

## IDENTIFYING HUMAN DISTURBANCE GRADIENT IN A NEOTROPICAL COASTAL PLAIN RIVER AFFECTED BY MULTIPLE STRESSORS

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**Keywords** – Anthropogenic stressors. Environmental conditions. Guaraguaçu River. Tropical coastal river. Water pollution.

### INTRODUCTION

Freshwater ecosystems are increasingly impacted by various anthropogenic activities such as changes in land use within drainage basins, removal of riparian vegetation, pollution, alterations in river flow, excessive exploitation of natural resources, introduction of invasive species, and the ongoing climate crisis (Dudgeon et al. 2006; Reid et al. 2019; Kefford, Nichols, Duncan, 2023). These pressures contribute to modifications in the natural flow of rivers, shifts in thermal conditions, and degradation of aquatic habitats. Additionally, they lead to elevated levels of nutrients, sediments, and pollutants, which negatively affect ecosystem health and ecological integrity (Dala-Corte et al. 2020; Karr, Larson, Chu, 2022; Fanelli, Cashman, Porter, 2022). In low-lying coastal regions, the degradation of wetlands and flat topography hinder effective water drainage during periods of intense rainfall, which prolongs flood events (Silva et al. 2008).

Urban development within watersheds further exacerbates hydrological disruption. The proliferation of impervious surfaces, construction of drainage systems, and artificial channelization of rivers decrease natural infiltration rates, modify water retention capacity, and alter flow patterns (Marçal, Lima, 2016; Shaikh et al. 2023). In addition, the presence of non-native species poses severe threats to local biodiversity (Clavero, García-Berthou, 2005; Guerin et al. 2018) and modifies aquatic habitat structure (Galvanese et al. 2022; Macêdo et al. 2024). These species also contribute to increased sedimentation and interference in flow dynamics (Yang, 1998; Leonard, Wren, Beavers, 2002; Temmerman et al. 2005; Zuo et al. 2025).

Faced with the current global environmental crisis, the United Nations declared the “Decade of Ocean Science for Sustainable Development” beginning in 2021 (Lopes, Rotta, 2022). This international initiative seeks to promote awareness about pollution in oceans and coastal basins and to support efforts to preserve marine resources (Lopes, Rotta, 2022). Achieving this objective requires continuous monitoring, evaluation, and spatial mapping of ecological conditions in coastal areas. Monitoring efforts are fundamental in identifying highly impacted sites and the principal stressors responsible for ecological decline (Allan, Levin, Kark, 2023; Herlihy et al. 2020). Furthermore, such efforts help to highlight less-disturbed areas with conservation potential (Fanelli, Cashman, Porter, 2022; Allan, Levin, Kark, 2023).

In Brazil, national legislation regulates the water quality standards for surface waters. CONAMA Resolution No. 357/2005 (National Environmental Council) establishes limits for physical (e.g., temperature, conductivity, turbidity), chemical (e.g., pH, dissolved oxygen, nitrate),

and biological (e.g., total coliforms, thermotolerant coliforms) variables to be assessed in monitoring programs. These limits are associated with the different classes of river conditions. For effective assessment of freshwater conditions, it also is essential to incorporate analysis of basin landscape and environmental conditions (Rosenfield; Müller, 2020). Several tools are currently available for comprehensive environmental monitoring of river basins (Tsatsaris *et al.* 2021), as water and sediment quality are commonly used approaches (Gutiérrez; Neill; Grand, 2004; Bownik; Włodkowic, 2021). The combination of land use, physical habitat and water quality variables enhance the evaluation of environmental conditions in areas with varying land uses, helping to identify key factors that contribute to either good or poor conditions (Zhang *et al.* 2023).

The coastal area of the state of Paraná in Brazil has notable economic relevance on both national and international scales due to the development of infrastructure and services that support global trade, particularly in sectors related to port operations for agribusiness and petroleum (Sezerino, Tiepolo, 2016). The Guaraguaçu River, located in this region, is a lowland coastal river that flows through the Lagamar region within the Atlantic Forest biome (Vitule, 2008; Reis *et al.* 2015). The Lagamar region, composed of a series of estuaries spanning the southern and southeastern coasts of Brazil, is among the best-preserved areas of the Atlantic Forest. Due to its exceptional ecological significance, the region is recognized both as a Biosphere Reserve and a UNESCO World Heritage Site (Galvanese *et al.* 2022). The river flows northward into Paranaguá Bay and forms an extensive floodplain surrounded by the coastal marine plain (Elste *et al.* 2019). The Guaraguaçu River basin provides a range of important ecosystem services, including fisheries, navigation, water supply, and leisure opportunities (Faria *et al.* 2021). However, the area is increasingly affected by pressures such as growing water demand, expansion of port infrastructure, rapid urban growth, lack of adequate wastewater treatment, and contamination from landfills (Marques, 2017). In addition, invasive aquatic species have been reported in the region, further threatening the ecological integrity of the river system (Bora, Thomaz, Padial, 2020; Faria *et al.* 2021).

Given this context, we conducted a comprehensive environmental evaluation of the lower stretch of the Guaraguaçu River. This integrated approach combined the application of a rapid assessment protocol, measurements of water and sediment quality, analysis of invasive aquatic plant presence, and an examination of land use patterns. Our primary goal was to describe the environmental gradient along the river's lower reaches and to provide insights into how multiple stressors are influencing the riverine environment.

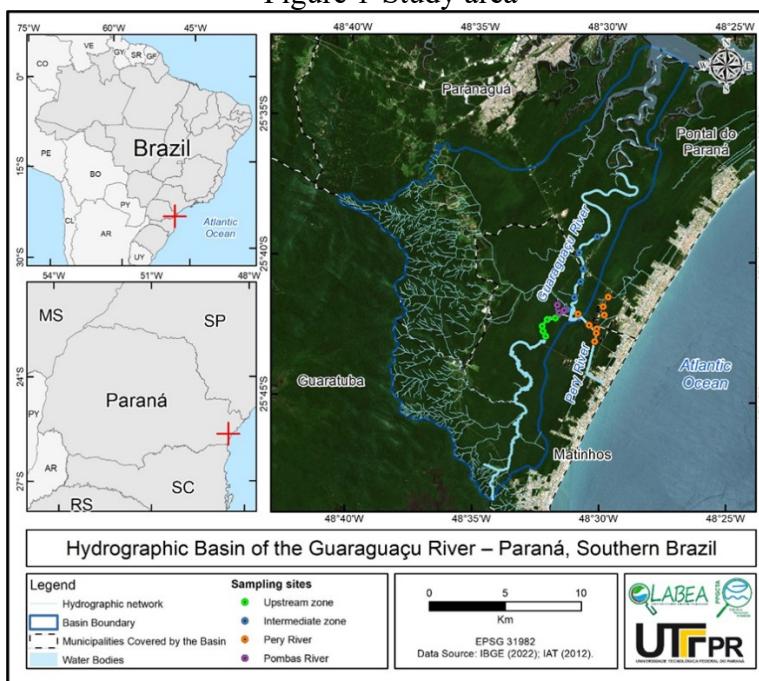
## MATERIAL AND METHODS

This study was carried out in the Guaraguaçu River Basin (BHRG), which is part of the Coastal Hydrographic Sub-Basin within the South Atlantic Hydrographic Region (Fig. 1). The basin is situated in the central portion of this region and includes both mountainous areas and lowland plains (Bigarella, 2009). The local climate is classified as humid subtropical (Abilhoa & Duboc, 2004), with an average annual precipitation of approximately 2,300 mm (Vanhoni & Mendonça, 2008). Rainfall is distributed throughout the year, although it exhibits seasonal variation, with winter being the driest season (around 280 mm of precipitation) and summer receiving the highest rainfall levels (around 800 mm; Vanhoni & Mendonça, 2008).

Sampling was conducted quarterly in March, June, September, and December 2024 at a total of 22 locations, categorized according to their sub-regions: Main channel (G1 to G11), Pery River (PER1 to PER6, POJ, and POM), and Pombas River (POO1, POO2, and POR; Table S1). Each sampling site encompassed a 50-meter stretch centered around the georeferenced point, allowing for an appropriate balance between capturing local environmental variability and ensuring logistical feasibility for sampling multiple locations (Downes *et al.*, 2002).

To evaluate the combined impact of land use, water pollution, physical habitat degradation, and biological invasion, data on RAP, land use, limnological variables, and the dominance of invasive aquatic plants were integrated to assess the environmental gradient of the Guaraguaçu River. Before combining the data, transformations were applied to minimize the impact of extreme values. Continuous positive data were transformed using the natural log ( $\ln+1$  for zero values), data with both positive and negative values were transformed using the cubic root, and percentage data were transformed using the arcsine of the square root (Johnson; Wichern, 2002). The pH values were not transformed. After transforming the other variables, they were standardized by their standard deviation to ensure equal contribution to the statistical analysis. A Euclidean distance matrix was then created to calculate the distances between the sites. Principal Coordinate Analysis (PCoA, Gower, 1966) was applied, with the "Lingoes" correction to adjust for negative eigenvalues, allowing for a Euclidean space representation of the relationships between sites based on the combined environmental variables. A correlation analysis between the standardized variables and the PCoA axes was used to identify the key variables influencing each principal coordinate.

Figure 1-Study area



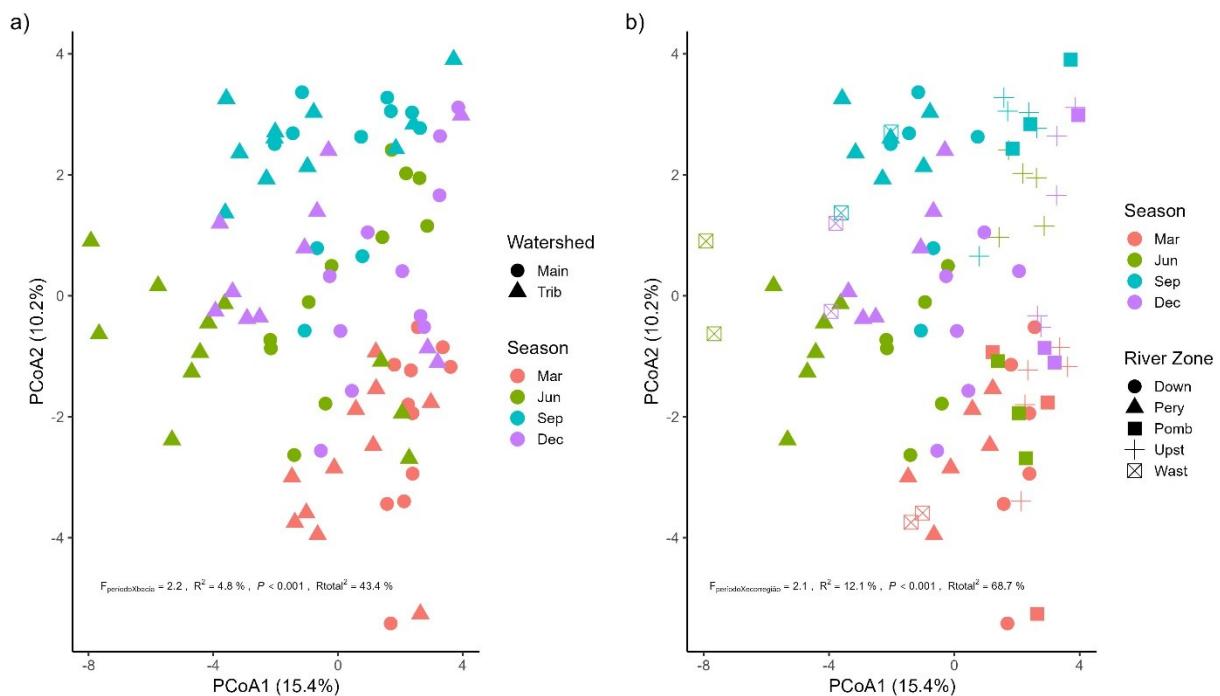
Permutational multivariate analysis of variance (Permanova; Anderson, 2001) was used to assess whether the environmental variables distance matrix could be explained by descriptor variables, including river zone, river type (main channel and tributary), dominance of invasive *U. arrecta*, and season, as well as the interaction between season and those variables. We assessed five zones: i) Guaraguaçu upstream (Upst): sites located upstream of the Pery River tributary; ii) Guaraguaçu downstream (Down): sites located downstream of the Pery River tributary; iii) Pery: sites on the Pery River; iv) Pombas: sites on the Pombas River; and v) Wastewater disposal (Wast): sites near the wastewater treatment plant. Regarding the dominance of the invasive *U. arrecta*, the term "yes" indicates that *U. arrecta* occupied more than 50% of the macrophyte cover at the site. The total  $R^2$  value of the model was used as an indicator of how well it explained the environmental variables. Analyses were conducted in R 4.4.1 (R Core Team, 2024) using the "vegan" package (Oksanen *et al.* 2015) for Permanova, data standardization, and distance matrices; "dplyr" (Wickham *et al.* 2020) for

data manipulation and transformation; "ape" (Paradis, 2012) for PCoA; and "ggplot2" (Wickham, 2011) for generating graphs.

## RESULTS AND DISCUSSION

Seasonal differences between the main channel and its tributaries were evident throughout the study period ( $F_{\text{Season} \times \text{Watershed}} = 2.2$ ;  $R^2 = 0.05$ ;  $P < 0.001$ ;  $R_{\text{total}}^2 = 0.43$ , Figure 2). The main channel's environmental conditions can be affected by substantial inflows of water, nutrients, and contaminants delivered by tributaries (Shi *et al.* 2014; Savi *et al.* 2020).

Figure 2- Principal Coordinate Analysis (PCoA) highlighting the differences between **a**) region of the basin (main channel and tributary) and sampling seasons, and **b**) sampling seasons and river zone and in which the sites were sampled (see description of the methods). Main= main channel of the Guaraguaçu River; Trib= Tributary of the Guaraguaçu River; Mar = March/2024; Jun = June/2024; Sep = September/2024; Dez = December/2024; Down= downstream; Pery= Pery River; Pomb= Pombas River; Upst= Upstream; Wast= Wastewater disposal region



Seasonal variation had a direct impact on abiotic variables across zones, thereby shaping the ecological conditions ( $F_{\text{Season} \times \text{River Zone}} = 2.1$ ;  $R^2 = 0.12$ ;  $P < 0.001$ ;  $R_{\text{total}}^2 = 0.69$ , Figure 2). Seasonal shifts in precipitation appear to play a significant role in modifying environmental variables such as nutrient concentrations, salinity, and turbidity (Mullen *et al.* 2023). For example, during the drier months of June and September, reduced rainfall likely diminished the river's capacity to dilute effluents, leading to elevated concentrations of pollutants in areas with greater human influence (Sampaio *et al.* 2023). In regions closer to the river mouth, decreased freshwater discharge likely caused salinity levels to rise due to the reduced capacity to dilute incoming seawater (Roy; Zahid, 2021). This salinity fluctuation may also be linked to tidal processes, particularly in estuarine zones, where tidal dynamics influence water circulation and saltwater intrusion (Sant'Ana; Cunha, 2022). However, such natural variability can be exacerbated by anthropogenic stressors, including the

discharge of untreated domestic sewage, urban runoff, alterations in land use, and diminished flows from water diversions (Araújo; Vitule; Padial, 2021). These factors can compromise the river's natural ability to assimilate and dilute pollutants (Sant'Ana; Cunha, 2022). Additionally, variations between wet and dry seasons influence not only the volume of water but also the extent to which nutrients and contaminants are diluted or concentrated, as well as the mobilization of sediments throughout the basin (Martins et al. 2021; Silva; Schwingel, 2021).

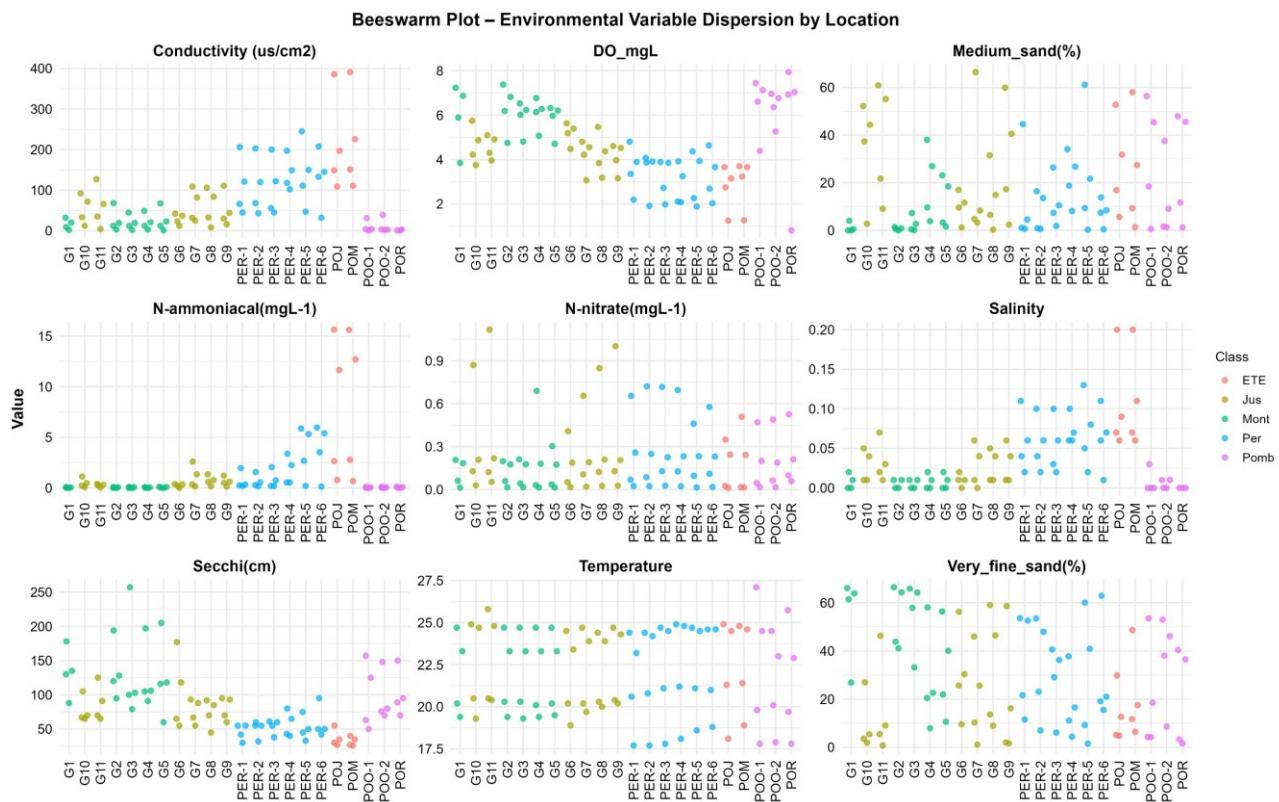
Seasonal variation may also impact the dynamics of species such as *Urochloa arrecta*, whose abundance in specific areas can be influenced by factors like rainfall (Galvanese et al. 2022). These seasonal changes may affect habitat suitability, nutrient availability, dispersal patterns, and interspecific competition along the river system (Weng et al. 2020). Despite these potential effects, the statistical test on *U. arrecta* dominance did not show significant associations with temporal or spatial variation ( $p > 0.13$ ). Instead, the proliferation and establishment of *U. arrecta* are more strongly associated with environmental conditions such as nutrient enrichment, water quality degradation, eutrophication, and physical habitat disturbance (Lindholm et al. 2020).

Overall, sites located in the lower reaches of the Guaraguaçu River (G7–G11) exhibited higher levels of conductivity, salinity, and nutrient concentrations (Figure 3). In contrast, upstream sites (G1–G5) were characterized by elevated levels of dissolved oxygen. Notably, high nitrate concentrations were observed at sites within the Pery River (PER1–PER6, POJ, POM) and in the lower Guaraguaçu, particularly at site G6, which is situated near the confluence of the Guaraguaçu and Pery Rivers. Excessive nitrogen input in aquatic environments is a key driver of eutrophication and plays a significant role in the decline of coastal ecosystem health (Esteves, 1998; Conley et al. 2009).

In the lower Guaraguaçu sites (G6–G11), nitrate concentrations were elevated (Figure 3), along with proportions of medium sand, very fine sand, and water temperature, while oxygen saturation levels were reduced. Sampling points along the Pery River (PER1–PER6) exhibited even higher nitrate concentrations, increased proportions of medium and very fine sand, elevated temperatures, and further reduced dissolved oxygen saturation, indicating heightened environmental disturbance in that region. In contrast, the Pombas River sites (POO1, POO2, and POR) showed higher levels of dissolved oxygen saturation, medium sand, and temperature, along with lower concentrations of nitrate and very fine sand, suggesting comparatively more favorable environmental conditions.

The spatial gradient in environmental conditions indicated that elevated nutrient levels were observed at some sites not directly associated with clear anthropogenic pollution sources. This pattern may be explained by tidal variations, which influence flow dynamics and can redistribute nutrients throughout different sections of the river. Tidal fluctuations are a key environmental driver within the basin, playing a central role in shaping habitat quality and regulating the functioning of floodplain ecosystems (Bilal et al. 2020; Regier et al. 2021; Sato, Costa, and Padial, 2021). These fluctuations significantly impact abiotic conditions, as variations in water levels and hydrological flow can differentially affect distinct segments of the river system (Bilal et al. 2020; Indivero, Myers-Pigg, and Ward, 2021).

Figure 3 - Beeswarm plots showing the environmental gradient. Each dot represents a single observation; colors indicate sampling sites regions. Variables include temperature, dissolved oxygen (DO), conductivity, salinity, nitrate and ammoniacal nitrogen concentrations, water transparency (Secchi depth), and sediment grain size composition (medium and very fine sand).



## CONCLUSION

The environmental status of the Guaraguaçu River was assessed through the integration of multiple indicators, including physicochemical characteristics of both water and sediment. Findings demonstrated a clear gradient of environmental conditions that fluctuated seasonally across distinct river zones. The most impaired sites were found in the Pery River tributary, an area heavily impacted by pollution, particularly from untreated wastewater inputs. Among the stressors identified, water pollution stood out as the main factor contributing to ecological degradation in the lower reaches of the Guaraguaçu River. Additionally, hydrological modifications introduced in the tributary to manage flow and improve effluent dilution capacity have altered the natural connectivity and functioning of the basin. Protecting these regions is essential to maintain the ecological integrity of the river system and to safeguard its biodiversity and the ecosystem services it provides. The outcomes of this study underscore the importance of incorporating scientific evidence into environmental management and policy decisions to avoid the accumulation of anthropogenic impacts that threaten the long-term sustainability of the Guaraguaçu River and its diverse uses.

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## ACKNOWLEDGEMENTS

This study was funded by the National Research Council CNPq (409593/2022-2). I would like to thank the Coordination for the Improvement of Higher Education Personnel (CAPES) for the master's scholarship granted. The Laboratory of Geoprocessing and Environmental Studies (LAGEAMB) for providing geospatial data.