Science of the Total Environment 651 (2019) 261–270

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Environmental health in southwestern Atlantic coral reefs: Geochemical, water quality and ecological indicators

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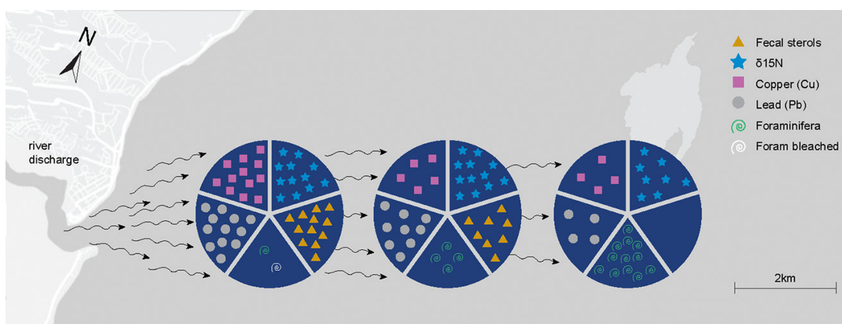
HIGHLIGHTS

- Runoff and pollution effects are understudied in South Atlantic coral reefs.
- Isotopes, sterols and metal levels indicated sewage contamination near the coast.
- Alterations in *Amphistegina* populations indicated detrimental ecological effects.
- Foraminifera as bioindicators of impacts of river influence
- Multidisciplinary indicators used are effective tools to assess reefs health.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 2 July 2018
Received in revised form 19 August 2018
Accepted 12 September 2018
Available online 13 September 2018

Editor: Kevin V. Thomas

Keywords:
Marine pollution
Runoff
Metals
Sewage
Symbiont-bearing foraminifera

ABSTRACT

Climate change, pollution and increased runoff are some of the main drivers of coral reefs degradation worldwide. However, the occurrence of runoff and marine pollution, as well as its ecological effects in South Atlantic coral reefs are still poorly understood. The aim of the present work is to characterize the terrigenous influence and contamination impact on the environmental health of five reefs located along a gradient of distance from a river source, using geochemical, water quality, and ecological indicators. Stable isotopes and sterols were used as geochemical indicators of sewage and terrigenous organic matter. Dissolved metal concentrations (Cu, Zn, Cd, and Pb) were used as indicators of water quality. Population density, bleaching and chlorophyll α content of the symbiont-bearing foraminifer *Amphistegina gibbosa*, were used as indicators of ecological effects. Sampling was performed four times during the year to assess temporal variability. Sediment and water quality indicators showed that reefs close to the river discharge experience nutrient enrichment and sewage contamination, and metals concentrations above international environmental quality guidelines. Higher levels of contamination were strongly related to the higher frequency of bleaching and lower density in *A. gibbosa* populations. The integrated evaluation of stable isotopes, sterols and metals provided a consistent diagnostic about sewage influence on the studied reefs. Additionally, the observed bioindicator responses evidenced relevant ecological effects. The water quality, geochemical and ecological indicators employed in the present study were effective as biomonitoring tools to be applied in reefs worldwide.

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https://doi.org/10.1016/j.scitotenv.2018.09.154
0048-9697/

1. Introduction

Human activities have reduced the environmental quality of marine ecosystems worldwide (Wilkinson, 2008). Coral reefs are highly productive and valuable ecosystems which are being severely affected by anthropogenic stressors at global and local scales (Ban et al., 2014; Hoegh-Guldberg, 2014; Wilkinson, 2008). Climate change, overfishing, pollution, and increased runoff due deforestation and urban development are some of the main drivers of degradation of coral reefs worldwide (Birkeland, 2015; Norstrom et al., 2016).

Rivers are the main sources of terrigenous sediment, nutrients and contaminants to inshore reefs (van Dam et al., 2011). A huge effort has been employed to assess the ecological effects of sediment and contaminant runoff in coral reef areas, such as the Caribbean and Great Barrier Reef. Also, strategies to minimize the loads of terrigenous material to these fragile ecosystems have been developed (Bartley et al., 2014; Brodie et al., 2017; Takesue et al., 2009; Takesue and Storlazzi, 2017). Nevertheless, the effects of runoff in South Atlantic reefs are poorly understood. Moreover, southwestern Atlantic reefs are naturally exposed to higher levels of terrigenous sediment compared to Caribbean and Indo-Pacific reefs (Castro et al., 2012; Castro and Pires, 2001; Leão et al., 2003; Rodríguez-Ramírez et al., 2008). There is an overall lack of knowledge about water quality, as well as a poor description of the chemical contaminants affecting South Atlantic reefs. Characterization of water and sediment quality parameters in these reefs is an important step for scientific and management purposes. Also, the use of reliable bioindicators integrated with measurements of abiotic parameters increases the ecological relevance of environmental impact assessments.

Foraminifera, as well as corals and other reef-dwelling species, can be adversely affected by river-related environmental stress, including sedimentation, changes in quality of organic matter (Barbosa et al., 2016; Dessandier et al., 2015), sewage input and metal contamination (Emrich et al., 2017; Prazeres et al., 2012a). In fact, foraminifera are widely used as monitors of environmental changes, including a well-established application of foraminifera as bioindicators of coral reef water quality. Monitoring of symbiont-bearing species is particularly relevant, since they have similar physiological requirements to corals, which are the main reef builders, but respond faster to environmental changes (Barbosa et al., 2016; Cooper et al., 2009; Emrich et al., 2017; Hallock et al., 2003; Marques et al., 2017; Ross and Hallock, 2014). *Amphistegina* spp. are diatom-bearing foraminifera found abundantly in reefs worldwide (Langer and Hottinger, 2000), that have been considered as reliable bioindicators of anthropogenic impacts (Marques et al., 2017; Prazeres et al., 2016, 2012b; Ross and Hallock, 2014).

In light of the background above, the aim of the present study was to identify and characterize the impact of terrigenous influence and contamination on the environmental health of five South Atlantic reefs located along a gradient of distance from a river mouth, using geochemical (stable isotopes and sterol levels), water quality (metals concentrations) and ecological (foraminifera population density, bleaching frequency and chlorophyll α content) indicators. Carbon and nitrogen stable isotopes are effective tools to evaluate the source of organic matter, reflecting river-ocean gradients, as well as nutrient contamination and environmental quality status (Claudino et al., 2015; Jona-Lasinio et al., 2015). Furthermore, stable isotopes can be effectively applied in combination with other techniques (Carreira et al., 2015b; Cordeiro et al., 2018; Derrien et al., 2017). Many organic compounds, such as steroids and aliphatic hydrocarbons, also known as molecular biomarkers, have been successfully applied to trace sources of organic matter and sewage contamination (Derrien et al., 2017; Emrich et al., 2017; Martins et al., 2014). In turn, concentrations of metals such as Cu, Zn, Cd and Pb can indicate the presence of some human activities. Additionally, they are also used to assess environmental health, based on criteria established by water quality guidelines (Martins et al., 2012; Prazeres et al., 2012b; Rocha et al., 2017). Finally, ecological effects of environmental stressors can be evaluated by assessing population and/or physiological responses in bioindicator species, such as the benthic foraminifera *Amphistegina* spp.

Our hypothesis is that markers of terrestrial influence and contamination, evaluated through stable isotopes, steroids and metals concentration, will be correlated with the distance between the reef and the river mouth, and will be related to lower *A. gibbosa* densities and higher bleaching frequency.

2. Material and methods

2.1. Study area

The Buranhém River is part of the largest hydrographic basin in southern region of the Bahia state (northeastern Brazil), flowing into the Atlantic Ocean at Porto Seguro coast (Sarmento-Soares et al., 2008), one of the most touristic area in South America and an important area for reef conservation (Seoane et al., 2012). Along the 148 km of river course, there are diffuse sources of untreated sewage input, as well as several points of potential water contamination with pesticides and industrial waste. These environmental pressures are related to the unplanned urban occupation and land use in the area, which involves activities such as agriculture, eucalyptus forestry, cellulose pulp industry and the severe ongoing deforestation of the Atlantic Forest (Bomfim, 2012; Oberling et al., 2013; Santos, 2013; Silva, 2016).

Previous studies suggested a gradient of influence of the Buranhém River plume in the coral reefs of the *Parque Natural Municipal do Recife de Fora* (PNMRF) (Leite et al., 2018; Seoane et al., 2012). The PNMRF is a Marine Conservation Area located 8 km offshore from Porto Seguro city (Bahia state, northeastern Brazil), with a complex reef system (17.5 km^2) composed mainly of sea grass beds, coralline algae, and corals (Seoane et al., 2012; Zilberberg et al., 2016).

The occurrence of an environmental quality gradient between reefs exposed to the Buranhém River plume was assessed by selecting five reef sites (S) between the river mouth area and the PNMRF region (Fig. 1). Three sampling sites were in reef patches outside the PNMRF [S1 (*Itassepocu Reef*): 16°26.0349′S–039°02.4140′W; S2 (*Pedra Carapindauba*): 16°25.7138′S–039°01.4990′W; and S3 (*Baixio Cerca*): 16°25.6938′S–039°00.4080′W]. The other two sampling sites were in reefs located inside the area of the marine park [S4 (*Recife de Fora-SW*): 16°25.0910′S–038°59.4502′W; and S5 (*Recife de Fora-NW*): 16°23.8428′S–038°59.0832′W]. Sampling was performed at the four different seasons in order to assess temporal variability (winter - August 2013; spring - December 2013; summer - February 2014; autumn - May 2014), carried out by scuba diving at mean depths of 5.9, 6.8, 7, 5.5 and 4 m for reefs 1, 2, 3, 4 and 5.

Air temperature and rainfall data during the period of study were acquired by an automatic station (code 86745) of the Brazilian National Institute of Meteorology (INMET) located in Porto Seguro (Bahia state, northeastern Brazil), and were expressed as daily means.

2.2. Collection methods

2.2.1. Sediment and seawater sampling

Sediment samples ($n = 3$, ~5 g of superficial sediment per sample) were collected and kept frozen (−80 °C) until analysis. Seawater ($n = 3$ per sampling site at each season, 10 mL each sample) were collected, filtered (45 μm), acidified (1% HNO_3 final concentration), kept in the dark and frozen (−20 °C) until analysis.

2.2.2. Foraminifera sampling

Pieces of reef rubble were manually collected at each sampling site, placed into labeled plastic bags ($n = 3$ per sampling site at each season) containing local seawater, transferred to the Coral Vivo Research facility at Arraial d'Ajuda (Porto Seguro, Bahia state, northeastern Brazil), and scrubbed with small brushes to detach the associated sediment and fauna. Residues obtained were distributed in 150-mm *Petri* dishes

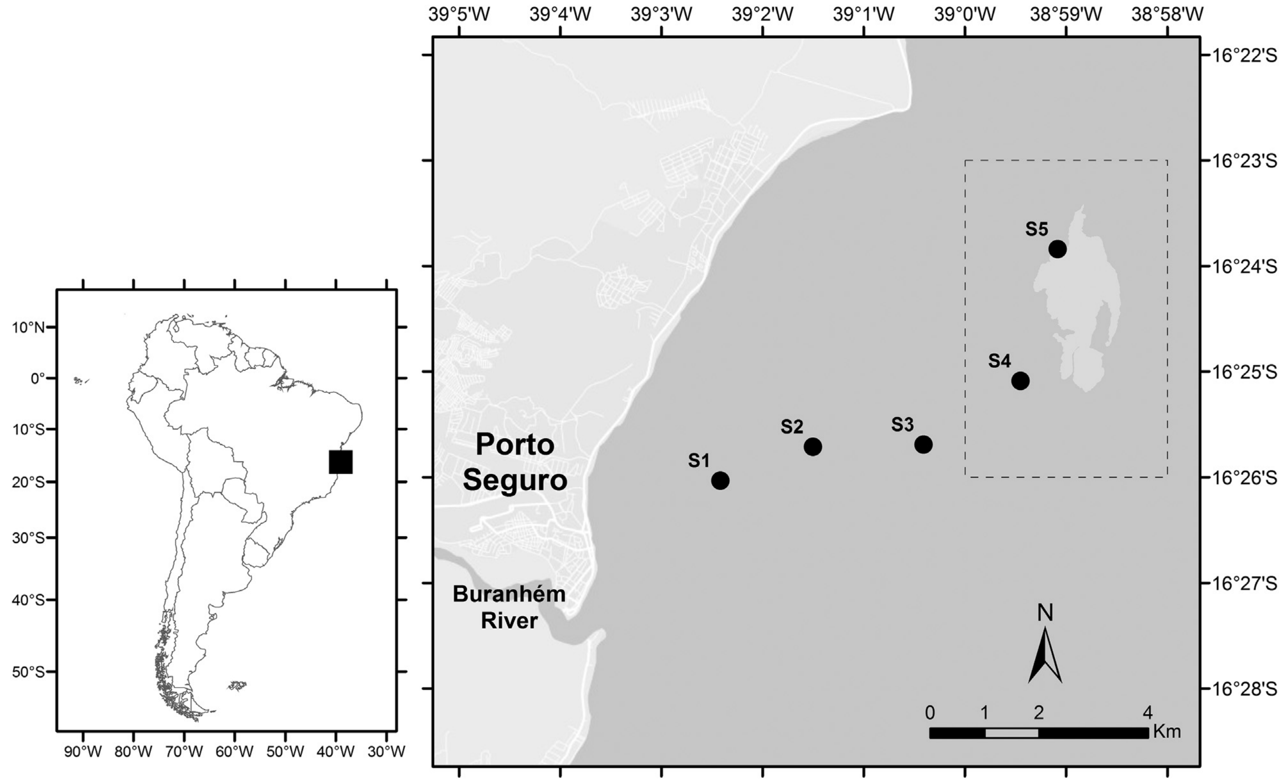

Fig. 1. Map showing the positions of the studied reef sites. Dashed square represents the area of the PNMRF.

containing seawater from the collection site, and sorted under stereomicroscope (Leica SD) for living *Amphistegina gibbosa* individuals.

2.3. Laboratory analysis

2.3.1. Carbon and nitrogen stable isotopes

Aliquots of sediment samples were dried (60 °C), ground to a fine powder with a mortar and pestle, weighed (~0.25 mg), and sent to Stable Isotope Core Laboratory (Washington State University) for determination of carbon and nitrogen stable isotopes values. For carbon stable isotope analysis, sample aliquots received acidification correction to remove inorganic carbon. Results were expressed in delta notation as $\delta^{13}C$ or $\delta^{15}N$ relative to Vienna Pee Dee Belemnite (VPDB) and atmospheric nitrogen, respectively.

2.3.2. Sterols

Considering the integrative and conservative feature of sediment matrix over time, sterol analysis was performed in samples collected in winter and autumn. Winter shows the highest rainfall and lowest temperature values, thus integrating the responses of a rainy season. In turn, autumn shows the lowest rainfall, thus integrating the responses of a dry season. Also, this season corresponds to the end of a period of intense touristic activities in the Porto Seguro area.

Aliquots of sediment samples were dried (45 °C), ground to a fine powder with a mortar and pestle, and weighed. Each sediment sample and blank (1 g) were spiked with surrogate, 5α-androstan-3β-ol, and then Soxhlet extracted for 12 h with 200 mL of hexane/dichloromethane (1:1) mixture, following the USEPA 3540 method. Extracts were concentrated down to 1 mL, using rotary evaporation and under gentle nitrogen stream. Sulfur was removed with activated copper. Clean-up and fractionation were performed by passing the extract through a silica/alumina column. Silica and alumina were activated at 160 °C for 4 h, and then partially deactivated with 5% Milli-Q water, following the modified USEPA 3640 method. Fractions containing steroids were evaporated to dryness and derivatized using 60 μL of a *N,O*-bis (trimethylsilyl) (BSTFA) trifluoroacetamide and trimethylchlorosilane (TMCS) mixture (9:1) for 90 min at 65 °C. Sterane 5-cholestane was added at the end of the derivatization process, as an internal standard.

Steroid analysis was performed using a Shimadzu GC with Flame Ionization Detector (model GC-17A FID). Compounds were identified based on the retention times of 6 sterols (coprostanol, epicoprostanol, cholesterol, cholestanol, stigmasterol and β-sitosterol) and 1 ketone (cholestanone). Calibration of the peak area to concentration was done within the range of 0.10 to 8.00 μg mL^{-1}, using the steroid standard in the derivatized form. The linear response was >0.995 for all compounds. The limit of detection (LDm, lowest concentration that can be detected and differentiated from the blank with a confidence level of 99%) of the method employed corresponded to 0.03 μg g^{-1} for all compounds.

Ratios between some sterols are considered good indicators of organic matter source and contamination level (Castellanos-Iglesias et al., 2018). The diagnostic indices chosen to improve our evaluation were: I. Coprostanol / (Coprostanol + Cholestanol); II. Fecal sterols / Total sterols ∗ (100); III. Cholestanol / (Cholesterol). Ratio I < 0.3 is indicative of a pristine environment, while Ratio I > 0.5 indicates sewage contamination (Grimalt et al., 1990; Leeming et al., 1998). Ratio II > 50% indicates high sewage contamination (Hatcher and McGillivary, 1979). Ratio III < 0.5 is indicative of fresh input of organic matter, while Ratio III > 0.5 indicates *in situ* reduction of cholesterol (Canuel and Martens, 1993; Chalaux et al., 1995).

2.3.3. Metals

Seawater samples were analyzed for dissolved metal (Cu, Zn, Cd, and Pb) concentrations. Samples were desalted according to Nadella et al. (2009), and metal concentrations were determined using a High-Resolution Continuum Source Graphite Furnace Atomic Absorption Spectrometry (HR-CS GF AAS, Analytic Jena, Germany).

Laboratory quality control (QC) for metal analysis included analysis of seawater reference material for trace metals NASS-6 (National Research Council of Canada NRC - CNRC). Blanks and spiked water samples were prepared and analyzed at intervals throughout the analytical procedure. Measurements were performed in triplicate. Analytical results of the quality control samples showed good agreement with the certified values, with recoveries ranging from 91.3 to 97.5% for the metals analyzed in seawater.

2.3.4. Density, bleaching frequency and chlorophyll α in A. gibbosa

Bleaching frequency and *A. gibbosa* density were assessed following the methods described by Hallock et al. (2003) and Prazeres et al. (2017a). Briefly, the number of individuals showing bleaching (mostly partial bleaching, ~60% of the test) was counted and divided by the total number of individuals analyzed. Dead specimens were differentiated from totally bleached individuals by complete bleaching with absence of protoplasm and lack of reticulopodal activity.

Rubble volume was estimated by volumetric assay. Therefore *A. gibbosa* density was calculated considering the total number of individuals counted in 1 cm^3 of substrate (Prazeres et al., 2017a).

Chlorophyll α (Chl a) concentration in *A. gibbosa* individuals was determined using the method described by Schmidt et al. (2011). Briefly, the pigment was extracted with ethanol 95% from each individual, and its concentration was measured using a microplate reader (ELX-800, BioTek, Winooski, VT, USA). For each of the replicates ($n = 3$), Chl a content was measured in a pool of 2 individuals, and was normalized by wet weight.

2.4. Data analysis

The association between environmental quality parameters (sediment, seawater and ecological) and the distance between reef and the Buranhém River mouth were analyzed by exploratory linear correlations (Pearson correlation) (Borcard et al., 2011).

The relationship between abiotic environmental quality markers and the ecological markers, as well as the identification of the potential drivers of the biological detrimental effects observed were analyzed by Redundancy Analysis (RDA). To minimize the number of explanatory variables and avoid multicollinearity, correlations and variance inflation factors were evaluated. Variables showing variance inflation factors above 10 were excluded. The statistical significance of the model was evaluated using Monte Carlo permutation tests (Borcard et al., 2011; Brauko et al., 2016). Statistical analyses were performed using R programming language (R Core Team, 2017).

3. Results

Rainfall and temperature means are described at Table S1. Winter collection had the lowest temperature and higher rainfall. Autumn collection was at the driest.

The environmental quality parameters evaluated were significantly correlated with the distance from the Buranhém River mouth (Tables 1a, 1b, 1c).

3.1. Geochemical markers

3.1.1. Carbon and nitrogen stable isotopes

Stable isotopes biplots (Fig. 2) showed that the sampling site 5 has an isotopic signature significantly different from all other sites, for all seasons.

A trend of seaward increase of $\delta^{13}C$ values was observed, as well as higher $\delta^{15}N$ in reefs closer to the river mouth.

3.1.2. Sterols

Stigmasterol and β-sitosterol were detected in samples from all sites, with the highest values (0.58 and 0.51 μg g^{-1}, respectively) observed at reef 1 in the rainy season. Also, the maximum value of total sterols (2.78 μg g^{-1}) was observed at the reef site 1 in the rainy season (Table 2). Coprostanol and epicoprostanol, indicators of domestic sewage input, were only observed in sampling sites 1, 2 and 3. These sampling sites are located outside the PNMRF and are the closer ones to the river mouth. The highest values were found at reef 1 in the dry season (0.43 and 0.29 μg g^{-1}, respectively), with decreasing values from sampling site 1 to 3 (Table 2).

Diagnostic indices showed an influence of sewage input (ratio I > 0.5) only at the sampling site 1, and no severe contamination (ratio II < 50%). Ratio III values showed input of fresh organic matter only at the sampling site 5 (Table 2).

3.2. Water quality analysis

3.2.1. Dissolved metals

Overall, results showed a trend of reduced metal concentrations at greater distances from the Buranhém River mouth. The highest values of metal concentrations were observed in winter at the reef site 1 (Fig. 3).

3.3. Ecological indicators

Amphistegina gibbosa density, bleaching frequency, and chlorophyll α content at each sampling site and season are shown in Fig. 4. The sampling site 5 was excluded, as no *A. gibbosa* was found.

For RDA, we selected only some abiotic parameters (selected parameters are illustrated in the RDA triplot; Fig. 5). This selection was based on the variance inflation factors and correlation results. The effect of abiotic environmental quality markers on the ecological indicators was statistically significant ($F = 9.05$; $p = 0.001$), and explained 58% of the model variability (adjusted R^2).

3.3.1. Amphistegina gibbosa density

Highest population density was observed at the sampling site 4 (Fig. 4a), located inside the PNMRF. The lowest population density was observed at the sampling site 2, for all seasons. Overall, lower densities were observed in the summer. Results from RDA (Fig. 5) indicated

Table 1a
Exploratory Pearson correlation matrix of geochemical markers and distance from the Buranhém River mouth. Significant ($p < 0.05$) correlations are in bold. Abbreviations: cop: coprostanol; epicop: epicoprostanol; sole.rol: cholesterol; cole.nol: cholestenol; estigm: stigmasterol; b.sito: β-Sitosterol; total.ols: total sterols sum; fecal.ols: cop and epicop sum.

	Distance	DeltaC	DeltaN	cop	epicop	cole.rol	cole.nol	estigm	b.sito	total.ols	fecal.ols
Distance	1	**0.42**	**−0.68**	**−0.90**	**−0.73**	**−0.58**	**−0.77**	**−0.52**	**−0.72**	**−0.83**	**−0.86**
DeltaC	**0.42**	1	**−0.69**	−0.34	−0.19	−0.01	−0.33	0.10	−0.12	−0.19	−0.29
DeltaN	**−0.68**	**−0.69**	1	**0.55**	**0.46**	0.13	**0.68**	0.14	**0.41**	**0.48**	**0.54**
cop	**−0.90**	−0.34	**0.55**	1	**0.84**	**0.72**	**0.79**	**0.50**	**0.69**	**0.90**	**0.97**
epicop	**−0.73**	−0.19	**0.46**	**0.84**	1	**0.62**	**0.84**	**0.56**	**0.65**	**0.88**	**0.94**
Cole.rol	**−0.58**	−0.01	0.13	**0.72**	**0.62**	1	**0.55**	**0.67**	**0.74**	**0.85**	**0.70**
Cole.nol	**−0.77**	−0.33	**0.68**	**0.79**	**0.84**	**0.55**	1	**0.41**	**0.61**	**0.84**	**0.84**
estigm	**−0.52**	0.10	0.14	**0.50**	**0.56**	**0.67**	**0.41**	1	0.91	**0.76**	**0.54**
b.sito	**−0.72**	−0.12	**0.41**	**0.69**	**0.65**	**0.74**	**0.61**	**0.91**	1	**0.88**	**0.70**
total.ols	**−0.83**	−0.19	**0.48**	**0.90**	**0.88**	**0.85**	**0.84**	**0.76**	**0.88**	1	**0.93**
fecal.ols	**−0.86**	−0.29	**0.54**	**0.97**	**0.94**	**0.70**	**0.84**	**0.54**	**0.70**	**0.93**	1

Table 1b
Exploratory Pearson correlation matrix of water quality markers and distance from the Buranhém River mouth. Significant ($p < 0.05$) correlations are in bold.

	Distance	Cu	Zn	Cd	Pb
Distance	1	**−0.47**	**−0.28**	**−0.35**	**−0.51**
Cu	**−0.47**	1	**0.53**	**0.77**	**0.43**
Zn	**−0.28**	**0.53**	1	**0.41**	0.22
Cd	**−0.35**	**0.77**	**0.41**	1	**0.52**
Pb	**−0.51**	**0.43**	0.22	**0.52**	1

that fecal sterols concentration and other variables related to domestic sewage were inversely related to *A. gibbosa* density values.

3.3.2. Bleaching frequency in Amphistegina gibbosa

In all seasons, the highest bleaching frequency was observed in foraminifera from reef 1 (Fig. 4b). Results from RDA suggested that Cu concentration was the main factor associated with foraminiferal bleaching responses (Fig. 5).

3.3.3. Chlorophyll α content in Amphistegina gibbosa

In a broad view, the higher values of chlorophyll α content were observed in foraminifera from the PNMRF (Fig. 4c). Individuals collected in summer showed the lowest value of chlorophyll α content. When live individuals were found, foraminifera collected at the reef site 1 usually had lower values of chlorophyll α content. RDA and exploratory correlations showed a strong relationship between chlorophyll α content and *A. gibbosa* population density, which was inversely related to sewage indicator parameters (Fig. 5).

4. Discussion

Our hypothesis that environmental quality parameters would correlate with distance from the coast was supported by results obtained. Foraminifera collected in reefs located near the Buranhém River mouth were exposed to higher concentrations of metals and fecal sterols, presented higher bleaching frequency, lower chlorophyll α content and lower population density.

To our best knowledge, no work to date has evaluated South Atlantic coral reef systems' environmental quality using a multi-marker approach. The integrated evaluation of stable isotopes, sterols, metals and ecological effects provided a consistent diagnostic indicating the terrestrial and anthropogenic influence in the reefs closer to the coast.

Distance from the river influenced all the markers assessed, with the strongest effects on nitrogen, fecal sterols, Cu, Pb and foraminifera bleaching. However, it is important to highlight that many factors associated with the distance from the river (including variables not assessed in the present work) may be involved in the observed responses.

4.1. Geochemical markers

Bulk sediment samples from sites 1, 2, 3, and 4 showed $\delta^{13}C$ values characteristic of marine organic matter, which are typically between −18 and −22‰ (Briand et al., 2015). Additionally, it was possible to differentiate the sampling sites by a relative seaward enrichment in $\delta^{13}C$ values, which was significantly higher at the sampling site 5. This offshore enrichment pattern was also reported by Claudino et al. (2015), with extremely high values of carbon stable isotope at reef beaches in an estuary-ocean gradient in northeastern Brazil. Autochthonous organic matter is usually associated with high $\delta^{13}C$ values (Carreira et al., 2015b; Meyers, 1997). Also, phytoplankton and macroalgae productivity preferentially removes ^{12}C, leaving the residual dissolved inorganic carbon (DIC) pool enriched with ^{13}C (Heikoop et al., 2000). Higher $\delta^{13}C$ values were observed in sedimentary organic matter collected in offshore reef flats with high biomass coverage of seagrasses in Japan (Umezawa et al., 2008), as well as in reef patches near macroalgae beds in Abrolhos (De Souza et al., 2013). Considering that sampling sites inside the PNMRF (sites 4 and 5) show higher biodiversity than those closer to the river mouth (sampling sites 1 to 3), data on $\delta^{13}C$ highlighted the autochthonous origin of organic matter inside the PNMRF. We must consider, however, the possibility of inorganic carbon influence on reef 5, irrespective of the acidification treatment.

The use of $^{15}N/^{14}N$ ratio as a biomarker of anthropogenic nutrient loading is suggested for coral reef monitoring (Heikoop et al., 2000; Moss et al., 2005). However, further studies are mandatory to define $\delta^{15}N$ threshold values (Moss et al., 2005). In the present study, there was a small enrichment with $\delta^{15}N$, indicating a higher N loading in the reefs closer to shore. Nutrients derived from sewage are generally enriched with $\delta^{15}N$, especially when they are derived from the anthropogenic dissolved inorganic nitrogen (DIN) present in groundwater (Heikoop et al., 2000). Costa Jr et al. (2006); Costa Júnior et al. (2008) discussed the occurrence and the detrimental effects of submarine groundwater discharge of wastewater on Porto Seguro reefs. Leite et al. (2018) effectively showed a gradient of elemental nitrogen and fecal coliforms, followed by clear responses in coral's microbiome, in the same reefs sampled for the present work. Therefore, we can consider that the enrichment with ^{15}N observed in the sampling sites located near the Buranhém River mouth is a reliable indicator of nutrient enrichment and sewage input.

Coprostanol is the most abundant sterol in human feces, and therefore can be used as a robust sewage tracer (Abreu-Mota et al., 2014; Carreira et al., 2015a; Martins et al., 2008, 2012, and references therein). Additionally, it is also an indicator of livestock sources of organic matter (Hatcher and McGillivary, 1979). Writer et al. (1995) classified sites with coprostanol concentration above 0.1 μg g^{-1} as being contaminated, while Readman et al. (2005) considered 0.5 μg g^{-1} as being an indicative of sewage contamination in sediments. The highest concentration of coprostanol observed in the sampled reefs was 0.41 μg g^{-1}. This concentration indicates contamination, but not heavily as in many estuaries (Cabral et al., 2018; Carreira et al., 2015a) and densely populated marine sites (Castellanos-Iglesias et al., 2018; Emrich et al., 2017). The coprostanol level was higher in the sampling sites near shore, and was not detected in the sampling sites located inside the PNMRF, indicating no detectable fecal contamination within the marine park area.

Although the thresholds for the abovementioned sterols are still a subject of discussion in literature (Martins et al., 2008, 2014), ratios between some sterols are widely used as diagnostic indices. The combined evaluation of coprostanol levels, ratios I and II, and $\delta^{15}N$ data indicated that sampling sites 1 and 2 are indeed exposed to sewage, while sampling sites located inside the PNMRF can be considered as being relatively pristine (Abreu-Mota et al., 2014; Briand et al., 2015; Grimalt et al., 1990; Hatcher and McGillivary, 1979; Leeming et al., 1998; Martins et al., 2014). Ratio III values corroborated with $\delta^{13}C$ data and are evidences of the presence of autochthonous organic matter in sampling site 5, indicating an input of fresh organic matter (Briand et al., 2015; Canuel and Martens, 1993; Chalaux et al., 1995; De Souza et al., 2013).

Most studies evaluating fecal sterols in Brazil have been carried out in southeastern, subtropical, estuarine systems (Carreira et al., 2015b; Martins et al., 2011, 2012, 2014). Carreira et al. (2016) analyzed sterols in the Camamu Bay (Bahia state, northeastern Brazil), a tropical estuary located approximately 280 km north of Porto Seguro, reportingvery low

Table 1c
Exploratory Pearson correlation matrix of ecological markers and distance from the Buranhém River mouth. Significant ($p < 0.05$) correlations are in bold. Marginally significant correlations are in italic.

	Distance	Density	Bleaching	Chl α
Distance	1	**0,51**	**−0,69**	*0,24*
Density	**0,51**	1	−0,09	**0,63**
Bleaching	**−0,69**	−0,09	1	0,19
Chl α	0,243	**0,63**	0,19	1

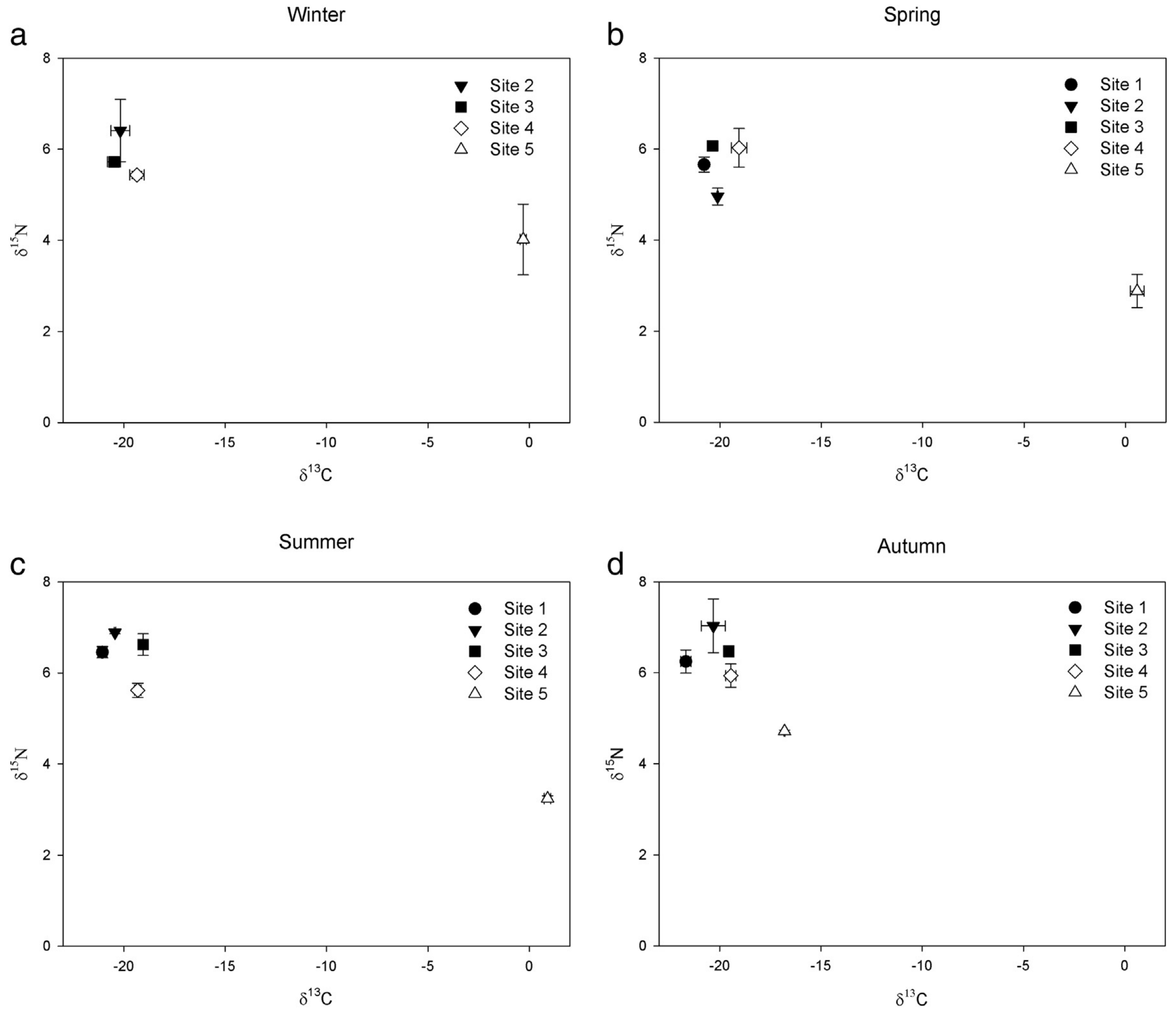

Fig. 2. Carbon and nitrogen stable isotopes biplots for each sampling site, at each season.

concentrations of coprostanol. Our study is the first one to report sterol analysis in South Atlantic coral reef systems.

Emrich et al. (2017) found moderate to severe sewage contamination in inshore coral reefs from Belize, with coprostanol levels above 0.1 μg g^{-1}, and sometimes even higher than 0.5 μg g^{-1}. Fecal sterols and diagnostic ratios in these Belize reefs were negatively correlated with foraminiferal diversity and densities of symbiont-bearing species, which corroborates with our findings regarding population density of *A. gibbosa*. In Cuban reefs, Castellanos-Iglesias et al. (2018) found coprostanol levels higher than 0.5 μg g^{-1}, associated with altered hydroid assemblages.

Sitosterol and stigmasterol are commonly related to higher terrestrial plants, tracing terrestrial influence in sedimentary organic matter (Derrien et al., 2017; Volkman, 1986). β-sitosterol, the 24β isomer of sitosterol, is better correlated with terrestrial/riverine influence (Canuel and Zimmerman, 1999; Carreira et al., 2015b, 2016; Martins et al., 2007; Saavedra et al., 2014; Volkman, 1986). Indeed, its concentration showed a strong relationship with the distance from the coast, as well

Table 2
Mean values of sterols (μg g^{-1}) and diagnostic indices at each season and sampling site.

Sampling site	Rainy season					Dry season				
	1	2	3	4	5	1	2	3	4	5
Coprostanol	0.267	0.263	0.173	0.000	0.000	0.410	0.307	0.170	0.000	0.000
Epicoprostanol	0.130	0.197	0.000	0.000	0.000	0.170	0.247	0.067	0.000	0.000
Colesterol	0.667	0.455	0.468	0.213	0.357	0.463	0.537	0.260	0.117	0.337
Cholestanol	0.440	0.567	0.397	0.220	0.107	0.377	0.693	0.377	0.267	0.090
Stigmasterol	0.553	0.288	0.142	0.168	0.260	0.297	0.387	0.260	0.217	0.260
β-Sitosterol	0.460	0.240	0.162	0.152	0.155	0.263	0.350	0.213	0.167	0.143
Cholestanone	<LDm	<LDm	<LDm	<LDm	<LDm	<LDm	<LDm	<LDm	<LDm	<LDm
Fecal sterols	0.397	0.460	0.173	<LDm	<LDm	0.580	0.553	0.237	<LDm	<LDm
Total sterols	2.520	2.007	1.340	0.757	0.877	1.957	2.517	1.340	0.763	0.840
ratio I	0.377	0.317	0.304	nc	nc	0.521	0.307	0.311	nc	nc
ratio II	15.741	22.924	12.935	nc	nc	29.642	21.987	17.662	nc	nc
ratio III	0.660	1.245	0.847	1.031	0.299	0.813	1.292	1.449	2.286	0.267

^{nc}: not calculated;; $^{<LDm}$: value detected, but smaller than the detection limit of the method; ^{LDm} = 0.03 μg g^{-1}.

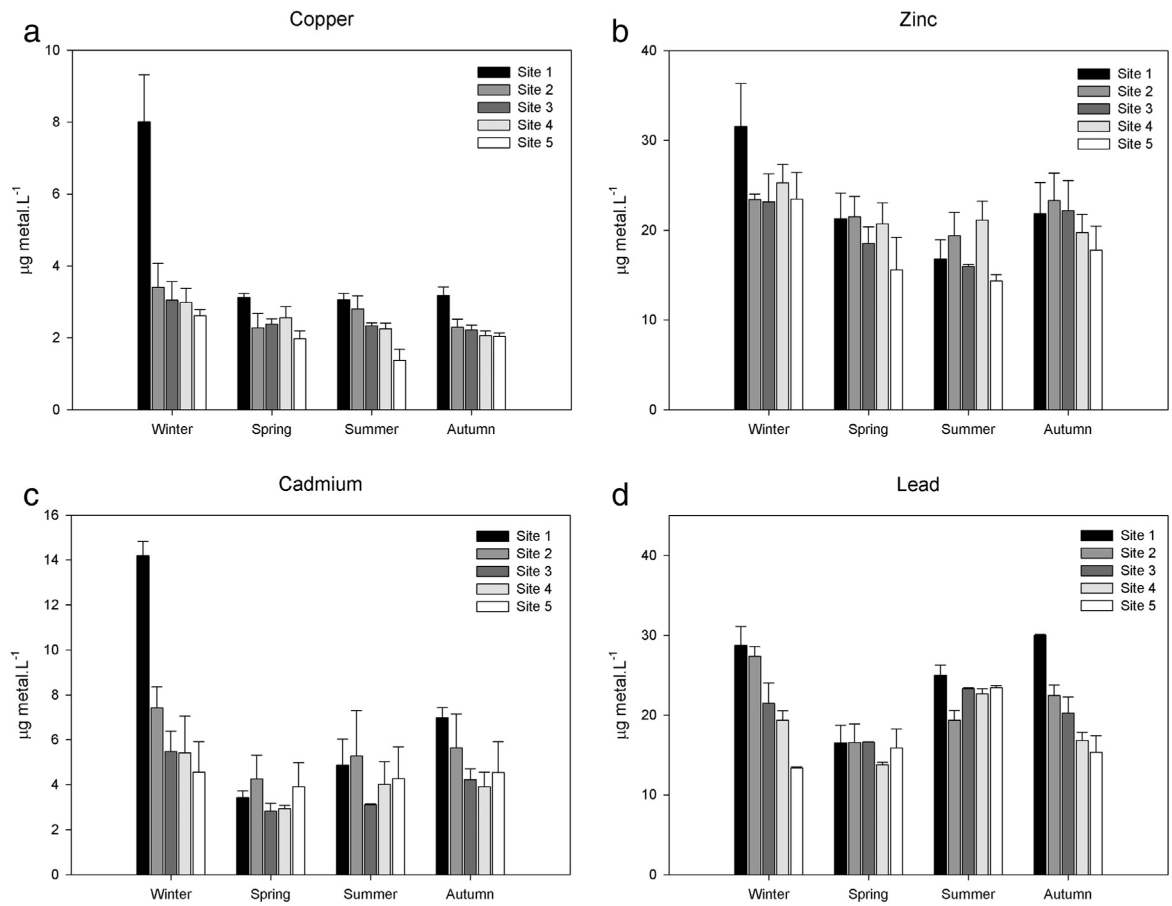

Fig. 3. Mean (±SE) values of metals concentrations at each reef site and season.

as other markers of terrestrial/riverine input analyzed in the present study. In turn, stigmasterol can also be produced by marine macroalgae and seagrasses (Derrien et al., 2017). This fact would explain the slight increase in its levels at the sampling site 5. It is important to consider that sampling site 5 may also receive some influence of other rivers, which are located north of the Buranhém River.

4.2. Water quality parameters

Peak concentrations of metals, were considerably related to the distance from the coast. This is an expected result, considering that rivers are one of the main drivers of metal contamination to coastal areas (Lopes-Rocha et al., 2017; Martins et al., 2012; Rocha et al., 2017; van Dam et al., 2011). Also, it corroborates with findings reported by other studies on gradients of river discharge distance (Lopes-Rocha et al., 2017; Monroy et al., 2014).

Regarding water quality guidelines, Cu and Cd concentrations in seawater collected at the sampling site 1 during winter were higher than the water quality criteria established by the current Brazilian (CONAMA, 2005) and USA (USEPA) environmental guidelines. This reinforces the hypothesis that the influence of the Buranhém River reduces water quality in coastal reefs of Porto Seguro in northeastern Brazil. Pb concentrations in seawater collected at all sampling sites and seasons were above the current water quality criteria established by the current Brazilian (CONAMA, 2005) and USA (USEPA) environmental guidelines. Considering the Great Barrier Reef Heritage Water Quality Guidelines, based on Australian Water Quality Guidelines for Fresh and Marine Waters (2010), all sampled reefs would be considered contaminated with at least one of the analyzed metals, in all seasons.

Metals can be toxic to key reef species, causing direct effects at cellular, organism, population, and community levels (Browne et al., 2015; Marangoni et al., 2017; Negri and Hoogenboom, 2011; Prazeres et al., 2011; Restrepo et al., 2016). Regarding symbiont-bearing foraminifera responses, Prazeres et al. (2012a, 2012b) reported enhanced bleaching frequency and biochemical responses indicative of oxidative stress in *Amphistegina* spp. from contaminated reefs showing Cu concentrations similar to the observed in the present study (10.3 µg L^{-1}). Prazeres et al. (2011) reported oxidative stress and bleaching in *A. lessonii* acutely exposed to Zn concentrations similar to the maximum concentration observed for this metal in the present study (41.1 µg L^{-1}). Cu concentrations lower than the water quality criteria established by the current Brazilian environmental guidelines (CONAMA, 2005) were shown to cause detrimental physiological effects in *A. gibbosa* only when combined to ocean acidification (Marques et al., 2017). It is therefore imperative to consider that the pressure imposed by multiple stressors can cause harmful effects in organisms exposed even to low metal concentrations. Considering that reefs located near the Buranhém River mouth are exposed to frequent and wide changes in environmental parameters, river water containing multiple contaminants, sediment and nutrient enrichment, they can be considered as being more vulnerable to metals exposure, even at concentrations lower than the current water quality criteria guidelines.

4.3. Ecological indicators

Population density of *A. gibbosa* was significantly reduced in summer. Several authors reported reduced symbiont-bearing species density during increased temperature periods, especially when associated with high UV radiance (Barbosa et al., 2016; Fujita et al., 2016; Kelmo and Hallock, 2013). Periods of elevated temperature and low rainfall, as the summer period in the present study, are expected to impose a severe thermal and photic stress to photosymbionts. Additionally,

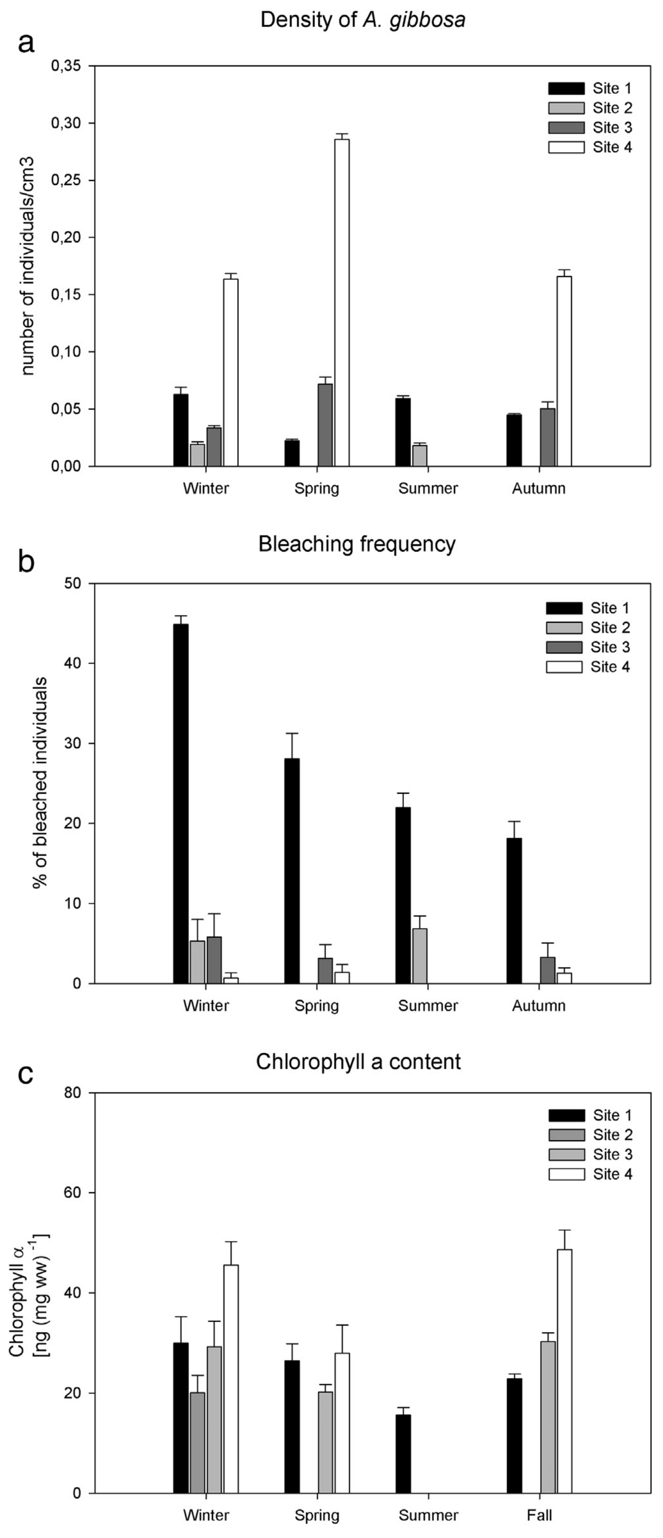

Fig. 4. Values (mean ± SE) of *Amphistegina gibbosa* (a) density, (b) bleaching frequency and (c) chlorophyll α content at each reef site and season.

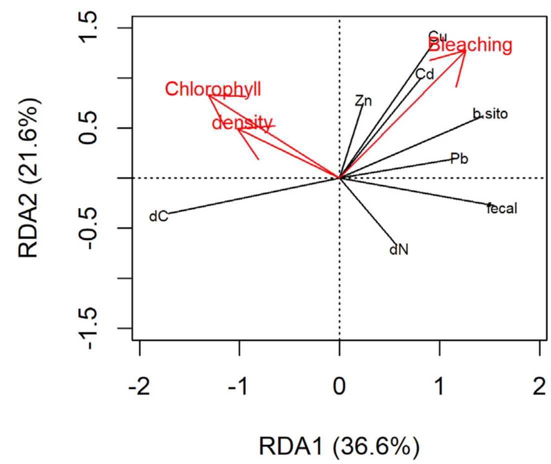

Fig. 5. RDA triplot for the relationship among selected geochemical/water quality indicators (black arrows) and ecological indicators (red arrows). Arrows indicate the parameter increase direction, and angles reflect their correlations. dC: $\delta^{13}C$; dN: $\delta^{15}N$; fecal: the sum of fecal sterols concentrations; b.sito: β-sitosterol concentration. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Amphistegina populations from shallow reefs can be dramatically reduced during heat waves once they are exposed to an even higher degree of photic stress (Baker et al., 2009). Sampling sites inside the PNMRF (reefs 4 and 5) are shallower than reefs 1–3, explaining the expressively lower numbers of *A. gibbosa* found in the offshore reefs during summer.

The ecological parameters analyzed were significantly correlated to the distance from the river. RDA showed some relationship between *Amphistegina* densities and sewage descriptors, similarly to Emrich et al. (2017) findings. The physiological mechanisms involved in the responses to sewage presence are poorly understood, so it is challenging to discuss the potential association between populational responses and sewage. However, they are likely related to nutrification and food availability affecting symbiosis.

A strong correlation between foraminiferal bleaching frequency and density was expected (Prazeres et al., 2012b), but this was not observed in the present study. Prazeres et al. (2016, 2017b) discussed how populations collected from different sites deal with temperature and nutrient stress differently, suggesting some kind of phenotypic plasticity among populations from different reefs. This may explain why higher bleaching frequencies were not strictly followed by reduced foraminifera population density. In this case, foraminifera populations from reef site 1 could be more adapted/acclimated to environmental changes.

The marked effect of dissolved Cu exposure on bleaching observed in the present study was also reported by Prazeres et al. (2012a, 2012b) in foraminifera from Fernando de Noronha reefs (Pernambuco state, northeastern Brazil). Cu can also cause oxidative stress in corals (Fonseca et al., 2017; Marangoni et al., 2017), which is one of the main causes of bleaching in these organisms (Downs et al., 2002).

Chlorophyll α content was higher in foraminifera from reef 4, which is located inside the PNMRF and shows the lowest level of anthropogenic influence. Chlorophyll level was strongly correlated with population density, but did not respond clearly to visual bleaching. The higher levels of chlorophyll α observed in foraminifera from reef 4, which had the lowest bleaching frequency, indicated a healthy population. In the present study, metal, sewage contamination and detrimental ecological effects were related to the distance from the river mouth. These effects were enhanced in the rainy season, suggesting land-based source pollution (Fabricius, 2005; Fabricius et al., 2013; Rocker et al., 2017). Therefore, adequate management of watersheds and land use is crucial for coral reef conservation (Brodie et al., 2017; Takesue and Storlazzi, 2017), especially when combined with coral reef monitoring. Our results show that an integrated and multi-marker approach can provide consistent diagnostics indicating anthropogenic influence in coral reefs. Also, they highlight that monitoring of symbiont-bearing foraminiferal populations is effective to detect environmental health degradation in this ecosystem.

5. Conclusions

Coral reef patches located near the Buranhém River mouth presented declined environmental health, evidenced by reduced water and sediment quality, as well as detrimental ecological effects in populations of the larger benthic foraminifera *A. gibbosa*. Reef sites located inside the PNMRF area can be considered as being nearly pristine, according to the water quality data (low concentrations of metals) and geochemical indicators responses. Populations of *A. gibbosa* in this area were healthier (lowest bleaching frequency) than those collected at the other reefs. On the other hand, sites located closer to the coast showed considerable concentrations of Cu, Pb, and sewage contamination, higher terrigenous influence, and foraminifera with the highest bleaching frequency. In summary, findings reported in the present study indicate that coastal Brazilian reefs are subjected to multiple anthropogenic stressors. Additionally, they point out that the water quality, geochemical and ecological indicators employed in the present study were effective as biomonitoring tools that can be used in reef areas worldwide.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.09.154.

Acknowledgments

Support for field research is acknowledged to the “Projeto Coral Vivo”, which is sponsored by the Petrobras Socio-environmental Program of Petróleo Brasileiro S.A. (Petrobras, Brazil) and the Arraial d'Ajuda Eco Parque (Brazil). Financial support is acknowledged to the International Development Research Centre (IDRC, Ottawa, Canada) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES - Programa Ciências do Mar II, Brasília, DF, Brazil). We thank Maria Gabriela Fernandes Dias for graphical abstract production. We thank Dr. Deborah Leite and Dr. Raquel Peixoto for sharing subsamples from the project. A. Bianchini is a research fellow from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; Brasília, DF, Brazil; Proc. # Proc. # 307647/2016-1) and supported by the International Canada Research Chair Program from IDRC, Canada. J.A. Marques is a graduate fellow from CAPES.

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