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Seasonal distribution of nanoflagellates and bacterioplankton and relationship with environmental factors in a brazilian semi-arid reservoir

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ABSTRACT. The semi-arid region of Rio Grande do Norte State, in northeast Brazil exhibits specific physical-climatic aspects: the climate is very hot, with mean annual rainfall of 550 mm, characterized by periodic drought and uneven distribution of rains. Due to these features, is important to study and preserve its water bodies. The purpose of this paper was to characterize and investigate bacterioplankton and heterotrophic nanoflagellates, and their seasonal variations in a reservoir located in the semi-arid region of Rio Grande do Norte State, in 2008. To this aim, four samplings were carried out in the rainy season and three in the dry season to determine the biotic and abiotic variables. Bacterial densities in the reservoirs ranged from 8.98×10^6 to 1.62×10^7 bac mL⁻¹, while total biomass varied between 207 and 262 µgCL⁻¹. The nanoflagellates showed a variation between 1,777 and 8,229 org mL⁻¹ in density, and between 3.0 and 9.6 µgCL⁻¹ in biomass. Statistical analyses presented non-standard results for both biological and physical-chemical variables throughout the study year, revealing the complexity of the reservoir with respect to the mechanisms controlling the microbial populations analyzed.

Keywords: microorganisms, dam, protozoa, abiotic variables.

Distribuição sazonal de nanoflagelados e do bacterioplâncton e relações com fatores ambientais em um reservatório do semiárido brasileiro

RESUMO. O semiárido norterriograndense apresenta aspectos físico-climáticos bastante específicos: o clima é muito quente, com média pluviométrica de 550 mm ano⁻¹, caracterizado pelo regime de escassez e desigual distribuição de chuvas. Por isso se torna imprescindível conhecer e preservar os seus corpos d'água. Este trabalho teve como objetivo a caracterização e o conhecimento sobre as comunidades bacterioplânctônica e de nanoflagelados heterotróficos e suas variações sazonais em um reservatório localizado na região semiárida do Estado do Rio Grande do Norte, durante o ano de 2008. Para isto, foram realizadas quatro coletas de amostras para verificar as variáveis bióticas e abióticas no período chuvoso e três no período seco. As densidades bacterianas nos reservatórios estudados variaram de $8,98 \times 10^6$ e $1,62 \times 10^7$ bac mL⁻¹, enquanto que as biomassas totais variaram de 207 a 262 µgCL⁻¹. Os nanoflagelados apresentaram variação de 1.777 e 8.229 org mL⁻¹ na sua densidade e de 3,0 a 9,6 µgCL⁻¹ na sua biomassa. As análises estatísticas apresentaram resultados não-padronizados tanto para as variáveis biológicas como para as físico-químicas no decorrer do ano de estudo, revelando a complexidade do reservatório no que se refere aos mecanismos controladores das populações microbianas analisadas.

Palavras-chave: microrganismos, açude, protozoários, variáveis abióticas.

Introduction

The poor quality of natural water is one of the most serious problems worldwide. In Brazil, around 90% of the population has access to drinking water of quality, but 47.8% of Brazilian municipalities still do not have sewage collection systems, and only 20.2% of those with such system have sewage treatment (FREITAS et al., 2008). Accordingly, efficient and easy-to-understand methods are needed to monitor water bodies. In this context

bioindicators have been widely used to understand and diagnose possible water quality problems.

Bioindicators are species chosen by their sensitivity or tolerance to a number of parameters, such as organic pollution or other types of pollutants (GOULART; CALLISTO, 2003). Since aquatic systems are subject to several disturbances, the aquatic biota reacts to both natural and anthropogenic stimuli (BUSS et al., 2003; SILVA et al., 2006; TOLEDO; NICOLELLA, 2002).

Aquatic microorganisms present close and mandatory relationships with other organisms, and with physical and chemical conditions of the environment (RONQUI et al., 2011). For this reason they have been used to evaluate environmental changes and to determine which type of change is occurring, since they have different degrees of sensitivity to diverse types of disturbances.

Most aquatic bacteria are heterotrophic, feeding on organic substances (ANACLETO; GOMES, 2006). In aquatic environments, the bacterial community can act as a link in the cycles of energy, carbon and nutrient for higher trophic levels (AZAM et al., 1983), since bacterioplankton and cyanobacteria are predated by protozooplankton, mainly heterotrophic flagellates, which are in turn predated by larger zooplankton organisms (ANACLETO; GOMES, 2006).

Protozooplankton consists of free-living protozoa incapable of maintaining their distribution independently of water masses movement. They exhibit considerable morphological and physiological diversity, occupying a large variety of ecological niches (AREAS et al., 2006; FENCHEL, 1987). Despite wide distribution, many protozoa can survive only within narrow environmental amplitudes. Species adaptation varies significantly and species successions often occur insofar as environmental conditions change (HICKMAN et al., 2001).

Although recognized by its high animal and plant diversity, Brazil has scarce data about microbial diversity, especially with respect to free-living protozoa. However, this situation has been changing in recent years, with governments increasingly supporting biodiversity research.

Owing to the important role of microorganisms as quality bioindicators in aquatic systems, this study aimed to determine the total density and biomass of bacterioplankton and protozooplankton, relating them to a number of physical-chemical variables, in order to accomplish a limnological characterization of a reservoir (Itans-Caicó) located in the semi-arid region of Rio Grande do Norte State.

Material and methods

Characterization of the study area

The Piranhas-Assu Hydrographic Basin (Figure 1) has a total drainage area of 43,681.50 km², and

40% (17,498.50 km²) of this area belongs to the State of Rio Grande do Norte (AESA, 2009; SEMARH, 2008).

Samplings, physical, chemical and biological analyses

Seven samplings were performed in 2008 during the rainy (March, May, June and July) and dry season (September, October and November), at two sites in the reservoir, one near the dam (P2), and other at the entrance of the river that feeds the reservoir (P1).

The samples were collected after integration of those obtained with a Van Dorn bottle (5 L) at surface, middle and bottom from each sampling point. The protozooplankton was fixed with a lugol-formaldehyde-sodium thiosulfate solution, as recommended by Sherr and Sherr (1993), and bacterioplankton with 2% formaldehyde (final concentration) in 50 mL amber flasks. The samples were transported under refrigeration to the laboratory and stored until analyses.

We also measured temperature, pH, dissolved oxygen, water transparency (Secchi) as well as total phosphorous and chlorophyll-*a*. Abiotic variables were compared through Carlson's Trophic State Index (TSI) to analyze the trophic level. This is a multidimensional concept that involves assessing oxygenation, transparency, eutrophying nutrients, biomass, microorganism composition and concentration, among others (MERCANTE; TUCCI-MOURA, 1999).

Determination of microorganism densities

Protozoan density was determined by sedimenting 10 mL of the sample in suitable chambers for at least 24 hours. The count was performed using an inverted microscope (Olympus IX70) at 500x magnification, as recommended by Lund et al. (1958).

Bacterial counts were carried out using 5 mL of each sample, stained with 200 µL of 0.01% acridine orange (AO) (HOBBIE et al., 1977) for five minutes and filtered through a black polycarbonate Millipore filter (pore size 0.2 µm). A cellulose acetate membrane with pore size of 0.45 µm (Poretics) was used under the black membrane to obtain better bacterial cell distribution. The filter was placed between the slide and the coverslip, using non-fluorescent immersion oil, and the count was performed under an epifluorescence microscope with 1,250x magnification (Olympus BX41). At least 300 bacteria were counted in 15 microscope fields.

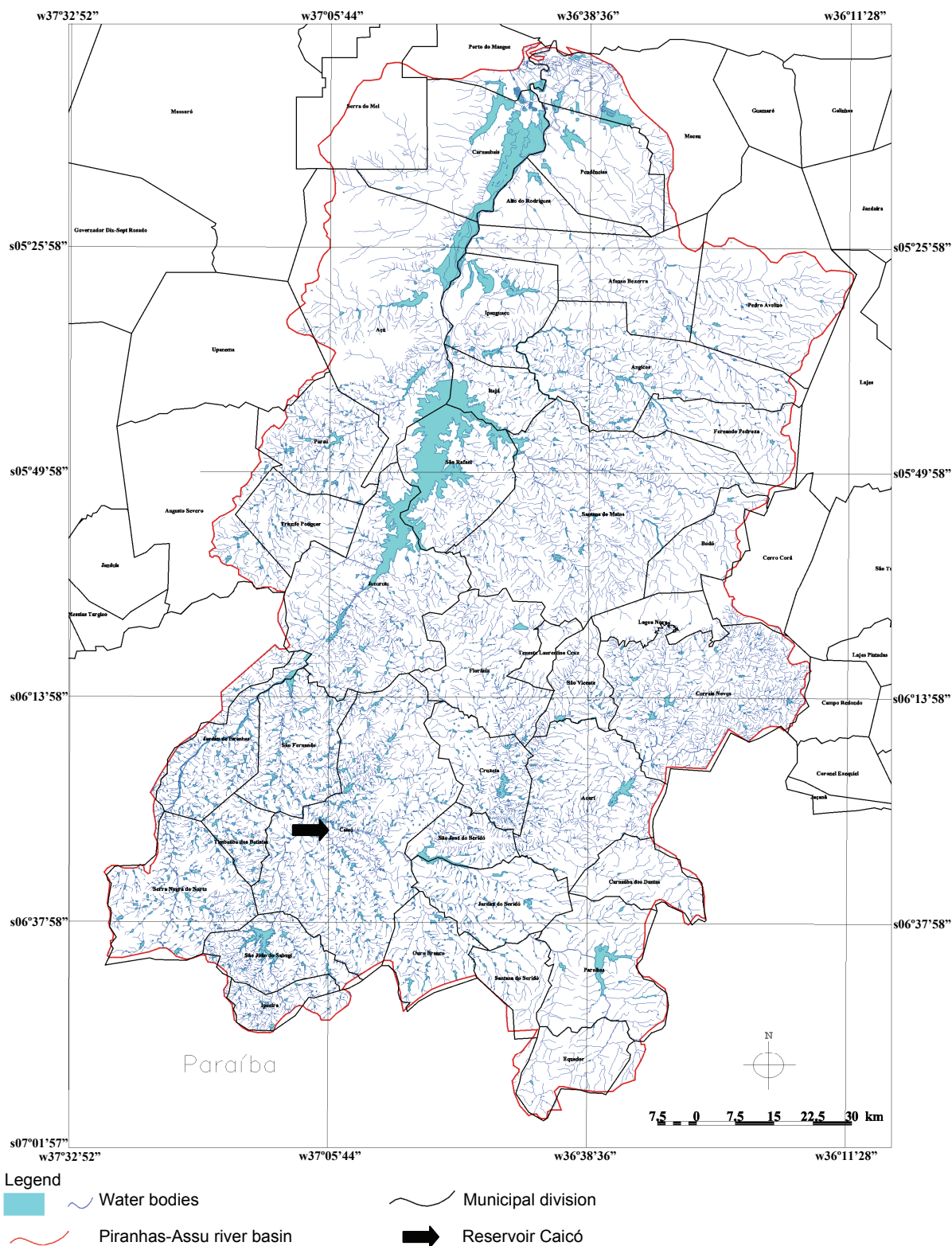


Figure 1. Itans reservoir in the Piranhas-Assu basin. Adapted by SEMARH-RN.

Determination of microorganism biomass

The biomass of heterotrophic nanoflagellates was calculated from the measure of at least 10 to 20 individuals from each group observed

(McCAULEY, 1984) and their biovolume (μm^3) was calculated from the approximate geometric shape (Table 1). Carbon content ($\mu\text{g L}^{-1}$) was estimated using the factor $1 \mu\text{m}^3 = 137 \text{ fgC}$ (FENCHEL,

1982). After the estimation of total biomasses of each shape, they were pooled and the result was expressed as total biomass of heterotrophic nanoflagellates, in μgCL^{-1} .

Table 1. Formulas of approximate geometric shapes used to calculate the biovolume of heterotrophic flagellates.

Geometric shapes	Volume formulas (μm^3)
cylinder	$V = \pi r^2 l$
sphere	$V = \pi d^3/6$

r = radius, l = length and d = diameter.

The bacterial cells were measured with an ocular micrometer and classified into morphological classes: cocci, bacilli, vibria or filaments. Biovolumes were calculated using the formula $(\pi/4) \times [W^2 \times (L - (W/3))]$, where W and L correspond to cell width and length, respectively. For coccoid cells $W = L$ (BRATBAK, 1993). An allometric conversion formula specifically elaborated for AO-stained samples was used to calculate cell carbon content - $\text{CC (fg C)} = 120 \cdot V^{0.72}$, where: CC = carbon content and V = cell volume (μm^3). This formula, proposed by Simon and Azam (1989) and recalculated by Norland (1993), is proposed by Posch et al. (2001) as the most appropriate for acridine orange-stained cells.

Statistical analysis

The significance of the differences in biotic data among the sampling points in each month, and at different times of the year was tested using the Student's t-test for independent variables at a significance level of $p < 0.05$. Pearson's correlations were used to determine the influence of abiotic and biotic factors on the densities and biomasses of bacteria and protozoa in the two periods studied. The statistical tests were comparatively analyzed to obtain more accurate results. All the analyses used STATISTICA software version 6.0 (STATSOFT Inc., 1998).

Results

After the obtaining of the concentrations of chlorophyll-a and total phosphorous (total P), they were pooled with the other variables. The mean values and standard deviations of the physical-chemical variables of the Itans Reservoir during the rainy and dry seasons are shown in Table 2.

Bacterial densities ranged from 8.68×10^6 to 1.66×10^7 bac mL^{-1} at P1, and from 9.28×10^6 to 1.59×10^7 bac mL^{-1} at P2. Mean annual variation was between 8.98×10^6 and 1.62×10^7 bac mL^{-1} . Figure 2 shows bacterial density variation in 2008 at the Itans Reservoir.

Table 2. Mean values and standard deviations of physical-chemical variables and concentrations of chlorophyll-a and total phosphorous in the Itans reservoir, in 2008.

Variables	Rainy		Dry	
	Mean	Deviation	Mean	Deviation
Temperature ($^{\circ}\text{C}$)	29.15	3.00	26.95	0.49
pH	6.17	0.52	6.90	0.42
DO (mg L^{-1})	8.48	6.41	3.85	0.49
Chlorophyll- a ($\mu\text{g L}^{-1}$)	74.74	26.89	27.56	13.85
Total P ($\mu\text{g L}^{-1}$)	270.82	44.55	108.02	45.81
Secchi depth (m)	0.61	0.36	0.50	0.14
Depth (m)	8.75	6.26	7.31	7.42

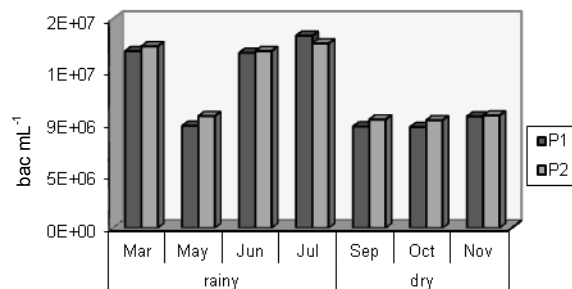


Figure 2. Bacterial density variation in 2008, in the Itans reservoir.

Bacterial biomass, excluding the filamentous forms, presented an annual mean between 207 and 262 μgCL^{-1} (Figure 3).

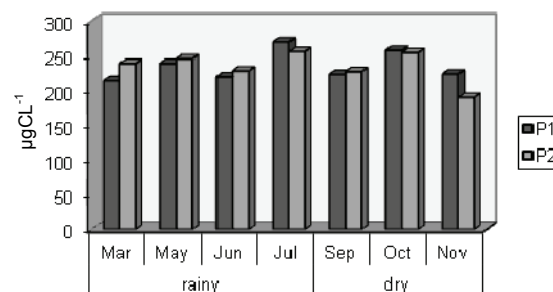


Figure 3. Bacterial biomass variation in 2008, in the Itans reservoir.

Since the filamentous forms had higher biomass, were analyzed separately to avoid influencing the biomass results of other forms. The mean annual biomass of this morphology type ranged from 253 to 926 μgCL^{-1} (Figure 4).

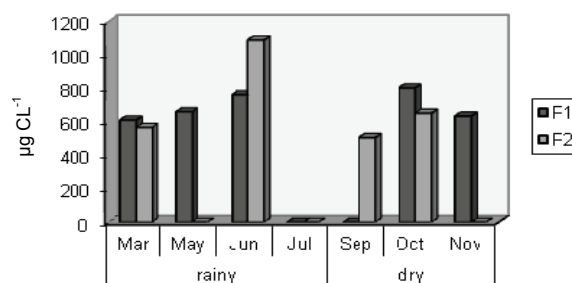


Figure 4. Biomass variation of the filamentous forms in 2008, in the Itans reservoir.

The heterotrophic nanoflagellates showed mean annual density between 1,777 and 8,229 org mL⁻¹ (Figure 5).

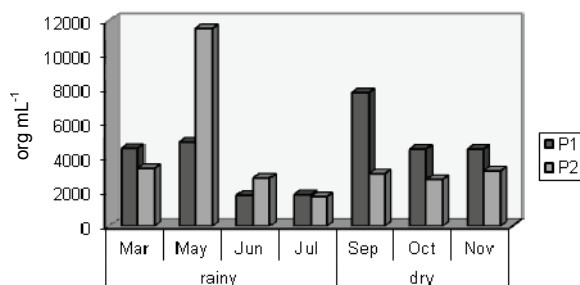


Figure 5. Flagellate density variation in 2008, in the Itans reservoir.

Mean annual biomass of nanoflagellate varied between 3.0 and 9.6 µgCL⁻¹ (Figure 6).

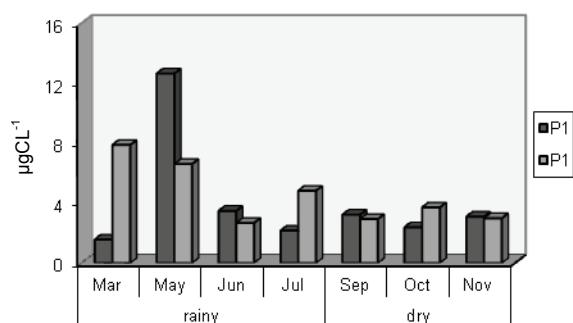


Figure 6. Flagellate biomass variation in 2008, in the Itans reservoir.

Negative correlations were verified during the rainy season between protozoan biomass and the following physical-chemical variables: rainfall ($r = -0.80$) depth ($r = -0.73$), and concentrations of chlorophyll-*a* ($r = -0.86$) and total phosphorous ($r = -0.75$). The higher the biomass values, the lower the values of these variables.

A negative correlation was observed in the dry season between bacterial biomass and bacterial density ($r = -0.76$), as well as between protozoan biomass and the physical-chemical variables pH ($r = -0.74$), total phosphorous ($r = -0.77$) and water transparency ($r = -0.74$).

Discussion

The physical-chemical variables presented large oscillations between the seasons, with a significant difference between the values of all the variables between rainy and dry seasons ($p < 0.05$). In the rainy season the concentrations of chlorophyll-*a*, total phosphorous and dissolved oxygen were much higher than in the dry season, probably due to the soil leaching promoted by rainfall during this period, which carry the nutrients to its interior.

However, regardless the climatic period and according to recent studies, Itans reservoir can be classified as hypertrophic regarding the levels of total phosphorous and chlorophyll-*a* (COSTA et al., 2009).

The values of abiotic variable recorded in this study are typical of reservoirs located in the semi-arid northeastern (BOUVY et al., 1999; ESKINAZI-SANT'ANNA et al., 2006; SODRÉ-NETO; ARAÚJO, 2008). These environmental conditions characterize the water stress in these regions, along with the degradation associated to climatic factors such as low rainfall and high evaporation, and anthropogenic factors such as lack of a basic sewage disposal system, and the high input of agricultural products into the reservoirs.

Moreover, the bacterial densities found in this study are comparable with those obtained in aquatic environments in northeast Brazil. Bouvy et al. (1998) verified levels of around 10^6 bac mL⁻¹ in the reservoirs of this region. Bacterial densities between 5×10^7 and 4×10^8 bac mL⁻¹ were registered in reservoirs from this same basin between 2002 and 2009 (AMORIM et al., 2009; ARAÚJO; COSTA, 2007; SODRÉ-NETO; ARAÚJO, 2008).

Few oscillations were observed between the two sampling points (P1 and P2) within a same month. This was evidenced by the t-test, which showed no significant difference between these locations in any of the studied months. However, during the rainy season, bacterial densities were much higher than in the dry season ($p = 0.0004$).

The high rainfall in the rainy season may have influenced the increased bacterial density. Although no correlation with abiotic variables, the high concentrations of nutrients might have caused the increase in bacterial populations, such as occurs with Cyanobacteria, whose development is favored by climatic changes and loss of water quality (COSTA et al., 2009).

The bacterial biomass, considered high, presented few oscillations throughout the study period, with no significant variation between the two seasons or two sampling points. Araújo and Godinho (2008a) found mean bacterial biomass ranging from 123 to 269 µgCL⁻¹ in a fluvial-lagunar system in the State of Rio Grande do Norte. Bouvy et al. (1998) obtained bacterial biomass of 51.8 µgCL⁻¹, considering it high, in a eutrophic reservoir located in the semi-arid of northeast Brazil. Amorim et al. (2009) recorded similar biomasses, ranging from 175 to 430 µgCL⁻¹ in two reservoirs of the Piranhas-Assu river basin between 2005 and 2007, and Sodré-Neto and Araújo (2008), in a study in the same basin in 2006, registered bacterial

biomasses oscillating from 47 to 122.9 μgCL^{-1} . These same studies observed high levels of filamentous biomass. Besides that, Amorim et al. (2009) obtained filamentous biomass between 800 and 1680 μgCL^{-1} , and Sodré-Neto and Araújo (2008), between 629.4 and 1295.7 μgCL^{-1} . Elevated biomass may be related to high trophic levels and the consequent availability of nutrients for microorganism growth.

By comparing bacterial density with filamentous biomass we can infer that, in the dry season the latter is related to the decrease in bacterial density during this period. This occurs because bacteria have a defense mechanism under extreme situations, during which they cluster to form filaments, becoming larger and more difficult to be predated by protozoa (MATZ; KJELLEBERG, 2005; SOMMARUGA; PSENNER, 1995; YOUNG, 2006). Nanoflagellate density underwent a number of oscillations throughout the year, although not significant. However, if we consider all the samplings, protozoan density varied considerably between the two points analyzed ($p < 0.05$).

Of the seven samplings carried out in 2008 in the Itans Reservoir, the number of protozoa was greater at point 1 in four of them, mainly during the dry season. The high number of protozoa at the entrance of the river may be related to the greater biodiversity of organisms at this point, since besides being shallower, the system can be considered lotic, favoring a higher mixture of nutrients through the water flow itself and the action of winds (BOZELLI; HUSZAR, 2003).

The heterotrophic nanoflagellate densities verified in this study are among the highest ever reported. Araújo and Godinho (2008b) found densities between 8 and 44 org mL^{-1} in a fluvial-lagunar system; and Gomes et al. (2007) obtained densities ranging from 2.23 to 614 org mL^{-1} in two coastal ecosystems. Furthermore, Areas et al. (2006) found densities oscillating between 2.28 and 4.8 org mL^{-1} .

Densities of up to 2,070 org mL^{-1} were observed in a study in reservoirs with different trophic degrees in Southern Brazil, while these densities ranged from 0.6 to 2.6 org mL^{-1} in the southeast. The semi-arid northeast showed total protozoan density between 13 and 1,482 org mL^{-1} from 2002 to 2004 (ARAÚJO; COSTA, 2007; PEREIRA et al., 2005). As observed in other studies, (ESKINAZI-SANT'ANNA et al., 2006; GOMES et al., 2007; PEREIRA et al., 2005), the results of the present study showed that high nutrient concentrations influences microorganism abundance (density and biomass).

Nanoflagellate biomass varied considerably throughout the year (2008) but, as observed for density, no significant difference was found between rainy and dry seasons. The t-test results of the two sampling points showed a significant difference during the last sampling, except for one of the months. Ronqui et al. (2011) registered protozoan biomass varying between 0.04 and 6.5 μgCL^{-1} , in a study conducted in a reservoir in the southeast of the country, and Pereira et al. (2005) observed biomass of up to 20.64 in reservoirs in the south of Brazil.

The nanoflagellate biomass did not exhibit a clear pattern of temporal elevation, nevertheless in the rainy season was slightly higher. This coincides with the higher biomass of filamentous forms, leading once again to the supposition that the bacteria become larger as a strategy to avoid predation by protozoa. Furthermore, higher nutrient concentrations may have provided better ecological conditions for the increased protozoan biomass.

Since no correlations were found between bacterioplankton biomass or density with chlorophyll-*a* or any other physical-chemical parameter, we can assume that the control of this component is not associated to the primary production of the system or to the factors that drive the phytoplankton. The literature confirms that in eutrophic environments bottom-up mechanisms are not typical, and that the bacteria are more likely to be controlled by predation (top down) in this type of environment (DUFOUR; TORRÉTON, 1996). Meanwhile, strong positive correlations were registered between heterotrophic nanoflagellates and rainfall, and chlorophyll-*a* with total phosphorous ($p < 0.05$), indicating that these forces govern the development of this group in the environment studied.

Conclusion

The Itans Reservoir presented non-standard results for both biological and physical-chemical variables throughout the study year. The rainy and dry seasons had distinct and atypical characteristics concerning the variables observed. The rainy season was characterized by high nutrient concentrations and increased bacterial biomass and density, whereas the dry season had lower nutrient concentrations, but higher nanoflagellate biomass and density. These findings support the idea that microorganism populations in the rainy season were controlled by the availability of nutrients, and in the dry season by competition and predation.

The obtained results reveal the complexity of the reservoir as regard the mechanisms controlling the examined microbial populations. A longer monitoring period is needed as well as experiments to obtain more thorough knowledge of the trophic relationships that take place in eutrophicated reservoirs in the semi-arid northeast Brazil.

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