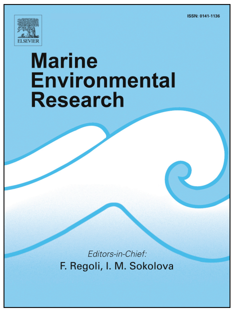

Assessment of the ecological quality status of the Sepetiba Bay (SE Brazil): When metabarcoding meets morphology on foraminifera

Fabrício Leandro Damasceno, Maria Virgínia Alves Martins, Fabrizio Frontalini, Jan Pawlowski, Kristina Cermakova, Inès Barrenechea Angeles, Luiz Guilherme Costa Santos, João Graciano Mendonça Filho, Fabio Francescangeli, Thaise M. Senez-Mello, Wellen Fernanda Louzada Castelo, Renata Cardia Rebouças, Wania Duleba, Silvia Helena de Mello e Sousa, Lazaro Laut, Luzia Antonioli, Members of the Project “Anthropized Coastal Systems”

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Authors contribution credit

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Assessment of the Ecological Quality Status of the Sepetiba Bay (SE Brazil): When metabarcoding meets morphology on foraminifera

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Abstract

In recent years, the region surrounding Sepetiba Bay (SB; SE Brazil) has become a hub of intense urban expansion and economic exploitation in response to ore transport and industrial and port activities. As a result, contaminants have been introduced into the bay, leading to an overall worsening of the environmental quality. The present work applies for the first time a foraminiferal morphology-based approach (M) and eDNA-based metabarcoding sequencing (G), along with geochemical data to assess the ecological quality status (EcoQS) in the SB. Principal component analysis shows that the eDNA and morphospecies diversity as well as most of the taxa relative abundance decline in response to the environmental stress (ES) gradient related to total organic carbon (TOC) and metal pollution. Based on ecological indices, Exp(H'_{bc}) (G), Exp(H'_{bc}) (M), foraminifera ATZI marine biotic index (Foram-AMBI), Foram Stress Index (FSI), and geochemical indices (TOC and Potential Ecological Risk Index), the lowest values of EcoQS (i.e., bad to moderate) are inferred in the innermost part of the SB. Despite minor discrepancies among the six EcoQS indices, an agreement has been found for 63% of the stations. To improve the agreement between the ecological indices, it is necessary to fill the gap in species ecology; information on the ecology of many species is still unknown. This work reinforces the importance of molecular analysis and morphological methods in environmental impact studies and confirms the reliability of foraminiferal metabarcoding in EcoQS assessment. This is the first study evaluating the EcoQS in the South Atlantic by using combined foraminiferal eDNA metabarcoding with morphological data.

Keywords: eDNA, metals, organic matter enrichment, pollution, Foram-AMBI, FSI, diversity

1. Introduction

Coastal areas have been significantly impacted over the last decades (Bervoets and Blust, 2003; Xiang et al., 2008; Mirlean et al., 2009). The high population density, the intensification of human occupation and activities, and the discharge of municipal, industrial and pharmaceutical effluents (Shola et al., 2022; Marinho et al., 2022) have led to environmental deterioration of these sensitive ecosystems (Zhang et al., 2007; Ribeiro et al., 2015). Transitional environments (TEs) such as coastal bays, lagoons, estuaries, and adjacent river areas have been among the most affected environments (Marques et al., 2022; Almeida et al., 2023), particularly in developing countries (Anyanwu et al., 2018).

According to IBGE (2011), 24.6% of the Brazilian population was concentrated in coastal areas in 2000. Currently, most of these areas face environmental problems, particularly in the southeast of Brazil. The negative impacts have been caused by strong industrial/economic development, such as alteration of natural geomorphological characteristics, deforestation, removal of mangroves, landfilling of water bodies, loss of biodiversity, eutrophication, and accumulation of pollutants (Souza et al., 2021; Silva et al., 2022). These environmental issues have led to the loss of biodiversity, changes in ecosystem functioning, and threats to the ecological integrity of Brazil's coastal areas (Hatje et al., 2021). Among the most impacted areas along the Brazilian coast, the Sepetiba Bay (SB), geographically located in a region of high demographic occupation and high economic, industrial, and port interest, has experienced a long-lasting history of environmental quality alteration (Kütter et al., 2021; Silva et al., 2022). The area sees the occurrence of 400 industries, the gigantic steelworks complex of Latin America, the highway known as the metropolitan arch of Rio de Janeiro State, the Santa Cruz air base and three ports, including the Port of Sepetiba/Itaguaí, which handles ≈51.7 million tons of iron ore per year (Docas, 2022). The intense anthropogenic activities and the release of industrial and domestic effluents have been causing the accumulation of potentially toxic elements (PTEs) both in sediments and biota (Ribeiro et al., 2015; Rodrigues et al., 2020; Tonhá et al., 2020; Souza et al., 2021).

For this reason, it is essential to monitor this bay and to develop and test new methodological tools for evaluating the ecological quality status (EcoQS). Among benthic components, macrofauna is widely used for biomonitoring coastal systems (Borja et al., 2000; Rosenberg et al., 2004; Borja and Dauer, 2008). Since benthic foraminifera have been proven to be excellent and reliable bioindicators (Martins et al., 2018, 2019; Rostami et al., 2023), they have been increasingly used to assess the EcoQS (Francescangeli et al., 2021, Frontalini et al 2020; Nunes et al., 2023). Several biotic indices, based on morphospecies analysis, such as the foraminifera-ATZI marine biotic index (Foram-AMBI: Alve et al., 2016; Jorissen et al., 2018; Bouchet et al., 2021), the Foram Stress Index (FSI: Dimiza et al., 2016), and diversity-based index ($Exp(H'_{bc})$) according to Bouchet et al. (2012) have been successfully developed, tested and applied for assessing the EcoQS (e.g., Bouchet et al., 2018; Nunes et al., 2023). Recently, the development of environmental metagenomic (eDNA metabarcoding) techniques (Pawlowski et al., 2016; 2022) has opened new possibilities for assessing the EcoQS in marine environments and extended the application of these ecological indices (e.g., Cavaliere et al., 2021; Al-Enezi et al., 2022; Barranchea Angeles et al., 2023).

In light of it, this work aims to document, for the first time in the Brazilian transitional waters, 1) the response of foraminiferal communities analyzed through morphological and metagenomic (eDNA metabarcoding) approaches to environmental stress (ES) gradient in the SB; 2) to apply several ecological indices based on benthic foraminiferal for both the morphospecies and amplicon sequence variants (ASVs) and geochemical ones such as total organic carbon (TOC) and Potential Ecological Risk Index (PERI), based on PTEs, to assess the EcoQS in the bay.

2. Study area

The Sepetiba Bay (SB) is located in the western region of the Rio de Janeiro State (Fig. 1) and covers an area of approximately 450 km^2. It is bordered to the south by the Marambaia barrier island formed during the marine regression after the last glaciation (Reis et al., 2020; Dadalto et al., 2022). It is connected to the Atlantic Ocean through its main opening located between the Ilha Grande Island and the tip of the Marambaia barrier island and in its eastern part through the tidal channels of the Guaratiba region (Reis et al., 2020; Dadalto et al., 2022). The Marambaia barrier island protects the SB from the high oceanic hydrodynamics (Carvalho et al., 2023). The SB receives fresh water from several rivers, such as the Guandu, the main water body, Guandu-Mirin, Lapa, Mazomba, and Sahy rivers.

The bathymetric profiles extracted from navigation sheets of the Directorate of Hydrography and Navigation of the Brazilian Navy (DHN, 2021) show that the marginal and internal zones of the SB are the shallowest (i.e., 2 to 5 m) areas. The external portion of SB has the deepest depths (i.e., 20 to 30 m) as well as the navigable channels that cross this bay. The circulation patterns result from the asymmetric influence between flood (more intense) and ebb (longer) tides and winds acting in the region (Cunha et al., 2006). According to Coelho et al. (2018), the bay exhibits a stationary tidal wave during the spring tide and a poorly stratified estuarine circulation pattern.

The SB is characterized by relatively high-temperature waters related to its position in a tropical region and to its shallow depth, particularly in its inner part. Despite the physical protection of the Marambaia barrier island that shelters the SB from direct contact with the ocean and the adjacent oceanic processes, cold waters (20°C) resulting from the continental shelf currents mainly flow in SB from October to April (Kjerfve et al., 2021). This process is associated with northeast synoptic winds and Ekman-induced coastal upwelling which are strongest from October to April (Kjerfve et al., 2021).

The natural conditions of the bay, as well as the hydrodynamics, meteorological, tidal variation, biogeochemical aspects, sediment distribution, and physicochemical factors, favor the accumulation of fine-grained sediments and the retention of organic matter and metals in its inner area (Carvalho et al., 2020; Souza et al., 2021; Silva et al., 2022).

As mentioned, in recent years, the SB has been the target of high environmental impact due to intense economic development (Rodrigues et al., 2020) due to ore transport and exportation and port activities (Trevisan et al., 2020). The impacts have been caused by several industries, such as the Companhia Siderúrgica Mercantil Ingá, Companhia Siderúrgica do Atlântico (CSA), Nuclebras Equipamentos Pesados S/A NUCLEP, USIMINAS, Guaíba Island Terminal, NUCLEP Port Terminal, Porto de Sepetiba, Porto Sudeste, Solid Bulk Terminal Mineração Usiminas S.A. and others (Fig. 1). From these companies, for example, Companhia Siderúrgica Mercantil Ingá, a large zinc smelting plant, went in operation in 1962, processing ore to produce high-purity zinc and generated large quantities of waste rich in heavy metals during the purification process, mainly cadmium and zinc. Although this company closed down in 1998, it left an environmental liability, a toxic lake with 390 thousand cubic meters of liquid effluents, which still affects the SB. The Port of Sepetiba was set up in the municipality of Itaguaí in 1976 and began to operate in 1982. According to the Itaguaí Town Council, it can be considered a highly productive port, responsible for around 70% of Brazil's Gross Domestic Product – GDP. Guaíba Island Terminal (Terminal da Ilha Guaíba -TIG), is a private port for the exclusive use of the company Minerações Brasileiras Reunidas S/A, today VALE. The TIG established in 1973 is located on Guaíba Island, in the Municipality of Mangaratiba, and consists of a private port connected to the mainland by a 1,765-meter railway bridge, with a shipping capacity of 50 million tons per year. It is currently used to receive and export iron ore extracted in Minas Gerais. NUCLEP Port Terminal – Nuclebrás Equipamentos Pesados S/A is for the exclusive use of the company for collecting, from abroad, tanks and accessories and for loading, in coastal shipping, heavy and large equipment manufactured by the company. Porto Sudeste, a private port, has operated since 2015 for the export of iron ore from producers in Minas Gerais. The Mineração Usiminas S.A company was founded in the Ilha da Madeira neighborhood in Itaguaí-RJ in 2010 and deals with iron ore extraction. The Companhia Siderurgica Nacional (CNS) has the Solid Bulk Terminal (Tecar) in Itaguaí (RJ); through this terminal, the CNS receives the mineral coal used in the Presidente Vargas Plant (UPV), one of the largest steel plants, and transports iron ore from Minas Gerais to the international market. A railway network connects the mines, UPV and Tecar.

In addition, it is currently estimated that ~10 million people live around the SB and many houses do not have a sewage system service. Domestic effluents and waste eventually end up without pre-treatment into the SB (Ribeiro et al., 2014). These problems have led to a significant environmental deterioration in this ecosystem (Wasserman et al., 2013; Ribeiro et al., 2015; Souza et al., 2021), mostly since the 1950s (Castelo et al., 2021 a, b; Silva et al., 2022).

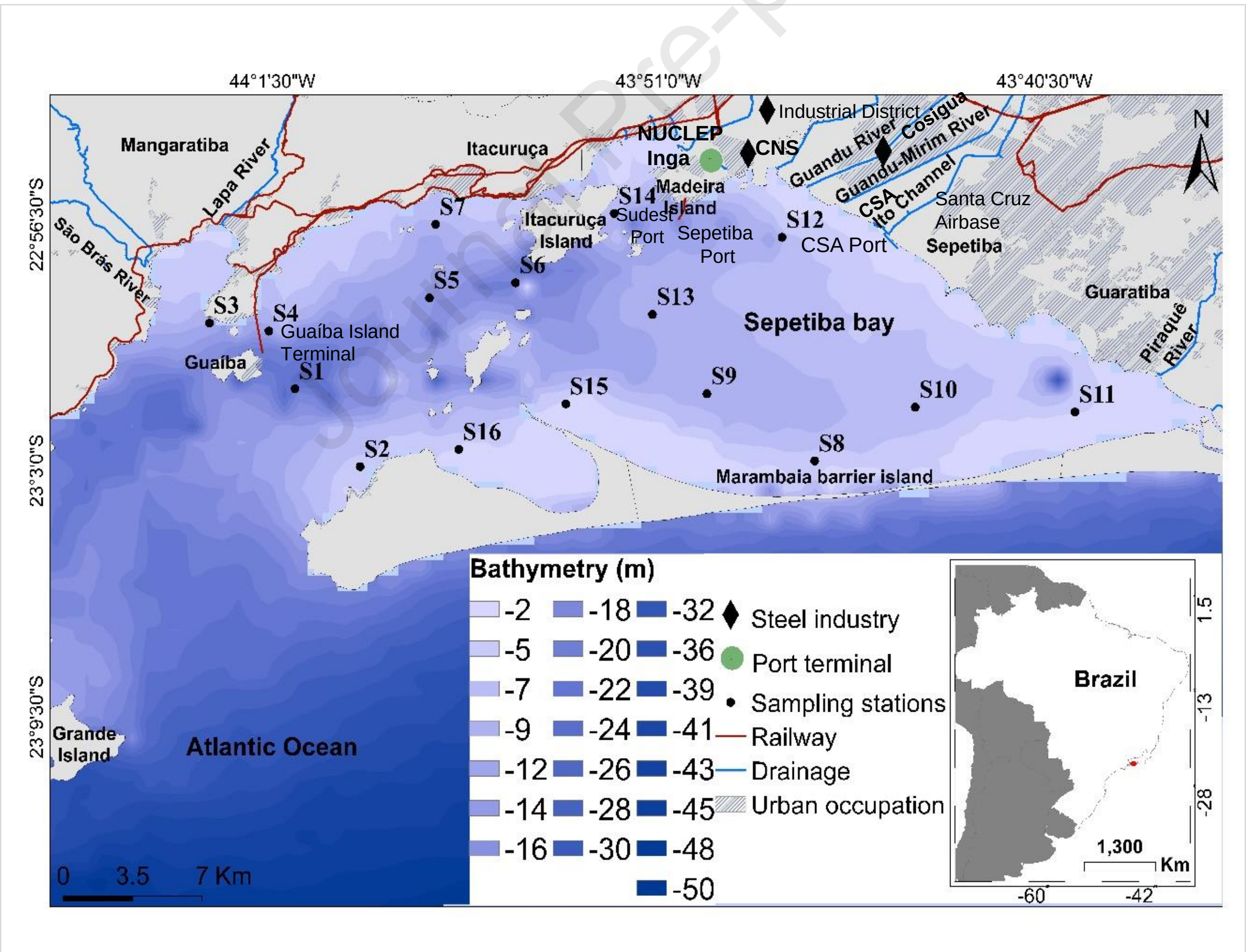


Figure 1. Location map of the Sepetiba Bay (Brazil) and the location of sampling sites. The most important ports and industries are singed (see the text).

3. Materials and methods

3.1 Sample collection and preparation

In May 2022, 16 surface sediment samples were collected in the SB on board a local fishing boat (Fig. 1; Appendix 1). For the location of each sampling station, a Global Position System (model GPSMAP® 78S) and coordinates (with WGS84 datum) were used (Appendix 1). The site depth was estimated with an echo sounder. At each sampling station, physicochemical data were recorded in the water over the sediment (temperature and salinity) and the sediment surface layer (pH and redox potential, Eh) with a multiparameter probe (Hanna Instruments). The multiparameter probe was calibrated with international standards for each variable according to ISO 7393-2:2017.

At each sampling station, at least three deployments of a box-corer (90 cm X 70 cm X 40 cm) were performed to have three independent replicates (labeled as RI, RII, and RIII) of sediment. From each replicate, the surface of the sediment was sampled with a spatula and a volume of sediment of about 50 ml was taken to study living benthic foraminifera (i.e., morphological analyses). These samples were preserved in a solution of rose Bengal (2 g of rose Bengal in 1000 ml of ethanol, 90º). The samples were preserved in this solution for 14 days, according to the recommendation of Schönfeld et al. (2012). Samples were then washed with tap water through a 63-µm sieve.

For metabarcoding analyses, the surface of the sediment was collected with a sterile spoon. Approximately 10 g of surface sediment (corresponding to the uppermost 1 cm) was collected (one sample for each station), placed in a cryogenic tube, and immediately frozen at -20 °C. The sediment for metabarcoding analyses was collected at the same station, but does not generally correspond to the same sediment in the morphological analyses (it was necessary to carry out multiple deployment to obtain enough sediment for all the analyses, considering the need to use only the surface sediment).

At each sampling station, aliquots of surface sediment from each replicate were collected, mixed and stored in zip lock bags for grain size and geochemical analyses (organic matter, carbonates and metals). The samples for these analyses were immediately frozen until they processed.

3.2 Sediment grain size and geochemistry

For grain size, about 250 g (in the case of sand) and 150 g (in the case of fines) of dry sediment were weighed and separated by sieving. The fines (<63 µm fraction) were separated from the coarse fractions of the sediment through a 63 µm sieve. Both fractions were stored in containers and oven-dried at about 60°C. The dry residue instead of both fractions was weighed. The >63 µm fraction was separated using a column of sieves (2000 µm, 1000 µm, 500 µm, 250 µm, 125 µm and 63 µm) and the sediment retained on each sieve was weighed. A Malvern Mastersizer 2000 Size (model hydro 2000MU) was used to determine the microgranulometry of the <63 µm fraction. The percentage of each particle size fraction was determined.

Total organic carbon (TOC) values were carried out using the ASTM D4239 (American Society for Testing and Materials - ASTM, 2017) and NCEA-C-1282 (United States Environmental Protection Agency-US EPA, 2002) methods. The sedimentary material was decarbonated using 1:1 HCl acid. The decarbonated sediments were dried at 1350°C and analyzed using the SC-144DR-LECO equipment at the Palynofacies and Organic Facies Laboratory of the Federal University of Rio de Janeiro (LAFO-UFRJ, Rio de Janeiro, Brazil).

For PTE concentrations (As, Cd, Co, Cr, Cu, Hg, Ni, Pb, Sn Zn), about ~10 g of total dry sediment of each sample was powdered in an agate mortar and sieved with a 63-μm mesh sieve. The sediments were treated with aqua regia, followed by ultra-trace analyses with inductively coupled plasma mass spectrometry (ICP-MS). The analysis was performed in Activation Laboratories Ltd. (Canada). The Pollution Load Index (PLI: Tomlinson et al., 1980) following Martins et al. (2014), as well as the enrichment factor (EF: Buat-Menard and Chesselet, 1979), the Geoaccumulation Index (Igeo: Müller, 1986) and the Potential Ecological Risk Index (PERI; Håkanson, 1980; Swarnalatha et al., 2013) were calculated in order to evaluate the degree of enrichment and pollution caused by PTEs.

The Pollution Load Index (PLI: Tomlinson et al., 1980) was estimated with the equation (1):

$$\text{PLI} = \sqrt[n]{CFm1\ x\ \text{CF}m2\ \times \ldots\ldots \times\ \text{CF}mi} \qquad (1)$$

where the contamination factor (CF) is the metal concentration (Cm) in the sample divided by its local baseline (Bm) value (Cm/Bm). The CF values were computed for each analyzed metal (*m1, m2, ... mi*). The baseline values used in the PLI calculation were estimated from the average of the lowest concentrations of 35 samples out of a total of 73 surface sediment samples from the SB (unpublished data); the average of the concentrations of metals at pre-industrial levels in the SB cores were also considered (Castelo et al., 2021a).

The EF was calculated using the equation Buat-Menard and Chesselet (1979) (2):

$$EF = \frac{\left(\frac{Cm}{Cn}\right) station}{\left(\frac{Cm}{Cn}\right) \text{baseline}} \qquad (2)$$

where Cm is the metal concentration and Cn is the normalizing element concentration; in this analysis the Sc was used as a normalizer, since it is related to fine-grained sediments and is a lithogenic element. The EF values show how much a metal is enriched in a sample above the natural value and whether the proportion of fines in the sample, where metal concentrations are generally higher, influences the EF values.

The Igeo was determined with the equation of Müller (1986) (3):

$$\text{Igeo} = \log_2 \left[\frac{\text{Cm}}{\text{Bm x 1.5}}\right] \qquad (3)$$

The Håkanson (1980) method was used to estimate the potential ecological risk index (PERI) (4)

$$\text{PERI} = \Sigma\text{RI} = \Sigma(T_{rf} \times \text{CF})$$

where the RI is the ecological risk index for each metal (5):

$$\text{RI} = T_{rf}\ \text{x CF} \qquad (5)$$

and CF is the Cm/Bm (concentration of the metal divided by its baseline value) and T_{rf} is the parameter of its toxicity response, as follows: Zn = 1, Cr = 2, Co = Cu = Ni = Pb = 5, As = 10, Cd = 30, and Hg = 40 (Håkanson, 1980; Huang et al., 2021; Liu et al., 2021).

3.3. Living foraminifera

3.3.1. Morphospecies analyses

Living foraminifera specimens were picked in the sediment fraction >125 μm following the FOBIMO protocol (Schönfeld et al., 2012). The foraminiferal specimens were identified using references, such as Brönnimann (1979), Boltovskoy et al. (1980), Poag (1981), Loeblich and Tappan (1987), and Alves Martins et al. (2019), as well as the Ellis and Messina (1940-2015) catalog and the World Register of Marine Species (WoRMS; Hayward et al., 2020).

The relative abundance of each species and some biotic parameters, such as the species richness (S), the Shannon index (H'; Shannon, 1948), and the equitability (J'; Magurran, 1988) were determined using Primer software (version 6.1.13, Plymouth, UK; Clarke and Gorley, 2006).

To indicate the sedimentary environmental oxygen scarcity and impact, the *Ammonia-Elphidium* Index was calculated according to Sen Gupta and Machian-Castillo (1993) and Sen Gupta et al. (1996): AEI = [NA / (NA + NE)] × 100, where NA and NE are the numbers of *Ammonia* spp. and *Elphidium spp.* specimens, respectively.

3.3.2. Metabarcoding analyses

The eDNA extraction, PCR amplification and high-throughput sequencing (HTS) have been performed as described in Cordier et al. (2019). Briefly, three extractions per sample were performed with DNeasy Power Soil Kit (Qiagen) following the manufacturer's instructions. PCR amplification of the hypervariable region of nuclear 18S rRNA gene (37 + 41f) targeting benthic foraminifera was performed using foraminiferal specific primers (forward F1 5'-AAGGGCACCACAAGAACGC-3' and reverse 17 5'-CGGTCACGTTCGTTGC-3'). The PCR comprised an initial denaturation step, 10 cycles of denaturation, annealing at 57°C for 30 s and elongation at 72°C for 45 s, followed by 30 cycles of denaturation, annealing at 47°C for 30 and elongation 72°C for 45 s, and a final elongation step at 72°C for 5 min. The PCR products were checked by agarose gel electrophoresis and then quantified by high-resolution capillary electrophoresis using QIAxcel System (Qiagen). The sequencing library was then prepared using the Illumina TruSeq® DNA PCR-Free Library Preparation Kit. The library was quantified by qPCR using the KAPA Library Quantification Kit. MiSeq instrument (Illumina) was used for 500 cycles of paired-end sequencing with a Standard v2 kit. The raw data (Fastq files) were processed using SLIM (Web application; Dufresne et al., 2019). The samples were first demultiplexed and the algorithm dada2 (Callahan et al., 2016) was applied to the quality filter, trim and merge reads and remove chimeras. After that, we obtained an ASV table and a fasta file. We removed the sequences not containing the foraminifera pattern AGGTGGTGCA. Then, LULU (Frøslev et al., 2017) curation was applied to remove PCR and sequencing artifacts. The sequences were then compared against a curated reference sequence database for taxonomic assignments. The relative abundance of ASV species was determined, as well as biotic indices: the number of ASVs (S) and Shannon index (H'), and equitability (J').

3.4 Ecological Quality Status

To evaluate the EcoQS three ecological indices, namely the Exp(H'_{bc}) (M) (Bouchet et al., 2012 for details), the Foram Stress Index (FSI) (Dimiza et al., 2016) and the Foram-AMBI (Alve et al., 2016; Jorissen et al., 2018; Bouchet et al., 2021) were calculated in the morphological dataset. Since the species were not yet assigned for the South Atlantic Ocean, the species assignments for the Foram-AMBI estimation were based on those of Atlantic TWs (according to Bouchet et al., 2021). The EcoQS classification was performed according to Nunes et al. (2023, and references herein). Additionally, the Exp(H'_{bc}) (G) was also computed for the molecular dataset. Since the present study represents the first attempt to evaluate the EcoQS based on molecular data on foraminifera in the South Atlantic and class boundaries have not been established, the ecological quality ratio (EQR) was used by standardize the value of diversity at each site to the maximum value recorded in the area. The values of EQR vary from 0 (i.e., bad EcoQS) to 1 (i.e., high EcoQS). Following

Al-Enezi et al. (2022), five class boundaries were defined (i.e., 1–0.8 high, 0.8–0.6 good, 0.6–0.4 moderate, 0.4–0.2 poor and 0.2–0 bad EcoQS).

The criteria used to infer the EcoQS were based on Exp(H'$_{bc}$) - Bouchet et al. (2018), Foram-AMBI - Parent et al. (2021), FSI - Dimiza et al. (2016), PERI - Håkanson (1980) and Swarnalatha et al. (2013) (Table 1). The TOC classes (standardized values) were established in this work.

To define the agreement/disagreement of the selected ecological indices (i.e., Exp(H'$_{bc}$) (G), Exp(H'$_{bc}$) (M), Foram-AMBI, and FSI), and geochemical indices (TOC and PERI), two EcoQS (i.e., 'Acceptable' or 'Not acceptable') were considered following Blanchet et al. (2008). The 'Acceptable' includes High or Good EcoQS and scores as 1, while 'Not acceptable' incorporates Moderate, Poor, or Bad EcoQS and scores as 0. The scores were then summed for each station and categorized in order to infer the level of agreement/disagreement (i.e., full agreement 0/6 or 6/6, partial agreement 1/6 or 2/6 and disagreement 2/6, 3/6 and 4/6) among indices.

Table 1. Ecological Quality Status (EcoQS) classification criteria. The criteria used to evaluate EcoQS were based on: Exp(H'bc) (M) Bouchet et al. (2018); Foram-AMBI Parent et al. (2021); FSI Dimiza et al. (2016); PERI Håkanson (1980) and Swarnalatha et al. (2013). The TOC and Exp(H'bc) (m) classes (standardized values) were established in this work. Legend: (M) - morphospecies; (m) - molecular results; stnd - standardized data.

Index	**Ecological Quality Status**				
	High	**Good**	**Moderate**	**Poor**	**Bad**
ExpH'bc (G) - stnd	0.8-1	0.6-0.8	0.4-0.6	0.2-0.4	0-0.2
Exp(H'bc) (M)	>15	11-15	7-11	3-7	<3
Foram-AMBI	<1.4	1.4-2.4	2.4-3.4	3.4-4.4	>4.4
FSI	10.0-9.0	9.0-5.5	5.5-2.0	2.0-1.0	1.0-0.0
TOC - stnd	0.2-0	0.4-0.2	0.6-0.4	0.8-0.6	0.8-1.0
PERI	< 150		150-300	300-600	> 600

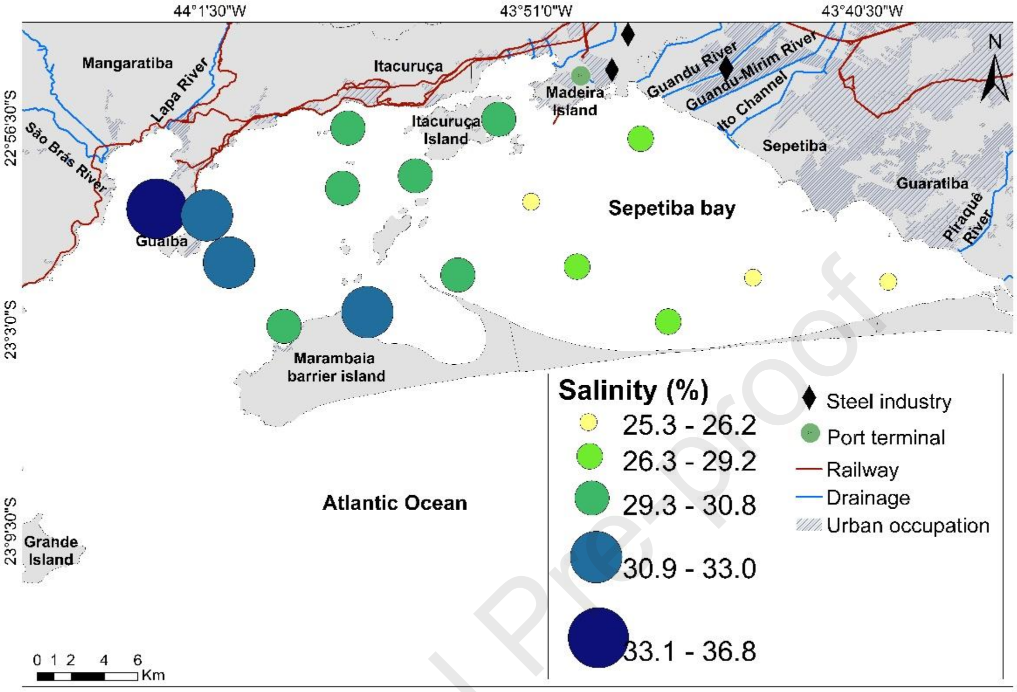
3.5 Statistical analysis

Data were logarithmically (log x+1) transformed before statistical analyses. Morphospecies with a relative abundance >5% and the assigned ASVs were selected for the statistical analysis. Principal component analyses (PCA) and Spearman Rank Order Correlations were performed using the software STATISTICA 13.5, based on selected biotic, ecological, and environmental parameters. The PCA analyses were used to relate the biotic variables with abiotic ones. In addition, Spearman Rank Order Correlations (p < 0.050) were also carried out to identify and analyze the distribution trends among the selected variables. These statistical analyses were performed using Primer software (version 6.1.13, Plymouth, U; Clarke & Gorley, 2006).

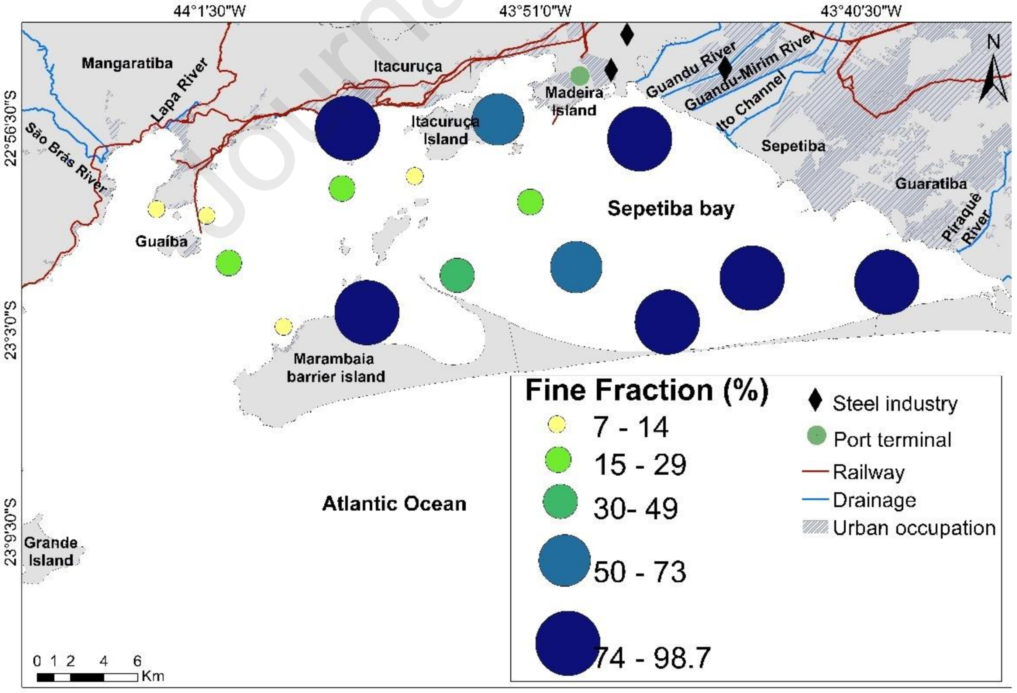
4. Results

4.1 Physicochemical parameter of water

Bottom water temperature ranged from 22.30-26.11 °C (mean 24.56±1.09 °C). The salinity varied from 25.3 to 36.80 with a mean of 29.93±2.95. The lowest salinity values were recorded in the inner sector of the SB, near the river mouths, whereas normal marine salinity values were found in the outermost part of the bay (Fig. 2A). The pH and Eh values varied between 7.94-8.20 (mean 8.10±0.09) and -65.70 mV and-51.30 mV (mean -60.33±4.64 mV), respectively.



A



B

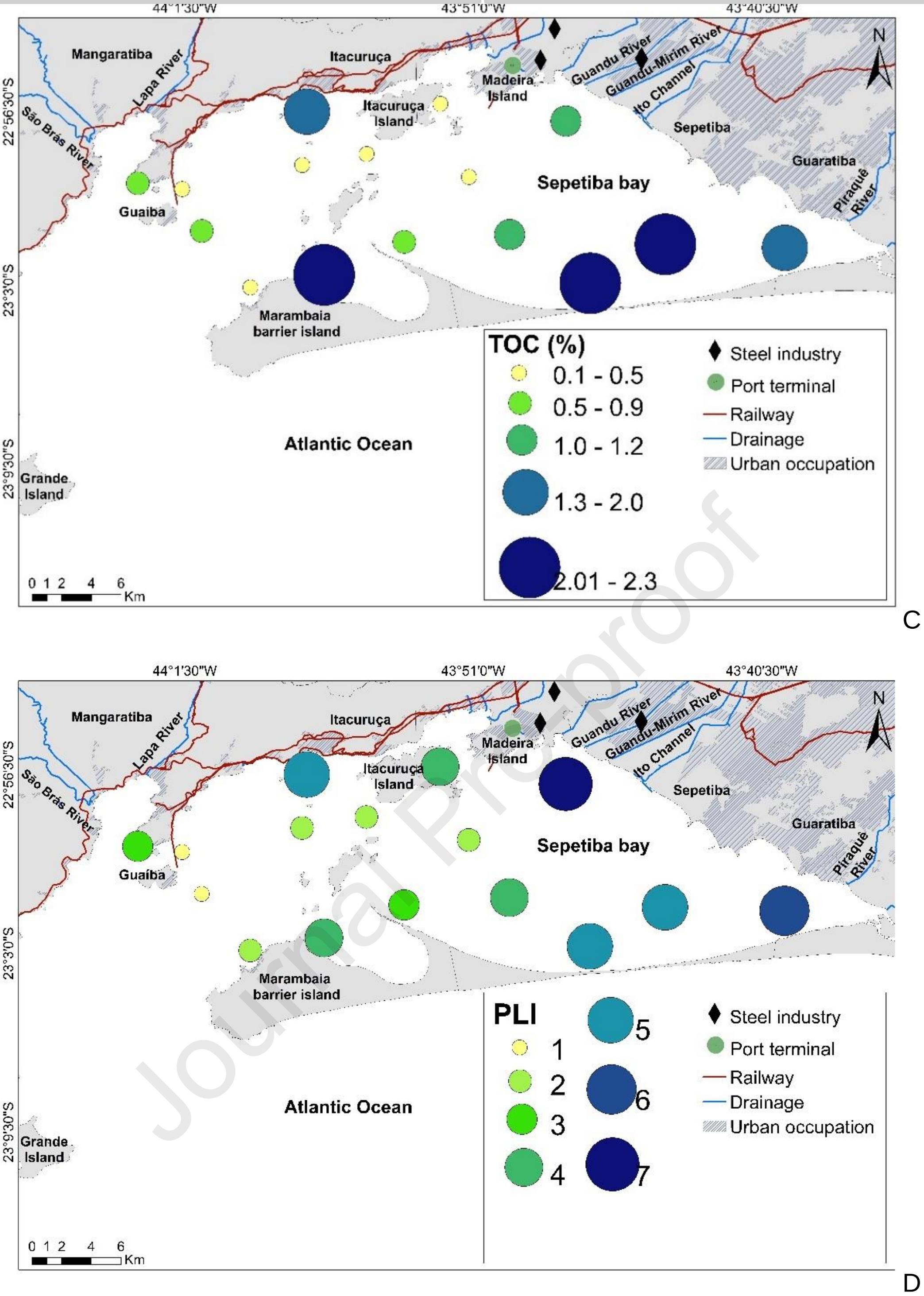


Figure 2. Distribution maps of A. salinity, B. fine fraction (%; <63 µm), C. TOC (%) and D. PLI values in the studied stations in the Sepetiba Bay.

4.2 Grain size and geochemical data

The studied sites were heterogeneous for sediment grain-size characteristics, with sand and fine fraction contents varying between 0.47-92.50% (mean 44.61±38.04%) and 7.18-98.70 % (mean 55.07±38.11 %), respectively. The sediment of one-third of the samples was composed of mud; the remaining samples consisted of sandy mud or muddy sand. The sandy samples mostly corresponded to stations located in the outer and more central sector of the bay or the vicinity of navigable channels. TOC contents ranged from 0.19 to 2.31% (mean 1.16±0.79 %). Stations with higher fine fraction and TOC contents were generally found in protected areas of the SB margins (Fig. 2 B, C).

The PLI values varied between 1.00-7.00 (mean 3.53±1.74). The highest PLI values were found at stations in the inner sector and close to the margins of the SB (Fig. 2 D).

The concentrations of the analyzed chemical elements were in decreasing order of maximum concentration: Zn (595.0 mg kg^{-1}) > Cr (65.0 mg kg^{-1}), >Cu (54.0 mg kg^{-1}) > Pb (31.7 mg kg^{-1}) > Ni (20.4 mg kg^{-1}) > Co 10.7 mg kg^{-1}) > As (9.7 mg kg^{-1}) > Sn (5.7 mg kg^{-1}) > Cd (2.0 mg kg^{-1}) > Hg (170.0 µg kg^{-1}). The range, mean, and standard deviation of these PTEs can be found in Appendix 1. The highest concentrations of Cd, Cr, Pb and Zn were found near the northern margin of the SB and along the Marambaia barrier island (Fig. 3 A-D). Similar distribution patterns were also observed for As, Co, Hg, Ni, and Sn.

The EF values varied between 0.8-3.4 (Appendix 2). Some metals reached EF values between $2<EF<5$, such as EF.Cd (3.4), EF.Sn (3.0), EF.Zn (2.5), EF.Cu (2.5), EF.Cr (2.1) EF.Co (2.1). The EF values for Ni>Pb>Hg>As varied between 0.8 and 2.0 (Appendix 2).

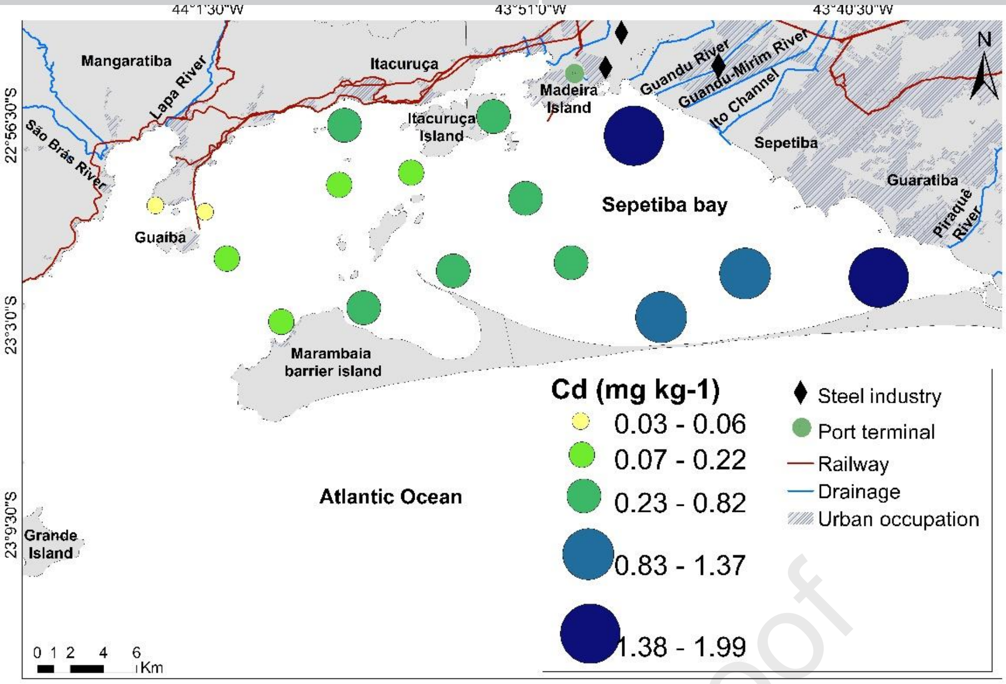
The Igeo values ranged between 0.9-3.6 (Appendix 2). The maximum Igeo values were reached by Sn (3.6) >Cd (3.5) >Zn (3.2) >Cr (2.7) > Co (2.5) >Pb (2.5) >Ni (2.5) >Cu (1.4) > Hg (1.3) >As (0.9). PERI values (131-831) also significantly varied (Appendix 2).

4.3 Morphological community

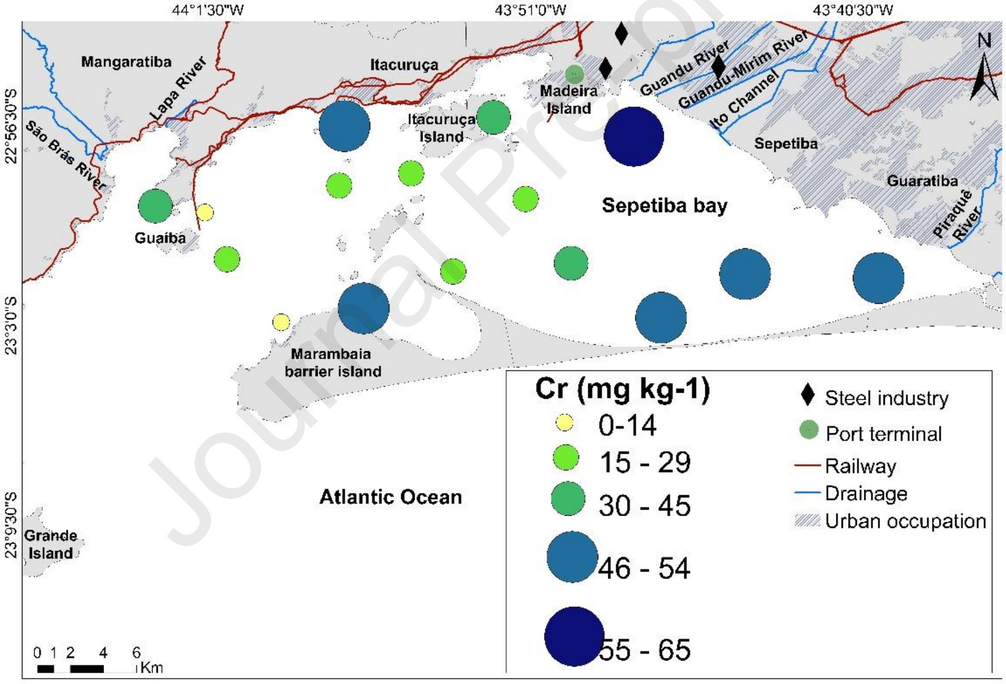
A total of 122 morphospecies (M) were identified (Appendix 3). The S (M) values varied between 4-52 (mean 18.19±16.21); J' (M) ranged between 0.19 and 0.89 (mean 0.55±0.24) and H' (M) diversity between 0.27-3.45 (mean 1.56±1.14). The lowest values of H' (M) were recorded in the inner and marginal areas of the bay, whereas the highest ones were found in the outer sector of the SB mostly in the same areas of H' (G) (Fig. 4 A, B). The Exp(H'_{bc}) (M) ranged from 1.4 to 110; the higher values of Exp(H'_{bc}) (M) were found in the outer area of the SB, as Exp(H'_{bc}) (G) (Fig. 5 A, B).

The AMBI and specifically Foram-AMBI can be applied when at least 50% of the taxa are assigned at each station. The assigned taxa to Ecological Groups in this study were on average 79%. The Foram-AMBI, FSI, and AEI ranges were 2.2-4.5, 1.1-5.9 and 64-100, respectively (Fig. 5 and Table 2). The AEI values (range: 64-100; mean 94±10) revealed the great dominance of *Ammonia* spp. over *Elphidium* spp. (Table 2). The highest Foram-AMBI and lowest FSI values were observed in the inner zone of the SB (Fig. 5 C, D).

The most abundant (i.e., > 5%) and most frequent (> 20% of the studied sites) taxa were: *Ammonia tepida* (Fig. 4 C), *Elphidium excavatum, Bolivina striatula* (Fig. 4 D)*, Buliminella elegantissima* (Fig. 4 E), *Trochammina hadai, Ammonia buzasi, Pararotalia sarmientoi, Nonionella auris, Bolivina ordinaria, Cancris auricula, Quinqueloculina bosciana, Rosalina globularis, Rosalina williamsoni* (Fig. 4F) and *Quinqueloculina seminulum* (Figure S1). *Ammonia tepida* dominated in more than 55% of the studied stations and reached higher relative abundances in the inner zone of the SB (Fig. 4 C and Figure S1). A similar distribution was found for *E. excavatum* and *A. buzasi*. Most of the other species, including *B. striatula, B. elegantissima* and *R. williamsoni*, were more frequent and reached higher relative abundances in the outer and central area of the SB (Fig. 4 C-F).


44°1'30"W
43°51'0"W
43°40'30"W
22°56'30"S
23°3'0"S
23°9'30"S
N
Mangaratiba
Lapa River
São Brás River
Itacuruça
Itacuruça Island
Madeira Island
Guandu River
Guandu-Mirim River
Ito Channel
Sepetiba
Guaratiba
Piraquê River
Sepetiba bay
Guaíba
Marambaia barrier island
Atlantic Ocean
Grande Island
0 1 2 4 6 Km
Cd (mg kg-1)
0.03 - 0.06
0.07 - 0.22
0.23 - 0.82
0.83 - 1.37
1.38 - 1.99
Steel industry
Port terminal
Railway
Drainage
Urban occupation

A


44°1'30"W
43°51'0"W
43°40'30"W
22°56'30"S
23°3'0"S
23°9'30"S
N
Mangaratiba
Lapa River
São Brás River
Itacuruça
Itacuruça Island
Madeira Island
Guandu River
Guandu-Mirim River
Ito Channel
Sepetiba
Guaratiba
Piraquê River
Sepetiba bay
Guaíba
Marambaia barrier island
Atlantic Ocean
Grande Island
0 1 2 4 6 Km
Cr (mg kg-1)
0-14
15 - 29
30 - 45
46 - 54
55 - 65
Steel industry
Port terminal
Railway
Drainage
Urban occupation

B

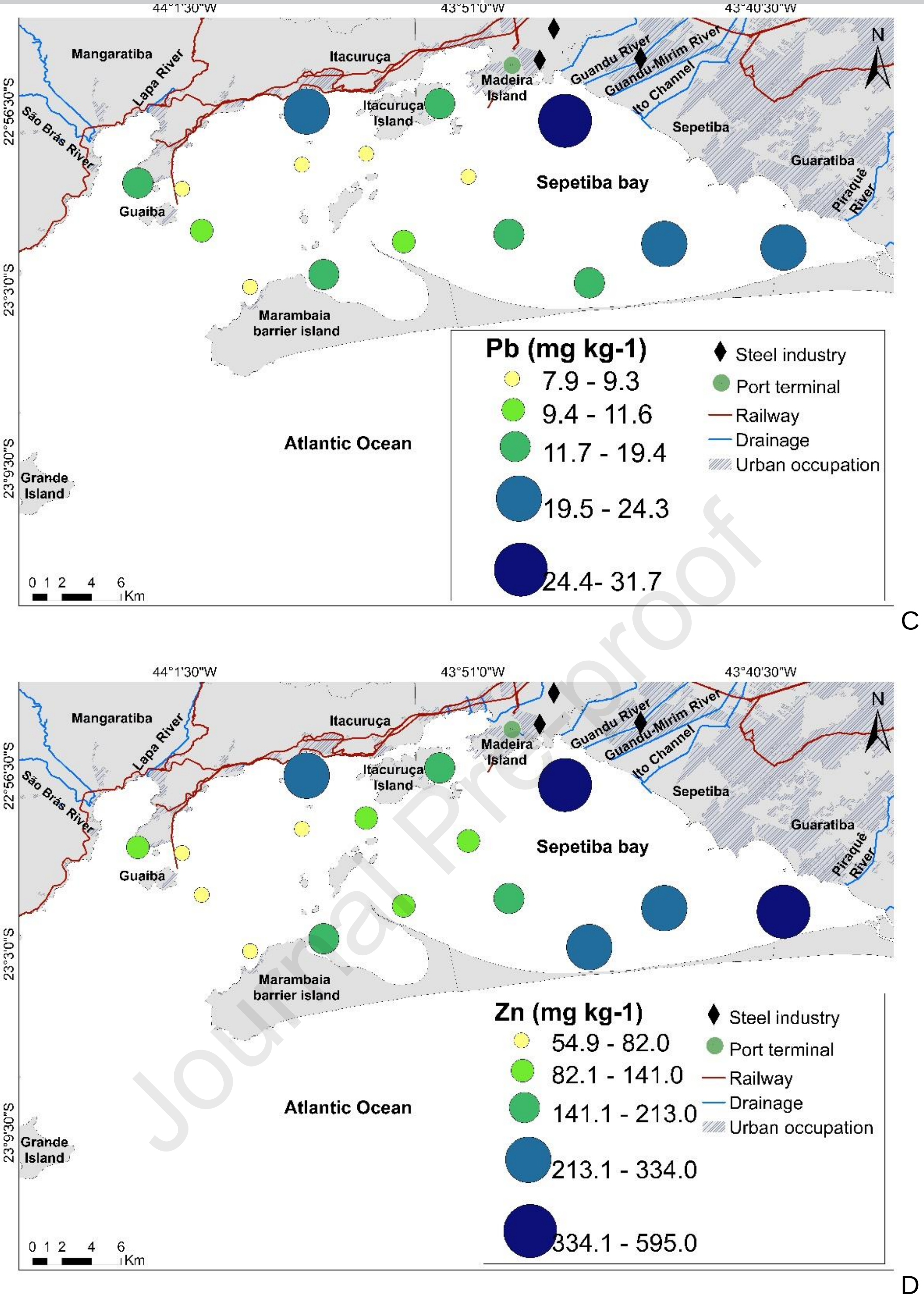


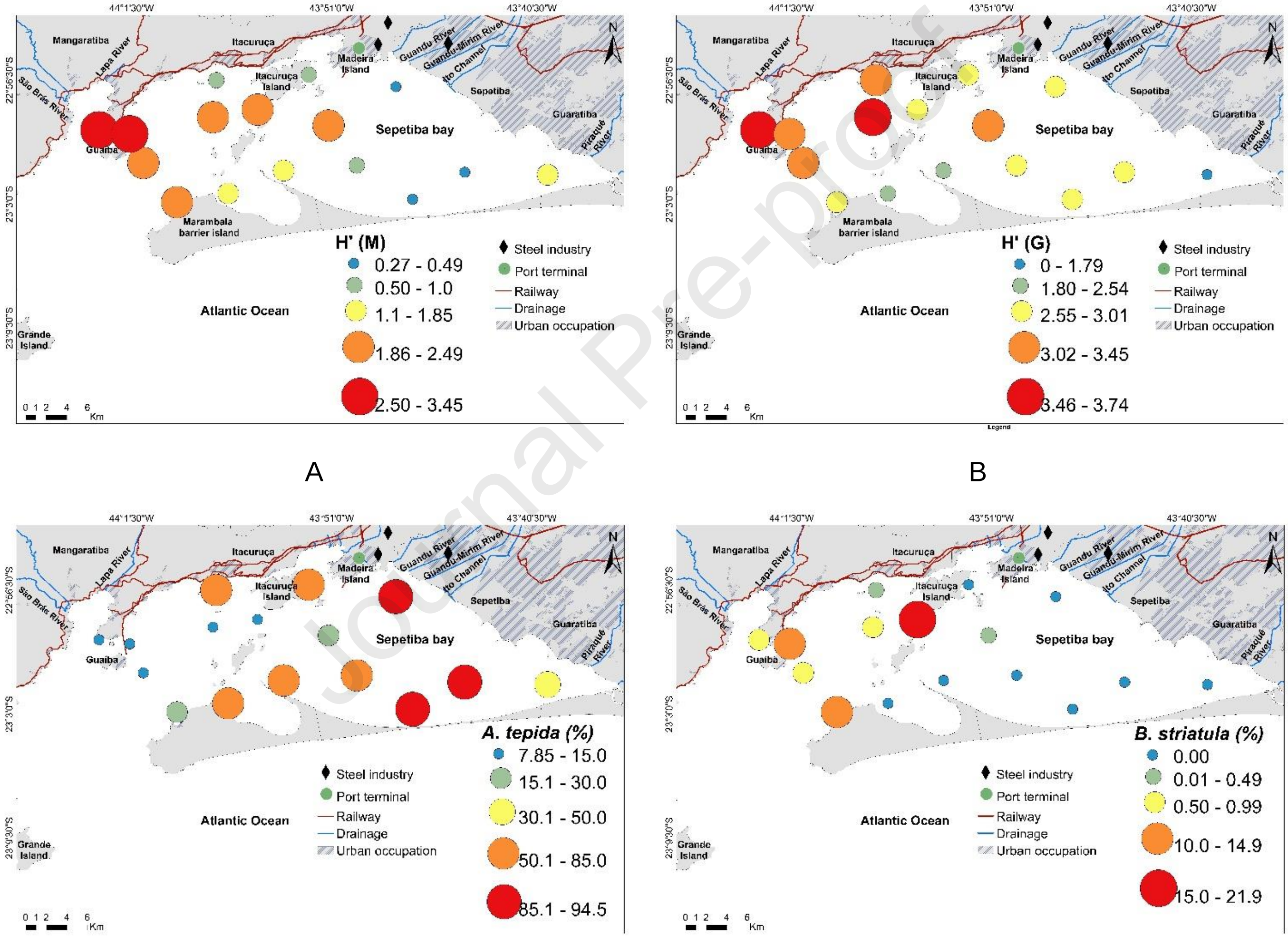
Figure 3. Distribution maps of A. Cd, B. Cr, C. Pb and D. Zn concentrations (mg kg^{-1}) in the studied stations of the Sepetiba Bay.

4.4 Molecular community

The total number of high-quality sequences (reads) of foraminiferal metabarcodes was 981,532. The raw data is available from the Sequence Read Archive public database under the accession number: PRJNA1043870. On overall, 398 ASVs (G) were identified through metabarcoding analyses (Appendix 4), of which 44 ASVs were assigned (11%) that corresponded to 85.31% of reads given the dominance of ASV1 (Saccaminidae) representing 79% of the reads.

The S (G) values varied between 28-97 (mean 57.44±21.52), J' (G) ranged between 0.50-0.84 (mean 0.74±0.09), and H' (G) between 1.80-3.73 (mean 2.98±0.50). The lowest H' (G) values were found in the inner and marginal areas of the SB, whereas the highest H' (G) values were associated with stations in the outer sector of the bay (Fig. 4 B). This diversity trend well corresponds with that identified for the morphological community (Fig. 4 A, B). However, the overall diversity of the molecular dataset is much higher as it includes soft-shelled taxa. The Exp(H'$_{bc}$) (G) species varied between 6.0 and 41.8 (Fig. 5 B).

The most frequent assigned ASVs were Saccaminidae, *Ammonia buzasi*, *Cylindrogullmia* sp., *Ammonia tepida*, *Vellaria pellucida,* Monothalamea spp., Monothalamea X squat *Hauerina, Buliminella elegantissima*, *Bathysiphon* spp*., Micatuba flexilis, Bathysiphon* sp. (17735.2), *Epistominella* sp., Clade F X saccamminids tail.1d; cDNA, *Micrometula* sp., *Buliminella tenuata*, *Nemogullmia longevariabilis*, *Quinqueloculina* sp. and *Quinqueloculina* sp. (14651.1), and *Bolivina* spp. Most ASV species exhibited a scattered distribution and a low relative abundance.

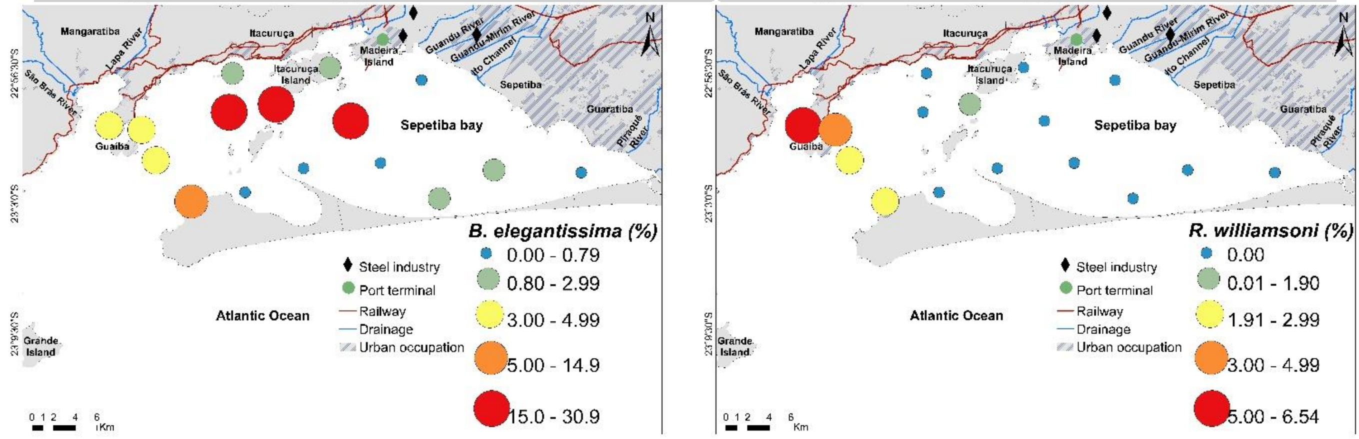


A

B

C

D



E F

Figure 4. Distribution maps of A. H' (M) (Shannon index based on morphospecies), B. H' (G) (Shannon index based on ASV species) and percentage of C. *Ammonia tepida,* D. *Bolivina striatula,* E. *Buliminella elegantissima,* and F. *Rosalina williamsoni* in the studied stations of the Sepetiba Bay.

Table 2. Values of PCA Factor 1 (Fig. 7) and the ecological indices used in this work to evaluate the EcoQs (G – genetical results, molecular community and; M – morphological community).

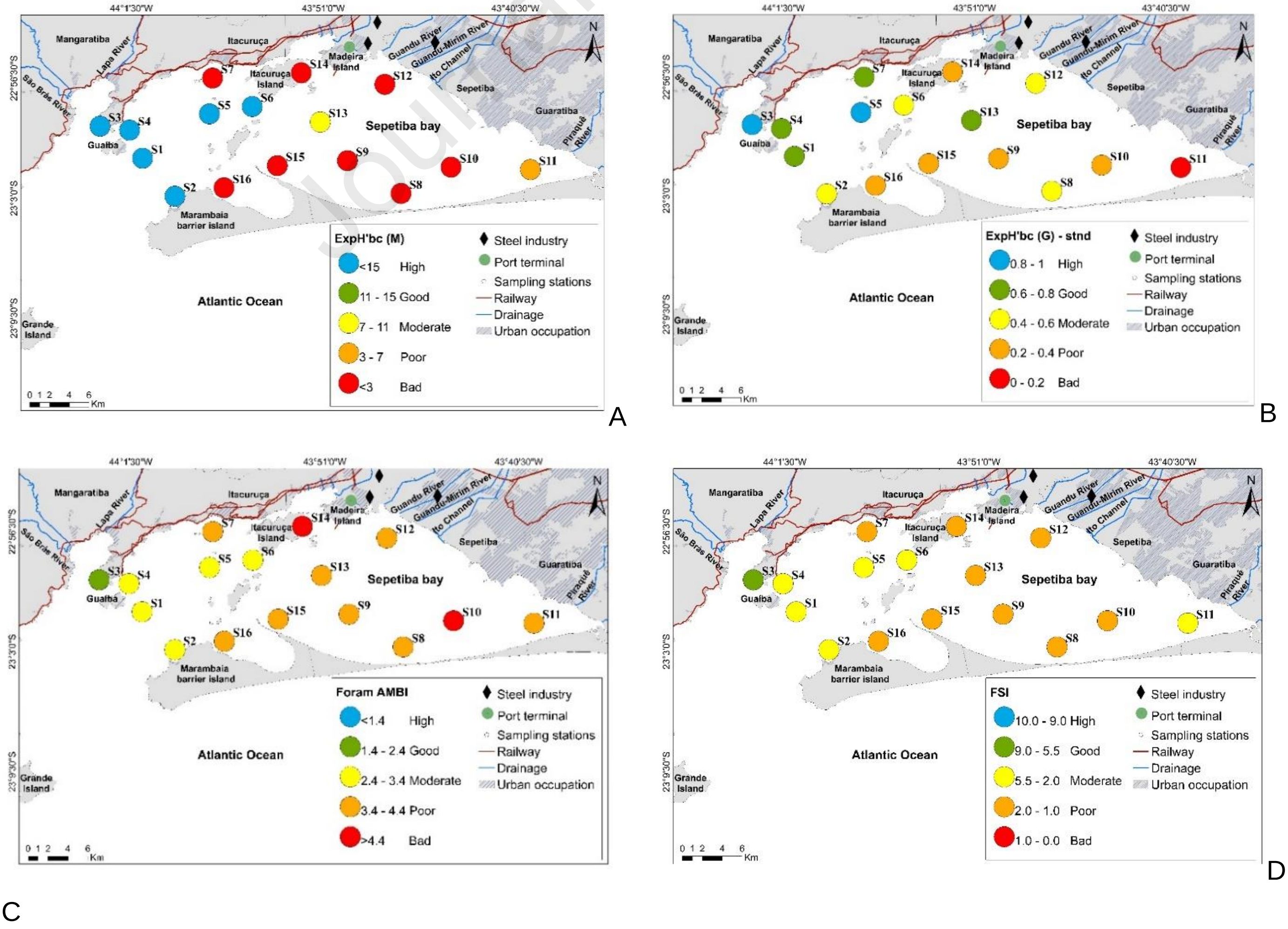
Stations	**PCA Score**	**ASVs (G)**	**Morphospecies (M)**				**Abiotic Parameters**	
	Factor 1	**ExpH'bc (G)**	**ExpH'bc (M)**	**Foram-AMBI**	**FSI**	**AEI**	**TOC**	**PERI**
S1	0.92539	28.4	26.0	3.0	3.7	100	0.92	177
S2	1.33803	19.8	26.0	3.1	2.7	100	0.25	156
S3	0.16418	39.0	63.8	2.2	5.9	92	1.01	234
S4	1.49536	31.6	110.4	2.6	4.8	74	0.19	131
S5	0.7939	41.8	37.2	3.0	2.1	100	0.51	182
S6	1.07898	20.3	25.1	3.2	2.0	100	0.22	186
S7	-0.95855	26.8	2.2	4.2	1.4	95	2.02	466
S8	-0.8045	19.1	1.8	4.3	1.4	98	2.13	516
S9	-0.35253	16.7	2.1	4.4	1.2	99	1.46	384
S10	-1.07264	15.5	1.4	4.5	1.1	100	2.31	597
S11	-1.2752	6.0	2.6	2.9	4.4	64	1.93	794
S12	-1.60085	17.8	1.7	4.2	1.6	93	1.64	831
S13	0.89499	28.2	8.0	3.7	1.7	100	0.45	204
S14	-0.36142	16.7	2.1	4.5	1.1	100	0.25	363

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
S16	-0.5194	10.3	2.3	4.4	1.2	98	2.11	331
Maximum		**41.8**	**110.4**	**4.5**	**5.9**	**100**	**2.31**	**831**
Minimum		**6.0**	**1.4**	**2.2**	**1.1**	**64**	**0.19**	**131**
Mean		**21.9**	**19.7**	**3.6**	**2.4**	**94**	**1.16**	**392**
Standard deviation		**10.0**	**30.0**	**0.8**	**1.5**	**10**	**0.79**	**222**

4.5 Ecological Quality Status

The EcoQS based on Exp(H'$_{bc}$) (M) and Exp(H'$_{bc}$) (G) varied between high and bad (Fig. 5 A, B). The EcoQS was worse in the inner part of the bay (north and south of the bay), whereas in the outer part of the bay and along the navigable channel the EcoQS was high to good. The EcoQS based on Foram-AMBI ranged from good to bad and exhibited a similar trend of the diversity-based indices (Fig. 5C). Similar to Foram AMBI, the FSI identified most of the stations with a bad-to-moderate EcoQS and only one station, in the outermost part of the SB as good conditions (Fig. 5 D).

The PERI revealed a lower (e.g., bad, poor, and moderate) EcoQS for all the stations except in S4 located in the outer part of the SB (Fig. 5 E). On the other hand, the EcoQS resulting from TOC exhibited relatively better (high and good) conditions for the stations along the navigable channels and at the bay entrance (Fig. 5 F).



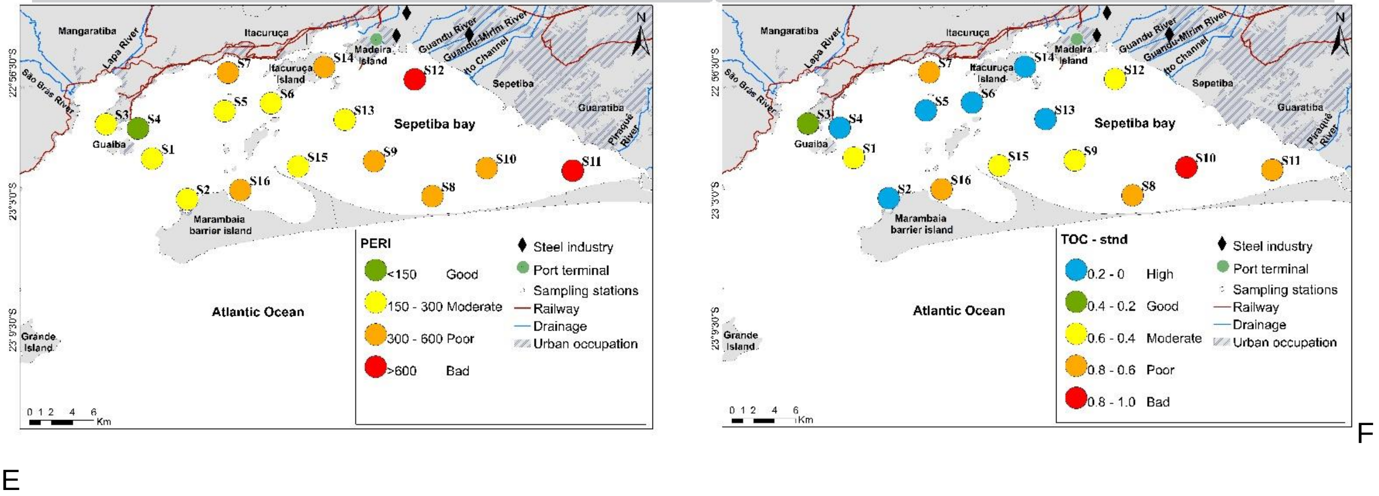


Figure 5. Distribution maps of ecological and abiotic indices' values used to classify the EcoQs: A. Exp(H'_{bc}) (M), B. Exp(H'_{bc}) (G), C. Foram-AMBI, D. FSI, E. PERI and F. TOC stnd (standardized). The classification scale was defined according to the criteria reported in Table 1.

A total of seven stations out of 16 (43.8%) showed a perfect agreement in the evaluation of the EcoQS when six indices were considered in the SB (Fig. 6) and a partial agreement in 3 stations (18.8%). Specifically, in the inner part of the SB, the EcoQS fully agreed (except for S13 and S14). The highest disagreement was found in the outer part of the SB and along the navigable channel, where molecular and morphological diversity showed acceptable conditions, but the Foram-AMBI, FSI, and PERI suggested mostly unacceptable ones (Fig. 5)

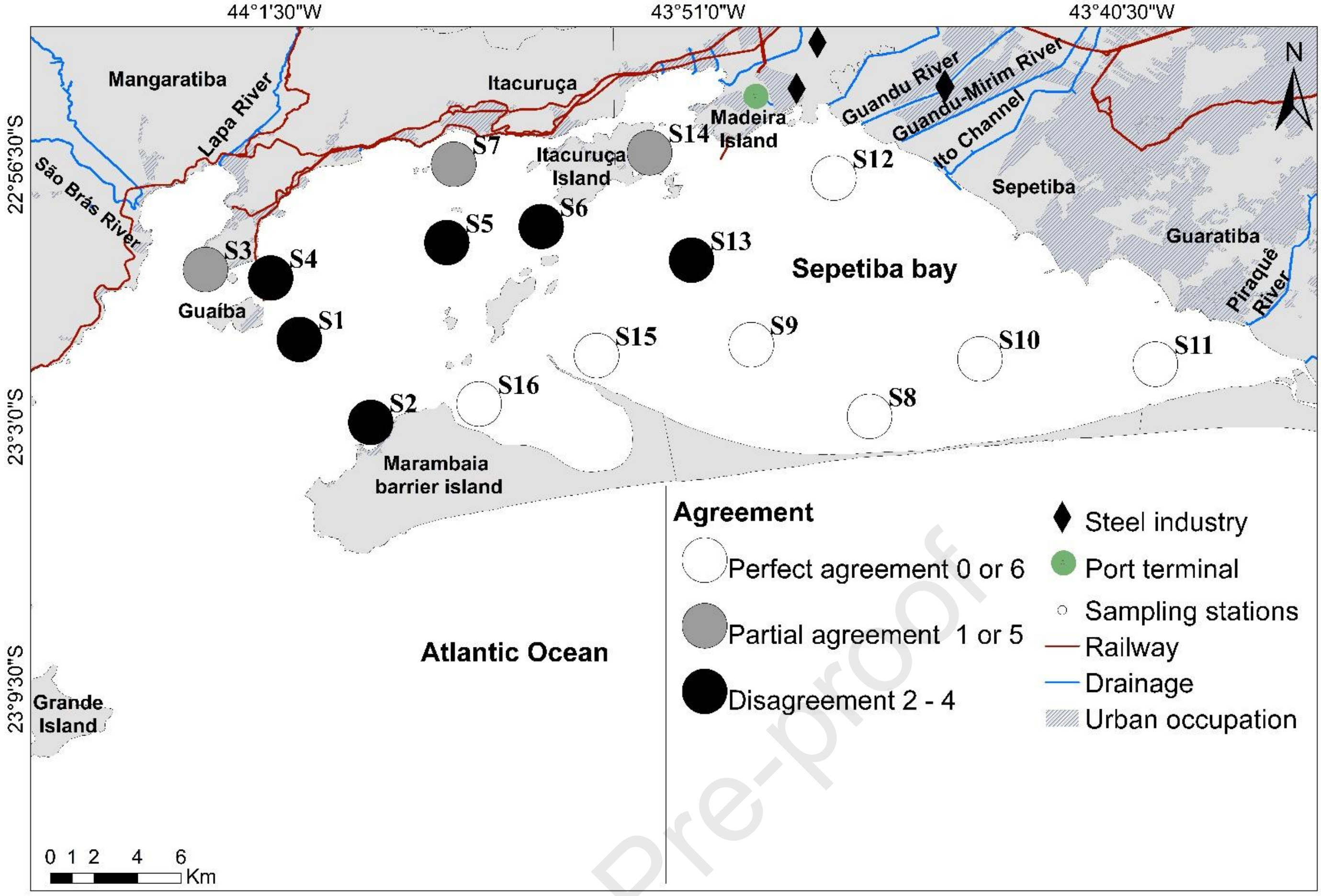


Figure 6. Agreement between the classification based on the ecological (ExpH'bc (G), ExpH'bc (M), Foram-AMBI, FSI, and AEI) and abiotic (TOC and PERI) indices used to estimate the Ecological Quality Status (EcoQS) according to the criteria of Table 1 and the values of Appendix 7.

4.6 Statistical results

The first two PCA factors (Factor 1: 71.74 %; Factor 2: 9.99 %) explained most of the data variability (81.73 %). The factor loading values of the first two PCA factors (listed in Appendix 5) and the factor score of each variable represented in the biplots of Figure 7A-D allowed us to infer that Factor 1 represents the environmental stress (ES) as it was strongly related to TOC, PLI, and PTEs, whereas Factor 2 was mainly associated with salinity and represented, therefore, the confinement gradient. Higher positive score values of Factor 1 (positive values of the PCA) were related to healthier environmental conditions, while negative ones represent worse environmental conditions (negative values of the PCA) and higher ES (Fig. 7 A-D).

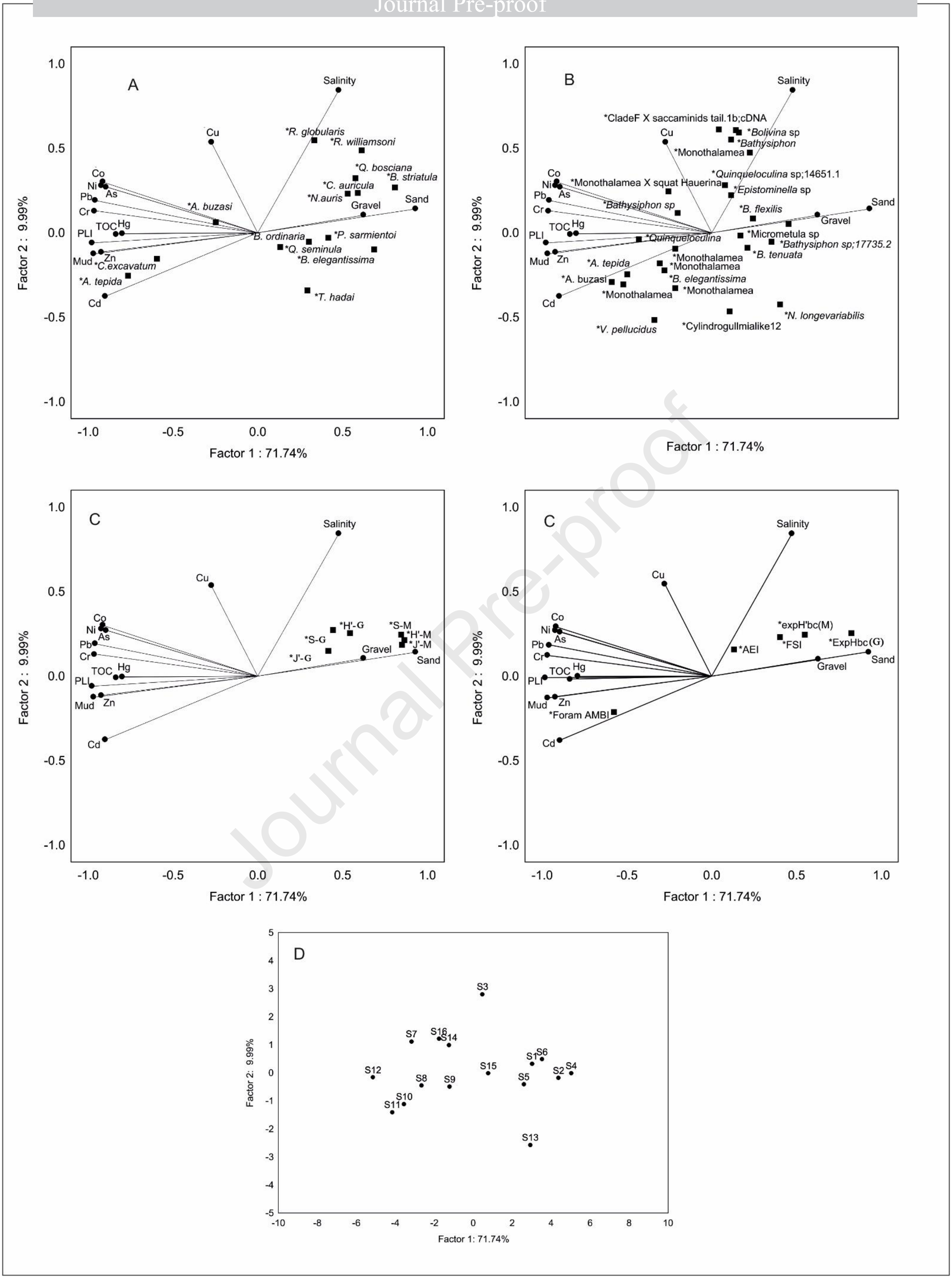


Figure 7. Biplots of the first two PCA factors (explaining 81.73% of the data variability) based on selected biotic and abiotic variables. The primary variables were the abiotic parameters (such as gravel, sand and mud, as well as TOC, salinity and PLI values) and as secondary variables were the biotic variables: A.

Percentages of the main morphospecies; B. Percentages of the main ASVs; C. Biotic indices: number of species per sample (S), Shannon diversity (H'), equitability (J') of the morphospecies (M) and ASV species (G); D. Ecological indices used to evaluate the EcoQS: Foram-AMBI, FSI, Exp(H'_{bc}) based on morphological (M) and molecular (G) analyses; E. plot of stations were related to the scores of the first two PCA factors.

Most of the frequent morphospecies (such as *B. elegantissima, B. striatula, T. hadai, P. sarmientoi, N. auris, B. ordinaria, C. auricula, Q. bosciana, R. globularis, Q. seminulum* and *R. williamsoni*) were associated with coarser sediments, lower TOC and PTE contents and partly to more saline waters (Fig. 7A). On the other hand, *A. tepida, A. buzasi* and *E. excavatum* were primarily related to high ES (Fig. 7B).

The Spearman Rank Order correlations between species abundance and the score values of Factor 1 (i.e., environmental stress or ES) showed that *B. elegantissima, B. striatula, N. auris, Q. bosciana, C. auricula* and *R. williamsoni* are significantly and positively correlated with the Factor 1. An opposite trend was found for *A. tepida* and *E. excavatum* (Appendix 5).

Similar to the morphological data, the PCA (Fig. 7B) and the Spearman Rank Order correlations on the molecular community showed that only monothalamids - Mono X 7742 was positively related to Factor 1 (negatively to ES), whereas negative relations with this factor were found for *A. buzasi, B. elegantissima* and *Monothalamea* spp. (Appendix 5). Although not significant, it is also worth mentioning the negative correlations of *Quinqueloculina* sp. and *Ammonia tepida* with Factor 1 and the positive ones of *Bolivina* sp., *Bathysiphon* sp. (17735.2) and *Nemogullmia longevariabilis* with Factor 1 (Appendix 5).

The PCA biplot revealed that the S, H' and J', as well as FSI and Exp(H'_{bc}) of morphological and molecular communities, were negatively related to the ES gradient, whereas an opposite trend was found for Foram-AMBI (Fig. 7 C, D). Some stations (S4, S2, and S6) were negatively related to the ES gradient, while others (i.e., S11, S12, S10) were positively related to it (Fig. 7E).

The ecological indices of the morphological (i.e., Foram-AMBI, FSI, and Exp(H'_{bc}), and molecular (Exp(H'_{bc})) community were all significantly correlated with the ES (Appendix 6). These results indicated that these indices respond to the environmental impact, corroborating the results of the PCA. The AEI had also a positive correlation with ES, although not significant (Appendix 6).

5. Discussion

5.1 Environmental parameters and pollution indices

The samples were collected in spring; during this season the sedimentary environment in the study area was characterized by relatively high temperatures (24.56±1.09ºC), and low salinities (29.93±2.95) when compared to the data published by Kjerfve et al. (2021) for May. Lower salinity values are mostly observed in the inner area of the SB under the influence of the river's outflow (Fig. 2A). The highest salinities are instead associated with the outer sector, though it can vary depending on the tidal phase and the rainfall and river runoff. According to rainfall data from the National Institute of Meteorology-INMET station A602 (Rio de Janeiro - Marambaia), about 388 mm of atmospheric precipitation (daily average 9.25 mm) was recorded from 01/04/2022 to 12/05/2022. These data reveal the high freshwater input in the SB, near and during the sampling period, and explain the low salinities in the inner part of the bay (Fig. 2A).

The sediment pH values are alkaline at all stations (mean 8.10±0.09). The lowest pH values slightly below 8.0 are recorded at the stations: S12 (7.94) near the Guandu, Guandu-Mirim, and Ito River mouths, S11 (7.95) near the Piraquê River mouth, S3 (7.98), in the region between the continent and Guaiba Island. The influence of continental waters and human activities may have contributed to the slight decrease in pH at these stations. pH does not show significant correlations with the biotic variables and most of the abiotic parameters except with very fine sand fraction and Eh values, with which it has negative correlations.

The sediment Eh values are negative at all stations, revealing low oxygenated conditions within the sediment. Additionally, Eh does not show significant correlations with particle size data or TOC contents (Appendix 6). Considering that the variability of Eh values is reduced (average -60.33±4.64 mV), the heterogeneity of particle sizes is significant (from sandy-gravel sediments to muds), and TOC contents are <2.31 %, it can be deduced that the oxygen consumption by living organisms is significant either at fine or sandy bottom sediments. Studies conducted in the east-southeast sector of the bay, near the Piraquê River, revealed dissolved oxygen values below the level (2.02 mg dm^{-3} DO) recommended in Brazilian legislation, indicating oxygen deficiency in the region (Alves Neto et al., 2014).

The positive correlations between TOC, mud fraction, and PTE concentrations (except Cu; Appendix 6) indicate that there is a strong influence of hydrodynamics on metals and organic matter accumulation since calmer areas allow the deposition of muddy sediments enriched in organic matter and PTEs (Carreira et al., 2009; Alves Neto et al., 2014; Carvalho et al., 2020). Based on the EF values and the classification criteria of Sutherland (2000), moderate enrichment of Cd, Sn, Zn, Cu, Cr, and Co was found at some sites such as: S8-S12 for Cd and for Zn (except S9 for Cd); S13 for Co and Cr; S4 for Sn and; S2 and S6 for Cu. The highest enrichment factors for Cd, Zn, Cr, and Co (EF between 2.1-3.4) are observed in the inner zone of the SB, while those of Cu (2.3-2.5) were found at outer stations, located near Marambaia Barrier Island. In contrast, the highest EF value for Sn (3.0) was found near Guaíba Island Terminal.

According to the Igeo values and following the classification of Müller (1986), some sites are: moderately polluted (Igeo: 1-2) by Cu and Hg, moderately to strongly polluted (Igeo: 2-3) by Cr, Co, Pb and Ni and, strongly polluted (Igeo: 3-4) by Sn, Cd, and Zn. The PLI values (Fig. 2 C; Appendix 1) suggest that in most of the studied sites, there is degradation caused by metals (PLI>1). The distribution patterns of PLI and TOC values suggest that environmental degradation is high in the inner sector and close to the margins of the SB (Fig. 2 C, D).

Metal pollution in the SB has been documented to affect living organisms. Relatively high concentrations were identified, for instance: by Cd and Zn in oyster tissues (Lacerda and Molisani, 2006), in shrimp *Litopenaeus schmitti* (Nascimento et al., 2017), in brown algae species, *Padina gymnospora* and *Sargassum stenophyllum* (Amado Filho et al., 1999) and in *Avicenna schaueriana* a mangrove species (Langenbach et al., 2022); by Zn, Cd, Ni, Pb, Cu, and Cr, in kidney and liver tissues of *Egretta thula* (a seabird species) (Ferreira, 2011); by As, Cu, Zn, and Pb in fishes, such as *Cathorops spixii, Genidens genidens* and *Trinectes paulistanus* (Kütter et al., 2021).

The risk of contamination of the species is expressed in the PERI values which show, following the criteria of Håkanson (1980) and Swarnalatha et al. (2013), moderate, high, and very high ecological risk in 50%, 37.5%, and 12.5% of the stations, respectively (Appendix 1). The highest PERI values (>600) indicate that the sites with very high potential ecological risk are at stations S12 and S11, located close to the Guandu and Piraque river mouths, respectively (Fig. 5E); this risk is mainly associated with Cd, Sn, Zn, Cr, Co, Pb, Ni, Hg, As and Cu pollution (according to the Igeo values; Appendix 2). PERI values between 300-600 are reached at stations S10>S8>S7>S9>S14>S16 (Fig. 5E) that denote high potential ecological risk caused mainly by Cd, Sn, Ni, Zn, Cr, Co and Pb (according to Igeo values; Appendix 2). A moderate potential ecological risk is found at stations S15>S3>S1>S6>S5>S1>S2 caused by Sn, Co, Ni, Cr, Pb, Cd and Zn pollution (Fig. 5E). Station S4 has the lowest potential ecological risk, although Sn pollutes it moderately; it should also be noted that this station has the highest EF-Sn value (Fig. 5E).

Previous studies in the region have shown that areas near the Guandu River and Madeira Island are the most affected by heavy metal contamination, such as Cu, Cr, Cd, Zn, Mn, and Pb (Lacerda et al., 1987). This work shows that Sn, Co, Ni can also become a concern. The dumping of municipal effluents is the main factor of contamination in the region's water bodies, particularly in the Guandu River, which is responsible for discharging a large load of metals (Mn > Zn > Cr > Pb > Cu > Cd) into the SB (Lacerda et al., 1987). In addition, high negative impacts have occurred between the installation of the Companhia Siderúrgica Mercantil Ingá in 1962 and its closure in 1998 (Veríssimo and Moura, 2021) which have made the inner zone of the SB the most degraded (Moreira et al., 2023). Other causes of this impact are the pollutants received in the SB from urban, port and industrial activities and from the tourism sector in the Mangaratiba region with mega hotel enterprises (Carvalho et al., 2021; Moreira et al., 2023).

5.2 Foraminiferal communities

The most frequent morphospecies were *A. tepida, E. excavatum, B. elegantissima, B. striatula, T. hadai* and *A. buzasi*. All these morphospecies have been identified in other coastal regions of Brazil (Duleba et al., 2019; Oliveira et al., 2022; Nunes et al., 2023; Filippos et al., 2023), except *A. buzasi*. *Ammonia buzasi* was described for the first time by Hayward et al. (2021) in the Caribbean Sea (Cuba).

Trochammina hadai morphospecies occurs at 31.3% of the stations. This species has been recently considered invasive in the Flamengo Inlet (Ubatuba, São Paulo State, SE Brazil) by Eichler et al. (2018) and in other parts of the world (e.g., Mcgann and Sloan, 1966, 1999; Mcgann et al., 2000; Pavard et al., 2023). This taxon is positively correlated with fine and very fine sand and relatively high Eh values. *Trochammina hadai* shows a negative (but not significant) correlation with the ES, which may reflect a preference for less-impacted coastal environments. *Ammonia tepida* and *E. excavatum*, the most abundant morphospecies at the studied stations, show significant positive correlations with fine sediment fractions, TOC, and PTEs (i.e., As, Cd, Co, Cr, Ni, Pb, Sn, and Zn). *Ammonia tepida* also correlates positively with Hg. Both taxa show positive correlations with the ES, suggesting their higher tolerance. This ecological behavior has been also reported in several regions, for example, Brazilian coastal areas (e.g. Belart et al., 2018; Alves Martins et al. 2020; Filippos et al., 2023; Nunes et al., 2023).

Other species, such as *B. elegantissima, B. striatula, T. hadai, A. buzasi, P. sarmientoi, N. auris, B. ordinaria, C. auricula, Q. bosciana, R. globularis, Q. seminulum* and *R. williamsoni*, are, in general, negatively related to mud, TOC and PTEs. So, their relative abundance increases in saltier waters and sandier and less impacted sediments by organic matter and metals. *Buliminella elegantissima, B. striatula, N. auris, Q. bosciana C. auricula,* and *R. williamsoni* have significant negative correlations with ES, revealing a clear preference for less impacted environments. These results suggest that these species prefer less impacted environments with a greater oceanic influence. The pre-industrial communities analyzed by Castelo et al. (2021a), in which most of these species were found, generally show similar characteristics.

Based on the PCA outcome, it is revealed that species richness, equitability, and Shannon diversity, based on the morphological analysis, decline in the most impacted areas, such as in stations S10, S11, and S12, located in the inner region of the SB, close to the continent. These results highlight the negative response of the morphological community to adverse environmental conditions.

Of the total number of identified ASVs (398), only 44 ASVs were assigned. These results suggest that the genetic sequences of most of the foraminiferal species in the study area, but also in the South Atlantic, are yet unknown. A similar situation has also been observed by Rodrigues et al. (2021) in the Ubatuba region (São Paulo State, SE Brazil), where the species are also genetically different from those assigned in other regions, such as in the Mediterranean area (e.g., Cavaliere et al., 2021).

Higher taxa (i.e., ASVs) were identified through the molecular approach rather than through morphological analysis. The molecular community shows that the studied stations have a much higher S (range 28-97; mean 57.44±21.52) than that recorded by morphological analysis (range 4-52; mean 18.19±16.21). The minimum and mean J' values provided by molecular analysis are also higher (range 0.50-0.84; mean 0.74±0.09) than those registered by morphological analysis (range 0.19-0.89; mean 0.55±0.24). The range of variation of H' is smaller for ASV species (0.27-3.45), but the mean value is higher (2.98±0.50) than that found for the morphological dataset (1.56±1.14). These variations may be related to differences in the analytical methods. It should be noted that the molecular analyses consider the eDNA content in the total sediment, while the morphological analyses were performed on the dry sediment fraction >125 µm. This means that the morphological analysis disregards the early stages of the species' development and smaller specimens and the organic and soft-walled species, as well as small-sized species (such as *Bolivina, Stainforthia, Epistominella, Rosalina, Discorbis, Neoconorbina*) dominant in fraction 63-125 µm, as well as chain-like fragile species such as *Hormosinella* and *Reophax* when wet picking is not done. When the sediment was dried, the foraminiferal specimens with organic protections and soft agglutinated tests were mostly destroyed as well as resting propagules, which may never develop from the sediment propagule banks but are picked up by eDNA (see for instance the presence of shallow water *Ammonia* in the deep fjord basin; Brinkmann et al., 2023). It is also worth mentioning that in the morphological analysis, only living specimens have been considered with dimensions >125 µm, whereas the eDNA comprises intra- and extra-organismal DNA from living and dead organisms (Greco et al., 2022).

The most abundant species in most stations is the Saccaminidae 2399-AJ307756, which reaches up to 98.86%. The dominance of a coastal foraminiferal association by monothalamids has been reported in other areas such as in Kuwait Bay (Arabian Gulf) (Al-Enezi et al., 2022) and in the coastal region of Ubatuba (South Atlantic, SE Brazil; Rodrigues et al., 2021). This work reveals that little is known about the current distribution and ecology of monothalamids and identifies a large gap in the entire Atlantic Ocean, as also observed by Rodrigues et al. (2021).

The PCA results show that the response of the ASVs to the abiotic parameters is quite heterogeneous. However, it is possible to observe that *A. buzasi*, *B. elegantissima*, and two monothalamid species display significant positive correlations with ES. It should also be noted that *A. tepida*, *Quinqueloculina* sp. and another monothalamid species also have positive but not significant correlations with ES. These data suggest that these ASVs are tolerant or indifferent to increased ES. On the other hand, *Bolivina* sp., *Bathysiphon* sp. (17735.2) and *Nemogullmia longevariabilis*, which show negative correlations with ES, can be considered more sensitive to environmental stress. Curiously, *A. buzasi* and *A. tepida* are recorded in both the morphological dataset and in the molecular one and consistently exhibit the same behavior, being both positively related to the ES gradient.

The PCA results, which evidence the significant negative correlation of H'-G with ES, suggest that the diversity of the molecular community declines in the most impacted areas (stations S10, S11, and S12; Fig. 7C). Therefore, the biotic indices of the molecular and morphological communities provide the same indication: the stations located in the inner region of the SB, close to the continent, with low diversity and equitability, are the most impacted and those with the most stressful environmental conditions for benthic foraminifera. In these areas, pioneer assemblages of foraminifera can be found, including mostly species capable of surviving in disturbed sedimentary environments, such as *A. tepida, E. excavatum* and *A. buzasi* (PCA Fig. 7A, B).

5.3 Molecular and morphological indices: comparison of their performance in EcoQS assessment

The ecological indices, such as Exp(H’$_{bc}$) (G), Exp(H’$_{bc}$) (M), Foram-AMBI, and FSI, and geochemical ones, such as TOC and PERI, have been used to estimate the EcoQS. The values and distribution maps of these biotic indices (Fig. 5 A-F) reveal a clear difference in the EcoQS within the study area. It is also clear that molecular index overestimates EcoQS due to a much higher diversity (dormant propagules + monothalamids), as compared to morphospecies, which based on our data follow pollution trends better.

It also should be noted that the Foram-AMBI and FSI were based on the ecological behavior of species from European coastal regions (North Atlantic), according to Bouchet et al. (2021). Although significant results have been reached in the SB, the method needs to be improved based on a better knowledge of the ecological requirements of foraminiferal species from the South Atlantic coastal regions.

Based on the EcoQS based on six biotic [(Exp(H’$_{bc}$) (G), Exp(H’$_{bc}$) (M), Foram-AMBI and FSI] and geochemical (TOC and PERI) indices and it is possible to observe an overall agreement for 62.5% of the stations (perfect agreement 44% and partial agreement 19%) and disagreement for 37.5% of the stations (Fig. 6; Table 3; Appendix 7).

Table 3: I. Classification of biotic and abiotic indices used to estimate Ecological Quality Status (ECoQ) according to the criteria presented in Table 1 (see also Appendix 7). II. The agreement between the six indexes was also estimated.

Stations	**I. Ecological Quality Status**						**II.**
	ExpH'$_{bc}$ (G) - stnd	**ExpH'$_{bc}$ (M)**	**Foram AMBI**	**FSI**	**TOC - stnd**	**PERI**	**Agreement**
S1	1.00	1.00	0.00	0.00	0.00	0.00	2.0
S2	0.00	1.00	0.00	0.00	1.00	0.00	2.0
S3	1.00	1.00	1.00	1.00	1.00	0.00	5.0
S4	1.00	1.00	0.00	0.00	1.00	1.00	4.0
S5	1.00	1.00	0.00	0.00	1.00	0.00	3.0

S6	0.00	1.00	0.00	0.00	1.00	0.00	2.0
S7	1.00	0.00	0.00	0.00	0.00	0.00	1.0
S8	0.00	0.00	0.00	0.00	0.00	0.00	0.0
S9	0.00	0.00	0.00	0.00	0.00	0.00	0.0
S10	0.00	0.00	0.00	0.00	0.00	0.00	0.0
S11	0.00	0.00	0.00	0.00	0.00	0.00	0.0
S12	0.00	0.00	0.00	0.00	0.00	0.00	0.0
S13	1.00	0.00	0.00	0.00	1.00	0.00	2.0
S14	0.00	0.00	0.00	0.00	1.00	0.00	1.0
S15	0.00	0.00	0.00	0.00	0.00	0.00	0.0
S16	0.00	0.00	0.00	0.00	0.00	0.00	0.0

I. Ecological Quality Status Classes				
High	**Good**	**Moderate**	**Poor**	**Bad**

II. Agreement Classification	Stations Nº	%
Perfect agreement 0 or 6	7	43.8
Partial agreement 1 or 5	3	18.8
Disagreement 2-4	6	37.5

The diversity indices [(Exp(H'$_{bc}$) (G) and Exp(H'$_{bc}$) (M)] applied to estimate the EcoQS have significant negative correlations with TOC and PERI, as well as with the ES based on the extracted factor score of the PCA Factor 1. These indices also reveal a reduction of diversity for both the morphological and molecular communities in response to the ES caused by an increase in organic matter and PTEs. A negative effect on biota caused by environmental stress is also observed for FSI and Foram-AMBI, with a negative and positive relation with this factor, respectively. These trends further support the response of the foraminiferal communities in terms of diversity and composition to the ES. Accordingly, an increase in the relative abundance of opportunistic species is related to enhanced disturbance, as observed by Castelo et al. (2021a) in a core that records the turnover of foraminiferal communities in pre-industrialization and post-industrialization Holocene environments in the Sepetiba Bay.

In coastal and transitional waters, benthic foraminifers have several other stressors than the ones mentioned above, such as: the instability and mobility of the bottom sediment caused by active hydrodynamic and/or bioturbation processes; the excessive accumulation or erosion of sediments that bury the organisms or disturb the biotopes; the variability of physicochemical parameters (e.g., salinity, oxygen availability, temperature, pH); the excessive availability or scarcity of nutrients and; biotic factors (predation competition, differentiated reproduction rates, distinct reproductive periods). The AEI has also a negative correlation with ES, although not significant. This could be ascribed to the reduced number of analyzed stations where both *Ammonia* spp. and *E. excavatum* co-occur, differentiated breeding periods of the species and low juvenile survival rate due to disturbances caused by the high frequency of rainfall and storms during the sampling period.

Table 4. Spearman Rank Order Correlations between the biotic indices estimated in this work to evaluate the EcoQs [$ExpH'_{bc}$ (G), $ExpH'_{bc}$ (M), Foram AMBI, FSI and AEI] and selected abiotic parameters (TOC, PERI) and environmental stress (ES - PCA Factor 1). The correlations marked in bold are significant ($p < 0.05$). Legend: (m – molecular community and M – morphological community), (ES) - environmental stress.

Correlations		Abiotic Parameters		
		TOC	PERI	ES
Molecular community	$ExpH'_{bc}$ (G)	**-0.52**	**-0.64**	**-0.58**
Morphological community	$ExpH'_{bc}$ (M)	**-0.67**	**-0.84**	**-0.78**
	Foram-AMBI	0.49	**0.56**	**0.50**
	FSI	-0.46	**-0.54**	**-0.50**
	AEI	-0.25	-0.29	-0.32

The correlation matrix shows that the biotic indices, namely $Exp(H'_{bc})$ (G), $Exp(H'_{bc})$ (M), Foram-AMBI and FSI (Fig. 5 A-D), clearly evidence the responses of the living foraminiferal community to a set of parameters (i.e., TOC, PERI, and ES) underlining the environmental impact, corroborating also the results of the PCA (Fig. 7D). Thus, it is possible to infer that the most impacted regions and stressful areas are located in the inner region of the SB, close to its North, South and East margins (Fig. 5A-F). It should be noted that urban centers, ports, and industrial areas are located along the North and Eastern margins of the SB; thus, it would be expected that they would be more impacted, as they receive higher PTEs and organic matter contents; however, the South region near the Marambaia barrier island is also highly contaminated despite being a protected and sparsely inhabited area. This indicates that the remobilization of pollutants caused by coastal dynamics (swell and currents in shallow areas) as well as probably dredging activities and disposal may contribute to reintroducing pollutants into the water column. Once resuspended, pollutants are dispersed by tidal currents and accumulate again in calm areas where they find barriers to their transit, for example, near the Marambaia barrier island.

The map of Figure 6 shows that the agreement among the six indices used to classify the EcoQS is higher in the inner region of the SB where most of the stations are characterized by unacceptable (i.e., moderate, poor, and bad) conditions. In contrast, a substantial disagreement has been identified at outer stations or those in correspondence with navigable channels. In the present study, the ~63% of agreement (sum of full and partial agreement) among the six indices can be considered fairly high and can be directly compared to Guanabara Bay (63% of agreement between two morphological indices, namely Foram AMBI and $Exp(H'_{bc})$) (Nunes et al., 2023), Kuwait Bay (71% of agreement between two diversity indices for morphological and molecular approaches) (Al-Enezi et al., 2022) and Bagnoli area (75% of agreement among seven indices for both morphological and molecular approaches) (Cavaliere et al., 2021).

The disagreement is mostly related to better EcoQS resulting from the foraminiferal diversity in both the molecular and morphological communities being strongly influenced by the occurrence of marine water. It is also worth mentioning that the species ecology from the most impacted TWs is now better known than those that tend to occupy environments under more significant marine influence. Therefore, the classification provided by the ecological indices is more accurate in the impacted regions. Thus, further studies should be conducted to understand better the ecology of species that occur mainly in biotopes of TWs under the greatest oceanic influence, as the species from Brazilian TWs are not the same as in Europe, it would also be important to know their ecology better.

6. Conclusion

The results of this work show that the studied stations in the SB are characterized by significant differences in physicochemical water parameters (e.g., salinity) and sediment characteristics (e.g., grain size, TOC and PTE contents). The Igeo values allowed us to identify heavily polluted areas by Sn, Cd, and Zn and moderately to heavily polluted by Cr, Co, Pb, and Ni. The PERI values indicate high to very high and moderate ecological risk in 50% and 44% of the stations, respectively. The molecular and morphological analyses reveal a congruent gradient of diversity for foraminiferal communities with a relatively high number of taxa at stations located in the outer part of the bay. The combination of six indices, based on both ecological ($\mathrm{Exp(H'_{bc})}$) (G), $\mathrm{Exp(H'_{bc})}$ (M), Foram AMBI and FSI and geochemical (TOC and PERI) indices, allows us to identify an inner area of the SB with a lower EcoQS mostly affected by organic matter and PTEs. Although differences were observed in the EcoQS classification at some stations, a relatively high level of agreement (i.e., 63%) between the six indices is recognized. These indices congruently identify the lowest EcoQS for the stations located in the innermost region of the SB close to its North, South, and East margins. The degree of agreement among biotic indices used to estimate the EcoQS can be improved in the forthcoming. The assignment of species based on their ecological response to ES has not yet been properly established for South Atlantic TWs due to the small number of studies based on living benthic foraminifera. However, the recent increase of foraminiferal works and the application of accurate methodologies will undoubtedly allow getting more information and better results for ecological indices, namely Foram-AMBI and FSI. This work represents the first study based on eDNA metabarcoding to evaluate the EcoQS based on foraminifera in the South Atlantic in comparison with morphological analysis of foraminifera. Our results also strongly support the great potential of eDNA metabarcoding as a standalone method for routine biomonitoring.

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References

Al-Enezi, E., Francescangeli, F., Balassi, E., Borderie, S., Al-Hazeem, S., Al-Salameen, F., Anwar, A.B., Pawlowski, J., Frontalini, F. 2022. Benthic foraminifera as proxies for the environmental quality assessment of the Kuwait Bay Kuwait, Arabian Gulf: Morphological and metabarcoding approaches. Science of The Total Environment, 833, 155093. https://doi.org/10.1016/j.scitotenv.2022.155093.

Almeida, M.P., Gaylarde, C., Pompermayer, F.C., Lima, L.S., Delgado, J.F., Scott, D., Neves, C.V., Khauê, K.S., Baptista Neto, J.A., Fonseca, E.M., 2023. The Complex Dynamics of Microplastic Migration through Different Aquatic Environments: Subsidies for a Better Understanding of Its Environmental Dispersion. Microplastics 2 (1), 62-77. https://doi.org/10.3390/microplastics2010005

Alve, E., Korsun, S., Schönfeld, J., Dijkstra, N., Golikova, E., Hess, C., Husum, K., Panieri, G., 2016. Foram-AMBI: A sensitivity index based on benthic foraminiferal faunas from North-East Atlantic and Arctic fjords, continental shelves and slopes. Marine Micropaleontology, 122, 1–12. https://doi.org/10.1016/j.marmicro.2015.11.001

Alves Martins, M.V., Hohenegger, J., Frontalini, F., Sequeira, C., Miranda, P., Rodrigues, M.A.C., Duleba, W., Laut, L., Rocha, F., 2019. Foraminifera check list and the main species distribution in the Aveiro Lagoon and Adjacent Continental Shelf Portugal. Journal of Sedimentary Environments, 4 (1), 1-52. https://doi.org/10.12957/jse.2019.39308

Alves Martins, M.V., Hohenegger, J., Martínez-Colón, M., Frontalini, F., Bergamashi, S., Laut, L., Belart, P., Mahiques, M., Pereira, E., Rene Rodrigues, R., Terroso, D., Miranda, P., Geraldes, M.C., Villena, H.H., Reis, T., Socorro, O.A.A., Sousa, S.H.M., Yamashita, C., Rocha, F., 2020. Ecological quality status of the NE sector of the Guanabara Bay (Brazil): a case of living benthic foraminiferal resilience. Marine Pollution Bulletin, 158, 111-449. https://doi.org/10.1016/j.marpolbul.2020.111449

Alves Neto, J.L., Barbosa, A.V.L., Medeiros, M.A., Casartelli, E.A., Guarino, A.W.S., Merçon, F., 2014. Transposition of cadmium and zinc from Sepetiba Bay, and its deposition in sediments of Cabuçu-Piraquê River Estuary Rio de Janeiro, Brazil. Revista Virtual de Química, 6, 1295-1315. https://doi.org/10.5935/1984-6835.20140085

Amado Filho, G.M., Andrade, L.R., Karez, C.S., Farina, M., Pfeiffer, W.C., 1999. Brown algae species as biomonitors of Zn and Cd at Sepetiba Bay, Rio de Janeiro, Brazil. Marine Environmental Research 48, 213–224.

Anyanwu, B.O., Ezejiofor, A.N., Igweze, Z.N., Orisakwe, O.E., 2018. Heavy metal mixture exposure and effects in developing nations: an update. Toxics. 6(4), 65. https://doi.org/10.3390/toxics6040065

Barrenechea Angeles, I.B., Romero-Martinez, M.L., Cavaliere, M., Varrella, S., Francescangeli, F. et al. 2023. Encapsulated in sediments: eDNA deciphers the ecosystem history of one of the most polluted European marine sites. Environment International, 172, 107738. https://doi.org/10.1016/j.envint.2023.107738.

Belart, P., Clemente, I., Raposo, D., Habib, R., Volino, E.K., Villar, A., Martins, M.V.A., Fontana, L.F., Lorini, M.L., Panigai, G., Frontalini, F., Figueiredo, M.S.L., Vasconcelos, S.C., Laut, L., 2018. Living and dead Foraminifera as bioindicators in Saquarema Lagoon System, Brazil. Latin American Journal of Aquatic Research, 46 (5), 1055-1072. http://dx.doi.org/10.3856/vol46-issue5-fulltext-18

Bervoets, L., Blust, R., 2003. Metal concentrations in water, sediment and gudgeon *Gobio gobio* from a pollution gradient: Relationship with fish condition factor. Environmental Pollution, 126:9–19, https://doi.org/10.1016/S0269-74910300173-8

Boltovskoy, E., Giussani, G., Watanabe, S., Wright, R., 1980. Atlas of Benthic Shelf Foraminifera of the Southwest Atlantic. The Hague, The Netherlands: Dr. W. Junk bv Publishers, 147p.

Borja, A., Dauer, D.M., 2008. Assessing the environmental quality status in estuarine and coastal systems: comparing methodologies and indices. Ecological Indicators, 8, 331–337. https://doi.org/10.1016/j.ecolind.2007.05.004

Borja, A., Franco, J., Pérez, V., 2000. A marine biotic index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. Marine Pollution Bulletin, 12, 1100–1114. https://doi.org/10.1016/S0025-326X(00)00061-8

Bouchet, V.M.P., Alve, E., Rygg, B., Telford, R.J., 2012. Benthic foraminifera provide a promising tool for ecological quality assessment of marine waters. Ecological Indicators, 23, 66–75. https://doi.org/10.1016/j.ecolind.2012.03.011

Bouchet, V.M.P., Frontalini, F., Francescangeli, F., Sauriau, P.-G., Geslin, E., Martins, M.V.A., Almogi-Labin, A., Avnaim-Katav, S., Di Bella, L., Cearreta, A., Coccioni, R., Costelloe, A., Dimiza, M.D., Ferraro, F., Haynert, K., Martínez-Colón, M., Melis, R., Schweizer, M., Triantaphyllou, M.V., Tsujimoto, A., Wilson, B., Armynot du Châtelet, E., 2021. Indicative value of benthic foraminifera for biomonitoring: assignment to ecological groups of sensitivity to total organic carbon of species from European intertidal areas and transitional waters. Marine Pollution Bulletin, 164, 112071. https://doi.org/10.1016/j.marpolbul.2021.112071

Bouchet, V.M.P., Goberville, E., Frontalini, F., 2018. Benthic foraminifera to assess Ecological Quality Statuses in Italian transitional waters. Ecological Indicators, 84, 130–139. https://doi.org/10.1016/j.ecolind.2017.07.055

Brinkmann, I., Schweizer, M., Singer, D., Quinchard, S., Barras, C., Bernhard, J.M. et al. (2023) Through the eDNA looking glass: Responses of fjord benthic foraminiferal communities to contrasting environmental conditions. Journal of Eukaryotic Microbiology, 70, e12975. https://doi.org/10.1111/jeu.12975

Brönnimann, P., 1979. Recent benthonic foraminifera from Brasil Morphology and ecology Part IV: Trochamminids from the Campos shelf with description of *Paratrochammina* n. gen. Paläontologische Zeitschrift, 531, 5-25. https://doi.org/10.1007/BF02987785

Buat-Menard, P., Chesselet, R., 1979. Variable influence of the atmospheric flux on the trace metal chemistry of oceanic suspended matter. Earth and Planetary Science Letters, 42 (3), 398–411.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods 13, 581–583. https://doi.org/10.1038/nmeth.3869.

Carreira, R.S., Ribeiro, P.V., Silva, C.E.M., Farias, C.O., 2009. Hidrocarbonetos e esterois como indicadores de fontes e destino de matéria orgânica em sedimentos da Baía de Sepetiba, Rio de Janeiro. Química Nova, 327, 1805–1811. https://doi.org/10.1590/S0100-40422009000700023

Carvalho, A. C.B., Moreira, V.A, Vicente, M.C, Bernardes, M.C, Bidone, E.D., Sabadini-Santos, E. 2021. Evolution of the sources contribution of aliphatic hydrocarbons and their fate in Sepetiba Bay, Rio De Janeiro, Brazil, Estuarine, Coastal and Shelf Science, 261, 107548. https://doi.org/10.1016/j.ecss.2021.107548.

Carvalho, A.C.B., Silva, L.J., Dick, D.P., Moreira, V.A., Vicente, M.C., Andrade, A.F.O., Bidone, E.D., Sabadini-Santos, E., 2020. Análise Espectroscópica Da Matéria Orgânica No Sedimento Superficial da Baía de Sepetiba, Rio de Janeiro, Brasil. Química Nova, 435, 552–557. https://doi.org/10.21577/0100-4042.20170518

Carvalho, B.C., Gomes, C.L.S., Guerra, J.V., 2023. Spatio-temporal morphological variability of a tropical barrier Island derived from the Landsat collection. Front. Remote Sens., 4, 1111696. https://doi.org/10.3389/frsen.2023.1111696

Castelo, W.F.L., Alves Martins, M.V., Martínez-Colón, M., Guerra, J.V., Dadalto, T.P., Terroso, D., Soares, M.F., Frontalini, F., Duleba, W., Socorro, O.A.A., Geraldes, M.C., Rocha, F., Bergamaschi, S., 2021 a. Disentangling natural vs. anthropogenic induced environmental variability during the Holocene: Marambaia Cove, SE sector of the Sepetiba Bay SE Brazil. Environmental Science and Pollution Research, 28, 22612–22640. http://dx.doi.org/ 10.1007/s11356-020-12179-9

Castelo, W.F.L., Alves Martins, M.V., Ferreira, P.A.L., Figueira, R., Costa, C.F., Fonseca, L.B., Bergamashi, S., Pereira, E., Terroso, D., Pinto, A.F.S., Simon, M.B., Socorro, O.A.A., Frontalini, F., Silva, L.C., Rocha, F., Geraldes, M., Guerra, J.G, 2021b. Long-term eutrophication and contamination of the central area of Sepetiba Bay SW Brazil. Environmental Monitoring and Assessment, 193, 100. https://doi.org/10.1007/s10661-021-08861-1

Cavaliere, M., Angeles, I.B., Montresor, M., Bucci, C., Brocani, L., Balassi, E., Margiotta, F., Francescangeli, F., Bouchet, V.M.P., Pawlowski, J., Frontalini, F. 2021, Assessing the ecological quality status of the highly polluted Bagnoli area Tyrrhenian Sea, Italy using foraminiferal eDNA metabarcoding, Science of The Total Environment, 790, 147871. https://doi.org/10.1016/j.scitotenv.2021.147871.

Clarke, K.R. & Gorley, R.N. (2006). PRIMER v6: User Manual/Tutorial (Plymouth Routines in Multivariate Ecological Research). PRIMER-E, Plymouth.

Coelho, A.L., Teixeira, G.L.G., 2018. Circulação e processos de mistura no estuário da Baía de Sepetiba nas proximidades da ilha de Itacuruçá: Condiçoes de Sizígia. Anais Hidrográficos / Dh3 / LXXIV. DHN – Diretoria de Hidrografia e Navegação. https://www.marinha.mil.br/dhn/sites/www.marinha.mil.br.dhn/files/anais/ah_2017.pdf

Cunha, C.L.N., Rosman, P.C.C., Ferreira, A.P., Monteiro, T.C.N. 2006. Hydrodynamics and Water Quality Models Applied to Sepetiba Bay. Continental Shelf Research, 26, 1940-1953. http://dx.doi.org/10.1016/j.csr.2006.06.010

Dadalto, T., Carvalho, B., Guerra, J., Reis, A., Silva, C., 2022. Holocene morpho-sedimentary evolution of Marambaia Barrier Island SE Brazil. Quaternary Research, 105, 182-200. http://dx.doi.org/10.1017/qua.2021.43

DHN - Diretoria de Hidrografia e Navegação. 2021. Catálogo de cartas e publicações - 14. ed. – Niterói RJ: A Diretoria, 2021-2025. Last access: 16/04/2023 < Carta Nautica Raster 1:40.122 – Baía de Sepetiba – Carta 1622 mar.mil.br.

Dimiza, M.D., Triantaphyllou, M.V., Koukousioura, O., Hallock, P., Simboura, N., Karageorgis, A.P., Papathanasiou, E., 2016. The Foram Stress Index: A new tool for environmental assessment of soft-bottom environments using benthic foraminifera. A case study from the Saronikos Gulf, Greece, Eastern Mediterranean. Ecological Indicators, 60, 611-621. https://dx.doi.org/10.1016/j.ecolind.2015.07.030.

Docas, 2022. Plano de Desenvolvimento e Zoneamento do Porto de Itaguaí. Porto de Itaguaí. https://www.portosrio.gov.br/pt-br/negocios/pdz. Accessed on: 10/05/2023

Dufresne, Y., Lejzerowicz, F., Perret-Gentil, L.A., Pawlowski, J., Cordier, T., 2019. SLIM: a flexible web application for the reproducible processing of environmental DNA metabarcoding data. BMC bioinformatics 20 (1), 1–6.

Duleba, W., Gubitoso, S., Alves Martins, M.V., Teodoro, A.C., Pregnolato, L.A., Prada, S.M., 2019. Evaluation of Contamination by Potentially Toxic Elements PTE of Sediments around the Petroleum Terminal Pipeline “Dutos e Terminais do Centro Sul DTCS”, SP, Brazil. Journal of Sedimentary Environments, 4 4, 387-402. /https://doi.org/10.12957/jse.2019.46539

Eichler, P.P.B., McGann, M., Rodrigues, A.R., Mendonça, A., Amorim, A., Bonetti, C., Cordeiro de Farias, C., Mello e Sousa, S.H., Vital, H., Gomes, M.P., 2018. The occurrence of the invasive foraminifera *Trochammina hadai* Uchio in Flamengo Inlet, Ubatuba, São Paulo State, Brazil. Micropaleontology, 64 5-6, pp. 391–402. https://www.jstor.org/stable/26759098

Ellis, B.F., Messina, A., 1940-2015. Catalogue *of* Foraminifera. Micropaleontology Press, American Museum of Natural History, New York.

Fatela, F., Taborda, R. 2002. Confidence limits of species proportions in microfossil assemblages. Marine Micropaleontology, 45 (2), 169–174.

Ferreira, AP. 2011. Assessment of heavy metals in Egretta thula. Case study: Coroa Grande mangrove, Sepetiba Bay, Rio de Janeiro, Brazil. Braz. J. Biol., 71, no. 1, p. 77-82

Filippos, L.S., Duleba, W., Hohenegger, J., Pregnolato, L.A., Bouchet, V.M.P., Alves Martins, M.V., 2023. Domestic sewage outfall severely altered environmental conditions, foraminiferal communities and ecological quality statuses in front of the nearshore Beach of Cigarras SE Brazil. Water, 15, 405, 1-16. https://doi.org/10.3390/w15030405

Francescangeli, F., Milker, Y., Bunzel, D., Thomas, H., Norbisrath, M., Schönfeld, J., Schmiedl, G., 2021. Recent benthic foraminiferal distribution in the Elbe Estuary North Sea, Germany: A response to environmental stressors. Estuarine, Coastal and Shelf Science, 251, 107198. https://doi.org/10.1016/j.ecss.2021.107198

Frontalini, F., Cordier, T., Balassi, E., Armynot du Chatelet, E., Cermakova, K., Perret-Gentil, L., Martins, M.V.A., Bucci, C., Scantamburlo, E., Treglia, M., Bonamin, V., Pawlowski, J., 2020. Benthic foraminiferal metabarcoding and morphology-based assessment around three offshore gas platforms: congruence and complementarity. Environ. Int. 144, 106049. https://doi.org/10.1016/j.envint.2020.106049

Greco, M., Lejzerowicz, F., Reo, E., Caruso, A., Maccotta, A., Coccioni, R., Jan Pawlowski, J., Frontalini, F., 2022. Environmental RNA outperforms eDNA metabarcoding in assessing impact of marine pollution: A chromium-spiked mesocosm test. Chemosphere, 298, 134239. https://doi.org/10.1016/j.chemosphere.2022.134239

Håkanson, L., 1980. Ecological risk index for aquatic pollution control. A sedimentological approach*. Water Research*, 14, 975-1001. https://doi.org/10.1016/0043-13548090143-8

Hatje, V., Andrade, R.B., Oliveira, C.C., Polejack, A., Gxaba, T., 2021 Pollutants in the South Atlantic Ocean: sources, knowledge gaps and perspectives for the Decade of Ocean Science. Front. Mar. Sci. 8:644569. https://doi.org/10.3389/fmars.2021.644569

Hayward, B.W., Holzmann, M., Pawlowski, J., Parker, J.H., Kaushik, T., Toyofuku, M.S., Tsuchiya, M., 2021. Molecular and morphological taxonomy of living *Ammonia* and related taxa Foraminifera and their biogeography. *Micropaleontology.* 67: 109-313.

Hayward, B.W., Le Coze, F., Vandepitte, L., Vanhoorne, B., 2020. Foraminifera in the World Register of Marine Species Worms taxonomic database. Journal of Foramininiferal Research, 503, 291-300. https://dx.doi.org/10.2113/gsjfr.50.3.291

Huang Z, Zheng S, Liu Y, Zhao X, Qiao X, Liu C, Zheng B, Yin D. Distribution, toxicity load, and risk assessment of dissolved metal in surface and overlying water at the Xiangjiang River in southern China. Sci Rep. 2021 Jan 8;11(1):109. doi: 10.1038/s41598-020-80403-0. PMID: 33420280; PMCID: PMC7794442.

IBGE (Brazilian Institute of Geography and Statistics). 2011. Atlas Geográfico das Zonas Costeiras e Oceânicas. At: http://biblioteca.ibge.gov.br/visualizacao/livros/liv55263.pdf [accessed on November 2023].

Jorissen, F.J., Nardelli, M.P., Almogi-Labin, A., Barras, C., Bergamin, L., Bicchi, E., El Kateb, A., Ferraro, L., McGann, M., Morigi, C., et al. Developing Foram-AMBI for Biomonitoring in the Mediterranean: Species Assignments to Ecological Categories. Mar. Micropaleontol. 2018, 140, 33–45.

Kjerfve, B., Dias, G.T., Filippo, A., Geraldes, M. 2021. Oceanographic and environmental characteristics of a coupled coastal bay system: Baía de Ilha Grande-Baía de Sepetiba, Rio de Janeiro, Brazil. Regional Studies in Marine Science, 41, 101594. https://doi.org/10.1016/j.rsma.2020.101594

Kütter, V. T., Almeida Moreira, V., Tavares Kütter, M., Vieira Silva-Filho, E., Duarte Marques, E., Garnier, J., & Dausacker Bidone, E., 2021. Metal bioavailability and distribution in the fish community in a tropical estuary, Sepetiba Bay, Rio De Janeiro, Brazil. Geochimica Brasiliensis, *35*1, 17–38. https://doi.org/10.21715/GB2358-2812.2021351017

Lacerda, L. D., Pfeiffer, W. C., Fiszman, M., 1987. Heavy metal distribution, availability and fate in Sepetiba bay, S.E. Brazil. The Science of the Total Environment, 65, 163-173. https://doi.org/10.1016/0048-9697(87)90169-0Lacerda, L.D., Molisani, M. M. 2006. Three decades of Cd and Zn contamination in Sepetiba bay, SE Brazil, Evidence from the mangrove oyster *Crassostraea rhizophorae*. Marine Pollution Bulletin, 52, 974-977. https://doi.org/10.1016/j.marpolbul.2006.04.007

Langenbach, T., Denise Maria Mano Pessoa, Mario Luiz Gomes Soares, Anna Carolina Ribeiro Mendes Magdaleno, Allen Hagler, Tácio Mauro Pereira de Campos, 2022. Zinc and cadmium contamination in an *Avicennia schaueriana* mangrove environment: Mass balance and tissue distribution. Marine Pollution Bulletin, 184, 114170, https://doi.org/10.1016/j.marpolbul.2022.114170.

Liu, D., Wang, J., Yu, H. *et al.* 2021. Evaluating ecological risks and tracking potential factors influencing heavy metals in sediments in an urban river. *Environ Sci Eur* **33**, 42. https://doi.org/10.1186/s12302-021-00487-x

Loeblich, A.R., Tappan, H., 1987. Foraminiferal Genera and their Classification. Van Nostrand Reinhold Company, New York, 970 pp.

LULU (Frøslev et al., 2017) Frøslev, T.G., Kjøller, R., Bruun, H.H., Ejrnæs, R., Brunbjerg, A.K., Pietroni, C., Hansen, A. J., 2017. Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates. Nature Communications 2017 8:1 8, 1–11. https://doi.org/10.1038/s41467-017-01312-x.

Magurran, A. E. 1988. Ecological Diversity and Its Measurement. University Press, Princeton, NJ.

Marinho, C.C., Breves, A., Rocha, A.M., Silva, E.C., Weerelt, V. et al., 2022. Long-term changes in sanitary conditions and zooplankton community in the Imboassica Lagoon, Rio de Janeiro, Brazil: effects of sandbar openings. Oecologia Australis, 26, 239-254. https://doi.org/10.4257/oeco.2022.2602.12

Marques, J.A., Simone, R., Costa, A.C. Maraschi, C.E.D., Vieira, P.G. et al. 2022. Biochemical response and metals bioaccumulation in planktonic communities from marine areas impacted by the Fundão mine dam rupture southeast Brazil. Science of The Total Environment, 806, Part 3, 150727. https://doi.org/10.1016/j.scitotenv.2021.150727

Martins, A.M.V., Pereira, E., Figueira, R.C.L., Oliveira, T., Pinto Simon, A.F.S., Terroso, D., Ramalho, J.C.M., Silva, L., Ferreira, C., Geraldes, M.C., Duleba, W., Rocha, F., Rodrigues, M.A., 2019. Impact of eutrophication on benthic foraminifera in Sepetiba Bay Rio de Janeiro State, SE Brazil. Journal of Sedimentary Environments, 4 (4), 480-500. https://doi.org/10.12957/jse.2019.47327

Martins, M.V.A., Frontalini, F., Laut, L.L.M., Silva, F.S., Moreno, J., Sousa, S., Zaaboub, N., El Bour, M., Rocha, F., 2014. Foraminiferal biotopes and their distribution control in Ria de Aveiro Portugal: a multiproxy approach. Environ. Monit. Assess. 186, 8875–8897. https://doi.org/10.1007/s10661-014-4052-7

Martins, M.V.A., Hohenegger, J., Frontalini, F., Laut, L.., Miranda, P., Rodrigues, M.A., Duleba, W., Rocha, F., 2018. Heterogeneity of sedimentary environments in the Aveiro Lagoon mouth Portugal: comparison between the dead and living benthic foraminiferal assemblages. Estuarine, Coastal and Shelf Science 213, 199–216. http://dx.doi.org/10.1016/j.ecss.2018.08.018

McGann, M. Sloan, D., 1996. Recent introduction of the foraminifer Trochammina hadai Uchio into San Francisco Bay, California, USA. Marine Micropaleontology, 128, 1–3.McGann, M., Sloan, D., 1999. Benthic foraminifers in the Regional Monitoring Program's San Francisco Estuary samples. In: 1997 Annual Report for the Regional Monitoring Program for Trace Substances in the San Francisco Estuary, 249-258. San Francisco Estuary Institute, Richmond, CA.

McGann, M., Sloan, D., Cohen, A.N., 2000. Invasion by a Japanese marine microorganism in western North America. Hydrobiologia, 421, 25–30.

Mirlean N., Medeanic, S., Garcia, F.A., Travassos, M.P., Baisch, P., 2009. Arsenic enrichment in shelf and coastal sediment of the Brazilian subtropics. Continental Shelf Research, 35, 129–136. https://doi.org/10.1016/j.csr.2012.01.006.

Moreira, V.A., Cravo-Laureau, C., de Carvalho, C.B.A., Baldy, A., Bidone, E.D., Sabadini-Santos, E., Duran, R. 2023. Microbial indicators along a metallic contamination gradient in tropical coastal sediments, Journal of Hazardous Materials, 443, Part B, 130244. https://doi.org/10.1016/j.jhazmat.2022.130244

Müller, V.G., 1986. Schadstoffe in Sedimenten - Sedimente als Schadstoffe. Mitteilungen der Österreichischen Geologischen Gesellschaft, 79, 107-126.

Nascimento JR, Sabadini-Santos E, Carvalho C, Keunecke KA, César R, Bidone ED. 2017. Bioaccumulation of heavy metals by shrimp (Litopenaeus schmitti): A dose-response approach for coastal resources management. Mar Pollut Bull. Jan 30;114(2):1007-1013. doi: 10.1016/j.marpolbul.2016.11.013. Epub 2016 Nov 20. PMID: 27876373.

Nunes, M., Alves Martins, M.V., Frontalini, F., Bouchet, V.M.P., Francescangeli, F., Hohenegger, J., Figueira, R., Senez-Mello, T.M., Castelo, W.F.L., Damasceno, F.L., Laut, L., Duleba, W., Mello e Sousa, S.H., Antonioli, L., Geraldes, M.C., 2023. Inferring the ecological quality status based on living benthic foraminiferal indices in transitional areas of the Guanabara Bay SE Brazil. Environmental Pollution, 320, 121003 https://doi.org/10.1016/j.envpol.2023.121003

Oliveira, T.R.S., Santos, L.D., Eichler, P. P. B., Barker, C.P., Barcellos, R.L., 2022. Benthic foraminifera of tropical estuarine-lagoonal-bays system, in the Suape Harbor, Brazil: a case study. Journal of Foraminiferal Research, 52, 4-20. https://doi.org/10.2113/gsjfr.52.1.4

Parent, B., Hyams-Kaphzan, O., Barras, C., Lubinevsky, H., Jorissen, F. 2021. Testing foraminiferal environmental quality indices along a well-defined organic matter gradient in the Eastern Mediterranean. Ecological Indicators, 125, 107498. https://doi.org/10.1016/j.ecolind.2021.107498

Pavard, J.-C., Richirt, J., Seuront, L., Blanchet, H., Fouet, M.P.A., Humbert, S. Gouillieux, B., Duong, G. & Bouchet, V.M.P. (2023). The great shift: The non-indigenous species *Ammonia confertitesta* (Foraminifera, Rhizaria) outcompetes indigenous Ammonia species in the Gironde estuary (France). Estuarine, Coastal and Shelf Science 289: 108378. https://doi.org/10.1016/j.ecss.2023.108378

Pawlowski, J., Bruce, K., Panksep, K., Aguirre, F.I., Amalfitano, S., Apothéloz-Perret-Gentil, L. et al. 2022. Environmental DNA metabarcoding for benthic monitoring: a review of sediment sampling and DNA extraction methods. Sci. Total Environ. 151783. https://doi.org/10.1016/j.scitotenv.2021.151783

Pawlowski, J., Esling, P., Lejzerowicz, F., Cordier, T., Visco, J.A., Martins, C.I.M., Kvalvik, A., Staven, K., Cedhagen, T., 2016. Benthic monitoring of salmon farms in Norway using foraminiferal metabarcoding. Aquac. Environ. Interact. 8, 371–386. https://doi.org/10.3354/aei00182

Poag, C.W., 1981. Ecologic Atlas of Benthic Foraminifera of the Gulf of Mexico. Hutchinson Ross, Marine Science International, Woods Hole, 175 pp.

Reis, A.T., Amendola, G., Dadalto, T.P., Silva, C.G., Tardin, R.G.C.P., Guerra, J.V., Martins, V., Cardia, R.R., Gorini, C., Rabineau, M., 2020. Architecture and depositional evolution of the latest Pleistocene–Holocene last ~20ky sedimentary succession of Sepetiba Bay RJ. Geociências, 39, 695–708.

Ribeiro, A.P., Figueiredo, A.M.G., Santos, J.O.d., Ferreira, P.A.d.L., Graudenz, G.S., Ruiz, M.S., Mahiques, M.M.d., Figueira, R.C.L., Wasserman, J.C.F.A. 2015. Effects of contamination with toxic metals on the environmental quality of Sepetiba Bay SE Brazil. The case of Inga Company. Management of Environmental Quality, 26 (4), 538-551. https://doi.org/10.1108/MEQ-06-2013-0074

Ribeiro, A.P., Figueiredo, M.G., A., Osman dos Santos, J., Dantas, E.S.K. et al., 2014. Enrichment diagram ED: A graphic approach for assessing inorganic enrichment and contamination in marine sediments of Sepetiba Bay SE Brazil. Management of Environmental Quality, 25 (4), 467-479. https://doi.org/10.1108/MEQ-02-2013-0013

Rodrigues, A.R., Pellizari, V.H., de Salles Almeida, C. et al. 2021. New phylotypes of foraminifera in subtropical Brazilian coastal waters revealed by environmental DNA metabarcoding. J. Sediment. Environ. 6, 13–23 https://doi.org/10.1007/s43217-021-00049-4

Rodrigues, S. K., Machado, W., Guerra, J. V., Geraldes, M., Morales, S., Vinzón, S.B., 2020. Changes in Cd and Zn distribution in sediments after closure of an electroplating industry, Sepetiba bay, Brazil. Marine Pollution Bulletin, 161, 111758. https://doi.org/10.1016/j.marpolbul.2020.111758

Rosenberg, R., Blomqvist, M., Nilsson, H. C., Cederwall, H., Dimming, A. 2004. Marine quality assessment by use of benthic species-abundance distributions: a proposed new protocol within the European Union Water Framework Directive, Marine Pollution Bulletin, 49 (9–10), 728-739. https://doi.org/10.1016/j.marpolbul.2004.05.013.

Rostami, A., Frontalini, F., Armynot du Châtelet, E., Francescangeli, F., Alves Martins, M.V. et al., 2023. Understanding the Distributions of Benthic Foraminifera in the Adriatic Sea with Gradient Forest and Structural Equation Models. Appl. Sci. 2023, 13, 794. https://doi.org/10.3390/app13020794

Schönfeld, J., Alve, E., Geslin, E., Jorissen, F., Korsun, S., Spezzaferri, S., Abramovich, S. et al., 2012. The FOBIMO FOraminiferal BIo-MOnitoring initiative – towards a standardised protocol for benthic foraminiferal monitoring studies. Marine Micropaleontology, 94–95, 1–13. http://dx.doi.org/10.1016/j.marmicro.2012.06.001

Sen Gupta, B.K., Machian-Castillo, M.L., 1993. Benthic foraminifera in oxygen-poor habitats. Marine Micropaleontology 203, 183-201. http://dx.doi.org/10.1016/0377-83989390032-S

Sen Gupta, B.K., Turner, R.E., Rabalais, N.N., 1996. Seasonal oxygen depletion in continental-shelf waters of Louisiana: Historical record of benthic foraminifers. Geology, 243, 227-230. https://doi.org/10.1130/0091-76131996024<0227:SODICS>2.3.CO,2

Shannon, C.E., 1948. A mathematical theory of communication. The Bell System Technical Journal, 27, 379–423 and 623–656.

Shola, D., Kayode-Afolayan, E., Ahuekwe, F., Nwinyi, O.C., 2022. Impacts of pharmaceutical effluents on aquatic ecosystems, Scientific African, 17, e01288. https://doi.org/10.1016/j.sciaf.2022.e01288.

Silva, L.C., Alves Martins, M.V., Castelo, W.F.L., Saibro, M.B., Rangel, D., Pereira, E., Bergamaschi, S., Sousa, S.H.M., Varela, J., Laut, L., Frontalini, F., Chaves, H., Reis, A.T., Aguilera, O., Zaaboub, N., Cheriyan, E., Geraldes, M.C., 2022. Trace metals enrichment and Potential Ecological Risk in sediments of the Sepetiba Bay Rio de Janeiro, SE Brazil. Marine Pollution Bulletin 177 2022 113485. https://doi.org/10.1016/j.marpolbul.2022.113485

Souza, A.M., Rocha, D.S., Guerra, J. V., Cunha, B. A., Martins, M. V.A., Geraldes, M.C., 2021. Metal concentrations in marine sediments of the Rio de Janeiro Coast Brazil: A proposal to establish new acceptable levels of contamination, Marine Pollution Bulletin, 165, http://dx.doi.org/10.1016/j.marpolbul.2021.112113

Sutherland, R.A., 2000. Bed sediment-associated trace metals in an urban stream, Oahu, Hawaii. Environmental Geology, 39, 611-627. https://doi.org/10.1007/s002540050473

Swarnalatha, K., Letha, J., Ayoob, S., 2013. Ecological risk assessment of a tropical lake system. Journal of Urban and Environmental Engineering, 7 (2), 323-329.

Tomlinson, D.L., Wilson, J.G., Harris, C.R., Jeffrey, D.W. Problems in the assessment of heavy metal level in estuaries and the formation of a pollution index. Helol. Meeresunters. 1980, 33, 566–575.

Tonhá, M. S., Araújo, D. F., Araújo, R., Cunha, B. C.A., Machado, W., Portela, J.F., Pr Souza, J., Carvalho, H. K., Dantas, E. L., Roig, H. L., Seyler, P., Garnier, J. 2021. Trace metal dynamics in an industrialized Brazilian river: A combined application of Zn isotopes, geochemical partitioning, and multivariate statistics, Journal of Environmental Sciences, 101, 313-325. https://doi.org/10.1016/j.jes.2020.08.027.

Trevisan, C.L, Vicente, M.C., Rocha, B.C.S., Wasserman, J.C., 2020. Development of a Dredging Sensitivity Index, applied to an industrialized coastal environment in Brazil. Science of the Total Environment, 748, 141294. doi.org/10.1016/j.scitotenv.2020.141294

Veríssimo, T.N.S, Moura, E.P. 2021. Impactos socioambientais e educação ambiental: breve debate a partir do exemplo da Ternium Siderúrgica em Santa Cruz - Rio de Janeiro. Espaço e Economia 22. https://doi.org/10.4000/espacoeconomia.21275

Wasserman, J.C., Barros, S.R., Lima, G.B.A., 2013. Planning dredging services in contaminated sediments for balanced environmental and investment costs. J. Environ. Manage. 121, 48–56. https://doi.org/10.1016/j.jenvman.2013.02.024

Xiang, R., Yang, Z., Saito, Y., Muhong Chen, D.F., Guo, Z., Chen, Z. 2008. Paleoenvironmental changes during the last 8400 years in the southern Yellow Sea: Benthic foraminiferal and stable isotopic evidence, Marine Micropaleontology, 67 (1–2), 104-119. https://doi.org/10.1016/j.marmicro.2007.11.002.

Zhang QF, Su RH, Jiang MX et al., 2007 The south-to-north water transfer project and its ecological security: the priorities. Res. Environ Yangtze Basin 3:217–221.

Figures:

Figure 1. Location map of Sepetiba Bay (Brazil) and the location of sampling sites.
Figure 2. Distribution maps of salinity, mud (%), TOC (%) and PLI values in the studied stations in the Sepetiba Bay.
Figure 3. Distribution maps of Cd, Cr, Pb and Zn concentrations (mg kg^{-1}) in the studied stations of the Sepetiba Bay.
Figure 4. Distribution maps of H'-ASVs (Shannon index based on ASV species) and H'-MA (Shannon index based on morphospecies) values and percentage of *Ammonia tepida, Bolivina striatula, Buliminella elegantissima* and *Rosalina williamsoni* in the studied stations of the Sepetiba Bay.
Figure 5. Distribution maps of biotic and abiotic index values used to classify the EcoQs. The classification scale used was defined according to the criteria reported in Table 1.
Figure 6. Agreement between the station's classification based on the biotic (ExpH'bc (m), ExpH'bc (M), Foram-AMBI, FSI and AEI) and abiotic (TOC and PLI) indices used to estimate the Ecological Quality Status (EcoQS) according to the criteria of Table 1 and the values of Appendix 7.
Figure 7. Biplots of the first two PCA factors (explaining 81.73 % of the data variability) based on selected biotic and abiotic variables. The primary variables were the abiotic parameters (such as gravel, sand and mud, as well as TOC, salinity and PLI values) and as secondary variables were the abiotic variables: A. percentage of the main morphospecies; B. percentage of the main ASV species; C. Number of species per sample (S), Shannon diversity (H'), equitability (J') of the morphospecies and ASV species.; D. Biotic indices used to evaluate the EcoQS: Foram-AMBI, FSI, Exp(H'bc) based on morphological and ASV analyses; finally, in the biplot E. the studied stations were related to the scores of the first two PCA factors, defined as a function of the primary and secondary mentioned variables.

Tables:

Table 1. Ecological Quality Status (EcoQS) classification criteria. The criteria used to evaluate EcoQS were based on: Exp(H'bc) (M) Bouchet et al. (2018); Foram AMBI Parent et al. (2021); FSI Dimiza et al. (2016); PERI Håkanson (1980) and Swarnalatha et al. (2013). The TOC and Exp(H'bc) (G) classes (standardized values) were established in this work. Legend: (M - morphological community and m - molecular community); stnd - standardized data.

Table 2. Values of PCA Factor 1 (Fig. 7) and the ecological indices used in this work to evaluate the EcoQs (m – molecular community and M – morphological community).

Table 3. Spearman Rank Order Correlations between the biotic indices estimated in this work to evaluate the EcoQs (ExpH'$_{bc}$ (G), ExpH'$_{bc}$ (M), Foram AMBI, FSI and AEI) and selected abiotic parameters: TOC, PERI and PCA Factor 1. The correlations marked in bold are significant ($p < 0.050$). Legend: (G) - genetical analysis; (M) - morphospecies; (ES) - environmental stress.

Supplementary material

Figure S1. Relative abundance (%) of the main species identified by morphological analysis.

Appendix 1. Physicochemical, sedimentological and selected biotic data.

Appendix 2. EF and Igeo values.

Appendix 3. Number and percentage of foraminiferal morphospecies per sample

Appendix 4. Number and percentage of foraminiferal of ASV species

Appendix 5. PCA- Factor score (related to Fig. 5) and Spearman Rank Order Correlations (significant level at p <0.05000) between the main morphological species and the ASV species (genetic data) and the PCA Factor 1 (Fig. 5) associated to the environmental stress.

Appendix 6. Spearman Rank Order Correlations between selected sedimentological and biotic data. The correlations marked in red are significant correlations for at p <0.05000).

Appendix 7. Estimation of the EcoQS (following the criteria presented in Table 1) based on biotic indices, such as Exp(H'_{bc}) (m), Exp(H'_{bc}) (M), Foram AMBI, and FSI and abiotic ones, such as TOC and PERI.

Highlights

Foraminiferal eDNA and morphology are used to infer EcoQS in coastal SE Brazil

Biotic and abiotic indices show poor/bad EcoQS in the inner area of the Sepetiba Bay

High/good EcoQS are found in the outer area of the Sepetiba Bay

Molecular and morpho-methods provide similar EcoQS

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: