



Ecological indexes and phytoplankton structure of urban reservoirs supplied by estuarine waters and under the effects of seasonality (Belém, Pará, Brazil)

Índices ecológicos e estrutura do fitoplâncton de reservatórios urbanos abastecidos por águas estuarinas e sob os efeitos da sazonalidade (Belém, Pará, Brasil)

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Ecological indices are used in the study of phytoplankton because they are related to their productivity and can describe the stresses caused by anthropogenic impacts on the aquatic environment. The aim of this study was to evaluate the phytoplankton structure and the application of ecological indices in the monitoring of water quality in public supply reservoirs in Belém (Pará). Monthly sampling occurred in four stations in the Bologna and Água Preta reservoirs from January to October/2019, to determine the abiotic and biotic factors and the calculation of ecological indices. Turbidity, transparency, precipitation and dissolved oxygen were the main abiotic variables that differentiated the reservoirs, evidencing the estuarine, seasonal and anthropogenic influence on species distribution. A total of 187 phytoplankton species were identified with representation of diatoms. In January, Água Preta reservoir presented lower diversity (2.14 ± 0.58 nats.ind⁻¹), equitability (0.57 ± 0.14) and higher dominance (0.25 ± 0.19) with the species *Trachelomonas volvocina* in higher density, contrary to June that presented higher diversity and equitability and no species dominance. The Bolonha reservoir showed lower richness (8.3 ± 1.9), diversity (1.2 ± 0.5 nats.ind⁻¹) and equitability (0.32 ± 0.12) with dominance of the species *Gonyostomum* sp. (0.55 ± 0.19). In this sense, the Água Preta reservoir was considered diverse with species well distributed in the environment, while the Bolonha reservoir showed low diversity and dominance of a phytoplankton species. Ecological indices describe well the spatio-temporal variation of phytoplankton in these reservoirs and are a good approach in monitoring environmental quality.

Key-words: diversity, bioindicators, tropical reservoirs.

Índices ecológicos são usados no estudo do fitoplâncton por apresentarem uma relação com sua produtividade, podendo descrever os estresses causados pelos impactos antrópicos no ambiente aquático. O objetivo deste estudo foi avaliar a estrutura do fitoplâncton e a aplicação de índices ecológicos no monitoramento da qualidade da água em reservatórios de abastecimento público em Belém (Pará). Amostragens mensais ocorreram em quatro estações nos reservatórios Bolonha e Água Preta de janeiro a outubro/2019, para determinar os fatores abióticos, bióticos e o cálculo dos índices ecológicos. A turbidez, transparência, precipitação e oxigênio dissolvido foram as principais variáveis abióticas que diferenciaram os reservatórios evidenciando a influência estuarina, sazonal e antrópica na distribuição das espécies. Foram identificadas 187 espécies do fitoplâncton com representatividade das diatomáceas. Em janeiro o reservatório Água Preta apresentou menor diversidade ($2,14 \pm 0,58$ nats.ind⁻¹) e equitabilidade ($0,57 \pm 0,14$) e maior dominância ($0,25 \pm 0,19$) com a espécie *Trachelomonas volvocina* em maior densidade, contrário a junho que apresentou maior diversidade e equitabilidade e sem dominância de espécies. O reservatório Bolonha apresentou menor riqueza ($8,3 \pm 1,9$), diversidade ($1,2 \pm 0,5$ nats.ind⁻¹) e equitabilidade ($0,32 \pm 0,12$) com dominância da espécie *Gonyostomum* sp. ($0,55 \pm 0,19$). Neste sentido, o reservatório Água Preta foi considerado diverso com as espécies bem distribuídas no ambiente, já o reservatório Bolonha apresentou baixa diversidade e dominância de uma espécie fitoplanctônica. Os índices ecológicos descrevem bem a variação espaço-temporal do fitoplâncton destes reservatórios e são uma boa abordagem no monitoramento da qualidade ambiental.

Palavras-chave: diversidade, bioindicadores, reservatórios tropicais.

1. INTRODUCTION

Estuarine environments are complex due to the action of several physical-chemical, climatic, hydrological factors, contribution and mixture of nutrients that interact and determine their dynamics and the maintenance of their biota [1, 2]. In the Amazon there are several estuarine systems of great socio-environmental importance, such as the Amazon Coastal Zone, between the states of Pará, Maranhão and Amapá, which interact with the ocean forming estuarine plumes that join the discharges of the Amazon River basin, considered the largest river in the world, having extensive estuarine regions that extend to 7×10^6 km from its mouth [3].

The hydrographic system of Belém is located in the central region of the Amazon Coastal Zone, in the state of Pará, together with the estuary of the Pará River, which has as main tributaries the rivers Guamá, Acará, Moju and Tocantins, which join the Amazon River through the Breves Strait [4, 5]. The action of these estuarine plumes together with the semi-diurnal tides influence the hydrodynamic regime of these tributaries that compose the Guajará Bay [4, 6], which is 1,646 km from the Amazon River basin [7] and approximately 120 km from the Atlantic Ocean [8].

Part of this estuarine system is inserted in the water supply system of the population of the Metropolis of Belém, through water abstraction from the mouth of the Guamá River, a tributary of the Pará River estuary and the Guajará Bay [4-6]. The public supply system of the Metropolitan Region of Belém, one of the largest metropolises in the Amazon, is formed by the Guamá River and the Água Preta and Bolonha reservoirs, is located in the urbanized part of the metropolitan region and, therefore, is vulnerable to the various impacts caused by disorderly population growth and lack of sanitation [9], critical conditions in Brazilian cities, especially in the Amazon.

In this scenario, sewage is discharged without prior treatment into rivers and the contaminated water is the main cause of several processes of ecological imbalance due to the loss of its quality and biodiversity [10]. The reservoirs of this supply system are dominated by aquatic macrophytes and their waters are eutrophic, with records of cyanobacteria blooms, which makes them unsafe for human consumption [11-14]. Due to the increasing scarcity of water, existing aquatic environments must be monitored in order to ensure safe water for supply and to predict processes that impact these environments and may endanger their ecological integrity. Therefore, phytoplankton has been requested as a good monitoring tool, since the community has the ability to respond to changes in the environmental gradient due to the short life cycle and diverse adaptive strategies [15-17].

In many countries, phytoplankton is used as a biomonitoring tool, where its application includes the study of phytoplankton biomass through chlorophyll concentrations and algal biovolume or the use of ecological indexes, through species abundance and diversity [18], for example the Water Framework Directive (2000, 60, EC), which provide the evaluation of the quality and protection of European waters [18, 19]. In Brazil, the legislation on the use of biological communities in water quality assessment refers to the Resolution CONAMA 357/2005 [20]. However, this resolution does not determine which indicators should be used or the type of approach to be applied. Currently, the Ordinance of the Ministry of Health of Brazil MS 888/2021 suggests the monitoring of waters intended for human consumption through the counting of phytoplankton, cyanobacteria, and chlorophyll-a concentration [21].

Ecological indexes are metrics commonly used in the study of phytoplankton, as they have a unimodal relationship with the productivity of these algae [22]. Furthermore, they can distinguish the extent and levels of stress caused by human impacts on the aquatic environment [18, 19], since they express local complexity, allow to identify the environmental elements and factors that provide for the maintenance of these species in a given habitat and infer on the functioning of the ecosystem [23, 24], and also, have good application when performed in hydrologically connected environments allowing their comparison [25].

Given this, as studies using phytoplankton are scarce for the study area, monitoring of these environments is necessary, as is knowledge of phytoplankton community structure and behavior, therefore, the aim of this study was to evaluate the phytoplankton structure and the application of ecological indexes in water quality monitoring in public supply reservoirs in Belém (Pará). For

this, we started from the applications of the indexes of Richness ($D\alpha$), Diversity (H'), Dominance ($1-D$) and Equitability (J') applied to phytoplankton.

2. MATERIAL AND METHODS

2.1 Study Area

The Metropolitan Region of Belém-MRB (Pará, Brazil) is located in the North of Brazil [26] and comprises an area of approximately 2,506,737 km², and an estimated population of 2,262,159 [27]. The climate of the region is hot and humid, with low thermal amplitudes (31°C to 33°C), high relative humidity (79.6% to 89.8%), monthly rainfall of 125.1 mm to 503.6 mm and average wind speed of 3.9 km.h⁻¹ to 6.7 km.h⁻¹. The rainy season comprises from January to May and the least rainy from July to November [28].

The water supply system of the Belém Metropolitan Region supplies about 70% of the MRB population, being formed by the mouth of the Guamá River and the Água Preta and Bolonha reservoirs [29]. As expected for the pattern of Amazonian estuarine waters, the Guajará Bay has its salinity ranging from 0 to 4.5 [30], while the Guamá River, responsible for about 90% of the hydric capacity of these reservoirs [10], has turbid and slightly acidic waters, with salinity variation from 0 to 0.04. This river receives untreated domestic and industrial sewage, which makes its waters vary from mesotrophic at high tide to hypereutrophic at low tide [5, 31]. The Água Preta and Bolonha reservoirs are located in environmental Conservation Unit (01°27'21"S; 48°30'15"W), being surrounded by a strip of primary and secondary forest with ombrophilous vegetation [32], however, the urban area added the deforested area is equivalent to 43% of the total area of the watershed. The Água Preta reservoir has an area of 3,116,860 m² and a volume of 9,905,000 m³, whereas the Bolonha reservoir has an area of 577,127 m² and a volume of 1,954,000 m³ [33]. The reservoirs are interconnected by a 5 km long channel, where the waters of Água Preta drain towards Bolonha.

In these reservoirs, water samples were collected in four sampling stations, for determination of abiotic and phytoplankton factors: AP1, AP2 and AP3, in the Água Preta reservoir (AP), these stations were established strategically because of the access to the lake, are equidistant stations and consist of the upstream, front and downstream entrance of the estuarine waters of the Guamá River, respectively. BL4 situated in the Bolonha reservoir (BL) is the intake station of the water treatment plant of the water supply system, in this reservoir there is only one sampling station due to the difficult access to the water mirror by the excess of macrophytes (Figure 1). Sampling was carried out in January, February, March, April, June, July and October 2019.

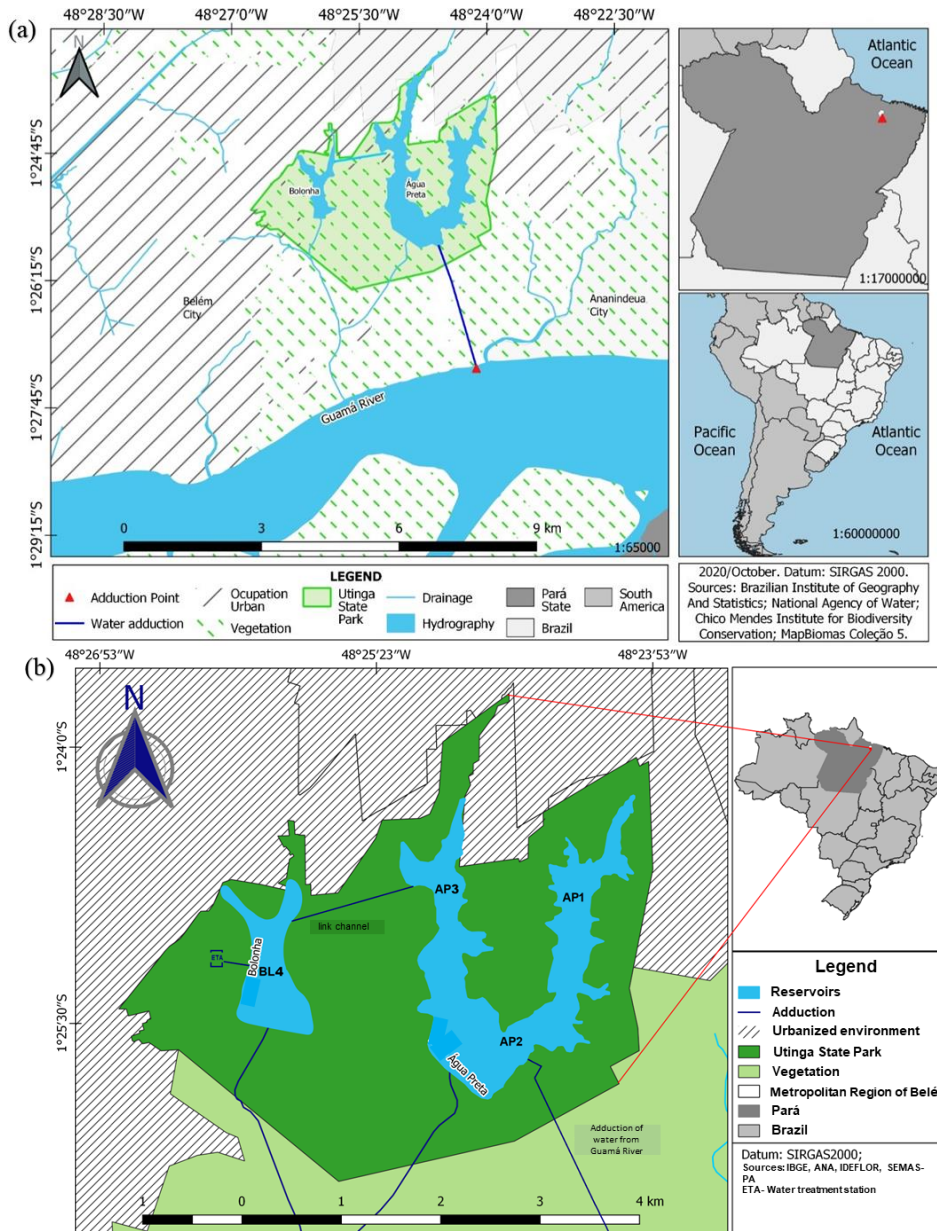


Figure 1. Study area and sampling stations (a): Water collection station from the Guamá River for the Água Preta and Bolonha reservoirs (b): Map of the location of the water supply system in the Metropolitan Region of Belém (Pará, Brazil) with the sampling stations: AP1, AP2, AP3 Água Preta reservoir and BL4 Bolonha reservoir.

2.2 Sampling and analysis of biotic and abiotic variables

The transparency (m) of the water was estimated using the Secchi disk and the limnological variables: water temperature ($^{\circ}\text{C}$), hydrogenic potential (pH), electrical conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$), dissolved oxygen ($\text{mg}\cdot\text{L}^{-1}$) and salinity ($\text{mg}\cdot\text{L}^{-1}$) determined using a multiparametric probe (HANNA HI9828, HANNA INSTRUMENTS, USA). Turbidity (NTU) was estimated using a turbidimeter (2100Q EPA 0-1000NTU FONTE/USB, HACH, PORTUGAL). Precipitation (mm) and wind ($\text{km}\cdot\text{h}^{-1}$) data were obtained from the Belém meteorological station ($1^{\circ}25'48.0''\text{S}$ - $48^{\circ}25'12.0''\text{W}$) and provided by the National Institute of Meteorology of Brazil [28].

The phytoplanktonic samples were collected directly from the subsurface of the water with polypropylene bottles (250 ml) and fixed with neutral formalin (4%) according to the 10200 B methodology of the Standard Methods for the Examination of Water and Wastewater [34]. For the quantification (density) of phytoplankton ($\text{cel}\cdot\text{mL}^{-1}$) the methodology 10200 F [34] was used.

The counting was performed in sedimentation chambers, and the analysis methodology consists in counting the individuals until 100 organisms of the most abundant species are reached, thus covering the entire area of the chamber, in an inverted optical microscope (AXIOVERT.A1, ZEISS, GERMANY) coupled to a camera (AXIOCAM ICC5, ZEISS, GERMANY) with measurement software, under 400x magnification and the values were converted into cells per milliliter. The identification, nomenclature and taxonomic classification of phytoplankton were carried out according to specialized literature [35-39].

2.3 Statistical analysis

Multivariate analyzes of variance were performed to identify spatio-temporal differences of limnological and environmental variables (rainfall and winds). These variables were standardized by ranging and transformed into fourth root or square root and subjected to the test of Mardia (1970) [40] and Doornik and Hansen (1994) [41] to verify multivariate normality. The data distribution was not normalized, so the One-way PERMANOVA analysis was applied using the Euclidean distance. The ANOVA (F) test was performed for parametric data and Kruskal-Wallis (H) for non-parametric data and the post-hoc comparison of Tukey and Dunn, respectively, to verify the spatio-temporal difference (months and sampling stations) of density and of the ecological indexes.

The ecological indexes were calculated from the density (cel.mL^{-1}) of species:

- Margalef's Richness ($D\alpha$) [42]: $D\alpha = (S - 1)/\ln(N)$, whereby S: number of species sampled; N: total number of individuals sampled; ln: logarithm in the neperian base.
- Shannon-Weaver's Diversity (H') [43]: $H' = [\mathbf{N} \cdot \ln(\mathbf{N}) - \sum_{i=1}^S ni \ln(ni)]/\mathbf{N}$, whereby: N: total number of individuals sampled; ni: number of sampled individuals of the i-th species; S: number of species sampled; ln: logarithm in the neperian base.
- Simpson's Dominance (D) [44]: $D = \sqrt{\sum_{i=1}^n (li - l^{-2})} - 1$; $D = 1 - l$ whereby: l: measure of dominance; C: Simpson dominance index; ni: number of sampled individuals of the i-th species; N: total number of individuals sampled; S: number of species sampled.
- Pielou's Equitability (J') [45]: $J' = H'/H'_{\max}$, whereby: $H'_{\max} = \ln(S)$: maximum diversity; S: number of species sampled.

Spearman's nonparametric correlation analysis was applied to determine the abiotic factor that was most related to phytoplankton and ecological indexes. For all tests, a significance lower than 5% ($p < 0.05$) was considered and calculations were performed using the software PAST 4.02 [46].

To evaluate the relationship between phytoplankton and abiotic factors, Redundancy Analysis - RDA was performed: considering the matrix of abiotic data and the matrix with the density of the most abundant species with the elimination of species with less than 5% of relative abundance, because species less abundant are considered undercounted [47]. The biological matrix was transformed by Hellinger [48]. The tests were performed using the CANOCO 4.5 for Windows program [49].

3. RESULTS AND DISCUSSION

3.1 Abiotic factors

Despite being relatively small, the reservoirs proved to be heterogeneous between the stations (PERMANOVA $F = 1.95$; $p = 0.03$) and the months of study (PERMANOVA $F = 11.37$; $p = 0.0001$) in relation to the limnological characteristics. March was different among the wettest months (January, February and April), with dissolved oxygen concentrations ($4.0 \pm 2.0 \text{ mg.L}^{-1}$), electrical conductivity ($54.0 \pm 11.3 \mu\text{S.cm}^{-1}$) and turbidity ($29.5 \pm 35.0 \text{ NTU}$) higher than the other rainy months. While June was different from October and presented lower turbidity ($12.8 \pm 8.1 \text{ NTU}$) while October ($23.3 \pm 14.5 \text{ NTU}$) presented higher turbidity (Table 1).

Table 1. Abiotic variables recorded for the public water supply system in Belém (Pará, Brazil).

	JAN	FEB	MAR	APR	JUN	JUL	OCT
	Min.-Max.	Min.-Max.	Min.-Max.	Min.-Max.	Min.-Max.	Min.-Max.	Min.-Max.
	(Ave.±SD)	(Ave.±SD)	(Ave.±SD)	(Ave.±SD)	(Ave.±SD)	(Ave.±SD)	(Ave.±SD)
Transparency (m)	0.4-1.4 (0.6 ± 0.6)	0.5-1.5 (1.0 ± 0.4)	0.3-1.7 (1.1 ± 0.7)	0.7-2.4 (1.7 ± 0.7)	1.5-2.2 (1.7 ± 0.5)	1.0-2.0 (1.5 ± 0.5)	0.7-1.8 (1.3 ± 0.5)
Temperature (C°)	28.6-29.7 (29.2 ± 0.6)	27.9-30.08 (29.2 ± 1.0)	27.6-28.1 (27.9 ± 0.2)	28.9-29.8 (29.3 ± 0.4)	29.7-30.8 (30.1 ± 0.5)	28.06-30.07 (29.5 ± 1.0)	28.9-30.4 (30.4 ± 1.1)
Dissolved Oxygen (mg.L⁻¹)	0.1-0.5 (0.3 ± 0.2)	0.0-0.9 (0.2 ± 0.5)	1.3-5.9 (4.0 ± 2.0)	0.8-9.4 (6.0 ± 3.6)	1.3-7.4 (4.0 ± 2.6)	6.1-9.09 (7.4 ± 1.4)	3.9-8.6 (6.4 ± 2.0)
Electric conductivity (µS.cm⁻¹)	36.0-52.0 (44.0 ± 7.7)	0.0-46.0 (20.0 ± 23.6)	37.9-63.5 (54.1 ± 11.3)	42.0-49.0 (44.3 ± 3.3)	56.4-74.4 (65.6 ± 7.5)	26.0-51.0 (37.5 ± 10.3)	56.0-70.0 (63.0 ± 5.7)
Salinity (mg.L⁻¹)	0.02-0.02 (0.02 ± 0.0)	0.0-0.02 (0.0075 ± 0.009)	0.01-0.02 (0.017 ± 0.005)	0.02-0.02 (0.02 ± 0.0)	0.03-0.04 (0.03 ± 0.005)	0.01-0.02 (0.015 ± 0.005)	0.03-0.04 (0.03 ± 0.005)
pH	5.6-6.4 (5.9 ± 0.3)	6.4-6.8 (6.6 ± 0.16)	6.1-6.5 (6.3 ± 0.14)	6.1-6.5 (6.3 ± 0.2)	6.5-7.03 (6.8 ± 0.2)	6.4-6.8 (6.6 ± 0.2)	6.4-6.8 (6.5 ± 0.19)
Turbidity (NTU)	7.0-31.0 (17.2 ± 10.9)	7.0-34.0 (16.0 ± 12.3)	9.0-82.0 (29.5 ± 35.0)	2.0-23.0 (9.2 ± 9.3)	6.0-24.0 (12.8 ± 8.1)	7.0-27.0 (14.0 ± 9.2)	8.3-42.0 (23.3 ± 14.5)
Precipitation (mm)	- (393.7 ± 92.4)	- (432.2 ± 107.08)	- (503.6 ± 129.5)	- (456.02 ± 86.2)	- (205.3 ± 71.2)	- (163.2 ± 69.2)	- (133.9 ± 59.8)
Wind speed (Km.h⁻¹)	- (4.3 ± 1.2)	- (4.08 ± 1.2)	- (3.9 ± 1.06)	- (3.9 ± 1.1)	- (6.6 ± 7.5)	- (5.5 ± 1.1)	- (6.7 ± 1.6)

Legend: Min.: Minimum; Max.: Maximum; Ave.: Average; SD: Standard deviation; JAN: January; FEB: February; MAR: March; APR: April; JUN: June; JUL: July; OCT: October.

On the other hand, AP1, AP2 and BL4 stations were different from each other, where AP2 presented greater turbidity (38.0 ± 21.0 NTU), since it is the catchment station of water from the Guamá River to the reservoirs and, therefore, with greater influence of the high turbidity of this river [5]. The AP1 showed higher values of transparency (1.7 ± 0.3 m) and electrical conductivity (56.4 ± 10 $\mu\text{S}\cdot\text{cm}^{-1}$) and lower turbidity (7.0 ± 2.3 NTU), located in the east direction, upstream of AP2 and, therefore, without the influence of the Guamá River. In this sense, it is essentially lacustrine, since the water velocity in this portion is practically null, as observed by Lima et al. (2015) [50].

While BL4, due to the characteristics of the reservoir, which has extensive mats of aquatic macrophytes, showed low concentrations of dissolved oxygen (2.0 ± 2.2 $\text{mg}\cdot\text{L}^{-1}$), this characteristic was also evidenced in other studies in the reservoir, which in addition to low dissolved oxygen concentrations, also showed higher turbidity values [9, 51]. Although the reservoirs are small, interconnected and suffer the same anthropic interference, they are heterogeneous in space and time, as previously observed by Vasconcelos and Souza (2011) [10] and Lima et al. (2015) [50].

Spearman's correlation evidenced seasonality acting on the dynamics of the reservoirs, where precipitation was negatively related to temperature ($r_s = -0.64$) and average wind speed ($r_s = -0.96$), in addition to rains, winds are positively related to the temperature ($r_s = 0.67$) and salinity ($r_s = 0.61$), since the highest temperatures and strongest winds occur in the less rainy season (Table 2). Turbidity and transparency are inversely proportional ($r_s = -0.73$) and are directly related to seasonality, as rainfall influences the suspension of solids in water [14]. While salinity is positively related to electrical conductivity ($r_s = 0.83$), due to the greater availability of ions in water (Table 2).

Table 2. Value of Spearman's significant correlation for the biotic and abiotic variables recorded for the public water supply system in Belém (Pará, Brazil).

VARIABLES	CORRELATION (r_s')
EC x Salinity	0.83*
Precipitation x Temperature	-0.64*
Precipitation x AWS	-0.96*
Salinity x AWS	0.61*
Temperature x AWS	0.67*
Transparency x Turbidity	-0.73*
Diversity (H') x Richness ($D\alpha$)	0.69*
Diversity (H') x Equitability (J')	0.96*
Dominancy (D) x Diversity (H')	-0.97*
Dominancy (D) x Richness($D\alpha$)	-0.62*
Dominancy (D) x Equitability (J')	-0.98*
Richness($D\alpha$) x Equitability (J')	0.61*
Richness($D\alpha$) x DO	0.57*
Richness($D\alpha$) x pH	0.6*

Legend: EC- Electric conductivity, AWS- average wind speed, DO- dissolved oxygen, *= $p < 0.05$.

3.2 Biotic factors

A total of 187 phytoplankton species were identified and distributed into 15 classes (Appendix 1 – available at: < [dx.doi.org/10.6084/m9.figshare.23557770](https://doi.org/10.6084/m9.figshare.23557770)>). The most representative classes were Chlorophyceae (41 spp.), Cyanophyceae (26 spp.), Euglenophyceae (25 spp.), Bacillariophyceae (21 spp.) and Coscinodiscophyceae (23 spp.), the other classes contributed in 27.3% of the composition. The composition of phytoplankton in reservoirs largely consists of

chlorophytes, cyanobacteria and diatoms [52, 53], therefore, similar to that recorded for the reservoirs of Belém, mainly diatoms and chlorophytes due to their wide distribution, since they are cosmopolitan [52].

The large amount of diatoms (Bacillariophyceae and Coscinodiscophyceae) in the reservoirs is due to the influence of the mouth of the Guamá River, since they are very abundant in this river [30]. Allied to this, we assume that the low depths of these reservoirs and the presence of macrophytes create environmental mosaics that favor the presence of epiphytic and benthic diatom species to the plankton.

There were no significant differences for density between months ($F= 0.52$, $p > 0.05$) and stations ($F= 2.7$, $p > 0.05$) for both reservoirs, in the Água Preta density range on average from $33.19 \text{ cel.mL}^{-1}$ in January to $90.06 \text{ cel.mL}^{-1}$ in October (Figure 2a) and between the stations it ranged from 16 cel.mL^{-1} at AP2 station to 32.4 cel.mL^{-1} at AP1 station (Figure 2c). For the Bolonha density range on average from $15.49 \text{ cel.mL}^{-1}$ in April to $39.62 \text{ cel.mL}^{-1}$ in July (Figure 2b).

Although this study did not observe a significant temporal variation of phytoplankton, other studies [30, 54, 55] verify the existence of a variation in phytoplankton density, with the highest values occurring in the less rainy season, due to the interaction of factors that influence the phytoplankton development, such as the entry of light into the water column, which is greater in the dry months [14].

Thus, it is relevant to mention that the density alone, for this study, was not a good parameter of the variations in the reservoirs. On the other hand, the phytoplankton classes showed a spatio-temporal variation, with the Zygnemaphyceae and Cryptophyceae classes (Figure 3a and 3c) being more representative in Água Preta reservoir in general and Raphidophyceae classe being representative in Bolonha reservoir (Figure 3b and 3c).

The Zygnemaphyceae class was more representative through the *Closterium* sp. 1, mainly in AP1 and AP3 stations, evidencing the similarity of these stations. This class is composed of organisms that inhabit environments ranging from oligotrophic to mesotrophic [56]. The classes Cryptophyceae (33%), mainly with the species *Rhodomonas minuta* (Skuja), and Euglenophyceae (34%), with the species *Trachelomonas volvocina* (Ehrenberg) Ehrenberg, dominated in July and October, respectively (Figure 3a). In this period, the classes in evidence are represented by the presence of two species that inhabit clear mesotrophic environments [15], mainly at AP1 station of the Água Preta reservoir, combined with the environmental characteristics of this portion of the reservoir, which is more lentic and transparent, these months represent the dry period where higher values of transparency and lower turbidity occur, factors that directly influence the entry of light into the water column and this condition is limiting to phytoplanktonic development [57].

The class Raphidophyceae was relevant throughout the months of study and mainly associated with the Bolonha reservoir (BL4) through the presence of the species of *Gonyostomum* sp., making up 75% of the relative density of this reservoir (Figure 3b). However, was also present during the month of April in the Água Preta reservoir at the AP2 station through the presence of the species *Vacuolaria* spp. (Figure 3a).

Such species are associated with eutrophic environments, with more acidic waters where there is the presence of aquatic macrophytes and greater turbidity, in this context, the Bolonha reservoir environment, which has extensive mats of aquatic macrophytes, favors the presence of individuals of the species *Gonyostomum* sp., on the other hand, AP2 station, which is the point of adduction of water from the Guamá River, has greater turbidity, favoring individuals of the species of the genus *Vacuolaria*.

Among the stations, there was a higher relative density of the classes Raphidophyceae, Treboxiophyceae and Euglenophyceae. In this aspect, the BL4 station was different from the other stations due to the dominance of Raphidophyceae with more than 80% of representation, mainly with the species *Gonyostomum* sp. (Figure 3c).

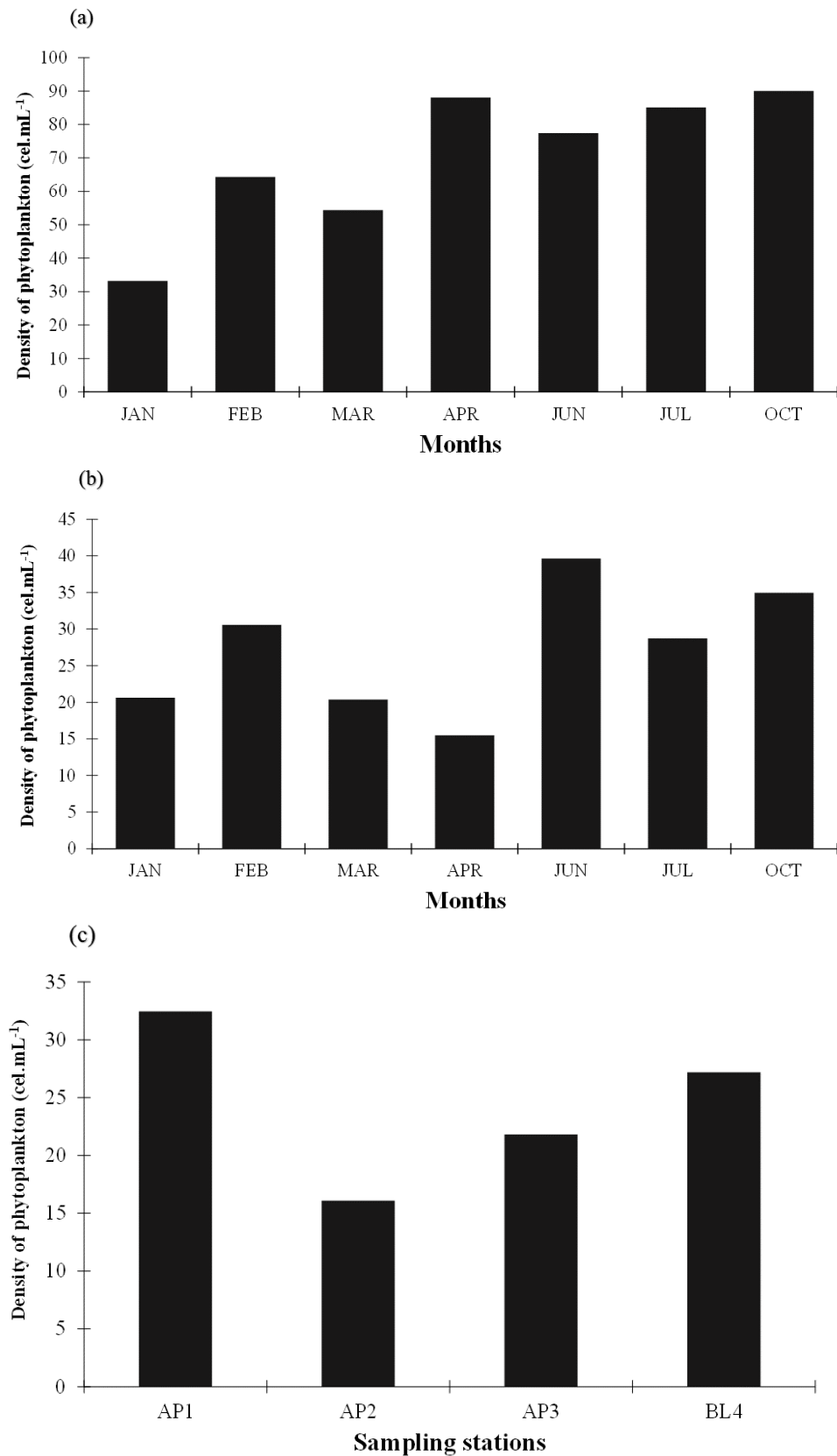


Figure 2. Spatio-temporal variation of density (cel.mL⁻¹) of phytoplankton recorded for the public supply system of Belém (Pará, Brazil) (a): Temporal variation of phytoplankton in Água Preta reservoir; (b): Temporal variation of phytoplankton in Bolonha reservoir; (c): Spatial variation of phytoplankton. Legend: JAN: January; FEB: February; MAR: March; APR: April; JUN: June; JUL: July; OCT: October; AP: Água Preta reservoir; BL: Bolonha reservoir.

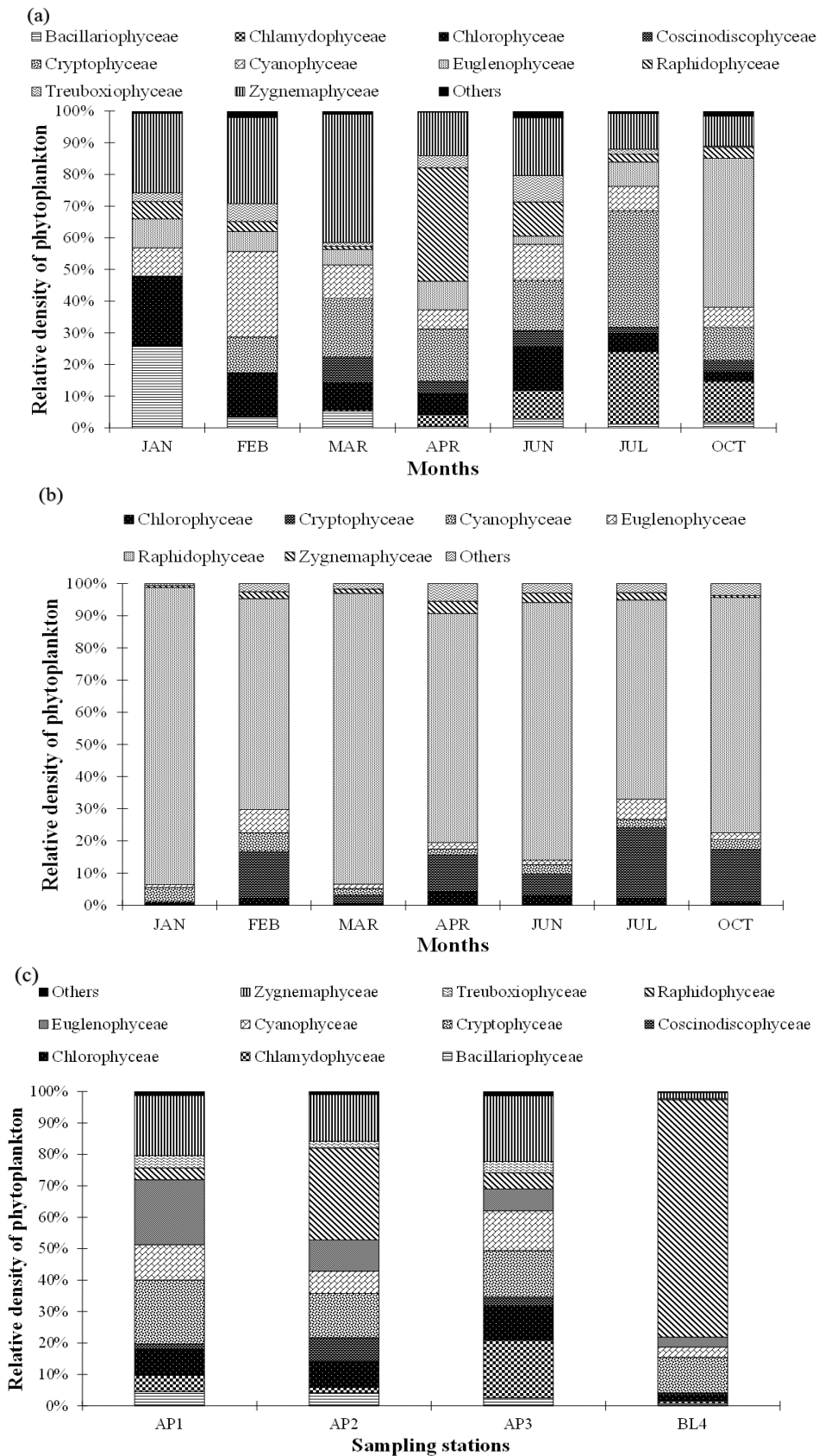


Figure 3. Relative density of phytoplankton recorded for public supply reservoirs in Belém (Pará, Brazil) (a): Temporal variation of the relative density of phytoplankton in Água Preta reservoir; (b): Temporal variation of the relative density of phytoplankton in Bolonha reservoir; (c): Spatial variation of the relative density of phytoplankton. Legend: JAN: January; FEB: February; MAR: March; APR: April; JUN: June; JUL: July; OCT: October; AP: Água Preta reservoir; BL: Bolonha reservoir.

The RDA established seasonal and spatial variation of samples in the reservoir, where environmental variables explained 34.5% of the species distribution (Figure 4), the first two axes explained 26% of this distribution. Axis 1 (18%) showed the samples as a function of the variables precipitation, transparency and dissolved oxygen (DO), where precipitation grouped samples from the rainy season associated with two species: *Crucigenia apiculata* (Lemmermann) Schmidle and *Gonyostomum* sp. Contrary to transparency and DO, which grouped samples from the less rainy months associated with the species *Vacuolaria* sp. 1, *Vacuolaria* sp. 2, *Mucidosphaerium tetrachotomum*, *Cosmarium sphagnicolum* West & G.S.West and *Closterium acutum* Brébisson. Axis 2 (8%) established a spatial pattern grouping all samples from AP2 station with turbidity, where this factor behaves similar to the result shown in the multivariate analysis, evidencing the spatial heterogeneity of the reservoir influenced by the Guamá River.

Climatic factors are important for phytoplankton development [58], where periods of high rainfall can provide nutrient inputs to aquatic ecosystems [59], as observed by Silva et al. (2019) [12] in the rainy season, the highest values of nitrogen and phosphorus occur in the reservoirs, providing the development of phytoplankton, such as the species *Gonyostomum* sp., which benefits from this condition [59]. Transparency limits the development of phytoplankton, as it is associated with the entry of light into the water column, mainly grouping species of freshwater chlorophytes, generally related to naturally clear environments [15], in this way, the high productivity of these organisms can act in the increase in dissolved oxygen concentration, as this variable is directly dependent on the photosynthetic process [9].

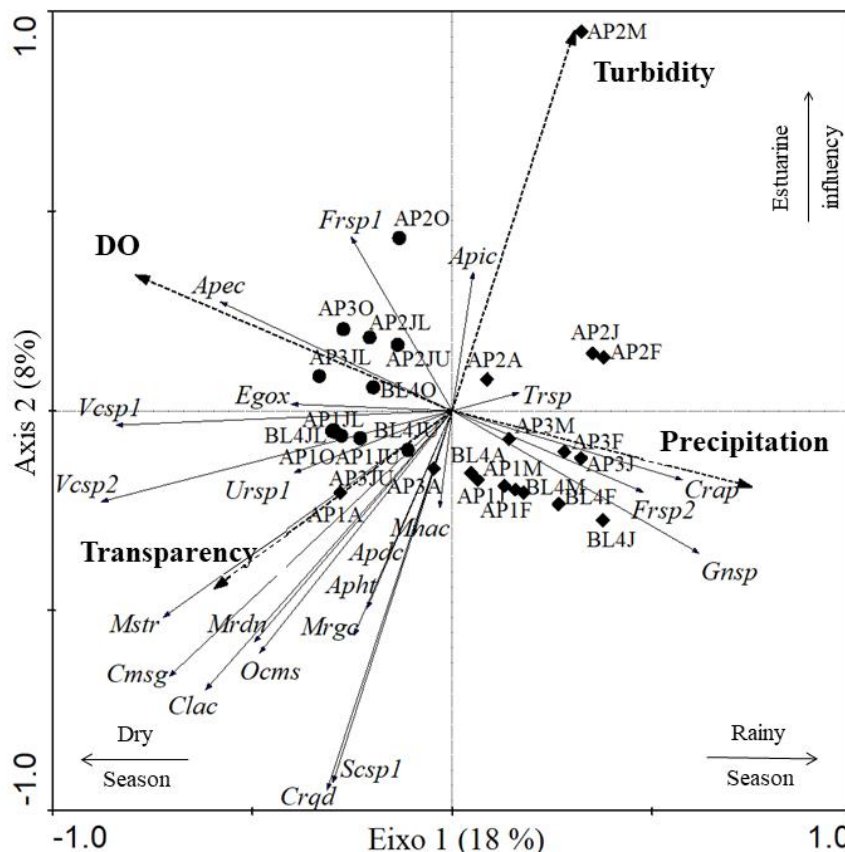


Figure 4: Triplet of the Redundancy Analysis - RDA for the phytoplankton of the Água Preta and Bolonha reservoirs. Legend: Apdc: *Aphanocapsa delicatissima*; Apec: *A. elachista*; Apic: *A. incerta*; Apht: *A. holsatica*; Crap: *Crucigenia apiculata*; Crqd: *C. quadrata*; Clac: *Closterium acutum*; Cmsg: *Cosmarium sphagnicolum*; Egox: *Euglena oxyuris*; Frsp1: *Frustulia* sp. 1; Frsp2: *Frustulia* sp. 2; Gnspl: *Gonyostomum* sp.; Mnac: *Monoraphidium arcuatum*; Mstr: *Mucidosphaerium tetrachotomum*; Mrdn: *Merismopedia danubiana*; Mrgc: *M. glauca*; Ocms: *Oocystis marsonii*; Scsp1: *Scenedesmus* sp. 1; Ursp1: *Urosolenia* sp. 1; Vcsp1: *Vacuolaria* sp. 1; Vcsp2: *Vacuolaria* sp. 2. Rhombus: rainy season; Circle: dry season. Sampling stations: AP1, AP2, AP3, BL4; Months: J- January, F- February, M- March, A- April, JU- June, JL- July, O- October.

3.1 Ecological indexes analysis

For the Água Preta reservoir richness ($D\alpha$) varied significantly between months ($F= 3.8$; $p < 0.05$), being lowest in January (8.7 ± 1.1) and highest in June (14.13 ± 1.5) (Table 3). Although the other indexes did not show significant temporal variations, oscillations were observed in the months of January and June. January showed lower diversity (H') (2.14 ± 0.58 nats.ind⁻¹), higher dominance (D) (0.25 ± 0.19) and lower evenness (J') (0.57 ± 0.14). Contrary to June, which presented greater diversity (3.07 ± 0.13 nats.ind⁻¹), less dominance (0.07 ± 0.01) and greater evenness (0.7 ± 0.02). While in Bolonha reservoir there were no significant differences between the indexes on the months ($H= 0.58$; $p < 0.05$), but January followed the pattern also observed in the Água Preta reservoir, presenting the lowest richness (4.52), diversity (0.46 nats.ind⁻¹) and equitability (0.14) values and the highest species dominance (0.83) values, however June only presented highest richness (10.37), while the highest diversity (1.88 nats.ind⁻¹), equitability (0.48) and lower dominance (0.35) occur on April (Table 4).

Ecological indexes have been widely used through the study of alpha diversity to show the spatio-temporal variations of the phytoplankton community [25, 60], their use plays an important role in understanding biodiversity and in elucidating measures for monitoring aquatic ecosystems, especially of reservoirs, also contributing to the conservation and maintenance of these environments [61]. Similar to this study, Badsı et al. (2012) [62] found seasonal variation in phytoplankton diversity, with the lowest values occurring in the rainy season. For the present study, this is due to the relationship between the indexes and the density of organisms, since January and April are the wettest quarter in the eastern Amazon region, and June is the transition month, where it rains 48% less than the others months. The BL4 station showed significantly ($p < 0.05$) lower richness (8.3 ± 1.9), lower diversity (1.2 ± 0.5 nats.ind⁻¹), higher dominance (0.55 ± 0.19) and lower evenness (0.32 ± 0.12), in relation to the other stations (Table 4).

Table 3. Temporal variation of ecological indexes recorded for Água Preta reservoir (Pará, Brazil).

	JAN		FEB		MAR		APR		JUN		JUL		OCT	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
	(Ave. ± SD)		(Ave. ± SD)		(Ave. ± SD)		(Ave. ± SD)		(Ave. ± SD)		(Ave. ± SD)		(Ave. ± SD)	
Dα	7.52	9.67	8.57	10.18	8.81	12.05	9.53	12.79	13.18	15.86	12.11	12.69	10.37	12.3
	8.7 ± 1.1		9.58 ± 0.87		10.67 ± 1.67		10.71 ± 1.8		14.13 ± 1.5		12.37 ± 0.29		11.1 ± 1.04	
H' (nats.ind ⁻¹)	1.46	2.48	2.10	2.68	1.92	2.67	1.72	3.09	2.91	3.17	2.23	2.47	1.76	2.54
	2.14 ± 0.58		2.47 ± 0.32		2.34 ± 0.38		2.44 ± 0.68		3.07 ± 0.13		2.32 ± 0.12		2.27 ± 0.43	
D	0.14	0.48	0.11	0.19	0.14	0.27	0.07	0.39	0.07	0.1	0.18	0.28	0.13	0.4
	0.25 ± 0.19		0.14 ± 0.04		0.18 ± 0.07		0.2 ± 0.17		0.08 ± 0.01		0.24 ± 0.05		0.22 ± 0.15	
J'	0.4	0.66	0.54	0.68	0.5	0.64	0.41	0.74	0.67	0.72	0.52	0.59	0.4	0.68
	0.57 ± 0.14		0.62 ± 0.07		0.6 ± 0.08		0.6 ± 0.16		0.7 ± 0.02		0.54 ± 0.03		0.57 ± 0.14	

Legend: D α : Margalef's richness; H': Shannon-Weaver diversity; D: Simpson dominance; J': Pielou's equitability; Min.: minimum; Max: maximum; Ave.: average; SD: standard deviation; JAN: January; FEB: February; MAR: March; APR: April; JUN: June; JUL: July; OCT: October.

Table 4. Temporal variation of ecological indexes recorded for Bolonha reservoir (Pará, Brazil).

	JAN	FEB	MAR	APR	JUN	JUL	OCT
Dα	4.52	8.94	7.57	9.48	10.37	9.97	7.9
H' (nats.ind ⁻¹)	0.46	1.5	0.67	1.88	1.42	1.69	1.22
D	0.83	0.42	0.78	0.35	0.54	0.37	0.54
J'	0.14	0.38	0.18	0.48	0.34	0.41	0.31

Legend: D α : Margalef's richness; H': Shannon-Weaver diversity; D: Simpson dominance; J': Pielou's equitability; JAN: January; FEB: February; MAR: March; APR: April; JUN: June; JUL: July; OCT: October.

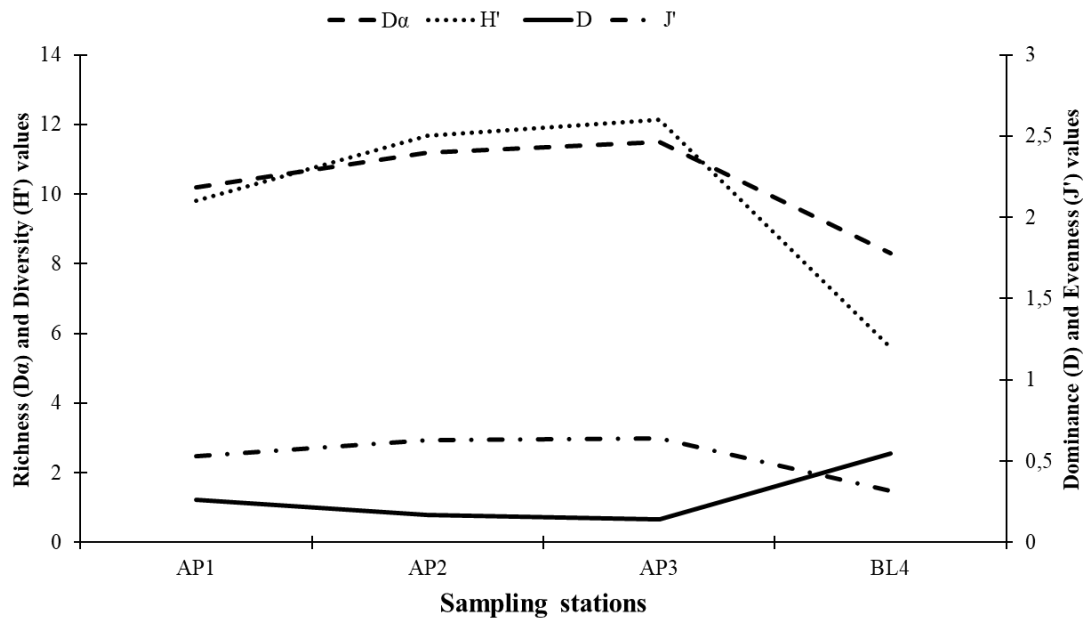


Figure 5. Spatial variation of ecological indexes recorded for the Água Preta and Bolonha reservoirs (Pará, Brazil). Legend: $D\alpha$: Margalef's richness; H' : Shannon-Weaver diversity; D : Simpson dominance; J : Pielou's equitability; AP1, AP2, AP3: Água Preta reservoir; BL4: Bolonha reservoir.

According to Flores-Lopes et al. (2010) [63] the integrity of an environment is related to high to regular values of species richness, diversity and equitability in relation to dominance values, as it is considered a balanced environment when the species composition is high and well distributed between groups. In this sense, the use of ecological indexes showed better responses of phytoplankton dynamics in the studied reservoirs when compared to the analysis of density alone, which did not show significant variations in the phytoplankton community, since the analysis through raw data is not able to demonstrate more complex variations of the community.

The negative correlation between the dominance index (D) with the indexes of richness ($D\alpha$), diversity (H') and evenness (J) ($r_s = -0.97$; $r_s = -0.62$; $r_s = -0.98$, respectively) and the positive correlation of diversity, richness and evenness ($r_s = 0.69$; $r_s = 0.96$; $r_s = 0.61$, respectively) (Table 2), evidenced the analogy between these indexes. Higher dominance values indicate lower species diversity in the environment, because when there is a prevalence of one species over others, whether this species benefits from an environmental or physicochemical variable, interspecific relationships tend to compete, and the environment ends up select the species that best adapts to the existing conditions, causing a possible decrease in the occurrence of other species.

Evenness (J) was also negatively related to density ($r_s = -0.39$), since this index indicates the degree of community organization [64] and, therefore, higher density values of unique species imply the homogeneous distribution of organisms in the environment. Richness ($D\alpha$) is positively correlated with the variables pH ($r_s = 0.6$) and DO ($r_s = 0.57$) (Table 2). The richness index ($D\alpha$) was the one that best demonstrated the phytoplankton variation in the reservoirs. According to Corte et al. (2013) [65] the Margalef Richness Index ($D\alpha$), measures alpha diversity, which takes into account the total number of individuals and specific richness, through the estimate of biodiversity, based on the numerical distribution of individuals of each species, where the higher its value, the greater the biodiversity of the area, in addition, its application is simple and seeks to compensate for sampling effects, dividing the richness and number of species found by the total number of individuals in the sample.

Other studies also show the Margalef richness index as more sensitive in the expression of diversity [65, 66], however, its weak point is to consider that all species are uniformly distributed [67], which is not true for many environments, so it is important that other analyzes accompany

the studies of the communities, such as the application of the diversity (H'), dominance (D) and equitability (J') indexes, which allow inferring the level of organization of the populations, through the analysis of patterns of distribution of individuals in the environment. In addition, the richness index ($D\alpha$) does not give weight to the species, where frequent and rare species have the same value, while the diversity and dominance indexes employ weight for the occurrence of these species, for example, the diversity index (H') deposits intermediate weight to rare species, whereas the dominance index (D) employs minimum weight [68].

Currently, this set of indexes has been applied in studies on environmental monitoring [23, 24, 60] in this way, this combination was efficient in the description of the dynamics of the phytoplankton community in the reservoirs of Belém, where the Água Preta reservoir was considered diverse and the species are well distributed in the environment. On the other hand, the vulnerability of this water system is evident, since the Bolonha reservoir presented low diversity and equitability, with dominance of species over others.

According to Zaghoul et al. (2020) [69], a low amount of species present in the environment demonstrates a high degree of stress to which the system is subjected. Due to the disorderly advance of urbanization around the reservoirs and allied to the low coverage of basic sanitation present in the region [8, 10], the supply reservoirs of Belém are in the process of eutrophication [5, 12, 70], which has contributed to the gradual decrease in the diversity of the aquatic environment [71], mainly in the Bolonha reservoir, which is dominated by aquatic macrophytes, and their proliferation is mainly associated with the increase of nutrients in the water [72], and this increase is possibly associated with the contribution of sewage discharges without prior treatment.

On the other hand, the presence of macrophytes is a factor that directly contributes to the decrease in the diversity of the phytoplankton community of this reservoir, as they are able to compete with phytoplankton for resources, in addition to providing microhabitats for zooplanktonic species [73], thus, also plays a role in the selection of organisms, such as the phytoplanktonic species *Gonyostomum* sp., tolerant to water acidity [74] caused by the release of fulvic and humic acids during macrophyte decomposition [75], and thus being responsible for the high values of dominance (D) in the Bolonha reservoir (BL4).

In addition, although the present study has some limitations, mainly regarding nutrient data, especially nitrogen and phosphorus, the presence of certain species in high density in the environments allows us to assess the sanitary conditions of the reservoirs. According to Menezes and Bicudo (2010) [59] the species of the genus *Gonyostomum* have been increasingly frequent in aquatic environments and not only associated with their acidity, but also with eutrophication, however they do not present greater risks to water treatment. In this scenario, another limitation of this work is related to the few months of study, this is due to the proliferation of macrophytes that depending on climatic conditions prevent access to the reservoirs, the large volume of macrophytes also indicates eutrophication, and the reservoirs of Belém are vulnerable to discharges of illegal domestic sewage from their surroundings, but the presence of macrophytes also contributes to a lower occurrence of unwanted organisms, such as cyanobacteria [73].

Thus, with the use of the indexes it is possible to assess that the reservoirs are in a state of vulnerability, due to the process of eutrophication that they have been suffering as a reflection of the anthropic impacts of their surroundings. Besides this, the low diversity, richness and equitability values together with high dominance values for Bolonha reservoir indicate that the quality of the environment is compromised, mainly by the great presence of macrophytes and planktonic organisms typical of eutrophic environments.

4. CONCLUSION

The spatial-temporal variation of phytoplankton was well evidenced by the phytoplankton classes and the ecological indexes, which were significant in representing the effects of environmental conditions on the reservoirs, among them the seasonality, the influence of the Guamá river mouth and the sanitary conditions of the Bolonha reservoir, which has been suffering with the impacts of urbanization of its surroundings. Thus, the monitoring of this environment is

essential, because the conditions in which it is submitted can bring future risks of lack of supply. Furthermore, this work provides subsidies for future studies of water quality monitoring in the Amazon region using phytoplankton ecological indexes.

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