve future attention.

Results of the current study, along with that of Berti et al. (1993a), contribute to the basic understanding of *An. aquasalis* and *An. oswaldoi* with respect to a variety of environmental conditions in northern Venezuela. In this area, *An. aquasalis* is the main malaria vector, whereas *An. oswaldoi* is a suspected malaria vector only in western Venezuela (Rubio-Palis et al. 1992). The results of this study allow us to stratify *An. aquasalis* breeding sites according to larval presence and abundance over time and space, which is an important component of control programs. Future studies should assess the species and habitat spatial distribution on a larger scale (regional level).

The proximity to and density of immature mosquitoes in habitats near human settlements is an important risk factor for malaria transmission by *An. aquasalis* in northwestern Sucre State in Venezuela (Berti et al. 1993b, Barrera et al. 1998). In this region, there is strong overlap between human populations and breeding sites, which increases the potential for vector–human contact and the risk of malaria. *An. aquasalis* is a species with low vectorial capacity. It needs to bite in large numbers for efficient disease transmission (Berti et al. 1993b); thus, abundance or larval occurrence, or both, of this species may be a good indicator of the need for vector control. In northeastern Sucre State, most of the high density breeding sites of *An. aquasalis* (i.e., mangrove and brackish herbaceous wetlands) are >5 km from the human villages (Berti et al. 1993b), although malaria transmission in the area has increased recently (Aché 1998). This suggests that factors other than the spatial location of breeding sites are influencing malaria transmission. The actual adult flight range of *An. aquasalis* is unknown (Fleming 1986); however, it would be useful to know if a large number of low density, but continuously productive freshwater anopheline habitats, contribute more than the single high density saline larval habitat to the adult density of *An. aquasalis* and the risk of malaria transmission. Adult longevity, host preference of *An. aquasalis,* and the availability and migration of human hosts also may be key factors to explore in future entomological and epidemiological studies in northeastern Sucre State.

Based on seasonal patterns observed here for *An. aquasalis* in northeastern Venezuela, vector control operations should be applied in the dry season rather the rainy season, to take advantage of natural regulation (density-dependent mechanisms) of the anopheline larval population. Previous studies (Berti et al. 1993a, Barrera et al. 1998) have already shown the relevance of *An. aquasalis* larval presence in permanent wetlands, during the dry season, to the maintenance of malaria transmission throughout the year.

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