# Impact of environmental manipulation for *Anopheles pseudopunctipennis* Theobald control on aquatic insect communities in southern Mexico

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ABSTRACT: Extraction of filamentous algae from river pools is highly effective for the control of *Anopheles pseudopunctipennis* in southern Mexico. We determined the magnitude of changes to the aquatic insect community following single annual perturbations performed over two years. In 2001, algae were manually removed from all the pools in a 3 km long section of the River Coatán, Mexico, while an adjacent section was left as an untreated control. In 2002, the treatments of both zones were switched and algal extraction was repeated. The abundance of *An. pseudopunctipennis* larvae + pupae was dramatically reduced by this treatment and remained depressed for two to three months. A total of 11,922 aquatic insects from ten orders, 40 families, and 95 genera were collected in monthly samples taken over five months of each year. Algal extraction did not reduce the overall abundance of aquatic insects in river pools, but a greater abundance and a greater richness of taxa were observed in 2002 compared to the previous year. This was associated with reduced precipitation and river discharge in 2002 compared to 2001. Shannon diversity index values were significantly depressed following algal extraction for a period of three months, in both years, before returning to values similar to those of the control zone. However, differences between years were greater than differences between treatments within a particular year. When insects were classified by functional feeding group (FFG), no significant differences were detected in FFG densities between extraction and control zones over time in either year of the study. Similarly, percent model affinity index values were classified as "not impacted" by the extraction process. Discriminant function analysis identified two orders of insects (Diptera and Odonata), water temperature, dissolved oxygen and conductivity, and river volume (depth, width, and discharge) as being of significant value in defining control and treatment groups in both years. We conclude that habitat manipulation represents an effective and environmentally benign strategy for control of *An. pseuduopunctipennis*. Variation in precipitation and river discharge between years was much more important in determining aquatic insect community composition than variation generated by the filamentous algal extraction treatment. ***Journal of Vector Ecology*** **32 (1): 41-53. 2007.**

*Keyword Index: Anopheles pseudopunctipennis*, aquatic insect community, environmental impact, habitat manipulation, Mexico.

## INTRODUCTION

Environmental management has proved to be highly effective for the control of populations of medically important species of mosquitoes (Keiser et al. 2005). Such measures usually involve environmental modification, which eliminates the habitats required for insect development, often on a permanent basis (e.g., draining of wetlands), or environmental manipulation, in which the existing habitat is changed to make it unsuitable or unattractive to mosquitoes (Rafatjah 1988, Ault 1994). Environmental management is generally favored over programs based on the periodic use of larvicidal chemicals applied to aquatic breeding sites (Wisner and Adams 2002).

Chemical-based mosquito control measures can adversely impact populations of non-target organisms, including aquatic invertebrates (Chavasse and Yap 1997) and vertebrates (Rozendaal 1997). Non-target impacts arising from environmental management, however, are usually restricted to aquatic species, such as invertebrates, amphibians, and fish, that cannot easily migrate to other suitable areas. As the central aim of mosquito control programs is to minimize vector populations, the effect of control measures on sympatric species, particularly communities of aquatic invertebrates, is rarely addressed in detail. This is an issue of concern because invertebrates exert an important influence on numerous ecological processes in aquatic systems, particularly the cycling of nutrients and the flow of carbon between trophic levels (Malmqvist 2002).

The extraction of filamentous algae from riverside pools in southern Mexico is a highly effective intervention for control of the malaria vector, *Anopheles pseudopunctipennis* Theobald, during the principal period of vector activity of this species (Bond et al. 2004). Filamentous algae of the genera *Spirogyra* and *Cladophora* represent an important larval food source and also provide mosquito larvae with physical refuges from aquatic predators (Bond et al. 2005). These characteristics render pools containing algae highly attractive to ovipositing females. As a consequence, some rural communities in malarial endemic zones of southern Mexico have started local programs of algal extraction during the dry season when algae proliferate in river pools (Chanon et al. 2003).

Temporally-discrete habitat manipulation procedures of this kind represent pulse perturbations of the aquatic system (Resh et al. 1996, Poulton et al. 2003, Harrison et al. 2004). The perturbation results in an instantaneous change in the availability of filamentous algae to the phytophagous community and the abundance and accessibility of prey items, particularly immature mosquitoes, to the predatory community (Pickett and White 1989).

A diversity of techniques has been employed to quantify anthropogenic perturbations of aquatic habitats, particularly the use of indicator species and biotic indices based on taxa richness or community functional structure (Lenat et al. 1980, Carlisle and Clements 1999, Rawer-Jost et al. 2000). Here, we examined in detail the consequences of the extraction of filamentous algae from river pools in southern Mexico on the taxa richness, diversity, and functional structure of the aquatic insect community associated with *An. pseudopunctipennis* immature development sites. Specifically, we used diversity metrics, functional trophic classes, and biotic indices to determine the magnitude of community changes and the recovery of the community following single annual perturbations performed over two years.

## MATERIALS AND METHODS

### Study area

The study was performed in a 6 km section of the Coatán River, 25 km northeast of the town of Tapachula, Chiapas, Mexico, between the villages of Unión Roja and La Boquilla at an altitude between 297 and 675 m above sea level. The river discharge is reduced during the dry season resulting in the formation of interconnected pools in which filamentous algae proliferate, thus favoring the development of *An. pseudopunctipennis* larvae. The climate is tropical with a wet season from May to October and a dry season from November to April. Average annual rainfall is 3,800 mm, annual temperature averages 25° C, and relative humidity is 50-90% most of the year.

### Experimental design

The 6-km section of river was divided into two 3-km zones. The experiment began halfway through two dry seasons, February - June 2001 and January - May 2002. In 2001, treatments were assigned randomly to each zone; the upstream zone was selected for extraction of filamentous algae (treated zone) from all river pools of water, whereas the downstream 3-km zone was left untreated (control zone). In 2002, the zonal treatments were switched and the process of algal extraction repeated. Extraction of floating mats of filamentous algae was performed over a seven-day period using garden rakes covered with mosquito netting. The bottom of the pools was not disturbed during this procedure. The start of the study was taken to be the first day after extraction of algae from all pools in the treated zone had been achieved.

### Insect sampling and identification

Aquatic insects were sampled monthly in each zone during five months of each year. Five samples were taken in each zone at intervals of ~500 m. During 2001, the first sample was taken prior to the extraction of algae, whereas during 2002, all samples were taken post-extraction. Estimates of the density of *An. pseudopunctipennis* larvae and pupae were obtained using a standard 850-ml cup with a flat outer side attached to a wooden pole. Samples were taken by carefully immersing the cup at the edges of aquatic vegetation, or by dragging the dipper over the water surface. Larvae were collected, counted, and returned to the pool to avoid population disturbances from sampling. Sampling effort was defined based on Taylor's Power Law (Taylor 1979). This consisted of five to 20 dips from each breeding site, depending of the numbers of larvae in the initial five dips which was used to determine the sample size (total number of dips). If the number of larvae captured in the first five dips averaged zero to five larvae per dip, a maximum of 20 dips were taken. If the average was five to 20 larvae, then 15 dips were taken, if 20 to 50 larvae, then ten dips were taken, and if >50 larvae per dip, then only five dips were taken. The larval density index was calculated by dividing the total number of larvae by the sampling effort (total number of dips). On each occasion, sub-samples of larvae were transported to the laboratory in sealed plastic bags for identification. Density index values were normalized by $\sqrt{(x + 1)}$ transformation and subjected to repeated measures ANOVA in SPSS ver. 10 (SPSS Inc., Chicago, IL). A pre-treatment sample taken in 2001 was excluded from the analysis.

Aquatic insects were collected with an aquatic entomological net of 24 cm x 46 cm and mesh size 0.9 mm situated to catch drifting insects at the rapidly flowing riffle zones at the outflow of the sampling area (Figure 1). Invertebrates were disturbed and encouraged to drift by agitating the substrate using the traveling kick method (Pollard 1981) for 5 min over a 5 x 1 m area of the pool. Sampled sites were marked on a nearby boulder and were never resampled. Insect samples were preserved in 95% ethanol, taken to the laboratory, and identified to genus using the appropriate keys (Lehmkuhl 1979, Contreras-Ramos 1999, González-Soriano and Novelo-Gutiérrez 1996, Merritt and Cummins 1996, Westfall and May 1996, Novelo-Gutiérrez 1997a, 1997b, Needham et al. 2000). A total of 23 non-insect invertebrates was found in samples (snails, spiders and isopods), which represented less than 0.2% of the total capture and was ignored in all analyses.

### Physico-chemical measurements

Water temperature and physico-chemical parameters were measured at the moment of sampling in each pool on each occasion. Dissolved oxygen, pH, and conductivity were measured using portable meters (Hanna Instruments

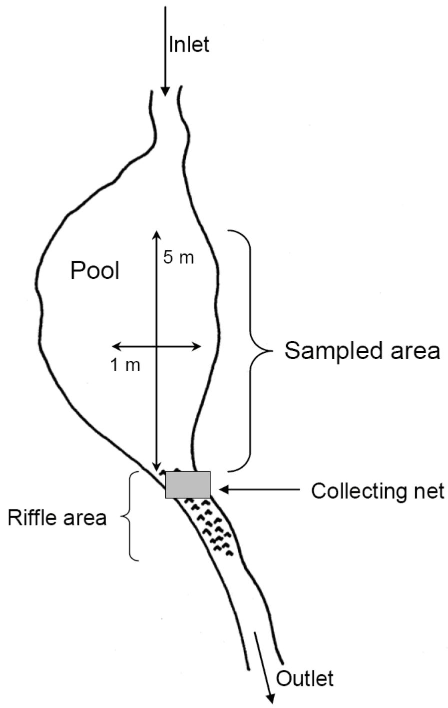


Figure 1. Area sampled and point of capture of aquatic insects disturbed in each river pool sampled during the study period.

Ltd., U.K.). Flow rate and river discharge were calculated using the method of Needham and Needham (1962) based on flow velocity, width, depth, and riverbed substrate. The statistical significance of differences between the mean physico-chemical parameters for the treatments of both years was determined by multivariate analysis of variance (MANOVA) and multiple comparisons with Tukey's HSD test. Meteorological data were obtained from a weather station located next to the village of El Retiro, approximately 1 km from the central dividing point between treated and untreated sections of the river.

**Taxa richness, community diversity, and functional structure**

Estimates of taxa richness and community composition by order were obtained for aquatic insects sampled prior to extraction of algae and at monthly intervals for four to five months in each treatment during two years of study. The abundance of insects captured at each sampling date was subjected to MANOVA following log $(x+1)$ transformation. The diversity of aquatic insects was estimated with the Shannon index. The accuracy of index values was estimated by jack-knifing, which permitted a reduction in the bias in our estimate and provided a standard error (Magurran 2004). Confidence intervals for the statistic were calculated by bootstrap with replacement. Comparisons of index values for the disturbed vs. untreated zone were performed by *t*-test (Hutcheson 1970) with degrees of freedom calculated by harmonic interpolation (Southwood and Henderson 2000). The Shannon index assumes that all taxa are represented in the overall sample. To validate this index, the cumulative number of genera observed during the sampling procedure each year was plotted against sampling effort (the number of samples taken per zone during each five-month sampling period).

Community structure was examined by classifying aquatic insects into functional feeding groups (FFG) (Cummins 1973, Wallace and Webster 1996). Accordingly, insects were classified as filterers, gatherers, predators, scrapers, piercers, and shredders, based on examination of mouthpart morphology, published literature, and when necessary, gut content examination, following the criteria described by Merritt and Cummins (1996). The number of individuals in each FFG was transformed to $\sqrt{(x+1)}$ to normalize the distribution and eliminate heteroscedasticity. The variance/covariance matrix of transformed FFG densities did not conform to sphericity assumptions and was therefore subjected to multivariate analysis of variance (MANOVA). F values were calculated based on Pillai's trace (Winer et al. 1991).

**Discriminant function analysis**

Differences between control and extraction zones in each year of the study (four groups in total) were compared by discriminant function analysis based on 18 biological and physico-chemical variables. These were (i) the abundance of the eight most common orders of insects, (ii) eight physico-chemical parameters (described above), and (iii) two biotic indices: EPT and FBI. The EPT index is based on the abundance and diversity of Ephemeroptera, Plecoptera, and Trichoptera on a scale of >10 = non-impacted, 6-10 = slightly impacted, 2-6 = moderately impacted and 0-2 = severely impacted (Lenat and Penrose 1996). The Family Biotic Index (FBI) is a measure of the abundance of insect families that differ in their tolerance to reduced oxygen and organic pollution (Hilsenhoff 1988). The FBI is calculated by multiplying the number of individuals of each family by an assigned tolerance value ranging from intolerance (0) to very tolerant (10), adding these products and dividing by the total number of individuals (Novak and Bode 1992, Mandaville 2002). Stepwise discriminant analysis was perfomed using an F-remove procedure to estimate the contribution of each variable to predicted group membership. The condition of the variance/covariance matrix was determined by checking tolerance values for each variable. Canonical correlation analysis was performed to determine whether the dimensionality of the matrix could be reduced by eliminating canonical roots.

**Percent model affinity index**

The percent model affinity is an index of macroinvertebrate community composition based on similarity to that of an undisturbed reference community, expressed as percent composition of seven major groups (Novak and Bode 1992). For the present study, the six most abundant orders of aquatic insects were considered as six groups and other orders as the seventh group. The ideal community on which the model was based was obtained by

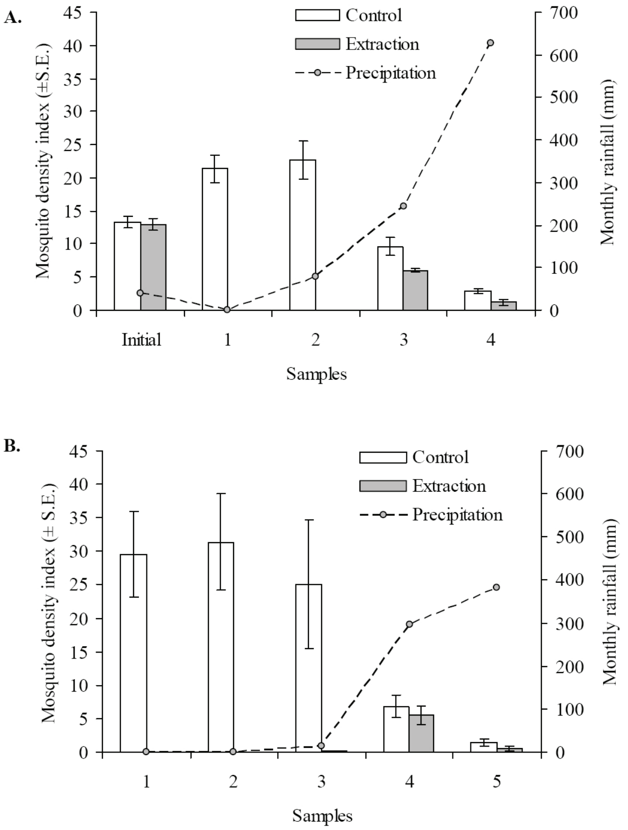


Figure 2. Density index (± S.E.) of *Anopheles pseudopunctipennis* larvae and pupae and monthly rainfall in riverside pools in (A) 2001 and (B) 2002. Sampling was performed prior to the extraction of filamentous algae (Initial) in 2001 and at monthly intervals thereafter. Pre-treatment sampling was not performed in 2002.

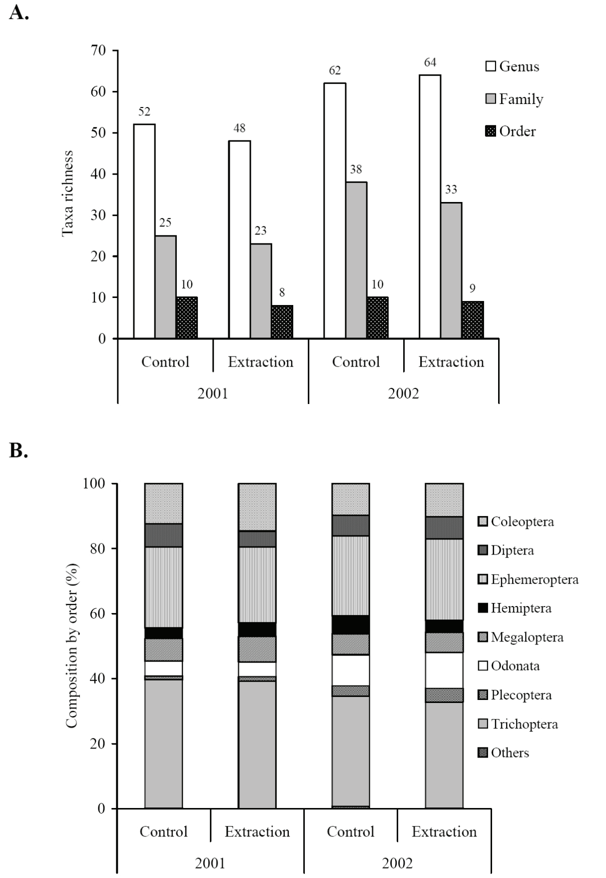


Figure 3. Composition of insect communities sampled in River Coatán, southern Mexico, in untreated control and algal extraction zones in 2001 and 2002 in terms of (A) taxa richness by order, family, and genus and (B) relative representation of orders (in percent).

Table 1. Mean ± S.D. physico-chemical parameter values recorded in control and extraction treatment zones of River Coatán, Chiapas, Mexico, during the experimental periods of 2001 and 2002.

| Year and Treatment | Temperature (°C) | Dissolved $O_2$ (mg/l) | pH | Conductivity (μS/cm) | Speed (m/sec) | Depth (m) | Width (m) | Discharge ($m^3$/sec) |
|---|---|---|---|---|---|---|---|---|
| 2001 | | | | | | | | |
| Control | 25.2 ±1.4a | 6.97 ±1.01a | 8.96 ±1.48a | 65.5 ± 12.1a | 0.19 ±0.02a | 0.26 ±0.06 a | 5.96 ±1.33a | 0.28 ±0.12a |
| Extraction | 25.2 ±1.3a | 6.13 ± 0.75a | 7.57 ±1.39b | 50.1 ±14.0b | 0.20 ±0.02a | 0.26 ±0.06 a | 6.24 ±1.51a | 0.31 ±0.15a |
| 2002 | | | | | | | | |
| Control | 27.3 ±1.7b | 8.25 ±1.99b | 7.51 ±0.94b | 60.4 ± 10.0a | 0.19 ±0.03a | 0.21 ± 0.04b | 4.67 ±0.73b | 0.16 ±0.05b |
| Extraction | 26.8 ±1.4b | 5.86 ±2.33a | 6.74 ±1.24b | 49.5 ±11.1b | 0.20 ±0.04a | 0.20 ±0.03b | 4.27 ±0.76b | 0.15 ±0.04b |

All means based on five pools on each of four sample dates in 2001 (pre-treatment measurements excluded) and five sample dates in 2002. Values followed by the same letters are not significantly different for comparisons within columns (Tukey HSD, P > 0.05). Table reproduced from Bond, J.G. 2005. Efectos de la extracción de las algas filamentosas sobre las poblaciones de *Anopheles pseudopunctipennis* (Diptera: Culicidae) y la comunidad de insectos acuáticos del Río Coatán, Chiapas. Unpublished Ph.D. thesis. El Colegio de la Frontera Sur, Tapachula, Mexico.

averaging the five months of samples taken in the control zone for each year. The mean number of individuals of each order was calculated for each sample (each month) and was compared with the composition of each month's sample from the treated zone.

## RESULTS

### Physico-chemical measurements

Mean water temperatures in 2002 were approximately 1.5 - 2° C greater than during 2001 (Table 1). Dissolved oxygen levels did not differ between treatment zones in 2001 but were higher in the control zone in 2002. Mean pH values in the control zone were significantly higher than in the treated zone in 2001 but not in 2002. Conductivity was significantly lower in extraction zones in both years compared to the control zone. Flow rate did not differ according to zone or year, but river discharge was significantly greater in 2001 compared to 2002. This was related to differences in rainfall: mean monthly precipitation during the experimental period was 288 mm in 2001 compared to 184 mm in 2002.

### Abundance of *Anopheles pseudopunctipennis* in pools

Prior to the extraction of filamentous algae, population density indices of *An. psedopunctipennis* larvae and pupae in river pools in 2001 were very similar in both sections of the river (Figure 2A). Extraction of algae resulted in a dramatic decrease in the density index in both years of the study (repeated measures Time*Treatment $F_{4,13} = 40.3$, $P < 0.001$; Time*Treatment*Year $F_{4,13} = 16.4$, $P < 0.001$) (Figures 2A,B). Immature mosquito population densities in treated pools did not return to values similar to those of untreated control pools until three months post-extraction (sample 3) in 2001 or four months post-extraction (sample 4) in 2002, reflected in a significant Time*Treatment*Year interaction term ($F_{4,13} = 50.3$, $P < 0.001$). In both years, increasing precipitation at the end of the sampling period resulted in a reduction in *An. pseudopunctipennis* populations in both control and treated pools.

### Taxonomic richness and composition

Sampling over the two-year period resulted in the identification of aquatic insects from ten orders, 40 families, and 95 genera (Figure 3A). The total number of individuals captured in each monthly sample ranged from 471 to 616 in 2001 and from 523 to 848 in 2002 (total N = 11,896). Multivariate analysis of variance indicated significant differences in the abundance of individuals from different aquatic insect orders ($F_{7,126} = 89.9$, $P < 0.001$), and between years ($F_{1,126} = 12.3$, $P < 0.001$), but the abundance of aquatic insects did not differ significantly between treatments ($F_{1,126} = 3.09$, $P = 0.081$), in either year of the study (treatment*year $F_{1,126} = 0.32$, $P = 0.569$). The richness of the aquatic insect community was reduced by one order and one to four families following the extraction of algae in both years. However, a greater total number of genera were identified in the extraction zone in 2002.

The relative abundance of orders was similar in treatment and control zones in both years with Trichoptera, Ephemeroptera, and Coleoptera, the most abundant insect orders, making up approximately 35, 25, and 10% of the sampled insects, respectively (Figure 3B). The less well-represented orders were Plectoptera (approximately one to four percent of sampled insects) and Lepidoptera and Hymenoptera, which together represented ≤0.8% (shown as "others" in Figure 3B).

### Diversity index

Plots of cumulative taxa curves against sampling effort reached a plateau in both years indicating that all taxa were represented in the sampling program (data not shown). Sampling prior to algal extraction in 2001 confirmed no difference in the diversity of aquatic insects in zones assigned to treatment vs. control (Figure 4A). Insect diversity in control pools remained unchanged during the sampling period. Manual extraction of filamentous algae resulted in a significant reduction in the diversity index which remained significantly depressed until the sample taken at four months post-treatment.

Pre-treatment sampling was not performed in 2002 (Figure 4B). Diversity index values were greater in 2002 than in the previous year; the difference between years was similar in magnitude to the difference between treatment and control zones. Diversity values in control and treatment zones also fluctuated more widely than in the previous year. However, the effect of algal extraction on insect diversity was similar to that observed in 2001 and persisted for the same duration of three months. Jack-knifing indicated that diversity index values were slightly underestimated in all cases. The magnitude of the error was minimal; 1.73 and 1.49% for the control and treated zones, respectively, in 2001, compared to 1.77 and 2.45% for the same zones, respectively, in 2002.

### Functional community structure

When classified into FFGs, filterers, gatherers, predators, and scrapers were observed in decreasing order of abundance in 2001 (Figure 5), whereas in 2002, predators were slightly more abundant than gatherers in both treatment and control zones. Piercers and shredders were observed in very low numbers only in the control zone and only in the second year of the study. Piercers and shredders represented just 0.16 and 0.06% of the individuals collected in 2002, respectively (not shown in Figure 5).

Marked variation in FFG density over time (excluding piercers and shredders) was detected ($F_{12,\ 189} = 3.69$, $P < 0.001$), which differed according to the year of study (time*year*FFG interaction $F_{12,\ 189} = 1.94$, $P = 0.032$) (Figure 6A-H). Densities of filterers and gatherers tended to be higher in 2002 than in 2001, as were the densities of predatory insects, possibly indicating a numerical response to prey densities. However, the densities of FFGs did not differ significantly between extraction and control zones over time in either year of the study ($F_{4,\ 61} = 2.17$, $P = 0.083$). Treatment had no significant interactions with any other sources of variation.

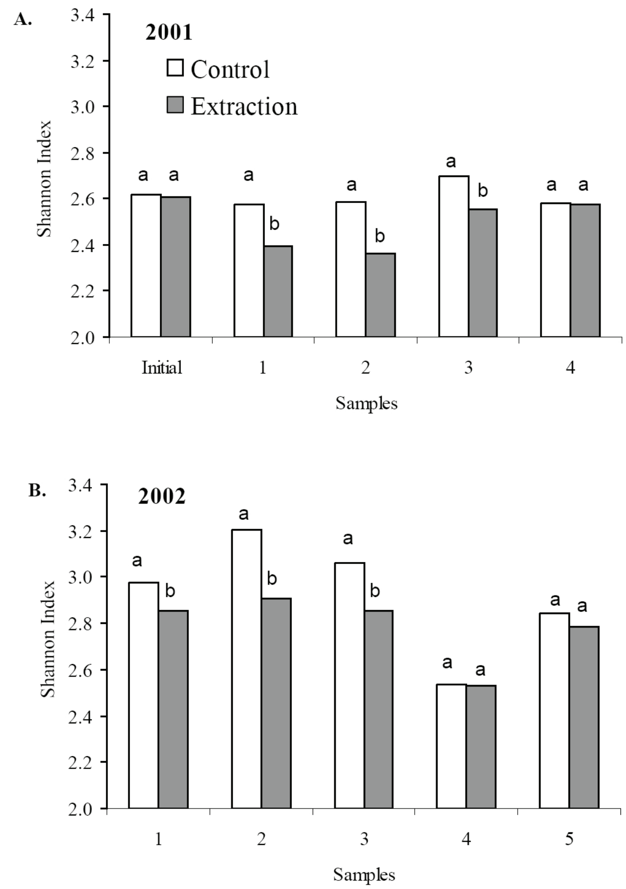


Figure 4. Shannon diversity index values calculated following extraction of filamentous algae from pools in the River Coatán, Mexico, in (A) 2001 and (B) 2002. Sampling was performed at monthly intervals. Initial indicates pre-treatment sample taken in 2001. Columns headed by identical letters are not significantly different for within-sample comparisons between treatments (t-test with degrees of freedom calculated by harmonic interpolation).

### Discriminant function analysis

Canonical analysis indicated significant differences between groups comprising control and treatment zones in both years (Wilks' lambda = 0.0734, $F_{54, 233} = 6.057$, $P < 0.001$) (Table 2). These differences were defined by two orders of insects (Diptera and Odonata), water temperature, dissolved oxygen and conductivity, and the volume of the river (depth, width, and discharge). Of these variables, dissolved oxygen, and river depth and width were the most influential. In contrast, the biotic indices (EPT, FBI), water pH, flow velocity, and the remaining six insect orders were of no significant value as predictive variables.

### Percent model affinity index

The composition of the insect community was compared as the percentage composition of seven groups (representing the six most abundant insect orders + one miscellaneous group) at each sample post-treatment with the model composition of the community of the control

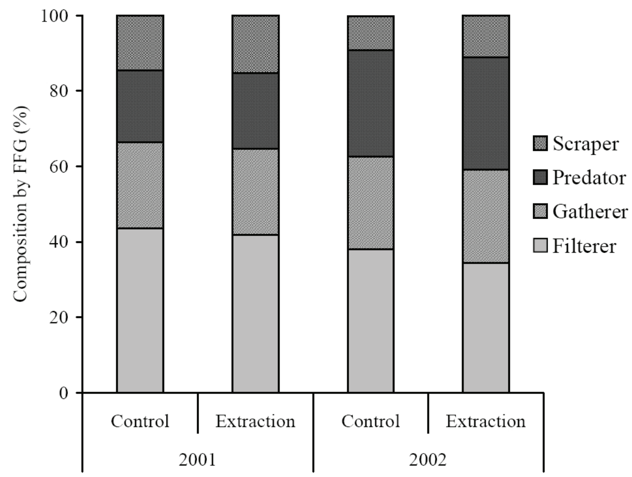


Figure 5. Overall functional composition of insect communities in River Coatán, southern Mexico, in untreated control and algal extraction zones in 2001 and 2002. Insects were classified by trophic guild corresponding to functional feeding groups (FFG). Shredders and piercers represented <0.2% of the insects sampled and are not shown.

zone. The community in the treated zone presented 88-97% affinity to the model community in 2001 and 84-95% affinity in 2002 (Table 3). Affinity index values did not change systematically during the sampling period in each year as would be expected from a gradual return to pre-disturbance status following a major perturbation. All samples from communities in the treatment zone were classified as not impacted by the extraction process based on the criteria of Novak and Bode (1992).

## DISCUSSION

Habitat manipulation involving the extraction of filamentous algae can successfully control the malaria vector, *An. pseudopunctipennis* for periods of two to three months in southern Mexico (Bond et al. 2004, and this study). In the present study, we examined this method of vector control and its impact on aquatic insect communities present in river pools subjected to the intervention.

With a total of 95 genera, 40 families, and ten orders registered in a total of ten months of sampling, the River Coatán appears to harbor an elevated diversity of aquatic insects. A comparable study in northern Mexico reported approximately half the number of genera and families associated with *An. pseudopunctipennis* breeding sites close to the border of Mexico with the United States (Delgado-Gallardo et al. 1994). This is likely due to a combination of a cup-dipping sampling technique that was unlikely to provide a representative sample of benthic invertebrates and the temperate geographical location of the region with correspondingly reduced taxa richness compared to the tropical southern portion of Mexico where the present study was performed. Accordingly, studies on aquatic insect

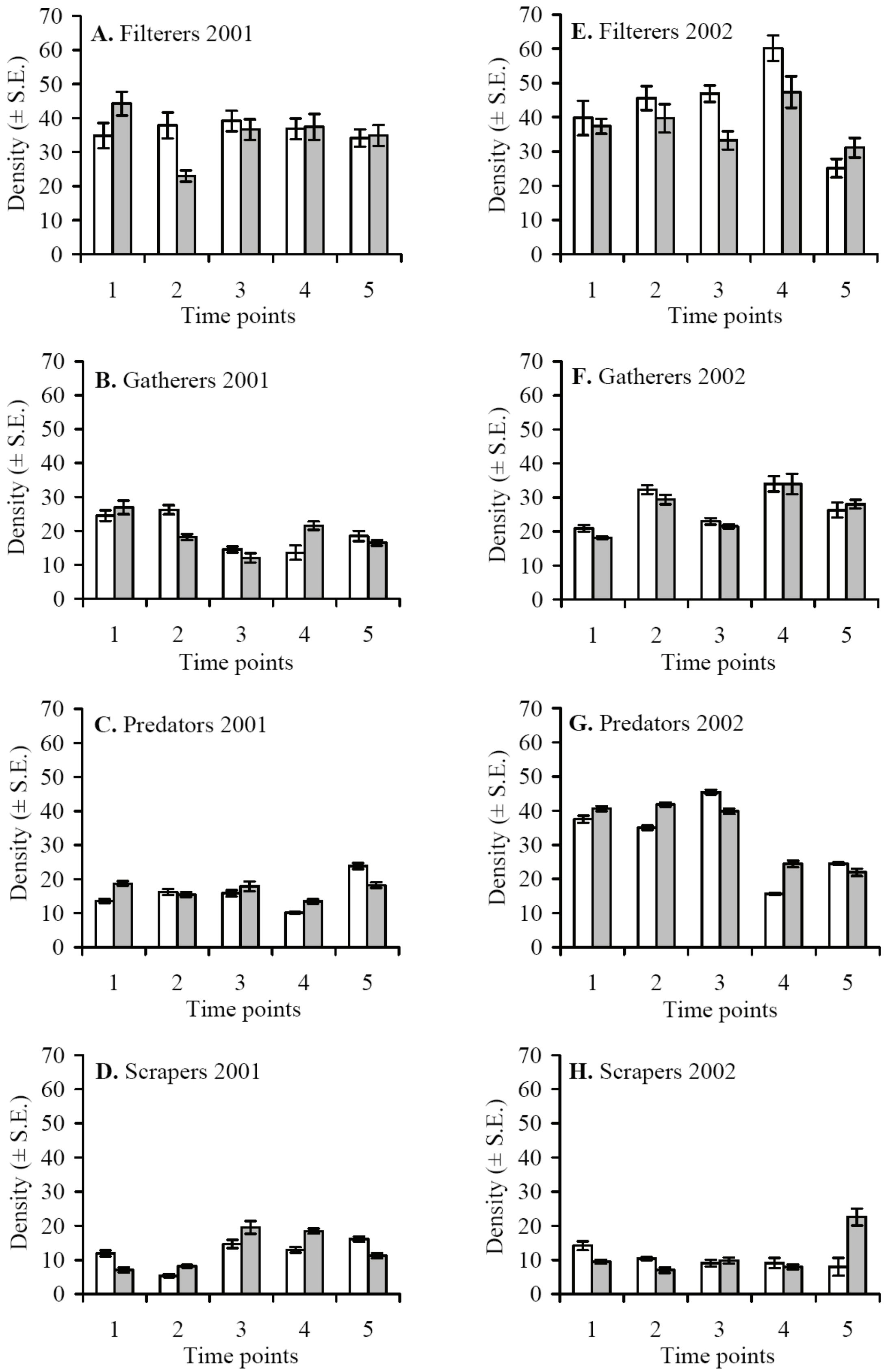


Figure 6. Mean (±S.E.) densities of functional feeding groups (insects/m$^2$) in control (unshaded columns) and algal extraction (gray columns) zones at five sample timepoints in (A-D) 2001 and (E-H) 2002 (excluding shredders and piercers). The first timepoint in 2001 was prior to applying the extraction treatment.

communities performed in single years in California and Chile have reported much lower numbers of genera and families than we observed (Rogers 1998, Figueroa et al. 2003).

A greater abundance and richness of aquatic insect taxa were observed in 2002 than in the previous year. Changes in taxonomic richness were associated with reduced precipitation and river discharge in 2002 compared to 2001. The dry season also ended several weeks earlier in 2001, with rainfall in March 2001 approximately six-fold greater than occurred in March 2002. Indeed, variation between years proved to be much more important in community composition than variation generated by the algal extraction treatment. This finding is consistent with previous reports that taxa richness measures are highly sensitive to ecosystem disturbance, such as the habitat manipulation intervention (Pratt and Bowers 1992, Niemi et al. 1993, Resh and Jackson 1993).

Despite the importance of algae in river ecology (Stevenson et al. 1996, Doi et al. 2006), this intervention resulted in minor but detectable changes in the diversity of the aquatic insect community associated with the outflow of riverside pools. In both years, Shannon index values were significantly depressed following algal extraction for a period of three months, before returning to values similar to those of the control zone. However, differences between years tended to be greater than differences between zones within a particular year, i.e. aquatic insect diversity varied more from year-to-year than it did between treated and untreated zones. Annual differences in river discharge are likely to have been the primary sources of the observed variation (Resh et al. 1998), as hydraulic conditions are often the principal determinants of the composition (Mérigoux and Dolédec 2004), abundance (Statzner et al. 1988, Cobb et al. 1992), and spatial distribution of benthic invertebrates (Quinn and Hickey 1994, Rempel et al. 2000), mainly due to physical removal and increased drift with increasing flow (Brittain and Eikeland 1988, Bond and Downes 2003). In this respect, connectivity between treated and untreated sections of the river appeared to be of little importance as very similar patterns in diversity and FFG composition were observed when algal extraction was performed upstream or downstream of the control section. River discharge also shapes the productivity (Cardinale et al. 2005) and functional processes (Hansen et al. 1991, Thomson et al. 2002) of benthic invertebrate communities. This is because the species that comprise FFGs respond differently to flow modification. For example, increased flow and variability

Table 2. Discriminant function analysis applied to the abundance of eight orders of insects, two biotic indices (EPT, FBI), and eight physico-chemical variables. Values shown in bold indicate significant predictors of groups comprising algal extraction and control treatments in each year of the study.

| | Wilks' Lambda | Partial Lambda | F(remove) | P | Tolerance | $1-R^2$ |
|---|---|---|---|---|---|---|
| *Insect orders* | | | | | | |
| Coleoptera | 0.0794 | 0.9248 | 2.1146 | 0.1051 | 0.6982 | 0.3018 |
| **Diptera** | **0.0842** | **0.8721** | **3.8133** | **0.0132** | **0.6932** | **0.3068** |
| Ephemeroptera | 0.0807 | 0.9094 | 2.5893 | 0.0588 | 0.8057 | 0.1943 |
| Hemiptera | 0.0798 | 0.9200 | 2.2595 | 0.0881 | 0.7783 | 0.2217 |
| Megaloptera | 0.0788 | 0.9316 | 1.9103 | 0.1348 | 0.5415 | 0.4585 |
| **Odonata** | **0.0844** | **0.8703** | **3.8734** | **0.0123** | **0.6649** | **0.3351** |
| Plecoptera | 0.0753 | 0.9745 | 0.6809 | 0.5664 | 0.6075 | 0.3925 |
| Trichoptera | 0.0751 | 0.9773 | 0.6045 | 0.6140 | 0.7505 | 0.2495 |
| *Biotic indices* | | | | | | |
| EPT Index | 0.0761 | 0.9644 | 0.9598 | 0.4161 | 0.8201 | 0.1799 |
| FBI Index | 0.0772 | 0.9516 | 1.3215 | 0.2734 | 0.6938 | 0.3062 |
| *Physico-chemical parameters* | | | | | | |
| **Temperature** | **0.0842** | **0.8721** | **3.8142** | **0.0132** | **0.7727** | **0.2273** |
| **Oxygen** | **0.1016** | **0.7224** | **9.9926** | **<0.0001** | **0.5147** | **0.4853** |
| pH | 0.0794 | 0.9249 | 2.1101 | 0.1057 | 0.6453 | 0.3547 |
| **Conductivity** | **0.0850** | **0.8640** | **4.0942** | **0.0094** | **0.5534** | **0.4466** |
| Speed | 0.0740 | 0.9925 | 0.1977 | 0.8977 | 0.2615 | 0.7385 |
| **Depth** | **0.0966** | **0.7598** | **8.2217** | **0.0001** | **0.0756** | **0.9244** |
| **Width** | **0.0901** | **0.8148** | **5.9111** | **0.0011** | **0.0842** | **0.9158** |
| **Discharge** | **0.0852** | **0.8622** | **4.1543** | **0.0087** | **0.0325** | **0.9675** |

D.F. for $F_{(remove)}$ = 3, 78.

Table 3. Percent Model Affinity index evaluation of the effects of extraction of filamentous algae on aquatic insect community composition, in a two-year experiment, River Coatán, southern Mexico. Criteria for classification of impact: 1) 65% similarity to model= non-impacted; 2) 50-64%= slightly impacted; 3) 35-49%= moderately impacted y 4) < 35%= severely impacted (Novak and Bode 1992).

| | Model | Stations | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | | 2 | | 3 | | 4 | | 5 | |
| | % Compos. | % | Diff. | % | Diff. | % | Diff. | % | Diff. | % | Diff. |
| 2001 | | | | | | | | | | | |
| Diptera | 7 | 1 | 6 | 9 | 2 | 3 | 4 | 7 | 0 | 4 | 3 |
| Trichoptera | 38 | 35 | 3 | 38 | 0 | 34 | 4 | 36 | 2 | 41 | 3 |
| Ephemeroptera | 26 | 27 | 1 | 27 | 1 | 28 | 2 | 20 | 6 | 17 | 9 |
| Plecoptera | 1 | 3 | 2 | 0 | 1 | 1 | 0 | 1 | 0 | 3 | 2 |
| Coleoptera | 12 | 15 | 3 | 12 | 0 | 19 | 7 | 18 | 6 | 14 | 2 |
| Megaloptera | 8 | 11 | 3 | 6 | 2 | 9 | 1 | 8 | 0 | 7 | 1 |
| Others | 8 | 9 | 1 | 7 | 1 | 6 | 2 | 10 | 2 | 13 | 5 |
| Σ difference | | | 19 | | 7 | | 20 | | 18 | | 25 |
| Σdiff. x 0.5 | | | 9.5 | | 3.5 | | 10 | | 9 | | 12.5 |
| 100–(Σdiff. x 0.5) | | | 90.5 | | 96.5 | | 90 | | 91 | | 87.5 |
| % model affinity | | | **91** | | **97** | | **90** | | **91** | | **88** |
| Level of impact | | | none | | none | | none | | none | | none |
| 2002 | | | | | | | | | | | |
| Diptera | **10** | 9 | 1 | 6 | 4 | 10 | 0 | 12 | 2 | 11 | 1 |
| Trichoptera | **24** | 17 | 7 | 20 | 4 | 23 | 1 | 25 | 1 | 35 | 11 |
| Ephemeroptera | **26** | 27 | 1 | 41 | 15 | 27 | 1 | 26 | 0 | 22 | 4 |
| Plecoptera | **2** | 3 | 1 | 3 | 1 | 5 | 3 | 4 | 2 | 3 | 1 |
| Coleoptera | **13** | 13 | 0 | 10 | 3 | 16 | 3 | 8 | 5 | 10 | 3 |
| Megaloptera | **6** | 5 | 1 | 5 | 1 | 4 | 2 | 7 | 1 | 7 | 1 |
| Others | **19** | 25 | 6 | 15 | 4 | 16 | 3 | 17 | 2 | 13 | 6 |
| Σ difference | | | 17 | | 32 | | 13 | | 13 | | 27 |
| Σdiff. x 0.5 | | | 8.5 | | 16 | | 6.5 | | 6.5 | | 13.5 |
| 100–(Σdiff. x 0.5) | | | 91.5 | | 84 | | 93.5 | | 94.5 | | 86.5 |
| % model affinity | | | **92** | | **84** | | **94** | | **95** | | **87** |
| Level of impact | | | none | | none | | none | | none | | none |

in the rapids zones of Swedish rivers were observed to affect grazers and predators negatively but not filterers and shredders (Englund and Malmqvist 1996).

The influence of river discharge on aquatic communities was readily detected by discriminant function analysis to a degree at which we conclude that year-to-year variation in river volume represents the most decisive factor regulating community diversity in the River Coatán. In contrast, the use of a biotic index, the percent model affinity index, was useful only when it confirmed that the algal extraction treatment resulted in a minimal perturbation of the insect community in the treated zone compared to that of the control zone. Evidently, biotic indices that are designed to detect major anthropogenic changes in water quality (Barton 1996), are not very sensitive to the subtle changes in functional community composition observed in the present study.

The importance of filamentous algae resides in their ability to act as refuges to predators (Sih 1986, Orr and Resh 1989), reduce current velocity (Hall 1972), provide direct and indirect food sources by harboring an important epiphytic microbial community (Wetzel and Søndergaard 1998, Stanley et al. 2003), and increase the physical heterogeneity of the habitat (Cardinale et al. 2002). Filamentous algae may also provide attractive cues to ovipositing mosquitoes (Orr and Resh 1992, Rejmankova et al. 1996), and their texture can positively influence macroinvertebrate community diversity, particularly for the Chironomidae and species of Trichoptera and Plecoptera (Downes et al. 2000).

Removal of filamentous algae resulted in two almost simultaneous biotic effects. First, the removal of algae eliminated a physical refuge and critical food source for developing *An. pseudopunctipennis* and other aquatic invertebrates that graze upon this resource (Bond et al. 2005). Second, without a food source a rapid and dramatic decline was observed in the population of immature *An. pseudopunctipennis* (Bond et al. 2004) with direct consequences for the populations of their predators. Probably for this reason, discriminant function analysis identified the abundance of Diptera (mosquito larvae) and Odonata (predatory dragonfly larvae) as the only significant predictor variables among the eight insect orders included in the analysis.

An important issue in the experimental design employed in this study is that of the balance between the scale of the experiment and the degree of replication of treatments. Replication in this experiment was only possible over time (years) because it was necessary to apply the extraction treatment on a large scale (3 km section of the river) to minimize the influence of invertebrate drift from untreated upstream habitats. The fact that we had formulated clear predictions regarding the direction of the change in diversity expected following extraction of algae, combined with the marked similarity of changes in diversity in the treated zone observed in each year of the study, give us confidence that the results of the study were not unduly influenced by the severe limitations on experimental replication.

With serious human health issues at stake and limited budgets for achieving the objectives of vector control programs, the impact of mosquito control practices on aquatic insect communities is almost invariably classified as being of minimal importance, and consequently rarely evaluated. The principal exception to this statement involves programs based on control of mosquitoes and blackflies by application of the δ-endotoxin of the bacterial pathogen *Bacillus thuringiensis* var. *israelensis* (Bti). This toxin shows selective toxicity to nematocerous Diptera (mosquitoes, blackflies, midges, tipulids, etc.) and the majority of field studies have reported no significant adverse effects on non-target aquatic invertebrate fauna following application of Bti (Boisvert and Boisvert 2000, Lacey and Merritt 2003). However, intensive use of Bti over several years may result in altered patterns in trophic relationships of aquatic invertebrate communities inhabiting wetlands (Hershey et al. 1998).

In conclusion, despite the importance of algae in river function and primary productivity, the algal extraction intervention employed for *Anopheles* control caused only minor changes in taxa richness, diversity, and functional structure of the aquatic insect community associated with river pools. Year-to-year variation in river discharge was far more influential than algal extraction in determining the abundance and composition of these communities. The algal extraction intervention represents a highly effective vector control measure with minimal consequences for the aquatic insect community associated with *An. pseudopunctipennis* breeding pools in southern Mexico.

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REFERENCES CITED

Ault, S.K. 1994. Environmental management: a re-emerging vector control strategy. Am. J. Trop. Med. Hyg. 50: 35-49.

Barton, D.R. 1996. The use of percent model affinity to assess the effects of agriculture on benthic invertebrate communities in headwater streams of southern Ontario, Canada. Freshwat. Biol. 36: 397-410.

Boisvert, M. and J. Boisvert. 2000. Effects of *Bacillus thuringiensis* var. *israelensis* on target and nontarget organisms: a review of laboratory and field studies. Biocontr. Sci. Technol. 10: 517-561.

Bond, J.G., J.I. Arredondo-Jiménez, M.H. Rodríguez, H. Quiroz-Martínez, and T. Williams. 2005. Oviposition habitat selection for a predator refuge and food source in a mosquito. Ecol. Entomol. 30: 255-263.

Bond, J.G., J.C. Rojas, J.I. Arredondo-Jiménez, H. Quiroz-Martinez, J. Valle, and T. Williams. 2004. Population control of the malaria vector *Anopheles* *pseudopunctipennis* by habitat manipulation. Proc. R. Soc. B. 271: 2161-2169.

Bond, N.R. and B.J. Downes. 2003. The independent and interactive effects of fine sediment and flow on benthic invertebrate communities characteristic of small upland streams. Freshwat. Biol. 48: 455-465.

Brittain, J.E. and T.J. Eikeland. 1988. Invertebrate drift - a review. Hydrobiologia 166: 77-93.

Cardinale, B.J., M.A. Palmer, A.R. Ives, and S.S. Brooks. 2005. Diversity-productivity relationships in streams vary as a function of the natural disturbance regime. Ecology 86: 716-726.

Cardinale, B.J., M.A. Palmer, C.M. Swan, S. Brooks, and N.L. Poff. 2002. The influence of substrate heterogeneity on biofilm metabolism in a stream ecosystem. Ecology 83: 412-422.

Carlisle, D.M. and W.H. Clements. 1999. Sensitivity and variability of metrics used in biological assessments of running waters. Environ. Toxicol. Chem. 18: 285-291.

Chanon, K.E., J.F. Méndez-Galván, J.M. Galindo-Jaramillo, H. Olguín-Bernal, and V.H. Borja-Aburto. 2003. Cooperative actions to achieve malaria control without the use of DDT. Int. J. Hyg. Environ. Hlth. 206: 387-394.

Chavasse, D.C. and H.H. Yap. 1997. *Chemical methods for the control of vectors and pests of public health importance.* 5th edn. document ref. WHO/CTD/WHOPES/97.2. World Health Organization, Geneva, Switzerland.

Cobb, D.G., T.D. Galloway, and J.F. Flannagan. 1992. Effects of discharge and substrate stability on density and species composition of stream insects. Can. J. Fish. Aquat. Sci. 49: 1788-1795.

Contreras-Ramos, A. 1999. List of species of Neotropical Megaloptera (Neuropterida). Proc. Entomol. Soc. Wash. 101: 273-284.

Cummins, K.W. 1973. Trophic relations of aquatic insects. Annu. Rev. Entomol. 18: 183-206.

Delgado-Gallardo, M.L., M.H. Badii, and H. Quiroz-Martínez. 1994. Diversidad ecológica de las comunidades acuaticas cohabitando con *Anopheles pseudopunctipennis* (Diptera: Culicidae) en el arroyo La Ciudadela en el municipio de Benito Juárez, Nuevo León, México. Southwest. Entomol. 19: 77-81.

Doi, H., I. Katano, and E. Kikuchi. 2006. The use of algal-mat habitats by aquatic insect grazers: effects of microalgal cues. Basic Appl. Ecol. 7: 153-158.

Downes, B.J., P.S. Lake, E.S.G. Schreiber, and A. Glaister. 2000. Habitat structure, resources and diversity: the separate effects of surface roughness and macroalgae on stream invertebrates. Oecologia 123: 569-581.

Englund, G. and B. Malmqvist. 1996. Effects of flow regulation, habitat area and isolation on the macroinvertebrate fauna of rapids in North Swedish rivers. Reg. Rivers Res. Manag. 12: 433-445.

Figueroa, R., C. Valdovinos, E. Araya, and O. Parra. 2003. Macroinvertebrados bentónicos como indicadores de la calidad de agua de ríos del sur de Chile. Rev. Chil. Hist. Nat. 76: 275-285.

González-Soriano, E. and R. Novelo-Gutiérrez. 1996. Odonata. In: J. Llorente, A. N. García, and E. González-Soriano (eds.) *Biodiversidad, Taxonomía y Biogeografía de Artrópodos de México: Hacia una Síntesis de su Conocimiento.* pp. 147-167. Universidad Autónoma de México, Mexico City..

Hall, T.F. 1972. The influence of plants on anopheline breeding. Am. J. Trop. Med. Hyg. 21: 787-794.

Hansen, R.A., D.D. Hart, and R.A. Merz. 1991. Flow mediates predatory prey interactions between triclad flatworms and larval black flies. Oikos 60: 187-196.

Harrison, S.S.C., J.L. Pretty, D. Shepherd, A.G. Hildrew, C. Smith, and R.D. Hey. 2004. The effects of instream rehabilitation structures on macroinvertebrates in lowland rivers. J. Appl. Ecol. 41: 1140-1152.

Hershey, A.E., A.R. Lima, G.J. Niemi, and R.R. Regal. 1998. Effects of *Bacillus thuringiensis israelensis* (Bti) and methoprene on nontarget macroinvertebrates in Minnesota wetlands. Ecol. Appl. 8: 41-60.

Hilsenhoff, W.L. 1988. Rapid field assessment of organic pollution with a family level biotic index. J. N. Am. Benthol. Soc. 7, 65-68.

Hutcheson, K. 1970. A test for comparing diversities based on the Shannon formula. J. Theor. Biol. 29: 151-154.

Keiser, J., B.H. Singer, and J. Utzinger. 2005. Reducing the burden of malaria in different eco-epidemiological settings with environmental management: a systematic review. Lancet Infect. Dis. 5: 695-708.

Lacey, L.A. and D.L. Merritt. 2003. The safety of bacterial microbial agents used for black fly and mosquito control in aquatic environments. In: H. M. T. Hokkanen and A. E. Hajek (eds.) *Environmental Impacts of Microbial Insecticides: Need and Methods for Risk Assessment.* pp. 151-168. Kluwer, Dordrecht, Netherlands.

Lehmkuhl, D.M. 1979. *How to Know the Aquatic Insects*. WC Brown Publishing Co., Dubuque, IA.

Lenat, D.R., L.A. Smock, and D.L. Penrose. 1980. Use of benthic macroinvertebrates as indicators of environmental quality. In: D. L. Worf (ed.) *Biological Monitoring for Environmental Effects*. pp. 97-112. D.C. Heath and Co., Lexington, MA.

Lenat, D.R. and D.L. Penrose. 1996. History of the EPT taxa richness metric. Bull. N. Am. Benthol. Soc. 13: 305-306.

Magurran, A.E. 2004. *Measuring Biological Diversity*. Blackwell, Oxford, U.K.

Malmqvist, B. 2002. Aquatic invertebrates in riverine landscapes. Freshwat. Biol. 47: 679-694.

Mandaville, S.M. 2002. Benthic macroinvertebrates in freshwaters-taxa tolerance values, metrics, and protocols. Soil and Water Conservation Society of Metro Halifax. Project H-1, Appendices A-B. Online: http://chebuco.ca/Science/SWCS/SWCS.html.

Mérigoux, S., and S. Dolédec. 2004. Hydraulic requirements of stream communities: a case study on invertebrates. Freshwat. Biol. 49: 600-613.

Merritt, R.W. and K.W. Cummins. 1996. *An Introduction to the Aquatic Insects of North America*. 3rd ed. Kendall-

Hunt, Dubuque, IA.

Needham, J.G. and P.R. Needham. 1962. *A Guide to the Study of Fresh-water Biology.* Holden-Day Inc., San Francisco, CA.

Needham, J.G., M.J.L. Westfall, and M. May. 2000. *Dragonflies of North America*. Scientific Publishers, Gainesville, FL.

Niemi, G.J., N.E. Detenbeck, and J.A. Perry. 1993. Comparative analysis of variables to measure recovery rates in streams. Environ. Toxicol. Chem. 12: 1541-1547.

Novak, M.A. and R.W. Bode. 1992. Percent model affinity: a new measure of macroinvertebrate community composition. J. N. Am. Benthol. Soc. 11: 80-85.

Novelo-Gutiérrez, R. 1997a. Clave para la separación de familias y géneros de las náyades de Odonata de México. Parte I. Zygoptera. Dugesiana 4: 1-10.

Novelo-Gutiérrez, R. 1997b. Clave para la identificación de familias y géneros de las náyades de Odonata de México. Parte II. Anisoptera. Dugesiana 4: 31-40.

Orr, B.K. and V.H. Resh. 1989. Experimental test of the influence of aquatic macrophyte cover on the survival of *Anopheles* larvae. J. Am. Mosq. Contr. Assoc. 5: 579-585.

Orr, B.K. and V.H. Resh. 1992. Influence of *Myriophyllum aquaticum* cover on *Anopheles* mosquito abundance, oviposition, and larval microhabitat. Oecologia 90: 474-482.

Pickett, S.T.A. and P.S. White. 1989. The ecological concept of disturbance and its expression at various hierarchical levels. Oikos 54: 129-136.

Pollard, J.E. 1981. Investigator differences associated with a kicking method for sampling macroinvertebrates. J. Freshwat. Ecol. 1: 215-224.

Poulton, B.C., M.L. Wildhaber, C.S. Charbonneau, J.F. Fairchild, B.G. Mueller, and C.J. Schmitt. 2003. A longitudinal assessment of the aquatic macroinvertebrate community in the channelized lower Missouri River. Environ. Monitor. Assess. 85: 23-53.

Pratt, J.R. and N.J. Bowers. 1992. Variability of community metrics: detecting changes in structure and function. Environ. Toxicol. Chem. 11: 451-457.

Quinn, J. M. and C.W. Hickey. 1994. Hydraulic parameters and benthic invertebrate distributions in two gravel bed New Zealand rivers. Freshwat. Biol. 32: 489-500.

Rafatjah, H.A. 1988. Malaria vector control: environmental management. In: W. H. Wernsdorfer and I. McGregor (eds.) *Malaria: Principles and Practice of Malariology*. pp. 1135-1172. Churchill Livingstone, Edinburgh, UK.

Rawer-Jost, C., J. Bohmer, J. Blank, and H. Rahmann. 2000. Macroinvertebrate functional feeding group methods in ecological assessment. Hydrobiologia 422/423: 225-232.

Rejmankova, E.H., M. Savage, D.R. Roberts, S. Manguin, K.O. Pope, J. Komárek, and R.A. Post. 1996. *Anopheles albimanus* (Diptera: Culicidae) and cyanobacteria: an example of larval habitat selection. Environ. Entomol. 25: 1058-1087.

Rempel, L.L., J.S. Richardson, and M.C. Healey. 2000. Macroinvertebrate community structure along gradients of hydraulic and sedimentary conditions in a large gravel-bed river. Freshwat. Biol. 45: 57-73.

Resh, V.H. and J.K. Jackson. 1993. Rapid assessment approaches to biomonitoring using benthic invertebrates. In: D.M. Rosenberg and V.H. Resh (eds.) *Freshwater Biomonitoring and Benthic Macroinvertebrates*. pp. 195-233. Chapman and Hall, NY.

Resh, V.H., A.V. Brown, A.P. Covich, M.E. Gurtz, H.W. Li, G.W. Minshall, S.R. Reice, A.L. Sheldon, J.B. Wallace, and R.C. Wissmar. 1998. The role of disturbance in stream ecology. J. N. Am. Benthol. Soc. 7: 443-455.

Resh, V.H., M.M. Myers, and M.J. Hannaford. 1996. Macroinvertebrates as biotic indicators of environmental quality. In: F.R. Hauer and G.A. Lamberti (eds.) *Methods in Stream Ecology*. pp. 647-667. Academic Press, London, U.K.

Rogers, C.D. 1998. Aquatic macroinvertebrate occurrences and population trends in constructed and natural vernal pools in Folson, California. In: C.W. Witham, E.T. Bader, D. Belk, W.R. Ferren Jr., and R. Ornduff (eds.) *Ecology Conservation and Management of Vernal Pool Ecosystems*. pp. 224-235. California Native Plant Society, Sacramento, CA.

Rozendaal, J.A. 1997. *Vector Control: Methods for Use by Individuals and Communities.* Wld. Hlth. Org. Geneva, Switzerland.

Sih, A. 1986. Antipredatory responses and the perception of danger by mosquito larvae. Ecology 67: 434-441.

Southwood, T.R.E. and P.A. Henderson. 2000. *Ecological Methods***.** 3rd ed., Blackwell, Oxford, U.K.

Stanley, E.H., M.D. Johnson, and A.K. Ward. 2003. Evaluating the influence of macrophytes on algal and bacterial production in multiple habitats of a freshwater wetland. Limnol. Oceanogr. 48: 1101-1111.

Statzner, B., J.A. Gore, and V.H. Resh. 1988. Hydraulic stream ecology: observed patterns and potential applications. J. N. Am. Benthol. Soc. 7: 307-360.

Stevenson, R.J., M.L. Bothwell, and R.L. Lowe. 1996. *Algal Ecology: Freshwater Benthic Ecosystems.* Academic Press, San Diego, CA.

Taylor, N.J. 1979. A rapid efficient area sampler for estimating absolute abundance of floodwater mosquito larvae. Environ. Entomol. 8: 1004-1006.

Thomson, J.R., P.S. Lake, and B.J. Downes. 2002. The effect of hydrological disturbance on the impact of a benthic invertebrate predator. Ecology 83: 628-642.

Wallace, J.B. and J.R. Webster. 1996. The role of macroinvertebrates in stream ecosystem function. Annu. Rev. Entomol. 41: 115-139.

Westfall, M.J. and M.L. May. 1996. *Damselflies of North America*. Scientific Publishers, Gainesville, FL.

Wetzel, R.G. and M. Søndergaard. 1998. Role of submerged macrophytes for the microbial community and dynamics of dissolved organic carbon in aquatic ecosystems. In: E. Jeppesen, M. Søndergaard, M. Søndergaard and K. Christoffersen (eds.) *The Structuring Role of Submerged*

*Macrophytes in Lakes.* pp. 133-148. Springer-Verlag, Berlin, Germany.

Winer, B.J., D.R. Brown, and K.M. Micheals. 1991. *Statistical Principals in Experimental Design*. McGraw Hill, New York.

Wisner, B. and J. Adams. 2002. *Environmental Health in Emergencies and Disasters. A Practical Guide.* World Health Organization, Geneva, Switzerland.