



Towards a more integrative approach for environmental decision-making in Brazilian transitional waters: improving biomonitoring surveys with a benthic foraminiferal biotic index

Vincent M. P. Bouchet¹, Silvia Helena de Mello e Sousa², Carla Bonetti³, Leticia Burone⁴, Pierre Belart⁵, Wania Duleba⁶, Fabio Francescangeli⁷, Fabrizio Frontalini⁸, Lazaro Laut⁵, Débora S. Raposo⁹, André R. Rodrigues¹⁰, Sibelle Trevisan Disaró¹¹, Daniel Vicente Pupo¹¹, Fabrício Leandro Damasceno¹², Jean-Charles Pavard^{1,13}, and Maria Virgínia Alves Martins^{12,14}

¹Univ. Lille, CNRS, IRD, Univ. Littoral Côte d'Opale, UMR 8187, LOG, Laboratoire d'Océanologie et de Géosciences, Station Marine de Wimereux, 59000 Lille, France

²Instituto Oceanográfico, Universidade de São Paulo (IOUSP), Pça. do Oceanográfico, 191, Butantã, São Paulo, 05508 120, Brazil

³Coordenadoria de Oceanografia, Universidade Federal de Santa Catarina (UFSC), Florianópolis, Santa Catarina, Brazil

⁴Cincytema, Sección Oceanología, Instituto de Ecología y Ciencias Ambientales, Facultad de Ciencias, Universidad de la República, Igua 4225, Montevideo 11400, Uruguay

⁵Departamento de Ciências Naturais, Universidade Federal do Estado do Rio de Janeiro – UNIRIO, Rio de Janeiro, Rio de Janeiro, Brazil

⁶Universidade de São Paulo (USP), Escola de Artes, Ciências e Humanidades (EACH), Rua Arlindo Bértio, 1000 – Ermelino Matarazzo, São Paulo – SP, 03828-000, Brazil

⁷Department of Geosciences, University of Fribourg, Chemin Du Musée 6, 1700 Fribourg/Freiburg, Switzerland

⁸Department of Pure and Applied Sciences (DiSPeA), University of Urbino “Carlo Bo”, Campus Scientifico Enrico Mattei, Località Crocicchia, 61029 Urbino, Italy

⁹German Federation for Biological Data – GFBio e.V., Mary-Somerville-Strasse 2–4, 28359 Bremen, Germany

¹⁰Universidade Federal do Espírito Santo, UFES, Vitória, ES, Brazil

¹¹Universidade Federal do Paraná, Museu de Ciências Naturais, Laboratório de Foraminíferos e Micropaleontologia Ambiental, Av. Cel. Francisco H. Dos Santos, 100 – Jardim Américas, CEP 81530-000, Curitiba, PR, Brazil

¹²Faculdade de Geologia, Departamento de Paleontologia e Estratigrafia, Universidade do Estado do Rio de Janeiro (UERJ), R. São Francisco Xavier, 524 – Lab 4037F – Maracanã, Rio de Janeiro 20550-900, Brazil

¹³Department of Marine Science, University of Gothenburg, Gothenburg, Sweden

¹⁴Department of Geosciences, Aveiro University, GeoBioTec, Campus de Santiago, 3810-197 Aveiro, Portugal

Correspondence: Vincent M. P. Bouchet (vincent.bouchet@univ-lille.fr) and Jean-Charles Pavard (jean-charles.pavard@gu.se)

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Abstract. This study represents the first attempt to determine the indicator values of benthic foraminiferal species in Brazilian transitional waters. It also uses a regionally adapted species list to explore the potential application of Foram-AMBI, a biotic index for ecological quality. To test this, we assigned 95 living (rose-bengal-stained) benthic foraminiferal species into five ecological groups (EGs), based on the weighted-averaging (WA)

optimum and tolerance to the total organic carbon (TOC) contents. Selected and published regional studies were used as the database, while independent Brazilian datasets from Sepetiba Bay and Guanabara Bay – the most polluted regions – were used to validate the ecological group assignments through ForAM-AMBI. Furthermore, ecological quality status (EcoQS) criteria adapted to Brazil were developed for ForAM-AMBI. The index accurately reflects the degraded environmental conditions in these two ecosystems, with moderate to poor ecological quality status in the most polluted areas. This was further confirmed by significant correlations between ForAM-AMBI and TOC in both bays. This study highlights the importance of developing regional species lists and EcoQS criteria for ForAM-AMBI, as the accuracy of the Brazilian list was better than that of the European list. While further research across broader pollution gradients is needed, our findings confirm the suitability and reliability of benthic foraminifera as biological indicators for assessing environmental quality in transitional waters.

1 Introduction

Brazil hosts the second-largest mangrove area in the world, which accounts for 13.5 % of the annually sequestered carbon in the world's mangroves (Rovai et al., 2022). In addition, blue carbon storage in the woody biomass of Brazilian mangroves represents 10 % of the carbon sequestration in mangrove woody biomass globally (Rovai et al., 2022). Unfortunately, coastal areas, with their high population density and associated activities, are often subject to major human impacts (Diaz and Rosenberg, 2008). Rapid urban development and industrialisation have directly promoted the widespread contamination of Brazilian coastal areas. Accordingly, solid urban waste, industrial effluents, and micro- and nanoplastics, among other factors, have altered the ecological quality, impaired ecological functions, and led to a loss in ecosystem goods and services (e.g. Baptista Neto et al., 2006; Souza et al., 2021a). These areas, like those of southeastern Brazilian bays, have also been negatively affected by the discharges of metals, hydrocarbons, and other organic compounds, resulting in a decline in water and sediment quality (e.g. Baptista Neto et al., 2000; Ribeiro et al., 2015).

In Brazil, the National Environment Council (CONAMA), subordinated to the Ministry of the Environment and Climate Change, is responsible for defining the guidelines and standards for the environmental monitoring of ecosystems, including coastal and marine areas (Brazil, 1981). The CONAMA Resolution 357/05 (Brazil, 2005) defines the water classes, uses, and quality standards for major pollutants. It also foresees the use of “biological indicators, when appropriate, and through the use of aquatic organisms and/or communities” to evaluate the quality of aquatic environments, thus formalising the legal framework to conduct an environmental survey based on biota. Benthic macro-invertebrates are widely used in environmental surveys (Dauvin et al., 2012). To facilitate the use of macro-invertebrates for biomonitoring, several biotic indices have recently been proposed, e.g. AMBI (Borja et al., 2000), BO2A (Dauvin and Ruellet, 2007), and BENTIX (Simboura and Zenetos, 2002).

Until now, few studies have been carried out to test and validate the performance of the widely used AMBI, BO2A, and BENTIX along the Brazilian coast (e.g. Brauko et al., 2016; Checon et al., 2018). These studies suggested that the inferred environmental quality based on these indices was, in general, satisfactory for the studied gradients in estuaries along the coasts of Brazil (Valença and Santos, 2012; Brauko et al., 2016; Checon et al., 2018; Souza et al., 2021b). However, some inconsistencies in the ecological classification provided by AMBI and other indices were reported (e.g. Brauko et al., 2016; Souza et al., 2021b). Specifically, a study in a subtropical estuary in Paranaguá Bay (Brazil) observed significant differences in the assignment of ecological quality status (EcoQS) among the tested indices (Brauko et al., 2016). The level of agreement in determining EcoQS varied depending on the index, and not all indices were accurately responsive to the varying levels of sewage contamination in the tidal flats (Brauko et al., 2016). This may be explained by the fact that the assignment of species to ecological groups (EGs) is based on taxa found in European waters (Borja et al., 2000; Simboura and Zenetos, 2002). Many species from Brazilian coastal and transitional ecosystems are not yet included in the database (Brauko et al., 2016; Muniz et al., 2005; Valença and Santos, 2012). Since these indices are based on the relative proportion of sensitive/tolerant taxa, assigning dominant species to one of the five EGs constitutes a prerequisite for the adequate evaluation of ecosystems, as Bigot et al. (2008) described. However, appropriate assignments are neither necessarily available (Muniz et al., 2005) nor easy to achieve beyond the coasts of Europe, for which the AMBI was developed (Borja et al., 2000). Along the Brazilian coasts, if macro-invertebrate taxa were used with the original European species list without modifying their EGs, most EcoQS scores would be wrongly evaluated (Valença and Santos, 2012). Hence, the applicability of these indices requires adjustments regarding some species' assignment in EGs (Checon et al., 2018; Muniz et al., 2005). Parallel to applying macro-invertebrate-based biotic indices to a broader extent, the complementary use of different indices and/or methods is recommended to confidently assess the EcoQS of coastal areas in Brazil (Muniz et al., 2005). Ef-

forts should focus on developing integrative tools to help the environmental decision-making process (Muniz et al., 2011). For instance, benthic foraminifera are complementary to marine macro-invertebrates for characterising the environmental health of benthic ecosystems in Europe (Alve et al., 2019; Bouchet et al., 2020, 2018a, b).

Due to their wide distribution, small size, high abundance even in small amounts of sediment sample, short life cycles and reproductive cycles, high biodiversity, and specific ecological requirements (see Review in Schönfeld et al., 2012), foraminifera are particularly sensitive and can be successfully applied as bioindicators of environmental change in a wide range of transitional and marine habitats (e.g. Alve, 1995; Armynot du Châtelet et al., 2004; Francescangeli et al., 2020). There is a long-lasting tradition of ecological studies on benthic foraminifera (total assemblages or living fauna) in Brazil (e.g. Vilela et al., 2004; Burone et al., 2006; Debenay et al., 1998; Eichler et al., 2018; Eichler et al., 2003; Duleba et al., 2018). Specifically, living foraminiferal fauna have been utilised as sensitive indicators of trace metal pollution (e.g. Raposo et al., 2022), organic matter (OM) enrichment (e.g. Laut et al., 2016), oil spill (e.g. Nunes et al., 2023), shellfish farming (e.g. Rudorff et al., 2012), and water sewage (e.g. Filippou et al., 2023). To meet the objectives of the varying marine legislation, biotic indices have been established to synthesise the ecological information provided by foraminiferal communities (O'Brien et al., 2021). Some of these indices are based on diversity (Alve et al., 2009; Bouchet et al., 2012, 2018a), while others, such as Foram-AMBI, i.e. an adaptation of AMBI to benthic foraminifera (Alve et al., 2016; Bouchet et al., 2021; Jorissen et al., 2018), TSI-Med (Barras et al., 2014), and FSI (Dimiza et al., 2016), rely on the response of species along a pollution gradient, commonly ascribed to enrichment in total organic carbon (TOC). These indices have been applied to monitor the EcoQS in the context of aquaculture (e.g. Bouchet et al., 2020), trace metal elements, and water sewage (e.g. Melis et al., 2019). It is worth mentioning that lists of ecological behaviour of benthic foraminiferal species have already been compiled for some geographical areas and specific habitats, such as in the Northeast Atlantic and Arctic fjords on continental shelves and slopes (Alve et al., 2016); in open waters in the Mediterranean Sea (Jorissen et al., 2018); in transitional waters (TWs) along the English Channel and European coasts (Bouchet et al., 2021), called hereafter the European TW Atlantic list, and along the Mediterranean sea coasts (Bouchet et al., 2021), called hereafter the European TW Mediterranean list; and in the Gulf of Mexico (O'Malley et al., 2021). These species lists can be used in the different sensitivity-based indices designed for benthic foraminifera that consider the relative proportion of sensitive, tolerant, and opportunistic species to assess EcoQS, particularly Foram-AMBI. As for AMBI, there are no species lists adapted to Brazil, and adjusting local species classification in EGs to improve the accuracy of Foram-AMBI is required.

Preliminary tests of Foram-AMBI in Brazilian TWs are scarce (Nunes et al., 2023; Damasceno et al., 2024; Martins et al., 2020). For example, in Guanabara Bay, the increase in environmental stress was well correlated with Foram-AMBI (Nunes et al., 2023). The assessment of the EcoQS based on living benthic foraminifera illustrated the elevated pollution level in this bay (Cotovicz Jr. et al., 2015), showing the high potential of benthic foraminiferal biotic indices for the evaluation of environmental health (Nunes et al., 2023). However, the percentage of agreement in terms of EcoQS assessment between the diversity index $\exp(H'_{bc})$ and Foram-AMBI was only $\sim 64\%$ (Nunes et al., 2023). The use of the European TW Atlantic list for computing Foram-AMBI may have limited the accuracy and reliability of the index in Brazilian waters, as observed for benthic macro-invertebrates (Muniz et al., 2005; Valença and Santos, 2012; Checon et al., 2018). According to Nunes et al. (2023), a better agreement between the indices can be attained with a specific Brazilian list of foraminiferal species' assignments to the EGs of Foram-AMBI. Overall, these results and previous studies are promising regarding the use of Foram-AMBI in TWs of Brazil; however, many local species remain unassigned or wrongly assigned to an EG. Indeed, geographical variability in benthic foraminiferal EG assignments has already been observed in Europe (Bouchet et al., 2021). Suitable application of Foram-AMBI demands further research on tolerance shifts of key indicator species in different geographical regions and habitats. This would significantly improve the EG assignment in the area, thereby increasing Foram-AMBI effectiveness and providing an important tool to monitor and preserve transitional ecosystems. Currently, only Norway recognises benthic foraminifera as a Biological Quality Element (BQE; Veileder, 2018). Although they have been widely used as accurate and reliable indicators of environmental conditions (O'Brien et al., 2021), they have not been included in the official Brazilian guidelines for environmental biomonitoring (Sousa et al., 2020).

In this context, this study aims to establish a foraminiferal species list adapted to TWs in Brazil to provide stakeholders with an improved and complementary method to benthic macro-invertebrates for environmental health assessment. To do that, we gathered and analysed published data from studies conducted in Brazilian TWs containing living foraminifera and environmental parameters. It was then possible to determine the indicative values of benthic foraminifera and to assign them to five EGs based on their responses to sediment TOC content, as described in the pioneering work on Foram-AMBI by Alve et al. (2016). The classification was tested by calculating Foram-AMBI with published foraminiferal data not used for the species assignment from two polluted transitional areas in Brazil, namely Sepetiba Bay (Damasceno et al., 2024) and Guanabara Bay (Nunes et al., 2023). We further compared species' assignments for those occurring in both Brazil and Europe to con-

firm any potential tolerance shift over a large geographical scale.

2 Materials and methods

2.1 Dataset selection for the Brazilian list

A literature collection was performed to find studies on living foraminifera and reporting relevant environmental parameters such as organic matter or total organic carbon content. We selected studies following the same criteria as those defined in previous work on the Foram-AMBI list (Alve et al., 2016; Bouchet et al., 2021; Jorissen et al., 2018). Only studies in coastal waters and TWs according to McLusky and Elliott (2007) were selected (Table 1). Furthermore, studies had to be solely based on living benthic foraminifera. Note that all datasets used in the present study are based on rose-bengal-stained fauna. The foraminiferal and TOC data had to be from the same station (less than 1 m from the sampling point). The TOC content data in some studies were derived from the measurement of loss on ignition (LOI; studies 1, 2, 3, 4, and 5 in Table A1). For these five studies, the TOC content was estimated by dividing by 3 the OM content obtained by LOI measurement as suggested for TW sediments from tropical areas (Leong and Tanner, 1999). Only samples containing at least 50 stained specimens were considered (Alve et al., 2016).

The selected studies were based on the > 63 or $> 125 \mu\text{m}$ fraction (see Appendix A). In total, 14 studies were considered (Fig. 1, Table A1). Detailed information on the selected datasets can be found in Appendix A. We gathered all data in a master table (available in Bouchet et al., 2025) containing relative species abundances and TOC concentrations in sediments (in %) at each studied station selected. Species names were homogenised among studies following the World Register of Marine Species (WoRMS; Hayward et al., 2020a). Only accepted scientific names from WoRMS are used in this study (Hayward et al., 2020b), and the unique AphiaID is reported for each species.

2.2 Assignment to ecological groups

To assign foraminiferal species, we followed Bouchet et al. (2021) and used an objective method for species assignments in order to avoid using “best expert judgement”. The weighted-averaging (WA) optimum and tolerance (Birks et al., 1990; Ter Braak, 1987) were computed on the master table data (see Sect. 2.1 for data selection) for each species to determine its response to TOC (%). A simple and ecologically reasonable estimate of a benthic foraminiferal species’ optimum is the average of all TOC values for intertidal areas and TWs in which the species occurs, weighted by the species’ relative abundance (WA regression). To summarise, the WA optimum method is rapid, easy to implement, theo-

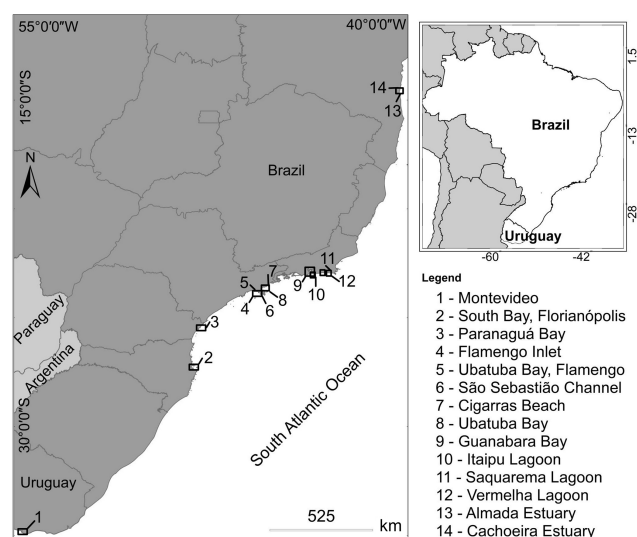


Figure 1. The geographical position of the 14 selected studies from which datasets were used to assign the species from Brazilian and Uruguayan transitional waters. More details in Appendix A.

retically sound, and robust (Birks et al., 1990), and it leads to an objective assessment of species-specific indicative values.

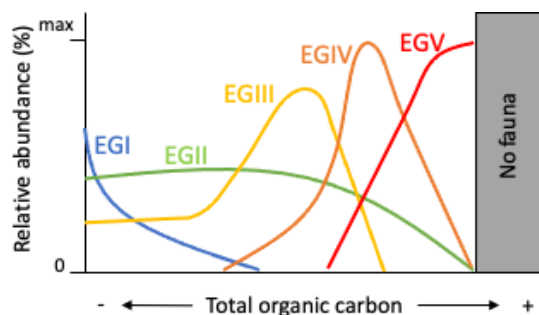
In detail, foraminiferal species were assigned to five EGs according to their response to organic carbon enrichment: (i) EGI – “sensitive species” are sensitive to TOC enrichment, and their relative abundance is highest at the lowest TOC values, dropping to zero as organic carbon concentration increases; (ii) EGII – “indifferent species” are unaffected by the organic carbon enrichment and never dominate the assemblage, occurring in low relative abundance over a broad range of organic carbon concentrations, only being absent at very high concentrations; (iii) EGIII – “tolerant species” are stimulated by the excess organic carbon enrichment, but they may occur at low TOC and are absent at very high organic carbon concentrations, being labelled as “third-order opportunistic species” by Jorissen et al. (2018); (iv) EGIV – “second-order opportunistic species” have a clear positive response to organic carbon enrichment with maximum abundances between the maxima of EGIII and EGV; and (v) EGV – “first-order opportunistic species” exhibit a clear positive response to excess organic carbon enrichment with maximum abundances at the higher level induced by organic load. A theoretical distribution of these EGs is represented in Fig. 2. Foraminifera are not able to survive extreme levels of TOC concentrations.

2.3 Datasets to test the Brazilian list

Published datasets from Sepetiba Bay (Damasceno et al., 2024) and Guanabara Bay (Nunes et al., 2023) were used to test the Brazilian list developed in the present work based on the datasets presented in Appendix A. Note that these two datasets from Sepetiba Bay and Guanabara Bay were

Table 1. Waterbody types in intertidal areas and transitional waters, according to McLusky and Elliott (2007). The “artificial waterbody” type (European communities, 2000, p. 6) was added to complete the original table.

Waterbody types	Natural features
Classical estuary	Tidally dominated at the seaward part; salinity is notably reduced by freshwater river inputs; riverine dominance landward.
Lentic non-tidal lagoon	Limited exchange with the coastal area through a restricted mouth; separated from the sea by sand or shingle banks, bars, coral, etc.; shallow area, tidal range < 50 cm.
Lentic microtidal lagoon	As above but with a tidal range > 50 cm.
Ria	Drowned river valley, some freshwater inputs; limited exchanges with coastal waters.
Delta	Low energy, characteristically shaped, sediment-dominated river mouth area; estuary outflow.
Coastal freshwater/brackish water plume	The outflow of estuary or lagoon, with notably diluted salinity and hence differing biota from the surrounding coast.
Semi-enclosed bay/lagoon	Low energy, notably limited exchange with the open sea waters.
Artificial waterbody	Human activities have created harbours and docks, constructed dredging pools, and connected coastal water bodies to the sea.

**Figure 2.** Theoretical behaviour of benthic foraminiferal species from the five ecological groups according to their response to organic carbon enrichment (modified from Dubois et al., 2021).

not used for the species assignments to avoid any circular argument. For the analysis of living benthic foraminifera in Sepetiba Bay and Guanabara Bay, three replicates of sediment (from three independent box-corer deployments) were collected at each site and pooled together. Sediments were sieved on a 63 µm (Guanabara Bay) and 125 µm (Sepetiba Bay) fraction. Then, living foraminifera (rose-bengal-stained) were identified and counted.

Sepetiba Bay is in the Rio de Janeiro State on the south-eastern Atlantic coast of Brazil (Damasceno et al., 2024). The main opening, located between Ilha Grande and the tip of the Marambaia barrier island, connects the bay to the Atlantic Ocean. Sepetiba Bay is one of Brazil's most polluted coastal areas (Kütter et al., 2021). In the surroundings of the bay, there is the largest steelworks complex of Brazil; the highway known as the metropolitan arch of Rio de Janeiro State; the Santa Cruz air base; and three ports, including the harbour of Sepetiba/Itaguaí, which handles ≈ 51.7 million tonnes of iron ore per year. In addition, intense industrial discharge and

domestic effluents occur in the area. Altogether, these anthropogenic activities have led to the contamination of the sediments and living organisms with potentially toxic elements (PTEs) (Ribeiro et al., 2015; Souza et al., 2021a). To investigate the foraminiferal response to environmental stress and to evaluate the EcoQS, 16 stations were sampled in Sepetiba Bay in May 2022 (Fig. 3). Environmental and foraminiferal data were published in Damasceno et al. (2024).

Guanabara Bay (Nunes et al., 2023) is located in the metropolitan region of Rio de Janeiro State (Fig. 4). Due to the massive presence of large urban centres and industries, the bay has experienced an enormous anthropogenic pressure (IBGE, 2014), which today represents a great environmental concern. The discharge of organic matter (OM), persistent organic pollutants, and heavy or trace metals has exerted significant pressures on this ecosystem (Baptista Neto et al., 2000, 2006, 2013). A total of 33 stations were sampled in the bay in the summer of 2018 (Fig. 4). Environmental and foraminiferal data were published by Nunes et al. (2023).

2.4 Calculation of Foram-AMBI

Foram-AMBI was applied to Sepetiba Bay and Guanabara Bay to evaluate the EcoQS. The foraminifera-specific criteria to assess EcoQS with Foram-AMBI are provided in Table 2 (Parent et al., 2021). If the count of living foraminifera assigned to EGs falls below 50 and/or the relative abundance of non-assigned species exceeds 20 %, the index was not calculated to prevent any biased estimation of the EcoQS (Borja and Muxika, 2005). The Foram-AMBI calculation follows the formula of Borja et al. (2000):

$$\text{Foram-AMBI} = [(0 \times \% \text{EGI}) + (1.5 \times \% \text{EGII}) + (3 \times \% \text{EGIII}) + (4.5 \times \% \text{EGIV}) + (6 \times \% \text{EGV})] / 100. \quad (1)$$

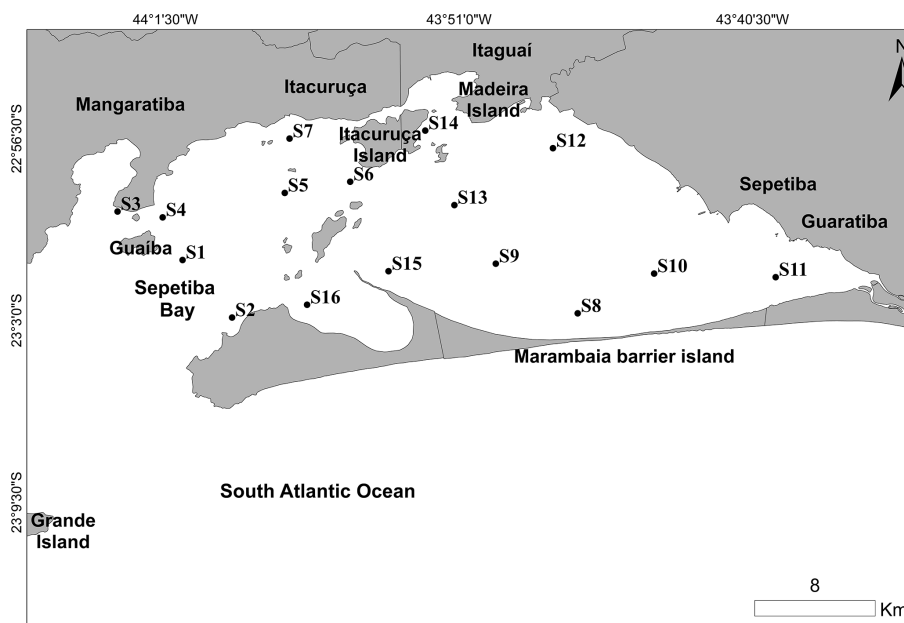


Figure 3. Sampling stations in Sepetiba Bay (modified after Damasceno et al., 2024).

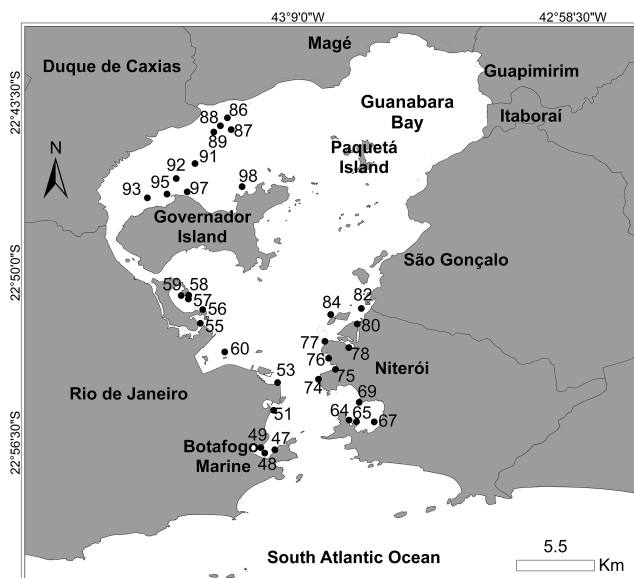


Figure 4. Sampling stations in Guanabara Bay (modified after Nunes et al., 2023).

To evaluate the relevance of developing a regional species list, we computed Foram-AMBI on the Sepetiba Bay and Guanabara Bay datasets with the Brazilian and European TW Atlantic lists. We also computed Foram-AMBI with both lists on two European datasets: one from the French Atlantic coast in southwestern France in the Pertuis Charentais (Bouchet, 2007; Bouchet and Sauriau, 2008) and another from the harbour of Cagliari along the Sardinian coast in Italy (Schintu et

al., 2016). The European Atlantic list, based on foraminiferal datasets from sites in the English Channel and other areas of the European Atlantic coast, reflects the ecology of species from the European TWs (Bouchet et al., 2021).

2.5 Statistical analyses

The weighted-averaging (WA) optimum and tolerance were computed for each species to determine its preference with respect to TOC (%) using the Analogue R package (Simpson and Oksanen, 2021). Since no criteria exist in the literature to infer EcoQS from TOC in Brazilian transitional areas, we used the ones from Bakke et al. (2010) and Viaroli et al. (2004) to assign foraminiferal species to EGs following the same procedure as in Bouchet et al. (2021). After optimum calculation with the WA method, species assignment to EGs was done as follows: if the species had an optimum in the TOC range of 0 %–2 %, it was assigned to EGI; in the range of 2 %–2.5 %, it was assigned to EGII; in the range of 2.5 %–3.4 %, it was assigned to EGIII; in the range of 3.4 %–4.1 %, it was assigned to EGIV; and above 4.1 %, it was assigned to EGV. This is based on TOC-derived EcoQS (see Table 2; Bakke et al., 2010; Viaroli et al., 2004). The outcome of this work is a list of species assigned to one of the EGs.

To further illustrate the classification, a typical example for each EG is shown to characterise species response along the TOC gradient. A locally weighted scatterplot smooth line (LOESS) was fitted to each scatterplot. Marginal plots were added to each scatterplot to show the frequency of distribution of occurrences along the TOC gradient. The median of the distribution of the occurrences was also computed.

Table 2. Criteria for determining the ecological quality status (EcoQS) according to Foram-AMBI (Parent et al., 2021) and with sediment TOC content (Bakke et al., 2010; Viaroli et al., 2004).

EcoQS	Bad	Poor	Moderate	Good	High
Foram-AMBI	> 4.4	3.4–4.4	2.4–3.4	1.4–2.4	< 1.4
Total organic carbon (%)	> 4.1	3.4–4.1	2.5–3.4	2.0–2.5	< 2.0

We then compared the agreement between the EcoQS assessment with $\exp(H'_{bc})$ (Nunes et al., 2023) and the one obtained with Foram-AMBI in Guanabara Bay in the present study. The contingency table was analysed using Cohen's kappa coefficient (Landis and Koch, 1977) from the package “irr” (Heinzel and Leisch, 2022). Two EcoQS categories (i.e. “acceptable” or “not acceptable”) were considered, where “acceptable” results from “High” or “Good” EcoQS and “not acceptable” results from “Moderate”, “Poor”, or “Bad” EcoQS (Blanchet et al., 2008; Bouchet and Sauriau, 2008). The following classification was used: (1) full agreement when both indices precisely identify the same EcoQS class, (2) partial agreement (i.e. different classes but the same category, namely acceptable and not acceptable), and (3) full disagreement when the two indices provide a different category in the EcoQS assessment.

Since data do not fit a normal distribution (Shapiro–Wilk test, $p < 0.05$), the correlations between Foram-AMBI and environmental parameters were investigated using nonparametric Kendall's coefficient of rank correlation (τ). Kendall's coefficient of correlation was used instead of Spearman's coefficient of correlation (ρ) because Spearman's ρ provides greater weight to pairs of ranks that are further apart, while Kendall's τ weights each disagreement in rank equally (Sokal and Rohlf, 1995).

3 Results

3.1 Foraminiferal species from southwestern Atlantic TW assignments to Foram-AMBI EGs

A total of 95 species from Brazilian and Uruguayan TWs were assigned: 77 in the sensitive EGI, 4 in the indifferent EGII, 5 in the tolerant EGIII, 6 in the second-order opportunistic EGIV, and 3 in the first-order opportunistic EGV (Fig. 5, Table B1). Figure 6 presents a typical example of species response curves for each of the five EGs, i.e. *Buliminella elegantissima* (EGI), *Ammonia parkinsoniana* (EGII), *Ammonia tepida* (EGIII), *Quinqueloculina seminulum* (EGIV), and *Criboelphidium gunteri* (EGV).

3.2 Test of the Brazilian TW list on independent datasets

Based on the Brazilian list (Fig. 7a), stations at the entrance of Sepetiba Bay had a High/Good EcoQS (S01, S02, S05, and S06). Conversely, stations from the inner part of the bay

were all in a Good EcoQS, except S13, which exhibited a High EcoQS. Foram-AMBI based on the Brazilian TW list significantly correlated with Cd ($p < 0.001$), silt + clay, Hg, Pb, Zn ($p < 0.01$), TOC, As, and Cr ($p < 0.05$; Fig. C1).

Based on the Brazilian list, 7 stations in Guanabara Bay had a High EcoQS, 21 had a Good EcoQS, 2 had a Moderate EcoQS, and 3 had a Poor EcoQS (Fig. 7b). Furthermore, Foram-AMBI computed with the Brazilian TW list significantly correlated with TOC, Zn in organic matter, total PTEs (PTEs.T), PTEs in organic matter (PTEs.OM), mud ($p < 0.01$; Fig. C2), and PTEs in Mn (PTEs.Mn) ($p < 0.05$; Fig. C2).

3.3 Comparison between Foram-AMBI and $\exp(H'_{bc})$ in Guanabara Bay

In Guanabara Bay, the Foram-AMBI values calculated with the Brazilian list were significantly correlated with $\exp(H'_{bc})$ according to Kendall's coefficient ($p < 0.05$; Fig. C2). However, the comparison of EcoQS values obtained in the present study – adopting Foram-AMBI criteria from Parent et al. (2021) – with those obtained with $\exp(H'_{bc})$ in Nunes et al. (2023) showed a slight level of agreement of only about 36 %, with 18 % full agreement, 18 % partial agreement, and 64 % disagreement (Fig. 8a). The Cohen's Kappa results ($p = 0.078$) suggest that the low observed agreement is random.

Given this rather low agreement, an attempt was made to intercalibrate EcoQS between $\exp(H'_{bc})$ and Foram-AMBI to establish specific criteria for Brazilian TWs for the latter. A linear regression plot between the two indices was created, and, considering the good agreement of $\exp(H'_{bc})$'s EcoQS with the environmental conditions in Guanabara Bay (Nunes et al., 2023), limits between EcoQS for Foram-AMBI were calibrated according to those of $\exp(H'_{bc})$ (Fig. D1). The new criteria are presented in Table 3, and the updated EcoQS is presented in Fig. 7c and d for Sepetiba Bay and Guanabara Bay, respectively.

According to the EcoQS established with the new Foram-AMBI criteria (Fig. 7c and d), a fair and statistically significant level of agreement of 70 % was obtained between Foram-AMBI and $\exp(H'_{bc})$, with 36 % full agreement, 33 % partial agreement, and 30 % disagreement ($p < 0.05$; Fig. 8b). These results, based on the Brazilian TW list, were better than the ones obtained comparing $\exp(H'_{bc})$ with Foram-AMBI calculated with the Atlantic TW European list,

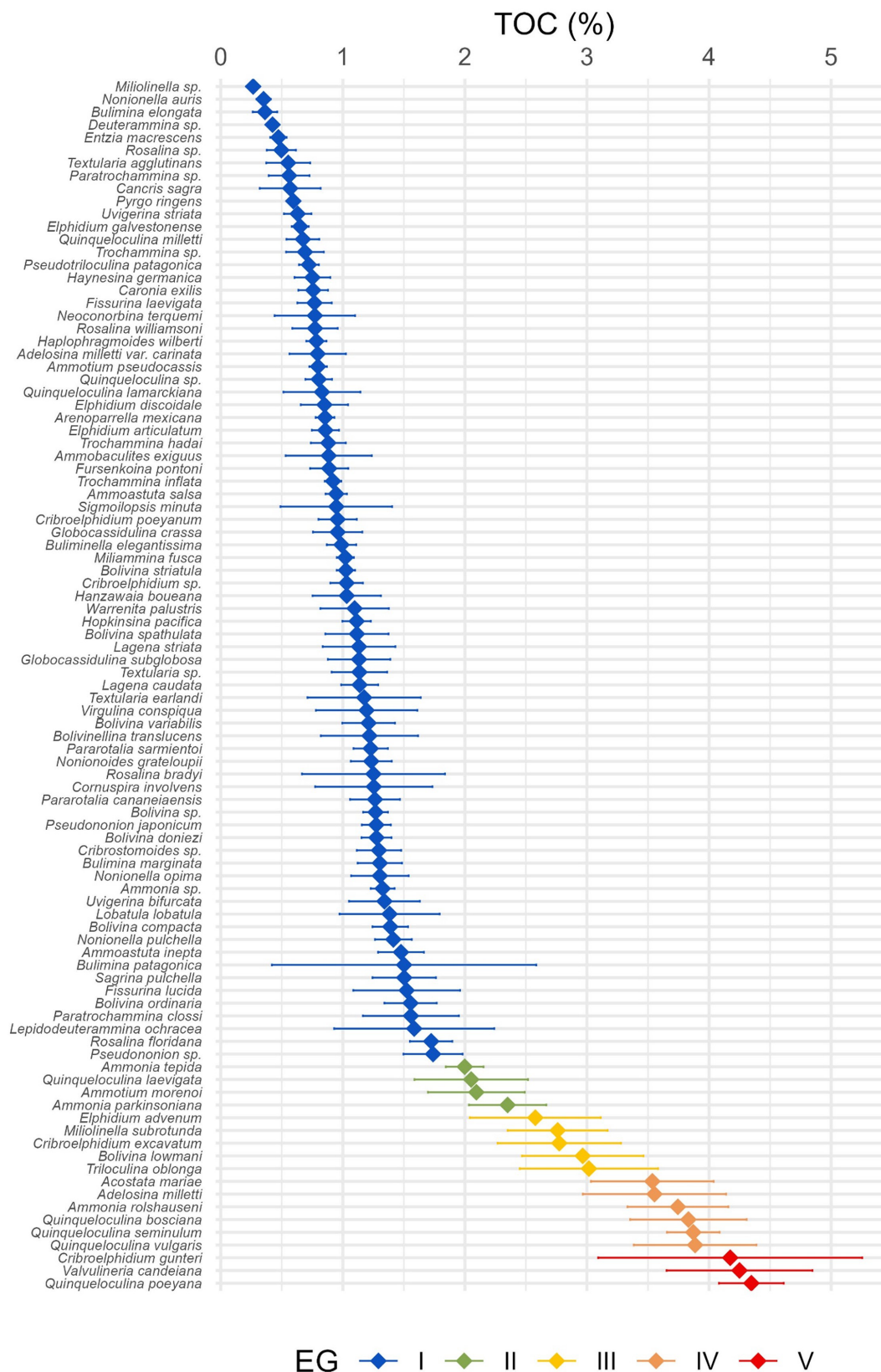


Figure 5. Caterpillar plot of the Brazilian species list: species optima and tolerance interval against TOC and their assignment to EGs.

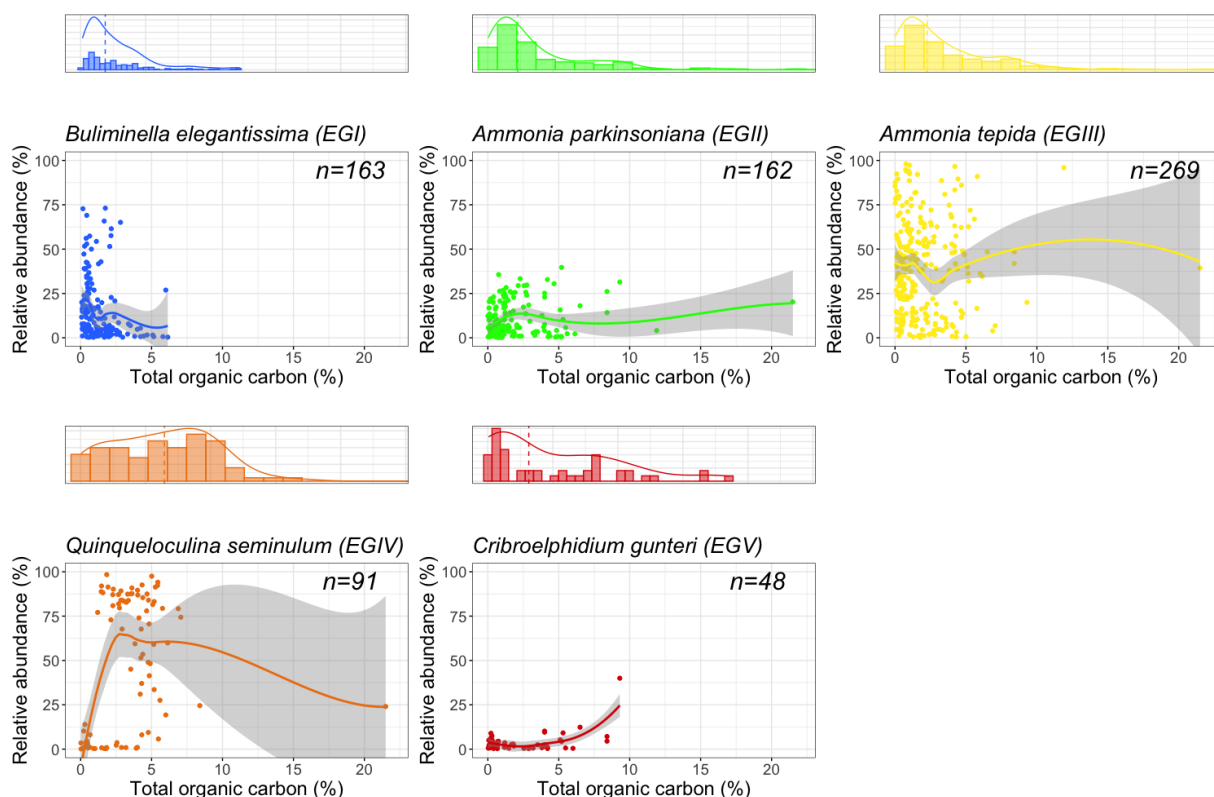


Figure 6. Scatterplots fitted with a locally weighted scatterplot smooth line (LOESS) to visualise species response patterns along the TOC (%) gradient. Typical responses are given for each of the five Foram-AMBI EGs from southwestern Atlantic coastal waters and TWs (the shaded area is the 95 % confidence interval). *Buliminella elegantissima* (EGI), *Ammonia parkinsoniana* (EGII), *Ammonia tepida* (EGIII), *Quinqueloculina seminulum* (EGIV), and *Criboelphidium gunteri* (EGV). Marginal plots show the frequency distribution of occurrences along the TOC gradient; the dashed line marks the median.

Table 3. New criteria for determining the ecological quality status (EcoQS) according to Foram-AMBI in Brazilian TWs.

EcoQS	Bad	Poor	Moderate	Good	High
Foram-AMBI	> 4	3–4	1.8–3	1.4–1.8	< 1.4

with a level of agreement of 64 %, with 15 % full agreement, 49 % partial agreement, and 36 % disagreement (Nunes et al., 2023).

3.4 Comparison between the Brazilian and the European Atlantic lists

The Brazilian list shares 29 species with the European TW Atlantic list. Except for 7 species (*Ammonia parkinsoniana*, *A. tepida*, *Ammotium morenoi*, *Arenoporella mexicana*, *Criboelphidium poeyanum*, *Elphidium advenum*, and *Lepidodeuteraochraea*), assignments in EGs differ between the two lists (Table 4).

Conversely to Foram-AMBI calculated with the Brazilian TW list, in Sepetiba Bay, Foram-AMBI was not correlated to any environmental parameter when using the European TW list ($p > 0.05$; Fig. C1). Note that, with the European TW

list, about 44 % of the stations had more than 20 % of species not assigned to an EG (only 12 % with the South American list). In Guanabara Bay, with the European Atlantic list, Foram-AMBI was only significantly correlated with TOC and Zn.OM, PTEs.OM, and mud ($p < 0.05$; Fig. C2). Note that correlations were weaker with the European TW list than with the Brazilian one.

When applying the Brazilian list to foraminiferal datasets from European sites, we did not find any significant correlation between Foram-AMBI and TOC in the Pertuis Charentais ($p > 0.05$) and in the harbour of Cagliari ($p > 0.05$), while it was significantly correlated with the European Atlantic list in the Pertuis Charentais ($p < 0.05$) and with the European Mediterranean list in Cagliari ($p < 0.01$). Note that, in the harbour of Cagliari, about 62 % of the sta-

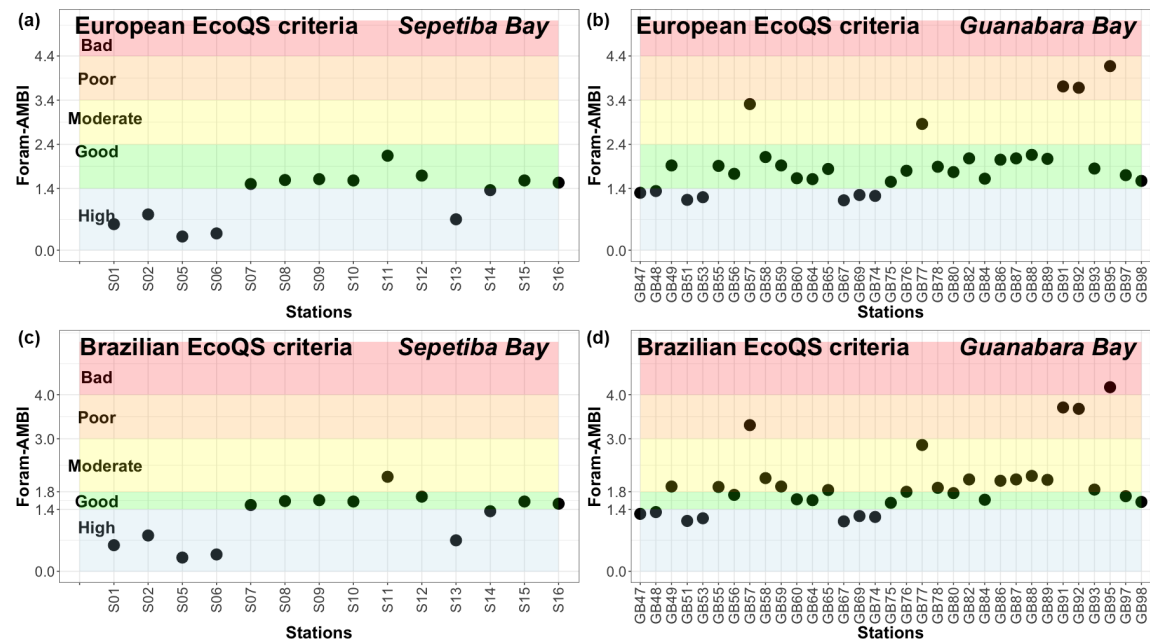


Figure 7. Foram-AMBI calculated on independent datasets with the South American list and EcoQS using Parent et al. (2021) criteria for the (a) Sepetiba Bay (Damasceno et al., 2024) and (b) Guanabara Bay lists (Nunes et al., 2023) and EcoQS using criteria developed in the present study for Brazilian TWs for (c) Sepetiba Bay and (d) Guanabara Bay.

A		Exp(H'_{bc})^*					
		NOT ACCEPTABLE			ACCEPTABLE		
		BAD	POOR	MODERATE	GOOD	HIGH	
Foram-AMBI_{EU}	NOT ACCEPTABLE	BAD					
		POOR	3	6			
		MODERATE	3		3		
	ACCEPTABLE	GOOD	15	21	18		9
		HIGH		3	6	3	9
Percentage agreement acceptable x not acceptable = 36.4 % Exp(H'_{bc})_{5 cl} x Foram-AMBI-EU_{5 cl} = 18.2%							
Cohen's Kappa (unweighted) Kappa_{accpt x not accpt} = 0.09 (p_{value} = 0.20) (slight agreement; may be random) Kappa_{Exp(H'_{bc}) x Foram-AMBI-EU} = 0.09 (p_{value} = 0.08) (slight agreement; may be random)							

B		Exp(H'_{bc})^*					
		NOT ACCEPTABLE			ACCEPTABLE		
		BAD	POOR	MODERATE	GOOD	HIGH	
Foram-AMBI_{BR}	NOT ACCEPTABLE	BAD	3				
		POOR	3	6			
		MODERATE	6	15	18		3
	ACCEPTABLE	GOOD	9	6	3		6
		HIGH		3	6	3	9
Percentage agreement acceptable x not acceptable = 69.7 % Exp(H'_{bc})_{5 cl} x Foram-AMBI-BR_{5 cl} = 36.4%							
Cohen's Kappa (unweighted) Kappa_{accpt x not accpt} = 0.36 (p_{value} = 0.02) (fair agreement; <i>not random</i>) Kappa_{Exp(H'_{bc}) x Foram-AMBI-BR} = 0.21 (p_{value} = 0.01) (fair agreement; <i>not random</i>)							

full agreement

partial agreement

full disagreement

* Nunes et al. 2023

Figure 8. The contingency table and Cohen's Kappa analysis results for the comparison of EcoQS obtained in Guanabara Bay with $\exp(H'_{bc})$ and Foram-AMBI with the Brazilian list (the present work). (a) With Foram-AMBI criteria from Parent et al. (2021) and (b) with Foram-AMBI criteria established in the present work.

Table 4. Comparison of EG assignments for species found in both the European TWs and the Brazilian TWs. NA: not assigned

Species	EG Brazilian TW list (this study)	EG European Atlantic TWs (Bouchet et al., 2021)
<i>Ammonia parkinsoniana</i>	II	II
<i>Ammonia tepida</i>	III	III
<i>Ammotium morenoi</i>	II	II
<i>Arenoparrella mexicana</i>	I	I
<i>Bolivina ordinaria</i>	I	II
<i>Bolivina spathulata</i>	I	III
<i>Bolivina striatula</i>	I	V
<i>Bolivina variabilis</i>	I	III
<i>Bolivinellina translucens</i>	I	III
<i>Bulimina elongata</i>	I	IV
<i>Bulimina marginata</i>	I	IV
<i>Buliminella elegantissima</i>	I	III
<i>Cornuspira involvens</i>	I	IV
<i>Criboelphidium excavatum</i>	III	I
<i>Criboelphidium gunteri</i>	V	III
<i>Criboelphidium poeyanum</i>	I	I
<i>Elphidium advenum</i>	III	III
<i>Elphidium articulatum</i>	I	V
<i>Entzia macrescens</i>	I	NA
<i>Fusulina lucida</i>	I	V
<i>Haynesina germanica</i>	I	III
<i>Hopkinsina pacifica</i>	I	V
<i>Lepidodeuterammina ochracea</i>	I	I
<i>Miliammina fusca</i>	I	II
<i>Miliolinella subrotunda</i>	III	II
<i>Quinqueloculina seminulum</i>	IV	V
<i>Rosalina bradyi</i>	I	II
<i>Textularia earlandi</i>	I	III
<i>Trochammina inflata</i>	I	NA

tions had more than 20 % of species not assigned to an EG when considering the Brazilian list.

4 Discussion

4.1 ForAMBI performance in Brazilian transitional waters

A key prerequisite for effectively evaluating Brazilian coastal ecosystems with ForAMBI was the assignment of dominant species to one of the five EGs. This study reports species indicator values of 95 foraminiferal species from coastal waters and TWs from Brazil and, for the first time, assigns them to one of the five EGs used in ForAMBI. This work contributes to the ongoing work aimed at acknowledging benthic foraminifera as an official BQE (Alve et al., 2016). Furthermore, the Brazilian list complements the five existing lists: those established for the Northeast Atlantic and Arctic fjords, continental shelves, and slopes (Alve et al., 2016); for open environments in the Mediterranean Sea (Jorissen et al., 2018); for the English Channel and European Atlantic TWs (Bouchet et al., 2021); for the TWs in the Mediterranean Sea

(Bouchet et al., 2021); and for the Gulf of Mexico (O'Malley et al., 2021).

The Brazilian list was tested on two independent datasets from Sepetiba Bay and Guanabara Bay to assess EcoQS with ForAMBI. Although we had significant correlations between ForAMBI and abiotic parameters, the EcoQS values were not in accordance with the known pollution in both bays. The criteria used for TOC and EcoQS were developed in and for TWs in Europe. Although recent works in Brazilian TWs have reported accurate biomonitoring with benthic foraminifera using these criteria (Nunes et al., 2023; Filippos et al., 2023), the use of local reference, hence local criteria, should be prioritised according to marine legislation. In Europe, the criteria to define EcoQS with the diversity index $\exp(H'_{bc})$ were adapted to open environment (Bouchet et al., 2012) and TWs (Bouchet et al., 2018a), and the same was done for ForAMBI by calibrating the values used for macro-invertebrates to benthic foraminifera in open marine environments in the Mediterranean Sea (Parent et al., 2021). The discrepancies observed between EcoQS derived from ForAMBI with Parent et al. (2021) criteria and abi-

otic and between $\exp(H'_{bc})$ and ForAMBI EcoQS assessment in the tested dataset from Guanabara Bay in terms of EcoQS actually highlight the fact that the criteria developed in Europe are not adapted for Brazilian TWs.

The EcoQS derived from ForAMBI with the new criteria developed in this study better reflect the high disturbances from human activities in these two ecosystems. There is a remarkable accumulation of metals in sediments in Sepetiba Bay (Ribeiro et al., 2015; Souza et al., 2021a), while Guanabara Bay exhibits a substantial anthropogenic impact from domestic sewage, industries, and oil spills (Baptista Neto et al., 2000, 2006, 2013). In Sepetiba Bay, the gradient from high to moderate EcoQS illustrates the intense pollution occurring in its inner part (Ribeiro et al., 2015; Damasceno et al., 2024). Furthermore, the moderate to poor EcoQS values in Guanabara Bay confirm the deleterious impact of pollution on the benthic habitats of this blue carbon ecosystem (Cotovicz et al., 2015). Significant correlations were also reported between ForAMBI and environmental parameters (e.g. TOC and metals). These results support the use of the list established in the present work and also confirm the reliability of ForAMBI for the evaluation of the environmental health in coastal and transitional areas in Brazil. The results of the tests showed that the WA optimum method allows an accurate assessment of the ecological requirement of each species along the TOC gradient as reported for European species from TWs (Bouchet et al., 2021). In Brazil, more tests on other independent datasets are, nevertheless, needed to further validate the findings of this work and most likely to improve the present study's list of species.

Furthermore, a significant correlation of ForAMBI with $\exp(H'_{bc})$ in Guanabara Bay, together with congruent EcoQS, is reported. The $\exp(H'_{bc})$ is known to be an accurate diversity index that can be used to monitor the health of benthic ecosystems in Brazil (Jesus et al., 2020; Filippou et al., 2023). However, in estuaries where diversity is naturally low, the use of $\exp(H'_{bc})$ may lead to a wrong EcoQS assessment (Fouet et al., 2022). It may also happen in oligotrophic areas (Barras et al., 2014; Dubois et al., 2021). The difficulties of interpreting diversity indices and associated EcoQS values have also been discussed for macro-invertebrates (Labruno et al., 2006; Blanchet et al., 2008; Lavesque et al., 2009). This may be explained by the lack of ecological considerations in diversity indices (Bouchet and Sauriau, 2008). Specifically, $\exp(H'_{bc})$ evaluates diversity, while ForAMBI is based on species tolerance level. In this context, and based on the present study results in Brazil, ForAMBI may serve, at a minimum, as a complementary index to $\exp(H'_{bc})$ or even as an alternative when the use of $\exp(H'_{bc})$ is not possible because of the natural environmental features of the studied ecosystems. Benthic foraminifera, as represented by the ForAMBI metric, may also complement benthic macro-invertebrates for a more exhaustive assessment of EcoQS, thereby contributing to the development of integrative tools to help the environmental decision-making process (Muniz

et al., 2011). In estuaries where AMBI (macro-invertebrates) fails to evaluate EcoQS (Valença and Santos, 2012), ForAMBI (foraminifera) may actually be prioritised.

The intercalibration of EcoQS boundaries of ForAMBI for Brazilian TWs allows improved assessment of the health of benthic habitats in both bays. However, one may consider that these new criteria were obtained with a rather limited dataset that may not encompass all the natural variability in Brazilian TWs. Hence, future works should focus on testing these new criteria and their accuracy and reliability in other TWs in Brazil.

4.2 Regionally specific lists are recommended

In their paper devoted to macro-invertebrate indicator species, Zettler et al. (2013) stressed that “the use of fixed reference lists needs to be reconsidered, especially in areas with strong salinity gradients, like estuaries or the Baltic Sea, or eutrophic systems like Mediterranean lagoons”. Some species are, indeed, flexible enough to adapt to their environment. Consequently, they may change their autecology requirements along environmental gradients (see Review in Zettler et al., 2013, and references therein). Species even exhibit different responses to disturbance depending on their habitat and the source of disturbances; they behave as sensitive species in some environmental settings, while they can be tolerant or opportunistic somewhere else or when facing a different perturbation (Zettler et al., 2013). The use of a unique sensitivity/tolerance list for different geographical areas (such as in AMBI and comparable methods based on benthic macro-invertebrates) is therefore not recommended (Grémare et al., 2009), since it integrates the ecological requirements from species behaviour over a span of geographical regions and subregions that is too large and not on a local scale. Local adaptation of species' ecological requirements may hence lead to wrong ecological group assignments if only one global species list is used (Dauvin et al., 2010; Zettler et al., 2013). Similar features were recorded for benthic foraminifera (see Review in Alve, 1995).

In the present study, the same assignment in EGs is only obtained for 5 species among the 29 shared between the Brazilian and the European TW Atlantic lists. Similar discrepancies were also observed in Europe, where offsets of up to four categories in foraminiferal species assignments to EGs, depending on habitat type (different water bodies in coastal waters and TWs) or latitudinal gradient, were reported (northern Europe to the Mediterranean Sea; Bouchet et al., 2021). Noticeably, *Haynesina germanica* is a sensitive species in Brazil, while it was assigned to the tolerant group in Europe. This species, introduced in South America (Calvo-Marcilese and Langer, 2010), may be less adapted and therefore more sensitive in subtropical estuaries compared to European settings in its natural range of distribution. Conversely, *Elphidium excavatum* is tolerant in the Brazilian list and sensitive in European TWs. These incongruent

assignments may be explained by (i) different populations with different ecological requirements for TOC content, suggesting plasticity in this species (see Discussion above), or by (ii) the occurrence of cryptic species. The presence of cryptic species in Elphidiidae is well documented (Darling and Wade, 2008), and we may hypothesise that *E. excavatum* from Brazil and Europe belongs to different phylotypes. Hence, the present findings can be the result of different cryptic lineages having different levels of tolerance to TOC. This hypothesis remains highly speculative, since the data used in this work are based on morphological identifications in the absence of any thorough molecular assessment of this species in Brazil and Europe. Phylogeographic studies may help to better distinguish and identify possible cryptic species to describe the distribution pattern of the different phylotypes. A combination of morphological and molecular taxonomy in large environmental surveys would clarify the ecological requirements of different cryptic lineages and help to assign appropriate indicator values to genetically distinct populations. Therefore, ecological studies on cryptic species in relation to the indicative value of species present a major topic for further scientific research projects. Indeed, a recent work on three phylotypes of *Ammonia* spp. in Europe suggested that they actually have different ecological requirements (Pavard et al., 2023).

The comparison between the Brazilian list (developed in this work) and the European Atlantic and Mediterranean TW lists (Bouchet et al., 2021) also provides compelling evidence that reliable ecological assessments can only be achieved when the local conditions are considered. In South America, the European Atlantic TW list was far less accurate than the Brazilian list to evaluate the EcoQS. In Europe, we have a significant correlation between Foram-AMBI and TOC only with the European TW lists and not with the Brazilian ones. The Brazilian list was particularly poorly adapted to most stations in the Mediterranean Sea in Europe, since more than 20 % of species were not assigned to an EG, leading to a non-robust and unreliable assessment of EcoQS (Borja and Muxika, 2005). The Mediterranean Sea encompasses completely different, mostly oligotrophic ecosystems, leading to different species responses to TOC compared to the more organic-rich TWs of the Atlantic and Brazilian coasts (Angel et al., 2000; Dubois et al., 2021; Hyams-Kazphan et al., 2009). By applying the European TW Atlantic list in Brazilian coastal habitats and the Brazilian list in European habitats, both lists show poor accuracy outside their biogeographic area. The same results were reported for benthic macro-invertebrates, and it was stressed that more studies are needed in Brazil to adjust local macro-invertebrate species classification in EGs to improve AMBI's performance (Checon et al., 2018; Muniz et al., 2005; Brauko et al., 2016). The present study, therefore, confirms the importance of validating EG classifications when transferring and applying them overseas, as previously observed for benthic macro-invertebrates (Aguado-Giménez et al., 2007; Borja

and Muxika, 2005; Keeley et al., 2012). A joint research effort is therefore necessary in order to recognise regional differences in species pool and ecological tolerances.

4.3 Limitations of the study

Various methods were used in the different selected studies to measure organic matter, from the old-fashioned loss on ignition (LOI) up to the more sophisticated use of an elemental analyser (EA). While the estimation by the latter is more accurate, the LOI is still largely used in large surveys because it allows a quick and cheap measurement of OM in sediments (Luczak et al., 1997). It is well acknowledged that the LOI may overestimate OM content compared to the EA (Barillé-Boyer et al., 2003); hence, OM values had to be converted to TOC. As a result, the TOC and the OM data from the different studies may be compared with caution. Although it may have introduced a bias in our species assignment, the problem is probably fairly limited, as previously suggested (Bouchet et al., 2021). Indeed, the selected sites yield a wide range of environmental conditions, ensuring that our dataset is representative of the natural variability occurring in transitional waters along the Brazilian coasts. The inclusion of datasets from hypersaline lagoons such as Vermelha Lagoon (Laut et al., 2017, 2022) may be questioned, as they represent a peculiar type of transitional ecosystem. Noticeably, they present harsh conditions for benthic foraminifera due to elevated and highly variable salinity and temperature and frequent hypoxia in bottom waters (Stal, 2012). These systems commonly contain fine, organic-rich sediments that promote the buildup of toxic byproducts such as hydrogen sulfide (Stal, 2012). Limited water exchange further amplifies these stressors, creating a selective environment where only a few opportunistic or euryhaline species can persist. Future works may explore if specific assignments should take place for foraminiferal species thriving in these peculiar lagoons.

The definition of a foraminiferal species list for the use of biotic indices in biomonitoring is based on the paradigm that TOC is one of the main constraining drivers of species distribution patterns in TWs (see Review in O'Brien et al., 2021). A further environmental parameter constraining foraminiferal species distribution is sediment grain size. In TWs, silt, clay, and OM, sedimentary contents are naturally high. The complex interplay between sediment grain size and TOC with foraminiferal species is complex. In their work in marginal environments from the Skagerrak and the Kattegat, Alve and Murray (1999) did not observe any clear trend in foraminiferal assemblage distribution patterns, TOC, and grain size. However, in the Canche estuary in the English Channel, both abiotic factors constrained foraminiferal assemblages, with sediment grain size being the limiting factor (Armynot du Châtelet et al., 2009). The fact that TOC in TWs may reach very high values, somewhat higher than in polluted environments, is due purely to the development of

healthy natural vegetation and associated fauna developing within that environment (Armynot du Châtelet et al., 2018). Commonly, OM is more lignified and not labile (Armynot du Châtelet et al., 2009) and may not be regarded as a stress for benthic foraminifera, explaining why sediment grain size is usually the most limiting factor. In fact, TOC only informs about the reservoir of organic carbon, but it does not give any information about the quality and the origin of organic matter (Pusceddu et al., 2003). Sediment grain size also reflects the hydrodynamic regime and is linked with variations in associated environmental parameters, such as nutrients (OM quality), pollutant accumulation, oxygen, and active biogeochemical reactions on the sea floor, to which foraminifera react (Martins et al., 2015). Hence, several natural stressors must be considered when evaluating species' response to TOC. Noticeably, a species' tolerance to a stressor or a cocktail of stressors of human origin depends on whether the prevailing natural environmental conditions fall well within its ecological requirements or lie near the margins of its ecological niche (Alve, 1995). A taxon may exhibit greater resilience on anthropogenic stressors when the surrounding environment is close to its optimal conditions, compared to situations where it is already near the limits of its natural distribution. To summarise, the natural environmental features of a studied site may be just as important as the type of pollution in determining which sensitive, tolerant, or opportunistic species dominate in the foraminiferal assemblage (Alve, 1995).

In the present study, different size fractions are used to monitor EcoQS in Sepetiba Bay and Guanabara Bay, i.e. $> 125 \mu\text{m}$ in the former and $> 63 \mu\text{m}$ in the latter. In Norwegian fjords, a recent study tested the effect of the studied foraminiferal size fraction $> 63 \mu\text{m}$ versus $> 125 \mu\text{m}$ on the EcoQS assignment with different biotic indices, i.e. Shannon index, Hurlbert rarefaction index, Foramin-AMBI, and Norwegian Quality Index (NQI; Klootwijk and Alve, 2022). The different size fractions had similar EcoQS values; hence they tended not to influence the Foramin-AMBI results, for instance. Although we did not test it in Brazilian transitional ecosystems, we assume from the results of the Norwegian example that the different size fractions in Sepetiba Bay and Guanabara Bay did not introduce a bias in our conclusions. This should, however, be further tested in future works.

Last but not least, it is true that different taxonomical schools co-exist in Brazil, like everywhere else. This may hence lead to some inconsistencies between research groups in terms of species identification. Previous studies stressed this issue by assigning benthic foraminifera in EGs (Alve et al., 2016; Jorissen et al., 2018; Bouchet et al., 2021). Hence, there is an urgent need to start organising taxonomical workshops to intercalibrate the different taxonomical schools. For instance, the French researchers recently met for a taxonomical workshop, and, using morphological and molecular assessment, they homogenised their taxonomy on benthic foraminifera from French TWs. The outcome of this effort is

a taxonomical guide (Jorissen et al., 2023) which serves as a reference at the French level and could serve as a basis to homogenise the taxonomy of foraminiferal species at Brazilian and international scales.

5 Conclusion

This work represents the first attempt to assign foraminiferal species to EGs from the southwestern Atlantic transitional waters and to investigate the potential applicability of Foramin-AMBI using a regional list. This list is a key step towards improving biomonitoring surveys conducted in Brazil with benthic foraminifera. This may contribute to this group being regarded as an official BQE by the National Environment Council in Brazil. In this study, we reveal differences in the classification of EGs between species occurring in Brazil and in Europe. The overall pattern of EcoQS at the sites analysed would be different depending on the assignment of the selected species/taxa. For instance, if these taxa were submitted to the European list without modification of their EG, most sites would result in a different EcoQS. Hence, the outcome of the present work strongly supports the rationale for (i) developing regional lists for Foramin-AMBI and (ii) establishing local criteria for EcoQS assessment. Our results further show that Foramin-AMBI is robust in detecting the effects of different contaminants in the area and reinforce the importance of the index as a tool for coastal management.

To summarise, these results point in a promising direction. However, despite the encouraging findings of this study, they do not yet justify the straightforward implementation of Foramin-AMBI in Brazilian TWs. To thoroughly assess the effectiveness of Foramin-AMBI, we require further studies across a broader range of pollution gradients and to thoroughly consider the natural variability in Brazilian TWs that lie from the equatorial/tropical to the early temperate zones of the Southern Hemisphere. As in the case of earlier studies assigning benthic foraminifera to EGs in Europe, we anticipate that future applications of the Brazilian lists on SW Atlantic coasts will lead to regular updates. This would greatly improve the ecological group classifications in the area, thereby increasing Foramin-AMBI effectiveness and providing an important tool to monitor and preserve Brazilian coastal ecosystems.

Appendix A

Table A1. Datasets from the southwestern Atlantic coastal waters and TWs used for species assignments. The location of the different areas is shown in Fig. 2. TOC: total organic carbon.

Dataset	Region	Country	Latitude (S)	Longitude (W)	Waterbody type	Reference	Number of samples	Foram size fraction	Method to analyse organic matter			Grain size	
									TOC method	Type	Range (%)	Sand (%)	Mud (%)
1	Montevideo	Uruguay	34°53′59.4″	56°13′53.0″	Seawater/brackish water plume	Burone et al. (2006)	24	0.500, 0.250 and 0.062 mm	Mass loss on ignition at 500 °C	OM	3.5–12.8	2.1–25.5	74.5–97.9
2	South Bay, Florianópolis	Brazil	27°43′13.4″	48°36′01.7″	Seawater/brackish water plume	Rudorff et al. (2012)	16	0.063 mm	Mass loss on ignition at 500 °C	OM	0.5–5.0	28–86	14–72
3	Paranaguá Bay	Brazil	25°27′11.2″	48°22′42.6″	Seawater/brackish water plume	Disaró unpublished	–	> 63 µm	Mass loss on ignition at 500 °C	OM	1.8–4.1	ND	ND
4	Flamengo Inlet	Brazil	23°30′26.4″	45°05′49.8″	Seawater/brackish water plume	Eichler et al. (2018)	18	0.500 and 0.063 mm	Mass loss on ignition at 500 °C	OM	2.0–10.0	4.5–86.6	13.4–94.5
5	Flamengo Inlet	Brazil	23°30′27″	45°05′50″	Seawater/brackish water plume	Rodrigues et al. (2014)	34	0.250 and 0.063 mm	Mass loss on ignition at 500 °C	OM	1.47–11.56	4.5–86.6	13.4–94.5
6	São Sebastião Channel	Brazil	23°49′03.7″	45°24′19.6″	Coastal freshwater/brackish water plume	Duleba et al. (2018)	20	0.500 and 0.063 mm	CHN analyser (LECO CNS 2000)	TOC	0.39–2.7	0.7–85.5	10.5–87.6
7	Ubatuba Bay	Brazil	23°26′21.7″	45°03′24.6″	Seawater/brackish water plume	Burone and Pres-Vamin (2006)	4	0.500, 0.250 and 0.062 mm	CHN analyser (LECO CNS 2000)	TOC	0.1–1.44	19–95.9	4.1–81
8	Cigarras Beach	Brazil	23°43′48.9″	45°23′57.4″	Seawater/brackish water plume	Filippos et al. (2023)	20	> 63 µm	CHN analyser (LECO CNS 2000)	TOC	0.50–2.70	29–77	23–69
9	Guanabara Bay	Brazil	22°45′53.4″	43°05′18.5″	Seawater/brackish water plume	Martins et al. (2020)	44	125–500 µm	CHN analyser (LECO CNS 2000)	TOC	1.0–6.1	0.4–73.1	19.8–88.6
10	Itaipu Lagoon	Brazil	22°57′37.4″	43°02′29.0″	Seawater/brackish water plume	Raposo et al. (2018)	12	0.500 and 0.063 mm	CHN analyser (LECO SC 144)	TOC	0.22–6.00	1.3–100	0–98.7
11	Saquarema Lagoon	Brazil	22°54′57.9″	42°33′35.1″	Seawater/brackish water plume	Belart et al. (2018)	22	0.500 and 0.063 mm	CHN analyser (LECO SC 144)	TOC	0.09–21.5	1.5–100	0–98.5
12	Vermelha Lagoon	Brazil	22°55′48.5″	42°23′07.8″	Hypersaline lagoon	Laut et al. (2022)	56	0.500 and 0.063 mm	CHN analyser (LECO SC 144)	TOC	0.28–7.10	ND	ND
13	Almada Estuary	Brazil	14°46′39.9″	39°4′22.0″	Classical estuary	Laut et al. (2021)	6	0.500 and 0.063 mm	CHN analyser (LECO SC 144)	TOC	0–0.345	87.1–99.6	0.4–12.9
14	Cachoeira Estuary	Brazil	14°45′00.0″	39°01′00.0″	Classical estuary	Raposo et al. (2022)	30	0.500 and 0.063 mm	CHN analyser (LECO SC 144)	TOC	0.01–0.05	0–99.8	0.2–100

Appendix B

Table B1. Assigned species from the Brazilian transitional waters per ecological group (EG). For each species, the accepted scientific name, the AphiaID (source: WoRMS), and the optimum and tolerance range of TOC are also reported.

Accepted scientific name	Accepted AphiaID	Optimum	Tolerance –	Tolerance +	EG	Accepted scientific name	Accepted AphiaID	Optimum	Tolerance –	Tolerance +	EG
<i>Adelosina milleti</i> var. <i>carinata</i>	525779	0.8	0.6	1.0	I	<i>Nonionella opima</i>	113603	1.3	1.1	1.5	I
<i>Ammonoastuta inepta</i>	417584	1.5	1.3	1.7	I	<i>Nonionella pulchella</i>	418050	1.4	1.3	1.6	I
<i>Ammonoastuta salsa</i>	417585	0.9	0.9	1.0	I	<i>Nonionoides grateloupiei</i>	418051	1.2	1.1	1.4	I
<i>Ammobaculites exiguus</i>	417589	0.9	0.5	1.2	I	<i>Pararotalia cananeiaensis</i>	556282	1.3	1.1	1.5	I
<i>Ammonia</i> sp.	112078	1.3	1.2	1.4	I	<i>Pararotalia sarmientoi</i>	1481637	1.2	1.1	1.4	I
<i>Ammotium pseudocassis</i>	736482	0.8	0.7	0.9	I	<i>Paratrochammina clossi</i>	817095	1.6	1.2	2.0	I
<i>Arenoparrella mexicana</i>	417609	0.9	0.8	0.9	I	<i>Paratrochammina</i> sp.	413976	0.6	0.4	0.7	I
<i>Bolivina compacta</i>	112970	1.4	1.2	1.5	I	<i>Pseudononion japonicum</i>	712062	1.3	1.2	1.4	I
<i>Bolivina doniezi</i>	522974	1.3	1.2	1.4	I	<i>Pseudononion</i> sp.	415894	1.7	1.5	2.0	I
<i>Bolivina ordinaria</i>	112978	1.6	1.3	1.8	I	<i>Pseudotriloculina patagonica</i>	492957	0.7	0.6	0.8	I
<i>Bolivina</i> sp.	112101	1.3	1.2	1.4	I	<i>Pyrgo ringens</i>	112597	0.6	0.5	0.6	I
<i>Bolivina spathulata</i>	112988	1.1	0.9	1.4	I	<i>Quinqueloculina lamarciana</i>	112643	0.8	0.5	1.1	I
<i>Bolivina striatula</i>	112989	1.0	1.0	1.1	I	<i>Quinqueloculina milleti</i>	1545320	0.7	0.5	0.8	I
<i>Bolivina variabilis</i>	112998	1.2	1.0	1.4	I	<i>Quinqueloculina</i> sp.	112040	0.8	0.7	0.9	I
<i>Bolivinellina translucens</i>	526512	1.2	0.8	1.6	I	<i>Rosalina bradyi</i>	113167	1.3	0.7	1.8	I
<i>Bulimina elongata</i>	933974	0.4	0.3	0.5	I	<i>Rosalina floridana</i>	113169	1.7	1.6	1.9	I
<i>Bulimina marginata</i>	113042	1.3	1.1	1.5	I	<i>Rosalina</i> sp.	112148	0.5	0.4	0.6	I
<i>Bulimina patagonica</i>	525526	1.5	0.4	2.6	I	<i>Rosalina williamsoni</i>	113182	0.8	0.6	1.0	I
<i>Buliminella elegantissima</i>	113747	1.0	0.9	1.1	I	<i>Sagrina pulchella</i>	417936	1.5	1.2	1.8	I
<i>Cancris sagra</i>	418011	0.6	0.3	0.8	I	<i>Sigmoilopsis minuta</i>	490002	0.9	0.5	1.4	I
<i>Caronia exilis</i>	723130	0.8	0.6	0.9	I	<i>Textularia agglutinans</i>	114264	0.6	0.4	0.7	I
<i>Cornuspira involvens</i>	112488	1.3	0.8	1.7	I	<i>Textularia earlandi</i>	114273	1.2	0.7	1.6	I
<i>Criboelphidium poeyanum</i>	113244	1.0	0.8	1.1	I	<i>Textularia</i> sp.	112394	1.1	0.9	1.4	I
<i>Criboelphidium</i> sp.	112159	1.0	0.9	1.2	I	<i>Trochammina hadai</i>	395080	0.9	0.7	1.0	I
<i>Cribrostomoides</i> sp.	112347	1.3	1.1	1.5	I	<i>Trochammina inflata</i>	114348	0.9	0.9	1.0	I

Table B1. Continued.

Accepted scientific name	Accepted AphiaID	Optimum	Tolerance –	Tolerance +	EG	Accepted scientific name	Accepted AphiaID	Optimum	Tolerance –	Tolerance +	EG
<i>Deuterammina</i> sp.	112401	0.4	0.4	0.5	I	<i>Trochammina</i> sp.	112412	0.7	0.5	0.8	I
<i>Elphidium articulatum</i>	113257	0.9	0.7	1.0	I	<i>Uvigerina bifurcata</i>	113764	1.3	1.1	1.6	I
<i>Elphidium discoidale</i>	418086	0.8	0.7	1.0	I	<i>Uvigerina striata</i>	710260	0.6	0.5	0.7	I
<i>Elphidium galvestonense</i>	582693	0.7	0.6	0.7	I	<i>Virgulina conspicua</i>	1553121	1.2	0.8	1.6	I
<i>Entzia macrescens</i>	742429	0.5	0.4	0.5	I	<i>Warrenita palustris</i>	417567	1.1	0.8	1.4	I
<i>Fissurina laevigata</i>	113205	0.8	0.6	0.9	I	<i>Ammonia parkinsoniana</i>	418095	2.3	2.0	2.7	II
<i>Fissurina lucida</i>	113206	1.5	1.1	2.0	I	<i>Ammonia tepida</i>	112857	2.0	1.8	2.2	II
<i>Fursenkoina pontoni</i>	417967	0.9	0.7	1.0	I	<i>Ammotium morenoi</i>	736481	2.1	1.7	2.5	II
<i>Globocassidulina crassa</i>	397221	1.0	0.8	1.2	I	<i>Quinqueloculina laevigata</i>	908565	2.1	1.6	2.5	II
<i>Globocassidulina subglobosa</i>	113091	1.1	0.9	1.4	I	<i>Bolivina lowmani</i>	112976	3.0	2.5	3.5	III
<i>Hanzawaia boueana</i>	113185	1.0	0.8	1.3	I	<i>Elphidium advenum</i>	1636051	2.6	2.0	3.1	III
<i>Haplophragmoides wilberti</i>	113955	0.8	0.7	0.9	I	<i>Elphidium excavatum</i>	113267	2.8	2.3	3.3	III
<i>Haynesina germanica</i>	113294	0.8	0.6	0.9	I	<i>Miliolinella subrotunda</i>	112564	2.8	2.4	3.2	III
<i>Hopkinsina pacifica</i>	113728	1.1	1.0	1.2	I	<i>Triloculina oblonga</i>	112764	3.0	2.4	3.6	III
<i>Lagena caudata</i>	849890	1.1	1.0	1.3	I	<i>Acostata mariae</i>	732417	3.5	3.0	4.0	IV
<i>Lagena striata</i>	113507	1.1	0.8	1.4	I	<i>Adelosina milleti</i>	1545320	3.6	3.0	4.1	IV
<i>Lepidodeuterammina ochracea</i>	114306	1.6	0.9	2.2	I	<i>Ammonia rolshauseni</i>	418097	3.7	3.3	4.2	IV
<i>Lobatula lobatula</i>	113118	1.4	1.0	1.8	I	<i>Quinqueloculina bosciana</i>	112620	3.8	3.4	4.3	IV
<i>Miliammina fusca</i>	114064	1.0	1.0	1.1	I	<i>Quinqueloculina seminulum</i>	112675	3.9	3.7	4.1	IV
<i>Miliolinella</i> sp.	112028	0.3	0.2	0.3	I	<i>Quinqueloculina vulgaris</i>	112690	3.9	3.4	4.4	IV
<i>Neoconorbina terquemi</i>	113697	0.8	0.4	1.1	I	<i>Cribroelphidium gunteri</i>	1026170	4.2	3.1	5.3	V
<i>Nonionella auris</i>	466467	0.4	0.3	0.4	I	<i>Quinqueloculina poeyana</i>	417712	4.3	4.1	4.6	V
						<i>Valvulineria candeiana</i>	557005	4.2	3.7	4.8	V

Appendix C

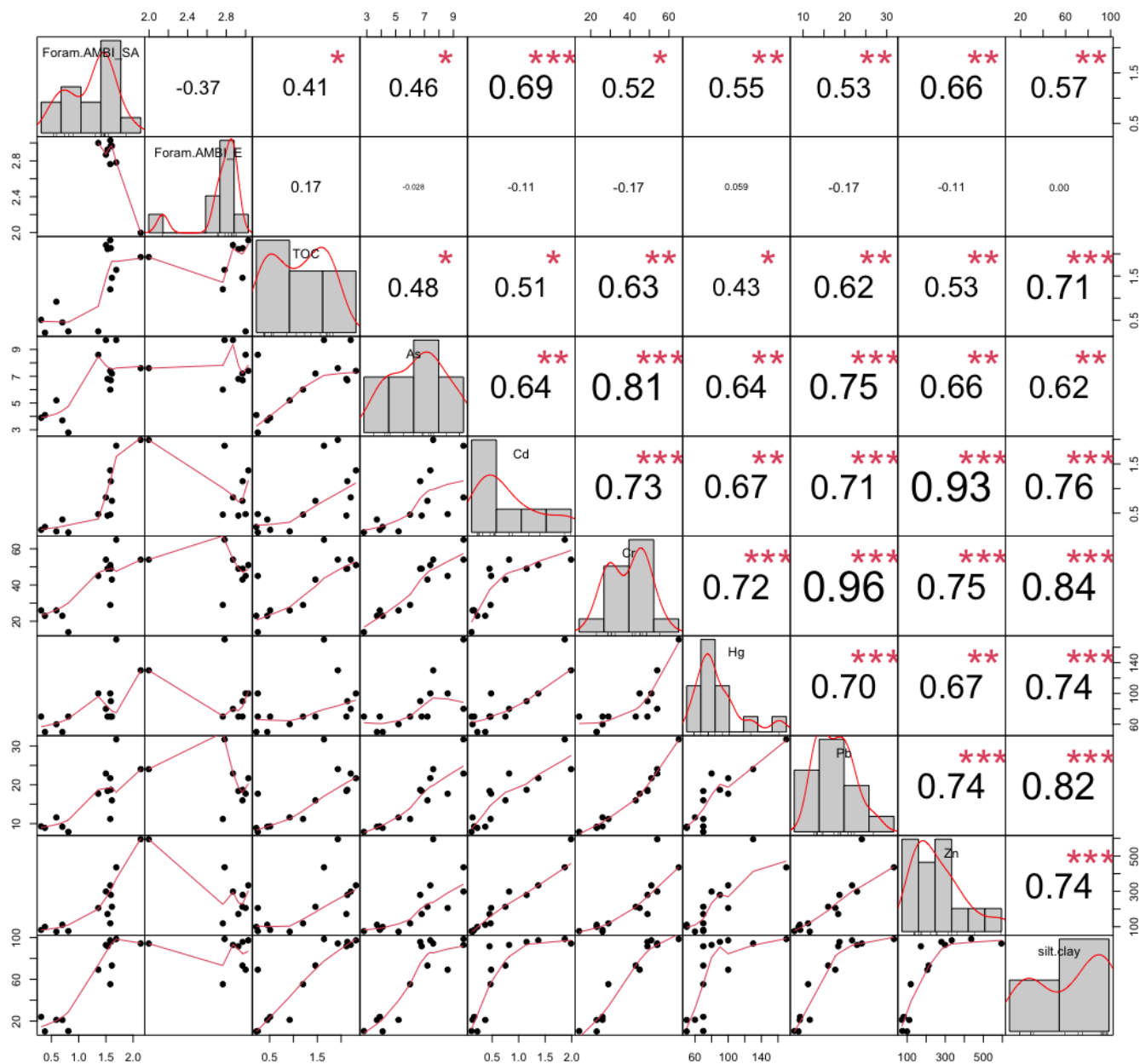


Figure C1. Kendall's coefficient of rank correlation (τ) between environmental parameters (from Damasceno et al., 2024) and Foram-AMBI calculated with the Brazilian TW list (Foram.AMBI_SA) and the European TW list (Foram.AMBI_E) in Sepetiba Bay.

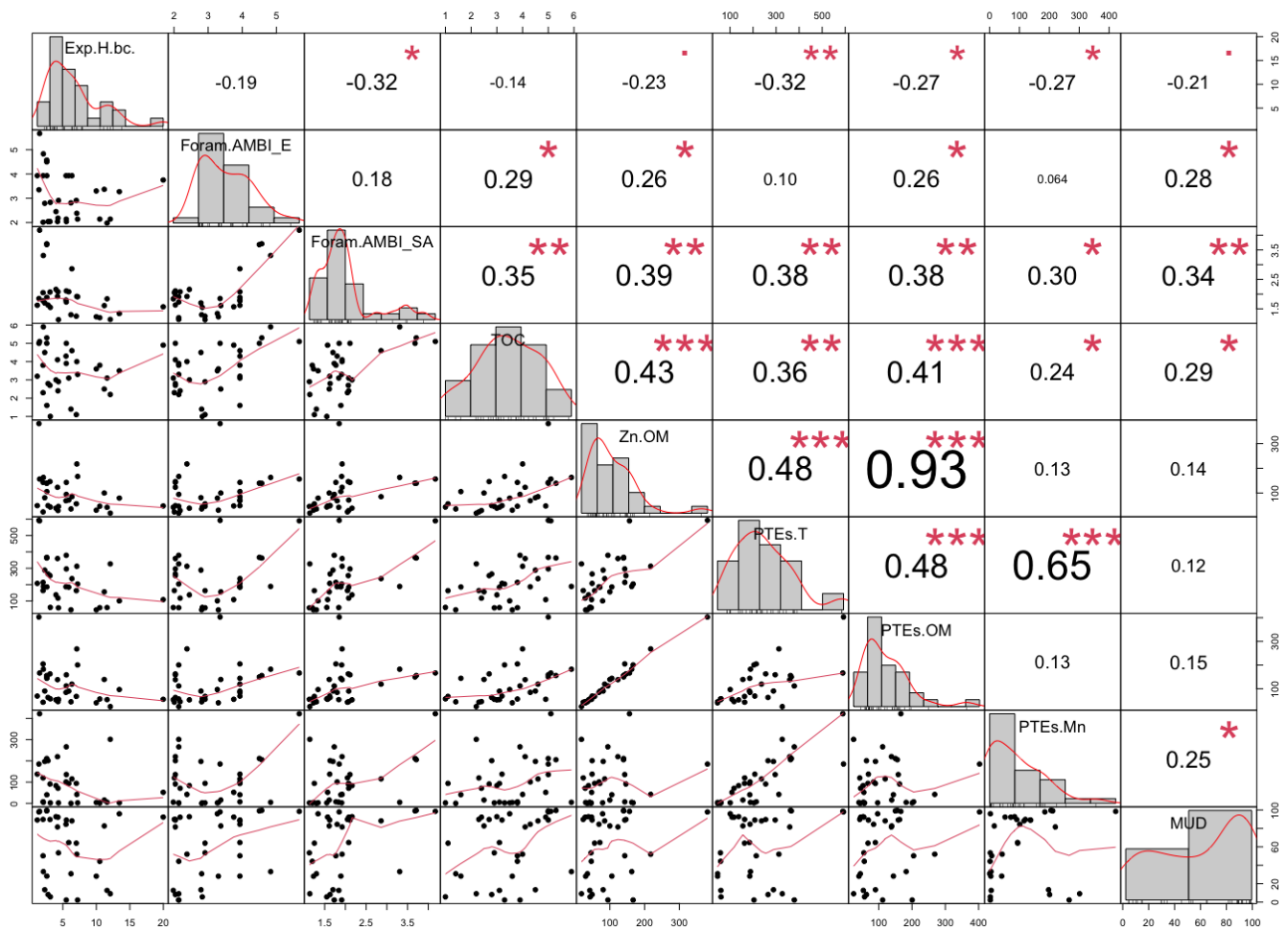


Figure C2. Kendall's coefficient of rank correlation (τ) between environmental parameters (from Nunes et al., 2023) and $\exp(H'_{bc})$ and Foram-AMBI calculated with the Brazilian list (Foram.AMBI_SA) and the European list (Foram.AMBI_E) in Guanabara Bay.

Appendix D

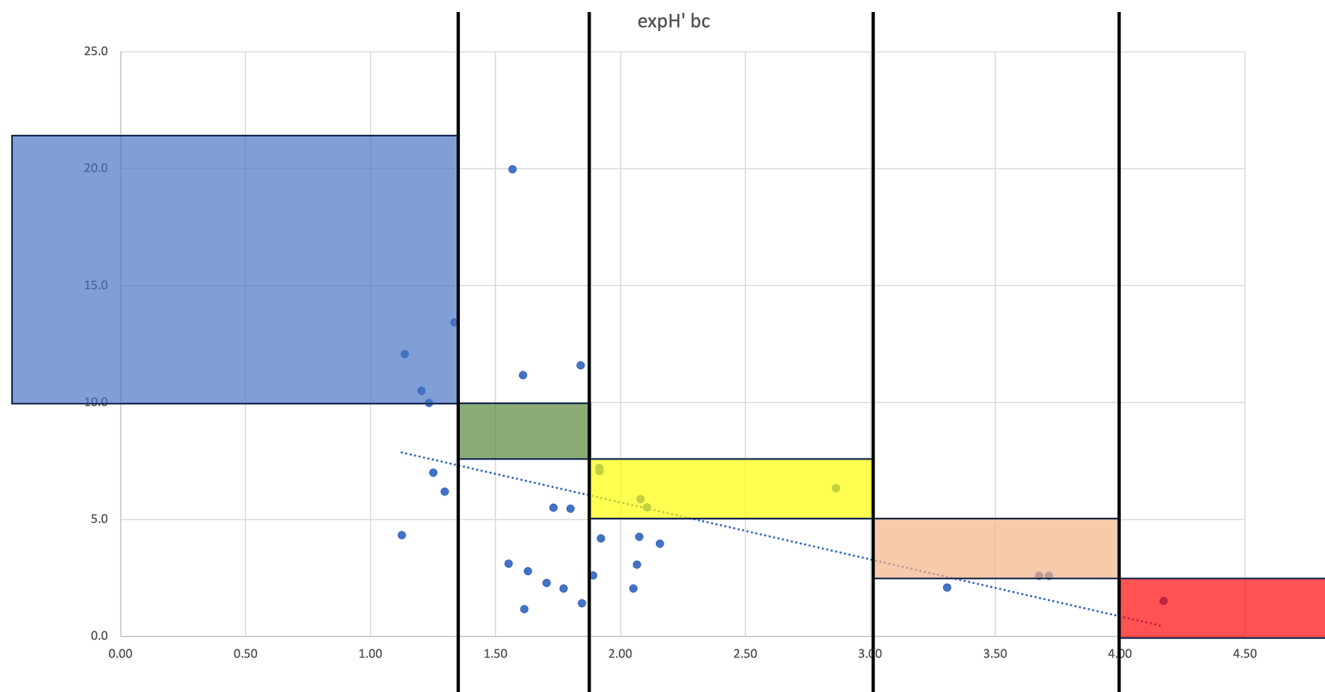


Figure D1. Calibration plot of $\exp(H'_{bc})$ and ForAMBI to establish Brazilian-adapted criteria for ForAMBI.

Data availability. The data are available in Mendeley: <https://doi.org/10.17632/nw4xd5ymgd.1> (Bouchet et al., 2025).

Author contributions. VMPB: conceptualisation, formal analysis, funding acquisition, methodology, visualisation, writing (original draft). SHdMS: conceptualisation, data curation, investigation, writing (original draft). PB: investigation, writing (review and editing). CB: investigation, formal analysis, visualisation, writing (review and editing). LB: investigation, writing (review and editing). WD: investigation, writing (review and editing). FFra: formal analysis, writing (review and editing). FFro: conceptualisation, data curation, methodology, writing (original draft). LL: investigation, writing (review and editing). DSP: investigation, writing (review and editing). ARR: investigation, writing (review and editing). STD: investigation, writing (review and editing). DVP: investigation, writing (review and editing). FLD: investigation, visualisation, writing (review and editing). JCP: data curation, writing (review and editing). MVAM: conceptualisation, data curation, investigation, writing (original draft).

Competing interests. The contact author has declared that none of the authors has any competing interests.

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