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Evaluation of a new sampling method for assessing Cladocera richness (Crustacea, Branchiopoda) in macrophyte-rich wetlands

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Abstract – The wetlands found in the Brazilian Cerrado are poorly studied environments regarding ecological aspects. Assessing the diversity of aquatic invertebrates in wetlands is a challenging task, since there are no standard sampling methods that minimize the spatial effects caused by macrophytes. The aim of this study was to evaluate the efficiency of a new sampling method for assessing Cladocera richness in macrophyte-rich wetlands of Brazilian Cerrado. In six wetlands, one transect was established which corresponded to a gradient of depth or change in aquatic vegetation. Samples containing cladocerans were collected using plankton net dragged among aquatic vegetation in the dry and rainy seasons. The species accumulation curves using non-parametric estimators and the overestimation of richness were used to determine the sampling efficiency. The species accumulation curves showed different asymptotic trends regarding the season and wetland studied. Especially in the rainy season, an asymptotic trend was not observed in two of the wetlands studied, which may reflect the influence of seasonality on Cladocera assemblage. Nevertheless, the overestimation of species richness showed that the method of sampling was able to find more than 60% of the estimated species richness, regardless of season or wetland studied. These results indicate that the method employed for sampling Cladocera in the Cerrado wetlands can be considered adequate.

Key words: Cerrado / microcrustaceans / preserved areas / rainy season / spatial heterogeneity

Introduction

In the current context of natural environment degradation, recognizing the biodiversity within ecosystems has become an indispensable tool for monitoring and maintenance of ecological processes. Undoubtedly, getting the number of species of a particular plot or ecosystem is the simplest and most widely used measurement to characterize the diversity of ecological assemblages (Magurran, 2004). For practical reasons, for any study of diversity it is almost impossible to record all the species present in an assemblage, because it requires an extensive sampling effort on temporal and spatial scales (Gotelli and Colwell, 2001; Gonzáles-Oreja *et al*., 2010; Glowacki, 2011).

Currently, several studies about diversity have used and recommended the non-parametric estimators of species richness to minimize the effects of biased sampling on the final results for the number of species (*e.g*., Petersen *et al*., 2003; Caterino, 2007; Merlo *et al*., 2010; Zagmajster *et al*., 2010). These methods for estimation can be used for comparisons of the analysis of ecological assemblages, since they are obtained through abundance or incidence data (Magurran, 2004); they can also be applied to biotic groups where the number of individuals is difficult to assess, especially in modular organisms, colonies and clones (Williams *et al*., 2007; Cruz *et al*., 2008; Gotelli and Colwell, 2010). Another important issue is that, hypothetically, the non-parametric estimators of species richness produce good estimates even for a small set of samples or individuals, and their results can be used as an assessment tool in strategies for species inventories (Magurran and Queiroz, 2010).

The Brazilian tropical savanna, known as Cerrado, is considered a hotspot widely recognized by its high level of endemism (Klink and Machado, 2005). This in turn suggests that wetlands included in this biome could harbor

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a high diversity of species, as shown by Reid (1984, 1987, 1993, 1994) to microcrustaceans of the suborder Copepoda. Their potential contribution to biodiversity puts the wetlands of the Brazilian Cerrado among the priority target areas for conservation. Nevertheless, the presence of any kind of aquatic ecosystem is not a major criterion in the choice of conservation areas, since they are designed through studies of terrestrial flora and fauna. Thus, aquatic biodiversity has not been the focus of conservation strategies, which can lead to a considerable loss of species richness of this region (Agostinho *et al.*, 2005).

According to the Ramsar Convention (information paper No. 1), wetlands that occur on upwelling groundwater or where there are water-saturated soils are categorized as paludal. This type of wetland is widely distributed in the Brazilian Cerrado, and it is considered a heterogeneous ecosystem due to the presence of large numbers of plant species with different life forms and morphological architectures. These characteristics favor the association of a high diversity of taxonomic groups for differential diversification of ecological niches and habitats (Thomaz and Cunha, 2010).

However, knowledge about any ecological aspect of the wetlands of the Brazilian Cerrado is largely neglected. In case of Cladocera, which the most species live in association with aquatic macrophytes (Forró *et al.*, 2008), the information is only preliminary (Elmoor-Loureiro, 2007; Sousa and Elmoor-Loureiro, 2008). These studies presented the species richness; however, there was no attempt to assess the sampling effort because no systematized methods was used for data collection.

Some sampling methods for different groups of microfauna associated with macrophytes, including cladocerans, have been proposed in the literature. Campbell *et al.* (1982) tested the use of a hand-operated vacuum pump for sampling Cladocera associated exclusively with *Chara* sp. and *Hydrolea ovata*. Sakuma *et al.* (2002) suggested two methods based on sampling of submerged macrophytes using bottles to obtain the phytophilous fauna. Ferreira *et al.* (2008) used three different methods to sample environments dominated by macrophytes (Jar, Manual Removal and Ekman Dredge) and concluded that the abundance and richness of the biota groups were dependent of the method used. Loutte *et al.* (2008) used a sampler tube for the collecting cladocerans in dominated macrophytes ponds. Standard methods for sampling zooplankton have also been used (Maia-Barbosa *et al.*, 2008; Kruk *et al.*, 2009). These sampling methods may not be appropriate for particular environments, such as shallow wetlands macrophytes rich, because greater effort would be needed to try to minimize the possible effects of spatial scales, such as depth, architecture and richness of macrophytes (Vieira *et al.*, 2007; Thomaz *et al.*, 2008; Hansen *et al.*, 2011; Lucena-Moya and Duggan, 2011).

Furthermore, other studies reveled the influence of structural complexity provided by macrophytes to small body animals (such as Cladocera). The main feature pointed is the density increasing because of the decline on predation rate in function of the refuge zones provides by aquatic vegetation (Thomaz *et al.*, 2008; Padial *et al.*, 2009; Thomaz and Cunha, 2010; Mormul *et al.*, 2011). This feature, undoubtedly influence the diversity of species and need to be considered in sampling methods that aim survey biodiversity data.

In the past 3 years, research projects on biodiversity of the Cerrado have focused on the standardization of sampling techniques for assessment in aquatic ecosystems in order to generate data that can be used in comparisons of richness and diversity. The aim of this study was to evaluate the efficiency of a new sampling method for assessing Cladocera richness in macrophyte-rich pristine wetlands of Brazilian Cerrado.

Methods

Study area

The study was carried out in Central Brazil, in two preserved areas of Cerrado: Brasília's National Park (BNP) and the Formosa's Instructional Camp (FIC) (Fig. 1). The BNP has a total area of 42 389 ha, making it the largest Conservation Unit in the Federal District, Brazil. The aquatic ecosystems of BNP belong to the upper Paraná River basin and are represented mainly by headstreams, but several natural wetlands formed by upwelling groundwater are also found. For this study, three paludal wetlands were sampled: Henrique Pond (HE) (15°41'18"S, 47°56'10"W), Exército Pond (EX) (15°44'44.3"S, 47°58'49.1"W) and Peito de Moça (PM) (15°45'05.8"S, 48°01'33.2"W). These wetlands have many species of macrophytes occupying more than 80% of the water surface. Examples of species commonly found are: *Rhynchospora globosa* (Kunth) Roem. & Schult, *Cyperus haspan* L. and *Cyperus articulatus* L. The richness of macrophytes is considered higher, with EX presenting 19 species, HE 26 species and PM 41 species (see Sousa, 2012).

The FIC is located in the state of Goiás and is a training camp for the Brazilian Army. It is bordered to the east by the Federal District and to the south by Minas Gerais state. The FIC is a large preserved fragment of Cerrado and has several types of aquatic ecosystems, such as lotic systems and wetlands, belonging to the upper São Francisco River basin. In this study, we sampled the following natural paludal wetlands: Cabocla I Pond (CBI) (15°48'16.6"S, 47°14'58.8"W), Cabocla II Pond (CBII) (15°48'22.6"S, 47°14'10.6"W) and Grande Pond (GR) (15°49'37.3"S, 4713'50.8"W). These wetlands also have numerous species of macrophyte, for example, *Eleocharis capillaceae* Kunth, *Eleocharis minima* Kunth, *Scleria hirtella* Sw. and *C. articulatus* L. In the total, CBI presented 44 species of macrophytes, CBII 16 species and GR 22 species (see Sousa, 2012).

The wetlands studied are classified as inland wetlands featuring a relatively stable water level (Source: National Institute of Science and Technology on Wetlands), but they are subject to small fluctuations

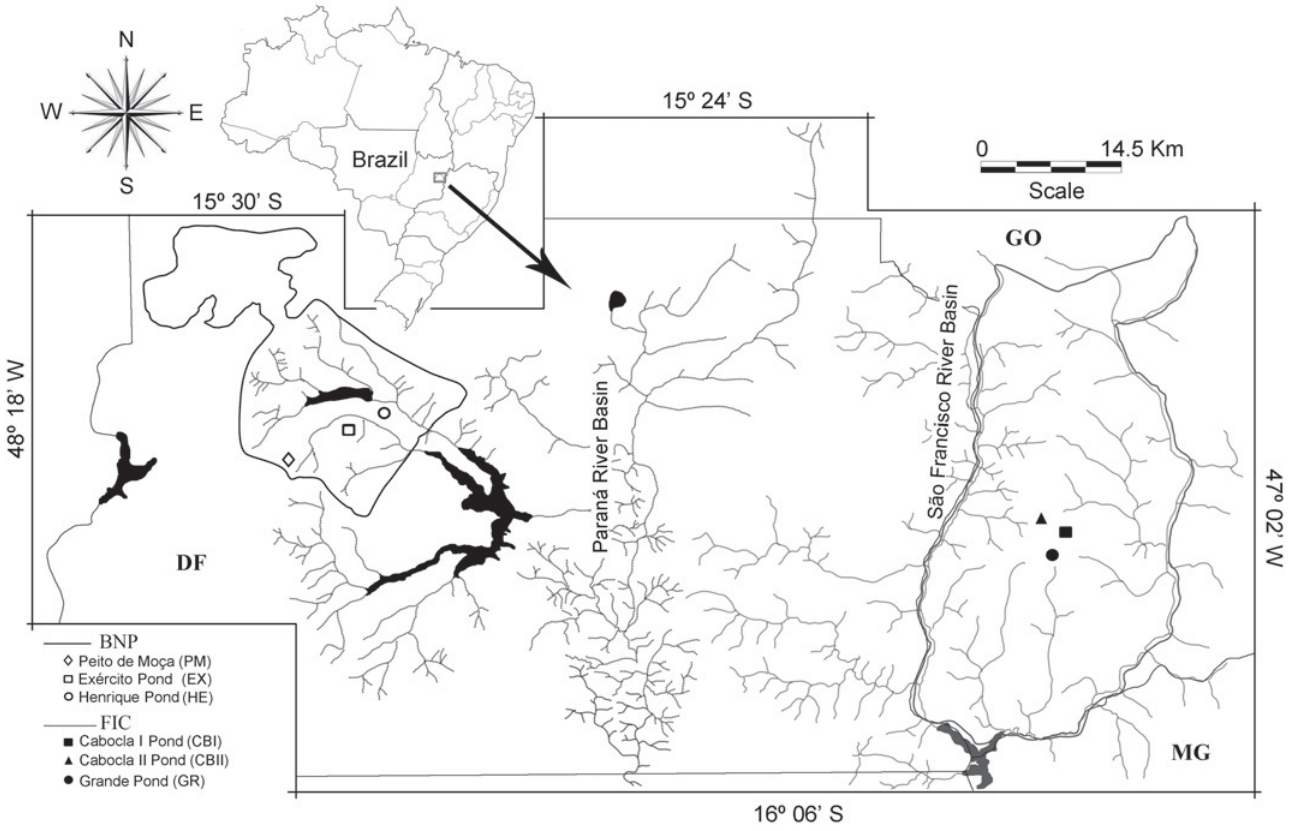

Fig. 1. Location of BNP and FIC, their drainage basins (Paraná and São Francisco) and paludal wetlands studied.

Table 1. Transects parameters in the studied wetlands: number of sampling points, depth average, maximum and minimum length of the transects. BNP – Brasília's National Park and FIC – Formosa's Instructional Camp.

		Transects				
Codes	Wetlands	Number of sampling points dry season	Number of sampling points rainy season	Depth (m) dry season	Depth (m) rainy season	Maximum length (m)
	BNP					
HE	Henrique Pond	5	5	0.58 (0.05–1.03)	0.73 (0.25–1.20)	46
EX	Exército Pond	5	5	0.19 (0.01–0.90)	0.33 (0.15–1.00)	50
PM	Peito de Moça	3	3	0.05 (0.05–0.05)	0.05 (0.05–0.05)	50
	FIC					
CBI	Cabocla I Pond	4	4	0.32 (0.03–0.62)	0.43 (0.18–0.71)	50
CBII	Cabocla II Pond	5	5	0.50 (0.11–0.96)	0.65 (0.30–1.14)	50
GR	Grande Pond	4	4	0.60 (0.26–1.17)	0.83 (0.42–1.30)	45

in the water level between seasons. These wetlands are represented by moist grasslands, earth mounds (*murundus*) and shallow ponds. The wetlands are pristine, oligothrophic and are located in grasslands physiognomies of the Cerrado. No information about the other biota groups for theses environments are available.

The two preserved areas are under typical climatic conditions of the Cerrado domain (Aw, rainy tropical, according to the Köppen classification), marked by strong seasonality. The dry season occur between May and September, presenting lower temperatures. The rainy season (warmer period) from October to April (Sano *et al.*, 2008). The range of temperature for this region was 19–23 °C and precipitation 4–272 mm, according to the National Institute of Meteorology; historical series between 1961 and 2009.

Inventory method

In each wetland, a transect was installed from the lowest depth of water table toward the center of the water body. Sampling points were established by changes in the depth or in macrophyte assemblage (Table 1). Therefore, the distance between sampling points were not fixed. In

Table 2. Mean, standard deviation and maximum and minimum values for limnological variables measured in the dry and rainy season in wetlands sampled in Brasília's National Park (BNP) and Formosa's Instructional Camp (FIC).

	BNP						FIC					
	Dry season			Rainy season			Dry season			Rainy season		
Variables	HE	EX	PM	HE	EX	PM	CBI	CBII	GR	CBI	CBII	GR
Temperature (°C)	22.7 ± 1.0 (21.2–23.9)	23.4 ± 1.2 (22.4–25.2)	22.7 ± 1.9 (20.6–24.1)	28.3 ± 0.5 (27.8–29)	25.2 ± 1.9 (22.3–27.3)	25.7 ± 3.4 (23.4–29.6)	24.5 ± 13.0 (22.9–26.2)	19 ± 1.5 (17.0–20.5)	23.3 ± 2.3 (205.0–25.2)	25.1 ± 0.7 (24.1–25.8)	25.7 ± 0.9 (24.5–26.7)	31.0 ± 0.3 (30.7–31.4)
pH	5.2 ± 0.2 (4.9–5.5)	5.3 ± 0.2 (5.2–5.5)	5.6 ± 0.6 (5.1–6.2)	5 ± 0.1 (4.9–5.2)	5.7 ± 0.6 (5.1–6.6)	5.3 ± 0.3 (5.1–5.6)	4.6 ± 0.5 (3.9–52)	5.2 ± 0.2 (4.9–53)	5.5 ± 0.3 (5.2–5.9)	5.4 ± 0 (5.38–5.43)	5.6 ± 0.2 (5.3–5.8)	5.9 ± 0.6 (5.6–6.8)
Dissolved oxygen ($mg.L^{-1}$)	4.8 ± 1.0 (3.6–6.3)	5.4 ± 0.3 (4.9–5.9)	4.9 ± 0.9 (4.1–5.8)	5 ± 0.6 (4.2–5.6)	5.6 ± 1.4 (3.8–7.0)	5.1 ± 0.1 (4.9–5.2)	5.3 ± 0.6 (4.7–6.0)	4.2 ± 0.7 (3.0–4.9)	5.2 ± 1.2 (5.9–3.5)	4.1 ± 0.4 (3.7–4.6)	3.3 ± 0.5 (2.7–38)	4.4 ± 1.1 (4.2–4.5)
Conductivity ($\mu s.cm^{-1}$)	5.3 ± 2.9 (2.8–8.9)	4.5 ± 0.9 (3.3–5.7)	6.7 ± 1.4 (5.2–7.9)	4.6 ± 0.8 (3.9–5.9)	5.1 ± 1.6 (3.7–6.9)	8.8 ± 3.9 (5.4–13.1)	6.2 ± 2.2 (4.8–9.4)	4.5 ± 0.6 (3.8–5.2)	4.4 ± 3.1 (2.7–9.0)	5.3 ± 0.7 (4.6–6.3)	5.7 ± 0.9 (4.8–6.7)	5.4 ± 1.7 (3.9–7.3)

See Table 1 for code names of the wetlands.

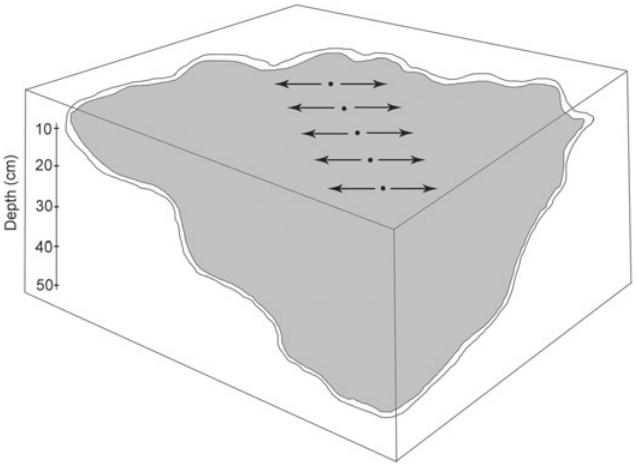

Fig. 2. Schematic diagram showing the formation of transects in each wetland studied. The points refer to the places where the limnological variables were measured and the arrows the range where the samples for Cladocera fauna were taken.

each sampling point, a perpendicular line up to 24 m was established for data collection (Fig. 2). Depth and some physical and chemical variables of water were measured (Table 2) and Cladocera assemblage was collected.

Samplings were conducted concentrated in the two seasonal periods (dry season, July and August 2009; and rainy season, November and December 2009). The ranges in size of the perpendicular lines to the sampling points were used in order to cover most of the spatial variability in the wetlands.

Collection, sorting and identification of Cladocera

The fauna was obtained using a plankton net with mesh size of 80 μm, dragged among the aquatic vegetation four times on the way up to 24 m at each point of the transect. As the wetlands studied are shallow, dragging net allowed to sample macrophytes with different life forms (rooted emergent plants, rooted submerged plants and floating plants) and structures that they offer for association of invertebrates, as roots, stems, petioles and leaves. Furthermore, the drag was used to capture species swimming in the water among vegetation, such as Daphnidae and some Sididae. The animals were anesthetized in carbonated water, and then fixed in ethanol with final proportion of 70%. At each point within transects, a sample was collected and aliquots with volume of 4 ml were examined under stereomicroscope until stabilization of species richness.

All individuals obtained were identified with the support of taxonomic references (Smirnov, 1992, 1996; Elmoor-Loureiro, 1997; Kotov *et al.*, 2004; Kotov and Štifter, 2006; Sinev and Elmoor-Loureiro, 2010; Van Damme *et al.*, 2010, 2011). The voucher specimens are deposited at the Laboratory of Aquatic Biodiversity of the Catholic University of Brasília.

Table 3. List of species of Cladocera (Crustacea) found in the studied wetlands in Brasília's National Park (BNP) and Formosa's Instructional Camp (FIC).

	Dry season						Rainy season					
Taxa	HE	EX	PM	CBI	CBII	GR	HE	EX	PM	CBI	CBII	GR
Sididae Baird, 1850												
Latonopsis australis-group					•	•	•				•	•
Daphniidae Straus, 1829												
Ceriodaphnia cornuta (Sars, 1886)					•		•					
Ceriodaphnia sp1					•						•	
Ceriodaphnia sp2											•	
Ilyocryptidae Smirnov, 1992												
Ilyocryptus spinifer (Herrick, 1882)	•	•	•	•	•	•	•	•	•	•	•	•
Macrothricidae (Norman and Brady, 1867)												
Macrothrix elegans (Sars, 1901)						•						•
Macrothrix paulensis (Sars, 1900)	•			•	•		•	•		•	•	•
Streblocerus pygmaeus (Sars, 1901)		•			•			•			•	•
Chydoridae Stebbing, 1902												
Acroperus tupinamba Sinev and Elmoor-Loureiro, 2010			•	•						•		
Alona dentifera (Sars, 1901)	•				•							
Alona glabra (Sars, 1901)											•	
Alona iheringula (Sars, 1901)	•	•	•	•	•	•	•	•		•	•	•
Alona intermedia (Sars, 1862)	•	•			•	•					•	•
Alona ossiani (Sinev, 1998)	•	•	•	•	•	•	•	•	•	•	•	•
Alona setigera (Brehm, 1931)	•	•	•	•			•	•			•	
Alona sp.			•									
Alonella clathratula (Sars, 1896)	•	•		•	•	•	•	•		•	•	•
Alonella dadayi (Birge, 1910)	•	•		•	•		•	•		•	•	•
Anthalona verrucosa (Sars, 1901)	•			•		•	•	•		•	•	•
Celsinotum candango Sinev and Elmoor-Loureiro, 2010	•						•	•				
Chydorus dentifer (Daday, 1905)				•		•						•
Chydorus eurynotus (Sars, 1901)				•	•	•	•	•		•	•	•
Chydorus pubescens (Sars, 1901)	•					•	•				•	•
Disparalona leptorhyncha Smirnov, 1996						•						•
Dunnhevedia odontoplax (Sars, 1901)				•								
Ephemeroporus sp.	•				•	•	•	•			•	•
Ephemeroporus barroisi (Richard, 1984)	•	•		•	•	•	•	•		•	•	•
Graptoleberis occidentalis (Sars, 1901)	•	•		•	•	•	•			•	•	•
Karualona muelleri (Richard, 1897)	•					•		•				•
Leydigiopsis curvirostris (Sars, 1901)	•						•				•	
Notoalona sculpta (Sars, 1901)	•											

See Table 1 for code names of the wetlands.

Data analyses

The number of species observed in each wetland was compared in the dry season and rainy season using one-way ANOVA – followed by Tukey test. These analyses were performed using the software PAST (Hammer *et al.*, 2001). In order to avoid violations the of normality and homoscedasticity assumptions, data were transformed using log $(x + 1)$.

Individual-based non-parametric estimators of species richness (ACE, Chao1 and first-order Jackknife) were calculated to assess the total richness in each wetland using EstimateS 8.2 (Colwell, 2009). In general, these estimators perform an approximation of total richness through comparisons between rare species (singletons) and species shared between at least two samples (doubletons). In this study, singletons species with less than ten individuals and doubletons species with less than ten individual shared between two samples were considered. The formulas for the calculation of the estimators are found in Gotelli and Colwell (2010).

Individual-based rarefactions were generated to accumulate the number of species for each wetland (Gotelli and Colwell, 2001). The individual-based rarefaction method was used to reduce possible effects of sampling. To assess whether the number of species was close to the total expected for the wetlands, species accumulation curves were constructed and trends to asymptote were analyzed (Colwell and Coddington, 1994; Gotelli and Colwell, 2010).

Results

A total of 31 species were observed, belonging to five families (Table 3). In the BNP, difference in mean richness

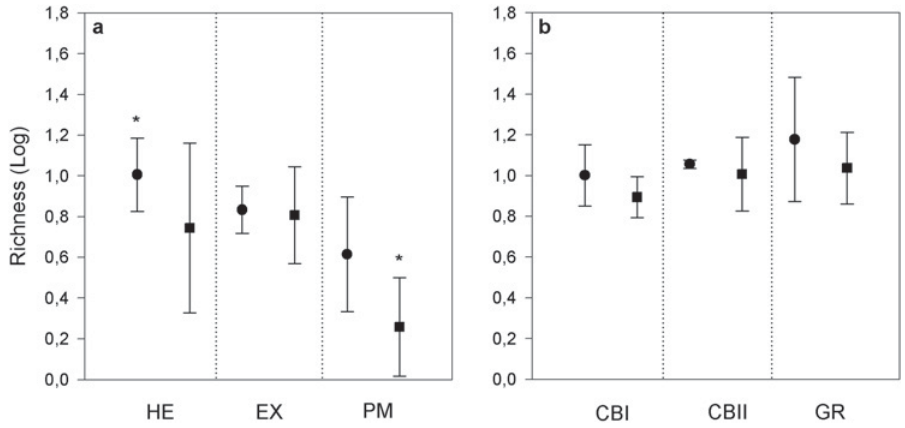

Fig. 3. Log of the mean and standard error of the number of species observed in the studied wetlands. (a) Wetlands of BNP and (b) wetlands of FIC. HE – Henrique pond; EX – Exército pond; PM – Peito de moça; CBI – Cabocla I pond; CBII – Cabocla II pond and GR – Grande pond. Dots – dry season; square – rainy season *significant difference in species richness $P < 0.05$.

between HE and PM was observed between dry and rainy seasons ($F = 3.642$; $P = 0.0287$; Tukey test: $P = 0.01$) (Fig. 3(a)). The trend of the species accumulation curves for the estimators was not similar between wetlands of the BNP. For example, in HE both estimators in the dry season reached the asymptote at just over 100 individuals sampled, where the curves of singletons and doubletons visibly declined with an increase in the number of individuals (Fig. 4(a)).

In EX, it was possible to observe the formation of the asymptote in the dry season at approximately 100 individuals, but, as the curve of singletons increased, the species accumulation curves corresponding to the estimators did not reach an asymptote and visibly tended to increase (Fig. 4(c)). In the rainy season, only Jackk1 approached asymptotic behavior (Fig. 4(d)). However, in the PM, Jackk1 was the only estimator to show asymptotic behavior in the dry season (Fig. 4(e)). Accumulation of species curves was not performed in the rainy season for PM because only 11 individuals were found for the set samples, while the presence of at least 20 individuals is recommended for the construction of these curves (Gotelli and Colwell, 2010).

In the wetlands of the FIC, there was no difference in mean richness observed (Fig. 3(b)) ($F = 1.27$; $P = 0.378$). In CBI, it was observed that the species accumulation curves reached asymptote at just over 200 individuals in the dry season (Fig. 5(a)). In the rainy season, asymptote was observed for all curves (Fig. 5(b)). In the dry season, CBII reached asymptote, and the singleton and doubleton curves showed differences, with an increasing tendency for singletons and visibly declining after 300 individuals for doubletons (Fig. 5(c)). In the rainy season, only the accumulation curve for Jackk1 reached asymptote, probably influenced by doubletons, which decreased (Fig. 5(d)).

Still for the FIC, the wetland named GR obtained asymptote only in the species accumulation curves for Jackk1, and singletons and doubletons declined after 250 individuals (Fig. 5(e)). In the rainy season, none of the richness estimators reached asymptote (Fig. 5(f)).

The application of estimators for the studied wetlands predicted that, depending on the area, on the dry and rainy seasons and the estimator used, between 5 and 35% of species estimated for the studied areas were not accessed (Table 4–6). This means that it was possible to access a sufficient number of species for wetlands studies here. According to Heck *et al.* (1975), a survey of species could be considered satisfactory when are obtained between 50 and 70% of species that potentially occur in a plot or ecosystem.

Discussion

Studies of wetlands have been conducted in Brazil on large floodplains, such as the Paraná River floodplain and the Pantanal (*e.g.*, Roberto *et al.*, 2009; Alho, 2011). These two large Brazilian wetlands have extremely rich Cladocera fauna, with estimates ranging between 60 and 85 species (Hollwedel *et al.*, 2003; Serafim-Júnior *et al.*, 2003; Junk *et al.*, 2006). However, it would be wrong to compare richness obtained in this study to the results of large wetlands, due to differences in spatial and temporal scales, since these large wetlands have been the focus of studies for over 20 years.

Also evaluating shallow wetlands that were densely covered by macrophytes in the Paranã River basin, Goiás state, Elmoor-Loureiro (2007) reported richness values between 4 and 19. In another study in shallow wetlands in the Brazilian Cerrado, Sousa and Elmoor-Loureiro (2008) found 11 species in the richest site and four in the poorest site in an inventory of Cladocera fauna conducted in the Emas National Park, also in Goiás state. Their results are quite similar to the ones reported by this study the BNP and FIC (Tables 4 and 5), and it is noticeable that the number of species is very similar. However, the lack of standardization in the sampling does not allow making comparisons with results found in Elmoor-Loureiro (2007) and Sousa and Elmoor-Loureiro (2008).

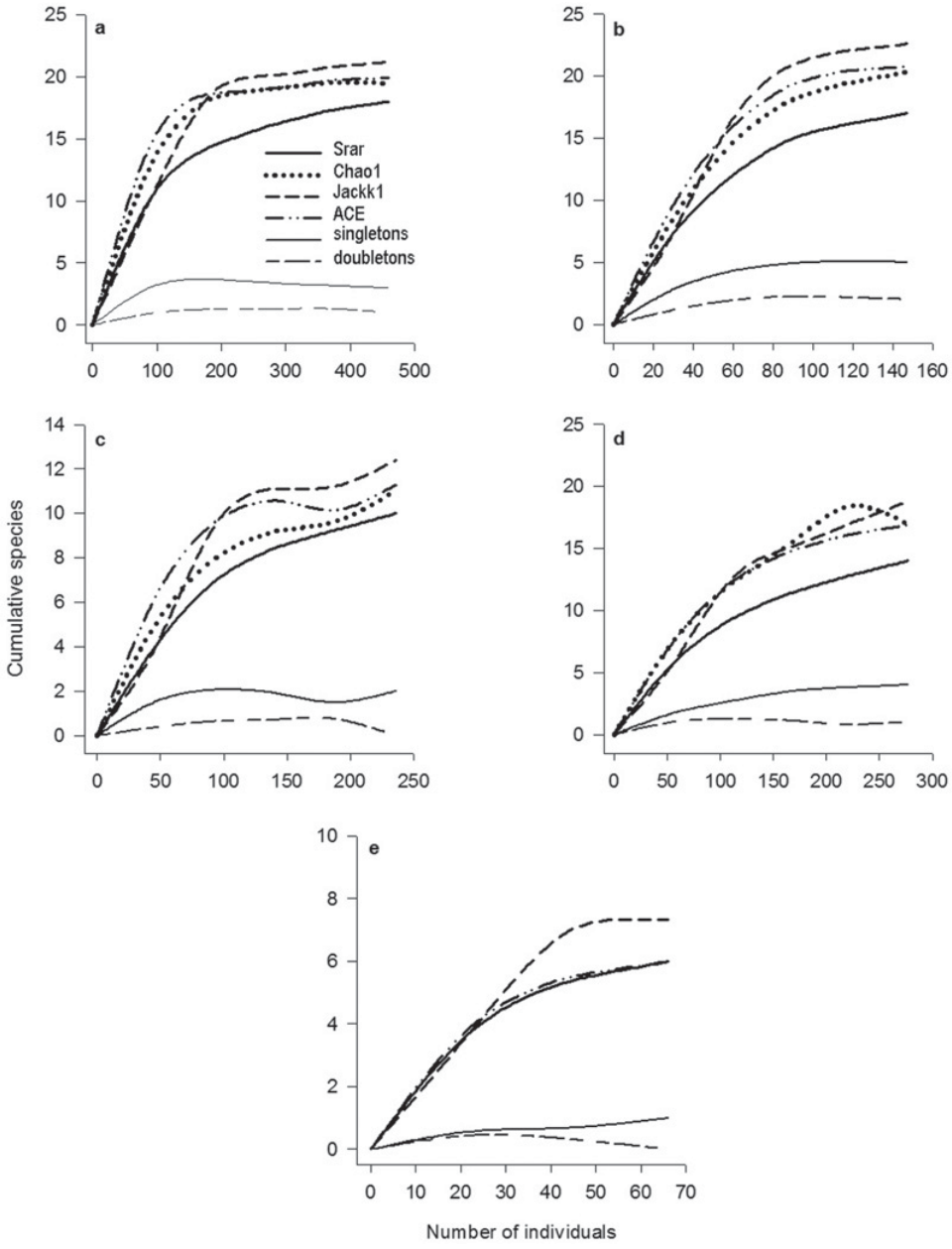

Fig. 4. Species accumulation curves for wetlands in BNP. (a) HE dry season, (b) HE rainy season, (c) EX dry season, (d) EX rainy season and (e) PM dry season.

Non-parametric estimators have been used to assess the richness of aquatic invertebrates, and the results are always given by overestimation of observed richness (Melo and Froehlich, 2001; Muirhead *et al.*, 2006; Turki and Turki, 2010), but the asymptotic trends of species accumulation curves for these estimators can indicate whether results are representative. We observed the asymptote of the species accumulation curves for the estimators in wetlands studied here (Figs. 4 and 5). The trend of the species accumulation curves for estimators is a function of the numbers of rare species (Melo, 2004). In all areas where curves reached asymptote at least for one estimator, it is possible to observe a decrease, or at least a stabilization of the curves of rare species (singletons and doubletons), indicating that the likelihood of new species being added is low, when sampling effort is increased (Melo, 2004).

The use of non-parametric estimators can also reveal failures in the methods of sampling, as some studies have already indicated (Muirhead *et al.*, 2006; Cardoso *et al.*, 2009; Zagmajster *et al.*, 2010) and as noted in the dry season for EX and in the rainy season for GR and CBII because of the non-formation of asymptote of species in accumulation curves. Specifically for wetlands, spatial heterogeneity seems to be an important factor that can lead to failures in sampling (Kaeser and Kirkman, 2009). For the wetlands studied here, a factor of heterogeneity are the macrophytes, which are responsible for increasing

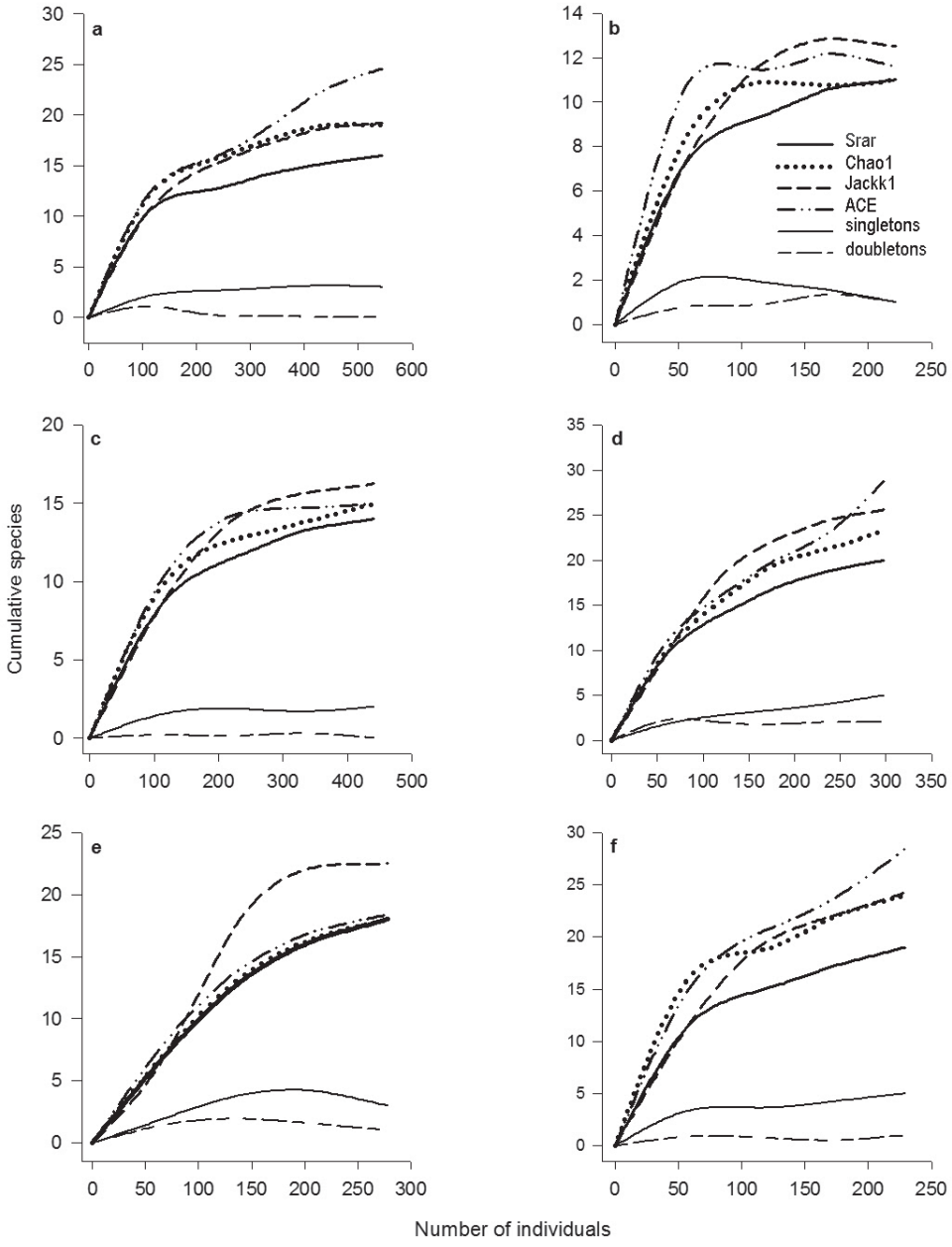

Fig. 5. Species accumulation curves for wetlands in the FIC. (a) CBI pond dry season, (b) CBI pond rainy season, (c) CBII pond dry season, (d) CBII pond rainy season, (e) GR pond dry season and (f) GR pond rainy season.

the spatial variability in aquatic environments, due to their high number of species, life forms and architecture (see Sousa, 2012). It is possible that we did not assess some aspect from the complexity provided by aquatic vegetation in the sampling method used.

Another important factor is the seasonal climate which is, admittedly, characteristic of the Cerrado biome and influences the biological assemblages (Ledru, 2002). Fluctuations in water level are responsible for driving successional process of vegetation in response to seasonality (Middleton, 2002; Odland and Moral, 2002). Fluctuations of small magnitude (lateral expansion of wetland and small changes in water level in the rainy season) are related to increased abundance of vegetation and formation of zones in different stages of succession (van der Valk, 2006; Moore, 2007). As the structure of invertebrates assemblages is linked to changes in vegetation, we considered that fluctuation in the depth, as well as the lateral expansion of the surface water, may favor the development of new species of macrophytes (van der Valk, 2006), expanding also the area of their colonization and, then, increasing the spatial variability in the rainy season.

Furthermore, the dilutive effect of water on the assemblage in the rainy season requires an increase in sampling effort in order to expand the number of collected species, because this probably affects the dispersion. The effect of the rainy season on sampling can be found in

Table 4. Species richness in wetlands sampled in Brasília's National Park (BNP), Federal District.

	BNP					
	Dry season			Rainy season		
	HE	EX	PM	HE	EX	PM
Samples	5	5	3	5	5	3
Individuals	459	263	66	147	277	11
Srar	18 (±1.27)	10 (±1.63)	6 (±0.64)	17 (±1.78)	14 (±2.18)	2 (±0.53)
Singletons	3	2	0	5	4	–
Doubletons	1	0	1	2	1	–
Chao1	19.50 (±2.60)	11 (±2.27)	6 (±0.01)	22.60 (±3.49)	17 (±4.18)	–
Jackk1	21.20 (±1.96)	12.40 (±1.60)	7.33 (±1.33)	20.33 (±4.13)	18.80 (±3.88)	–
ACE	19.91	11.27	6	20.77	16.88	–

See Table 1 for code names of the wetlands. Srar – rarefied richness; Jackk1 – first-order Jackknife.

Table 5. Species richness in wetlands sampled in the Formosa's Instructional Camp (FIC), Goiás.

	FIC					
	Dry season			Rainy season		
	CBI	CBII	GR	CBI	CBII	GR
Samples	4	5	4	4	5	4
Individuals	441	544	278	222	298	229
Srar	14 (±1.02)	16 (±1.37)	18 (±1.24)	11 (±0.60)	20 (±1.98)	19 (±1.92)
Singletons	2	3	1	1	5	5
Doubletons	0	0	3	1	2	1
Chao1	15 (±2.29)	19 (±4.55)	18 (±0.53)	11 (±0.25)	23.30 (±4.13)	24 (±6.05)
Jackk1	16.25 (±1.44)	19.20 (±1.50)	22.50 (±1.50)	12.50 (±0.87)	25.60 (±2.04)	24.25 (±2.56)
ACE	15	24.57	18.39	11.60	28.75	28.40

See Table 1 for code names of the wetlands. Srar – rarefied richness; Jackk1 – first-order Jackknife.

Table 6. Mean percentage and standard deviation of overestimation for each estimator in the studied wetlands in the Cerrado of Central Brazil.

	Mean % overestimation					
	Dry season			Rainy season		
	Chao1	Jackk1	ACE	Chao1	Jackk1	ACE
HE	7.69 (±0.19)	15.09 (±0.29)	9.60	24.77 (±0.52)	16.37 (±0.67)	18.15
EX	9.09 (±0.20)	19.35 (±0.30)	11.26	17.65 (±0.73)	25.53 (±0.99)	17.06
PM	0	18.14 (±0.24)	0	–	–	–
CBI	6.66 (±0.15)	13.84 (±0.19)	6.66	0	12 (±0.10)	5.17
CBII	15.78 (±0.71)	16.66 (±0.24)	34.87	14.96 (±0.61)	21.87 (±0.44)	30.43
GR	0	20 (±0.30)	0	20.83 (±1.26)	21.64 (±0.55)	33.09

See Table 1 for code names of the wetlands. Srar – rarefied richness; Jackk1 – first-order Jackknife.

Table 6, where the percentage of richness overestimated was higher than in the dry season in most wetlands. Thus, we can indicate that seasonality is quite relevant to assessing the species richness of Cladocera in wetlands.

In conclusion, all results achieved showed efficiency in obtaining a good set of data using the sampling method described here, since that percentage of overestimation of richness showed that the sampling was adequate. This result can be related to two main features that reduced the effects of spatial variability found in the wetlands studied: (1) sampling in depth gradients and (2) sampling in different substrates involved with Cladocera association (macrophytes with complexity and life forms different). Although observed efficiency in the sampling method, the absence of asymptote in some species accumulation curves indicates the need for increased sampling effort, possibly as a consequence of spatial heterogeneity and seasonality. This can be minimized by increasing the length of transects, increasing the number of sampling points per transect, as well as increasing the distance traveled to drag the plankton net and obtain fauna. These procedures may be necessary during the rainy season, when there is an increase in spatial variability.

There are no standardized methods for collection of data in studies of microcrustacean fauna in wetlands, and different sampling techniques have been used (see Campbell *et al.*, 1982; Sakuma *et al.*, 2002; Ferreira *et al.*, 2008; Loutte *et al.*, 2008; Maia-Barbosa *et al.*, 2008; Kruk *et al.*, 2009). The usage of the same sampling method is an important strategy, since the lack of standardization in

data collection limits comparisons in studies of biodiversity. Although, developed for assessment of the Cladocera fauna in shallow wetlands, the sampling method proposed and evaluated in this study has great potential for assessing the richness of other groups of aquatic biota as Copepoda, Rotifera and Amoeba Testacea. The sampling method can be tested in ecosystem presenting higher cover aquatic vegetation, such as temporary ponds, littoral zone in reservoirs, lakes and shallow lakes.

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