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Evaluation of sampling methods for periphytic fauna in macrophytes at the Espinhaço Mountain Range Biosphere Reserve, Minas Gerais State, Brazil

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ABSTRACT. The methods "Jar", "Manual Removal" and "modified Ekman Dredge" were evaluated for sampling periphyton fauna associated with aquatic macrophytes. Sixty-three samples were collected from five lentic and three lotic water bodies at the Espinhaço Mountain Range Biosphere Reserve (Minas Gerais State, Brazil). Anova and Tukey statistical tests were performed for Protista, Rotifera and Crustacea richness, whereas the abundance of Protista, Rotifera, Crustacea, Gastrotricha, Tardigrada and Nematoda was evaluated by percentage. Of the three methods, the Dredge is less indicated for different water bodies systems in which there is interest in analyzing various microinvertebrate groups. The Protista and Rotifera represent 80% of the total abundance and richness in the invertebrate community. In the ecosystems evaluated, all methods are relevant for Protista analysis; on the other hand, Crustacea analysis required the Jar method. Manual Removal and Dredge methods are appropriate for Rotifera analysis. Gastrotricha and Tardigrada abundance presented better results with the Jar method; Nematoda with the Dredge method. The three methods are appropriate for periphyton fauna sampling in both water body systems; nevertheless, it is important to be aware that for each fauna community in a specified ecosystem, there is a specific method for best performance.

Key words: Biosphere Reserve, ecotone, littoral region, lentic and lotic ecosystems, periphyton fauna.

RESUMO. Avaliação dos métodos de amostragem para fauna perifítica em macrófitas na Reserva da Biosfera, Serra do Espinhaço, Estado de Minas Gerais, Brasil. Os métodos "Jarra", "Remoção Manual" e "Draga de Eckman modificada" foram avaliados para amostrar a fauna perifítica associada à macrófitas aquáticas. Foram coletadas 63 amostras em cinco ambientes lênticos e três lóticos na reserva da biosfera da Serra do Espinhaço (Estado de Minas Gerais, Brasil). Os testes estatísticos Anova e Tukey foram feitos para riqueza de Protista, Rotifera e Crustacea, enquanto para a abundância de Protista, Rotifera, Crustacea, Gastrotricha, Tardigrada e Nematoda foram avaliados os percentuais. Os protozoários e rotíferos representaram 80% da abundância e riqueza da comunidade. Nos ecossistemas avaliados todos os métodos foram relevantes para Protista, por outro lado, o método da Jarra foi o mais adequado para a análise de Crustacea. Entre os métodos, a Draga foi menos indicada para os grupos de microinvertebrados nos ecossistemas aquáticos. Os métodos Remoção Manual e Draga foram apropriados para analisar Rotifera. A abundância de Gastrotricha e Tardigrada demonstrou melhores resultados pelo método da Jarra e Nematoda pelo método da Draga. Os três métodos são apropriados para amostragem da fauna perifítica em ambos os sistemas aquáticos. Entretanto, é importante estar ciente de que para cada tipo de ecossistema a amostragem da comunidade faunística requer um método específico para obter a melhor performance.

Palavras-chave: Reserva da Biosfera, ecótone, região litorânea, ecossistemas lóticos e lênticos, fauna perifítica.

Introduction

The aquatic macrophyte diverse biological forms with physiological and ecological adaptations add heterogeneity to the inland ecotone littoral region, increasing the interstitial spaces and supporting a specific biota (Junk, 1970; Wetzel, 1983; Esteves, 1998). The heterogeneity and abundance of the littoral region habitat hampers and restricts microinvertebrate quantitative samples, resulting in scarcity of ecological information (Wetzel and

Likens, 1991). Nogueira *et al.* (2003) also emphasized the need for intensification of methodological tests in this biotope.

There are various procedures of microbiota sampling associated with macrophytes. Pennak (1962) recommends flexible tubes or pipes. Bicudo (1990a and b); Hardoim and Heckman (1996) and Rodrigues (1998) propose the mechanical extraction by scraping the adhered or associated organisms after manual removal of the plant or parts of its structures. Torres and Schwarzbold (2002) design a container for rhizoids sampling and Nogueira *et al.* (2003) tested several apparatus for the evaluation and follow-up of certain populations. Other authors (Boltovskoy, 1995; Bonecker and Lansac-Tôha, 1996; López *et al*., 1996; Rocha *et al*., 2000; Wisniewski *et al*., 2000) adapt methods used for limnetic plankton sampling, such as pumps, plankton net dragging and the Schindler-Patalas trap.

The present paper evaluates the applicability and the performance of three sampling methods – Jar, Manual Removal and modified Ekman Dredge – for microfauna and meiofauna adhered or associated with aquatic macrophytes in the inland ecotone littoral zone of Espinhaço Mountain Range Biosphere Reserve, Minas Gerais state, Brazil.

Material and methods

Inland ecotone littoral zone

The study region comprises eight areas situated within 20°21'56"S, 43°26'02"W and 14°58'54"S, 42°30'10"W (Espinhaço Mountain Range Biosphere Reserve, Minas Gerais, Brazil) (Figure 1). These areas were selected through cartographical analysis (IBGE, 1977) to define field surveys. The first criterion was to choose areas that represent the three river basins of Espinhaço Mountain Range – São Francisco, Doce and Jequitinhonha (Table 1), with a wider distribution within each watershed, and situated above 600 m. Conservation status, antropical pressures, occurrence of aquatic macrophyte banks and easy access were decided in the field.

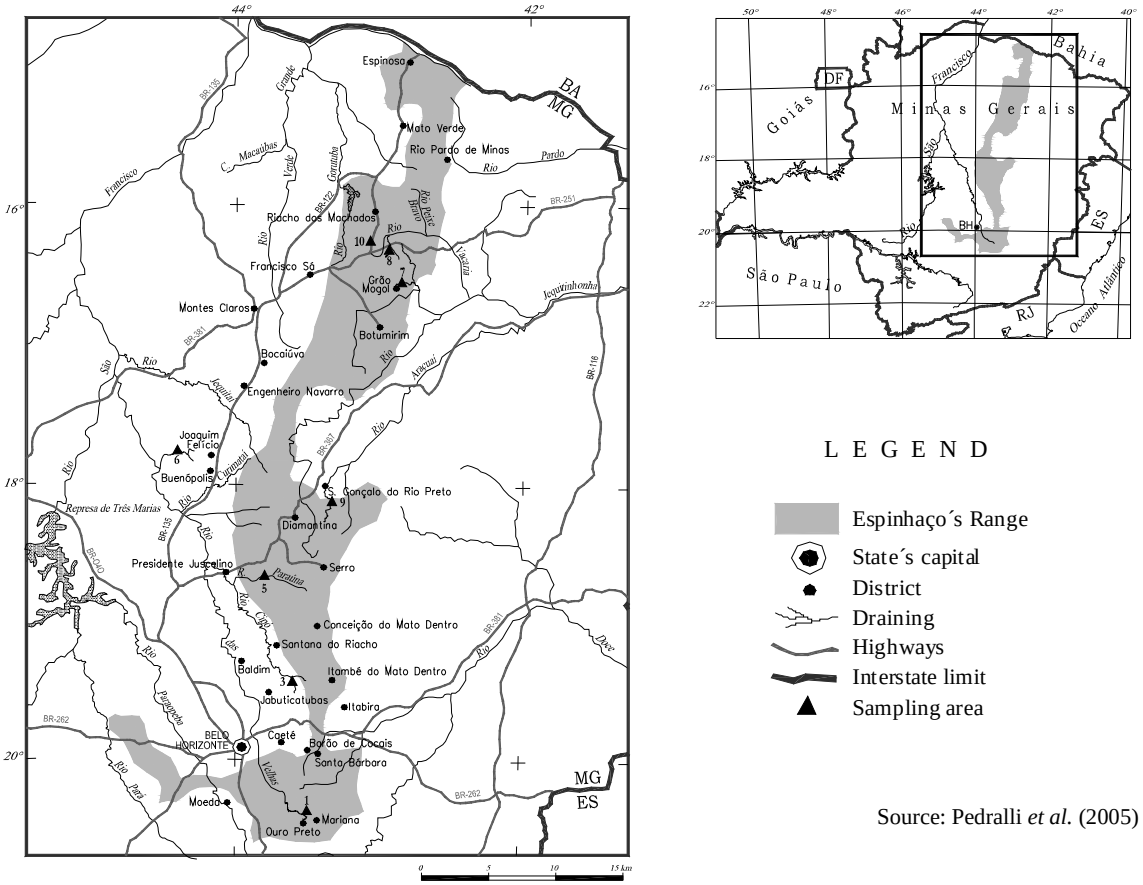

Figure. 1. Map showing locations of sampling areas at Espinhaço Mountain Range Bosphere Reserve, Minas Gerais State, Brazil.

Table 1. Ecotone locality identification: ecosystem, watershed and sample quantities.

Ecosystem	Locality	Watershed	Sample (Nº)
Lentic	Tanque da Fazenda lake	Doce river	7
	Comprida lake	São Francisco river	8
	Arame Farpado lake		8
	Estivinha reservoir	Jequitinhonha river	9
	Americana reservoir		8
Lotic	Taquaral stream		9
	Preto stream		7
	Corrento stream	São Franscisco river	7

The regional climate is mesothermal, with mild and rainy summer. Mean temperature varies from 17.4 to 19.8ºC and mean annual rainfall is about 1,500 mm (Giulietti *et al.*, 1987; Araujo *et al.,* 2005; Pedralli *et al.*, 2005).

Sampling

In December 2001 and March 2002, the microinvertebrates were sampled in homogeneous and representative aquatic macrophyte banks. The three methods – Jar, Manual Removal and modified Ekman Dredge – were used in a delimited area of 1.0 m². The sampled material was immediately sent to the Hydrobiology and Hydrology Laboratory (Technological Foundation Center of Minas Gerais – CETEC, Water Resources Sector – SAA) in Belo Horizonte, Minas Gerais state.

Jar method

Periphyton was sampled by adapting the procedures described by Dioni (1967) and Dabés and Velho (2001), using a rigid plastic, graduated jar (15 cm diameter, 1.0 L capacity). The jar (Figure 2a) was filled six times, being dredged among the floating and submersed macrophytes and their rhizoids. Each time, the collected water was filtered through the CETEC/SAA plankton net (20 μm pore mesh).

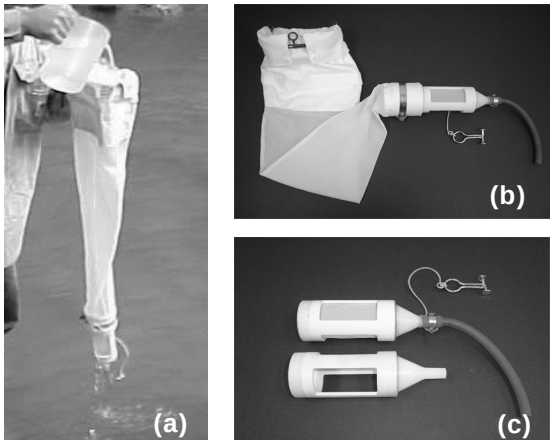

Figure 2. Jar (a), net (b) and detail of filtration cup (c).

The filtered material was conditioned in a 250 mL plastic container, fixed with 4% formaldehyde for laboratory analysis.

The CETEC/SAA plankton net consisted of three different parts: a holding ring, variable-pore mesh net and a container. The holding ring (Figure 2b) is a metal ring with or without a shaft. The cotton net top (Figure 2b) has three flaps button closing, which embrace the metal ring, and therefore facilitates transportation, cleaning, reuse and contributes to its durability. The cylindrical PVC container (5.5 mm diameter and 12.0 cm height) (Figure 2c) has three 2.5 cm wide and 7.0 cm high apertures covered with the same plankton net mesh, net screwed and topped with a plastic funnel connected to a latex or silicone hose, pressed by Mohr tweezers (Ferreira *et al.*, 1997).

Manual Removal and modified Ekman Dredge methods

APHA (1995) suggests sampling macrophytes by either the Manual Removal or Ekman dredge methods. In order to probe epiphyton and pseudoepiphyton (Sládečková, 1962; Wetzel, 1983; Esteves, 1998; Pompêo and Mochini-Carlos, 2003) microinvertebrates from macrophytes non-senescent vegetative structures, both methods were used. Ekman dredge was adapted by CETEC/SAA: 190 cm² area, 80 cm height shaft and an upper end manual closing system (Figure 3a).

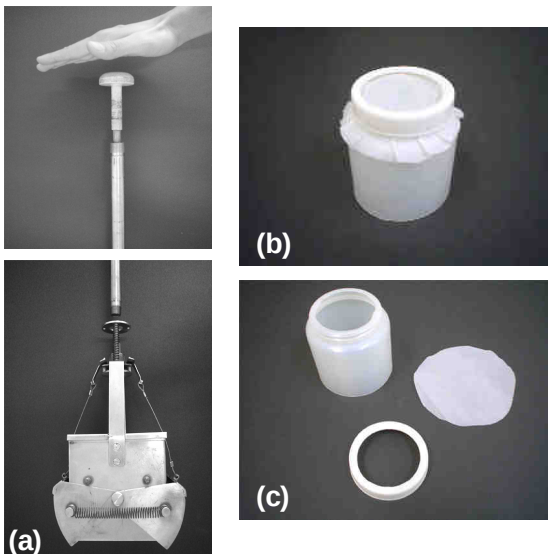

Figure 3. Ekman-type dredge adapted for CETEC/SAA with detail of its disarms in the superior part (a) and cup of filtration (b) with details of the parts (c).

Two macrophyte lots were sampled by each

method and conditioned in plastic bags with 30 μm filtered surrounding water. One was kept *in natura* under refrigeration and the other was preserved in 4% formaldehyde solution.

In the laboratory, the periphyton was removed from the macrophytes using a thin bristle brush, followed by agitation in magnetic agitator. CETEC/SAA adapted rigid plastic containers as a filtration apparatus with a membrane of the same net pore mesh used in the fieldwork (Figures 3b and c) to concentrate the samples for analysis. The *in natura* macrophytes were preserved in FAA solution for taxonomical identification (Bicudo, 1990b). Their principal typologies (Pedralli, 1990) were amphibian, emergent, fixed submersed and fixed floating plants (Table 2).

Table 2. Macrophyte* by ecotone littoral type.

Ecotone littoral type	Macrophyte typologies	Macrophyte families
Lentic	Emergent	Alismataceae
	Emergent, fixed submersed	Cyperaceae
	Fixed floating	Menyanthaceae
	Fixed submersed	Najadaceae
	Fixed floating	Nymphaeaceae
	Emergent	Pontederiaceae
	Emergent	Typhaceae
Lotic	Emergent, fixed submersed	Eriocaulaceae
	Emergent	Sphagnaceae
Lentic/lotic	Amphibian, emergent, fixed submersed	Poaceae
	Fixed submersed	Mayacaceae

*López and Ferreira (2005) and Pedralli *et al.* (2005) present detailed identification of the macrophytes.

Sample analysis and data treatment

The organisms were counted by Sedgewick-Rafter method (APHA, 1995). The sixty-three samples analyzed and statistically treated for periphyton richness and abundance were grouped by two ecosystem typology: lentic and lotic (Table 1).

Gastrotricha, Tardigrada and Nematoda were difficult species to identify; therefore they were analyzed only for methods performance in relation to fauna density. Protista, Rotifera and Crustacea, on the other hand, were analyzed both for fauna density and richness.

Density data were treated to express fauna relative abundance (%), while richness statistics used box plot plus Anova, respected Kolmogorov-Smirnov normality and Levene homoscedasticity assumptions, followed by Tukey test at 5% (Triolla, 1999).

Results

Abundance of Protista, Rotifera, Crustacea, Gastrotricha, Tardigrada and Nematoda

The mean percentage abundance of the invertebrate groups from the lotic and lentic ecosystems is shown in Figure 4. Protista showed the highest abundance, followed by Rotifera, in all samples. However, Nematoda and Crustacea answered differently to the sampling methods used. Jar and Manual Removal methods showed predominance of Nematoda in the lotic ecosystem and Crustacea in the lentic one. Nematoda was more abundant in both ecosystems through modified Ekman Dredge method. Despite the natural low presence of Tardigrada and Gastrotricha, the Jar method detected them in higher concentrations than in the other two.

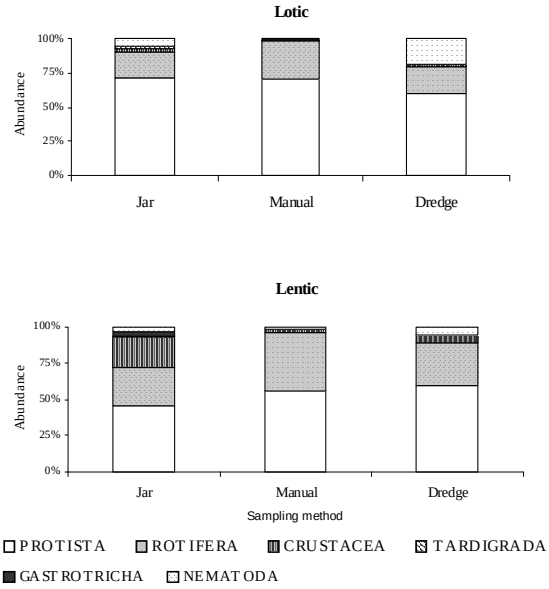

Figure 4. Relative abundance of invertebrate groups in the lotic and lentic ecosystems. Espinhaço Mountain Range Biosphere Reserve, Minas Gerais, Brazil, 2001, 2002.

The Jar method was the most appropriate for sampling Crustacea with 4 to 5 times higher percentages, in both ecosystems; conversely, the Manual Removal method was the least efficient, showing low density percentages: below 1% in the streams and only 2.8% in lakes/reservoirs. Nonetheless, this method was the best option for Rotifera sampling in both water body systems, reporting abundances between 30 to 40% of their total density, values 1.5 higher than with the other methods. The Nematoda total density was 4% and 8%, respectively, in the lotic and lentic ecosystems, the group percentage abundance was six times higher in lotic water bodies sampled by modified Ekman Dredge method.

Richness of Crustacea, Protista and Rotifera

The community richness (Figure 5) showed significant differences amongst the sampling methods, between the ecosystems (Table 3). Jar and Dredge methods presented differences between lentic and lotic, however the Manual Removal revealed similar response for both ecosystems.

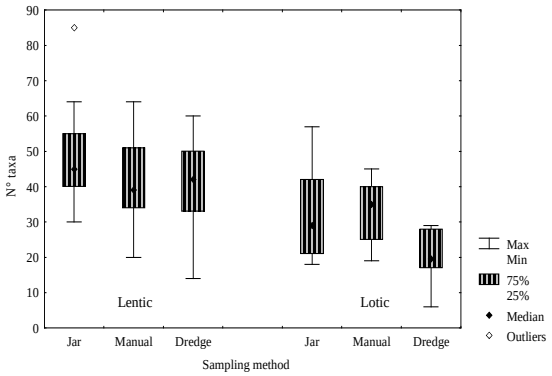

Figure 5. Invertebrate community richness (taxa number) for sampling method and aquatic ecosystems. Espinhaço Mountain Range Biosphere Reserve, Minas Gerais, Brazil, 2001, 2002.

Table 3. Mean values (taxa number) of invertebrate community richness (Protista, Rotifera and Crustacea) for sampling method and aquatic ecosystems. Espinhaço Mountain Range Biosphere Reserve, Minas Gerais, Brazil, 2001, 2002.

Sampling method	*n*	Lentic (*n* = 40)	*n*	Lotic (*n* = 23)
Jar	17	47,5 ± 14,4 Aa	11	31,3 ± 12,2 Ba
Manual removal	13	42,5 ± 13,0 Aa	6	33,2 ± 9,9 Aa
Dredge	10	40,3 ± 14,1 Aa	6	19,8 ± 8,4 Ba

Values followed by different capital letters in the lines and small letters in the columns differ from one another by the Tukey test (5%).

For the lentic ecosystem, the chosen methods revealed significant differences, with the Jar method behaving distinctly from the others in relation to Crustacea richness. In the lotic ecosystem, modified Ekman Dredge method presented not only the lowest values for richness but demonstrated the greatest limitations, mainly for Crustacea sampling. Protista and Rotifera richness was similar in both water bodies systems except for Rotifera sampled by the Jar method in streams (Table 4; Figure 6).

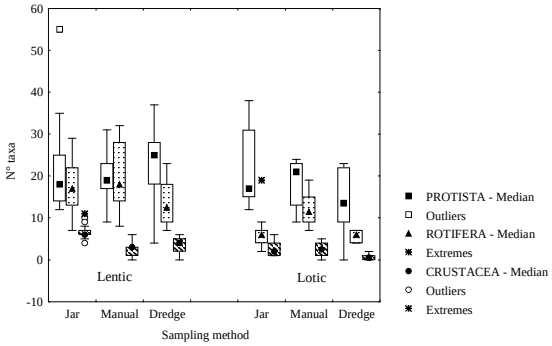

Figure 6. Protista, Rotifera and Crustacea richness (taxa number) for sampling method and aquatic ecosystems. Espinhaço Mountain Range Biosphere Reserve, Minas Gerais, Brazil, 2001, 2002.

Discussion

Among the three methods, Dredge is less indicated for different water bodies systems when there is interest in analyzing various microinvertebrate groups. The Rotifera and Protista are both 80% of the total invertebrate community abundance and richness. Both Dredge and Manual Removal methods are appropriated for Rotifera analysis. For Protista analysis in both ecosystems, all methods are relevant; on the other hand, Crustacea analysis in both ecosystems demands Jar methods. The abundance of Tartigrada and Gastrotricha demonstrated better results for the Jar method, and Nematoda for the Dredge method.

For sampling communities associated to sediments, such as Nematoda, the Dredge is the best method; however for very mobile communities, such as Crustacea, this is the worst, suggesting that the organisms can escape.

The three methods are appropriated for fauna sampling in both water body systems; nevertheless is important to be aware that for each fauna community in a specified ecosystem there is always a specific method for best performance.

Table 4. Mean values (taxa number) of Protista, Rotifera and Crustacea richness, for sampling method and aquatic ecosystems Espinhaço Mountain Range Biosphere Reserve, Minas Gerais, Brazil, 2001, 2002.

Sampling method	Protista		Rotifera		Crustacea	
	Lentic (*n* = 40)	Lotic (*n* = 23)	Lentic (*n* = 40)	Lotic (*n* = 23)	Lentic (*n* = 40)	Lotic (*n* = 23)
Jar	22,6 ± 11,5 Aa	21,5 ± 8,9 Aa	18,1 ± 6,3 Aa	6,9 ± 4,4 Ba	7,0 ± 2,0 Aa	2,8 ± 1,7 Ba
Manual removal	20,0 ± 6,0 Aa	18,5 ± 6,1 Aa	19,8 ± 8,2 Aa	12,2 ± 4,4 Aa	2,5 ± 1,8 Ab	2,5 ± 1,9 Aa
Dredge	22,6 ± 10,2 Aa	13,5 ± 8,6 Aa	14,0 ± 5,6 Aa	5,7 ± 1,4 Aa	3,7 ± 2,0 Ab	0,7 ± 0,8 Ba

Values followed by different capital letters in the rows and lowercase letters in the columns differ by the Tukey test (5%).

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