Sampling sufficiency for estimating zooplankton diversity in neotropical floodplain lakes

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Abstract

This study focuses on determining how many samples are needed to effectively assess the species richness of a community. Zooplankton samples at 40 sampling sites distributed among four lakes in the floodplain of the middle Araguaia River (Central-West region of Brazil) were evaluated to determine the effect of the accumulation of collecting points by lake on species richness estimates. The results indicated the zooplankton community has high spatial heterogeneity. Thus, using a single sampling unit per lake would not be sufficient to accurately estimate their diversity (i.e. the zooplankton composition in these cases would be represented mainly by abundant species). Sampling designs that include a minimum of seven sampling sites in each lake are needed to record 70% of the total species richness. It is recommended, therefore, that researchers use a larger number of sampling sites per lake or, alternatively, that the water obtained and filtered through plankton nets is extracted from a wide area and in different lake compartments, rather than from a single site.

Key words

Araguaia River, biological diversity, sampling effort, species–area.

INTRODUCTION

Two recurring questions for field ecologists are as follows: (i) how many sampling units are needed for accurate assessments (Walker 2003; Santos *et al.* 2008; Fischer & Paukert 2009) and (ii) how should the samples be distributed in space and/or time (Rhodes & Jonzén 2011)? The species–area relationship is one of the most studied ecological patterns and predicts that the number of species increases with the area sampled (Rosenzweig 1995). Thus, observed richness is asymptotically related to the number of samples (i.e. relationship between species and sampling effort) once the occurrence of new species decreases with an increased number of samples (Cam *et al.* 2002). Because species–area and species–sampling effort relationships are driven by different processes, it is not a straightforward exercise to incorporate both processes into designing diversity studies. While the species–area effect is related to an increased environmental heterogeneity concomitant with an increased geographical area, for example, the species–sampling effort effect is related to the diversity of taxa in a region (Azovsky 2011).

Sampling designs allowing more accurate estimates of species richness at both local and regional scales are particularly important within the context of a growing extinction crisis (Regan *et al.* 2001; Hutchings & Reynolds 2004; Pimm *et al.* 2014). Habitat destruction is the main cause for this crisis, reducing abundances and species richness across the globe (Pearson & Shine 2005; Lin & Liu 2006; Pimm *et al.* 2014). Thus, quantifying biological diversity and describing the spatial patterns of species distribution are critical steps to support conservation efforts (Balmford & Gaston 1999). Nevertheless, information about biological diversity and distribution of species is scarce, hindering accurate estimates of extinction rates (Brito 2004). Scarce information about diversity and distribution of species is not restricted to groups that are difficult to survey (e.g. microorganisms), but also affect relatively well-known and conspicuous

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taxa such as birds, reptiles and mammals (Brito 2004; Whittaker *et al.* 2005).

One of the complicating factors for creating effective sampling protocols is that the diversity and/or structure of communities of various taxa may be spatially structured at the microscale (Maia-Barbosa *et al.* 2008). This is particularly common in aquatic environments in which both abiotic and biotic characteristics may be strongly spatially and temporally allocated over very fine spatial scales, causing complex distribution patterns of fauna and flora. Certain zooplankton species, for example, occupy shallow water zones of lakes at certain times of the day, seeking refuge from predation within aquatic macrophytes stands (Burks *et al.* 2002; Iglesias *et al.* 2007; Meerhoff *et al.* 2007). Thus, effectively assessing lentic zooplankton community structure requires knowledge of the spatial structure of the community in the environment.

In practical terms, decisions about sample size are influenced by a trade-off between the objectives of the studies and time availability, human and financial resources (Tista & Fiedler 2011). Interactions between practical constraints and sampling strategies that do not consider spatial and temporal structuring of communities lead to poorly designed surveys that may significantly underestimate diversity. Monitoring zooplankton communities, for example, is usually based on a relatively small number of samples obtained in the central and/or littoral regions of aquatic environments, regardless of the size of the environment and its spatial heterogeneity. Such sampling effort may be insufficient even if samples are from a single environment.

This study focuses on the results of a survey of floodplain lakes of the Araguaia River (Central-West region of Brazil), with the following objectives: (i) to compare the zooplankton community structure between littoral and pelagic regions, particularly to test the prediction that littoral areas have higher species richness and densities because of their higher spatial heterogeneity, and (ii) to evaluate the effects of the accumulation of collecting points by lake on the estimated species richness.

MATERIALS AND METHODS

Study area

Zooplankton samples were obtained from 40 sites distributed in four lakes belonging to the Middle Araguaia River floodplain, located in the municipality of São Miguel do Araguaia, Goiás (Brazil), during September 2010 (see Table 1 for main characteristics of each lake).

Eight sites (four sites in littoral zone and four sites in pelagic zone) were established in lakes Comprido (S12°51′18.92″, W50°34′40.78″), Varal (S13° 2′6.45″, W50°37′4.41″) and Luiz Alves (S13°13′42.72″, W50°34′21.42″). Sixteen sites (eight sites in littoral zone and eight sites in pelagic zone) were sampled in the Brito Lake (S13°10′10.28″, W50°35′0.47″). The spatial distribution of sites within each lake was paired, with an average distance of 97 m between littoral and pelagic sites. All samples were taken at a depth of 0.5 m.

Zooplankton

Three hundred litres of water were filtered through a plankton net (68 μm) at each site, concentrating the samples in 100 mL and fixing them with 4% formaldehyde. The species richness was analysed with the aid of a Sedgewick–Rafter chamber until stabilization of the number of species. Subsampling was performed for abundance with a Hensen-Stempel pipette (2.0 mL), with at least 50 individuals being counted for each group (testate amoebas, copepods, cladocerans and rotifers). Organism counting was based on the method described by Bottrell *et al.* (1976), using three subsamples for each sample. The density was expressed in terms of individuals m^{-3}.

Data analysis

Non-metric multidimensional scaling NMDS (Legendre & Legendre 2012) was used to ordinate samples according to the zooplankton community. The Bray–Curtis coefficient was used for this analysis. Abundance data were previously log-transformed.

Diversity profiles were calculated for each lake following the Rényi series (Equation 1) for the parameters α 0.5, 1, 2, 4 and ∞, as follows:

$$H_\alpha = \frac{\ln \sum_{i=1}^{S} p_i^\alpha}{1-\alpha}, \qquad (1)$$

where H_α = diversity for a given value of α; α = parameter defining the weight of the dominant species; S = total number of species; and p_i = proportion of individuals of species i in relation to total number of individuals in the sample (Tóthmérész 1995). The Rényi series can be considered as a generalization of the diversity indexes and can be more advantageous than choosing a specific index (e.g. Shannon or Simpson) wherein an arbitrary choice (implicit in each index) of the relative weights given to the dominant species is made. With the use of this series, the α parameter represents this weight, with diversity values being calculated over a range of α. As a reference, the values of α equal to 0, 1, 2 and ∞ represent total

Table 1. Mean and standard deviation (SD) of main characteristics of the studied lakes

			Temperature (°C)		Turbidity (NTU)		Transparency (cm)		Depth (m)		Dissolved oxygen concentration (mg L^{-1})	
Lakes	Area (km^2)	Regions	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Brito	0.555	Littoral	28.2	0.8	53.8	25.0	27.6	7.4	0.6	0.3	5.5	0.7
		Pelagic	28.1	1.2	53.3	27.1	31.0	9.7	1.3	0.7	5.5	0.8
Comprido	0.426	Littoral	29.7	1.0	14.3	3.0	65.8	5.7	1.1	0.3	2.8	0.5
		Pelagic	29.4	0.2	13.8	2.3	70.0	12.6	2.7	1.3	2.5	0.5
Luiz Alves	0.633	Littoral	30.4	0.3	29.3	7.2	37.5	4.1	0.7	0.3	6.1	0.4
		Pelagic	30.0	0.5	31.0	6.1	37.3	1.7	1.4	0.4	6.2	0.1
Varal	0.877	Littoral	29.6	1.3	25.1	10.5	40.5	7.6	1.0	0.5	2.3	0.3
		Pelagic	30.1	1.0	20.9	9.6	49.8	10.3	2.5	0.9	2.3	0.2

richness, Shannon, Simpson and Berger–Parker indexes, respectively (Kindt *et al.* 2006).

Paired *t*-tests were used to compare species richness and zooplankton density for each group (testate amoebas, rotifers, cladocerans and copepods) using all 20 pairs of sampling points (pelagic and littoral regions). The assumption of normally distributed differences between pairs was tested before each analysis.

The number of species observed in relation to density was presented by species accumulation curves, which were constructed by sample-based rarefaction methods considering the expected species richness to be found, with a random sample of each possible sample size until the total sample size is reached. The equation for calculating the expected species richness ($E(S_n)$) is given by Gotelli and Graves (1996). The 95 % confidence interval was estimated according to Colwell *et al.* (2004). Two statistical approaches were selected to evaluate the expected species richness of the lakes. The nonparametric first-order jackknife estimator (Magurran 2004) was first used to compare it with the observed richness (using the 95% confidence intervals as a proxy of statistical differences). It is important to note the jackknife method does not always generate reliable estimates, as when the number of rare species continues to increase with the sampling effort – commonly observed in tropical environments – it may provide biased results (Melo 2004). Even considering this potential bias, this approach was retained in this study because it is widely used in community ecology. To evaluate the effects of an increased sampling effort, a method of extrapolating the observed total richness by a negative binomial estimator (based on the abundance distribution model of the same name) was then used, which is capable of generating accurate and non-biased estimates of species richness (Melo *et al.* 2007). For this approach, the expected total richness for sampling efforts that are 25%, 50%, 75% and 100% greater than those actually performed was calculated.

RESULTS

A total of 111 species and 3 805 549 zooplankton individuals were sampled from 40 sites. Cladocerans, copepods, rotifers and testate amoebas were represented by 16, 15, 61 and 19 species, respectively (Supporting Information).

The result of NMDS indicated a high compositional dissimilarity between lakes (Fig. 1). Comprido Lake and Brito Lake were relatively homogeneous in terms of community structure, while Luiz Alves Lake and Varal Lake were more heterogeneous. However, no difference in species composition between pelagic and littoral regions was observed.

Comprido Lake had the lowest species diversity (Fig. 2), independently of the value of α, despite the similarity to Varal Lake in terms of species richness (49 and 48 species, respectively). The other lakes had more similar diversity profiles. Starting from the parameter $\alpha = 4$, the diversity is greater in Brito and Varal than Luiz Alves. The diversity values from Brito lake, however, might be overestimated compared to the others, as it comprised twice the sampling effort used for the other three lakes. Thus, the values should be used solely as descriptors of the species diversity, but should not be directly compared to the values found for the other lakes.

Neither the number of species nor the abundances of the groups showed significant differences between the lakes' pelagic and littoral regions. The only exception was the density of copepods ($t = 2.4$; gl = 19, $P = 0.024$), which exhibited an average of 10 143 ind. m^{-3} (about

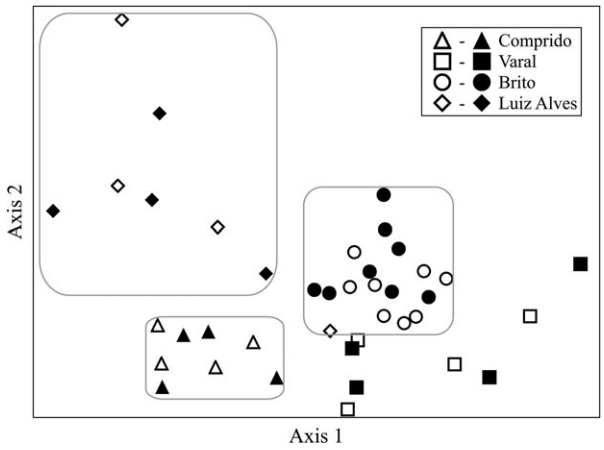

Fig. 1. Ordination by NMDS of 40 sites in relation to zooplankton community structure (open circles = pelagic region; filled circles = littoral region; STRESS = 0.177).

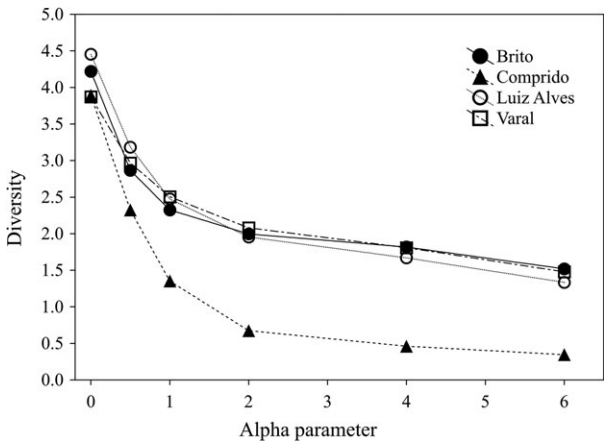

Fig. 2. Diversity profiles (Rényi series) for each lake (values from Brito lake should not be compared to the others because of the differences in sample sizes (see Results for details)).

35%) more in the pelagic regions, compared to the littoral regions. If the Bonferroni correction is applied, however, the new significance level will be 0.00625, suggesting that there is no strong evidence of differences in copepod density between both regions.

According to the jackknife estimator, the total species richness in lakes Comprido, Varal, Luiz Alves and Brito is 66, 59, 83, and 114 species, respectively. Based on these values, 75%, 81%, 82% and 75% of the total species richness expected in lakes Comprido, Varal, Luiz Alves and Brito were sampled, respectively (Fig. 3).

The species accumulation curves and their subsequent extrapolation (Fig. 4) exhibited a very similar result to that provided by the jackknife estimates. In this case, the richness found for lakes Comprido, Varal, Luiz Alves and Brito would be 66, 57, 79 and 112, respectively. Thus, sampling designs comprising only one site per lake were able to obtain, on average, only about 35% of the existing richness, while three sites would sample about 54% of the total species richness. Finally, at least seven sites would be needed to obtain sample approximately 70% of the total species richness for a lake.

DISCUSSION

The composition of zooplankton varied idiosyncratically among lakes, as they exhibited different compositions and species diversity patterns. Even though located on the same floodplain, watercourse distance between the four lakes studied is large, ranging between 10.5 km (lakes Luis Alves and Brito) and 68 km (lakes Luis Alves and Comprido), with an average distance of 39.2 km. Moreover, the lakes exhibit different levels of environmental preservation, with two being more impacted (Luis Alves and Brito) because they are located near the district of Luis Alves – Araguaia, with the other two being more preserved (Comprido and Varal, located near Bananal Island). Nevertheless, all the lakes belong to the Environmental Preservation Area of the Araguaia River Meanders.

Aquatic macrophytes in littoral regions increase the area for colonization, slow the current flow and increase detritus and epiphytic growth, thereby increasing environmental heterogeneity (Taniguchi *et al.* 2003; Vieira *et al.* 2007; Thomaz *et al.* 2008). Thus, it is predicted that littoral regions containing large quantities of macrophytes will have a greater zooplankton diversity than limnetic areas. The results of the present study, however, do not support this prediction as the richness and density of the

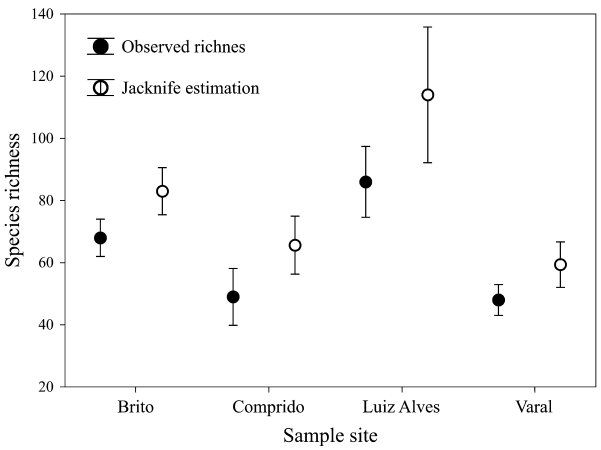

Fig. 3. Observed species richness (filled circles) and richness predicted by jackknife method (open circles) for four sampled lakes (whiskers = 95% confidence interval).

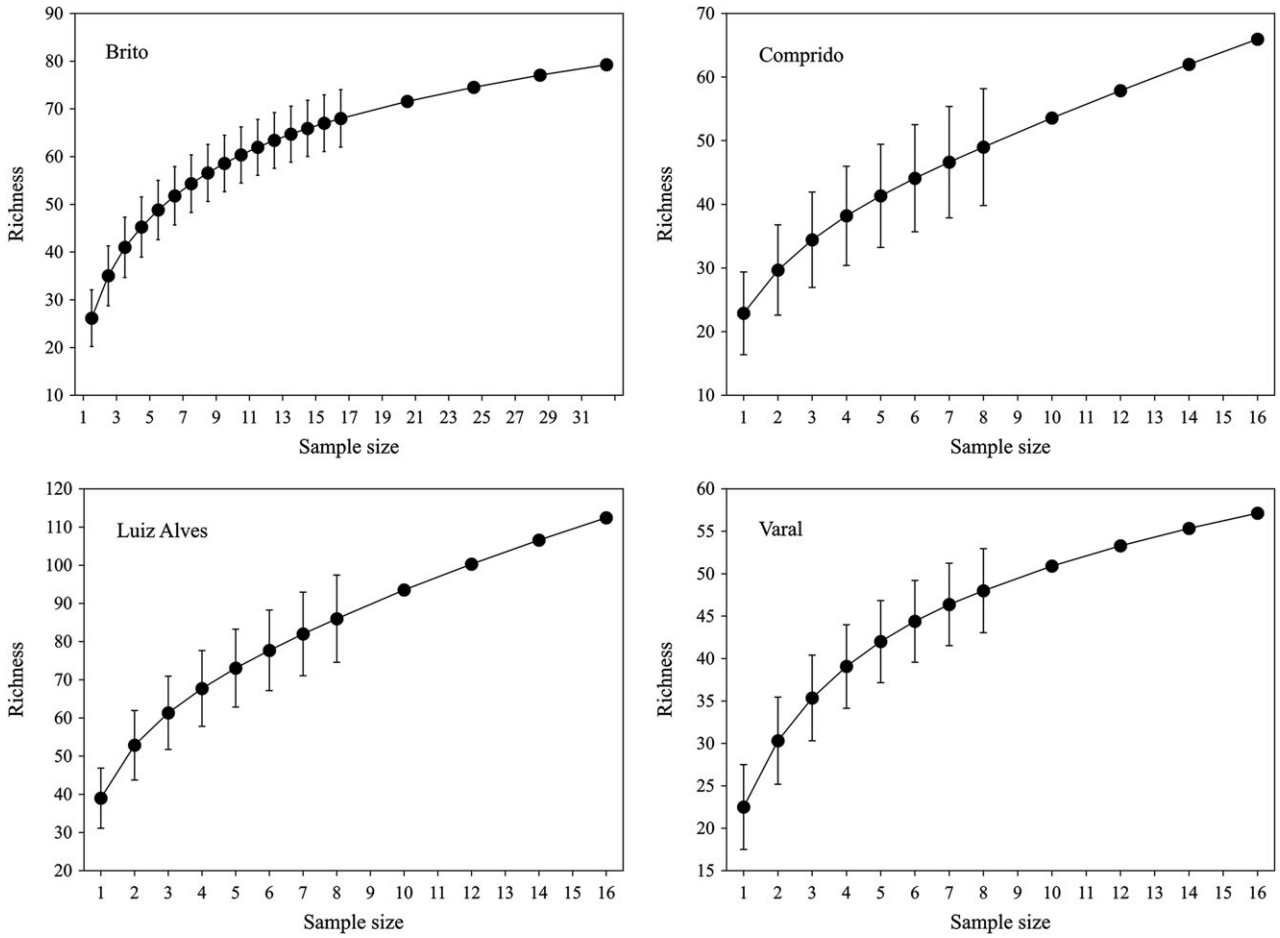

Fig. 4. Species accumulation curves constructed by rarefaction method (circles with 95% confidence interval) and extrapolation of expected richness for sampling efforts 25%, 50%, 75% and 100% higher than the observed values, as estimated by negative binomial estimator (circles without 95% confidence interval).

organisms did not differ between the littoral and pelagic regions. This was somewhat a surprising result, as Gasith and Hoyer (1998) suggested that even small paths of aquatic plants may be important to structure local aquatic communities.

The spatial structure of the zooplankton community, both in a local and regional context, can be explained by a combination of dispersal processes and environmental filtering caused by variations in local environmental conditions (Beisner *et al.* 2006; Lopes *et al.* 2011). Environmental and spatial predictors, however, are often unable to explain, for example, large proportions of the variance in community structure (Beisner *et al.* 2006). High residual values may be attributed to the sampling effort used to characterize the zooplankton community. The present study indicates the zooplankton community has a high spatial heterogeneity and, therefore, one site per lake would be insufficient to estimate the species richness and properly represent the community structure. Nevertheless, the use of a single site per lake is still a standard procedure in most studies involving zooplankton (e.g. see Górski *et al.* 2013; Vadadi-Fülöp 2013). According to our results, only sampling designs that include at least seven sites in each lake in the Araguaia floodplain would be enough to sample 70% of the total species richness.

The results of the present study also suggest that studies with low sampling efforts run the risk of significantly underestimating zooplankton diversity. A larger number of sites per lake is recommended, therefore, or, alternatively, water subsamples from different lake compartments should be filtered (e.g. as seen for Medley & Havel 2007).

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Zooplankton data. SD = standard deviation.