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Article

Estuary Adjacent to a Megalopolis as Potential Disrupter of Carbon and Nutrient Budgets in the Coastal Ocean

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The goals were to estimate nutrients and carbon flow rates between Guanabara Bay and the adjacent coastal waters, to characterize the provenance of the exported/imported organic matter. Samples were collected from different depths over 25 h in two seasons at the bay entrance. Measurements included physicochemical parameters, nutrients, chlorophylls, dissolved organic carbon (DOC), particulate organic carbon (POC), particulate nitrogen (PN), carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic composition, sterols in suspended particulate matter (SPM) and bacterioplankton. Most variables showed higher values in ebb tide events. The flow rates calculated on daily basis and estimated on annual basis revealed the exportation to the continental shelf of 1.27×10^4 kmol year^{-1} dissolved inorganic nitrogen (DIN), 9.52×10^2 kmol year^{-1} dissolved inorganic phosphorus (DIP), 2.65×10^4 t year^{-1} DOC, 1.96×10^4 t year^{-1} POC, and 2.96×10^4 t year^{-1} PN. The estimates show the bay contributes with 0.01% of the total global carbon influx to the ocean.

Keywords: organic carbon, nutrients, flow rates, sterols, carbon and nitrogen isotopes

Introduction

Coastal environments play an important role in the biogeochemical cycles and budgets of carbon and nutrients on a global scale.[1] The changes of these cycles and budgets result in high rates of primary and secondary production, and also increase the flux and transformation rates of organic matter (OM) along the river/estuary/coastal-sea continuum.[2-5]

The cycle of OM in coastal systems, as in estuaries and bays, is highly dynamic and complex. In part, this results from the constant change in the relative contribution and distribution of OM from autochthonous and allochthonous sources, which is modulated by variations in freshwater regime and tidal forcing.[6-9] Turbulent mixing of fresh- and sea-water can also generate sudden changes in temperature, salinity, turbidity, pH and bioactive element concentrations in time scale of seconds to months.[7]

Superimposed to the aforementioned natural factors, in many urbanized areas raw or only partially treated sewage is released directly into adjacent aquatic systems. The introduction of these nutrient rich effluents into water bodies with restricted water circulation can cause eutrophication.[10,11] It is also the cause of less evident problems including high economic losses,[11] changes in the ecological structure[12] and decreased levels of dissolved oxygen (DO).[13,14] In addition to alterations directly affecting the receiving body, the nutrient load may be transported to inner and mid shelf areas, in the dissolved and particulate forms, and thereby changing the carbon balance and fixation rates in the marine system.[15] It has been considered, for instance, that nutrient and carbon exports originating from areas contaminated by sewage may be directly related to ocean acidification processes, and associated with climatic changes.[1,15,16]

A number of studies have attempted to relate environmental changes in estuaries to the increased input of nutrients and OM.[17-20] However, these studies usually

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addressed qualitative aspects, whereas a quantitative approach including, for instance, the influence of material transferred along the estuarine gradient is less frequently observed.[21-23]

Selman *et al.*[24] provide strong evidences of the growing global impact of eutrophication, identifying 415 eutrophic and hypoxic coastal systems around the globe, including the coastal region of Rio de Janeiro and other areas in the south-southeastern coast of Brazil.

The warm and nutrient poor waters of the Brazil current dominate a large portion of the Brazilian inner and mid shelf. In the mid shelf area off Rio de Janeiro, on the other hand, the upwelling of South Atlantic central waters (SACW) is a strong seasonal phenomenon that creates areas of enhanced primary production. Besides the fertilization by upwelling, the region receives nutrient and OM inputs from the eutrophic Guanabara Bay (GB), whose hydrographic basin houses nearly 12×10^6 inhabitants.[25] GB is considered to be one of the most polluted coastal systems in Brazil, where the foremost contamination derives from untreated domestic effluents.[26-28]

The high eutrophication of GB results in enhanced local rates of primary production, reaching values of 0.17 mol C m^{-2} day^{-1}.[29] The annual input of 3.2×10^{10} mol P and 6.2×10^{10} mol N, mostly originated from untreated sewage discharge, has been estimated by Wagener.[28] A fraction of the highly available nutrients and OM derives from industrial activities producing about 150 tons of effluents *per* day.[27,30]

In the present study, GB was taken as a model system to investigate the potential impact of highly eutrophic environments on the nutrient and carbon global balance in coastal zone and to estimate its relevance as a source of these materials to the inner continental shelf.

For this purpose, physicochemical and biogeochemical properties of the water column were studied over tidal cycles in the dry and wet seasons providing also information on the origin of particulate organic carbon (POC) through the use of elemental (C and N) and isotopic ($\delta^{13}C$ and $\delta^{15}N$) composition. Sterols were also considered as additional indicators of the sources of OM in the studied region.

Experimental

Study area

Guanabara Bay (Figure 1), one of the largest bays in the Brazilian coast, is circumscribed by the metropolitan area of Rio de Janeiro (22°40'-23°00' S, 43°00'-43°20' W) serving as estuary for more than 45 rivers and streams, of which only six are responsible for 85% of total annual freshwater discharge. The surface area of the bay is approximately 384 km^2 and the average volume of water is around 1.87×10^9 m^3. The average depths in the shallowest area are of 3 m, and in the central portions water depth ranges from 30 to 40 m.[31-33]

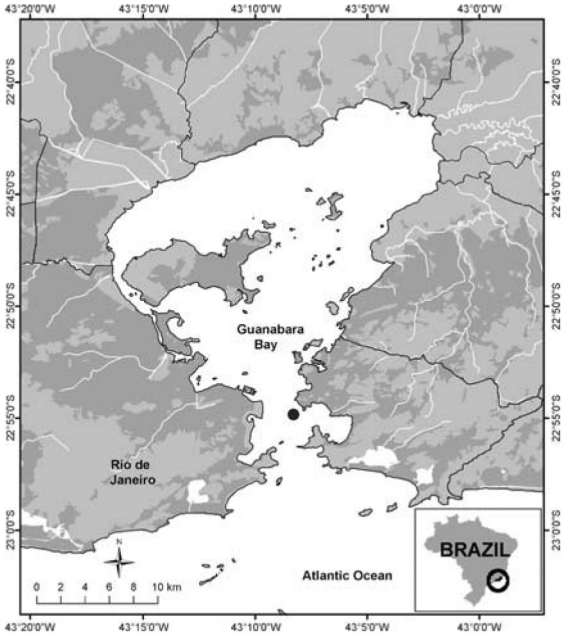

Figure 1. Map of Guanabara Bay showing the sampling station.

The influx of fresh water to GB is not sufficient to modify the flow pattern of the water in the bay because the ratio of fresh water runoff to tidal prism for a period of 24 h is less than 5%.[33] However, riverine inputs are important for determining some patterns and water mass exchanges. The effects of riverine contribution on the receiving system depend on the use and occupation of the drainage basin. In systems adjacent to highly industrialized areas, such as GB, in addition to nutrient inputs, environmental conditions are significantly altered due to releases of trace metals, hydrocarbons, other potentially toxic substances and OM.[33,34]

The tidal influence is very important in the bay especially due to the intensity of the current velocities, which range from 80 to 150 cm s^{-1} at the bay mouth decreasing to 30-50 cm s^{-1} in the inner areas. The average tidal amplitude is of 0.7 m, varying from 1.1 m in spring tide to 0.3 m in neap tide.[35] Salinity varies in the range of 29.5 ± 4.8 and reported average surface water temperature is 24.2 ± 2.6 °C.[33]

Sampling

A sampling station (Figure 1) was strategically positioned as to allow the observation of tidal influence and

exchange of materials between the bay and the open coastal area. Two sampling campaigns (the dry season sampling (C1) was in June 2011, and the wet season campaign (C2) occurred in November 2011) were performed, and the total monthly precipitation for C1 was 32.7 mm and for C2 it was 93.3 mm. Both samplings occurred in spring tide due to the higher amplitude variation (3.4 m for both) between low and high tides. Water samples were taken at each 2 h from three different depths: surface (S, < 1 m), mid-depth (M, 5 m) and close to the bottom (F, 25 m). Sampling was conducted aboard the parcel ship and the local depth varied from 28 to 30 m.

Salinity, temperature and depth were monitored through a conductive, temperature and depth (CTD) system (SBE, model 19 plus V2 SEACAT; frequency 4 Hz) to obtain a full water profile characterization. The data were processed on MatLab (R2008a) using only the data collected when the instrument was submerged and the average calculation for each 0.5 m of depth. The pressure inversions data were removed and also all the unrealistic values were removed based on the thermohaline index for the local water mass. The temperature and salinity data were filtered in order to remove peaks that exceeded values 3 times the standard deviation around the mean. An acoustic doppler current profiler (ADPC) WorkHorse 600 KHz was installed to obtain current data. Current components were corrected for magnetic decline and a small rotation of 22° W in relation to the geographic North. DO was measured with Alfakit AT 150 oximeter (range 0.01 to 20.00 mg L^{-1}) and the pH was measured with Thermo Scientific Orion 3-Star (range –2 to 19.999), both *in situ*.

Water samples for organic compounds (sterols), elemental and isotopic compositions (C and N) of the suspended particulate matter were collected at each depth by using an amber glass bottle that was inserted closed into the water column. Bottles containing samples were kept under ice until filtration in the lab. For nutrients and chlorophyll determinations 4L Go-Flo bottles were used for sampling and samples were stored in 1.5 L polyethylene bottles under refrigeration. Samples for bacterioplankton counting (Bact) were stabilized with *p*-formaldehyde immediately after sampling and thereafter kept in liquid N_2. Samples for ammonium (NH_4^+) determination were stored in 50 mL glass tubes with screw cap after fixation *in situ* as described in Grasshoff *et al*.[36]

For separation of the particulate matter samples were filtered in the lab. Those intended for sterol determination were filtered in Macherey-Nagel 142 mm diameter, 0.7 µm porosity glass filters. Samples for POC, particulate nitrogen (PN), $\delta^{13}C$ and $\delta^{15}N$ were filtered in Macherey-Nagel 47 mm, 0.7 µm glass filters. For chlorophyll determination, filtration through cellulose acetate filters was applied before filter immersion in 90% acetone-water solution following Parsons.[37] The filtrate was stored at –20 °C in polyethylene bottles for dissolved inorganic nutrient analyses.

Analytical procedures

Nutrients (nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), inorganic dissolved phosphorus (P_{inorg})) were determined following the methods described by Grasshoff *et al*.[36] Chlorophylls (Chl-*a*, Chl-*b* and Chl-*c*) were determined using the method of Parsons[37] and equations given by Jeffrey and Humphrey.[38] A DMS 100 UV-Vis spectrophotometer (Intralab, PerkinElmer) was used and all variables were determined in duplicate. The analytical variability was < 2%. Quality control included calibration curves with $r > 0.99$. Blanks were treated as samples for zero calibration in the spectrophotometer. Limits of detection (LOD) for nutrient determinations were the lowest concentration detected with precision 0.01 µmol L^{-1} for NO_3^- and NO_2^-; 0.1 µmol L^{-1} for NH_4^+; and 0.02 µmol L^{-1} for P_{inorg}. Bact was analyzed by flow cytometry.[39,40]

Dissolved organic carbon (DOC) was determined in a Shimadzu total organic carbon (TOC)-VCPN analyzer (samples determined in duplicate). The LOD was equal to 3 × standard deviation (SD) of 10 blanks / slope of the calibration curve = 0.35 mg L^{-1} and limit of quantification (LOQ) = 3.33 × LOD was 1.15 mg L^{-1}.

The filters used for particulate carbon and nitrogen were sub sampled and only the ones used for POC and $\delta^{13}C$ analysis were treated with HCl fumes for over 18 h to remove carbonates and dried at 60 °C overnight. Filter blanks were treated in the same way as the samples. The standard reference material (SRM) 1944 of The National Institute of Standards and Technology (NIST) (New York/ New Jersey Waterway Sediment; TOC: 4.4 ± 0.3% d.w.) was used for accuracy testing and for n = 4 the average TOC concentration obtained was of 4.65 ± 0.29% d.w. The accepted variation coefficient was 0.06%; when higher values were obtained for duplicates sample analysis was repeated. A Thermo Scientific Flash 2000 elemental analyzer was used for POC and PN determination. Instrumental calibration was performed with standard aspartic acid (C = 36.09% d.w., N = 10.52% d.w.) and only calibration curves showing r = 0.999 or higher were accepted. LOD and LOQ were calculated using the smallest detectable amount of the standard and n = 7. As the minimum mass was used in these measurements LOQ was considered equal to LOD: 0.003 mg C and 0.01 mg N. At each 10 samples batch a standard was analyzed to check the calibration.

$\delta^{13}C$ and $\delta^{15}N$ of the particulate organic matter was determined in a Thermo Scientific Flash EA 1112 Delta V Plus isotope-ratio mass spectrometer (IRMS). For reference gases calibration and determination of LOD and LOQ the standard reference material U. S. Geological Survey (USGS) 40 (*L*-glutamic acid) purchased from the International Atomic Energy Agency (IAEA) (–26.389 ± 0.042‰ Vienna Pee Dee Belemnite (VPDB) for $\delta^{13}C$ and –4.5 ± 0.1‰ air N_2 for $\delta^{15}N$) was used. Reported values are the mean of triplicate determinations and those considered as valid measurements were generating pulses > 500 mV for carbon and > 800 mV for nitrogen.

Extraction of sterols was based on the U. S. Environmental Protection Agency (EPA) 3540C method.[41] Filters containing the suspended particulate matter (SPM) were freeze dried and weighed before addition of the surrogate standard (5α-androstan-3β-ol) and Soxhlet extracted with 250 mL of dichloromethane over 24 h. Following extract concentration in a rotary evaporator, solvent was exchanged to hexane before fractionation in a glass column (7 g alumina, 2% deactivated silica gel and 1 g Na_2SO_4) to isolate aliphatic (F1) and aromatic hydrocarbons (F2) not considered in the present work. Only the fraction of interest, containing the sterols (F3), was then cleaned up using dichloromethane:methanol (9:1). To F3, evaporated to dryness under N_2 flow, 250 µL of acetonitrile and 100 µL of bis(trimethylsilyl)trifluoroacetamide (BSTFA) were added for derivatization under 80 °C for 1 h. Lastly, the extracts were dried and the final volume adjusted to 1 mL with dichloromethane after addition of cholestane (2500 ng) as internal standard.

Sterols were determined in a Finnigan Focus DSQ gas chromatography-mass spectrometry (GC-MS) system operated in the electron ionization (EI, 70 eV) and full scan (*m/z* 50-550) modes. A DB-5 type column (5% methyl-phenyl siloxane, 30 m × 0.32 mm × 0.25 µm film) was used. Quantification in the GC-MS was performed using a calibration curve (100 to 5,000 ng mL^{-1}) with commercial standards [cholest-5-en-3β-ol ($27\Delta^5$), 5α-22-cholestan-3β-ol ($27\Delta^0$), 24-methylcholest-5-en-3β-ol ($28\Delta^5$), 24-ethylcholesta-5,22-dien-3β-ol ($29\Delta^{5,22}$) and 24-ethylcholest-5-en-3β-ol ($29\Delta^5$)] and considering the peak areas of key ions (*m/z* 129 and 215 for unsaturated and saturated sterols, respectively; *m/z* 370 for coprostanol (5β-cholestan-3β-ol) and *m/z* 368, 394 or 396 for sterols with double bond in C_{22}). Similar response factors for key ions were assumed for structurally related compounds for which standards were not commercially available. GC-MS compound assignments were based on the full scan mass spectra obtained from the available standards (see above) or by comparison with mass spectra found in the literature for the other compounds. Cholestane (5α-cholestane, *m/z* 217) was used as quantification internal standard and androstanol (5α-androstan-3β-ol) was the surrogate standard quantified by the *m/z* 333. LOD (LOD = 3 × SD of 10 blanks / slope of the calibration curve) and LOQ (first point of the calibration curve > 3.33 × LOD) were 0.01 and 100 ng mL^{-1}, respectively.

Statistical data evaluation

The software Statistica 11 was used for data evaluation through Pearson correlation test, Kruskal-Wallis test, and principal component analysis. Further information is given in Results and Discussion. All discussions referring to differences or similarities are based on the results of the statistical analysis using $p < 0.05$ for decision.

Results and Discussion

Characterization of the water column and physicochemical properties

The average values, standard deviations and range for all physicochemical and geochemical parameters are given in Table 1.

The temperature (T) and salinity (Sa) diagram revealed distinct water column stratification especially in C2 (Figure 2).

In C1, temperatures were around 22 °C at all depths and the salinity varied slightly in the range of 32 to 34.5, with lower values in the surface. These values indicate that coastal waters, which are characterized by Sa < 33, dominated most samples collected in C1 at all depths. In C2, there was a linear correlation between T and S, marked by predominance of cold (T < 18 °C) and saline waters (Sa > 34.5) at the bottom and in mid-depth. These properties indicate the influence of SACW identified in the bottom by temperatures below 20 °C and salinities in the range of 34.6 to 36.2.[42] The plot of salinity against temperature for C1 and C2 can be seen in Supplementary Information Figure S1. The current intensity and direction is shown in Figure 3.

The pH values were higher in the surface due to interaction between respiration, production and water-atmosphere exchange processes. The consumption of CO_2 by photosynthesis causes an increase in pH, while the production of CO_2 during respiration leads to a decrease in pH. In general pH was more elevated in C2 ($p < 0.05$), especially at the bottom, due to the intrusion of SACW.[43] DO saturation varied from 131% at the surface to 89% at the bottom in C1 and from 75 to 39% in C2. The significant positive linear correlations between DO and

Table 1. Means, standard deviations (SD), medians and ranges for all physicochemical and geochemical parameters in surface, mid-depth, and bottom layers in C1 and C2

Parameter		Mean ± SD			Median			Range		
		Surface (S)	Mid-depth (M)	Bottom (F)	Surface (S)	Mid-depth (M)	Bottom (F)	Surface (S)	Mid-depth (M)	Bottom (F)
Temperature / °C	C1	21.81 ± 0.16	21.82 ± 0.13	21.77 ± 0.04	21.81	21.82	21.77	21.56-22.06	21.67-22.02	21.72-21.88
	C2	21.40 ± 0.82	20.13 ± 1.10	16.89 ± 0.56	21.69	20.21	16.83	19.31-22.15	17.71-21.34	15.97-17.92
pH	C1	8.14 ± 0.11	8.11 ± 0.10	8.05 ± 0.19	8.11	8.11	8.06	7.98-8.42	7.87-8.34	7.53-8.37
	C2	8.38 ± 0.11	8.32 ± 0.08	8.18 ± 0.10	8.37	8.30	8.18	8.24-8.64	8.22-8.48	8.02-8.46
DO / (mg L^{-1})	C1	7.91 ± 0.86	7.71 ± 0.57	7.17 ± 0.56	7.50	7.52	7.13	7.02-9.44	6.95-8.88	6.40-8.51
	C2	4.62 ± 0.43	4.26 ± 0.39	3.60 ± 0.45	4.24	4.29	3.45	4.01-5.48	3.44-4.72	3.14-4.79
DO / %	C1	109 ± 11.85	106.80 ± 7.79	99.48 ± 7.80	105.45	106.34	98.79	96.24-130.80	95.82-122.94	88.90-118.16
	C2	63.43 ± 5.94	57.38 ± 5.38	45.83 ± 5.90	61.26	57.83	44.94	54.35-75.40	46.13-63.81	39.49-61.68
Salinity	C1	32.38 ± 0.44	32.65 ± 0.42	33.98 ± 0.30	32.23	32.51	34.00	31.74-33.10	32.11-33.27	33.49-34.39
	C2	33.31 ± 0.37	33.75 ± 0.47	34.79 ± 0.09	33.33	33.77	34.76	32.82-34.18	33.14-34.75	34.69-34.98
Chl-*a* / (μg L^{-1})	C1	35.01 ± 19.92	32.77 ± 16.30	18.66 ± 15.09	32.04	28.13	11.60	10.78-88.43	11.92-63.37	7.12-49.70
	C2	38.94 ± 22.55	24.75 ± 11.75	12.37 ± 6.49	29.92	22.65	10.15	11.47-86-45	6.12-47.34	5.88-30.76
Chl-*b* / (μg L^{-1})	C1	5.99 ± 4.15	6.73 ± 4.93	4.76 ± 4.55	6.47	5.50	3.02	1.60-10.05	0.74-18.57	0.78-17.70
	C2	2.67 ± 4.16	1.12 ± 2.38	0.77 ± 1.10	0.27	0.12	0.38	0.53-13.56	0.25-8.97	0.19-4.25
Chl-*c* / (μg L^{-1})	C1	9.81 ± 4.95	10.83 ± 7.03	7.40 ± 6.09	9.07	8.33	6.12	4.35-17.30	1.81-27.62	1.52-25.18
	C2	11.76 ± 9.84	8.05 ± 5.70	4.21 ± 2.74	7.86	7.54	3.84	0.10-31.53	0.96-20.80	0.10-10.47
Bact / (10^5 cells mL^{-1})	C1	33.80 ± 5.20	34.10 ± 13.90	24.70 ± 15.40	32.75	28.98	18.90	2.73-4.41	2.17-4.78	1.49-2.94
	C2	38.6 ± 8.39	61.50 ± 4.63	54.20 ± 10.80	36.27	61.77	56.19	2.93-5.66	4.99-6.84	3.30-7.32
NH_4^+ / (μmol L^{-1})	C1	31.37 ± 5.60	32.09 ± 6.49	21.63 ± 9.22	31.89	33.87	20.72	20.27-43.52	22.80-46.07	8.80-44.99
	C2	13.33 ± 3.48	12.49 ± 3.97	11.03 ± 3.67	13.42	11.77	11.15	6.81-19.16	7.36-22.11	6.22-21.81
NO_2^- / (μmol L^{-1})	C1	2.66 ± 0.29	2.36 ± 0.28	2.04 ± 0.43	2.73	2.43	1.92	2.11-3.24	1.91-2.85	1.45-2.69
	C2	3.44 ± 0.82	2.97 ± 0.83	1.88 ± 0.43	3.55	3.17	1.82	1.54-4.99	1.48-4.17	1.25-2.86
NO_3^- / (μmol L^{-1})	C1	2.36 ± 1.02	2.68 ± 1.45	2.07 ± 1.45	2.03	2.44	1.55	1.45-5.08	1.61-7.06	0.79-6.37
	C2	1.81 ± 1.55	1.80 ± 1.35	1.63 ± 1.26	1.27	1.50	1.32	0.49-6.10	0.03-4.42	0.03-3.38
DIN / (μmol L^{-1})	C1	36.40 ± 5.99	37.13 ± 6.33	25.74 ± 9.3	36.61	38.06	25.14	24.13-49.51	27.15-50.99	13.40-50.12
	C2	18.60 ± 4.26	17.27 ± 4.20	14.56 ± 4.30	18.56	16.60	13.80	10.45-27.82	10.88-25.86	8.30-27.18
P_{inorg} / (μmol L^{-1})	C1	0.54 ± 0.19	0.58 ± 0.12	0.58 ± 0.10	0.55	0.61	0.58	0.25-0.85	0.37-0.76	0.47-0.72
	C2	1.88 ± 0.15	1.99 ± 0.19	2.15 ± 0.28	1.85	1.96	2.20	1.75-2.21	1.65-2.44	1.35-2.43
SPM / (mg L^{-1})	C1	16.25 ± 5.25	14.04 ± 2.30	12.51 ± 3.60	13.86	14.08	12.53	8.50-28.67	9.80-18.30	7.36-17.80
	C2	36.28 ± 12.23	33.74 ± 7.63	34.05 ± 9.23	31.38	30.57	32.00	24.50-68.24	24.00-49.40	23.40-52.93
DOC / (mg L^{-1})	C1	49.71 ± 116.25	18.56 ± 58.40	30.97 ± 72.64	2.58	2.58	1.98	1.54-415.04	1.75-212.95	1.49-226.32
	C2	55.51 ± 155.26	2.82 ± 1.31	36.10 ± 107.75	3.52	2.43	2.19	2.06-561.30	1.70-5.75	1.37-392.30
POC / (mg L^{-1})	C1	1.34 ± 0.52	1.11 ± 0.35	0.53 ± 0.16	1.28	1.28	0.51	0.52-2.30	0.51-1.99	0.35-0.95
	C2	1.30 ± 0.35	1.09 ± 0.26	0.63 ± 0.18	1.21	1.07	0.59	0.83-1.94	0.73-1.63	0.33-0.91
PN / (mg L^{-1})	C1	0.25 ± 0.09	0.21 ± 0.05	0.10 ± 0.05	0.22	0.22	0.09	0.10-0.44	0.10-0.33	0.07-0.28
	C2	0.24 ± 0.06	0.20 ± 0.04	0.11 ± 0.04	0.22	0.20	0.11	0.17-0.36	0.14-0.29	0.01-0.17

SD: standard deviation; C1: dry season campaign; C2: wet season campaign; DO: dissolved oxygen; Chl-*a*, Chl-*b*, Chl-*c*: chlorophylls *a*, *b* and *c*; Bact: bacterioplankton; DIN: dissolved inorganic nitrogen; P_{inorg}: inorganic phosphorus; SPM: suspended particulate matter; DOC: dissolved organic carbon; POC: particulate organic carbon; PN: particulate nitrogen.

Chl-*a* (r = 0.635; $p < 0.01$), of DO and bacteria counting (r = 0.550; $p < 0.01$), as well as of Chl-*a* and bacteria counting (r = 0.423; $p < 0.01$) only in C2 may be taken as an indication of respiration activity (bacteria and other grazing) exceeding primary production in the entire water column including the photic zone.[44] This may occur subsequent to the incidence of an algae bloom. Algae blooms have been frequently observed in the outer bay region as well as in the adjacent coastal region.[44] DO and pH are correlated in the linear data fits (C1: DO = –17.98 + 3.15 pH, r = 0.603, $p < 0.05$; C2: DO = –27.21 + 3.78 pH, r = 0.807, $p < 0.05$) demonstrating dominance of biochemical process[45] as driver of DO and pH values from surface to bottom waters. Similar slopes but very different intercepts (C1 = –17.9 and C2 = –27.2) also support that in C2 respiration exceeded primary production thereby generating a greater decrease in DO than in pH. In the internal areas of the bay DO usually reaches saturation > 100% in surface waters, while in many occasions the bottom is suboxic. The photic zone in the bay varies from 0.5 m in the inner areas to 2.5 m in the central channel mostly due to the autochthonous production of particulate matter.[29,46] Kjerfve *et al.*[33] reported DO data obtained by the state environmental agency from 1980-1993 for a site in the central channel, which are similar (surface:

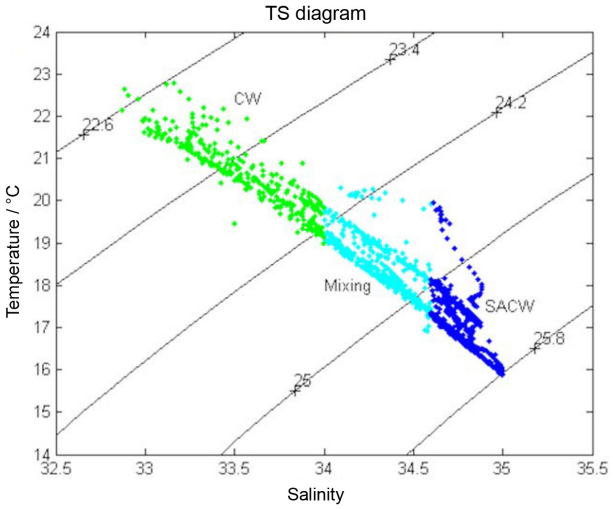

Figure 2. CTD full water profile characterization in C1 and C2 samplings. CW: Coastal water; SACW: South Atlantic central waters.

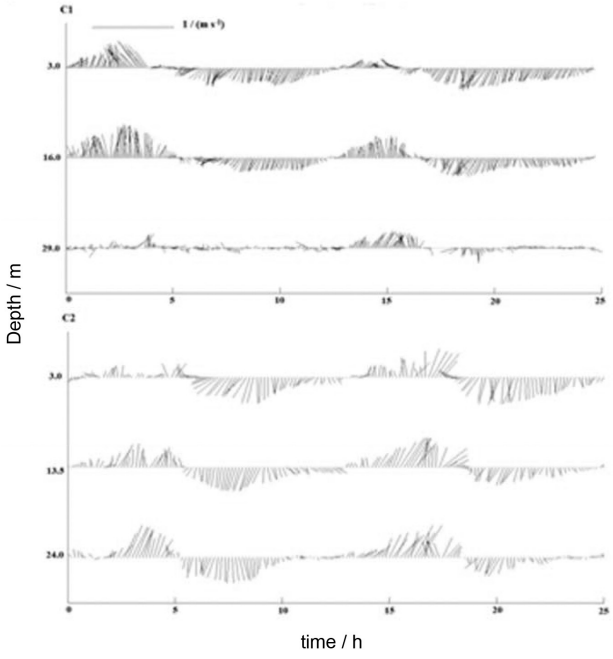

Figure 3. Current intensity and direction in C1 and C2 samplings.

124%; bottom: 73%) to those found in C1. The lower DO levels in C2 compared to the 13 years average reveal occasions of eutrophic conditions aggravation in the outer region of the bay specially during the wet period.

The intensification of eutrophic conditions in areas of the bay with higher circulation is confirmed by the nutrient concentrations which were up to three times higher than those reported previously for the same area.[33,47] This should be expected due to the combined effect of a submarine outfall installation in 2003 downstream of the sampling station and the population growth in recent years.[25] NH_4^+ was the most abundant nitrogen species, which showed strong seasonal variations with higher concentrations in C1 ($p < 0.05$). In both samplings concentrations of ammonium were more elevated at mid-depth during ebb tide proving that OM degradation in the water column just below the euphotic zone is very intense as observed by Wagener[28] and that the inner areas of the bay are important source of NH_4^+ derived from sewage release. Nitrite showed low seasonal variation being more elevated in surface waters in C2, while for C1 higher nitrate concentration occurred during flood tide (Figure 4) and higher concentration of NH_4^+ occurred in the ebb tide (Figure 4). Higher nitrate concentrations (3.7 μmol L^{-1}) were reported in the past by Rebello *et al.*[29] for surface waters and by Kjerfve *et al.*[33] for bottom waters (average for 13 years: 4.5 ± 3.4 μmol L^{-1}). Such change is an additional indicator of a tendency towards further decay of the system with increasing NH_4^+.

P_{inorg} predominated in C2 especially at the bottom and variations in both samplings occurred without tide dependence. Higher concentrations in C2 bottom waters reveal the input from the SACW but a contribution from the sediments cannot be discarded in both C1 and C2.[33,48]

The ratio dissolved inorganic nitrogen (DIN) / dissolved inorganic phosphorus (DIP) was calculated considering all DIN species and the results are given in Table 2. By using the Redfield ratio N / P (16:1) it is possible to estimate that P is the limiting nutrient in C1 and also that the remobilization of P from sediments is substantial as the sharp DIN / DIP decrease from surface to bottom waters, especially in C1, and the trend to higher concentrations in the bottom suggest. In C2 N / P values were often below 16 indicating a strong relative P consumption.[49]

SPM was higher in C2 ($p < 0.05$) (see Table 1) possibly due to the intense continental runoff during the rainy season. Kalas *et al.*[50] found in the sampling region values for SPM in the range reported here.

The intense primary production in the bay is represented by the high Chl-*a* concentrations during the samplings, with concentrations reaching more than 80 μg L^{-1} (Table 1). According to the Organisation for Economic Cooperation and Development (OECD),[51] Chl-*a* concentrations above 25 μg L^{-1} are typical of eutrophic systems. The presence of higher values in C1 may be ascribed to the lower SPM found in the dry season since, as observed by Rebello *et al.*,[29] primary production in the bay is limited by the water transparency. Chl-*c* is also a significant component of the photosynthesized pigments and such observation concurs with the reported abundance of dinoflagellates in the bay.[52,53]

Heterotrophic bacteria are important for the structure and dynamics of food chains, as well as for the biogeochemical cycles, being responsible for the inter-conversion of DOC and POC in the carbon cycle.[54] Bacterioplankton

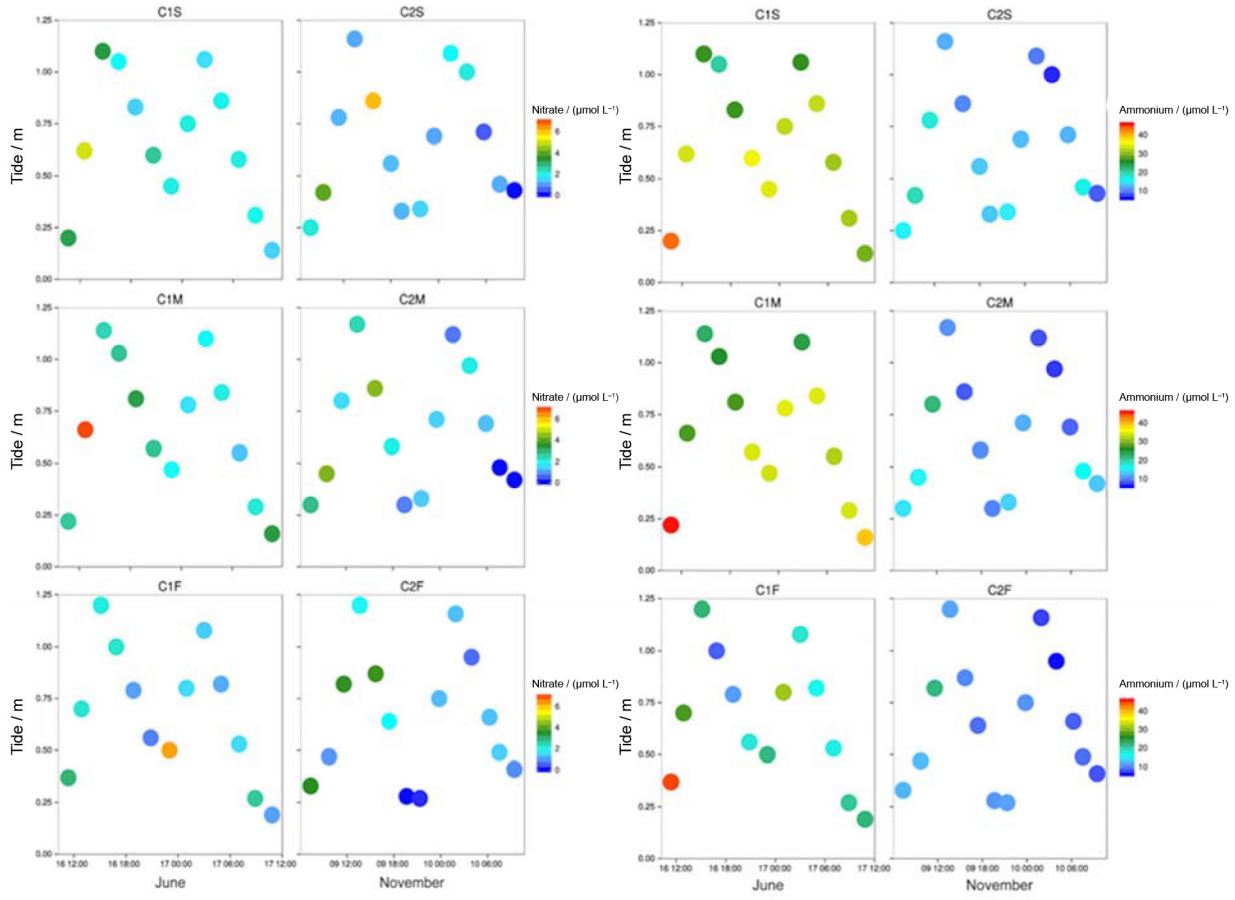

Figure 4. NO_3^- and NH_4^+ variation during 25 h tide in C1 and C2.

Table 2. Ranges and medians for DIN / DIP ratio, C / N ratio, $\delta^{13}C_{VPDB}$ (‰) and $\delta^{15}N_{air}$ (‰) at the surface, mid-water and bottom in C1 and C2

		Range			Median		
		Surface (S)	Mid-depth (M)	Bottom (F)	Surface (S)	Mid-depth (M)	Bottom (F)
DIN / DIP	C1	41-136	44-124	27-70	74.00	60.32	44.82
	C2	6-15	5-11	4-20	10.27	8.38	6.24
C / N	C1	4.5-5.8	5.0-6.1	3.4-6.5	5.32	5.22	5.47
	C2	5.0-5.9	5.1-6.1	5.0-6.0	5.22	5.48	5.46
$\delta^{13}C_{VPDB}$ / ‰	C1	20.34-23.43	20.08-23.39	20.58-22.45	20.94	21.13	21.65
	C2	16.40-20.03	17.01-20.59	17.90-20.44	17.63	18.07	19.40
$\delta^{15}N_{air}$ / ‰	C1	3.86-7.11	2.17-6.57	3.74-6.44	5.08	4.67	5.55
	C2	7.63-11.08	6.19-10.48	6.25-9.53	9.88	8.99	7.95

C1: dry season campaign; C2: wet season campaign; DIN: dissolved inorganic nitrogen; DIP: dissolved inorganic phosphorus; $\delta^{13}C$ and $\delta^{15}N$: carbon and nitrogen isotopic composition; VPDB: Vienna Pee Dee Belemnite.

abundance was similar in C1 and C2 as indicated by the Kruskal-Wallis test (Table 1) although variations with tide were very intense as indicative of the heterogeneity of the system. Maximum abundance was present at mid-depth, below the photic zone, as expected due to the fast recycling of organic matter typically occurring in the bay down in the water column.[55] In C2 bacterioplankton abundance was significantly correlated to NH_4^+ ($r = 0.764$, $p = 0.001$) and to POC ($r = 0.500$, $p = 0.001$). A low correlation is observed with respect to $\delta^{13}C$ ($r = 0.398$, $p = 0.01$, $s = 1.83$). These associations during periods of water stratification when vertical transport is hindered as in C2 prove the intensity of bacterial activity at mid-depth resulting in residual organic material enriched in ^{13}C and liberation of NH_4^+.

DOC was on average higher at the surface. The very elevated concentrations sporadically present (up to 400 mg C L^{-1}) demonstrate the time variability of the system due to the influence of diffuse sources of sewage around the bay and to the sewage outfall located downstream of the sampling site (Figure 5).

Guenther *et al.*[44] reports DOC values for the outer bay area similar to those found in the present work except for the extremely high concentrations (overall DOC average and standard deviation after removing the extremes: 2.19 ± 0.48 mg L^{-1}, n = 34 out of 39 samples in C1; 2.39 ± 0.68 mg L^{-1}, n = 31 out of 39 samples in C2). Also, similar values are reported for the Pearl River in China.[56] In polluted European estuaries, as the Sheldt estuary in Belgium and the Thames estuary in England, average concentrations are in the range of 5.8 to 6.8 mg C L^{-1} while in less altered estuaries, such as those of Gironde in France and of Douro in Portugal, DOC concentrations are from 2.5 to 3.1 mg C L^{-1}.[57]

The data for Guanabara Bay fits well in the latter range in spite of being highly polluted. This can be understood on the basis of the fast degradation of organic matter under high temperatures and microbial activity, and the intensity of water exchange. According to Kjerfve *et al.*[33] the time for renewal of 50% of the water volume (ca. 10^9 m^3) in Guanabara Bay is only 11.4 days and this would explain the better water conditions in the most water-circulated areas, such as those near the bay mouth where the sampling point is located.

POC was more elevated at the surface during daytime in both samplings but in C1 the variations with depth were more prominent (Figure 5). The overall average POC concentration was of 1.00 ± 0.50 mg L^{-1} in C1 and 1.01 ± 0.39 mg L^{-1} in C2. In both samplings, DOC correlates significantly with POC if extreme DOC values are removed. The linear correlation parameters in C1 are: s = 0.41, r = 0.680, $p < 0.05$; in C2: s = 0.79, r = 0.715, $p < 0.05$.

POC and PN are very strongly correlated even if the entire data collection is considered (r = 0.997; $p < 0.05$). A linear slope of 5.1 indicates predominance of autochthone organic matter in both campaigns.[50] The C / N ratio of 4.4 (range 3.4 to 6.2; Table 2) indicates marine phytoplankton (C / N between 4 and 10)[51] and bacterioplankton (C / N ca. 5) predominance.[58,59]

Carbon and nitrogen stable isotopes

The plot of $\delta^{13}C$ *vs.* $\delta^{15}N$ (Figure 6) highlights the different properties of the system in the two samplings

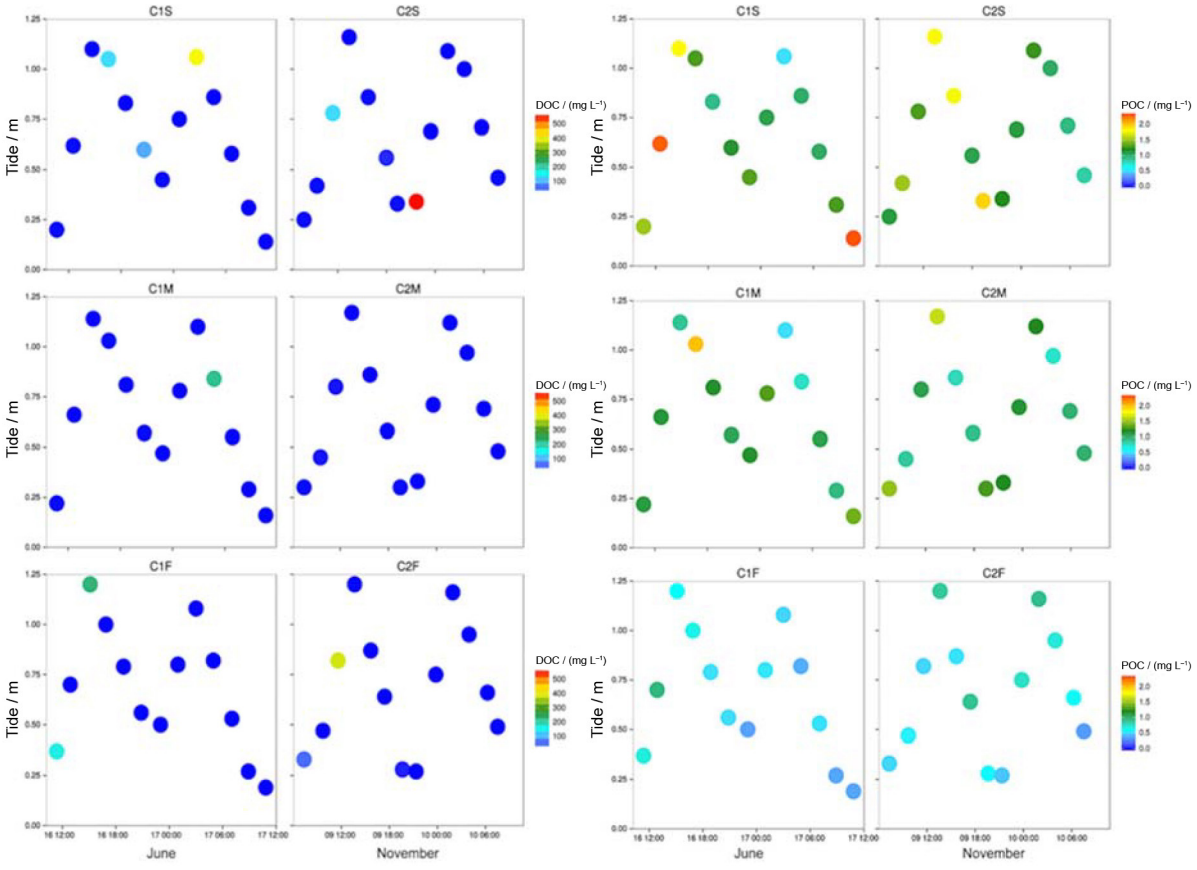

Figure 5. DOC and POC variation during 25 h tide in C1 and C2.

and the significant linear correlation between the two isotopic ratios. C1 shows lower values for both $\delta^{13}C$ and $\delta^{15}N$ predominantly in the range of phytoplankton origin, however, the terrestrial influence was greater than in C2. C2 shows high values for both $\delta^{13}C$ and $\delta^{15}N$ indicating marine influence.

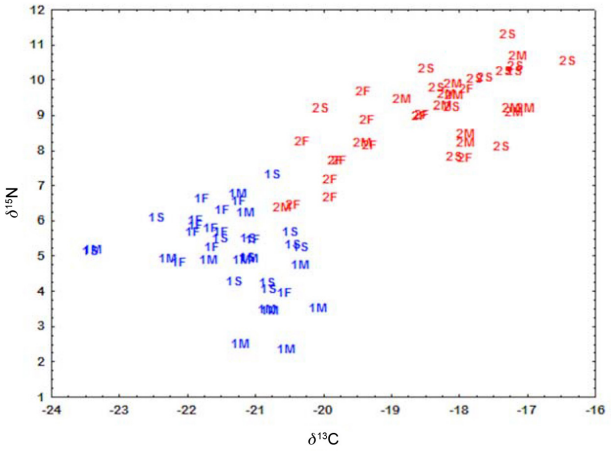

Figure 6. $\delta^{13}C$ and $\delta^{15}N$ in particulate organic matter from C1 (1S, 1M, 1F; in blue) and C2 (2S, 2M, 2F; in red) samplings.

SPM, $\delta^{13}C$ and $\delta^{15}N$ also demonstrated a strong phytoplankton contribution to the organic matter pool (Table 2). In C1 the average for $\delta^{13}C$ was –21.35 ± 1.75‰ with only two values appearing below –23‰. These isotopic shifts are typically reported for autochthone OM (–19 to –25‰), according to Meyers.[60] The presence of sewage-derived organic carbon, whose $\delta^{13}C$ is approximately –24‰,[34] cannot be discarded; however, as observed by Carreira and Wagener,[61] under the high regional temperatures the turnover rate of sewage OM and release of nutrients occur largely before reaching the marine environment. Kalas *et al.*[50] reported $\delta^{13}C$ of –21.30 to –15.10‰ for particulate organic matter in GB, similarly to the range found here in C1. In C2, the average for $\delta^{13}C$ shifted to –18.41 ± 1.11‰ and OM enriched in ^{13}C ($\delta^{13}C$ = –16.40‰) was present. Such enrichment may result from fast fixation rates and utilization of enriched dissolved inorganic carbon (DIC) produced during intense phytoplankton activity. Cifuentes *et al.*[62] reported for periods of elevated primary production and during algae blooms carbon isotopic shift in the range of –18 to –12‰. The presence of POC enriched in ^{13}C and other indications of phytoplankton predominance in C2 suggest utilization of dissolved carbon enriched in ^{13}C and seem to reinforce the possible occurrence of an algae bloom just prior to the sampling. The significant correlation between the POC, made up mainly of autochthonous OM, and $\delta^{13}C$ ($r = 0.632$; $p < 0.05$, $s = 1.80$) only in C2 may also be considered an indication of ^{13}C enrichment and primary fixation rates association.

C1 and C2 average values for $\delta^{15}N$ were 4.92 ± 1.13 and 8.79 ± 1.20‰, reaching a maximum of 7.11 and 11.08‰, respectively. In the marine environment, the predominant nitrogen incorporation mechanism includes nitrate reduction that generates nitrogen isotopic shifts between –2 and 4‰.[63] According to Fogel and Cifuentes,[64] $\delta^{15}N$ between 7 and 11‰ occurs principally when nitrate is the limiting nutrient, since in periods of elevated primary production phytoplankton consumes the available nitrate leading to enrichment in the heavier nitrogen isotope in the dissolved form.[65] In coastal areas the contribution of dissolved nitrogen derived from anthropogenic activities ($\delta^{15}N$ of 7 to 30‰) has strong influence on the $\delta^{15}N$ of the SPM. Also, sediment trap studies have shown that microbial degradation of phytoplankton leads to increase in ^{15}N in the residual OM.[66,67] The ^{15}N enriched OM in C2, as compared to C1, may result from the algae bloom occurring before the sampling day. The lower nitrate concentration and OM $\delta^{15}N$ close to 9-10‰ in C2 surface waters may derive, in addition, from nitrate consumption by dinoflagellates, the presence of which is confirmed by elevated Chl-*c* concentrations. Low values of $\delta^{15}N$ in the range of –3 to 1‰, typical of N_2 fixation,[64] were not observed here. This is expected due to the abundance of dissolved nitrogen species in both samplings.

A factor analysis (FA) with varimax rotation was carried out with and without data normalization. As described in Massone *et al.*,[68] the standard normalization is intrinsic to principal components analysis (PCA) as long as it is based on correlation matrix. The variables were grouped in four factors totalizing of 73% of variance. Salinity and temperature were very distinct in the two samplings and therefore were not used in the FA to avoid obscuring the differentiation of chemical properties. In the first factor (F1, 32% of the variance) are all variables that discriminate the two samplings: P_{inorg}, DO, SPM, $\delta^{13}C$, $\delta^{15}N$, NH_4^+ and pH. Samples with higher concentration of P_{inorg}, SPM, $\delta^{13}C$, $\delta^{15}N$ and pH were positively correlated in F1, while those negatively correlated in F1 showed lower values of DO and NH_4^+. In the second factor (F2, 21% of variance) PN and POC are strongly correlated, which separates the samples according to the sampling depth. A third factor (F3, 12% of variance) includes DOC and POC and distinguishes the few samples appearing with unusually high concentration. The three chlorophylls are strongly correlated in F4 (8% of variance) and were not efficient in separating groups of samples, possibly due to the influence of depth and tidal variations. Figure 7 shows the graphical representation for the cases.

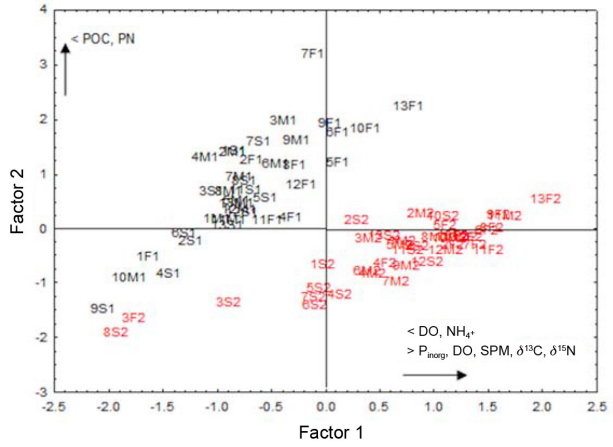

Figure 7. Factor analysis including P_{inorg}, DO, SPM, $\delta^{13}C$, $\delta^{15}N$, NH_4^+, pH (F1), and PN and POC (F2) samplings for C1 (S1, M1, F1) and C2 (S2, M2, F2) samplings.

Sterols

Table 3 shows the results obtained for phytol and 15 sterols identified in the SPM. The average concentration of total sterols for C1 was 2,728 ± 2,058 ng L^{-1}, which was slightly higher than the average value measured in C2, of 2,381 ± 1,006 ng L^{-1}. Only 4 out of the 15 identified compounds made up the majority of the total sterols found in the present study. Cholest-5-en-3β-ol (cholesterol or 27Δ^5) was one of the most abundant sterols, representing, on average, 20.8 ± 24.2 and 15.5 ± 18.8% of the total sterols in C1 and C2, respectively. There was no clear evidence of 27Δ^5 concentration gradient with depth: the highest average value was at the mid-depth in C1 (800.5 ± 613.4 ng L^{-1}) and in bottom water in C2 (408.3 ± 244.9 ng L^{-1}). 27Δ^5 has been usually observed in natural waters at high concentrations due to its widespread occurrence in planktonic organisms and other animals.[69] It is also found in raw sewage,[70] and thus in contaminated coastal waters, such as in GB,[26] the input of domestic effluents can be a relevant additional source of this sterol.

The sterol 24-methylcholesta-5,22-dien-3β-ol (28Δ^{5,22}) was also abundant, averaging 12.8 ± 12.2% of the total sterols in C1 and up to 26.3 ± 44.2% in C2. In fact, in C2 the 28Δ^{5,22} exhibited the highest concentration amongst all sterols, with 998.2 ± 452.4 ng L^{-1} at the surface, 571.0 ± 325.2 ng L^{-1} at mid-depth and 283.16 ± 173.01 ng L^{-1} at the bottom. 28Δ^{5,22} is produced by diatoms, dinoflagellates and prymnesiophytes.[69-72] Another sterol associated with diatoms, such as *Thalassiosira* and *Skeletonema*,[73,74] is 24-methylcolesta-24(28)-dien-3β-ol (28Δ$^{5,24(28)}$). In the present study, 28Δ$^{5,24(28)}$ accounted for 14.4 ± 17.9% of the total sterols in C1, similarly to 28Δ^{5,22}; however, in the second sampling, 28Δ$^{5,24(28)}$ accounted only for 9.9 ± 14.3% of the total sterol; it is a lower contribution as compared to 28Δ^{5,22}.

Another important sterol was 24-ethylcholest-5-en-3β-ol (29Δ^5), which represented 22.6 ± 29.7 and 9.9 ± 14.3% of the total sterols in C1 and C2, respectively. This sterol is generally considered typical of vascular (generally land) plants but is also present in some species of microalgae (prymnesiophytes) and it is possibly produced by cyanobacteria.[50,69,70,75,76]

Other phytosterols, such as 24-methylcholest-5-en-3β-ol (28Δ^5) and 27-nor-24-methylcholestan-5,22-dien-3β-ol (27Δ^{5,22}), accounted on the average for 5-6% of the total sterols. On the other hand, 4α,23,24-trimethyl-5α-colest-22(e)-en-3β-ol (30Δ^{22}) made a remarkably low contribution (3.3 ± 3.4 and 22 ± 2.2% in C1 and C2, respectively) to the total sterols. 30Δ^{22} is considered a consistent marker for dinoflagellates, although can also be found in some species of diatoms.[77,78] Kalas *et al.*[50] reported the absence of 30Δ^{22} in the SPM at the entrance of Guanabara Bay. Such feature is consistent with the occurrence of dinoflagellates especially in the northern part of the bay.[79]

The saturated sterols 5α-cholestan-3β-ol (27Δ^0), 24-methyl-5α-cholestan-3β-ol (28Δ^0) and 24-ethyl-5α-cholestan-3β-ol (29Δ^0) were on the average below 2% of the total sterols in both samplings. The fecal sterol coprostanol contributed with 2.4 ± 3.3 and 3.1 ± 3.5% of the total sterols in C1 and C2, respectively. Similar values were also found for coprostanone, a fecal ketone.

Sterols as source assignments of OM

Sterols and phytol were used as a complimentary tool, in addition to bulk properties and isotopic composition of POC (as previously discussed), for source assignments of OM in the studied site. As these compounds have multiple and, in some cases, non-specific sources[80] the sources assignment of OM using sterols was attained after a specific FA. The factor analysis applied to the entire sterol data set after normalization was not efficient in discriminating sampling campaigns and samples. Normalized data from C1 and C2 were then analyzed separately.

In both samplings, two factors accounted for 65-67% of the total variance (Figure 8). In Figure 8, the bi-dimensional plot of factor loadings (i.e., variables) shows similar groups of sterols (with some exceptions). For example, the major phytosterols discussed above (27Δ^5, 28Δ^{5,22}, 28Δ$^{5,24(28)}$ and 29Δ^5) appear negatively correlated in factor 1 in C1 (except 28Δ^{5,22}) whereas in C2 they are positively correlated in this factor. Similar pattern was observed for coprostanol and for other less abundant sterols.

It is interesting to note in Figure 8 that in spite of 27Δ^5 being positioned distant from the other most abundant phytosterols, it appears always correlated with 27Δ^{5,22} and

Table 3. Means, SD and range for the 15 determined sterols in C1 and C2 samples

Sterol		Mean ± SD / (ng L^{-1})			Range / (ng L^{-1})		
		Surface	Mid water	Bottom	Surface	Mid water	Bottom
Phytol	C1	137.8 ± 102.9	163.4 ± 174.2	30.63 ± 22.56	< LOD-333.0	< LOD-556.6	< LOD-64.03
	C2	193.2 ± 84.30	140.4 ± 50.18	81.49 ± 41.30	76.02-374.8	70.64-255.4	< LOD-155.9
Coprostanol	C1	44.76 ± 20.24	60.34 ± 34.25	92.13 ± 109.2	14.12-86.76	< LOD-139.1	21.95-435.9
	C2	65.34 ± 30.52	59.38 ± 27.40	100.7 ± 36.31	29.12-110.5	30.42-104.2	52.74-154.5
Coprostanone	C1	23.05 ± 7.93	28.06 ± 10.96	40.20 ± 34.65	< LOD-34.28	< LOD-44.13	18.13-147.6
	C2	36.84 ± 8.98	32.23 ± 13.16	46.08 ± 8.06	27.04-59.86	< LOD-52.39	30.06-57.24
Cholest-5-en-3β-ol ($27\Delta^5$)	C1	568.5 ± 467.9	800.5 ± 613.4	330.5 ± 264.7	14.70-1535	113.18-2281	78.19-845.3
	C2	343.0 ± 150.0	350.3 ± 175.2	408.3 ± 244.8	59.42-602.9	57.91-658.2	86.92-940.8
27-Nor-24-methylcholestan-5,22-dien-3β-ol ($27\Delta^{5,22}$)	C1	144.4 ± 118.0	196.7 ± 121.9	82.68 ± 71.59	12.80-371.1	32.93-477.4	23.54-263.1
	C2	95.26 ± 57.30	92.71 ± 57.91	99.63 ± 68.05	25.60-201.41	24.98-198.7	27.46-228.5
24-Methylcholest-5-en-3β-ol ($28\Delta^5$)	C1	190.1 ± 146.3	261.02 ± 168.19	82.83 ± 69.45	13.34-512.0	47.26-651.5	22.34-249.3
	C2	151.7 ± 85.77	139.5 ± 76.90	140.4 ± 82.90	35.57-306.7	32.58-273.5	36.56-292.2
24-Methylcolesta-24(28)-dien-3β-ol ($28\Delta^{5,24(28)}$)	C1	479.2 ± 381.8	555.7 ± 405.9	135.1 ± 111.8	< LOD-1312.73	82.93-1481	25.45-299.1
	C2	473.5 ± 204.3	382.7 ± 194.3	258.8 ± 119.3	129.63-889.0	104.3-720.9	85.41-417.3
Cholestanone	C1	39.96 ± 11.91	47.05 ± 21.25	50.80 ± 30.46	18.36-67.02	< LOD-80.33	26.37-143.6
	C2	54.25 ± 13.94	46.53 ± 17.39	48.25 ± 7.17	29.71-80.86	< LOD-63.55	34.67-57.86
5α-Cholestan-3β-ol ($27\Delta^0$)	C1	32.48 ± 15.73	44.12 ± 26.36	36.47 ± 17.43	< LOD-55.88	< LOD-78.85	17.50-70.20
	C2	58.85 ± 13.55	51.32 ± 10.55	49.52 ± 11.48	30.37-75.92	28.13-64.47	29.50-73.94
24-Methyl-5α-cholestan-3β-ol ($28\Delta^0$)	C1	34.48 ± 17.66	42.76 ± 26.36	35.72 ± 19.66	< LOD-67.14	< LOD-79.66	< LOD-70.68
	C2	61.13 ± 14.93	54.65 ± 16.35	52.00 ± 14.56	29.86-82.70	27.45-90.92	28.68-75.68
24-Ethylcholest-5,22E-dien-3β-ol ($29\Delta^{5,22}$)	C1	40.89 ± 23.36	49.92 ± 29.54	35.65 ± 16.28	< LOD-80.62	< LOD-97.57	19.07-64.57
	C2	43.16 ± 11.78	37.56 ± 14.06	40.40 ± 8.37	25.05-58.23	< LOD-58.69	25.72-39.86
24-Ethylcholest-5-en-3β-ol ($29\Delta^5$)	C1	697.4 ± 511.0	938.1 ± 773.4	213.3 ± 164.8	12.26-1856	178.3-3087	45.42-490.6
	C2	271.7 ± 166.7	258.5 ± 145.4	171.23 ± 96.15	49.16-710.80	57.18-522.4	17.54-362.9
4α,23,24-Trimethyl-5α-colest-22(e)-en-3β-ol ($30\Delta^{22}$)	C1	96.14 ± 69.33	120.36 ± 83.98	50.71 ± 36.41	< LOD-196.67	< LOD-262.9	< LOD-117.97
	C2	67.48 ± 22.61	51.23 ± 22.37	36.10 ± 5.92	34.24-103.4	< LOD-97.45	28.10-46.68
24-Ethyl-5α-cholestan-3β-ol ($29\Delta^0$)	C1	23.23 ± 11.22	25.86 ± 14.93	26.31 ± 13.07	< LOD-40.13	< LOD-54.94	< LOD-46.07
	C2	37.20 ± 13.68	31.84 ± 10.58	34.44 ± 4.40	< LOD-59.01	< LOD-42.54	25.72-39.86
24-Methylcholesta-5,22-dien-3β-ol ($28\Delta^{5,22}$)	C1	343.6 ± 195.6	424.8 ± 321.2	286.3 ± 214.9	14.70-689.6	113.19-1152	78.19-765.1
	C2	998.1 ± 452.4	571.0 ± 325.2	283.1 ± 173.0	192.5-1523	140.9-1063	84.11-662.6

SD: standard deviation; C1: dry season campaign; C2: wet season campaign; LOD: limit of detection.

$28\Delta^5$. Such grouping is not easy to explain; it might well reveal the occurrence of grazing, as $27\Delta^5$ is more abundant in zooplankton and/or fecal pellets.[79] Anyway, there are few samples (scores represented in Figures 8c and 8d) associated with this group. The grouping of $29\Delta^5$ with the phytosterols in both samplings suggest an autochthonous origin for this sterol in the present study.

Whereas the FA aided to evaluating the origin of some sterols, the most relevant outcome of the analysis was obtained from the scores (Figures 8a and 8b). Most of the surface and mid-depth samples are positioned in the same quadrant as the main phytosterols (excluding $27\Delta^5$). Although observed for some C1 samples (Figure 8c), this feature is strikingly evident for C2 surface samples (Figure 8d) and is in agreement with a post-bloom scenario in C2 as indicated by the physicochemical and isotopic data.

Finally, another important outcome of the FA scores is the position of the bottom samples, which are in most of the cases in the quadrant where coprostanol dominates. This suggests the presence of sewage particles. The ratio 5β / (5β + 5α) sterol proposed by Grimalt *et al.*[81] was calculated in order to check out the sewage contamination using coprostanol. The threshold value of 0.6 proposed by Carreira *et al.*[26] was considered for indicating sewage contamination. Figure 9 shows that in almost all bottom samples the ratio is > 0.6. The higher incidence of sewage-derived OM in bottom samples can be explained by the nearby discharge of the sewage outfall and the presence of settling aggregated particles; the sample highlighted with a red circle in Figure 9 showed the highest coprostanol concentration and the highest 5β / (5β + 5α) ratio.

Flow rates

Flow rates were calculated based on water mass transport data obtained from the current intensity and

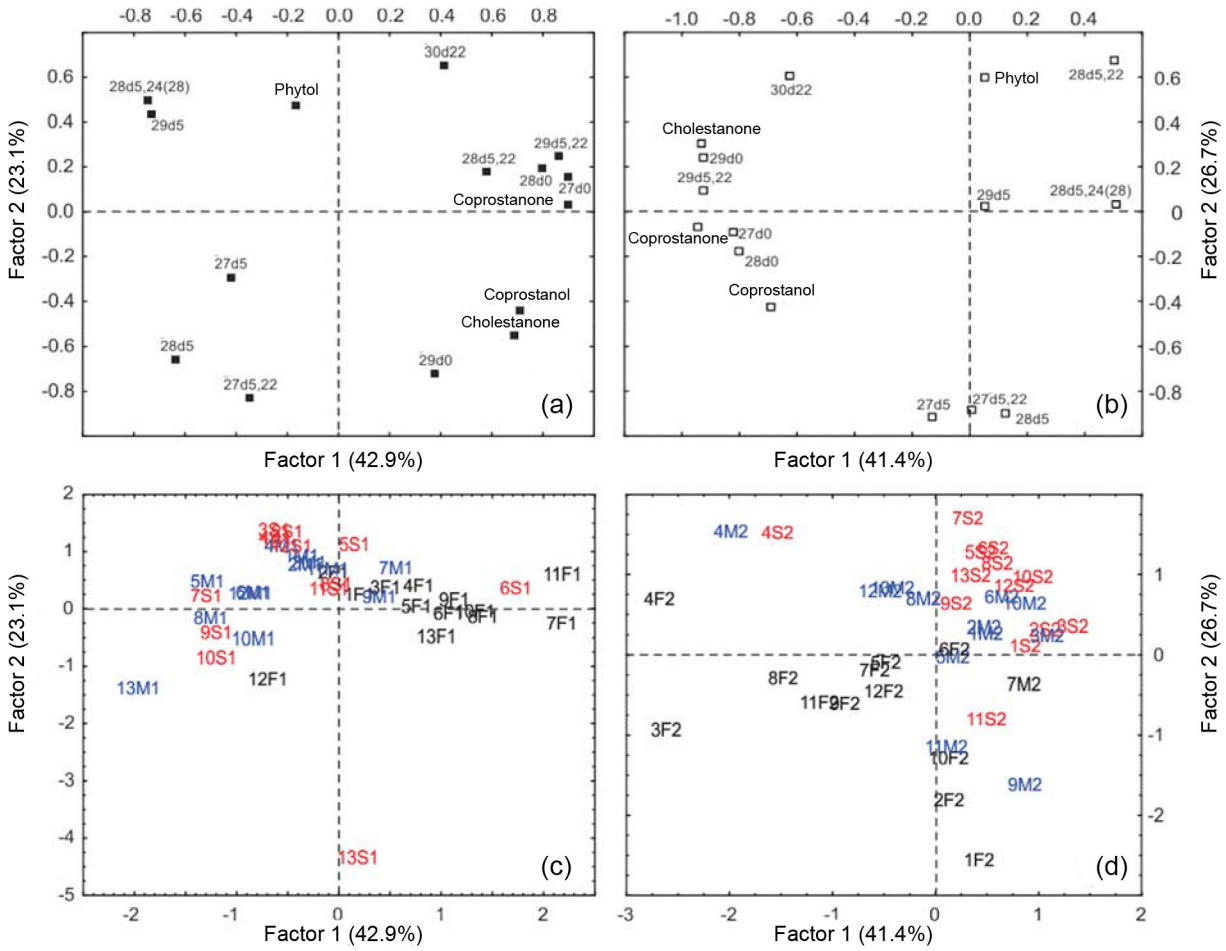

Figure 8. Factor analysis including sterols in surface (S, in red), mid-depth (M, in blue) and bottom (F, in black) samples; (a) and (c) stand for C1 and (b) and (d) stand for C2 sampling.

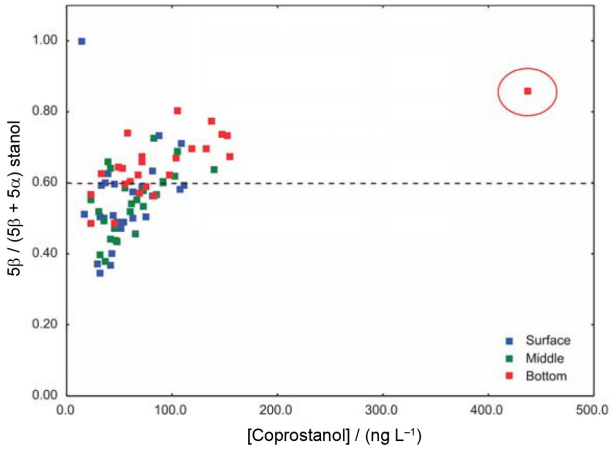

Figure 9. 5β / (5β + 5α) sterol ratio as a function of coprostanol concentration. The broken line highlights the threshold for indication of sewage contamination.

direction measurements. Physical data were integrated for the surface-mid-depth and mid-depth-bottom sectors and then multiplied by the mean concentration of each chemical variable in the same sectors, as follows:[82]

$$F = \Sigma((T(s\text{-}m)_i \times M(s\text{-}m)_i) + (T(m\text{-}f)_i \times M(m\text{-}f)_i)) \quad (1)$$

where: T = transport, M = mean concentration, s-m = integration of surface-mid-depth data, m-f = integration of mid-depth-bottom data, and i = sampling.

The flow rates calculated on a daily basis are given in Table 4 and indicate net export of materials to the inner shelf in both samplings, except for DOC in C2. Exports of DIN, Chl-*b* and Chl-*c* were of the same order in both campaigns; those of DIP, Chl-*a* and sterols predominated in C2 while SPM, DOC, POC and PN were more important in C1. DOC importation in C2 may derive from the inflow during high tide of organic carbon associated with the suggested algae bloom event prior to the sampling day and also from influence of the sewage outfall located closer to the bay mouth with respect to the sampling station. The widespread delivery of untreated sewage into the bay causes variability in chemical properties of the system and difficulties of source identification.

As the two samplings were performed in distinct conditions typically found during the dry and wet period,

Table 4. Flow rates for SPM, DOC, POC, PN, Σ sterols, NH_4^+, DIN, DIP, Chl-*a*, Chl-*b*, Chl-*c* obtained on a daily basis for C1 and C2 samplings. Negative values represent transport from Guanabara Bay to the continental shelf

	SPM / (g day^{-1})	DOC / (g day^{-1})	POC / (g day^{-1})	PN / (g day^{-1})	Σ sterols / (g day^{-1})	NH_4^+ / (mol day^{-1})	DIN / (mol day^{-1})	DIP / (mol day^{-1})	Chl-*a* / (g day^{-1})	Chl-*b* / (g day^{-1})	Chl-*c* / (g day^{-1})
C1	-1.83×10^7	-1.45×10^8	-1.07×10^8	-1.62×10^8	-7.37×10^1	-3.18×10^4	-3.49×10^4	-5.72×10^2	-6.74×10^4	-1.63×10^4	-3.25×10^4
C2	-7.91×10^4	1.17×10^5	-1.35×10^3	-2.98×10^2	-5.82×10^3	-1.95×10^4	-3.46×10^4	-4.65×10^3	-1.36×10^5	-1.31×10^4	-5.18×10^4

SPM: suspended particulate matter; DOC: dissolved organic carbon; POC: particulate organic carbon; PN: particulate nitrogen; DIN: dissolved inorganic nitrogen; DIP: dissolved inorganic phosphorus; Chl-*a*, Chl-*b*, Chl-*c*: chlorophylls *a*, *b* and *c*; C1: dry season campaign; C2: wet season campaign.

obtained data on daily basis can be used for estimating the magnitude of the exchanges on an annual basis as necessary for comparison with literature data. The export rates to the inner continental shelf so far estimated are: 1.27×10^4 kmol year^{-1} DIN; 9.52×10^2 kmol year^{-1} DIP; 2.65×10^{10} g year^{-1} DOC; 1.96×10^{10} g year^{-1} POC; and 2.96×10^{10} g year^{-1} PN.

Souza *et al.*[83] report the following integrated flow rates for 24 rivers in the northeast and southeast coast of Brazil: average DIN ca. 3.0×10^2 kmol day^{-1} and DIP = 4.4 and 37 kmol day^{-1} in the months of September and December 2000, respectively. These flow rates are approximate since they were calculated based on published data (National Water Agency). Figueiredo *et al.*[84] evaluated the outputs of C and N from the Paraiba do Sul River (hydrographic basin: 22,000 km^2, average drainage: 900 m^3 s^{-1}) to the Atlantic Ocean and found 48×10^9 g year^{-1} particulate carbon (PC), 83×10^9 g year^{-1} DOC and 8×10^8 g year^{-1} PN. The data of the present work reveal that on a regional scale GB contributes with about 10% of the dissolved N and P that enters into the continental shelf through river inputs of the Northeast and Southeast Brazil. If compared to the Paraiba do Sul River estimates, GB contributes with an export of DOC of the same order of magnitude and with 24 times higher PN output.

The international program Land-Ocean Interactions in the Coastal Zone (LOICZ) produced numerous studies targeting nutrient flow rates to the oceans in coastal areas. Table 5 shows the results for nutrient flow rates from several systems in the Baltic Sea.[85]

Table 5. Flow rates for DIN and DIP of some ecosystems of the Baltic Sea

Ecosystem	DIN / (kmol year^{-1})	DIP / (kmol year^{-1})
Lulealven	6.5×10^4	1.2×10^3
Neva	4.3×10^6	1.2×10^5
Gulf of Riga	5.8×10^6	7.0×10^4
Curonian Lagoon	7.5×10^5	2.5×10^4
Gulf of Gdansk	3.3×10^6	1.8×10^5
Sczeczin Lagoon	6.3×10^6	6.3×10^4

DIN: dissolved inorganic nitrogen; DIP: dissolved inorganic phosphorus.

Dupra[86] estimated flow rates in the range of 10^2 to 10^6 kmol year^{-1} DIN and 10^2 to 10^5 kmol year^{-1} DIP for 30 ecosystems in the Southeast Asia region. Wepener,[87] by using the LOICZ model, found the following average flow rates for the Nhlabane, Thukela and Mvote Estuaries, respectively: 0.076, 15, and 0.634 kmol day^{-1} for DIP and 1.7, 399, and 0.015 kmol day^{-1} for DIN. Arndt *et al.*,[88] using mechanistic model, estimated for the Scheldt Estuary, Belgium, a DIN flow rate of the order of 10^5 kmol year^{-1}. The GB exports of DIN and DIP are of the order of one thousandth of the outputs into the Baltic Sea, and are in the center of the flow rate range reported for the South Asia Sea. He *et al.*[6] estimated a mangrove-derived POC flux of 5.3×10^5 to 1.0×10^6 kg year^{-1} POC from the Shark River into the Gulf of Mexico.

Meybeck[89] estimated the following global nutrient transport to the oceans using data from the 1970s: 13×10^6 kmol year^{-1} (natural load) DIP; 26×10^6 kmol year^{-1} (natural + anthropic loads) DIP; 320×10^6 kmol year^{-1} (natural load) DIN; and 480×10^6 kmol year^{-1} (natural + anthropic loads) DIN. The comparison of these loads with the reported for the 1990s by Smith *et al.*[90] of 21×10^6 kmol year^{-1} (natural load) DIP; 74×10^6 kmol year^{-1} (natural + anthropic loads) DIP; 400×10^6 kmol year^{-1} (natural load) DIN; and $1{,}350 \times 10^6$ kmol year^{-1} (natural + anthropic loads) DIN shows an increase of up to 3 times in the global nutrient anthropic load over the period of the 20 years so far considered. More recently, Liu *et al.*[91] estimated the net global organic carbon input from rivers into the oceans as 0.4 Pg C year^{-1}.

The estimated global nutrient loads from rivers are of 0.32-0.64 Tmol N year^{-1} for DIN and 13-27 Gmol P year^{-1} for DIP. The contribution of GB on a global scale may be estimated using these flow rates and turns out to be of 1.2×10^{-2}% for organic carbon, 2.6×10^{-3}% DIN and 4.7×10^{-3}% DIP.

Conclusions

The present results show that GB comprises one of the most relevant systems in the southeastern Brazilian coast as

far as the export of organic carbon and nutrient species is concerned. The comparison of the obtained flow rates with regional and global data demonstrates the magnitude of potential impact on the biogeochemistry of the inner shelf. The major source of nutrient and productivity enrichment is sewage input derived from densely populated municipalities in the hydrographic basin.

The use of sterols and stable isotopes as markers of organic matter origin successfully indicated the system variability due to interaction with ocean waters, seasonality and tidal oscillations. The study shows that anthropogenic inputs to the bay are rapidly mineralized and used to produce autochthonous material, which is then exported to the inner shelf. Such POC, differently from the natural terrestrial organic carbon, is of high nutritional value and thus may contribute significantly to the ecology of the pelagic and benthic secondary producers in the shelf. Moreover, undesirable effects, such as red tides occurring in the open coastal area off Guanabara Bay and appearance of large bacteria community may result in indirect effects upon the health of the coastal oceans.

The discharge of raw or insufficiently treated sewage directly into estuaries is not unique of Rio de Janeiro but often occurs in developing as well as in developed countries. Therefore, the global impact of these sources on the carbon and nutrient cycles must be better quantified and understood.

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br as PDF file.

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