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Application of Rapid Bioassessment Protocols (RBP) for Benthic Macroinvertebrates in Brazil: Comparison between Sampling Techniques and Mesh Sizes

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Aplicação de Protocolos de Bioavaliação Rápida para Macroinvertebrados Bentônicos no Brasil: Comparação entre Métodos de Coleta e entre Malhas

RESUMO - Este estudo é parte do esforço para o estabelecimento de um Protocolo de Bioavaliação Rápida (PBR) utilizando macroinvertebrados bentônicos como bioindicadores da qualidade de água de riachos no Sudeste do Brasil. Foi analisada a relação custo/eficácia de procedimentos de coleta frequentemente utilizados em PBRs, amostradores do tipo Surber e *Kick-net*, e de três malhas, 125, 250 e 500 μm . Foram coletados e identificados 126.815 macroinvertebrados, representando 57 famílias. As amostras coletadas com *Kick-net* apresentaram número de taxa e de valores do índice BMWP significativamente maiores do que as coletadas com Surber, sem um aumento significativo no esforço amostral medido em tempo despendido para análise da amostra. Não houve diferenças significativas quanto à relação custo/eficácia entre os coletores. Quanto ao tamanho da malha, em 125 μm e 250 μm foram encontradas maiores abundância de macroinvertebrados e necessário mais tempo para processar as amostras, no entanto sem produzir diferenças significativas no número de taxa e em valores do índice BMWP. Assim, a malha de 500 μm foi considerada mais eficiente do que as malhas mais finas. Portanto, recomenda-se o uso de coletores do tipo *kick* usando uma malha de 500 μm , para PBRs ao nível taxonômico de família em rios de características similares às deste estudo no Brasil.

PALAVRAS-CHAVE: Avaliação ambiental, inseto aquático, ecologia de rios, biomonitoramento, bioindicador

ABSTRACT - This study is part of the effort to test and to establish Rapid Bioassessment Protocols (RBP) using benthic macroinvertebrates as indicators of the water quality of wadeable streams in south-east Brazil. We compared the cost-effectiveness of sampling devices frequently used in RBPs, Surber and Kick-net samplers, and of three mesh sizes (125, 250 and 500 μm). A total of 126,815 benthic macroinvertebrates were collected, representing 57 families. Samples collected with Kick method had significantly higher richness and BMWP scores in relation to Surber, but no significant increase in the effort, measured by the necessary time to process samples. No significant differences were found between samplers considering the cost/effectiveness ratio. Considering mesh sizes, significantly higher abundance and time for processing samples were necessary for finer meshes, but no significant difference were found considering taxa richness or BMWP scores. As a consequence, the 500 μm mesh had better cost/effectiveness ratios. Therefore, we support the use of a kick-net with a mesh size of 500 μm for macroinvertebrate sampling in RBPs using family level in streams of similar characteristics in Brazil.

KEY WORDS: Environmental assessment, aquatic insect, freshwater ecology, biomonitoring, bioindicator

The original Rapid Bioassessment Protocols (RBPs) were designed as inexpensive screening tools for determining if a stream is supporting or not supporting a designated aquatic life use. However, RBP tools can also be applied to other program areas, like characterizing the

existence and severity of impairment to the water resource; helping to identify sources and causes of impairment; evaluating the effectiveness of control actions and restoration activities; supporting use attainability studies and cumulative impact assessments and characterizing

regional biotic attributes of reference conditions (Barbour *et al.* 1999).

Given its cost-effective principle, RBPs have been widely used in many countries to assess biological water quality and ecological health of aquatic ecosystems (Plafkin *et al.* 1989, Chessman 1995, AQEM 2002).

Successful biological monitoring and assessment depend on rigorous quality control, starting from the design and execution of field studies to proper laboratory procedures and data analyses (Doberstein *et al.* 2000). Wadeable streams RBP methodologies intend to be efficient, effective, low in cost and easy to use (Resh & Jackson 1993, Resh *et al.* 1995), but significant differences exist between sampling techniques, forms of processing samples and metrics used.

Biologists choose study sites and plan data analyses based on study objectives. However, the steps in between the collection of samples, the separation of organisms from the substrate and the level of identification, are often a product of tradition or convenience. Decisions concerning the choice of sampling device, where to take samples, whether to subsample, and how to sort samples, may greatly influence study conclusions and subsequent management considerations (Carter & Resh 2001).

Many organisms have been tested in RBPs (Karr 1981, Barbour *et al.* 1999), and benthic macroinvertebrates are the most used group around the world (Rosenberg & Resh 1993, Chessman 1995). Therefore, it is a reasonable choice for use in the Neotropical region. However, the systematic development of RBP tools on river basins using benthic macroinvertebrates in Brazil is recent (Marques & Barbosa 2001, Buss *et al.* 2003, Maltchik & Callisto 2004), and very few studies dealt with testing methods in this region.

This study is part of the effort to test and standardize RBP methods using macroinvertebrates as bioindicators of water quality in streams and rivers. The aims of this study were to compare the cost-effectiveness of two sampling techniques frequently used in macroinvertebrate RBPs, Surber and Kick screen net, and the cost-effectiveness of three mesh sizes, 125, 250 and 500 μm . In order to propose the application of wadeable stream RBPs in Brazil, other studies are being conducted for testing subsampling methods, taxonomic sufficiency, and the development of multimetric indices to assess biological water quality (Buss 2001, Silveira *et al.* 2005, Baptista *et al.* 2007).

Material and Methods

Field and laboratory procedures. The study took place in the municipality of Guapimirim, Rio de Janeiro state, one of the best preserved Atlantic Forest areas in the state. This area was an ideal place for a rigorous testing of sampling methods because of high macroinvertebrate diversity and because ecological patterns and taxonomy are well known in this region (Buss *et al.* 2002, 2004).

Three streams were chosen for this study. The main objective was to make an intense effort to compare samples within each stream. Two stream sites (A and B) were classified as reference areas, with dense riparian vegetation (75% stream cover), more than 50% of the upstream area

forested, with no visible anthropogenic impacts, and excellent environmental integrity according to the "Habitat Assessment Field Data Sheet – High Gradient Streams" (Barbour *et al.* 1999). Both sites were located at the border of the Serra dos Órgãos National Park, one at the Soberbo River (site A; 22° 29' 52" S, 42° 59' 36" W) and other at the Bananal River (site B; 22° 30' 44" S, 43° 00' 17" W). The third site (C; 22° 32' 36" S, 42° 59' 00" W) was close to the urban area, where silt was common, with scarce riparian vegetation and marginal environmental integrity according to the "Habitat Assessment Field Data Sheet – High Gradient Streams" (Barbour *et al.* 1999). All sites were at 3rd order streams and at about the same altitude (between 40 and 100 m.a.s.l.).

Macroinvertebrates were collected two times during the dry season, in October 2001 and 2003. This period was chosen because it is the period of higher macroinvertebrate richness and diversity in this river basin (Buss *et al.* 2004). Two samplers were used: Kick screen net (1 m² net size, 250 μm mesh size, sampled area of approximately 1 m²) and Surber (fixed sampling area of 30 x 30 cm; 125 μm mesh size). All samples were collected and sorted by the same team of experts to avoid differences in procedures. Six samples in riffle areas were taken with each sampler at the three streams at each sampling period. Samples were preserved in 80% ethanol and packed for examination in the laboratory.

To compare methods, samples collected with Surber were sieved in a 250 μm mesh and these data were used in the comparison with samples collected with Kick-net. To compare mesh sizes, Surber samples were sieved in successive sieves of 500 μm , 250 μm and 125 μm meshes and data were recorded. All biological samples were fully examined under a stereoscopic microscope. Macroinvertebrates were identified mostly at family level using the available taxonomic keys.

Data analysis. For each sample, macroinvertebrate abundance, richness, BMWP score (modified by Alba-Tercedor & Sanchez-Ortega 1988), and the time necessary to process a sample (*i.e.* collect and sort the macroinvertebrates) were registered. The BMWP index is based on 1-10 scores for each family based on their sensitivity/tolerance to anthropogenic impacts (lower scores for more tolerant taxa), the water quality is based on the sum of scores of all families in a site and a table is used to determine the water quality of the site (Alba-Tercedor & Sanchez-Ortega 1988).

Since sampled areas used in Kick and Surber procedures were different, to compare taxa richness obtained by the two methods we used rarefaction to construct individual-based species accumulation curves. Rarefaction procedures have been largely discussed in the recent literature, and many authors agree that comparisons should be standardized by individuals rather than sampled area (McCabe & Gotelli 2000, Gotelli & Colwell 2001, Costa & Melo 2008). We followed these recommendations and calculated rarefied richness for samples based on the smallest sample (in number of individuals) found at each stream. Additionally, we calculated the ratio 'Time for processing sample/Richness', in order to represent a best estimate of the necessary effort (time required for processing a sample) to collect a new taxa (based on total richness of the same sample). By doing it, we excluded the effect of the different sampled areas and

we were able to compare the cost/effectiveness of the two sampling devices.

To compare samplers (Kick vs. Surber), t-tests for independent samples were performed for each stream, using rarefied richness and the ratio 'Time for processing sample/Richness'. To compare mesh sizes (500 µm vs. 250 µm; 500 µm vs. 125 µm; 250 µm vs. 125 µm), t-tests for dependent samples were performed, using abundance, richness, BMWP scores, time for processing sample and the ratio 'Time for processing sample/Richness'. Box-and-whisker plots were used to represent distributions graphically.

Results

Comparison between sampling devices. A total of 126,815 benthic macroinvertebrates, representing 57 taxa, were collected, sorted and identified in this study. Almost all taxa were collected by both samplers, with exception of a few rare taxa (≤ 2 individuals), exclusively found in Kick samples (Dryopidae, Tabanidae, Gyrinidae, Hydraenidae, and Collembola) or in Surber samples (Ptilodactilidae and Staphylinidae).

In general, Kick sampler reflected better the macroinvertebrate assemblage than Surber. Surber often underestimated macroinvertebrate richness (5-25% less families than in Kick). At the three streams, Kick samples had higher total and mean richness (Table 1). Attesting that richness numbers were not simply an artifact of sampling effort, rarefied richness, calculated as the number of species expected to be found in samples if they had the same number of individuals than the smallest sample at each stream (Stream A, 269 individuals; Stream B, 140 individuals; Stream C, 92 individuals) was also significantly higher in Kick than in Surber (Stream A, t-value = 3.18, P = 0.004;

Stream B, t-value = 3.27, P = 0.003; Stream C, t-value = 4.74, P = 0.0001).

To determine the cost-effectiveness of sampling methods, we analyzed taxa richness (a measure of "efficacy") and the time necessary to collect and sort biological samples (a measure of "cost"). The cost/effectiveness thus represents the necessary time (in minutes) to collect a "new" taxa. This ratio was not significantly different between Kick and Surber samples at all streams (all t-tests, P > 0.05), although Kick samples had lower values (i.e. best cost-effectiveness ratio), at site C (Fig. 1).

Differences in BMWP scores between Kick and Surber samplers were sufficient to result in different biological water quality classification at the three streams (Table 1). In most sites, classification based on BMWP scores of Kick and Surber samples varied one class, but in two times, differences were of two classes (stream A 2001, Kick: Class 1 – "very clean"; Surber: Class 3 – "some sign of contamination" and stream C 2001, Kick: Class 2 – "clean"; Surber: Class 4 – "contaminated"). Only in stream A (2003) both sampling methods assigned a site the same biological quality class. Thus, the assessment of water quality using the BMWP system was dependent on the sampling technique used in this study.

Comparison between mesh sizes. A total of 80,775 macroinvertebrates were retained in the 125 µm mesh, 65,581 in the 250 µm mesh, and 54,144 in the 500 µm mesh. Despite differences in abundance numbers, the 250 µm and the 500 µm meshes retained all 50 taxa that were collected with the 125 µm mesh.

In general, the finer mesh tended to retain smaller individuals of species already represented in coarser meshes and demanded more time to sort. By its turn, the coarser mesh demanded less time for sorting because of

Table 1. Total richness, mean richness, mean rarefied richness, mean ratio 'time for processing sample/richness', BMWP scores and water quality classification according to the BMWP system for Kick and Surber at each stream.

	Stream A		Stream B		Stream C	
	Kick	Surber	Kick	Surber	Kick	Surber
Total richness	39	37	40	33	40	30
Mean richness	22.4 (4.3)	16.8 (6.4)	22.3 (4.8)	17.2 (4.6)	16.7 (6.7)	12.4 (6.5)
Mean rarefied richness	17.2 (3.4)	12.8 (3.3)	14.9 (2.1)	11.6 (2.8)	8.6 (1.7)	4.7 (2.3)
Mean time (min.) for processing sample	240.9 (84.8)	179.3 (101.4)	327.1 (110.4)	244.9 (58.8)	311.3 (153.8)	335.6 (202.3)
Mean ratio 'time for processing sample/richness'	10.8 (3.6)	10.8 (5.0)	14.8 (4.2)	14.8 (3.4)	18.7 (6.5)	33.1 (24.9)
BMWP scores						
2001 samples	127	82	146	107	104	53
2003 samples	135	129	138	101	123	117
BMWP water quality class						
2001 samples	1	3	1	2	2	4
2003 samples	1	1	1	2	1	2

Rarefied species richness refers to the number of species expected to be found in each sample if they had the same number of individuals than the smallest sample at each stream (see text for details). Between brackets, standard deviations.

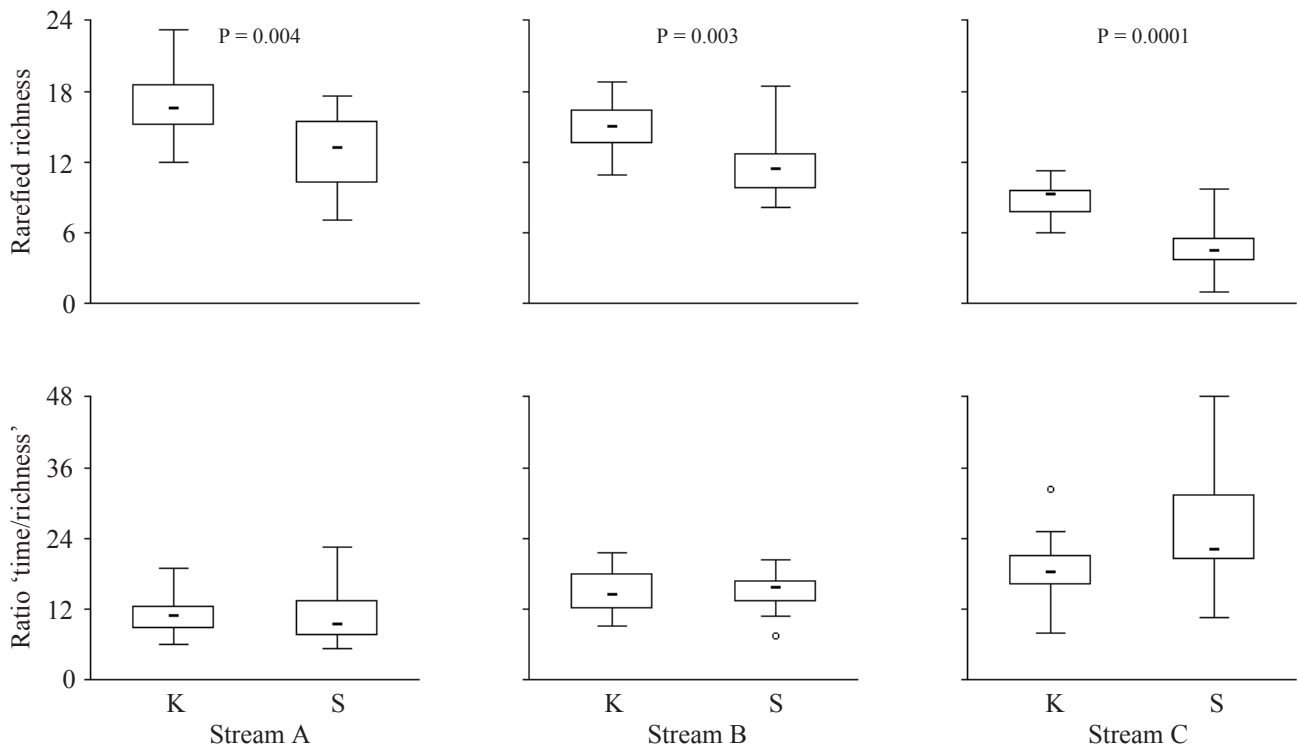


Fig. 1. Box-and-Whisker plots of rarefied richness and ratio `time for processing sample/richness` for each sampling device (K, Kick; S, Surber) at each stream. P-level is indicated where t-tests were significant. In the Box-plots, the signal (-) indicate the median, borders of the box indicate the 25% and 75% percentiles, the extremities indicate minimum and maximum numbers and the signal (o) indicate outlier values.

Table 2. Total richness, abundance, time to process sample, mean ratio `time for processing sample/richness`, BMWP scores and water quality classification according to the BMWP system for each mesh size (125, 250 e 500 µm) at each stream. (Between brackets, standard deviations)

	Stream A			Stream B			Stream C		
	125	250	500	125	250	500	125	250	500
Total richness	39	39	37	33	33	33	32	30	29
Mean richness	17.2 (6.2)	16.7 (6.4)	15.2 (6.2)	18.1 (5.7)	17.1 (4.6)	16.5 (4.5)	13.9 (5.7)	13.4 (5.6)	12.7 (5.8)
Mean abundance	735.9 (393.3)	603.0 (318.6)	430.8 (225.7)	916.3 (770.3)	776.1 (653.7)	622.3 (570.2)	5540.1 (5743.4)	4456.1 (4624.6)	3764.4 (3804.2)
Mean time to process sample (min.)	231.7 (159.3)	179.3 (101.4)	109.0 (47.3)	301.8 (87.9)	236.3 (52.7)	175.4 (60.6)	414.0 (227.1)	384.4 (193.4)	274.0 (154.1)
Mean ratio `time for processing sample/richness`	13.0 (6.1)	10.8 (5.0)	7.3 (3.1)	17.4 (4.4)	14.4 (3.4)	11.0 (3.0)	29.8 (8.3)	26.4 (11.7)	22.0 (9.2)
BMWP score	141	141	136	123	123	123	119	112	112
BMWP water quality class	1	1	1	1	1	1	2	2	2

lower numbers of individuals in each sample (Table 2). No significant differences were found considering taxa richness and BMWP index, indicating that even coarser mesh sizes were able to retain invertebrates' groups. At all streams, significant differences (all paired t-tests, $P < 0.05$) were found between mesh sizes considering time for processing samples

and abundance (Table 2). In summary, total abundance was 17-30% lower when using the 500 µm in relation to the 125 µm mesh size, representing 35-53% less time required for processing the samples, with no significant loss regarding to taxa richness. As a consequence, the 500 µm mesh size had the best performance, showing lower cost-effectiveness ratio than

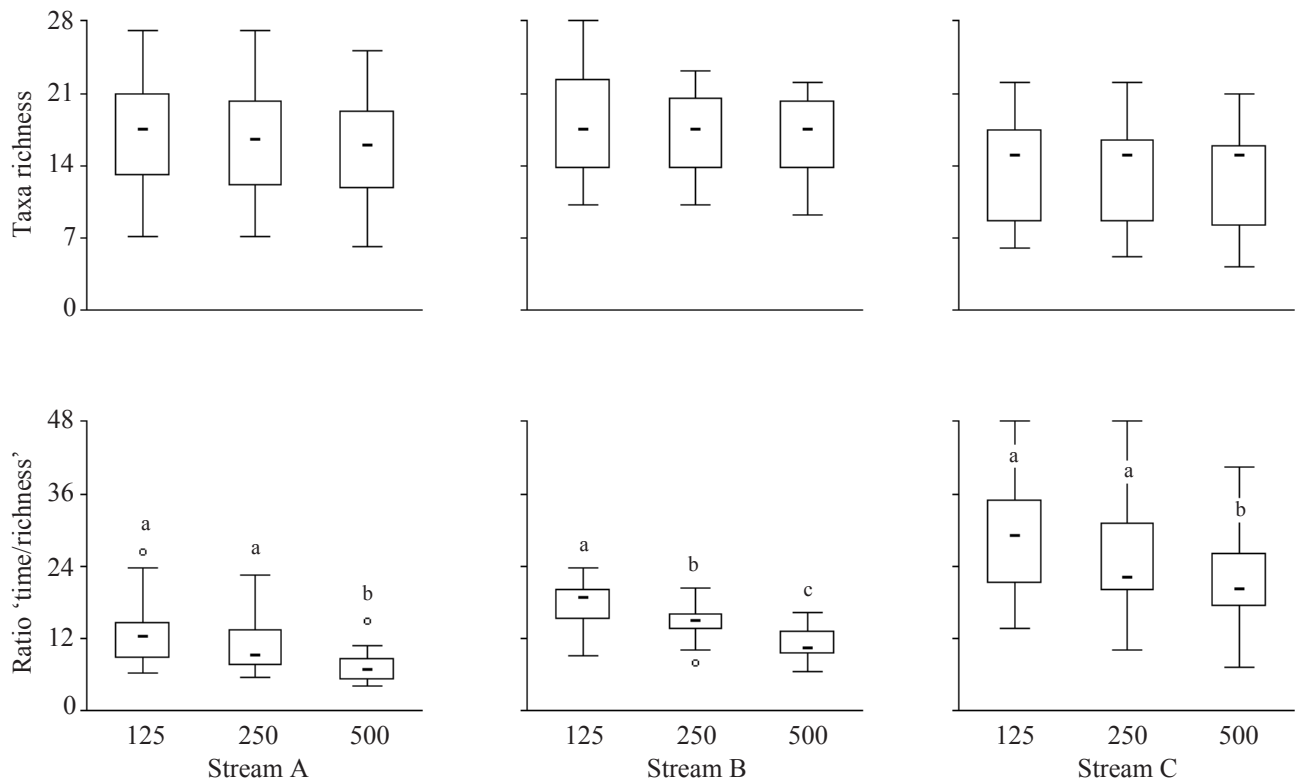


Fig. 2. Box-and-Whisker plots of taxa richness, abundance, time for processing samples and ratio 'time for processing sample/richness numbers' for each mesh size (125, 250 and 500 µm) at each stream. Different letters (a, b, c) indicate significant differences at paired t-tests ($P < 0.05$). In the Box-plots, the signal (-) indicate the median, borders of the box indicate the 25% and 75% percentiles, the extremities indicate minimum and maximum numbers and the signal (o) indicate outlier values.

the finer meshes at all sampling sites and periods (Fig. 2). This ratio was significantly different between 125 µm and 500 µm meshes (Stream A, t -value = 3.65, $P = 0.004$; Stream B, t -value = 5.46, $P = 0.0002$; Stream C, t -value = 5.63, $P = 0.0002$) and between the 250 µm and 500 µm meshes (Stream A, t -value = 3.58, $P = 0.004$; Stream B, t -value = 4.39, $P = 0.001$; Stream C, t -value = 4.27, $P = 0.001$) confirming the efficiency of using the 500 µm compared to the finer meshes.

Discussion

Comparison between sampling devices. Decisions about the best sampling device to use in RBPs have been highly discussed in the literature, and Kick nets have been preferred in front of Surber samplers (Storey *et al.* 1991). Kick methods have been recommended in biomonitoring surveys providing semiquantitative or qualitative data, and many environmental agencies decided to use it in their national RBP programmes. Carter & Resh (2001) noted that kicktype samplers were the most used sampling device for biomonitoring by state agencies in the USA (64.5%; where 35.6% of the total used a D-frame kick sampler). Other studies used fixed quadrat like Surber (8.9%) artificial substrates (13.3%) and grabs (2.2%). Agencies worldwide also indicated the use of kicktype samplers in their biomonitoring programmes (Europe – AQEM 2002; United

States – Barbour *et al.* 1999, Canada – Rosenberg *et al.* 1998, New Zealand – Stark *et al.* 2001, among others).

Desired methods for RBPs are those with favorable cost/effectiveness ratio, where ideally samples demand little time for processing and are representative of the macroinvertebrate fauna. In this study, samples collected with Kick method had significantly higher total richness and BMWP scores than Surber, but no significant differences were found between sampling devices when considering the time necessary for processing samples.

Our findings are in accordance with other studies that found higher richness numbers in samples collected with Kick in comparison to Surber sampler (Horning & Pollard 1978, Mackey *et al.* 1984). However, these studies did not consider the time necessary for processing samples, a critical aspect for determining the cost/effectiveness of methods in a RBP. In our study, Kick and Surber samplers had similar mean ratio 'Time for processing samples/Richness' (a cost/effectiveness ratio) and no significant differences were found between methods, although Kick samples had better ratios in stream C.

Before deciding on a sampling device, one should consider the number of samples that should be taken from the field and processed. According to Resh & Jackson (1993), 85% of the studies using Surber collected between three and five samples for each site. In our study, we collected

six Surber samples in each site and they seemed insufficient to represent the macroinvertebrate fauna: we found a loss of 5-25% of the taxa when comparing to samples collected with the Kick method. Considering biological conservation programs, that information alone could be used for deciding in favor of a Kick sampler. Our findings indicate that in order to use Surber in RBPs, more than six samples should be collected and processed, increasing the total time necessary for an adequate assessment of the biological quality of the stream. New studies are necessary to test the number of samples for each sampling device to best represent the benthic macroinvertebrate assemblage and we suggest that the time necessary for processing the samples should be registered in order to calculate the cost/effectiveness ratio of each sampling method.

Considering the logistic in the field, both samplers used in this study are low in cost and easy to use. One difference is that Kick screen net should be handled by two persons, while sampling with Surber can be performed by a single person. Another advantage of the Surber method is its fixed-area, allowing the calculation of an absolute (rather than relative) measure of density per taxon (Resh & McElravy 1993, Klemm *et al.* 1990, Carter & Resh 2001). On the other hand, Kick method may be used to collect samples in depths greater than Surber (which are restricted to stream depths of < 30cm; Barbour *et al.* 1999), and its main advantage is the gain of biological information as shown in this and other studies. A summary of the advantages and disadvantages of each method are described in Table 3.

Comparison between mesh sizes. The mesh size of nets used to collect benthic macroinvertebrates is of crucial importance to the effectiveness of the sampling method. Coarser mesh nets allow smaller animals to pass, and thereby may bias the sample and underestimate the real density of benthic organisms. Fine-meshed nets, on the other hand, may be affected by clogging and retain large quantity of detritus and

a greater numbers of small organisms, resulting in samples that are time-consuming to sort and identify compared with those from larger-mesh nets (Environment Canada 1993).

An ideal mesh size is the one with favorable cost/effectiveness ratio, in which samples demand little time for processing and are representative of the macroinvertebrate fauna. By convention, macrobenthos is defined as organisms that are easily visible to the naked eye, corresponding roughly to around 0.5 mm (Nalepa & Robertson 1981, Bachalet 1990). A mesh size of 500 μm (0.5 mm) has been proposed by the sampling standardization normative ISO in Europe (AQEM 2002). A review on sampling methods used in RBP programs indicated it was the most used in the US (Carter & Resh 2001), and was indicated by the US-Environmental Protection Agency to be the standard mesh size for benthic sampling (Klemm *et al.* 1990).

In this study, the higher abundance found in the 125 μm and 250 μm meshes (in relation to the 500 μm mesh) was not followed by an increase in taxa richness. There was no significant difference between the three mesh sizes considering taxa richness or BMWP scores. This higher abundance occurred in great part due to small individuals of Chironomidae, Hydropsychidae and Elmidae, which were also represented in the 500 μm mesh. We cannot tell, however, if these individuals found in the finer-mesh size are early instars of the same species of the ones found with the coarser-mesh or if they represent different species. Other studies have shown that a mesh of 500 μm could retain from 100% for large-bodied chironomids to as low as 21% for small-bodied ones (Nalepa & Robertson 1981). Bunn (1995) stated that it would be more practical to use that effort to collect more coarse-mesh samples whose members can be identified, and thereby derive better population estimates of larger organisms and later instars.

Other drawback of using finer meshes is that early instars, better retained by fine mesh nets, are not the best indicators of environmental conditions, because these organisms have not been in place long enough to respond

Table 3. Comparison between Kick Screen net and Surber procedures.

	Kick screen net sampler	Surber sampler
Number of persons for sampling	Two persons	One person
Sampling method	One person disturb substrates using foot and/or hand while the other person holds the sampler downstream	One person holds the sampler with one hand and disturb substrates within the frame area upstream using the other hand
Sampling area	Approximately 1m ²	Fixed area of 30 x 30 cm
Maximum suitable water depth for sampling	Up to approximately 1m	Shallow waters, normally < 30 cm
Use in the field	Heavier, but usable in any stream bottom condition	Easier to transport, but its frame may difficult fitting the sampler in streams with cobble/pebble bottom
Taxa richness	In this study, significantly higher	In this study, significantly lower
Rarefied richness	In this study, significantly higher	In this study, significantly lower
Abundance	In this study, not significantly higher	In this study, not significantly lower
Time for processing samples	In this study, not significantly higher	In this study, not significantly lower
'Time for processing samples/richness' ratio	In this study, lower in most cases (better ratio)	In this study, higher in most cases (worse ratio)

to chronic conditions. Ferraro *et al.* (1994) found that site assessments can be confounded and unnecessarily costly if the animals in the smallest size class are primarily ephemeral, patchily distributed juveniles. Since the use of finer mesh implicates more time to process samples, given that most biomonitoring studies have limited budgets, increasing the sample processing time reduces the total number of sampling sites or the number of replicates at each site.

The largest mesh-size to be used in a RBP should be defined based on the size of the 'target' assemblage or population. In the case of a RBP based on macroinvertebrate assemblages identified at family level, a mesh size > 500 µm could underestimate the fauna (Silva *et al.* 2005), compromising the results.

In this study, as a consequence of similar taxa richness and significant differences in time necessary for processing samples, the 500 µm mesh was considerably more efficient than 250 µm and 125 µm meshes. Therefore, 500 µm appears to be a reasonable choice of net mesh size for macroinvertebrate sampling in RBP programs using family level in streams of similar characteristics in Brazil.

In conclusion, our study showed that both sampling devices had similar cost/effectiveness, but using Kick sampler we sampled 5-25% higher richness than Surber sampler with non-significant increase in time for processing samples (treated as "cost"). Other feature we considered was that Surber sampler is not an adequate device for streams with more than 30 cm depth and also because of difficulties in handling it in rocky, cobble/pebble bottom streams, commonly found in Atlantic Forest mountainous areas. We therefore support the use of a Kick sampler for Wadeable Stream Rapid Bioassessment Protocols in this region. Also, we support the use of 500 µm mesh size in a RBP working in family taxonomic level because we found a better cost/effectiveness ratio than finer meshes, allowing a substantial reduction in costs with relatively no loss in biological information.

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