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Evaluation of quantitative sampling methods in pleuston: An example from ostracod communities

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ABSTRACT

We address two methodological questions related to sampling pleuston in floodplain lakes, using ostracod as model group: (1) we test the homogeneity of ostracod communities by comparing samples from the edge and from the centre of the floating *Eichhornia crassipes* patches, (2) we test if there is a clear difference between two methods of sampling. According to the ecological attributes evaluated, there was no significant difference between edge and centre of the patches, while both sampling methods were equally efficient to represent the ostracod communities.

Ostracoda are small bivalved crustaceans (c. 0.2–8 mm) in which the carapace totally encloses the body and appendages (Horne et al., 2002). These organisms live in a wide variety of aquatic and even (semi-) terrestrial habitats. In freshwater environments, ostracods are abundant in the benthic and periphytic communities (Martens and Behen, 1994).

Aquatic macrophytes provide refuge against visual predators to the invertebrate communities that are associated with it (Padial et al., 2009; Stansfield et al., 1997). In addition, the submerged parts of floating aquatic macrophytes retain small sediment particles and thus form a substrate for hiding, feeding (periphyton) and reproduction (Poi de Neiff and Neiff, 2006; van den Berg et al., 1997).

Ostracod communities are abundant in the microcosms in roots systems and other submerged parts of floating macrophytes, called pleuston (Higuti et al., 2007; Poi de Neiff and Carignan, 1997; Rocha-Ramírez et al., 2007). Such floating plants are common in South American floodplain systems. They mostly occur in small to medium-sized patches to sometimes very large stands in a variety of habitats in river systems: either in the river itself, or in associated channels, open and closed lakes. It is unclear if abundance, species richness and composition are homogenous throughout such patches of floating plants. For example, are species richness, diversity and abundances comparable at edges and in the centre of a patch, so that point sampling of a few plants is representative of the entire patch?

Basically, two types of methods have been used to sample invertebrates associated with floating macrophytes: (1) removing the plant from the water by hand and washing the root systems in a bucket, after which the remaining material is washed in a hand net and the washed roots are then dry-weighted to provide quantitative samples (e.g. Higuti et al., 2007; Liberto et al., 2012) or (2) sampling the macrophytes (roots and/or submerged vegetation) in situ, using tools such as boxes and cores (quantitative) (e.g. Kiss,

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Table 1
Geographic coordinates and some abiotic variables of the studied lakes in the Upper Paraná River floodplain.

Locality name	Coordinate	Macrophyte	Site	T (°C)	pH	EC (μS cm^{-1})	DO (mg L^{-1})
Guaraná	S 22°43′13.4”	Patch 1	Edge	31.0	5.8	53.3	3.2
	W53°18′13.8”		Centre	31.0	6.0	30.8	2.0
Guaraná	S 22°43′2.01”	Patch 2	Edge	30.6	6.1	51.7	3.4
	W53°18′18.5”		Centre	30.5	5.9	26.6	3.2
Porcos	S 22°42′6.8”	Patch 3	Edge	30.9	6.4	42.0	2.1
	W53°14′43.7”		Centre	31.0	6.2	56.4	1.8
Porcos	S 22°42′11.4”	Patch 4	Edge	32.2	6.1	38.6	3.8
	W53°14′44.7”		Centre	31.8	6.0	31.5	1.9
Maria Luiza	S 22°40′9.4”	Patch 5	Edge	30.5	6.4	34.5	4.3
	W53°12′53.3”		Centre	31.0	6.2	33.5	1.7
Maria Luiza	S 22°39′57.8”	Patch 6	Edge	30.0	5.9	30.3	2.4
	W53°12′36.1”		Centre	29.5	5.8	28.1	1.1

2007) and hand nets (qualitative or Catch per Unit Effort) (e.g. Karan-Žnidaršič and Petrov, 2007; Rossetti et al., 2004).

For ostracods, especially the first method has been used in Brazilian floodplains. Two variations to the method can be distinguished. The first one lifts the plant out of the water and places it in the bucket outside of the water. In this method, some water drips out of the root systems of the plants and there is a risk that some fauna is lost that way. In the second method, the bucket is placed in the water and the plants are arranged in the bucket without leaving the water. This has the risk that the bucket will contain animals that were in the open water, not in the root systems.

The present study addresses two methodological questions related to sampling pleuston (with ostracod as model group) of floating plants in the floodplain of the Upper Paraná River (Paraná, Brazil).

1. We compare samples from the edge and from the centre of the patches of floating *Eichhornia crassipes* (Mart.) Solms (the common water hyacinth), to test the homogeneity of ostracod communities in open lakes (=connected to the river or to a channel) in a floodplain system.
2. We test if there is a clear difference in the result of the two methods of “bucket” sampling, both used at the edge of patches of floating macrophytes.

The study was conducted in the three lakes (Guaraná, Porcos and Maria Luiza) of the Upper Paraná River floodplain (22°40′–22°50′S and 53°10′–53°24′W). This floodplain is 230 km long and can reach a width of 20 km, and includes several secondary channels and open and closed lakes (Agostinho et al., 2004).

All samples were collected in January 2016. In order to assess the abiotic environment, several chemical and physical variables were measured in situ, such as dissolved oxygen (mg L^{-1}) and water temperature (°C) (both with YSI oximeter 550A), pH and electrical conductivity (μS cm^{-1}) (both with YSI multiparameter 63).

Firstly, we tested the possible differences in results using two “bucket” methods, both at the edge of the macrophyte patches. Method “bucket 1”: *Eichhornia crassipes* were sampled removing the plants from the water by hand and the plants were immediately placed in a plastic bucket; Method “bucket 2”: The bucket was placed in the water, beneath the roots to collect *E. crassipes*.

Secondly, in order to test for the homogeneity of floating macrophyte patches we collected from the edge and from the centre of the patches, in both cases using “bucket” method 1.

Roots of the *E. crassipes* plants were separated and the remainder of the plants was disregarded. The roots were washed in the bucket and this residue was thoroughly washed through a hand net with 160 μm mesh size. Samples were preserved in 70° ethanol while roots were dried and weighted.

A parametric analysis of variance (ANOVA) was performed to test for significance of differences in species richness, density (ind g^{-1} DW), Shannon diversity and evenness of ostracods between the two methods and between the two sites. Rarefaction curves plot species richness against increasing sampling units (specimens) at comparable levels of density in both “bucket” methods and at the edge and in the centre of the macrophytes patches (Gotelli and Colwell, 2001).

Principal Coordinate Analyses (PCoA), using a density matrix, were performed to compare the composition of ostracod communities between the two methods and between the two sites in the macrophytes patches. The PCoA axes were retained using the criterion of Broken-Stick. The differences between samples in both methods and sites were tested using a one-way ANOVA applied to the scores of the PCoA axes.

The beta diversity was analysed by means of a dispersion homogeneity test (PERMDISP; Anderson et al., 2006), which tested the variability of the composition of ostracod species between the two methods and between the two sites in the macrophytes patches. This analysis calculates a centroid for each method and site, and determines the Bray-Curtis distance of each sampling locality to the centroid. The higher average distance to the centroid is the largest dispersion in the species composition, meaning an increase in the beta diversity. The significance level ($p < 0.05$) of beta diversity was calculated using a permutation test with 999 permutations.

PCoA and PERMDISP were performed using the software R 3.2.4 (R Development Core Team 2016) with the vegan (Oksanen et al., 2016) and permute packages (Simpson, 2016). The one-way analysis of variance (ANOVA) was performed using the STATISTICA software (StatSoft Inc., 2005).

The values of abiotic variables in the edge and centre sites of the macrophytes patches are shown in Table 1. In general, higher electrical conductivity, dissolved oxygen and pH values were recorded at the edge, when compared to the values of the centre. However, only the dissolved oxygen showed significant differences ($F = 1.46$; $p = 0.02$) between the sites.

A total of 30 species of ostracods associated with *E. crassipes* were recorded during the January 2016 sampling in the three lakes of the Baía River system (Upper Paraná River floodplain). The ostracod communities comprised four families: Cyprididae, Candonidae, Limnocytheridae and Darwinulidae. Of these 30 species, 29 occurred at the edge of macrophyte patches (28 and 25 taxa were recorded with the “bucket 2” (in the water) and the “bucket 1” (out of the water) methods, respectively) and 24 species were observed in the centre of macrophyte patches with the “bucket 1” method.