Journal of the Marine Biological Association of the United Kingdom, 2019, 99(1), 9–18.
doi:10.1017/S0025315417001795

Are biochemical composition parameters of sediment good tools for assessing the environmental quality of estuarine areas in tropical systems?

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The present study aims to assess the environmental quality of six estuaries in north-eastern Brazil, using biochemical composition and quantity of organic matter in tropical sediments. Samples were collected monthly during spring low tide from August 2011 to July 2012, in the mid-littoral. Concentrations of organic matter and its biochemical composition reflected the degree of anthropogenic pressure. Although total concentrations of proteins and carbohydrates were similar among estuaries with contrasting levels of anthropogenic pressure, the protein to carbohydrates (PRT : CHO) ratio effectively reflected the trophic state of these areas. PRT : CHO ratios >1 were observed in all studied areas suggesting eutrophic conditions or an initial stage of eutrophication in all of them. Low Chl-a/Phaeo ratios may be associated with the level of eutrophication of each area, however it may also reflect the natural productivity or the presence of mangrove debris. These results were compared with those obtained in previous environmental assessments of the same estuarine areas using the AZTI Marine Biotic Index and monitoring reports showing good agreement. The present study confirmed for the first time in tropical estuarine areas that the biochemical approach can be successfully used to assess the trophic state of the benthic compartment.

Keywords: Mangrove sediments, trophic state, protein/carbohydrates ratio, eutrophication, phytopigments

Submitted 21 February 2017; accepted 3 October 2017; first published online 6 November 2017

INTRODUCTION

Mangroves are among the most productive environments in the world and are of great economic and ecological importance (Kristensen, 2008; Alongi, 2009; Giri *et al.*, 2011). However, tropical estuarine ecosystems are being globally affected by pressure caused by human activities. According to Duke *et al.* (2007), 1–2% of mangrove forests is lost per year, a rate greater than or equal to the reduction of adjacent highly threatened coral reef or tropical rainforest systems.

Coastal eutrophication is one of the most important phenomena to threaten estuarine environments. This is the result of excessive nutrient inputs, especially nitrogen and phosphorus (Nixon, 1995; Cloern, 2001). Increased levels of nutrients stimulate the production of dissolved and particulate organic matter and lead to reduced oxygen concentration due to intense bacterial activity decomposing organic matter in the water (Gray *et al.*, 2002). Responses to low oxygen concentration include the escape or mortality of demersal fish and benthic organisms, and a subsequent loss of biodiversity (Gray, 1992; Cloern, 2001).

Organic compounds in sediment are the main energy source for benthic organisms. Besides being food for higher trophic levels, benthic organisms also influence biogeochemical cycles through bioturbation (Danovaro *et al.*, 1999; Pearson, 2001). Phytopigment concentrations in the sediment are tracers of the amount of organic matter produced by photosynthesis, so chlorophyll-*a* (Chl-*a*) is frequently used as a descriptor of the trophic state and productivity of estuarine systems (Cahoon & Cooke, 1992; de Jong & de Jonge, 1995; Lucas *et al.*, 2000; Manini *et al.*, 2001).

The trophic state of marine ecosystems is usually assessed through measures of chemical variables (e.g. nitrogen and phosphorus) and the amount of algal biomass in the water column (Stefanou *et al.*, 2000). However, these variables can fail to detect the effects of nutrient increase on benthic systems (Dell'Anno *et al.*, 2002). Recently, it has been shown that the trophic state of the benthos in marine systems is a function not only of organic carbon quantity, but also of its biochemical composition and bioavailability (Pusceddu *et al.*, 2009). In fact, due to the conservative nature of the sedimentary organic carbon, changes in the trophic state of sediments can be more evident in terms of organic matter composition (e.g. ratio of proteins to carbohydrates) than in terms of the concentration of organic matter (Fabiano *et al.*, 1995; Danovaro *et al.*, 1999).

In impacted areas, nutrient increase can result in large amounts of protein-rich organic material entering the benthic system (Dell'Anno *et al.*, 2002). Proteins are more labile and represent the most important nitrogen source for

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benthic organisms, whereas the carbohydrate pool consists mostly of refractory compounds characterized by low degradation rates (Vezzulli & Fabiano, 2006). Thus, sediments with high protein concentrations in relation to carbohydrates may indicate eutrophic conditions, while low protein and high carbohydrate concentrations suggest oligotrophic environments (Dell'Anno *et al.*, 2002; Vezzulli & Fabiano, 2006). This relationship has been used in the literature as a tool for assessing the environmental quality of subtropical and temperate estuaries, where protein to carbohydrate ratio values greater than 1 are common in eutrophic areas, and values less than 1 are typical of meso-oligotrophic areas (Dell'Anno *et al.*, 2002; Pusceddu *et al.*, 2003, 2011; Vezzulli & Fabiano, 2006; Signorini *et al.*, 2008; Garcia-Rodriguez *et al.*, 2011; Muniz *et al.*, 2011; Venturini *et al.*, 2012).

Despite being legally protected areas along the coast of Brazil, mangroves are the coastal environments most affected by anthropogenic influence, as they are close to urban centres. Along the coastline of Pernambuco (north-eastern Brazil), the removal of native mangrove forests and dredging of rivers to allow the construction of port complexes and for land use for sugar cane plantations, the establishment of different industries (textile, metallurgy, food, soap, paper, among others) in nearby estuaries and the drainage of agricultural and domestic effluents untreated into the shoreline are the most frequent estuarine threats (CPRH, 2006).

Nevertheless, in tropical areas, few studies have assessed the environmental quality of estuarine systems, and no studies exist that use protein and carbohydrates concentrations to assess the trophic state. Considering that those responsible for managing and protecting the environment require scientifically based information to assist in decision making and to help in defining strategies for management and conservation, the present study aims (1) to assess the benthic trophic state of six estuaries in north-eastern Brazil, using for the first time the quantity and biochemical composition of organic matter in tropical sediments and (2) to evaluate if the biochemical composition of the sedimentary organic matter, as well as the benthic trophic status, reflect the different levels of human impact in the studied estuaries.

Fig. 1. Location of areas studied along coastline of Pernambuco, north-east Brazil.

MATERIALS AND METHODS

Study areas and sampling

Samples were collected monthly during spring low tide from August 2011 to July 2012, in the mid-littoral of six estuarine areas along the north-eastern Brazilian coast (Figure 1). The selected areas are subjected to different pollution sources (Table 1) based on monitoring reports of estuaries in the state of Pernambuco (CPRH, 2006).

The climatic characterization of north-eastern Brazil is complex, with large spatial and temporal variability of precipitation (Braga *et al.*, 2012). According to Molion & Bernardo (2002), the climate of the north-eastern Brazilian coast is hot and humid with total rainfall ranging from 600 to 3000 mm. The annual cycle of precipitation shows a distinct rainy season that generally occurs from March to July, with peak rainfall in May (Marques *et al.*, 1983; Molion & Bernardo, 2002). In this study the rainfall peak occurred in June and July of 2012 with a lower peak in January and February of the same year (Figure 2).

Sedimentary variables, parameters and analytical methods

Three replicates for proteins and carbohydrates analysis were collected from the top 2 cm of the sediment with a cylindrical corer and sampled twice during the study (December 2011, which is among the months with lower rainfall and June

Table 1. Study area and summary of their main disturbance sources (CPRH, 2006).

Study area (geographic coordinates)	Sources of disturbance	Subjective scale of impact
Paripe (07°48′38.76″S 34°51′23.28″W)	Subsistence fishing (anthropic action is minimal)	0
Timbó (07°51′18.72″S 34°50′33.96″W)	Fishing activities, domestic sewage, industrial effluents (mainly steel and textile mills), urban occupation	++
Pina Basin (08°5′27.0″S 34°53′11.64″W)	Intense urban occupation, domestic and industrial effluents and input from polluted tributaries	+++
Ipojuca-Merepe (08°24′39.66″S 34°58′28.62″W)	Mangrove deforestation and construction of Suape Industrial Port Complex	+++
Maracaípe (08°32′21.42″S 35°00′21.72″W)	Tourism	++
Mamucabas (08°46′41.81″S 35°06′27.46″W)	Agricultural activities	+

0, null impact; +, minimal impact; ++, moderate impact; +++, high impact.

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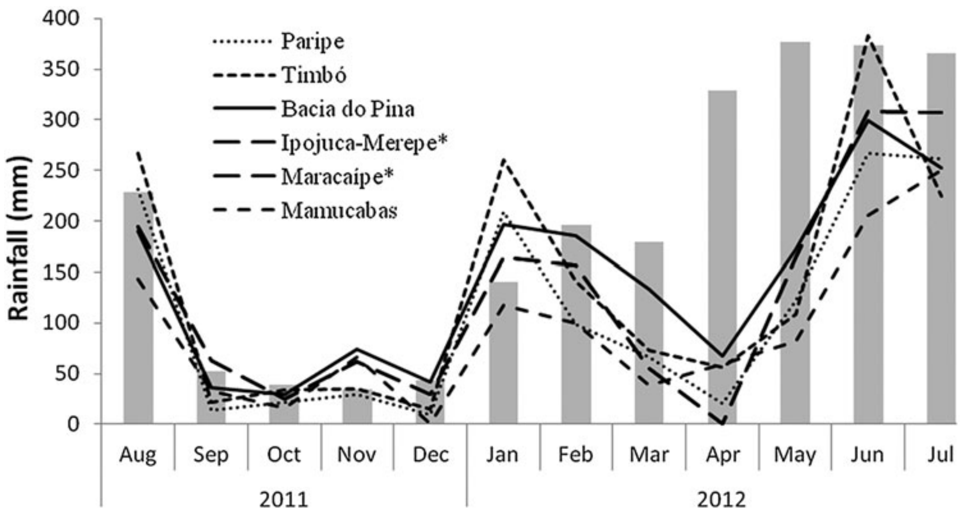

Fig. 2. Monthly rainfall (mm) in the six areas studied. (*) Values of the rainfall monitoring station are the same for both areas. The grey bars indicate historical precipitation.

2012 which is the month with the highest precipitation level). Total organic matter and phytopigment content were also collected in three replicates in the top 2 cm of the sediment, but were sampled monthly throughout the study period.

Total organic matter (TOM) was determined as the difference between the dry weight (60°C, 24 h) of the sediment and the weight of the residue after combustion (495°C, 5 h) according to Wetzel & Likens (1990). Phytopigment concentration of the sediments (Chl-*a* and phaeopigments) determination followed the method described by Colijn & Dijkema (1981) with modifications, and the calculations were performed using the equations of Lorenzen (1967). Pigments were extracted with 90% acetone (24 h in the dark at 4°C). The supernatant was used to determine the functional Chl-*a* and acidified with 0.1 N HCl to estimate the amount of phaeopigments (Phaeo).

Sediment protein was solubilized using an ultrasonic homogenizer and then centrifuged (4000 rpm, 30 min). Protein (PRT) analyses followed the BCA method (Smith *et al.*, 1985), modified from the Lowry method, and concentrations were reported as albumin equivalent. Carbohydrates (CHO) were analysed according to Gerchacov & Hatcher (1972) and expressed as glucose equivalents. The method is based on the same principle as the widely used method of Dubois *et al.* (1956), but is specifically adapted for carbohydrate determination in sediments. The determination of the quality of the organic matter in the sampled areas was based mainly on the protein to carbohydrates ratio: eutrophic areas were considered those with a ratio of PRT/CHO > 1, and meso-oligotrophic areas were those with PRT/CHO < 1. The phytopigment concentration of the sediments and relevant data from literature on the areas were also used to support the assessment of the trophic state of the areas studied.

Redox potentials (Eh) were measured with an oxidation–reduction potential (ORP) platinum electrode at 2 and 10 cm in the sediment. Final readings were corrected by adding the potential of a calomel reference electrode (+244 mV). Dissolved oxygen (measured with portable oximeter), interstitial salinity (measured with refractometer) and temperature were measured *in situ*. Granulometric composition (silt and clay percentage) was determined according to Suguio (1973) and precipitation rates were obtained from the *Laboratório de Meteorologia de Pernambuco* (LAMEPE).

Statistical analysis

The Friedman test, equivalent to the non-parametric two-factor ANOVA, was used (since variances were heteroscedastic even after transformation) to test differences in the concentrations of Chl-*a* and phaeopigments among the six areas during the 12 months of collection. The non-parametric Kruskal–Wallis test, equivalent to the one-factor ANOVA, was used to test statistical differences in the total organic matter (TOM) among the six areas and the Dunn's post-hoc test compared the differences between each pair of areas.

RESULTS

Abiotic variables

Sediment temperature and interstitial salinity varied little throughout the collection period (Figure 3, Table 2). The annual average redox potential ranged from 225 mV in Maracaípe to 391 mV in Timbó at 2 cm depth. At 10 cm depth redox potential ranged from 55 mV in Paripe to 402 mV in Ipojuca-Merepe (Table 2). Dissolved oxygen was very low in the Pina Basin (2.83 ± 2.39 mg l^{-1}), but was higher than 5 mg l^{-1} in the remaining areas: 5.13 ± 1.41 mg l^{-1} in Timbó, 5.2 ± 0.72 mg l^{-1} in Ipojuca-Merepe, 6.6 ± 1.45 mg l^{-1} in Mamucabas, 6.7 ± 1.08 mg l^{-1} in Maracaípe and 7.03 ± 2.17 mg l^{-1} in Paripe river (these values represent the mean ± standard deviation of May, June and July 2012).

Overall, low proportions of silt and clay were found (1.5% in Ipojuca-Merepe, 2.1% in Timbó, 3.1% in the Pina Basin, 3.2% in Mamucabas and 4.8% in Paripe river), except in Maracaípe which reached 15.5% of silt and clay.

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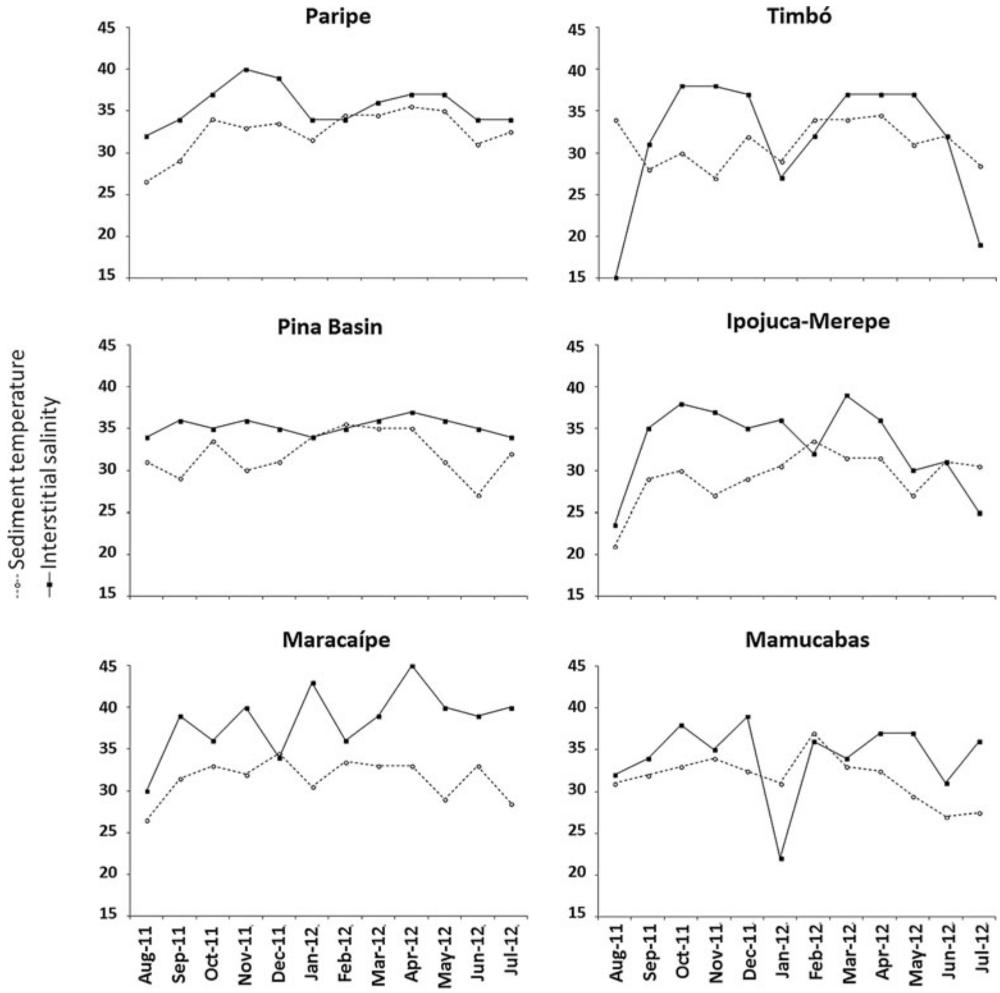

Fig. 3. Temporal variation of interstitial salinity and sediment temperature throughout the study period in the six areas collected.

Composition of organic matter in sediment

The annual average proportion of total organic matter (TOM) was significantly different among the estuaries (Kruskal–Wallis, $H_{(5)} = 148.97$; $P < 0.001$), with significantly higher values in the Maracaípe river (2.74 ± 0.62%) compared with other studied areas. In Paripe the second highest percentage of TOM (1.17 ± 0.43%) was found, significantly higher than all other estuaries, except of the Mamucabas (0.89 ± 0.23%) and the aforementioned Maracaípe. Timbó (0.50 ± 0.25%), Ipojuca-Merepe (0.44 ± 0.14%), Pina Basin (0.42 ± 0.13%) and Mamucabas did not present statistically significant differences between them (Figure 4).

Chl-*a* concentrations (annual average) were significantly different ($Fr_{(5)} = 28.94$; $P < 0.05$) among rivers (Table 3). Phaeopigment concentrations also showed significant differences ($Fr_{(5)} = 36.89$; $P < 0.05$) among rivers with Paripe, Timbo and Pina Basin showing higher concentrations of both chl-*a* and phaeopigments. The annual Chla-*a*/Phaeo ratio varied little among estuaries with Maracaípe presenting the lowest value and Ipojuca-Merepe presenting the highest value (Table 3).

Protein total (PRT) concentrations ranged from 429.3 ± 24.7 $\mu g\ g^{-1}$ in Mamucabas to 4195.2 ± 1161.1 $\mu g\ g^{-1}$ in the Pina Basin, in June 2012. In December 2011, protein concentrations varied little along the estuaries, with the highest value found in the Pina Basin (699.5 ± 182.4 $\mu g\ g^{-1}$) and the lowest in Maracaípe (155.5 ± 78.5 $\mu g\ g^{-1}$). The carbohydrate (CHO) concentration was also highest in the Pina Basin (858.7 ± 382.6 $\mu g\ g^{-1}$), but the lowest concentration was found in Ipojuca-Merepe (158.5 ± 148.1 $\mu g\ g^{-1}$) in June 2012. In December 2011, Ipojuca-Merepe showed the highest concentration of carbohydrates (243.49 ± 83.46 $\mu g\ g^{-1}$), and Mamucabas had the lowest concentration (39.62 ± 7.89 $\mu g\ g^{-1}$). In both sampling dates, all studied areas had a PRT/CHO ratio >1. The total concentrations of proteins and carbohydrates were higher in June 2012 compared with December 2011 in most areas, except for Ipojuca-Merepe, which showed slightly higher values in December 2011 (Figure 5).

DISCUSSION

Despite the fact that estuaries are generally characterized by highly variable salinities, due to the mixing of seawater and fresh water, the collection areas were predominantly characterized as euhaline zones. The average annual values of Eh obtained in this study were always positive, both at 10 and 2 cm depth, indicating oxidative conditions. According to Araújo *et al.* (2012), values of Eh ~ 100 mV indicate

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Table 2. Monthly variation in average of the parameters measured in the sediment in each study area.

Monthly	*T* (°C)	*S*	Eh (2 cm)	Eh (10 cm)	Chl-*a* (μg cm^{-2})	Phaeo (μg cm^{-2})	TOM (%)	*T* (°C)	*S*	Eh (2 cm)	Eh (10 cm)	Chl-*a* (μg cm^{-2})	Phaeo (μg cm^{-2})	TOM (%)	*T* (°C)	*S*	Eh (2 cm)	Eh (10 cm)	Chl-*a* (μg cm^{-2})	Phaeo (μg cm^{-2})	TOM (%)
	Paripe							*Timbó*							*Pina Basin*						
Aug	26.5	32	263	21	6.83	20.31	0.84	34	15	359	404	1.75	11.88	0.29	31	34	387	437	6.49	13.74	0.27
Sep	29	34	38	3	2.99	26.60	0.56	28	31	231	97	5.69	9.20	0.33	29	36	357	415	2.23	17.08	0.36
Oct	34	37	306	243	1.23	17.99	1.11	30	38	409	332	0.81	8.29	0.64	33.5	35	407	467	2.04	5.39	0.43
Nov	33	40	206	−75	2.42	22.53	1.10	27	38	323	425	3.06	6.50	0.36	30	36	389	369	1.92	6.19	0.35
Dec	33.5	39	375	−72	16.21	15.08	1.28	32	37	442	474	6.54	3.88	0.69	31	35	413	464	2.32	6.04	0.30
Jan	31.5	34	157	−15	8.18	20.04	1.05	29	27	406	407	2.13	13.59	1.18	34	34	354	368	1.99	2.32	0.37
Feb	34.5	34	246	209	2.65	28.10	1.10	34	32	428	284	2.23	11.04	0.36	35.5	35	391	405	2.23	13.37	0.35
Mar	34.5	36	34	−61	0.90	7.79	2.01	34	37	397	320	1.66	5.71	0.61	35	36	57	60	4.03	6.79	0.42
Apr	35.5	37	431	88	2.65	14.50	0.96	34.5	37	443	320	2.70	3.07	0.34	35	37	416	429	2.46	13.96	0.45
May	35	37	406	304	2.51	17.03	0.73	31	37	382	340	2.89	2.48	0.25	31	36	393	404	9.57	9.83	0.34
Jun	31	34	363	−39	7.11	29.35	1.67	32	32	487	506	4.93	8.08	0.35	27	35	462	481	4.17	8.87	0.76
Jul	32.5	34	na	na	3.32	15.39	1.77	28.5	19	na	na	2.70	12.36	0.49	32	34	na	na	3.22	16.55	0.60
Average	32.5	35.7	256.8	55.09	4.75	19.56	1.18	31.2	32	391.5	355.36	3.09	8.01	0.49	32	35.3	366	390.82	3.56	10.01	0.42
	Ipojuca-Merepe							*Maracaípe*							*Mamucabas*						
Aug	21	23.5	360	296	1.04	5.32	0.19	26.5	30	26	−86	3.41	5.98	2.19	31	32	381	396	1.66	0.86	0.49
Sep	29	35	403	381	1.52	4.70	0.25	31.5	39	363	333	1.75	11.45	1.94	32	34	385	394	0.62	3.27	0.40
Oct	30	38	337	381	0.85	4.39	0.36	33	36	278	14	0.81	11.53	2.95	33	38	398	364	1.33	4.81	0.87
Nov	27	37	385	415	1.75	3.12	0.32	32	40	300	95	0.28	9.62	2.14	34	35	400	445	1.33	7.83	0.98
Dec	29	35	369	406	3.32	3.15	0.52	34.5	34	297	68	4.60	7.21	2.95	32.5	39	209	−145	3.18	2.65	1.02
Jan	30.5	36	364	383	1.56	1.39	0.59	30.5	43	268	−24	2.46	8.38	2.76	31	22	236	285	1.47	3.34	0.94
Feb	33.5	32	na	na	0.28	4.03	0.32	33.5	36	na	na	1.23	8.85	2.14	37	36	411	436	0.81	11.32	0.98
Mar	31.5	39	377	400	1.23	1.27	0.68	33	39	−42	−53	0.95	5.89	2.76	33	34	365	314	0.90	7.79	1.26
Apr	31.5	36	382	419	0.90	3.31	0.51	33	45	123	15	0.66	5.74	2.63	37	37	385	400	0.38	3.14	0.89
May	27	30	431	454	1.00	3.73	0.42	29	40	391	332	0.95	5.16	2.49	37	37	393	428	2.18	3.33	0.99
Jun	31	31	488	487	2.99	1.10	0.52	33	39	249	198	0.76	8.27	4.40	31	31	445	463	0.52	7.09	0.94
Jul	30.5	25	na	na	2.70	7.55	0.51	28.5	40	na	na	0.43	5.09	2.95	36	36	na	na	0.95	8.81	0.91
Average	29.3	33.1	389.6	402.2	1.60	3.59	0.43	31.5	38	225.3	89.2	1.52	7.76	2.69	33.7	34.3	364.4	343.64	1.28	5.35	0.89

T, temperature; Eh, redox potential; Chl-*a*, Chlorophyll-*a*; Phaeo, phaeopigments; TOM, total organic matter; na, not available.

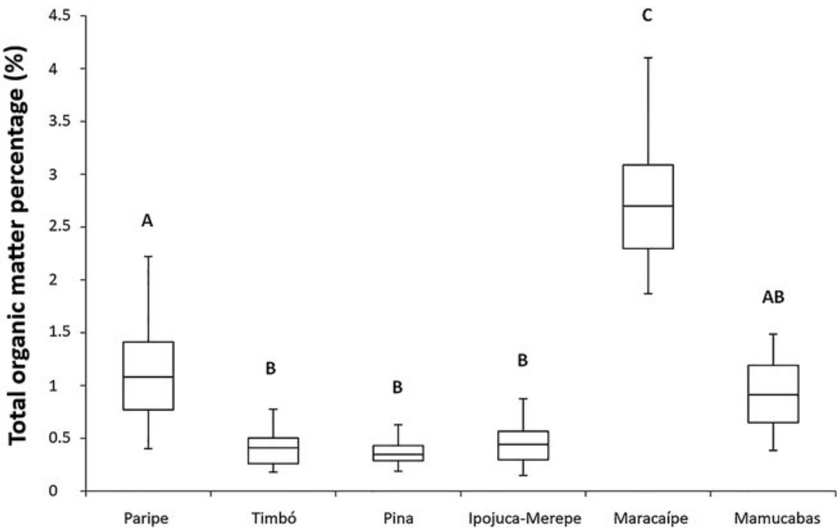

Fig. 4. Box-plot showing the annual organic matter concentrations among studied areas. Different letters indicate statistically significant differences.

Table 3. A posteriori test among the studied areas showing the differences in phaeopigment and chlorophyll-*a* concentrations (±SD).

	Chl-*a*			Phaeo			Chl-*a*/Phaeo
	μg cm^{-2}	(±SD)	Differences	μg cm^{-2}	(±SD)	Differences	
Paripe	4.75	4.31	A	19.55	6.31	A	0.24
Timbó	3.09	1.73	AB	8.01	3.75	ABC	0.38
Pina Basin	3.55	2.32	AB	10.01	4.82	AB	0.35
Ipojuca-Merepe	1.59	0.93	BC	3.58	1.85	C	0.44
Maracaípe	1.52	1.32	BC	7.76	2.29	ABC	0.19
Mamucabas	1.27	0.79	C	5.35	3.12	BC	0.23

Different letters indicate statistically significant differences.

suboxic conditions while Eh values < 50 mV indicate anoxic conditions. Redox potential can be influenced by the grain size, soil aeration and amount of organic matter (Brady, 1990; Grizzle & Penniman, 1991). Thus, the high levels of TOM and small grain size in Paripe, and especially in Maracaípe, may be responsible for lower values of redox potential in these estuaries.

In most areas (except the Pina Basin), dissolved oxygen levels were found within normal limits for Brazilian estuaries (>5 mg l^{-1}), suggesting poor water quality in the Pina Basin estuarine area. Low dissolved oxygen is a critical factor that affects marine organisms (Gray *et al*., 2002). Low oxygen values are common in urbanized areas subjected to organic enrichment and may represent a good indicator of stressful levels of eutrophication (Gray *et al*., 2002; CPRH, 2006; Pinckney, 2006), however they are not a good indicator of early stages of eutrophication.

The use of indices based on chemical properties and organic enrichment of the sediment to assess the environmental quality of estuaries has increased in recent years (Angelidis

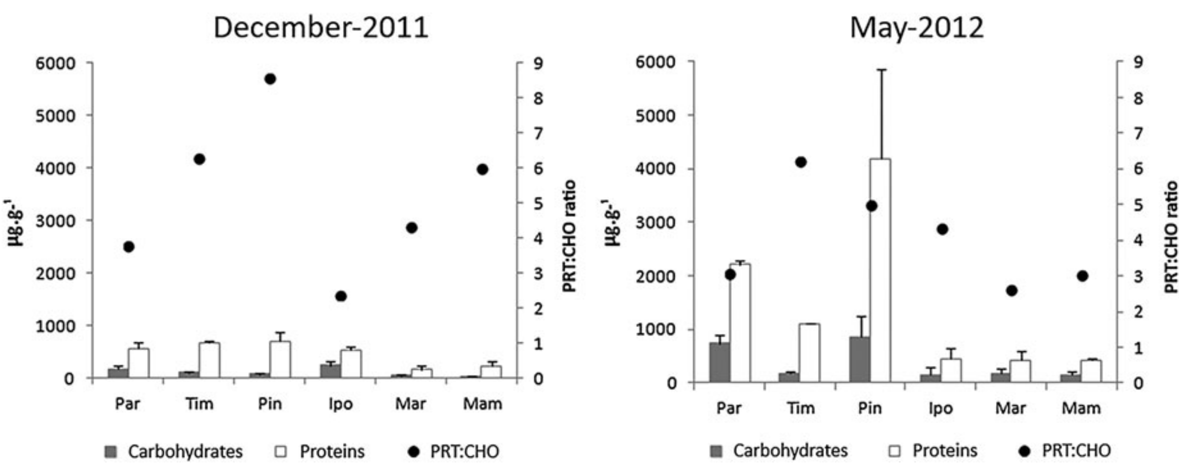

Fig. 5. Protein and carbohydrates concentrations in the sediments along the studied areas in December 2011 and May 2012.

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& Aloupi, 2001; Peterson, 2001; Dell'Anno *et al.*, 2002; Kucuksezgin *et al.*, 2006). The biochemical composition of the sediment can be considered a simple and efficient tool to classify the trophic state of coastal and marine systems and has been widely used in the coasts of Italy (Dell'Anno *et al.*, 2002; Vezzulli & Fabiano, 2006; Signorini *et al.*, 2008), Spain (Cotano & Villate, 2006), Uruguay (Garcia-Rodriguez *et al.*, 2011; Muniz *et al.*, 2011; Venturini *et al.*, 2012), New Caledonia (Pusceddu *et al.*, 2011) and in the Mediterranean Sea (Pusceddu *et al.*, 2003).

In our study the concentrations of organic matter and its biochemical composition indicated the trophic state of the coastal benthic sediment reflecting some degree of anthropogenic pressure. PRT : CHO ratios >1 were observed in all studied areas suggesting hypertrophic or eutrophic conditions. The phytodetritus accumulation and anthropogenic waste associated with smaller organic nitrogen degradation rates may contribute to the prevalence of PRT on the environment and contribute to high values of this ratio (Vezzulli & Fabiano, 2006; Venturini *et al.*, 2012). However, it is also common to ascribe values of PRT : CHO > 1 to deposition of fresh materials of recent formation or of autochthonous origin (Danovaro *et al.*, 1993).

Timbo and Pina Basin were the areas with the highest ratios of PRT : CHO in both December 2011 and June 2012, suggesting eutrophic conditions. These estuaries are known to be subjected to considerable anthropogenic pressure, are included in a metropolitan area and have suffered a serious distortion of the landscape over time (Cabral *et al.*, 2006; CPRH, 2006). According to Somerfield *et al.* (2003), Pina Basin is considered hypereutrophic and organically polluted, corroborating our findings. The tributaries of the Pina basin flow through urban areas without sanitation carryng a range of pollutants, especially high levels of nutrients from domestic wastewater. Valença & Santos (2012) assessing the environmental quality of the same areas using the AZTI Marine Biotic Index (AMBI) found similar results (conditions 'moderately disturbed' in the Pina Basin and 'slightly disturbed' in Timbó).

The Paripe and Maracaípe are always among the three estuaries with lower values of PRT : CHO ratio, although this value is still >1, indicating a beginning of the eutrophication process. These estuaries are far from urban centres and agricultural activities, and human impact on this area is basically fishing and tourism (CPRH, 2006). However, in recent years the Maracaraípe is experiencing a strong process of real estate expansion and tourism, getting more and more domestic effluents and solid waste, beyond the landfill of mangroves (Vila Nova & Torres, 2012). The high input of organic matter and debris associated with an increase in the discharge of domestic sewage in Maracaípe probably determined the ratio PRT : CHO > 1 and consequently its eutrophication state, since this process is mainly due to the volume of inputs of nitrogen and phosphate compounds derived from domestic and industrial effluents and agricultural activities. Paripe, on the other hand, is an area with minimal human intervention with well-preserved mangroves (Monteiro & Coelho-Filho, 2004), which explains one of the lowest values of PRT : CHO ratio among the studied areas. It is likely that this ratio is influenced by the natural input of organic matter and mangrove debris, since this area also has the highest levels of TOM and Chl-*a*. Natural eutrophication occurs very slowly, through inputs of nutrients brought by rain, surface water and resuspension processes. Valença & Santos (2012) classified Paripe as an 'Undisturbed' estuary, but classified Maracaípe as 'moderately disturbed' through the AMBI, highlighting the limitation of the index in ecosystems where the organic matter of the sediment is naturally high, as is the case in Maracaípe.

Ipojuca-Merepe is an area subjected to heavy anthropogenic pressure, according to CPRH (2006) reports. This river receives a high volume of pollutants of agribusiness activities (power plants, distilleries and sugarcane) and its estuarine area suffered severe changes in recent years due to the installation of the Port Industrial Complex. Moreover, the dredging and channels construction, common in ports construction, represent a significant source of suspended sediment, which can promote phytoplankton growth and increased turbidity, leading to reductions in light penetration (Erftemeijer & Lewis, 2006; de Jonge, 2011). Thus the PRT : CHO ratio >1 in Ipojuca-Merepe river may be the result primarily of the contribution of allochthonous substances from the dredging activities. Corroborating our findings, Valença & Santos (2012) using the AMBI, classified Ipojuca-Merepe as a 'heavily disturbed' estuary. In this case, the agro-industrial pollution, besides the eutrophication process, may have had an important role in the quality of the local macrofauna, making this one of the most disturbed estuaries according to the AMBI.

Mamucabas is considered a quasi-pristine estuary (CPRH, 2006), with few human interventions along its route. However, the PRT : CHO ratio suggests that Mamucabas is also an area with eutrophic conditions. There are no previous studies that assess environmental quality indicators (e.g. concentration of Chl-*a*) in this estuary, except for Valença & Santos (2012) who used the AMBI to classify this estuary as 'heavily disturbed'. However, the authors also discuss the fragility of AMBI in the assessment of areas with low salinity (Borja & Muxika, 2005) as was the case in the study area at the time/area that they conducted their collections. It is possible that the Mamucabas is still at an early stage of eutrophication, especially because among the studied areas, it has the lowest concentrations of Chl-*a* and phaeopigments.

High Chl-*a* and phaeopigment concentrations in the benthic compartment are commonly found in eutrophic systems under human influence with high primary production levels (Muniz *et al.*, 2011; Venturini *et al.*, 2012). Nevertheless, in the present study a clear pattern was not observed in the phytopigments concentrations related to the level of human pressure exerted on the studied areas. For instance, in both Pina Basin (an area with high human pressure) and Paripe (an area with low human pressure) high Chl-*a* and phaeopigments concentrations were found compared with other studied estuaries. These variations are probably related to the natural productivity and conditions of each area. In addition to Chl-*a*, Paripe also showed high levels of carbohydrates, proteins and TOM, which leads us to believe that this is a naturally highly productive estuary. In Timbo, Chl-*a* concentrations similar to Pina basin were found. These high values are possibly associated with high levels of anthropogenic impact in both estuaries. Spatial and temporal distribution of microphytobenthos is generally controlled by light penetration, nutrient availability, foraging pressure (Bianchi & Rice, 1988), turbidity (Lehman, 1992) and sediment type (Colijn & Dijkema, 1981; Davis & McIntyre, 1983).

The Chl-*a*/Phaeo ratio <1 could indicate the prevalence of detritus in the sediments (Venturini et al., 2012). The predominance of phaeopigments may be attributed to high water column turbidity, chemical contamination or any other factor affecting the photosynthetic potential (Bhadauria *et al.*, 1992; Dell'Anno *et al.*, 2002). This ratio is currently used as an indicator of the ecosystem trophic state (Dell'Anno *et al.*, 2002; Muniz *et al.*, 2011; Pusceddu *et al.*, 2011), nevertheless, it may also reflect both the physiological condition of the algae and/or the input of detritus and partially degraded organic matter (Cadée & Hegeman, 1974). The Chl-*a*/Phaeo ratio was lower than 1 in all studied areas. Despite the ratio varying little, it is observed that these values were lower in estuaries with less human impact, such as the Mamucabas, Maracaípe and even the Paripe which showed high concentrations of Chl-*a*. Low values of the Chl-*a*/Phaeo ratio may be related to natural low productivity or to anthropogenic impacts on the estuary (Muniz *et al.*, 2011). Is also possible that little productive or meso-oligotrophic estuaries present a low ratio of Chl-*a*/Phaeo due to low amounts of Chl-*a*. Besides, the limitation of light penetration by turbidity has been frequently regarded as a factor controlling primary production in estuaries (Lehman, 1992).

According to Dell'Anno *et al.* (2002), protein concentrations were sufficient to identify differences in the trophic state of the studied areas. Furthermore, Dell'Anno *et al.* (2002) proposed the use of trophic thresholds, which have already been used in the assessment of the trophic state of estuarine areas (Vezzulli & Fabiano, 2006; Garcia-Rodriguez *et al.*, 2011; Muniz *et al.*, 2011; Venturini *et al.*, 2012), on the basis of both protein and carbohydrates concentrations: hypertrophic (PRT > 4.0 mg g^{-1}; CHO > 7.0 mg g^{-1}) eutrophic (PRT $= 1.5-4.0$ mg g^{-1}; CHO $= 5.0-7.0$ mg g^{-1}) and meso-oligotrophic (PRT < 1.5 mg g^{-1}; CHO $<$ 5.0 mg g^{-1}). However, the total protein and carbohydrates concentrations in the present study were well below the values proposed by Dell'Anno *et al.* (2002), and would classify all estuaries in meso-oligotrophic levels. In general, Chl-*a* and phaeopigment concentrations were also very low, in relation to the aforementioned works and to other works carried out in the north-east of Brazil (Murolo *et al.*, 2006). These results suggest that Chl-*a*/Phaeo and PRT/CHO ratios could be used as ecological indicators of environmental quality instead of isolated concentrations, to allow for the influence of system functioning differences. Pusceddu *et al.* (2007) also concluded that the assessment of the environmental quality of transitional ecosystems cannot be achieved using a single indicator.

Generally, distribution patterns of organic matter in sediments and biochemical compounds are influenced by granulometric composition (Naidu, 1982; Cotano & Villate, 2006). Organic compounds in the sediment are usually related to small particle size due to the high adsorption capacity of organic molecules by fine particles (Naidu, 1982). In the present study, the sediment was predominantly sandy (with low silt and clay content), whereas in previous studies (Murolo *et al.*, 2006; Garcia-Rodriguez *et al.*, 2011; Muniz *et al.*, 2011; Venturini *et al.*, 2012), silt and clay represented at least 50% of the total granulometric composition. Those results support the low concentrations of proteins, carbohydrates and also of phytopigments content in the present study and suggest the need for using concentration ratios.

Detecting anthropogenic impact in estuarine environments is complex, mainly because of the large natural variability of such ecosystems (Elliott & Quintino, 2007; Borja *et al.*, 2008). The need to combine multiple tools for impact assessment is increasingly evident, but even then the interpretation of the results still requires a minimum of subjectivity, as considered in the 'estuarine quality paradox' discussion (Elliott & Quintino, 2007; Dauvin & Ruellet, 2009). The PRT/CHO ratio in this study showed consistent results as an indicator of the health of the environment and was effective in assessing the trophic state of tropical estuaries, mainly when combined with the literature about the environmental quality of these areas, like the AMBI results (Valença & Santos, 2012), CPRH (2006) reports and Chl-*a* and phaeopigments concentrations. Nevertheless, more studies are required on the use of biochemical indicators (mainly proteins and carbohydrates) to assess the environmental quality of tropical estuaries in other regions and to allow a comparative assessment and, if appropriate, an adaptation of the thresholds proposed by Dell'Anno *et al.* (2002). Furthermore, considering the natural variability of estuarine systems, holistic studies which use multiple and integrative approaches of environmental quality assessment are needed to circumvent these limitations.

Despite all the studied estuaries showing eutrophic conditions with PRT/CHO ratios always >1, spatial differences in this ratio were clearly associated with the level of disturbance of each area. This trophic threshold together with both AMBI results (Valença & Santos, 2012) and monitoring reports (CPRH, 2006), allowed more consistent conclusions about the environmental quality of these areas. Phytopigment concentrations were partly related to the level of anthropogenic pressure exerted on studied areas. The low Chl-*a*/Phaeo ratio reflected well the estuarine eutrophication status according to what was found in the PRT/CHO ratio. However the small variations of this ratio among the areas are probably influenced by the natural productivity and prevalence of mangrove detritus in the sediments. More studies focusing on the biochemical composition of sediment organic matter in tropical estuarine areas are needed in order to allow direct comparisons and a possible adaptation of the threshold values previously proposed by Dell'Anno *et al.* (2002). In summary, the results of the present study confirm that the biochemical approach can be successfully used to assess the trophic state of the tropical estuarine areas.

ACKNOWLEDGEMENTS

We are grateful to the staff of the Laboratório de Dinâmica de Populações de Invertebrados Bentônicos of the Universidade Federal de Pernambuco for assistance in fieldwork and to Mr James A. Young for English revision.

FINANCIAL SUPPORT

This work was supported by a graduate grant (IBPG-0369-2.04/10) for Fred B. Silva from Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE) and by a research fellowship (507274/2010-5) to Paulo J. P. Santos from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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https://doi.org/10.1017/S0025315417001795 Published online by Cambridge University Press