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Bacteria and Phytoplankton Production Rates in Eight River Stretches of the Middle Rio Doce Hidrographic Basin (Southeast Brazil).

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ABSTRACT

Measurements of bacterial secondary production (BSP), together with primary phytoplanktonic production (PPP) were conducted during dry and rainy seasons, in eight rivers of different orders submitted to different degrees of human impacts (different trophic degree). We aimed to determine and compare the importance of BSP and PPP in carbon fixation in these different lotic ecosystems. Our results showed that the Ipanema River was the most modified system by anthropogenic effluents inputs. These inputs altered the trophic degree and BSP rates of these streams and rivers.

Key words: Bacterioplankton, carbon, phytoplankton, rivers, trophic state

INTRODUCTION

Assessments of carbon incorporation by microorganisms (bacterial and phytoplanktonic algae) are very important to characterize and quantify the sources of authocthonous carbon to the lotic ecosystems, especially when bacterioplankton is considered together with phytoplankton. By using simultaneous measurements of bacterial secondary production (BSP), phytoplanktonic primary production (PPP) and the BSP/PPP ratios, it is possible to estimate the potential specific contribution of each community for carbon fixation, making it available to heterotrophic organisms (Cole et al., 2002).

Studies of BSP, together with PPP in lotic ecosystems, and of the factors that affect these processes are very scarce (Hobbie, 1988; Currie, 1990; di Sierve et al., 1995), especially in Brazil. For example, the importance of rains in determining seasonality in tropical aquatic ecosystems is well known (Norris and Thoms 1999), but the effects of this functioning force upon BSP and PPP in tropical rivers are poorly understood. The same can be considered about the longitudinal patterns of BSP and PPP in lotic ecosystems. According to the River Continuum Concept (Vannotte et al., 1980), an increase in the heterotrophic activity, relative to primary productivity, is expected in low order streams (1^{st} – 3^{rd} orders), whereas the opposite is expected in streams of intermediate order (4^{th} – 6^{th}

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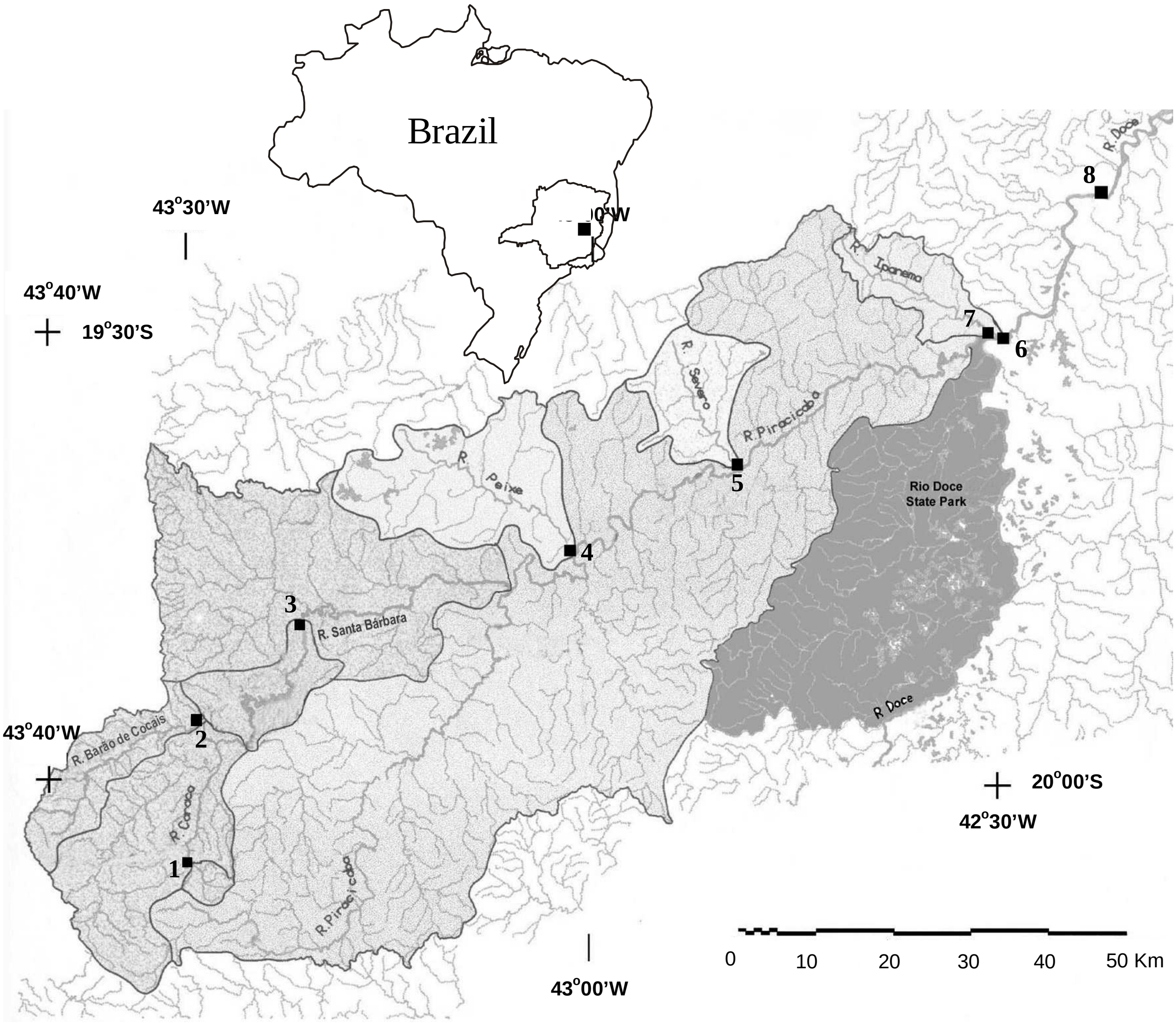
orders). Hypothesis like this one are still to be tested in tropical lotic ecosystems.

In this study, BSP and PPP measurements were conducted during dry and rainy seasons, in eight rivers of different orders submitted to different degrees of human impacts, aiming to compare production rates and infer the importance of bacterial and phytoplanktonic algae in carbon fixation in these different conditions.

STUDY AREA

This investigation was carried out in the medium stretch of the Rio Doce basin, State of Minas Gerais, southeast Brazil. Water samples were collected in eight stations, representative of the sub-basins of the rivers: 1- Caraça (20°06’00”S; 43°29’09”W), 2- Barão de Cocais (19°57’27”S; 43°28’24”W), 3- Santa Bárbara (19°50’01”S; 43°21’14”W), 4- Peixe



Source: Paula et al. (1997) modified.

Figure 1- Location of the sub-basin and respective sampling stations in the middle Rio Doce basin, Minas Gerais State-Brazil.

(19°44'35"S; 43°01'16"W), 5- Severo (19°36'57"S;42°50'50"W), 6- Piracicaba (19°29'25"S; 42°31'08"W), 7- Ipanema (19°28'14"S; 42°32'01"W) and 8- Doce (19°19'12"S; 42°21'52"W).

These ecosystems (Fig. 1) were chosen because they permited comparative studies, given that strong differences have been found in the environmental characteristics of the sampling stations, due to different degrees of human activities (Paula et al., 1997). At the sampling points, the rivers orders were: Caraça second order river; Barão de Cocais, Severo and Ipanema third order; Peixe fourth order; Santa Bárbara fifth order; Piracicaba sixth order; and Doce seventh order, according to the method of Strahler (Gordon et al., 1992).

The Caraça river flows almost entirely inside an Environmental Protection Area (Caraça Natural Park) and it is considered as a "reference" to the basin, given it is submitted to minimum anthropogenic activity. The other rivers are submitted to different types of human influence, such as cattle raising, mining, domestic and industrial effluents. Extensive areas with *Eucalyptus* spp plantations are found in the basin of some environments. Ipanema river has great part of its course canalized and receive domestic and industrial effluents and Doce river receives discharges of all other rivers. The sampling station in this river was localized downstream the Escura falls, which according to Barbosa et al. (1997) is very important to its water quality, mainly through an efficient re-oxygenation of the water.

METHODS

Two samplings were carried out in dry (July 1999 and 2000) and two samplings in rainy (January 2000 and 2001) seasons. Water temperature, electrical conductivity, pH and dissolved oxygen were measured *in situ* with a multiprobe apparatus (Horiba, mod. U-22) and total alkalinity by titration (Mackereth et al., 1978). Water samples were taken from sub-surface in the morning (8-12 am) and carried to the laboratory to determine dissolved organic carbon (TOC-5000 Schimadzu). Total nitrogen, nitrate-nitrogen, nitrite-nitrogen, reactive silicate (Mackereth et al., 1978), ammonium-nitrogen (Grasshorf, 1976), total phosphorus and soluble reactive phosphorus (Golterman et al., 1978) were also determined. Chlorophyll-a concentrations were measured in the material retained in membranes GF 52-C Schleicher and Schuell (∅ 47 mm), extracted with acetone 90% (Lorenzen, 1967).

The PPP was measured *in situ* using ^{14}C incorporation method (Steemann-Nielsen, 1952). Incubations were carried out in dark and transparent 70 ml flasks for 3-4 hours periods in the morning, with 0.5 ml of $NaH^{14}CO_3$ (2 µCi), after filtration in membranes ME 25 Schleicher and Schuell (∅ 25; 0.45 µm). BSP values were measured right after sampling by incubating 1.3 ml water samples in the dark with L-[4,5-^{3}H] Leucine (TRK 510, 142Ci/mmol), final concentration of 10 nM for 40 minutes and C-incorporation was estimated after multiplying protein (estimated through leucine incorporation) by 0.86. The final concentration of 10 nM was chosen after a saturation x concentration curve and the incubation time (40 min) was fixed according to Smith and Azam (1992). Activity (DPM) for both BSP and PPP was measured in Bray cocktail (Bray, 1960) in a Liquid Scintillation Analyzer (Packard, Tri-carb 2100TR).

The effects of seasons (dry x rainy season) and river orders (2, 3, 4, 5, 6 and 7) upon BSP and PPP, as well as the interaction between both factors, were tested with a factorial ANOVA (General Linear Model of the package Statistica). Data were log-transformed before analyses to meet assumptions. Since two tests were performed, Bonferroni criteria for multiple tests were used to minimize possibilities of type I error and the accepted level of significance became 0.05/2 = 0.025 (Sokal and Rohlf, 1995).

Principal component analysis (PCA) was applied in order to reduce the dimensionality of the abiotic data (Manly, 1994) and to search for a gradient in trophic conditions. The following variables were used in the matrix: temperature, pH, electrical conductivity, dissolved oxygen, TP, P-PO_4^{3-}, TN, N-NH_4^+, N-NO_3^-, N-NO_2^-, silicate, DIC, DOC and chlorophyll-a. Before analyses, all data except pH, were log-transformed to achieve linear relationships. The Broken-Stick method (Jackson, 1993) was used as a stopping-rule in the PCA. Possible effects of trophic gradient (represented by first component principal – see Results) upon BSP and PPP were assessed using correlation analyses (package Statistica).

RESULTS

During the sampling periods, precipitation varied between 0.0 mm (July 1999, 2000) and 59.2 mm (January 2000) (Data obtained from Instituto Mineiro de Gestão das Águas – IGAM/SIMGE). The results of the physical and chemical variables analyzed in the rivers are shown in Table 1. Considerable differences in water temperature were registered in the rivers, reflecting the differences in altitude (Caraça river: 1,200 m and Doce river: 230 m). Values fluctuated between 14 and 23 °C during dry season (winter) and between 19 and 29 °C during the rainy season (summer). pH values remained acidic in Caraça river and varied from slightly acidic to slightly alkaline (6.0 to 7.4) at the other sites. Higher values of the electrical conductivity (455 µS/cm) and alkalinity (1.0 meq CO_2/L) were measured in the Ipanema river, whereas in the others, the values fluctuated from 17.7 to 164.1 µS/cm and 0 to 0.42 meq CO_2/L, respectively. Ipanema was the only river exhibiting low oxygen concentrations (0.4-2.0 mg/l).

Table 1 - Minimum and maximum values of temperature, pH, electrical conductivity, dissolved oxygen, total alkalinity, chlorophyll-a and % of light penetration in the water in the eight rivers, during the dry (Jul/1999 and Jul/2000) and rainy (Jan/2000 and Jan/2001) periods.

Ecosystems	Temp °C	pH	Conduct. µS/cm	D.O mg/L	Alkal. meq/L	Chl.-a µg/L
	min-max	min-max	min-max	min-max	min-max	min-max
Caraça	12.7 - 19.3	4.0 - 6.4	9.2 - 17.7	6.5 - 9.9	0.003 - 0.02	2.9 - 5.1
Barão de Cocais	20.0 - 22.5	7.0 - 8.3	27.0 - 94.2	5.0 - 8.5	0.3 - 0.9	1.3 - 20.8
Santa Bárbara	18.0 - 24.5	6.1 - 7.1	37.0 - 58.3	5.0 - 8.7	0.1 - 0.4	6.7 - 28.3
Peixe	15.4 - 24.8	6.3 - 7.5	25.0 - 63.0	6.1 - 9.5	0.2 - 0.4	1.6 - 18.7
Severo	15.5 - 24.5	6.0 - 7.4	19.0 -27.9	3.2 - 10.0	0.1 - 0.3	2.7 - 53.5
Piracicaba	21.6 - 27.5	6.2 - 7.6	75.0 - 164	3.8 - 7.6	0.2 - 1.0	1.6 - 27.5
Ipanema	21.1 - 29.0	6.4 - 7.5	283 - 455	0.4 - 2.0	0.2 - 2.1	0.7 - 12.3
Doce	21.4 - 28.3	6.1 - 7.6	54.0 - 107	3.2 - 9.0	0.2 - 0.4	6.4 - 15.5

Low chlorophyll-a concentrations were expected in lotic systems, but some sites had high concentrations and the values fluctuated from 1.6 and 63.5 µg/L (Table 1). Ipanema river exhibit high concentrations of dissolved organic carbon (28.5 mg/L), total nitrogen (6,762 µg/L) and total phosphorus (1,595 µg/L) during the sampling period (Tables 2 and 3). Caraça river exhibited low total nitrogen (103.0 µg/L) and total phosphorus (4.7 µg/L) during the same period.

Considering only the phosphorus concentration, and following the typology presented by Salas and Martino (1991) for tropical lakes, presented a wide range of trophy. Ipanema river could be considered eutrophic, Caraça oligotrophic, Barão de Cocais, Peixe, Piracicaba and Doce rivers from meso to eutrophic and Santa Bárbara and Severo oligotrophic during dry season and eutrophic during rainy season (Table 2).

The highest BSP value (5.7 mg $C.m^{-3}.h^{-1}$) was recorded in the Ipanema river and the lowest in the Santa Bárbara river (0.004 mg $C.m^{-3}.h^{-1}$) (Fig. 2a). The BSP values were slightly higher during the rainy period, except in the Barão de Cocais and Ipanema rivers in the first year. Results of the factorial ANOVA showed that the effects of seasons ($F = 0.081$; $p = 0.778$) and river orders ($F = 1.191$; $p = 0.348$) upon BSP were not significant. The same was true for the interaction seasons/river order ($F = 0.923$; $p = 0.486$).

The highest PPP value (374.7 mg $C.m^{-3}.h^{-1}$) was recorded in the Piracicaba river in the dry season of 2000 and the lowest (0.01 mg $C.m^{-3}.h^{-1}$) in the Caraça river in the dry season of 2000 (Fig. 2b). Results of the factorial ANOVA showed that PPP values did not differ between seasons ($F = 0.548$; $p = 0.468$) and river order ($F = 2.647$; $p = 0.054$). The interaction seasons/river order was also not significant ($F = 1.397$; $p = 0.268$).

A wide range of the ratios BSP:PPP was found (from 0.0005 to 1.297) and in only two occasions PPP was smaller than BSP, in the Caraça and Severo rivers (Table 4). This ratio was higher in five rivers during the rainy period.

The first two axes of the PCA performed with abiotic variables accounted for 60.2 % of the total variance. The dissolved oxygen concentrations were negatively correlated and the temperature, pH and nutrients (except $N\text{-}NO_3^-$ and $N\text{-}NO_2$) positively correlated with the first axis (45.6 %, eigenvalues = 6.382). $N\text{-}NO_3^-$ and $N\text{-}NO_2^-$ concentrations were positively correlated with axis 2 (14.6%, eigenvalues = 2.048) (Fig. 3a). Only the principal component 1 was significant, according to the Broken-Stick model (eigenvalues = 3.252). If the broken-stick eigenvalue is less than the actual eigenvalue for an axis, then that axis contains more information than expected by chance and should be considered for interpretation (Jackson, 1993). Thus, the principal component 1 was retained in the analyses to explain BSP and PPP.

The first principal component scores represents the contrast between rich, more eutrophic, and poor rivers, in terms of nutrients (Fig. 3b). Chlorophyll-a, a variable indicating phytoplankton biomass was weakly correlated with both axes (Fig. 3a).

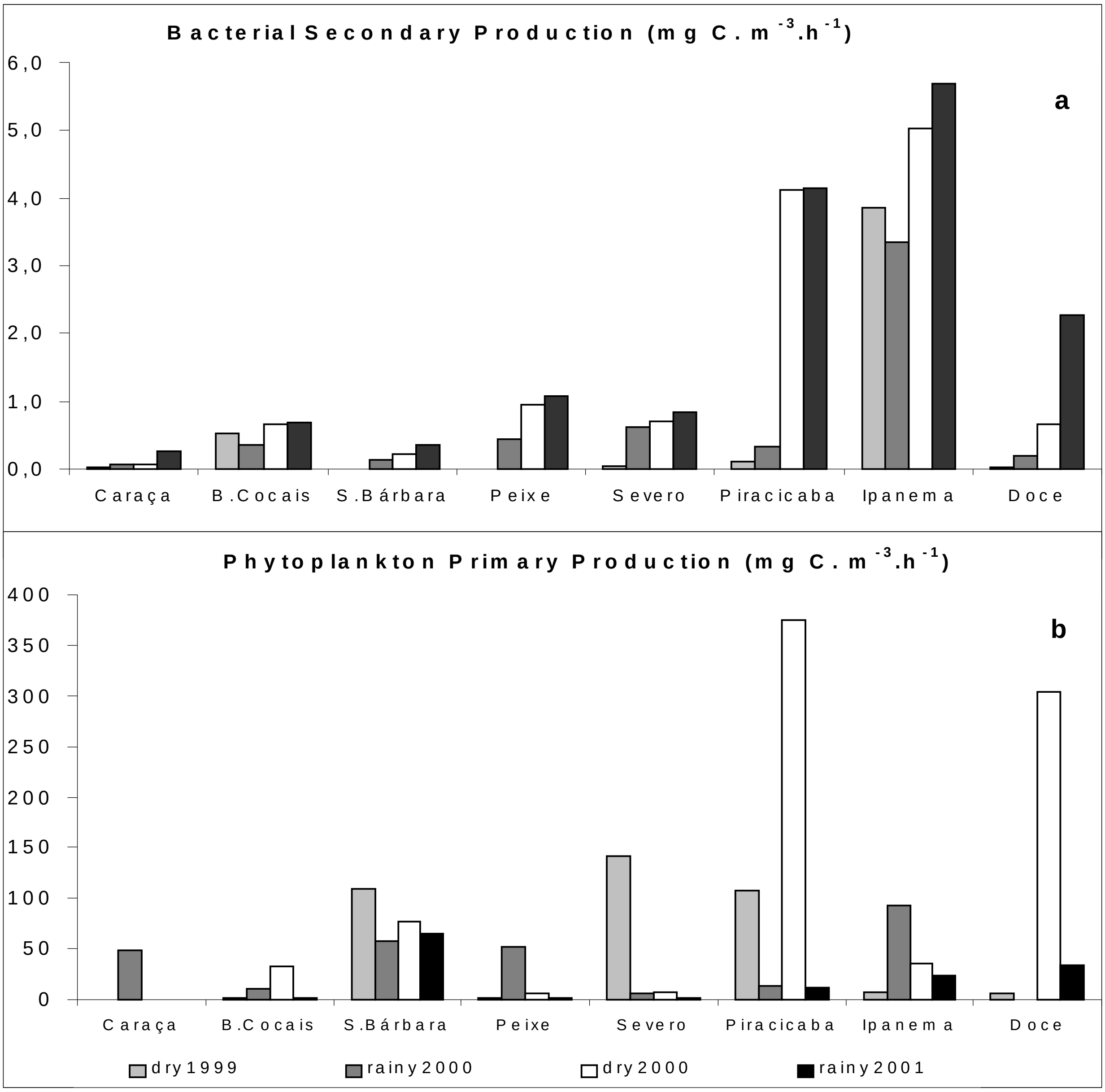


Figure 2 - Bacterial secondary production (a) and phytoplankton primary production (b) values, during the dry (Jul/1999 and Jul/2000) and rainy (Jan/2000 and Jan/2001) seasons.

Table 2 - Minimal and maximal concentrations of total and soluble reactive phosphorus; silicate and dissolved organic and inorganic carbon in the eight rivers, during the dry and rainy periods (1999-2001).

Ecosystem	Tot-P µg/L	PO_4-P µg/L	Silicate mg/L	DIC mg/L	DOC mg/L	Tropic State* (Salas and Martino, 1991)
	min – max	min-max	min-max	min-max	min-max	
Caraça	4.7 - 14.5	2.0 - 4.4	0.3 - 0.8	0.2 - 0.7	1.2 - 12.4	Oligotrophic
Barão de Cocais	56.3 - 141	14.9 - 54.9	2.6 - 34.3	5.1 - 6.8	1.4 - 4.0	Meso-Eutrophic
Santa Bárbara	17.4 - 154	1.3 - 12.6	2.1 - 27.9	2.0 - 3.5	1.4 - 3.9	Oligo-Eutrophic
Peixe	52.9 - 170	11.1 - 62.1	1.8 - 38.7	1.0 - 2.5	1.1 - 7.5	Meso-Eutrophic
Severo	14.7 - 469	1.2 - 7.8	4.0 - 16.9	0.1 - 1.5	0.7 - 10.0	Oligo-Eutrophic
Piracicaba	78.0 - 240	10.8 - 23.7	3.3 - 46.7	0.4 - 3.2	1.9 - 4.8	Meso-Eutrophic
Ipanema	760 - 1,595	68.7 - 1,035	4.8 - 67.7	0.1 - 13.5	9.3 - 28.5	Eutrophic
Doce	45.7 - 318	5.0 - 33.1	3.5 - 44.0	0.1 - 3.8	1.7 - 5.1	Meso-Eutrophic

* For trophic status classification was considered only the total phosphorus concentrations.

Table 3 - Minimal and maximal concentrations of total nitrogen, ammonium-, nitrate-, nitrite-nitrogen, and N/P ratios in the eight rivers, during the dry and rainy periods (1999-2001).

Ecosystem	Total-N µg/L	NH_4-N µg/L	NO_3-N µg/L	NO_2-N µg/L	N/P
	min-max	min-max	min-max	min-max	min-max
Caraça	103 - 963	5.4 - 565	9.0 - 36.6	1.4 - 4.2	13.6 - 98.1
Barão de Cocais	627 - 852	42.1 - 697	2.9 - 400	5.8 - 42.1	5.5 - 15.3
Santa Bárbara	393 - 590	22.6 - 129	34.5 - 430	4.1 - 8.5	3.8 - 29.9
Peixe	1,181 - 2,019	26.3 - 473	87.5 - 852	13.6 - 171	7.9 - 22.3
Severo	234 - 1,836	5.5 - 32.6	23.3 - 229	2.0 - 11.8	3.9 - 15.9
Piracicaba	1,197 - 2,461	67.2 - 308	693 - 790	9.5 - 32.2	5.0 - 31.5
Ipanema	5,341 - 6,762	1,547 - 3,320	0.5 - 41.5	2.7 - 28.3	4.2 - 7.4
Doce	806 - 1,240	23.5 - 235	92.4 - 445	6.3 - 29.1	3.9 - 13.7

Table 4 - Bacterial Secondary Production / Phytoplankton Primary Production (BSP/PPP) rates in the eight rivers, during the dry and rainy periods (1999-2001).

Ecosystem	BSP/PPP
	Minimal - Maximal
Caraça	0.001 - 1.297
Barão de Cocais	0.020 - 0.574
Santa Bárbara	0.000 - 0.005
Peixe	0.005 - 0.733
Severo	0.000 - 1.093
Piracicaba	0.001 - 0.348
Ipanema	0.036 - 0.514
Doce	0.002 - 0.382

An overlap among the rivers sampled was observed in the winter and summer. In this way, different seasons were not clearly separated by both axes. BSP was affected simultaneously by a group of factors, since this variable was significantly correlated with PC1 ($R = 0.63$; $P < 0.001$; $N = 32$) (Fig. 4a). On the other hand, the correlation between PPP and PC1 was not significant ($R = 0.20$; $P = 0.259$; $N = 32$) (Fig. 4b).

DISCUSSION

A wide range of the BSP and PPP values were registered in the rivers investigated. In the Ipanema river, the most eutrophic habitat, BSP was considerably high compared with data from temperate lakes and rivers (Goosen et al., 1999; Vicent et al., 1996; Vrede, 1996; Le et al., 1994). In tropical ecosystems, at coastal environments in the south of Brazil, Cesar and Abreu (2001) recorded a wide range of variation with higher values than the ones recorded in the present study (0.11 to 6.55 mg C m^{-3} h^{-1}). On the other hand, PPP values were lower relative to other studies carried out in temperate rivers (Goosen et al., 1999; Vicent et al., 1996).

Despite the high variability in BSP and PPP results, seasons did not affect the values of both processes. The reasons leading to the absence of seasonal differences were not clear, but some possibilities could be considered. It was likely that the differences of trophic states among rivers investigated were so high that such differences overcame the effects of seasonality. This explanation was reinforced in the region due to the high human impact observed in some of the rivers. Moreover, the differences in temperature and solar radiation, smaller in tropical than in temperate regions, might have not be sufficient to lead to metabolism differences in the investigated rivers and their microbiota. The observed lack of seasonal difference could well be a consequence of the low number of samples.

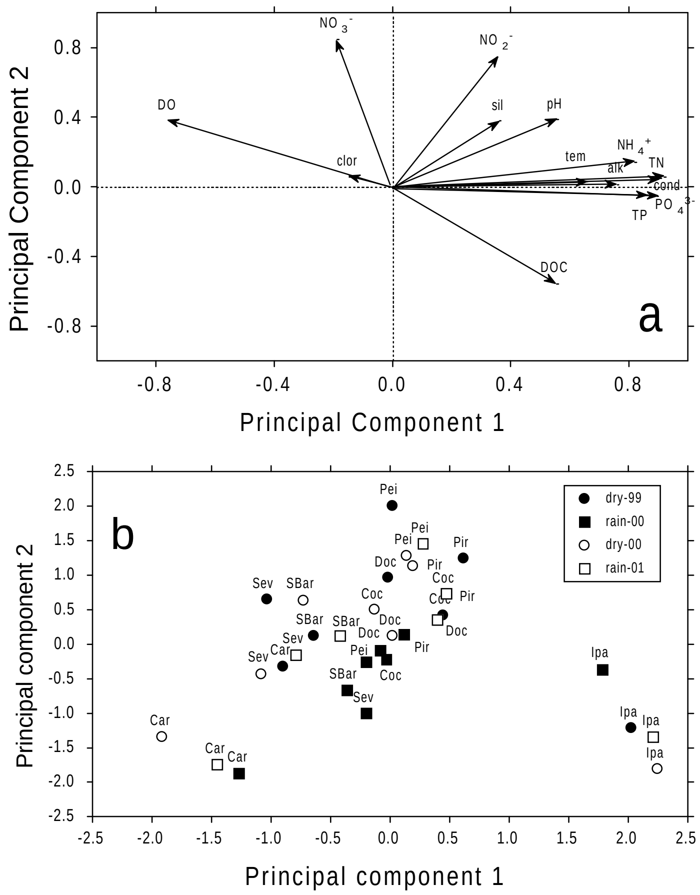


Figure 3 - Correlations of physico-chemical parameters with the first two axes of principal component analysis (a) and score distributions of streams sampled in different seasons along the first two principal components axes (b). Car = Caraça; SBar = Santa Barbara; Coc = Barão de Cocais; Pei = Peixe; Sev = Severo; Pir = Piracicaba; Ipa = Ipanema; Doc = Doce.

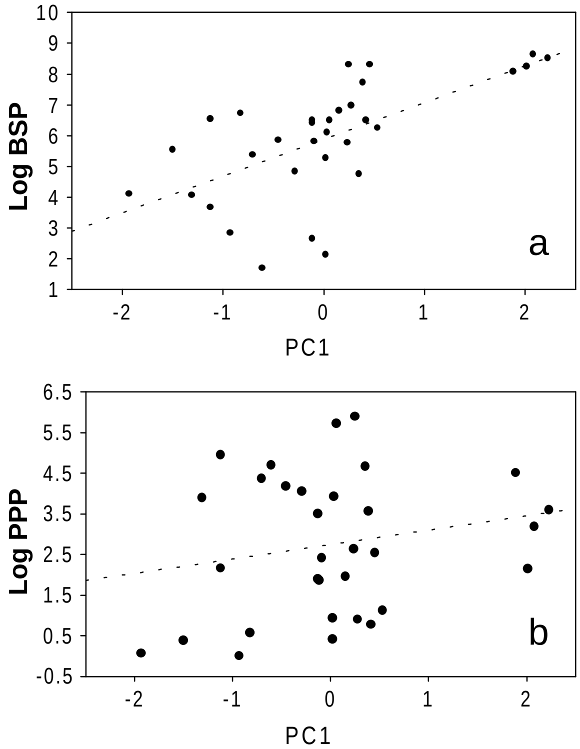


Figure 4 - Effect of Principal Component 1 on Bacterial Secondary Production (BSP) (a) and on Phytoplanktonic Primary Production (PPP) (b).

The BSP was always lower than the PPP, except in the Caraça river in the dry season of 1999 and in the Severo river in the rainy season of 2001. The high PPP relative to BSP production was maintained even during the rainy season, when increased inputs of allochthonous material were expected. Inputs of allochthonous material in general increase the trophy of the studied habitats, especially those more subjected to human activities, leading these ecosystems to exhibit more eutrophic features. BSP values were significantly correlated with the degree of trophy, as evidenced by the relationship between this variable and the scores of the first principal component. These results strongly suggested that BSP was very sensitive to changes in physical and chemical characteristics associated with trophy (N and P levels and conductivity). Thus, it could be considered an alternative indicator of trophy states in tropical rivers and streams.

This was demonstrated through the higher BSP values (Fig. 2) and higher DOC, total-P, PO_4-P, NH_4-N and total-N values (Tables. 2 and 3) recorded at the Ipanema river.

Phosphorus was considered one of the main limiting factors to BSP in the set of environments investigated, except in the Ipanema river, where N:P ratio was smaller than 8. In the rainy period, with higher inputs from outside, or from sediments upwelling, the rivers had higher phosphorus concentrations and nitrogen probably became the main limiting factor (N:P < 8.0). The ratio N:P was considered at a weight basis and the limit 8:1 determined according to Salas and Martino (1991). Phosphorus limitation had also been demonstrated as a limiting factor for BSP in several temperate lakes (Le et al., 1994; Vrede, 1996). At lower degree, DOC seemed to be also important to explain BSP, since this variable was positively correlated with the component principal 1. Nevertheless, it has to be emphasized that in the case of rivers, chlorophyll-a was poorly related with first principal component (Fig. 2a) and thus with BSP, suggesting that DOC was basically allochthonous originated. In a similar investigation carried out in Amazonian lakes and rivers, DOC was also among the most important explanatory factors of BSP (Thomaz et al., 1998).

Differently from the BSP, the PPP was not significantly correlated with degree of trophy, suggesting that other factors not considered here (e.g., turbidity, flow velocity) were more important than nutrients. Moreover, the lack of correlation could be due to the fact that PPP in this study refereed only to the particulate one (retained on filters) not including the smaller fraction which normally showed a good correlation (Le et al., 1994; Vrede, 1996).

In addition to seasons and degree of trophy, river order has been considered important in organizing the structure and dynamic rivers communities (Vannote et al., 1980). According to the River Continuum Concept (RCC), an increase of PPP in medium size rivers could be expected and an increase of BSP (i.e., heterotrophic activity) in the smaller order rivers. For our data set, river orders were not significantly correlated with either PPP or BSP. The same explanation used to the absence of seasonality could be applied in this case, that is, the high human impact observed in some rivers might be obscuring a pattern of variation in response to river order. Ipanema river was a good example considering that this was a third order river but exhibited the highest BSP values due to allochthonous material, originated from human activities. The values of BSP obtained simultaneously with the PPP were inedited to this stretch of the River Doce basin. Together with physical, chemical and other biological characteristics, these results were important elements to propose strategies aiming at restore and preserve such rivers. The present investigation could be considered a tentative of using river community processes, such as heterotrophic and autotrophic production rates, as a means of measuring the degree of impact derived from human activities and degree of trophy.

In conclusion, the results of our study supported the view that nutrients inputs, probably from anthropogenic effluents, altered the degree of trophy of the rivers considered, mainly in Ipanema river, what was directly reflected by the BSP measurements. It also suggested that human activity obscured the RCC concept and BSP could be considered a potential indicator of degree of trophy and as a consequence, an additional tool for rivers monitoring. On the other hand, the lack of a clear pattern concerning PPP variation prevented this variable of being used in this sense. Nevertheless, the inclusion of other explanatory variables not considered in the present research (e.g., flow velocity and underwater radiation), as suggested by Norris and Thoms (1999), would certainly improve the idea proposed, and probably bring new conclusions concerning PPP and BSP measurements.

RESUMO

Estudos das taxas de produção secundária bacteriana (PSB), junto com as de produção primária fitoplanctônica (PPF) em ecossistemas lóticos são escassos na literatura. Estimativas de PSB e de PPF foram realizadas durante os períodos de chuva e seca em 8 rios de diferentes ordens e submetidos a diferentes graus de impactos antrópicos (diferentes graus de trofia). O objetivo foi quantificar e comparar PSB, PPF e as taxas PSB/PPF para inferir sobre a importância destas comunidades na fixação de carbono nestes ecossistemas lóticos. Os resultados deste estudo permitem concluir que o rio Ipanema é o ambiente mais alterado pelo aporte de efluentes de origem antrópica, e que este aporte altera o grau de trofia em todos os ambientes, refletindo diretamente nas estimativas da PSB.

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