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Assessment of the trophic status in a tropical estuarine system

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ABSTRACT

Estuaries are extremely valuable ecosystems and have been affected by several anthropogenic activities from urban growth and industrial development, such as the release of untreated effluents which decrease water quality. This study aimed to evaluate the seasonal and temporal trophic status variation in the Formoso River Estuarine System (FRES), a tropical estuary. Three campaigns were carried out in each season (rainy and dry), during which surface water was sampled for abiotic parameter analyses. The TRIX index was used for trophic status assessment. For the temporal evaluation, a review of literature addressing the matter in the study area was conducted. Between 2017 and 2018, the TRIX index varied from a mean of 6 ± 0.2 upstream to 5 ± 0.4 downstream during the rainy season, indicating eutrophic conditions throughout most of the FRES extension, especially near the Formoso River, and from 6 ± 1 to 3 ± 0.8 during the dry season. Temporal analyses showed that in 2002 the overall mean TRIX index was 5.72 ± 0.4, decreasing in 2005 and 2014, and followed by an upwards trend. We conclude that the FRES is impacted by the release of untreated effluents such as sewage and fertilizers from the city of Rio Formoso and from various agricultural activities, which are more intense during the rainy season. Nevertheless, over time there were more critical scenarios pertaining to the trophic status. The present diagnosis reinforces the importance of public sanitation policies in estuarine regions and spatial planning instruments for pollution control in fishery areas.

Descriptors: Water quality, Dissolved inorganic nutrients, Multivariate index, Environmental impacts.

INTRODUCTION

Estuaries are complex and dynamic hydrological environments and are considered one of the most productive areas in the biosphere (Gattuso et al., 1998; Borges, 2011; Filho et al., 2020). This ecosystem is rich in organic matter, solutes, and nutrients, representing an important site for material exchange with the atmosphere, associated wetlands, and especially the ocean, due to the marine-freshwater interaction. Estuarine systems also provide a nursery for several organisms such as fish assemblages and thus represent an area of social importance, especially considering the local artisanal fisher population (Vasconcelos et al., 2011; Dantas et al., 2012; Whitfield, 2017).

Rising urbanization and land use are major threats to coastal environments worldwide (Hader et al., 2020). Estuaries have been directly

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affected by anthropogenic activities, including the release of fertilizer and untreated sewage (Alves et al., 2013; Cabral and Fonseca, 2019). These effluents are loaded with organic nutrients, especially nitrogen and phosphorus, and are generally associated with heavy metals and other potential pollutants (Clark et al., 2017; Zhang et al., 2017; Chen et al., 2021).

From the mid-twentieth century until recently, the loads of reactive nutrients of anthropogenic origin reaching estuarine systems increased significantly. For instance, nitrogen and phosphorus input increased from 34 Tg N yr^{-1} to 64 Tg N yr^{-1} and from 5 Tg P yr^{-1} to 9 Tg P yr^{-1} (Beusen et al., 2016; Malone and Newton, 2020), significantly impacting water quality and especially the eutrophication process (Guenther et al., 2014). Harmful algae bloom (HAB) triggered by an increased nutrient input is characteristic of this, causing dissolved oxygen depletion, creating dead zones that impact local biota through community phase shifts, and an increase in mortality of fishes, crustaceans, and several calcifying organisms (Barros et al., 2017; Lemasson et al., 2017; Morelli et al., 2018). Such impacts may be greater in regions highly influenced by seasonality, as is the case for tropical estuaries (Gaspar et al., 2018; Taillardat et al., 2020).

Estimates indicate long-term ecological and economic impacts for eutrophicated estuaries through the end of this century, with losses in excess of US$1 billion annually in several sectors such as recreational water use and fishing (Dodds et al., 2009; Schoen et al., 2017). Because of this, several modeling and multiparameter indices were designed to measure and determine the trophic status of coastal marine environments, as tools for water quality monitoring and improvement (Vollenweider et al., 1998; Vargas-Gonzáles et al., 2014).

In this study, we aimed to assess temporal and seasonal variations in the trophic status of the Formoso River Estuarine System (FRES), northeast Brazil, using TRIX as a multivariate index. We test the hypotheses that the system's trophic status significantly increases during the rainy season, and that there has been an improvement in water quality over the years.

METHODS

Study area

The FRES is located in Northeastern Brazil (8º37'45.34''S, 35º05'04.33''W to 8º44'02.85''S, 35º06'17.06''W), state of Pernambuco. It is part of the Guadalupe Marine Protected Area (EPAG), which spans more than 120 km^2 and was created to protect and conserve the rich local biodiversity and water resources as well as to improve the quality of life of the local community, which relies mostly on subsistence fishing (Lima, 2016; Pinto, 2016).

The climate in the area is wet tropical, with a rainy and dry season spanning from March to August and September to February, respectively. Annual mean precipitation is 1977 mm, of which 76% occurs during the rainy season. The estuarine system is a 27.24 km^2 hydrographic basin (Lira et al., 1979; Silva et al., 2009). It is composed of three main coastal rivers: the Formoso River and Passos River in its upstream portion and the Ariquindá River as the main tributary in its downstream portion, resulting in a main channel 12km in extension (Figure 1) and a mean flow rate of 4.95 m^3 s^{-1}, which significantly increases during the rainy season (Lira et al., 1979).

The lower estuary is mainly influenced by seawater and marine ecosystems such as reef barriers and seagrass meadows (Lima, 2016). In contrast, the upper and middle estuaries are characterized by high-density mangrove forests and sediments with more than 20% of organic matter (Lira et al. 1979; Silva et al., 2009), influenced by the urban zone of Rio Formoso city. This city has an area of 222,458 km^2 and population of over 23,500 inhabitants, for a demographic density of 97.39 inhabitants km^{-2}. Its nearly 5,000 households rely on farming and fishing activities as a main source of income (IBGE, 2010).

Sampling strategy and data compilation

Between 2017 and 2018, six campaigns were carried out in the FRES: three during the dry season (December 2017, September and December 2018) and three during the rainy season (May and

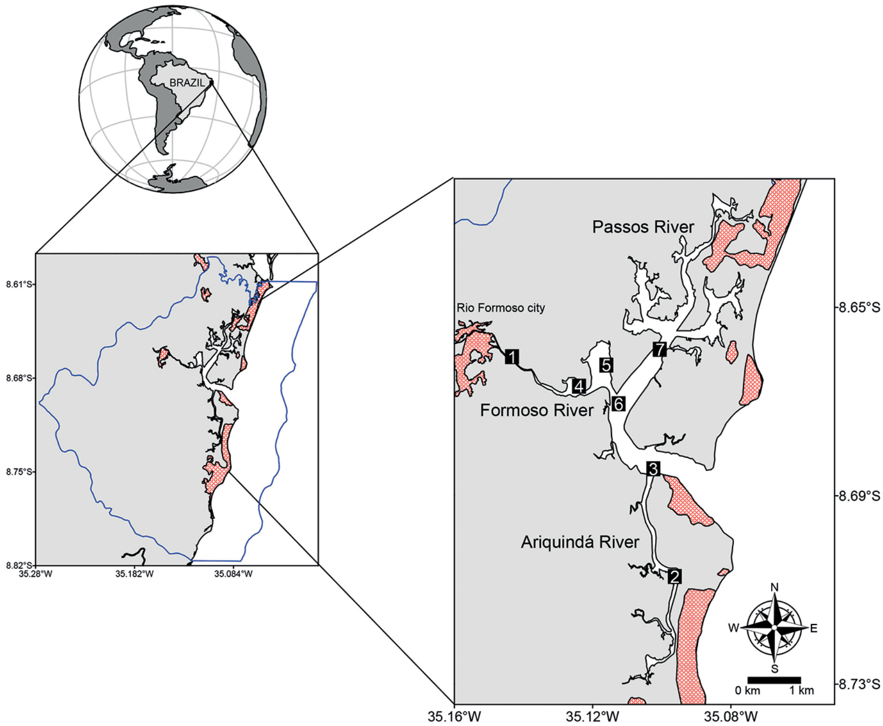

Figure 1. Study area and sampling strategy. Red plaid areas represent the main urban centers, the blue line is the EPAG boundaries, and black squares are the sampling stations and their respective numbers.

July 2017, July 2018). Seven sampling stations were established along the FRES (Figure 1), with stations 1, 2, and 7 representing the upper estuary, stations 4, 5, 6 the middle estuary, and station 3 the lower estuary. Surface water samples were collected during low tide using a 2-L Niskin bottle to further analyze dissolved inorganic nutrients, dissolved oxygen (DO), and chlorophyll-*a* (Chl-*a*). *In situ* measurements of salinity in surface waters were obtained using an RBR® C.T.D.

A review of literature addressing the hydrology and phytoplankton biomass in the FRES was carried out to assess trophic status and its temporal variability. Only articles, dissertations, and doctorate theses with the same sampling strategy used in this study and all the necessary parameters to calculate the multivariate index of trophic status (TRIX) were included.

Chemical analyses and trophic status assessment

All dissolved inorganic nutrients were analyzed using spectrophotometric methods. Soluble reactive phosphorus (SRP) and reactive silicate (SRSi) were analyzed per Grasshoff et al. (1983), with the formation of a blue complex due to the use of ascorbic acid ($C_6H_6O_6$) as a reducing reagent and analytical errors of 0.008 µmol L^{-1} and 0.3 µmol L^{-1}, respectively. Nitrate ($N\text{-}NO_3^-$) and nitrite ($N\text{-}NO_2^-$) were analyzed as described by following the García-Robledo et al. (2014) sulfanilamide/N-(1--naphthyl)-ethylenediamine method, using vanadium III chloride (VCl_3) in the reduction of NO_3^- to NO_2^-. Ammonium ($N\text{-}NH_3 + NH_4^+$) followed Bower and Holm-Hansen (1980), in which indophenol blue is produced through the reaction of $N\text{-}NH_3 + NH_4^+$ with salicylate-hypochlorite, and dissolved inorganic nitrogen (DIN) values were obtained by adding the $N\text{-}NO_3^-$, $N\text{-}NO_2^-$, and $N\text{-}NH_3 + NH_4^+$ concentrations, with a mean error of 0.02 µmol L^{-1}.

Chl-*a* is a direct indicator of phytoplankton biomass and was also analyzed through spectrophotometry according to UNESCO (1966). Dissolved oxygen (DO) was measured through the modified Winkler method (Strickland and Parsons, 1972), using manganese sulfate ($MnSO_4$) and potassium iodide (KI) to form a precipitate equivalent to the DO concentration, followed by a titration with sodium thiosulfate ($Na_2S_2O_3$). Its saturation (DO%) was calculated according to the UNESCO table (1973).

The TRIX index was originally used to assess the trophic status of the Adriatic Sea (Vollenweider et al., 1998). It has now been applied to several coastal ecosystems, including estuaries (e.g., Flores-Montes et al., 2011; Seisdedo et al., 2014; Vargas-Gonzáles et al., 2014; Sá et al., 2021; Silva et al., 2021). Here, this index was calculated according to the Vollenweider et al. (1998) equation, based on Chl-*a*, DO%, DIN, and SRP values:

$$TRIX = (log_{10}[Chl - a \times DO\% \times DIN \times SRP] + k) \quad m \qquad (1)$$

In which Chl-*a*, DIN, and SRP are expressed in µg L^{-1} and *k* and *m* are constants to adjust the TRIX against a eutrophication scale of 0 to 10, with values of 1.5 and 1.2, respectively. The classification of the trophic status and water quality followed Vollenweider et al. (1998) (Table 1).

Complementary data and statistics

Recent (2017 to 2018) and historical (1970 to 2018) rainfall data were obtained from the Pernambuco Water and Climate Agency (APAC). Because data were not normally distributed and lacked homoscedasticity, analyses were carried out using non-parametric tests. The Spearman test was used to investigate correlations between measured parameters, and a Kruskal-Wallis test was used to analyze seasonal and temporal differences. In all cases, a significance level of $p < 0.05$ was used. General mean distribution was presented when a parameter was not significantly different between seasons.

Table 1. TRIX classification, following Vollenweider et al. (1998).

TRIX	Trophic status	Water quality
0 ≤ 4	Oligotrophic	High
> 4 ≤ 5	Mesotrophic	Good
> 5 ≤ 6	Eutrophic	Bad
> 6	Hypereutrophic	Poor

RESULTS

Rainfall

In 2017, rainfall patterns varied significantly between seasons ($p < 0.05$). The annual accumulation was 2,740 mm, with May, June, and July accounting for 66%, varying significantly ($p < 0.05$) from their respective historical means (Figure 2). During 2018, most of the months were below historical levels, and no significant seasonal differences were detected ($p > 0.05$), with an overall monthly mean of 144 ± 121 mm.

Salinity and DO

In the FRES, salinity increased downstream across the entire sampling period (Figure 3a, b), exhibiting significant seasonal differences ($p < 0.05$). During the rainy season, values ranged from a mean 6 ± 5 g kg^{-1} close to the Formoso River to 24 ± 8 g kg^{-1} in the lower estuary (Figure 3a) and were negatively correlated with precipitation ($rs = -0.47$, $p < 0.05$). During the dry season, the distribution was homogenous throughout most of the FRES, with a lower mean of 12 ± 10 g kg^{-1} recorded upstream (Figure 3b). DO was positively and significantly correlated with salinity ($rs > 0.4$, $p < 0.05$), with no intra-season variation, an overall minimum of 99 ± 48 µmol kg^{-1}, and a maximum of 219 ± 29 µmol kg^{-1} (Figure 3c).

Trophic status

Dissolved inorganic nutrients were negatively correlated with salinity ($rs = < -0.5$, $p < 0.05$). Their respective means are summarized in Table 2. DIN, SRP, and SRSi exhibited seasonal differences ($p < 0.05$) and a similar spatial distribution, with higher concentrations upstream during the rainy season, especially in July 2017, decreasing according to seawater influence (Table 2).

For Chl-*a*, the highest mean of 7 ± 5 µg L^{-1} was obtained next to the Formoso River, and the lowest of 3 ± 1 µg L^{-1} was recorded downstream (Figure 4a), with a significant positive correlation with SRSi ($rs > 0.3$, $p < 0.05$) and no significant seasonal differences. Following this spatial distribution pattern, the TRIX index varied from a mean of 6 ± 0.2 to 5 ± 0.4 during the rainy season

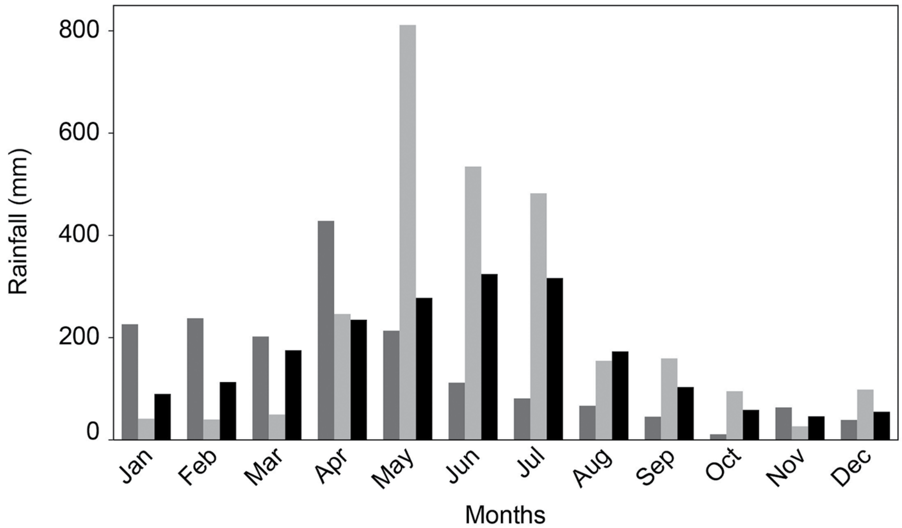

Figure 2. Monthly rainfall data from the Rio Formoso city meteorological station. Light and dark gray bars represent data from 2017 and 2018, respectively. Black bars represent the historical mean from 1970 to 2018.

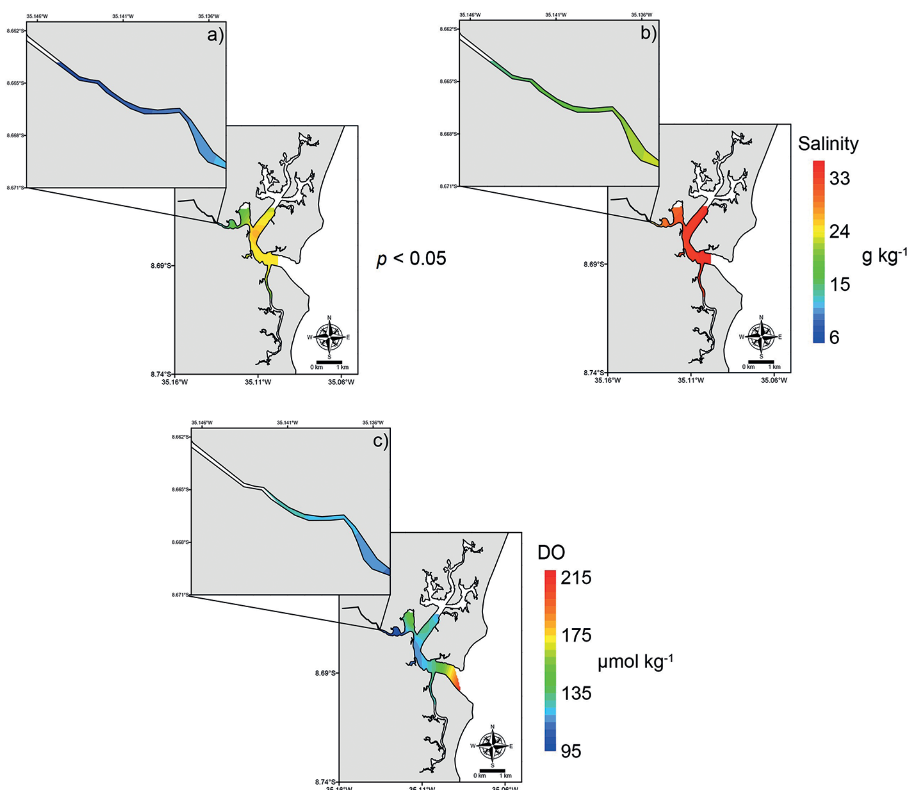

Figure 3. Mean salinity distribution in the FRES during the rainy (a) and dry (b) seasons, and overall DO (c).

(Figure 4b), indicating a eutrophic status in most of the FRES, and from 6 ± 0.9 to 3 ± 0.8 in the dry season (Figure 4c). Both Chl-*a* and TRIX values were positively correlated with rainfall ($rs > 0.4$, $p < 0.05$) and negatively correlated with salinity ($rs > 0.5$, $p < 0.05$) during all sampled periods.

Table 2. Minimum, maximum, and mean concentrations of DIN, SRP and SRSi in the FRES, according to season and location.

Nutrient	Estuary	Season					
		Dry			Rainy		
		Min	Max	Mean	Min	Max	Mean
DIN (μmol L^{-1}) n = 42	Upper	0.02	13.7	0.31	2.23	30.5	4.87
	Middle	0.03	1.21	0.18	1.32	27.1	6.25
	Lower	0.24	0.55	0.36	1.27	4.28	1.99
SRP (μmol L^{-1}) n = 42	Upper	0.01	0.69	0.07	0.03	1.07	0.25
	Middle	0.01	0.42	0.06	0.02	0.74	0.17
	Lower	0.01	0.34	0.07	0.05	0.19	0.11
SRSi (μmol L^{-1}) n = 42	Upper	1.4	176.8	23.58	7.42	158.0	50.66
	Middle	5.1	81.2	27.18	24.7	121.6	60.29
	Lower	12.5	27.6	21.04	21.3	53.4	30.11

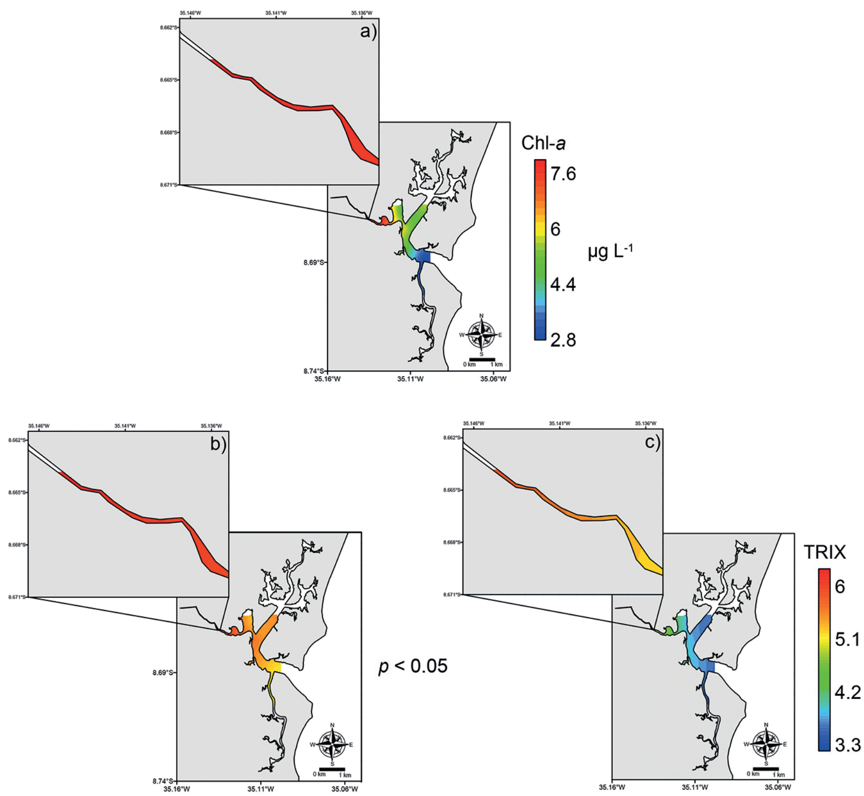

Figure 4. Overall mean Chl-a distribution (a) in the FRES, and seasonal TRIX mean during the rainy (b) and dry (c) seasons.

In addition to the data provided herein, an additional three studies (Silva et al., 2009 Grego, 2010; Lima, 2014) conducted in the FRES were identified, which allowed us to evaluate temporal TRIX variations (Figure 5). Significant temporal changes were observed ($p < 0.05$). In 2002, TRIX values ranged from 5 downstream to 6 upstream, with an overall mean of 6 ± 0.4. In the three years that followed, the TRIX decreased to 4.5 ± 0.5. No data is available between 2006 to 2013. In 2014,

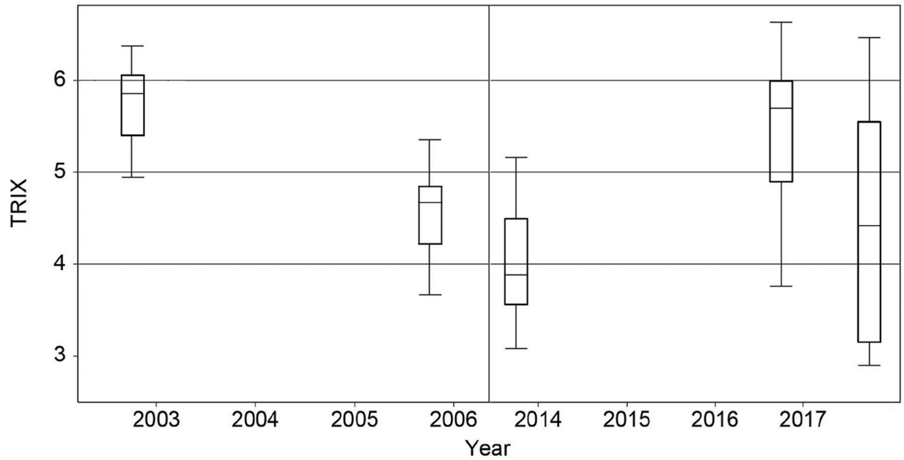

Figure 5. Box plot of TRIX variation over time in the FRES. Horizontal lines represent TRIX values of 4, 5, and 6, corresponding to mesotrophic, eutrophic, and hypereutrophic conditions, respectively.

an overall mean of 4 ± 0.65 was reported, which increased to > 5 by 2017, followed by a decreasing trend (Figure 5).

DISCUSSION

Seawater intrusion in an estuarine system is a determinant factor for local biotic and abiotic processes, acting mainly by diluting solutes, influencing biota distribution, oxygenating the water, and consequently regulating its quality (Santiago et al., 2010; Guenther et al. 2014; Lima, 2016). The FRES is classified as well-mixed, and its geomorphology results in euryhaline conditions throughout most of its extension, especially in periods of low river discharges (Lira et al., 1979; Batista and Flores-Montes, 2013), as in the dry season (Figure 3b).

Seasonality is an important factor for abiotic and biotic parameters in the FRES by regulating the distribution and abundance of phytoplankton and zooplankton, salinity, organic matter and nutrient concentrations in the water according to precipitation levels (Silva et al., 2009; Santos, 2016; Costa et al., 2018). During the rainy season, the influx of terrestrial nutrients and freshwater into coastal waters increases significantly due to an intensification in continental runoff resulting from precipitation, which lowers water salinity (Figure 3a), increases concentrations of dissolved inorganic nutrients in estuarine systems, and thus increases Chl-*a* levels (Cordeiro et al., 2018; Rubio-Cisneros et al., 2018; Silva et al., 2021). This process may be intensified by climatic events such as the El Niño-Southern Oscillation (ENSO), which can trigger temperature and precipitation anomalies (Sathicq et al., 2015; Andrade et al., 2016).

During 2017, a moderate La Niña was reported, which may have led to higher precipitation rates in northern and northeastern Brazil (Grimm, 2004; Andreoli and Kayano, 2007; Grimm and Tedeschi, 2009; Rodrigues et al., 2017). This could explain the precipitation above historical levels observed in May, June, and July 2017 (Figure 2) and the higher nutrient concentrations measured in the FRES during the same period (Pereira et al., 2013). Under these conditions, continental runoff increases significantly and ecological impacts can be seen, such as the reduction in copepods diversity and, consequently, the occurrence of zooplanktivorous fish. This may lead to an increase in local trophic status, affecting local water quality (Sahu et al., 2013; Andrade et al., 2016; Desmit et al., 2018).

In addition, non-naturals processes, such as the release of untreated effluents and nonpoint source pollution (excess fertilizers) in estuaries, also lead to water nutrient enrichment at a higher scale (Marreto et al., 2017; Dewi et al., 2018). The TRIX is a multivariate index commonly used worldwide to assess trophic status and water quality (Seisdedo et al., 2014; Vargas-Gonzáles et al., 2014; Sá et al., 2021; Silva et al., 2021). It showed that the FRES is under anthropogenic pressure, especially during the rainy season and near the

Formoso River, where hypereutrophic conditions were recorded even disregarding dissolved organic fractions of phosphorus and nitrogen, which could further increase TRIX values by ± 0.4 points (Vollenweider et al., 1998).

Dissolved inorganic nutrients presented a typical distribution for tropical estuaries, with values decreasing according to seawater-freshwater mixing (Jales et al., 2012; Cordeiro et al., 2014; Anguiano-Cuevas et al., 2015). Silicious (Si) is one of the main compounds found in the continental crust, which is why the SRSi is the most abundant dissolved nutrient in estuaries. Its correlation with Chl-*a* indicates the predominance of diatoms that use silica ($Si(OH)_4$) to build cells walls, as noted in prior studies conducted in the FRES (Silva et al., 2009; Grego, 2010; Aquino et al., 2012; Lima, 2016).

Regarding DIN and SRP, concentrations were higher in the upper and middle portions of the FRES due to organic matter remineralization, which is more intense in regions surrounded by mangrove forests and sediments rich in organic carbon, mainly during rainy seasons (Chen et al., 2018; Taillardat et al., 2020). Moreover, the high TRIX values near the Formoso River were similar to those reported in extremely polluted estuaries (Flores-Montes et al., 2011; Batista and Flores-Montes et al., 2013; Guenther et al., 2014; Fang and Wang, 2020). This may reflect the proximity to several agriculture and aquaculture facilities, the urban center of Rio Formoso city, and the influence of a Sewage Treatment Station (STS).

Over 80% of the entire Rio Formoso city territory is occupied by farming, mainly sugarcane, fish, and shrimp, which are the most profitable locally (IBGE, 2010). The use and inadequate release of fertilizers are the main sources of anthropogenic nitrogen in estuaries, with a global input of 118×10^9 kg N yr^{-1} (Lu and Tian, 2017; Malone and Newton, 2020). In addition to the resuspended sediments, excess residual organic matter from farming and the use of pesticides significantly decrease water quality in the effluent-receiving ecosystem (Barraza-Guardado et al. 2014; Cabral and Fonseca, 2019). Adding to that is the fact that only 32% of the city's sewage is properly treated (IBGE, 2010).

Urban growth and low sewage treatment rates are the second main sources of nitrogen and phosphorus input into the estuarine system (Beusen et al., 2016), increasing the local eutrophication status (Flores-Montes et al., 2011; Cordeiro et al., 2014; Monteiro et al., 2016; Sá et al., 2021). The STS next to Rio Formoso stores effluents in containment lagoons which overflow during extreme rainy seasons, such as during 2017 (Figure 3), carrying the sewage into the upper estuary (Melo, 2018). This has a direct effect on local organisms (Guenther et al., 2014; Araujo et al., 2016; Barroso et al., 2018) and can be associated with a reduced ichthyofaunal diversity, which is frequently reported by the local community (Barros et al., 2017; Lemasson et al., 2017).

The EPAG was created in 1997 with the primary aim of protecting and conserving natural systems essential to biodiversity, especially water resources. It classified the FRES as an integrated protected zone, where the release of untreated effluents is strictly prohibited. However, at the beginning of the 21st century, the STS in Rio Formoso city was not entirely effective, and domestic and industrial waste flowed directly into the estuarine system (Figure 5) (Paiva, 2012).

The high eutrophication status in the 2000s produced dead zones that resulted in a significant increase in the mortality of fishes and crustaceans and a strong media backlash (Paiva, 2012). As a result, in 2005, the STS started to use biological filters to reduce organic matter and nutrient excess, generating positive outcomes (Figure 5) such as reducing phytoplankton biomass by more than 90%, which then improved fishing activities (Paiva, 2012).

According to previous studies (Silva et al., 2009; Grego, 2010; Lima, 2016) and local residents (Melo, 2018), from 2014 to the present day the main causes behind decreases in water quality and declines in FRES fishing resources are the STS's inadequate disposal of sewage and intensive farming. Nevertheless, compared to other tropical estuaries, the FRES is generally still in good condition, particularly during the dry season (Batista and Flores-Montes et al., 2013; Nascimento-Filho et al., 2013; Guenther et al., 2014).

CONCLUSIONS

We show that the FRES is a highly dynamic environment with two trophic status conditions, influenced by seasonal variations. During the dry season, the main channel of the estuary is mostly oligotrophic to mesotrophic. However, during the rainy season, almost all of the estuary is considered eutrophic with poor water quality, especially in the Formoso River, negatively impacting local subsistence activities.

Rainfall is a determinant factor for water quality and anthropogenic pressures, because agricultural and urbanization activities release untreated effluents into the Formoso River year round, albeit more intensely under high precipitation levels. By using a multivariate trophic index (TRIX), we observed that the local traditional community has faced worse scenarios over the past 20 years and might be forced to turn into complementary activities for additional income due to increasing pollution rates.

Integrated management of the EPAG, with the control and inspection of effluent release and treatments by farms and the STS, and a continuous water monitoring are important tools to improve water quality in the FRES. In addition, scientific research must be increasingly aligned with the local community's needs with a view towards creating public policies that could improve the system's conservation and protection.

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AUTHOR CONTRIBUTIONS

B.J.S.: Writing – original draft; Investigation; Conceptualization; Visualization; Formal Analysis; Validation;

C.A.L: Writing – original draft; Investigation; Conceptualization;

P.W.M., N.M., F.L.G.: Writing – review & editing; Investigation; Conceptualization;

M.E.A.: Writing – review & editing; Conceptualization; Funding acquisition; Project administration; Supervision;

M.J.F-M.: Writing – review & editing; Conceptualization; Resources; Validation; Supervision.

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