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**Distribuição dos foraminíferos bentônicos e caracterização dos metais  
dissolvidos nas águas da Laguna Tramandaí-Armazém, Planície Costeira do  
Rio Grande do Sul (Brasil)**

São Leopoldo

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**Resumo:**

Neste trabalho apresentamos um estudo avaliativo da distribuição dos foraminíferos bentônicos juntamente com análises de parâmetros físico-químicos da Laguna Tramandaí-Armazém, no estado do Rio Grande do Sul (Brasil). A distribuição dos foraminíferos juntamente com análises de parâmetros físico-químicos da água, sugerem que a dinâmica ambiental na laguna é impulsionada por uma interação de mecanismos naturais e também antropogênicos. Os foraminíferos bentônicos na laguna se apresentam com uma dominância de espécies aglutinantes próximas às margens da laguna, enquanto espécies hialinas calcárias, com preferências por salinidades mais elevadas, dominam na porção central da laguna. As concentrações de metais dissolvidos nas águas da laguna estão dentro dos limites estabelecidos para águas salobras classe 2, destinada a pesca amadora e à recreação de contato secundário, de acordo com as normas brasileiras (CONAMA e CONSEMA) e internacionais. No entanto, o aumento do carbono orgânico, oriundo do aporte fluvial e dos esgotos de cidades próximas, e a oxigenação da água, apresentada pelo pH, interferem na distribuição de foraminíferos na Laguna Tramandaí-Armazém. Embora as assembleias de foraminíferos bentônicos tenham permanecido relativamente estáveis desde a década de 1960, quando foram descritos pela primeira vez na laguna, no presente trabalho registra a primeira ocorrência do gênero *Bulimina*. Adicionalmente, nossos resultados ressaltam a importância do monitoramento contínuo e dos esforços de conservação na Laguna Tramandaí-Armazém e corpos d'água adjacentes no sul do Brasil.

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## **Apresentação**

Ambientes marinhos marginais são caracterizados por uma variabilidade significativa nos parâmetros ambientais devido à interação entre influências continentais, como o influxo de água doce dos rios, e processos marinhos, como regimes de marés e ondas. Essa variabilidade também é observada nos parâmetros físico-químicos da água, caso da salinidade, temperatura e disponibilidade de oxigênio, que estão relacionados a flutuações nos teores de matéria orgânica e metais dissolvidos na água (Tabajara et al., 1997; Leipnitz et al., 2014; Santana et al., 2015; Silva et al., 2016).

Ambientes costeiros, além da sua variabilidade natural, também são frequentemente afetados por atividades antrópicas, devido à densidade demográfica ao longo dessas áreas em todo o mundo. Como resultado da influência urbana, a contaminação da água por metais pesados e esgoto doméstico e industrial acaba afetando a vida marinha (Frontalini et al., 2009; Rodrigues et al., 2013; Stankovic et al., 2013; Santana et al., 2015; Bouchet et al., 2018; Martins et al., 2018).

A avaliação da qualidade e das condições ambientais em áreas costeiras, geralmente, é realizada através de medições dos parâmetros físicos e químicos, como temperatura, pH, salinidade, concentrações elementares orgânicas e inorgânicas. Na biosfera, os efeitos da contaminação ambiental dependem de fatores como exposição e biodisponibilidade, taxa de absorção e a resposta metabólica das espécies presentes no ambiente contaminado. As implicações podem se apresentar em todos os níveis dos ecossistemas, desde alterações moleculares (bioquímicas e genéticas), individuais (fisiológicas, morfológicas, reprodutivas e comportamentais), até em assembleias e comunidades bióticas (Santana et al., 2015).

Dessa forma, as respostas biológicas aos efeitos da contaminação se apresentam como biomarcadores, indicando variações nos parâmetros metabólicos à medida que esses organismos são expostos aos contaminantes (Santana et al., 2015). Assim a utilização de organismos no controle ambiental ajuda com respostas sobre a real influência dos contaminantes nos ecossistemas (Bouchet et al., 2018; Martins et al., 2018). Organismos bioindicadores, como os foraminíferos bentônicos, são um bom exemplo de utilização no controle ambiental. Por serem sensíveis às variações do ambiente, fornecem uma resposta rápida e consistente acerca das contaminações. Essas respostas podem se apresentar como alterações na diversidade e densidade das espécies de foraminíferos, bem como alterações morfológicas (Coccioni et al., 2009; Frontalini et al., 2009).

As anormalidades morfológicas em testas de foraminíferos vêm sendo observadas desde os últimos dois séculos, e em trabalhos com depósitos sedimentares recentes as carapaças deformadas se apresentam ainda mais abundantes e geralmente estão relacionadas à contaminação por oligoelementos, esgoto doméstico e outros produtos químicos, como hidrocarbonetos e pesticidas (Frontalini et al., 2009; 2018; 2019 Coccioni et al., 2019).

Além da deformação nas carapaças, ambientes poluídos também interferem na densidade, diversidade e preservação das espécies encontradas, se apresentando como um estresse ambiental, interferindo nas comunidades de foraminíferos bentônicos, mas também na conservação das testas pós-morten (Leipnitz et al., 2014). O aumento de indivíduos de espécies oportunistas também pode ser relacionado a poluição (Murray, 2006; Raposo et al., 2018; Coccioni et al., 2019).

Na Laguna Tramandaí-Armazém, localizada na Planície Costeira do Rio Grande do Sul, Brasil, e foco do presente estudo, os foraminíferos são registrados desde a década de 1960, onde primeiramente foram caracterizados, por Closs et al. (1967), de acordo com a tolerância das espécies às variações da salinidade. Espécies como *Miliammina fusca*, *Trilocularena patensis*, *Ammotium salsum*, *Trochammina salsa*, e *Elphidium pyriformis* foram encontrados na parte mais interna da laguna, sendo *Miliammina fusca* a espécie mais abundante encontrada por Closs et al. (1967). Já na zona do canal de desembocadura para o mar foram encontrados espécimes com maior afinidade a ambientes marinhos, como *Elphidium discoidale*, *Quinqueloculina seminulum*, *Nonionella atlantica* e *Ammonia beccarii*. Pequenas modificações morfológicas também foram observadas nos foraminíferos à medida que se avançou em sentido norte da laguna, com alterações na espessura da parede das testas, na ornamentação, na disposição das câmaras e no aspecto da abertura (Closs et al, 1967). As variações de salinidade tendem a controlar a distribuição dos foraminíferos bentônicos dentro da laguna (Closs e Madeira, 1967, Leipnitz et al., 2014), em águas salobras a formação e preservação de testas calcárias é facilitada, de tal forma que a presença de testas aglutinantes se torna mais comum em ambientes pouco salinos e ácidos, característicos de zonas de transição (Murray, 2006).

Em 2014, Leipnitz et al. apresentaram 14 espécies de foraminífero bentônico na laguna Tramandaí-Armazém, sendo elas *Ammonia beccarii*, *Ammoscalaria pseudospiralis*, *Ammotium salsum*, *Arenoparrella mexicana*, *Elphidium excavatum*, *Elphidium gunteri*, *Haplophragmoides wilberti*, *Miliammina earlandi*, *Miliammina fusca*, *Protoschista findens*, *Quinqueloculina seminula*, *Trilocularena patensis*, *Trochammina salsa* e *Trochammina inflata*. Todas as espécies encontradas foram reconhecidas como típicas de ambientes de transição raso (Leipnitz et al, 2014), onde os espécimes em sua maioria possuem testas aglutinantes e as suas assembleias são pouco diversificadas, refletindo as rápidas variações físico-químicas (e.g., salinidade) encontradas na laguna, que ora estão influenciados pela entrada de água doce do Rio Tramandaí, ora estão sob influência marinha do Oceano Atlântico. As maiores quantidades de foraminíferos bentônicos foram encontrados na área de desembocadura para o mar, sendo menos observados nos ambientes mais internos, com predomínio da água doce (Leipnitz et al, 2014). Além disso, segundo Leipnitz et al. (2014), o predomínio de formas aglutinantes seria facilitado pelo crescimento urbano no entorno da

laguna, responsável por promover um aumento da deposição de material orgânico, acidificando as águas que dissolveriam as formas calcárias *post-mortem*.

O último trabalho dedicado ao estudo dos foraminíferos bentônicos na Laguna Tramandaí-Armazém é predecessor ao presente escrito, e foi publicado em 2021 (Martins et al., 2021). Nele foram recuperadas cinco espécies durante o período de um ano, *Ammotium salsum*, *Haplophragmoides wilberti*, *Miliammina earlandi*, *Trochammina inflata* e *Trochammina salsa*. Durante o ano de coleta, foram identificadas variações de abundância nas assembleias de foraminíferos, fortemente influenciadas pela oscilação da salinidade e dos nutrientes aportados na laguna por drenagens. Também foram identificados espécimes com deformações e um aumento de indivíduos deformados durante os meses de alta temporada, janeiro e março, indicando um possível desequilíbrio causado pela interação urbana.

As mudanças sazonais na abundância de foraminíferos bentônicos na Laguna Tramandaí-Armazém correlacionam-se também com variações nos parâmetros hídricos e meteorológicos (Martins et al., 2021). O número de foraminíferos recuperados por Martins et al. (2021) aumentou durante março e julho de 2019, fator relacionado com o aumento da precipitação acumulada. Durante estes meses de maior precipitação, as assembleias de foraminíferos foram dominadas pela espécie *Ammotium salsum*, conhecida por tolerar uma ampla gama de valores de salinidade da água (Murray, 1991). Além disso, *A. salsum* é um táxon infaunal (Murray, 2006) que provavelmente prosperou sob o aumento da oferta de alimentos (por exemplo, fitodetritos) trazido para a Laguna durante o escoamento superficial das chuvas.

Neste contexto, de crescimento e influência urbana em um ambiente com marcada dinamicidade, o presente escrito busca dar sequência ao monitoramento das associações de foraminíferos bentônicos da Laguna de Tramandaí-Armazém. São investigados os parâmetros que controlam a distribuição de foraminíferos bentônicos na laguna, integrando dados taxonômicos e parâmetros físico-químicos da água, ambos coletados durante o inverno de 2022. Identificamos a interação entre os fatores naturais e antropogênicos na dinâmica ambiental da laguna, e constatamos algumas mudanças na composição das assembleias de foraminíferos bentônicos recuperados (quando comparadas a estudos anteriores), fomentando a utilização destes organismos como bioindicadores eficazes das condições ambientais. O artigo apresentado a seguir será submetido para avaliação no periódico científico *Ocean and Coastal Research*, Qualis CAPES A4.

# Environmental factors controlling foraminiferal distribution in the Tramandaí-Armazém Lagoon, southern Brazil

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## Abstract

Foraminiferal assemblages of coastal transitional environments are affected by highly dynamic fluctuations of water salinity, oxygenation, pH, among other natural and human-induced factors. We present a comprehensive study to foraminiferal distribution in surface sediments of the Tramandaí-Armazém Lagoon, southern Brazil. Our multidisciplinary approach, combining foraminiferal distributions and analyses of physicochemical water parameters, suggests that environmental dynamics in the lagoon are driven by natural and, likely, anthropogenic factors. Benthic foraminiferal distribution in the lagoon depicts a dominance of agglutinated species close to the lagoon's shores, while calcareous hyaline species, with preferences for higher salinities, dominate in the central portion of lagoon. This overall distribution pattern allowed us to clearly characterize a high-salinity wedge entering the lagoon from the South Atlantic Ocean. Dissolved concentrations of metals in the water of the lagoon are within acceptable limits, according to Brazilian and international standards. However, increased organic carbon input from riverine input, and likely sewage disposal from nearby cities, seem to affect water, oxygenation, dissolved metal contents, pH, and, consequently, foraminiferal distributions in the Tramandaí-Armazém Lagoon. Even though benthic foraminiferal assemblages remained relatively stable since the 1960s, we recorded for the first-time occurrence of the genus *Bulimina*, supporting a modern increase of organic carbon supply and reduced water oxygenation in the Tramandaí-Armazém Lagoon. Our results underscore the importance of continuous monitoring and conservation efforts in the Tramandaí-Armazém Lagoon and adjacent water bodies in southern Brazil.

## **Introduction**

Marginal marine transitional environments are characterized by high-amplitude variability of environmental parameters due to the interplay between continental influences, such as freshwater riverine inflow, and marine processes, such as tidal and wave patterns (Gupta, 1999; Murray, 2006; Bouchet et al., 2018). This variability is depicted by physical and chemical water parameters, such as salinity, temperature, and oxygen levels, which are coupled with fluctuations in organic matter and dissolved metals contents in the water (e.g., Coccioni et al., 2009; Frontalini et al., 2009). Additionally, the distribution of living organisms is also affected by changing environmental conditions in transitional settings, with protists like foraminifera being generally used groups to track water physical and chemical parameters (e.g., El Baz, 2017; Coccioni et al., 2009).

Besides their natural variability, marginal marine transitional environments are usually impacted by anthropogenic activities, due to high-density urban occupation along coastal areas worldwide. Examples of anthropogenic impacts in those environments are water contamination by heavy metals (e.g., Coccioni et al., 2009; Frontalini et al., 2009), and domestic and industrial sewage (e.g., Rodrigues et al., 2014), which can affect living organisms depending on factors such as exposure and absorption rates, bioavailability, and metabolic responses (Santana et al., 2015; Yang et al., 2017; Frontalini et al., 2018). Bioindicators, such as benthic foraminifera, are usually applied as environmental monitoring tools (e.g., Murray, 1991; Rodrigues et al., 2014; Raposo et al., 2018). They are sensitive to environmental variations, providing a rapid and consistent response to environmental changes. These responses may manifest as alterations in species diversity and distributions, as well as morphological and metabolic changes (Jorissen et al., 1995; Murray, 1991; Coccioni et al., 2009; Frontalini et al., 2009; Bouchet et al., 2018; Martins et al., 2018).

Here we integrate benthic foraminiferal assemblages from surface sediments and water physical and chemical properties measurements to assess environmental factors controlling foraminiferal distribution at the Tramandaí-Armazém Lagoon, southern Brazil. Our results reveal that selected trace metal concentrations in the water show significant correlations with benthic foraminiferal taxa indicative of salinity and water oxygenation changes, highlighting the application of the latter as bioindicators in modern environments, and paleoenvironmental proxies in the fossil record.

## **Methodology**

### **Study area**

Covering 18 km<sup>2</sup>, distributed over two contiguous lagoonal bodies, the Tramandaí-Armazém Lagoon is one of the links with the Atlantic Ocean of a chain of lakes running along the northern coast of Rio Grande do Sul, southern Brazil. The Lagoon has an average depth of 1.0 m, reaching a maximum

depth of 1.4 m in the Tramandaí sector (northern direction). Oligohaline waters of the Tramandaí-Armazém Lagoon are dominated by continental influence, due to the outflow of the Tramandaí River and other drainages that interconnect several lakes. A secondary marine influence, which invades the lagoon through its outflow channel to the South Atlantic Ocean, is driven by an astronomical micro-tide regime with amplitude of ~30 cm (Tabajara and Dillenburg, 1997; Leipnitz et al., 2014).

Sedimentation in the region is characterized by coastal Quaternary deposits, dominated by sandy sediments deposited by marine and aeolic processes. Fine-grained sediments in the lagoon are mainly delivered by the Tramandaí River and generated by weathering of volcanic sedimentary rocks of the Paraná Basin, as well as of the crystalline basement (Barboza et al., 2008). Accumulation of fine-grained sediments is concentrated in the central region of the lagoon, while coarser sediments are concentrated closer to the margins (Tabajara and Dillemburg, 1997). This pattern of sediment distribution is controlled by waves action, lagoon currents and the prevailing NE winds (Tabajara and Dillemburg, 1997).

The coastal cities of Imbé and Tramandaí surround the Lagoon and have considerable increases of population density during summer months, reaching 300% in Imbé and 200% in Tramadaí (Zuanazzi and Bartels, 2016). Additionally, consolidated data on water and sewage at the municipalities of Tramandaí and Imbé show that in 2021, only 28% and 0.6% of sewage, respectively, were treated (National Information System on Sanitation - SNIS, 2021).

### **Sampling strategy**

With the aid of a small boat, sediment and water samples were collected in the Tramandaí-Armazém Lagoon in July 2022. We selected this period so that the greatest number of benthic foraminiferal specimens could be recovered, as suggested by the longitudinal study, with a one-year sampling period, of Martins et al (2021). These authors reported higher foraminiferal abundances during the wetter months of March and July. Sampling stations were selected to track environmental gradients and possible anthropogenic influences surrounding the lagoon. Figure 1 shows sampling stations, identified as p01 to p08, which are located close to: A petrochemical storage terminal (a), agricultural cultivation areas (b), urban areas (c), the channel of the lagoon connected to the Atlantic Ocean (d), and the outflow area of the Tramandaí River (e).

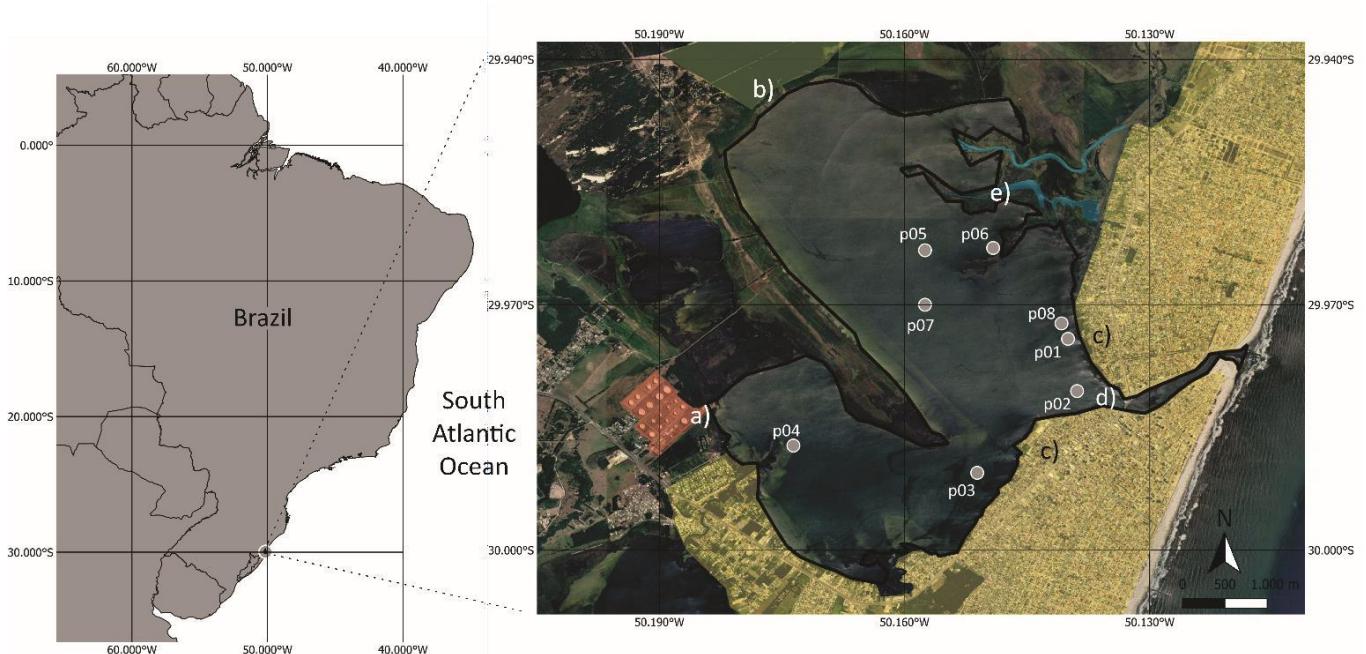


Figure 1: Map of the Tramandaí-Armazém Lagoon with the sampling stations in grey, and main natural and landscape usage features. Satellite images obtained from Google Earth.

Surface sediments were collected with an Eckmann sampler and conditioned in 50 ml Falcon tubes. Sediments were collected directly from the surface of the recovered sediments. At each sampling station, two samples were taken for taxonomic analysis, and preserved in a solution of ethanol and Rose Bengal, allowing us to recognize organisms that were alive or dead at the time of sampling. All samples were refrigerated between 4 °C and 11 °C until they were prepared and analyzed.

Water samples were collected manually using 200 ml polyethylene bottles, which had previously been washed and sterilized in nitric acid ( $\text{HNO}_3$ ) 1:1, in accordance with the norm NBR-9.898, 1987 of the Brazilian Association of Technical Standards (ABNT). Cleaned bottles were first washed with water from the sampling stations, then emptied, and water was eventually collected at a depth of approximately 30 cm in the water column. Water samples were also kept at temperatures between 4 °C and 11 °C until they were chemically characterized.

### Water analysis

Water salinity, conductivity and temperature were measured in-situ with a Hanna Salinometer (HI98319), and pH was measured with pH strips at the sampling stations. All in-situ measurements

were performed at the water surface. Trace metals concentrations in water samples were measured with an Inductively Coupled Plasma Optical Emission Spectrometer (ICP OES; iCAP 7200 Duo) at the Technological Institute for Paleoceanography and Climate Change (itt OCEANEON, UNISINOS University). Initially, samples underwent acid digestion based on method 3015A of the Environmental Protection Agency (EPA). Water aliquots of 45 ml were mixed with 5 ml of concentrated nitric acid ( $\text{HNO}_3$ ) and digested at a temperature of  $170 \pm 5^\circ\text{C}$ , with a 15-minute heating ramp and a 15-minute digestion plateau, in an ETHOS UP (Milestone) microwave oven. After digestion, samples were measured with ICP-OES, and instrumental blanks were subtracted from measurement intensities. To ensure reproducibility of results, we used the standard ERM-CA615 for calibrating intensities to concentrations (in parts per million, ppm) and quality control. We also performed measurements as triplicate, with mean values reported. We analyzed Ag, Al, As, Ba, Be, B, Cd, Co, Cu, Cr, Fe, Li, Mg, Mn, Ni, P, Pb, Sb, Se, U, V and Zn, which correspond to the main contaminant metals found in water bodies near cities (e.g., Frontalini et al., 2009; Coccioni et al., 2009; Bouchet et al., 2018).

### **Foraminifera analysis**

Sediment samples were washed over 38  $\mu\text{m}$ , 63  $\mu\text{m}$  and 125  $\mu\text{m}$  mesh sieves, removing organic residues, and mud before picking foraminiferal specimens under stereomicroscope, no type of flotation being carried out in preparation for the picking. Benthic foraminifera were only found in grain-size fractions  $>125 \mu\text{m}$ , and the total number of individuals was recovered. To confirm taxonomic identifications and identify dissolved and deformed specimens, we imaged selected benthic foraminiferal specimens with a Zeiss EVO MA 15 scanning electron microscope (SEM) at itt OCEANEON. Taxonomic identifications of the recovered species were mainly based on previous regional studies (e.g., Closs et al., 1967; 1971; Leipnitz et al., 2014; Ferreira et al., 2015; Martins et al., 2021), as well as original descriptions in the Ellis and Messina Catalog.

### **Data analysis**

We statistically explored our new data sets with Pearson correlation indices, clustering and rarefaction curves using the software PAST - Palaeontological Statistics, version 1.81 (Hammer et al., 2008). Pearson correlations were calculated between all foraminiferal counts and water physical and chemical parameters, those where  $p < 0.75$  are considered relevant correlations. For foraminiferal count, we performed two-ways (Q and R mode) cluster analysis, using the Bray-Curtis similarity coefficient (Bray and Curtis, 1957), and calculated rarefaction curves for each sampling station.

Spatial data analysis was carried out using the Qgis 3.28 software. Heat maps of benthic foraminiferal abundances were generated using the Kernel interpolation algorithm distribution in Qgis, where the estimation is calculated based on the number of points in a location, with larger numbers of clustered points resulting in larger values. Heatmaps allow easy identification of hotspots and

clustering of points. The radius measurement used to create the heat maps was 0.026 and the pixel size was 0.001, so that the entire surface of the lagoon was represented, despite the few sample points collected.

## Results

### Foraminiferal diversity and abundance patterns

Fifteen foraminiferal species were recovered in the Tramandaí-Armazém Lagoon (Table 1; Figure 2). Of these, eight have hyaline calcareous tests, corresponding to the species *Ammonia tepida*, *Ammonia parkinsoniana*, *Ammonia rolshauseni*, *Buccella frigida*, *Bulimina* sp. cf. *B. marginata*, *Elphidium* sp., *Nonionella atlantica* and *Globigerinoides ruber*, the latter being the only planktonic species recovered. The remaining seven species are characterized by agglutinating tests, identified as *Ammoscalaria pseudospiralis*, *Ammotium salsum*, *Haplophragmoide wilberti*, *Miliammina* sp. *Textularia* sp., *Trochammina inflata* and *Trochammina salsa*. Most of the specimens identified (~95%) were dead when samples were collected (Table 1). Even though dead foraminiferal assemblages may be allochthonous, there may be significant time averaging involved (Martin, 1993; Glenn-Sullivan and Evans, 2001; Martin et al., 2002). Therefore, we only considered total (living + dead) abundances, and restricted our interpretations to general environmental patterns, instead of evaluating environmental quality at the moment of samples collection.

In total, 2022 foraminiferal specimens were recovered at the eight sampling stations, with peak abundances at stations p04, p06 and p07 (Figure 3). Assemblages at these stations were dominated by *Ammotium salsum* (55,2%) and *Ammoscalaria pseudospiralis* (5,9%) at stations p04 and p06, and *Ammonia parkinsoniana* (17,5%), *Ammonia tepida* (5,6%) and *Elphidium* sp. (6,6%) at station p07. Diversity peaks can also be seen at stations p01 and p02 (Figure 3), located close to the connection with the Atlantic Ocean. At station p01, located near to urban occupations of Imbé, there are agglutinating species such as *A. pseudospiralis*, *A. salsum*, *H. wilberti*, *Miliammina* sp., *T. inflata* and *T. salsa*. At station p02, located in the channel leading into the South Atlantic Ocean, hyaline calcareous species are found, such as *B. frigida*, *A. tepida*, *A. parkinsoniana*, *A. rolshauseni*, *B. sp. cf. B. marginata*, *Elphidium* sp., *N. atlantica* and a planktonic specimen of *Globigerinoides ruber* (Figure 4).

All sampling stations, except for station p03, yielded living specimens, which correspond to just 5% of the total number of recovered individuals, mostly assigned to *A. salsum*, *A. pseudospiralis*, *A. tepida*, *A. parkinsoniana*, and *Elphidium* sp.. A smaller number of individuals with signs of dissolution and deformities on their tests were also identified, mainly at sampling stations p02, p05 and p07, being represented by the species *A. tepida*, *A. parkinsoniana* and *Elphidium* sp., and

corresponding to 1,2% of the total number of recovered individuals (Table 1).

Table 1: Census counts of benthic foraminiferal species recovered at each sampling station in the Tramandaí Armazém Lagoon, with the total number of living (L) and dead (D) specimens. Counts of dissolved or deformed specimens are relative to the total amount of recovered foraminiferal specimens at each station.

Station	p01		p02		p03		p04		p05		p06		p07		p08		% total assemblage
Species (living or dead)	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	
<i>Ammoscalaria pseudospiralis</i>	20	-	-	-	16	-	52	5	-	-	23	1	2	-	-	-	5,9%
<i>Ammotium salsum</i>	161	35	5	-	104	-	433	20	-	-	300	11	13	2	28	5	55,2%
<i>Buccella frigida</i>	-	-	51	-	-	-	1	-	-	-	-	-	-	-	-	-	2,6%
<i>Ammonia tepida</i>	2	1	10	-	-	-	-	1	5	-	-	9	83	2	-	-	5,6%
<i>Ammonia parkinsoniana</i>	-	-	12	2	4	-	-	1	84	11	-	1	233	6	-	-	17,5%
<i>Ammonia rolshauseni</i>	-	-	12	-	-	-	-	-	-	-	-	-	-	-	-	-	0,6%
<i>Bulimina sp. cf. B. marginata</i>	-	-	23	-	-	-	-	-	-	-	-	-	-	-	-	-	1,1%
<i>Elphidium sp.</i>	-	-	84	4	-	-	-	-	4	-	-	7	34	-	-	-	6,6%
<i>Haplophragmoidea wilberti</i>	3	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	0,2%
<i>Miliammina sp.</i>	10	1	2	-	-	1	14	1	-	-	11	1	1	-	4	-	2,3%
<i>Nonionella atlantica</i>	-	-	22	1	-	-	-	-	-	-	-	-	-	-	-	-	1,1%
<i>Trochamminita salsa</i>	2	-	-	-	2	-	-	-	-	-	-	-	1	-	-	-	0,2%
<i>Trochammina inflata</i>	4	-	4	-	4	-	-	-	-	-	-	-	-	-	1	-	0,6%
<i>Textularia sp.</i>	-	-	1	-	-	-	-	-	-	-	-	-	4	-	-	-	0,2%
<i>Globigerinoides ruber</i>	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	0,04%
Total	237		231		130		529		103		364		382		37		2022
Deformed/ dissolved	2		4		2		1		4		0		11		0		1,2%

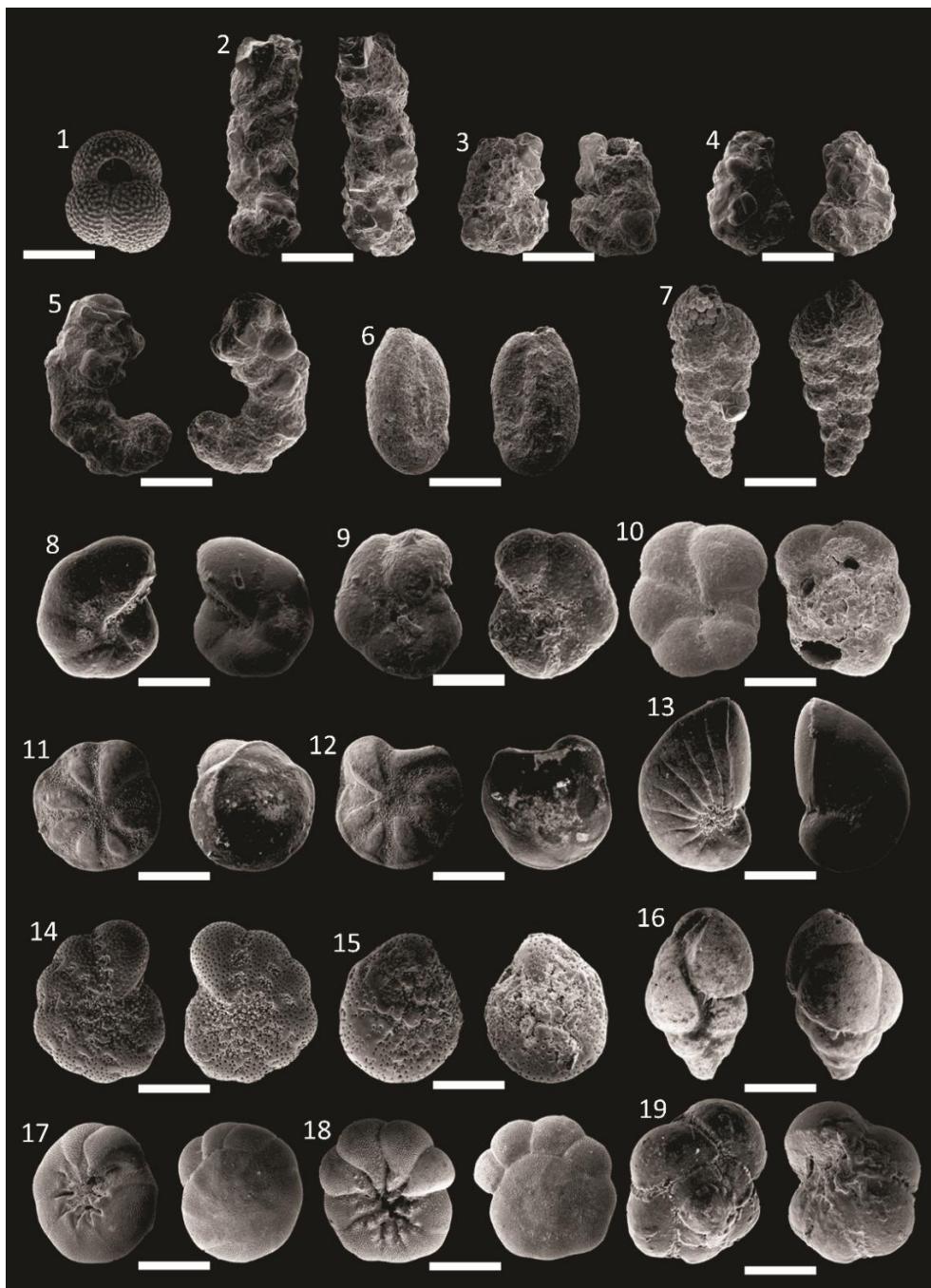


Figure 2: Scanning electron micrographs of benthic foraminifera recovered from the Tramandaí-Armazém Lagoon. 1: *Globogerinoides ruber* (d'Orbigny, 1839) found at p02; 2 - 4: *Ammotium salsum* (Cushman and Brönnimann, 1948) present at all stations, except for p05; 5: *Ammoscalaria pseudospiralis* (Williamson, 1858), present at stations p01, p03, p04 and p06; 6: *Milliammina* sp. (Loeblich and Tappan, 1955) found at stations p01, p04 p06 and p08; 7: *Textularia* sp. (d'Orbigny, 1839) found at p07; 8: *Haplophragmoides wilberti* (Andersen, 1953) present at stations p01 and p02; 9: *Trochammina salsa* (Cushman and Brönnimann, 1948) found at stations p01 and p02; 10: *Trochammina inflata* (Montagu, 1931) found at stations p01, p02 and p03; 11 - 12: *Buccella frigida* (Cushman, 1922) found at station p02 and p04; 13: *Nonionella atlantica* (Cushman, 1947) found at station p02; 14 - 15: *Elphidium* sp. (Terquem, 1875) found in p02, p05 and p07; 16: *Bulimina* sp. cf. *B. marginata* (d'Orbigny, 1826) found only at station p02; 17: *Ammonia tepida* (Cushman, 1926) found at all stations except at p03 and p08; 18: *Ammonia Parkinsonana* (d'Orbigny, 1839) found at stations p02, p03, p04, p05, p06 and p07; 19: *Ammonia rolshauseni* (Cushmas and Bermúdez, 1946) found at station p02. Scale bars represent 100 µm.

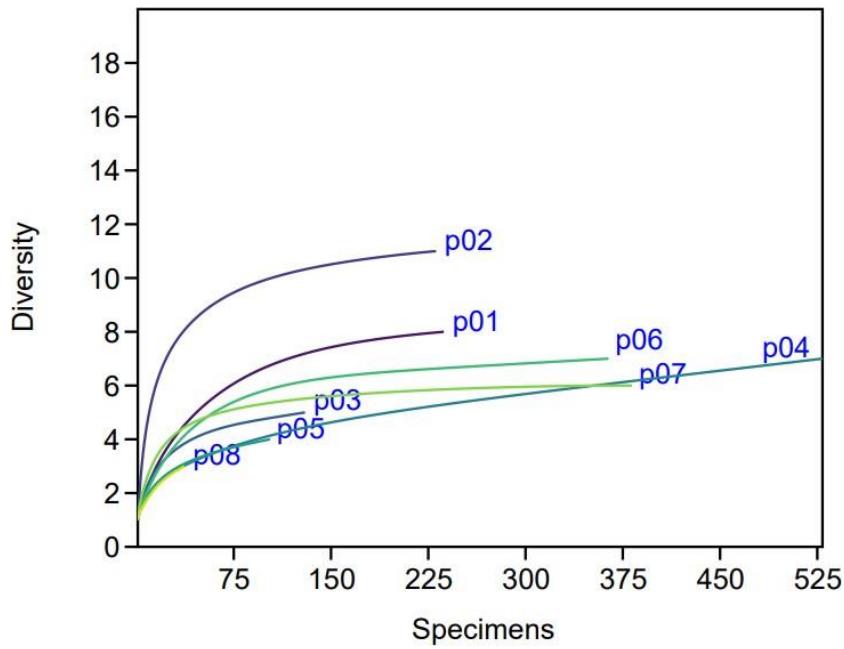


Figure 3: Rarefaction curves showing the relationship between the number of specimens (total abundance) and the number of recovered species (diversity) at each sampling station in the Tramandaí-Armazém Lagoon. Curves tend to stabilize above  $\sim 75$  recovered specimens recovered, except for station p04, suggesting that our sampling is representative of the foraminiferal diversity in the lagoon.



Figure 4: Foraminiferal distributions in the Tramandaí-Armazém Lagoon. Calcareous foraminifera dominate stations characterized by higher water conductivity and salinity (central green shaded area), and agglutinated foraminifera dominate stations further away from the channel connecting the lagoon to the South Atlantic Ocean (blue shaded areas).

## Water physicochemical analyses

Physicochemical conditions of surface water in the lagoon, measured during sampling, showed minor to moderate variations between sampling stations (Table 2). pH was slightly acidic, ranging from 5 at stations p01, p02 and p07, 6 at stations p03, p04, p06 and p08, and 7 at sampling station p05. Surface water temperature also varied slightly, from 18.8 °C at station p08 to 22 °C at station p01. Conductivity and salinity varied the most between the sampling stations, ranging from 1.4 to 19.3, respectively, with the highest values at stations p04, p05, p06 and p07, in the innermost region of the lagoon.

Analyses of dissolved metals in the water showed small variability between sampling stations, with values dominantly below the quantification limit of the method (Table 3). Relatively higher concentrations in the water occur for Al, B, Fe and Mg (Table 3). It is possible to observe a subtle increase in the dissolved Al content at stations p01, p02, p06, p07 and p08, closer to urban occupations of the city of Imbé, and the Tramandaí River delta. Dissolved Fe contents were higher at stations p01, p02 and p08, closer to the urban occupation of Imbé. Dissolved Mg and B contents were higher at stations p04, p05, p06 and p07, in the innermost region of the lagoon. Concentrations of Ag, As, Be, Cd, Co, Cr, Hg, Ni, Pb, Sb and Se did not vary between sampling stations, and were usually below our analytical detection limit.

Considering dissolved metal concentrations in the water of the Tramandaí-Armazém Lagoon, no measured metal was above the standards established by the Brazilian National Environmental Council (CONAMA) and State Environmental Council (CONSEMA) agencies, as reported in the CONAMA resolutions 20/1986, 357/2005, 396/2008, 397/2008, and CONSEMA resolution 355/2017. For a broader comparison, we also compared our measured values with threshold values for water quality standards of the United States Environmental Protection Agency (EPA), as well as the Dutch List of Soil and Groundwater Quality Values - 6530, for which dissolved metal concentrations of the Tramandaí-Armazém Lagoon are still within accepted values. Therefore, our water dissolved metals data indicate that water of the Tramandaí-Armazém Lagoon is not currently affected by anthropogenic heavy metals contamination, as supported by low frequencies of abnormal/deformed foraminiferal tests (e.g., Frontalini et al., 2009; Coccioni et al., 2009; Bouchet et al., 2018). Minor morphological modifications were already observed by Closs et al. (1971), as one moves northward in the lagoon, with changes in test wall thickness, ornamentation, chamber arrangement, and aperture appearance.

Table 2: Measurements of physicochemical water parameters of surface waters at each sampling station in the Tramandaí-Armazém Lagoon.

<b>Physicochemical parameters</b>	<b>p01</b>	<b>p02</b>	<b>p03</b>	<b>p04</b>	<b>p05</b>	<b>p06</b>	<b>p07</b>	<b>p08</b>
<b>pH</b>	5	5	6	6	7	6	5	6
<b>Temperatures (° C)</b>	22	19,4	20,1	19,4	19,6	20,1	20,9	18,8
<b>Conductivity (S/m)</b>	82	767	877	1720	1620	1775	1330	720
<b>Salinity (‰ p. eq. NaCl)</b>	1,4	7,7	8,9	18,6	17,4	19,3	14,1	7,2

Table 3: Concentrations of metals dissolved in water (ppm) measured by ICP-OES for each sampling station in the Tramandaí-Armazém Lagoon. Where <Q.L. means that concentrations did not reach quantification limits of the method.

<b>Sample</b>	<b>p01</b>	<b>p02</b>	<b>p03</b>	<b>p04</b>	<b>p05</b>	<b>p06</b>	<b>p07</b>	<b>p08</b>
<b>Analyte</b>	ppm							
<b>Ag</b>	0,002	<Q.L.						
<b>Al</b>	0,052	0,346	0,435	0,289	0,196	0,257	0,354	0,384
<b>As</b>	0,017	<Q.L.						
<b>Ba</b>	0,012	0,013	0,018	0,031	0,027	0,033	0,026	0,023
<b>Be</b>	0,001	<Q.L.	<Q.L.	0,001	<Q.L.	<Q.L.	<Q.L.	<Q.L.
<b>B</b>	0,025	0,034	0,120	0,152	0,529	0,208	0,296	0,199
<b>Cd</b>	0,001	<Q.L.						
<b>Co</b>	0,008	<Q.L.						
<b>Cu</b>	0,007	0,007	0,009	0,006	0,007	0,007	0,007	0,007
<b>Cr</b>	0,017	<Q.L.						
<b>Fe</b>	0,163	1,213	1,232	0,990	0,404	0,865	0,931	0,996
<b>Li</b>	0,521	0,001	0,005	0,098	0,013	0,009	0,012	0,008
<b>Mg</b>	0,152	6,615	32,517	44,914	157,249	63,802	89,864	58,981
<b>Mn</b>	0,056	0,039	0,040	0,033	0,021	0,029	0,033	0,033
<b>Ni</b>	0,003	0,003	0,003	0,003	<Q.L.	<Q.L.	0,003	0,003
<b>P</b>	0,085	<Q.L.	0,098	<Q.L.	<Q.L.	<Q.L.	<Q.L.	<Q.L.
<b>Pb</b>	0,049	<Q.L.						
<b>Sb</b>	0,011	<Q.L.						
<b>Se</b>	0,024	<Q.L.						
<b>U</b>	-	-	-	-	-	-	-	-
<b>V</b>	0,033	0,005	0,014	0,017	0,028	0,021	0,025	0,016
<b>Zn</b>	0,253	0,010	0,014	0,017	0,011	0,030	0,020	0,018

### **Statistical analysis of the data**

Pearson's coefficients ( $r$ ) between faunal data and water parameters were significant for abundances of *Ammoscalaria pseudospiralis*, *Ammotium salsum*, *Ammonia rolshauseni*, *Bulimina* sp. cf. *B. marginata*, *Elphidium* sp., *Miliammina* sp., *Nonionella atlantica*, *Globigerinoides ruber*, *Textularia* sp., *Trochammina inflata*, for the water salinity, and for the water dissolved contents of Cu, Fe, Mg, Mn, P and V (appendix). Significant correlations were those whose linear relationships were closer to 1 or -1 (strong positive/ negative correlation). Among foraminiferal abundances, it is possible to establish positive correlations between *A. pseudospiralis* with the species *A. salsum* (0.96), *B. frigida* with *B.* sp. cf. *B. marginata* (0.99), *B. frigida* with *Elphidium* sp. (0.93), *B. frigida* with *N. atlantica* (0.99) and *B. frigida* with *G. ruber* (0.99). The correlation presented between *A. rolshauseni* and *B. Frigida* abundances was also high (0.99), as well as correlations between *B.* sp. cf. *B. marginata* and *Elphidium* sp. (0.93), *B.* sp. cf. *B. marginata* and *N. atlantica* (0.99), *B.* sp. cf. *B. marginata* and *A. rolshauseni* (1.00), *A. rolshauseni* with *N. atlantica* (0.99), *A. tepida* and *Textularia* sp. (0.98) and *B.* sp. cf. *B. marginata* and *G. ruber* (1.00). *Elphidium* sp. also showed significant correlations with *A. rolshauseni* (0.93), and. with *N. atlantica* (0.92), the latter also depicting strong correlation with *G. ruber* (0.92).

Water salinity and conductivity showed high correlation with each other (0.99), as expected. Among water dissolved metal contents, Cu showed a high correlation with the species *B. frigida* (0.91), with *B.* sp. cf. *B. marginata* (0.91), with *A. rolshauseni* (0.91), with *Elphidium* sp. (0.88) with *N. atlantica* (0.91) and with *G. ruber* (0.91). Dissolved Fe content showed a strong negative correlation with water B content (-0.95). Unlike Mg content, which showed a strong positive correlation with B (0.99), but a negative correlation with Fe (-0.95). Dissolved Mn showed a strong positive correlation with Fe (0.98), and a strong negative correlation with Mg content (-0.92) and with B content (-0.91). Dissolved P showed a strong positive correlation with *B. frigida* (0.99), with *A. rolshauseni* (1.00), with *B.* sp. cf. *B. marginata* (1.00), with *N. atlantica* (0.99) and with *G. ruber* (1.00). Finally, it is still worth highlighting the correlations between V and conductivity (0.94), V and salinity (0.94), V and B (0.91), and between V and Mg (0.92).

It is important to highlight that some strong correlations, such as the correlations between dissolved P in water with the species of *B. frigida*, *A. rolshauseni*, *B.* sp. cf. *B. marginata*, *N. atlantica* and *G. ruber*, due to the recovery of these species, occurred only at sampling point p02, the station where dissolved P was presented in larger quantities, which would explain the high correlations.

## **Discussion**

### **Salinity constrains on foraminiferal distributions in the Tramandaí-Armazém Lagoon**

Lagoons, estuaries, and deltas environmental dynamics are heavily controlled by river inlets, waves action and tidal regimes that shape these transitional settings. The interplay between fluvial and marine regimes impacts physical, chemical, and biological processes in these environments (Murray, 2006). Among foraminifera, this balance is depicted by the substitution between species, since each taxon has a specific environmental niche, and can be conditioned by several factors, including food availability, oxygen concentrations, environmental stability, as well as predation and competition (e.g., Jorissen et al., 1995; Murray, 2001). From Figure 4, which shows the distribution of benthic foraminiferal species in the Tramandaí-Armazém Lagoon, it is possible to establish a clear distribution pattern for the recovered foraminiferal taxa.

In the Tramandaí-Armazém Lagoon we also observe a variation in sedimentary deposition, evidenced by Tabajara and Dillenburg (1997), where larger sediments, such as sand, would be located close to the banks, whereas finer sediments, such as silts and clays, would be arranged in the portions central areas of the lagoon, transported mainly due to the action of winds directed towards the NE and the influence of the tide into the lagoon (Tabajara et al., 1997). This sedimentation pattern can influence the distribution of benthic foraminifera found in the lagoon, as these organisms use the sediment available in the environment to manufacture their tests.

Our new foraminiferal distribution and water properties data collected in the Tramandaí-Armazém Lagoon clearly depicts a wedge of high salinity water influx, defined by stations p02, p05, and p07 (Figure 6). These stations are aligned with the channel connecting the lagoon to the South Atlantic Ocean, and exhibit relatively high salinity and conductivity values, as well as dissolved B contents, the latter tending to increase with increasing water salinity (Wei and Algeo, 2019; 2020). Additionally, dissolved Mg contents are high at stations p05, p06, and p07, which could be due to the fact that Mg is usually present as a salt ( $MgCl_2$ ) in ocean waters (Lopez et al., 2009). Foraminiferal assemblages characterized by the occurrence of fully marine taxa, such as *G. ruber* and *Bulimina* sp. cf. *B. marginata*, occur at station p02, and taxa with mixohaline preferences occur at stations p05 and p07 (Murray 2001; Gupta, 2003). Hyaline calcareous species at stations p05 and p07, the innermost points of the lagoon, correspond mainly to *Ammonia* and *Elphidium* species, with high tolerance to changes in salinity, oxygen availability, and variations in sediment composition (Murray, 2006). However, despite their high tolerance to dissolved oxygen variations, they are sensitive to the availability of organic carbon, which may regulate their occurrences in the innermost part of the lagoon (Sen Gupta et al., 2006; see section below). These water physicochemical settings are comparable to those previously documented by Wurdig (1987), Kapusta (2005), and Correa et al. (2021) for the Tramandaí-Armazém Lagoon.

Modern benthic foraminifera with agglutinated tests are highly adapted to transitional environmental conditions, such as high-amplitude changes in salinity or organic matter accumulation. Agglutinated multilocular, planispiral/trochospiral, lenticular forms are particularly common on shelf and in marine marginal environments (Murray et al., 2011). Elongated organisms, such as milliamminids, are characteristic of marshes and lagoons (Murray, 1991; Sen Gupta et al., 2003). Agglutinated benthic foraminifera occupy mainly the margins of the Tramandaí-Amazém (Figures 5, 6), close to urban areas, where salinity is lower due to fresh-water input. In addition, sediments deposited close to the margins of the lagoon are of coarser granulometry (Tabajara and Dillemburg, 1997), which would favor the occurrence of coarsely agglutinating tests, such as those of *A. salsum* (Figure 2).

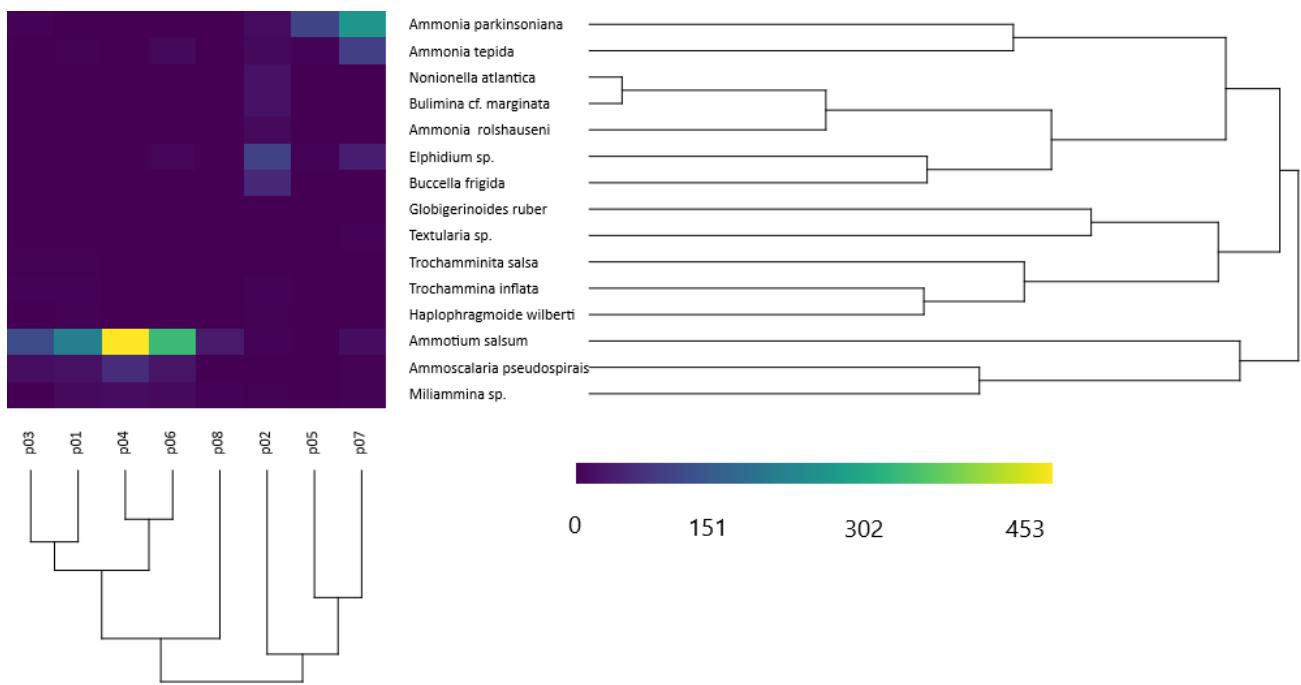


Figure 5: Cluster analyses of foraminiferal counts in the Tramandaí-Armazém Lagoon. We present clustering in Q and R modes, using the Bray-Curtis similarity coefficient (Bray and Curtis, 1957). Color scheme represents foraminiferal counts at each station.

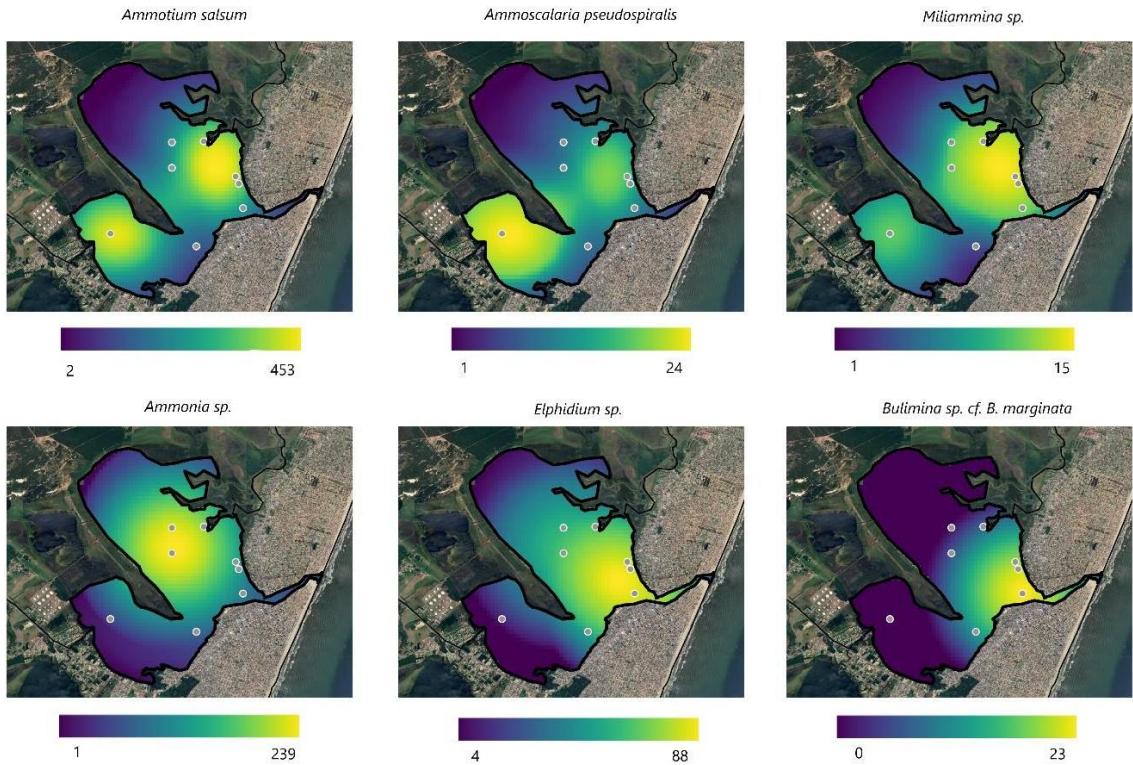


Figure 6: Heat maps, using Kernel interpolation, showing the abundances (total counts) of the species *A. salsum*, *A. pseudospiralis*, *Miliammina* sp., *Ammonia* sp., *Elphidium* sp., and *B.* sp. cf. *B. marginata* in the Tramandaí-Armazém Lagoon.

#### Dissolved oxygen constrains on foraminiferal distributions in the Tramandaí-Armazém Lagoon

Besides changes in salinity, water oxygenation, and pH are likely major environmental forcings in the lagoon. The amount of food supply (organic carbon) available in the water influences the availability of dissolved oxygen and water pH. Thus, environments with a high influx of organic material consume significant amounts of dissolved oxygen in the process of oxidizing organic carbon, leading also to water acidification (Jorissen et al., 1995). This scenario agrees well with our observations in the Tramandaí-Armazém Lagoon, as in areas with slightly acidic waters agglutinated benthic foraminifera tend to dominate and calcareous tests show signs of dissolution. Additionally, infaunal foraminifera with greater tolerance to reduced oxygenation and increased food supply also tend to occur in these environmental settings (Sen Gupta et al., 2006; Jorissen et al., 1995; 2007).

Water pH measurements indicate more acidic waters near the urban shoreline of Imbé, at stations p01, p02, and p07, at the time of collection. This observation could be attributed to increase riverine runoff and/or discharge of domestic sewage, both enriched in organic carbon, directly into the lagoon, which would lead to water acidification (e.g., Koutsoukos et al., 1990; Murray, 2001; Caratelli et al., 2023). In fact, the highest frequencies of calcareous tests with signs of dissolution

occurred at stations p02, p05, and p07, which are also characterized by the occurrence of infaunal taxa, such as *B.* sp. cf. *B. marginata* and *N. atlantica*. These more elongated serial benthic foraminifera are usually assigned to infaunal microhabitats and thrive under increased food supply and/or reduced bottom water oxygenation (Boltovskoy et al., 1991; Sen Gupta, 2003; Murray, 2006; Jorissen et al., 1995; 2007). Our first report of *Bulimina* sp. cf *B. marginata* at station p02 in the Tramandaí-Armazém Lagoon is striking. This species typically inhabits shelf environments, with salinities varying between 25 and 35 psu, it is capable of withstanding significant oxygenation changes associated with substantial inputs of organic carbon, and it thrives in stressful environments (Murray, 1991; Eichler et al., 2014). Increased rates of organic carbon oxidation at stations p02, p05, and p07 would decrease water oxygenation, which is supported by relatively high dissolved Al, Cu, and Fe contents (Byrd et al., 1990), since these precipitation of these elements as oxi-hydroxides would not be favored under these conditions.

*Ammotium salsum* is the most abundant species near the shores of the lagoon, in areas characterized by more acidic waters, with higher concentrations of dissolved metals, like Al, Fe and Mn. It is known to tolerate a wide range of water salinity and increased organic matter flux (Murray,

1991; Sen Gupta et al., 2003). Furthermore, it is an infaunal taxon (Jorissen et al., 1995; Murray, 2006) that probably thrived under increased food supply (e.g., phytodetritus) brought to Tramandaí-Armazém Lagoon by riverine input and/or anthropic influence (Martins et al., 2021). It is also possible to observe the presence of *Miliammina* sp. at station p01, p04, p06 and p08, species that, as previously described, are characteristic of the lagoonal environments (Sen Gupta et al., 2003). Furthermore, the prevalence of agglutinated benthic foraminifera in the Tramandaí-Armazém Lagoon would be facilitated by urban growth around, which promotes increased organic material deposition and acidifying waters, leading to dissolution calcareous forms post-mortem (Leipnitz et al., 2014).

### Concluding remarks

We presented an integrated benthic foraminiferal and water physicochemical study to assess environmental factors controlling foraminiferal distributions in the Tramandaí-Armazém Lagoon, southern Brazil. Our data suggests that environmental dynamics in the lagoon are driven by an interplay of marine, continental, and, likely, anthropogenic factors. Foraminiferal distributions and concentrations of dissolved metals in the water are highly influenced by the inflow of a high-salinity wedge in the central region of the lagoon, as well as by enhanced organic carbon flux, the latter leading to water deoxygenation and acidification.

Besides occurrences of *B.* sp. cf. *B. marginata*, we cannot find marked faunal composition changes when comparing the species recovered herein with previous studies of the Tramandaí-Armazém Lagoon. Since the 1960's, there are reports of assemblages dominated by agglutinated species, such as *Ammotium salsum*, *Trochammina salsa*, *Miliammina fusca* (Closs et al., 1967; Leipnitz et al., 2014; Ferreira et al., 2015, Martins et al., 2021), *Trilocularena patensis* (Closs et al., 1967; Leipnitz et al., 2014; Ferreira et al., 2015), *Ammoscalaria pseudospiralis*, *Haplophragmoides wilberti*, *Miliammina earlandi*, *Trochammina inflata* (Leipnitz et al., 2014). Among calcareous species, *Ammonia* and *Elphidium* species dominate, together with *Nonionella atlantica* (Closs et al., 1967; Leipnitz et al., 2014; Ferreira et al., 2015).

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## **Síntese integradora**

Nossos resultados mostram que a diversidade de foraminíferos bentônicos na Laguna Tramandaí-Armazém é influenciada por fatores como a disponibilidade de alimentos e a salinidade da água. Observamos uma dominância de espécies aglutinantes próximas à costa da laguna, enquanto espécies calcárias hialinas, que preferem salinidades mais altas, dominam a porção central da laguna.

As concentrações de metais dissolvidos na água da laguna estão dentro dos limites aceitáveis de acordo com padrões brasileiros e internacionais. No entanto, identificamos que o alto aporte de carbono orgânico, derivado de aporte fluvial e/ou descarte de esgoto das cidades próximas, parece afetar a oxigenação e pH da água, o conteúdo de metais dissolvidos e, consequentemente, a distribuição de foraminíferos na Laguna Tramandaí-Armazém. Mesmo que as associações de foraminíferos bentônicos tenham permanecido relativamente estáveis desde a década de 1960, registramos pela primeira vez a ocorrência do gênero *Bulimina*, o que sugere um aumento recente no suprimento de carbono orgânico e uma redução na oxigenação na laguna.

Nossos resultados destacam a importância de um monitoramento contínuo e dos esforços de conservação na Laguna Tramandaí-Armazém e nos corpos d'água adjacentes no sul do Brasil. À medida que as áreas urbanas próximas continuam a crescer e o tratamento inadequado de esgoto persiste, é essencial tomar medidas para proteger esses valiosos ecossistemas costeiros e garantir suaqualidade ambiental para as gerações futuras.

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