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Biodiversity and interannual variation of cyanobacteria density in an estuary of the brazilian Amazon

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Abstract: The influence of environmental variables on planktonic biodiversity is widely known. However, the absence of information about the cyanobacterial community in tropical estuarine regions motivated this work, whose objective was to investigate the spatio-temporal variation of cyanobacterial density related to physicochemical factors in a Brazilian Amazonian estuary. For the qualitative and quantitative study of cyanobacteria and physicochemical variables, samples were collected in April/July/2009 and April/August/2010. We identified 31 species of the orders Chroococcales, Oscillatoriales and Nostocales. Species of the genera *Aphanocapsa*, *Dolichospermum*, *Komvophoron*, *Microcystis*, *Pseudanabaena* and *Merismopedia* were frequent and abundant throughout the study period. Some of the found genera have already been described as potential toxin producers. The dynamics of cyanobacteria were temporal, the highest densities occurred in 2010 (average= 1080.86 ± 702.86 cells.mL^{-1}) mainly influenced by the high values of pH, temperature, electrical conductivity, total dissolved solids, ammonium nitrogen which led cyanobacteria to present different responses in terms of richness, density and diversity between the years.

Key words: Diversity, Phytoplankton, Chroococcales, Amazon.

INTRODUCTION

The occurrence of toxic cyanobacterial blooms in aquatic ecosystems are usually consequences of eutrophication, which result from complex interactions between physicochemical and biological factors, and have also been attributed to climate change (Pearl & Huisman 2008, Reichwaldt & Ghadouani 2012). Excessive increase in cyanobacterial cell numbers causes a number of ecological and public health consequences. Further, toxin release can lead to reduced species diversity, altered water quality, and the production of inadequate water for consumption and recreation (McQueen & Lean 1987, Falconer 2005, Visser et al. 2005, Lee 2008).

Cyanobacterial blooms also occur in marine and brackish environments, with occurrence and dominance reported via the phenomenon of potentially toxic species blooming in estuarine environments (Rocha et al. 2002, Robson & Hamilton 2003, Taş et al. 2006, Lehman et al. 2017). In Brazil, one of the most commonly recurring blooms within an estuarine environment occurs in Patos lagoon (Yunes 2009).

The Pará River estuary, located on the northern coast of Brazil (Eastern Amazon), it is a complex aquatic environment with high biological productivity (Nittrouer et al. 1991, Santos et al. 2008) and is one of the largest estuaries of Brazil. Water within the estuary has great potential to be used in the development of port activities, including industrial fishing (R.P. Rosário, unpublished data). Since it is bordered by several cities, whose surrounding populations are mainly riverside communities, a large number of people depend on its waters for survival and use it for consumption, recreation and fishing.

On the bank (01° 32'37.2" S, 48° 44' 47.4" W) of the Pará River, downstream from an industrial complex implemented in the 1980s and 1990s, is the largest port terminal in the Pará State, Vila do Conde Port, (Lima et al. 2011). In the region, numerous environmental accidents have been reported involving both port and industrial activity. In 2003 and 2007 there was contamination by caustic soda in the Pará River and also in 2007 there was a leak of more than 200 thousand m^3 of kaolin that covered 19 km in two rivers in the region, reaching the Pará River (E. Carmo et al., unpublished data). In 2015, a wreck involving a cargo ship carrying 5000 oxen resulted the release of both biological material (carcasses, bones and fluids), and fuel (approximately 700,000 L of diesel oil) into the Pará River. The spill caused fluctuations in the structure of the zooplankton community, species composition, density and abundance and facilitated the emergence of opportunistic species (Pinheiro et al. 2019).

Studies in this region (Amazon) on cyanobacteria and physicochemical factors influencing the river are scarce. And, as in other regions, cyanobacterial studies have focused largely on lentic environments. According to Bukaveckas et al. (2018), harmful algal blooms in lotic environments receive less attention possibly because these blooms are less apparent and may be masked due to the mixing of waters and transport along rivers.

The present article is pioneering because it considers the cyanobacterial community in terms of factors including species richness, frequency and density that are associated with environmental variables in an Amazonian estuary. We answer the following questions: 1) Do spatial and temporal variations in abiotic and tidal factors affect the structure of cyanobacterial communities? 2) What are the main factors that influence the richness, density and diversity of this group? 3) In terms of density, do cyanobacteria from the Pará River estuary (Amazon, Brazil) pose potential environmental and public health risks?

The objective of this study was to investigate relationship between physicochemical factors and spatiotemporal variation in the density of cyanobacterial species in the Pará River estuary (Amazon, Brazil).

MATERIALS AND METHODS

Study area

The study was conducted in the Pará River estuary located in northern Brazil (Eastern Amazon, Pará State), in the region where the Vila do Conde Port has been built. It is part of the Vila do Conde Industrial Port Complex, Barcarena and Abaetetuba (1°41'25.03"S, 48°53'15.47" W; 1°34'59.07" S, 48°47'29.14" W; 1°30'46.48" S, 48°43'46.04" W, respectively).

The Pará River, the second largest river in the Amazon drainage system, is over 300 km long, 20 km wide and has an estimated flow rate of 10^4 m^3/s. The river receives input from the Tocantins, Guamá and Acará-Moju rivers, communicates with the Amazon River through the Furos de Breves and is influenced by seawater (Atlantic Ocean) via rainfall variation and tidal factors (Gregório & Mendes 2008, Santos et al. 2008, Rosário et al. 2016). Tidal propagation reaches up to several km inland, ranging from 5 m at the mouth of the estuary to 3 m about 140 km upstream of the estuary (Cavalcante et al. 2010). The climate of the region is hot and humid Am

(Koppen classification), with an average annual temperature of 26°C, relative air humidity above 80% and average annual rainfall of 2,800 mm. The rainiest months are February, March, and April, and months receiving the least rain include August, September, and October (Moraes et al. 2005).

Sample collection

Samples were collected during April and July of 2009 and April and August of 2010 during flood and ebb tides from three sampling stations distributed along the port complex, which is located upstream (RPA 1), in front of (RPA 2) and downstream (RPA 3) from the port of Vila do Conde (Figure 1).

Precipitation and wind velocity data (1979-2010) from the area studied were obtained from the Instituto Nacional de Meteorologia of Brazil (INMET 2018). Characteristics such as temperature, pH, electrical conductivity (EC), total dissolved solids (TDS), salinity and dissolved oxygen (DO) were obtained *in situ* using a portable multiparameter meter (HI 9828 HANNA®) and the water transparency was estimated using a Secchi disk. Water samples were collected to determine turbidity, total suspended solids (TSS), chemical oxygen demand (COD), biochemical oxygen demand (BOD), and nitrite (NO_2^-), nitrate (NO_3^-), ammonium nitrogen (NH_4^+) and phosphate (PO_4^{-3}) content. To determine chlorophyll *a* concentration, samples were collected using 300 mL plastic containers. For the qualitative study of cyanobacteria, samples were collected using a plankton net (20 µm) and were preserved in Transeau's solution (Bicudo & Menezes 2018). Samples used for the quantitative study of algae

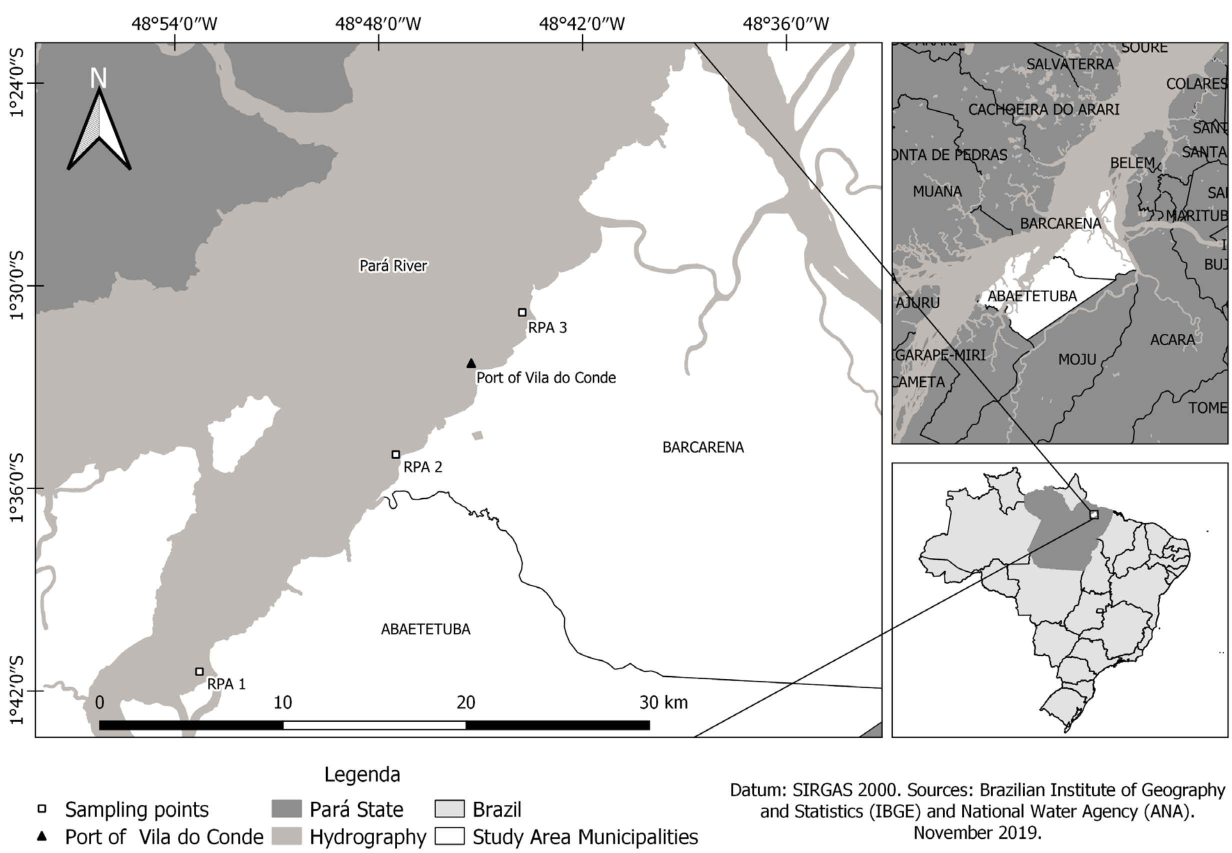

Figure 1. Location and sampling stations in the Pará River estuary (Amazon, Brazil). RPA: Pará River.

were collected using 250 mL plastic containers and preserved in Lugol's solution.

Sample analysis

Turbidity, TSS, and COD were determined by UV-VIS spectrophotometry (HACH DR 2400). BOD was determined according to procedures outlined in APHA (2005); and NO_2-, NO_3^-, NH_4^+ and PO_4^{3-} ion levels were determined using an ion chromatography system (ICS DUAL 2000 DIONEX, USA). Chlorophyll *a* was analyzed by the spectrophotometric method outlined by Parsons & Strickland (1963) using a Hanna D2000 Spectrophotometer.

Qualitative cyanobacterial samples were visualized using temporary slides under a biocular microscope (Axiostarplus, Carl Zeiss, Germany) with camera-attached eyepieces for measuring samples (Axiocam MRc, Carl Zeiss). Identification and naming of the species identified were performed according to Komárek & Anagnostidis (1999, 2005), and Komárek & Zapomelová (2007). To determine sample density, 10 mL of each sample was analyzed according procedures outlined in APHA (2005) using an invertoscope (Axiovert 40c-Carl Zeiss) and results were given in cells.mL^{-1}. The frequency of occurrence and relative abundance of species were estimated according to Matteucci & Colma (1982) and Lobo & Leighton (1986), respectively. Shannon diversity (1948), Margalef richness (1967) and Pielou evenness (1977) indices were also calculated.

Data analysis

One-way analysis of variance (one-way ANOVA) was used to compare normal and homoscedastic variance within physicochemical factors such as density, diversity, richness and evenness of species abundance at different sampling stations, tidal cycle, study months, seasonal periods and years. For nonparametric data, the Kruskal-Wallis test was used. For all analyses, a significance level of 0.05 was adopted. All analyses were performed using the STATISTICA 8.0 program.

A principal component analysis (PCA) was performed to identify temporal and spatial patterns and a canonical redundancy analysis (RDA) was performed to explain the pattern of variation of cyanobacterial species as a function of the spatiotemporal variation of environmental variables. Densities were transformed via square root and Hellinger transformation (Legendre & Gallagher 2001). RDA data were subjected to a 10% fit, highlighting the species with the best significance level and improving visualization of ordering. For these analyses we used CANOCO 4.5 software (Ter Braak & Šmilauer 2002).

RESULTS

Variation of environmental factors

The average precipitation during the months of April, July 2009 and April, August 2010 were 469.9 mm, 193.1 mm, 450 mm and 188.1 mm, respectively. The total precipitation in 2009 (3463.6 mm) was higher than the average value determined for the last 30 years (3065.3 mm). Winds blew most intensely during July of 2009, at an average of 4 km/h, while other months studied had average windspeeds of 3 km/h. The windspeed has been below the average of the last 30 years (6 km/h).

April of 2009 waters with the highest BOD value (12.25 ± 2.50 mg.L^{-1}) and lowest turbidity (8.83 ± 2.04), and NH_4^+ (0.03 ± 0.02 mg.L^{-1}) and PO_4^{-3} (0.03 ± 0.03 mg.L^{-1}) concentrations. The month of July of the same year was characterized by waters with a higher concentration of NO_2^- (0.03 ± 0.01 mg.L^{-1}L), lower temperature (29.26 ± 0.29° C), more acidic pH (5.82 ± 0.19), and lower salinity values (0.01 ± 0.004), electrical conductivity (EC) (33.67 ± 1.86 µS.cm^{-1}), TDS (17.00 ± 0.89 mg.L^{-1})

NO_3^- (0.08 ± 0.02 mg.L^{-1}) and PO_4^{-3} (0.03 ± 0.03 mg.L^{-1}) (Table SI - Supplementary Material).

April of 2010 had significantly higher levels of salinity (0.02 ± 0.01) and turbidity (21.00 ± 6.45). The month also had greater concentrations of NO_3^- (0.72 ± 0.07 mg.L^{-1}), NH_4^+ (0.60 ± 0.04 mg.L^{-1}) and PO_4^{-3} (0.19 ± 0.05 mg.L^{-1}) and lower DO (6.02 ± 0.69 mg.L^{-1}) and BOD (3.83 ± 3.25 mg.L^{-1}) values. August was characterized by increased temperatures (30.74 ± 0.32°C), neutral pH (7.62 ± 0.30); and increased EC (50.50 ± 3.39 µS.cm^{-1}), TDS (25.17 ± 1.60 mg.L^{-1}) and DO (9.13 ± 0.84 mg.L^{-1}). Throughout the month, NO_2^- values were below the detection limit of the equipment used (Table SI).

Between the years, the temperature (30.57 ± 0.38 °C), pH (7.57 ± 0.26), EC (49.50 ± 10.77 µS.cm^{-1}), TDS (24.75 ± 5.33 mg.L^{-1}), salinity (0.02 ± 0.01) and concentrations of NO_3^- (0.68 ± 0.08 mg.L^{-1}), NH_4^+ (0.55 ± 0.06 mg.L^{-1}) and PO_4^{-3} (0.18 ± 0.05 mg.L^{-1}) were higher in 2010 than 2009. However, BOD (10.29 ± 2.70 mg.L^{-1}) and NO_2^- (0.03 ± 0.02 mg.L^{-1}) levels were higher in 2009 than 2010.

Seasonally, the highest DO values (7.86 ± 1.55 mg.L^{-1}) and water transparency (61.7 ± 9.37 cm) were observed in dry periods. Between seasons, the highest COD was observed at RPA 3 (19.03 ± 4.44 mg.L^{-1}) and the lowest was observed at RPA 1 (13.46 ± 5.64 mg.L^{-1}). Chlorophyll produced an opposite trend to density in which the values were observed at RPA 1 (11.33 ± 6.54 µg.L^{-1}) and the lowest values were observed at RPA3 (4.03 ± 1.44 µg.L^{-1}).

The two axes of principal component analysis explained 61.9% of total variance observed between environmental factors in the Pará River estuary and established two distribution patterns for the variables, which confirmed results obtained via univariate analysis (Figure 2). PC1 (39.1%) separated the April and July 2009

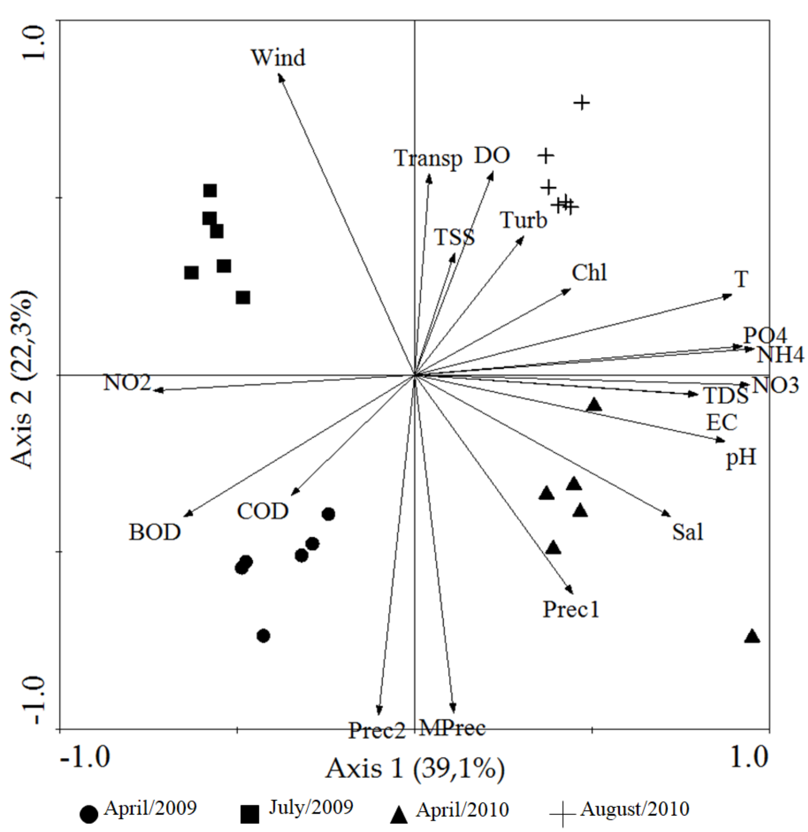

Figure 2. Principal component analysis for the environmental variables of the Pará River estuary (Amazon, Brazil). Mprec- Monthly precipitation; Prec1- precipitation one week prior to sample collection; Prec2- precipitation during sample collection; Wind; Transp- Transparency; T- Temperature; pH; EC- electrical conductivity; TDS- Total dissolved solids; TSS- Total suspended solids; Turb- Turbidity; Sal- Salinity; DO- dissolved oxygen; COD- Chemical oxygen demand; BOD- Biochemical oxygen demand; NO3- Nitrate; NO2- Nitrite; NH4- Ammonium nitrogen; PO4- Phosphate; Chl- Chlorophyll *a*

stations from the April and August 2010 stations, and established a temporal pattern between the years. PC1 was comprised of NH_4^+ (0.96), NO_3^- (0.94) and PO_4^{-3} (0.92), temperature (0.89), pH (0.87), TDS (0.80), EC (0.80), salinity (0.72), NO_2^- (-0.74) and BOD (-0.65).

PC2 (22.3%) established a seasonal pattern, separating the rainy season (April 2009, 2010) from the dry season (July 2009 and August 2010). PC2 was comprised of climatic variables including precipitation during sample collection (0.96), monthly precipitation (0.95), precipitation one week prior to sample collection (0.62), wind (-0.85), transparency (-0.57) and DO (-0.57).

Cyanobacterial community structure

A total of 31 cyanobacterial species belonging to eight families were identified. The most highly represented belonged to the Merismopediaceae (22.6%), Pseudanabaenaceae (19.4%) and Microcystaceae (16.1%). The genera *Aphanocapsa* Nägeli and *Microcystis* Kützing ex Lemmermann had the largest number of species identified (5). The largest number of species (27 spp.) were identified in April of 2010, and the fewest were identified in April of 2009 (17 spp.) (Table SII). Non-filamentous colonial species (Chroococcales) were predominant in different months and years studied.

Aphanocapsa elachista, *Dolichospermum* sp.1, *Komvophoron* sp.2, *Microcystis aeruginosa*, *Microcystis protocystis*, *Microcystis wesenbergii*, *Pseudanabaena mucicola*, *Pseudanabaena galeata* and *Merismopedia tenuissima* were identified very frequently. In fact, *Merismopedia tenuissima* was present in 100% of the samples obtained (Table SII). Regarding relative abundance, no particular species dominated. *Microcystis aeruginosa*, *Microcystis protocystis*, *Aphanocapsa elachista*, *Aphanocapsa holsatica*, *Aphanocapsa incerta*, *Merismopedia tenuissima* and *Pseudanabaena* sp. were abundant throughout 2009 and 2010. *Microcystis wesenbergii* was only identified in 2009 and *Aphanocapsa delicatissima* was only found in 2010 (Table SII).

In the Pará River estuary, cyanobacterial density showed temporal variation. The minimum density observed was 136.26 cells.mL^{-1}, which was recorded in April 2009 (RPA 1), and the maximum density observed was 4303.28 cells.mL^{-1}, which was recorded in August 2010 (RPA 2) (Figure 3). Cell density within the month of August was significantly higher ($H = 14.58$; $p<0.05$) (average = 1508.33 ± 724.29 cells.mL^{-1}) than April of 2009 (average = 203 ± 116.87 cells.mL^{-1}), but did not significantly differ from other months. Cell density within 2010 was significantly ($H= 7.05$; $p< 0.05$) higher (average = 1080.86 ± 702.86 cells.mL^{-1}) than 2009 (average of 436.36 ± 279.61 cells.mL^{-1}). There was also seasonal variation observed ($H= 7.36$; $p<0.05$) in which the highest cell densities were recorded during the dry season (average = 1049.91 ± 654.36 cells.mL^{-1}) and the lowest cell densities were recorded in the rainy season (average = 428.20 ± 362.23 cells.mL^{-1}).

Among the classes that contributed more highly to total density were Merismopediaceae and Microcystaceae (Figure 2, Table SII). Merismopediaceae were responsible for 41.1% of cyanobacterial density in April of 2009, and the contribution of the class increased in other months to over 60%. Microcystaceae were responsible for more than 50% of total density in April 2009 and the contribution of the class was reduced over the remaining period assessed.

Between examined months, significant differences in species diversity and richness were observed ($F = 4.50$, $p < 0.05$; $F = 6.43$, $p < 0.05$). When years were considered values assessing species diversity were also significantly different ($F = 12.27$, $p < 0.05$; $F = 11.05$, $p < 0.05$). Diversity values (H') measured were highest in August of 2010 (2.66 ± 0.26 bits/cell) and were also higher

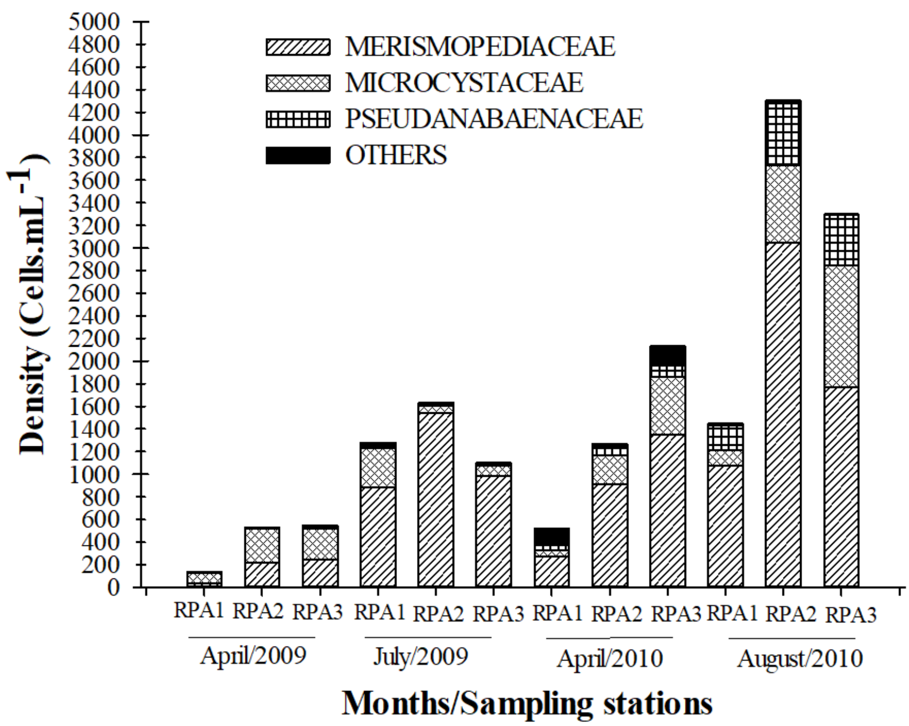

Figure 3. Spatio-temporal variation in the density of cyanobacteria (family) present in the Pará River estuary (Amazon, Brazil).

in 2010 than 2009 generally (2.53 ± 0.59 bits/cell). The richness of cyanobacteria species (Dm) was increased in April of 2010 (1.80 ± 0.62) relative to other months. Further, Dm was greater in 2010 (1.56 ± 0.50) than 2009 (1.00 ± 0.29). Equitability (J) showed no spatio-temporal variation (Table SII).

Relationship between species density and environmental factors (RDA)

Redundancy analysis (RDA) showed that environmental factors accounted for 38.4% of the observed variation of cyanobacterial species in the Pará River estuary, and the two axes explained 30.5% of this variation (Figure 4). The results of the RDA were similar to those of the PCA.

Axis 1 (20.5%) established a temporal pattern as a function of year. The left of this axis included station groupings that were sampled in 2010. These correlated with the density, colonial species (*Aphanocapsa holsatica* and *Aphanocapsa delicatissima* and the filamentous *Pseudanabaena* sp.1) and had high concentrations of NH_4^+ (-0.89), NO_3^- (-0.83) and PO_4^{-3} (-0.80) ions. The group also had higher temperatures (-0.79), EC (-0.60), TDS (0.60), a more neutral pH (-0.56) and low concentrations of NO_2^- (0.62) and BOD (0.73). The other collection stations belonging to the year 2009 were grouped on the right side of the diagram and were represented mainly by the species *Microcystis wesenbergii*.

Axis 2 (10%) also established a temporal pattern that depended on seasonality. The wind (0.90) and density of the species of *Aphanocapsa elachista* and *Aphanocapsa incerta* ordered samples collected in July 2009 and August 2010. While samples collected in April of 2009 and April of 2010 were ordered according to monthly precipitation (-0.82), precipitation during sample collection (-0.72), precipitation one week prior to sample collection (-0.53) and the density of the species *Pseudanabaena mucicola*.

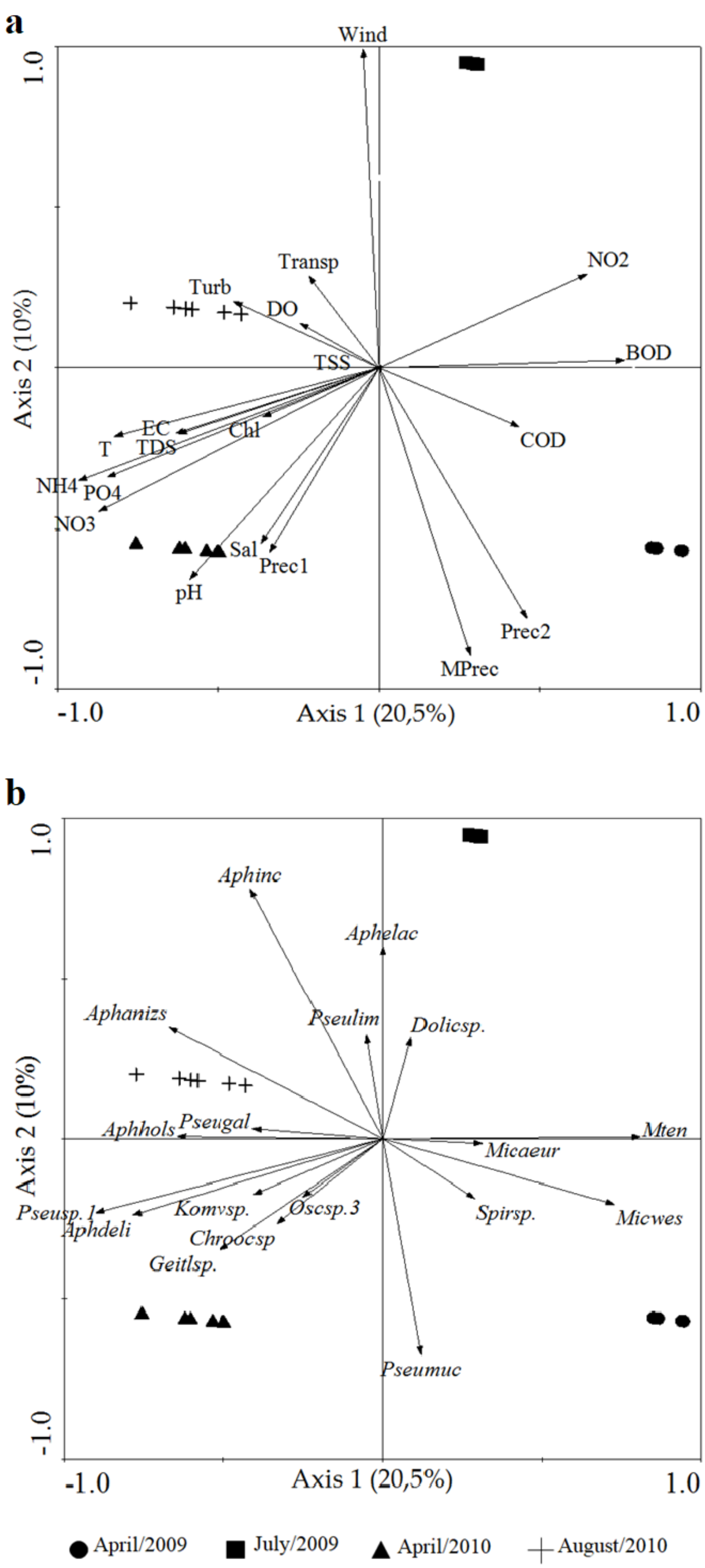

Figure 4. Redundancy analysis (RDA) showing relationships between species and environmental variables of the Pará River estuary (Amazon, Brazil). a, environmental variables: Prec1- precipitation one week prior to sample collection; Prec2- precipitation during sample collection; Wind; Transp- Transparency; T- Temperature; pH; EC- electrical conductivity; TDS- Total dissolved solids; TSS- Total suspended solids; Turb- Turbidity; Sal- Salinity; DO- dissolved oxygen; COD- Chemical oxygen demand; BOD- Biochemical oxygen demand; NO3- Nitrate; NO2- Nitrite; NH4- Ammonium nitrogen; PO4- Phosphate; Chl- Chlorophyll *a*. b, species legend: Aphdeli- *Aphanocapsa delicatissima*; Aphelac- *Aphanocapsa elachista*; Aphinc- *Aphanocapsa incerta*; Aphhols- *Aphanocapsa holsatica*; Aphanizs- *Aphanizomenon* sp.; Chroocsp- *Chroococcus* sp.; Dolicsp- *Dolichospermum* sp.; Geitlsp- *Geitlerinema* sp.; Komvsp- *Komvophoron* sp.2; Mten- *Merismopedia tenuissima*; Micaeur- *Microcystis aeruginosa*; Micwes= *Microcystis wesenbergii*; Oscsp3- *Oscillatoria* sp.3; Pseussp.1- *Pseudanabaena* sp.1; Pseugal- *Pseudanabaena galeata*; Pseulim- *Pseudanabaena limnetica*; Pseumuc- *Pseudanabaena mucicola*; Spirsp- *Spirulina* sp.

DISCUSSION

In intertropical regions the temporal dynamics of phytoplankton is influenced by external factors such as precipitation, together with wind and solar radiation. Due to the intense rainfall regime that exists within the region of the Amazon, many of the studies conducted investigating estuarine ecosystems in the Pará State pointed to precipitation as one of the main climatic factors responsible for seasonal variation of physicochemical and biological parameters (planktonic community) such as those performed by Costa et al. (2011), Matos et al. (2011) and Costa et al. (2016a).

The rainfall and wind intensity observed in this study determined a typical rainfall distribution pattern in the Amazon, with two well-defined seasonal periods: April of 2009 and 2010, which were rainy, and July of 2009 and August of 2010, which were dry. The first component of our PCA analysis revealed the differences between the years and the second component indicates the differences between the season (dry and rainy). The differences in the physical-chemical composition of the water found between the years are greater than those recorded between the dry and rainy periods, which can be explained by the greater rainfall recorded in 2009 compared to 2010. In 2010, a scenario occurred that was characterized by warmer waters, neutral pH and increased nutrient availability, which included increased levels of nitrate, ammonium nitrogen, phosphate, elevated EC and increased concentrations of TDS. These physicochemical factors affected the cyanobacterial community of the Pará River estuary.

A difference of one degree Celsius in the water temperature between years is likely associated with the sampling time, presence of clouds, winds, among other factors that can regulate the temporal scales of variation of this parameter, which influences on the chemistry and physics of the water, in addition to exert influence on biological activities and the growth of aquatic organisms (Esteves 2011).

The rivers of this region of the Amazon have a tendency to be slightly acidic even when there has not been damage to aquatic environment (Esteves 2011). This acidity may result from the leaching of acidic soils and the large amount of organic matter that decomposes to form organic acids (SEMA, unpublished data). However, throughout 2010 a neutral pH was observed in the Pará River estuary.

The increase in EC and TDS in 2010 may indicate higher concentrations of Ca^{2+}, Mg, K^{+} and Na^{+} that have important roles in the productivity of aquatic ecosystems and have been recognized as having a great degree of influence on EC (Esteves 2011), since the presence of these ions in the waters of the study region is common.

Nutrient input to aquatic ecosystems usually occurs via runoff. Nutrients are transported via rainfall, and also are derived from point sources, and wastewater from urban or industrial areas (Moruzzi et al. 2012). In 2010, there was a more than a 100% increase in nitrate and phosphate ions and a more than a 1000% increase in ammonium nitrogen when compared with 2009. Further, this increase in nutrients occurred within the two months of the year, April (rainy month) and August (dry month), indicating that input aside from precipitation was affecting the system. In the region under study, industrial and port activities occur. In fact, the region houses the Vila do Conde port-industrial complex. Costa et al. (2016a, b) suggest in their studies that the zooplankton community of the Pará River and other rivers near this region were affected by effluent derived from both industrial activities and domestic sources.

Regarding the cyanobacterial community of the Pará River estuary, most species that stand out in terms of their frequency of occurrence, relative abundance or density are described as bloom or potentially toxic species. For example, species of the genus *Microcystis* and *Aphanocapsa* were very common in the study area. Most toxic cyanobacterial blooms consist of *Microcystis* species, like those found by Lehman et al. (2017) in the São Francisco River estuary. The genus *Aphanocapsa* rarely produce dense blooms, they are often abundant in the phytoplankton and has been described as a potential toxin producer (Sant'Anna et al. 2004, Calijuri et al. 2006). Other genera identified in this study have been described as toxic, including *Oscillatoria*, *Pseudanabaena* (Oudra et al. 2002), *Phormidium* (Mez et al. 1997), *Aphanizomenon* and *Dolichospermum*. The occurrence of these species in the Amazon estuary provides warning for a potential risk to human health, information which is important for municipal managers.

Of the 31 species identified, over 80% are planktonic and freshwater species, which may have been favored as a result of the low levels of salinity observed in the estuary. The area of this study is located close to the two main water contributors, the Tocantins and Amazonas rivers (across the Breves Straits), according to Y.O. Prestes (unpublished data). The average fluvial discharge of these rivers into the estuary during the rainy season is 10.987 m^3/s and 8.495 m^3/s, respectively, which explains the low salinity observed.

Of the species identified, *Microcystis novacekii* (Komárek) Compère, *Aphanocapsa holsatica* (Lemmermann) G. Cronberg & J. Komárek, *Chroococcus distans* (G.M. Smith) and the genus *Komvophoron* Anagnostidis & Komárek have been newly found in the Northern Region. Costa et al. (2014) estimated that in the Pará State, cyanobacteria constitute the 3rd phytoplankton class (except Bacillariophyceae), and have the highest degree of taxonomic representation, which is composed of 89 infrageneric taxa. This diversity may be underestimated since most studies conducted in the region are not centrally focused on the characterization of the group.

During the study period, this environment wasn't characterized by blooms, since the values observed for density were below the values established by Brazilian law CONAMA 357/2005 for class 2 freshwater in which limits are 50.000 $cells.mL^{-1}$ (Brasil 2013). However, it was observed that the density values in 2010 doubled (increase of more than 100%) in relation to 2009, with this difference being observed in the density of cyanobacteria between the years significant, which demonstrates that the water quality in the Pará River changes over time, which reinforces the need for continuous monitoring in the region.

The results also showed that environmental conditions in 2010 allowed for the development of a larger number of species when compared to 2009, which also was reflected in increased levels of diversity and richness of cyanobacterial species in the Pará River estuary. The highest values of richness and diversity observed during 2010 were the result of the density contribution of species mainly belonging to the genera *Aphanocapsa* (*Aphanocapsa delicatissima* and *Aphanocapsa holsatica*), *Merismopedia* (*Merismopedia tenuissima*), *Microcystis* (mainly represented by *Microcystis protocystis*), *Pseudanabaena*, among others, while in 2009, the largest contributions were from *Microcystis aeruginosa*, *Microcystis wesenbergii* and *Merismopedia tenuissima*.

In the present study, the RDA analysis confirmed that the cyanobacteria community in the Pará River estuary varied annually in terms of density and diversity and that, although it was not the predominant phytoplankton group in that estuary, the variation in physical-chemical parameters was important in structuring of the cyanobacteria community, in the Pará River estuary. The results of the RDA revealed that the physical–chemical variables explained 38.4% of the variations this community. This was especially apparent in 2010, when the RDA biplot diagram shows that cyanobacteria were well adapted to high levels of nutrients (ammonium nitrogen, nitrate and phosphate), temperature, EC, TDS and pH.

There are factors that act together and/or in isolation, capable of causing ideal conditions in the waters for the growth of cyanobacteria: among these factors, nutrient availability plays an important role in the control of phytoplankton communities, affecting species composition and productivity. Increased cyanobacterial density observed in 2010 coincided with increased nutrient availability (mainly nitrogen, including NH_4^+). Ammonium nitrogen, in terms of energy (use with lower energy cost), is the main form of nitrogen assimilable by primary producers (Esteves 2011). Phosphorus is traditionally considered to be the main nutrient that favors the formation of cyanobacterial blooms, however, recent studies have shown that nitrogen can also be an important factor controlling the occurrence of blooms, especially with regard to non-diazotrophic species (Gobler et al. 2016). Davis et al. (2010) compared the effect of phosphorus and nitrogen on *Microcystis* strains obtained during a bloom and found that *Microcystis* species were more frequently stimulated by nitrogen than phosphorus.

Temperature together with nutrients also are considered factors that greatly affect cyanobacterial dominance. The factors are especially important since the vast majority of cyanobacterial species have maximum growth rates at high temperatures (above 20° C) and possess efficient light capture mechanisms which favors the predominance of this cyanobacterial species over other phytoplankton groups (Carey et al. 2012). The year 2010 had the highest average water and air temperatures considered. Nobles & Manning (2017) reinforced the idea that cyanobacterial blooms will intensify with global warming, as toxic cyanobacteria usually thrive in warm, nutrient-rich environments.

Increased TDS and EC suggest the presence of dissolved ions in the water that enable cyanobacteria to flourish. Sousa et al. (2015), in studies examining the phytoplankton community in the Charapucu State Park (Marajó Archipelago), observed a positive correlation between the density of this community and TDS, EC and possibly the availability of macronutrients. Another important factor identified was pH. Cyanobacteria grow optimally at neutral to alkaline pH (Pearl & Paul 2012). Higher cyanobacterial densities also were observed along with the occurrence of neutral pH in 2010. pH directly affects cell membrane permeability processes and interferes with ionic transport between organisms and environment (Esteves 2011).

Cyanobacteria have adopted different adaptive strategies. The species *Merismopedia tenuissima* has been shown to be well adapted to conditions of the Pará River estuary. During 2009 it comprised about 30% of the total density of species in the region and was competitive even in the face of resource limitation (lower nutrient availability). In 2010, the density of the species doubled under the best environmental conditions observed that year, but there was a decrease in the percentage of contribution (20%) of the species since other organisms flourished under conditions observed in 2010.

RDA analysis also showed that colonial species, *Aphanocapsa delicatissima* and *Aphanocapsa holsatica,* and the filamentous species, *Pseudanabaena* sp., were competitive under the favorable conditions observed in 2010. Together they contributed to 40% of the total cell density that year, while in 2009 their contribution was only 1%. *Microcystis aeruginosa* and *Microcystis wesenbergii* species contributed to 15% of the total cell density of cells in 2009. In that year, according to Wetzel (2001), the presence of aerotopes (gas vesicles) in the species allowed for migration within the water column, which could provide access to light and nutrients under nutrient limiting conditions. In 2010, the density of *Microcystis aeruginosa* species increased, however, its contribution decreased (8%), while *Microcystis wesenbergii* species made up only 1% of the total cell density present.

CONCLUSION

Cyanobacterial growth in the Pará River estuary is temporally dynamic and is influenced by limnological conditions that fluctuate from year to year. Of time periods examined, 2010 was the most favorable year with respect to the growth of cyanobacterial species. Elevated nutrient availability, followed by temperature, pH, as well as EC and TDS were among the physicochemical factors that most influenced cyanobacterial dynamics (richness, density and diversity) in 2010.

Although no bloom was observed during the period studied, the Pará River estuary should be constantly monitored because potentially toxic species were both qualitatively and quantitatively important in the region. The human population living in areas surrounding the estuary depends almost exclusively on its waters for survival, both for consumption and fishing for subsistence or recreation. The present study advances our knowledge regarding the dynamics of cyanobacterial growth in the region of the Amazon, which may guide future studies related to the toxicity and physiology of cyanobacterial species that grow within the estuary.

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SUPPLEMENTARY MATERIAL

Tables SI and SII

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A Gomes carried out fieldwork, analyzed and interpreted the results and wrote the manuscript. C Cunha conducted the fieldwork and contributed to the critical review of the text. M Lima and E Sousa helped in data analysis and contributed to the critical review of the text. V Tavares helped with the experimental design, data analysis and contributed to the critical review of text. J Martinelli-Lemos conceived and designed the experiments, helped with statistical analysis, supervising and contributed to the critical review of text. All authors read and approved the final manuscript.