Effects of shrimp on periphyton and sediments in Atlantic forest streams: an exclusion experiment.

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ABSTRACT: Effects of shrimp on periphyton and sediments in Atlantic forest streams: an exclusion experiment. **Recent studies have shown various cases in which shrimp and fish detritivores strongly influence the benthic community of Neotropical streams. We studied the effects of atyid (*Potimirim glabra*) and palaemonid (*Macrobrachium olfersi*) shrimp in two fishless third-order forest streams at Ilha Grande, RJ, Brazil. We used cages to exclude shrimp from stones and mesh substrate and compared the deposition of sediments and growth of algae (chlorophyll-a) with substrates in open cages and without cages. After 6 and 24 days, stones protected from shrimp inside closed cages had more sediment than those in open cages and without cages. Chlorophyll-a on stones did not significantly vary with experimental treatment, but mesh substrates developed less chlorophyll-a in the absence of shrimps than in the other treatments. We hypothesize that either increased sediments inhibit periphyton growth or that, in the absence of shrimp, ephemeropteran grazers are more active. We conclude that atyid shrimps significantly remove sediments in the pools and slow-current sites.**
Key-words: **ecosystem engineering, strong interactors, sediments, periphyton, benthic community, stream ecology.**

RESUMO: Efeitos de camarões sobre perifiton e sedimentos em córregos de Mata Atlântica: um experimento de exclusão. **Estudos recentes têm mostrado vários casos em que camarões e peixes detritívoros influenciam fortemente a comunidade bentônica de córregos neotropicais. Estudamos os efeitos de camarões das famílias Atyidae (*Potimirim glabra*) e Palaemonidae (*Macrobrachium olfersi*) em dois córregos de terceira ordem, sem peixes, na Ilha Grande, RJ, Brasil. Usamos gaiolas para excluir camarões de substratos de pedras e telas, e comparamos a deposição de sedimentos e o crescimento de algas (clorofila-a) com substratos em gaiolas abertas e sem gaiolas. Após 6 e 24 dias, pedras protegidas de camarões dentro de gaiolas fechadas tinham mais sedimento comparado com as dentro de gaiolas abertas e sem gaiolas. A clorofila-a sobre pedras não variou significativamente entre os tratamentos, mas substratos de tela desenvolveram menos clorofila na ausência de camarões comparado com os outros tratamentos. Levantamos a hipótese de que ou a maior quantidade de sedimento inibiu o crescimento de perifiton, ou na ausência de camarões, os efemerópteros herbívoros são mais ativos. Concluímos que camarões atiídeos removem significativamente sedimentos nos remansos e lugares de baixa correnteza.**
Palavras-chave: **engenharia de ecossistema, sedimentos, perifiton, comunidade bentônica, camarão atiídeo, ecologia de córrego.**

Introduction

In recent years, various studies have shown large effects of omnivorous fish and shrimp in altering the sediments and benthic community in Neotropical rivers and streams. Flecker (1996, 1997) used an exclusion experiment to show that the detritivoral fish *Prochilodus mariae* significantly "cleaned" the substrate of a Venezuelan piedmont river, provoking a substantial change to the benthic algal community. Pringle and colleagues have shown that atyid shrimp (Pringle, 1996; Pringle & Blake, 1994; Pringle et al., 1993) in Puerto Rico and shrimp and fish in Costa Rica

Pringle & Hamazaki, 1998) remove sediments and benthic algae. The phenomena have been cited as examples of "ecosystem engineering" (Flecker, 1996) and the organisms called "strong interactors" (Pringle & Hamazaki, 1998). Power (1997) discusses the phenomena and the use of these terms.

Coastal streams of south-eastern Brazil often have abundant shrimps of the families Atyidae and Palaemonidae (Moulton, 1998; Moulton & Parslow, 1994; Silveira & Moulton, 1998), and we can expect that they might act similarly to their counterparts in Central American coastal streams. We have begun exclusion experiments to test this. Siviero & Moulton (1998) used cages to exclude shrimp and observed a reduction in the quantity of algae (evidenced by chlorophyll-a) on artificial substrates protected from shrimp, which was the opposite trend to that encountered by Pringle (Pringle, 1996; Pringle & Blake, 1994). Further experiments using exclusion by electricity revealed an important role of baetid ephemeropteran nymphs as strong interactors with benthic sediments and periphyton (Silveira & Moulton, 2000; Silveira, 2002). Furthermore, there appeared to be a "trophic cascade" effect of palaemonid shrimp (*Macrobrachium olfersi*) inhibiting the ephemeropterans (Silveira & Moulton, 2000; Silveira, 2002).

Modern experimental ecology often uses exclusion (a type of "press perturbation") to elucidate the interactions of communities. Such perturbation always represents an artifact, and the method and design of the experiment must reduce the collateral effects to the minimum and control for them. The technique of electrical exclusion has the advantage of minimizing the changes to flow and sedimentation regime while excluding the desired organisms (Pringle & Blake, 1994), unlike cages which suffer by altering the flow regime inside the cage and being subject to destruction in high flow events. The electrical exclusion technique works successfully in situations in which we have tried it in streams of Rio de Janeiro (Silveira & Moulton, 2000; Silveira, 2002). However, the experimental design is restricted to the number of electrifying devices and the situations in which they can be used. In the present experiment, we chose exclusion by cages in order to investigate widely separated sites and localities in which electrical apparatus might have suffered vandalism. The electrification device, battery and solar panel of the electrification technique are more obvious and attractive than underwater cages; our sites on Rio Barra Pequena were particularly vulnerable because of a road close by.

In the study of benthic communities and processes, many researchers choose artificial substrates in order to reduce the heterogeneity which is always found in natural substrates. This creates questions of artifacts and naturalness. In this experiment we chose to work with natural substrates of stones selected from the stream bed at the sites of the experiment and we supplemented these with observations of an artificial substrate – pieces of nylon mesh.

Our objective in this study was to test whether shrimp affected the quantity of sediments and periphyton on stones and nylon mesh substrate in pools at four sites in two streams.

Materials and methods

Study site

We studied two streams at Ilha Grande, Município de Angra dos Reis, RJ: Rio Barra Pequena and Rio Andorinha are third order streams which flow to the sea at either end of Vila Dois Rios, where the research centre CEADS is situated. We chose four sampling points in each stream (Tab. 1). The points were associated in pairs, approximately 5 m apart and each pair separated by ca. 500 m. Rio Barra Pequena has abundant atyid (*Potimirim glabra*) and palaemonid (*Macrobrachium olfersi*) shrimps but only low density of one species of fish (*Characidium japuhybensis*). In Rio Andorinha, we chose sites above the large waterfall which forms a barrier to all fish species apart from *Characidium*; this stream also has high densities of shrimp. The

sampling points were situated in pools with slow but not zero current; although current was undetectable by a current meter at the position of some of the cages and substrates (Table 1), there was always visible water movement above the positions. We measured water current using a current meter (Teledyne-Gurley, "Pygmy" model, Troy, NY, U.S.A.) and depth at the position of each cage and substrate, and recorded substrate characteristics of the site (Table 1).

Table I: Characteristics of the points at which replicates of the experiment were located

	Rio Barra Pequena				Rio Andorinha			
Site	Bridge		Upstream		Mãe D'água		Upstream	
Point	1	2	3	4	5	6	7	8
Current, m/sec								
Closed cage	0.14	0	0.03	0	medium	slow	slow	slow
Open cage	0	0	0	0	medium	slow	slow	slow
Without cage, stone	0.03	0	0.07	0	medium	slow	slow	slow
Without cage, mesh	0.03	0	0.03	0	medium	slow	slow	slow
Depth, cm								
Closed cage	66	35	37	44	48	41	55	41
Open cage	50	49	35	31	66	55	59	67
Without cage, stone	43	30	33	40	58	53	36	56
Without cage, mesh	58	40	31	19	30	52	42	57
Substrate characteristics	rocks, litter and sand	rocks, litter and sand	rocks and sand	rocks, litter and sand	contin-uous bedrock	rocks and bedrock	rocks and sand	mainly rocks

Experimental procedure

The shrimps were excluded from cages 45 x 25 x 25 cm made from a wire frame and covered with material with a mesh size of ca. 3 mm. The smallest *Potimirim* could pass through this mesh, but the majority of the population were excluded. We controlled for cage effects using cages of the same material with an opening of 5 cm diameter at either end. The cages had a zipper for access. In the third treatment, the substrates were placed in a similar position to the other treatments but without a cage. At each point we collected stones and chose three stones, ca. 15 cm diameter and similar in appearance and apparent colonization by algae. These were allocated randomly one to each treatment. They were sampled for sediments and periphyton at the start of the experiment (day 0, 8/3/2001) and after 6 and 24 days. The sampling apparatus comprised a 10 mL plastic syringe with a brush made from a toothbrush fitted to the nozzle and a circular flange of rubber to contain the sample. The apparatus was applied to the stone underwater, scrubbing the stone surface with the brush and simultaneously sucking up the dislodged material. Each sample was of ca. 5 cm^2 of substrate area. Two or three such samples were taken from each stone at each sampling and the sampling positions were marked to prevent resampling the same position. On day 0, each sample was analysed; on days 6 and 24 the samples of each stone were pooled before analysis.

We used an indirect method for assessing sediments: we measured the turbidity of the sample and converted this to an estimate of the dry mass of material of the sample. In previous work we obtained a good fit to a linear relationship between turbidity and total dry mass of the sample as measured from a volume filtered and dried on glass fibre filter. We measured turbidity using the nepthalometric setting of a hand-held fluorometer (model "Aquafluor 8000", Turner Designs, Sunnyvale, California, USA). We measured the chlorophyll-a content of the sample directly in the hand-held fluorometer and also using spectrophotometry. For spectrophotometry, the sample was filtered onto a 25 mm Whattman GF/F glass-fibre filter paper, which was maintained frozen and sealed from light until extracted overnight in 1 mL 90% acetone for 24 hours in a freezer. The extract was read at 750 and 664 nm and the concentration of chlorophyll-a and pheopigments in the substrate ($\mu g/cm^2$) calculated

by the equation: 26.7*(0.7/1.7)*Abs[664-750]*x*V/(v*A), where Abs[664-750] was the corrected absorbance, x the volume of the extract in litres, V the volume of the original sample (L), v the volume of the sample filtered (L), and A the area of the sampling device in cm^2 (Hauer & Lamberti, 1996; Nusch, 1980). We did not acidify the extract to differentiate between chlorophyll-a and pheopigments.

We used squares of nylon mesh as substrate for algal colonization. They were 8 x 8 cm, 200 m mesh size, and three squares were suspended inside the cages or tethered to the stream bed depending on the treatment. They were set up on day 0 and sampled by removing one square on days 6 and 24. We took 48 cm^2 of the square for extraction of chlorophyll-a. The squares were stored in a refrigerator until extracted in 5 mL 80% ethanol overnight in a freezer. The extracts were read in a spectrophotometer as above, except that the chlorophyll-pheopigments concentration in the extract was calculated as: 29.6*(0.7/1.7)*Abs[664-750]*5/48 to allow for the change in solvent (Nusch, 1980).

We calibrated the hand-held fluorometer against the measurements of chlorophyll-pheopigments obtained by spectroscopy. For the measurement of in-situ chlorophyll-a, we included the turbidity of the sample as a possible covariate; that is, we regressed estimated chlorophyll-a per area against fluorescence and turbidity of the sample. Turbidity did not show a significant relationship, so we discarded it as a factor. We then regressed chlorophyll-a per area against fluorescence of the sample without a constant (intercept) and excluding outliers and data with large leverage to arrive at the definitive relationship. The conversion factor, 0.00201, was intrinsic to our fluorometer calibration and volume and area of the syringe device.

The relationship between fluorescence and chlorophyll-a content of the ethanol extract of the mesh substrate was similarly determined, except that turbidity of the extract was not present. We express the result per area of mesh.

We report the estimates of chlorophyll-a obtained with the fluorometer because the instrument appeared to be more sensitive at low chlorophyll-a concentrations, and at high chlorophyll-a concentrations the spectrophotometric method appeared to be non-linear. The qualitative conclusions we reach are not affected by this choice – the spectrophotometric determinations showed the same trends.

Statistical design

The experimental design was of two sample points nested within site and two sites nested within stream, with three treatments at each sample point. However, since we were not primarily interested in differences between sites or between streams, and since the sample points displayed as much variability within site as between sites, we treated the 8 sample points as blocks of a two-way anova of treatments x points. We analysed the three sampling days separately; repeated measures analysis was invalidated by the different pattern of results on different days. Data were transformed to logarithms to homogenize variance. The two-way anova without replicates was performed with the GLM module of SYSTAT 7 (SPSS Inc., USA).

Results

During the 24 days of the experiment, there were no large rainfall events and the stream conditions were relatively constant. Shortly after the 24-day sampling a large spate destroyed the experiment. On day 24 the closed cage of point 1 had been removed from the water, apparently as an act of vandalism or curiosity. None of the other cages showed signs of human disturbance.

The results of the sampling of the stones at the start of the experiment show variability of sub-samples taken from each stone, variability of the three stones (treatments) within sample point and differences between the stones chosen at different sampling points (Fig. 1). We did not try to select stones to be uniform between

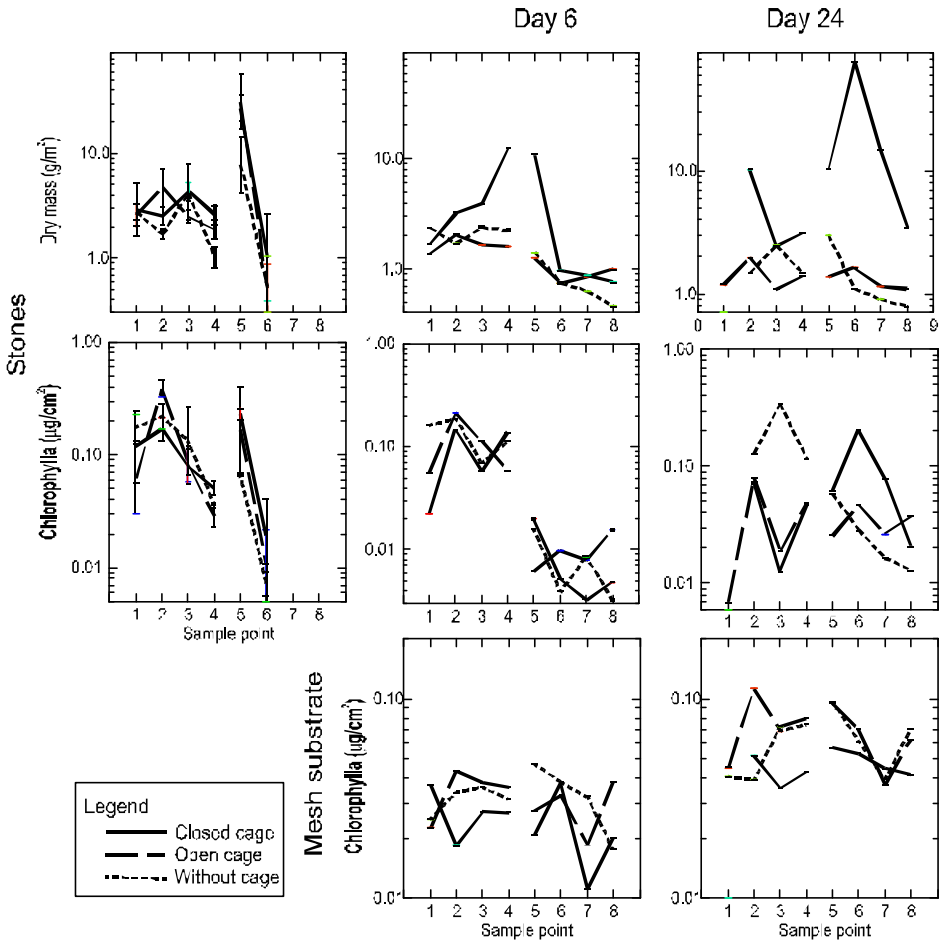

Figure 1: Mass of sediments and chlorophyll-a content on stones and chlorophyll-a content of mesh substrates of exclusion experiment. Sample points 1 to 4 are in Rio Barra Pequena; 5 to 8 are in Rio Andorinha. Points 7 and 8 were not sampled on day 0. Mesh substrate was uncolonized on day 0. The closed cage of point 1 was lost on day 24. Day 0 data display the standard error of the mean of 3 samples per stone; on subsequent days, subsamples were pooled before measurement (note the log scale of the ordinate).

points, but tried to match them within points. The three stones chosen at point 5 (at site "Mãe D'água", Rio Andorinha) contained more sediment than those at other sites, a fact that was obvious to us at the time of choosing. Chlorophyll-a also varied significantly between points.

After 6 and 24 days of experiment, the stones in the closed cages had significantly more sediment than those of the other treatments ($P<0.05$ on day 6; $P<0.001$ on day 24) (Fig. 1). The results were not uniform between points, nor consistent between sampling days, and we cannot relate the observed variability to any particular factor other than the natural variability of sediments over time and over sites. The results from open cages generally followed those from stones outside cages, which indicates no obvious effect of caging on sedimentation.

The chlorophyll-a sampled from stones on days 6 and 24 showed no association with experimental treatment.

The chlorophyll-a extracted from mesh substrates was significantly less (2-way anova, $P<0.01$) in the closed-cage treatment compared to the others on day 24. The trend was similar on day 6, but the result was not statistically significant. The

values for day 24 were significantly higher than those for day 6, indicating algal growth between samplings. Again, the results were variable between points. The substrates in the open-cage and without-cage treatments were not significantly different, indicating no cage effect.

Discussion

The experimental results imply a strong interaction of shrimps with sediments on rocky substrate in pools and slow-moving water in the two streams studied. When we excluded shrimp, sediments accumulated more compared to rocky substrate that was exposed to shrimps. It is unlikely that this difference was an artifact of the experimental method. We could expect there to have been different sedimentation inside the cages compared to outside, due to the altered water current produced by the mesh of the cages. Because the 5 cm openings of the open cages were only a small fraction of the total area of the mesh of the cages (<0.01%), we expected that the sedimentation environment inside both types of cages would be very similar. The results show that sediment on stones was more similar (and not significantly different) between stones in open cages and stones without cages compared to stones in closed cages. Thus the "cage effect" on sedimentation was apparently small and the effect of caged out shrimps was large.

We cannot attribute this phenomenon to either one or the other of the shrimp species, but suspect from the visible abundance of *Potimirim* on cages and substrate that this species rather than *Macrobrachium* was primarily responsible for the effect. *Potimirim*, as with other members of the family Atyidae, has modified chelae with abundant long setae which are used to sweep detritus towards its feeding appendages. *Macrobrachium* on the other hand has chelae adapted for picking up objects and does not sweep the substrate in the same way as *Potimirim*.

The result was different to those of electrical exclusion experiments conducted at the site Mãe D'água on Rio Andorinha (Silveira & Moulton, 2000; Silveira, 2002), where baetid ephemeropteran larvae have been shown to be important agents in the removal of sediments and periphyton, rather than shrimps. We suspect that the difference was due to the depth and current velocity of the electrical exclusion experiments, which were conducted at points which were more shallow (3 to 30 cm) and with faster current (0.2 – 0.4 m/s), in which conditions ephemeropterans were abundant and *Potimirim* rare. Points 5 and 6 of this study were at Mãe D'água, but in deeper locations with slow or undetectable current (Table 1).

The response of the periphyton, as evidenced by chlorophyll-a, was different to those reported for atyids (*Atya lanipes*) in Puerto Rico (Pringle & Blake, 1994) and Costa Rica (Pringle & Hamazaki, 1998), which tend to reduce the quantity of periphyton by their foraging activities (in previous observations, Pringle et al., 1993, reported enhancement of periphyton by atyids). We observed no significant difference on stones and significantly more chlorophyll-a attributable to the action of shrimp on mesh substrate (Fig. 1). This corroborated the earlier cage experiment (Siviero & Moulton, 1998) which also used mesh substrate, but not stones. We cannot distinguish between two hypotheses for this result: (i) sediments that accrue in the absence of shrimp inhibit periphyton (Biggs, 1996, cites cases in which periphyton is inhibited by sediments), (ii) ephemeropterans act more strongly as herbivores in the absence of shrimp, as seen in other experiments (Silveira & Moulton, 2000; Silveira, 2002). Ephemeropterans were abundant at all sites, however we could not observe ephemeropterans inside the cages and, because they are quite mobile, they were impossible to sample on mesh substrate or within the cages (an advantage of the electrical exclusion technique is that it permits direct observation of the substrate). We offer no explanation of the difference in behavior of the two substrates, but note that the chlorophyll-a concentrations on stones were much more variable in time and space than those on the mesh substrates.

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