Nauplius
THE JOURNAL OF THE BRAZILIAN CRUSTACEAN SOCIETY

e-ISSN 2358-2936
www.scielo.br/nau
www.crustacea.org.br

SHORT COMMUNICATION

Evaluating two sampling methodologies for shrimp density and biomass estimates in streams

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SUBMITTED 22 April 2020
ACCEPTED 30 July 2020
PUBLISHED 20 November 2020

DOI 10.1590/2358-2936e2020042

ABSTRACT

Freshwater shrimp can reach high biomasses, affecting ecosystem processes. It is important to define the most accurate methodology to estimate their densities and biomass. We studied two species of different sizes, *Potimirim brasiliana* Villalobos F., 1960 and *Macrobrachium olfersii* (Wiegmann, 1836), in three sites of a coastal stream in Ilha Grande, Rio de Janeiro, Brazil. We evaluated the efficiency of two infrequently used sampling methods: a depletion method using electrofishing, and a substrate method using benthic samplers. The methodologies provided different estimates, especially for *M. olfersii,* of density (electrofishing 1.27 ± 6.3 ind/m^2; substrate sampling 0.1 ± 5.05 ind/m^2) and biomass (electrofishing 1.66 ± 8.5 g/m^2; substrate sampling 0.11 ± 1.16 g/m^2). The median size of *M. olfersii* was higher for electrofishing, while the median size of *P. brasiliana* was higher for substrate sampling. Electrofishing is good at collecting bigger individuals that are not possible to catch with the substrate method and it samples a bigger area that includes many different microhabitats. The substrate method is cost-effective, especially for *P. brasiliana*, but by sampling a small area, its estimates can be highly affected by local heterogeneity. The substrate method is not recommended for *M. olfersii*, as it underestimated both its density and biomass.

Keywords

Electrofishing, freshwater shrimp, *Macrobrachium olfersii, Potimirim brasiliana,* substrate sampling

Species with high biomasses often play important roles as ecosystems engineers (Jones *et al.*, 1996). These species have a major impact, like promoting environmental heterogeneity (Flecker and Taylor, 2004; Spooner *et al.*, 2013) and affecting ecosystem processes such as leaf decomposition (Moulton, 2006), algal accrual (Moulton *et al.*, 2010a), and nutrient recycling (Atkinson *et al.*, 2013). In streams, shrimp can reach very high density and can control several ecosystem functions and potentially act as ecosystems engineers (Flecker, 1996). These organisms can influence organic and inorganic sediment accumulation on substrates (Pringle and Blake, 1994), allochthonous material processing (March *et al.*, 2001; Crowl *et al.*, 2006; Moulton *et al.*, 2010b), and periphyton dynamics (Moulton *et al.*, 2010a; Lourenço-Amorim *et al.*, 2014).

Shrimps occupy several types of substrate in freshwater ecosystems and during the day they can hide under stones, rocks, leaf-litter and others sediments (Pringle and Hamazaki, 1998). Several shrimp species are found in southeastern Brazil streams, among them, *Macrobrachium olfersii* (Wiegmann, 1836) (Caridae: Palaemonidae) and *Potimirim brasiliana* Villalobos F., 1960 (Caridae: Atyidae) (Moulton *et al.*, 2004). They can reach very high densities (Moulton and Parslow, 1994) and they require brackish water for their larval development (Ammar *et al.*, 2001; Da Rocha *et al.*, 2013). These two species have different life habits occupying different substrate types and distinct positions in the food web (Moulton *et al.*, 2004; Neres-Lima *et al.*, 2016). *Macrobrachium olfersii* hides under different kinds of substrates during the day, and feeds at night (Moulton *et al.*, 2004). *Potimirim brasiliana* has diurnal habits and can be found in every substrate in the stream (De Souza and Moulton, 2005). This species has a direct influence on the substrate by removing sediments through ingestion and bioturbation (De Souza and Moulton, 2005), and indirectly by participating in a trophic cascade (Moulton *et al.*, 2004). For a better understanding of the ecosystem relevance and population dynamics of these species, it is necessary to have precise estimates of their densities and biomass.

Several methods can be used for shrimp sampling, the most common being baited traps, visual census (*e.g.*, snorkeling and cameras), and hand netting (Ng, 2017). Baited traps can be very effective depending on the context and goals, for instance for long-term monitoring (Covich *et al.*, 1991; Hein *et al.*, 2011). Although widely used (Covich *et al.*, 1991; Rachman and Husin, 2014), they can present some problems if used for density and biomass estimates. For instance, baited traps are difficult to use in fast flowing parts of the streams, they can be conditional on the shrimp behavior and their food preference (*e.g.,* the bait might not be optimal) and they work better for bigger sized individuals (Osawa *et al.*, 2015). Baited traps work well to catch *Macrobrachium* Spence Bate, 1868 species that are difficult to capture with hand nets because they often hide in refuges under rocks (Hein *et al.*, 2011). However, they have not proven efficient for some smaller species, such as *Potimirim* Holthuis, 1954 species, that are not attracted to the bait (T.P. Moulton, pers. comm., but see Hein *et al.*, 2011 for the use of baited traps for small species such as *Xiphocaris elongata* (Guérin-Méneville, 1855)). Small individuals can also be killed by bigger ones, unless traps with parts that segregate shrimp by size are used (*e.g.*, Taiwanese traps; Ng, 2017). Small individuals are better caught with "refuge traps" (Osawa *et al.*, 2015) and they might work for *P. brasiliana*. For quantitative estimates one needs a high deployment of traps and it is rather time consuming when used to calculate density with a depletion method. However, baited traps can be very effective for tracking seasonal and inter-annual change in relative abundance (Hein *et al.*, 2011). Visual censuses, from inside and outside the water, also have several limitations, as they are only possible when the water is clear (Ng, 2017) and can only be deployed in certain substrates, such as rocks and sand (Moulton *et al.*, 2004), but not in riffles or in leaf packs, where shrimp are very active and can hide under the leaf layers. In particular, the census method is not efficient for *Macrobrachium* species, because the bigger individuals are often hiding during the day. However, observations during the night with the use of flashlights were more efficient to count shrimp, including *Macrobrachium* species, than during the day, because shrimp eyes reflect the light (Johnson and Covich, 2000; Ng, 2017).

Methodologies used to catch benthic macroinvertebrates (*e.g.*, Hess and Surber sampler) are not commonly deployed for shrimp density estimates. Benthic samplers are easy to use, inexpensive and sampling can be performed on different types of substrate (rocks, sand, and leaf-litter). However, benthic samplers also have some limitations, since shrimp can easily escape before the sampling procedure and they do not catch hidden individuals. Additionally, benthic samplers are restricted to depths of approximately 60 cm and they require a large number of samples to represent all types of substrates and to minimize the variance in density estimates due to the patchy distribution of animals (Lancaster *et al.*, 1991; Pringle and Ramirez, 1998).

Electrofishing is not commonly deployed for sampling shrimp (Fièvet *et al.*, 1996; 1999; Rabeni *et al.*, 1997; King and Crook, 2002), but its use can be very promising. Electrofishing generates different voltages that can capture different sizes of animals: the stronger the voltage, the smaller the animals caught. One of its advantages is sampling effectiveness, due to the large number of individuals affected by the electric charge and because it allows the sampling of hidden organisms and from different microhabitats, including those difficult to reach. On the other hand, it is directly dependent on the visibility of the stunned individuals, as well as on water velocity and shrimp size (Fièvet *et al.*, 1999). For density estimates and mark-recapture studies, electrofishing shows more accurate results than baited traps (Perry and Acosta, 2000) or hand netting (Rabeni *et al.*, 1997), as it allows sampling of a defined area.

The main objective of this study is to evaluate the efficiency of two methodologies in collecting freshwater shrimp: by depletion using electrofishing and by substrate, using benthic samplers. We expect electrofishing to be a better methodology in estimating shrimp density and biomass, because of its capacity to: 1) sample several types of substrate; 2) catch individuals hidden in refugia; and 3) catch individuals of many different sizes, including the bigger ones, which are those that more greatly affect biomass estimates. We also expect benthic samplers to be better at sampling small individuals that might not be as sensitive to electrical charge.

The present study was carried out in the Barra Pequena stream, a third order stream in Ilha Grande State Park, Rio de Janeiro, Brazil (23°04' 23°14'S, 44°05' 44°23'W). It is located in an area covered by a dense and well-preserved rainforest (Moulton *et al.*, 2010a). The Barra Pequena riverbed is located in a steep region with a drainage basin that occupies approximately 566 ha (Aguiar *et al.*, 2018). The annual mean temperature is 23.2 °C and the precipitation is 2,071 mm (data collected from the nearest meteorological station between December/2015 and November/2016 in the municipality of Angra dos Reis).

The data were collected in November 2018 during the day. Three sites were selected in the river, observing as reference the distance from the mouth of Barra Pequena stream. The first sampled site (close) was located at 300 meters distance from the mouth, the second site (intermediate) was approximately 410 meters from the mouth and the third site (distant) was 1,210 meters from the mouth.

In order to compare methodologies, samples were taken by substrate defined area and by electrofishing of *P. brasiliana* and *M. olfersii* species. For substrate sampling, three samples per substrate type were taken in different areas of each site, in order to estimate the density of each reach. For the sampling by electrofishing, an area was selected at each site to be representative of the stream reach. The sampling areas were close to each other, but they were not overlapping.

Substrate sampling was done on leaf litter, sand, and rock. Leaf litter associated shrimp were sampled using a "stovepipe sampler", which consisted of a steel cylinder (60 cm high and 20 cm in diameter: 0.0314 m^2). The Surber sampler (0.09 m^2) was used to sample the shrimp on rocks and sand. All the collected animals were stored in plastic bags containing water from the collection site and taken to the laboratory for counting, identification and measurement. Subsequently, the shrimps were returned to the stream.

In each of the three sampled sites, electrofishing was performed using the same equipment and voltage (600V, Smith Root, LR-24 Backpack Electrofisher). Each sampled area included different types of substrates and was delimited by nets to prevent the entrance and exit of organisms. The sampled area in the "close" site was 22.726 m^2, in the "intermediate" site was 16.267 m^2 and in the "distant" site was 37.612 m^2. Four removals of 15 minutes each were performed in each of the three areas sampled, with the same sampling effort (the same three researchers). The collected individuals were housed in a container with stream water, counted and measured in the field. We randomly selected fifty specimens of *P. brasiliana* and all individuals of *M. olfersii* for length measurements (total length – from the ocular orbit to the distal end of the telson) (Lima *et al.*, 2006). After the procedure, the shrimps were returned to the river. Population densities were estimated using the catch-per unit effort method (Krebs, 1989).

Biomass was estimated from the length of 316 individuals of *P. brasiliana* and 132 *M. olfersii* using an exponential mass-length equation: $Y=a^*X^b$, where Y=weight (g) and X=Length (mm). The weight of all the shrimps was used to estimate the biomass, which was then divided by the area sampled to obtain the shrimp biomass per area.

To make the two methodologies comparable, a density estimate was performed for the substrate sampling. The density estimate by substrate was related to the proportion of substrates (sand, rock, and leaf litter) present in the area where the electrofishing was carried out. In this way, it was possible to estimate the density of the area assigned for the electrofishing according to the sampling by the substrate. We reported descriptive statistics for the two methodologies and for the two species as median density and median biomass.

A paired t-Student test ($p<0.05$) was performed to test the difference in average size captured between methodologies for each species separately. The statistical analysis was performed in R version 3.3.3 software.

In total, we collected 2,738 individuals of *P. brasiliana* and 121 individuals of *M. olfersii* with electrofishing, and 198 individuals of *P. brasiliana* and 11 *M. olfersii* with substrate sampling. The estimated median density across sites using electrofishing was 1.27 ± 6.3 *M. olfersii*/m^2 and 29.8 ± 41.5 *P. brasiliana*/m^2. In the substrate sampling, 0.1 ± 5.05 *M. olfersii*/m^2 and 19.99 ± 23.8 *P. brasiliana*/m^2 were estimated (Fig. 1). The estimated median biomass using electrofishing was 0.62 ± 0.84 g/m^2 of *P. brasiliana* and 1.66 ± 8.5 g/m^2 of *M. olfersii*. In the substrate sampling, the estimated median ± standard deviation biomass was 0.42 ± 0.53 g/m^2 of *P. brasiliana* and 0.11 ± 1.16 g/m^2 of *M. olfersii*. There was a big variation in density and biomass values between the three sampled sites. The "intermediate" site showed the highest values of density and biomass for both species. The abundance of *M. olfersii* in the distant site was very low, while the abundances of *P. brasiliana* were similar between the "distant" and "close" site.

Collected individuals of *P. brasiliana* ranged from 4 to 21 mm in length, and *M. olfersii* ranged from 13 to 55 mm. The paired Student's t-test showed that the mean size of *P. brasiliana* differed significantly between substrate sampling (M = 14.28 mm, SE = 0.21) and sampling by electrofishing (M = 12.98 mm, SE = 0.30) ($t_{(247)} = 5.5$, $p < 0.0001$)) (Fig. 2). There was also a significant difference in the mean size (mm) of *M. olfersii* individuals between the substrate sampling (M = 18.3 mm, SE = 1.97) and the sampling by electrofishing (M = 31.8 mm, SE = 0.72) ($t_{(126)} = -3.7$, $p = 0.007$) (Fig. 2). Substrate sampling caught more individuals of *P. brasiliana* in the size classes between 16–18 mm, while electrofishing caught a higher number of individuals between the size class 10–12 mm and 13–15 mm (Fig. 2). Electrofishing caught a high number of individuals of *M. olfersii* in the size classes between 28–30 mm and 34–36 mm, while the substrate methodology mostly caught individuals in the smaller size class, between 13–15 mm (Fig. 2).

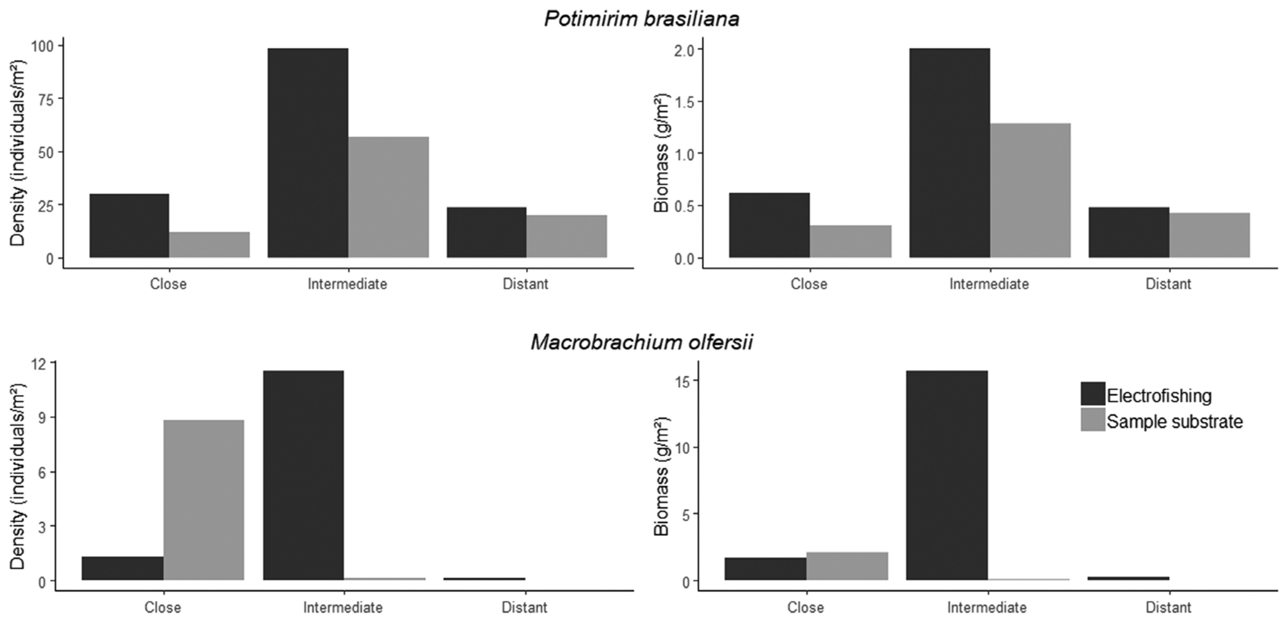

Figure 1. Density (individuals/m²) and biomass (g/m²) estimated by electrofishing (dark grey) and sample substrate (light grey) methodologies for *Potimirim brasiliana* (above) and *Macrobrachium olfersii* (below).

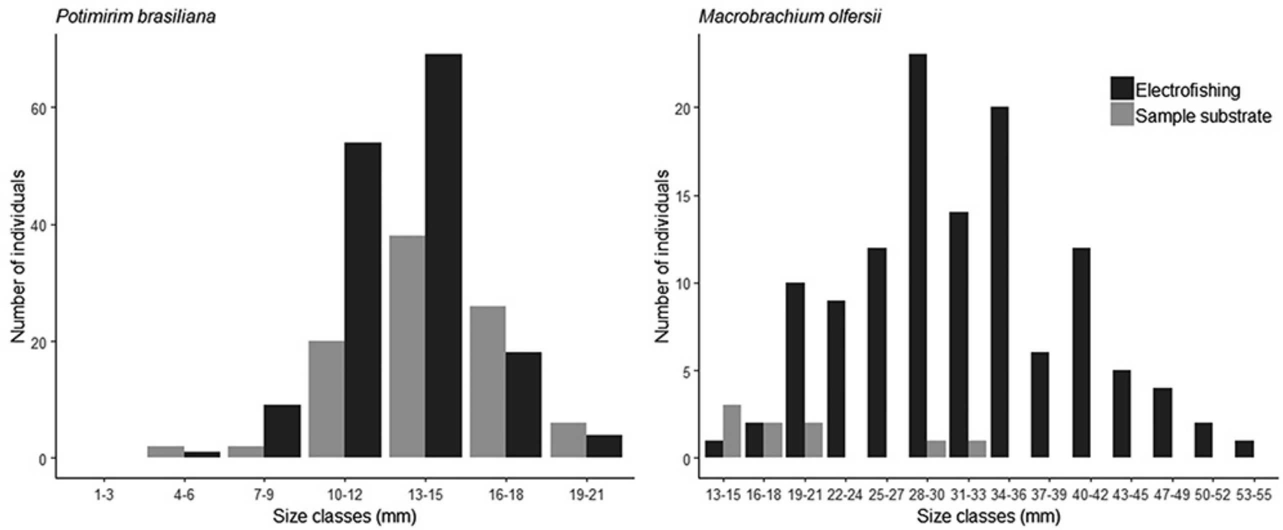

Figure 2. Size classes of *Potimirim brasiliana* (left) and *Macrobrachium olfersii* (right) in absolute number for electrofishing (dark grey) and sample substrate (light grey).

Electrofishing and substrate sampling with benthic samplers led to different results, mostly for the bigger shrimp species, *M. olfersii*. While benthic samplers caught relatively few and only small *M. olfersii*, electrofishing caught individuals of all size classes, thus characterizing more accurately the population size distribution, density and, especially, biomass of this species. Electrofishing also led to higher density and more accurate biomass estimates for *P. brasiliana*, but the differences between the methodologies were not as striking as for *M. olfersii*. In addition, *P. brasiliana* size distributions obtained with the two methodologies were similar.

Substrate sampling could underestimate density estimates due to the presence of the person sampling the organisms (Fièvet *et al.*, 1999) and, in particular, Surber samplers, being open, might allow shrimp to escape prior to collection (Taylor *et al.*, 2001).

Electrofishing instead stuns animals and, because they are in a confined area, makes them easier to catch (Mazzoni *et al.*, 2000). It is interesting to note that the two methodologies performed similarly for *P. brasiliana*, but electrofishing performed much better for *M. olfersii*. This could have been due to their different habits, *P. brasiliana* being very active during the day (Oliveira-Cunha *et al.*, 2018), while *M. olfersii* being mostly nocturnal (Moulton *et al.*, 2004). *Macrobrachium olfersii* often hide during the day and forage mostly at night, which might have hindered collection with the benthic samplers during the day. Electrofishing instead potentially allows the capture of those organisms hidden in shelters, which are not collected with the substrate methodology. However, we do not know how efficient electrofishing is in capturing these hidden animals and other methodologies, such as baited traps, could be just as effective. A comparison between these two methodologies would be useful to test their relative efficiencies.

Considering that *Macrobrachium* species are generally more active at night, electrofishing and substrate sampling should be tested both during the day and during the night. Collections performed at night might lead to a higher number of individuals collected, but darkness can hamper the visibility of animals stunned by electricity. It is possible that differences in density estimates conducted at night between the two methodologies are smaller, because *M. olfersii* are not hiding and can thus be collected with benthic samplers more easily. Other methods, such as visual census, could be just as effective during the night, as shrimp eyes are highly visible with flashlights (Johnson and Covich, 2000).

Potimirim brasiliana do not show a big variation in size, ranging approximately from 6 to 20 mm in length, while *M. olfersii* ranged approximately from 15 to 55 mm (Fig. 2). Due to the small variation in size in *P. brasiliana*, the patterns of density and biomass data were similar, which was not true for *M. olfersii* (Fig. 1). Previous studies have shown that electrofishing would tend to capture larger animals (Reynolds, 1996; Fièvet *et al.*, 1999; King and Crook, 2002) and would not be highly effective at capturing small individuals (Fièvet *et al.*, 1996). We were thus expecting electrofishing to collect bigger animals and the substrate sampling to be better at catching smaller ones. However, the two species showed opposite patterns, where electrofishing caught bigger individuals for *M. olfersii*, but smaller *P. brasiliana* when compared to benthic samplers. The average length of *P. brasiliana* was in fact higher for substrate sampling (14.28 mm) in comparison with electrofishing (12 mm). Larger animals have a better swimming ability, and therefore, can more easily escape from the electric current, making it more difficult to catch them. However, this is probably not the case for *P. brasiliana* and the bigger average size of individuals found with substrate sampling could have just been a spurious result due to the low number of individuals caught with this methodology. Benthic samplers sample a very small area thus being highly affected by habitat heterogeneity and the patchy distribution of animals. In this way a small number of replicates can bias the results.

Electrofishing performed particularly well for *M. olfersii*, collecting an order of magnitude more animals and a bigger size range. It was also effective at collecting small size shrimp, even if electrical charge is not considered effective for small sized individuals (Fièvet *et al.*, 1996). Rabeni *et al.* (1997) showed that electrofishing performed better by not selecting the size of the shrimp when compared with hand netting and quadrat sampler method, which captured the biggest and the smallest ones respectively (Rabeni *et al.*, 1997). Our study showed that benthic samplers were ineffective at collecting *M. olfersii* and were thus highly underestimating their densities and biomass.

Shrimp density and biomass show different patterns between the three sites (Fig. 1). The intermediate site showed higher density and biomass values for both species, while the close and distant sites had similar but lower values. The abundance distribution observed is expected for migratory shrimp species (Ammar *et al.*, 2001; Da Rocha *et al.*, 2013), as the site close to the river mouth might be mostly a passage zone, where shrimp pass through to get to more upstream sites, but do not establish. In the most distant site, shrimp density could be low simply because of the great distance they need to travel to get there, while in the middle is where most individuals establish.

Overall, electrofishing proved a very effective methodology to estimate shrimp density and biomass. However, there are some caveats and open questions.

For instance, there is no knowledge of the possible negative effects of electrical charge on shrimp, especially when the intensity of electric shocks needs to be high (*i.e.*, in low-conductivity streams). This is of concern especially when frequent sampling is required in long-term monitoring. Also, electrofishing backpacks are expensive to buy, heavy to carry and need more manpower to be employed. They are thus more time-consuming than substrate sampling, which requires less effort and is cheaper. Substrate sampling is more cost-efficient than electrofishing for sampling *P. brasiliana* and provides reasonable estimates of size distribution. Substrate sampling through benthic samplers can be useful for making comparisons between streams in terms of substrate preference, but it is not as good for density and biomass estimates. Also, it is necessary to get many replicates in order to get more reliable estimates, as they sample a very small area and they can thus be strongly affected by unrepresentative samples and habitat heterogeneity. The efficiency of multiple sampling methodologies needs to be compared in the same stream and in different streams to help distinguish which methods can be most useful for different types of short-term and long-term studies. For instance, baited traps are a cheap alternative widely used for long-term shrimp monitoring and, maybe, a less harmful way than electrofishing to monitor shrimp populations when monthly collection is needed (Harrison *et al.*, 1986; Covich *et al.*, 2003; Hein *et al.*, 2011). Their efficiency should be tested for density and biomass estimates compared to substrate sampling and electrofishing. We thus suggest performing more comparative studies for shrimp density estimates using different methodologies and choosing the methodology according to the variable of interest. These studies are necessary to guarantee precise density and biomass assessments of these taxa, which have essential roles in controlling ecosystem processes.

Acknowledgements

We thank the staff of Centro de Estudos Ambientais e Desenvolvimento Sustentável (CEADS) for their support and assistance at Ilha Grande. This research is the result of a class project developed during the field course "Tropical Stream Ecology" at UERJ. We would like to thank Jonatas de Souza Merced, Priscila de Oliveira Cunha, Ana Carolina Aguiar and Karoline E.F. Lacerda for their help in the field. We would like to thank Prof. Timothy Moulton for all the theoretical support during the class and the field. We also thank Dr. Alan Covich, Dr. Vinicius Neres de Lima and an anonymous reviewer for their comments and suggestions. The research was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Prociência - UERJ, the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and the Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ).

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