Algal blooms and trophic state in a tropical estuary blocked by a dam (northeastern Brazil)

Ana Karoline Duarte dos Santos Sá[1], Marco Valerio Jansen Cutrim[2,*], Denise Santos Costa[2], Lisana Furtado Cavalcanti[1], Francinara Santos Ferreira[2], Amanda Lorena Lima Oliveira[2], Jefferson Horlley Feitosa Serejo[2]

[1] Universidade Federal de Pernambuco - Departamento de Oceanografia - Laboratório de Fitoplâncton (Av. Arquitetura, s/n. - Cidade Universitária 50740-550 - Recife - PE - Brazil)
[2] Universidade Federal do Maranhão - Programa de Pós-Graduação em Oceanografia - Laboratório de Ficologia (Avenida dos Portugueses s/n Cidade Universitária Dom Delgado - 65080-805 - São Luís - MA -Brazil)

* Corresponding author: marco.cutrim@ufma.br

ABSTRACT

The Bacanga River Estuary is socioeconomically important due to artisanal fishing and aquaculture. It is blocked by a dam and is under human pressure along its drainage basin, intensifying the eutrophication process. This study reports on the occurrence of phytoplankton blooms and trophic state (TSI and TRIX) at six sampling sites during the annual cycle. The estuary was divided into downstream and upstream regions. Higher salinity, turbidity, depth, and lower dissolved oxygen levels were found downstream; whereas, high levels of chlorophyll a and nutrient concentrations were observed in both regions. There were blooms of *Leptocylindrus danicus* (1.45×10^6 cells L^{-1}) and *Skeletonema costatum* (1.89×10^6 cells L^{-1}) downstream; whereas phytoflagellate proliferation, such as those of *Chlamydomonas* sp. (13.17×10^6 cells L^{-1}), *Euglena gracilis* (7.84×10^6 cells L^{-1}), and *Euglena proxima* (1.03×10^6 cells L^{-1}) were recorded upstream, with *Chlamydomonas* sp. as the discriminant species of this zone. Both trophic indices (TSI; TRIX) indicated elevated trophic conditions for the estuary, classifying it as hypereutrophic. Nevertheless, TSI only showed a significant relationship with some specific phytoplankton blooms. Thus, TSI seems to be the trophic index with a better response in the assessment of estuarine ecological functioning.

Descriptors: Bacanga estuary, *Chlamydomonas* bloom, TSI, TRIX, Maranhão, macrotidal regime.

INTRODUCTION

Eutrophication in estuaries has been a major ecological problem that is caused by the inputs of natural and anthropogenic nutrients through the discharge of sediments, the atmosphere, the ocean, or even through the exchange of nutrients with the coastal region (Ferreira et al., 2011; Statham, 2012; Cloern et al., 2014; Hayn et al., 2014; Monteiro et al., 2016). The estuarine ecosystems are under heavy anthropogenic pressures that compromise their water quality and biodiversity, causing a loss of habitat and a reduction in the quality of life of riverside communities. Because of these impacts, it is necessary to characterize and assess the current conditions of these environments based on an ecosystemic and trophic approach (Cloern, 2001; Herrera-Silveira et al., 2009; Smith and Schindler, 2009; Ferreira et al., 2011; Cotovicz-Júnior et al., 2012a).

Therefore, it is important to measure the excessive growth of phytoplankton, also known as algal blooms, because these events are associated with increased eutrophication caused by anthropogenic activities (Napiorkowska-Krzebietke et al., 2017).

The use of trophic indices to assess eutrophication status has been recommended by the Expert Group on the Scientific Aspects of Marine Environmental Protection (GESAMP) (Moncheva et al., 2001), as they can reflect the anthropogenic influence on water quality and the ecological functioning of rivers, lakes, and reservoirs (Cunha et al., 2013).

Trophic status indices provide information on how the availability of nutrients and light controls the development of phytoplankton, and how they can serve as useful tools for monitoring and protecting coastal ecosystems (Lemley et al., 2015). For example, the trophic state index (TSI) is used to evaluate the degree of eutrophication in tropical environments (Toledo et al., 1983), and the following variables are used to calculate the index: concentrations of chlorophyll *a*, total phosphorus, and Secchi depth. This index, according to the external inputs of nutrients, such as domestic sewage, industrial, and agricultural waste, contribute to the classification of water bodies according to the degree of eutrophication (Maia et al., 2015).

The trophic index (TRIX) is used to determine the trophic status of coastal waters based on biological (chlorophyll-a) and physical-chemical (oxygen, nutrients) metrics (Nasrollahzadeh et al., 2008). TRIX was originally developed for Italian coastal waters and then applied in many European seas. It is currently used in Brazilian coastal waters (Cotovicz-Júnior. Et al., 2013; Nascimento-Filho et al., 2013; Monteiro et al., 2016; Araújo et al., 2017). This trophic evaluation with phytoplankton indicator species and algal blooms contributes to the comprehensive assessment of phytoplankton, one of the biological quality elements in coastal waters (Pettine et al., 2007; Garmendia et al., 2013; Brugnoli et al., 2019).

These indices assume that the eutrophication processes depend mainly on algal biomass (Giordani et al., 2009), which considered a key element in determining water quality. Structural changes in their community and are often expressed by ecological indices (Spatharis and Tsirtsis 2010). Quantifying eutrophication in estuarine environments in Brazil has been a challenge. There are no conceptual models and standards to determine the trophic status according to the national environmental legislation (Cotovicz-Júnior et al., 2012b; Nascimento-Filho et al., 2013).

Based on this assumption, this study was carried out in a shallow, urbanized, and dammed tropical estuary. A large amount of sewage is discharged into the estuary daily, leading to contamination by toxic substances, thus, putting aquatic life at risk (Duarte-dos-Santos et al., 2017). This study aimed to relate the occurrence of phytoplankton blooms and trophic state using the TSI and TRIX indices to assess the ecological functioning of the Bacanga River Estuary (BRE) and the effects of nutrient enrichment on the phytoplankton community.

The estuary of the Bacanga River is inserted in the basin of the same name. It is considered the second largest in length and demographic density on São Luís Island, with a deficient sewage network, totaling 850 discharge points of untreated domestic sewage. It is worth mentioning that potentially polluting industrial plants are located in this basin, such as a port complex, beyond the surrounding population using the water resources available in the area. The effects on the eutrophication process in the phytoplankton communities were explored to identify the relationships of such indexes with nutrients concentrations in the tropical estuary blocked by a dam.

METHODS

STUDY AREA AND SAMPLING DESIGN

The Bacanga River basin is in the northwestern part of the city of São Luís, in the Brazilian state of Maranhão, between 2°32′26″ and 2°38′07″ S and 44°16′00″ and 44°19′16″ W. The basin occupies an area of 11,030 ha, which accounts for 12.33% of the island of São Luís, and it is divided into five sub-watersheds. There are approximately 58,844 households and a population estimate of 223,343 inhabitants distributed across 41 neighborhoods according to the São Luís Institute of City, Research and Urban and Rural Planning (INCID). It is worth mentioning that part of the Bacanga River basin is an environmental preservation area, according to State Decree nº 7545, on March 2, 1980.

The Bacanga River estuary (BRE) has a length of 22 km from its source to São Marcos Bay (Figure 1). This estuary is an inlet that penetrates the continental region near the city of São Luís and has two tributaries, Bicas and Gapara. Despite its perennial hydrological regime, Bacanga river flow is discrete (low flow) and is
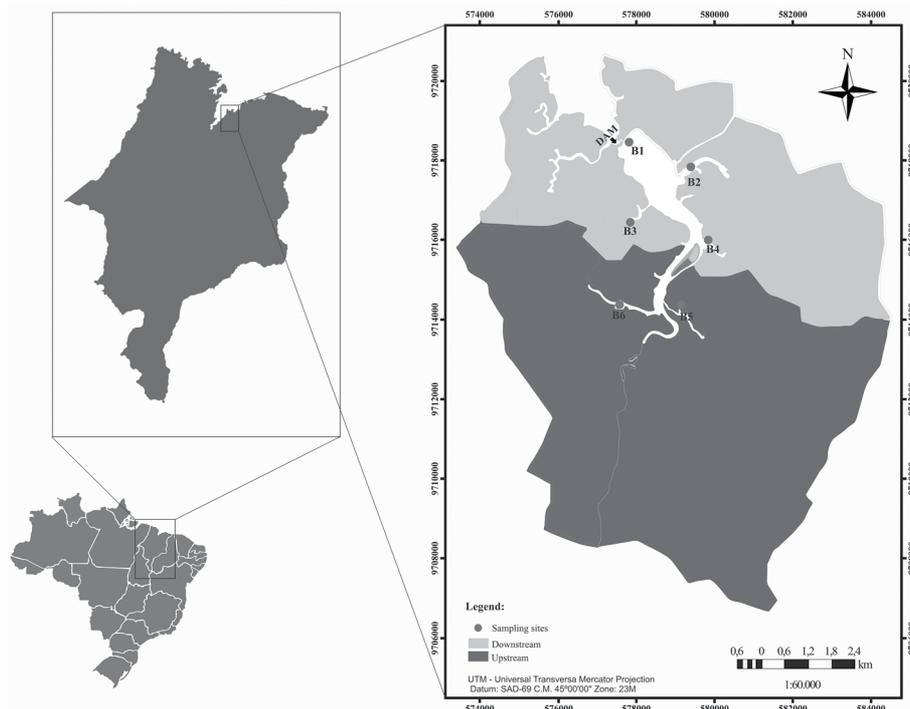
Figure 1. Bacanga River Estuary and sampling sites (B1, B2, B3, B4, B5, B6), where: DAM - region of estuary blocked.

subject to variations according to the season, which favor the advance of marine waters that periodically flood the wide fluvial-marine plain, reaching basically the low and medium course of the river. As a result, the water body has estuarine characteristics and behaves according to the semidiurnal tidal regime, with tidal heights ranging between 4 and 6 m.

The connection with the sea is blocked by a dam that controls the entrance and the level of the seawater up to the 4-m level, in addition to reducing the inflow of the estuary waters. The hydraulic movement follows a circular, unidirectional, and irreversible pattern, which begins in the floodgates and moves upstream through the left side, returning through the right side of the dam. This behavior is repeated in both tidal periods (Pitombeira and Morais, 1977).

According to the rainfall historical average of the ten years (2003-2013), the seasonal cycle is well defined. The rainy season is from January to July, and the dry season is from August to December. During the sampling campaigns, the highest monthly rainfall rate was registered in February (296 mm) and the lowest in September (2.8 mm), according to the Brazilian Institute of Meteorology (INMET).

The samples were collected from six locations (Figure 1) along the estuary during the annual cycle between 2012 and 2013. The sampling sites B1, Bicas stream (B2), Jambeiro stream (B3), and Coelho stream (B4) are located downstream, influenced mostly by seawater. On the other hand, sites B5 and B6 are located upstream, influenced mostly by freshwater. Six bimonthly samplings were carried out, three of them during the rainy season (April 2012, June 2012, and February 2013) and three during the dry season (August 2012, September 2012, and December 2012). The sampling was carried out from the downstream to the upstream region during the flood tide, according to the lunar cycle and the macrotidal dynamics in the BRE.

PHYSICAL AND CHEMICAL PARAMETERS

The assessment of water quality was based on physical and chemical parameters, such as water temperature, pH, salinity and total dissolved solids (TDS), measured in the surface layer (50 cm deep) with a multiparametric probe (HI-9828). The water depth was measured with a digital probe (Speedtech), the transparency of the water with a Secchi disk, and the turbidity with a turbidimeter (Lamotte-2020).

Dissolved oxygen and biochemical oxygen demand (BOD_5) were determined according to the chemical method of Winkler, modified by Golterman et al. (1978) with 5 days of incubation at 20°C for BOD_5.

NUTRIENT MEASUREMENTS

For the analysis of nutrients, 2 liters of water was collected in the surface layer (50 cm deep) with a Van Dorn bottle. The quantification of total phosphorus (TP) and ammonium ion (NH_4^+) was made following the methodology described by Koroleff (1983). Nitrate (NO_3^-) and nitrite (NO_2^-) were determined according to Strickland and Parsons (1972); silicate (SIO_2^-) and orthophosphate (PO_4^-) according to Grasshoff et al. (1983). Dissolved inorganic nitrogen (DIN) was obtained from the sum of NH_4^+-N, NO_2^--N, and NO_3^--N; Dissolved inorganic silicate (DIS) from the SIO_2^-, and for dissolved inorganic phosphorus (DIP) only the PO_4^{-3} was used. To evaluate the stoichiometric limitations, Redfield ratios were used, according to Pavlidou et al. (2004) and Xu et al. (2008).

PHYTOPLANKTON BIOMASS AND ABUNDANCE

The phytoplankton biomass was estimated by chlorophyll *a* concentration. Water samples (2L) for chlorophyll *a* (µg L^{-1}) determination were filtered through Whatman GF/F glass fiber filters, and pigment extraction was performed with 90% acetone. The volume of the filtrate ranged from 0.10 to 0.25 L. Pigment concentration was measured by spectrophotometry (Parsons et al., 1984; Greenberg et al., 1992) and calculations were done according to Strickland and Parsons (1972).

To analyze the structure of the phytoplankton community and the phytoplankton abundance, sub-surface water samples (250 mL) were collected with a Van Dorn bottle and fixed with Lugol solution. The identification and counting of phytoplankton cells were carried out with an inverted microscope at 400 × magnification, following Utermöhl's technique (Utermöhl, 1958). At least 100 fields were counted using sedimentation chambers of 25 ml . The phytoplankton community at each sampling point was analyzed taxonomically, at the lowest taxonomic level, according to the specialized literature and updated according to AlgaeBase database. The phytoplankton were considered a bloom when a particular taxon reached or exceeded a density of 1×10^6 cells L^{-1} (Livingston, 2007).

TROPHIC STATE INDEXES

The trophic index (TRIX) was adapted by Cotovicz-Júnior et al. (2012b), where a correction was made for a temporal data series. Vollenweider et al. (1998) recommended the mean ± 2.5 standard deviation as an appropriate statistical tool to define the upper and lower limits of the parameters used in the TRIX index. Thus, extreme or inconsistent values were excluded. The values were converted to log base 10. The TRIX for BRE was calculated according to the following formula (1):

$$\mathrm{TRIX} = \frac{log\left[Chla.\ aD\%O.\ DIN.\ DIP\right] - [k]}{m} (1)$$

Where *Chla* is the chlorophyll *a* concentration (µg L^{-1}); *aD%O* is the absolute deviation of dissolved oxygen saturation; DIN is the dissolved inorganic nitrogen, DIP is the dissolved inorganic phosphate, *k* is equal to 1.5, and *m* is equal 1.2. Trophic scales and descriptors for water quality were defined according to Giovanardi and Vollenweider (2004), Penna et al. (2004), Nasrollahzadeh et al. (2008) and Cotovicz-Júnior et al. (2012b). Numerically, the index is scaled from 0 to higher than 8, covering a wide range of trophic conditions from ultra-oligotrophic to hyper-eutrophic (Table 1).

The trophic state index (TSI) was proposed by Carlson (1977) and was modified for tropical environments in this paper. The formulas used for calculating the TSI for lotic environments are given below (2 and 3):

$$\mathrm{TSI\ Chla} = \frac{10x\left(6 - \left((-0.7 - 0.6x\left(\mathrm{In\ Chla}\right)\right)\right.}{\mathrm{In\ 2}} - 20 (2)$$

$$TRI\ TP = \frac{10x\left(6 - \left((0.42 - 0.36x\left(\mathrm{In\ TP}\right)\right)\right.}{\mathrm{In\ 2}} - 20 (3)$$

where TP is the total phosphorous concentration measured at the water surface (µg L^{-1}); Chla is the Chlorophyll *a* concentration measured at the water surface (µg L^{-1}); *In* is the natural logarithm. Thus, the results are equal to the arithmetic average of the total phosphorous and chlorophyll *a* indices that were used to classify the estuary into different trophic

Table 1. Classification of trophic status for estuarine waters according to the trophic index (TRIX) model.

TRIX	Conditions	Trophic State
< 2	Water very poorly productive and very low trophic status	Excellent (Ultra-Oligotrophic)
2-4	Water poorly productive and low trophic status	High (Oligotrophic)
4-5	Water moderately productive and medium trophic status	Good (Mesotrophic)
5-6	Water moderate to very productive and high trophic status	Moderate (Mesotrophic to Eutrophic)
6-8	Water very productive and high trophic status	Poor (Eutrophic)
>8	Water highly productive and highest trophic status	Very Poor (Hypereutrophic)

categories, including ultra-oligotrophic (TSI<47), oligotrophic (47<TSI≤52), mesotrophic (52<TSI≤59), eutrophic (59<TSI≤63), supereutrophic (63<TSI≤67) and hypereutrophic (TSI>67), according to the classification scheme from Cunha et al. (2013).

STATISTICAL ANALYSIS

For the numerical analysis and spatial graphical representation, the mean of the spatial data from both downstream and upstream in the estuary in an annual cycle was used. Before these analyses, one-way PERMANOVA was performed to determine the significant differences ($p<0.05$) in the physical-chemical factors between the downstream and upstream regions. To test the differences ($p <0.05$) of the environmental variables in relation to the locations, parametric (ANOVA-F unilateral) or nonparametric (Kruskal-Wallis-H) tests were performed according to normality (Kolmogorov-Smirnov) and homogeneity (Levene's test) of variances. Based on the correlations established between the trophic indices (TSI and TRIX) and the environmental variables ($R > 0.7$), a Cluster analysis was performed using the cluster group average method to determine the dissimilarity, based on Euclidean distance (square root transformed).

Non-metric multidimensional scaling (nMDS) was applied to determine the similarity of the trophic states concerning phytoplankton blooms. A SIMPER analysis, based on phytoplankton abundance, was also applied to identify the "characterizing species" and the "discriminating species" with regards to similarity and dissimilarity, respectively (Clarke and Gorley 2006), and then an ANOSIM was applied to test the significance of the similarities. The multiple linear regression model (GLM), with a progressive forward selection of the variables (using p<0.05), was applied to assess the relationships between the trophic indices and the algal blooms. Trophic indices (TRIX and TSI)

were used as predictors. The statistical analyses were performed using SPSS (version 24.0), STATISTIC (version 10.0), and Primer (version 6.0).

RESULTS

PHYSICAL AND CHEMICAL PARAMETERS

The spatial distribution of the physical and chemical parameters of the water monitored in the estuary are shown in Figure 2 and Table 2. Significant differences were registered between the downstream and upstream regions of the study area based on the one-way permutational multivariate analysis of variance (PERMANOVA) analysis (F = 3.64, $p = 0.02$).

This difference between the upstream and downstream regions was observed for salinity (F = 10.02 and $p = 0.003$) and local depth (H = 3.92, $p = 0.04$), with higher values found downstream. However, the dissolved oxygen (H = 1.62 and $p = 0.20$), pH (F = 0.78 and $p = 0.38$), water temperature (F = 3.21, $p = 0.08$), turbidity (F = 1.19 and $p = 0.28$), water transparency (F = 0.31 and $p = 0.58$), and total dissolved solids (F = 0.31 and $p = 0.90$) did not have significant differences among the sampling sites, showing homogeneous distribution along the two regions of the BRE. The five-day biochemical oxygen demand (BOD_5) values characterized the environment as polluted, according to national resolution CONAMA 357/05, for regions with concentrations above 10.0 mg L^{-1}, with highest concentrations downstream (H = 0.94 and $p = 0.33$) (Figure 2).

NUTRIENT MEASUREMENTS

The dissolved inorganic nitrogen (DIN) concentrations were significantly higher downstream (112.26 ± 113.39 µmol L^{-1}), with significant differences (H = 7.94 and p = 0.04) determined by high NH_4^+ concentrations (H = 7.57 and $p = 0.005$) among the sampling sites, mainly at B2 (Bicas stream). NO_2^- (H = 1.15 and
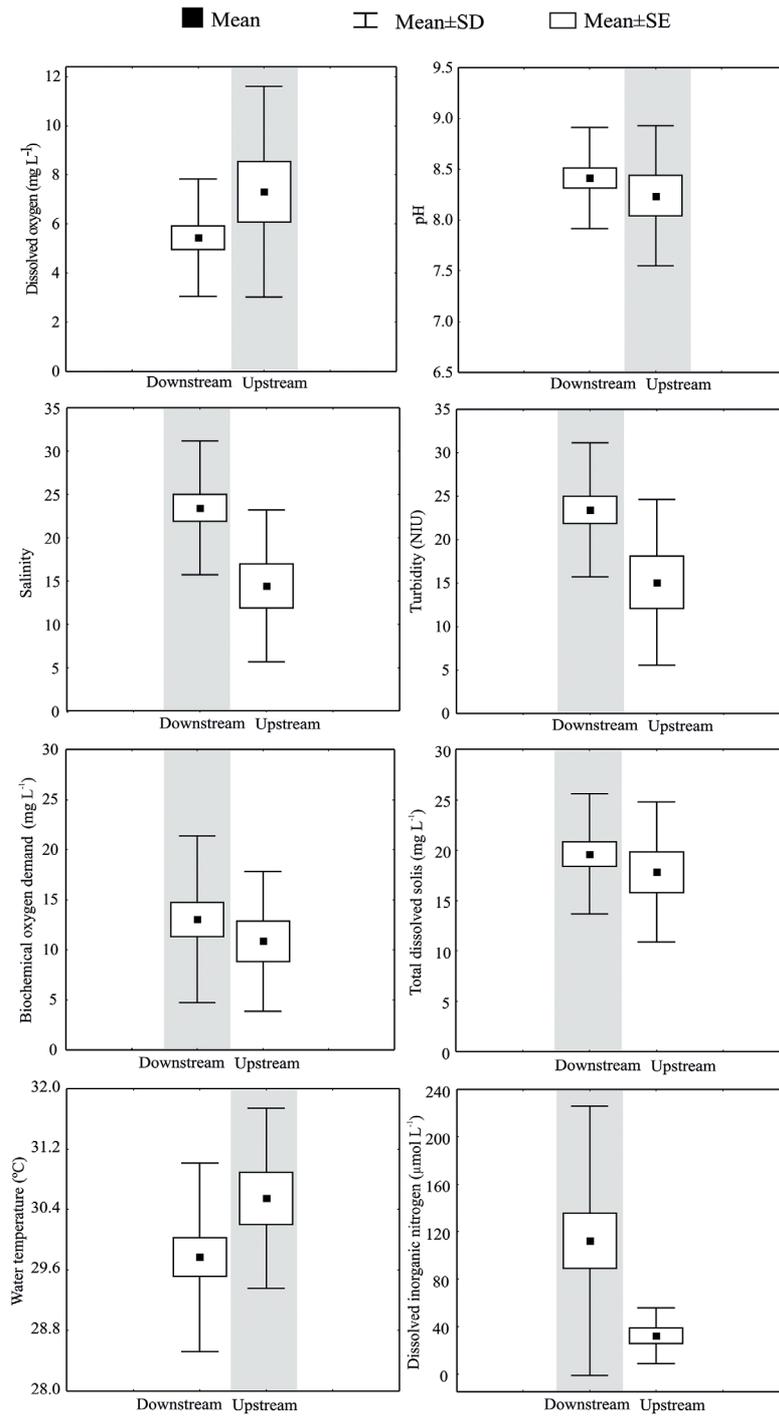
Figure 2. Spatial variations of the physical and chemical parameters (Mean ± standard deviation) observations for each site in the Bacanga River Estuary (BRE).

Table 2. Descriptive statistics of the data collected in the Bacanga River Estuary (BRE).

Variable	Unit.	Downstream	Median	Upstream	Median
DO	mg L^{-1}	5.44±2.39	4.95	7.32±4.28	5.08
pH	-	8.41±0.49	8.38	8.24±0.68	8.38
Temperature	°C	29.77±1.24	29.98	30.34±1.19	29.77
Salinity	-	23.44±7.70	24.72	14.41±8.77	12.5
Turbidity	NTU	23.45±7.70	24.71	15.09±8.77	12.5
Secchi	m	0.78±0.24	0.70	0.79±0.28	0.79
Depth	m	3.05±2.52	2.71	0.82±1.13	0.55
NO$_3^-$	µmol L^{-1}	0.20±0.98	0.01	ND	ND
NO$_2^-$	µmol L^{-1}	0.49±0.39	0.43	0.42±0.30	0.28
NH$_4^+$	µmol L^{-1}	111.57±113.49	80.68	31.80±23.25	30.22
DIN	µmol L^{-1}	112.26±113.39	83.53	32.23±23.51	30.55
DIP	µmol L^{-1}	26.53±47.06	0.32	26.53±48.13	0.32
DIS	µmol L^{-1}	60.46±82.38	17.98	77.81±96.58	33.29
TP	µmol L^{-1}	6.13±5.74	4.84	3.79±5.44	3.95
BOD$_5$	mg L^{-1}	13.03±8.33	10.79	10.86±6.98	7.00
DIN:DIP	-	211.38±257.20	162.26	57.71±78.40	27.02
DIN:DIS	-	3.05±2.52	2.71	1.09±1.38	0.83
DIS:DIP	-	158.66±272.15	52.69	224.48±320.83	79.03
Chlorophyll *a*	µg L^{-1}	34.26±32.70	26.54	33.20±46.33	14.15

ND =Non-detected concentration

$p = 0.27$) and NO$_3^-$ (H = 0.54 and $p = 0.36$) were very low downstream and upstream, with no significant differences. In relation to downstream and upstream zones, no significant differences were observed to DIP concentrations (PO$_4^{-3}$) (H = 0.02 and $p = 0.87$), total phosphorus (TP) (H = 0.17 and $p = 0.6$) or DIS concentrations (SiO$_2^{-3}$) (H = 0.70 and $p = 0.38$) (Table 2).

When considering the Redfield ratio in BRE, the DIN:DIP ratio was higher downstream (F = 4.04 and $p = 0.05$), specifically in B2. Likewise, the DIN:DIS ratio was higher in the lower reaches of the estuary (F = 5.36, $p = 0.02$), mainly in B4. Regarding the DIS:DIP ratio, there were no significant differences (F = 0.28 and $p = 0.58$) among the sampling sites, with lower values at B1 (near the dam) and B6 (upper region) (Table 2).

PHYTOPLANKTON COMMUNITY

A total of 66 species of phytoplankton belonging to five major taxonomical groups were identified: Bacillariophyta (41 species), Euglenophyta (nine species), Cyanobacteria (eight species), Dinophyta (four species), and Chlorophyta (four species). Diatoms were the dominant group in both downstream and upstream zones, followed by cyanobacteria with seven species identified downstream, and Euglenophyta with nine species identified upstream.

The estuary showed a significant variation in the density of phytoplankton (H = 8.30 and p = 0.003), lower downstream (0.02 ± 0.22 × 10^6 cells L^{-1}) and higher upstream (0.19 ± 0.15 × 10^6 cells L^{-1}). The density peaks (>10^6 cells L^{-1}) downstream were associated with diatom blooms of *Leptocylindrus danicus* with 1.45. × 10^6 cells L^{-1} (H = 0.92 and $p = 0.32$) and *Skeletonema costatum* with 1.89 × 10^6 cells L^{-1} (H = 8.30 and $p = 0.003$). In the upstream zone, high densities of phytoflagellates of the genus *Chlamydomonas* sp. 13.17 × 10^6 cells L^{-1} (H = 3.69 and p = 0.04), *Euglena gracilis* with 7.84 × 10^6 cells L^{-1} (H = 8.30 and $p = 0.003$), and *Euglena proxima* with 1.03 × 10^6 cells L^{-1} (H = 0.92 and $p = 0.32$) contributed to phytoplankton proliferation (Table 3).

The similarity percentages (SIMPER) analysis of the density of the phytoplankton species revealed a similarity contribution of 33.86%, with high similarity between the species observed downstream (30.57%), while the upstream percentage was only

Table 3. Summary of phytoplankton blooms dynamic. and representative species density in the Bacanga River estuary (BRE).

Species Density (x 10^6 cells L^-1)	Downstream				Upstream	
	B1	B2	B3	B4	B5	B6
Chlamydomonas sp.	0.00	0.00	0.00	0.00	0.00	13.17
Cylindrotheca closterium	0.00	0.00	0.01	0.00	0.06	0.11
Dinophysis sp.	0.18	0.02	0.02	0.05	0.04	0.00
Euglena gracilis	0.00	0.00	0.00	0.00	0.01	7.84
Euglena proxima	0.00	0.00	0.00	0.00	1.03	0.14
Leptocylindrus danicus	0.23	1.45	0.10	0.07	0.00	0.00
Protoperidinium sp1	0.18	0.07	0.03	0.04	0.02	0.00
Skeletonema costatum	1.89	1.49	0.05	0.00	0.01	0.01
Melosira varians	0.01	0.11	0.14	0.08	0.14	0.10
Trachelomonas sp.	0.00	0.00	0.00	0.00	0.76	0.00

3.29%. In the downstream area, the group with the highest similarity included *S. costatum* (29.12%), *Thalassiosira* sp. (21.20%), and *L. danicus* (20.57%) diatoms; and the second contained the dinoflagellates *Protoperidinium* sp1 (9%) and Dinophysis sp. (5%). In the upstream region, the main species were *E. proxima* (33.59%), *Melosira varians* (24.45%), and *Cylindrotheca closterium* (15.04%) (Table 4). The percentage contribution of the discriminant species to dissimilarity was 92.39%. The upstream discriminating species were *Chlamydomonas* sp. (29.41%), *E. proxima* (14.68%), and *Trachelomonas* sp. (10.59%); in the downstream region, only *L. danicus* (5.70%) was considered discriminant. *Skeletonema costatum* (9.33%) and *E. gracilis* (17.55%) were discriminant species upstream as well as downstream (Table 4).

In addition to the SIMPER analysis, the analysis of similarity (ANOSIM) global R test determined the level of significance of the differences in the structure of the phytoplankton community between the BRE zones. The results revealed a dissimilarity between the downstream and upstream regions with a global R of 0.71 and a significance level of 6.7%. The chlorophyll *a* concentration was not significantly different along the BRE (F = 0.006 and *p* = 0.33), with values ranging from 34.26 ± 32.70 µg L^-1 downstream to 32.20 ± 46.33 µg L^-1 upstream (Table 2).

TROPHIC STATE INDICES

The TRIX ranged from 8.39 ± 0.23 (upstream) to 8.52 ± 0.32 (downstream), suggesting hypereutrophic

conditions, characterizing the BRE as a very poor environment in terms of water quality. There was no significant difference between the downstream and upstream regions (F = 0.06 and p = 0.80). The TSI ranged from 67.23 ± 0.23 (upstream) to 68.28 ± 1.10 (downstream), suggesting high trophic conditions (hypereutrophic), with no significant difference between the two regions (F = 0.29 and *p* = 0.59) (Figure 3).

The TSI for chlorophyll *a* varied from 74.65 ± 1.51 (upstream) to 77.03 ± 0.75 (downstream), showing the same hypereutrophic conditions (F = 0.57 and *p* = 0.45). The TSI for total phosphorus ranged from 59.40 ± 2.12 (downstream) to 59.98 ± 0.36 (upstream), changing to a eutrophic state (F = 0.13 and *p* = 0.72). Site B3 showed mesotrophic conditions in relation to the TSI for total phosphorus with a value of 57.45 ± 2.61 (Figure 3).

RELATION OF THE TROPHIC STATE AND ALGAL BLOOMS

The association between the sampling sites through the hierarchical cluster analysis with the application of Euclidean distance showed the formation of two groups with a cut-off value of 0.73. The combinations indicated a similarity (0.42) among the sites B1, B2, B3 and B4 (downstream) forming group A, and between B5 and B6 (0.73) forming group B (upstream). The estuary is divided into two regions, with group A representing the downstream region and group B (Figure 4) in the upstream region, considering the variables of dissolved oxygen, salinity, turbidity, depth, and trophic state (TRIX and TSI).

Table 4. Phytoplankton *characterizing species* and *discriminating species* with mean density (x10⁶ cells L⁻¹) of species that contribute to the maximum dissimilarity between the assemblages (Bacanga River Estuary – BRE). Av. Density = Average Abundance; Av. Sim. = Average Similarity; Contrib% = Contribution Percentage; Cum.% = Cumulative Percentage. and Av. Diss. = Average Dissimilarity.

	Characterizing species	Av.Density x10⁶ cells L⁻¹	Av.Sim	Contrib%	Cum.%
Downstream Average similarity: 30.57%	*Skeletonema costatum*	0.86	8.90	29.12	29.12
	Melosira varians	0.11	6.48	21.20	50.32
	Leptocylindrus danicus	0.46	6.29	20.57	70.89
	Protoperidinium sp₁	0.08	2.70	9.00	79.71
	Dinophysis sp.	0.07	1.60	5.00	84.94
	Chaetoceros lorenzianus	0.02	0.83	2.70	87.65
	Chaetoceros rostratus	0.01	0.73	2.38	90.03
	Coscinodiscus sp₁	0.01	0.59	1.94	91.96
	Chaetoceros teres	0.03	0.44	1.44	93.4
	Trachelomonas armata	0.01	0.31	1.03	94.43
	Chaetoceros simplex	0.00	0.20	0.67	95.00
Upstream Average similarity: 3.29%	*Euglena proxima*	0.59	1.11	33.59	33.59
	Melosira varians	0.12	0.80	24.45	58.05
	Cylindrotheca closterium	0.09	0.49	15.04	73.09
	Pleurosigma sp.	0.04	0.21	6.27	79.36
	Trachelomonas armata	0.05	0.14	4.31	83.67
	Phacus longicauda	0.04	0.12	3.53	87.19
	Chaetoceros subtilis	0.01	0.08	2.35	89.55
	Chaetoceros similis	0.33	0.07	2.06	91.60
	Skeletonema costatum	0.01	0.06	1.76	93.37
	Euglena gracilis	3.92	0.05	1.67	95.03

Discriminating species	Av.Density Downstream x10⁶ cells L⁻¹	Av.Density Upstream x10⁶ cells L⁻¹	Av.Diss.	Contrib%	Cum.%
Chlamydomonas sp.	0.00	6.59	27.18	29.41	29.41
Euglena gracilis	0.00	3.92	16.22	17.55	46.96
Euglena proxima	0.00	0.59	13.56	14.68	61.65
Trachelomonas sp.	0.00	0.38	9.78	10.59	72.23
Skeletonema costatum	0.86	0.01	9.43	10.2	82.43
Leptocylindrus danicus	0.46	0.00	5.26	5.69	88.13
Chaetoceros similis	0.03	0.33	1.61	1.75	89.87
Cylindrotheca closterium	0.01	0.09	0.93	1.01	90.88
Protoperidinium sp1	0.08	0.01	0.76	0.82	91.70
Dinophysis sp.	0.07	0.02	0.65	0.70	92.41

The contribution of phytoplankton species (characterizing and discriminating) and the trophic status are represented in the non-metric multidimensional scaling (nMDS) with a stress level of 0.01 (Figure 5).

The analyses showed a well-defined trophic state (TRIX and TSI) in both zones, with evident blooms of *S. costatum* and *L. danicus* downstream, specifically at B1 and B2 (Bacanga Dam). In the upstream
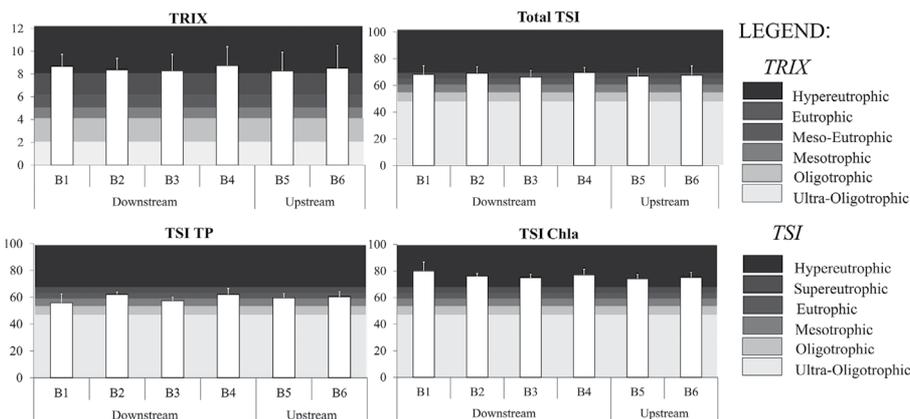
Figure 3. Trophic state indexes (TSI and TRIX) spatial variations in the Bacanga River Estuary (BRE).
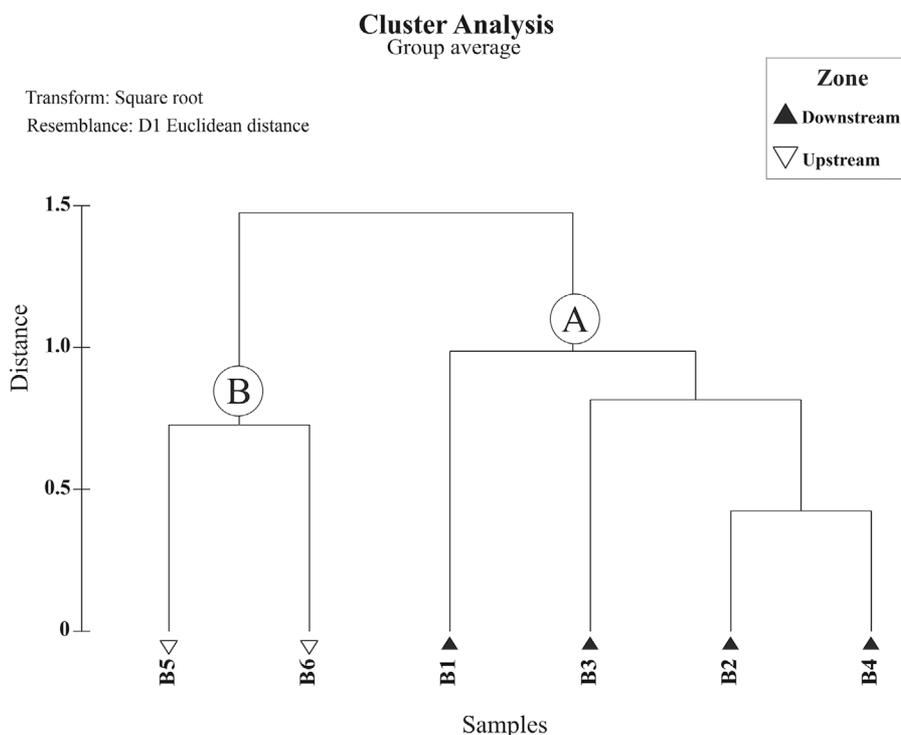

Figure 4. Dendrogram based on the Euclidean distance of the physical-chemical parameters ($p < 0.05$), and trophic state indexes (TSI and TRIX) for the Bacanga River Estuary (BRE) showing the arrangement of the sites in two groups (A and B), where: B1, B2, B3 and B4 are located in the downstream region, and B5 and B6 are located in upstream area.

region, a high density of *E. proxima* and dominance of *Trachelomonas* sp. were observed at B5. The proliferation of *Chlamydomonas* sp. and *E. gracilis* is specific to site B6.

RESPONSES OF ALGAL BLOOMS TO THE TROPHIC STATE

The generalized linear models (GLM) showed that the bloom-forming species of phytoplankton in the

BRE did not respond to the trophic state indicated by the TRIX index, with low Adjusted Squared Multiple (R = 0.21) and non-significant correlation (F = 2.29, $p = 0.05$). However, *S. costatum*, *Protoperidinium* sp., *E. gracilis*, *Chlamydomonas* sp., and *L. danicus* showed a direct and significant correlation (F = 9.17, $p = 0.000007$) with the total trophic status index (TSI Total) and an adjusted squared multiple of
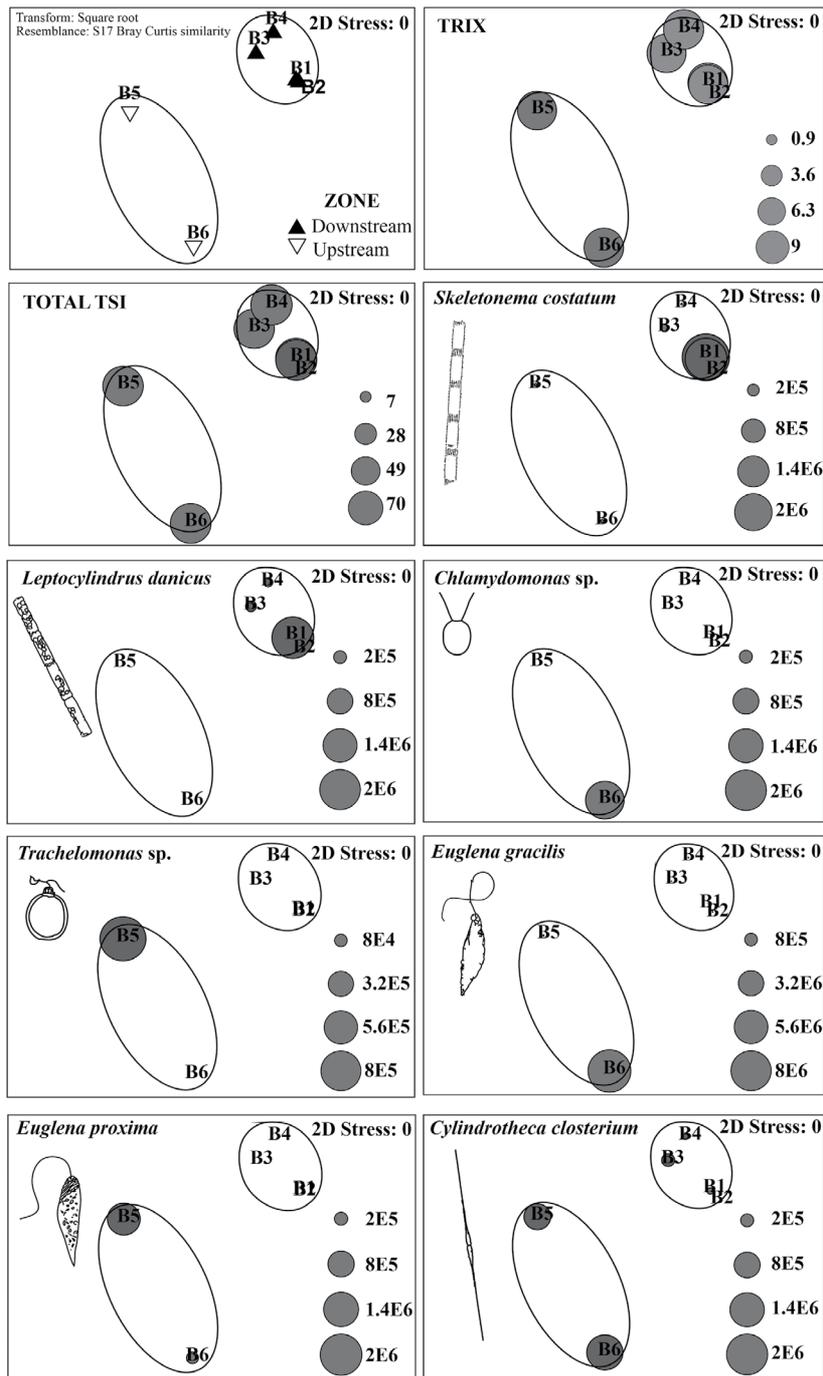
Figure 5. Bubble plot of density (cells L^{-1}) of main species of phytoplankton, and the trophic indexes in the BRE, superimposed on the nMDS ordinal.

0.62. All these blooms are directly related to the TSI-chlorophyll *a*, except for *Melosira varians*, which had a significant correlation only with TSI-total phosphorus (Table 5).

DISCUSSION

INTERACTION BETWEEN NUTRIENT LOAD AND TROPHIC STATE

Tropical estuaries are subject to many impacts that affect biodiversity and ecosystem services (Herrera-Silveira et al., 2009; Cloern et al., 2014; Monteiro et al., 2016; Lepšová-Skácelová et al., 2018). These impacts are mainly owing to discharges of domestic sewage, industrial wastewater, and runoff from agricultural fertilizers that pollute many estuaries around the world (Pei et al., 2019). The damming of these environments is another factor that strongly modifies water biogeochemistry and flow patterns, which compromise their ecological and biological dynamics (Lima et al., 2020).

The impacts mentioned can be seen along the drainage basin of the Bacanga River; according to the trophic indices, the BRE has been classified as hypereutrophic, which is a relevant factor for the formation of algal blooms. Both TRIX and TSI had the same behavior in the BRE, with no significant differences between zones (downstream-upstream).

Thus, the TRIX index can be considered the one that best defines the eutrophication conditions of the BRE, owing to the high concentrations of NH_4^+ that follow the low oxygen saturation levels throughout the estuary. This leads to a highly productive environment in terms of water quality (Cotovicz-Júnior et al., 2012b) in response to the interdependent relationship between nutrient availability and biomass and consequent higher transference energy to various trophic levels (Cloern et al., 2014).

Based on the Redfield molar ratio (N:P and N:Si), this pattern can be expressed by the co-limitation

Table 5. Model accounting for the observed variation in the TRIX index in BRE according to the results of the multiple linear regression model analysis (GLM) (number of cases = 36).

		TRIX		
	Adj R² = 0.21		**F=2.29**	**p=0.05**
Effect	**R square**	**Tolerance**	**t**	**p-level**
Skeletonema costatum	0.07	0.93	0.28	0.78
Protoperidinium sp.	0.24	0.76	-1.74	0.09
Euglena gracilis	0.99	0.01	1.97	0.06
Chlamydomonas sp.	0.99	0.01	-1.78	0.09
Cylindrotheca closterium	0.02	0.98	0.00	1.00
Leptocylindrus danicus	0.91	0.09	0.44	0.67
Melosira varians	0.22	0.78	0.91	0.37
		TSI TOTAL		
	Adj R² = 0.69		**F=9.17**	**p=0.0000007**
Effect	**R square**	**Tolerance**	**t**	**p-level**
Skeletonema costatum	0.07	0.93	3.43	0.00
Protoperidinium sp.	0.24	0.76	-4.75	0.00
Euglena gracilis	0.99	0.01	3.46	0.00
Chlamydomonas sp.	0.99	0.01	-3.58	0.00
Cylindrotheca closterium	0.02	0.98	0.37	0.72
Leptocylindrus danicus	0.91	0.09	2.49	0.02
Melosira varians	0.22	0.78	-0.57	0.57

of phosphorus and silicate, to the excess of anthropogenic nitrogen (NH_4^+) observed downstream (Pavlidou et al., 2004; Xu et al., 2008). This excess is a response to an increase in the DIN concentrations, which was probably the result of industrial wastewater and domestic sewage discharge in the study area, in addition to the indiscriminate use of fertilizers upstream that contributed to an increase in phosphorus in that region (Silva et al., 2014; Duarte-dos-Santos et al., 2017). Therefore, the limitation of phosphorus may have a low impact on the reduction of the trophic level of the water body, being compensated by the conservative behavior of nitrogen concentrations as a function of salinity (Anderson et al., 2002; Cloern et al., 2014; Paula-Filho et al., 2020).

The TSI was the index that best responded to phytoplankton blooming events. The TSI showed the spatial variation of total phosphorus and the high concentrations of chlorophyll *a*, promoting interaction between the conditioning factor and the response variable. In lotic environments usually rich in phosphorus, the shorter residence time and low light availability may restrict the development of phytoplanktonic biomass (Cunha et al., 2013; Lemley et al., 2015; Madhu et al., 2017).

Therefore, the TSI results are satisfactory because the estuary has characteristics much more similar to that of a reservoir. The BRE was homogeneous with respect to the trophic indices owing to the shorter time of water renewal. Residence time in the BRE is longer because of the dam, providing conditions and facilitating decisions about water management (Cunha et al., 2013, Pitombeira and Morais, 1977). The presence of the dam causes the environment to have a common lentic, water-body behavior, in which the salinity depends on the control and administration of the gates that regulate the freshwater outflow and entry of seawater (Duarte-dos-Santos et al., 2017).

According to Kang et al. (2019), the construction of a dam in estuaries affects water quality owing to slower water flow rates, providing an increase in water residence time and primary productivity because of retention of anthropogenic constituents, including nutrients and organic compounds in estuarine reservoirs.

RESPONSES OF ALGAL BLOOMS TO THE TROPHIC STATE

Diatoms dominated downstream, being responsible for *S. costatum* blooms in this part of the estuary, followed by *Melosira varians* and *L. danicus*. In the upstream region, the proliferation of phytoflagellates, such as *Chlamydomonas* sp., *E. proxima,* and *E. gracilis,* was observed, under conditions of low salinity and phosphorus availability. According to Lepšová-Skácelová et al. (2018) and Cloern et al. (2014), the phytoplankton community responds to salinity tolerance and nutritional preferences, which could be used as key indicators of ecological status in eutrophic estuarine environments (Napiorkowska-Krzebietke et al., 2017).

Considering the occurrences of these blooms, it can be observed that the salinity and total phosphorus concentrations were the main factors that determined the pulses of *S. costatum* and *L. danicus* downstream, and *Chlamydomonas* sp., *E. gracilis*, and *E. proxima* upstream, organisms which could potentially be considered as key species and indicators of the trophic state in BRE.

According to the GLM, the TRIX does not respond well to the occurrences of these algal blooms in the BRE; values of this index are weakly correlated and insignificant. However, the total TSI showed a moderate correlation, including with the main bloom-forming species (*S. costatum*, *Protoperidinium* sp., *E. gracilis, Chlamydomonas* sp., and *L. danicus*). The adaptive strategies presented by phytoplankton, in response to the limitations of nutrients and light that control their growth, divides the community into two groups, namely, the bloomers and the survivors. Algal bloomers are microphytoplankton such as diatoms and larger dinoflagellates, which grow in eutrophic systems. Survivalists, however, are predominantly smaller algae that grow in low-nutrient conditions (Franz et al., 2012; Pei et al., 2019).

The mixture of freshwater and seawater created two environmental gradients (downstream and upstream), with specific physical-chemical characteristics, considering salinity and depth as the most important characteristics. Under these conditions, microalgae, such as chain-forming diatoms, dominated the lower region of the estuary. This group of

species characterizes the downstream region, which is subject to high salinity, greater depths, and low transparency as well as a high organic matter load (BOD_5), alkaline pH, and high concentrations of nutrients of anthropogenic origin (NH_4^+).

CONCLUSION

The Bacanga River estuary is a coastal system with variations in salinity, depth, and algal blooms in the two estuarine regions (downstream and upstream). The high load of nutrients of anthropogenic origin and restricted circulation of BRE waters may have contributed to the increase in chlorophyll *a* levels and bloom events.

The density peaks downstream were associated with blooms of *L. danicus* and *S. costatum*, which were limited by silicate. Meanwhile, in the upstream zone, phytoplankton proliferation was caused by a high density of phytoflagellates of the genus *Chlamydomonas*, as well as *E. gracilis,* and *E. proxima*, limited by phosphorus.

The TRIX was the index that best reflected the anthropogenic influence on the water quality of BRE, providing information on reduced nitrogen surplus. However, the TSI showed the trophic state as a function of chlorophyll-*a* concentrations, an evident response to phytoplankton growth and the formation of specific algal blooms. This index proved to be an excellent tool that better assesses the ecological functioning of a dammed estuary, with the confinement of nutrients caused by anthropogenic actions.

AUTHOR CONTRIBUTIONS

A.K.D.S.S.: Conceptualization, Methodology, Validation, Formal analysis, Writing - original draft, Writing - review & editing.

M.V.J.C.: Conceptualization, Methodology, Validation, Writing - review & editing, Supervision.

D.S.C.: Conceptualization, Methodology, Validation, Formal analysis, Writing - review & editing.

L.F.C.: Conceptualization, Methodology, Writing - review & editing.

F.S.F.: Conceptualization, Methodology, Writing - review & editing.

A.L.L.O.: Conceptualization, Methodology, Writing - review & editing.

J.H.F.S.: Conceptualization, Methodology, Writing - review & editing.

REFERENCES

ANDERSON, D. M., GLIBERT, P. M. & BURKHOLDER, J. M. 2002. Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. *Estuaries*, 25(4), 704-726, DOI: https://doi.org/10.1007/bf02804901

ARAÚJO, A. V., DIAS, C. O. & BONECKER, S. L. C. 2017. Differences in the structure of copepod assemblages in four tropical estuaries: importance of pollution and the estuary hydrodynamics. *Marine Pollution Bulletin*, 115(1-2), 412-420, DOI: https://doi.org/10.1016/j.marpolbul.2016.12.047

BRUGNOLI, E., MUNIZ, P., VENTURINI, N., BRENA, B., RODRÍGUEZ, A. & GARCÍA-RODRÍGUEZ, F. 2019. Assessing multimetric trophic state variability during an ENSO event in a large estuary (Río de la Plata, South America). *Regional Studies in Marine Science*, 28, e100565, DOI: https://doi.org/10.1016/j.rsma.2019.100565

CARLSON, R. E. 1977. A trophic state index for lakes. *Limnology and Oceanography*, 22(2), 261-269, DOI: https://doi.org/10.4319/lo.1977.22.2.0361

CLARKE, K. R. & GORLEY, R. N. 2006. *PRIMER V6: user manual/tutorial*. Plymouth: PRIMER-E.

CLOERN, J. E. 2001. Our evolving conceptual model of the coastal eutrophication problem. *Marine Ecology Progress Series*, 210, 223-253, DOI: https://doi.org/10.3354/meps210223

CLOERN, J. E., FOSTER, S. Q. & KLECKNER, A. E. 2014. Phytoplankton primary production in the world's estuarine-coastal ecosystems. *Biogeosciences*, 11(9), 2477-2501, DOI: https://doi.org/10.5194/bg-11-2477-2014

COTOVICZ JUNIOR, L. C., BRANDINI, N., KNOPPERS, B. A., FRIEDERICHS, W., SOUZA, L. D. E. & MEDEIROS, P. R. P. 2012a. Comparação de modelos e índices para avaliação do estado trófico do Complexo Estuarino-Lagunar Mundaú-Manguaba (AL). *Geochimica Brasiliensis,* 26(1), 7-18.

COTOVICZ JUNIOR, L. C., BRANDINI, N., KNOPPERS, B. A., MIZERKOWSKI, B. D., STERZA, J. M., OVALLE, A. R. C. & MEDEIROS, P. R. P. 2012b. Assessment of the trophic status of four coastal lagoons and one estuarine delta, eastern Brazil. *Environmental Monitoring and Assessment*, 185(4), 3297-3311, DOI: https://doi.org/10.1007/s10661-012-2791-x

CUNHA, D. G. F., CALIJURI, M. C. & LAMPARELLI, M. C. 2013. A trophic state index for tropical/subtropical reservoirs (TSI$_{tsr}$). *Ecological Engineering,* 60, 126-134, DOI: https://doi.org/10.1016/j.ecoleng.2013.07.058

CUTRIM, M. V. J., FERREIRA, F. S., DUARTE-DOS-SANTOS, A. K., CAVALCANTI, L. F., ARAÚJO, B. O., AZEVEDO-CUTRIM, A. C. G. & OLIVEIRA, A. L. L. 2019. Trophic state of an urban coastal lagoon (northern Brazil), seasonal variation of the phytoplankton community and environmental variables. *Estua-

rine, Coastal and Shelf Science, 216, 98-109, DOI: https://doi.org/10.1016/j.ecss.2018.08.013

DUARTE-DOS-SANTOS, A. K., CUTRIM, M. V. J., FERREIRA, F. S., LUVIZOTTO-SANTOS, R., AZEVEDO-CUTRIM, A. C. G., ARAÚJO, B. O., OLIVEIRA, A. L. L., FURTADO, J. A. & DINIZ, S. C. D. 2017. Aquatic life protection index of an urban river Bacanga basin in northern Brazil, São Luís - MA. *Brazilian Journal of Biology*, 77(3), 602-615, DOI: https://doi.org/10.1590/1519-6984.01016

FERREIRA, J. G., ANDERSEN, J. H., BORJA, A., BRINCKER, S. B, CAMP, J., SILVA, M. C., GARCÉS, E., HEISKANEN, A. S., HUMBORG, C., IGNATIADES, L., LANCELOT, C., MENESGUEN, A., TETT, P., HOEPFFNERM, N. & CLAUSSEN, U. 2011. Overview of eutrophication indicators to assess environmental status within the European Marine Strategy Framework Directive. *Estuarine, Coastal and Shelf Science*, 93(2), 117-131, DOI: https://doi.org/10.1016/j.ecss.2011.03.014

FRANZ, J., KRAHMANN, G., LAVIK, G., GRASSE, P., DITTMAR, T. & RIEBESELL, U. 2012. Dynamics and stoichiometry of nutrients and phytoplankton in waters influenced by the oxygen minimum zone in the eastern tropical Pacific. *Deep Sea Research Part I: Oceanographic Research Papers,* 62, 20-31, DOI: https://doi.org/10.1016/j.dsr.2011.12.004

GARMENDIA, M., BORJA, A., FRANCO, J. & REVILLA, M. 2013. Phytoplankton composition indicators for the assessment of eutrophication in marine waters: present state and challenges within the European directives. *Marine Pollution Bulletin*, 66(1-2), 7-16, DOI: https://doi.org/10.1016/j.marpolbul.2012.10.005

GIOVANARDI, F. & VOLLENWEIDER R. A. 2004. Trophic conditions of marine coastal waters: experience in applying the Trophic Index TRIX to two areas of the Adriatic and Tyrrhenian seas. *Journal of Limnology*, 63(2), 199-218, DOI: https://doi.org/10.4081/jlimnol.2004.199

GOLTERMAN, H. L., CLYMO, R. S. & OHNSTAD, M. A. M. 1978. *Methods for physical and chemical analysis of freshwater*. Oxford: Blackwell Scientific Publications.

GRASSHOFF, K., KREMLING, K. & EHRHARDT, M. 1983. Methods of seawater analysis. 2nd ed. New York: Verlag Chemie.

GREENBERG, A. E., CLESCERI, L. S. & EATON, A. D. 1992. *Standard methods for the examination of water and wastewater.* 18th ed. Washington: American Public Health Association.

GUIRY, M. D. & GUIRY, G. M. 2020. *AlgaeBase. Worldwide electronic publication* [online]. Galway: National University of Ireland. Available at: http://www.algaebase.org [Accessed: 08 Feb. 2020].

HAYN, M., HOWARTH, R., MARINO, R., GANJU, N., BERG, P., FOREAMN, K. H., GIBLIN, A. E. & MCGLATHERY, K. 2014. Exchange of nitrogen and phosphorus between a shallow lagoon and coastal waters. *Estuaries and Coasts*, 37(1), 63-73, DOI: https://doi.org/10.1007/s12237-013-9699-8

HERRERA-SILVEIRA, J. A. & MORALES-OJEDA, S. M. 2009. Evaluation of the health status of a coastal ecosystem in southeast Mexico: assessment of water quality, phytoplankton and submerged aquatic vegetation. *Marine Pollution Bulletin*, 59(1-3), 72-86, DOI: https://doi.org/10.1016/j.marpolbul.2008.11.017

INMET (Instituto Nacional de Meteorologia). 2017. *Portal INMET* [online]. Brasília: INMET. Available at: http://www.inmet.gov.br/portal/ [Accessed: 31 Jul. 2017].

KOROLEFF, K. 1983. Determination of phosphorus. *In*: Grasshoff, K., Ehrhardt, M. & Kremling, K. (eds.). Methods of sea water analysis. 2nd ed. Weinhein: Verlag Chemie, pp. 125-39.

LEMLEY, D. A., ADAMS, J. B., TALJAARD, S. & STRYDOM, N. A. 2015. Towards the classification of eutrophic condition in estuaries. *Estuarine, Coastal and Shelf Science*, 164, 221-232, DOI: https://doi.org/10.1016/j.ecss.2015.07.033

LEPŠOVÁ-SKÁCELOVÁ, O., FIBICH, P., WILD, J. & LEPŠ, J. 2018. Trophic gradient is the main determinant of species and large taxonomic groups representation in phytoplankton of standing water bodies. *Ecological Indicators,* 85, 262-270, DOI: https://doi.org/10.1016/j.ecolind.2017.10.034

LIMA, M. A. L., DORIA, C. R., CARVALHO, A. R. & ANGELINI, R. 2020. Fisheries and trophic structure of a large tropical river under impoundment. *Ecological Indicators*, 113, e106162, DOI: https://doi.org/10.1016/j.ecolind.2020.106162

LIVINGSTON, R. J. 2007. Phytoplankton bloom effects on a gulf estuary: water quality changes and biological response. *Ecological Applications*, 17(sp5), S110-S128, DOI: https://doi.org/10.1890/05-0769.1

MADHU, N. V., MARTIN, G. D., HARIDEVI, C. K., NAIR, M., BALACHANDRAN, K. K. & ULLAS, N. A. 2017. Differential environmental responses of tropical phytoplankton community in the southwest coast of India. *Regional Studies in Marine Science*, 16, 21-35, DOI: https://doi.org/10.1016/j.rsma.2017.07.004

MAIA, A. A. D., CARVALHO, S. L. & CARVALHO, F. T. 2015. Comparação de dois índices de determinação do grau de trofia nas águas do Baixo Rio São José dos Dourados, São Paulo, Brasil. *Engenharia Sanitária Ambiental*, 20(4), 613-622, DOI: https://doi.org/10.1590/s1413-41522015020040121258

MONCHEVA, S., GOTSIS-SKRETASB, O., PAGOUB, K. & KRASTEVA, A. 2001. Phytoplankton blooms in black sea and Mediterranean coastal ecosystems subjected to anthropogenic eutrophication: similarities and differences. *Estuarine, Coastal and Shelf Science,* 53(3), 281-295, DOI: https://doi.org/10.1006/ecss.2001.0767

MONTEIRO, M. C., JIMÉNEZ, J. A. & PEREIRA, L. C. C. 2016. Natural and human controls of water quality of an Amazon estuary (Caeté-PA, Brazil). *Ocean and Coastal Management*, 124, 42-52, DOI: https://doi.org/10.1016/j.ocecoaman.2016.01.014

MOSER, G. A. O., CIOTTI, A. M., GIANNINI, M. F. C., TONINI, R. T. & HARARI, J. 2012. Changes in phytoplankton composition in response to tides, wind-induced mixing conditions, and freshwater outflows in an urbanized estuarine complex. *Brazilian Journal Biology,* 72(1), 97-111, DOI: https://doi.org/10.1590/S1519-69842012000100012

NAPIORKOWSKA-KRZEBIETKE, A., DUNALSKA, J. A. & ZEBEK, E. 2017. Taxa-specific eco-sensitivity in relation to phytoplankton bloom stability and ecologically relevant lake state. *Acta Oecologica*, 81, 10-21, DOI: https://doi.org/10.1016/j.actao.2017.04.002

NASCIMENTO-FILHO, G. A., FLORES-MONTES, M. J., GASPAR, F. L., PAULO, J. G. & FEITOSA, F. A. N. 2013. Eutrophication and water quality in a tropical Brazilian estuary. *Journal of Coastal Research*, 65(spe1), 7-12, DOI: https://doi.org/10.2112/SI65-002.1

NASROLLAHZADEH, H. S., DIN, Z. B., FOONG, S. Y. & MAKHLOUGH, A. 2008. Trophic status of the Iranian Caspian Sea based on water quality parameters and phytoplankton diversity. *Continental Shelf Research*, 28(9), 1153-1165, DOI: https://doi.org/10.1016/j.csr.2008.02.015

PARSONS, T. R., MAITA, Y. & LALLI, C. M. 1984. *A manual of chemical and biological methods for seawater analysis*. Oxford: Pergamon Press.

PAULA-FILHO, F. J., MARINS, R. V., CHICHARO, L., SOUZA, R. B., SANTOS, G. V. & BRAZ, E. M. A. 2020. Evaluation of water quality and trophic state in the Parnaíba River Delta, northeast Brazil. *Regional Studies in Marine Science*, 34, e101025, DOI: https://doi.org/10.1016/j.rsma.2019.101025

PAVLIDOU, A., KONTOYIANNIS, H. & PSYLLIDOU-GIOURANO-VITS, R. 2004. Trophic Conditions and stoichiometric nutrient balance in the Inner Saronikos Gulf (Central Aerean Sea) affected by the Psittalia Sewage Outfall. *Fresenius Environmental Bulletin,* 13(12B), 1509-1514.

PEI, S., LAWS, E. A., ZHU, Y., ZHANG, H., YE, S., YUAN, H. & DING, X. 2019. Nutrient dynamics and their interaction with phytoplankton growth during autumn in Liaodong Bay, China. *Continental Shelf Research,* 186, 34-47, DOI: https://doi.org/10.1016/j.csr.2019.07.012

PENNA, N., CAPELLACCI, S. & RICCI, F. 2004. The influence of the Po River discharge on phytoplankton bloom dynamics along the coastline of Pesaro (Italy) in the Adriatic Sea. *Marine Pollution Bulletin*, 48(3-4), 321-326.

PETTINE, M., CASENTINI, B., FAZI, S., GIOVANARDI, F. & PAGNOTTA, R. 2007. A revisitation of TRIX for trophic status assessment in the light of the European Water Framework Directive: application to Italian coastal waters. *Marine Pollution Bulletin,* 54(9), 1413-1426, DOI: https://doi.org/10.1016/j.marpolbul.2007.05.013

PITOMBEIRA, E. S. & MORAIS, J. O. 1977. Comportamento hidrodinâmico e sedimentológico do estuário do Rio Bacanga (São Luís, Estado do Maranhão, Brasil). *Arquivos de Ciências do Mar*, 17(2), 165-174.

RANZ, J., KRAHMANN, G., LAVIK, G., GRASSE, P., DITTMAR, T. & RIEBESELL, U. 2012. Dynamics and stoichiometry of nutrients and phytoplankton in waters influenced by the oxygen minimum zone in the eastern tropical Pacific. *Deep Sea Research Part I. Oceanographic Research Papers,* 62, 20-31, DOI: https://doi.org/10.1016/j.dsr.2011.12.004

SILVA, G. S., SANTOS, E. A., CORRÊA, L. B., MARQUES, A. L. B., MARQUES, E. P., SOUSA, E. R. & SILVA, G. S. 2014. Avaliação integrada da qualidade de águas superficiais: grau de trofia e proteção da vida aquática nos rios Anil e Bacanga, São Luís (MA). *Engenharia Sanitária e Ambiental*, 19(3), 245-250, DOI: https://doi.org/10.1590/s1413-41522014019000000438

SMITH, V. H. & SCHINDLER, D. W. 2009. Eutrophication science: where do we go from here? *Trends in Ecological and Evolution,* 24(4), 201-207, DOI: https://doi.org/10.1016/j.tree.2008.11.009

SPATHARIS, S. & TSIRTSIS, G. 2010. Ecological quality scales based on phytoplankton for the implementation of water framework directive in the Eastern Mediterranean. *Ecological Indicators*, 10(4), 840-847, DOI: https://doi.org/10.1016/j.ecolind.2010.01.005

STATHAM, P. J. 2012. Nutrients in estuaries - an overview and the potential impacts of climate change. *Science of the Total*