



Taxonomic and morphofunctional phytoplankton response to environmental variability in rivers from different hydrographic basins in Southern Brazil

Resposta taxonômica e morfofuncional do fitoplâncton à variabilidade ambiental em rios de diferentes bacias hidrográficas no sul do Brasil

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Abstract: Aim: Urbanization, agriculture, and deforestation are the main anthropogenic factors that modify the soil, altering the quality of water, and influencing limnological aspects and the aquatic biota in rivers. We investigated the morphology-based taxonomic and functional response (MBFG) of the phytoplankton community among different public supply rivers in distinct hydrographic basins with ultraoligotrophic, oligotrophic, and mesotrophic characteristics. **Methods:** We sampled the phytoplankton community and environmental variables in nine rivers along three hydrographic basins in western Paraná. In order to evaluate the taxonomic and functional relationship of the community with the environmental variables, we applied both variance and redundancy analyses. **Results:** Differences in temperature, pH, turbidity, total phosphorus, chemical oxygen demand, and total dissolved solids were identified among river basins and/or trophic states. The highest taxonomic contributions to richness and biovolume were from green algae and diatoms, while the highest functional contributions were from MBFG IV (algae without specialized traits), MBFG V (unicellular flagellated algae), MBFG VI (algae with a siliceous exoskeleton) and MBFG (large colonial algae). The taxonomic approach was sensitive to environmental variability in the rivers, while for the functional approach no relationship to environmental variability was identified. **Conclusions:** The taxonomic approach of the phytoplankton community was more sensitive to the environmental variability of the studied rivers than the functional approach based on morphology. Therefore, we reinforce the importance of biological indicators for understanding the dynamics in aquatic ecosystems, providing crucial information for the management of water resources used for public supply.

Keywords: lotic environments; bioindicators; MBFG; water quality.



Resumo: Objetivo: A urbanização, a agricultura e o desmatamento são os principais fatores antropogênicos que modificam o solo, alterando a qualidade da água e influenciando os fatores limnológicos e a biota aquática em rios. Nós investigamos a resposta taxonômica e funcional baseada na morfologia (GFBM) da comunidade fitoplanctônica entre diferentes rios de abastecimento público em distintas bacias hidrográficas com características ultraoligotróficas, oligotróficas e mesotróficas. **Métodos:** Amostramos a comunidade fitoplanctônica e as variáveis ambientais em nove rios ao longo de três bacias hidrográficas da região oeste do Paraná. Para avaliar a relação taxonômica e funcional da comunidade com as variáveis ambientais nós aplicamos análises de variância e análises de redundância. **Resultados:** A maior contribuição taxonômica para a riqueza e biovolume foram de algas verdes e diatomáceas, enquanto as maiores contribuições funcionais foram dos GFBM IV (algas sem traços especializados), GFBM V (algas unicelulares flageladas), GFBM VI (algas com exoesqueleto silicoso) e GFBM (grandes algas coloniais). Apenas a abordagem taxonômica foi sensível a variabilidade ambiental dos rios, enquanto que para a abordagem funcional não foi identificada nenhuma relação com a variabilidade ambiental. **Conclusões:** A abordagem taxonômica da comunidade fitoplanctônica foi mais sensível a variabilidade ambiental dos rios estudados do que a abordagem funcional baseada na morfologia. Portanto, nós reforçamos a importância dos indicadores biológicos para compreensão das dinâmicas em ecossistemas aquáticos, fornecendo informações cruciais para a gestão dos recursos hídricos utilizados para abastecimento público.

Palavras-chave ambientes lóticos; bioindicadores; MBFG; qualidade da água.

1. Introduction

Water consumption in Brazil has increased by approximately 80% in the last two decades and is projected to increase by 23% until 2030, with urban water supply being the second largest use of Brazilian water bodies (ANA, 2020). Urban supply is lower only when compared to use in agriculture, which represents a service of exceptional economic value, and due to the intensification of agricultural activities, competition has been created between supply and regulation services in productive landscapes (Jia et al., 2014). However, the high demand for water resources, whether for supply or for agriculture, stemming from population growth and economic development, drastically affects the sustainability of aquatic ecosystems (Gleick, 2018). Therefore, assessing the environmental quality of rivers is of utmost importance for sustainable hydrographic basin management since the anthropic influence and exploitation of the surrounding landscape affects water quality and its multiple uses (Kashaigili, 2008; Santos et al., 2018).

Currently, the main landscape-modifying anthropic activities are related to urbanization, agriculture, and deforestation (Vörösmarty et al., 2010; Yu et al., 2013; Kim et al., 2019), directly influencing ecosystems at local, regional, and global scales (Wan et al., 2014). These activities generate impacts on the limnological characteristics of rivers, increasing the concentration of nutrients and pollutants (fertilizers, pesticides, and sewage flows), and altering water quality (Schulz & Martins-Junior, 2001; Bussi et al., 2016; Xiao et al.,

2019; Zhang et al., 2019), and, consequently, aquatic biota (Medeiros et al., 2020). Thus, in addition to the assessment of physical and chemical water characteristics, complementary methods using biological indicators (Soofiani et al., 2012; Wang et al., 2021) are efficient strategies for understanding how anthropic activities and hydrographic basin uses influence river water quality and are important for establishing monitoring strategies.

There are still few studies that evaluate the phytoplankton of rivers and its relationship to environmental dynamics when compared to lakes and reservoirs (Bolgovics et al., 2017), especially in Brazil's extensive hydrographic network. The ecological knowledge of phytoplankton dynamics in rivers has intensified since the first half of the 20th century. From the studies of Colin Reynolds (2006), who identified that river discharge and turbidity were the main barriers to the development and maintenance of phytoplankton in unidirectional flow, more studies have been developed (Abonyi et al., 2021). However, due to the intense global changes that aquatic ecosystems have been facing, additional studies should be developed in order to establish a relationship between phytoplankton communities and environmental variability (Bolgovics et al., 2017).

The phytoplankton community is an important component of aquatic ecosystems, contributing significantly to primary productivity, and being a key link in nutrient cycling (Litchman et al., 2015). Algae and cyanobacteria from this group

are commonly used as bioindicators to assess anthropogenic impacts in freshwater environments (Kim, et al., 2019). These organisms reflect different responses (e.g., physiological responses, changes in abundance, changes in community structure and productivity) depending on the intensity of stressors and anthropogenic changes (Salmaso & Tolotti, 2021). Phytoplankton responds quickly and efficiently to changes in water conditions, such as levels of nutrients and toxic contaminants, electrical conductivity, turbidity, and pH (Triest et al., 2012; Castro-Roa & Pinilla-Agudelo, 2014; Jia et al., 2019). Their short life cycle, representative population size, easy sampling, and storage (Litchman & Klausmeier, 2008; Kruk et al., 2017) also facilitate their use in monitoring programs.

Changes in the occurrence and distribution of taxa, as well as in their population size, morphology, and physiology, are some of the main responses as a function of anthropogenic activities on the ecosystem (Casé et al., 2008; Abonyi et al., 2020; Zohary et al., 2021). Thus, phytoplankton species composition, richness, and abundance represent important measures for assessing the health and water quality of rivers (Soofiani et al., 2012; Santana et al., 2016; Zhang et al., 2020), as phytoplankton directly reflect ecosystem functioning (Borics et al., 2021). The use of functional groups has also been applied and shown to be efficient in water quality assessment (Salmaso et al., 2015; Graco-Roza et al., 2021).

Therefore, in order to understand the structure and functionality of phytoplankton and the patterns of their dynamics in aquatic environments, the phytoplankton community can be grouped based on morphological similarity of species (e.g., volume, size, presence or absence of flagella) (Kruk et al., 2010; Kruk & Segura, 2012). In addition to taxonomic characteristics and traditional measures of community structure, morphology-based functional measures provide crucial information about environmental variability in aquatic ecosystems, being relatively easy to measure.

In view of the need to evaluate the water quality of catchment rivers and their use for public supply, this study aims to evaluate the phytoplankton community based on the taxonomic and morphofunctional approaches as models of response to the environmental variability of rivers in southern Brazil. Thus, we investigate the water quality of nine supply rivers with surrounding urbanization and agricultural characteristics and relate it to phytoplankton distribution. Therefore, we have as central questions of our study: i) how does

the taxonomic and morphofunctional distribution of phytoplankton relate to the environmental conditions of the supply rivers, considering their different trophic states and different hydrographic basins, and ii) how this relationship can indicate the environmental quality of these rivers.

2. Materials and Methods

2.1. Selection, location, and characterization of the study sites

The state of Paraná has 16 hydrographic basins (Resolution n. 024/2006/SEMA) (Paraná, 2006): Litorânea, Iguassu, Ribeira, Itararé, Cinzas, Tibagi, Ivaí, Paranapanema I, II, III and IV, Pirapó, Paraná I, II and III, and Piquiri. The western region of the state, which covers 54 municipalities, is bordered by the Paraná III, Piquiri, and Iguassu basins (AMOP, 2018). This region has an economy focused on agricultural activities, and a large part of the municipalities are dominated by extensive monoculture areas and urbanized areas (PNUD, 2018), including the most populous municipalities, such as Cascavel (336,073 inhabitants), Foz do Iguaçu (257,971 inhab.), Toledo (144,601 inhab.), and Medianeira (46,940 inhab.) (IBGE, 2010).

We selected nine rivers used for water withdrawal for public supply in the western region of Paraná, along the hydrographic basins of the Lower Iguassu River (BI), Paraná III (PIII), and Piquiri (PQ), which are distributed in nine municipalities: Guaraniaçu (GUAR), Catanduvas (CTD), Três Barras do Paraná (TBP), Boa Vista Aparecida (BVA), Foz do Iguaçu (FOZ), Medianeira (MED), Santa Tereza do Oeste (STO), Cascavel (CVEL), and Toledo (TOL) (Figure 1). Information about the sampling sites, main anthropic activities in the region, trophic state, morphometric and hydrological characteristics are presented in Table 1.

The water samples for physicochemical and biological analysis were taken in two locations in each river, one at the headwaters and another at the point of the catchment used for water supply, during the summer of 2020, totaling 18 samples. All samples were deposited in the herbarium of UNIOESTE - Universidade Estadual do Oeste do Paraná - UNOPA, Cascavel campus, linked to the Brazilian Herbaria Network, and the data were computerized and made available on Species Link (2021).

2.2. Sampling and analysis of environmental variables in rivers

Precipitation data (Pre) were provided by the Paraná Meteorological Institute (Simepar). Data

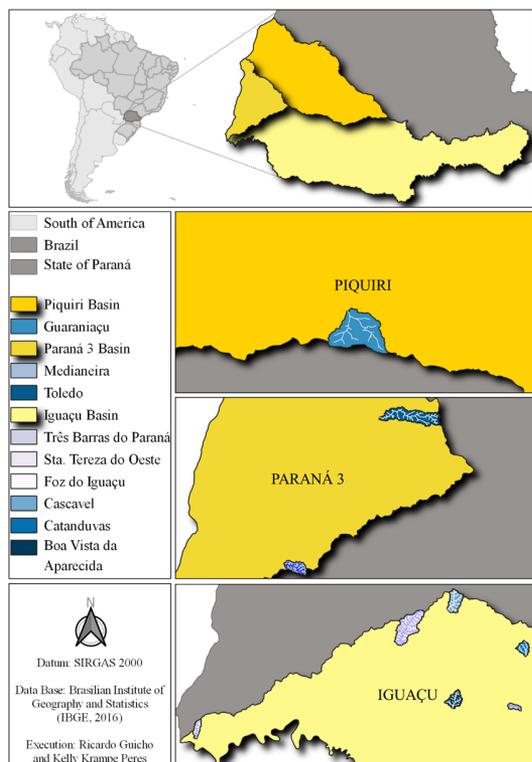


Figure 1. Municipalities in western Paraná, Brazil, selected to evaluate the water quality of rivers used for withdrawal and urban supply.

on maximum depth (Z_{\max} - cm), water temperature (Temp - °C), dissolved oxygen (DO - mg L^{-1}), pH, electrical conductivity (Conduct - mS cm^{-1}), and turbidity (Turb - NTU) were measured at the time of sampling using a Horiba U-5000 multiparameter probe. Data regarding flow ($\text{m}^3 \text{s}^{-1}$) and maximum depth were collected in situ using a ruler, measuring tape, and a floating object. The flow was calculated by multiplying the average speed resulting from the displacement of the object and the cross-sectional area where the stone was collected, measured in situ (Table 1).

Chemical analyses were measured based on water samples collected by sub-surface immersion of polyethylene bottles, properly cooled, and kept in the dark until their destination. The oxygen consumption that occurred due to chemical oxidation was evaluated through the chemical oxygen demand (COD - mg L^{-1}) and organic matter was evaluated through the biochemical oxygen demand (BOD - mg L^{-1}) and was estimated following the methods described in Standard Methods (APHA, 2017). Concentrations of nitrate (NO_3^- - mg L^{-1}), ammoniacal nitrogen (N-NH_3 - mg L^{-1}), total phosphorus (TP- mg L^{-1}),

orthophosphate (PO_4^- - mg L^{-1}), chlorophyll *a* (CLa - mg L^{-1}), and total dissolved solids (TDS - mg L^{-1}) were also estimated (APHA, 2017).

2.3. TSI – Trophic State Index

The Trophic State Index presented and used in the calculation of the Aquatic Life Protection Index (ALPI), was composed of the Trophic State Index for phosphorus - TSI(PT) and the Trophic State Index for chlorophyll *a* - TSI(CL), modified by Lamparelli (2004), being established for lotic environments, according to the Equations 1 and 2:

- Rivers

$$TSI(CL) = 10x \left(6 - \left((-0,7 - 0,6x(\ln CL)) / \ln 2 \right) \right) - 20 \quad (1)$$

$$TSI(PT) = 10x \left(6 - \left((0,42 - 0,36x(\ln PT)) / \ln 2 \right) \right) - 20 \quad (2)$$

where:

TP: total phosphorus concentration measured at the water surface, in $\mu\text{g L}^{-1}$; CL: chlorophyll *a* concentration measured at the water surface, in $\mu\text{g L}^{-1}$; ln: natural logarithm.

For the classification of Trophic State for rivers the Carlson Index (Carlson, 1977) modified by Toledo et al. (1983) was used.

2.4. Sampling and analysis of the phytoplankton community

The qualitative analyses were based on phytoplankton samples collected using a plankton net of $\sim 20 \mu\text{m}$ mesh size and preserved in Transeau solution (Bicudo & Menezes, 2017), in order to concentrate the phytoplankton material and facilitate taxonomic studies. The qualitative study of phytoplankton was performed in an Olympus CX41 photomicroscope, with an Olympus SC30 camera attached, and the morphometry of the taxa was performed at 400 \times and 1000 \times magnification. We followed the classification system of Round (1965, 1971) proposed by Bicudo & Menezes (2017), to group the algae at the Class level.

For the quantitative analyses, the phytoplankton community was collected directly with 300 mL flasks at the subsurface and fixed in situ with acetic Lugol. The quantitative analysis was estimated according to the methodology described by Utermöhl (1958), using an Olympus inverted microscope, model CKX41. The sedimentation volume was defined according to the concentration of algae and/or detritus present in the sample, with the sedimentation time being equivalent

Table 1. Geographic location of the sampled rivers in different hydrographic basins in southern Brazil (1 - headwaters; 2 - water catchment point), main anthropic activities, trophic level according to Lamparelli (2004), and morphometric and hydrological characteristics.

Hydrographic basins	Municipality	River	Sampling sites	Latitude Longitude	Anthropic activity	Trophic state	Depth (cm)	Width (m)	Flow (m ³ /s)
Piquiri	Guaraniaçu	Baú River	GUARP1	25°40'56"S	Agricultural production PP	Mesotrophic	14	5.78	0.05
				52°53'29"W	Agricultural production PP	Ultraoligotrophic	18	4.69	0.11
Paraná III	Medianeira	Alegria River	MEDP1	52°53'20"W	Urban	Oligotrophic	8	2.90	0.04
			MEDP2	25°18'35"S	Agricultural production GP	Oligotrophic	34	3.70	0.09
	Toledo	Toledo River	TOLP1	54°40'35"W	Urban	Mesotrophic	37	4.31	0.62
			TOLP2	24°45'49"S	Agricultural production GP	Mesotrophic	52	7.90	3.00
Lower Iguassu River	Cascavel	Cascavel River	CVELP1	53°39'50"W	Urban	Mesotrophic	52	3.70	0.54
			CVELP2	53°42'40"W	Agricultural production GP	Ultraoligotrophic	30	4.80	0.28
	Catanduvas	Arroio Passo Liso River	CTDP1	52°53'20"S	Agricultural production PP	Ultraoligotrophic	18	9.50	0.05
			CTDP2	53°08'18"W	Agricultural production PP	Oligotrophic	17	7.25	0.30
	Boa vista da Aparecida	Jacutinga River	BVAP1	25°12'38"S	Agricultural production PP	Ultraoligotrophic	24	6.90	0.27
			BVAP2	53°07'51"W	Agricultural production PP	Oligotrophic	28	3.50	0.18
	Foz do Iguauçu	Tamanduá River	FOZP1	25°25'17"S	Urban	Mesotrophic	9	2.86	0.06
			FOZP2	53°26'17"W	Urban	Mesotrophic	30	3.40	0.15
	Santa Tereza do Oeste	Gonçalves Dias River	STOP1	54°31'50"W	Urban	Oligotrophic	19	1.50	0.16
			STOP2	25°32'13"S	Agricultural production GP	Oligotrophic	21	5.00	0.23
Três Barras do Paraná	Itaguaçu stream	TBPP1	25°20'29"S	Agricultural production GP	Oligotrophic	40	2.40	0.06	
		TBPP2	53°36'14"W	Agricultural production PP	Oligotrophic	25	3.20	0.15	
				53°11'17"W	Agricultural production PP				
				25°26'21"S	Agricultural production PP				
				53°10'50"W	Agricultural production PP				

PP = small-scale production; GP = large-scale production.

to the height of the counting chamber used (Margalef, 1983). Counting was performed in random transects, and the counting limit was established by the species rarefaction curve, basing the counting of individuals on the form they occur in nature: cells, colonies, coenobium, or filaments (Uhelinger, 1964). Phytoplankton density was calculated according to APHA (2017), and results were expressed as individuals per milliliter (ind. mL⁻¹). The phytoplankton biovolume was calculated by multiplying the density of each taxon by its respective volume. The cell volume was calculated from geometric models, according to the shape of the cells (Sun & Liu, 2003). Total phytoplankton richness was defined by the number of taxa found in each quantitative sample.

We classified the phytoplankton taxa recorded in the quantitative samples into morphology-based functional groups (MBFG), according to Kruk et al. (2010) and Reynolds et al. (2014). This classification uses continuous (individual volume, surface area, maximum linear dimension, mean, and range) and categorical (presence and frequency of aerotopes, flagella, mucilage, heterocysts, siliceous exoskeletal structures, and mean frequency) variables for grouping taxa into MBFGs. Thus, according to these criteria, phytoplankton organisms were classified into MBFG I - small organisms with high individual volume to surface area ratio. Species in this group are small-sized with rapid individual growth rate and high numerical abundance; MBFG II - small flagellate organisms with siliceous exoskeletal structures. They have low biomass and do not pose a significant threat to water quality; MBFG III - species with large filaments and aerotopes. They are large and slow-growing organisms, but their high volume/surface area ratio gives them greater tolerance to light limiting conditions; MBFG IV - medium-sized organisms with no specialized traits and a moderate tolerance for resource limitation; MBFG V - medium to large unicellular flagellates. The medium size and volume to surface area ratio, the presence of flagella, and the production of cysts allow these organisms to tolerate low levels of nutrients; MBFG VI - non-flagellated organisms with silica exoskeleton. Represented only by diatoms, containing high cell density; MBFG VII - large mucilaginous colonies; MBFG VIII - nitrogen-fixing cyanobacteria. The high size and volume and the low volume/surface area ratio tend to make the species of this group sensitive to low resource supply. They can produce toxins and allelopathic substances.

2.5. Data analysis strategies

We compared possible statistical differences of environmental variables among the trophic states of the rivers and hydrographic basins evaluated, applying an analysis of variance (two-way ANOVA). In addition, the environmental variables were submitted to Principal Component Analysis (PCA), aiming to characterize and order the sampling sites according to environmental variability. For the PCA the following variables were used: water temperature, dissolved oxygen, pH, electrical conductivity, turbidity, maximum depth, total phosphorus, orthophosphate, nitrate, ammoniacal nitrogen, chemical oxygen demand, biochemical oxygen demand, and total dissolved solids.

Differences in biovolume and richness of taxa and MBFGs among hydrographic basins, as well as river trophic status, were assessed using a non-parametric permutational analysis of variance (PERMANOVA; Anderson, 2001). Subsequently, to evaluate the biovolume relationship of the phytoplankton community (taxonomic and morphofunctional matrix) with environmental variables, a Redundancy Analysis (RDA) was performed. The community biovolume data underwent Hellinger's transformation (Borcard et al., 2011). The collinearity of the environmental variables (water temperature, dissolved oxygen, pH, electrical conductivity, turbidity, maximum depth, total phosphorus, orthophosphate, nitrate, ammoniacal nitrogen, chemical oxygen demand, biochemical oxygen demand and total dissolved solids) was tested using the VIF (Variance Inflation Factor - VIF > 10 were removed – Oksanen et al., 2017) and then the selection procedure (Ordstep) was applied ($p \leq 0.05$) (Rao, 1964). All analyses were performed using the R language and environment for computational statistics (R Development Core Team, 2014), with the Vegan package (Oksanen et al., 2017).

3. Results

3.1. Environmental characterization of rivers

We observed differences between the mean values of temperature, pH, COD, and TDS among the hydrographic basins, while differences between pH, turbidity, and TP were identified among the trophic states of the rivers (Table 2). Mean values and standard deviation of environmental variables are also presented in Table 2.

The principal component analysis (PCA) summarized for the first axis 39%, and for the

Table 2. Mean, standard deviation, and ANOVA results (among hydrographic basins and river trophic status) applied to the environmental variables sampled in different supply rivers in the southern region of Brazil.

Municipalities Basins	BVA Lower Iguassu River	CTD Lower Iguassu River	CVEL Lower Iguassu River	FOZ Lower Iguassu River	GUAR Piquiri	MED Paraná Ill	STO Lower Iguassu River	TBP Lower Iguassu River	TOL Paraná Ill	Basins p	Trophy p	Basins x Trophy p
Environmental variables												
Temp (°C)	23.22 ± 1.08	19.45 ± 0.26	20.9 ± 1.00	22.41 ± 0.98	17.93 ± 0.61	21.93 ± 0.42	22.52 ± 1.26	21.04 ± 0.05	20.83 ± 0.20	0.018	0.45	0.94
DO (mg L ⁻¹)	3.43 ± 0.32	4.94 ± 0.35	10.74 ± 0.35	2.58 ± 0.54	4.84 ± 0.22	2.8 ± 0.57	2.53 ± 0.78	4.47 ± 0.35	10.59 ± 0.29	0.64	0.05	0.12
pH	7.41 ± 0.28	7.31 ± 0.01	5.47 ± 0.52	6.32 ± 0.13	6.79 ± 0.44	6.66 ± 0.10	6.64 ± 0.31	7.27 ± 0.09	5.94 ± 0.26	0.004	0.009	0.19
Conduct (mS cm ⁻¹)	0.08 ± 0.01	0.08 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.08 ± 0.01	0.03 ± 0.01	0.01 ± 0.01	0.08 ± 0.01	0.02 ± 0.01	0.03	0.97	0.73
Z _{max} (m)	0.26 ± 0.02	0.17 ± 0.01	0.41 ± 0.15	0.19 ± 0.15	0.16 ± 0.03	0.21 ± 0.18	0.2 ± 0.01	0.32 ± 0.10	0.44 ± 0.10	0.33	0.48	0.79
Turb (NTU)	9.21 ± 0.46	6.07 ± 2.31	9.67 ± 2.31	13.65 ± 3.06	13.25 ± 1.20	8.91 ± 1.20	10.53 ± 1.51	9.61 ± 0.21	22.25 ± 1.90	0.099	0.04	0.28
BOD (mg L ⁻¹)	1.30 ± 0.36	1.00 ± 0.01	1.68 ± 0.82	1.75 ± 1.06	1.76 ± 0.14	1.05 ± 0.08	1.45 ± 0.64	1.00 ± 0.01	1.72 ± 0.17	0.31	0.07	0.77
COD (mg L ⁻¹)	67.50 ± 3.53	58.33 ± 2.35	41.66 ± 9.42	16.66 ± 11.78	63.33 ± 14.14	61.67 ± 14.14	71.66 ± 2.35	77.50 ± 5.89	25.83 ± 3.53	0.04	2.52	0.25
TP (mg L ⁻¹)	0.02 ± 0.01	0.02 ± 0.02	0.01 ± 0.01	0.02 ± 0.02	0.02 ± 0.05	0.02 ± 0.02	0.01 ± 0.02	0.03 ± 0.06	0.04 ± 0.02	0.34	0.01	0.81
NO ₃ ⁻ (mg L ⁻¹)	0.09 ± 0.07	1.00 ± 0.42	1.25 ± 0.49	1.25 ± 0.40	0.80 ± 0.42	1.00 ± 0.01	0.55 ± 0.07	1.75 ± 0.49	1.80 ± 0.14	0.32	0.47	0.26
N-NH ₃ (mg L ⁻¹)	0.001 ± 0.01	0.01 ± 0.01	0.05 ± 0.07	0.17 ± 0.25	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.67	0.37	0.84
PO ₄ ⁻ (mg L ⁻¹)	0.01 ± 0.01	0.01 ± 0.02	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.06	0.26	0.59
TDS (mg L ⁻¹)	77.25 ± 6.72	71.00 ± 4.95	40.00 ± 3.53	41.50 ± 6.36	67.50 ± 12.02	48.75 ± 5.30	40.50 ± 8.48	71.75 ± 10.96	21.57 ± 2.47	0.003	0.13	0.18
Precipitation (mm)	5.02	3.81	3.81	2.87	2.6	2.54	3.81	5.02	2.84	-	-	-
Flow (m ³ s ⁻¹)	0.22 ± 0.68	0.17 ± 0.17	0.41 ± 0.18	0.10 ± 0.06	0.08 ± 0.04	0.06 ± 0.03	0.19 ± 0.04	0.1 ± 0.06	1.81 ± 1.68	-	-	-

Significant differences ($p < 0.05$) are highlighted in bold. Temp: water temperature, DO: dissolved oxygen, pH, Conduct: electrical conductivity, Z_{max}: maximum depth, Turb: turbidity, BOD: biochemical oxygen demand, COD: chemical oxygen demand, TP: total phosphorus, NO₃⁻: nitrate, N-NH₃: ammoniacal nitrogen, PO₄⁻: orthophosphate e TDS: total solids. BVA: Boa Vista da Aparecida, CTD: Caranduvas, CVEL: Cascavel, FOZ: Foz do Iguauçu, GUAR: Guaraniaçu, MED: Medianeira, STO: Santa Tereza do Oeste, TBP: Três Barras do Paraná, and TOL: Toledo.

second axis 16% of the environmental variability among the rivers of the different hydrographic basins (Figure 2). The spread of the scores of the sampled sites in these axes evidenced a separation in the diagram of the sampled basins, especially some rivers located in the Lower Iguassu River and Paraná III basins (Figure 2). The first axis of the PCA

explained positively the environmental variability mainly in relation to turbidity (0.34) and negatively with pH (-0.38), TDS (-0.39), COD (-0.34) and PO_4^- (-0.31). The second axis was positively related to the variable $N-NH_3$ (0.53), isolating one of the rivers of the Lower Iguassu River basin, and negatively with Condu t (-0.51) and NO_3^- (-0.48).

3.2. Phytoplankton community characterization and distribution in rivers

A total of 67 taxa were recorded along all rivers, distributed in eight taxonomic classes: Dinophyceae (1 taxon), Cyanophyceae (1 taxon), Coscinodiscophyceae (3 taxa), Trebouxiophyceae (3 taxa), Zygnematophyceae (8 taxa), Euglenophyceae (8 taxa), Bacillariophyceae (10 taxa) and Chlorophyceae (33 taxa). *Desmodesmus* (R.Chodat) S.S.An, T.Friedl & E.Hegewald and *Ankistrodesmus* Corda were the most representative genera, with 11 and 7 taxa, respectively.

Considering taxa richness, the highest values were recorded in the rivers of the Lower Iguassu River and Paraná III basins (Figure 3a). Regarding phytoplankton biovolume, low values were recorded in most rivers, however, three rivers from the Lower Iguassu River basin presented higher values ($>1 \text{ mm}^3 \text{ L}^{-1}$) (Figure 3b). Green algae, diatoms, and euglenophyceans were the taxonomic groups with the greatest contribution in terms of richness and biovolume.

When we classified the species according to morphological characteristics, we recorded four MBFG: MBFG IV, MBFG V, MBFG VI, and MBFG VII. Most rivers were represented by the presence of MBFG IV, V, and VI (Figure 4a).

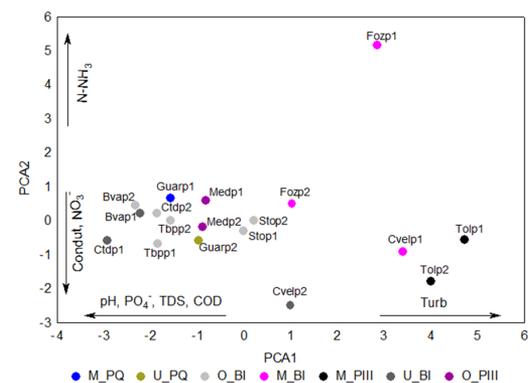


Figure 2. Principal Component Analysis (PCA) for the environmental variables analyzed in the rivers of the three hydrographic basins evaluated in western Paraná, Brazil (Condu t : electrical conductivity, pH, Turb: turbidity, COD: chemical oxygen demand, NO_3^- : nitrate, $N-NH_3$: ammoniacal nitrogen, PO_4^- : orthophosphate, and TDS: total dissolved solids. Mesotrophic - Piquiri (M_PQ); Ultraoligotrophic- Piquiri (U_PQ), Oligotrophic – Lower Iguassu River (O_BI); Mesotrophic - Lower Iguassu River (M_BI); Mesotrophic – Paraná III (M_PIII); Ultraoligotrophic - Lower Iguassu River (U_BI), Oligotrophic - Paraná III (O_PIII); Bva - Boa Vista da Aparecida; Ctd – Catanduvas; Cvel – Cascavel; Foz - Foz do Iguaçú; Guar – Guaraniçú; Med – Medianeira; Sto - Santa Tereza do Oeste; Tbp - Três Barras do Paraná; Tol – Toledo; 1 - headwaters; 2 - water catchment point).

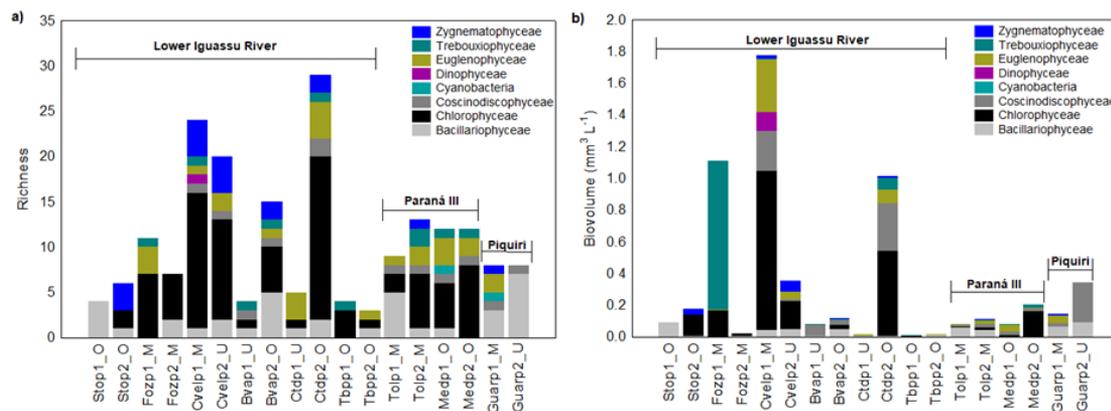


Figure 3. (a) Species richness and (b) biovolume ($\text{mm}^3 \text{ L}^{-1}$) of phytoplankton in rivers from different hydrographic basins in western Paraná, Brazil (1- headwaters; 2 – water catchment point; O – oligotrophic; M – mesotrophic; U – ultraoligotrophic).

Regarding the biovolume of MBFG, the rivers from the Lower Iguassu River basin also showed the highest values, especially due to the presence of MBFG IV (Figure 4b).

According to PERMANOVA, differences were only seen for species richness between hydrographic basins ($F = 0.19865$; $p = 0.030$). For species biovolume and MBFG richness and biovolume, no differences were verified between the hydrographic basins or between the trophic state of the rivers (Table 3).

3.3. Phytoplankton community and environmental variability of rivers

The Redundancy Analysis (RDA) performed with the species biovolume as a function of

environmental variables resulted in explanatory power of 25%, also evidencing that the species matrix is significantly related to the selected variables ($F = 1.63$, $p = 0.007$; Figure 5). Only the RDA1 axis (RDA1: $F = 24.513$, $p = 0.019$; RDA2: $F = 15.833$, $p = 0.203$) was significant. The variables selected as explanatory in the model were turbidity, orthophosphate and nitrate. However, NO_3^- ($F = 21.511$; $p = 0.004$) and PO_4^- ($F = 17.124$; $p = 0.039$) were significant. It was possible to observe a clear spatial separation between the basins, especially of the Piquiri basin, while the Lower Iguassu River and Paraná III basins were divided into three groups separating the rivers. RDA did not identify any variable explaining the presence of MBFG in the rivers of the different hydrographic basins.

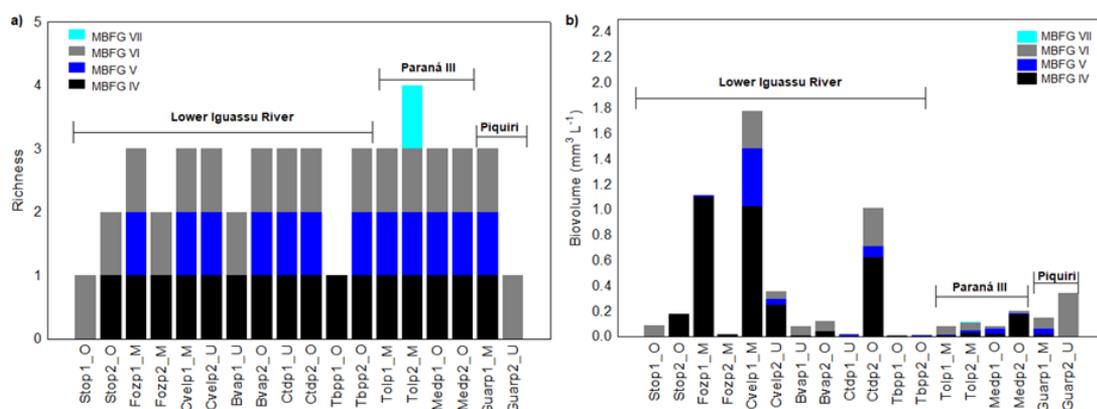


Figure 4. (a) MBFG richness and (b) MBFG biovolume ($\text{mm}^3 \text{L}^{-1}$) in rivers from different river hydrographic basins in western Paraná, Brazil (1- headwaters; 2 – water catchment point; O – oligotrophic; M – mesotrophic; U – ultraoligotrophic).

Table 3. PERMANOVA results applied to richness and biovolume of taxa and MBFG data to evaluate differences between hydrographic basins and river trophic states (Significance level = $p < 0.05$, highlighted in bold).

Hydrographic basin				
Taxa		DF	F	p
MBFG	Richness	2	0.19865	0.030
	Biovolume	2	0.15331	0.114
	Richness	2	0.18809	0.306
	Biovolume	2	0.12170	0.487
Trophic state				
Taxa		DF	F	p
MBFG	Richness	2	0.08837	0.752
	Biovolume	2	0.08023	0.931
	Richness	2	0.02975	0.751
	Biovolume	2	0.03927	0.984

DF: degree of freedom.

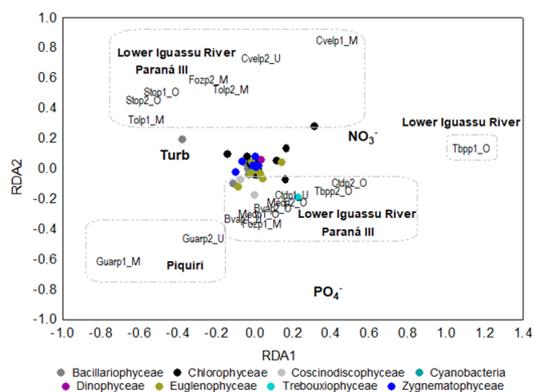


Figure 5. Redundancy analysis performed with biovolume species, rivers sampled and environmental variables (Nitrate - NO_3^- ; turbidity - Turb; orthophosphate - PO_4^- ; Mesotrophic - M; Oligotrophic - O; Ultraoligotrophic - U; Bva - Boa Vista da Aparecida; Ctd - Catanduvás; Cvel - Cascavel; Foz - Foz do Iguaçu; Guar - Guaraniáçu; Med - Medianeira; Sto - Santa Tereza do Oeste; Tbp - Três Barras do Paraná; Tol - Toledo; 1 - headwaters; 2 - water catchment point).

4. Discussion

Our results pointed out a distinction between the rivers of the different hydrographic basins as a function of the environmental variables that characterized the water quality, which is reflected in the phytoplankton community. Urban supply rivers are sensitive to anthropogenic activities, such as the continuous growth of urban populations and agricultural activities, which affect surface waters (Kim et al., 2019; Silva et al., 2020). Moreover, these environments play an important role in maintaining biodiversity and sustaining ecosystem products and services that are also essential for human well-being (Zhang et al., 2019). The trophic state of the different supply rivers evaluated and the main environmental conditions were reflected in the biological response of phytoplankton, which proved to be a good indicator of environmental quality, especially when treated at the taxonomic level.

Relating the community to the environmental conditions of the rivers, the taxonomic approach proved to be more sensitive than the MBFGs. MBFGs have been applied in different studies, and their responses to environmental variability have also been satisfactory (Bohnenberger et al., 2018; Cupertino et al., 2019; Yang et al., 2020; Trindade et al., 2021). However, in our study, MBFGs were not related to the environmental variability of rivers. This is likely associated with

the low phytoplankton biovolume in most of the rivers evaluated, as well as the simple and objective refinement of the MBFGs, allowing to fit the algae and cyanobacteria into few groupings, which consequently did not relate to any environmental filter. Thus, our results suggest that the small number of MBFG was not sensitive to environmental variability in rivers, captured by the taxonomic approach alone (Cupertino et al., 2019). The response of MBFGs in lotic environments and their weak relationship to environmental variability has already been reported (Bortolini et al., 2014), and thus the taxonomic approach may still be more sensitive for river biomonitoring when treated on a local scale and with small numbers of samplings.

We identified in this study a higher contribution of green algae, diatoms, and euglenophyceans to richness and biovolume along the rivers. These algae show high sensitivity to environmental changes and are used as bioindicators of organic pollution and water eutrophication in most aquatic ecosystems (Lobo et al., 2016; Barnard et al., 2021).

Green algae belonging to Chlorophyceae presented the highest contribution in biovolume and richness in most of the sampled rivers, which has already been recorded in other studies (Rodriguez et al., 2007; Descy et al., 2011; Zhao et al., 2012; Yang et al., 2019). These algae are considered opportunistic since some species contain small size and fast growth, besides being found in various environments, from oligotrophic waters to polluted environments (Bicudo & Menezes, 2017). They are also resistant to environmental variations and competition with other species (Pan et al., 2011; Domingues & Torgan, 2012). Additionally, several species have accessory structures such as spines or mucilage, helping them to avoid longitudinal carriage and favoring their permanence longer in the water column (Kruk & Segura, 2012). In our study, this group was correlated with ultraoligo- to mesotrophic environments, from the Paraná III and Lower Iguassu River basins, as well as higher concentrations of NO_3^- and PO_4^- . High levels of these variables in rivers usually occur due to surface runoff and wastewater discharges, influencing water quality, and promoting species selection (Kelly et al., 2015; Shi et al., 2017).

Diatoms in turn have genera related to urban growth associated with organic pollution conditions, containing indicator species of environmental impacts (Moresco & Rodrigues, 2014; Rangel et al., 2017). These aspects are best visualized through ecological guilds for the group. Passy (2007)

classified larger species, such as those in the genus *Pinnularia*, as high profile, commonly found in the phytoplankton and poorly resistant to turbulence, but favored by nutrient enrichment. The genus *Navicula* is included in the motile guild and is considered an adapted genus to turbulence and variations in nutrient concentrations (Passy, 2007). Previous studies corroborate our results, demonstrating that diatoms are representative contributors to taxonomic richness and abundance in the phytoplankton community (Bolgovics et al., 2017; Conceição et al. 2021) of ultraoligo- to mesotrophic, and agricultural or urban rivers, with low flow and distinct nutrient concentrations (Passy, 2007; Simić et al., 2015).

Euglenophyceae have high metabolic flexibility and, exploiting diverse organic carbon sources under different conditions, (Cordoba et al., 2021). This adaptation confers an advantage in oligotrophic or organic matter-rich water bodies, strongly light-limited (Bicudo & Menezes, 2017). The representativeness of this group in our study may be related to their flagellar motility, which facilitates survival in different environmental conditions, such as shallow waters and with little turbulence (Brasil & Huszar, 2011).

The variation in species biovolume among river trophic states reflects the environmental variability among aquatic environments. Thus, the abundance of taxa was associated with environmental conditions such as turbidity, temperature, pH, and nutrient concentrations. Temperature is considered one of the main environmental factors as it has major impacts on phytoplankton growth, leading to seasonal variation in algal abundance and composition (Fietz et al., 2005; Lv et al., 2020). Water quality can also be altered by nutrient concentrations and turbidity, promoting the selection of a single species or a few dominant species that can survive in this stressful environment (Yang et al., 2021). Thus, changes in physical and chemical water conditions indicate impacts from increasing urbanization as well as agricultural activities (Lötjönen & Ollikainen, 2019), regional characteristics adjacent to the rivers evaluated here. Such conditions comprise major ecological axes that define the niche of species and influence reproduction, resource acquisition, and predation prevention (Litchman & Klausmeier, 2008), reflecting in higher contributions of green algae, diatoms, and euglenophyceans in the phytoplankton community.

We evaluated the limnological characteristics of rivers in different hydrographic basins distributed in nine municipalities of western Paraná, based on physical, chemical, and biological parameters, verifying distinct environmental characteristics among the studied environments. In our study, the taxonomic approach used for the phytoplankton community was more sensitive in responding to the environmental variability of the studied rivers than the MBFG. In general, the biovolume of the classes grouped the environments, with Bacillariophyceae related to ultraoligotrophic environments of the Piquiri and Paraná III basins and mesotrophic environments of the Piquiri and Lower Iguassu River basins, Chlorophyceae related to ultraoligo- to oligotrophic environments of the Paraná III basin and mesotrophic environments of the Lower Iguassu River, and Euglenophyceae related to oligotrophic environments of the three basins evaluated. Our data indicate environments with intermediate stages of degradation, but facing effects of urbanization and agricultural expansion. The integrity of these rivers is gradually being affected, reflected in the phytoplankton community, showing that these activities can compromise water quality used for public supply. Thus, comprehending the environmental variability and the response of biological indicators is essential to understanding the functions of aquatic ecosystems, as well as the provision of ecosystem services.

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