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Isotopic characterization of the energy autotrophic sources at Grande lake complex in Amazonian

Jesaias Ismael da Costa[1], Daniel Pinto Borges[2], Fabiane de Almeida Santos[3], Ana Cristina Belarmino de Oliveira[4]

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ABSTRACT

The flooded areas of white water rivers, floodplains, are the most productive among the different environments of the Amazon basin and account for about 90 % of total fish caught for subsistence and commercial fishing. From the technique of stable isotopes can indicate which energy sources are incorporated by fish and quantify its importance for the maintenance of fish stocks. This study identified and characterized isotopically the autotrophic energy sources of the fish of the Grande lake, situated to the left at Solimões river and the right at Manacapuru river. Were collected and characterized isotopically in C and N, 59 samples of primary energy sources in low water and high, represented as seston phytoplankton (n = 11), riparian vegetation of the flooded forest (n = 12) and macrophytes (n = 22). The phytoplankton was the source with the lowest values in $\delta^{13}C$ and C4 macrophytes with the highest values showing the variation that exists in values isotopic of the plants that utilize different carbon sources and different photosynthetic pathways. The values of $\delta^{15}N$ are influenced by many factors, but the interaction with nitrogen fixing organisms causes the plants present values in $\delta^{15}N$ smaller than the others.

Key words: carbon isotope, nitrogen isotope, primary producers, floodplain lake

Caracterização isotópica das fontes autotróficas de energia do complexo do lago Grande na Amazônia. As áreas alagadas dos rios de água branca, as várzeas, são as mais produtivas dentre os diferentes ambientes da bacia Amazônica e responsáveis por cerca de 90% do total de peixes capturados pela pesca de subsistência e comercial. A partir da técnica de isótopos estáveis é possível indicar quais fontes de energia são incorporadas pelo peixe e quantificar sua importância para a manutenção dos estoques pesqueiros. Este trabalho identificou e caracterizou isotopicamente as fontes autotróficas de energia das assembleias de peixes do lago Grande, situado à margem esquerda do rio Solimões e a direita do rio Manacapuru. Foram coletadas e caracterizadas isotopicamente em C e N 59 amostras de fontes primárias de energia na cheia e seca, representadas como fitoplâncton-seston (n=11), vegetação ripária da floresta alagada (n=26) e macrófitas aquáticas (n=22). Os resultados de composição isotópica foram submetidos à análise estatística descritiva de média e desvio padrão com auxilio do software R. O fitoplâncton foi a fonte com os menores valores de e macrófitas C4 com os maiores valores, mostrando que há variação nos valores isotópicos das plantas que utilizam diferentes fontes de carbono e diferentes caminhos fotossintéticos. Os valores de $\delta^{13}C$ são influenciados por muitos fatores, mas a interação com organismos fixadores de nitrogênio faz com que as plantas apresentem valores em $\delta^{13}C$ menores que os outros.

Palavras chaves: isótopos de carbono, isótopos de nitrogênio, produtores primários, lago de várzea

[1] Discente UFAM - Faculdade de Ciências Agrárias, Departamento de Ciências Pesqueiras, Avenue General Rodrigo Octávio, 6200, Coroado I, 69077-000 - jesaiaslbr@gmail.com
[2] Discente mestrado CIPET - Faculdade de Ciências Agrárias, Departamento de Ciências Pesqueiras, Avenue General Rodrigo Octávio, 6200, Coroado I, 69077-000 - danielpborges@hotmail.com
[3] Discente mestrado CIPET - Faculdade de Ciências Agrárias, Departamento de Ciências Pesqueiras, Avenue General Rodrigo Octávio, 6200, Coroado I, 69077-000 - pygocentrus_bia@yahoo.com.br
[4] Docente UFAM - Faculdade de Ciências Agrárias, Departamento de Ciências Pesqueiras, Avenue General Rodrigo Octávio, 6200, Coroado I, 69077-000 - acbolive@ufam.edu.br

1. Introduction

Among the different environments of the Amazon basin, wetlands white water rivers, floodplains, are the most productive, accounting for about 90% of total fish caught by commercial and subsistence fisheries (BAYLEY and PETRERE, 1989). These areas have a specific floristic composition and strongly influenced by variations in water level. In periods of high waters flooded forest participates as a primary source of importance to the fish fauna, however macrophyte are responsible for most primary productivity (JUNK, 1984). The alternation between periods of rising and receding water seems to be fully understood by the biota, because the bodies of these areas interrelate evenly between the periods of greatest abundance and diversity and slumps with little available water sources energy (LOWE-MCCONNELL, 1987).

Floodplain lakes are still considered fishes natural creators and are not protected from frequent and intense anthropic actions due to increasing human occupation. Deforestation of flooded forests, which changes ecological conditions and compromises the biodiversity of plants and animals, advances in the increasingly important areas for system maintenance and renewal of fish stocks. Statistics indicate the last decades a considerable decrease in fish landings in the region (BATISTA, 1998; IBAMA, 2007).

Given this reality, it is necessary to know how much and how the biomass of fish can be influenced by changes in the primary sources of the medium. This information is important for planning effective management of fish stocks, including the factors that control its production, in other words, the availability of energy (ARAUJO-LIMA et al., 1986). A key step for this research is the study of some aspects of carbon dynamics in the system and biota in the system based on the identification of autotrophic carbon sources incorporated bodies. It is necessary to both identify and isotopically characterize the primary energy sources of floodplain lakes, generating information that supports the understanding of trophic relationships that support the food web in these aquatic ecosystems.

The isotopic method is based on varying the ratio between the heavy and the light isotope of a given element in organic matter, organic matter where each has a reason "specifies" with predictable isotopic fractionation as it is transformed, either by physical, chemical or biological actions (BOUTON, 1991). The isotopic composition of organic matter is expressed by δ (‰) notation of the reference element. This methodology has been used in ecological studies of fish mainly using the isotopes of carbon and nitrogen as indicators of primary sources incorporated by the animal, and the measurement of their trophic position (FORSBERG et al., 1993; POST, 2002).

Autotrophic sources (primary producers), isotopically differ depending on the form of CO_2 fixation, with three different routes: pathway C3 (cycle Calvim), pathway C4 (Hatch Slack cycle) and Metabolism of Fatty Crussaláceas (CAM). The bodies of C3 pathway the most discriminating ^{13}C relative to ^{12}C, resulting in impoverished $\delta\ ^{13}C$ values averaging -27 ‰. The photosynthetic pathway of C4 plants discriminate less ^{13}C to ^{12}C ratio, resulting in more enhanced values $\delta^{13}C$ compared to C3 plants, on average -12 ‰. Pathway CAM plants have a system of fixing CO_2 specialized, intended primarily to maintain a relatively positive carbon balance in the tissues, resulting in intermediate ranges in $\delta\ ^{13}C$ values for those of the other cycles (MARTINELLI et al., 2009) .

In Amazonian aquatic ecosystems, several authors characterized isotopically autotrophic energy sources. Along the channel of the Amazon/Solimões river, Araujo-Lima et al (1986), found values of $\delta^{13}C$ for C4 macrophytes, C3 macrophytes, periphyton and riparian trees of -12.9 ‰, -27.6 ‰, -26 8 ‰ and -27.6 ‰, respectively. Martinelli (1988) obtained isotope values in $\delta^{13}C$ to C3 and C4 plants of the lowland areas of the Amazon on average -12 ‰ to -27 ‰, respectively. Forsberg et al. (1993) studied the major primary energy sources for fish of commercial importance in the Central Amazon, indicated values of $\delta^{13}C$ of 33.3‰, -28,8‰, -26.2‰, -27.6‰ and -12.8‰ for phytoplankton, flooded forest, periphyton, C3 macrophytes and C4 macrophytes, respectively. Leite et al. (2002) studied food webs of fish larvae in four Amazonian lakes found average values in $\delta^{13}C$ of -37 ‰, -30.0 ‰, -25.8 ‰ and -12.8 ‰ for phytoplankton, leaves of C3 tree, C3 macrophytes and C4 macrophytes, respectively. Oliveira et al. (2006) studied the autotrophic energy sources for fish in the lake Camaleão-AM characterized isotopically fruits and seeds of C3 plants ($\delta^{13}C$= -29.3 ‰ and $\delta^{15}N$ = 4.5 ‰), C4 macrophytes

($\delta^{13}C$ = -12.9 ‰ and $\delta^{15}N$ = 6.6 ‰) and seston phytoplankton ($\delta^{13}C$ = -36.1 ‰ and $\delta^{15}N$ = 6.6 ‰). In the basin river Paraná-PR-BR, Lopes et al. (2006) characterized isotopically terrestrial riparian vegetation ($\delta^{13}C$ = -30.1 ± 1.32 ‰ and $\delta^{15}N$ = 2.09 ± 2.46 ‰), C4 macrophytes ($\delta^{13}C$ = -13.0 ± 0.87 ‰ and $\delta^{15}N$ = 3.51 ± 3.17 ‰) and C3 macrophytes ($\delta^{13}C$ = -28.42 ± 2.73 ‰ and $\delta^{15}N$ = 4.15 ± 3.88 ‰).

Although these results contribute to the knowledge of the primary sources of Amazonian aquatic ecosystems, do not release more detailed investigations of these sources, in view of the diversity and specificity in floristic composition found in these environments and the wide range of variation that can display fonts in their different environments water (FRANCE, 1995).

The Grande Lake is a floodplain lake in the Amazon region with specific characteristics, which depending on the hydrological period, can be considered a lakeside complex (SOARES, 2009). The fishing activity in this lake has economic, social and cultural importance to its traditional populations. Is located in a region of influence of the route of the pipeline Coari/Manaus, which configures an area susceptible to environmental change risk and therefore demand more information to knowledge and understanding of the complex relationships between organisms that make up these ecosystems in order to subsidize their sustainable exploitation.

In this context, this work has identified and characterized isotopically autotrophic energy sources that support the biomass of fish assemblages in the Grande lake, contributing information to the study of energy flow in floodplain lakes, as well as subsidizing the environmental monitoring of this aquatic ecosystem.

2. Material and Methods

The present study was realized in lakeside complex of Grande lake, on the left margin of the Manacapuru river and right margin of the Solimões river, consisting of riverbanks, holes, creeks and small lakes that are all interconnected (SOARES, 2009).

The primary sources of energy in the Grande lake are represented by phytoplankton, plants flooded forest and aquatic weeds. The samples were collected in periods of extreme fluctuation of the water level in the months of November and December 2006 (Drougth), and May and July 2007 (high water).

The samples of phytoplankton were performed using plankton nets of 20 μm, thrown into the water for the sub-surface drag at low speed and obtaining water samples concentrated in approximately 20 L. In the laboratory the water samples were submitted to a filtering process on a battery of eight networks (500 μm; 330 μm, 120 μm, 80 μm, 60 μm, 30 μm, 20 μm and 10 μm) in order to separate phytoplankton impurities resulting drag. Considering the phytoplankton samples from those filtering on meshes of 20 μm and 10 μm. The difficulty of separating algal materials and debris, these samples were designated seston phytoplankton (CALHEIROS, 2003).

The plants present in the ecotone between terrestrial and aquatic system portion of leaves, stems, roots and inflorescence composing a homogeneous sample of the plant were removed. Herbarium specimens were prepared for correct species identification by experts and comparison of materials from the INPA and UFAM herbarium. By importance of fruits in the diet of fish (MAIA and CHALCO, 2002), when present were collected composing the reference plant sample.

The samples were dried in a forced circulation at 55 ° C and ground in a mortar and pestle to a fine powder. The crushed samples were stored in small plastic containers and sent to the Centro de Energia Nuclear para Agricultura da Universidade de São Paulo (CENA-USP) for analysis of isotopic composition of carbon and nitrogen.

The results of the isotopic composition of the sources were submitted to descriptive statistics of mean and standard deviation with the aid of the software R.

3. Results

Were collected and characterized isotopically 59 samples autotrophic energy sources in low water and high water, represented as seston phytoplankton (n = 11), riparian vegetation of the flooded forest (n = 23) and macrophytes (n = 22) (Table 1).

In the low water, the sources are represented by C3 macrophytes (mean values of ($\delta^{13}C$= -29.32‰ and $\delta^{15}N$ = 5.10‰), C4 macrophytes (mean values of $\delta^{13}C$=-12.69‰ and

δ^{15}N= 8.43 ‰) and seston phytoplankton (mean values of δ^{13}C= -33.67‰ and δ^{15}N= 6.46 ‰). The macrophyte *Aeschynomene* sp. showed the least amount of enriched in δ^{15}N (-0.78 ‰), while *Echinochloa polystachya* showed the most enriched value (10.05 ‰) (Table 1).

In high water the increase in water level creates new environments, providing a greater number of niches and sources to be exploited by the fish fauna. In this period autotrophic sources were represented by C3 macrophytes, with average values of δ^{13}C = -29.27 ‰ and δ^{15}N=3.46 ‰; C4 macrophytes with δ^{13}C = -11.29 ‰ and δ^{15}N= 6.62 ‰; riparian vegetation of the flooded forest-RVFF with δ^{13}C= -29.19 ‰ and δ^{15}N= 3.30‰; and seston phytoplankton with δ^{13}C= -33.01‰ and δ^{15}N= 6.64 ‰ (Table 1). The macrophytes *Azolla filiculoides*, *Neptunia plena*, *Acosmium nitens*, *Elvasia calophylla*, *Ipomoea* sp. and *Tabebuia barbata* showed isotopic composition values in δ^{15}N less than 1 ‰.

Among the sources collected, seston phytoplankton was the source less enriched in δ^{13}C for the two hydrological periods, with values of -33.01 ‰ and -33.67 ‰ for high water and low water, respectively.

Between high water and low water periods, there was an isotopic enrichment in the values of δ^{15}N of 1.64 ‰ to C3 macrophytes, 1.81% to C4 macrophytes and 0.22 ‰ to seston phytoplankton. For the values of δ^{13}C was the reverse, lowering the value of 0.05 ‰ for δ^{13}C macrophytes, 1.40 ‰ to C4 macrophytes and 0.66 ‰ for seston phytoplankton.

4. Discussion

The wide inter-relationship between the aquatic and terrestrial environments observed in lowland offers a wide variety of food items (SANTOS and FERREIRA, 1999). In the high water, this is more evident because the flooded forest is responsible for the availability of many food sources, among which stand out the fruits and seeds. In the period of retraction of water, low water, floodplain lakes are isolated from forest and phytoplankton and macrophytes become important sources of carbon and nitrogen (JUNK et al., 1997; SAINT-PAUL et al., 2000).

In the low water, the average values of δ^{15}N in *E. polystachya* found in this work are much more enriched than the mean values of 5.4 ‰ and 6.6 ‰ of δ^{15}N found by Leite et al. (2002) and Oliveira et al. (2006) respectively, for a sample of *P. repens* and *E. polystachya.*

Less enriched values of δ^{15}N found in the macrophytes *Aeschynomene sp., Azolla filiculoides, Neptunia plena, Acosmium nitens, Elvasia calophylla, Ipomoea sp.* and *Tabebuia barbata*, is associated with the fact that these plants interact with nitrogen fixing organisms that remove nitrogen from the air in gaseous form and turns it into absorbable forms (MARRENCO and LOPES, 2005). As the isotope ratio of the nitrogen from the air is close to zero, these plants have values less δ^{15}N enriched compared to other plants. And the greater the degree of interaction between bacteria and higher plant efficiency in nitrogen fixation. This fact was evidenced by Martinelli et al. (1992) in the Amazon River, that observed difference in isotope values of nitrogen in leguminous plants (4.4 ‰) and *Echinochloa polystachya* (10.04 ‰) in the low water season.

The wide variation in δ^{15}N found for plants in general in this work can also be a function of nitrogen pools that regulate the values of δ^{15}N. Because the input and output of this nutrient in the environment, the change in nitrite and ammonium are -5‰ a 10‰ giving a wide variation in values in δ^{15}N of the plants. Other factors that may influence the values of δ^{15}N are: seasonal variations that affect the degree of nitrogen fixation influencing the isotope values of plants (LOPES and BENEDITO-CECILIO, 2002); occurrence of fractionation based on the dynamics of the system (LOPES and BENEDITO-CECILIO, 2002), the form of inorganic nitrogen (N_2 , N_2O , NO_3) used (LOPES and BENEDITO-CECILIO, 2002), the environment in the study because of different characteristics environments may have different values of δ^{15}N in producers primary (LOPES and BENEDITO-CECILIO, 2002), and the concentration of CO_2 in the atmosphere (BASSIRIRAD et al., 2003).

Autotrophic sources of the Grande lake-AM complex also showed a wide variation in the values of δ^{13}C, what can this relates to both the form of inorganic carbon fixation as the variety of substrates used by plants (CO_3, atmospheric CO_2, biogenic CO_2) in the process photosynthetic (LOPES and BENEDITO-CECILIO, 2002). For aquatic plants, Farquhar et al. (1982) attributed the variation in carbon isotopic values of the three factors: the enzymatic isotope discrimination during carbon fixation, the rate of diffusion of

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CO_2 and isotopic composition of the "pool" of dissolved inorganic carbon (DIC).

Table 1. Mean values of $\delta^{13}C$ and $\delta^{15}N$ of autotrophic sources collected at Grande lake in the high water (May and July 2007) and low water period (November and December 2006).

ID	Source	High Water		Drougth	
		$\delta^{15}N$ (‰)	$\delta^{13}C$(‰)	$\delta^{15}N$ (‰)	$\delta^{13}C$(‰)
	Macrophytes C_3 (*n=12,**n=4)	3.46	-29.27	5.10	-29.32
1	*Aeschynomene sp.* Benth	A	A	-0.78	-31.73
2	*Azolla filiculoides* Lam.	-0.59	-29.94	A	A
3	*Cabomba sp.*	4.91	-32.04	A	A
4	*Ceratopteris pteridoides* (Hook.) Hieron	4.06	-29.42	A	A
5	*Cyperus gardneri* Ness.	3.34	-29.33	A	A
6	*Eichhornia crassipes* (Mart.) Solms	A	A	8.51	-28.37
7	*Leersia hexandra* Sw.	3.97	-28.65	A	A
8	*Ludwigia elegans* (Cambess.) Hara	2.84	-30.69	A	A
9	*Ludwigia helmintorrhiza* (Mart.) Hara	3.43	-28.34	A	A
10	*Ludwigia sp.*	5.36	-28.58	A	A
11	*Luziola sp.*	A	A	5.49	-28.80
12	*Neptunia plena* (L.) Benth.	0.87	-30.69	A	A
13	*Pistia estratiotes* L.	4.43	-28.56	7.19	-28.39
14	*Salvinia auriculata* Aubl.	4.66	-29.34	A	A
15	*Oryza sp.*	4.26	-25.67	A	A
	Macrophytes C_4 (*n=2,**n=4)	6.62	-11.29	8.43	-12.69
16	*Cyperus esculentus* L. var. leptostachyus Boeck.	A	A	8.80	-12.23
17	*Echinochloa polystachya* (H.B.K.) Hitchc	8.74	-11.31	10.05	-13.12
18	*Gymnocoronis sp.*	A	A	7.41	-12.77
19	*Paspalum sp.*	4.51	-11.27	7.45	-12.67
	Riparian vegetation of the flooded forest (*n=3,**n=0) C_3	3.30	-29.19	A	A
20	*Acosmium nitens (Vog.)Yakoulev*	0.70	-26.92	A	A
21	*Arrabidaea sp.*	3.94	-31.08	A	A
22	*Calyptranthes cuspidatum D.C.*	2.08	-29.97	A	A
23	*Campsiandra comosa var. laurifolia (Benth.)Cowan*	3.26	-29.25	A	A
24	*Caperonia castaneifolia (L.) St. Hil*	3.94	-31.86	A	A
25	*Cassia leiandra Benth*	5.19	-28.18	A	A
26	*Cassia occidentalis L.*	3.91	-31.13	A	A
27	*Cassia reticulata Willd*	2.70	-28.07	A	A
28	*Cissus hassleriana Chod.*	4.44	-27.74	A	A
29	*Dioclea sp.*	4.09	-26.77	A	A
30	*Elvasia calophylla D. C.*	0.69	-27.41	A	A
31	*Hevea spruceana Muell. Arg.*	2.46	-27.68	A	A
32	*Hiraea schultesii Cuatrec*	2.77	-30.20	A	A
33	*Hymenachne amplexicaulis (Rudge) Nees*	4.80	-28.62	A	A
34	*Ipomoea sp.*	-2.43	-28.67	A	A
35	*Nectandra amazonun Ness*	3.09	-30.43	A	A
36	*Phoradendron sp.*	3.34	-31.31	A	A
37	*Phthirusa stelis*	5.98	-28.65	A	A
38	*Phyllanthus fluitans Muell. Arg.*	4.82	-29.54	A	A
39	*Piranhea trifoliata Baill*	5.06	-28.61	A	A
40	*Ruprechtia tangara Standl.*	4.54	-29.73	A	A
41	*Securidaca rinvinaefolia St. Hill var. pavifolia Benn*	2.72	-28.42	A	A
42	*Tabebeuia barbata (E.Mey) Sandwith*	0.92	-27.20	A	A
43	Seston phytoplankton (*n=3,**n=8)	6.24	-33.01	6.46	-33.67

A: absent, were not collected or samples found in the period; * high water; ** Drougth

The $\delta^{13}C$ values of *Eichhornia crassipes*, *Pistia estratiotes* and *Oryza* sp are similar to that found by Leite et al. (2002) that grouped to form a sample of C3 macrophytes, with an average of -28.8 ‰. Were the same, the values of $\delta^{13}C$ in C4 macrophytes this study are similar to those reported by Forsberg et *al*. (1993), Leite et al. (2002) and Oliveira et al (2006) for *Paspalum repens* and *E. polystachya* that grouped in C4 macrophytes and had average values of -12.9 ‰, -12.8 ‰ and -12.9 ‰ respectively.

The seston phytoplankton was the source less enriched in $\delta^{13}C$, differing from the other plants that perform metabolism by the C3 photosynthetic pathway. This depletion of seston phytoplankton in relation the other C3 plants is due to phytoplankton preferentially use the dissolved inorganic carbon and the other plants of this photosynthetic pathway using atmospheric CO_2 thus delaying the substrate using (BOUTTON, 1991). The values of $\delta^{13}C$ of seston phytoplankton may still be influenced by the concentration and origin of inorganic carbon fixed temperature and species studied (LOPES and BENEDITO-CECILIO, 2002). The $\delta^{13}C$ values for seston phytoplankton were similar to -33.3 ‰ found by Forsberg et al. (1993) in Central Amazonia. However, differ from the average value of -37 ‰ found by Leite et al. (2002) in four Amazonian lakes, and -36.6 ‰ found by Oliveira et al. (2006) in lake Camaleão-AM.

5. Conclusions

The phytoplankton was the source with the lowest values in $\delta^{13}C$ and C4 macrophytes with the highest values showing the variation that exists in values isotopic of the plants that utilize different carbon sources and different photosynthetic pathways. The values of $\delta^{15}N$ are influenced by many factors, but the interaction with nitrogen fixing organisms causes the plants present values in $\delta^{15}N$ smaller than the others.

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