Research Square

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Bacterial community structure along the estuary–ocean gradient based on 16S rRNA gene profiling

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Abstract

Microorganisms inhabiting the estuarine and marine zones of the Patos Lagoon estuary play key ecological roles and warrant attention due to their potential implications for human health. The biochemical interactions that occur in this transitional environment contribute to the uniqueness of one of the world's largest choked lagoons. This study determines the bacterial composition in the Patos Lagoon estuary and adjacent marine area using 16S rRNA amplicon sequencing. It represents the first application of high-throughput sequencing to comprehensively assess the estuarine–marine microbiota of this system.

Variations in temperature, salinity, dissolved oxygen, and nutrient concentrations were the primary drivers of community structure. Higher temperatures combined with reduced oxygen levels were associated with increased microbial density and a predominance of Proteobacteria, particularly in areas subjected to greater anthropogenic influence. Conversely, regions under lower human impact exhibited more stable environmental conditions, lower microbial densities, and a more balanced taxonomic distribution.

These findings underscore the strong influence of environmental variability on microbial communities along the estuary–ocean continuum. The seasonal shifts and site-specific differences observed highlight the dynamic nature of coastal ecosystems and reinforce the importance of continuous microbial monitoring.

INTRODUCTION

Estuaries and adjacent oceanic areas are highly dynamic and productive environments, crucial for sustaining upper trophic levels with food resources at rates hard to replicate in terrestrial settings. Bacterial life is abundant and widely distributed, colonizing even the harshest environments and maintaining continuous cycles. Despite the vast microbial diversity present, representing almost 99% of all non-cultivable microorganisms in any given ecosystem (Bobrova, Kristoffersen, Oulas, & Ivanytsia, 2016 ; Shah, Tang, Doak, & Ye, 2011), there are still limited studies focusing on the richness and native microbial variety of these ecosystems. Salinity, temperature, and nutrient concentration vary according to seasonal rainfall and wind regimes, generating well-defined gradients that influence microbial distribution and metabolic activity.

Bacteria are estimated to constitute up to 20% of the Earth's total biomass (DeLong & Pace, 2001), and many of their ecological roles remain largely unknown. Most microorganisms are challenging to study in culture, as they fail to grow in laboratory conditions, depend on specific environmental conditions, or may have even gone extinct without being discovered (Malik, Beer, Megharaj, & Naidu, 2008; Tringe & Rubin, 2005). In estuarine and marine ecosystems, these bacterial communities play critical roles in biogeochemical processes, contributing to the balance between production and consumption of substances in these regions (Bobrova et al., 2016). However, human activities increasingly impact these habitats, reinforcing the importance of developing monitoring and assessment tools that can detect

early ecological disturbances and support evidence-based actions to preserve ecosystem functionality rather than merely assuming balance can be maintained.

Genomic data offer a comprehensive view of the marine environment, revealing information on genes, regulatory elements, and functional regions, which allows a deeper understanding of microbial systems (Bourlat, Borja, & Gilbert, 2013). Yet, defining what constitutes a species remains challenging, and various approaches, such as Operational Taxonomic Units (OTUs), are used to describe indirectly detected organisms (They & Motta-Marques, 2019). 16S rRNA amplicon-based profiling, through Next-Generation Sequencing (NGS) technologies, provides an accurate method for exploring microbial community organization, species diversity, and functional variety, based on the amplification of the rrs gene encoding for the 16S rRNA subunit, especially in hypervariable regions (Bukin et al., 2019; Y. Wang & Qian, 2009).

Efforts toward the sustainable use of coastal waters are assessed through an integrative approach, encompassing ecosystem components, physico-chemical parameters, pollutant elements, and potentially harmful substances such as antimicrobials and resistance genes (Q. Wang et al., 2021). The 16S rRNA gene profiling allow for mapping genes or proteins with specific functions, providing valuable insights into uncultivated microbial communities typically analyzed only through marker genes (Tringe & Rubin, 2005).

This study provides an overview of the bacterial composition in the Patos Lagoon estuary and adjacent marine area using 16S rRNA 16S rRNA metabarcoding. By examining bacterial diversity along an estuary-to-ocean gradient, this pioneering research establishes a foundational understanding of the microbial communities in the region. The results offer critical insights into both current and potential future environmental threats arising from anthropogenic activities and natural factors, providing a valuable reference point to support future coastal management and conservation strategies.

METHODS

Study Sites

Samples were collected monthly from July of 2021 to October 2022 in three stations on the Patos Lagoon estuary and oceanic adjacent area (Estuarine Area; Patos Lagoon Bar; and Marine Area - Cassino beach) (Fig. 1). 1- Upper Estuary Area, 2- Lower Estuary (LEA) and 3- Adjacent Marine Coast (AMC) (Fig. 1)(M. A. Rodrigues, Ortega, & Dincao, 2019; M. A. D. I. Rodrigues, F, 2015). EA is the most anthropized location of all three, for its proximity with the urbanized area of the city of Rio Grande, in Southern Brazil. PLB is the point where estuary and marine area encounter, with the discharge of the Patos Lagoon estuary on the top water layer, and the high salinity marine water entering in the bottom layer.

The Patos Lagoon estuary represents a complex transitional ecosystem influenced by both freshwater inflow and marine intrusion, resulting in marked spatial and seasonal gradients of salinity, temperature,

dissolved oxygen, and nutrient concentration. These physico-chemical parameters shape microbial community structure and function, favoring taxa capable of tolerating fluctuations in osmotic stress, oxygen availability, and organic matter load. In the estuarine area, anthropogenic inputs from urban and agricultural activities contribute to higher levels of organic pollutants, heavy metals, and antimicrobial residues, which in turn may promote selective pressure for bacteria carrying antimicrobial resistance genes. Such environmental heterogeneity creates microhabitats that drive bacterial adaptation and diversification along the estuary-to-ocean gradient, influencing the composition and resilience of local microbiomes.

Samples

Briefly, 1-liter amber sterilized bottles were used to store the water from each of the three sampling points and transported in ice to the NUPEMM – FURG Laboratory, and sterile filtered with 0,22µm pore membrane by vacuum pump, and stored in a solution with 50% ethanol and 50% MiliQ water. Total DNA was extracted with a commercial kit according to manufacturer's instructions (FastDNA Spin Kit for Soil), and the extracted DNA was analyzed through metabarcoding for the 16S rDNA/V4 (Bacteria).

Abiotic parameters

Abiotic data was measured on each of the sampling points, with a Multiparameter (YSI ProDSS, Yellow Springs, OH. USA), and consisted of salinity (PSU), dissolved oxygen (mg/L), pH and Total Dissolved Solids (mg/L).

Construction of Metabarcoding Libraries

A metabarcoding library was constructed with 200k reads using the MiSeqTM (Illumina) platform, and a taxonomic report (relative abundance: phylum to genera) was elaborated and analyzed. The taxonomic inference for phylum, class, order, family and genus was generated, and data from relative abundance and diversity were compiled. The more informative data was from phylum and class, and therefore it is showed in Results session.

Relative Abundance

Relative bacterial abundance was estimated based on the number of high-quality reads assigned to each taxonomic group after 16S rDNA sequencing. The abundance of each taxon was expressed as the percentage of total reads per sample, representing the relative contribution of that group to the microbial community. Abundance data were transformed using the Aitchison log-ratio approach and analyzed by Principal Component Analysis (PCA) to explore patterns in community structure in relation to abiotic variables (temperature, salinity, dissolved oxygen, pH, and total dissolved solids)

RESULTS

General characteristics of the sampling points analyzed.

The abiotic data variation followed the seasonal predictors, and therefore Principal Component Analysis (PCA) samples were grouped according to each of the seasons. Despite that, some winter months were grouped in both Autumn and Spring samples, for the similarities in the parameters measured. Dissolved oxygen was low in summer months, and salinity grouped the samples into two groups: low and high salinity.

Bacterial composition of the samples obtained.

A total of 52 phyla, 52 classes, 309 orders, 503 families, and 895 genera were identified from all samples analyzed (Table 1). In the estuarine area, the most abundant phyla in all samples were Proteobacteria, followed by Bacteroidota and Actinobacterota, and these three phyla comprised almost 80% of all the encountered abundance. Other important phyla encountered are described in Fig. 2.

When the three sampled locations were analyzed seasonally, the abundance varied and some phyla that were encountered in high abundances in the estuarine area are low in the marine area and vice versa (Fig. 2)

Table 1

Temp	**Spring*(1)**	**Winter(2)**	**Summer(3)**	**Autumn*(4)**
	16.3 ± 3,11	**14.9 ± 3.27**	**23.9 ± 2.47**	**16.6 ± 3.73**
Sal psu	5.5 ± 3.4	27.0 ± 1.7	32.8 ± 1.3	15.0 ± 7.9
OD %mg/L	9.0 ± 3.55	8.5 ± 7.31	6.6 ± 7.33	8.2 ± 3.99
pH	7.55 ± 0.3	7.98 ± 0.06	7.85 ± 0.1	7.84 ± 0.2
TDS (mg/L)	6.10^{-3} ±3.10^{-3}	27.10^{-3} ± 1.10^{-3}	32. 10^{-3} ±1. 10^{-3}	15. 10^{-3} ±7.10^{-3}

DISCUSSION

Understanding the bacterial diversity in marine and coastal environments through genomic tools offers a valuable approach to accurately and efficiently assess ecological changes (Bourlat et al., 2013). Continuous monitoring of bacterial communities in the Patos Lagoon estuary and adjacent marine areas is essential for detecting shifts in microbial diversity. This knowledge is important for evaluating and monitoring the health of these ecosystems and for implementing timely responses to environmental impacts.

The physicochemical and biological characterization of the three sampling sites revealed spatial and seasonal variations in microbial diversity associated with environmental gradients. Temperature, salinity, dissolved oxygen, and nutrient concentrations were the main factors influencing community composition. Higher temperatures and lower oxygen levels favored increased microbial density and the predominance of Proteobacteria in more anthropized areas. In contrast, area under lower anthropic influence showed more stable and mixed conditions, with lower microbial density but greater taxonomic balance. These results demonstrate that environmental variability strongly shapes microbial communities along the estuary–ocean interface and highlight the importance of continuous characterization of physicochemical parameters to understand ecological dynamics in coastal ecosystems under changing climatic conditions

In this study wide diversity of phyla was observed, with the most abundant belonging to groups Proteobacteria, Bacteroidota, and Actinobacterota. Members of Proteobacteria and Bacteroidota phyla were abundant in the summer in EA and PLB and less abundant in MA. These areas are the closest to the cities and with high anthropogenic interactions.

Proteobacteria is highly diverse and abundant phylum in marine and estuarine environments, including the Patos Lagoon and adjacent coastal ocean. They play a central role in nutrient cycling, especially in the degradation of organic matter and in nitrogen and sulfur cycling, which are critical for maintaining ecosystem health (Mou, Sun, Edwards, Hodson, & Moran, 2008). Some groups within Proteobacteria, including species of *Vibrio* and *Escherichia*, are known to harbor pathogenic strains that can impact human and animal health. Increased pollution and warmer temperatures can enhance the proliferation of pathogenic Proteobacteria, posing risks to public health through waterborne diseases (Colwell, 1996). Monitoring these bacterial communities can provide early warnings of potential health threats, supporting the interconnected health of humans, animals, and ecosystems. Indeed, Proteobacteria are often among the first bacterial responders to pollutants, including heavy metals, hydrocarbons, and nutrients from agricultural runoff. Further, exposure to pollutants can lead to the emergence of antibiotic-resistant strains, posing risks to both human and animal health (Gillings et al., 2015; Martínez, Coque, & Baquero, 2015). In the Patos Lagoon, where anthropogenic pollution is a concern, monitoring Proteobacteria can reveal the presence of pollutant-degrading communities as well as emerging threats from resistant bacteria.

Bacteroidota are often linked to the decomposition of organic materials and are considered key players in the remineralization of organic matter, which supports ecosystem stability and productivity (Thomas, Hehemann, Rebuffet, Czjzek, & Michel, 2011). Their presence in environments like the Patos Lagoon estuary reflects their adaptability to various ecological niches and their role in supporting food webs by converting organic compounds into forms usable by other organisms (Zhang & Lo, 2015). Furthermore, the activity of Bacteroidota is impacted by pollution and climate change, both of which can alter the availability and type of organic matter in estuarine and marine environments. Pollution, particularly from nutrient-rich runoff and heavy metals, can change the microbial community structure, often resulting in shifts that favor organisms capable of degrading pollutants, including some Bacteroidota species

(Cottrell & Kirchman, 2000). Climate change can also affect these communities by altering water temperatures, salinity, and organic input patterns. As primary decomposers, Bacteroidota are sensitive to these changes, and their response may impact the overall resilience of microbial communities in the face of climate change, potentially affecting carbon cycling and, consequently, increasing greenhouse gas emissions (Kirchman, 2002; Thomas et al., 2011).

Members of the phylum Actinobacteriota were found in all three environments, with higher prevalence during the spring. Interestingly, in the EA, microorganisms from this phylum were also detected in significant abundance during the winter. Actinobacteriota are gram-positive bacteria that, due to their complex structures and ecological roles, were long misclassified as fungi. These bacteria play crucial roles in soil and aquatic ecosystems by decomposing organic matter and cycling nutrients, particularly carbon and nitrogen (Ventura et al., 2007). The relevance of Actinobacteriota in the One Health context is significant. These bacteria are found in soil, water, and the microbiomes of animals and humans, making them integral to the interconnected health of ecosystems, animals, and humans (McDaniel, Tiemann, & Grandy, 2021). Changes in Actinobacteriota communities due to climate change and pollution can disrupt these microbial networks, potentially leading to a decrease in biodiversity, the spread of antimicrobial resistance, and impacts on food security and human health (Fierer, 2017). Understanding and monitoring Actinobacteriota in various environments thus provides valuable insights for addressing challenges related to climate change, pollution, and public health within a One Health framework.

The point EA is the most anthropized sampling site in terms of organic matter charge and DBO, thermotolerant coliforms (*pers obs.*). The variation in the abundance observed reflects the annual variation (Fig. 2). The point PLB is a highly dynamic environment, and marked changes occur, in short time, mainly due to movements of water, pushed by northeasterly and southerly winds (Castelao, 2006). The point MA is comprised of constant high salinity, thus affecting the bacterial composition and the abundance (Fig. 2).

The bacterial diversity follows the changes in salinity, temperature and other abiotic parameters measured. The variation in the abundance observed reflects the annual variation of the abiotic parameters. The Patos Lagoon Bar is a highly changing environment, and these changes occur mainly by movements of water, pushed by winds from the Northeastern and South quadrants and the Marine area is comprised of constant high salinity, thus affecting the bacterial composition and the abundance (Seeliger, 2001).

CONCLUSION

This study provides the first 16S rRNA amplicon-based mapping of estuarine and marine microbiota associated with the Patos Lagoon complex, revealing a high bacterial diversity shaped by seasonal dynamics and abiotic gradients. The predominance of Proteobacteria, Bacteroidota, and Actinobacteriota underscores the functional relevance of these groups in organic matter degradation and nitrogen and sulfur cycling, both fundamental for sustaining ecosystem homeostasis.

Our findings demonstrate that microbial community structures rapidly respond to variations in salinity, temperature, and nutrient availability, indicating that microorganisms can serve as sensitive bioindicators of environmental change and anthropogenic pressure. The higher abundance of Proteobacteria in the most impacted sites further supports their potential as biological markers of human influence and possible risks to environmental and public health associated with antimicrobial resistance gene dissemination.

Beyond expanding the understanding of coastal microbial ecology, this work establishes an unprecedented baseline for long-term environmental monitoring and for integrating One Health strategies in estuarine systems. The combined use of 16S rRNA amplicon approaches and environmental analyses opens new pathways for detecting, in near real-time, microbial responses to pollution, climate change, and extreme events, enabling early anticipation of ecological imbalances in one of the most representative lagoon systems of the South Atlantic.

Declarations

Author Contribution

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Figures

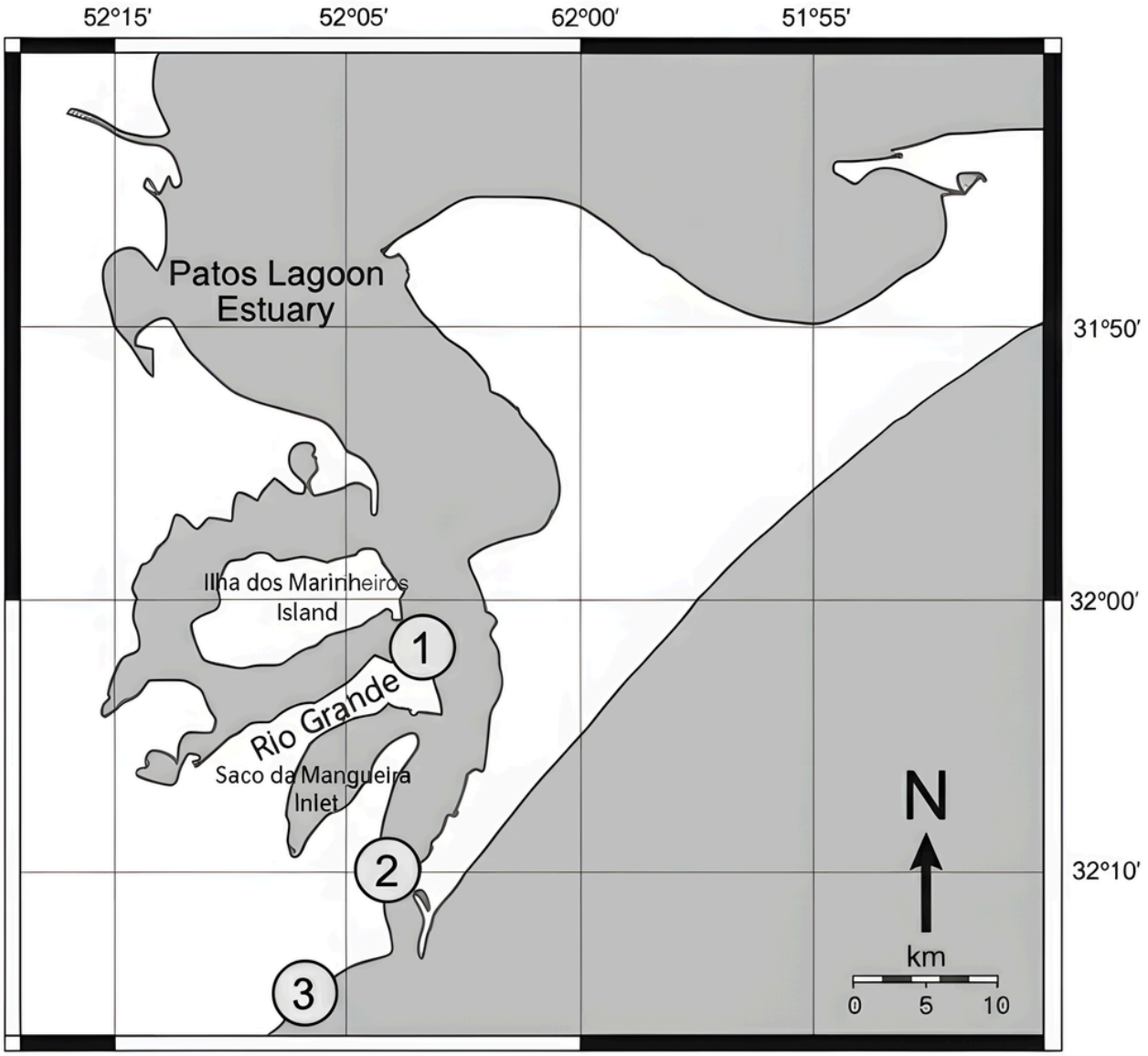


Figure 1

Map of The Patos Lagoon estuary and oceanic adjacent area. The points are marked as it follows: 1 – Estuarine area (EA); 2 – Estuary to ocean area (PLB – Patos Lagoon Bar); and 3 – Oceanic area (MA – Marine Area). Map adapted from Rodrigues et al., 2015 ((Rodrigues, Ortega, and Dincao 2019))

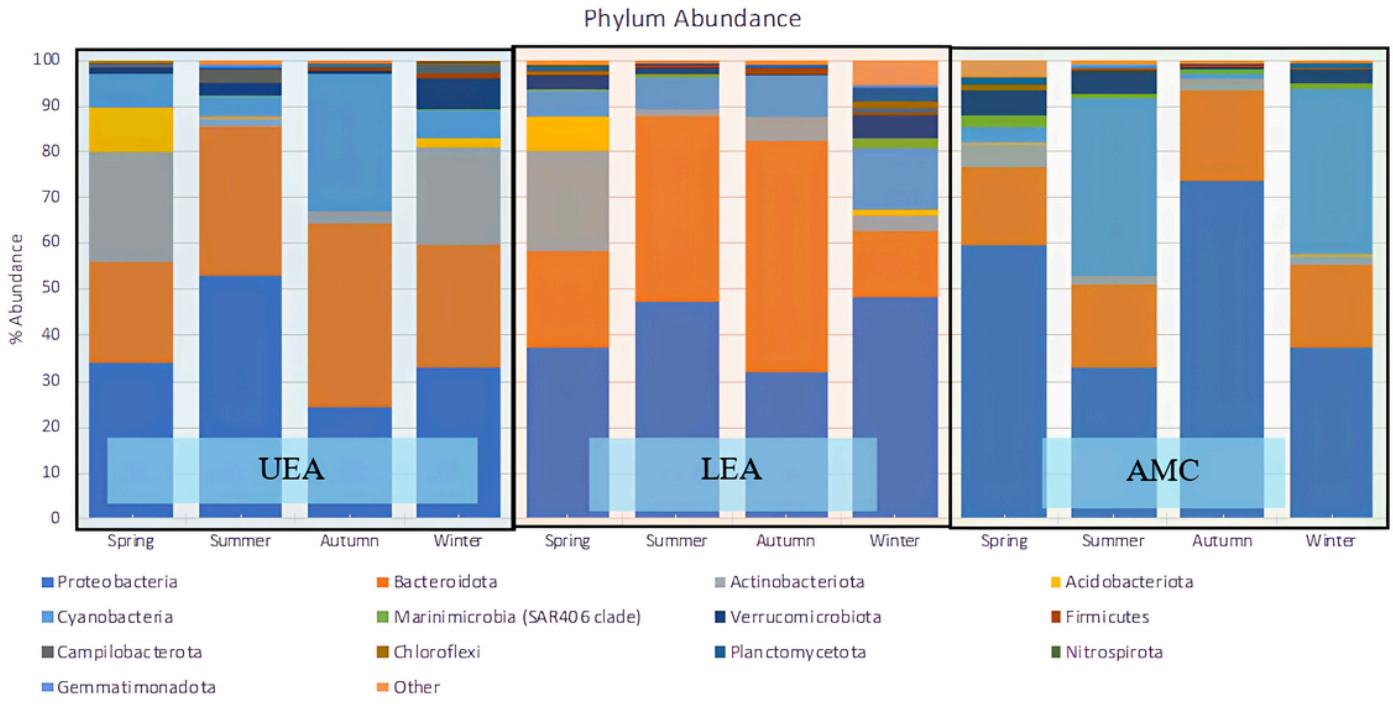


Figure 2

Abundance distribution of the most identified Phyla in the three sampled points according to seasonal grouping (EA – Estuarine Area; PLB – Patos Lagoon Bar; MA – Marine Area). Abundance data is presented in percentage.