

***Simulium metallicum* cytospecies E larval habitat characterization in the Altamira focus of onchocerciasis, northern Venezuela**

MARIA EUGENIA GRILLET, ROBERTO BARRERA

and JAN CONN* Instituto de Zoología Tropical, Universidad Central de Venezuela,
Caracas, Venezuela, and *University of Florida, Gainesville, Florida, U.S.A.

Abstract. *Simulium metallicum* sibling species E was identified cytotaxonomically from an onchocerciasis focus at Altamira in northern Venezuela. *S. metallicum* E larvae were sampled monthly from two small mountain streams over a 15-month period (July 1990 to September 1991) and eleven habitat variables were measured at two altitudes. One stream consistently harboured much higher densities of *S. metallicum* E larvae than the other, with three annual peaks of abundance: during the dry season and at the beginning and end of the rainy season. These peak densities were correlated with high rainfall 4 months previously. Larvae were most abundant on submerged rocks and fallen leaves, in small shallow areas characterized by slow water current, high conductivity and sparse terrestrial vegetation cover. Stream variables which best explained the temporal changes in abundance were water discharge and conductivity. The population dynamics of *S. metallicum* E appeared to be influenced primarily by interactions between stream discharge and substrate stability. Relevance of these results to vector control with larvicides is discussed.

Key words. *Simulium metallicum*, onchocerciasis, vector ecology, cytotaxonomy, cytotype, larval population abundance, larval microdistribution, habitat factors, stream variables, limnology, Venezuela.

Introduction

Certain species of *Simulium* blackflies (Diptera: Simuliidae) are the vectors of *Onchocerca volvulus* (Leuckart) (Nematoda: Onchocercidae), the parasite causing human onchocerciasis. Cytotaxonomic studies of larval populations of morphologically defined blackfly taxa have revealed many complexes of reproductively isolated sibling species, referred to as cytospecies or cytotypes (Rothfels, 1987). Vector control problems may arise when sympatric members of a species complex differ in their vectorial capacity (Shelley *et al.*, 1986) or habitat preferences (Adler, 1987). Taxonomic and ecological studies of *Simulium* vectors of onchocerciasis can help to interpret the dynamics of disease transmission, and are necessary in order to

formulate appropriate control strategies. Unfortunately the comparative ecology of blackfly sibling species remains poorly understood for neotropical onchocerciasis vectors.

Simulium metallicum Bellardi *sensu lato* is the main vector of onchocerciasis in northern Venezuela, where it is mainly anthropophilic (Lewis & Ibañez de Aldecoa, 1962). In Mexico and Guatemala, *S. metallicum s.l.* is primarily zoophilic and plays only a secondary role in the transmission of *O. volvulus* (Collins, 1979). Cytotaxonomic studies revealed that *S. metallicum s.l.* comprises a complex of at least eleven cytotypes, two of which (D and E) have been found in Venezuela (Hirai, 1985; Conn *et al.*, 1989). *S. metallicum* cytotypes D and E differ by chromosomal inversions IIL-B+A being fixed in cytospecies E but polymorphic and sex-linked ($Y_0 = \text{IIL-std/std}$; $X_2 = \text{IIL-B+A/std}$) in cytotype D (Conn *et al.*, 1989).

Previous studies on populations of the *S. metallicum* complex have led to reports on their bionomics (Dalmat, 1955), vector competence (Collins, 1979; Takaoka *et al.*, 1984; Grillet *et al.*, 1994), seasonality (Porter & Collins,

Correspondence: Dr M. E. Grillet, Laboratory of Vector Biology, Instituto de Zoología Tropical, Facultad de Ciencias, Universidad Central de Venezuela, Apartado 47058, Caracas 1041-A, Venezuela.

1988a, b) and cytotaxonomy (Hirai, 1985; Conn *et al.*, 1989), but not the ecological factors influencing population dynamics of the *S. metallicum* complex. We therefore investigated the distribution patterns of immature stages of *Simulium metallicum s.l.* found in the north-central focus of onchocerciasis in Venezuela, in order to determine the key habitat factors associated with seasonal changes in population density of this vector blackfly.

Materials and Methods

Study area. Immature stages of the *S. metallicum* complex were collected from two first-order mountain streams (Stream I and Stream II) near Altamira (10°00'N, 67°47'W), Carabobo State (Fig. 1), an onchocerciasis endemic village in northern Venezuela, where *S. metallicum s.l.* is the predominant human-biting blackfly species. Vegetation of this area is a mixture of lowland wet forest, semi-deciduous forest and land cleared for agriculture. Annual mean temperature is 24°C and total rainfall 1100 mm, with a wet season from May to October and a dry season from November to April (Ewel *et al.*, 1976).

Streams I and II were 500 m apart with moderate to steep slopes along their routes. They consisted of alternating pools and riffles of clear, slightly alkaline water (pH rarely exceeding 8.0), with the bed composed predominantly of stones (1–40 cm in diameter). Two sampling stations, at

800 and 900 m altitude, were selected for each stream approximately 150 m apart for Stream I and 100 m apart for Stream II. Riparian vegetation along Stream I was relatively undisturbed, with the canopy dominated by deciduous trees: *Triplaris caracasana* Cham. (Polygonaceae), *Acacia glomerosa* Benth. (Leguminosae), *Erythrina glauca* Willd. (Leguminosae), *Bursera simaruba* (L.) Sarg. (Burseraceae) and *Genipa americana* L. (Rubiaceae), and the understorey dominated by *Ipomoea carnea* Jacq. (Convolvulaceae), *Clusia cuneata* Benth. (Guttiferae), *Piper nigrum* L. (Piperaceae), *Aphelandra glabrata* Willd. (Acanthaceae) and *Heliocarpus popayanensis* H.B.K. (Tiliaceae). Along the margins of Stream II, riparian vegetation was dominated by cultivated fruit trees and coffee shrubs which formed a partial canopy, consisting primarily of *Mangifera indica* L. (Anacardiaceae), *Annona muricata* L. (Annonaceae), *Musa paradisiaca* L. (Musaceae) and *Coffea arabica* L. (Rubiaceae). Stream I had less human disturbance than Stream II, which was closer to the village of Altamira.

Sampling. Larvae and pupae of *S. metallicum s.l.* were sampled monthly, July 1990 to September 1991. Because random sampling is inefficient when the distribution of organisms is highly aggregated (Elliot, 1977) as for larval simuliids (Adler & Kim, 1984), the sample sites were located selectively at the riffles of the stream. The sample area was a quadrat of 0.50 m² (50 × 50 cm). On each occasion, eight to ten samples were taken per sample

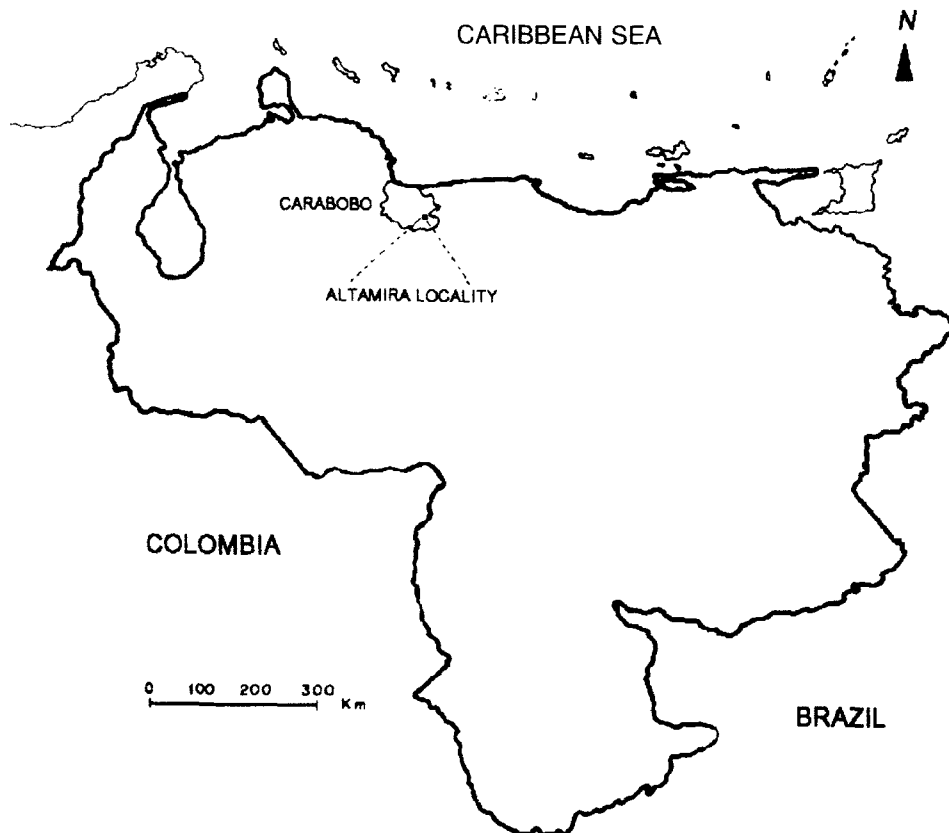


Fig. 1. Location of the study area in north-central Venezuela.

station, starting downstream to minimize disturbance. The larvae were removed with forceps from the surface of natural substrates within the quadrat, blotted on paper and preserved in two changes of fresh Carnoy's fixative (1 part of glacial acetic acid and 2 parts of absolute ethanol) for later cytotoxic identifications. Mature pupae were collected and placed on moist paper to allow for the emergence of adults. The substrate rock was sampled *in situ*, while the vegetal substrate (leaf litter, twigs, roots) was placed into labelled plastic bags kept on ice for transportation to the field laboratory, where larvae and pupae were removed and the vegetation was dried and weighed.

Environmental variables. The hypothesis for this study was that the distribution of immature stages of the *S. metallicum* complex is associated with certain habitat variables. We studied eleven chemical/physical stream variables (width, depth, current velocity, discharge, temperature, pH, dissolved oxygen, conductivity, vegetal biomass, canopy cover and substrate type), most of which are known to be associated with the larval distribution of other Simuliidae (Colbo & Wotton, 1981; Ross & Merritt, 1987). These variables were measured (three replicates) for each collection event and at each sampling site. Stream width and depth measurements were made with a metre-stick. Because the streams were shallow (2–5 cm) with irregular topography, the current velocity (cm/s) was estimated by recording the time required for a small piece of cork to travel 100 cm. Discharge was determined on the basis of depth, width and mean current velocity readings taken at three points across the stream (Newbury, 1984). The water temperature, conductivity, dissolved oxygen and pH were determined with portable apparatus. Percentages of canopy cover and of substrate cover (leaf litter, rocks, roots, twigs) within each quadrat sampling area were estimated consistently by the same person.

Data analysis. Density data were transformed using $\log(x+1)$ to normalize the frequency distribution of the counts (Elliot, 1977). Relative density estimates were expressed as number of larvae per square metre. A three-way analysis of variance (ANOVA) was performed to examine the simultaneous and interactive effects of the stream type (I or II), altitude (800 or 900 m) and sample months on the relative density of *S. metallicum*. Because the analyses showed a significant effect of each factor (stream: $F = 12.78$, $P < 0.05$; altitude: $F = 2.91$, $P < 0.05$; month: $F = 8.87$, $P < 0.05$) and their interactions (stream \times altitude \times month: $F = 3.74$, $P < 0.05$), we also analysed the separate effects of stream and elevation. Simple correlation analyses were used to examine the relationship between larval abundance and the stream variables, except Spearman non-parametric analysis was used to correlate rainfall with the relative density of *S. metallicum* in each stream. Two-tailed *t*-tests were used to compare the means of stream variables (including the mean density of larvae) between altitudes for each stream. To determine whether the distribution of *S. metallicum* larvae differed between substrates, a χ^2 test was conducted with the substrate distribution in the habitat as the null hypothesis. Multiple regression analyses were performed for larval abundance

against the eleven independent environmental variables for each stream. Stream variables not showing symmetrical distribution were transformed using the \log_{10} transformation (width, depth, current velocity, discharge, vegetal biomass and conductivity) or the angular transformation (canopy cover and substrate type) prior to analysis.

Species identification. Larvae and pupae of the *S. metallicum* complex were identified as conforming with morphological descriptions by Ramírez-Pérez (1983). For cytotoxicity, mature larvae (with fully developed pupal respiratory histoblasts) were fixed in Carnoy's and stored as 4°C until they could be processed. Slide preparations of salivary gland polytene chromosomes ($2n = 6$) were stained using the Feulgen method (Rothfels & Dunbar, 1953) and microscopically examined at $\times 100$ magnification. Using photomicrographs, the polytene chromosomal banding patterns of each individual specimen were compared with known cytotoxicity patterns of the *S. metallicum* complex (Hirai, 1985; Conn *et al.*, 1989). Chromosome mapping conventions and inversion nomenclature follow those set out by Conn *et al.* (1989), with the use of numbers instead of capital letters when new rearrangements were observed.

Voucher specimens of *S. metallicum* complex larvae (putatively cytospecies E) from Altamira Streams I and II were deposited in the Natural History Museum, London, and in the Entomological Museum at the Central University of Venezuela, Caracas.

Results

Simuliid fauna

Simuliidae recorded from Stream I were predominantly *S. metallicum* s.l., *S. ochraceum* Walker, *S. paynei* Vargas, *S. horacioi* Okazawa & Onishi and some undetermined species. Only the first three of these morphospecies were found in Stream II.

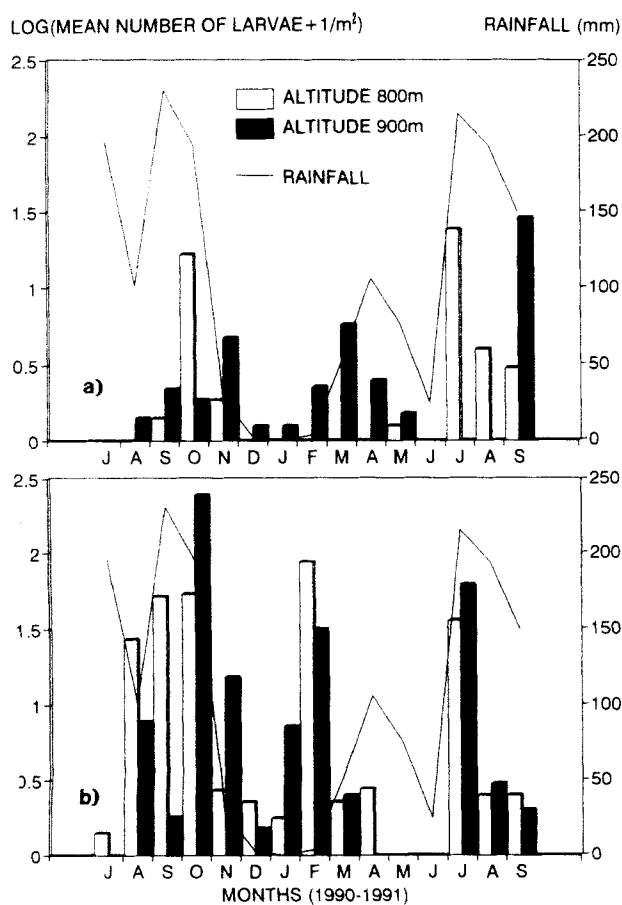
Cytotoxicity

Among forty mature larvae of the *S. metallicum* complex examined cytotoxicity, only seventeen specimens gave preparations of the polytene chromosomes in which all six arms could be read completely. Banding patterns of readable polytene chromosome arms in the remaining larvae (data not shown) agreed with the results reported here.

All seventeen individuals examined were identified as cytospecies E of the *S. metallicum* complex, with eight fixed inversion rearrangements from the standard polytene sequence, as originally described by Hirai (1985), including the diagnostic IIL-B+A inversion homozygote in both sexes of larva. No sex-linked polymorphisms were detected on any chromosomes in our samples of nine male and eight female larvae; any IIL-B+A/std heterozygous females would have been classified as cytotype D. Five floating (polymorphic) inversions were observed in our seventeen specimens of cytospecies E, with the terminal floating

Table 1. Comparisons between aquatic variables of Streams I and II (mean \pm SE) at 800 m and 900 m altitude (two-tailed Student's *t* test).

Variable	800 m altitude		900 m altitude	
	Stream I	Stream II	Stream I	Stream II
Depth ^a (cm)	4.2 \pm 0.2	2.9 \pm 0.1*	4.9 \pm 1.9	2.5 \pm 1.4*
Current velocity ^a (cm/s)	30.3 \pm 1.4	28.1 \pm 0.8	28.2 \pm 10.0	27.0 \pm 13.0
Width ^a (cm)	67.4 \pm 4.7	46.9 \pm 3.1*	58.8 \pm 40.6	42.3 \pm 27.4
Discharge ^a $\times 10^3$ (cm ³ /s)	5.55 \pm 0.6	2.36 \pm 0.4*	4.91 \pm 6.01	1.77 \pm 2.15*
Temperature ($^{\circ}$ C)	20.3 \pm 0.1	20.4 \pm 0.1	20.3 \pm 1.1	21.0 \pm 1.2*
Conductivity ^a (μ S/cm at 21 $^{\circ}$ C)	307.0 \pm 2.9	296.4 \pm 2.2	270.8 \pm 17.1	298.0 \pm 26.2*
Dissolved oxygen (mg/l)	8.2 \pm 0.0	8.1 \pm 0.0	8.1 \pm 0.3	8.0 \pm 0.3
pH	8.1 \pm 0.0	7.9 \pm 0.0	8.1 \pm 0.3	8.1 \pm 0.2
Vegetal biomass ^a (g)	7.5 \pm 0.8	5.6 \pm 0.5	6.6 \pm 5.1	5.0 \pm 5.0*
Canopy cover ^b (%)	59.7 \pm 2.5	59.3 \pm 2.8	84.5 \pm 18.4	41.2 \pm 30.6*
Larval substrate ^b (%)				
Rocks	81.3 \pm 1.4	86.9 \pm 1.3	83.4 \pm 1.4	84.5 \pm 1.5
Fallen leaves	14.4 \pm 1.2	11.7 \pm 1.2	12.6 \pm 1.1	12.0 \pm 1.1
Roots	2.9 \pm 0.7	0.4 \pm 0.2	2.6 \pm 0.2	1.8 \pm 0.7
Stems	1.4 \pm 0.3	1.2 \pm 0.3	1.5 \pm 0.3	0.8 \pm 0.2

* $P < 0.05$ for comparison between streams at same altitude.^a log transformation; ^b angular transformation.**Fig. 2.** Rainfall and density of *Simulium metallicum* cytospecies E larvae in Streams I (a) and II (b) at Altamira, June 1990 to September 1991.

inversion IS-A at a frequency of 35% (12/34 chromosomes), significantly higher than 7–18% in eastern Venezuela (Hirai, 1985). Four new floating inversions were found once each: IS-6 (spanning sections 6B–11A), IIII-16, IIII-17 and IIII-18 spanning sections 83A–85B, 87A–90B, and 95B–99B, respectively. Polytene chromosome arms IS, IIS and IIIS in all our specimens were identical to the standard for *S. metallicum* (Conn *et al.*, 1989). Inversions IIIS-A and IIIS-B, reported by Hirai (1985) to be polymorphic in cytospecies E, were fixed in the standard homozygous arrangement in our seventeen larvae.

Abundance and seasonality

Simulium metallicum E larvae were significantly more abundant ($t = -3.14$, $P < 0.05$) in Stream II (mean density $21.1 \pm \text{SE } 4.2/\text{m}^2$) than in Stream I ($2.9 \pm 0.7/\text{m}^2$). Larvae were slightly more abundant in Stream II at 900 m ($24.6 \pm 5.8/\text{m}^2$) than at the 800 m altitude ($17.5 \pm 2.5/\text{m}^2$, $t = 0.61$, $P > 0.05$) (Figs 2a, b). In Stream II, *S. metallicum* E exhibited three seasonal peaks of larval abundance (Fig. 2b): in the dry season (February 1990), at the beginning of the rainy season (July 1990) and the main peak at the end of the rainy season (October 1990), alternating with periods of few or no larvae at all (e.g. March–June 1991). Larval abundance was best correlated with 4-month lagged monthly rainfall ($r_s = 0.56$, $n = 15$, $P < 0.05$, Stream II).

Associated stream variables

Data for stream variables are summarized in Table 1. The mean depth, width and discharge of Stream I were

Table 2. Proportions of *Simulium metallicum* E larvae associated with differential ranges of stream variables.

Stream variable	Observed range of variable	Proportion of larvae (%) (<i>n</i> = 2582)
Depth (cm)	0.5–6	89.1
	7–12	10.8
Current velocity (cm/s)	9–20	42.4
	21–40	33.9
	41–125	23.6
Width (cm)	10–60	66.8
	61–120	25.7
	121–210	7.4
Conductivity ($\mu\text{S}/\text{cm}$ at 21°C)	220–300	31.3
	305–350	68.7
Vegetal biomass (g)	0–11	80.9
	11.1–22	18.9
	22.1–50	0.1
Canopy cover (%)	0–30	47.9
	31–61	21.1
	62–100	31.0

significantly greater than for Stream II at 800 m altitude. At 900 m, the mean depth, discharge, vegetal biomass and canopy cover were significantly greater for Stream I, whereas conductivity and temperature values were significantly greater in Stream II at the higher altitude. No other significant differences between streams were found in the variables measured.

Simulium metallicum E larvae were collected most frequently in sites (microhabitats) where the stream was narrowest, shallowest and with slowest current velocity, associated with least vegetal biomass and canopy cover (Table 2), although these values were not significantly correlated with larval abundance. Conductivity showed a significant positive correlation with larval abundance ($r = 0.27$, $n = 109$, $P < 0.05$). Most of the larvae were found attached to submerged rocks (49%) or fallen leaves (47%), rarely on roots or twigs (2% each; $\chi^2 = 88.5$, $df = 3$, $n = 4$, $P < 0.05$). Larvae predominated on rocks during the rainy season and on submerged fallen leaves during months of lowest rainfall (data not shown). Multiple regression analyses of monthly *S. metallicum* E larval abundance with the eleven aquatic habitat variables for Stream II resulted in significant, positive partial regression coefficients for discharge ($b = 0.14$, $R^2 = 0.11$, $F = 16.15$, $P < 0.01$) and conductivity ($b = 0.01$, $R^2 = 0.10$, $F = 11.30$, $P < 0.01$) at 900 m altitude ($R^2 = 0.31$, $F = 6.42$, $P < 0.01$), and for discharge ($b = 0.12$, $R^2 = 0.11$, $F = 14.90$, $P < 0.01$) at 800 m altitude ($R^2 = 0.25$, $F = 4.78$, $P < 0.05$).

Discussion

Simulium metallicum cytospecies E was originally described by Hirai (1985) from larvae collected in eastern Venezuela,

where this blackfly appears to be the primary vector of onchocerciasis (Takaoka *et al.*, 1984). The present study revealed the presence of this cytospecies at Altamira, within the north central focus of onchocerciasis where *S. metallicum s.l.* appears to be the vector based on vector competence studies (Duke, 1970; Grillet *et al.*, 1994).

The importance of studying local variation in the distribution and abundance of *S. metallicum* complex was shown by comparing Streams I and II at Altamira. Larvae predominated in Stream II, although both streams were close to each other and similar in their aquatic habitat variables. Differences between these two small confluent streams were mainly structural: Stream I being wider and deeper, with greater discharge. Stream II is closer to a village and has more signs of human intervention, i.e. sparse canopy cover, higher conductivity of the water (probably reflecting pollution), lower species richness of Simuliidae, but greater abundance of midge larvae (Chironomidae: *Phaenopsectra* spp.) (M. E. Grillet, unpubl. data). It would be interesting to investigate whether *S. metallicum* E is more abundant in Stream II because of its proximity to the village, where this anthropophilic species could more readily obtain bloodmeals. The differential distribution of blackfly larvae between streams may arise from the female's oviposition behaviour (Welton *et al.*, 1987; Pistrang & Burger, 1988; McCreadie & Colbo, 1992) interacting with structural and physicochemical features of streams to determine habitat selection. Alternatively, biotic interactions – such as predation and competition (cf. Dudley *et al.*, 1990; Hansen *et al.*, 1991) – might have prevented *S. metallicum* E from thriving in Stream I, in particular if disturbance has influenced the natural occurrence of competitors or predators in Stream II, as reported by Adler & Kim (1984) for the *S. vittatum* complex.

Variations in micro-habitat factors such as food availability (Hart, 1987), heterogeneity of flow (Eyman, 1993) and substrate size variation (Rabeni & Minshall, 1977) have been associated with blackfly larval micro-distribution and probably influenced the non-random distribution of *S. metallicum* E larval population that we observed. However, the greater abundance of *S. metallicum* E larvae in Stream II at 900 m rather than at 800 m altitude could not be accounted for simply on the basis of the habitat factors studied.

Larvae of *S. metallicum* E predominated in small, shallow reaches of the stream with slow water current, high conductivity and sparse vegetation cover. Determining whether these are the preferred conditions for this species would require more extensive sampling (Grunewald, 1976). The positive correlation between *S. metallicum* E and conductivity may indicate a preference for nutrient-enriched sites (Gordon & Cupp, 1980; Corkum & Currie, 1987). Also, a greater abundance of aquatic organisms at open sites in streams has been associated with higher quality of food (Behmer & Hawkins, 1986). The association of *S. metallicum* E with slow water current (<40 cm/s), which has also been reported for some North American blackflies (Osborne *et al.*, 1985), may involve the simultaneous

action of several factors. For example, although water flow is required for feeding by most blackfly species, there may be a trade-off between a good flow for feeding and the presence of potential competitors and predators (Newbury, 1984; Orth & Maughan, 1983). The apparent preference of *S. metallicum* E larvae for shallow water (≤ 10 cm), also reported for some blackflies in the temperate zone (Reisen, 1977; McCreadie & Colbo, 1992), could be related to turbulence, which is important for larval feeding (Orth & Maughan, 1983) and is usually inversely related to depth (Reisen, 1977; Morin *et al.*, 1986).

This study revealed seasonal fluctuations in the pre-adult population of *S. metallicum* E. Three distinct peaks of density occurred within the year: the highest at the end of the rainy season, with smaller pulses at the beginning of the rainy season and during the dry season. Since we did not examine the age structure of the population, it could not be determined whether those peaks represented discrete generations, but they are indicative of multivoltinism. The stream variable which best explained temporal changes in larval abundance of *S. metallicum* E was water discharge, as correlated with seasonal fluctuations of Simuliidae by Cobb *et al.* (1992). Discharge can influence substrate suitability and stability as well as feeding and respiration rates (Orth & Maughan, 1983; Minshall, 1984; Newbury, 1984). The larvae of *S. metallicum* E were mostly attached on rocks in the rainy season and on fallen leaves in the dry season, implying a seasonal change of substrate availability in our study streams. A low discharge in the dry season reduced the availability of rock substrate and coincides with an input of leaves from the adjacent deciduous forest. Thus, substrate availability and stability varied over time with stream discharge which, in turn, is a function of fine seasonal distribution of rainfall. The fact that *S. metallicum* E larvae mainly occupied two contrasted substrates (rocks and dead leaves) suggests that this is a larval generalist species. The positive association between stream discharge and *S. metallicum* E abundance may be related to several variables, such as turbulence and oxygenation, increased abundance of fine particulate organic matter (Bunn, 1986), and availability of various substrates.

In a Guatemalan study, *S. metallicum* s.l. showed less fluctuations in time and space, with larvae associated mainly with plant substrates in streams of various types and sizes (Dalmat, 1955). Different findings between that study and ours are not surprising because of the contrasted cytotypes of *S. metallicum* s.l. in Guatemala and Venezuela (Hirai, 1985; Conn *et al.*, 1989). Interspecific differences in spatial and temporal distribution, as well as in habitat and substrate preferences, are well known within species complexes of blackflies (Grunewald, 1976; Adler & Kim, 1984; Adler, 1987; Ciborowski & Adler, 1990; McCreadie & Colbo, 1991, 1992). Considering that there are at least eleven cytotypes in the *S. metallicum* complex (Conn, 1990), ecological contrasts between them are to be expected.

The pronounced seasonality of *S. metallicum* E and its predominance in Stream II at this locality help to focus vector control operations using larvicides, which should be applied in the dry season (e.g. February) rather than the

rainy season, in order to take advantage of possible natural regulation (density-dependent mechanism) in the simuliid larval population (Huffaker *et al.*, 1984).

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