ABUNDANCE AND ISOTOPIC COMPOSITION OF PLANKTONIC MICROCRUSTACEANS IN A CENTRAL AMAZON FLOODPLAIN LAKE: IMPLICATIONS FOR THE TROPHIC DYNAMICS OF THE PLANKTON COMMUNITY

Abundancia y composición isotópica de los microcrustáceos planctónicos en un lago de inundación de la Amazonía Central: implicaciones para la dinámica trófica de la comunidad planctónica

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ABSTRACT

During the hydrological year from December 2007 to November 2008, monthly samplings in the pelagic, littoral and macrophytes zones were conducted in the *Lago Catalão*, a floodplain lake receiving a mixture of water from *Negro* and *Solimões Rivers*, in front of Manaus city. Taxonomic composition and their relative abundance of the planktonic microcrustaceans community was studied. Natural abundances of carbon (C) and nitrogen (N) stable isotopes were measured to indicate energy sources. Cladocerans were the most abundant, with a relative abundance of 60%, followed by the calanoid and cyclopoid copepods with relative abundances of 29% and 11%, respectively. *Diaphanosoma* spp. was the dominant cladoceran group during all the sampling periods. Cladocerans were also represented by *Moina* spp., *Ceriodaphnia* spp. and *Daphnia gessneri*. Three genera of calanoid copepods were found: *Notodiaptomus spp*, *Rhacodiaptomus* spp., and *Argyrodiaptomus* spp. The genus *Mesocyclops* spp. was identified among the cyclopoid copepods. Zooplankton $\delta^{13}C$ values indicated that the aquatic macrophyte zone was distinct, with a mean of -27.31‰, which was more enriched than zooplankton in the pelagic and littoral zones, where they had mean $\delta^{13}C$ values of -33.11 and -34.66‰, respectively. Overall, analysis of stable isotopes showed that regardless of the pathways, the initial source of carbon for the zooplankton was phytoplankton, with a minimal participation of heterotrophic bacteria.

Key words. Amazon, planktonic community, stable isotopes.

RESUMEN

Durante el año hidrológico de diciembre 2007 a noviembre de 2008, se realizaron muestreos mensuales del zooplancton en las regiones pelágica, litoral y de macrófitas acuáticas, en el lago Catalão, un lago de aguas mixtas en la región de confluencia de los ríos Negro y Solimões, frente a la ciudad de Manaus. La comunidad de los microcrustáceos planctónicos se estudió en términos de su abundancia relativa, así como la abundancia natural de los isótopos estables de carbono y nitrógeno para definir las fuentes de energía. Los cladóceros fueron más abundantes en todas las regiones

estudiadas, presentando una media de 60%, seguido por los copépodos calanoideos con 29% y los ciclopoideos con 11%. Los cladóceros estuvieron representados por *Moina* spp*., Ceriodaphnia* spp*., Daphnia gessneri* y como grupo dominante, *Diaphanosoma* spp. durante todo el período de estudio. Sólo los copépodos adultos fueron contados e entre estos, tres géneros de calanoideos fueron identificados, *Notodiaptomus* spp*., Rhacodiaptomus* spp. y *Argyrodiaptomus* spp., sin embargo la abundancia individual de los géneros no fue evaluada. Entre los ciclopoideos, fue identificado el género *Mesocyclops spp*. La abundancia de $\delta^{13}C$ en las tres regiones estudiadas presenta a la región de macrófitas acuáticas como un área diferenciada en términos de la alimentación del zooplancton, con una media de -27,31‰, que es más enriquecida que las regiones litoral y pelágica, con valores de -33,11 e -34,66‰ respectivamente. El uso del análisis de isótopos estables de carbono y nitrógeno permitió definir que, independiente de las vías por las cuales el carbono este circulando, la fuente inicial de carbono para el zooplancton es principalmente el fitoplancton, con una mínima participación de las bacterias heterotróficas.

Palabras clave. Amazonia, comunidad planctónica, análisis de isótopos estables.

INTRODUCTION

The central role of the zooplankton in the carbon flow to upper trophic levels in lakes is well established, but the origin of the carbon (energy) that supports zooplankton is less well understood. Studies carried out in lakes of temperate zones have shown that zooplankton use carbon from both phytoplankton and terrestrial sources as energy sources (Carpenter *et al.* 2005), which would include aquatic macrophytes and flooded forests in the case of many tropical regions. The major uncertainty involves the role of detrital organic carbon, which may originate from one or more of the autotrophic sources and may be obtained either directly from the consumption of particulate organic carbon or indirectly via consumption of bacteria or fungi which are in turn dependent on detrital organic matter (Porter 1996). This uncertainty is due in part to the diversity of potential zooplankton dietary sources including phytoplankton, detritus, bacteria, and protozoans, the relative importance of which depends on the community species composition and environmental conditions including, in the case of floodplain lakes, the seasonal flood pulse.

In lakes, zooplankton communities are ecologically diverse and temporally and spatially dynamic, and specifically in floodplain lakes, the composition and abundance of zooplankton communities change frequently under the influence of abiotic and biotic factors (Torres-Bejarano *et al.* 2013). In floodplain lakes, zooplankton populations has been shown to be influenced by predation by fishes and by invertebrates such *Chaoborus* (Twombly & Lewis 1989). Evidence of top-down control of zooplankton comes from the analysis of the size distributions of zooplankton in lakes of Central Amazon presented by Trevisan & Forsberg (2007), who found predominantly small zooplankton in all lakes they studied, which indicates high predation pressure. Nevertheless, the same authors found a significant correlation between the abundance of zooplankton and phytoplankton indicating that bottom-up effects of resource limitation were also important. A study of the zooplankton population dynamics in a floodplain lake in the Orinoco River in Venezuela showed high birth rates throughout the hydrological year, which were indicative of high predation rates (Twombly & Lewis 1987, 1989). Based on *in situ* experiments

in a lake in Bolivia, Rejas *et al.* (2005) also found a constant birth rate, however, their results suggest an alternating bottom-up and top-down control of the zooplankton, especially those of greater size. It is likely that the abundance of the zooplankton in all of these systems is controlled simultaneously by the top-down (predation) and bottom-up effects, as shown for phytoplankton in several lakes of the Northern hemisphere (Mazumder 1994). However, the top-down effect apparently predominates in the majority of the floodplain lakes of Central Amazon that have been studied.

Population dynamics analysis conducted by different researchers in floodplain lakes have shown a higher zooplankton density during low water period (Brandorff & Andrade 1978, Lindholm & Hessen 2007), but this does not necessarily imply greater abundance because these lakes vary seasonally in volume. Considering the enormous fluctuations of the water level in Amazon lakes, which for instance during 2007, was 18 m in the *Rio Negro*, the zooplankton populations may increase in number of individuals as they decrease in density (Twombly & Lewis 1987). This was observed in the *Lago Calado* by Caraballo (1992), who used the estimates of zooplankton density together with the data of Lesack (1988) to calculate the volume of that lake, and express the results in terms of zooplankton abundance, demonstrating the highest zooplankton abundance during the flooding, even though densities were lower.

The relation between zooplankton population dynamics and hydrological phases is not equal for all components of the zooplankton community, which includes four large groups: protists, rotifers, cladocerans and copepods. In general, the species richness is greatest among the protists and smallest among the copepods. In fact, the trophic position of the zooplankton varies with the taxa, the hydrological phase, and size of the organisms. For example,

Trevisan & Forsberg (2007) compared abundance and zooplankton size distributions in floodplain lakes associated with the *Rio Solimões*, *Rio Negro* and a floodplain lake receiving water from both of those rivers (*Lago Catalão*). They found a mean density of 19.8 ind./L from which only 17.8% were large, corresponding to mesozooplankton following Sieburth *et al.* (1978) classification, which comprises organisms of sizes ranging 200-2000 μm, in which the cladocerans and copepods dominate. Contrasting abundances and sizes were found in lakes of *Rio Solimões* (47.4 ind./L and 14.5% large) and *Rio Negro* (3.7 ind./L and 4.9% large). In *Lago Catalão*, they found a mean of 185 ind./L total, of which 25% were large.

Zooplankton community responses to the seasonal changes in the floodplain lakes have been documented in terms of composition and abundance (Brandorff & Andrade 1978, Lindholm & Hessen 2007), but without a clear explanation of how such zooplankton communities respond to seasonal variations in the spatial distribution of their energy resources in the lakes. Looking for an explanation, Yoshioka *et al.* (1994) used stable isotopic analysis of the zooplankton and its autotrophic sources of carbon in a Japanese lake. Since the C isotopic ratio (expressed as $\delta^{13}C$) of a consumer reflects the isotopic composition of its diet (Post 2002) and the nitrogen isotopic ratio ($\delta^{15}N$) indicates the trophic position of an organism (Vander Zanden & Rasmussen 1996), the simultaneous analysis of C and N isotope ratios can reveal how the carbon is transferred from primary energy sources to upper trophic levels (Smyntek *et al.* 2007).

This study analysed the zooplankton community of *Lago Catalão*, an Amazon floodplain lake, using stable C and N isotopes to reveal the relative importance of different autotrophic sources of carbon to the zooplankton.

MATERIAL AND METHODS

Area of study. *Lago Catalão* is located on the floodplain near the confluence of *Solimões* and *Negro* Rivers (3°10'04''S and 59°54'45''W), 3 km from Manaus (Fig. 1). The lake lies on a peninsula that has a series of interconnected water bodies that can become a continuous water surface during inundation, but become isolated from each other and may dry completely at low water levels, normally in Oct-Nov (Brito 2006). Normally, *Lago Catalão* is connected to the *Rio Negro* directly by a channel and it is seasonally connected to *Rio Solimões* through a wide area during flooding. Thus, it is a lake with alternating dominance of *Rio Negro* in the low-water period, and the *Rio Solimões* at the end of the high water period.

The lake presents extreme environmental variation between the low water and flood phases. Brito (2006) and Almeida & Melo (2009) observed values of conductivity up to 400 µS/cm in the low-water phase, which decreased with the entrance of water from the *Rio Negro* down to 30 µS/cm, and increased again to 70 µS/cm, after the entrance of water from the *Rio Solimões*, becoming similar n chemistry to that river during flooding. The abundance of phytoplankton was strongly related to conductivity, presenting mean values of 9.0 µg/L of total chlorophyll and primary productivity of 29.1 ± 17.9 mg C/m³/h during the flooding (Brito 2006), being dominated by the cyanophyceae, specially *Synechocystis aquatilis*, *Synechococcus elongates*, and *Planktothrix isothrix* (Almeida & Melo 2009).
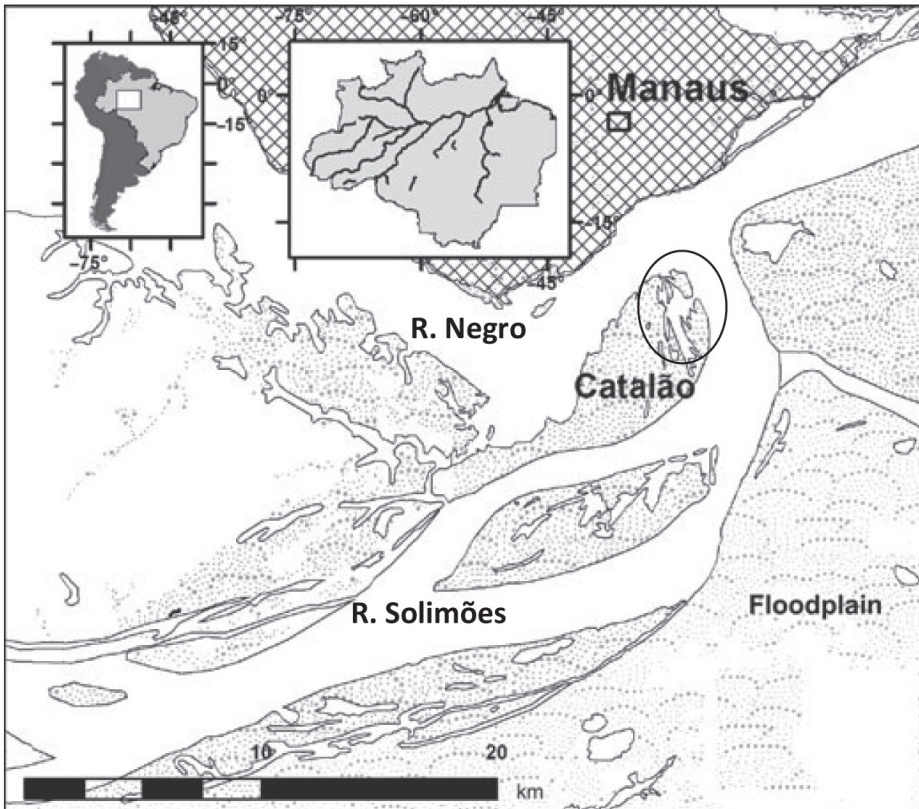
Figure 1. Location of *Lago do Catalão*, Amazônia State, Brazil. The lake is within the circle, 3°10'04''S and 59º54'45''W. (modified from Santos *et al.* 2007).

The hydrological phases of isolation, filling, flooding, and falling waters are well-defined in the lake, as they are the direct result of the predictable flooding regime of both rivers. Figure 2 presents the dynamic of the water level at the Port of Manaus, which corresponds to the water level of the lake. On November 22nd 2007, when the level of the *Rio Negro* at Manaus was 18.9 m, the lakes started to receive water from this river, and when the level reached 25.6 m on April 27th 2007, the lake started to receive water from the *Rio Solimões* through its northern channel. The flooding phase occurred between June and July, followed by the falling water phase between August and September, and the isolation phase between October and November, when the lake did not receive water from any of the adjacent rivers, and became much reduced in area.
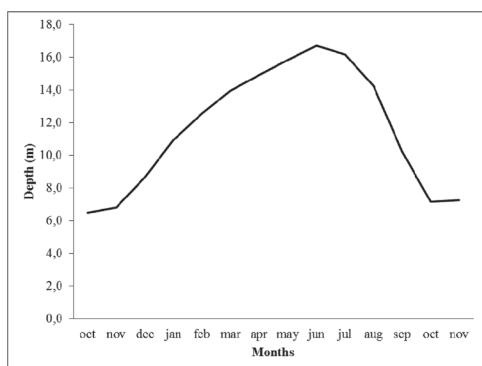
Figure 2. Variation of depth of the *Lago Catalão* between October 2007 and December 2008.

Low transparency (0.3 m Secchi depth) and high conductivity characterize the isolation phase in the lake, when it resembled other kinds of isolated lakes. At the end of November, the inflow began with the input of water from the *Rio Negro*, diluting the water of the lake, increasing the transparency and decreasing the conductivity. The minimal conductivity was reached at the end of April (24 µS/cm); at the same time, the transparency reached its maximum level (1-1.2 m; Brito 2006). Such levels remained stable until the

beginning of May, when the lake reached 12 metres of depth and began to receive input of water from the *Rio Solimões*, promoting a homogenization of the lake due to the mixture of the waters. In June-July, the flooding phase occurred, with smaller fluctuations of the water level and maximum transparency of the water (1.3 m). By the end of this phase, the floating macrophytes had disappeared from the lake.

Sampling. Monthly samplings were conducted from December 2007 to November 2008 in the open water, littoral, and macrophyte zones of the lake. In all environments, independent of the sampling technique, 30 L of water were filtered through a 65 µm sieve; the organisms retained by the sieve were preserved in 4% formalin. PVC tubes of 10 cm diameter and 6 m length were used for sampling the pelagic and coastal regions. The depth was always three times deeper than Secchi depth. The tube had a device that allows the entrance of water when it is lowered into the water column, and hinders the entrance of water when it is pulled to the surface, which results in a vertically integrated sample of the entire photic zone. At the shallower littoral zone, the tube was lowered diagonally until it approached the bottom, without touching the sediment. In the sampling within the floating meadows, a 10 L bucket was filled and the sample filtered through 200 µm mesh to remove residuals of roots and other debris before the final collection of zooplankton on a 65 µm sieve.

The relative abundance was calculated based on counting, under a stereoscopic microscope, of 50% of the volume of the samples. Fish and insect larvae, especially from the littoral and aquatic macrophyte environments, were not counted, but removed from the samples for individual analysis of stable isotopes.

Zooplankton samples for stable isotope analyses were collected using a conical net of 65 µm mesh, 50 cm in diameter, and 1.1

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m long, taking horizontal and vertical tows in the open water zone and horizontal tows in the littoral zone, and collecting water with a bucket below the aquatic macrophytes. In all cases, sampling was carried out until a visible amount of organisms was captured. These organisms were kept in distilled water up to 3 hours, changing the water every hour, to clear their intestinal contents (Smyntek *et al.* 2007).

Preparation of the samples for stable isotope analysis (SIA). Samples were filtered onto GF/C glass-fiber filters that had been pre-combusted for one hour at 450ºC and dried at 60ºC for 12 hours. Depending on the amount of the sample, the filter or the total zooplankton mass was kept in desiccator until the shipping for isotope ratio mass spectrometric analysis (IRMS/EA) at the Environmental Stable Isotope Centre of the Bioscience Institute/ Unesp/ Botucatu, Brasil. The samples, which were free from impurities and contaminants, were dried in a forced-air oven at 50ºC and then pulverized to ≤250 μm in a liquid nitrogen-based cryogenic mill (-196ºC). To avoid cross-contamination, a mass of 0.5 mg is enough for $^{13}C/^{12}C$ ratio isotopic analysis with two replicates, and an error below 0.2‰.

In general, isotopic analyses were conducted on samples of total zooplankton mass. In cases of large abundance of a specific group, separate samples were prepared as well. The isotopic analysis of the potential feeding sources of the zooplankton included the C3 and C4 aquatic macrophytes, phytoplankton according to the methodology proposed by Forsberg *et al.* (1993), and detritus and heterotrophic bacteria according to Coffin *et al.* (1989), modified by Caraballo *et al.* (2012). As complementary information, monthly assessments of depth and transparency were carried in the open water zone of the lake.

Variability of d $\delta^{13}C$ and $\delta^{15}N$ values is presented as an average values ± standard deviation (sd) and analyzed using a non-parametric Kruskal-Wallis test, following of a Kolmogorov-Smirnov test. The significance level was p<0,05.

RESULTS

Lago Catalão is a "mixed water" floodplain lake, influenced alternately by two of the largest rivers in the world. In the beginning of the riverine inflow in November 2007, the lake received black water from the *Rio Negro*, lasting through July. However, at the end of April, when the lake had reached a depth of 12 m, the *Rio Solimões* began to bring white water through the northern part of the lake, in a process that can block the intake of the water from *Rio Negro*, whose entry channel became dammed by the lake. During this flood pulse period, besides depth, strong variations in transparency were observed (Fig. 2). The highest levels of transparency were found in the months of January and February, when the lake received water exclusively from the *Rio Negro*. The level of transparency decreased with the inflow of water of *Rio Solimões* in April, then increased later in the high water period (Caraballo *et al.* 2014).

After the second sampling, the low number of rotifers in the samples suggested that the sieves were not retaining this group, and therefore they were not counted. This observation was supported by the low biomass and the small contribution of rotifers to samples for stable isotope analysis. The cladocerans were represented by *Moina* spp., *Ceriodaphnia* spp., *Daphnia gessneri*, and particularly by the dominant genus *Diaphanosoma* spp. during the entire sampling period. Only the adult copepods were counted; among them, three genera of calanoids were found – *Notodiaptomus* spp., *Rhacodiaptomus* spp. and *Argyrodiaptomus* spp. – however, the individual abundance of the genera was not assessed. Among the cyclopoids, we identified the genus *Mesocyclops*. Other organisms such *Chaoborus*, fish larvae and insects of the

family Corixidae appeared occasionally in the samples – while we disregarded them from the zooplankton counting, they were included in the isotopic analysis. In the case of the larvae of *Chaoborus*, night samples were performed exclusively for its sampling.

The cladocerans were the most abundant organisms in all of the sampled zones, presenting a mean of 60%, followed by the calanoid with 29% and cyclopoid with 11%. Such zooplankton abundance was higher in the open water zone and lower in the aquatic macrophyte zone (Fig. 3). *Diaphanosoma* was the only genus that was present in all months of sampling, while *Daphnia gessneri* only appeared at the end of the flow and flood, in both cases being associated with low levels of turbidity in the water.

Comparing the three zones of the lake, the largest dominance of the cladocerans was found in the open water zone (Fig. 4B),

and the smallest found at the macrophyte zone, where cladoceran abundance was still >50%. In the littoral zone, calanoid copepods dominated temporarily at the beginning of the inflow (Fig. 4A), together with a strong presence of larval fish and insects of the family Corixidae. At the aquatic macrophyte zone, the proportion of cyclopoid copepods became larger during the falling water phase (August and September), when the plants were in senescence (Fig. 4C). In fact, during October and November (isolation phase), there were not floating macrophyte in the *Lago Catalão*.

During this study, the values of δ^{13}C in the zooplankton were between -21.79‰ (at the aquatic macrophyte zone in May) and -38.48‰ (littoral zone in September). The largest range in zooplankton δ^{13}C (11.43‰) was observed in the littoral zone. The open water zone was more stable, showing a range of 8.01‰ in zooplankton δ^{13}C. The
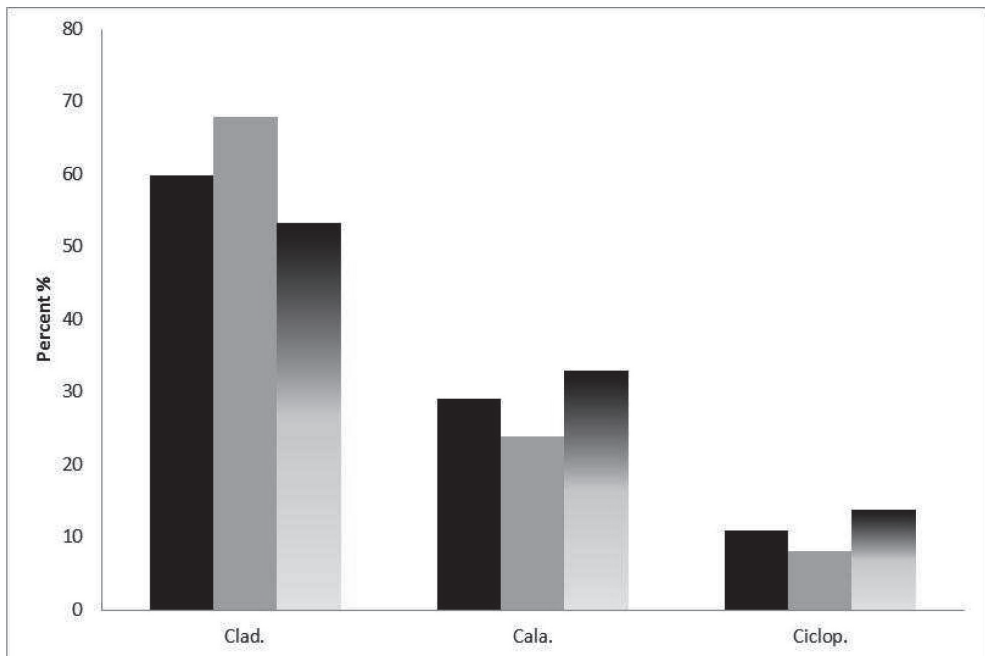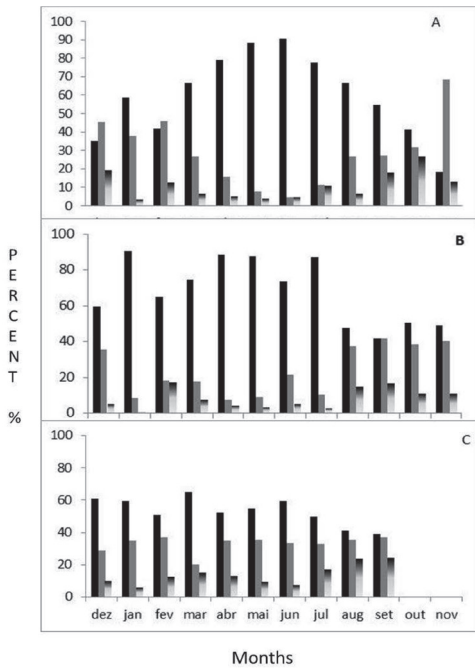

Figure 3. Relative abundance of the three groups of zooplankton studied in the different ecological zones of the *Lago Catalão*. Littoral zone (black), open water zone (grey), and aquatic macrophyte zone (graded).
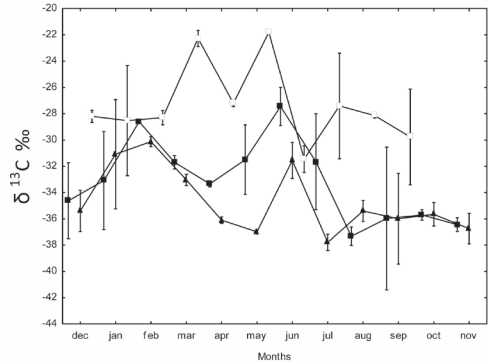
aquatic macrophyte zone was more dynamic (p < 0.05) when compared to the other two zones, showing a mean zooplankton $\delta^{13}C$ of -27.31‰, which is more ^{13}C-enriched than the values from the littoral and open-water zones, which averaged -33.11 and -34.66‰, respectively (Fig. 5).

In the other hand, zooplankton $\delta^{15}N$ showed more uniform values among the three zones, varying from 9.73‰ (December, in the open-water zone) to 4.71‰ (March, in the aquatic macrophyte zone). The littoral zone had the lowest variability in zooplankton $\delta^{15}N$ (range, 2.88‰), whereas the aquatic macrophyte zone showed the highest variability (4.17‰). A seasonal oscillation of the zooplankton $\delta^{15}N$ values was observed in all three zones, but

a strong and general decrease was evident in the period between December and April, which corresponds to flooding with water from the *Rio Negro*. An increase in $\delta^{15}N$ (until June) was evident with the input of the water from the *Rio Solimões* at the end of April, decreasing again in August and September (Fig. 6).

Figure 5. Seasonal variation (mean and standard deviation) of $\delta^{13}C$ of the zooplankton in the littoral zone (filled boxes), open-water zone (filled triangles), and macrophyte zone (empty boxes) in *Lago Catalão* during the hydrological year from December 2007 to November 2008.

Figure 4. A, B and C. Percent relative abundance of the mesozooplankton in *Lago Catalão* between December 2007 and November 2008. A) littoral zone; B) open-water zone; and C) aquatic macrophyte zone. Dark bars are cladocerans, grey bars are calanoid copepods, and graded bars are cyclopoid copepods.
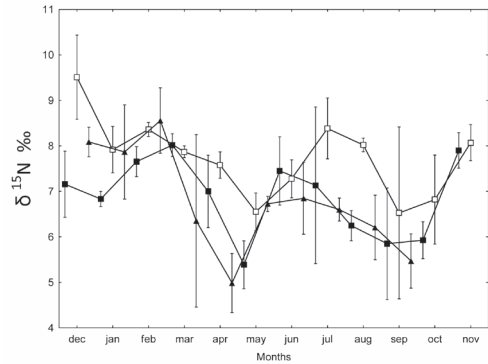
Figure 6. Values of the mean and standard deviation (bars) of $\delta^{15}N$ of the zooplankton in littoral zone (filled boxes), open-water zone (filled triangles), and macrophyte zone (empty boxes) in *Lago Catalão* during the hydrological year from December 2007 to November 2008.
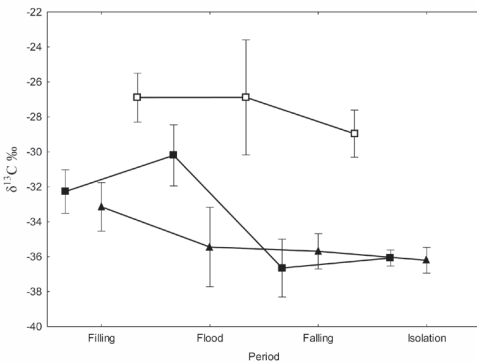
Values of zooplankton δ¹³C identified the aquatic macrophytes as a differentiated zone regarding zooplankton feeding behaviour, with a mean of -27.31‰, much more enriched than the zooplankton of the littoral and pelagic zones, with had values of -33.11 and -34.66‰, respectively (Fig. 7). Zooplankton in the littoral zone was enriched in ¹³C during the flood phase, and later presented minimal values during falling water and isolation phases. Such a tendency, even less pronounced, was also observed for the open-water zone, which presented the lowest variability in zooplankton δ¹³C. Highest variability was found in the open-water and aquatic macrophyte zones during the flooding phase. The values of zooplankton δ¹³C were more negative in the open-water and littoral zones during the falling water and isolation phases.

Values of zooplankton δ¹⁵N showed high variability, being relevant the case of the aquatic macrophyte zone during the flow, with values ranging from 4.71-8.88‰, suggesting a range across two trophic levels if the basal δ¹⁵N source were the same (Fig. 8). The highest values of δ¹⁵N were found in the open-

water zone during the flooding phase and the lowest in the aquatic macrophyte zone during the falling water phase.
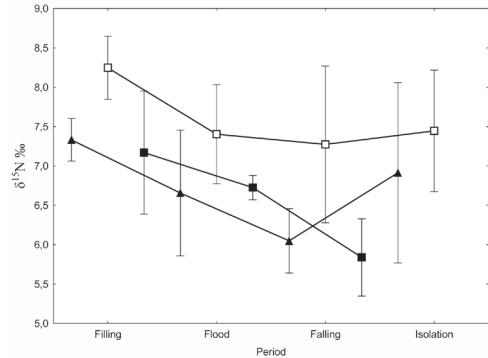
Figure 8. Values of the mean and standard deviation of δ¹⁵N of the zooplankton in the littoral zone (filled boxes), open-water zone (filled triangles), and macrophyte zone (empty boxes) in *Lago Catalão* during the hydrological phases of filling, flood, falling water, and isolation.

Samples of cladocerans, calanoid and cyclopoid copepods, insects of the family Corixidae, fish larvae and *Chaoborus* spp. (Table 1) were analysed separately for stable isotope ratios. Among them, the highest value of δ¹³C was registered for cladocerans from the aquatic macrophyte zone and the lowest value was registered for *Chaoborus*, sampled at night in the open-water zone. In terms of variation of δ¹³C within the same group, which reflects its trophic plasticity, cladocerans and calanoid copepods presented ranges of 4.61 and 4.34‰, respectively. Regarding δ¹⁵N, the highest values were observed for fish larvae from the open-water zone, and the lowest for calanoid copepods in the littoral zone. Fish larvae belonged to the Engraulididae and Characidae families, not including five specimens from the genus *Pyrrulina* (Lebiasinidae) sampled in August 2008 at the aquatic macrophytes zone, which presented δ¹³C values of -23.46‰ ± 0.04 and 4.82‰ ± 0.02.

Figure 7. Values of the mean and standard deviation of δ¹³C of the zooplankton in the littoral zone (filled boxes), open-water zone (filled triangles), and macrophyte zone (empty boxes) in *Lago Catalão* during the hydrological phases of filling, flood, falling water, and isolation.

Table 1. Mean and standard deviation for the natural abundance of stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in specific groups of zooplankton, corixids, and larval fishes collected from three zones in the *Lago Catalão* from December 2007 to November 2008.

Zone	Group	$\delta^{13}C$ ‰	sd	$\delta^{15}N$ ‰	sd
Littoral	Calanoid	-32.67	1.12	5.24	1.38
Open water	Calanoid	-34.52	1.31	7.93	1.68
Aquatic Macrophyte	Calanoid	-28.33	0.79	8.42	1.20
Open water	Cyclopoid	-30.34	0.44	7.90	0.38
Littoral	Cyclopoid	-28.56	0.20	7.62	0.04
Littoral	Corixidae	-33.68	1.26	5.85	1.60
Aquatic Macrophyte	Corixidae	-31.26	0.68	6.93	0.34
Littoral	Fish Larvae	-33.53	2.46	9.48	1.03
Open water	Fish Larvae	-33.54	1.35	14.73	2.20
Aquatic Macrophyte	Fish Larvae	-30.30	1.88	10.24	2.21
Littoral	Cladoceran	-31.93	0.19	7.92	0.42
Open water	Cladoceran	-30.61	0.48	8.50	0.52
Aquatic Macrophyte	Cladoceran	-27.32	0.39	6.36	0.33
Open water	*Chaoborus*	-34.84	1.67	12.31	0.27

Values of $\delta^{13}C$ and $\delta^{15}N$ from different zooplankton feeding sources were taken from Caraballo *et al.* (2012) and are presented in Figure 9, showing that phytoplankton were the most ^{13}C-depleted source, and the aquatic macrophytes the most enriched. Zooplankton tended to be more depleted in 13C than any of the sources, but not too far from phytoplankton. However, these are the mean values, which do not reflect the variation of such sources across the hydrological year.

DISCUSSION

The hydrological dynamics of *Lago Catalão*. In the complex hydrological cycle of a floodplain lake, gradients of temperature, light, conductivity, and nutrients interact affecting quality, quantity, and composition of zooplankton food sources. A rapid increment in the zooplankton richness is the regular result of the beginning of the process of flooding in
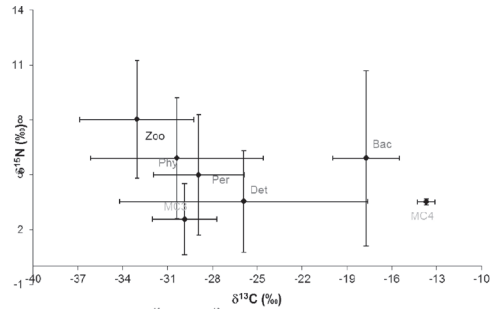
Figure 9. Relation between the $\delta^{15}N$ and the $\delta^{13}C$ (mean and standard deviation) of the potential food sources of the zooplankton in *Lago Catalão* during the period from December 2007 to November 2008: Zoo is zooplankton; Phy is phytoplankton; Per is periphyton; Det is detritus; MC3 are C3 macrophytes; MC4 are C4 macrophytes and Bac are bacteria (taken from Caraballo *et al.* 2012).

tropical lakes (Brandorff & Andrade 1978, Twombly & Lewis 1989, Lindholm & Hessen 2007). The water input is associated with the increase of food resources (Torres-Bejarano et al. 2013), what is especially true in *Lago Catalão*, where the response of the macrophytes *Echinochloa polystachya* and *Paspalum repens* is immediate, covering broad areas that provide refuge for some organisms of the zooplankton (Hamilton 1990; Hamilton *et al.* 1992). Thus, the water input of the *Rio Negro* at the end of November begins the expansion of the trophic niches, which reaches its maximum in the flood, creating an inundated forest zone (locally called igapó) around the lake. In this process, the lake inundates the floodplain vegetation, including the wastes of the buffaloes that graze during the low waters, that has been associated with the increase of nutrient availability (Brito 2006).

Relative abundance of the Zooplankton. The exclusion of rotifers and copepod larval stages is undoubtedly one of the reasons why in our results there was a strong

dominance of cladocerans at the three studied environments. It is possible to affirm, based on Trevisan & Forsberg (2007), that only 25% of the zooplankton community in terms of number of individuals was analysed, which corresponds to the larger sized organisms. In fact, the majority of the assessments of tropical zooplankton that include rotifers have shown them to be the dominant group in richness and abundance (Keppeler 2003). However, when counts of larval stages of the copepods were included, this group was found to have the highest abundance (Waichman *et al.* 2002).

Cladoceran dominance was more evident during the high water period in *Lago Catalão*. During the low water period, copepods showed an increase, and calanoids were the dominant group in the littoral zone during the isolation phase. Such changes may be associated with the presence of fish larvae at the littoral zone during low water resulting in a strong predation pressure upon cladocerans – a situation observed by Leite *et al.* (2006) in the same lake. On the other hand, considering the abundance of calanoid copepods in this work.

In general, the distribution of zooplankton relative abundance in the *Lago Catalão* presented an absolute dominance of two groups, mainly herbivorous, and a lower abundance of the cyclopoids, which are carnivorous zooplankters. *Mesocyclops* – the genus of cyclopoid identified in *Lago Catalão* – is known to feed on rotifers, cladocerans, nauplii of copepods, and protozoans (Williamson 1980). Carbon and nitrogen stable isotope ratios showed that the cladocerans *Diaphanosoma spinolosum* and *Ceriodaphnia cornuta* consumed predominantly phytoplankton (Caraballo *et al.* 2011), which had already been observed for the calanoid copepods by Yoshioka *et al.* (1994). The herbivorous efficiency of a zooplankton community is proportional to the mean size of the species presented (Matthews & Mazunder 2005b). For example, the variation in the feeding behavior of calanoid species may be higher than the difference in the same behavior between two species of *Daphnia* (Matthews 2005).

Based on analysis conducted in 2466 Norwegian lakes, Walseng *et al.* (2006) demonstrated that the microcrustaceans may be divided into three groups: strictly coastal-benthic species, species that are present at the coastal and pelagic regions, and a smaller group comprised of strictly pelagic species. This classification, although broad, does not encompass the characteristics of some large species, as those from the family Chydoridae, which normally are present in the samples collected within the aquatic macrophytes. Such large species are considered to inhabit littoral zones (Walseng *et al.* 2006), but are not present in our littoral zone samples, presumably because of high predation pressure by the fish larvae.

Seasonal variation in natural abundance of δ^{13}C and δ^{15}N. The analysis of seasonal variation of δ^{13}C of the zooplankton from the different zones showed a more ^{13}C-enriched mean value for samples collected from aquatic macrophytes (Figure 5), suggesting two sources of carbon, one originated from C3 plants, which generates more ^{13}C-depleted values in the pelagic and coastal samples; and the other from C4 plants, which occur mainly in the aquatic macrophyte zone. Prior research carried out in the Amazon region considered the phytoplankton as C3 plants in addition to the C3 vascular plants, and the only significant C4 plants were considered to be the floating aquatic grasses, especially *Paspalum repens* and *Echinochloa polystachya* since they are the most abundant in biomass (Forsberg *et al.* 1993). However, an alternative explanation for the 13C-enriched zooplankton in the macrophyte zone would be a trophic linkage to attached algae (epiphyton), which are known

to be variable in d13C but often relatively 13C-enriched (Hamilton *et al.* 1992).

The mesozooplankton consumes the phytoplankton, but also different groups of heterotrophic protists, which connects the microbial (detrital) trophic chain with the classical fish-zooplankton-algae trophic chain (Porter 1996). Generally, the copepods are considered efficient predators of ciliated microbes and the cladocerans efficient predators of bacteria (Perga *et al.* 2006), what could explain the patterns of $\delta^{13}C$ of the zooplankton in the littoral and macrophyte zones obtained in this research. In the open-water zone, the values of $\delta^{13}C$ of the zooplankton are closer to those of the phytoplankton, which agrees with the results of Oliveira *et al.* (2006). Green and chrysophyte algae of sizes smaller than 20 µm were the most abundant in the stomach content analysis of the fishes *Argyrodiaptomus furcatus* and *Notodiaptomus iheringi*, therefore algae were considered as an important source of food for these species in a reservoir at south-eastern Brazil (Rietzler *et al.* 2002). Therefore, it is possible to affirm that the mesozooplankton in *Lago Catalão* presents high feeding plasticity, grazing over a $\delta^{13}C$ range of 11.43‰, 8.01‰ and 9.95‰ in the littoral, open-water, and macrophyte zones, respectively. Of course, it may be the result of a punctual activity of a species (Carpenter *et al.* 2005, Matthews 2005), which would mask the inferences made about dietary sources. However, it is more likely that those species find the same resources in the three environments. Or it could be also the result of the intrinsic variability of the resource, as the result of the biogeochemical variation associated with the hydrological dynamics.

Except in May and July, when the lake experienced a cooling event, locally called "friagem" (Caraballo *et al.* 2014), the lowest values of $\delta^{13}C$ were found during the months when copepods dominated (Fig. 4).

Low values of $\delta^{13}C$ may be associated with the consumption of methanogenic carbon (Bastviken *et al.* 2003) that has been widely demonstrated in the hypolimnion of floodplain lakes, as well as its input associated with the upper layers of the water column during the mixing events. The consumption of methanotrophic bacteria was linked to ^{13}C depletion in consumers down to -50.3‰ by Kankaala *et al.* (2006). Thus, taking into account that the minimal values of $\delta^{13}C$ for phytoplankton (-34 and -33‰ in the coastal and pelagic zones, respectively), the consumption of methanotrophic bacteria may be more important than has been considered, as suggested by Calheiros (2003) in her research at the Pantanal region. On the other hand, the degree of 13C depletion observed in zooplankton in this study could also be explained by consumption of 13C-depleted phytoplankton; such negative δ 13C values would be expected in waters high in dissolved carbon dioxide (Hamilton *et al.* 1992).

The values of zooplankton $\delta^{15}N$ did not show high spatial variation, showing means of 6.88, 7.74 and 6.77‰ for the littoral, open-water, and macrophyte zones, respectively. The zooplankton $\delta^{15}N$ did show variation associated with the flood pulse, with more ^{15}N-depleted values during April and September, and more enriched values during December and July, corresponding with the hydrological phases. The variability of the values of $\delta^{15}N$ suggested, assuming a trophic fractionation of 3.4‰ (Post 2002), that the zooplankton fed on just one trophic level. According to Matthews (2005), copepods are omnivorous, and therefore a higher value of $\delta^{15}N$ is expected; however we did not observe ^{15}N enrichment relative to the cladocerans.

Natural Abundance of $\delta^{13}C$ and $\delta^{15}N$ in relation to the hydrological phases. Zooplankton $\delta^{13}C$ values from the aquatic macrophyte zone during the hydrological cycle of the lake were more ^{13}C-enriched

than values from the other two zones (Figure 5); however, within each zone they did not differ much across the three limnological phases (Figure 7). Besides particulate organic detritus from the C4 grasses, another source of C4 carbon may be heterotrophic bacteria that rely on C4 organic matter, the importance of which has been demonstrated by Waichman (1996) and Caraballo *et al.* (2012).

The mean values of zooplankton $\delta^{13}C$ in the littoral and open-water zones showed similar degrees of ^{13}C depletion during the filling and falling water phases, but became more 13C-enriched during the flood phase, which is considered the period of the year in which *Lago Catalão* (Almeida & Melo 2009) and generally the other floodplain lakes (Torres-Bejarano et al. 2013) become more homogeneous. During the high-water period, the lake is subject to a disturbance of variable magnitude and intensity caused by the "friagem", which mixes the water column, and can bring dissolved methane from the bottom waters to the surface waters (Caraballo *et al.* 2014). This mixing can in turn generating more ^{13}C-depleted phytoplankton by the fixation of CO_2 created from the oxidation of the isotopically lighter methane), as proposed by Kankaala *et al.* (2006). This phenomenon may have affected first the zooplankton of the open-water zone, and later, in the the coastal region during the falling water and isolation phases. The different dynamic of the macrophyte zone, could be explained if the mixing process by the cooling of the upper part of the column had been hindered by the plants.

In general, the mean values of $\delta^{15}N$ were not significantly different ($p > 0.05$) for the three zones, but each showed similar seasonal variation (Figure 6). Is this seasonal variation a consequence of the seasonal change of the feeding habits of the zooplankton, or variation in the isotopic composition and structure of the resource? Or in other words, is it a trophic shift or just baseline isotopic variation? On the one hand, there is evidence that individual zooplankton species have the ability to exploit different resources, depending on the environmental conditions (Porter 1996). On the other hand, biogeochemical variability that could affect the isotopic composition of the food resources has been observed not only at seasonal time scale but also in diel cycles (Boëchat & Giani 2008). Seasonal variation in $\delta^{15}N$ of *Daphnia* was defined by Lehmann *et al.* (2004) as dependent on the seasonal variation in $\delta^{15}N$ of the dietary sources, which in turn varied as a consequence of the processes in the nitrogen cycle. The huge variability of the system and the trophic plasticity of mesozooplankton make it impossible to answer this question with the available information.

During the hydrological year studied, the phytoplankton had a mean $\delta^{13}C$ value of $-30.37‰ \pm 5.79$ including the variable contribution of the size fractions <60 μm, <30 μm and <10 μm (Caraballo *et al.* 2014). It is known that the $\delta^{13}C$ of phytoplankton may vary with depth and with the size of cells. Even considering that cladocerans are efficient consumers of bacteria, Perga *et al.* (2006), based on the proportion of lipids of phytoplanktonic and bacterial origin, have not shown a significant contribution of bacterial C in the production of *Daphnia*. In this study, carbon and nitrogen stable isotope analysis showed that, independent of the pathways through which the carbon is circulating, the initial source of carbon and energy for the zooplankton community is mainly phytoplankton.

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