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SHORT COMMUNICATION

Opportunities and challenges in incorporating benthic foraminifera in marine and coastal environmental biomonitoring of soft sediments: from science to regulation and practice

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Abstract

Scientific studies have demonstrated the usefulness of benthic foraminifera as bioindicators to assess the health of marine, coastal, and transitional ecosystems. Similar to macrofauna, these organisms are reliable proxies for biomonitoring. Despite recent scientific advances, Brazilian official monitoring plans using biotic indices on coastal and ocean environments with soft sediment areas remain mostly restricted to a few types of organisms. Therefore, to include benthic foraminifera in Brazilian biomonitoring routines regulated by national environmental guidelines and standards, the paper presents the challenges, which must be overcome, some recommendations and steps to move forward to implement foraminifera as bioindicators in biomonitoring routines. In light of it, it is essential to consider the contribution of the Brazilian foraminiferal research in this implementation process, improving ecological quality indices, adapting methods, and applying genetics tools. This paper is a step in this direction, which aims to strengthen the role of benthic foraminifera as a reliable tool in Brazilian biomonitoring.

Keywords Benthic foraminifera · Bioindicator · Biomonitoring · Conservation biology

1 Introduction

Since about 20 years, benthic invertebrates are the most widely used biological quality element to assess the ecological quality status (Borja et al., 2000; Dauvin & Ruellet, 2007; Bouchet & Sauriau, 2008). Although benthic foraminifera are a relevant component of the benthic fauna and hold many ecological traits ideal for environmental biomonitoring, they have not yet been included as biotic indicators in the Brazilian environmental guidelines. Benthic foraminifera are ameboid protists that: (a) constitute the most abundant and widespread unicellular organisms of the meiofauna in the modern oceans (Murray, 2007; Sen Gupta, 1999), inhabiting from the deep sea to transitional environments (brackish water lagoons, estuaries, mangroves, saltmarshes) and even rarely in freshwater streams and lakes (Boltovskoy et al., 1980; Siemensma et al., 2017; Wylezich et al., 2014); (b) play a key role in the functioning of the benthic environment, contributing to bioturbation, seafloor ventilation, carbon and nitrogen geochemical cycle (Groß, 2002; Cesbron et al., 2016; Piña-Ochoa et al., 2010); (c) generally have a short reproductive and life cycle, and therefore, their responses to environmental change are rapid, being considered environmental sentinels (Kramer & Botterweg, 1991; Schönfeld et al., 2012); (d) some species can tolerate adverse environmental conditions, while others are more sensitive (Bouchet et al., 2007; ; Jayaraju et al., 2011; Martins et al., 2017; Prazeres et al., 2017; Vidović et al., 2014); (e) are easy and cheap to collect and process, and are often found in high density in small samples (a few cm^3), providing an adequate basis for statistical studies (Armynot du Châtelet et al., 2004); and (f) their tests can be preserved in the sediments. This not only makes them a possible tool for paleoecological and paleoenvironmental reconstructions (Hayward et al., 2004; Alve et al., 2009; Dolven et al., 2013; Armynot du Châtelet et al., 2018a, b) but also as an indicator

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of pre-impact conditions (e.g., Alve, 1991; Alve et al., 2009; Dolven et al., 2013; Francescangeli et al., 2016).

Since the first studies which applied foraminifera as proxy indicators to assess pollution conditions were published (e.g., Resig, 1960; Watkins, 1961), new methods, approaches, and analytic tools have been established and refined. However, no standardized methods have been globally followed until Schönfeld et al. (2012) that proposed an international protocol for homogenizing method concerning sample acquisition, sampling devices, replication, sub-sampling, preservation and staining, sample preparation, analysis, and documentation of foraminifera. This protocol guarantees the reproducibility and comparability among investigations. It has been used in monitoring studies of environments characterized by soft sediments, being sometimes locally adapted depending on the environment. The purpose of an international standardized protocol was only possible due to the Foraminiferal BIo-MOnitoring (FOBIMO) workshop, held in 2011 at Fribourg (Switzerland) which assembled 37 scientists from 13 countries.

Many studies have demonstrated the potential of foraminifera to reflect environmental changes of coastal and open marine systems on soft sediments (e.g., Alve, 1995; Burone et al., 2006; Mojtahid et al., 2006; Frontalini et al., 2009; Frontalini & Coccioni, 2011; Martins et al., 2013, 2016, 2019; Bergamin et al., 2019). In Norwegian fjords, it has been shown that benthic foraminiferal communities significantly correlate with benthic macrofaunal communities, confirming that foraminifera can be as good sentinels of environmental conditions as macrofaunal (Bouchet et al., 2018a). Furthermore, foraminiferal indices based on diversity (Alve et al., 2009; Bouchet et al., 2012; 2013) and on the sensitivity of species to organic pollution (Barras et al., 2014; Dimiza et al., 2016; Alve et al., 2016; Jorissen et al., 2018) have been designed, and successfully applied to assess ecological quality statuses (Bouchet et al., 2012; Dolven et al., 2013; Barras et al., 2014; Francescangeli et al., 2016; Dijkstra et al., 2017; Bouchet et al., 2018b; Jesus et al., 2018; Alve et al., 2019; Melis et al., 2019). Alve et al. (2019) also demonstrated that foraminifera and macrofauna have similar indicator efficiency by applying a foraminiferal multimetric index (NQIf) as an alternative, which is an adaptation of the Norwegian Quality Index (NQI), an internationally intercalibrated macrofauna index. Based on their results, Alve et al. (2019) recommended the inclusion of foraminifera as Biological Quality Element within the European Water Framework Directive's guidelines (WFD). According to these authors, foraminifera bring additional information to characterize the ecological quality status (EcoQS) compared with macrofauna, mainly in certain conditions such as oxygen-depleted ecosystems. Furthermore, biotic indices based on foraminifera may give better results that macrofaunal ones in estuarine and transitional waters (Bouchet et al., 2018b).

Despite these scientific advances, official monitoring of Brazilian coastal and ocean environments with soft sediment areas based on biotic indices remains mostly restricted to a few types of organisms, mainly specific groups of bacteria, such as *Enterococcus* and *Escherichia coli* (Brasil, 2000; 2005). New methods that broaden the scope of biotic indices and complement information by considering other organisms, such as foraminifera, should be tested in a joint effort by the scientific community and regulatory authorities. In Brazil, the National Environment Council (CONAMA), subordinate to the Ministry of the Environment, is responsible for defining the guidelines and standards applied in environmental monitoring of ecosystems, including coastal and marine (Brasil, 1981). The Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA) is the federal agency responsible for carrying out initiatives of national environmental policies related to environmental quality control, authorization for the use of natural resources and environmental surveillance and monitoring, in accordance with the resolutions regulated by CONAMA (Brasil, 1989; 2007). IBAMA is also responsible for the environmental licensing of enterprises and activities with significant national or regional environmental impact, such as in the territorial sea, on the continental shelf and the exclusive economic zone (Brasil, 1997).

To evaluate the application of foraminifera in biomonitoring in Brazil, a Workshop on Biomonitoring in Brazil: Foraminifera and their use in environmental assessment—BioFom—was held in São Paulo (Brazil) in May 2019. The workshop assembled 83 participants, including researchers, professionals from public and private companies, graduate and undergraduate students from eight countries (Germany, Brazil, France, Italy, Norway, Peru, Portugal, and Switzerland). This workshop was also an excellent opportunity to perceive the status of the Brazilian scientific research about the application of foraminifera in biomonitoring of different ecosystems and to reinforce the connection of the growing foraminiferal community in Brazil to the international network FOBIMO.

The paper presents the primary outcomes of the workshop: (1) the challenges to overcome; (2) the recommendations and steps to move forward to implement foraminifera as bioindicators in biomonitoring routines; and (3) the potential contribution of the Brazilian foraminiferal research to national environmental guidelines and standards. This paper can be considered a step to the acceptance of benthic foraminifera as a reliable tool in Brazilian biomonitoring studies.

2 Challenges

Some difficulties recognized by the scientific community in foraminiferal studies rely on the standardization of methods and on systematics to ensure reproducibility and comparability among studies. Researchers have addressed these issues in specific papers through the last decades, but the protocol established by FOBIMO's group (Schönfeld et al., 2012) and its application indeed represent a significant effort to create an international protocol to standardize methods in monitoring studies.

Although most of the recommendations from FOBIMO may be considered straightforward to follow, several biomonitoring studies within the Brazilian territory do not meet such recommendations. Sampling, for example, is often conducted by environmental agencies according to officially regulated procedures (Gubitoso et al., 2008; Teodoro et al., 2009), which are not always prone to follow detailed protocols or modify theirs. Many foraminiferal biomonitoring studies are carried out using grab as sampling device (Barbosa and Suguio, 1999; Gubitoso et al., 2008; Teodoro et al., 2009; Eichler et al., 2015; Laut et al., 2016); some others employed bottom samplers opening on top or adapted equipment such as the mega van Veen with upper openings that has the functionality and efficiency of a typical box corer, capable of penetrating coarser sediment bottoms (Disaró et al., 2006, 2017; Ribeiro-Ferreira et al., 2017). Depending on the sampling device and due to the use of samples for multiple variables/studies, the volume of sediment for foraminiferal analysis commonly does not reach 50 cm^3. Sometimes, this volume is adequate to obtain more than 100 living foraminiferal specimens; nevertheless, in some cases, it is not enough to reliably characterize the structure of foraminiferal assemblages. In cases of fluffy surface sediments, where most of foraminifera live, we have also to consider the possibility of foraminifera been flushed away during the sampling.

Additionally, according to some authors (Semensatto-Jr. & Dias-Brito, 2007; Schönfeld et al. 2012), the flotation step in monitoring studies should be avoided when possible to prevent biased data on species richness and abundances, but it is still a common practice in studies conducted in Brazil (and other countries), mostly in cases when terrigenous grains dilute the number of foraminifera tests in the sample. Nevertheless, when executing the flotation step, it is worth to mention the need for revising the sediment residue, especially for samples collected in highly hydrodynamics environments. Studies carried out on living foraminifera in the southeastern Brazilian continental margin revealed the presence of many attached and encrusting foraminifera in the sunk fraction, which must be included to better evaluate the density and biomass of these organisms (Disaró et al., 2017). Parent et al. (2018) put forward an optimized method (sodium polytungstate, SPT) to concentrate living benthic foraminifera that works well in sandy substrate. Thus, the FOBIMO protocol can be considered a starting but not the conclusive point, so once new methodologies are developed and tested, they should be accounted for.

Another issue that should draw attention is the adoption of replicate sampling. Former foraminiferal studies conducted in Brazilian coastal environments rarely mention or adopted this procedure, which began to be more common in the last years, although there is no longer much doubt about its importance for better data accuracy (Schönfeld et al., 2012; Armynot du Châtelet et al., 2018b; Kukimodo and Semensatto, 2019). The challenge, in this case, is to promote the establishment of this sampling procedure in biomonitoring studies in Brazil. As replicate sampling increases the effort of collection, processing, and analysis by at least three-fold, researchers may improve the sampling design for the quantity and distribution of samples in space and time to maintain the desired resolution of information and ensure the representativeness of the potential environmental indicator (Schönfeld et al., 2012). Replicates enable data processing through statistical comparisons, which crucially contributes to monitoring data analysis to determine whether or not a particular indicator has varied over time and space according to given statistical confidence.

Benthic foraminifera in Brazil have long been used as environmental indicators such as ocean currents and circulation (Boltovskoy, 1959; Lançone et al., 2005), water stratification (Debenay et al., 1998), marine influence (Debenay et al., 2001; Duleba and Debenay, 2003; Eichler-Coelho et al., 1997), and hypoxia (Eichler et al., 2015). Nevertheless, the employment of benthic foraminifera as a biotic proxy in ecological quality status (EcoQS) of coastal and marine ecosystems evaluation remains almost unexplored in Brazil. In 2004, CETESB (Environmental Agency of the State of São Paulo) and the Institute of Geosciences from the University of São Paulo (USP) established a partnership to monitor the environmental quality around the vicinities of submarine sewage outfalls in the coastal zone of São Paulo (Gubitoso et al., 2008; Teodoro et al., 2009). The distribution and the composition of living benthic foraminifera assemblages were associated with geochemical analysis (calcium carbonate, organic carbon, total nitrogen, and sulfur concentrations) to assess sediment quality. Results revealed that the regions with the highest concentrations of nutrients and organic matter flux were associated with reducing environments, the lower oxygen concentration in the interstitial pore water, and the lower benthic foraminiferal species richness and diversity. They concluded that the accumulation of materials from the sewage outfall, whose nature is predominantly organic and under intense anaerobic decomposition, was taking place in the area. Therefore, the authors suggested improvements in the sewage treatment system, because the disposition was inadequate to guarantee the environmental quality required by the official guidelines (CETESB, 2007).

The Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA) have some mandatory requirements for monitoring the ecological quality status of the Brazilian coastal ecosystems and to assess the environmental impact caused by polluting projects and activities. Macrofauna is traditionally employed in such evaluations. Foraminifera, on the other hand, is not included in the official guidelines, but its potential to evaluate impact caused by pollution was demonstrated in the Environmental Monitoring Program of Potiguar Basin, in the northeastern Brazilian continental margin (Disaró et al., 2006), and in the Environmental Monitoring Project for the Sergipe—Mar Subsea Outfall of the Active Production (Pregnolato et al., 2018).

3 How to move forward ?

Although some of the earliest studies on foraminifera regarded essentially taxonomic aspects, its harmonization remains an issue to be solved; species descriptions are rare and not always easily found in papers. Identification of foraminifera using genetic tools is just beginning in Brazil; nevertheless, genetic data has revealed a more considerable cryptic diversity (Pawlowski et al., 2018). We recognized that there are some factors and actions to consider to make foraminifera identification easier, such as the availability of more reference books (e.g., Murray, 2006), catalogs (e.g., Ellis and Messina, 1940 et seq.; Kaminski and Gradstein, 2005; Loeblich and Tappan, 1988), collections, websites (e.g., www.marinespecies.org—World Register of Marine Species, WoRMS) and identification keys, which will enable an easier identification of species and provide taxonomic consistency.

Some of the biotic indices rely on an ecological classification of species found within the foraminifera assemblage. We still lack ecological information about the species found on the Brazilian coast and still depend on the also rare international works addressing species-specific ecological aspects. Therefore, we must make efforts to identify and describe species from Brazilian environments and their relationships with other biotic and abiotic components of ecosystems and to investigate the similarity of ecological traits of species found elsewhere.

There are several researches in Brazil that examined the potential of foraminifera for estimating pollution impacts, which concentrated their analyses on assemblage composition and structure (species richness, dominance, diversity and evenness) or morphological abnormalities caused by pollution stress (Eichler et al., 2006, 2015; Gubitoso et al., 2008; Laut et al., 2016; Teodoro et al., 2009; Vilela et al., 2004, 2011). To the best of our knowledge, only the FORAM index for coral reef areas was tested for Brazilian environments (Barbosa et al., 2009, 2012, 2016; Oliveira-Silva et al., 2012; Moura et al., 2016). Therefore, evaluating the performance of existing biotic indices, i.e., Diversity, expressed as the effective number of species—Exp (H'bc) (Bouchet et al., 2012), Foram Stress Index (Dimiza et al., 2016) and Foram Marine Biotic Index—Foram-AMBI (Alve et al., 2016; Jorissen et al., 2018) on Brazilian environments also represents a challenge to tackle, including determining the suitability of these indices and the eventual adaptations to the Brazilian reality. Once the protocols for biotic indices used in environmental monitoring are validated within the Brazilian context, it will be possible to move forward to include them in national environmental guidelines and standards.

It is essential to keep doing science to improve environmental quality indices, to adapt methods, to improve ecological knowledge and to apply genetics tools. We should also emphasize the importance of networking among research groups and non-academic institutions, aiming at the same objectives, as standards and harmonization of methods, which make possible the inter-calibration among research areas, scientific groups and environmental data (e.g., tropical, temperate). This inter-calibration will also enable us to establish the most suitable environmental quality indices, according to distinct ecosystems, promoting interdisciplinary research and integrating macrofauna and foraminifera (Alve et al., 2019).

Increasing the interaction between scientists and policymakers is imperative for a better understanding of modern challenges such as those associated with *Environmental Pollution*, climate change, and biodiversity loss. Science is one of the main tools of influence to protect and promote interests within the government. Technical information, scientific data, and environmental impact analyses provide input for more consistent discussions. Bringing in-depth analysis of different points of view to the debate enables public authorities to make more qualified decisions. In this sense, broad access to the largest amount of reliable scientific evidence on a given issue would broaden public managers' choice, thus contributing to more effective policymaking.

4 Brazilian Foraminiferal research and national guidelines

Two legal instruments nationally regulate the transitional, coastal and oceanic water quality in Brazil: CONAMA Resolution Nº 357/05 (Brasil, 2005), which defines the water classes, their designated uses and quality standards for major pollutants, and CONAMA Resolution Nº 274/00 (Brasil, 2000), which deals specifically with the bathing water quality. CONAMA Resolution 357/05 includes in its Article 8º,

§3º the use of biological indicators to evaluate the quality of aquatic environments "when appropriate, using aquatic organisms and or communities", thus formalizing the legal aspect of the application of biomonitoring.

As previously mentioned, guidelines for environmental impact assessments and monitoring programs of aquatics environments include benthic macrofauna and some microorganisms, such as certain groups of bacteria and algae. The use of benthic foraminifera as a quick and efficient assessment tool, complementing macrofauna-based monitoring, would improve and benefit environmental monitoring programs in Brazil and promote more effective management and conservation policies.

CETESB, for example, operates regularly two specific seawater monitoring programs, according to their designated uses:

- "*Bathing water quality and water quality of beach streams*", which evaluates water quality for primary contact recreation, such as swimming and diving;
- "*Coastal Network*", which assesses water quality for other designated uses, such as aquaculture, domestic sewage, and industrial discharges, port activities, protected areas, among others.

The Coastal Network was created in 2010 to monitor the quality of saline and brackish water intended for use beyond recreation. Microbiological, physical, chemical, and ecotoxicological analyses are performed semiannually in sediment and water samples. Since foraminifera are well-known bioindicators of anthropogenic stresses in coastal waters, this group, in addition to characterizing present-day ecological status, could complement the Coastal Network monitoring program.

Accumulated knowledge about benthic foraminiferal responses to environmental changes and impacts is already satisfactory to propose sampling and analysis protocols that allow the inclusion of this group in Marine Monitoring Plans. The first BioFom workshop has already been an excellent opportunity to discuss methodological approaches aiming to improve the standardization. Despite the questions to solve, the growing number of researchers working on foraminiferal physiology, ecology, and genetics, as well as the diversification of methodological research and study areas, are driving a rapid scientific and technological advance on environmental applications of recent foraminifera. The II BioFom, which is already planned and will occur in Paraná/Brazil in 2021 will be another good opportunity to join Brazilian groups, to discuss methodological approaches, and to try to establish an EcoQS index suitable for polluted coastal areas, such as Santos estuary and Guanabara Bay, in the southeastern Brazilian coast.

Finally, it is worth to mention that although the researchers are still discussing some foraminiferal analysis methods, there is a good understanding of the implications and restrictions of the options adopted. The present knowledge ensures conditions to critically analyze the results, draw reliable conclusions, and consequently to propose to the Brazilian regulatory authorities to consider the inclusion of foraminifera in marine environmental biomonitoring.

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