

## SEASONAL VARIATION IN THE DISTRIBUTION AND ISOTOPIC COMPOSITION OF PHYTOPLANKTON IN AN AMAZON FLOODPLAIN LAKE, BRAZIL

### Variación estacional de la distribución y composición isotópica del fitoplancton en un lago de inundación en la Amazonia, Brasil

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#### ABSTRACT

To evaluate the seasonal variation and isotopic composition of phytoplankton, water samples were collected monthly between October 2007 and November 2008 in Lake Catalão, a floodplain lake at the confluence between rivers Negro and Amazon. Analyses of total chlorophyll concentration and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic abundances were made from particulate size fractions of 30-60, 10-30 and  $<10\ \mu\text{m}$  in the littoral, pelagic, and floating meadows regions. Chlorophyll concentration was found to be inversely associated to lake depth, and high concentrations of chlorophyll in the floating meadows zone were significant. The fraction  $<10\ \mu\text{m}$  was the most abundant representing in average more than 40 % of the particulate matter. The  $\delta^{13}\text{C}$  values were relatively constant during the study ( $-25.1\ ‰ \sim -34.0\ ‰$ ), whereas the  $\delta^{15}\text{N}$  values showed strong variability ( $15.6\ ‰ \sim 2.4\ ‰$ ), which has been attributed to the resuspension of sediments during mixing of the water column. Mixing associated to the sudden drop in temperature during the rising water period was an important event in the trophic and isotopic dynamics of the lake. Variations in chlorophyll content were generally associated with the dilution process, in which concentration was inversely correlated to the water level, whereas abundance was directly correlated to the water level.

**Keywords:** Amazon, floodplain lake, size-fractionated phytoplankton, stable isotopes.

#### RESUMEN

Con el propósito de evaluar la variación estacional de la abundancia isotópica ( $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$ ) del fitoplancton, muestreos mensuales fueron realizados entre octubre de 2007 y noviembre de 2008 en el lago Catalão, un lago de inundación en la zona de confluencia de los ríos Negro y Solimões, ubicado frente a la ciudad de Manaus (AM, Brasil). Análisis de la clorofila total y evaluaciones de la abundancia natural de  $\delta^{13}\text{C}$  y  $\delta^{15}\text{N}$  fueron realizados en las fracciones particuladas de 30-60, 10-30 y  $<10\ \mu\text{m}$  en las zonas litoral, pelágica y de macrófitas acuáticas. La concentración de clorofila presentó una relación inversa con la profundidad del lago, siendo relevantes las altas concentraciones encontradas dentro del tapete de macrófitas acuáticas. La fracción  $<10\ \mu\text{m}$  fue la que presentó la mayor concentración de clorofila, representando más del 40% del material particulado. Los valores de  $\delta^{13}\text{C}$  fueron relativamente constantes durante el período de estudio ( $-25,1\ ‰ \sim -34\ ‰$ ), mientras que la abundancia natural de  $\delta^{15}\text{N}$  presentó una amplia variación ( $15,6\ ‰ \sim -2,4\ ‰$ ), que fue atribuida al proceso de resuspensión de los sedimentos en los procesos de mezcla de la columna de agua. En este contexto, el fenómeno de la mezcla asociada con

la friaje durante el período de aguas altas, fue un evento importante en la dinámica trófica e isotópica del lago. En general las variaciones de la concentración de clorofila fueron asociadas al proceso de dilución, en el cual la concentración es inversa y la abundancia es directamente relacionada con el nivel del agua.

**Palabras clave:** Amazonas, lago de inundación, fitoplancton, isótopos estables.

## INTRODUCTION

Phytoplankton is one of the main energy sources of autotrophic energy for the ichthyofauna in various tropical fluvial ecosystems (Araujo-Lima *et al.*, 1986; Hamilton *et al.*, 1992; Forsberg *et al.*, 1993). It is the same with Central Amazon floodplains where phytoplankton represents a great part of the carbon content of the main species of commercial fish, both in adult (Forsberg *et al.*, 1993; Benedito-Cecilio *et al.*, 2002) and larval stages (Leite *et al.*, 2002) despite making up only 2 % of the regional primary production (Melack and Forsberg, 2001). Thus, phytoplankton is a key element in the food chain of the fishing industry, and since fish is the main source of protein of the riverine population (Santos *et al.*, 2006), the study of phytoplankton is critical to regional development.

Since phytoplankton is composed of several species with different sizes and spatial-temporal distributions, density measurements provide little information on the dynamics of these communities. Chlorophyll concentration, in contrast, is the integrated biomass estimation that best represents the dynamics and productivity of the phytoplankton community in aquatic ecosystems (Wetzel, 2001).

The Amazon floodplain lakes are very productive systems, as was demonstrated by Schmidt (1973) in Lake Camaleão, and by Fisher and Parsley (1979) in Lake Calado. The phytoplankton productivity in these lakes is related to the availability of phosphorus and nitrogen (Forsberg *et al.*, 1988; Huszar *et al.*, 2006), which in turn depends on variable fractions that come from the river and have different geochemical properties depending on the local drainage basin. This chemical and hydrological variability creates difficulties in the study of chlorophyll dynamics and phytoplankton production in the studied environments. Among the few existing studies, it is worth mentioning the research conducted by Schmidt (1973) due to its duration and scope and, among other things, the inverse relation found between the water level of the lake and the concentration of chlorophyll, a pattern frequently observed in floodplain lakes (Carvalho *et al.*, 2001). Research on chlorophyll concentrations in the Amazon has generally demonstrated that the largest concentrations of phytoplankton usually occur in the dry season and the lowest concentrations in the flood season, as shown in the following studies: Fisher (1978) on Lake Janaucá and Rio Solimões

(Amazon River), Lopes and Bicudo (2003) on lakes in the Acre river basin; Camargo and Myai (1988) on Trombetas River; Schmidt (1982) on Tapajós River; Ibañez (1998) on Lake Camaleão, and Brito *et al.* (2014) on Lake Catalão.

The concentration of chlorophyll in a lake varies according to the physiological state of the cells, the species composition, the depth, and the environment studied (Santos *et al.*, 1999). This might make the assessment of the availability of phytoplankton for zooplankton based on chlorophyll not valid, particularly in systems with significant limnological variations over short periods of time. This assessment may be further complicated because the phytoplankton includes non-digestible forms and other forms that cannot be assimilated by the zooplankton (Runge and Ohman, 1982). Even so, chlorophyll has been found to be useful in the monitoring of phytoplankton in aquatic ecosystems, as has been demonstrated in lakes and rivers in North America (Carlson and Simpson, 1996). Since phytoplanktonic populations have different photosynthetic and demographic behavior according to their size, analysis of size-fractionated chlorophyll is a quick method (unlike cell count) to follow population changes more efficiently.

The study of size-fractionated phytoplankton makes it possible to investigate the contribution of each fraction to the community biomass, in the population dynamics and particularly in the phytoplankton-zooplankton relationship in the water bodies (Rai, 1982; Romero and Arenas, 1990), and as a useful tool for understanding the herbivore trophic chain. The size classifications of planktonic organisms, in relation to phytoplankton, have important physical and physiological implications. The smaller cells have a lower velocity of sedimentation and are more efficient in terms of nutrient assimilation, growth, breathing and photosynthetic rate (Greisberger *et al.*, 2008). This clearly shows the relative importance of the different size fractions that integrate a phytoplanktonic community, particularly in an ecosystem such as Lake Catalão, where a predominance of nanoplanktonic algae is found, especially Cyanobacteria and Chlorophyceae, in the low and high water periods of 2006-2007, respectively (Almeida and Melo, 2011). This predominance of the Cyanophyceae in aquatic ecosystems is associated with a relative contribution of picophytoplankton (<2 $\mu$ m) in the aquatic primary production higher than 70 % (Richardson and Jackson 2007). On the other hand, Romero and Arenas (1990) have determined that 57 % of primary production comes from the <10 $\mu$ m fraction. In turn, Ansotegui *et al.* (2003), who assessed a 60-80 % production of chlorophyll in the <8 $\mu$ m fraction suggest that the predominance of this fraction is normal in oligotrophic systems, whereas a predominance of microplankton occurs in eutrophic systems. The predominance of one or other fraction generally defines the route through which the carbon flows to higher levels. When there is a predominance of the smaller fraction, the main route, according to Adame *et al.* (2008), is carbon

recycling through the microbial loop in the photic zone. One of the most efficient ways of studying the assimilation of phytoplankton carbon by organisms from other trophic levels is through the study of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) stable isotopes. In fact, the assessment process would be simple if there was not the methodological problem of sorting the phytoplankton from the other seston particles. Thus, Hamilton and Lewis (1992) proposed the sorting of detrital material by means of centrifugation of the sample in a silica solution, which was not very successfully when performed by Calheiros (2003), but being later improved (Hamilton *et al.*, 2005). However, so far this method has not been largely used. Most studies of the isotopic composition of phytoplankton have analyzed all particulate organic matter smaller than 50-60  $\mu\text{m}$ , under the assumption that the isotopic value produced represents the phytoplanktonic populations in the environment. One alternative method used in some studies is the measurement of isotopic ratio of dissolved inorganic carbon (Marty and Planas, 2008).

The purpose of the present study was to investigate the seasonal and spatial variation of phytoplankton in Lake Catalão, including the variations in chlorophyll concentration and isotopic composition ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) associated with different fractions of phytoplankton particulate sizes.

## MATERIAL AND METHODS

### Study Area

Lake Catalão is a floodplain lake in the final portion of land that separates the rivers Negro and Amazon (Solimões), across from the city of Manaus, at  $3^{\circ} 10' 04'' \text{ S}$  and  $59^{\circ} 54' 45'' \text{ W}$  (Fig. 1). The lake is influenced by the Rio Negro at the start of the flood period and by the Amazon at the end of the flooding and during the high water periods. During the low water period the lake can be isolated from the two rivers, though it is usually linked to the Rio Negro by a small channel. The hydrologic balance of Lake Catalão is greatly influenced by the relative magnitudes of the inflows of rivers Solimões and Negro, and can be described as a variable mixture of

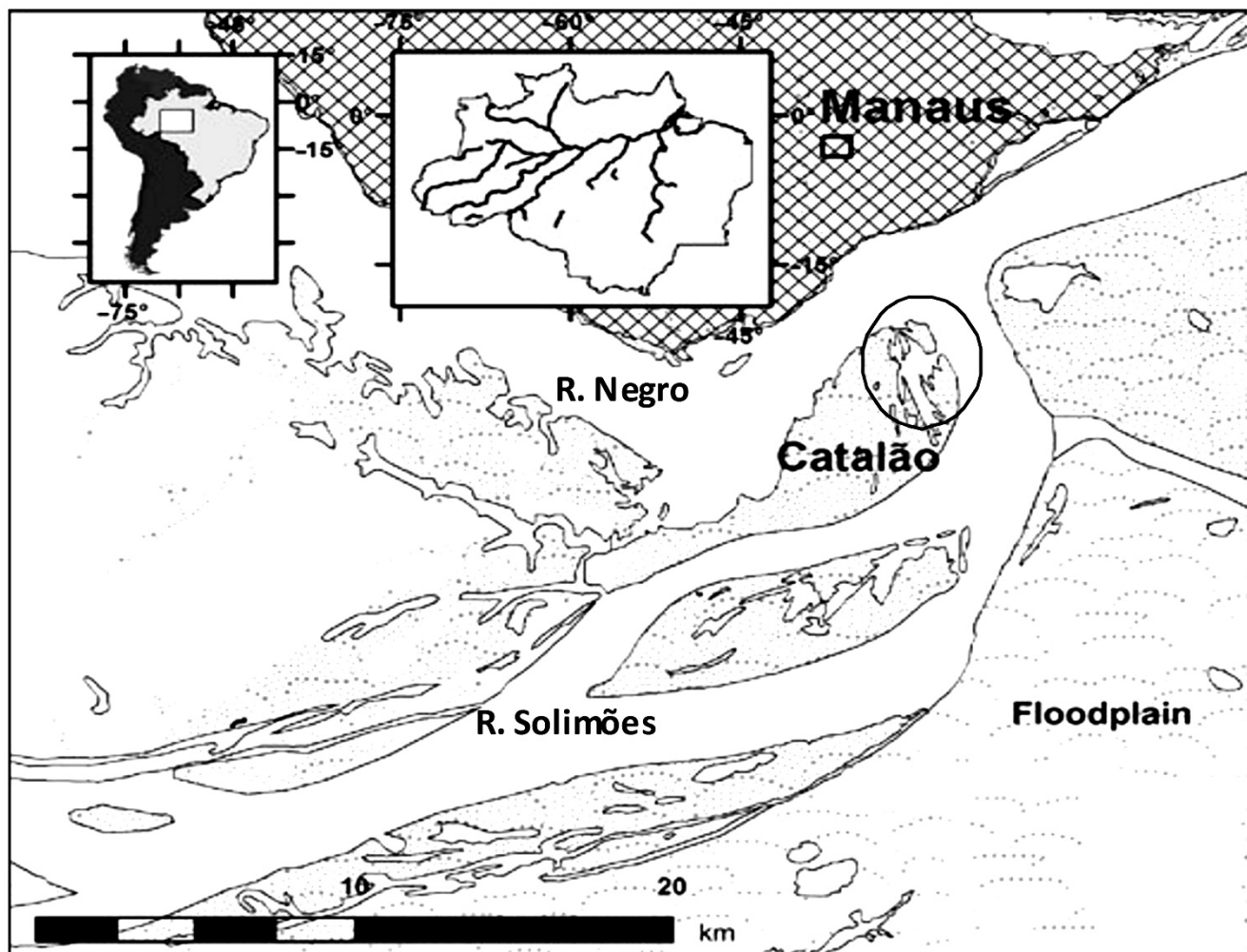


Figure 1. Map of the region including the Lake Catalão zone and the Manaus city. (modified from Neves dos Santos *et al.*, 2008).

these chemically different sources (Brito *et al.*, 2014; Almeida and Melo, 2011). This variable mixture results in a very particular temporal and spatial distribution of physical and chemical characteristics of the lake.

The limnological periods of low, flooding, flood and falling waters are well defined in the lake, as they are the direct result of the dynamics of two large rivers with a predictable flooding pattern. Figure 2 shows the water level in the port of Manaus, which corresponds to the water level of the lake. On November 22, 2007, when the water level of Rio Negro in the port of Manaus was 18.9 meters, the water began to flow into the lake and when the water level was 25.6 meters in the Port, water from Rio Solimões began to flow into the northern part of the lake. The flood period occurred between June and July, the falling water period between August and September, and the dry period between October and November. In the dry period, there is no water flow into the lake from the adjacent rivers, and the lake area is reduced.

In a study based on samples collected during the dry and flood periods, an inventory of 235 taxa of phytoplanktonic algae was registered by Almeida and Melo (2011): these algae belonged to 10 classes, with the greatest diversity of species found for class Chlorophyceae, and the greatest abundance Cyanophyceae, particularly the species *Synechocystis aquatilis*, *Synechococcus elongatus* and *Planktothrix isothrix*. The seasonal dynamics of the phytoplanktonic biomass of Catalão Lake were characterized by Brito *et al.* (2014) as follows: a

period of low density (rising and high water period), with the average values of chlorophyll in the photic zone varying between 3.4  $\mu\text{g/l}$  to 13.5  $\mu\text{g/l}$ , followed by the falling and low water phases when increase in the photic zone was observed, with average chlorophyll concentrations of 12.2  $\mu\text{g/l}$  to 24.7  $\mu\text{g/l}$ . On the other hand, studies of reproductive ecology of fish suggest that the areas of confluence between rivers of clear or dark waters are used for the reproduction of migrating Characiform species (Leite *et al.*, 2002). Therefore, the coast of Lake Catalão at Rio Solimões, and Lake Catalão itself, is a region of special interest to the study of various aspects of the trophic food webs.

### Field Sampling

Sample collection was carried out every fifteen days, through integrated samples from the littoral, pelagic and floating meadows zones. The littoral region was defined as the area with sparse vegetation and no macrophytes; pelagic region was the area of open waters; floating meadows region was the area in the middle of the floating macrophytes. In all cases 20 L of water was collected and taken to the laboratory in less than three hours. In the pelagic region a three inch wide PVC pipe with water-tight closing mechanism was used, which allows water passage when the pipe is lowered down and is closed when the tube is pulled up. The depth to which the pipe was taken down was determined in situ as three times Secchi depth value, thus ensuring sampling within the entire euphotic zone

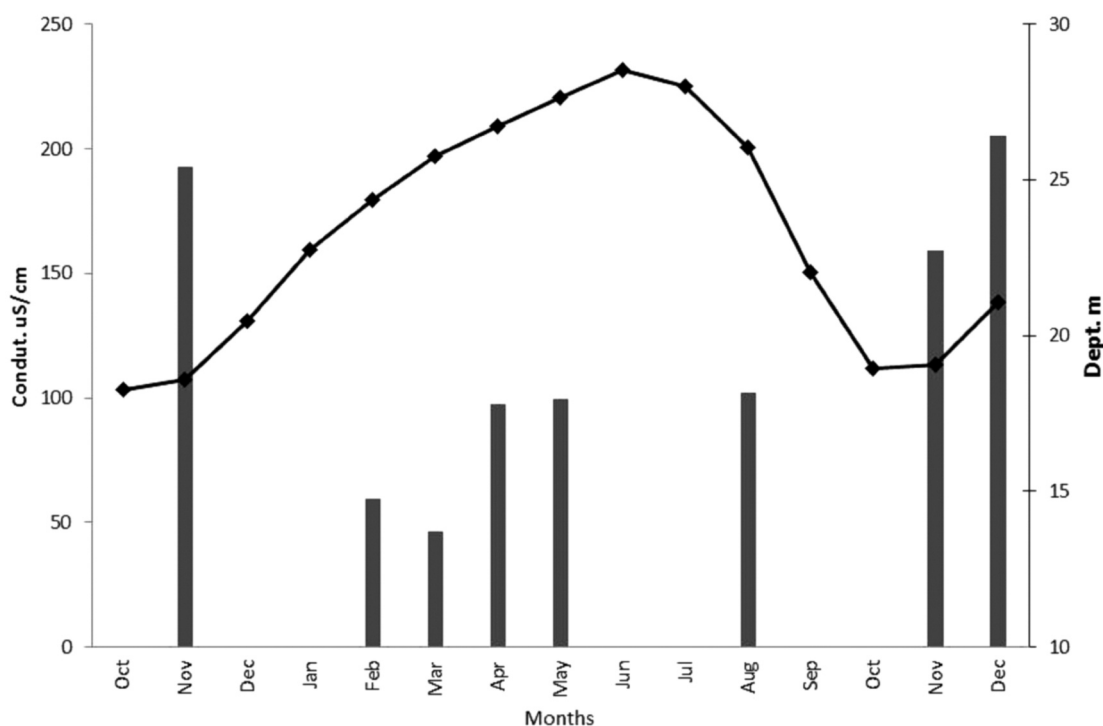


Figure 2. Average electrical conductivity (bars) of the water column in the pelagic zone and depth of water in the Rio Negro (line), from November 2006 until December 2007.



according to extinction coefficient values estimated by Brito *et al.* (2014). In the littoral region, water was sampled using plastic buckets and care was taken to avoid collection of sediments. For sampling inside the floating meadow, water was first filtered in the boat using a series of three filters as follows: 500 – 300 – 120 µm, to remove macroinvertebrates, small fish and fragments of plant roots.

*In situ* assessments of temperature, dissolved oxygen and electric conductivity were performed in the three environments studied with the use of a Thermo Scientific Orion 5-Star Plus device calibrated before every measurement. The OD polarographic sensor has a 0.1 – 0.01 mg/l resolution and a relative accuracy of ±0.2 mg/L. All parameters in the limnetic region were measured meter by meter to the bottom of the water column. In the other regions measurements were taken at the surface and at the bottom.

### Size-Fractionation of Phytoplankton

Three particle-size fractions were assessed for chlorophyll and carbon and nitrogen stable isotopes based on the studies of Rai (1982) and Romero and Arenas (1990): the <10µm fraction, usually called ultraplankton nanoplankton, the fraction between 10-30 µm called nanoplankton, and the fraction between 30-60 µm and a 30µm mesh plankton. The < 2µm fraction (autotrophic picophytoplankton) was not sampled in this study because the major phytoplanktonic species found in Lake Catalão were more than 2µm Cyanophyceae (Almeida and Melo, 2011).

Twenty liters of water were filtered through a 60 µm mesh to remove the large zooplankton represented by Copepoda and Cladocera. The values for the chlorophyll content obtained in this fraction are called total chlorophyll content or total pigment content. The second fraction was obtained in the laboratory through the filtration of 10 liters using a 30µm mesh. Five liters were analyzed and the values are called 10-30 µm total chlorophyll content or <30 µm total pigment content. The remaining five liters were filtered with 10 µm filter paper and the values obtained are called <10 µm total chlorophyll content or < 10 µm total pigment content.

### Chlorophyll Determination

The concentration of total pigment content was determined bimonthly in three different particle sizes (<10, 10-30 and 30-60 µm) according to the extraction technique using acetone at 90 % described in Golterman *et al.* (1978). For each sample 200 ml of filtered water with 60, 30 and 10 µm were collected with the use of GF/F glass filters, which are the most efficient devices for retaining algal cells. The filters were kept in aluminum foil bags in the freezer at -20 °C. For the extraction of chlorophyll-a, the filters were macerated in aqueous solution of 90 % acetone. The bulk was centrifuged at 3,500 rpm for 20 minutes and kept in a cool and dark environment for 24 hours. Then, absorption of the supernatant solution was determined in a quartz cuvette of 1 cm

optical path length, at 663 and 750 nm wavelengths, by means of a FEMTO 700S spectrophotometer. Then, the solution was acidified with HCl 1N, and the readings were repeated at the same wavelengths.

Determination of the total pigment content or total chlorophyll content was provide, as described by Carlston and Simpson (1996), which comprises of all pigments and degradation products that are absorbed at 665 nm. The equation of Golterman *et al.*, (1978) was used in this study for the calculations of total chlorophyll:  $B (\mu\text{g/L})_n = (106 U.Ve) / (kc. Vf)$ . The extinction coefficient used was 89.

### Stable Isotopes Analysis

A total of 138 isotope analyses of carbon and nitrogen in phytoplankton were performed, including three size fractions (<60 µm, <30 µm and <10 µm) and three environments (littoral-lit, pelagic-pel and macrophyte-mac).

Isotopic analyses were provided in 500 ml of water from the euphotic zone previously filtered (previously burnt for 1h at 450 °C) with 60 µm, 30 µm and 10 µm mesh nets. With reference to the difficulty reported by Hamilton *et al.* (2005) involving the presence of particulate matter different from algae in the samples, the criterion of Araujo-Lima *et al.* (1986) and Forsberg *et al.* (1993) was adopted, according to which the samples filtered with a 60 µm mesh net for removing zooplankton are representative of the amount of phytoplankton that exists in the environment.

The GFF filters with sample were dried at 60 °C for 24 hours, placed in 5 ml Eppendorf pipette and sent for isotopic analysis at UNESP's Center of Stable Isotopes in Botucatu, São Paulo. The samples were double analyzed by mass spectrometer (IRMS/EA), with an analytical error of 0.2 %. The <sup>13</sup>C/<sup>12</sup>C and <sup>15</sup>N/<sup>14</sup>N ratios were assessed using as reference the PDB standard (Pee Dee Belemnite in South Carolina, USA) for carbon and the atmospheric nitrogen for this isotope. The values are expressed in delta per thousand (δ ‰), as the result of the equation:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = R_{\text{sample}} = \{(R_{\text{sample}} - R_{\text{standard}}) - 1\} \times 10^3$$

where R is the <sup>13</sup>C/<sup>12</sup>C or <sup>15</sup>N/<sup>14</sup>N isotope ratio for sample and standard.

One test of filtering efficiency was done three times, always using water from the limnetic region of the lake. The isotope ratios of phytoplankton lower than 60 µm, lower than 30 µm, lower than 10 µm, lower than 5 µm and lower than 0.2 µm were analyzed. The data are presented as average values ± standard deviation (sd) and analyzed using Anova. The homoscedasticity was also tested.

## RESULTS

Temperature of Lake Catalão during the study was on average 28.9 °C ± 0.9, with maximum and minimum values of 32.3 – 27.6 °C. The hottest month was November, with an average

temperature of 30.03 °C and the coldest month was May, with an average temperature of 27.9 °C, with this value influenced by a phenomenon called “friagem” (sudden drop in temperature) that affected all the region. The electrical conductivity variation in the lake during the four limnological periods reflects the variation in the proportion of the mixture of waters from Rios Negro and Solimões in the lake (Fig. 2). At the dry period the conductivity values are on average  $193 \mu\text{S} \pm 4.0$ . In December, the waters from the Rio Negro flooded into the lake reduce the conductivity value to  $34 \mu\text{S} \pm 6.6$  in April, the period of major influence of Rio Negro. At the end of April the waters from Rio Solimões flowed in and the conductivity increased until reaching the typical values for this river in May. In August these values increased when the fall of water level began, and the values obtained were again those found in November 2007 at the dry season, though a little lower because they were measured in the first week of the month.

The results of assessments of total pigments in the three environments studied are shown in figure 3. The average value of chlorophyll content in the entire environment studied in the lake was  $10.42 \mu\text{g/L}$ , with the largest concentration ( $29.8 \mu\text{g/L}$ ) detected in the pelagic region in December, at the beginning of the flooding with waters from Rio Negro. The lowest concentration was assessed in the two months of flood, May and June, when, except for the floating meadows zone, the values were on average  $2.8 \mu\text{g/L}$ . During the same months, the total chlorophyll content in the floating meadows

zone was  $6.3 \mu\text{g/L}$ , which are the highest concentrations in the lake for this period. In the months of August and September, which correspond to the falling waters period, the concentration of chlorophyll increased, reaching the greatest values in November when the sampled period finished. It is important to stress that there were no floating macrophytes in the lake in the dry season, which explains why we do not have results for this environment during this period. The pelagic region of the lake always showed the lowest pigment values, but the tendency of variation of pigment values was generally the same in the three environments.

Despite the higher value of chlorophyll content in December, at the beginning of the flooding of the lake, the highest concentration occurred in the two dry periods, and the highest average value was found in 2007 (Fig. 3). The lowest concentrations corresponded to the high waters period and the intermediate values were observed in the flooding and falling water periods. The fact that the highest concentrations of chlorophyll were found in the floating meadows zone during the flooding and flood is significant. Therefore, the concentrations detected in the littoral region were highly variable during the whole study period, though they were the highest during the 2007 dry season. On the other hand, this value was lower in the same period in 2008, corresponding to near half the value found in the pelagic region.

The three fractions studied in all the regions showed different average values of chlorophyll content during the study period ( $p < 0.05$ ) as indicated in figure 4. The largest concentrations

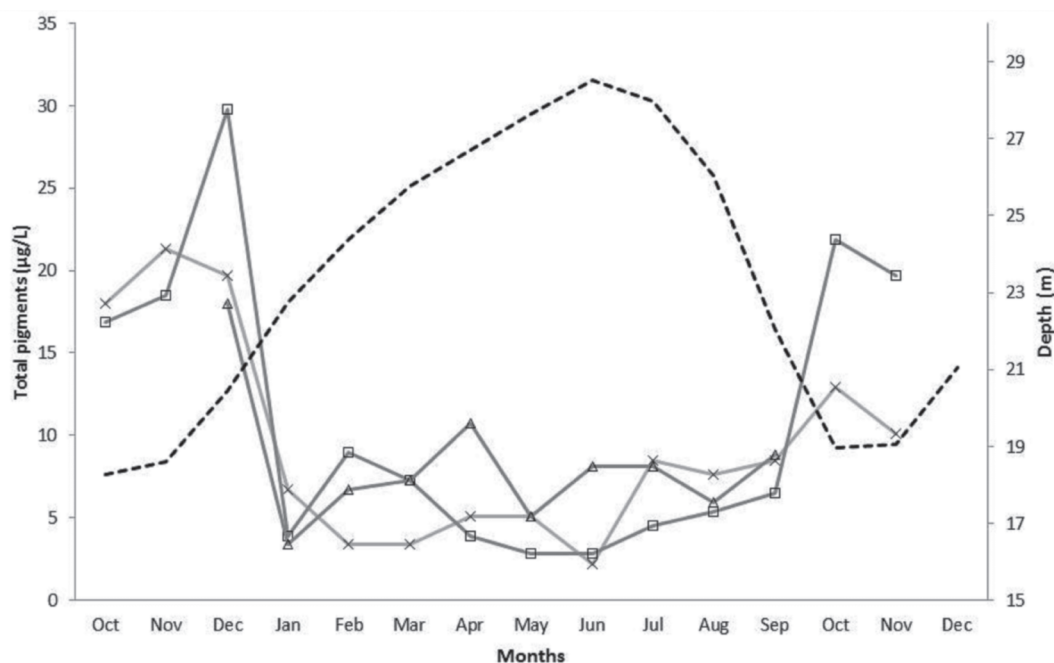


Figure 3. Monthly total pigments concentration in samples filtered through a net of 60  $\mu\text{m}$  at three environments in the Lake Catalão: Pelagic (squares), littoral (X) and floating meadows zone (triangle) vs. the depth of the Lake Catalão (line with  $\square$ ).

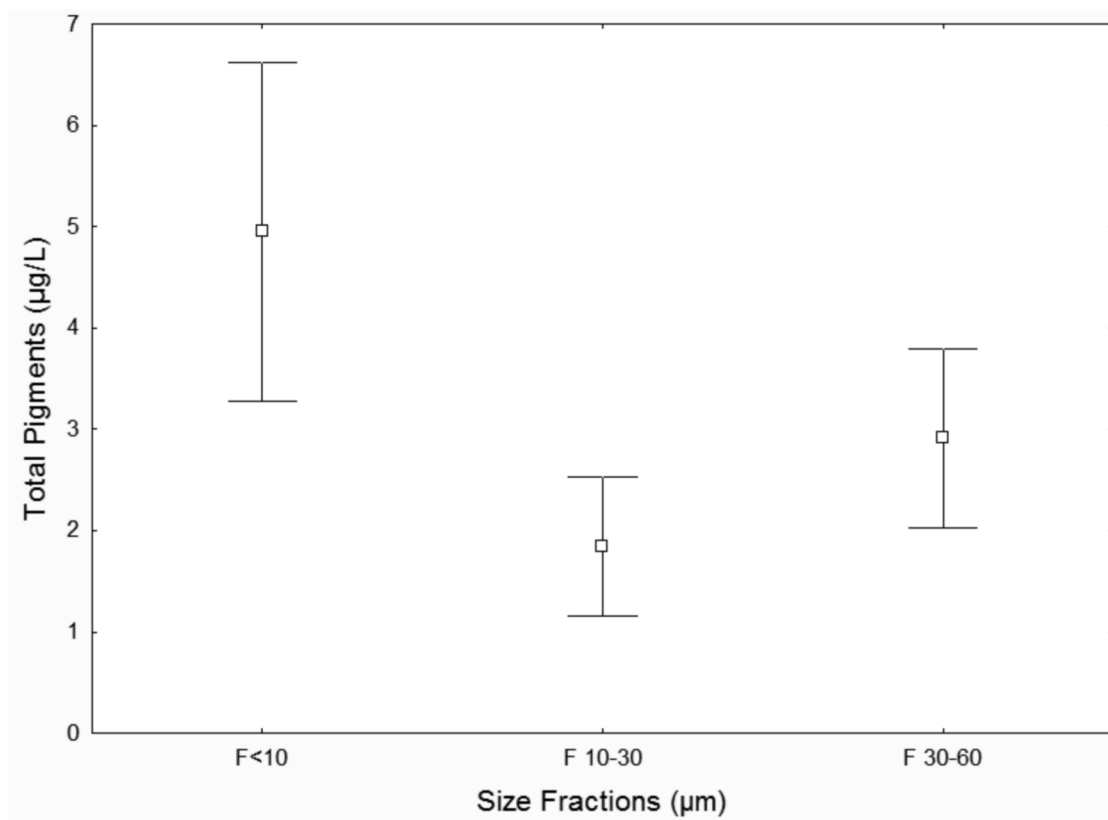


Figure 4. Average concentration of total chlorophyll in the size fractions studied in the Lake Catalão: <10 µm, <30 µm and <60 µm.

were found in the <10 µm fraction and the smallest concentrations in the less than 30µm fraction. On the other hand, no difference was detected between the concentrations of pigments in the three studied regions.

The seasonal variations in the percentage of the three chlorophyll fractions considered here (60-30, 30-10 and <10 µm) are shown in figure 5. It is important to stress the significant contribution of the total chlorophyll content of the <10 µm fraction in the three environments, which were respectively 51 %, 44 % and 41 % for the coastal region, pelagic region and floating meadows zone. The highest values are associated with to the dry season during the months of October, November and December, the latter corresponding to the beginning of the flooding. In this last period, the <10 µm fraction represented 87 % of phytoplankton in the coastal region, 63 % in the pelagic region and 78 % in the floating meadows zone. The 60-30 µm fraction had an average contribution of 30 % and the average contribution of the intermediate fraction (30-10 µm) was 22 % of the total chlorophyll content in the lake. This last fraction showed the most variable behavior throughout the year in the three environments, with the values varying from 0.0 % in the coastal and pelagic regions during the high and falling waters, to 63 % in the floating meadows zone s in the same period. The 60-30 and 30-10 fractions were unstable in the

pelagic and coastal regions, and one of them even disappeared for a few months.

With respect to the floating meadows zone, the three size fractions were present throughout the months of the studies, and the lowest participation of the <10 µm fraction was found in this environment.

#### Phytoplankton Stable Isotopes Analysis

The average value of  $\delta^{13}\text{C}$  for all the fractions in the three regions studied was  $-30.37\text{‰} \pm 5.79$  with a maximum value of  $-25.14\text{‰}$  (in the floating meadows zone, in April) and a minimum value of  $-33.95\text{‰}$  (pelagic region, in September). The general values of phytoplankton  $\delta^{13}\text{C}$  in the three environments studied are shown in figure 6. There is a significant correlation ( $p = 0.02$ ;  $r^2 = 0.62$ ) between the  $\delta^{13}\text{C}$  values of the pelagic and littoral regions (Fig. 6A), which does not occur between these variables and the values obtained for phytoplankton in the floating meadows zone. It is important to stress that no macrophytes occurred during the dry season in the lake, and when they occurred at the beginning of the flood, they represented a mere extension of the coastal and pelagic regions, with similar chlorophyll concentrations.

The maximum  $\delta^{15}\text{N}$  values for integrated samples were measured in the coastal and limnetic regions in the 2007 dry

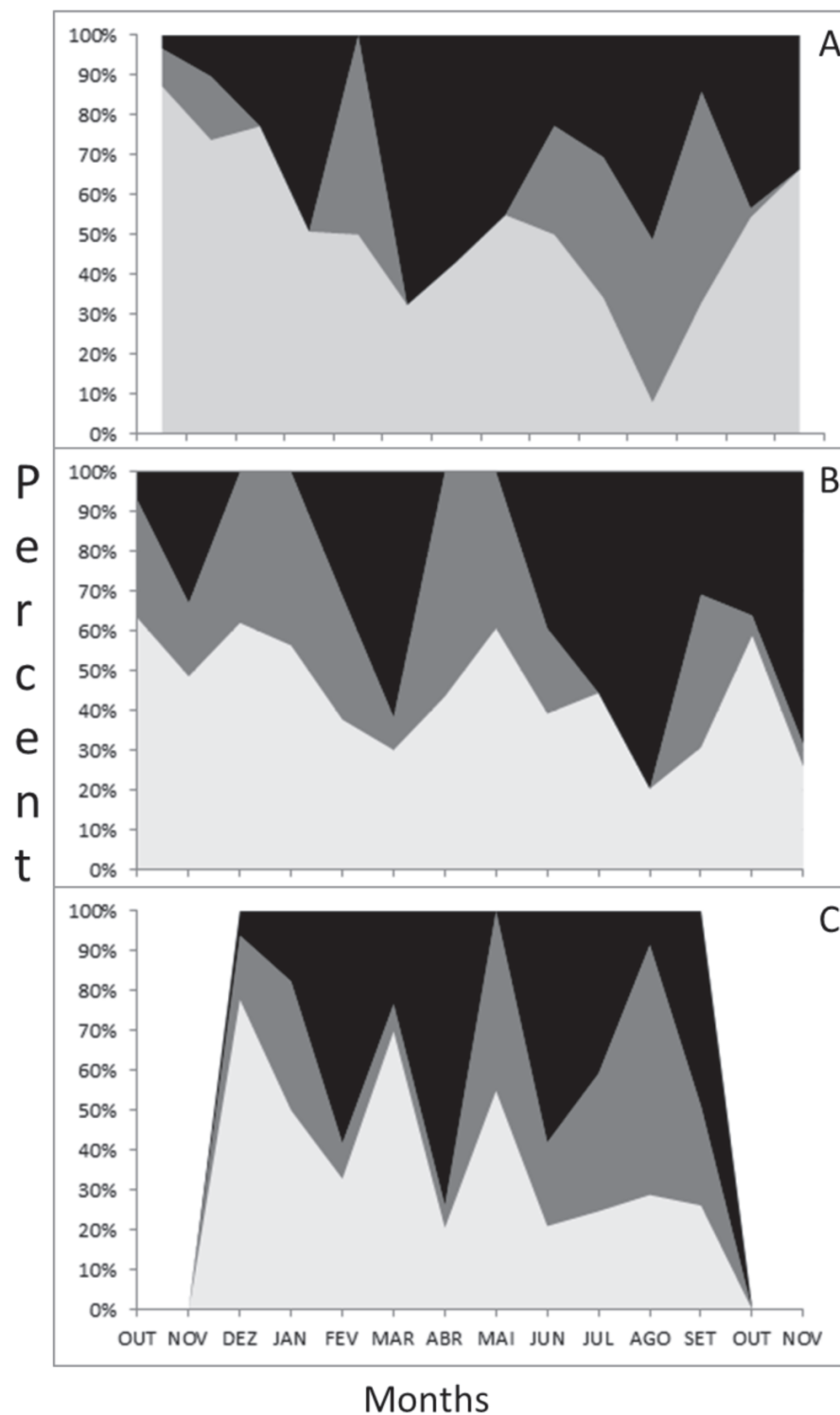


Figure 5. Percentage contribution of size fractions of the total chlorophyll in the three environments studied. A: littoral; B: pelagic and C: floating meadows zone. Black (<10 μm); Grey (10-30 μm) and white (30-60 μm).

season, and an average value of 14.88 ‰ was obtained. High values were also observed in June (12.08 ‰) in the three environments, and also in July (12.58 ‰) in the pelagic region,

after the first phenomenon of sudden drop in temperature that occurred in the lake that year. Excluding these three months when the lake was formed by a mixture of waters from the ana-



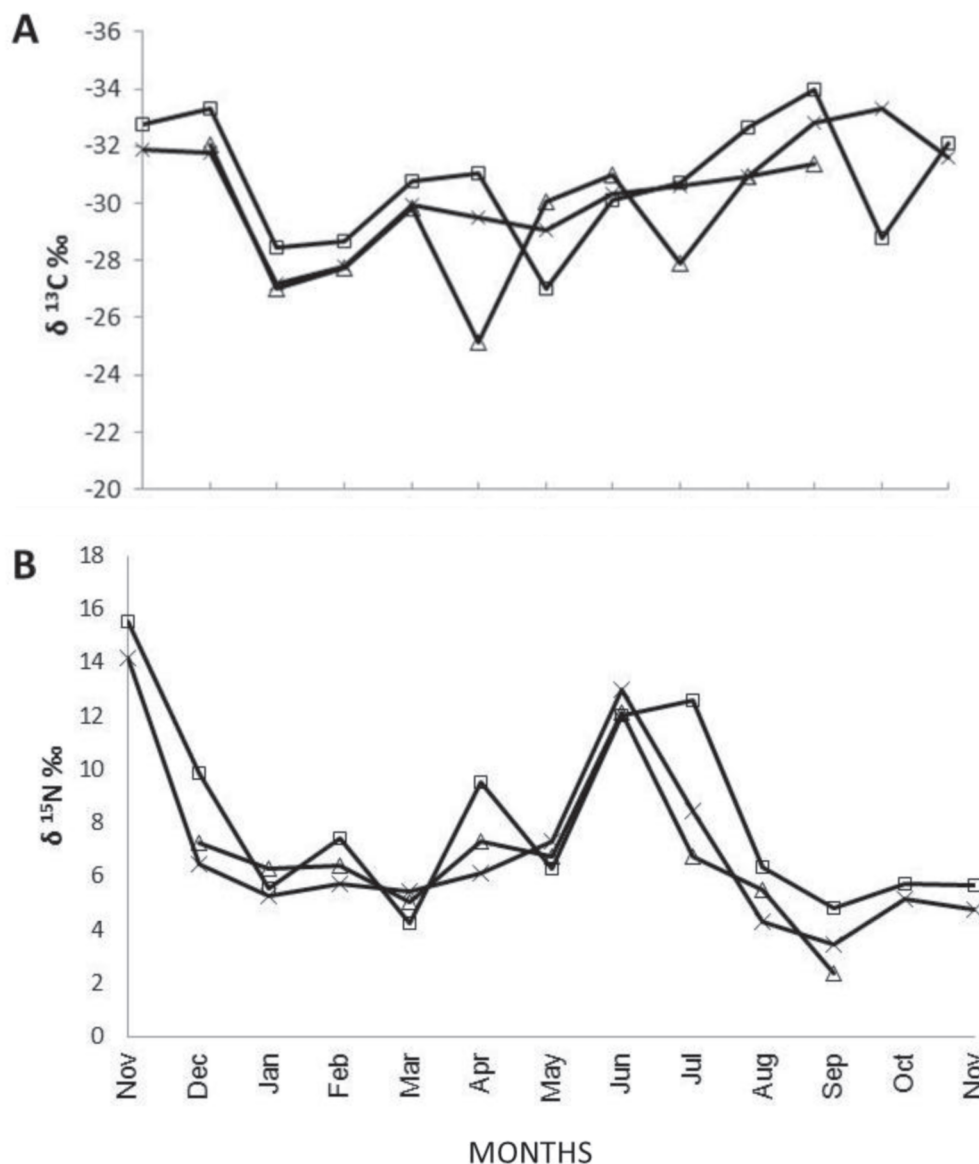


Figure 6. A and B. Monthly values of  $^{13}\text{C}$  ‰ (A) and  $^{15}\text{N}$  (B) in the three studied environments of the Lake Catalão. Floating meadows zone (triangle); Littoral (X); Pelagic (squares).

lysis, the average  $\delta^{15}\text{N}$  value for the phytoplankton was  $8.32 \text{ ‰} \pm 1.58 \text{ sd}$ . The  $\delta^{15}\text{N}$  data for the three studied environments are presented in figure 6B.

On the other hand, the three size fractions studied showed no significant difference between them at the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  measured values. The  $\delta^{15}\text{N}$  values had a minimum value of  $-1.19 \text{ ‰}$  in the fraction  $<60 \mu\text{m}$  from the floating meadows zone in September, and maximum value of  $6.37 \text{ ‰}$  in the fraction  $<60 \mu\text{m}$  from the littoral region, in August. In general, the  $\delta^{15}\text{N}$  values in the three environments and fractions studied showed great variability.

The  $\delta^{13}\text{C}$  values for the three fraction showed a high value of  $-26.51 \text{ ‰}$  in the fraction  $<30 \mu\text{m}$  from the floating meadows and a minimum value of  $-39.71 \text{ ‰}$  in the fraction  $<60 \mu\text{m}$  from

the floating meadows zone. Although without a significant difference in the global values, there is a difference higher than  $1 \text{ ‰}$  at  $\delta^{13}\text{C}$  between the values of floating meadows zone, which are richer than the values of the coastal and pelagic regions.

## DISCUSSION

Analysis of data showed no difference between the total of chlorophyll content in the three studied zones, excluding the data concerning the macrophytes in the dry period when this habitat did not exist. The abundance of pigments, associated with phytoplankton, in the aquatic macrophyte communities was surprising for a floodplain lake, although a similar situation was observed by Zocatelli *et al.* (2012) in a lagoon of the Brazilian northeast. During rising, high and falling water

periods, the concentrations of chlorophyll in the floating meadows zone were not different from those in the limnetic and coastal regions. These regions are considered poor or without significance in terms of phytoplankton because they are covered by floating vegetation. According to Melack and Forsberg (2001) phytoplanktonic communities in lakes are mostly restricted to open waters where there is more light (according to Lewis *et al.*, 2001). Due to the constant movement of water through the aquatic macrophytes, a great part of phytoplanktonic production remains in this region and is used to feed invertebrates. These authors affirmed that the macrophytes act like a filter that avoids the excessive growth of algae in the pelagic region. This explanation of phytoplankton production in macrophytes is consistent with the fact that the concentrations assessed in the beginning of the flood period were similar to those found in the coastal and limnetic regions (Fig. 3).

The chlorophyll contents in the floating meadows zone might change the projection of the primary production of phytoplankton made by Melack and Forsberg (2001), which is exclusively based on the production in open waters of the lakes. Brito *et al.* (2014) had already found that the pelagic regions of floodplain lakes are not always the most productive in terms of primary production. This occurs because the sunlight in the lake is significantly reduced by the concentration of particles dissolved and in suspension, which reduce the photic zone to around 3.0 m. Thus, trees and macrophytes can be more able to capture sunlight than phytoplankton (Melack and Forsberg, 2001), unless phytoplankton grows amidst this vegetation cover, by taking advantage of intermittent illumination (Diehl, 2002) calls.

Unlike the total chlorophyll contents, the analysis of the chlorophyll contents showed differences among the three size fractions studied. Phytoplankton of <10  $\mu\text{m}$  fraction was predominant in the three zones during the whole study period. This fraction, that includes the picophytoplankton and part of the nanoplankton, is mostly composed of cyanophyceae, chlorophyceae, bacteria and protozoa. Phytoplankton contents of <10 $\mu\text{m}$  fraction and had an average concentration of  $4.95 \pm 5.08 \mu\text{g/L}$ , which are almost double and triple from the 30-60 and 10-30 fractions, respectively. However, during the flood period the ratio of the 30-60 fraction increased and prevailed even during the falling waters period. This behavior correlates to the values of nutrients determined by Brito *et al.*, (2014), where the highest concentrations of nitrogen and phosphorus occurred during the dry season, in contrasts with the behavior observed in temperate lakes where the <10 $\mu\text{m}$  fraction is larger in oligotrophic waters and smaller in eutrophic waters (Adame *et al.*, 2008). Therefore two important elements must be considered: 1. the decreased concentration of nutrients during the flood period determined by Brito *et al.* (2014) which would limit the growth of cells, particularly the larger cells, and is less efficient for the assimilation of nutrients (Grisberger *et al.*, 2008), 2. Accord-

ing to Lampert and Trubetskova (1986), the herbivorous zooplankton is more active in the less than 30  $\mu\text{m}$  fraction, which was demonstrated by Caraballo *et al.*, (2011) for two cladoceran species in an in situ lake experiment. As a result, we suggest that more than a limitation in nutrients, the phytoplanktonic communities in the lake are effectively controlled by the zooplankton which is limited in the dry period due to the extreme turbidity of waters, which in turn reduces the filtration rate of Cladocera and constrains the presence of species such as the *Daphnia gessneri*, as demonstrated in laboratory and in situ experiments (Caraballo and Hardy, 1995).

Carbon isotopic data of the three studied fractions (<10, <30 and <60  $\mu\text{m}$ ), corroborate to the data of Araujo-Lima *et al.* (1986), Forsberg *et al.* (1993), Hamilton and Lewis (1992). No difference was detected between the isotopic values of the three study fractions, although the average value of the  $\leq 10$  fraction was higher in almost 2 ‰ than in the two other fractions. According to Burkhardt *et al.* (1999), evidence has been found on the difference between the size of algae and the taxonomic groups, on global phytoplankton isotope fractionation. Since it is unlikely that these groups of algae use different sources of  $\text{CO}_2$ , the isotope difference can only be explained by different fractionation rates during photosynthesis.

Except for the extreme values of  $\delta^{13}\text{C}$  found in the samples collected in the floating meadows zone, the values tended to be more negative in the dry period when there is a predominance of the <10  $\mu\text{m}$  fraction and richer in the flood when the <10  $\mu\text{m}$  phytoplankton rate is reduced. This tendency may reflect changes in the  $\delta^{13}\text{C}$  of the  $\text{CO}_2$  fixed by the phytoplankton, associated to the seasonal variation in the isotope characteristics of the organic material metabolized in the system. The lower value of  $\delta^{13}\text{C}$  for the phytoplankton in the dry period may reflect the greater contribution of organic material derived from phytoplankton for the community metabolism during this period. The volume of waters in the lake is reduced during the dry period, the influence of marginal habitats is lower and the chlorophyll concentration reaches its peak value. Since the  $\delta^{13}\text{C}$  of the phytoplankton is relatively negative compared to other groups of aquatic plants (Forsberg *et al.*, 1993), this would also result in more negative values for  $\text{CO}_2$  and phytoplankton in the referred period.

Amazon waters are usually supersaturated in  $\text{CO}_2$  (Richey *et al.*, 2002), then, the effect of the limitation of carbon isotope fractionation is minimum. Carbon isotope variation observed in submerged plants such as algae depends mainly on the isotope characteristics of the  $\text{CO}_2$  fixed *in situ* by respiration rates and its  $\delta^{13}\text{C}$  depends on the isotope characteristics of the organic material metabolized by the aquatic biota (Mayorga *et al.*, 2005). In general, a change in the specific composition of the phytoplanktonic community is not expected to produce a significant alteration in the  $\delta^{13}\text{C}$ , which would be the result of different assimilation of bicarbonate in face of  $\text{CO}_2$  deficiency by the different species.

The highest values of  $\delta^{15}\text{N}$  were found in the samples from the first flooding period and in the subsequent flood, exactly after the “friagem” phenomenon that occurred in the lake in the end of May (INPE, 2007). This behavior is associated total mixing of the water column that occurred in these two periods, in November 2007, as a consequence of the winds and low water level, and in May as a result of a convection process that resulted in the cooling of the water surface. During the second dry period studied, which was not as intense as the first, the  $\delta^{15}\text{N}$  values found were as low as the ones found during the flood period. According to Calheiros (2003), a great variation in the phytoplankton  $\delta^{15}\text{N}$  may occur during the assimilation of the different forms of nitrogen ( $\text{N}_2$ , ammonium, nitrate and nitrite).

The base line of the aquatic trophic network, defined as the isotope composition of carbon and nitrogen of their primary sources of food includes phytoplanktonic and non-phytoplanktonic sources that occasionally contribute to the secondary production (Perga and Gerdeaux 2006; Lehmann *et al.*, 2004). This explains the great variability observed in the  $\delta^{15}\text{N}$  of phytoplankton in Lake Catalão: the mechanisms that define the isotope ratio of nitrate in the surface are a response to the processes of nitrate assimilation by the algae, denitrification in the bottom and mixture of the water column (Lehmann *et al.*, 2004). Consequently, during the periods of lake stratification, when the anoxic hypolimnion is defined, the  $\delta^{15}\text{N}$  product of denitrification is enriched, and since the mixture of the water column re-suspends the substances of the anoxic hypolimnion, enrichment of the  $\delta^{15}\text{N}$  of phytoplankton can occur through the consumption of enriched ammonium (Perga and Gerdeaux, 2006). However, other processes can alter the  $\delta^{15}\text{N}$  of phytoplankton, including the direct  $\text{N}_2$  fixation, mostly by cyanobacteria, microzooplankton grazing, protein hydrolysis and microbial decomposition. The latter is traditionally associated with the increment of  $15\text{N}$  of residual organic material, but this is far from being indisputable (Lehmann *et al.*, 2004).

## CONCLUSIONS

The relatively high values of total pigment encountered in floating meadows were surprising, considering the traditional association of phytoplankton with pelagic environments only. This phytoplankton may be derived from periphyton communities (diatomaceae and chlorophyceae), which are common in this habitat and can become detached, forming an “accidental” plankton community. It may also represent pelagic phytoplankton which can be concentrated in macrophyte beds as lake water flows through them.

Some authors have associated the inverse relationship observed in most floodplain lakes between the water level and the phytoplankton concentration with seasonal variations in herbivore density and the availability of nutrients and light. Our more cautious analysis suggests that this relationship may reflect a simple process of dilution resulting from large

seasonal variations in lake volume associated with the riverine flood pulse. When variations in nutrient and phytoplankton concentrations are considered together with changes in lake volume, the natural production in these systems may actually be greater at high water.

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