



Spatial-temporal distribution of preimaginal blackflies in Neotropical streams

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Abstract

The current study investigates the spatial-temporal distribution of preimaginal blackflies in three *a priori* established, adjacent areas of Brazil and Venezuela. First we tested the hypothesis that the distribution of individual simuliid species, over two geographic scales, were predictable on the basis of several habitat parameters. Secondly, we determined if local species assemblages remain constant over two spatial axes, i.e., were species assemblages predictable within and between defined regions. Finally we compared the species assemblage between Venezuelan streams sampled in both the wet (October) and dry (February) seasons. As stream conditions changed across a north–south gradient, so did the occurrence of the most common species. This change in species composition from upland to lowland areas is consistent with the patterns of faunal change seen in North American simuliid assemblages. Discriminant Function Analysis showed a strong association between regional demarcations and stream site conditions. These results were paralleled by the strong correspondence between species assemblages and these regions. Thus each region presented both distinct stream environments and species assemblages. Monte Carlo analysis suggests that some if not all species are not only present in both the wet and dry season but that they are continuing to use the same stream sites. Three broad contingent rules for blackfly species assemblages in new world streams are put forward; (i) the ubiquitous nature of simuliids; (ii) species often distribute themselves along an axis of stream size and; (iii) there is a strong and consistent correspondence between regional characteristics of streams and the species assemblage.

Introduction

Community ecologists have argued that patterns of species distribution and abundance may provide insight into the mechanisms that structure species assemblages (e.g., Tokeshi, 1990; Begon et al., 1996). However, it has also been suggested that different mechanisms may lead to similar patterns of community structure (e.g., Lawton, 1999). More disturbing is Lawton's statement that "community ecology is a mess" with regards to the establishment of useful

rules or patterns of species assemblages. The species is considered the fundamental unit of ecological study (Krebs, 1994) and it has been argued that ecological interpretations have little meaning in the absence of taxonomic rigor (e.g., McCreadie & Adler, 1998). Thus, difficulties in establishing general laws or even contingent rules governing species assemblages (Lawton, 1999), may partially stem from poor taxonomic knowledge. A significant obstacle to the study of lotic insects has been the difficulty of species-level identifications. This point is well illustrated by the paucity of

species-level descriptions of immature stages of many common groups of aquatic insects, even in well studied areas such as North America (e.g., McCafferty et al., 1990; Wiggins, 1990). The situation is, of course, much worse in the tropics.

Because the taxonomy of North American blackflies (Diptera: Simuliidae) is well developed, integrating morphotaxonomic, cytotoxic, and ecological approaches, these ubiquitous organisms serve as model subjects for the study of lotic community structure (Adler & McCreadie, 1997). Likewise, recent studies have advanced both the taxonomy and ecology of the neotropical blackfly fauna (Grillet & Barrera, 1997; Hamada, 1997; Hamada & McCreadie, 1999; Hamada & Grillet, 2001; Hamada et al., 2002). For example, Hamada et al. (2002) showed that the preimaginal distributions of four of six amazonian species of blackflies examined were significantly associated with stream size or the presence of impoundments. These factors are also powerful predictors of species distribution in Nearctic and Palearctic regions (Ciborowski & Adler, 1990; McCreadie et al., 1995; Adler & McCreadie, 1997; Malmqvist et al., 1999). The importance of stream size and impoundments in Amazonas supports the contention that certain predictors of blackfly distribution may be common to all running-water ecosystems (Adler & McCreadie, 1997; Hamada & McCreadie, 1999) i.e., consistent habitat axes along which blackfly communities are assembled.

Accordingly, we asked a basic question regarding the relationship of preimaginal blackflies to the neotropical stream environment: Is the distribution of individual species and species assemblages predictable on the basis of select habitat conditions? We then compared our results to previous studies to determine if there are general, or at least contingent, assemblage rules for simuliid communities. Regression analysis was used to link the distribution of individual species to stream site conditions both across the three regions examined – Gran Sabana, Boa Vista, Pacaraima – and within a single region, Gran Sabana. DFA was used to determine if the three study regions differed significantly with respect to stream site conditions and blackfly fauna. In addition, we examined temporal distribution of species in one region (Gran Sabana) and asked whether the assemblage of preimaginal blackflies changed between the wet and dry seasons using a Monte Carlo approach. Hence, the current study investigated the spatial-temporal distribution of preimaginal blackflies in three distinct, but adjacent, areas of Brazil and Venezuela.

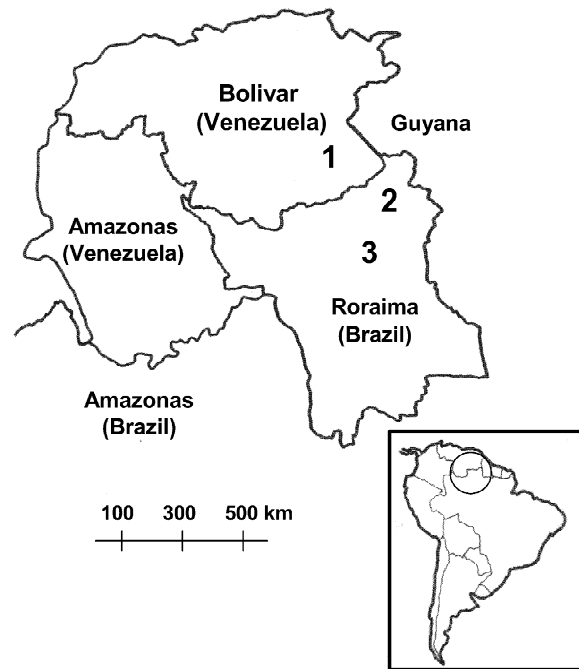


Figure 1. Map showing location of the three regions sampled: 1 = Gran Sabana, 2 = Pacaraima, 3 = Boa Vista. Circle on insert of South America shows approximate location of the area covered by the map.

Study area

Our intent was to sample a variety of streams in three distinct, but adjacent, regions. Accordingly, streams were sampled over 445 km and 140 km, north-south and east-west axes, respectively (Fig. 1). The first region sampled was Gran Sabana, Camaima National Park, Bolivar, Venezuela. Details of Gran Sabana can be found in Dezzee (1994) and Hamada & Grillet (2001). This region is an upland undulating plain, approximately 30 000 km² in size, in the southern most region of the state of Bolivar. It is dominated by treeless savannas with pockets of montane and gallery forests. Most of the region has a humid sub-montane climate with an annual rainfall between 2000 and 3000 mm. Streams were sampled between 4.45–5.99° N and 60.96–61.75° W and from 600–1400 m asl (above sea level). The second region investigated was in the vicinity of Pacaraima, Brazil. Collections were taken between 3.62–4.45° N and 60.89–61.73° W at elevations from approximately 100–700 m asl. The dominant vegetation form was low canopy forests and brush. The climate is lower montane with an annual rainfall of 1000–2000 mm.

The third region sampled was in the vicinity of Boa Vista, Brazil. The climate here is described as a hot-climate domain with distinct wet and dry seasons (Nimer, 1975); annual rainfall varies between 1500 and 2000 mm. Much of the land in this region is used for agriculture. Collections were taken between 1.98–2.75° N and 60.52–61.29° W and 50–100 m asl. Details about these latter two regions can be found in Hamada (1997).

Methods

Sampling and preimaginal identification

Sampling was conducted on the main roads and trails during October (wet season) of 1996. The number of streams sampled in Gran Sabana, Pacaraima, and Boa Vista was 37, 16, and 12, respectively. In February, 1998 (dry season), 17 streams were re-sampled in Gran Sabana; remaining streams in this region were dry in 1998. Each stream site was sampled by hand-collecting larvae and pupae from all available natural substrates. A mean ($\pm 99\%$ CI) of 30.0 ± 4.3 min was spent at each site. Rationale for this sampling protocol is given in McCreadie & Colbo (1991). As in similar studies (e.g., McCreadie & Adler, 1998; Hamada et al., 2002), it was assumed that species found in samples were representative of local occurrences.

Stream width, depth (2–5 equidistant measurements across stream), velocity, temperature (hand-held thermometer), pH (Cole-Parmer, pH Testr 2), conductivity (Oakton Con 10), position (Garmin GPS), elevation (Garmin GPS), dominant streambed particle size, canopy cover, and riparian vegetation were measured at the time of each collection. Where possible, water velocity was estimated by two methods: (i) the time a cork took to move a prescribed distance, and (ii) the distance water moved up the wide face of a steel ruler using the formula $U = \sqrt{2gd}$, where U = water velocity ($U \text{ ms}^{-1}$), g = the force due to gravity (ms^{-2}), and d = the distance (m) the water moved up the ruler (Craig, 1987). In some streams it was only practical to use either the cork or the ruler method, for example, in large, fast rivers or small trickles, only the cork method could be employed. Hamada & McCreadie (1999) showed that both methods produced similar estimates of velocity ($r = 0.931$, $p < 0.001$). Depth (d), width (w) and velocity (U) measurements were used to estimate discharge (i.e., $Q \text{ m}^3 \text{ s}^{-1} = d \times w \times U$). Ranked measurements of the dom-

inant substratum (mud, sand, small stones, rubble, boulders, bedrock; ranked 1–6), riparian vegetation type (open, brush, forest; ranked 1–3), and canopy cover (open, partial, complete; ranked 1–3) followed McCreadie & Colbo (1991). Presence/absence of water impoundments within 100 m upstream of each collecting site were also noted at each site.

Larvae were placed in acetic ethanol (1:3). This fixative was changed twice during the subsequent 12 h. Larvae were then refrigerated (4°C) until needed. Pupae with pharate adults were maintained alive in plastic vials provided with wet filter paper to obtain adults; after emergence the adults and pupal castings were preserved in 80% ethanol. Identification of specimens followed Hamada & Grillet (2001) as well as established chromosome maps (e.g., Hamada, 1997). Voucher specimens of all species examined are deposited in the Invertebrate collections of the Laboratorio de Biología de Vectores (MLBV), Instituto de Zoología Tropical, Universidad Central de Venezuela, Caracas, Venezuela and the Instituto Nacional de Pesquisas da Amazonia (INPA), Manaus, Amazonas, Brazil.

Data analysis

Species data from each site was expressed on a binary scale, i.e., presence /absence data. Previous studies (e.g., Corkum, 1989; McCreadie & Adler, 1998; Feminella, 2000) have shown that binary data from single-point collections is robust enough to detect faunal differences among streams. All statistical tests were considered significant at $p < 0.01$. To eliminate possible seasonal effects, only data collected in October 1996 ($n = 65$) was used to examine spatial distribution patterns. Potential collecting bias was examined by a rank correlation of species richness to sampling effort (i.e., time spent collecting at each site). Correlation analysis showed no significant relationship between sample species richness and sampling effort in either 1996 ($r = -0.263$; $n = 65$; $p = 0.044$) or 1998 ($r = 0.406$; $n = 17$; $p = 0.119$); indicating collecting methodology resulted in no substantial bias.

Discriminant Function Analysis (DFA) was used to examine the correspondence between stream conditions and region. Specially, DFA was used to first determine if the three regions differed significantly based on selected stream site conditions (velocity, depth, temperature, pH, conductivity, stream-bed particle size, canopy cover) and second, to determine which variables contribute most to this separation. A jack-knife approach was used to estimate classification-

error (Norusis 1985) and the standardized coefficients for discriminant function 1 were used to determine the contribution of each stream variable to region separation (Quinn & Keough, 2002). The most important assumption of DFA is that the within-region variance-covariance matrices are homogeneous among regions (Quinn & Keough, 2002). However, this assumption is not readily tested (Tabachnick & Fidell, 1996). Hence, subjective detection of heterogeneity across within-region variance-covariance matrices was made by plotting DFA scores for the first two discriminant functions and examining the spread of points among regions. To reduce any potential problem of heteroscedasticity, variables (except pH which is already transformed) were square root transformed. In addition the Pillai's trace statistic was used to test the significance of the DFA as it is known to be robust with respect to both departures from homogeneity and multivariate normality (Johnson & Field, 1993; Tabachnick & Fidell, 1996). If heterogeneity was determined to be a concern, based on the subjective score plots, then a quadratic DFA was performed (which does not assume homogeneity) and both the significance of the Pillai trace statistic and standardized coefficients for discriminant function 1 were compared to the linear DFA. If interpretations were similar for both the linear and quadratic DFA, it was assumed heterogeneity did not have a significant effect on the interpretation of the linear DFA. To determine if outliers had any influence on the DFA, potential outliers, as judged from DFA plot, were removed and the DFA recalculated. If both the significance of the Pillai trace statistic and the standardized coefficients were comparable to the saturated DFA, then outliers were judged to have no significant influence.

Regression analysis was used to link the distribution of individual species to stream site conditions. Because the response variable for each species was binary (0 = species absence, 1 = species presence in a sample), forward logistic multiple regression was used to estimate the probability of a species being present at a site, given measured site conditions. Entrance of each variable into the model was arbitrarily set at $p = 0.05$, with the significance of each predictor ($p < 0.01$) assessed using maximum likelihood estimation (Hosmer & Lemeshow, 1989). The occurrence of water impoundments (coded 0,1) was entered into the regression as a dummy variable. Only species that occurred at more than 20% of sites – either within a region when examining within-region distribution or across all regions when examining across

region distribution – were subject to regression analysis. Preliminary analysis indicated that using species occurring at a frequency less than 20% resulting in regressions with a lack of power (type II error) due to the large number of zero values. Thus, uncommon species were not considered in this analysis.

Stream variables are often highly intercorrelated (e.g., discharge and width, $r = 0.908$, $p < 0.001$). Such multicollinearity affects confidence intervals and significance tests of regression coefficients, hence, resulting models are unreliable (Neter et al., 1990). To avoid problems associated with multicollinearity, principal component analysis (PCA) was used to transform stream variables into a set of statistically independent principal components or PCs (McCreadie & Adler, 1998; Quinn & Keough, 2002). The use of PCA can also allow broader ecological interpretations of habitat variables (e.g. McCreadie & Adler 1998; Hamada et al., 2002). Thus, PCs with eigenvalues greater than 1.0 (Norusis, 1985), replaced the original stream variables as predictors in regression analyses. Variables not normally distributed were subjected to appropriate transformations (\log_{10} , square root) before entering a PCA. Interpretation of PCs was based on rank correlations between each PC and original stream variables (Ludwig & Reynolds, 1988). Concordance was used to assess the fit of regression models to the observed data. Concordance pairs all values of the response variable that are different (i.e., 0,1), and then counts the number of times that the member of a pair with the higher predicted probability of a species being present was correct (SAS, 1987). Results are expressed as a proportion of the total number of pairs compared.

Monte Carlo simulations were used to determine if species assemblages among streams within a region were similar. Accordingly, three separate simulations were undertaken, one for each region sampled in 1996. In contrast to regression analyses, all species were considered in this procedure. The test statistic, total number of co-occurrences within a region, was generated using 1000 Monte Carlo simulations (Manly, 1991) and compared to the observed total co-occurrences in that region (McCreadie et al., 1997). For example, if site A and B had two species in common, site B and C had three species, and sites A and C had six species, then the total number of observed co-occurrences among sites would be 11. This observed number of total co-occurrences was then compared with the generated distribution and, if the p value of the observed co-occurrence was low (i.e., $p < 0.01$), then the observation was judged to be significant. A

pattern of species co-occurrence at a frequency greater or less than that expected by a random model would indicate a predictable community structure. Total number of species collected in a region was considered to be the species pool from which simulated samples were drawn. For each species selection probability in the simulation was linked to its observed frequency of occurrence among streams. McCreadie et al. (1997) showed that this constraint produces robust and unbiased results. In addition, species richness at each stream in the simulation was set to equal observed richness.

A DFA was used to determine if each region supported a distinct fauna. This analysis was considered a measure of the similarity of the fauna within each region and the divergence of the fauna across regions. As above, a jackknife approach was used to estimate classification-error and the standardized coefficients from discriminant function 1 were used to determine the contributions of each species to region separation (Quinn & Keough, 2002). The Pillai's trace statistic was used to test the significance of the DFA and a DFA plot was used to look for heterogeneity. For this analysis all species found were used to maximize regional separation.

A Monte Carlo analysis was also used to examine temporal changes in the species assemblage in Gran Sabana between the wet (October, 1996) and dry (February, 1998) seasons. Specifically, we were interested in whether species co-occurrences between seasons within a site occurred at a frequency different than that expected by a random model (i.e., $p < 0.01$). All species observed from streams sampled in both seasons was considered to be the species pool. For each site, the same number of species as observed for each season was randomly drawn from the total species pool and the number of co-occurrences between seasons noted. This was repeated for all remaining streams after which the total number of co-occurrences was summed. The probability distribution (i.e., p values) for total species co-occurrences between seasons was generated by a 1000 simulations and the observed number of co-occurrences was then compared with the generated distribution.

A DFA was used to determine if stream conditions changed significantly between 1996 and 1998. It was reasoned that if stream sites could be correctly assigned to each season on the basis of velocity, discharge, depth, width, temperature, pH, and conductivity, then this would indicate significant seasonal differences in the stream habitat. It was assumed

that the remaining measured stream variables (e.g., streambed particle size) would show little seasonal change. As with the above DFA's a jackknife approach was used to estimate classification-error rates (Norusis, 1985) and the Pillai's trace statistic was used to test the significance.

Results

Stream fauna

Preimaginal blackflies were found at all 65 sites sampled in 1996; a total of 21 species were recorded (Table 1). The most frequently collected species were *Simulium lutzianum* Pinto, *Simulium quadridum* Lutz, *Simulium subpallidum* Lutz, *Simulium incrustatum* Lutz, *Simulium perflavum* Roubaud and *Simulium maroniense* Floch & Abonnenc. Three species, *Simulium covagarciai* Ramírez-Pérez et al., *Simulium guianense s.l.* Wise and *Simulium rubrithorax* Lutz, were each collected at only a single site. Mean (\pm 99% CI) number of species per site was 3.3 ± 0.4 and there was no significant difference in species richness among the three regions ($F = 0.22$; $df = 2,64$; $P = 0.807$). All 14 species that were found in Gran Sabana during February of 1998 were also recorded in 1996. The mean number of species per site in 1998 was 3.9 ± 1.1 .

Site conditions

In addition to a distinct elevation gradient from Gran Sabana to Boa Vista, correlation analysis (Pearson) indicated that temperature ($r = -0.519$), pH ($r = -0.412$) and conductivity ($r = -0.584$) decreased with distance northward ($n = 65$; $p < 0.001$), whereas streambed particle size ($r = 0.398$) increased. DFA showed that most streams could be correctly assigned to their region of origin on the basis of site conditions (Table 2). The Pillai's trace statistic was highly significant ($p < 0.001$) and the overall success of region separation was 86% (Jack-knifed estimate of error). In Gran Sabana 95% of sites were correctly assigned, whereas 75% of both Boa Vista and Pacaraima streams were correctly identified as to region of origin. Standardized coefficients for the first discriminant function indicated that temperature and conductivity were the variables that contributed most to stream separation (Table 2). The DFA plot shows no gross heterogeneity. Both the quadratic DFA, and the linear DFA in which

Table 1. Frequency of larval black fly species collected in Gran Sabana (Venezuela), Pacaraima (Brazil) and Boa Vista (Brazil), during October, 1996. Percent occurrence for each species was calculated as the number of stream sites in which a species was found, divided by the number of streams sampled (*n*)

| Species | % occurrence | | | |
|---|---------------------------------|-------------------------------|-------------------------------|-------------------------------|
| | Gran Sabana (<i>n</i> = 37) | Pacaraima (<i>n</i> = 16) | Boa Vista (<i>n</i> = 12) | All sites (<i>n</i> = 65) |
| <i>Simulium bipunctatum</i> Malloch | 0 | 25.0 | 0 | 4.9 |
| <i>S. cauchense</i> Floch & Abonnenc | 24.3 | 6.3 | 0 | 24.4 |
| <i>S. covagarciai</i> Ramírez-Pérez, Yarábal, Takaoka, Tada & Ramírez | 0 | 6.3 | 0 | 1.3 |
| <i>S. exiguum</i> Roubaud | 0 | 6.3 | 8.3 | 2.4 |
| <i>S. goeldii</i> Cerqueira & Nunes de Mello | 10.8 | 0 | 25.0 | 11.0 |
| <i>S. guianense</i> s.l. Wise | 0 | 6.3 | 0 | 3.7 |
| <i>S. ignacioi</i> Ramírez-Pérez & Vulcano | 5.4 | 0 | 0 | 3.7 |
| <i>S. inaequale</i> Paterson & Shannon | 18.9 | 31.3 | 0 | 43.9 |
| <i>S. incrustatum</i> Lutz | 45.9 | 6.3 | 66.7 | 23.2 |
| <i>S. iracouboense</i> Floch & Abonnenc | 0 | 12.5 | 8.3 | 3.7 |
| <i>S. kabanayense</i> Ramírez-Pérez & Vulcano | 16.2 | 0 | 0 | 7.3 |
| <i>S. lutzianum</i> Pinto | 21.6 | 31.3 | 0.0 | 17.1 |
| <i>S. maroniense</i> Floch & Abonnenc | 64.9 | 50.0 | 0.0 | 48.8 |
| <i>S. metallicum</i> s.l. Bellardi | 2.7 | 6.3 | 0 | 3.7 |
| <i>S. perflavum</i> Roubaud | 37.8 | 37.5 | 66.7 | 42.7 |
| <i>S. quadrifidum</i> Lutz | 10.8 | 31.3 | 66.7 | 21.0 |
| <i>S. rorotaense</i> Floch & Abonnenc | 0 | 18.8 | 0 | 3.7 |
| <i>S. rubrithorax</i> Lutz | 0 | 6.3 | 0 | 1.3 |
| <i>S. spinibranchium</i> Lutz | 16.2 | 0 | 0 | 12.2 |
| <i>S. suarezi</i> Ramírez, Perez, Rassi & Ramírez | 16.2 | 0 | 0 | 12.2 |
| <i>S. subpallidum</i> Lutz | 29.7 | 25.0 | 50.0 | 36.6 |

potential outliers were removed (analyses not shown), produced significant Phillai trace statistics as well as interpretations of standardized coefficients that were comparable to the saturated linear DFA. Thus, we concluded that stream sites in each region were distinct with respect to the stream variables measured.

Species distributions

When all streams sampled in 1996 were considered, four principal components had eigenvalues >1.0 and together accounted for 79.7% of the variability among sampling stations (Table 3). PC-1, largely a measure of position, explained 30.7% of the intersite variability. Higher PC-1 values identified more southern streams with the associated lower elevation and higher temperature, pH and conductivity. Sites with higher PC-1 values were also larger (higher velocity, depth, discharge), more open streams, with smaller streambed

particles. PC-2, accounting for an additional 24.7% of site variability, was largely a measure of stream size. Sites with higher PC-2 values were smaller (lower velocity, depth, width, discharge) and warmer than sites with lower PC-2 values. Higher PC-2 values also tended to be further south than sites with lower PC-2 values. PC-3 accounted for another 14.9% of site variability. PC-3 was a measure of stream cover and size; sites with high scores were more open and smaller than sites with lower scores. PC-4 explained another 9.4% of variability. High PC-4 scores indicated cooler, covered streams with low streambed particle size.

Five of the six regressions calculated for species distributions (Table 4) were significant ($p \leq 0.002$) and the fraction of concordance was >70% for these regressions. The distribution of *S. marionenses* and *S. quadrifidum* had a significant association with PC-1. Thus, *S. marionenses* was most likely to be found in

Table 2. Results of discriminant function analysis (DFA) of the correspondence between (i) stream site conditions and region from which streams originated and; (ii) stream fauna and stream origin. Stream site variables used in the analysis were velocity, depth, pH, temperature, conductivity, canopy cover and streambed particle size. Stream fauna included all blackflies species found at all streams examined ($n = 65$)

| Summary Statistics | Discriminating Variables | | | |
|--|--------------------------|--------|-----------------------|--------------------|
| | Stream site conditions | | Stream fauna | |
| % correct (N) | | | | |
| Gran Sabana (37) | 95 | | 74 | |
| Parcaraima (16) | 75 | | 56 | |
| Boa Vista (12) | 75 | | 92 | |
| Total (65) | 86 | | 74 | |
| Standardized coefficients¹ | | | | |
| | Velocity | 0.165 | <i>inaequale</i> | 1.021 ² |
| | Depth | 0.048 | <i>maroniense</i> | 0.754 |
| | Temperature | 0.593 | <i>perflavum</i> | 0.372 |
| | pH | 0.107 | <i>quadrifidum</i> | 0.720 |
| | Conductivity | 0.888 | <i>rorotaense</i> | 0.700 |
| | Streambed | -0.311 | <i>spinibranchium</i> | 0.463 |
| | Canopy Cover | -0.018 | <i>subpallidum</i> | 0.455 |
| Pillai's Trace | | | | |
| <i>F</i> | 7.98 | | 4.14 | |
| <i>df</i> | 14, 114 | | 40, 88 | |
| <i>P</i> | <0.001 | | <0.001 | |
| Eigenvalues (%) | | | | |
| Function 1 | 92.4 | | 54.1 | |
| Function 2 | 7.8 | | 45.9 | |

¹Standardized coefficients are for the first discriminant function.

²For purposes of presentation only the top seven standardized discriminant function 1 coefficients are given for species.

small northern streams with lower temperature, pH and conductivity. It was also less likely to be found at sites with impoundments than at sites without impoundments. In contrast, *S. quadrifidum* was most commonly found in large southern streams with higher temperature, pH and conductivity. *Simulium lutzianum* and *S. perflavum* were significantly associated with PC-2. *Simulium lutzianum* was most likely to occur in large cool northern streams, whereas *S. perflavum* was most common in small warmer southern streams. *Simulium subpallidum* was significantly associated with PC4, i.e., it was most common in warm open sites with large streambed particles.

Only Gran Sabana had sufficient collections for analysis of species distributions within a region. Three principal components had eigenvalues >1.0 and together accounted for 67.6% of the variability among sampling stations (Table 3). PC-1, largely a measure

of stream size, explained 35.2% of the intersite variability. Higher PC-1 values identified smaller, open streams with lower conductivity and higher temperature than sites with lower PC-1 scores. PC-2 accounted for an additional 19.8% of variability. High PC-2 values identified covered, low elevation narrow streams with small streambed particles. PC-3, accounting for another 12.6% of variability, was only correlated to streambed particle size and riparian vegetation. Only three of the six regressions calculated for species distributions in Gran Sabana (Table 4) were significant ($p \leq 0.007$) and the fraction of concordance was >86% for all regressions. *Simulium cauchense* Floch & Abonnenc and *S. lutzianum* show a significant negative association with PC1, thus, these species were most likely to be found in the relative larger streams of Gran Sabana. The negative association of the *S. subpallidum* with PC-2 indicated is was most frequently

Table 3. Results of PCA and Spearman's rank correlation analysis between stream variables and derived principal components (PC's) for all collections ($n = 65$) taken during October, 1996

| Variables | Stream Sites | | | Principal components | | | |
|--|--------------|-------------------------|------------------|----------------------|----------|----------|----------|
| | Min | Max | Mean (\pm SE) | PC-1 | PC-2 | PC-3 | PC-4 |
| All sites | | | | | | | |
| Velocity ($m s^{-1}$) | 0.23 | 1.50 | 0.76 ± 0.03 | 0.322* | -0.558** | 0.154 | -0.108 |
| Discharge ($m^3 s^{-1}$) | 0.01 | 12.76 | 1.38 ± 0.46 | 0.394* | -0.829** | -0.365* | -0.106 |
| Depth (m) | 0.02 | 0.80 | 0.20 ± 0.02 | 0.580** | -0.568** | -0.346* | 0.185 |
| Width (m) | 0.2 | 65.0 | 5.2 ± 1.1 | 0.147 | -0.816** | -0.410** | -0.098 |
| Temperature °C | 20.1 | 31.0 | 25.1 ± 0.3 | 0.363* | 0.559** | -0.134 | -0.409** |
| pH | 3.5 | 7.0 | 5.4 ± 0.1 | 0.607** | 0.199 | -0.003 | -0.312 |
| Conductivity ($\mu S cm^{-1}$, 25 °C) | 3.5 | 42.7 | 14.7 ± 1.1 | 0.803** | 0.237 | 0.074 | 0.010 |
| Elevation (m) | 55 | 1426 | 724 ± 56 | -0.852** | -0.374* | 0.020 | -0.113 |
| Degrees N of equator | 1.978 | 5.982 | - | -0.873** | -0.385* | 0.040 | -0.133 |
| Streambed ¹ | | mud – bedrock | | -0.339* | -0.249 | 0.266 | -0.820** |
| Riparian vegetation ¹ | | open – forest | | 0.361* | 0.290 | 0.732** | 0.052 |
| Canopy ¹ | | none – complete | | 0.289 | 0.127 | 0.818** | 0.232* |
| % variance explained | | | | | | | |
| Proportion | | | | 30.6 | 24.7 | 14.9 | 9.4 |
| Cumulative | | | | 30.7 | 55.4 | 70.3 | 79.7 |
| Gran sabana | | | | | | | |
| Velocity ($m s^{-1}$) | 0.23 | 1.27 | 0.74 ± 0.04 | -0.732** | -0.152 | -0.150 | |
| Discharge ($m^3 s^{-1}$) | 0.01 | 12.00 | 1.15 ± 0.38 | -0.887** | -0.299 | 0.204 | |
| Depth (m) | 0.02 | 0.80 | 0.18 ± 0.03 | -0.705** | -0.078 | 0.398 | |
| Width (m) | 0.4 | 18.0 | 4.9 ± 0.8 | -0.770** | -0.467* | 0.137 | |
| Temperature °C | 20.1 | 30.0 | 23.9 ± 0.4 | 0.633** | -0.024 | -0.094 | |
| pH | 3.5 | 6.4 | 5.0 ± 0.1 | -0.217 | 0.314 | -0.352 | |
| Conductivity ($\mu S cm^{-1}$ at 25 °C) | 3.5 | 18.5 | 8.6 ± 0.6 | -0.502* | 0.407 | 0.064 | |
| Elevation (m) | 653 | 1426 | 1069 ± 35 | 0.029 | -0.719** | -0.262 | |
| Streambed ¹ | | mud/silt – bedrock | | -0.015 | -0.711** | -0.544** | |
| | | open – forest | | | | | |
| Riparian vegetation ¹ | | none – partial/complete | | -0.480* | 0.466 | -0.562** | |
| Canopy ¹ | | | | -0.488* | 0.524* | -0.460 | |
| % variance explained | | | | | | | |
| Proportion | | | | 35.2 | 19.8 | 12.6 | |
| Cumulative | | | | 35.2 | 55.0 | 67.6 | |

¹Ranked variables: stream bed 1–6, riparian vegetation 1–3; canopy 1–3. Rankings followed McCreadie & Colbo, 1991.

* $p < 0.01$; ** $p < 0.001$.

found in wide, open, high elevation streams with large streambed particles.

Species assemblages

Total species co-occurrences among streams for both Gran Sabana and Boa Vista occurred at a rate higher ($p < 0.01$) than expected by random, i.e., the assemblage structure in each region showed significant

redundancy among streams (Figure 3). In contrast, in Pacaraima, the total number of observed species co-occurrences did not occur at a frequency different from that expected under a random model (Fig. 3).

DFA showed that most streams could be correctly assigned to region of origin on the basis of the blackfly fauna (Table 2). In Boa Vista only one streams was incorrectly classified; 56% of Pacaraima streams and 76% of Gran Sabana sites were correctly identified

Table 4. Regression analyses for distributions of *Simulium* species collected at all sites ($n = 65$) and Gran Sabana, during October, 1996

| Species | Regression Coefficients ¹ | | | | | | P | Concordance |
|-----------------------|--------------------------------------|--------|--------|------|--------|------------------|--------|-------------|
| | B ₀ | PC-1 | PC-2 | PC-3 | PC-4 | Imp ² | | |
| All sites | | | | | | | | |
| <i>S. incrustatum</i> | – | – | – | – | – | – | >0.050 | – |
| <i>S. lutzianum</i> | –1.803 | – | –0.737 | – | – | – | <0.001 | 79.7% |
| <i>S. maroniense</i> | 0.322 | –0.391 | – | – | – | –1.714 | 0.001 | 76.7% |
| <i>S. perflavum</i> | –0.331 | – | 0.493 | – | – | – | 0.002 | 70.1% |
| <i>S. quadrifidum</i> | –1.193 | 0.435 | – | – | – | – | 0.007 | 70.8% |
| <i>S. subpallidum</i> | –0.882 | – | – | – | –0.929 | – | 0.001 | 74.2% |
| La Gran Sabana | | | | | | | | |
| <i>S. cauchense</i> | –2.181 | –1.264 | – | – | – | – | <0.007 | 92.5% |
| <i>S. incrustatum</i> | – | – | – | – | – | – | >0.050 | – |
| <i>S. lutzianum</i> | –2.609 | –1.342 | – | – | – | – | <0.001 | 93.1% |
| <i>S. maroniense</i> | – | – | – | – | – | – | >0.050 | – |
| <i>S. perflavum</i> | – | – | – | – | – | – | >0.050 | – |
| <i>S. subpallidum</i> | –1.448 | – | –1.288 | – | – | – | <0.001 | 86.4% |

¹ Under the logistic function $p_i = e^L / (1 + e^L)$ and $L = B_0 + B_1 X_{1i} + \dots + B_j X_{ji}$. p_i is the probability that a species is present at the i th site, $X_{1i} \dots X_{ji}$ are the predictor (independent) variables (PCs and nominal variable), and $B_1 \dots B_j$ are the regression coefficients for the linear combination of predictors. Intercept for equation is B_0 .

² Imp = Impoundment.

as to region of origin. The Pillai's trace statistic was highly significant ($p < 0.001$) and the overall success of region separation was 74% (Jack-knifed estimate of error). Standardized coefficients for the first discriminant function indicated that four species – *S. inaequale*, *S. quadrifidum*, *S. rorotaense*, and *S. maroniense* – contributed most to stream separation (Table 2; also see Table 1). The DFA plot (Fig. 2) showed no gross heterogeneity. Both the quadratic DFA and the linear DFA in which potential outliers were removed (analyses not shown) produced significant Pillai trace statistics and standardized coefficients comparable to the saturated linear DFA. Thus, species assemblages, at least for Boa Vista and Gran Sabana, were distinct from each other.

The Monte Carlo analysis used to generate the test statistic for local species assemblages between the wet (October) and dry (February) seasons for Gran Sabana is given in Figure 4. In this case the test statistic is the total number of species co-occurrences between seasons in each stream sampled. As observed co-occurrences between the two seasons was significantly greater ($p < 0.01$) than expected from a random model; suggesting that the local assemblage found in both seasons were similar. DFA showed that most streams in Gran Sabana could be correctly assigned

to season (wet or dry) on the basis of site conditions. The Pillai's trace statistic was highly significant ($p = 7.75$, $df 7,23$; $p = 0.001$) and the overall success of separation was 87% (Jack-knifed estimate of error). In Gran Sabana during the wet season 82% of sites were correctly assigned; during the dry season 93% of were correctly identified to season. Standardized coefficients for the first discriminant function indicated that width, velocity and temperature were the variables that contributed most to stream separation. Hence, it was concluded that stream conditions differed between the wet and dry season.

Discussion

Species distribution patterns

The area sampled in our study showed distinct elevation and vegetation changes from a low plain dominated by agriculture, to low elevation forested mountains to an undulating upland savanna with patches of montane and gallery forests. A strong association was found between these *a priori* established regional demarcations and stream site conditions. Thus, each region studied (Gran Sabana, Pacaraima, Boa Vista)

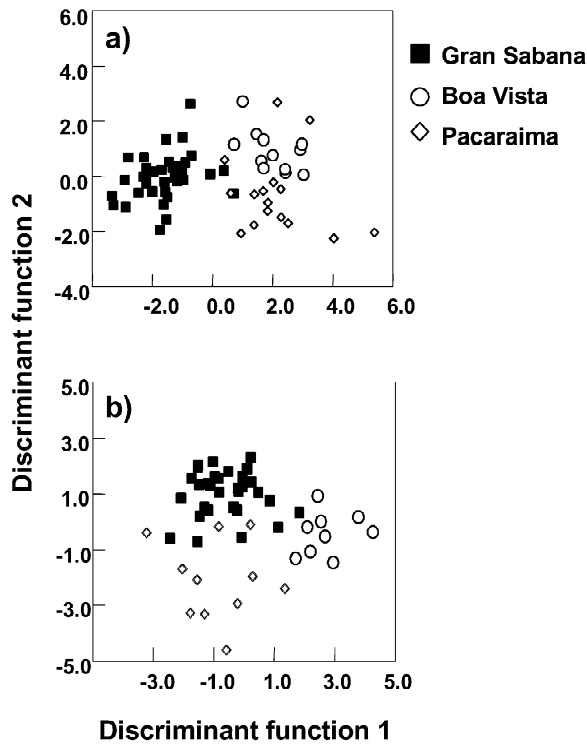


Figure 2. Plot of discriminant function scores for the first two discriminant functions. (a) DFA between stream conditions and regions from which streams originated. (b) DFA between black fly fauna and region.

presented distinct stream environments. It was also shown that species occurrence was not independent of region.

Five of the six species distributions examined across regions were significant. Accordingly, as stream conditions changed across a north-south gradient, so did the occurrence of the most common species. This upland to lowland faunal change is consistent with the patterns of change seen in North American assemblages (Adler & McCreadie, 1997). For example, in South Carolina, U.S.A., as one moves from the Appalachian mountains to the low elevation plains, streams become slower, warmer, and darker (with tannic acid), and the streambed changes from rock to silt. These changes in stream conditions are also accompanied by dramatic shifts in the simuliid assemblage (McCreadie & Adler, 1998). Changes in species composition of other lotic insects along longitudinal or elevational stream gradients is well known (Vannote et al., 1980; Minshall et al., 1985).

Predicting the distribution of species within a region (Gran Sabana) was somewhat less successful,

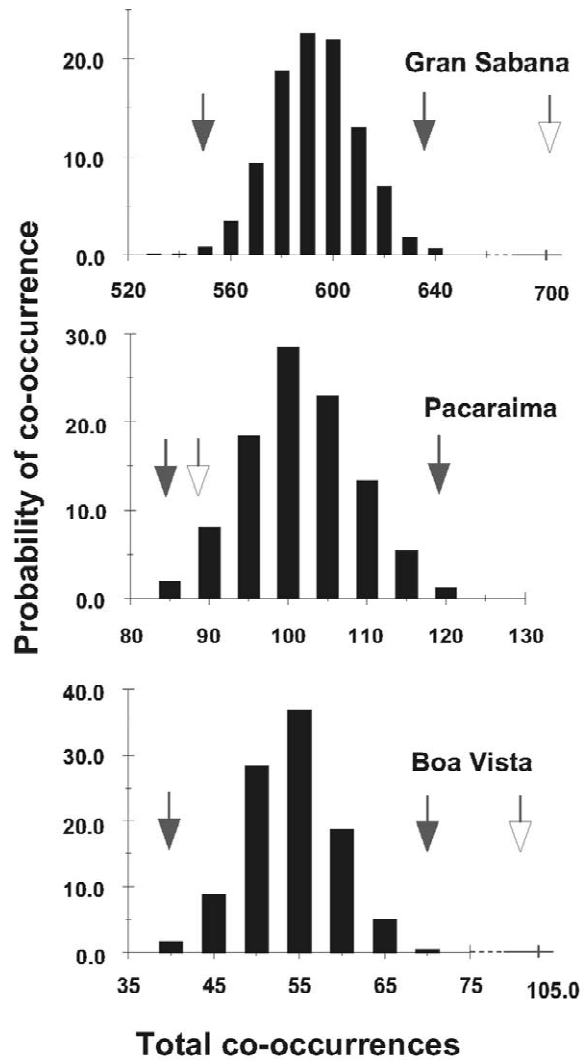


Figure 3. Results of 1000 Monte Carlo simulations for data collected in 1996 from Gran Sabana, Pacaraima and Boa Vista. The test statistic for this simulation is total number of co-occurrences between each pair of streams for each region. Co-occurrence refers to a species common to any pair of streams. Closed arrows indicate the critical values at the 99.0% level. The observed total number of co-occurrences for each region is shown with the open arrow. Probability of co-occurrence is expressed as a percent.

with only half of the six regressions calculated significant ($p < 0.01$). This is particularly apparent for both *S. maroniense* and *S. perflavum* – the distribution of both species was predictable when examined across regions, but not within a region (Table 4). Changes in predictive ability with scale has been noted by McCreadie & Alder (1998). These authors suggested that differences in physical and chemical characteristics of streams among regions (ecoregions) contrasts with

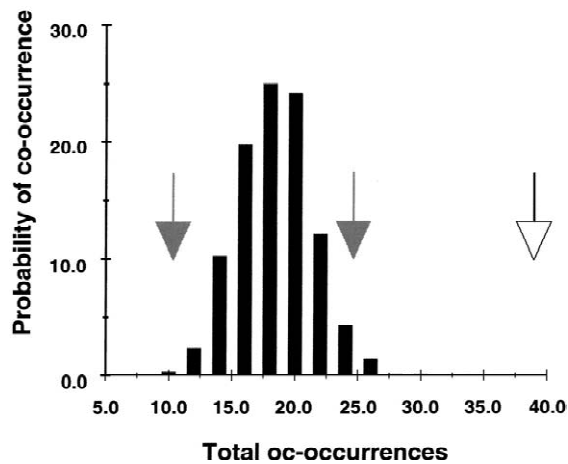


Figure 4. Results of 1000 Monte Carlo simulations comparing the species assemblage in Gran Sabana, Venezuela, during October of 1996 and February 1998. The test statistic for this simulation is total co-occurrences between 1996 and 1998. Co-occurrences refer to the number of species common to a stream between seasons. Closed arrows indicate the critical values at the 99.0% level. The observed total number of co-occurrences is indicated with an open arrow. Probability of co-occurrence is expressed as a percent.

the more homogeneous stream-site conditions within a region. As such, stream sites become more homogeneous, distributions appear more random, and predictive capability decreases. In the current study, DFA clearly demonstrated greater homogeneity of stream conditions within regions than across regions.

Although many factors, both biotic and abiotic, have been associated with preimaginal distributions of blackflies (Ross & Merritt, 1987; Adler & McCreadie, 1997), the few studies conducted at the species-level show that occurrence and abundance are strongly associated with stream-site conditions, particularly stream size and the presence of outlets (Ciborowski & Adler, 1990; McCreadie & Colbo, 1991, 1992; McCreadie et al., 1995; McCreadie & Adler, 1998; Hamada et al., 2002). In the current study, the distribution of three species, *S. cauchense*, *S. maroniense*, and *S. perflavum* showed a significant association with stream size on at least one scale of study. However, only one species, *S. maroniense*, showed a significantly associated with impoundments.

Species assemblages

It has been suggested that species assemblages or other biological properties of streams can be inferred from attributes of the region within which the system flows (e.g., Hawkins & Norris, 2000). The idea that aquatic

biota should vary at landscape spatial scales is based on a long held view that local ecological attributes of streams are influenced by their catchments (Hynes, 1975). Although some authors have reported high correspondence of stream invertebrate assemblages to ecoregions in both Nearctic (e.g., Feminella, 2000) and Palearctic (e.g., Heino et al., 2002) streams, correspondence between the aquatic biota and regional classifications has usually been met with limited success (Hawkins et al., 2000). Our study showed a strong correspondence between species assemblages and *a priori* established regional demarcations (74%). This finding is even more compelling if one considers our ability to discern regional differences in streams was based on single larval collections from a single taxon, Simuliidae.

Monte Carlo analyses suggested that the species assemblage among streams at both Gran Sabana and Boa Vista was, to some extent, repeatable. Species co-occurrences among stream sites at a frequency greater than that expected by a random model, by definition, indicates a predictable species assemblage. Such a repetitive species-assemblage pattern among streams could result from the cumulative effect of numerous species requiring similar or co-varying environmental conditions (McCreadie et al., 1997). Facilitation (*sensu* Connell & Slatyer, 1977) could also give rise to a repetitive assemblage. Although facilitation in feeding efficiency has been demonstrated within a species of blackflies (Chance & Craig, 1986), this has not been shown to occur among species. Significant faunal repeatability within a region would also account for the high correspondence between fauna and region for both Gran Sabana (74%) and Boa Vista (92%).

In contrast to Gran Sabana and Boa Vista, the amount of species overlap among streams in Paracaima was not greater than that expected under a random model. The only comparable study to these results is that of McCreadie et al. (1997). Using Monte Carlo simulations, these authors examined the blackfly assemblage among 18 small, homogeneous streams in Yellowstone National Park, Wyoming, U.S.A. Results indicated that blackfly assemblages among similar streams were largely unpredictable in terms of species co-occurrence. Species co-occurrences among streams at a rate no different than that expected by a random model would suggest that faunal assemblages are largely stochastic. Streams subject to a series of disturbance and recolonization events could produce such a random pattern (Reice, 1994; McCreadie et al., 1997). Thus, it is possible that disturbance might

play a larger role in structuring biotic assemblages in Pacaraima than either Boa Vista or Gran Sabana.

If species assemblages were strongly influenced by competition among closely related species, then species co-occurrence should occur at a rate significantly less than that expected by chance (Begon et al., 1996). Neither here, nor North American investigations (McCreadie et al., 1997) have produced such results, which support the contention that competition may have less importance in structuring lotic communities than other ecosystem parameters (Power et al., 1988; Allan, 1995).

We suggest that there are three broad contingent rules for blackfly species assemblages that can now be put forward. First, is the ubiquitous nature of simuliids. Paralleling the situation in North America, all the streams we examined in 1996 had preimaginal simuliids. With rare exceptions, flowing Nearctic streams, in which water quality has not been severely compromised by pollution or siltation, yield blackflies (Adler & McCreadie, 1997). Other contingent rules that can be gleaned from this study are that many species of simuliids distribute themselves along an axis of stream size and that there is a strong and consistent correspondence between regional characteristics of streams and the species assemblage. However, it should be pointed out that these latter rules, however useful, are still contingent because there are a significant number of exceptions. We suspect these exceptions may, in part, be the result of trying to explain the distribution of the aquatic stage of an organism while ignoring aspects of its terrestrial phase. Larvae occur in particular streams because females choose these sites to deposit their eggs (McCreadie, 1991). This has rarely been considered when examining the distribution of blackflies (and other lotic insects) and almost certainly represents a significant source of error in predicting larval distributions. Clearly, the most evolutionary stable strategy for an adult female would be to oviposit in streams where the likelihood of larval survival is maximized. However, our knowledge of simuliid ovipositional cues is rudimentary (Crosskey, 1990) and hence investigators have instead relied on easily measured site conditions as predictors of larval occurrence. Presumably, some of these habitat parameters might serve as oviposition cues for adults – on the other hand they may not. As the link between habitat parameters measured in the field and cues used by the female to select optimal larval habitats weaken, so does our ability to predict species distributions among sites. Until we have a clear understanding of site se-

lection by adults, we may have to accept a significant amount of error in predicting larval distribution patterns, i.e., our rules of species assemblage will remain contingent.

Seasonality

It is important to note that the Monte Carlo analysis of seasonal change in the Gran Sabana fauna compared each stream site to itself over two seasons. Accordingly, significant results not only indicate that most species were present in both the wet and dry season, but also that each species showed a high degree of site fidelity. An important implication of this result is that a single sample, at a single point in time may produce a reasonable picture of the species assemblage throughout the year. Typically studies examining the distribution of larval blackflies (e.g., McCreadie & Adler, 1998; Hamada et al., 2002) or other lotic insects (e.g., Quinn & Hickey, 1990) over large spatial scales have been based on single-point samples in time. The justification for such a sampling regime is that single point samples are the only logistic means of sampling the large number of streams required to examine spatial distributions across different scales of study. The question that arises in such studies is whether these samples are an accurate reflection of local species occurrences. Clearly, in the case of at least Gran Sabana, the Monte Carlo analysis strongly suggests that such a sampling protocol is reasonable.

Based on limited data it has been generally assumed that aquatic insects in tropical environments develop quickly, are multivoltine, and reproduce throughout the year (e.g., Jackson & Sweeney, 1995) though exceptions to this have been documented (Sweeney et al., 1995). Results presented in the current study also suggest blackfly species, like other aquatic insects, are present throughout the year. However, this conclusion is based on only two sets of data collected in October 1996 and February 1998 and although a number of sites were used there is no replication with regards to wet and dry seasons over time. Therefore, comparisons between wet and dry season should be repeated, both over time and in different locations, before any definitive statement can be made about seasonality.

In contrast to what the above results suggest, blackflies in temperate Nearctic locations show distinct seasonal patterns of larval occurrence. Typically larvae of univoltine species are found during the winter or early spring and those of multivoltine species

found during the summer and fall (Ross & Merritt, 1987). In the southern areas of the Nearctic, however, larvae of many species of blackflies can be found throughout the year (e.g., Moulton & Adler, 1995). Thus, it is perhaps expected that tropical simuliids should be found throughout the year.

Conclusions

It has been suggested that factors influencing community composition and species richness in temperate streams are likely to be important in neotropical streams (Covich, 1988). Thus, the similarity in factors influencing larval blackfly assemblages in both Nearctic and Neotropical streams should not be surprising. For example, both the importance of stream size as a predictor of species distributions and faunal changes from the upland to lowland areas were found in both temperature and tropical stream communities. In summary, three broad tenants for blackfly species assemblages are suggested; (i) the ubiquitous nature of simuliids; (ii) species distribute themselves along an axis of stream size and most likely other continuous aspects of their physical environment and; (iii) there is a strong and consistent correspondence between regional characteristics of streams and the species assemblage.

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References

Adler, P. H. & J. W. McCreadie, 1997. The hidden ecology of black flies: sibling species and ecological scale. *Am. Entomol.* 43: 153–161.

Allan, J. D., 1995. *Stream Ecology: Structure and Function of Running Waters*. Chapman & Hall, New York: 388 pp.

Begon, M., J. L. Harper & C. R. Townsend, 1996. *Ecology: Individuals, Populations and Communities*, 3rd edn. Blackwell Science, Cambridge, Massachusetts: 1068 pp.

Chance, M. M. & D. A. Craig, 1986. Hydrodynamics and behaviour of Simuliidae larvae (Diptera). *Can. J. Zool.* 64: 1295–1309.

Ciborowski, J. J. H. & P. H. Adler, 1990. Ecological segregation of larval black flies (Diptera: Simuliidae) in northern Saskatchewan, Canada. *Can. J. Zool.* 68: 2113–2122.

Connell, J. H. & R. O. Slatyer, 1977. Mechanisms of succession in natural communities and their role in community stability and organization. *Am. Nat.* 111: 1119–1144.

Corkum, L. D., 1989. Patterns of benthic invertebrate assemblages in rivers of northwestern North America. *Freshwat. Biol.* 21: 191–205.

Covich, A. P., 1988. Geographical and Historical comparisons of neotropical streams: biotic diversity and detrital processing in highly variable habitats. *J. N. Am. Benthol. Soc.* 7: 361–368.

Craig, D. A., 1987. Some of what you should know about water or K.I.S.S. for hydrodynamics. *Bull. N. Am. Benthol. Soc.* 35: 178–182.

Crosskey, R. W., 1990. *The Natural History of Blackflies*. Wiley & Sons, Chichester, U.K., 711 pp.

Dezzeo, N., 1994. *Ecología de la Altiplanicie de la Gran Sabana (Guayana Venezolana) I. Investigaciones sobre la dinámica bosque-sabana en el Sector SE: subcuencas del rios Yuraní, Arabopó y Alto Kukenán*. *Scientia Guaianae* 4: 1–205.

Feminella, J. W., 2000. Correspondence between macroinvertebrate assemblages and 4 ecoregions of the southeastern U.S.A. *J. N. Am. Benthol. Soc.* 19: 442–461.

Grillet, M. E. & R. Barrera, 1997. Spatial and temporal abundance, substrate partitioning and species co-occurrence in a guild of Neotropical blackflies (Diptera: Simuliidae). *Hydrobiologia* 345: 197–208.

Hamada, N., 1997. *Cytotaxonomy and ecology of four species in the Simulium perflavum species group and associated black flies in Central Amazonia, Brazil*. Ph.D. thesis. Clemson University, Clemson, South Carolina.

Hamada, N. & M. Eugenia Grillet, 2001. Black flies (Diptera: Simuliidae) of the Gran Sabana (Venezuela) and Pacaraima Region (Brazil): Distributional data and identification keys for larvae and pupae. *Entomotropica* 16: 29–49.

Hamada, N. & J. W. McCreadie, 1999. Environmental factors associated with the distribution of *Simulium perflavum* (Diptera: Simuliidae) among streams in Brazilian Amazonia. *Hydrobiologia* 397: 71–78.

Hamada, N., J. W. McCreadie & P. H. Adler, 2002. Species and Richness and Spatial Distributions of Black Flies (Diptera: Simuliidae) among Streams of Central Amazonia, Brazil. *Freshwat. Biol.* 47: 31–40.

Hawkins, C. P. & R. H. Norris, 2000. Performance of different landscape classifications for aquatic bioassessments: introduction to the series. *J. N. Am. Benthol. Soc.* 19: 367–369.

Hawkins, C. P., R. H. Norris, J. Gerritsen, R. M. Huges, S. K. Jackson, R. K. Johnson & R. J. Stevenson, 2000. Evaluation of the use of landscape classifications for the prediction of freshwater biota: synthesis and recommendations. *J. N. Am. Benthol. Society* 19: 541–556.

Heino, J., T. Muotka, R. Paavola, H. Hämäläinen & R. Koskenniemi, 2002. Correspondence between regional delineations and spatial patterns in macroinvertebrate assemblages of boreal headwater streams. *J. N. Am. Benthol. Soc.* 21: 397–413.

Hosmer, D. W. & S. Lemeshow, 1989. *Applied logistic regression*. Wiley Interscience, Chichester, U.K., 368 pp.

Hynes, H. B. N., 1975. The stream and its valley. *Ver. Theor. angewan. Limnol.* 19: 1–15.

- Jackson J. K. & B. W. Sweeney, 1995. Research in tropical streams and rivers: introduction to a series of papers. *J. N. Am. Benthol. Soc.* 14: 2–4.
- Johnson, C. R. & C. A. Field, 1993. Using fixed effects model multivariate analysis of variance in marine biology and ecology. *Oceanogr. mar. biol. Ann. Rev.* 31: 177–221.
- Krebs, G. J., 1994. *Ecology: the Experimental Analysis of Distribution and Abundance*, 3rd edn. Harper & Row, New York: 307 pp.
- Lawton, J. H., 1999. Are there general laws in ecology? *Oikos* 84: 177–192.
- Ludwig J. A & J. F. Reynolds, 1988. *Statistical Ecology: a Primer on Methods in Computing*. John Wiley & Sons, New York: 368 pp.
- Malmqvist, B., Y. Zhang & P. H. Adler, 1999. Diversity, distribution, and larval habitats of North Swedish blackflies (Diptera: Simuliidae). *Freshwat. Biol.* 42: 301–314.
- Manly, B. F. J., 1991. *Randomization and Monte Carlo Methods in Biology*. Chapman & Hall, New York: 281 pp.
- McCafferty, W. P. B. P. Stark & A. V. Provonsha, 1990. Ephemeroptera, Plecoptera, and Odonata. In Kosztarab M. & C. W. Schaefer (eds), *Systematics of the North American Insects and Arachnids: Status and Needs*. Virginia Agricultural Experimental Station Information Series 90-1: Blacksburg, Virginia. 43–58.
- McCreadie, J. W., 1991. Ecological characterization of larval cytotypes of the *Simulium venustum/verecundum* complex (Diptera: Simuliidae) on Newfoundland's Avalon Peninsula, Ph.D. thesis. Memorial University, St. John's, Newfoundland.
- McCreadie, J. W. & P. H. Adler, 1998. Scale, time, space, and predictability: species distributions of preimaginal black flies (Diptera: Simuliidae). *Oecologia* 114: 79–92.
- McCreadie, J. W., P. H. Adler & J. F. Burger, 1997. Species assemblages of larval black flies (Diptera: Simuliidae): random or predictable? *J. N. Am. Benthol. Soc.* 16: 760–770.
- McCreadie, J. W., P. H. Adler & M. H. Colbo, 1995. Community structure of larval black flies (Diptera: Simuliidae) from the Avalon Peninsula, Newfoundland. *Ann. Entomol. Soc. Am.* 88: 51–57.
- McCreadie, J. W. & M. H. Colbo, 1991. Spatial distribution patterns of larval cytotypes of the *Simulium venustum/verecundum* complex (Diptera: Simuliidae) on the Avalon Peninsula, Newfoundland: factors associated with occurrence. *Can. J. Zool.* 69: 2651–2659.
- McCreadie, J. W. & M. H. Colbo, 1992. Spatial distribution patterns of larval cytotypes of the *Simulium venustum/verecundum* complex (Diptera: Simuliidae) on the Avalon Peninsula, Newfoundland: factors associated with cytotypic abundance and composition. *Can. J. Zool.* 70: 1389–1396.
- Minshall, G. W., K. W. Cummins, R. C. Petersen, C. E. Cushing, D. A. Bruns, J. R. Sedell & R. L. Vannote, 1985. Developments in stream ecosystem theory. *Can. J. Fish. Aquat. Sci.* 42: 1045–1055.
- Moulton, J. K. & P. H. Adler, 1995. Revision of the *Simulium jenningsi* species-group (Diptera: Simuliidae). *Trans. Am. Entomol. Soc.* 121: 1–57.
- Neter, J., W. Wasserman & M. H. Kutner, 1990. *Applied Linear Statistical Models. Regression, Analysis of Variance and Experimental Design*, 3rd edn. Irwin, Homewood, Illinois: 1181 pp.
- Nimer, E., 1975. *Climatologia do Brasil*. IBGE, Rio de Janeiro.
- Norusis, M. J., 1985. *SPSSX. Advanced Statistics Guide*. McGraw Hill Books, New York: 505 pp.
- Power, M. E., R. J. Stout, C. E. Cushing, P. P. Harper, F. R. Hauer, W. J. Matthews, P. B. Moyle, B. Statzner & I. R. Wais De Badgen, 1988. Biotic and abiotic controls in a river and stream communities. *J. N. Am. Benthol. Soc.* 7: 480–502.
- Quinn, G. P. & M. J. Keough, 2002. *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge, U.K.: 537 pp.
- Quinn, J. M. & C. W. Hickey, 1990. Characterisation and classification of benthic invertebrate communities in 88 New Zealand rivers in relation to environmental factors. *New Zealand J. mar. Freshwat. Res.* 24: 387–409.
- Reice, S. R., 1994. Nonequilibrium determinants of biological community structure. *Am. Sci.* 82: 424–435.
- Ross D. H. & R. W. Merritt, 1987. Factors affecting larval black fly distributions and population dynamics. In Kim, K. C. & R. W. Merritt (eds), *Blackflies: Ecology, Population Management, and Annotated World List*. Pennsylvania State University, University Park: 90–108.
- SAS, 1987. *Users Guide: Statistics*, 6th edn. SAS Institute, Cary North Carolina: 956 pp.
- Sweeney, B. W., J. K. Jackson & D. H. Funk, 1995. Semivoltinism, seasonal emergence, and adult size variation in a tropical stream mayfly (*Euthyplocia hecuba*). *J. N. Am. Benthol. Soc.* 14: 131–146.
- Tabachnick, B. & L. Fidell, 1996. *Using Multivariate Statistics*, 3rd edn. Harper and Row, New York: 932 pp.
- Tokeshi, M., 1990. Niche apportionment or random assortment: species abundance patterns revisited. *J. Anim. Ecol.* 59: 1129–1146.
- Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell & C. E. Cushing, 1980. The river continuum concept. *Can. J. Fish. Aquat. Sci.* 37: 130–137.
- Wiggins, G. B., 1990. Systematics of North American Trichoptera: present status and future prospect. In Kosztarab M. & C. W. Schaefer (eds), *Systematics of the North American Insects and Arachnids: Status and Needs*. Virginia Agricultural Experimental Station Information Series 90-1: 203–210. Blacksburg, Virginia.