

# Spatial and temporal fluctuations in bacterioplankton and correlated abiotic variables in eutrophic environments of the Brazilian semi-arid region

Flutuações espaciais e temporais do bacterioplâncton e variáveis limnológicas em ambientes eutrofizados de região semi-árida brasileira

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**Abstract:** During the dry and rainy periods, fluctuations on bacterioplankton and trophic state indexes were studied in lotic and lentic environments of the Piranhas-Assu river hydrographic basin. Mean total bacterial densities varied in a magnitude from  $10^6$  to  $10^7$  organisms  $\text{mL}^{-1}$  and, they were higher in the rainy period (*t*-Test:  $p = 0.0025$ ). No significant variation of bacterioplankton occurred between sampling sites. Cocci bacteria were numerically predominant in all sampling stations, affecting the total abundance of bacterioplankton. Total bacterial biomass varied from 659 to  $1,997.3 \mu\text{gC.L}^{-1}$  due to the high values of filament cellular volume. Total phosphorus amounted to  $108 \mu\text{g.L}^{-1}$  and presented a positive correlation with chlorophyll “a” ( $r = 0.94$ ;  $p < 0.05$ ) during dry periods, when presented higher concentrations. Low transparency of water and phosphorus and chlorophyll concentrations indicated eutrophic or hypereutrophic levels in the studied stations of reservoir. Considering this condition, associated with high density and biomass values found, it is necessary a constant monitoring of the semi-arid aquatic ecosystems, since the quality of water is affected by drought occurrence.

**Keywords:** water quality, bacterioplankton, eutrophic environments, semi-arid.

**Resumo:** Variações no bacterioplâncton e nos índices de estado trófico em ambientes lótico e lêntico de trechos da bacia hidrográfica do rio Piranhas-Assu foram estudadas nos períodos seco e chuvoso. As médias das densidades bacterianas totais oscilaram na ordem  $10^6$  a  $10^7$  organismos por mL de amostra, sendo mais elevadas no período de chuva (Teste-*t*:  $p = 0,0025$ ). Não houve variação espacial significativa. As células em forma de cocos foram numericamente predominantes em todos os locais, influenciando a abundância total do bacterioplâncton. A biomassa bacteriana total variou de 659 a  $1997,3 \mu\text{gC.L}^{-1}$  em função dos altos valores de biovolume dos filamentos. O fósforo total atingiu  $108 \mu\text{g.L}^{-1}$  e teve uma correlação positiva com a clorofila “a” ( $r = 0,94$ ;  $p < 0,05$ ) no período de estiagem, quando estas duas variáveis apresentaram concentrações mais elevadas. A baixa transparência da água e as concentrações de fósforo e clorofila indicaram níveis eutrófico ou hipereutrófico para os locais estudados no reservatório. Considerando esta condição, associada aos altos valores de densidade e biomassa encontrados, faz-se necessário um freqüente monitoramento dos ecossistemas aquáticos de região semi-árida visto que a qualidade da água é afetada pela ocorrência de seca.

**Palavras-chave:** qualidade de água, bacterioplâncton, ambientes eutrofizados, semi-árido.

## 1. Introduction

Accumulated knowledge on the climate of the Brazilian semi-arid region points to variations in the rainfall distribution during the year, associated with high rates of evaporation due to very high thermal coefficients, determines severe drought periods which ravage the regional population.

Riverine communities of the semi-arid region of north-eastern Brazil are affected by water shortage imposed by the annual long periods of drought. Therefore, the maintenance

of the water quality in rivers and reservoirs of the region contribute significantly to improvement on life quality of the population. Water shortage during drought periods highlights the problem of water quality loss that is used by people to care of their basic needs. The use of hydric resource with poor quality results in a growing serious threat to the health and welfare of the people who have direct contact with the polluted and eutrophic waters of

rivers and reservoirs in the semi-arid region (Soares et al., 2001; Proença et al., 2003).

The presence and diversity of microorganisms are important factors to the operation of these environments. The organisms are a basic component in the aquatic food webs and they contribute significantly to nutrients cycling and flow of energy (Azam et al., 1983; Bettez et al., 2002; Gurung et al., 2002). They also maintain vital and refined relationships with other organisms in superficial water bodies (Rosado and Duarte, 2002) and, play a key role in the conservation and biological restoration of environmental degraded areas (Young, 1997).

The bacterioplankton is an abundant component (Lewis et al., 1986; Lindström, 2001) and ecologically important both, in the oceans and in the inland waters (Lindström and Bergström, 2005). Heterotrophic planktonic bacteria are associated with the carbon metabolism in pelagic environments (Gurung et al., 2002) and a large part of dissolved organic matter can be consumed by these organisms (Bouvy et al., 1998). However, the excessive degradation of organic carbon by bacteria can reduce the dissolved oxygen concentrations to levels harmful to other organisms (Eiler and Bertilsson, 2004).

Microorganisms in aquatic systems vary qualitatively and quantitatively over long periods or in a short time scale (Regali-Seleghim, 1992). Thus, temporal variations affect the ecology of specific populations and modify the structure and function of the microbial community (Liu and Leff, 2002).

The study on bacterial composition alterations can lead to the elucidation of important variations in the density and biomass of bacterioplankton in aquatic systems (Kirschner and Velimirov, 1997). Furthermore, the analysis of the trophic state indicators as total phosphorus, chlorophyll-*a*, and water transparency may show relations between biotic and abiotic factors (Matthews et al., 2002). In this regard, monitoring of aquatic ecosystems may be extremely relevant to know the functioning of these systems.

This work aimed to detect spatial and temporal fluctuations in density and biomass of bacterioplankton in upstream and downstream sites of the Engenheiro Armando Ribeiro Gonçalves dam and in dry and wet periods considering the variation on trophic state of the reservoir.

Variations of bacterioplankton have been the focus of investigation in many works (Kirschner and Velimirov, 1997; Gurung et al., 2002; Liu and Leff, 2002; Eiler and Bertilsson, 2004; Lindström and Bergström, 2005). But such studies are related, to a large extent, to the aquatic environments of temperate regions.

## 2. Material and Methods

### 2.1. Study area

The Piranhas-Assu river hydrographic basin, which supplies 148 cities of the Rio Grande do Norte and Paraíba

states, is entirely located in the Brazilian semi-arid region, where there is extreme irregularity of rainfalls and, high evaporation rates.

The annual average temperature is around 28 °C, with a maximum of 33 °C in the hottest months and a minimum of 21 °C during the coolest ones. Precipitations are extremely concentrated between February and May with an annual average of about 600 mm (Figure 1).

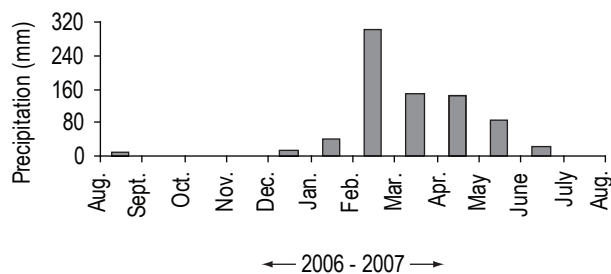
In the Rio Grande do Norte state, it is located a great reservoir with a total capacity of 2.4 billion cubic meters of water (Figure 2). The Engenheiro Armando Ribeiro Gonçalves dam is located geographically by coordinates 05° 40' 09.48" S and 36° 52' 44.34" W, in northeastern Brazil.

The annual average of water pH varies from 8.0 to 8.5 and dissolved oxygen in the dam presents an annual average concentration of approximately 5.5 mg.L<sup>-1</sup> (Costa et al., 1998). Characteristics of the drainage basin and dam are shown in Table 1.

### 2.2. Sampling

Samples were carried out in two dry periods (Sept./06 and Nov./06) and two rainy periods (Mar./07 and June-July/07), considering the annual distribution of precipitation (Figure 1).

Water samples were collected from two stations in the dam (at 5° 40' 31.70" S and 36° 53' 00.01" W, and at 05° 47' 50.38" S and 36° 55' 33.38" W), and, one on a stretch of the river (at 05° 36' 16.43" S and 36° 53' 41.99" W). The sampling stations were located at proximity of São Rafael, Itajá and Assu cities, respectively.

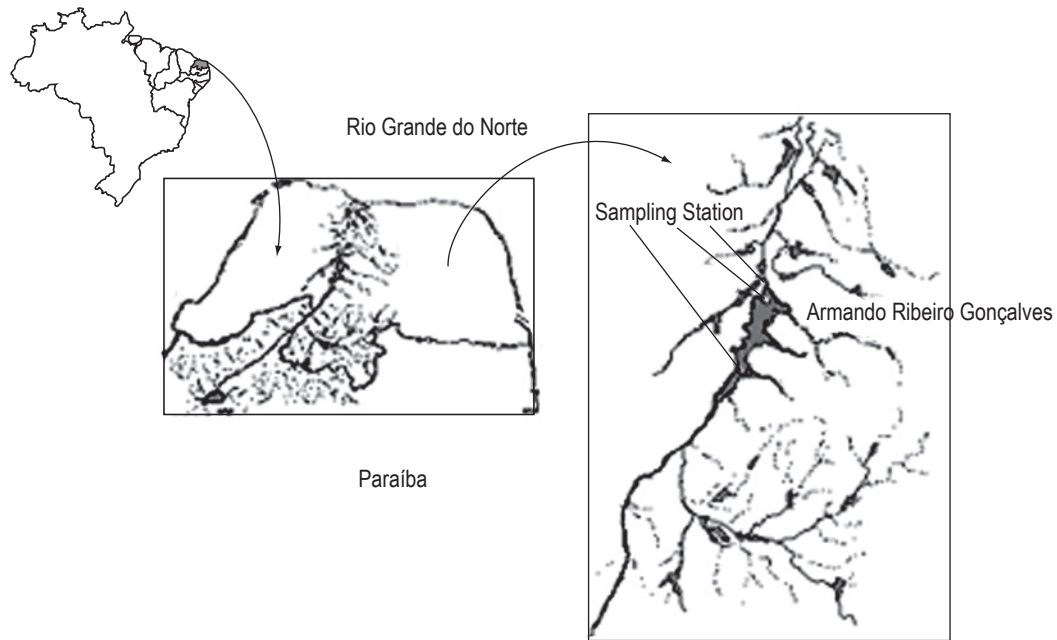


**Figure 1.** Monthly variation on precipitation from Aug./06 to July/07. Source: EMPARN

**Table 1.** Hydrologic and morphometric characteristics of drainage basin and dam.

Drainage Basin	36,770 Km <sup>2</sup>
Hydraulic Basin	19,500 ha
Maximum operational level of dam	35 m
Output of water (max)	13,200 m <sup>3</sup> /s
Water input (max)	47.5 m <sup>3</sup> /s
Maximum volume of dam	2.4 x 10 <sup>9</sup> m <sup>3</sup>
Flood area	19,200 ha

Source: DNOCS.



**Figure 2.** Piranhas-Assu river hydrographic basin and sampling stations.

Samples were collected on the surface, in the middle, and on the bottom of water column using a 0.5 L Van Dorn bottle. Subsamples were taken from integrated samples for analysis of bacterioplankton, total phosphorus, and chlorophyll-*a*. Water transparency was measured at each site using a 30 cm Secchi disk.

Total phosphorus was determined by colorimetry, using the method of ascorbic acid after digestion of the samples in potassium persulfate (APHA, 2000). For the extraction of chlorophyll-*a*, concentrated ethanol (90 to 95%) was used (Marker et al., 1980; Jespersen and Christoffersen, 1987).

### 2.3. Bacterioplankton analyses

Duplicated samples for bacterial analysis were fixed with tamponated formaldehyde (2%; pH 7.4). Subsamples (1 or 2 mL) were stained with acridine orange (0.01%) (Hobbie et al., 1977) and filtered in a black polycarbonate membrane filter (Millipore, GTBP; 0.2  $\mu\text{m}$  pore size), using a support filter (Poretics; 0.45  $\mu\text{m}$ ) to distribute the samples uniformly. Filters were mounted between the cover and the coverslip, covered with a non-fluorescent immersion oil and counted in an epifluorescence microscope (Olympus BX41; magnification  $\times 1250$ ; dichroic mirror DM500; excitation filter BP460-490 and barrier filter BA520IF). At least 300 bacterial cells were counted in each filter in 15 to 20 microscopical fields. Length and width of cells were obtained with a micrometric ocular. The bacteria were classified in four groups, on the basis of their morphology: coccid, rods, vibrios, and filaments. The cellular volumes were calculated through the formula:

$(\pi/4) \times [W^2 \times (L - (W/3))]$ , where *W* is width and *L* is the length of the cell. For the coccid cells, *W*=*L* (Bratbak, 1993). An allometric conversion formula was used in the calculation of the cellular carbon content:  $CC \text{ (fgC)} = 120 \cdot V^{0.72}$ , where: *CC* = carbon content and *V* = cellular volume ( $\mu\text{m}^3$ ). This formula, proposed by Simon and Azam (1989) and modified by Norland (1993), was considered by Posch et al. (2001) to be better adjusted to cells dyed with acridine orange.

### 2.4. Trophic state in the dam

Total phosphorus and chlorophyll *a* concentrations and water transparency in the dam were used to compute the OECD (Organization for Economic Cooperation and Development) indexes (Tundisi et al., 1988), Trophic State Indexes (TSI; Carlson, 1977), and the TSI indexes (Cullen and Small, 1981).

### 2.5. Statistical analysis

Pearson correlation matrices were constructed to determine the relations between different biotic and abiotic variables. The *t*-test was used to show significant differences ( $p < 0.05$ ) in the variables of reservoir and river between the dry and rainy periods.

## 3. Results

### 3.1. Bacterioplankton

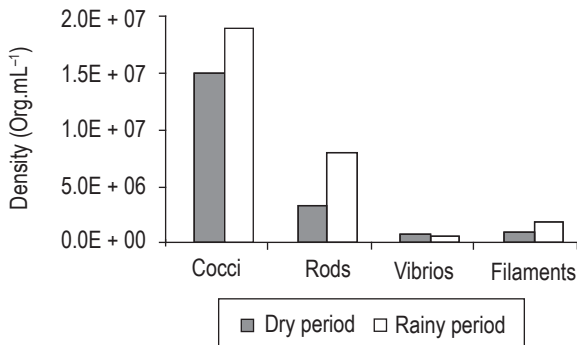
Total bacterial density varied significantly between the dry and rainy periods ( $p = 0.0025$ ). It was slightly higher in the lentic than in the lotic environments. Mean numbers of

organisms per mL of sample ranged from  $1.85$  to  $2.45 \times 10^7$  in the river and, from  $1.95$  to  $3.17 \times 10^7$  in the reservoir.

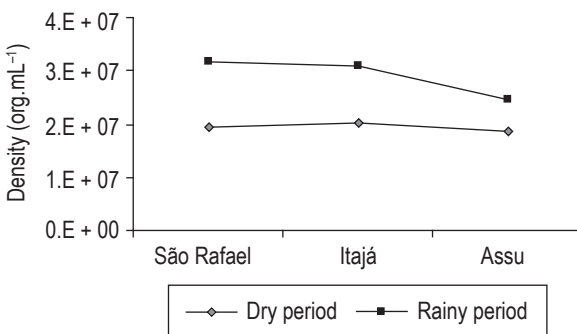
Bacterial morphotypes density did not vary significantly among sampling sites, but evident fluctuations occurred between dry and rainy periods. Cocci cells were numerically predominant at all the stations, affecting considerably the total abundance of bacterioplankton. The rods were the second most abundant group, followed by filaments and vibrios (Figure 3). The cocci and vibrio bacteria densities did not vary significantly; on the other hand, rods and filaments presented a strong temporal variation, ( $p = 0.006$ ) and ( $p = 0.0047$ ), respectively.

In both systems, the bacteria amount was higher in the rainy period (Figure 4), when the chlorophyll *a* and total phosphorus concentrations were lower. During the drought, when the bacterial abundance was lower, the concentrations of chlorophyll *a* and total phosphorus were higher. A positive correlation was found between these two variables in the dry period ( $r = 0.94$ ;  $p \leq 0.05$ ).

Mean values of bacterial biomass are shown in Figure 5. Filaments predominated and, influenced remarkably the high values of total biomass. Cocci, rods, and vibrio biomass ranged from  $47.0$  to  $122.9 \mu\text{gC.L}^{-1}$ , and for the filament the oscillation was from  $629.4$  to  $1,295.7 \mu\text{gC.L}^{-1}$ . These means were higher during the drought.



**Figure 3.** Bacterial morphotype density means in dry and rainy periods.



**Figure 4.** Total density of bacterioplankton in the three sampling sites.

Cocci biomass varied between  $31.7$  and  $75.3 \mu\text{gC.L}^{-1}$  and had the lower means among the bacterial morphotypes in the three sampling stations, in spite of appearing with higher density values. The opposite occurred with the filaments, which, despite their lower densities, presented the highest biomass values, reaching  $1,734.1 \mu\text{gC.L}^{-1}$  due to their high cellular volumes. Filament biomass varied significantly between the dry and rainy periods ( $p = 0.0011$ ) and the same occurred for the rod ( $p = 0.0099$ ) and vibrio biomasses ( $p = 0.0015$ ).

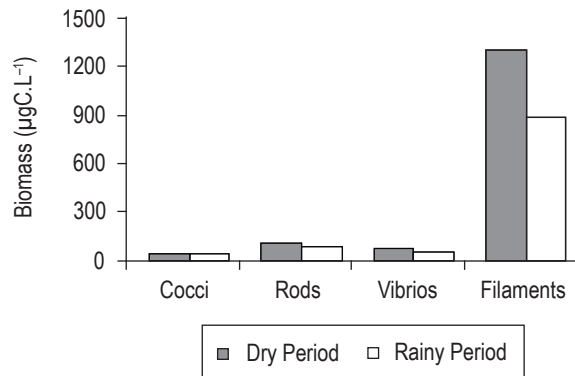
**3.2. Total Phosphorus, chlorophyll *a*, and water transparency**

During the sampling periods, total phosphorus and chlorophyll *a* concentrations and water transparency values were above the Trophic State Indexes (TSI) presented by Carlson (1977), and Cullen and Small (1981) to eutrophic aquatic environments, thus indicating eutrophic or hypereutrophic levels in Engenheiro Armando Ribeiro Gonçalves reservoir.

Total phosphorus varied from  $41.3$  to  $91.3 \mu\text{g.L}^{-1}$  in the rainy period and from  $99.7$  to  $108.0 \mu\text{g.L}^{-1}$  in the dry period ( $p = 0.0009$ ). Chlorophyll-*a* concentrations were between  $39.6$  and  $70.7 \mu\text{g.L}^{-1}$  during the drought and between  $25.3$  and  $47.9 \mu\text{g.L}^{-1}$  in the rainy period. Water transparency was lower in the dry period ( $p = 0,0028$ ), coinciding with the occurrence of stronger winds and high levels of total phosphorus and chlorophyll-*a* at this time. Means, standard deviations, and *t*-test values of the measured variables are shown in Table 2.

**4. Discussion**

The bacterial density found in this study ( $10^7$  org.mL<sup>-1</sup>) was higher than the values presented by Bouvy et al. (1998) for seven reservoirs in northeastern Brazil and it is among the highest cited by studies carried out in freshwater systems (Jugnía et al., 1998). Generally, the bacterial densities range between  $10^5$  and  $10^8$  organisms.mL<sup>-1</sup> and increase according to trophic state of the environment (Bouvy et al.,



**Figure 5.** Biomass of bacterial morphotypes in dry and rainy periods.



**Table 2.** *t*-Test comparing the variables in dry and rainy periods.

<i>t</i> -Test	Dry period		Rainy period		<i>t</i> value	p value
	mean	SD	mean	SD		
Total density (org.mL <sup>-1</sup> )	1.93E + 07	9.04E + 06	3.03E + 07	9.17E + 06	-3.327	0.0025*
Cocci density (org.mL <sup>-1</sup> )	1.48E + 07	1.05E + 06	1.88E + 07	5.53E + 06	-1.748	0.11
Rod density (org.mL <sup>-1</sup> )	3.17E + 06	4.16E + 05	7.83E + 06	3.26E + 06	-3.467	0.006*
Vibrio density (org.mL <sup>-1</sup> )	6.06E + 05	4.75E + 05	5.49E + 05	3.29E + 05	0.243	0.81
Filament density (org.mL <sup>-1</sup> )	7.53E + 05	3.61E + 05	1.75E + 06	5.71E + 05	-3.620	0.0047*
Total biomass (µg C.L <sup>-1</sup> )	1,310.4	327.6	895.5	248.2	3.909	0.0005*
Cocci biomass (µg C.L <sup>-1</sup> )	49.1	8.3	49.0	15.7	0.026	0.98
Rod biomass (µg C.L <sup>-1</sup> )	114.3	20.4	95.0	17.8	2.767	0.0099*
Vibrio biomass (µg C.L <sup>-1</sup> )	76.1	18.4	58.0	7.5	3.524	0.0015*
Filament biomass (µg C.L <sup>-1</sup> )	1,070.9	317.3	693.5	246.1	3.639	0.0011*
Total Phosphorus (µg.L <sup>-1</sup> )	102.3	4.1	77.0	19.8	3.955	0.0009*
Chlorophyll <i>a</i> (µg.L <sup>-1</sup> )	53.9	12.4	43.1	14.5	1.777	0.092
Water transparency (m)	0.9	0.1	1.2	0.12	-4.243	0.0028*

\* Significant ( $p < 0.05$ ).

1998). The aquatic communities may change in quantity and quality as a result of the eutrophication unleashed by the growth of the discharge of domestic sewage in water bodies (Biudes and Camargo, 2006). The highest density values found in March can be attributed to the contribution of allochthonous matter introduced to the aquatic systems by the rains of February. The absence of significant spatial variation is linked to the proximity of lentic and lotic studied environments that are affected by the same types of effluents.

The fluctuations observed in composition and density of microorganism populations in aquatic ecosystems are the results of the interaction of several factors such as the available food, the presence of predators, parasites, the allochthonous sources of nutrients, and microorganisms brought by soil erosion and washing and, by effluents of human activities, in addition to the local physical and chemical conditions. The possible competition between organisms that live in the same environment must also be considered.

The competition between phytoplankton and bacterioplankton seems to be less favorable to the latter, because the phytoplankton is more able to take the phosphorus in aquatic ecosystems with high availability of light (Farjalla et al., 2001). This may have influenced a lower bacterial density during the dry period in the present study.

In the study of Rodrigues et al. (2002), the phytoplankton biomass, estimated as chlorophyll-*a*, presented an inverse relation with the water level. In that work, low pigment and nutrient concentrations were detected when the water level was higher.

Bacterial biomasses of present work ( $>1,700 \mu\text{gC.L}^{-1}$ ) were very high in relation to the values published by Bouvy et al. (1998). These authors considered high the biomass ranging around  $51 \mu\text{gC.L}^{-1}$  in a eutrophic reservoir in the semi-arid region in northeastern Brazil. Kirschner and Velimirov (1997) found maximum value of  $122 \mu\text{gC.L}^{-1}$

in an aquatic environment in a temperate region. Erikson et al. (1999) and Farjalla et al. (2001) detected maximum biomass values of  $930 \mu\text{gC.L}^{-1}$  in Xolotlán Lake (Nicaragua), and  $1,432 \mu\text{gC.L}^{-1}$  in lagoons of Rio de Janeiro state, respectively. In a eutrophic lake of northeastern Poland, Kalinowska (2004) found bacterial biomass around  $1,700 \mu\text{gC.L}^{-1}$ . Wille et al. (1999) found  $15.3 \mu\text{gC.L}^{-1}$  as the maximum value of bacterial biomass in an oligotrophic lake in Austria.

The high values of biomass in the present study can be associated with the high temperatures, such as it was also considered by Araújo and Godinho (2008) that detected strong correlations between the temperature and the bacterial cellular volume.

Total phosphorus values were higher than those published by Costa et al. (2006), who found  $41.5 \mu\text{g.L}^{-1}$  in the Armando Ribeiro Gonçalves Reservoir. High concentrations of total phosphorus are probably related to the nutrients input from human activities. Intensive use of fertilizers is responsible for the increase of the phosphorus concentrations (Santos et al., 2004). In addition, human activities along the Piranhas-Assu river basin contribute to the dam eutrophication (Costa et al., 2006).

Chlorophyll-*a* concentrations were lower than the range (from 71 to  $110 \mu\text{g.L}^{-1}$ ) detected by Costa et al. (1998) in the same reservoir that were related to the high cyanobacteria concentrations. According to Bouvy et al. (1998), Costa et al. (1998), Rodrigues et al. (2002), and Ribeiro et al. (2005), chlorophyll-*a* is linked to the phytoplankton presence, and its use to estimate the trophic state is recommended because the index is based directly on algal biomass (Matthews et al., 2002).

The low values of water transparency in the reservoir are due to the influence of the continuous transport of allochthonous matter by the river (Moschini-Carlos et al., 1998), and by sediment resuspension caused by wind ac-

tion (Araújo et al., 2000; Rodrigues et al., 2002; Becker and Motta Marques, 2004). Water transparency decrease in tropical aquatic ecosystems is also due to the high sediment leaching, which directly affects the light penetration (Moschini-Carlos et al., 1998; Guarino et al., 2005; Ribeiro et al., 2005).

In summary, quantitative variations in bacterial density and biomass were influenced by the precipitation and trophic state of the environment. The limnological conditions of both environments and the high densities and biomass of bacterioplankton indicated a high microbial activity probably affected by effluents from human activities.

Data analysis indicates the need of a frequent monitoring of the water quality in Piranhas-Assu river basin. The results can be used in the development of actions directed to the conservation of rivers and reservoirs, since those waters from the reservoir are very important for coastal populations.

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