

## Autotrophic carbon sources for heterotrophic bacterioplankton in a floodplain lake of central Amazon

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### Abstract

The relative contribution of autotrophic carbon sources (aquatic macrophytes, flooded forest, phytoplankton) for heterotrophic bacterioplankton was evaluated in a floodplain lake of the Central Amazon. Stable carbon isotopes ( $\delta^{13}\text{C}$ ) were used as tracers. Values of  $\delta^{13}\text{C}$  of different autotrophic sources were compared to those of dissolved organic carbon (DOC) and those of bacterially produced  $\text{CO}_2$ .

The percentage of carbon derived from  $\text{C}_4$  macrophytes for bacterially produced  $\text{CO}_2$  was the highest, on average 89%. The average  $\delta^{13}\text{C}$  value of  $\text{CO}_2$  from bacterial respiration was  $-18.5 \pm 3.3\text{‰}$ . Considering a fractionation of  $\text{CO}_2$  of 3‰ by bacterial respiration,  $\delta^{13}\text{C}$  value was  $-15.5\text{‰}$ , near  $\text{C}_4$  macrophyte  $\delta^{13}\text{C}$  value ( $-13.1\text{‰}$ ).

The average value of total DOC  $\delta^{13}\text{C}$  was  $-26.8 \pm 2.4\text{‰}$ . The percentage of  $\text{C}_4$  macrophytes carbon for total DOC was on average 17%. Considering that bacteria consume mainly carbon from macrophytes, the dominance of  $\text{C}_3$  plants for total DOC probably reflects a faster consumption of the former source, rather than a major contribution of the latter source.

Heterotrophic bacterioplankton in the floodplain may be an important link in the aquatic food web, transferring the carbon from  $\text{C}_4$  macrophytes to the consumers.

### Introduction

The role of heterotrophic bacterioplankton in aquatic food chains has been intensively investigated, in the sea (Pomeroy, 1974; Azam et al., 1983; Ducklow et al., 1986; Sherr et al., 1987; Fenchel, 1988; Pomeroy & Wiebe, 1988; Furhman, 1992) and in temperate lakes (Hessen, 1985; Tranvik & Hölfe, 1987; Salonen et al., 1992; Tranvik, 1992; Hessen, 1992). In some ecosystems the 'microbial loop' (Azam et al., 1983) seems to represent the main pathway for the decomposing autotrophic carbon; however, it is not clear if bacteria are a significant source of food for the metazoan food web or a carbon sink through respiration losses in all ecosystems (Ducklow et al., 1986; Sherr et al., 1987; Wylie & Currie, 1991).

The role of pelagic heterotrophic bacterioplankton in tropical lakes has been less studied. The oxygen sub-

saturation and the predominance of community respiration over photosynthetic production of  $\text{O}_2$  frequently found in floodplain lakes of the Amazon (Wissmar et al., 1981; Melack & Fisher, 1983; Richey et al., 1988, Quay et al., 1995), the dominance of bacterial biomass over phytoplankton (Rai, 1979; Rai & Hill, 1980, 1984; Benner et al., 1995), and the high uptake rates of radiolabeled leucine and thymidine (Benner et al., 1995) are indicative of the importance of bacterial processes in these systems. Besides the organic matter production by phytoplankton, it is possible that the energy of other sources of autotrophic carbon, such as aquatic macrophytes, flooded forest and 'terra firme' forest, may be utilized by consumers of the food chain. The major part of the carbon of those sources would enter into the system as dissolved organic matter and could be transferred to the consumers by heterotrophic bacterioplankton.

The most abundant source of autotrophic carbon in Amazonian floodplains is aquatic macrophytes, mainly  $C_4$  metabolic grasses, which constitute 52% of total primary production, followed by flooded forest (33%), periphyton (8%) and phytoplankton (8%) (Junk, 1985). Because they are the main primary producers, aquatic macrophytes potentially are an important carbon source for the aquatic food web in the floodplain. Energy studies on the aquatic food web indicate controversy about which carbon source is being more utilized by consumers. Bayley (1983) and Junk (1985) suggest that aquatic macrophytes contribute more carbon for the aquatic food web due to their high productivity and decomposition rate. This production may enter in the aquatic food web mainly as detritus, rather than through herbivory (Fenchel & Jorgensen, 1977). Stable isotopic studies of carbon dynamics into the detritivore food chain in floodplains suggest that carbon derived from  $C_4$  macrophytes is less important. Araújo-Lima et al. (1986) and Forsberg et al. (1993) did not find a significant contribution of carbon from  $C_4$  macrophytes in adult detritivorous fish. Padovani (1992) determined that even though shrimps use aquatic macrophytes as habitat, they obtain only a small amount of carbon from  $C_4$  macrophytes. On the other hand, Fernandez (1993) showed that a significant fraction of carbon used by juvenile fish of *Semaprochilodus insignis* and *Prochilodus nigricans* is derived from  $C_4$  macrophytes. The great number of rotifers and bacteria in their stomach contents, suggests the importance of 'microbial loop' for juvenile fish. Rotifers probably acquire carbon from bacteria, which in turn are using mainly carbon from  $C_4$  macrophytes. The objective of the study was to investigate the importance of  $C_4$  macrophytes for heterotrophic bacterioplankton in a floodplain lake.

### Study area

Calado Lake ( $3^{\circ}15'S$ ,  $60^{\circ}34'W$ ) is located on the north bank of Solimões River, about 80 km upriver from its confluence with Negro River. It is a typical floodplain lake, of mixed water (Rai & Hill, 1980), medium sized and dendritic, with a single channel connecting to the Solimões river during the whole year (Figure 1). The area of the lake oscillates between 2 and 8 km<sup>2</sup>, and the maximum depth varies between 3 and 12 meters (Melack & Fisher, 1983).

The drainage basin of the lake has an area of nearly 58 km<sup>2</sup>. The part of the lake parallel to the marginal

ridge that separates Calado Lake from Solimões river, receives white water from the river, during flooding season. The part where the lake acquires the dendritic outline, receives input of water from small streams that originate in 'terra firme' (upland) forest.

The three major environments of the Amazon: floodplain, floating meadows, flooded forest and open waters are present in the lake. The portion closer to the Solimões River, during the high water season, is covered mainly by floating meadows (Junk, 1970). The portion of the lake which extend into the 'terra firme' forest has open waters. Their edges are covered by the flooded forest and minor floating meadows.

During the rise, Solimões waters penetrate into the lake and together with the local runoff, stream waters and rain, fill the lake. The entrance of 'white' waters from Solimões, rich in nutrients, permit a high production of aquatic macrophytes (Junk, 1970).

The floating meadows are mainly composed of by rooted grasses (*Paspalum repens*, *Echinochloa polystachya*, *Oryza perennis* e *Leersia hexandra*), and is surrounded by free species (*Eichornia crassipes*, *Azolla* sp, *Ludwigia natans*, *Pistia stratiotes*, *Salvinia auriculata*, and others (Junk, 1970; Junk, 1973). *Paspalum repens* is the dominant species in the floating meadows and jointly with *Echinochloa polystachya*, both with  $C_4$  metabolism, represent 80 to 90% of the macrophyte biomass (Junk, 1970). The periphyton has good conditions for developing while the water is rising because the abundant macrophyte roots and the leaves of the flooded forest serve as suitable substrate (Junk, 1970; Padovani, 1992).

The water temperature varies between 28 to 34 °C. Dissolved oxygen is usually undersaturated (Melack & Fisher, 1983, 1991), and planktonic community respiration exceeds phytoplanktonic production by a factor of about 2 fold.

### Materials and methods

One approach to study the bacterial utilization of the different sources of DOC in lakes is through stable carbon isotopes. This approach is particularly useful in ecosystems where there are at least two sources of carbon with distinct isotopic values. In floodplain lakes DOC may come from phytoplankton, periphyton,  $C_3$  and  $C_4$  aquatic macrophytes, flooded forest and 'terra firme' forest.

The isotopic ratio between carbon stable isotopes, <sup>13</sup>C and <sup>12</sup>C in plants varies due to physicochemical

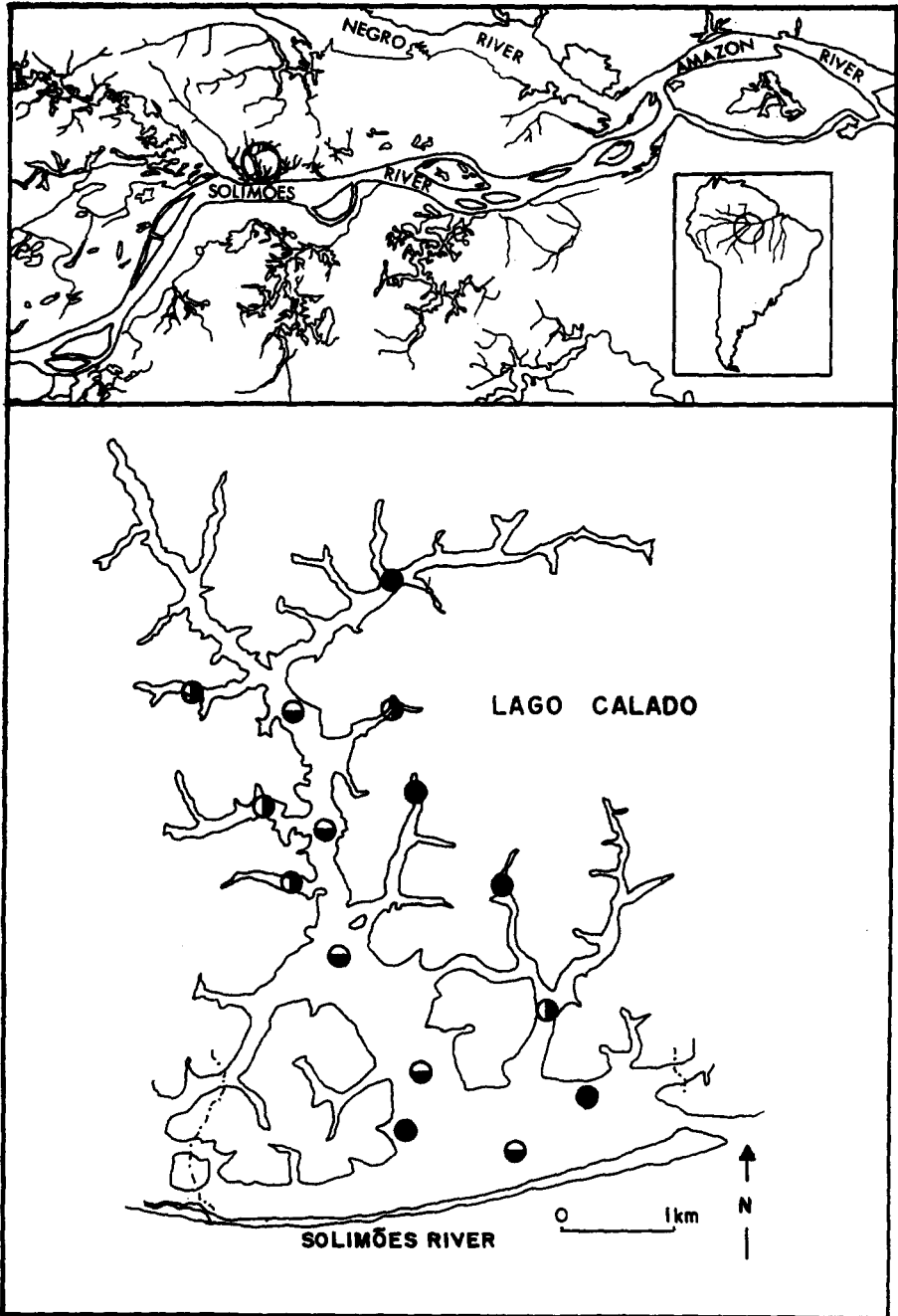


Figure 1. Location of Calado Lake in South America and Calado Lake in dry season showing sampling stations. ● Floating meadows. ● flooded forest, ◐ open waters

and metabolic processes that produce isotopic fractionation. The isotopic ratio between carbon stable isotopes is represented by the symbol  $\delta^{13}\text{C}$ , expressed as parts per thousand (‰). The ratio between these isotopes is expressed as the difference between the isotope ratio of the sample and the ratio of a calcium carbonate standard known as PDB (*Belemnitella americana* fossil of the Peedee formation, USA) and it is calculated as:

$$\delta^{13}\text{C}\text{‰} = \frac{{}^{13}\text{C}/{}^{12}\text{C}_{\text{sample}} - {}^{13}\text{C}/{}^{12}\text{C}_{\text{standard}}}{{}^{13}\text{C}/{}^{12}\text{C}_{\text{standard}}} \times 1000.$$

The different photosynthetic pathways in plants produce characteristic patterns of isotopic fractionation resulting from the action of enzymes with different selectivity for  $^{12}\text{C}$  and  $^{13}\text{C}$ . The difference of  $\delta^{13}\text{C}$  in different plant groups is maintained through the food web (De Niro & Epstein, 1978; Fry & Sherr, 1984), and is an efficient tool for investigating the carbon transfer from the primary producers to consumers, and can provide clues about the origin and transformation of organic matter (Fry & Sherr, 1984; Peterson & Fry, 1987; Hobbie, 1992).

To determine carbon sources for bacteria in Calado Lake it was necessary: (1) to obtain detritus-free bacterial cultures which can grow in the lake's DOC; (2) to conduct bioassay to determine bacterial  $\delta^{13}\text{C}$ ; (3) to determine DOC  $\delta^{13}\text{C}$  to demonstrate that the isotopic composition of bacteria reflects the isotopic composition of DOC used as substrate; and (4) to demonstrate that the isotopic composition of DOC reflects the isotopic composition of the parent plant.

Between February and April of 1994, during the flooding season, 5 plots consisting of floating meadows, flooded forest and open waters were chosen with a minimal distance of 200 m from the edge of the lake or the floating meadows. In each field trip one plot per habitat was randomly selected for sampling.

In each plot, 10 liters of surface water were collected, in Nalgene carboy previously rinsed with 10% HCl and distilled water. Of these 10 liters, 3 liters were used to determine  $\delta^{13}\text{C}$  and concentration of DOC, 3 liters were used to prepare the growth medium and 1 liter used to prepare the bacterias inoculum. The samples were chilled for several hours until further processing in the laboratory could be undertaken.

From the 10 liters sampled, 6 liters of the water were centrifugated in a model T-1 continuous flow Sharples centrifuge, to eliminate particulate material, sterilized by filtration through Gelman A/E glass fiber filters and subsequently filtered through a 0.22  $\mu\text{m}$  polycarbonate Nucleopore filters. All glassware used

in the filtration were rinsed with 10% HCl and autoclaved. Glass fiber filters were precombusted at 550 °C for 3 hours, and the polycarbonate filters were boiled 3 times for 15 minutes and autoclaved to avoid contamination with organic compounds.

The total DOC was concentrated by rotovaporation at 45 °C up to a volume of 10 ml. The samples were transferred to precombusted glass vials for drying with nitrogen gas and storage.

At the Centro de Energia Nuclear para a Agricultura (CENA), in Piracicaba, São Paulo, the organic matter of the sample was converted to  $\text{CO}_2$ , by combustion in pyrex sealed tubes with 5 mg of CuO (precombusted at 550 °C for 3 hours), at 550 °C for 12 hours. The  $\text{CO}_2$  was separated from the other combustion compounds by cryogenic distillation in a high vacuum system. In this system all the water vapor was collected in a trap containing an ethanol-dry ice mixture (−86 °C) and the  $\text{CO}_2$  collected in a trap containing liquid nitrogen (−196 °C). The  $\delta^{13}\text{C}$  of the  $\text{CO}_2$  was analyzed in a Micromass model 602-E mass spectrophotometer fitted with double inlet and double collector systems, with precision of 0.1‰. For each sample two measurements were made.

To determine the DOC concentration 20 ml of total DOC were collected in precombusted glass vials, and preserved with 0.1 ml of saturated  $\text{HgCl}_2$ . In the Pontificia Universidade Católica de Rio de Janeiro, the DOC concentration was measured on a Dohrmann DC-190 carbon analyzer, with precision of  $\pm 5\%$ .

#### *Determination of bacteria $\delta^{13}\text{C}$*

The main problem in the determination of bacterial  $\delta^{13}\text{C}$  is to discriminate bacterial  $\delta^{13}\text{C}$  from the  $\delta^{13}\text{C}$  of other particles of the same size contained in the water samples (Hobbie, 1992). Using the method proposed by Coffin et al. (1989) it is impossible to separate bacteria by filtration from small particles of colloids or detritus. The alternative method for the determination of  $\delta^{13}\text{C}$  of bacterial DNA and RNA (Coffin et al., 1990) was not accessible to us. For this reason a new method was employed to determine the isotopic ratio of the  $\text{CO}_2$  produced by the respiration of bacteria cultivate in the lake's water, as an equivalent alternative to measuring  $\delta^{13}\text{C}$  of bacteria (Waichman, 1995).

The bioassay were made using 1000 ml glass bottles, closed with a silicone stopper with a stainless steel cannula with a reel to permit the input of the inoculum, nitrogen gas etc, and a glass tube with a reel to connect the glass bottle to the high vacuum system.

Growth medium was made filtering 3 liters of lake water through Gelman A/E glass fiber filter to remove large particles, and through 0.22  $\mu\text{m}$  polycarbonate Nucleopore filters to remove bacteria. All glassware used in the filtration and the filters, were submitted to the sterilization procedure describe above.

The inoculum was prepared by filtering 1 liter of lake water through 3  $\mu\text{m}$  polycarbonate Nucleopore filters to remove zooplankton and through 1  $\mu\text{m}$  polycarbonate Nucleopore filters to remove phytoplankton.

Growth medium was acidified to pH 2 with 0.1 N HCl and the preexistent  $\text{CO}_2$  sparged with filtrated nitrogen gas. This change in pH may release organic substances that in other ways are not available as bacterial substrate. However, Moran & Hodson (1990) demonstrated that pH modification in waters used in bacterial culture medium, does not modify there quality. Subsequently, the pH was adjusted to the original value. For avoid the entrance of  $\text{CO}_2$  when the preexistent  $\text{CO}_2$  was sparged, all the connection were closed and created a positive pressure with  $\text{N}_2$ . A seringe containing NaOH was adjusted to stainless steel cannula, and the reel was open. NaOH was injected, the reel closed, the sample stirred, and with the same seringe, a subsample was taken to measure the pH. For each sample 3 cultures were made. Growth medium was inoculated with 60 ml of the inoculum and bubbled with artificial air (without  $\text{CO}_2$ ) for 10 minutes in order to aerate the growth medium because during the pre-existent  $\text{CO}_2$  remotion some  $\text{O}_2$  was eliminated. The cultures were incubated in a dark at room temperature for 24–48 hours.

The  $\text{CO}_2$  produced by bacteria was purified and collected in a high vacuum system using the method described above. The glass bottles with the cultures were attached to the vacuum system, the cultures being acidified to pH 2 with concentrated ortophosphoric acid, and the  $\text{CO}_2$  released sparged through the system with nitrogen gas, at 150  $\text{ml min}^{-1}$  for 2 hours. The purified  $\text{CO}_2$  was sent to CENA for mass spectrophotometer analysis.

#### Plant values of $\delta^{13}\text{C}$

The  $\delta^{13}\text{C}$  plant data used in this work were from material collected along the Solimões-Amazon river, their tributaries and marginal lakes, and at Calado Lake, during all seasons of the year.

Table 1.  $\delta^{13}\text{C}$  (‰) values of  $\text{C}_3$  and  $\text{C}_4$  plants collected at Calado Lake, Solimões-Amazonas river, their tributaries, and marginal lakes.  $N$ = number of samples,  $SD$ = standard deviation,  $Min$ = minimum value and  $Max$ = maximum value

Plants	$N$	Mean	$SD$	Min	Max
Phytoplankton ( $\text{C}_3$ )	13	-33.0	3.50	-38.1	-27.6
Periphyton ( $\text{C}_3$ )	22	-27.4	3.25	-34.8	-21.5
F. Forest (leaves $\text{C}_3$ )	49	-30.7	1.55	-33.5	-26.7
$\text{C}_3$ Macrophytes	33	-29.1	2.26	-34.7	-24.7
$\text{C}_4$ Macrophytes	68	-13.1	0.99	-15.7	-11.7
$\text{C}_3$ Plants	117	-29.7	2.83	-38.1	-21.5

#### Statistical analysis

Statistical analysis followed Wilkinson (1990) and Zar (1984). Non-parametric statistics were used due to the heteroscedasticity of data. The Kruskal-Wallis test was used to test the significance of differences between bacterial  $\delta^{13}\text{C}$  values in the different environments, in DOC  $\delta^{13}\text{C}$  values, and in DOC concentrations in the different environments. When necessary, multiple comparisons were made using the Student-Newman-Keuls or Tukey test. The significance level adopted for statistical tests was  $\alpha = 0.05$ .

#### Results

The value of  $\delta^{13}\text{C}$  of bacterially produced  $\text{CO}_2$  varied between -27.7 and -13.5‰, with a mean and standard error of  $18.5 \pm 3.3\%$  (Figure 2). The  $\delta^{13}\text{C}$  value of the carbon sources varied between -33.0‰ and -13.1‰ (Table 1). Values of  $\delta^{13}\text{C}$  of  $\text{C}_3$  plants have a large overlap and were significantly different than the  $\delta^{13}\text{C}$  mean value obtained for  $\text{C}_4$  macrophytes (Student-Newman-Keuls test.  $P < 0.05$ ). The values of  $\delta^{13}\text{C}$  of bacterially produced  $\text{CO}_2$  were intermediate between  $\text{C}_3$  plant values and macrophyte  $\text{C}_4$  values, with predominance of carbon derived from  $\text{C}_4$  macrophytes (Figure 3). The difference in  $\delta^{13}\text{C}$  values permitted an evaluation of the relative contribution of each plant group ( $\text{C}_3$  and  $\text{C}_4$ ) as a carbon source for bacteria, using the 'two end-member mixing model' (Martinelli, 1986; Forsberg et al., 1993). All  $\text{C}_3$  plants, i.e. phytoplankton, periphyton, flooded forest and  $\text{C}_3$  macrophytes were grouped as one end member and  $\text{C}_4$  macrophytes constituted the other end member:

$$\% \text{C}_4 = 1 - \frac{\delta^{13}\text{C}_{\text{bacteria}} - \delta^{13}\text{C}_{\text{C}_4}}{\delta^{13}\text{C}_{\text{C}_3} - \delta^{13}\text{C}_{\text{C}_4}} \times 100$$

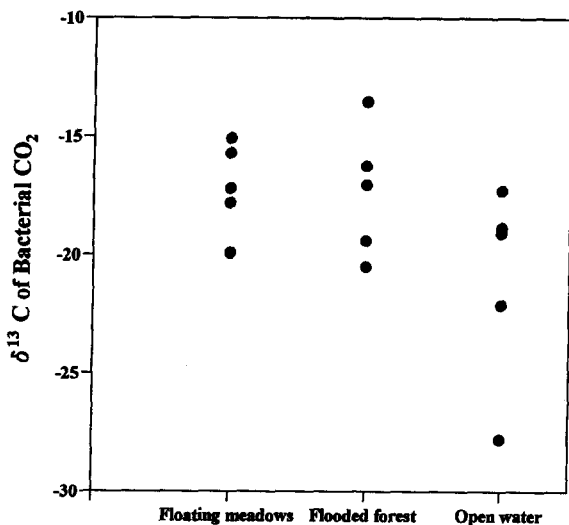


Figure 2.  $\delta^{13}\text{C}$  values of bacterially produced  $\text{CO}_2$  in the different environments.

Table 2. Percentage of carbon derived from  $\text{C}_4$  macrophytes used by bacteria in the different environments

Environment	% Mean	% Maximum	% Minimum
Floating meadows	75	79	70
Flooded Forest	72	76	66
Open water	54	62	46

where:

$\delta^{13}\text{C}_{\text{bacteria}}$  is the average  $\delta^{13}\text{C}$  value of bacterially produced  $\text{CO}_2$

$\delta^{13}\text{C}_3$  is the average  $\delta^{13}\text{C}$  value for the  $\text{C}_3$  plant group and

$\delta^{13}\text{C}_4$  is the average  $\delta^{13}\text{C}$  value for the  $\text{C}_4$  macrophytes

The first calculation to determine the contribution of carbon from  $\text{C}_4$  macrophytes at different environments was made using the average  $\delta^{13}\text{C}$  value of bacterially produced  $\text{CO}_2$  obtained in the isotope analysis without considering isotopic fractionation of  $\text{CO}_2$ . The correction for trophic level was not made because of the difficulty in determining the trophic level of bacteria. This is a conservative decision, because no consideration of these effects will decrease the percentage of  $\text{C}_4$  macrophytes estimated by the equation.

According to Blair et al. (1985) fractionation exits during aerobic metabolism of heterotrophic bacteria. One fractionation process occurring when pyruvate is

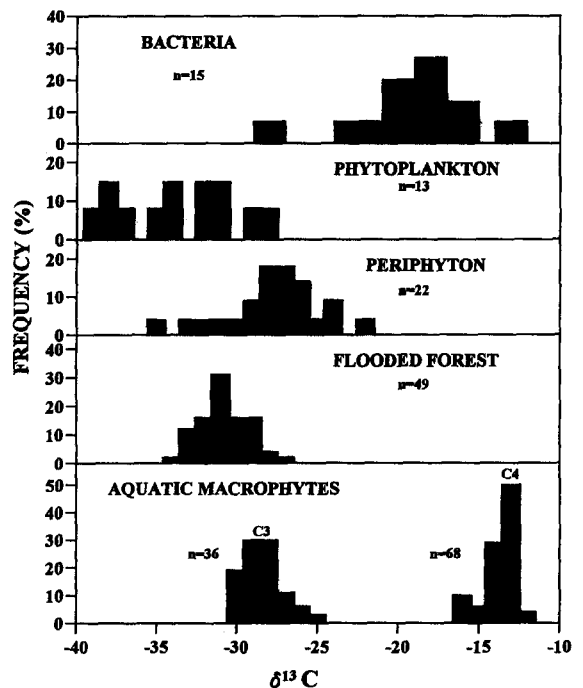


Figure 3. Frequency distribution of  $\delta^{13}\text{C}$  (‰) values of bacterially produced  $\text{CO}_2$  and of phytoplankton, periphyton; flooded forest,  $\text{C}_3$  aquatic macrophytes and  $\text{C}_4$  aquatic macrophytes collected at Calado Lake and Solimões-Amazonas river, their tributaries and marginal lakes. (Modified from Forsberg et al., 1993).

converted to Acetyl-CoA and the  $\text{CO}_2$  released during this reaction is depleted in  $^{13}\text{C}$ . In this way the Acetyl-CoA enters the Krebs cycle depleted in  $^{13}\text{C}$ . A second fractionation process occurs during the decarboxylation of the  $\alpha$ -ketoglutarate in the Krebs cycle, producing  $\text{CO}_2$  depleted in  $^{13}\text{C}$ , resulting in a total  $\text{CO}_2$  fractionation of 3‰. For this reason, a second calculation was made based on this fractionation. Due to the high variation of  $\delta^{13}\text{C}$  among  $\text{C}_3$  plants, the percentage of  $\text{C}_4$  carbon used by bacteria was calculated within a confidence interval with minimum and maximum values. To calculate the maximum contribution of  $\text{C}_4$  carbon for bacteria, the average  $\delta^{13}\text{C}$  value of phytoplankton – the most negative group – was utilized. The average  $\delta^{13}\text{C}$  value of periphyton – the most positive  $\text{C}_3$  plant – was used to calculate the minimum  $\text{C}_4$  contribution. The minimum value of carbon from  $\text{C}_4$  macrophytes used by bacteria ranged from 46% in open waters to 70% in the floating meadows (Table 2). However, when a fractionation of 3‰ was considered, the minimum contribution ranged from 67% in open waters to 92% in the floating meadows (Table 3).

Table 3. Percentage of carbon derived from C<sub>4</sub> macrophytes used by bacteria in the different environments considering a fractionation of 3‰ (Blair et al., 1985)

Environment	% Mean	% Maximum	% Minimum
Floating meadows	93	94	92
Flooded Forest	90	92	88
Open water	73	77	67

Table 4. Mean and standard deviation of concentration of total DOC (mg/l) and values of  $\delta^{13}\text{C}$  (‰) in the different environments

Environment	mg/l	$\delta^{13}\text{C}$ (‰)
Floating meadows (n=5)	9.3 ± 3.5	-27.27 ± 3.4
Flooded Forest (n=5)	8.3 ± 3.9	-27.87 ± 1.6
Open water (n=5)	6.9 ± 1.3	-26.28 ± 2.8

The maximum contribution of C<sub>4</sub> carbon to bacteria, without considering isotopic fractionation, was 79% in the floating meadows, and considering a fractionation of 3‰ was 94%. This percentage of utilization of carbon derived from C<sub>4</sub> macrophytes was the highest found in studies of consumers made in Central Amazon. The utilization of the different sources (C<sub>3</sub> and C<sub>4</sub>) was similar in the floating meadows and flooded forest. These results indicate that the main source of carbon for heterotrophic bacterioplankton independently of the environment, was the carbon originated from C<sub>4</sub> macrophytes. However, in open water there is a moderated utilization of carbon from C<sub>3</sub> plants, probably derived from phytoplankton it was not possible to distinguish among C<sub>3</sub> plants, which are utilized in a most significant way by heterotrophic bacterioplankton, because of the large overlap of their isotopic values.

There was a significant difference between  $\delta^{13}\text{C}$  values of bacteria for the different environments (Kruskal-Wallis test,  $P < 0.001$ ) (Figure 2). The  $\delta^{13}\text{C}$  value of bacterially produced CO<sub>2</sub> in open water was significantly smaller than the values obtained for bacterially produced CO<sub>2</sub> in floating meadows and flooded forest, that had not significant differences between them (Tukey multiple comparison test,  $P < 0.05$ ).

The total DOC concentration in Calado Lake averaged  $8.8 \pm 2.9 \text{ mg l}^{-1}$ , and the  $\delta^{13}\text{C}$  value  $-26.8 \pm 2.4\text{‰}$ . The total DOC concentration and the  $\delta^{13}\text{C}$  value were not significantly different among the environments studied (Kruskal-Wallis test,  $P = 0.306$ ) (Table 4). To calculate the percentage of C<sub>4</sub> plants car-

Table 5. Percentage of carbon derived from C<sub>4</sub> macrophytes to total DOC in the different environments.

Environment	% Mean	% Maximum	% Minimum
Floating meadows	17	31	1
Flooded Forest	14	28	0
Open water	21	34	5

bon for total DOC, the 'two end member mixing model' was used. The C<sub>4</sub> percentage for DOC was not higher than expected, including in the floating meadows. The maximum percentage of C<sub>4</sub> macrophytes carbon for DOC was in open waters and the minimum contribution was in flooded forest (Table 5).

## Discussion and conclusions

The results presented here indicate that C<sub>4</sub> macrophytes are the main carbon source for heterotrophic bacterioplankton in Calado Lake. The percentage of utilization of carbon derived from C<sub>4</sub> macrophytes by bacteria was the highest found in studies of consumers made in Central Amazon.

Considering that bacteria consume mainly carbon from macrophytes, the dominance of C<sub>3</sub> plants for total DOC probably reflects a faster consumption of the carbon of C<sub>4</sub> macrophyte rather than a major contribution of the C<sub>3</sub> plants. Quay et al. (1992) suggest a selective utilization of organic matter derived from C<sub>4</sub> macrophytes to explain differences between the  $\delta^{13}\text{C}$  of respired CO<sub>2</sub> and  $\delta^{13}\text{C}$  of organic matter in the main channel of the Solimões river. After analyzing carbohydrates and amino acids of organic matter from the Solimões-Amazon river, Hedges et al. (1994) demonstrated that DOC was basically constituted by the decomposing litter of the flooded forest. This DOC can not maintain the high respiration rates measured in the river (Richey, 1990; Benner et al., 1995). Another substrate should be maintaining these high respiration rates even though these could not be measured and identifying, due to their rapid turn over. The difference between bacterial  $\delta^{13}\text{C}$  and  $\delta^{13}\text{C}$  of DOC indicates that bacteria selectively used carbon derived from C<sub>4</sub> macrophyte in Calado Lake.

In open water the consumption of DOC derived from C<sub>3</sub> plants was higher than in the other environments. It is possible that in this environment the carbon derived from phytoplankton is relatively important. Due to the low light penetration in the flooded

forest and in the floating meadows the phytoplankton production is almost inexistent, and bacteria utilized mainly DOC derived from C<sub>4</sub> macrophytes. Phytoplankton production in Calado Lake is small (Melack & Fisher, 1983) and can support only a modest bacterial production. However, the area under influence of macrophytes and flooded forest varies greatly among lakes in the Amazon, and, in some cases, phytoplankton or periphyton may be a major carbon source for bacteria.

The utilization of C<sub>4</sub> macrophyte's DOC by heterotrophic bacterioplankton probably represent an important carbon flow, making this portion of the primary production available to the metazoa food web as bacterial biomass, and releasing CO<sub>2</sub> into the environment.

Although C<sub>4</sub> macrophytes are the dominant primary producers in the Amazon floodplain (Junk & Howard-Williams, 1984; Junk, 1985), the carbon derived from these plants as particulate detritus is not assimilated by most animals because of its poor digestibility and lower nutritional value (Forsberg et al., 1993; Padovani, 1992). The bulk of the C<sub>4</sub> macrophyte's biomass probably is processed through the microbial food web, originating with bacteria and fungi. Aquatic macrophytes' decomposition in floodplain lakes reduces 75% of the initial biomass in two weeks, mainly by leaching of organic substances, especially easily degradable carbohydrates and amino acids (Junk & Furch, 1991; Howard-Williams & Junk, 1977). Macrophytes detritus are less digestible due to their high fiber content and low nutritional value when compared to C<sub>3</sub> plants (Forsberg et al., 1993), however, the decomposition processes may increase their nutritional characteristics and may convert it to an optimum substrate for bacteria (Howard-Williams & Junk, 1976).

Araujo-Lima et al. (1986) and Forsberg et al. (1993) demonstrated that phytoplankton production in floodplain lakes is essential to sustaining commercial fish production, and C<sub>4</sub> macrophyte carbon are relatively unimportant. However, changes in the food web structure and dynamics occurs during the life cycle of fish. The trophic dynamics of larval and juvenile fish differ significantly than the adult fish (Bayley, 1983; Araujo-Lima & Hardy, 1987), and the former may use carbon derived from C<sub>4</sub> macrophytes. In the initial stage of development, 42.6% of the carbon consumed by *Semaprochilodus insignis* and 35.8% of the carbon consumed by *Prochilodus nigricans*, came from C<sub>4</sub> macrophytes (Fernandez, 1993). Those juveniles feed

mainly on rotifers (Fernandez, 1993), that may feed on bacteria (Turner & Tester, 1992) or bacterivores such as flagellates and ciliates (Starkweather, 1980; McManus & Furhman, 1988).

The bacterial utilization of DOC from C<sub>4</sub> macrophytes may be the major pathway of carbon flow in the floodplain. When heterotrophic bacterioplankton process DOC from C<sub>4</sub> macrophytes not only do they incorporate carbon in cellular biomass, but they also release CO<sub>2</sub> through respiration, that in turn may diffuse to the atmosphere or be fixed by phytoplankton. The bacterial biomass may remain as particulate organic carbon (POC), transported to the main channel of Solimões river, incorporated into the sediments or probably transferred to higher trophic levels in the aquatic food web via bacterivores.

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### References

- Araujo-Lima, C. A. R. M., B. R. Forsberg, R. Victoria & L. Martinelli 1986. Energy Sources for detritivorous fishes in the Amazon. *Science* 34: 1256-1258.
- Araujo-Lima, C. A. R. M. & E. Hardy. 1987. Aspectos biológicos de peixes amazônicos. VIII. A alimentação dos alevinos do laraqui, *Semaprochilodus insignis*. *Amazoniana* 5: 127-136.
- Azam, F., T. Fenchel, J. Field, J. S. Gray A, L. A. Meyer-Reil & F. Thingstad, 1983. The ecological role of water-column microbes in the sea. *Mar. Ecol. Prog. Ser.* 10: 257-263.
- Bayley, P. B., 1983. Central Amazon fish populations: biomass, production and some dynamics characteristics. Ph. D. Thesis, Dalhousie Univ. Nova Scotia, Canada, 330 pp.
- Benner, R., S. Opsahl, G. Chin-Leo, J. E. Richey & B. R. Forsberg, 1995. Bacterial carbon metabolism in the Amazon River system. *Limnol. Oceanogr.* 40: 1262-1270.



- Blair, N., A. Leu, E. Muñoz, J. Olse, E. Kwong & D. Des Marais, 1985. Carbon isotopic fractionation in heterotrophic microbial metabolism. *Appl. envir. Microbiol.* 50: 996–1001.
- Coffin, R. B., D. J. Velinsky, R. Devereux, W. A. Price & L. A. Cifuentes, 1990. Stable Carbon Isotope analysis of nucleic acids to trace sources of dissolved substrates used by estuarine bacteria. *Appl. Envir. Microbiol.* 56: 2012–2020.
- Coffin, R. B., B. Fry, B. J. Peterson & R. T. Wright, 1989. Carbon isotope composition of estuarine bacteria. *Limnol. Oceanogr.* 34: 1305–1310.
- De Niro, M. J. & S. Epstein, 1978. Influence of diet on distribution of carbon in animals. *Geochim. Cosmochim. Acta* 42: 495–506.
- Ducklow, H. W., D. A. Purdie, P. J. L. B. Williams & J. M. Davies, 1986. Bacterioplankton: a sink for carbon in a coastal plankton community. *Science* 232: 865–867.
- Fenchel, T., 1988. Marine plankton food chains. *Annu. Rev. Ecol. Syst.* 19: 19–38.
- Fenchel, T. M. & B. B. Jorgensen, 1977. Detritus food chains of aquatic ecosystems, the role of bacteria. In Alexander, M. (ed.), *Advances in microbial ecology*. Plenum, New York: 1–58.
- Fernandez, J. M., 1993. Fontes autotróficas de energia em juvenis de jaraqui *Semaprochilodus insignis* (Schomburgk, 1841) e curimatã, *Prochilodus nigricans* (Agassiz, 1829) (Pisces:Prochilodontidae) da Amazônia central. M.Sc. Thesis, Instituto Nacional de Pesquisas da Amazônia/Universidade Federal do Amazonas, Manaus, 58 pp.
- Forsberg, B. R., C. A. R. M. Araujo-Lima, L. A. Martinelli, R. L. Victoria & J. A. Bonassi, 1993. Autotrophic carbon sources for fish of the central Amazon. *Ecology* 74: 643–652.
- Furhman, J. A., 1992. Bacterioplankton roles in cycling of organic matter: the microbial food web. In Falkowsky, P. G. & A. D. Woodhead (eds), *Primary productivity and biogeochemical cycles in the sea*. Plenum Press, New York: 361–383.
- Fry, B. & E. B. Sherr, 1984.  $\delta^{13}\text{C}$  measurements as indicators of carbon flow in marine and freshwater ecosystems. *Cont. Mar. Sci.* 7: 13–47.
- Hedges, J. I., G. L. Cowie, J. E. Richey, P. D. Quay, R. Benner, M. Strom & B. R. Forsberg, 1994. Origins and processing of organic matter in the Amazon River as indicated by carbohydrates and amino acids. *Limnol. Oceanogr.* 39: 743–761.
- Hessen, D. O., 1985. The relation between bacterial carbon and dissolved humic compounds in oligotrophic lakes. *FEMS Microbiol. Ecol.* 31: 215–223.
- Hessen, D. O., 1992. Dissolved organic carbon in a humic lake: effects on bacterial production and respiration. *Hydrobiologia* 229: 115–123.
- Hobbie, J. E., 1992. Microbial control of dissolved organic carbon in lakes: research for the future. *Hydrobiologia* 229: 169–180.
- Howard-Williams, C. & W. J. Junk, 1976. The decomposition of aquatic macrophytes in the floating meadows of a central Amazonian várzea lake. *Biogeographica*: 115–123.
- Howard-Williams, C. & W. J. Junk, 1977. The chemical composition of central Amazonian aquatic macrophytes with special reference to their role in the ecosystem. *Arch. Hydrobiol.* 79: 446–464.
- Junk, W. J., 1970. Investigations on the ecology and production biology of the 'floating meadows' (Paspalo-echinochoetum) on the middle Amazon. I. The floating vegetation and its ecology. *Amazoniana*: 449–495.
- Junk, W. J., 1973. Investigations on the ecology and production biology of the 'floating meadows' (Paspalo-echinochoetum) on the middle Amazon. II. The aquatic fauna in the root zone of the floating vegetation. *Amazoniana* 4: 9–102.
- Junk, W. J., 1985. The Amazon floodplain – a sink or source for organic carbon? *Mitt. Geol. Paläont. Inst. Univ. Hamburg. SCOPE/UNEP Sonderbd.* 58: 267–283.
- Junk, W. J. & C. Howard-Williams, 1984. Ecology of aquatic macrophytes in Amazonia. In Sioli, H. (ed.), *The Amazon, limnology and landscape ecology of might tropical river and its basin*. Junk Publisher, The Hague, 296–293.
- Junk, W. J. & K. Furch, 1991. Nutrient dynamics in Amazonian floodplain: decomposition herbaceous plants in aquatic and terrestrial environments. *Verh. int. Ver. Limnol.* 24: 2080–2084.
- Martinelli, L. A., 1986. Composição química e isotópica ( $\delta^{13}\text{C}$ ) de sedimentos de várzea e suas interações com alguns rios da bacia Amazônica. M.Sc. Thesis, Universidade de São Paulo, 214 pp.
- McManus, G. B. & J. A. Furhman, 1988. Control of marine bacterioplankton populations: Measurement and significance of grazing. *Hydrobiologia* 159: 51–62.
- Melack, J. M. & T. R. Fisher, 1991. Comparative limnology of tropical lakes with an emphasis on the central Amazon. *Acta Limnol. Brasil.* 3: 1–46.
- Melack, J. M. & T. R. Fisher, 1983. Diel oxygen variations and their ecological implications in Amazon floodplain lakes. *Arch. Hydrobiol.* 98: 422–442.
- Moran, M. A. & R. E. Hodson, 1990. Bacterial production in humic and nonhumic components of dissolved organic carbon. *Limnol. Oceanogr.* 35: 1744–1756.
- Padovani, C. R., 1992. Determinação das fontes autotróficas de carbono para camarões em um lago de várzea da Amazônia Central utilizando isótopos estáveis de Carbono. M.Sc. Thesis Instituto Nacional de Pesquisas da Amazônia/Universidade Federal do Amazonas, Manaus, 72 pp.
- Peterson, B. J. & B. Fry, 1987. Stable isotopes in ecosystem studies. *Annu. Rev. Ecol. Syst.* 18: 293–320.
- Pomeroy, L. R., 1974. The ocean's food web: a changing paradigm. *BioScience* 24: 499–504.
- Pomeroy, L. R. & W. J. Wiebe, 1988. Energetics of microbial food webs. *Hydrobiologia* 159: 7–18.
- Quay, P., D. O. Wilbur, J. E. Richey, J. I. Hedges, A. H. Devol & R. Victoria, 1992. Carbon cycling in the Amazon: implication from the  $^{13}\text{C}$  composition of particles and solutes. *Limnol. Oceanogr.* 37: 857–871.
- Quay, P., D. O. Wilbur, J. E. Richey, H. Devol, R. Benner & B. R. Forsberg, 1995. The  $^{18}\text{O}$ : $^{16}\text{O}$  of dissolved oxygen in rivers and lakes in the Amazon Basin: Determining the ratio of respiration to photosynthesis rates in freshwaters. *Limnol. Oceanogr.* 40: 718–729.
- Rai, H., 1979. Microbiology of Central Amazon lakes. *Amazoniana* 6: 583–599.
- Rai, H. & G. Hill, 1980. Classification of central Amazon lakes on the basis of their microbiological and physico-chemical characteristics. *Hydrobiologia*: 85–99.
- Rai, H. & G. Hill, 1984. Microbiology of amazonian waters. In Sioli, H. (ed.) *The Amazon, limnology and landscape ecology of might tropical river and its basin*. Dr W. Junk Publishers, The Hague, 413–444.
- Richey, J. E., A. H. Devol, S. C. Wofsy, R. L. Vitoria & M. N. Goes-Ribeiro, 1988. Biogenic gases and the oxidation and reduction of carbon in Amazon river and floodplain waters. *Limnol. Oceanogr.* 33: 551–561.
- Richey, J. E., A. H. Devol, P. D. Quay, R. L. Vitoria, L. A. Martinelli & B. R. Forsberg, 1990. Biogeochemistry of carbon in the Amazon River. *Limnol. Oceanogr.* 35: 352–371.
- Salonen, K., P. Kankaala, T. Tulonen, T. Hammat, M. James, T. R. Metsälä & L. Arvola, 1992. Planktonic food chains of highly

- humic lake. I. A mesocosm experiment during the spring primary production maximum *Hydrobiologia* 229: 25–142.
- Sherr, E. B., B. F. Sherr & L. J. Albright. 1987. Bacteria: Link or sink? *Science* 235: 88–89.
- Starkweather, P. L., 1980. Aspects of the feeding behavior and trophic ecology of suspension feeding rotifers. *Hydrobiologia* 73: 891–908.
- Tranvik, L., 1992. Allochthonous dissolved organic matter as an energy source for pelagic bacteria and the concept of the microbial loop. *Hydrobiologia* 229: 107–114.
- Tranvik, L. & M. G. Höfke, 1987. Bacterial growth in mixed cultures on dissolved organic carbon from humic and clear waters. *Appl. envir. Microbiol.* 53: 482–488.
- Turner, J. T. & P. A. Tester, 1992. Zooplankton feeding ecology: bacterivory by metazoan microzooplankton. *J. exp. mar. Biol. Ecol.* 160: 149–167.
- Waichman, A. V., 1985. Fontes autótroficas de carbono para bactérias em um lago de várzea da Amazônia Central. M.Sc. Thesis, Instituto Nacional de Pesquisas da Amazônia/Universidade Federal do Amazonas, Manaus, 75 pp.
- Wilkinson, L., 1990. SYSTAT: The system for statistics. Systat, Inc Evanston, IL.
- Wissmar, R. C., J. E. Richey & R. F. Stallard, 1981. Plankton metabolism and carbon processes in the Amazon River, its tributaries, and floodplain waters, Peru-Brazil. May–June 1977. *Ecology* 62: 1622–1633.
- Wylie, J. L. & D. J. Currie, 1991. The relative importance of bacteria and algae as food sources for crustacean zooplankton. *Limnol. Oceanogr.* 36: 708–728.
- Zar, J. H., 1984. Bioestatistical analysis. Prentice Hall Inc., Englewood Cliffs, New Jersey, 718 pp.