Variability of d^{13}C and d^{15}N in Terrestrial and Aquatic Sources in The Upper Paraná River Basin, Paraná, Brazil.

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ABSTRACT - Variability of d^{13}C and d^{15}N in Terrestrial and Aquatic Sources in The Upper Paraná River Basin, Paraná, Brazil. The isotopic variability of riparian vegetation, C_3 and C_4 aquatic macrophytes, phytoplankton, periphyton and particulate organic carbon (POC) was investigated during the rainy (February and March 2000) and dry (August and September 2000) seasons in the Upper Paraná River Basin, including the Paraná River floodplain (Paraná, Baía and Ivinheima subsystems) and Itaipu Reservoir (lentic and lotic stretch). The mean values for the d^{13}C of riparian vegetation (–30.1 ± 1.32‰), C_4 aquatic macrophytes (–13.0 ± 0.87‰) and periphyton (–25.0 ± 3.41‰) were significantly different. The mean values for C_3 aquatic macrophytes (–28.42 ± 2.73 ‰) and POC (–27.2 ± 2.75‰) did not present significant differences between each other, they were significantly different from the riparian vegetation, C_4 aquatic macrophytes, periphyton and phytoplankton. Spatial variability of d^{13}C was identified in phytoplankton and POC. Seasonal influences were identified to phytoplankton and periphyton. Regarding d^{15}N values, riparian vegetation (2.09 ± 2.46‰) presented a significantly different isotopic mean from C_3 aquatic macrophytes (4.15 ± 3.88‰), phytoplankton (5.28 ± 2.43‰), periphyton (5.56 ± 3.13‰) and POC (4.78 ± 2.59‰). Significant spatial differences were recorded for the riparian vegetation and aquatic macrophytes. Seasonal effects occurred in phytoplankton. The terrestrial and aquatic sources investigated may be used as a tool to understand the origin and of the resources of the food chains in the Upper Paraná River since was considered the seasonal and spatial variability.

Key-words: isotopic variability, primary producer, Paraná River floodplain, Itaipu reservoir.

Resumo - Variabilidade de d^{13}C e d^{15}N em Fontes Aquáticas e Terrestres na Bacia do Alto Rio Paraná, Paraná, Brasil. A variabilidade isotópica da vegetação ripária, macrófitas aquáticas C_3 e C_4, fitoplâncton, perifíton e carbono orgânico particulado (COP) foi investigada durante as fases de chuva (fevereiro e março de 2000) e seca (agosto e setembro de 2000) na Bacia do alto rio Paraná, incluindo a planície de inundação do alto rio Paraná (subsistemas Paraná, Baía e Ivinheima) e Reservatório de Itaipu (trechos lêntico e lótico). Constatou-se que os valores médios de d^{13}C da vegetação ripária (–30,1 ± 1,32‰), macrófitas aquáticas C_4 (–13,0 ± 0,87‰) e perifíton (–25,0 ± 3,41‰) foram significativamente diferentes. Os valores médios das macrófitas aquáticas C_3 (–28,42 ± 2,73‰) e COP (–27,2 ± 2,75‰) não apresentaram diferenças significativas entre si, mas foram diferentes dos valores registrados para a vegetação ripária, macrófitas C_4, perifíton e fitoplâncton. A variabilidade espacial de d^{3}C foi identificada para o fitoplâncton e COP. Influências sazonais foram observadas para o fitoplâncton e perifíton. Considerando os valores de d^{15}N, a vegetação ripária (2,09 ± 2,46‰) foi isotopicamente diferente das macrófitas aquáticas C_3 (4,15 ± 3,88‰), fitoplâncton (5,28 ± 2,43‰), perifíton (5,56 ± 3,13‰) e COP (4,78 ± 2,59‰). Diferenças significativas foram observadas, espacialmente, para a vegetação ripária e macrófitas aquáticas enquanto para o fitoplâncton constatou-se efeito da sazonalidade. Conclui-se que os valores isotópicos das fontes terrestres e aquáticas podem ser utilizados como mais uma ferramenta útil ao entendimento da origem das fontes mantenedoras das cadeias alimentares do alto rio Paraná, desde que sejam consideradas suas variabilidades espaciais e sazonais.

Palavras-chave: variabilidade isotópica, produtores primários, planície de inundação do alto rio Paraná, Reservatório de Itaipu.

Introduction

The analysis of stable isotopes has been used to improve our understanding of the dynamic the nitrogen and carbon flow in complex food webs (Fry, 1988; Yoshioka et al., 1988; Forsberg et al., 1993). Several organisms from the food chain reflect the d^{13}C of their energy sources, increasing from 0.2 to 1‰ in each trophic level (Fry, 1988). Nitrogen stable isotopic composition, is consistently fragmented along the food chain in about 3.4‰ per trophic level, allowing inferences about the trophic position of consumers (Vander Zanden et al., 1997).

The use of stable isotopes as energy flow markers in ecosystems is possible only when the potential sources available to the consumers are isotopically different (DeNiro & Epstein, 1978). Differences in isotopic are a signatures result of physical–chemical and biological reactions that promote the discrimination of one of the isotopes (Martinelli et al., 1988). Infortunably, in some cases only a single collection of samples is often used to infer about isotopic compositions of the primary producers in aquatic environments. Boon & Bunn (1994) and Benedito-Cecilio et al. (2000) investigated temporal and spatial variations in the d^{13}C and d^{15}N values of aquatic plants, riparian vegetation and fishes. They concluded that variations were sufficiently great within important consequences for interpretation of aquatic food webs.

The floodplain of the Upper Paraná River basin, the second major river system of South America, presents a range of aquatic and ecotonal habitats that differ in connectivity and hydrodynamics with the Paraná River (Thomaz et al., 1991). It is possible that primary producers show some variability. The energy sources potentially available for use in aquatic food webs in this system include: C_3 riparian vegetation, C_3 and C_4 aquatic macrophytes, phytoplankton, periphyton, and particulate organic carbon (POC) (Manetta et al., 2003). The above authors did not take into account spatial and seasonal variation in the isotopic composition of plants.

In the present paper, in contrast to previous studies, we identify isotopic variations in the terrestrial and aquatic primary producers in food chains from Upper Paraná River Basin. The hypothesis tested was that: a) isotopic variation in the primary producers is distinguishable; and b) its seasonal and spatial variations must be considered in the interpretations of results in food webs.

Material and methods

The study was conducted in the Upper Paraná River Basin, including the Upper Paraná River floodplain (22° 40' and 22° 50'S; 53° 10'and 53° 40'W) and Itaipu Reservoir (24°05' and 25°33'S; 50° 00' and 50° 30'W). The study area was subdivided according to physical and chemical characteristics (Agostinho & Zalewski, 1996) into the following subsystems: a) Paraná subsystem: main channel of the Paraná River, located near Porto Rico city and Pau Véio lagoon; b) Baía subsystem: main channel of the Baía River; c) Ivinheima subsystem: main channel of the Ivinheima River and Finado Raimundo lagoon; and d) Itaipu Reservoir subsystem: in the lentic (near Santa Helena city) and lotic (near Guaíra) stretches (Fig. 1).

Biological material was collected during the rainy (February and March 2000) and dry (August and September 2000) seasons. Terrestrial sources consisted of 61 samples of riparian plants that fixed carbon with the Calvin photosynthetical pathway (C_3) (Tab. I). Aquatic sources consisted of 95 samples of aquatic C_3 macrophytes, 39 aquatic samples using the Hatch–Slack pathway (C_4), 40 samples of zooplankton, 40 samples of particulate organic carbon (POC) and, 42 samples of periphyton.

Periphyton was obtained by scratching the substratum (leaves or stems of plants) and the material on a pre–combusted (400°C; 4hs) fiber glass filter (GF/F Whatman). Samples of zooplankton (mainly cladocerans and adult calanoid copepods) were collected with a zooplankton net (53mm mesh size) and when necessary, with a suction pump. Samples were packed in tin foil. Isotopic values for phytoplankton were determined from zooplankton, considering the fractionation of 1‰ per trophic level for d^{13}C (Tiezen et al., 1983) and 3.4‰ for d^{15}N (Vander Zanden et al., 1997). Particulate organic carbon (POC) was retained in a glass fiber filter (GF/F Whatman), also previously submitted to combustion.

Samples were rinsed with a 1N HCl solution to remove carbonates, then were dried in an oven (50°C) and sent to the Centro de Energia Nuclear na Agricultura (CENA–

USP) in Piracicaba, São Paulo State, for determination of isotopic carbon and nitrogen ratios in mass spectrometer (IRMS) Finnigan MAT Delta Plus. Precision for this machine was ±0.05‰, and replicates for standards were usually within 0.1‰. The isotopic standard for carbon was Belemnintella americana of formation Pee Dee (PDB) and for nitrogen was atmospheric air.

Graphic and statistical analyses were conducted using STATISTIC 5.0™ Isotopic values (‰) were investigated using a two-way Analyses of Variance (ANOVA), comparing the differences among photosynthetical pathways, seasons and sites. In order to identify differences among sampled materials, we used a one-way variance analysis and post hoc Tuckey's test in the identification of such differences (Zar, 1999).

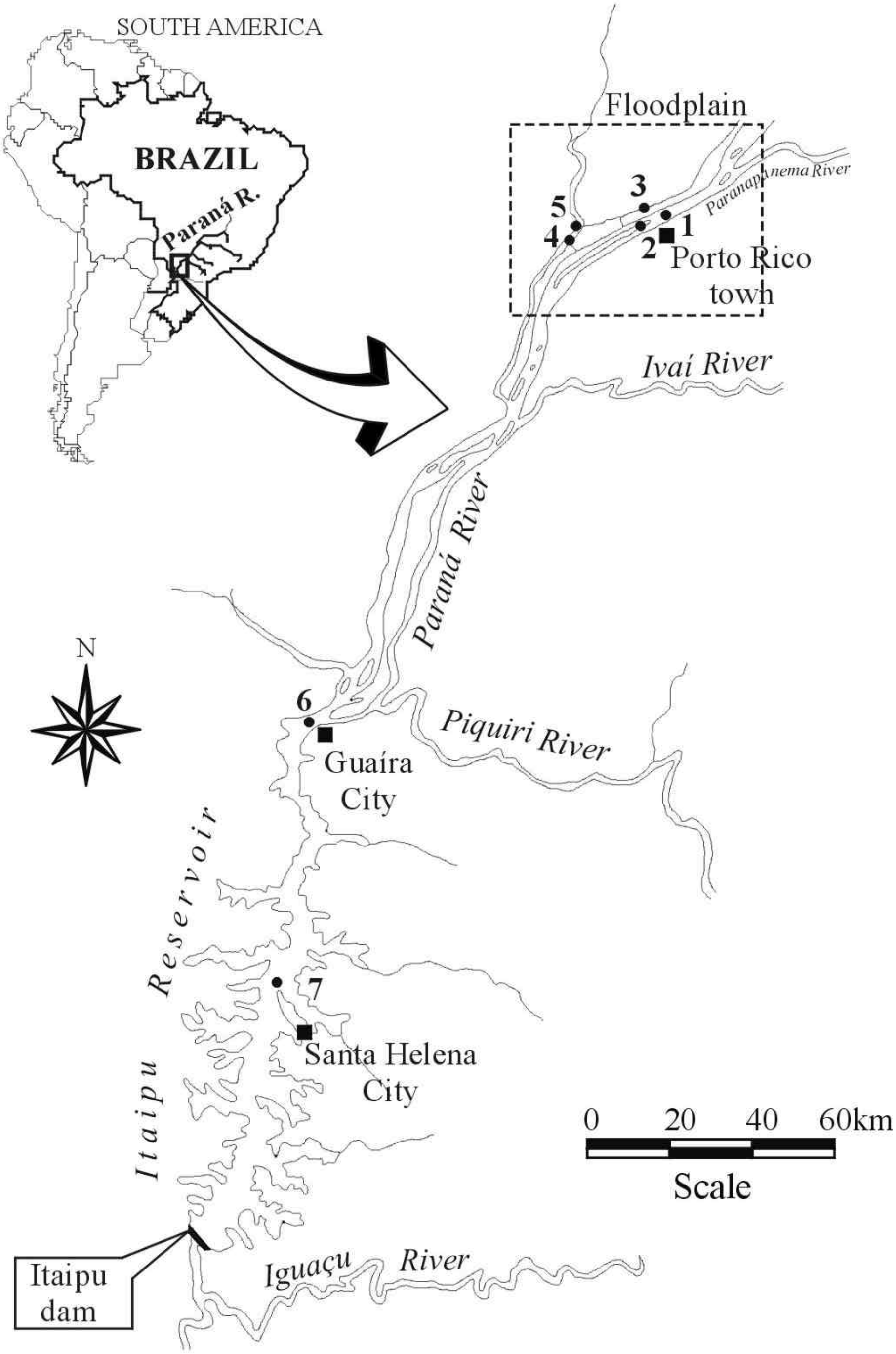

Figure 1: The study area sampling stations in the studied subsystems (Paraná = Paraná River (1) and Pau Véio lagoon (2); Báia = Báia River (3); Ivinheima = Ivinheima River (4) and Finado Raimundo lagoon (5), Itaipu Reservoir = lotic (6) and lentic stretches (7).

Table I: List of vascular plants sampled in Upper Paraná River Basin.

Riparian vegetation		Aquatic macrophythe	
Family/ Species	Photosynthetical Pathways	Family/ Species	Photosynthetical Pathways
Calathea sp.	C_3	Cyperaceae	C_3, C_4
Cecropia pachystachya Trèl	C_3	Cyperus gardini	C_3
Colubrina retusa (Pittier) R.S. Cowan	C_3	Eichhornia azurea (S.w) Kunth	C_3
Cróton urucurana	C_3	Hydrocotyle umbellata L.	C_3
Ingá sp.	C_3	Poaceae	C_3, C_4
Lauraceae	C_3	Paspalum sp	C_3
Leguminosae	C_3	Paspalum repens P.J. Bergius	C_4
Machaerium sp.	C_3	Polygonum sp	C_3
Meliaceae	C_3	Nymphaea sp	C_3
Myrtaceae	C_3	Eichhornia crassipes (Mart.) Solms	C_3
Ocotea diospyrifolia (Meisn.) Mez	C_3	Salvinia auriculata Aubl.	C_3
Trema. Micrantha L.) Blone	C_3	Egeria najas Planck	C_3
Tiliaceae	C_3		
Zygia cauliflora (Willd) Killip x Record	C_3		

Results

Variability among sources

The isotopic composition of the $d^{13}C$ was significantly different among terrestrial riparian vegetation (–30.1 ± 1.32‰), C_4 aquatic macrophytes (–13.0 ± 0.87‰), and periphyton (–25.0 ± 3.41‰) (ANOVA: df = 4; F = 258.02; P ‹0.05). Mean values for C_3 aquatic macrophytes (–28.42 ± 2.73‰) and POC (–27.2 ± 2.75‰) differ significantly from other primary producers (Fig. 2A). According to the $d^{15}N$ values, terrestrial riparian vegetation (2.09 ± 2.46‰) presented an isotopic mean value significantly different from the other materials (C_3 aquatic macrophytes: 4.15 ± 3.88‰; periphyton: 5.56 ± 3.13‰; and, POC: 4.78 ± 2.59‰), except in relation to C_4 macrophytes (3.51 ± 3.17‰), which differed only from periphyton (ANOVA: df = 4; F = 10.7250; P ‹0.05) (Fig. 2B).

Spatial and seasonal variability

Significant spatial differences in $d^{15}N$ were recorded for the riparian vegetation and aquatic macrophytes (Tab. II and Fig. 2B). The mean isotope values for the riparian vegetation in the Ivinheima (lowest values: 1.1 ± 1.19‰) and Paraná (highest values: 3.5±3.67‰) subsystems differed significantly from each other. The isotopic composition of the aquatic macrophytes was most negative in the Baía subsystem (1.2 ± 2.32‰). The Ivinheima subsystem (3.8 ± 2.93‰) differed from the Baía and from Itaipu Reservoir (6.1 ± 3.88‰) (Fig. 3B). During the rainy season, the plants from Itaipu Reservoir subsystem (7.6 ± 3.87‰) were significant different from those the Paraná (3.4 ± 3.21‰) and Baía (1.7 ± 1.80‰) subsystems. In the dry season, mean values recorded in the Baía subsystem (0.5 ± 2.492.49‰) were significantly different from those obtained in the Paraná (5.5 ± 3.93‰) and (5.4 ± 4.57‰) Itaipu Reservoir subsystems. Between the study seasons there were differences in the Baía subsystem and in rainy season in relation to the Paraná and Itaipu Reservoir subsystem in dry season.

Spatial differences for the phytoplankton were found in the $d^{13}C$ values of the Baía (–35.6 ± 2.33‰) and Paraná (–32.4 ± 3.86‰) subsystems in relation to the other subsystems (Tab. III, Fig. 4A). Furthermore,

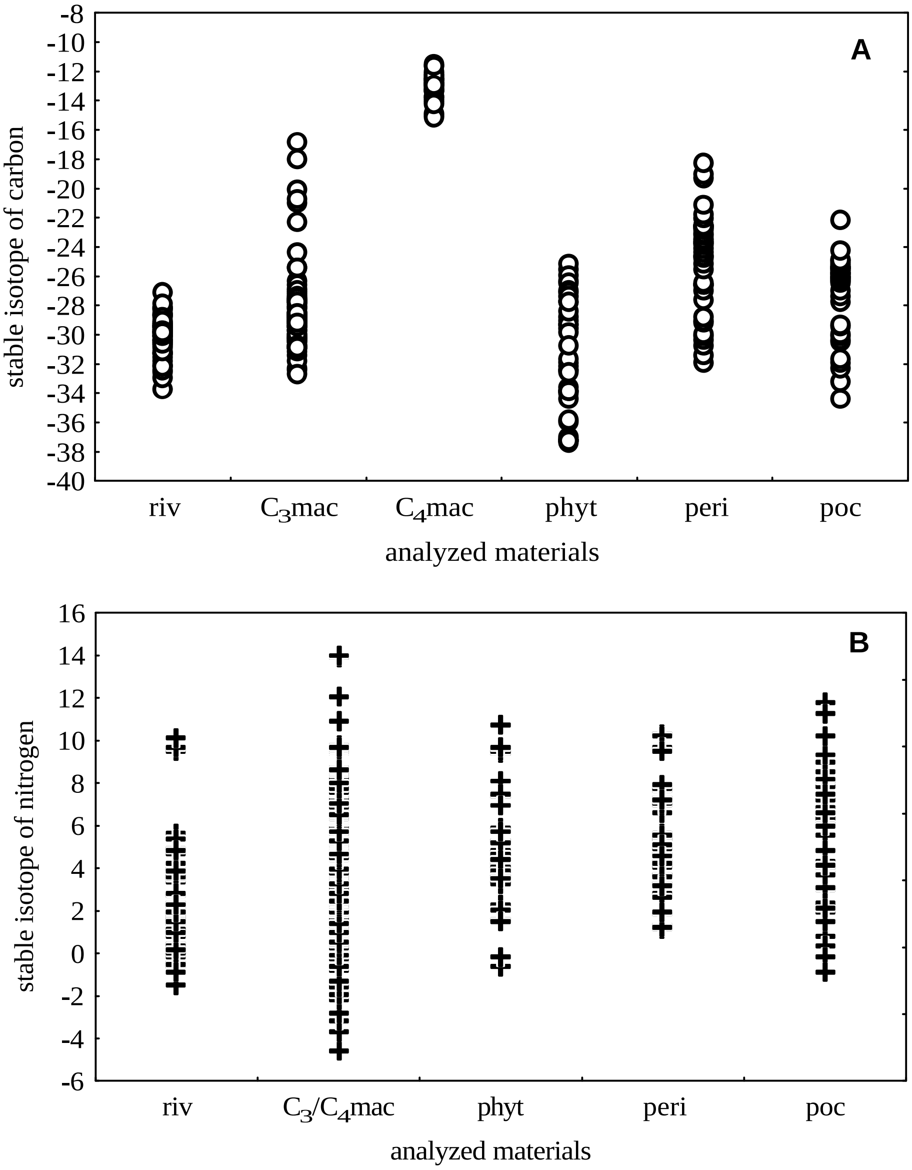

Figure 2: Stable isotope carbon (A) and nitrogen (B) ratios of riparian vegetation (riv), C_3 aquatic macrophytes (C_3mac), C_4 aquatic macrophytes (C_4mac), phytoplankton (phyt), periphyton (peri) and Particulate Organic Carbon (poc) (✦ = significant spatial variability and • = significant seasonal variability).

Table II: Two-way ANOVA results of $d^{15}N$ of primary producers, POC and periphyton among subsystems and between dry and wet seasons * denotes significant values (p< 0,05).

Source variation	**Spatial**			**Seasonal**			**Spatial x Seasonal**		
	df	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**
Riparian vegetation	3	3.4789	p<0.05*	1	2.1262	0.1507	3	0.5700	0.6372
Aquatic macrophytes	3	13.6199	p<0.05*	1	0.0542	0.8164	3	3.2488	p<0.05*
Periphyton	3	10.9570	p<0.05*	1	0.0489	0.8263	3	1.8129	0.1634
Phytoplankton	3	16.4413	p<0.05*	1	5.3081	p<0.05*	3	6.9393	p<0.05*
POC	3	2.6222	0.0670	1	2.1804	0.1493	3	3.5506	p<0.0*

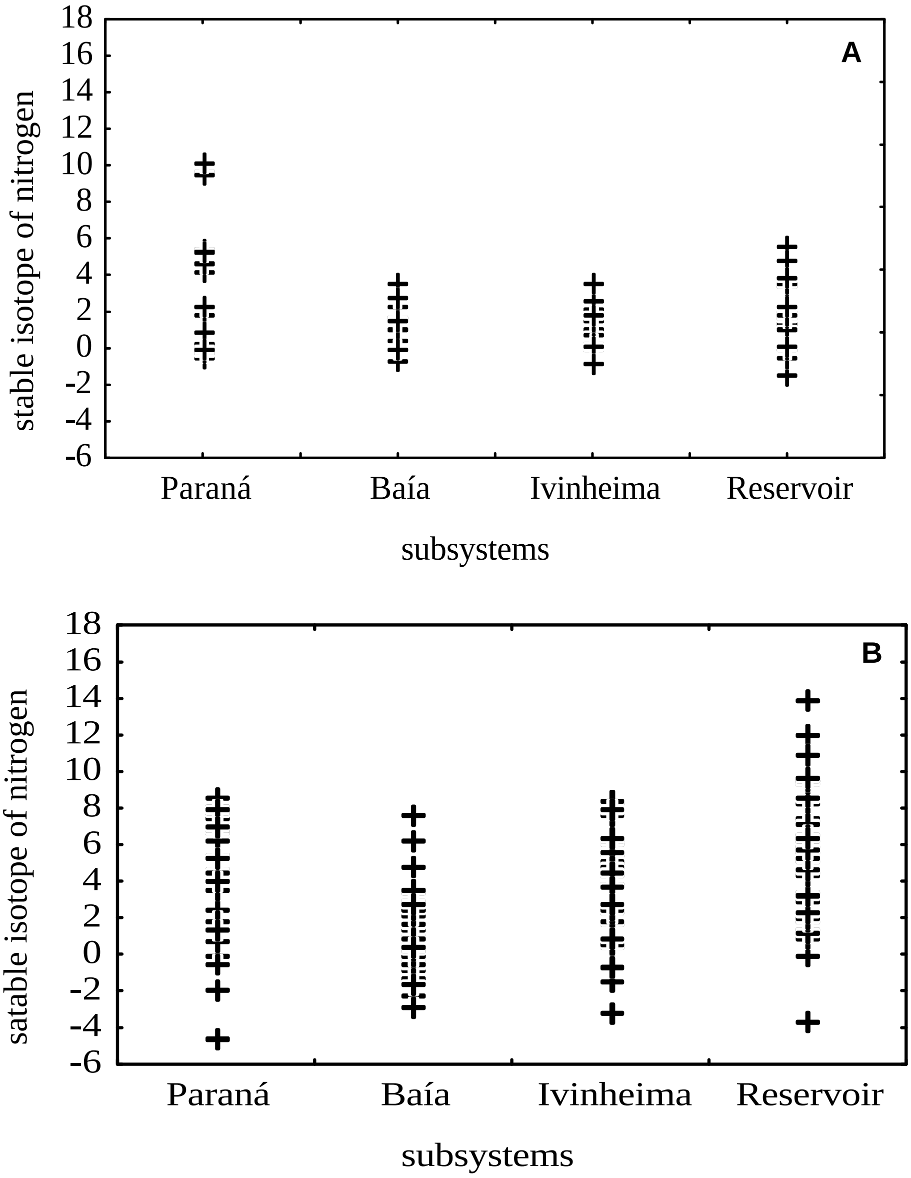

Figure 3: Stable isotope nitrogen ratio for the riparian vegetation (A) and aquatic macrophytes (B) in each subsystem

Table III: Two-way ANOVA results of d^{13}C of primary producers, POC and periphyton among subsystems and between dry and wet seasons * denotes significant values.

Source variation	**Spatial**			**Seasonal**			**Spatial x Seasonal**		
	df	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**
Riparian vegetation	3	2.6676	0.0570	1	0.0362	0.8497	3	0.9155	0.4398
C_3 aquatic macrophytes	3	1.0975	0.3547	1	0.5816	0.4477	3	0.0309	0.9927
C_4 aquatic grasses	3	1.9207	0.1468	1	0.0196	0.8896	3	0.9206	0.4424
Periphyton	3	11.2595	$p<0.05$*	1	8.5291	$p<0.05$*	3	1.0500	0.3840
Phytoplankton	3	25.1269	$p<0.05$*	1	46.2081	$p<0.05$*	3	7.7592	$p<0.05$*
POC	3	23.1136	$p<0.05$*	1	0.0600	0.8080	3	2.9171	$p<0.05$*

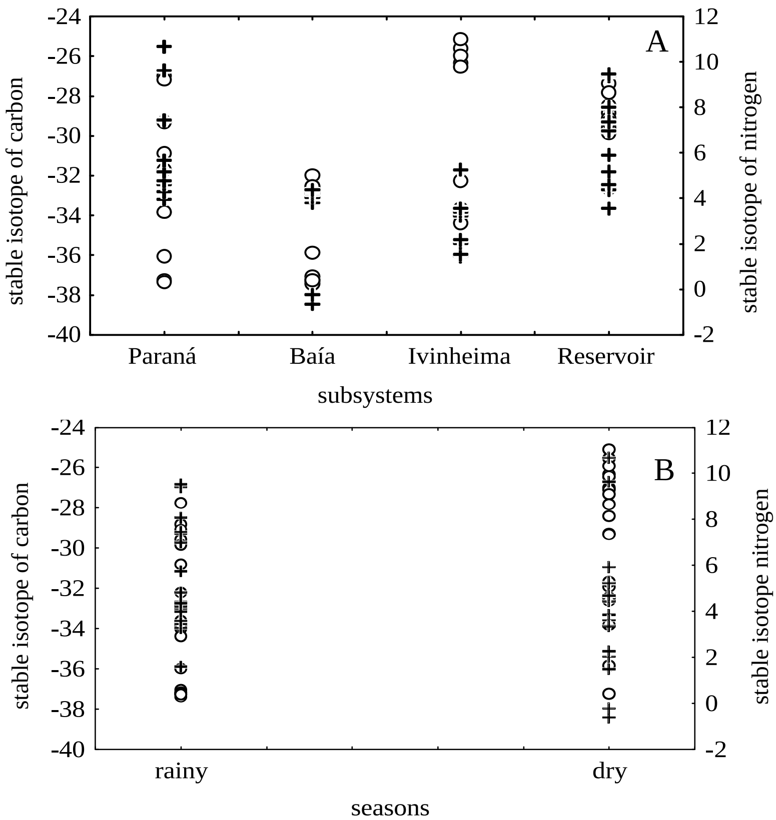

Figure 4: Stable isotope carbon (o) and nitrogen (+) ratios for the phytoplankton in each subsystem (A) and season (B).

values also had variations associated with seasonality (Fig. 2A). In the rainy season, the mean isotopic value was –33.7 ± 3.37‰ and in the dry season, –29.7 ± 3.59‰ (Fig. 4B). In the rainy season, the Itaipu Reservoir (–29.0 ± 0.81‰) subsystem was different from the other subsystems. In the dry season, the Baía (–34.0 ± 2.39‰) subsystem was isotopically different from the Paraná (–29.8 ± 2.97‰), Ivinheima (–25.9 ± 0.56‰) and Itaipu Reservoir (–29.1 ± 2.11‰) subsystems. These two latter subsystems were even different from each other. Spatial differences between the study seasons were verified in all subsystem in the rainy season in relation to the Paraná, Ivinheima and Itaipu Reservoir subsystems in the dry season.

$d^{15}N$ mean values for the phytoplankton in the Ivinheima and Baía subsystems were different from the other subsystems (Tab. II and Fig. 2B). These $d^{15}N$ mean values were highest in the Paraná (6.3 ± 2.42‰) and in the Itaipu Reservoir (6.5 ± 2.10‰) subsystems (Fig. 4A).

Seasonal differences were recorded for the $d^{15}N$ values of the phytoplankton (Tab. II and Fig. 2B). In the rainy season, the mean isotopic nitrogen value (5.2 ± 2.17‰) was 1‰ higher than in the subsequent season (4.2 ± 3.03‰) (Fig. 4B). In the rainy season the Itaipu Reservoir (8.3 ± 1.15‰) subsystem was different from the Baía (4.3 ± 0.13‰) and Ivinheima (3.1 ± 0.85‰) subsystems. The Baía subsystem was also different from the Paraná (5.3 ± 1.38‰). In the dry season the same isotopic tendency was observed in the Baía (1.0 ± 2.46‰) subsystem; whereas the Paraná (7.6 ± 3.04‰) subsystem differed from the Ivinheima (2.8 ± 1.70‰). The following differences were recorded between the study seasons: i) in Itaipu Reservoir/rainy season in relation to the Baía, Ivinheima and Itaipu Reservoir subsystems/dry season, ii) between the Paraná/rainy season and the Baía/dry season and iii) between the Ivinheima/rainy season and the Paraná/dry season.

In general, the Baía subsystem presented the most negative mean values for periphyton (–28.7 ± 2.70‰) and POC (–30.9 ± 2.45‰), while the most positive values (–23.12 ± 2.57‰) were found in the Ivinheima for periphyton and in the Paraná for POC (–25.8 ± 1.92‰). Significant spatial differences were found for

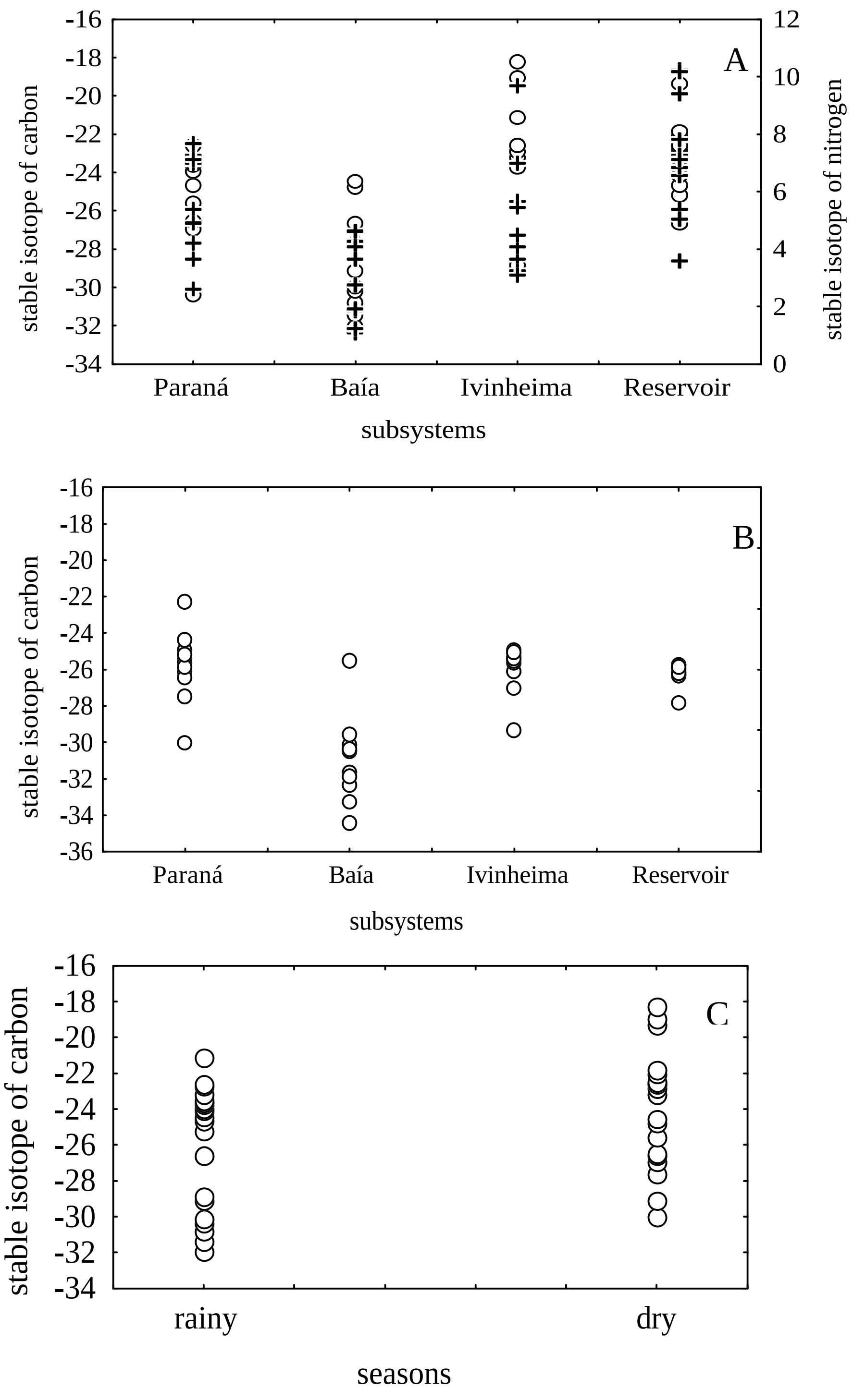

Figure 5: Stable isotope carbon (o) and nitrogen ratios (+) for the periphyton (A) and stable isotope carbon for the POC (B) in each subsystem and stable isotope carbon values for the periphyton in each season (C).

periphyton and POC (Tab. III and Fig. 2A); whereas the isotopic values obtained in the Baía subsystem were significantly different from those of the other subsystems (Fig. 5A and B). Significant seasonal variability was found in the isotopic composition of periphyton (Tab. III and Fig. 2A), whose mean values were –26.0 ± 3.31‰ in the rainy season and –23.9 ± 3.24‰ in the dry season (Fig. 5C).

Significant seasonal interactions were found for POC (Tab. III). During the rainy season, there were isotopic differences in the carbon mean values obtained in the Baía (–29.7 ± 3.06‰) subsystem in relation to those observed in the Paraná (–25.2 ± 2.89‰)

and Itaipu Reservoir (–24.5 ± 1.66‰) subsystems. In the dry season, the Baía (–27.6 ± 2.06‰) subsystem presented the same isotopic tendency, in addition to being different from the Ivinheima (–21.1 ± 2.25‰). Spatial differences between seasons were found in the Baía in the rainy season in relation to the Paraná (–24.9 ± 1.98‰) and Ivinheima in the dry season; whereas the mean value found in the Baía subsystem during the dry season was different from the values for the Paraná, Ivinheima (–25 ± 3.60‰) and Itaipu Reservoir subsystems/rainy season.

The highest nitrogen mean values for periphyton were observed in the Itaipu Reservoir (7.1 ± 2.09‰), Paraná (5.8 ± 1.84‰;) and Ivinheima (5.2 ± 2.01) subsystems, and the lowest values were found in the Baía (2.7 ± 1.38‰). Significant spatial variability differences for the Baía subsystem in relation to the other subsystems were recorded for periphyton (Tab. III and Fig. 2B and 5A). However, significant interactions among seasons and subsystems were recorded for the stable nitrogen isotope values for POC (Tab. II). In the dry season the Baía (–32.1 ± 1.43‰) subsystem was different from the Paraná (–24.9 ± 1.93‰) and Itaipu Reservoir (–26.5 ± 0.72‰) subsystems.

Discussion

Considering that interspecific genetic characteristics may have influenced the amplitude of isotopic variation in the primary producers, it is important to mention that the diversity of the species/families of C_3 plants analyzed was high. These factors were more determinant than the specific characteristics of the biological fixation of CO_2 to each plants group.

Riparian vegetation did not show significant spatial variation in the $d^{13}C$ composition along the Paraná River; however, the isotopic values in the lower stretch (Itaipu Reservoir subsystem) were a little more negative than those found in the upper stretch (Paraná subsystem), reflecting the reduced influence of biogenic CO_2 from the river, possibly resulting from the short distance (not more than 400 km) between these two subsystems (Agostinho & Zalewski, 1996). This area is relatively smaller than the areas of other ecosystems where the biogenic effect is evident (Martinelli et al., 1991).

The composition of $d^{15}N$ in terrestrial vegetation, as well as its variations, are related to the nitrogen pools that are determined by the input and output of this element in the environment and by the isotopic fractionations that might occur during nitrogen cycling (Nadelhoffer & Fry, 1994). A range of nitrogen isotopic variability in leaves (–8 to 3‰) was refereed by Peterson & Fry (1987). The values obtained for the plants used in this study remained within this range, as observed in Cecropia pachystachya, Ingá sp., Maechaerium sp. and Zygia cauliflora; nevertheless, some species such as Croton urucurana and Calathea sp. presented more enriched $d^{15}N$ values, near those commonly found for soil (–10 to 15‰) (Nadelhoffer & Fry, 1994). The isotopic differences between the Paraná and Ivinheima subsystems are due to the higher $d^{15}N$ mean values for plants from the Paraná subsystem, as verified for Croton urucurana, whose mean isotopic composition was about 5‰ more enriched in nitrogen than the plants of the same species collected in the Ivinheima subsystem.

The analyzed macrophytes of C_3 and C_4 photosynthetical pathways were significantly different from each other. C_4 macrophytes presented more positive $d^{13}C$ values. These macrophytes use the enzyme RuBP carboxylase, typical of the C_3 pathway, and use the enzyme phosphoenolpyruvate carboxylase (PEP–carboxylase), which reduces CO_2 to aspartic acid, discriminating less against ^{13}C (fractionation of –3.6 to 5.7‰). Thus, these plants present more positive $d^{13}C$ values, which extend from about –9 to –16‰ (Ribeiro et al., 1998).

There were no significant spatial and temporal differences in the $d^{13}C$ values for macrophytes C_3 in the study area, the opposite of what has been found in the studies of Boon & Bunn (1994) on the Murray River floodplain (Australia).

In addition to the riparian vegetation, the aquatic macrophytes of the Paraná River floodplain and Itaipu Reservoir have not experienced relevant influence from the biogenic effect of the river. The mean isotopic composition of C_3 macrophytes from the upper stretch of the Paraná River was 1‰ more enriched in $d^{13}C$ than that from the lower stretch; however, the opposite was recorded for C_4 macrophytes, which presented a $d^{13}C$ mean value about 0.4‰ more positive in the lower stretch of the

Paraná River. Thus, C_4 plants, represented by the emergent macrophyte ecological group, use a carbon source distinct from the CO_2 depleted in ^{13}C along the Paraná River. Martinelli et al. (1991) found spatial variances in C_4 macrophyte Echinochloa polystachya, which presented a decrease in the isotopic values downstream. This depletion resulted from the dilution of carbonate originating from the Andes by biogenic CO_2, produced by the respiration in the river.

The ^{15}N isotopic ratio of primary aquatic producers is dependent on the abundance of nitrogen forms of inorganic nutrients, as well as the occurrence of fractionation processes in the environment. Therefore, these factors can be influencing on spatial differences in the isotopic composition of nitrogen for aquatic macrophytes.

The $d^{13}C$ mean value for phytoplankton, estimated using the zooplankton, was the most negative among the primary producers investigated in this study. Using isotopic determinations from several biological groups collected on the Orinoco River floodplain (Venezuela), Hamilton & Lewis (1992) observed that the phytoplankton community had the most depleted values in ^{13}C (–34 to –37.2‰). The presence of low phytoplankton values in freshwater habitats are reported by Wada et al. (1997) in lakes in Médio Vale do rio Doce, and by Araujo–Lima et al. (1986) and Martinelli et al. (1994) along the Amazon River. These more depleted ^{13}C values are related to the influence of the detritus of C_3 riparian vegetation (–26‰), biogenic CO_2 and fractionations between the carbon source and phytoplankton.

The spatial variability observed in the phytoplanktonic organisms in this work is probably related to isotopic composition, and the concentration and origin of the inorganic form of the carbon fixed in the different subsystems.

The $d^{13}C$ values for these organisms were also influenced by seasonal changes introduced by environmental conditions (e.g. temperature and water level) and physiological effects over the study period. Great depletion in the ^{13}C mean value in the rainy season showed the incorporation of biogenic CO_2 provided by the decomposition processes of autochthonous and allochthonous vegetation, more accelerated in this season. However, Benedito–Cecilio et al. (2000), who investigated the carbon sources in fisheries between Vargem Grande and Óbidos (Amazon region) during lower and upper water periods from 1983 to 1998, demonstrated that the $d^{13}C$ of phytoplankton (using zooplankton) was not seasonally different along the river. So, this condition seems to be specific to each ecosystem and needs to be considered.

$d^{15}N$, as well as $d^{13}C$, presented both spatial and seasonal variability and significant interaction between the two variables, indicating the sensitivity of these organisms to determinant processes of isotopic composition, with different magnitudes over the study seasons and along the subsystems.

Exposure to low water turbulence in the littoral area of the studied subsystems promoted the occurrence of more positive isotopic ^{13}C values in the periphytic community than in the phytoplanktonic. Along the Amazon River, the periphytic community presents the greatest ^{13}C isotopic variability (–36.7‰ to –21.0‰) among the pools of carbon investigated (Martinelli et al., 1994). Such results are due to the great diversity of species that constitute the periphyton or even to the use of biogenic CO_2 derived from the substratum to which the periphyton is adhered.

The effect of spatial and seasonal variations on the periphytic community (and their interaction in the $d^{13}C$ values) possibly resulted from changes in primary producer composition (vascular plants and phytoplankton). Studies based on the chlorophyll–a determinations in the Baía and Ivinheima subsystems showed the predominance of autotrophic components in both seasons (UEM. Nupélia/Peld/Cnpq, 2001).

The $d^{13}C$ values of POC change spatially because of alterations in primary production, respiration and the solubility of carbonates (Angradi, 1993). The isotopic analysis of $d^{13}C$ for POC between the subsystems revealed trends similar to those observed in phytoplankton and periphyton. The mean isotopic values in the Baía subsystem for these two biological groups were different from those recorded in the other subsystems. These were more depleted of ^{13}C, which might be likened to the greater incorporation of ^{13}C from terrestrial vegetation, phytoplankton or biogenic CO_2 derived from respiratory processes. In addition, the range of POC isotopic variability remained intermediate for phytoplankton, periphyton, range riparian vegetation and C_3 macrophytes. So, this material is not only constituted in great part by phytoplankton, but also by other C_3 sources. On the other hand, POC did not present seasonal trends similar to those of phytoplankton and periphyton, remaining without relevant isotopic differences over the seasons.

Ecological studies using isotopic analysis are only emerging, with a vast field to be explored, tested and explained, mainly concerning freshwater environments. Floodplain ecosystems usually contain several groups of primary producers responsible for the maintenance of upper trophic levels, which are part of complex food webs. Studies using stable isotopes are only useful when the plant groups are isotopically distinct (Jepsen, 1999). It is also possible to evaluate the relative nutritional importance in different communities (Fry & Sherr, 1984). However, in many cases, these producers overlap isotopically (Forsberg et al., 1993).

The carbon isotope mean values for riparian vegetation, C_3 and C_4 aquatic macrophytes, phytoplankton, periphyton and POC can be an additional tool used to understand the origin and destiny of the autotrophic resources of food chains in the study area, although C_3 plant variability may overlap in some cases.

The isotopic composition of phytoplankton and periphyton seems to be more sensitive to the variations in the spatial and temporal conditions. Spatial and temporal variations for primary producers were registered for the isotope of nitrogen but no for the one of carbon. Thus, the combined use of these two isotopes in researches of isotopic ecology is recommended. Besides here, it was observed also that terrestrial plants as well as the aquatic can present variabilities that should be considered in study of isotopic ecology.

In this study, still the spatial and seasonal variability in the values for stable isotopes for energy sources showed that results at one site can be invalid for others. Site–specific studies together with an interpretation of the functional processes of the ecosystem are thus necessary.

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