Human Onchocerciasis in the Amazonian Area of Southern Venezuela: Spatial and Temporal Variations in Biting and Parity Rates of Black Fly (Diptera: Simuliidae) Vectors

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ABSTRACT   We investigated some entomological factors underlying altitudinal prevalence variation in the Venezuelan Amazonia human onchocerciasis focus. Spatial and temporal variation in relative abundance, daily biting rate, proportion of parous flies , and monthly parous biting rate were studied for the three main simuliid vectors (based on their vectorial competence: *Simulium oyapockense* s.l. Floch & Abonnenc ≈ *S. incrustatum* Lutz ≪ *S. guianense* s.l. Wise). Yanomami villages were selected among sentinel communities of the ivermectin control program, representing hypo- to hyperendemicity conditions of infection. Spatial variation was explored via increasing village altitude on two river systems (A: Ocamo-Putaco and B: Orinoco-Orinoquito). Temporal variation was studied between 1995 and 1999 by sampling the biting population during dry and rainy months. Environmental variables included monthly rainfall and maximum river height. Simuliid species composition itself varied along the altitudinal and prevalence gradient. *S. oyapockense* s.l. prevailed below 150 m. Above this altitude and up to 240 m, *S. incrustatum* and *S. guianense* s.l. became more frequently and evenly collected along A but not along B, where *S. incrustatum* remained absent. The daily biting rate of *S. oyapockense* s.l. was higher during the dry season along A, whereas the converse took place along B. Daily biting rate of *S. incrustatum* was lowest during early rains. By contrast, the daily biting rate of *S. guianense* s.l. was highest during this period. There was a significant negative cross-correlation between proportion of parous of *S. oyapockense* s.l. and river height (2 and 3 mo lagged), whereas this variable (1 and 2 mo lagged) was positively correlated with the proportion of parous flies for *S. incrustatum.* Monthly parous biting rate values suggest that the months contributing most to onchocerciasis transmission in the area are likely to be the dry season and the transition periods between seasons.

KEY WORDS   *Simulium oyapockense, Simulium incrustatum, Simulium guianense*, biting rate and parity, onchocerciasis, Amazonas

HUMAN ONCHOCERCIASIS IN the Amazonian area of southern Venezuela is found between the lowlands and the highlands of the Orinoco, Caura, and Siapa river basins (Rassi et al. 1977, Godoy 1982, Botto et al. 1997), affecting mainly the indigenous Yanomami Amerindian group. In the highlands of this area, *Simulium guianense* Wise s.l. is a very efficient vector of *Onchocerca volvulus* Leuckart, whereas in the lowlands *S. oyapockense* Floch & Abonnenc s.l. plays a lesser role (Shelley et al. 1987; Basáñez et al. 1988, 1995). Recently, onchocerciasis prevalence has been demon-strated to increase with altitude along two fluvial systems of the Amazonian area (Vivas-Martínez et al. 1998), confirming previous observations (Rassi et al. 1977, Yarzábal et al. 1983). This positive association could be partially explained by clinal variation in the *Simulium* species composition, black fly abundance, and vectorial efficiency along the altitudinal gradient (Shelley et al. 1997, Vivas-Martínez et al. 1998). In addition to *S. guianense* s.l. and *S. oyapockense* s.l., there are other anthropophilic black fly species such as *S. incrustatum* Lutz, whose vectorial role remains to be determined (Basáñez et al. 1988, Shelley et al. 1997).

The hypothesis of a clinal variation in onchocerciasis prevalence has been tested, from an epidemiological perspective, using altitude and simuliid human-biting rate as explanatory variables of *O. volvulus* microfilarial status (Vivas-Martínez et al. 1998). This study found that *S. oyapockense* s.l. prevails in the lowlands (<150 m above sea level) in association with hypo- to mesoendemic levels of human infection (prevalence <60%), whereas *S. incrustatum* and *S. guianense* s.l. occur above this elevation and are asso-

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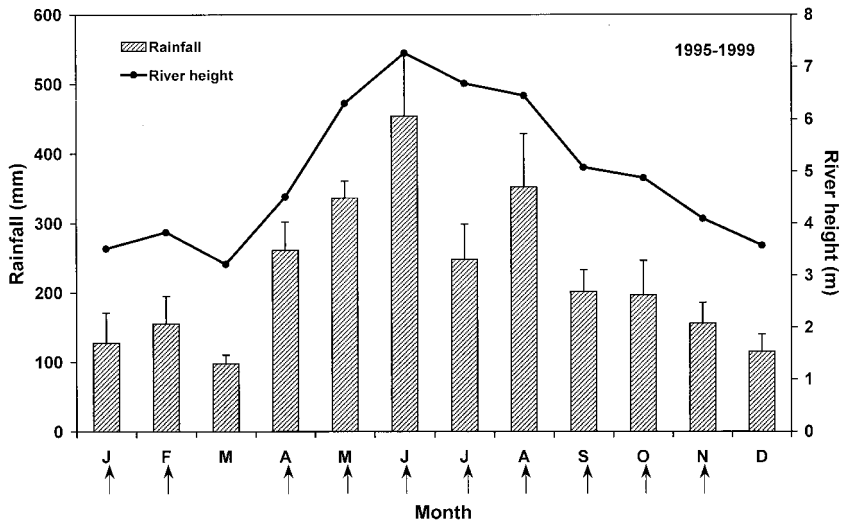
Fig. 1. Total monthly rainfall (hatched and standard error bars), and monthly maximum river height (solid line and markers) at Ocamo (southern Venezuela), averaged from 1995 to 1999. Drier months are October through March; April marks the transition from dry to rainy season; wettest months are May through August, and September corresponds to the rainy–dry season transition. The vertical arrows indicate months in which sampling took place.

ciated with hyperendemic areas (prevalence $\geq 60\%$). However, conclusions were based on collections that did not investigate temporal variations in relative abundance, biting and parity rates, or vectorial competence. Further entomological testing of the clinal hypothesis of onchocerciasis prevalence is, therefore, needed in the Amazonian area.

Vector density, vector competence, and vector survival are all important components of vectorial capacity, which may vary spatially and seasonally. Their quantification is important in the context of control programs (Dye 1994). A recently implemented ivermectin-based control campaign in the Amazonian area has required investigation of entomological indicators and risk factors along the identified altitudinal gradient to provide baseline data on the association of endemicity levels with common indices of onchocerciasis transmission intensity, i.e., biting rate, infective biting rate, and transmission potential (Walsh et al. 1978).

The results presented here form part of a more extensive study in southern Venezuela with the purpose of obtaining entomological, parasitological, and clinical indicators appropriate to the evaluation of onchocerciasis endemicity and severity, and for epidemiological modeling (Botto et al. 1997, 1999; Grillet et al. 1998; Basáñez et al. 1998, Basáñez and Boussinesq 1999; Vivas-Martínez et al. 1998, 2000a, 2000b). The entomological component of this project aimed at quantifying, before and during mass ivermectin distribution, the spatial and seasonal variations of various components of vectorial capacity: biting rate on humans, parity rate, vector competence, and vector survival, as well as natural infection levels in the less studied species of the Amazonian area, namely, *S. oyapockense* s.l. and *S. incrustatum,* and at poorly

known localities of intermediate altitude. This article presents information on the seasonal variation in relative abundance, human-biting rates, and parity rates of *S. oyapockense* s.l., *S. incrustatum* and *S. guianense* s.l. in sentinel localities up to 240 m asl within the control area. Variations in the biting activity of anthropophilic black fly species, and, in particular, of the parous component of the population, determine risk factors of exposure and transmission according to locality and season (Renz 1987; Renz and Wenk 1987; Porter and Collins 1988a, 1988b).

Materials and Methods

Study Area. The work was carried out in six sentinel riverine Yanomami communities of the onchocerciasis control program: Ocamo (2° 47′ N, 65° 12′ W), Maweti-theri (2° 55′ N, 64° 45′ W), Mahekoto-theri (2° 25′ N, 64° 54′ W), Awei-theri (2° 49′ N, 64° 30′ W), Hasupiwei-theri (2° 15′ N, 64° 32′ W), and Pashopeka-theri (2° 47′ N, 64° 28′ W) situated along the Ocamo-Putaco (A) and Orinoco-Orinoquito (B) river systems (for their geographical location see Fig. 1 of Vivas-Martínez et al. 1998). These river systems are interconnected as the Ocamo River joins the Orinoco at the locality of Ocamo. The study villages covered a range of endemicity levels representative of the study area (one hypoendemic, two mesoendemic, and three hyperendemic communities) ranging from 90 to 240 m asl (Table 1). The villages were reached by boat and on foot, or by air. The area is characterized by an average temperature of 26–27°C and 3,750–5,000 mm annual rainfall, lying in a very humid tropical forest (Ewel et al. 1976, Huber et al. 1984). The rainier months are those from May through August, and the drier include October through March, with a transi-

Table 1. Total number of sampling events, sampling days, and flies (all three species together) collected from 1995 to 1999 in the dry (September through March) and rainy (April through August) seasons in the study localities of the southern Venezuelan onchocerciasis focus

Locality (abbreviation)	River system	Altitude (m asl)	Endemicity (mf prevalence, %)[a]	Sampling	Season	
					Dry	Rainy
Ocamo (Oc)	A	90	Hypoendemic (2.4)	No. visits	—	2[b]
				No. days	—	8
				No. flies	—	9,263
Maweti-theri (Mw)	A	140	Mesoendemic (24.3)	No. visits	2	2
				No. days	5	10
				No. flies	11,967	18,541
Mahekoto-theri (Mk)	B	140	Mesoendemic (41.4)	No. visits	2[b]	1
				No. days	8	5
				No. flies	6,487	7,195
Awei-theri (Aw)	A	162	Hyperendemic (66.7)	No. visits	3	3
				No. days	14	10
				No. flies	5,505	3,961
Hasupiwei-theri (Ha)	B	200	Hyperendemic (78.4)	No. visits	2[b]	2
				No. days	7	10
				No. flies	2,182	9,519
Pashopeka-theri (Pa)	A	240	Hyperendemic (79.8)	No. visits	4	2
				No. days	19	10
				No. flies	5,096	2,524
			Total	No. visits	13	12
				No. days	53	53
				No. flies	31,237	51,003

[a] Parasitological data from Vivas-Martínez et al. (1998).
[b] Includes data from Vivas-Martínez et al. (1998) for which parity status was not assessed.
A, Ocamo-Putaco river system; B, Orinoco-Orinoquito river system.

tion between the dry season and the rains taking place roughly during April, and a transition between the rains and the drier months taking place in September. Maximum river water levels occur from May through August and the minimum at the end of the dry season, i.e., January through March (Fig. 1). A more detailed description of the study area, including epidemiological and some anthropological aspects, can be found in Vivas-Martínez et al. (1998).

Entomological Methods. In each community, and during one to five consecutive days per visit, all the black fly females that landed on two human attractants from the village were caught with manual aspirators by a team of two collectors (working during alternate hours) during the first 30 min of each hour, from 0700 to 1830 hours. This amounted to 12 half-hour intervals per collecting day. Because females were collected before procuring a blood meal, what we refer to as *biting rates* are more truly *landing rates*. The latter may overestimate the former because feeding success is not measured. In addition, what we report as *biting rate per person* would be more correctly referred to as *biting rate per team*, where each team consists of both attractant and collector catching flies on one person. Whenever possible, the collectors were the same throughout the study to minimize variations resulting from individual differences in catching ability. In the field, all hourly-caught flies were anesthetized with chloroform vapor, identified to species, counted, and dissected for parity status according to criteria suggested in table 1 of Wenk (1981). Taxonomic identification to morphospecies followed the keys by Ramírez-Pérez et al. (1982) and Shelley et al. (1997).

Parity assessment was always carried out by the member of our team with the most experience in this technique (N.V.). Parous females were preserved in 80% ethanol for subsequent determination of natural infection rates (Nelson 1958); this information will be presented elsewhere. Collections took place between 1995 and 1999, with most communities visited more than once to cover both dry and rainy seasons for each community (Table 1). Collection protocols, number of days per visit, and number of visits per village were, however, determined by the need to optimize logistic and traveling costs, and to accompany teams of clinicians and parasitologists who carried out simultaneous epidemiological evaluations in this remote area. Rainfall and river height data were obtained for 1995–1999 from the meteorological station closest to our study area in the locality of Ocamo.

Variables of Interest. We focused on the relative abundance of each simuliid species in each locality, the daily and monthly biting rates, the percentage of parous flies in the total dissected (proportion of parous flies), and the monthly parous biting rate. Relative abundance was calculated for both dry and rainy seasons as the percentage of each species in the total collected at each locality. Daily biting rate was computed by multiplying the number of flies caught in each 30-min interval by 2 (with the exception of the 1800–1830 hours), adding up the 12 hourly estimates and averaging the daily totals per month and locality. Arithmetic means were used for this purpose because there was little variation between days regarding such totals; the standard error of the mean ($\pm$SE) was used as a measure of dispersion. Thus, the catching day is

used as the basic unit in the current study (Renz and Wenk 1987). Monthly parous biting rate (*MPBR*) was obtained for each locality from the formula: MBPR = number of flies caught × number of days in month/ number of catching days, according to Walsh et al. (1978). Proportion of parous flies and its 95% CL was determined as the percentage of parous females in the total collected at each visit. Monthly proportion of parous fly values for each locality were also computed. Monthly parous biting rate was obtained by multiplying monthly parous biting rate by the corresponding proportion of parous fly value. The standard deviation of the monthly parous biting rate was calculated as the square root of the variance of the product monthly parous biting rate (X) times proportion of parous flies (Y) using the method of statistical differentials (Kotz et al. 1988),

$$\text{var}(X.Y) = [\sigma_X^2 + (\mu_X)^2][\sigma_Y^2 + (\mu_Y)^2] - (\mu_X)^2(\mu_Y)^2$$

where the variance of proportion of parous flies was estimated using the normal approximation.

Data Analysis. Relative abundance was compared between rainy and dry seasons (within localities and species) by Yates corrected chi-square tests. Spatial (locality) and seasonal variation of daily biting rate ($\log_{10} [x + 1]$ transformed) for each simuliid species was explored by analysis of variance (ANOVA) (two-factor ANOVA) and multiple regression (with locality, season, and river height as categorical variables). Only river height entered the analysis due to the highly significant positive correlation between this variable and monthly rainfall ($r = 0.71$, $n = 54$, $P < 0.001$). Seasonal variation in relative abundance and daily biting rate was examined by grouping the observations into two main seasons: dry (collections of October 1995; January, February, and September 1997; September and November 1998), and rainy (May and June 1995, July and August 1997, and April 1999). This division may hide potential variations within each season (e.g., early- and late-dry or rainy season) but it was necessary to produce a balanced design for statistical analyses in ANOVA. For similar reasons, the locality of Ocamo (only rainy season sampling) was excluded from all ANOVA but not from multiple regression analyses. To normalize the distribution of the residuals, the ANOVA for each simuliid species included only those localities in which the species in question was present. Multiple regression analyses included all localities. Because the latter technique does not require a strictly balanced design, a finer season subdivision was possible, with October through March as dry season, April as early rains, May through August as rainy season, and September as early dry. To detect the extent to which the data series of monthly proportion of parous fly values exhibited concordant periodic variation with rainfall and river height, cross-correlation analyses were conducted between these variables, shifting the series of monthly rainfall and river height with respect to that of proportion of parous fly values with time-lags of k months ($k = 0$-4) and using Spearman's r (Legendre and Legendre 1998). Only the significant results of this

analysis are presented. All statistical analyses were carried out using Statistica for Windows Package (StatSoft 1997) and were considered significant at $P < 0.05$.

Results

Relative Abundance. A total of 82,240 flies was collected during 106 sampling days between 1995 and 1999 (Table 1). Along the Ocamo-Putaco river system, *S. oyapockense* s.l. was the only anthropophilic black fly species present at 90 and 140 m asl (Ocamo and Maweti, respectively), and the most frequent (>90%) at 140 and 200 m (Mahekoto and Hasupiwei, respectively) along the Orinoco-Orinoquito river system, followed by *S. guianense* s.l. (Fig. 2). Along the former river system, a greater species richness was observed, with *S. incrustatum* and *S. guianense* s.l. being also present at 162 and 240 m (Awei-theri and Pashopeka, respectively), with a more even representation of the three simuliid species. Regarding seasonal variation of relative abundance, *S. guianense* s.l. was more frequently collected during the rainy season in both Awei-theri ($\chi^2 = 705.82$, df = 1, $P < 0.001$) and Pashopeka ($\chi^2 = 1,580.95$, df = 1, $P < 0.001$), whereas the converse was observed in the Orinoco-Orinoquito rivers, where *S. guianense* s.l. was more frequent during the dry season at Hasupiwei ($\chi^2 = 57.13$, df = 1, $P < 0.001$). No difference was observed at Mahekoto, where this species was rare. *S. incrustatum* was more frequently collected in the dry season at Pashopeka ($\chi^2 = 265.27$, df = 1, $P < 0.001$), whereas there was no difference at Awei-theri. *S. oyapockense* s.l. was more frequently collected during the dry season both in Awei-theri ($\chi^2 = 643.29$, df = 1, $P < 0.001$) and Pashopeka ($\chi^2 = 411.78$, df = 1, $P < 0.001$), whereas the converse took place in Hasupiwei ($\chi^2 = 57.13$, df = 1, $P < 0.001$).

Daily Biting Rates. Figure 3 shows daily biting rate per locality and month for each simuliid species. *S. oyapockense* s.l. reached the highest values in the lowlands (Ocamo, Maweti and Mahekoto) with 1,000–8,000 bites/person-day, whereas both *S. incrustatum* and *S. guianense* s.l. were well below 1,000 in all localities and months above 150 m (Awei-theri, Pashopeka, and Hasupiwei). *S. guianense* s.l. was found biting in Mahekoto at a much lower rate (6–16 bites/ person-day).

Biting rate of the three black fly species varied among localities and seasons. In a two-factor ANOVA, the effect of locality (a surrogate of altitude) on daily biting rate was highly significant for all simuliid species. Biting rate of *S. oyapockense* s.l. decreased with altitude ($F = 76.24$, df = 4, $P < 0.001$). In contrast, biting rate of both *S. incrustatum* ($F = 19.86$, df = 1, $P < 0.001$) and *S. guianense* s.l. ($F = 9.75$, df = 3, $P < 0.001$) increased with altitude.

In those communities that were visited during more than four different months throughout the collection years (Maweti, Awei-theri, and Pashopeka), we observed that the biting rates of *S. oyapockense* s.l. tended to increase toward the end of the rains and beginning
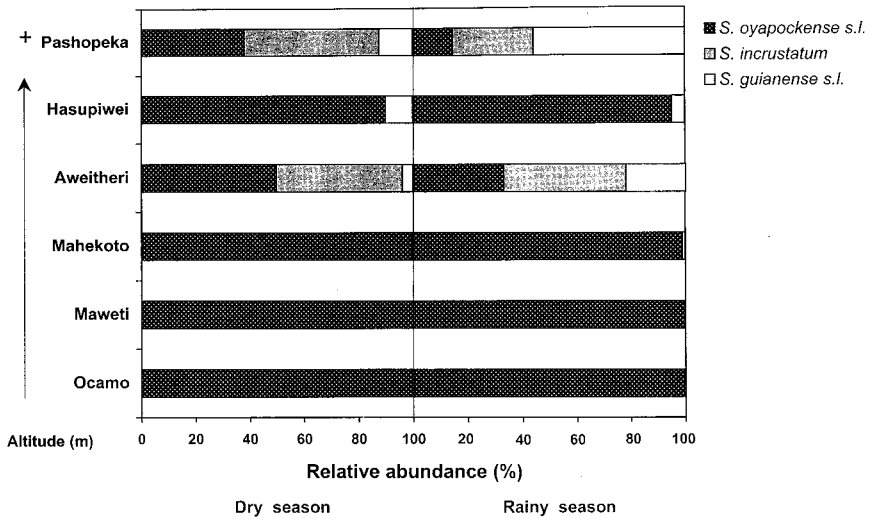
Fig. 2. Relative abundance of the three simuliid species according to locality and season. Vertical arrow indicates the direction of increasing altitude. *S. oyapockense* s.l.: dark bars; *S. incrustatum:* gray bars; *S. guianense* s.l.: white bars.

of the dry season, i.e., September–November. Nevertheless, when taking all localities together, differences between dry and rainy months were not significant in ANOVA ($F = 0.06$, df $= 1$, $P = 0.81$). However, there was a significant interaction effect between locality

and season ($F = 5.17$, df $= 4$, $P = 0.001$). This was due to the fact that Awei-theri and Pashopeka (on Ocamo-Putaco) biting rates of *S. oyapockense* s.l. were higher in the dry than in the rainy season, the converse was observed in Mahekoto and Hasupiwei (on Orinoco-
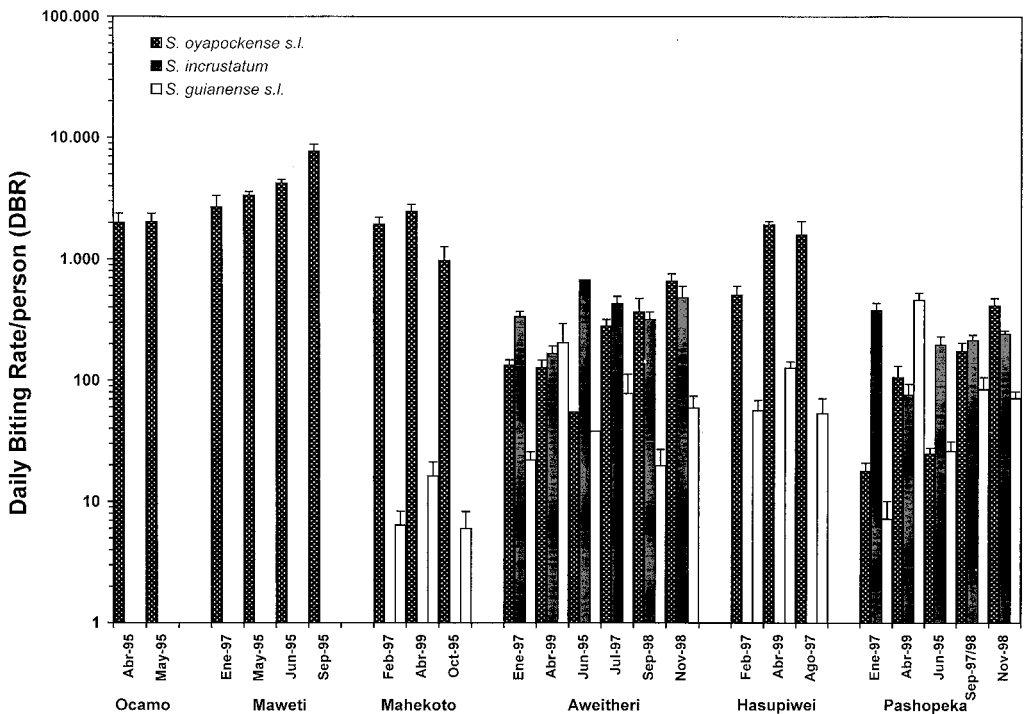

Fig. 3. Mean daily human-biting rates (daily biting rate and standard error) of the three anthropophilic simuliid species presented by locality, month, and year of collection in the Amazonian onchocerciasis focus of southern Venezuela. *S. oyapockense* s.l. (dark bars), *S. incrustatum* (gray bars), and *S. guianense* s.l. (white bars). June 1995: one sampling day only. Altitude of localities increases from left to right (see Table 1).
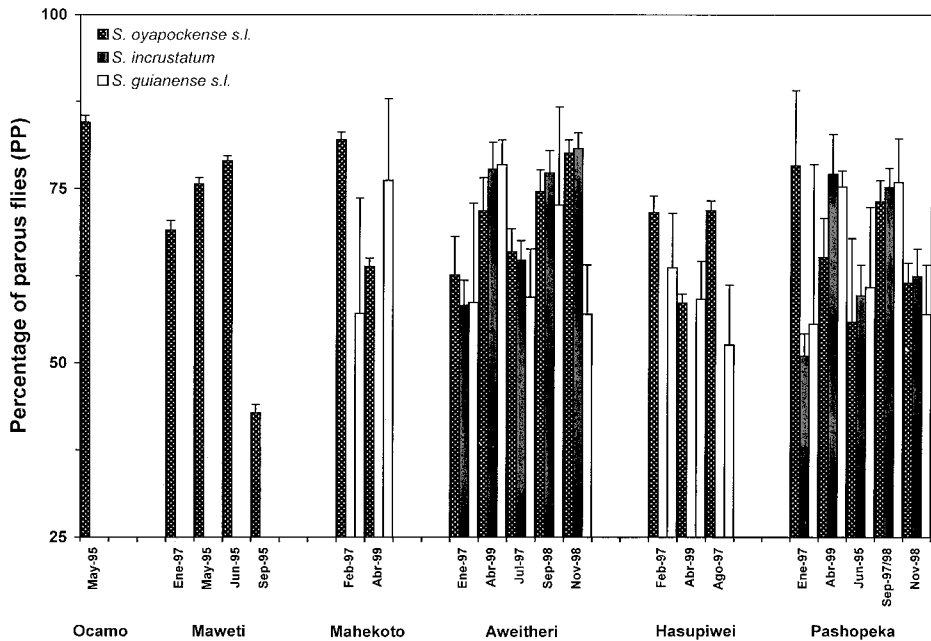
Fig. 4. Percentage of parous flies (proportion of parous flies ± 95% CL) for the three anthropophilic simuliid species presented by locality, month, and year of collection in the Amazonian onchocerciasis focus of southern Venezuela. *S. oyapockense* s.l. (dark bars), *S. incrustatum* (gray bars), and *S. guianense* s.l. (white bars).

Orinoquito). This is in agreement with the results of relative abundance. For *S. incrustatum*, daily biting rate was significantly higher in the drier months ($F = 13.35$, df = 1, $P = 0.001$) for both Awei-theri and Pashopeka (locality × season interaction was not significant). *S. guianense* s.l. showed highest daily biting rate during the early rainy season (April) compared with other months at all localities, and this translated into a significant season effect ($F = 10.72$, df = 1, $P = 0.002$) without significant locality × season interaction.

The effects of locality and season as explanatory variables on the biting rate variable were further explored by multiple linear regression. In this analysis, all villages were compared with Ocamo as base-line locality, and the seasonal subdivisions contrasted with the dry period. Regression models were significant for each species (*S. oyapockense* s.l.: $n = 104$, $r ± SE = 0.89 ± 0.34$, $R^2 = 0.78$; $F = 43.09$; df = 8, 95; $P < 0.001$; *S. incrustatum*: $n = 104$, $r ± SE = 0.99 ± 0.15$, $R^2 = 0.99$; $F = 799.58$; df = 8, 95; $P < 0.001$; and *S. guianense* s.l.: $n = 104$, $r ± SE = 0.90 ± 0.37$, $R^2 = 0.82$; $F = 53.42$; df = 8, 95; $P < 0.001$) and the results confirm the spatial trends described by ANOVA, permitting fine-tuning identification of temporal patterns. For instance, the daily biting rate of *S. oyapockense* s.l. was significantly lower in Awei-theri ($t = -6.60$, df = 95, $P < 0.001$) and Pashopeka ($t = -9.91$, df = 9, $P < 0.001$) with respect to that in Ocamo, and significantly higher in the early dry season compared with that in the dry season ($t = 2.88$, df = 95, $P < 0.005$). This result was maintained even when the analysis was repeated, excluding the month of September at Maweti (see Fig. 3). During

the early rains (April) the biting rate of *S. incrustatum* was significantly lower ($t = -5.95$, df = 95, $P < 0.001$) and that of *S. guianense* s.l. significantly higher ($t = 6.21$, df = 95, $P < 0.001$) than during other periods.

Parity. A less clear pattern emerged when the proportion of parous flies was plotted by locality and month for the three simuliid species (Fig. 4). In *S. oyapockense* s.l., proportion of parous fly values were at their lowest during April at Mahekoto and Hasupiwei, and during September at Maweti, and at their highest during February at Mahekoto, May at Ocamo, and November at Aweitheri. In *S. incrustatum*, parity was lowest in January (one of the drier months). In *S. guianense* s.l., the proportion of parous flies was, in most cases, higher in April (early rains) and September (early dry) than in the remaining months.

The results of the lag-correlation analyses, summarized in Table 2, may help to interpret the observed

Table 2. Results of cross-correlation analyses between the proportion of parous flies (*PP*) and river height measured at Ocamo, southern Venezuela, from 1995 to 1999

Lag in months k	*S. oyapockense* s.l.			*S. incrustatum*		
	n	r_S	P-value	n	r_S	P-value
0	17	−0.119	0.649	10	0.031	0.933
1	18	−0.265	0.288	9	0.678	0.043[a]
2	18	−0.546	0.019*	9	0.746	0.021*
3	18	−0.582	0.011*	9	0.322	0.398
4	19	−0.012	0.960	10	−0.282	0.429

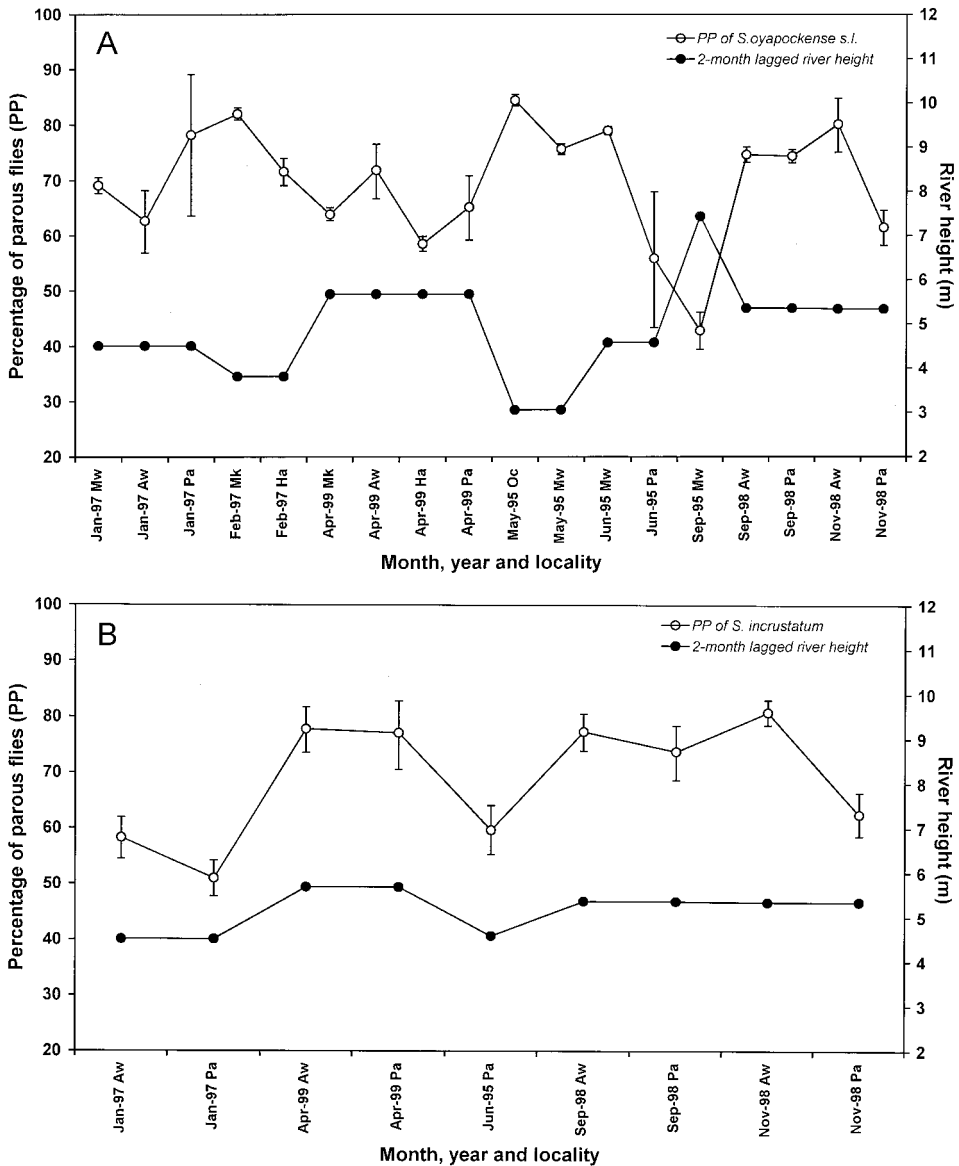* Indicates significance.
[a] Borderline significance.

Fig. 5. Percentage of parous flies presented by month, year, and locality for (A) *S. oyapockense* s.l.; (B) *S. incrustatum.* Open circles and error bars are, respectively, the observed parity values and the 95% CL. Closed circles are the 2 mo lagged river height values measured at Ocamo. Locality abbreviations as in Table 1. Connecting lines are not to be interpreted as reflecting a continuous temporal pattern from which intermediate values can be interpolated but rather as visual aids to facilitate inspection of the pattern.

parity patterns. For *S. oyapockense* s.l., the proportion of parous fly values were significantly and negatively correlated with river height for $k = 2$ and 3 mo (Fig. 5A), whereas for *S. incrustatum* the correlation was positive and significant for $k = 1$ and 2 (Fig. 5B), albeit $k = 1$ was just borderline significant. None of the correlations were significant in the case of *S. guianense* s.l. Correlations between proportion of parous fly values and rainfall were not significant for any of the three simuliid species.

Monthly Parous Biting Rate. Table 3 and Fig. 6 present, for each species, monthly parous biting rate at the six localities and for those months with information on both biting and parity. The monthly parous biting rate summarizes the host-seeking density of the epidemiologically important section of the biting population. The risk of being bitten by potentially infected/infective flies increases, in general, toward the rainy-dry transition and early dry season for *S. oyapockense* s.l. along Ocamo-Putaco; during the dry season

Table 3.   Monthly parous biting rates of the three simuliid species collected from 1995 to 1999 in the study localities of the southern Venezuelan onchocerciasis focus

| Locality	Species	January	February	April	May	June	July	August	September	November
Oc	*S. oyapockense*				53,213					
Mw	*S. oyapockense*	57,637			79,023	100,213			98,804	
Mk	*S. oyapockense*		44,602	47,212						
Mk	*S. guianense*		110	370						
Aw	*S. oyapockense*	2,609		2,770			5,778		8,230	15,889
Aw	*S. incrustatum*	5,890		3,913			8,427		7,409	11,697
Aw	*S. guianense*	387		4,830			1,392		431	1,174
Ha	*S. oyapockense*		10,156	33,834				35,462		
Ha	*S. guianense*		1,078	2,260				839		
Pa	*S. oyapockense*	432		2,077		416			3,833	7,694
Pa	*S. incrustatum*	5,795		1,762		3,547			4,850	4,582
Pa	*S. guianense*	120		10,436		478			1,566	1,224

Locality abbreviations as in Table 1.

for *S. incrustatum*, and during the dry-rainy transition for *S. guianense* s.l. at all localities.

Discussion

In the Amazonian onchocerciasis area, the biting rates of the three main anthropophilic simuliid species showed a general pattern of variation significantly related to a spatial component (altitude) and seasonal distribution of rainfall. Our study confirms that in southern Venezuela, *S. oyapockense* s.l. is the most abundant species below 150 m asl (Vivas-Martínez et al. 1998) exhibiting very high human-biting rates, which increase toward the beginning of the dry season, particularly along the Ocamo-Putaco rivers (Maweti, Awei-theri, Pashopeka). The opposite trend was observed along the Orinoco-Orinoquito (Mahekoto and Hasupiwei), which generated a significant interaction between locality and season. Above 150 m, and particularly along Ocamo-Putaco, species richness increased, with *S. incrustatum* and *S. guianense* s.l. becoming more frequently and evenly collected, exhibiting, respectively, highest daily biting rate in the dry and in the early rainy season. Although daily biting
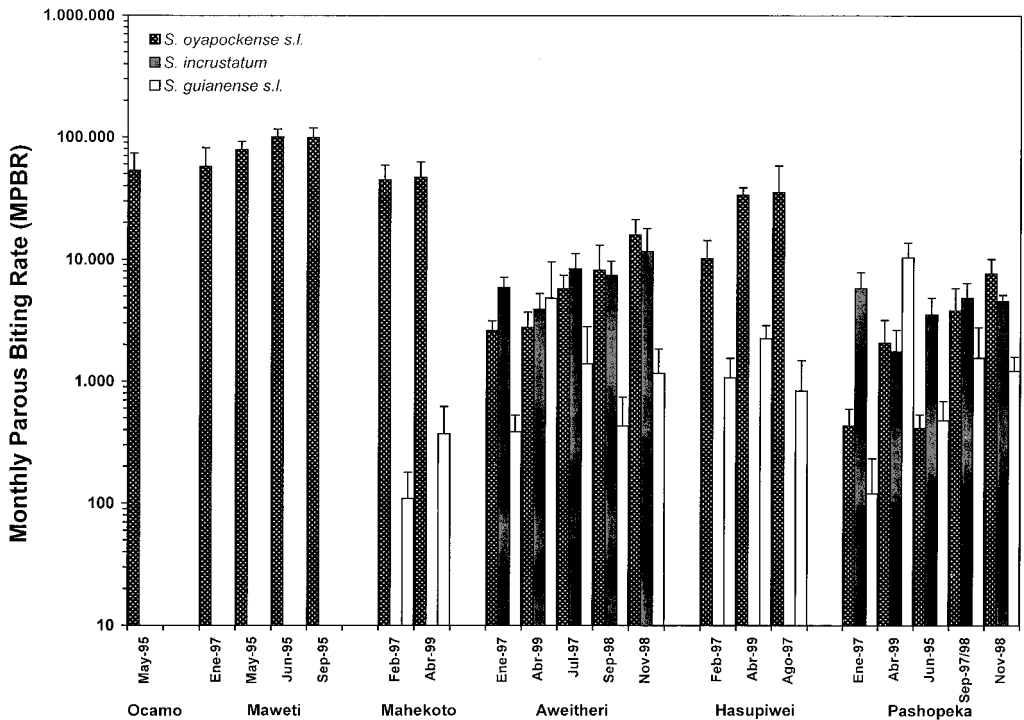
Fig. 6. Monthly parous biting rates of the three anthropophilic simuliid species presented by locality, month, and year of collection in the Amazonian onchocerciasis focus of southern Venezuela. *S. oyapockense* s.l. (dark bars), *S. incrustatum* (gray bars), and *S. guianense* s.l. (white bars). Error bars are the standard deviation of *monthly parous biting rate* calculated using the method of statistical differentials (see text).

rate of *S. incrustatum* was highest in the drier months, its proportion of parous fly values were generally lowest during this period. *S. incrustatum* occurred only in Awei-theri and Pashopeka, reaching parous host-seeking densities similar to or higher than those of *S. oyapockense* s.l. at these localities. Both daily biting rate and proportion of parous flies of *S. guianense* s.l. were highest in April, which resulted in this month showing the highest monthly parous biting rate at all localities for this species. This is in agreement with the less detailed observations of Shelley et al. (1997) in the Brazilian part of the Amazonian area. The host-seeking density of *S. guianense* s.l. increased with altitude and became as important as that of *S. oyapockense* s.l. in the locality of Pashopeka. This trend becomes even more pronounced as the altitudinal gradient approaches the Parima highlands (Vivas-Martínez et al. 1998). Results of daily biting rate, proportion of parous flies, and monthly parous biting rate for *S. guianense* s.l. in localities of Orinoquito and Sierra Parima (≥250 m) will be presented elsewhere. In this work, as well as in most of the published literature, human-biting (landing) rates possibly represent a maximum or an overestimated value of human exposure to black fly bites. Flies are collected on a passive subject and there is a nonquantified combined attractiveness of two people collecting on one. More work is needed on measuring exposure heterogeneities and the relationship between vector density and host biting for quantitative frameworks of vector-borne disease epidemiology (Kelly and Thompson 2000).

The patterns of spatial and temporal occurrence and abundance, as well as the variation in parity observed for the three anthropophilic simuliids studied here could be partially explained by an interplay between favored breeding sites, their proximity to villages, breeding site dynamics, and temporal distribution of rainfall and river level. Parous rates of adult biting populations, ignoring sampling biases, encompass both vector survival and possible age-related differential dispersion. Also, parous rates in the current study may reflect the effect of environmental variables affecting the immature populations at some time in the past. In *S. oyapockense* s.l., river height (2 and 3 mo lagged) was negatively correlated with parity status. By contrast, a positive correlation between river height (1 and 2 mo lagged) and proportion of parous fly values was detected in *S. incrustatum*, possibly reflecting the very different nature of the breeding sites of these two species.

Although the specific location and distribution of breeding sites for the three simuliids discussed here are not yet well known in southern Venezuela, lessons can be learned from work in Brazil (Shelley et al. 1997) and a few studies in Venezuela (Ramírez-Pérez et al. 1982, Takaoka et al. 1985). *S. oyapockense* s.l. breeds in large and perennial lowland rivers like the Ocamo and Orinoco, the volume of which increases considerably during the rains. A negative relationship between percentage of parous flies and maximum river flow has been observed in savanna populations of *S. damnosum* s.l. in northern Cameroon (Renz 1987),

a species that also tends to breed in large rivers. This decrease in parity could be due to the flooding of breeding sites during strong rains with the consequent increase of preadult mortality or a reduction of adult survival. Notwithstanding the fluctuations in parity, *S. oyapockense* s.l. exhibits sustainedly high host-seeking densities. This species possibly uses widely distributed submerged vegetation as preferred substrates, rather than being confined to specific stretches of river. These breeding sites are less prone to drastic flow reductions than smaller rivers and more likely to remain productive in terms of simuliid immature populations throughout the year, being responsible for more stable adult biting populations, with less marked seasonal fluctuations. By contrast, *S. incrustatum* breeds in smaller tributaries of highland rivers (e.g., the Parima-ü, tributary of the Putaco River), which makes it more susceptible to seasonal variation with respect to both biting and parity rates. Lastly, *S. guianense* s.l. breeds in rapids and waterfalls (e.g., the Goaharibos and Peñascal rapids in the Upper Orinoco), with the end of the dry season−start of the rains marking increased availability of larval habitats and improved adult survival. It is also possible that adult populations at the tail end of the dry season consist mainly of old flies, contributing to the increase of parity observed for this species during March in the Brazilian part of the study area (Shelley et al. 1997) and during April in the Venezuelan part.

These results suggest, however, the importance of the interaction between rainfall pattern and stream flow regime, with the transition periods (dry−rainy or rainy−dry) providing the most favorable conditions for preadult population establishment, in agreement with previous studies of Neotropical black fly ecology (Dalmat 1955; Grillet and Barrera 1997). However, our observations highlight the importance of considering the effect, on present adult black fly dynamics, of environmental events that, we suggest, may have affected immature populations in the near past, i.e., the importance of time-lags when analyzing snap-shot population patterns in the present. In another example, larval abundance of *S. metallicum* Bellardi s.l. was found to be best correlated with a 4-mo lagged rainfall in the northern Venezuela onchocerciasis focus (Grillet et al. 1995). Finally, a positive association between adult survival and mild climatic conditions (transition periods) has also been suggested for adult *S. ochraceum* Walker s.l. as a possible explanation for the highest parity rates found in Guatemala during such periods (Porter and Collins 1988a).

Our results can also be related to the epidemiological patterns of the sentinel communities investigated. In those villages where *S. oyapockense* s.l. is the only or nearly the only biting species (Ocamo, Maweti, and Mahekoto), microfilarial prevalence reaches at most mesoendemic levels (<60%), with mean infection intensities ranging from 0.06 to 6.97 mf/mg of skin (Vivas-Martínez et al. 1998). The highly anthropophilic nature of this species compensates for its low vector competence (Shelley et al. 1987; Basáñez et al. 1988, 1995), and generates transmission levels compatible

with observed endemicity (Basáñez et al. 2000). We have found *S. oyapockense* s.l. naturally infected with third-stage larvae indistinguishable from *O. volvulus* in the studied area (Grillet et al. 1997). The presence of *S. guianense* s.l., whose high vectorial efficiency is well established (Takaoka et al. 1984; Basáñez et al. 1988, 1995) is associated with prevalence values >60% (Hasupiwei-theri, Awei-theri, and Pashopeka), with the previous location of Hasupiwei (at a higher elevation) being characterized by a higher relative abundance of *S. guianense* (48.2%) than that at its present location (Fig. 2). Our results suggest that *S. incrustatum* must be playing a vectorial role in the hyperendemic localities of intermediate altitude, where this species has been found to support successful *O. volvulus* larval development and to harbor natural *O. volvulus* infections (Grillet et al. 2000, Basáñez et al. 2000).

Our monthly parous biting rate results suggest that the dry season and the transitions between season periods may constitute the most critical times with respect to exposure to parous black fly bites. Preliminary analyses of natural infection data suggest that these periods may indeed be the ones contributing the most to onchocerciasis transmission in southern Venezuela. Seasonal fluctuations in transmission expressed as the infective biting rate, which themselves correspond to changes in parous biting rate have been found in Guatemala (Porter and Collins 1988b) and west Africa (Duke 1968, Barbiero and Trpis 1984). More detailed analyses, in the context of ivermectin control programs, of the vector competence, fecundity, survival, and transmission potentials of the three simuliid species here studied will be the subject of subsequent publications.

Acknowledgments

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