

Effects of ephemeropterans and shrimps on periphyton and sediments in a coastal stream (Atlantic forest, Rio de Janeiro, Brazil)

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Abstract. We investigated the importance of shrimps (Atyidae and Palaemonidae) and ephemeropterans (Baetidae) in the removal of periphyton and sediments in a Neotropical stream. The experimental site was open with homogeneous bedrock, shallow depth (8–15 cm), and intermediate water current velocity (0.2–0.3 m/s). We used 2 intensities of electrical current to exclude both shrimps and ephemeropterans (high-intensity treatment) or only shrimps (low-intensity treatment) from fixed areas (180 × 30 cm) of bedrock. When both ephemeropterans and shrimps were excluded in 2 experiments, matter accumulated on the bedrock to 5 and 20× the level in controls; when only shrimps were excluded, no accumulation was observed. Chlorophyll *a* increased significantly in the high-intensity exclusion, but most of the accumulation was fine organic and inorganic matter. In experiment 1, benthic matter and chlorophyll *a* decreased by an order of magnitude in areas in which ephemeropterans increased ~40×. The increase in ephemeropterans was associated with a reduction in shrimp activity. In experiment 2, no increase in ephemeropterans was observed. Palaemonid shrimps (*Macrobrachium olfersi*) were more common than atyid shrimps (*Potimirim glabra*) in the study area. Therefore, we assumed that *Macrobrachium*, rather than *Potimirim*, interacted negatively with ephemeropterans and that *Macrobrachium* did not remove periphyton. We concluded that baetid ephemeropterans (particularly *Americabaetis* sp.) were the most important grazers and removers of benthic matter in this system.

Key words: strong interactors, detritivory, periphyton dynamics, sediments, biofilm, Ephemeroptera, Atyidae, Palaemonidae, electrical exclusion, Neotropical stream.

Herbivores in freshwater lotic systems often reduce the biomass of periphyton and alter its composition and structure (Feminella and Hawkins 1995, Steinman 1996, Hillebrand 2002). In North America, most studies addressing the effects of herbivores on periphyton have been conducted in temperate streams and have concentrated on snails and aquatic insects; studies of effects of fish and larger crustaceans such as crayfish are less common (Feminella and Hawkins 1995, Steinman 1996). In certain cases, predators have strong negative effects on herbivores and indirect positive effects on algae (Power 1992).

In apparent contrast to work in North America, studies of the effects of grazing fish and decapod shrimps are well represented in the literature from Neotropical streams. Loricariid catfish enhanced algal production at intermediate levels of grazing in Panama (Power 1990).

The detritivorous fish *Prochilodus mariae* reduced sediments and altered the composition and quantity of periphyton in a Venezuelan piedmont river (Flecker 1996). Palaemonid and atyid shrimps interacted strongly with periphyton and sediments of streams in Puerto Rico (Pringle and Blake 1994, Pringle 1996, Pringle et al. 1999, March et al. 2002) and Costa Rica (Pringle and Hamazaki 1998). In these studies, periphyton and sediments increased when shrimps were excluded from small areas (~0.2 m²) of substrate using electricity. Such strong interactions were reduced or absent in streams where the abundance of shrimps was lower (Pringle et al. 1999) or in lowland parts of the stream where snail grazing was dominant (March et al. 2002).

The role of small macroinvertebrates (such as Trichoptera and Ephemeroptera) was not directly investigated in the experiments in Costa Rica and Puerto Rico because the smaller macroinvertebrates were not excluded from the substrate by the experimental techniques. Shrimps (Pringle and Blake 1994, Pringle 1996, Pringle et

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al. 1999, March et al. 2002) and fish (Pringle and Hamazaki 1998) had large effects on sediments and periphyton, and the investigators in these studies inferred from their results that small macroinvertebrates were not important. In some studies, exclusion of shrimps caused increases in chironomid larvae (Diptera) by reducing predation or increasing favorable microhabitat (Pringle et al. 1993, Pringle et al. 1999, March et al. 2002). In contrast, the more-mobile ephemeropterans were more abundant in treatments with shrimps than in the exclusions (Pringle et al. 1993, March et al. 2002). None of the experiments showed a trophic cascade of strong negative effects of shrimps or fish on smaller macroinvertebrates such that the effect of insects on periphyton was altered, as has been reported for North American temperate streams (Power 1992). The existence of potentially strong grazing pressure by small herbivores was not established in the Puerto Rico and Costa Rica experiments, but the authors ascribed the absence of trophic cascades to the omnivory of the dominant fauna of shrimps and fish, which fed at >1 trophic level and, therefore, should not cause a trophic cascade (Pringle and Hamazaki 1998).

The shrimps *Macrobrachium* spp. (Decapoda: Palaemonidae) and *Potimirim* spp. (Decapoda: Atyidae) often are a conspicuous part of the fauna of coastal streams of the southeast of Brazil (Moulton and Parslow 1994, Moulton 1998, Silveira and Moulton 1998). These genera belong to the same families of amphidromous shrimps that are involved in strong interactions with the substrate in the Caribbean and in Central America. Experiments at our study site in Brazil had results opposite from those expected on the basis of studies in the Caribbean and Central America. Chlorophyll *a* decreased when shrimps were excluded with cages (Siviero and Moulton 1998). Inorganic sediments, ash-free dry mass (AFDM), and chlorophyll *a* decreased when shrimps were excluded using electricity (Silveira and Moulton 2000, Silveira 2002). At our study site, we observed that baetid ephemeropterans were abundant during the day, but disappeared from the exposed surface of the bottom substrate concomitant with the appearance of *Macrobrachium* at nightfall. We hypothesized that ephemeropterans were responsible for the decrease in chlorophyll *a* and sediments when shrimps were excluded from bottom substrate. We tested this hypothesis experimentally

using different intensities of electricity to exclude only shrimps (low intensity) or to exclude both shrimps and ephemeropterans (high intensity). We expected that periphyton would increase when ephemeropterans were excluded and decrease when ephemeropterans increased in numbers, if they were important grazers. Our experimental method could not isolate the effect of ephemeropteran grazing from the effect of potential interactions between ephemeropterans and shrimps because both groups were excluded by high-intensity electricity. Nevertheless, we expected that the dynamics of the ephemeropteran-periphyton relationship would provide strong inference for causality. That is, we could infer that ephemeropterans were the principal grazers if increases or decreases in ephemeropterans corresponded with respective decreases or increases in periphyton that were independent of, or in contrast to, the dynamics caused by the shrimps.

Periphyton is a complex association of microalgae and heterotrophic microorganisms. It is intimately associated with extracellular organic matter derived from the organisms of the periphyton, sedimentation, and the surrounding water. Inorganic material also settles out of the water column and becomes incorporated in the periphyton matrix. Periphyton commonly is quantified by removing a sample, drying and weighing it to determine total dry mass, and combusting it to determine organic content as AFDM (Steinman and Lamberti 1996). The method does not distinguish between components that were produced by in situ biological processes and those that accumulated by sedimentation. We used this method and analysis of chlorophyll *a* to characterize the material that accumulated on rock substrate in our experiments. Therefore, we use the term, periphyton, in the broad sense to describe all fine material that accumulated on rock substrate in our experimental area.

Grazers interact with periphyton by ingesting material selectively or nonselectively and by dislodging material that is then carried away by the water current (Scrimgeour et al. 1991). We did not quantify the forms of removal of periphyton in our experiments, but we inferred that the material that accumulated after exclusion of grazers was material that grazers would have removed had they been present. We use the term, grazing, to describe the action of removal of ma-

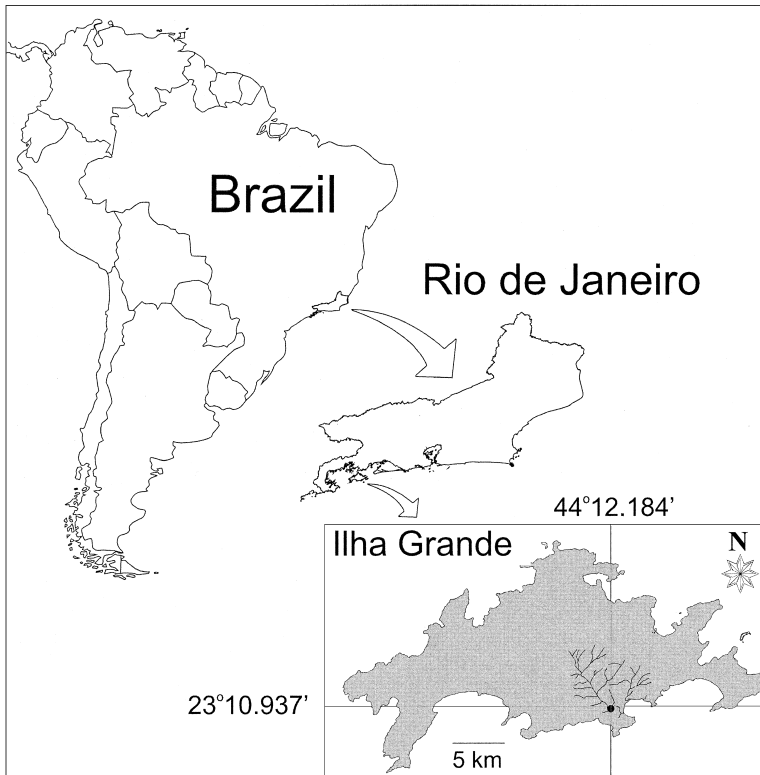


FIG. 1. Study site in Rio de Janeiro state, on the coastal island Ilha Grande on the stream Rio Andorinha.

terial, whether by ingestion or by dislodgement (nonconsumptive loss sensu Scrimgeour et al. 1991, or bioturbation sensu Pringle and Hamazaki 1998).

Methods

Study site

We conducted our experiments in a 3rd-order stream, Rio Andorinha, near Vila Dois Rios, on the coastal island Ilha Grande, Rio de Janeiro state (lat 23°10.9'S, long 44°12.2'W) (Fig. 1). The vegetation of the catchment is Atlantic rainforest in a good state of preservation, and the area is protected as a state park. We measured 2166 mm of rain at the experimental site in 2000; rainfall in the mountains of the catchment probably was higher. The baseflow discharge of Rio Andorinha at the study site was 80 L/s. The bedrock is Pre-Cambrian granite gneiss. The streambed consisted of large boulders and well-embedded rocks, so little substrate movement

occurred during floods, although scour from coarse sand appeared to be important.

Our study site is known locally as Mãe D'água and consists of an area of continuous bedrock. Immediately below the site is a waterfall, which restricts fish from our site. Above the site is a large pool. We placed our experiments downstream of the pool on an area of almost-level bedrock before the inclined face of the waterfall. The stream broadened at this point and the riparian forest did not overhang the stream, so no shading occurred at high sun angles.

The study area was poor in species compared to neighboring habitats of the stream. The fauna consisted of the shrimps *Macrobrachium olfersi* (Decapoda:Palaemonidae) and *Potimirim glabra* (Decapoda:Atyidae), 4 species of baetid ephemeropteran nymphs (*Americabaetis* sp., *Baetodes* sp., *Cloeodes* sp., and *Dactylobaetis* sp.), and 4 morphospecies of chironomid dipteran larvae. We rarely observed other macroinvertebrates such as Plecoptera, Trichoptera, Coleoptera, and flatworms in the study area, although they were

common on the inclined rockface immediately downstream (Moulton and Souza 1998). The periphyton community also appeared sparse compared to the downstream rockface; it consisted of blue-green algal turf, diatoms, and occasional filamentous green algae. One species of fish (*Characidium japyhybensis*) was present at very low density at the site; we never saw it in or near the experiments. We did not observe any snails in the stream.

Electrical exclusion

We excluded fauna using electric pulses, following the principle developed by Pringle and Blake (1994). Animals are affected by electric fields in proportion to their body size, i.e., large animals are affected at lower intensities than small animals. We developed the appropriate degrees of electrical exclusion by trial and error, using commercially available electric fence chargers. We altered the design of the electrified area and the settings of the electrifier until the desired response of the target animals was observed. The response to electric shock was readily observable; the shrimps and ephemeropterans contracted or twitched at each pulse of the charger. They either jumped out of the electrified area or, more often, lost their grip on the substrate and were washed out of the area by the water current. They did not appear to detect the electrified area from a distance and avoid it. The relatively weak electrifier Ballerup® (Alfa S.A., São Paulo, São Paulo, Brazil) visibly affected shrimps (minimum size ~25 mm) without causing a reaction in ephemeropterans (0.2–5 mm). The electrifier Speedrite® (model SB5000, Tru-Test Ltd., Palmerston North, New Zealand) at its highest setting (rated as 5 J) caused reactions in both ephemeropterans and shrimps with the configuration of electrodes we designed. Chironomids were too small to observe directly, but we presume from their population dynamics in the various treatments that they were not affected by the electrical shocks of the high-intensity treatment.

We set up the electrodes by fixing copper wire directly to the bedrock, either to screws drilled into the bedrock or to fixtures glued to the bedrock with epoxy. In previous experiments, we had observed that the electrical shock extended for some distance beyond the area between the anode and cathode, potentially af-

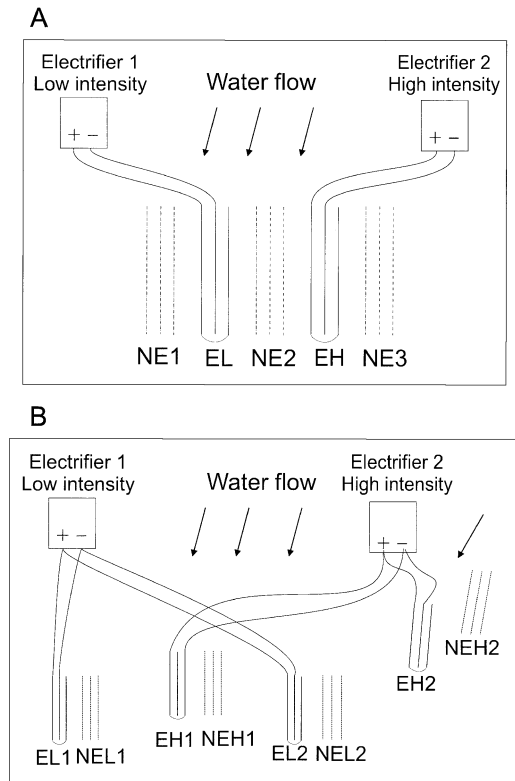


FIG. 2. Schematic drawings of the layout of the electrified and control quadrats in experiment 1 (A) and experiment 2 (B). Electrified quadrats are shown with solid lines; not-electrified quadrats with dashed lines. Drawing is not to scale.

fecting the nearby control areas. We reduced this problem by configuring a central anode or cathode and placing the other electrode on both sides and relatively close to the central electrode (15 cm). Nevertheless, the number of electrified areas we could set up in the same area was constrained by discharge between electrified areas.

We carried out 2 experiments. In experiment 1, we set up 5 fixed quadrats (180 × 30 cm), aligned parallel to each other, 60 cm apart, and with the long axis in line with the stream. We assigned 2 quadrats to be electrical exclusion treatments, either high-intensity (EH) or low-intensity (EL), and 3 quadrats to be not-electrified controls (NE1, NE2, NE3) (Fig. 2A). In experiment 2, we set up 4 pairs of quadrats, in which the quadrats were ~50 cm apart and were matched for depth and water current; each pair was isolated from the others by at least 2 m. We used the same high-intensity or low-intensity

electrification as in the first experiment, and we replicated intensity $2\times$ (Fig. 2B). In each pair, one quadrat was electrified (EH or EL) and one was not (NEH or NEL). A statistically more efficient design for experiment 2 would have used blocks of the 3 treatments (high-intensity, low-intensity, and no electrification), but we used the paired design to reduce possible interference between electrical treatments and to test that the effects of high-intensity electrified quadrats on their adjacent controls did not differ from the effects of low-intensity electrified quadrats on their adjacent controls.

We tested the electrified quadrats at the start of the experiment and observed that shrimps were affected by the shocks and excluded from the quadrats. We visited the study site during both experiments to verify that the electrifiers were working and that the organisms were responding as expected. After day 12 of experiment 2, we did not revisit until day 58. We assumed that the low-intensity electrification excluded shrimps during the whole experiment.

Sampling

Experiment 1 began on 22 January 2000, and we sampled on days 6 and 15 postelectrification. Experiment 2 began on 7 October 2000, and we sampled on days 7, 12, and 58 postelectrification.

We sampled periphyton and sediments with an apparatus consisting of a kitchen-sink plunger with a brush attached to the handle inside the plunger cup and a 60-mL plastic syringe inserted into the side of the plunger. We collected samples by pressing the plunger firmly to the substrate, scrubbing the substrate with the brush, and filling the syringe with the contents of the plunger cup. The volume of the syringe was larger than that of the plunger, so we retrieved all of the material suspended by scrubbing. The plunger sampled 40.6 cm² of the bedrock. We collected 2 periphyton/sediment samples adjacent to each Surber sample (see below) on each sampling occasion. We did not collect Surber samples on days 12 and 58 of experiment 2, so we collected 2 pairs of samples/quadrat. We combined the samples from each quadrat and used $\frac{1}{2}$ of the combined sample for estimating periphyton dry mass and the other $\frac{1}{2}$ for determining chlorophyll *a*. We determined the masses of organic and inorganic material by fil-

tering the sample through a pre-ashed, 47-mm, Whatman GF/A filter, drying at 60°C to constant mass, weighing to ± 0.001 g, ashing at 500°C for 1 h, and reweighing. We also used turbidity to estimate total mass of material; we measured the turbidity of the plunger sample in a colorimeter (model SMART, LaMotte, Chestertown, Maryland) and regressed the turbidity (FTU) against the measurement of total mass obtained by weighing (total mass = $0.0847 \times$ turbidity, $F_{1,59} = 114$, $p < 0.0001$, $r^2 = 0.66$). We used this relationship to recover the data from day 12 of experiment 2, after the filters for that day were weighed incorrectly.

In experiment 1, we measured chlorophyll *a* by filtering the sample through a 25-mm, Whatman GF/D filter, which was subsequently frozen until analysis. We extracted the chlorophyll in 1 mL 90% acetone overnight in a freezer and measured chlorophyll *a* using a spectrophotometer at 664 nm with correction for pheophytin using acidification (Nusch 1980).

We sampled the benthos immediately before electrifying quadrats in both experiments. We used a grid placed over each quadrat to locate random samples and to prevent sampling the same point twice. We used a 10 cm \times 10 cm Surber apparatus to collect aquatic insects. We collected 4 samples/quadrat on each sampling date in experiment 1 and 2 samples/quadrat on days 0 and 7 in experiment 2. We preserved insects in 80% alcohol and sorted them using a stereomicroscope at 40 \times magnification.

We also quantified the presence of shrimps and aquatic insects in the experimental quadrats by direct observation during the day and at night in experiment 1 (on days 3, 5, 6, 7, 8, and 9, and nights 0, 1, 3, 4, 7, and 8). We divided the quadrats into 4 equal areas (45 \times 30 cm) for counting. We rarely saw shrimps in the experimental quadrats during the day; at night, we shone a spotlight on the experimental area and counted the number of animals in each $\frac{1}{4}$ -quadrat before they retreated from the light. The aquatic insects that were visible were almost exclusively ephemeropterans. We passed a bent wire close to the substrate to induce them to move to make them more visible. We were able to recognize the 2 shrimp species, but we could not differentiate the ephemeropteran species, although we were confident of our counts even at night. We made 2 counts of each $\frac{1}{4}$ -quadrat area on each occasion; the counts were spaced by at

least 30 min. We did not count shrimps and ephemeropterans during floods and rain.

We used a ruler and a Teledyne® Pygmy model current meter to measure the depth and current velocity at each potential sampling position (16 positions/quadrat in experiment 1, 8/quadrat in experiment 2) in each quadrat on one day during each experiment. We monitored water level at a position close to the experimental areas using a depth logger (Global Water, Forestry Suppliers, Jackson, Mississippi). We also monitored rainfall using a tipping bucket rain gauge (Davis Instruments coupled to Hobo® event logger, Onset Instruments, Pocasset, Massachusetts) and water and air temperatures using a data logger (Stowaway®, Onset Instruments, Pocasset, Massachusetts).

Statistics and experimental design

In experiment 1, we tested for differences in variables (densities, mass) among quadrats (EH, EL, NE1, NE2, and NE3) using the replicate measures within quadrats in 1-way analysis of variance (ANOVA) or analysis of covariance (ANCOVA) (when depth and current were introduced as covariates). Thus, we inferred differences among quadrats, but treatments were not replicated, so the inference that such differences were caused by the treatments was weak. In experiment 2, we replicated the treatments, and we used the mean value from each quadrat to test for differences among the treatments. We used 2-way ANOVA to compare pairs of high-intensity electrification treatments with high-intensity control treatments and low-intensity electrification treatments with low-intensity control treatments. We used 1-way ANOVA to test for differences among all treatments, keeping the high-intensity and low-intensity controls separate to test differences between them.

We log-transformed data before ANOVA ($\log[x+1]$ for density data). We tested differences among means using Tukey's a posteriori Multiple Range Test.

We also analyzed the data using repeated-measures ANOVA with time as the repeated variable. However, this analysis assumed that the influence of the treatments was constant over time (Underwood 1997). We have biological reasons to doubt that this assumption was true in our experiments, i.e., grazing pressure could have varied through time. Therefore, we present

the ANOVAs for each day, including day 0 (before electrification), separately. We applied a Bonferroni adjustment to the probabilities of the separate-day analyses to correct for repeated tests; $p = 0.025$ for $\alpha = 0.05$ in experiment 1 (2 sampling days) and $p = 0.0137$ for $\alpha = 0.05$ in experiment 2 (3 sampling days). In almost all cases, the separate-day and repeated-measures analyses yielded the same statistical results.

Results

Experiment 1

Characteristics of the quadrats before electrification.—Stream-water variables were as follows: conductivity $\approx 25 \mu\text{S}/\text{cm}$, pH = 6.6, total P = $0.2 \mu\text{M}$, and total N = $5 \mu\text{M}$. Mean water depths in the quadrats were between 8.3 cm (NE2 and EH) and 14.8 cm (NE3) during baseflow. Depth varied systematically in each quadrat from deeper upstream to shallower downstream. The range of depths overlapped among quadrats and varied from 20 cm (NE1) to 9 cm (NE2 and EH). The mean current velocity at baseflow was between 0.21 m/s (NE1) and 0.32 m/s (NE2 and EH) and varied inversely with depth. On day 0, total sediment mass did not differ among quadrats (Table 1) and was not significantly related to current (ANCOVA of quadrats as fixed factors and current as covariate). NE3 and EH had significantly more AFDM than NE1 (Table 1). AFDM was not correlated with current. Chlorophyll *a* did not differ among quadrats (Table 1), but it was inversely related to depth.

The mean number of *Macrobrachium* was 2.33/sampling area ($17.3/\text{m}^2$) on the night before the electrification began. Their average length was ~ 3 cm. The mean number of *Potimirim* was 0.5/sampling area ($3.7/\text{m}^2$); it was not recorded in the quadrats after day 1. *Americabaetis* sp. was less dense in NE1 than in the other quadrats (ANOVA, $p < 0.05$). *Baetodes* sp. and the most common morphospecies of chironomid did not differ among quadrats, and the other species were too rare for analysis.

After electrification.—Water temperature was between 20.3 and 21.1°C during the experiment. Intense rain and a spate occurred on day 5. Chlorophyll *a* decreased in all quadrats over time (Fig. 3A). Chlorophyll *a* was significantly higher in EH than in EL and NE2 on day 15; levels in NE1 and NE3 were intermediate (Table

TABLE 1. Experiment 1: Analysis of variance (ANOVA) of differences in periphyton among quadrats (see text for details of analyses). Significant differences among means ($p < 0.05$) were identified using Tukey's Multiple Range Test; treatments with the same superscript were not significantly different. Probabilities for days 6 and 15 were corrected for repeated tests using the Bonferroni adjustment. AFDM = ash-free dry mass, EH = high-intensity electrification, EL = low-intensity electrification, NE = not electrified. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. – = analysis not done.

Day	Dry mass	AFDM	Chlorophyll <i>a</i>
0	$F_{4,15} = 2.8$	$F_{4,15} = 3.5^*$ NE3 ^a EH ^a NE2 ^{ab} EL ^{ab} NE1 ^b	$F_{4,15} = 0.58$
6	$F_{4,15} = 13.9^{***}$ EH ^a NE1 ^b EL ^b NE3 ^b NE2 ^b	$F_{4,15} = 11.1^{***}$ EH ^a NE1 ^b EL ^b NE3 ^b NE2 ^b	$F_{4,15} = 1.47$
15	$F_{4,15} = 24.8^{***}$ EH ^a NE1 ^b NE3 ^b NE2 ^c EL ^c	$F_{4,15} = 22.1^{***}$ EH ^a NE1 ^b NE3 ^b NE2 ^c EL ^c	$F_{4,15} = 4.74^*$ EH ^a NE1 ^{ab} NE3 ^{ab} EL ^b NE2 ^b
6–15	Quadrat: $F_{4,15} = 34.2^{***}$	Quadrat: $F_{4,15} = 29.8^{***}$	–
Repeated measures	Day: $F_{1,15} = 42^{***}$ Quadrat × day: $F_{4,15} = 11.8^{***}$	Day: $F_{1,5} = 16.9^{***}$ Quadrat × day: $F_{4,5} = 7.1^{**}$	

1). The quantity of sediment (dry mass and AFDM) increased in EH and decreased in EL. By day 15, dry mass was 30× greater in EH than EL (Table 1, Fig. 3B). Dry mass and AFDM also decreased in NE2 by approximately the same amount as in EL (to ~3% of the original). Dry mass and AFDM did not change in NE1 and NE3. The ratio of AFDM to chlorophyll *a* was much higher (~30,000) in EH on day 15 than it was at the start of the experiment (~3000) (Table 1, Fig. 3C). The ratio in the other quadrats did not increase, except in NE3 where it increased by day 15 (Table 1, Fig. 3C).

Very few ephemeropterans were seen inside EH (Fig. 4A, B), ephemeropterans appeared unaffected by EL (Fig. 4A, B), and no shrimps were seen in EH and EL (Fig. 4C). Night counts of ephemeropterans increased by more than an order of magnitude during the first 8 d of electrification (Fig. 4B). Fewer ephemeropterans were found in NE1 than in NE2, NE3, and EL at night (Fig. 4B; ANOVA, $p < 0.001$). This pattern appeared to be associated with a higher density of *Macrobrachium* in NE1 than in NE2 and NE3 (Fig. 4C). *Americabaetis* sp. was rare or absent in EH and dense in EL on both sampling days after electrification (Fig. 5A). *Baetodes* sp. was less dense in EH than in other quadrats on day 6, but densities did not differ among quadrats on day 15 (Fig. 5B). Densities of both species increased substantially in the first 6 d of the experiment.

Experiment 2

Characteristics of the quadrats before electrification.—Mean water depths in the quadrats were between 9.5 and 15 cm; current velocity was 0.22 to 0.26 m/s. The treatments were distributed between pairs of quadrats such that treatments did not differ with respect to depth and current. On day 0, total sediment mass did not differ among treatments. Sediment mass was not correlated with depth or current. *Americabaetis* density did not differ among treatments; the other species of ephemeropterans were too rare to be analyzed.

After electrification.—No high rainfall events occurred in the first 12 d of the experiment. Water temperature varied from 18.5 to 21.3°C. Dry mass increased in the EH treatment after electrification (Fig. 6). By day 7, it was ~5× greater than on day 0 and significantly greater in EH than in the other treatments (Fig. 6, Table 2). The difference remained significant on day 58 (Table 2). Dry mass in the EL treatment did not differ from NEL or NEH, and NEL did not differ from NEH, indicating no apparent lateral effects of the electrification (Table 2).

Surber samples on day 0 and day 7 confirmed that EH excluded *Americabaetis* (Fig. 7). *Americabaetis* density did not differ between days or among treatments other than EH. *Baetodes* sp., *Cloeodes* sp., and *Dactylobaetis* sp. were too rare to analyze statistically.

TABLE 1. Extended.

Day	AFDM/chlorophyll <i>a</i>
0	$F_{4,15} = 2.73$
6	$F_{4,13} = 13.9^{***}$ EH ^a NE3 ^b NE1 ^b EL ^b NE2 ^b
15	$F_{4,15} = 5.73^{**}$ EH ^a NE3 ^{ab} NE1 ^b EL ^b NE2 ^b
6–15	Quadrat: $F_{4,14} = 14.4^{***}$
Repeated measures	Day: $F_{1,5} = 6.7^*$ Quadrat × day: $F_{4,15} = 0.4$

Discussion

In this system, ephemeropterans appeared to have a strong negative effect on the quantity of periphyton and sediments. Periphyton increased 5× and 20× when ephemeropterans were excluded in the EH treatments of experiments 1 and 2, respectively (Figs 3, 6). This result implies that, at their normal density of 100 to 200/m², ephemeropterans maintained periphyton at levels well below its possible maximum. In experiment 1, when ephemeropterans increased ~40× at night, periphyton decreased by an order of magnitude. In experiment 2, ephemeropterans did not increase in any treatment, and periphyton did not decrease.

Ephemeropterans are important herbivores of periphyton, although they generally have smaller effects than other grazing taxa such as snails and Trichoptera (Feminella and Hawkins 1995, Lamberti et al. 1987, Steinman 1996). Hill and Knight (1987) found that an ameletid ephemeropteran significantly reduced periphyton mass and structure at their normal density of ~200/m². On the other hand, Lamberti et al. (1987) found that *Centropilium elsa* did not have a strong effect on periphyton AFDM and chlorophyll *a* at a density of 500/m² in an artificial stream. Brown et al. (2000) used electricity to inhibit macroinvertebrate grazing. They found that macroinvertebrate numbers and biomass increased in electrified areas, but ephemeropteran numbers decreased. Ephemeropterans were not totally excluded by the electrification, but

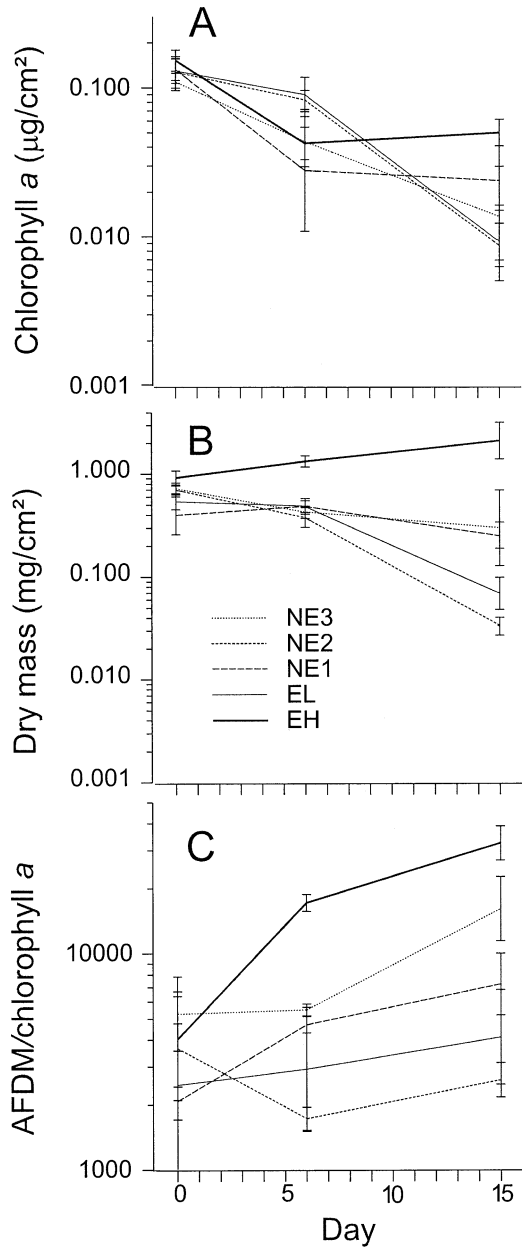


FIG. 3. Mean (±1 SE) periphyton chlorophyll *a* (A), dry mass (B), and ash-free dry mass (AFDM)/chlorophyll *a* ratio (C) in electrified and not-electrified quadrats in experiment 1. *n* = 4. EH = high intensity electrification, EL = low intensity electrification, NE = not-electrified.

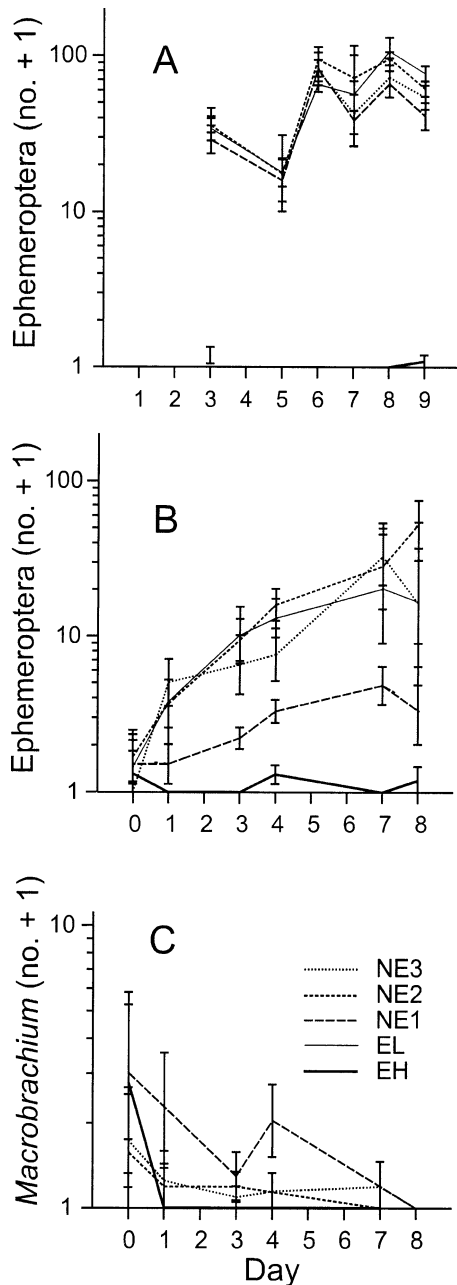


FIG. 4. Mean (± 1 SE) counts of Ephemeroptera during the day (A) and at night (B) and counts of *Macrobrachium* at night (C) in electrified and not-electrified quadrats. Counts were made by direct observation during experiment 1. *Macrobrachium* was absent from all quadrats during the day. $n = 8$ estimates/quadrat. Abbreviations are as in Fig. 3.

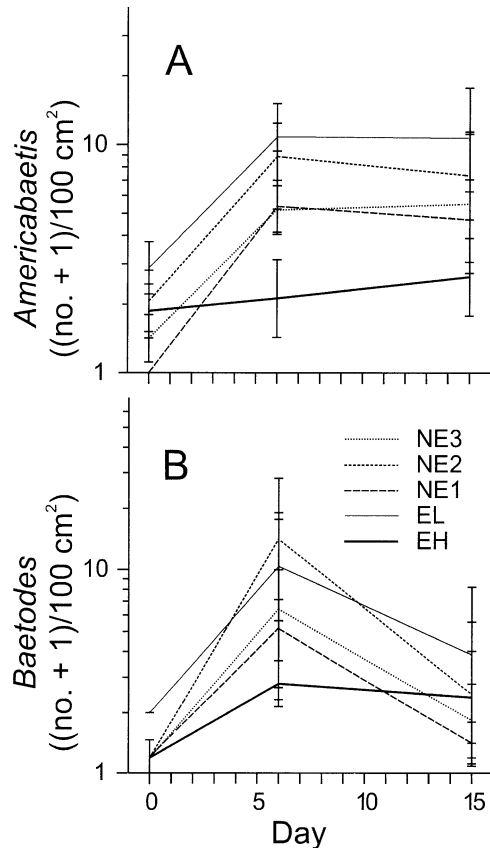


FIG. 5. Mean (± 1 SE) density of *Americabaetis* sp. (A) and *Baetodes* sp. (B) collected by Surber sampler in electrified and not-electrified quadrats during experiment 1. $n = 4$. Abbreviations are as in Fig. 3.

apparently their grazing activity was reduced by the treatment because chlorophyll *a* and AFDM increased in electrified treatments. The density of ephemeropterans was relatively high in these experiments: 8000/m² in the first experiment and 2000/m² in the second. In our experiments, the larger taxa of ephemeropterans were significantly reduced in density, and we speculate that the smallest genus, *Baetodes*, could have been inhibited by the electricity even though it was not completely excluded (Fig. 5). Taylor et al. (2002) extended the use of electricity to a reach-scale manipulation of streams in the Rocky Mountains, Colorado. They found a 57% increase in periphyton biomass when macroinvertebrates were reduced in numbers by 86%. The principal grazer in their streams was

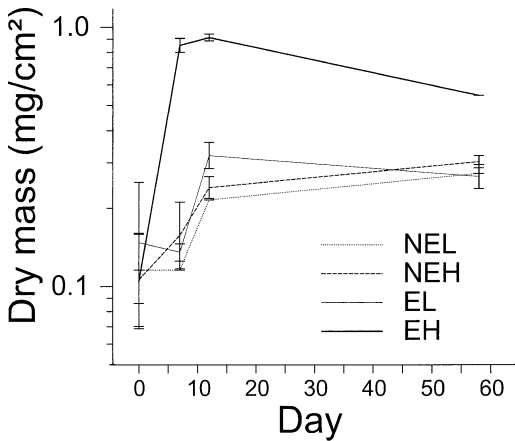


FIG. 6. Mean (± 1 SE) periphyton dry mass in electrified and not-electrified treatments in experiment 2. $n = 2$. EH = high-intensity electrification, EL = low-intensity electrification, NEH = not-electrified treatment associated with EH (high-intensity electrification control), NEL = not-electrified treatment associated with EL (low-intensity electrification control).

Baetis bicaudatus, which occurred in densities between 300 and 1000/m².

We observed that the digestive tracts of individuals of all 4 species of baetid ephemeropterans contained amorphous organic and inorganic material and algae (FAMK, unpublished data).

They appeared to ingest the benthic material nonselectively and possibly wasted much of the material by dislodging it into the current (Scrimgeour et al. 1991, FAMK, unpublished data).

Effect of shrimps on ephemeropterans

Our observations suggest that *Macrobrachium* had a negative effect on ephemeropterans. During the day in experiment 1, *Macrobrachium* were rarely seen in the exposed area where the experiments were carried out, whereas ephemeropterans were plentiful (Fig. 4) (Koblitz 2003). At sunset, the ephemeropterans disappeared, apparently traveling distances of several meters to find refuge. They appeared agile and able to evade *Macrobrachium*, but presumably underwent this movement to avoid predation by *Macrobrachium* at night. Avoidance behaviors of ephemeropterans are well researched (e.g., Peckarsky and McIntosh 1998, Muotka et al. 1999); our case is unusual in that the supposed predator is nocturnal, and the ephemeropterans are active during the day. Ephemeropterans were much less dense at night when *Macrobrachium* was active, and this effect was stronger in quadrat NE1 which had high activity of *Macrobrachium* (Fig. 4C). Ephemeropterans increased at

TABLE 2. Experiment 2: Analysis of variance (ANOVA) of differences in periphyton dry mass among electrification treatments (see text for details of analyses). Significant differences among means ($p < 0.05$) were identified using Tukey's Multiple Range Test; treatments with the same superscript were not significantly different. Probabilities for days 7, 12, and 58 were corrected for repeated tests using the Bonferroni adjustment. EH = high-intensity electrification, NEH = not-electrified high-intensity control, EL = low-intensity electrification, NEL = not-electrified low-intensity control. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Day	2-way ANOVA		1-way ANOVA
	High-intensity treatment vs control	Low-intensity treatment vs control	Among all treatments
0	$F_{1,2} = 0.002$	$F_{1,2} = 0.23$	$F_{3,4} = 0.57$
7	$F_{1,2} = 105^{**}$ EH > NEH	$F_{1,2} = 0.18$	$F_{3,4} = 29.7^{**}$ EH ^a EL ^b NEH ^b NEL ^b
12	$F_{1,2} = 584^{**}$ EH > NEH	$F_{1,2} = 2.66$	$F_{3,4} = 58.0^{***}$ EH ^a EL ^b NEH ^b NEL ^b
58	$F_{1,2} = 380^{**}$ EH > NEH	$F_{1,1} = 0.075$	$F_{3,3} = 74.9^{**}$ EH ^a EL ^b NEH ^b NEL ^b
7-158	Treatment: $F_{1,2} = 218^{**}$ EH > NEH	Treatment: $F_{1,1} = 3.40$	Treatment: $F_{3,3} = 115^{***}$
Repeated measures	Day: $F_{2,4} = 11.2^*$ Treatment \times day: $F_{2,4} = 57^{***}$	Day: $F_{2,2} = 1920^{***}$ Treatment \times day: $F_{2,2} = 113^{**}$	Day: $F_{2,6} = 125^{***}$ Treatment \times day: $F_{2,6} = 48^{***}$

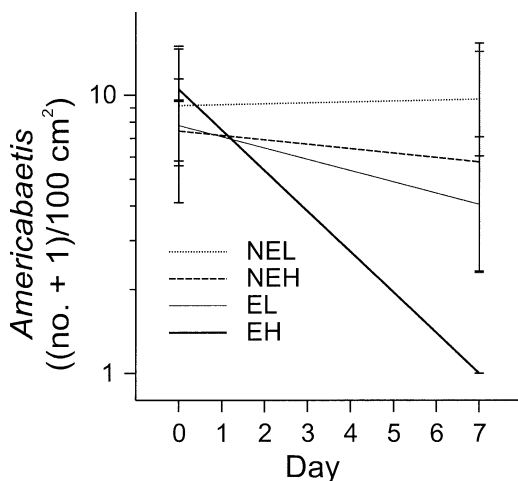


FIG. 7. Mean density (± 1 SE) of *Americabaetis* sp. collected by Surber sampler in electrified and not-electrified treatments during experiment 2. $n = 2$. Abbreviations are as in Fig. 6.

night by $\sim 40\times$ in quadrats EL, NE2, and NE3, where *Macrobrachium* activity decreased. We think that NE1 maintained relatively high density of shrimps because of the configuration of the quadrats with respect to the flow of water (Fig. 2A). Water arrived at the EL quadrat at an oblique angle, such that NE1 was downstream of the electrification. Shrimps that approached EL from downstream reacted to the electric shock and were swept back downstream towards NE1 and the area surrounding it. On the other hand, shrimps approaching EL and EH from the upstream reacted to the electric shock and often were swept downstream on the NE1 side. Thus, the area surrounding NE2 and NE3 appeared to become depleted of shrimps.

In experiment 2, we did not observe the inhibition of ephemeropterans by shrimps. The treatment in which shrimps were excluded, EL, was not significantly different from the controls in terms of periphyton dry mass (Fig. 6) and ephemeropteran density (Fig. 7). However, we had observed that exclusion of shrimps led to increased ephemeropteran density with a concomitant reduction in periphyton mass and chlorophyll *a* in a previous experiment in the same area of bedrock when the electrified area was longer and broader (Silveira and Moulton 2000, Silveira 2002). Thus, ephemeropterans may not be released from the inhibitory effects

of the shrimps when shrimps are excluded from relatively small areas, as in experiment 2.

Effects of shrimps on periphyton

Shrimps by themselves apparently do not remove periphyton and sediments in our system. Periphyton did not increase in the shrimp-only (EL) exclusion treatment in either experiment. Ephemeropterans could not be excluded without also excluding shrimps, so our experimental design could not distinguish between 2 possible actions of shrimps on periphyton: 1) shrimps had a negative effect on periphyton, but the effect of ephemeropteran grazing was much stronger and masked it, or 2) shrimps and ephemeropterans interacted synergistically such that their combined absence from EH treatments allowed periphyton to increase.

Our results were unexpected. Shrimps remove periphyton and sediments in Puerto Rico (Pringle and Blake 1994, Pringle 1996, Pringle et al. 1999, March et al. 2002) and Costa Rica (Pringle and Hamazaki 1998). Atyid shrimps are thought to be more active in this process than palaemonids. *Macrobrachium* has pincer-type chelae, which are used for picking up food; *Potimirim*, in common with most species of Atyidae, has chelae with long hairs, which it uses for sweeping the substrate and filtering fine particles. Thus, atyids appear to be more adapted to cleaning the substrate than palaemonids, and we might conclude that the observed lack of strong interaction of shrimps with periphyton in our system could have been caused by the predominance of *Macrobrachium* over *Potimirim* at our site. Evidence contrary to this line of reasoning comes from March et al. (2002) who found significant ($\sim 6\times$) accumulation of sediments on artificial tiles protected from *Macrobrachium* and *Xiphocaris* at midaltitude sites without atyids. The densities of *Macrobrachium* ($1.31/\text{m}^2$) and *Xiphocaris* ($2.09/\text{m}^2$) in their study were much lower than the densities of *Macrobrachium* ($17/\text{m}^2$) and *Potimirim* ($3.7/\text{m}^2$) in our study; however, the sizes of the animals in their study were similar to the sizes of the animals in our study.

The fact that *Potimirim* was not recorded in the quadrats of experiment 1 after day 1 may indicate that the presence of the experiment caused it to move away from the area. This unexplained absence was a potential artifact of the

experiment, but it cannot explain the observed result. If *Potimirim* were responsible for removing periphyton at the site, its absence should have provoked increased periphyton in control and EL quadrats, whereas we observed no change or a decrease.

Our experiments took place at shallower depths and faster water velocity than those of March et al. (2002) and Pringle and Hamazaki (1998). Baetid ephemeropterans are common in these conditions. *Potimirim* is more common in deeper sites with more substrate heterogeneity. Souza (2002) used low-intensity electricity to exclude shrimps from artificial substrates in a deeper site upstream from the present experiment. He found a 4× increase in periphyton AFDM and inorganic material in the absence of shrimps. Chlorophyll *a* was not significantly altered by the electrification, although diatoms were more abundant in the absence of shrimps. Visoni and Moulton (2003) used cages to exclude shrimps from natural cobbles at 2 sites in 2 streams, including a deeper place on the same area of bedrock as the present study. They observed a significant increase in dry mass and no difference in chlorophyll *a* on the cobbles in the absence of shrimps. On the other hand, chlorophyll *a* was reduced on mesh substrates in the absence of shrimps in the experiment of Visoni and Moulton (2003) and in a similar cage exclusion of Siviero and Moulton (1998). These results implied either the action of smaller grazers that passed through the mesh of the cages (e.g., ephemeropterans) or an inhibitory effect of the sediments that built up in the absence of shrimps.

Generality of our results

We have shown that baetid ephemeropterans exerted a strong negative effect on benthic material at a particular site in one stream. The particular site was unusual in its large area of shallow, homogeneous rock, but other smaller areas of shallow rock are common in streams of the region. Such areas appear similar to the study site in that they often have abundant baetid ephemeropterans and sparse periphyton and sediments.

Our results imply that shrimps do not always act as large-sized omnivores (sensu Pringle and Hamazaki 1998), which mask the effect of smaller herbivore-grazers in other Neotropical

streams (Pringle and Blake 1994, Pringle 1996, Pringle et al. 1999, March et al. 2002). At our site, *Macrobrachium* appeared to act as a potential predator of the abundant and active ephemeropteran grazers (Silveira and Moulton 2000, Silveira 2002, Koblitz 2003). *Macrobrachium* and the less-abundant *Potimirim* apparently do not exert strong grazing or bioturbation effects at the site, although *Potimirim* had strong negative effects on periphyton in a deeper, more physically heterogeneous site upstream (Souza 2002). Mathematical modeling of the community matrix (Silveira and Moulton 2000, Silveira 2002) showed that the potential trophic cascade among shrimps, ephemeropterans, and periphyton was critically dependant on the interaction strength between shrimps and periphyton. If the interaction strength exceeded a critical value, the trophic cascade was destroyed and the system changed to one composed of large-sized omnivores without intermediate grazers (Silveira and Moulton 2000, Silveira 2002). It is possible that the species of shrimp at our study site, *Macrobrachium olfersi*, is inherently more predaceous and less omnivorous than the species studied in Puerto Rico and Costa Rica. However, we think it more likely that the particular site conditions of medium to fast flow, shallow depth, and homogeneous substrate determined the interactions. We suggest that site-dependant changes in community interactions may be common in Neotropical coastal streams, as was seen for sites at different altitudes by March et al. (2002) and for different substrates in a temperate stream by Power (1992).

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