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Structure of microphytoplankton community and environmental variables in a macrotidal estuarine complex, São Marcos Bay, Maranhão - Brazil

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ABSTRACT

São Marcos Bay is an estuarine complex with semidiurnal tides that can reach more than 7 m during equinoctial spring tides. It is situated in the second largest Brazilian coastline and is subjected to continuous human activities holding an important port complex of Latin America. In order to contribute to the knowledge about the structure of the phytoplankton in a macrotidal systems, this study aims to evaluate the phytoplankton community and its relationship with environmental conditions in the São Marcos Bay. Five surveys in 2010 and 2011 were carried out on four sampling points during the rainy and dry seasons. Samples were taken during the flood and ebb phases at neap tides. Hydrological parameters were correlated with biological data (phytoplankton composition, abundance and biomass) using statistical analysis. The phytoplankton biomass (chlorophyll *a*) characterized the bay as quite productive (10.43±7.62 mg m^{-3}) with the nanophytoplankton as the dominant fraction. Seasonal variation was observed in phytoplankton abundance with higher values (34,262±18,422 cells L^{-1}) in the rainy season. Diatoms were the most important phytoplankton group, pointing out *Nitzschia* sp., *Diploneis weissflogii* and *Synedra* sp. as the dominant species. This study revealed that the composition of phytoplankton community was mainly influenced by the local dynamics, governed by macrotides, and precipitation regime that mostly contributed to the seasonal fluctuations of the environmental conditions, such as salinity, dissolved oxygen, and nutrients.

Descriptors: Macrotidal estuaries, Phytoplankton abundance, Chlorophyll *a*, Nutrients.

INTRODUCTION

Transitional and coastal water bodies such as estuaries and coastal lagoons are complex environments that link freshwater and marine systems (Bazin et al., 2014). They also go through continuous changes in response to their dynamic and natural processes. Because of their hydrodynamic characteristics, estuarine systems are among the most productive and resourceful aquatic ecosystems around the world (Sathicq et al., 2017).

These systems receive rich riverine supplies of nutrients and provide important ecosystem services (e.g., food production, nutrient cycling), becoming crucial to the maintenance of marine life and humanity (Costanza et al., 1997; Attrill and Rundle, 2002). Furthermore, these ecosystems are excellent ecological study sites due to their biotic and abiotic mechanisms varying over space and time, which control the distribution of organisms in the whole system (Sin et al., 2015). However, due to their proximity to land, estuaries have been more vulnerable to anthropogenic activities, such as domestic and industrial effluent contamination, which, under extreme conditions, can affect their productivity and consequently the water quality (Carstensen et al., 2011; Cloern et al., 2014).

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In macrotidal zones, physical processes control the system dynamics with large amounts of organic and inorganic compounds being reworked and transported within the estuary (Burford et al., 2008). In turbid estuaries under strong mixing conditions, tidal force is the most dominant factor, which can vary over time (diurnally, fortnightly or seasonally) (Azhikodan and Yokoyama, 2016). Wind force also promotes continuous vertical mixing of the water column allowing the translocation of oxygen-saturated water and protecting proximate coastal water bodies from eutrophication since macrotidal currents decrease the response of primary production to enhanced nutrient inputs (Cloern, 2001).

The biological indicators are commonly used to assess the water quality detecting changes in the biotic structure of the communities over spatial and temporal scales (Katsiapi et al., 2016). Phytoplankton plays a crucial role in the aquatic environment because they are the dominant primary producers and balance the overall food web dynamics (Katz et al., 2004; Chai et al., 2016). In addition to holding ecological services, biomass, composition, and community structure of phytoplankton can be considered an efficient bio-indicator of water quality as their distribution strongly correlates with several factors (e.g., physical, chemical, and biological) as well as interactions among them (Paerl et al., 2003; 2010). This variability may be reflected in population dynamics, especially those of phytoplankton populations thriving in coastal systems.

Studies addressed the dynamics of phytoplankton community in estuaries along the north coast of Maranhão - Brazil, especially in the São Marcos Bay, are scarce (Teixeira et al., 1988; Lavôr-Fernandes, 1988; Azevedo et al., 2008; Rodrigues and Cutrim, 2010; Duarte-dos-Santos et al., 2017). The analysis of the seasonal and tidal distribution of phytoplankton community and the complex relationships of these distribution patterns with environmental factors in the São Marcos Bay can identify potential indicative species of ecological conditions as well as provide relevant scientific evidence to water quality assessment and pollution control. Thus, the aim of this present study is to contribute to the knowledge about the structure of the phytoplankton community in terms of composition, abundance, chlorophyll *a* and evaluate its relationship with environmental conditions in a macrotidal estuarine complex of the São Marcos Bay.

MATERIAL AND METHODS

Study area

São Marcos Bay is a wide estuarine complex located in the Maranhão state (Figure 1), holding the second largest Brazilian coastline with about 640 km of extension. It has a semidiurnal macrotide with tidal range that can reach more than 8 m in some zones during equinoctial spring tides and has tidal currents with velocities higher than 1.1m s^{-1} (maximum tidal currents of 2.42 m s^{-1}) (González-Gorbeña et al., 2015).

São Marcos Bay together with São José Bay forms the geologic unit known as the Maranhense Gulf which encloses estuaries, straits, inlets, small rivers, and many islands. The bay is surrounded by an extensive mangrove area, such as a large mangrove island, named Caranguejos Island, that is situated in its southern half portion (Souza-Filho et al., 2005; El-Robrini et al., 2006). The three main rivers of the watershed are Pindaré, Mearim, and Grajaú, whose waters discharge at the southernmost region of the São Marcos Bay being responsible for the suspended solid loads, primarily rich in clay and silt (annual average of ~250 mg L^{1-}) (Morais, 1977).

The entrance of the bay presents a width of ~55 km, which narrows to 1.5 km at the intersection of Pindaré and Mearim rivers. The bay has a central channel with depths up to 90 m, which works as a waterway for the second most important port complex of Latin America including São Luís port, Itaqui port, Ponta da Madeira terminal, and Alumar terminal (Amaral and Alfredini, 2010). Ponta da Madeira terminal is undergoing an expansion to increase its export capacity to 235 millions of tons per year of iron mineral, becoming the port with the largest volume of cargo in Brazil (González-Gorbeña et al., 2015).

With regard to the climate in the Maranhão state, where São Marcos Bay is located, it is tropical and humid with equatorial air mass influence and intrinsic characteristics such as: high temperatures throughout the year (27 °C - 31 °C) and two distinct seasonal periods strongly marked by the precipitation: rainy season (January-June) and dry season (July-December) (Rios, 2001).

Field sampling

Five surveys were carried out during the rainy season (April 2010, January 2011, March 2011) and the dry season (August 2010, November 2010) at four sampling points (P1 - P3 with average depth up to 22 m and P4 with

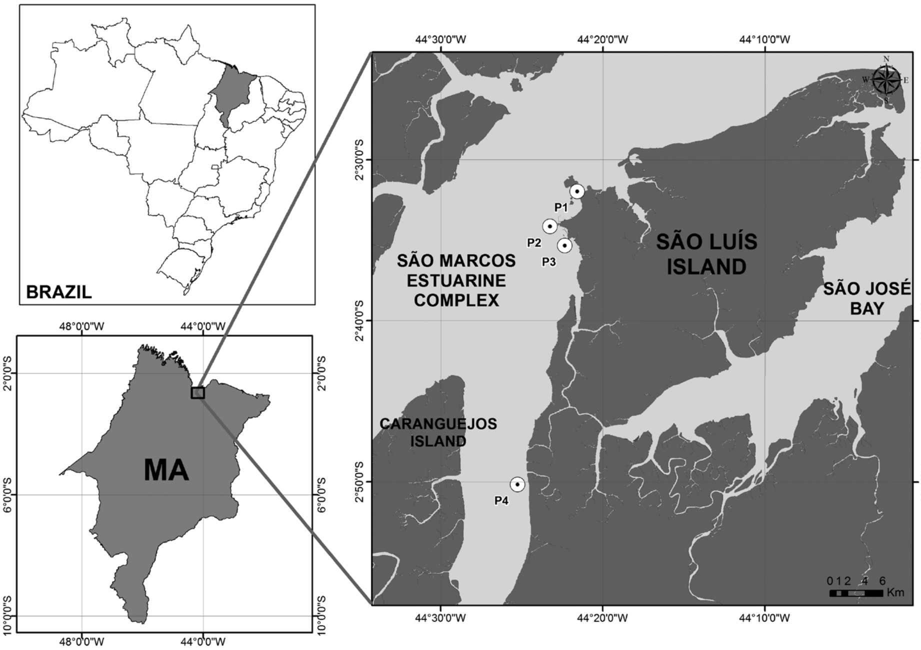

Figure 1. Position of the sampling sites and location of the estuarine complex of the São Marcos Bay, Maranhão - Brazil.

5 to 10 m; González-Gorbeña et al., 2015) along the São Marcos Bay (Figure 1). All the samples were taken during the flood and ebb tides at neap tide, comprising a total of forty samples (n=40).

METEOROLOGICAL AND HYDROLOGICAL ANALYSIS

Hydrological variables were measured *in situ*, including temperature (ºC), salinity, pH, dissolved oxygen (ml L^{-1}) and oxygen saturation (%), using a multiparametric probe (Hanna 9828). From the temperature and salinity data, we constructed a TS spread diagram, in which the plane T-S represented the water masses distribution using predetermined intervals of salinity and temperature (Miranda, 1985). The performance area for each TS pair was set according to its depth and grid point. After establishing the thermohaline indices for each water type, the mixing straight method, initially described by Mamayev (1975), was used to obtain the percentage of each water type present in a representative sample of a mixture in the stationary condition. In estuarine systems, and particularly in shallow layers, the properties of temperature and salinity are not stationary. Nevertheless, this method was used considering that the timescales involved in the variations of temperature and salinity were larger than the duration of the hydrographic surveys.

The application of this method requires the identification of thermohaline indices (T_1, S_1 and T_2, S_2), which is usually performed using the T-S spread diagram. The percentage quantities of each water type (m_1, m_2) in a given sample with the temperature T and salinity S are obtained by resolution of the linear system illustrated in the following equation:

$$m_1T_1 + m_2T_2 = mT$$
$$m_1S_1 + m_2S_2 = mT$$
$$m_1 + m_2 = 1$$

In addition, water transparency (Secchi depth) was measured by using a Secchi disc and turbidity (NTU) by a turbidity meter (Lamotte, 2020). For nutrient analysis, 2 liters of water were collected at the surface layer (50 cm depth) using a van Dorn bottle. Quantification of NH_4^+, NO_2^-, NO_3^-, and PO_4^{-3} followed the methodologies described in "Standard Methods for Water and Wastewater" (APHA, 2012). The 30 years historical average precipitation in São Luís city (São Luís Island - São Marcos Bay) and the wind speed for sampling months were obtained from the National Institute of Meteorology - INMET (http//www.inmet.gov.br).

Microphytoplankton composition

To analyze qualitatively the microphytoplankton composition in the São Marcos Bay, sampling was conducted with plankton net (mesh size 45 µm). Three-minute horizontal surface hauls were carried out in order to collect the biological material. After the collection, samples were preserved with 4% formalin. In the laboratory, identification was based on the specialized phytoplankton literature. Thus, microalgae have been identified at the lowest possible taxonomic level. AlgaeBase was used to classify and update the taxa information (Guiry and Guiry, 2017).

Phytoplankton abundance and biomass

To analyze quantitatively the phytoplankton community (phytoplankton abundance and biomass), surface water samples were collected using van Dorn bottle.

Samples were fixed with a Lugol's iodine solution to determine the phytoplankton abundance, which followed the Utermöhl method (Ferrario et al., 1995). The phytoplankton cells were counted using inverted microscopes (ZEISS Axiovert 100) with 400x magnification. At least 100 fields were counted in the deposit algae contained in 10 mL in each sample. In addition, the number of cells was applied to the equation of Villafañe and Reid (1995), expressing results as unit of cells per liter (cells L^{-1}).

To determine the phytoplankton biomass, estimated as size-fractionated chlorophyll *a* concentration, surface water samples were filtered onto 47 mm Whatman GF/F filters (0.7 µm porosity). In order to separate the microphytoplankton (>20 µm) from the nanophytoplankton (<20µm) fraction, each water sample was filtered through 20 µm net. With a volume of 250 mL, the chlorophyll *a* filters were dried and stored in a freezer (-18 °C) until the chlorophyll pigment extraction using spectrophotometric method (UNESCO, 1966) in accordance with Parsons and Strickland (1963).

Data analysis

Two Way Analysis of Variance (ANOVA - Two Way) was performed in order to determine the significant differences (p<0.05) of the hydrological and biological variables among seasonal periods and tides. To analyze the structure of the microphytoplankton community, some indexes were applied: a) the Constancy index, on which the taxa recorded in more than 50% of the samples was considered "RESIDENT," "VISITOR" when it was recorded in 25-50% of the samples and "ACCIDENTAL" when recorded in less than 25% of the samples (Dajoz, 1983); b) diversity index of Shannon (H') (Shannon, 1948), evenness (J) (Pielou, 1966), richness (S) (Margalef, 1958) and dominance (Y) (Sun et al., 2004) indexes, which were calculated based on the following equations:

Shannon index

$$H' = -\sum_{i=1}^{s} \mathrm{Pi} \log_2 \times \mathrm{Pi} \qquad \mathrm{Pi} = Ni/N$$

Pielou evenness index

$$J = H' / \log_2 S$$

Margalef richness index

$$S = (s-1) / \ln \cdot N$$

Dominance index

$$Y = (n_i/N) x \, fi$$

where Ni is the individual amount of the species organism, N is the total individual amount, S is the total species at any sampling point, and f_i is the individual frequency in the samples.

The non-metric multidimensional scaling (nMDS) and cluster analysis, based on the Euclidian distance, were applied to determine the dissimilarity of sampling sites with respect to the abundance of dominant species, precipitation, and salinity (square root transformed). SIMPER analysis was used to find out the discriminating species between the seasons, where it was used the Bray-Curtis similarity coefficient to determine the similarity of dominant species through their cell abundance, defined by the dominance index. Principal Component Analysis (PCA) was computed based on a matrix formed by precipitation, wind speed, hydrological data, chlorophyll *a* concentrations, and phytoplankton abundance in order to explore the relationship among them. The data used were standardized to normalize their distribution. In addition, Pearson's Correlation Analysis was applied to correlate the phytoplankton abundance in relation to different physical-chemical factors analyzed. Data analyses were carried out using the software IBM SPSS Statistics 24.0, STATISTIC 10.0 and PRIMER 6.0.

RESULTS

Meteorological and hydrological variables

Precipitation showed an annual average of 2290.2 mm, varying from 1755.6 mm in 2010 to 2713.3 in 2011. The historical average precipitation data and wind speed revealed two well-defined seasonal periods: the rainy season spanning from January to June, which can extend until July, with the highest precipitation in April 2011 (621.7 mm) and the lowest wind speed (2.1 m s^{-1}) registered in January 2011. The second is the dry season spanning from July to December, where September and October did not register any precipitation value while the wind speed revealed the highest values in November 2010 (4.5 m s^{-1}) (Figure 2).

Seasonal and tidal variations of hydrological variables in the São Marcos Bay during the study period are shown in Table 1. In general, water temperature ranged from 28.4 ºC to 30.24 ºC (overall mean 29.19±0.52 ºC) and the highest value was recorded during flood tide in the rainy season. Salinity ranged from 20.7 to 37.4 (overall mean 31.71±4.48) with higher values being recorded in the dry season during ebb tide (Table 1). Significant differences ($p<0.01$) were observed only between seasonal periods (rainy and dry seasons) in terms of water temperature and salinity.

The TS diagram pointed out two water masses in the São Marcos Bay (2010-2011) based on the criteria adopted by Dias et al. (2013) (Figure 3). The water masses were well characterized seasonally, with the presence of warmer waters (T > 29 ºC) with lower salinity (S < 30) compatible with the river water mass during the rainy season, mainly through the months of March 2010 and April 2011. During the dry season, the thermohaline indices were characteristic of coastal water with a marked increase in salinity (31<S<37). In January, a mixing between the two water masses was the predominant feature.

The dissolved oxygen varied from 3.08 mg L^{-1} to 6.5 mg L^{-1} (overall mean 4.38±1.06 mg L^{-1}) revealing a seasonal pattern with significant differences ($p<0.01$) between rainy and dry seasons with higher values observed in the dry season and ebb tide. A similar trend was observed in relation to the oxygen saturation rate, which ranged from 40% to 86% (overall mean 57.9±13.94 %) (Table 1). No significant difference ($p>0.05$) was observed between tidal periods (ebb tide and flood tide) in terms of dissolved oxygen and oxygen saturation.

Water transparency varied from 6 cm to 77 cm (overall mean 39±16 cm) with higher values recorded during ebb tide in the rainy season (Table 1). A significant difference occurred between flood and ebb tides ($p<0.05$). Turbidity ranged from 14 NTU to 390 NTU (overall mean 89.90±73.33 NTU) with higher values recorded in the rainy season and significant difference ($p<0.01$) between tidal periods. In general, water transparency and turbidity did not show significant differences ($p>0.05$) between dry and rainy seasons. The pH values presented higher than 7.0 in 95% of the samples ranging from 6.7 to 8.15 (overall mean 7.65±0.32) with no significant differences ($p>0.05$) between seasons and tidal periods.

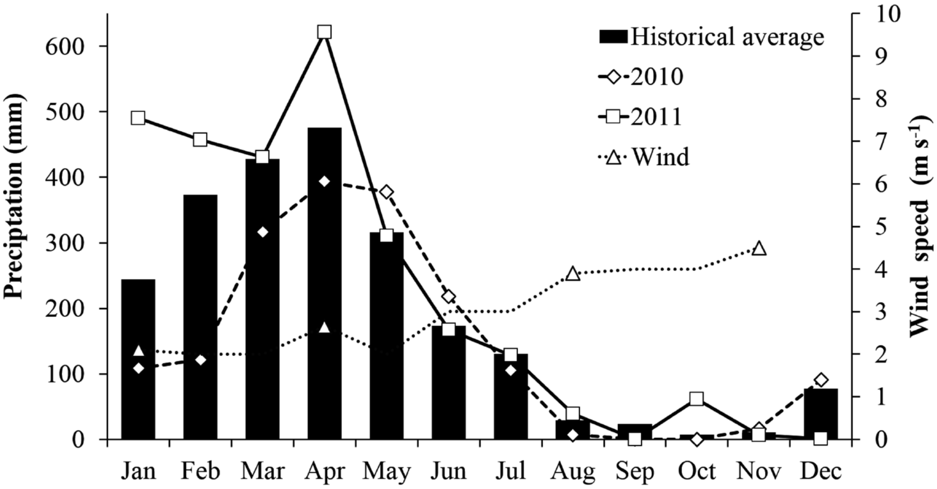

Figure 2. Historical average of precipitation (based on the last 30 years), total monthly precipitation registered in 2010 and 2011 and wind speed for the sampling months.

Table 1. Seasonal and tidal variation of hydrological variables with mean values, standard deviation (SD) and minimum and maximum (Min/Max) in the São Marcos Bay.

Hydrological Variables	**Rainy**				**Dry**			
	Flood		**Ebb**		**Flood**		**Ebb**	
	Mean ± SD	Min/Max	Mean ± SD	Min/Max	Mean ± SD	Min/Max	Mean ± SD	Min/Max
Temperature (ºC)	29.45±0.61	28.56/30.24	29.42±0.46	28.72/30.0	28.74±0.19	28.4/29.04	28.92±0.25	28.6/29.4
Salinity	28.91±4.05	20.7/34.78	29.45±3.95	21.09/34.8	35.40±1.81	31.76/37.3	35.63±1.85	32.07/37.4
Dissolved Oxygen (ml L^{-1})	3.55±0.37	3.08/4.5	3.69±0.28	3.4/4.16	5.42±0.68	4.5/6.3	5.65±0.68	4.7/6.5
Oxygen Saturation (%)	47.33±5.03	40/60	48.58±4.10	40/55	71.25±9.07	59/83	74.38±9.02	62/86
Secchi depth (cm)	35±13	6/50	43±23	8/77	42±9	27/56	36±15	22/72
Turbidity (NTU)	91.29±101.35	32/390	74.08±51.85	14.8/190	102.22±67.11	62.1/260	72.50±37.57	29.9/130
pH	7.66±0.44	6.7/8.15	7.7±0.35	7.0/8.11	7.52±0.18	7.3/7.8	7.69±0.13	7.43/7.8
NH_4^+ (µmol L^{-1})	0.33±0.17	0.07/0.61	0.31±0.12	0.17/0.61	0.50±0.30	0.15/0.98	0.16±0.06	0.09/0.25
NO_2- (µmol L^{-1})	0.10±0.03	0.06/0.16	0.11±0.05	0.05/0.26	0.33±0.74	0.03/2.16	0.11±0.06	0.06/0.26
NO_3- (µmol L^{-1})	1.16±0.05	1.08/1.24	1.06±0.35	0.13/1.35	1.19±0,05	1.10/1.27	1.23±0.04	1.13/1.29
PO_4^{-3} (µmol L^{-1})	1.32±0.11	0.03/0.46	0.23±0.04	0.17/0.33	0.23±0.10	0.03/0.34	0.25±0.04	0.17/0.33

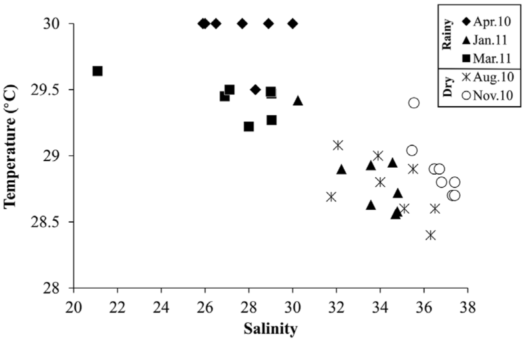

Figure 3. T x S Diagram for the samplings in the 4 sites at São Marcos Bay-MA in 2010 and 2011.

The concentrations of NH_4^+ ion varied from 0.07 µmol L^{-1} to 0.98 µmol L^{-1} (overall mean 0.32±0.20 µmol L^{-1}) with higher values being recorded in the dry season during the flood tide. In a similar way, the concentrations of NO_2^- showed the highest values recorded in dry season during the flood tide and ranged from 0.034 µmol L^{-1} to 2.16 µmol L^{-1}(overall mean 0.15±0.33 µmol L^{-1}). However, the highest value (1.36 µmol L^{-1}) of NO_3^- was recorded in the rainy season during the ebb tide (overall mean 1.15±0.20 µmol L^{-1}). In this study, the nitrogen compounds did not show significant differences (p>0.05) between seasons and tidal periods. The concentrations of PO_4^{-3} varied from 0.02 µmol L^{-1} to 0.46 µmol L^{-1} (overall mean 0.26±0.08 µmol L^{-1}) with higher values observed in the rainy season during the flood tide, however, these values were not significant (p>0.05) regarding the seasonal and tidal variations.

MICROPHYTOPLANKTON COMPOSITION

From the qualitative analysis of the microphytoplankton composition, a total of 178 species were identified including 7 varieties and 3 forms (Table 2). Six divisions were distinguished: Bacillariophyta (90.45%), Miozoa (5.62%), Cyanobacteria (1.12%), Chlorophyta (1.69%), Euglenophyta (0.56%) and Charophyta (0.56%).

The largest group was Bacillariophyta including 68 genera and 161 taxa. The principal genus of this division was *Chaetoceros* (18 taxa) followed by *Coscinodiscus* (11 taxa), *Nitzschia* (10 taxa) and *Actinoptychus* (8 taxa). Miozoa was the second most common group with 6 genera and 10 taxa with *Tripos* (4 taxa) being the dominant genus.

Seasonal variation of microphytoplankton showed a number of 156 taxa identified in the rainy season and 140 in the dry season. In both seasons, diatoms were the more abundant group. During the rainy season, there were 140 diatoms (89.74%), 10 dinoflagellates (6.41%), 2 cyanobacteria (1.28%), 3 chlorophytes (1.92%) and 1 charophyte (0.64%). By comparison, the dry season included 130 diatoms (92.86%), 7 dinoflagellates (5.00%), 1 cyanobacteria (0.71%) and 1 chlorophyte (0.71%). Euglenophytes were observed only in the rainy season with one taxon (0.71%).

Table 2. List of taxa identified in microphytoplankton samples collected in the São Marcos Bay with percentage of occurrence (%) (n = 40). Note: TO = Total Occurrence (n = 40), classification by Constancy Index (CI) with the categories: R = resident, V = visitor, and A = accidental, and occurrence by season.

Taxonomy Category	TO (%)	CI	Occurence by season	
			Dry	Rainy
Cyanobacteria				
Oscillatoria spp.	53	R	*	*
Spirulina sp.	3	A		*
Euglenophyta				
Euglena sp.	3	A		*
Miozoa				
Ceratium sp.	5	A	*	*
Peridinium sp.	8	A	*	*
Prorocentrum sp.	5	A		*
Protoperidinium conicum	13	A	*	*
Protoperidinium spp.	40	V	*	*
Pyrophacus steinii	10	A	*	*
Tripos furca	20	A	*	*
T. fusus	5	A		*
T. lineatus	33	V	*	*
T. macroceros	3	A		*
Bacillariophyta				
Achnanthes brevipes	3	A	*	
Actinocyclus curvatulus	3	A		*
Actinocyclus sp.	3	A	*	
Actinoptychus annulatus	85	R	*	*
A. aster	5	A		*
A. minutus	5	A		*
A. octonarius	10	A	*	*
A. senarius	78	R	*	*
A. splendens	60	R	*	*
A. vulgaris	10	A		*
Actinoptychus sp.	10	A	*	*
Alveus marinus	3	A	*	
Amphora sp.	3	A		*
Asterionellopsis glacialis	58	R	*	*
Asteromphalus sp.	3	A	*	
Aulacodiscus margaritaceus	3	A	*	
Bacillaria paxillifera	63	R	*	*
Bacteriastrum delicatulum	35	V	*	*
B. hyalinum	45	V	*	*
Bellerochea malleus	80	R	*	*
Biddulphia biddulphiana	3	A	*	
B. tridens	8	A	*	*
Caloneis permagna	38	V	*	*
Cerataulina sp.	3	A	*	
Chaetoceros aequatorialis	15	A	*	*
C. affinis	38	V	*	*
C. atlanticus	3	A		*
C. atlanticus f. *audax*	10	A	*	*
C. brevis	5	A		*
C. coarctatus	8	A	*	*
C. compressus	15	A	*	*
C. curvisetus	13	A	*	*
C. didymus	5	A		*
C. gracilis	8	A	*	
C. lorenzianus	40	V	*	*
C. pendulus	20	A	*	*
C. peruvianus	55	R	*	*
C. simplex	25	V	*	*
C. subtilis	3	A		*
C. subtilis var. *abnormis*	53	R	*	*
C. teres	5	A		*
Chaetoceros sp.	5	A		*
Coscinodiscopsis jonesiana	3	A	*	
Coscinodiscus asteromphalus	40	V	*	*
C. centralis	70	R	*	*
C. concinnus	23	A	*	*
C. curvatulus	3	A		*
C. gigas	33	V	*	*
C. granii	15	A	*	*
C. oculus-iridis	98	R	*	*
C. radiatus	85	R	*	*
C. rothii	60	R	*	*
Coscinodiscus sp.	28	V	*	*
Craticula cuspidata	8	A	*	*
Cyclotella meneghiniana	10	A	*	*
C. striata	43	V	*	*
C. stylorum	60	R	*	*
Cylindrotheca closterium	23	A	*	*
Diploneis bombus	20	A	*	*
D. gruendleri	30	V	*	*
D. interrupta	5	A	*	
D. weissflogii	13	A	*	*
Ditylum brightwellii	98	R	*	*
Entomoneis alata	35	V	*	*
Entomoneis sp.	3	A	*	
Eupodiscus antiquus	18	A	*	*

Taxonomy Category	TO (%)	CI	Occurence by season	
			Dry	Rainy
E. radiatus	10	A		*
Eupodiscus sp.	3	A	*	
Fragilaria sp.	8	A	*	
Frustulia interposita	33	V	*	*
F. rhomboides	15	A	*	*
Grammatophora marina	5	A	*	
G. oceanica	5	A	*	*
Guinardia flaccida	13	A	*	*
Gyrosigma attenuatum	10	A		*
G. balticum	83	R	*	*
G. hippocampus	13	A	*	*
Gyrosigma sp.	3	A		*
Hantzschia amphioxys	3	A		*
Helicotheca tamesis	88	R	*	*
Hemiaulus indicus	3	A		*
Leptocylindrus danicus	18	A	*	*
L. minimus	3	A		*
Lithodesmium undulatum	88	R	*	*
Lyrella lyra	3	A	*	
Melchersiela hexagonalis	50	V	*	*
Melosira moniliformis	23	A	*	*
M. nummuloides	73	R	*	*
Navicula sp.	13	A	*	*
Neocalyptrella robusta	10	A		*
Nitzschia fasciculata	23	A	*	*
N. longa	18	A	*	*
N. longissima	20	A	*	*
N. longissima f. *parva*	3	A	*	
N. obtusa	5	A		*
N. obtusa var. *scalpelliformis*	85	R	*	*
N. palea	8	A	*	*
N. sigma	48	V	*	*
N. tryblionella	5	A	*	*
Nitzschia sp.	18	A	*	*
Odontella aurita	55	R	*	*
O. longicruris	60	R	*	*
O. turgida	15	A	*	*
Odontella spp.	23	A	*	*
Paralia sulcata	60	R	*	*
Petroneis humerosa	13	A	*	*
Pleurosigma angulatum	10	A	*	*
P. formosum	3	A	*	
P. normanii	8	A	*	*
Pleurosigma spp.	50	V	*	*
Pleurosira laevis	3	A	*	
Podocystis adriatica	3	A	*	
Proboscia alata	3	A		*
Psammodictyon constrictum	8	A	*	*
P. panduriforme	8	A	*	*
Pseudo-nitzschia pungens	30	V	*	*
Pseudo-nitzschia spp.	28	V	*	
Pseudosolenia calcar-avis	10	A	*	*
Rhaphoneis amphiceros	50	V	*	*
Rhizosolenia hebetata	8	A	*	*
R. imbricata	8	A		*
R. imbricata var. *shrubsolei*	3	A		*
R. setigera	70	R	*	*
R. styliformis	5	A		*
Skeletonema costatum	68	R	*	*
S. tropicum	75	R	*	*
Surirella fastuosa	5	A	*	*
S. febigeri	45	V	*	*
S. gemma	25	V	*	*
S. minuta	3	A	*	
Synedra ulna	30	V	*	*
S. ulna var. *ulna*	28	V	*	*
Synedra sp.	20	V	*	*
Tabularia fasciculata	45	V	*	*
Terpsinoe americana	8	A	*	*
T. musica	30	V	*	*
Thalassionema frauenfeldii	100	R	*	*
T. nitzschioides	35	V	*	*
T. nitzschioides var. *capitulatum*	5	A	*	*
T. nitzschioides var. *claviforme*	3	A		*
T.nitzschioides var. *lanceolatum*	3	A		*
Thalassionema sp.	20	A		*
Thalassiosira eccentrica	80	R	*	*
T. leptopus	85	R	*	*
T. lineata	13	A	*	*
T. rotula	13	A	*	*
T. simonsenii	3	A		*
T. subtilis	98	R	*	*

Taxonomy Category	TO (%)	CI	Occurence by season	
			Dry	Rainy
Thalassiosira sp.	38	V	*	*
Triceratium dubium	3	A	*	
T. favus	90	R	*	*
T. favus f. *quadrata*	48	V	*	*
T. pentacrinus	3	A		*
T. robertsianum	5	A		*
Triceratium sp.	15	A	*	*
Trieres mobiliensis	100	R	*	*
T. regia	100	R	*	*
T. sinensis	60	R	*	*
Tryblionella angustata	3	A	*	
Tryblioptychus cocconeiformis	23	A	*	*
Zygoceros ehrenbergii	93	R	*	*
Chlorophyta				
Oedogonium sp.	3	A		*
Dimorphococcus sp.	33	V	*	*
Scenedesmus sp.	5	A		*
Charophyta				
Staurastrum sp.	3	A		*

According to the Constancy Index analysis (Table 2), 62.36% of the species were classified as ACCIDENTAL (98 diatoms; 8 dinoflagellates; 1 cyanobacteria; 2 chlorophytes; 1 charophyte; 1 euglenophyte), 19.66% as RESIDENT (34 diatoms; 1 cyanobacteria), and 17.98% as VISITORS (29 diatoms; 2 dinoflagellates; 1 chlorophyte).

A total of 17 diatoms showed high occurrence with a percentage varying between 80% and 100% of total occurrence. *Trieres mobiliensis, Trieres regia,* and *Thalassionema frauenfeldii* were the most frequent diatoms, as they were registered in 100% of the samples (n=40) (Table 2).

Phytoplankton abundance and chlorophyll *a* concentration

Phytoplankton abundance was lower in the dry season (25,097±9,244 cells L^{-1}) than in the rainy season (34,262±18,422 cells L^{-1}) with significant seasonal difference ($p<0.05$). In relation to tidal periods, the cell abundance was significantly higher ($p<0.01$) in flood tide (35,289±18,953 cells L^{-1}) than in ebb phase (25,903±10,731 cells L^{-1}) (Figure 4). Only diatoms were recorded through the counting of phytoplankton cells. Based on the cell abundance, the dominance index (Y) revealed 8 diatoms as the dominant species, which *Nitzschia* sp., *Synedra* sp., and *Diploneis weissflogii* showed higher abundance in both seasons (Table 3). The rainy season registered higher numbers of species; nevertheless, some species existed throughout the entire study period.

Diversity was lower in the dry season (0.81±0.15 bits cell^{-1}) and ebb tide (0.79±0.15 bits cell^{-1}), and higher in the rainy season (0.83±0.14 bits cell^{-1}) and flood tide (0.86±0.13 bits cell^{-1}). Likewise, richness was lower in the dry season (2.82±0.70) and ebb tide (2.82±0.76), and higher in the rainy season (3.00±0.79) as well as flood tide (3.03±0.75), but some species (e.g. *T. mobiliensis, T. regia,* and *T. frauenfeldii*) occurred throughout both seasons. Evenness was lower in the rainy season (0.94±0.05) and flood tide (0.94±0.05), and higher in the dry season (0.96±0.03) and ebb tide (0.96±0.04) (Figure 4). However, no significant differences ($p>0.05$) were observed between tidal periods and seasons in terms of diversity, richness, and evenness.

Chlorophyll *a* concentration was lower in the dry season (9.85±8.02 mg m^{-3}) and ebb tide (9.85±5.11 mg m^{-3}) and higher in the rainy season (10.81±7.50 mg m^{-3}) and flood tide (11.01±9.62 mg m^{-3}) (Figure 5). However, no significant differences ($p>0.05$) were observed between tidal periods and seasons. Concerning the contribution of phytoplankton size class, nanophytoplankton was dominant (90% of total samples) with high values in the rainy season (7.59±4.59 mg m^{-3}) during flood tide (7.36±6.07 mg m^{-3}), followed by microphytoplankton (10% of total samples) which was higher in the dry season (4.33±3.88 mg m^{-3}) during ebb tide (3.68±4.08 mg m^{-3}).

Statistical analysis

The non-metric multidimensional (nMDS) two-dimensional plot (stress 0.07) based on the abundance of dominant species, precipitation and salinity was done from cluster analysis (Euclidian distance). This analysis confirmed the formation of three major groups according to seasonality, with no overlapping between them (Figure 6). Among the three major assemblages of sampling months, groups I and III arranged mainly samples from the rainy season, except November (flood tide). In contrast, group II comprised exclusively samples from dry season (August - ebb tide, August - flood tide, and November - ebb tide).

To visualize the contribution of dominant species, in terms of cell abundance (cells L^{-1}), associated with

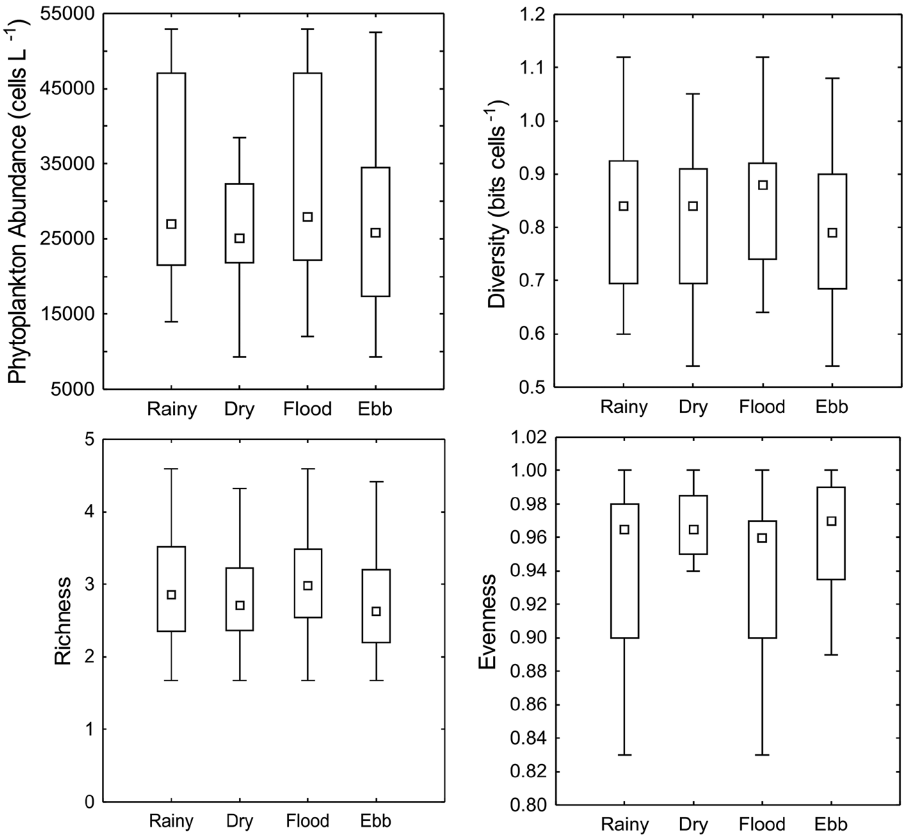

Figure 4. Boxplots of phytoplankton abundance (cells L^{-1}), diversity index (bits $cell^{-1}$), richness, and evenness in the São Marcos Bay.

Table 3. Cell abundance (cells L^{-1}) and dominance index (*Y*) of the most representative species of total phytoplankton in the São Marcos Bay. Note: F=Flood; E=Ebb.

Species	**Rainy**							**Dry**					
	apr./10		**jan./11**		**mar./11**			**aug./10**		**nov./10**			
	F	**E**	**F**	**E**	**F**	**E**	**Y**	**F**	**E**	**F**	**E**	**Y**	**∑**
Nitzschia sp.	99530	17525	13289	60390	46312	4039	**0.162**	27297	15853	16902	16591	**0.064**	**3.2 x 10^5**
Synedra sp.	32144	5708	9874	18512	58561	6381	**0.068**	11780	4039	4258	15791	**0.022**	**1.6 x 10^5**
Diploneis weissflogii	4376	4011	3725	20167	31379	7742	**0.044**	34736	22753	13046	14768	**0.062**	**1.6 x 10^5**
Thalassiosira sp.	4376	6017	5862	16661	15180	7405	**0.030**	2188	2188	5890	0	**0.003**	**6.6 x 10^4**
Cyclotella stylorum	14002	3702	4011	5862	4012	6045	**0.020**	2188	0	4193	2188	**0.002**	**4.6 x 10^4**
Thalassiosira leptopus	0	0	10028	10757	4011	4039	**0.014**	4813	6564	4376	0	**0.004**	**4.5 x 10^4**
Coscinodiscus sp.	9626	18543	4011	18822	9626	6381	**0.014**	4376	6227	0	2188	**0.004**	**7.9 x 10^4**
Eupodiscus radiatus	0	2006	0	2006	19253	1851	**0.003**	12218	4376	0	8751	**0.008**	**5.0 x 10^4**

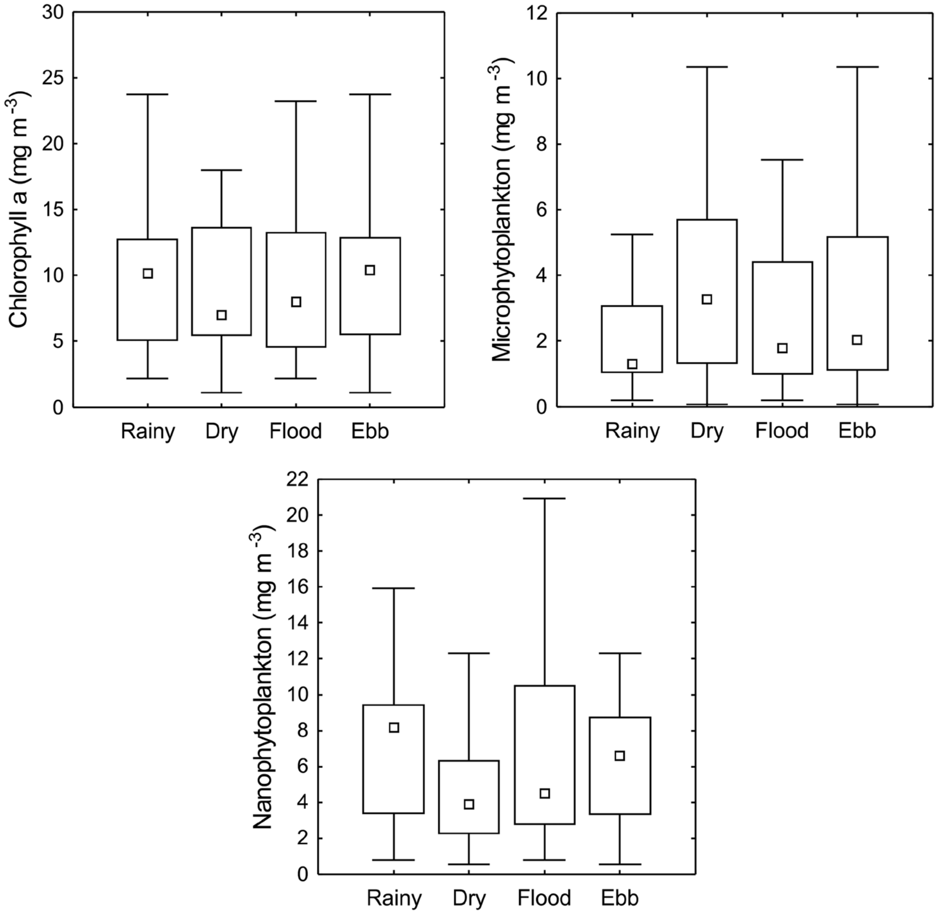

Figure 5. Seasonal and tidal variation of chlorophyll *a* (mg m^{-3}), microphytoplankton (mg m^{-3}) and nanophytoplankton (mg m^{-3}) in the São Marcos Bay.

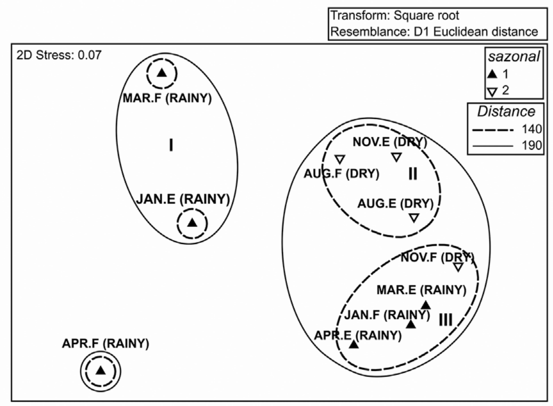

Figure 6. Representation of seasonal periods and sampling months by nMDS ordination based on phytoplankton abundance, precipitation, and salinity showing the arrangement of sampling months in three major groups (I, II, and III). Note: 1=rainy season, 2=dry season, JAN=January, APR=April, MAR=March, AUG=August, NOV=November and F=flood tide and E=ebb tide.

the variation in precipitation and salinity the data was represented as bubbles in the nMDS graph (Figure 7). The analysis showed a clear seasonal pattern with salinity values varying on account of the of precipitation regime. In response to the environmental fluctuations, the abundance of *Nitzschia* sp., *Synedra* sp., and *Coscinodiscus* sp. were higher in the sampling months of higher precipitation and lower salinity, while *Diploneis weissflogii* had higher abundance in the period of higher salinity (dry season).

The SIMPER analysis showed an overall average dissimilarity of 48.62% in relation to the discriminative species that differentiates their distribution between the rainy season and dry season. Among the discriminative species, *Nitzschia* sp. revealed the predominant diatom in this study with 29.12% of contribution and higher mean abundance in the rainy season (4.02 x 10^4 cells L^{-1}) followed by *Diploneis weissflogii, Synedra* sp.,

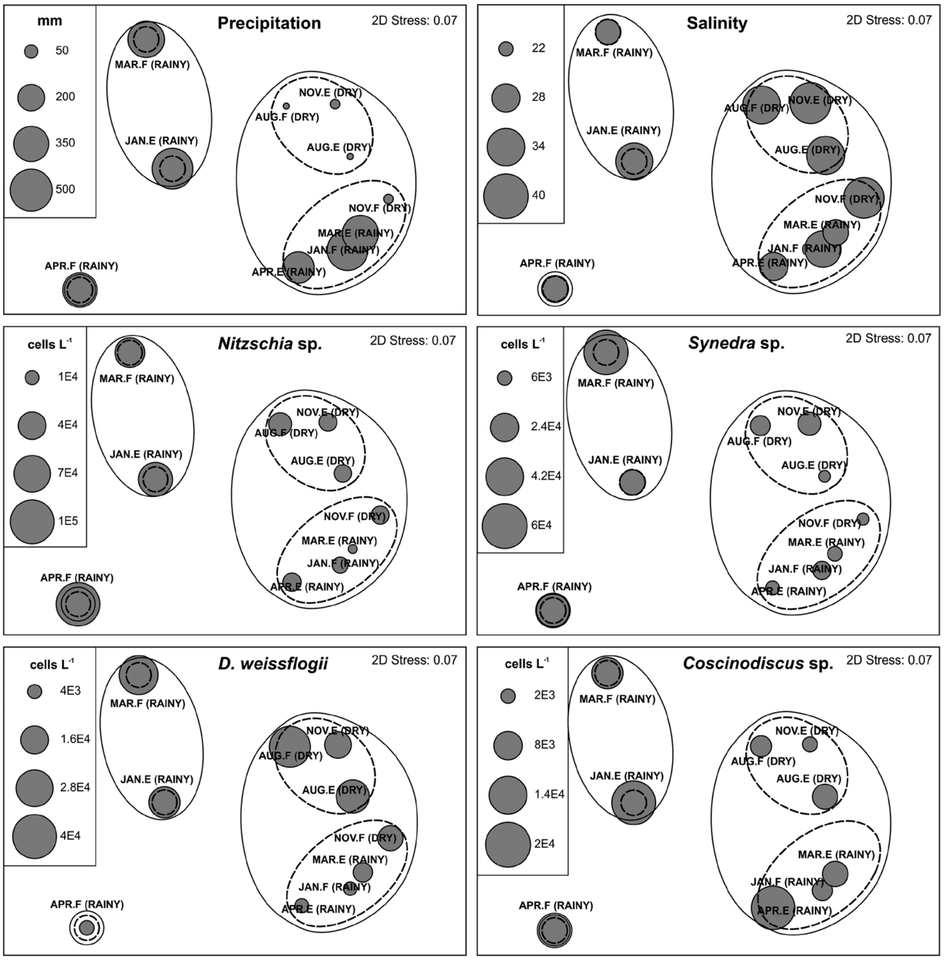

Figure 7. Bubble plot of precipitation, salinity and cell abundance (cells L^{-1}) of dominant species pointed out by Dominance Index (Y) in the São Marcos Bay superimposed in the nMDS ordination.

Coscinodiscus sp. and *Eupodiscus radiates*, which had elevated cell abundance in the rainy season, exceptionally *D. weissflogii* (Table 4).

The PCA explained 51.88% of the total variance (Factor 1=3.44; Factor 2=2.28; Factor 3=1.53) showing the relation between the precipitation regime and principal variables in the São Marcos Bay (Figure 8). Factor 1 explained 24.57% of the variance, where precipitation (0.93) was negatively correlated with wind speed (-0.93), dissolved oxygen (-0.89), salinity (-0.71). Chlorophyll *a* concentration (0.36) was directly correlated with turbidity (0.80) and inversely correlated with Secchi depth (-0.74) and pH (-0.68) in the factor 2 (16.34%). Phytoplankton abundance was directly correlated with PO_4^{-3} (0.59) and inversely correlated with NH_4^+ (-0.65) in factor 3 (10.97%).

Regarding the linear correlation, the phytoplankton abundance correlated positively with precipitation (0.53) which was negatively correlated with wind speed (-0.95) and dissolved oxygen (-0.81). Salinity was directly correlated with dissolved oxygen (0.62) and inversely with temperature (-0.84). The abundance of *Nitzschia* sp. was correlated with higher values of turbidity (0.41) and lower nitrate concentrations (-0.39). *Diploneis weissflogii* showed relationship with dissolved oxygen (0.43). In terms of chlorophyll *a* concentration, nanophytoplankton group showed higher correlation (0.84) (Table 5).

DISCUSSION

The dynamics of phytoplankton community in tropical estuarine complex, dominated by macrotides, are influenced mainly by the seasonal conditions of the winds, tides, precipitation patterns, the discharge of rivers and the availability of nutrients; or by the combination of these factors together (Eskinazi-Leça et al., 2004; Sousa et al.,

Table 4. Principal taxa contributing to the mean dissimilarities between rainy (1) and dry (2) seasons, as determined by the SIMPER analysis.

Taxa	Av. dissim	Contrib. %	Cumulative %	Mean abund. 1	Mean abund. 2
Nitszchia sp.	14.16	29.12	29.12	4.02E+04	1.92E+04
Diploneis weissflogii	8.981	18.47	47.59	1.19E+04	2.13E+04
Synedra sp.	7.764	15.97	63.56	2.19E+04	8.97E+03
Coscinodiscus sp.	5.053	10	73.95	1.12E+04	3.20E+03
Eupodiscus radiatus	3.946	8.117	82.07	4.19E+03	6.34E+03
Thalassiosira sp.	3.707	7.625	89.69	9.25E+03	2.57E+03
Thalassiosira leptopus	2.644	5.437	95.13	4.81E+03	3.94E+03
Cyclotella stylorum	2.368	5	100	6.27E+03	2.14E+03

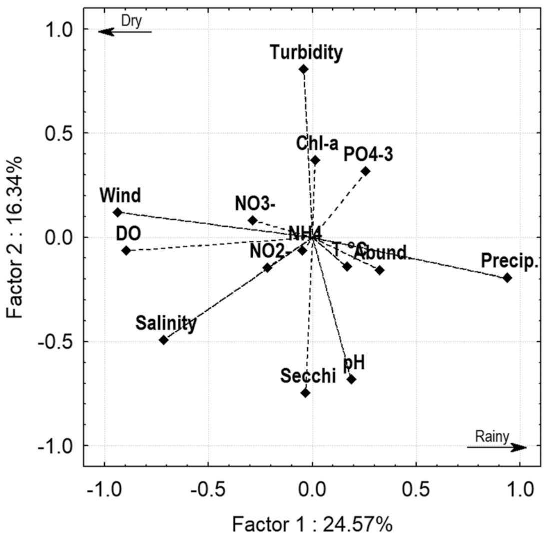

Figure 8. Principal components analysis performed on major variables in the São Marcos Bay. Note: Precip.=Precipitation; Wind=Wind speed; T °C=Water temperature; Sal.= Salinity; pH; Secchi=Secchi depth; Turb.=Turbidity; DO =Dissolved oxygen; NH_4^+=ammonium concentrations; NO_2^-=Nitrite concentrations; PO_4^{-3}=Orthophosphate concentrations; NO_3^-=Nitrate concentrations; Chl-a=Chlorophyll *a* and Abund.=Phytoplankton abundance.

2009; Chowdhury et al., 2017). In the São Marcos Bay, the well-defined precipitation regime is an important regulator of phytoplankton structure and chlorophyll *a* as well as some hydrological variables, for example, associated with the river discharge increasing may contribute mainly to salinity, dissolved oxygen and nutrients lowering.

The precipitation regime in the state of Maranhão is controlled by the Intertropical Convergence Zone (ITCZ), which produces the highest rainfall in northeastern Brazil between the months of January and May. The ITCZ, during the following months of the year, is much less intense and results in a dry season (Dias et al., 2016; Funceme, 2016). This seasonal pattern was confirmed along the study period and it was in accordance with previous studies for the northeastern region (Azevedo et al., 2008; Rodrigues and Cutrim, 2010; Duarte-dos-Santos et al., 2017).

In the São Marcos Bay, the salinity showed a clear seasonal variation with values lower in the period of higher precipitation and high phytoplankton abundance. In tropical coastal systems, salinity acts as an ecological barrier and regulator for aquatic life distribution, since temperature and sunlight have slight variation through the year (Santos-Fernandes et al., 1998; Attrill and Rundle, 2002). In addition, freshwater influx, seawater incursion and industrial as well as domestic effluent discharges may result in well-marked longitudinal gradients of salinity and nutrients that led to significant spatio-temporal changes in the structure of the phytoplankton community (Chowdhury et al., 2017).

The spread TS diagram analysis for the sampling period reveals an increase in salinity observed during the dry season. It can be explained by the fluvial flux decreasing (Mearim and Pindaré Rivers) and marine waters influence on the São Marcos Bay, which is probably due to the decrease in the aspect ratio determined by free surface variation and surface gravity wave propagation (Dias et al., 2013).

The variation of dissolved oxygen obtained in the bay are common to marine systems of Maranhense coast in natural conditions (Azevedo et al., 2008; Rodrigues and Cutrim, 2010). It may be explained by the tidal regime (macrotides) and consequently high local hydrodynamic that combined can accentuate the ocean-atmosphere interface and the oxygenation of the water (Monteiro et al., 2009; Matos et al., 2016). Furthermore, precipitation influences the fluctuations in the oxygen dissolved, because can lead to the increase of dead organic matter decreasing its concentration during the oxidation process (Kress et al., 2002). The undersaturation found in the bay did not indicate evidence of eutrophication events and polluted

Table 5. Matrix of the Pearson correlation between environmental parameters and phytoplankton abundance. Note: Precip.=Precipitation; T °C =Water Temperature; Sal.= Salinity; pH; Secchi=Secchi depth; Turb.=Turbidity; DO=Dissolved Oxygen; NH_4^+=ammonium concentrations; NO_2-=Nitrite concentrations; PO_4^{-3}=Orthophosphate concentrations; NO_3^-=Nitrate concentrations; Micro = Microphytplankton group; Nano=Nanophytoplankton group; Chl-a=Chlorophyll *a*; Abund.=Phytoplankton Abundance; Wind=Wind speed; Nitzs.=*Nitzschia* sp.; Syned.=*Synedra* sp.; Dipl.=*Diploneis weissflogii*; Thal.=*Thalassiosira* sp. Significant correlations are indicated in bold text.

	Prec.	**T °C**	**Sal.**	**pH**	**Secchi**	**Turb.**	**DO**	**NH_4^+**	**NO_2-**	**PO_4^{-3}**	**NO_3^-**	**Micro**	**Nano**	**Chl-a**	**Abund.**	**Wind**	**Nitzs.**	**Syned.**	**Dipl.**	**Thal.**
Prec.																				
T °C	0.32																			
Sal.	**-0.52**	**-0.84**																		
pH	0.40	**-0.49**	0.24																	
Secchi	0.06	-0.34	0.40	0.20																
Turb.	-0.13	0.25	-0.30	-0.39	**-0.58**															
DO	**-0.81**	-0.53	**0.62**	-0.11	0.07	0.04														
NH_4^+	0.02	-0.13	0.06	0.12	0.03	0.15	-0.06													
NO_2^-	-0.15	-0.10	0.06	0.01	0.07	-0.05	0.28	-0.06												
PO_4^{-3}	0.11	0.38	-0.30	-0.29	-0.09	0.06	-0.13	-0.31	-0.11											
NO_3^-	-0.25	-0.07	0.07	-0.14	-0.05	-0.01	0.17	0.06	0.01	-0.14										
Micro	-0.24	0.20	-0.01	-0.44	-0.28	**0.47**	0.03	-0.09	-0.07	-0.03	0.18									
Nano	0.18	-0.01	0.00	0.04	0.10	0.00	-0.30	0.04	-0.07	-0.11	-0.05	0.34								
Chl-a	-0.02	0.10	-0.01	-0.22	-0.10	0.27	-0.18	-0.03	-0.09	-0.09	0.06	**0.79**	**0.84**							
Abund.	0.53	0.21	-0.24	-0.09	0.16	-0.20	-0.18	-0.13	0.07	0.09	0.08	-0.15	-0.12	-0.16						
Wind	-0.95	-0.39	**0.57**	-0.23	-0.11	0.07	**0.78**	0.03	0.09	-0.16	0.25	0.22	-0.22	-0.02	-0.38					
Nitzs.	0.17	0.25	-0.21	-0.17	-0.06	**0.41**	-0.17	-0.19	0.02	0.35	**-0.39**	0.27	0.18	0.28	0.16	-0.23				
Syned.	0.20	0.10	-0.18	0.04	-0.09	-0.07	-0.22	-0.23	-0.01	0.27	-0.27	-0.09	-0.03	-0.07	**0.54**	-0.17	0.50			
Dipl.	-0.22	-0.24	0.10	0.08	-0.04	0.15	**0.43**	0.19	0.04	0.07	-0.10	-0.15	0.00	-0.08	0.06	0.21	0.24	0.21		
Thal.	0.13	-0.07	-0.01	0.13	0.13	-0.06	-0.02	-0.12	-0.03	0.02	-0.33	-0.10	-0.15	-0.15	**0.54**	-0.10	0.43	0.67	0.31	

zones which is expected in urbanized areas (Macêdo and Costa, 1978). Seasonal variation of dissolved oxygen was also observed in other tropical estuaries (Borges et al., 2012; Otsuka et al., 2016).

In estuarine systems, light availability and water turbidity can limit phytoplankton growth (Demers et al., 1986; Domingues et al., 2011). In the São Marcos Bay, high turbidity results in a narrow euphotic zone due to sunlight attenuation with photosynthetic process (chlorophyll *a*) restricted to surface layers (Teixeira et al., 1988; Azevedo et al., 2008). Turbidity of water is usually caused by the presence of suspended matter (organic and inorganic matter) in water column that may decrease the light intensity and affect the phytoplankton development. Nevertheless, the elevated turbidity associated with high nutrient loading may enhance the growth of phytoplankton in high hydrodynamic systems (Rai and Rajashekhar, 2014).

In this study, turbidity showed a positive relationship with chlorophyll *a*, wind and tidal currents (macrotides). This relationship indicates an intense estuarine mixing, which promotes high resuspension of sediments and ticopelagic species such as *Nitzschia* sp. with high abundance along the present study. In terms of contribution of phytoplankton biomass (chlorophyll *a*), the autotrophic plankton was mainly composed by nano-picoplankton. This fact was also reported by Guenther et al. (2012), Kocum and Sutcu (2014), and Sathicq et al. (2017). Guenther and Bozelli (2004) state that as small cells show high surface/volume ratio, they are capable of remaining in suspension longer and the lateral transport did not become size-selective.

In general, riverine nutrients are essential sources of production of phytoplankton in coastal areas (Sin et al., 2015). Variation in nitrate and its reduced inorganic compounds are predominantly the results of biologically activated reactions (Kathiravan et al., 2013). Usually, high levels of nitrate are recorded in the rainy period (Symader and Bierl, 1998). It is due to the runoff and enrichment of terrogenous deposit with a lot of nutrients, together with high temperature and water mixing might favor the nitrate replenishment mechanism (Kalaiarasi et al., 2012). The efficient assimilation by phytoplankton and enhancement by surface runoff results in large scale spatio-temporal variation of nitrate in the coastal areas (Noriega et al., 2005).

The characterization of phytoplankton communities has become an important tool to understand the functionality of ecosystems (Soria-Píriz et al., 2017; Santana et al., 2018). The phytoplankton community composition in the São Marcos Bay revealed diatoms as the most successful phytoplankton group (e.g., growth and abundance). The wild occurrence of diatoms in coastal ecosystems is based on their euryhaline nature and affinity for favorable high nutrient environments (Paiva et al., 2006; Rodrigues and Cutrim, 2010; Zhou et al., 2016).

Depending upon the adaptability to salinity gradients, euryhaline species, which occur throughout estuarine systems in varying density, and stenohaline species can show elevate abundance when the environmental conditions are favorable (Chowdhury et al., 2017). The presence of some riverine phytoplankton in the rainy season shows the succession through the seasons (Honorato da Silva et al., 2009; Santiago et al., 2010) and its importance to the bulk biomass, due to the increase of river discharge. However, the dominance of diatoms *Nitzschia* sp., *Diploneis weissflogii* and *Synedra* sp. confirms their capacity to be successful in dynamic environments.

CONCLUSIONS

The high hydrodynamics found in the São Marcos Bay, governed by macrotidal regimes, may be considered as the main driving force that leads to an ecological stability scenario for the phytoplankton community. The precipitation regime was also an important factor capable of driving favorable hydrological conditions to phytoplankton development, promoting seasonal variations in terms of salinity, dissolved oxygen, turbidity, and nutrient concentrations. Hence, the composition of phytoplankton community dominated by diatoms showed *Nitzschia* sp., *Diploneis weissflogii* and *Synedra* sp. as the most representative species and confirmed their capacity to be successful in dynamic environments such as macrotidal estuaries. In addition, the phytoplankton biomass characterized the bay as quite productive with the nanophytoplankton as the dominant fraction in both seasons and tides. Thus, these results are supported by high hydrodynamics associated to precipitation regime of the São Marcos Bay and consequently, does not allow the rapid phytoplankton growth that could contribute to the eutrophication processes and poor water quality.

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