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Phytoplankton functional groups associated with the trophic state of tropical reservoirs

Grupos funcionais do fitoplâncton associados ao estado trófico de reservatórios tropicais

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Abstract: Aim: The study investigated functional groups representing reservoirs of different trophic states, identifying eutrophication indicators. **Methods:** Water samples were collected to evaluate physical and chemical characteristics and the phytoplankton composition in five reservoirs during dry and rainy periods. **Results:** Low concentration of dissolved nutrients (nitrite, orthophosphate, and total dissolved phosphorus) and total phosphorus described the oligotrophic and mesotrophic reservoirs, and the opposite for the eutrophic and hypertrophic reservoirs. Twenty-four functional groups were identified, eight of which were considered descriptors due to high biomass. Functional groups were influenced by the trophic state, and secondarily by seasonality. Typical functional groups represented the hypertrophic, eutrophic, and mesotrophic reservoirs. However, an oligotrophic reservoir was represented by a functional group commonly associated with environmental eutrophic. **Conclusions:** The functional groups were representative of the trophic state of systems and detected signs of early eutrophication.

Keywords: Brazil; eutrophication; environmental factors; functional classification.

Resumo: Objetivo: O estudo investigou grupos funcionais representativos de reservatórios com diferentes estados tróficos e avaliou a ocorrência dos que indicassem eutrofização. **Métodos:** Amostras de água de cinco reservatórios foram coletadas para avaliar as características físicas e químicas e a composição do fitoplâncton durante os períodos de seca e chuva. **Resultados:** Baixas concentrações de nutrientes dissolvidos (nitrito, ortofosfato e fósforo total dissolvido) e fósforo total descreveram os reservatórios oligotrófico e mesotrófico; e o oposto para os reservatórios eutrófico e hipertrófico. Vinte e quatro grupos funcionais foram identificados, oito dos quais foram considerados descritores de reservatório devido à alta biomassa. Os grupos funcionais foram influenciados pelo estado trófico e, secundariamente, pela sazonalidade. Grupos funcionais típicos representaram os reservatórios hipertrófico, eutrófico e mesotrófico. No entanto, o reservatório oligotrófico foi representado por um grupo funcional comumente associado à eutrofia ambiental. **Conclusões:** Os grupos funcionais foram representativos do estado trófico dos sistemas e detectaram sinais de eutrofização precoce.

Palavras-chave: Brasil; eutrofização; fatores ambientais; classificação funcional.

1. Introduction

Phytoplankton is a diverse group of organisms presenting different characteristics and adaptive strategies that influence its ability to withstand environmental disturbances (Hu et al., 2013). Consequently, understanding phytoplankton dynamics is an important tool for understanding freshwater ecology. Phytoplankton classification into functional groups has been significant in helping to understand the relationships between structural and functional properties of the freshwater enviromment (Salmaso & Padisák, 2007). The functional group is composed of organisms that share ecological traits and attributes related to the environmental characteristics of lakes and reservoirs (Cunha & Calijuri, 2011). In addition, grouping species based on their functional attributes may provide a clear characterization of the habitat (Salmaso et al., 2015).

The ecological classification of functional groups proposed by Reynolds et al. (2002) assemble phytoplankton populations into associations based on morphometry, phenology, physiology, ecology, trophic state, and affinities that allow species to live in each environment. The paper by Padisák et al. (2009) updated the Reynolds functional groups and included new groups, consolidating the classification. There are 41 codons according to the functional groups approach, and two ideas support the functional theory: (1) functionally well-adapted species may more successfully tolerate restrictive conditions of nutritional deficiency than less-adapted species; and (2) a habitat limited by some factor may probably be inhabited by species with adequate adaptations to survive there, however, do not imply that the species will be there (Padisák et al., 2009). Phytoplankton classification based on the functional approach assembles parameters or indicator characteristics capable of representing the environment (Bomfim et al., 2019). In addition, the functional approach may describe the phytoplankton community spatial variations and assess its ecological status (Kosten et al., 2012).

Classification of functional groups is been used worldwide in ecological studies of freshwater phytoplankton and applied to different types of environments (Aquino et al., 2018). The relationship between phytoplankton and trophy has been successfully applied in tropical (Silva & Costa, 2015; Bortolini et al., 2016; Santana et al., 2017) and temperate environments (Gallego et al., 2012). The functional approach, due to having groups that respond and tolerate environmental conditions,

allows a better understanding of phytoplankton variations to environmental changes (Burliga, 2010). This approach represents a necessary tool for understanding species-environment relationships (Salmaso & Padisák, 2007). Based on this perspective, we evaluated the representativeness of the functional group's '*sensu*' Reynolds approaches across a trophic gradient of five tropical reservoirs. We specifically evaluated the relationship between the functional groups and the reservoir trophy, aiming at identifying trophy indicators. This study seeks to understand the functional groups responses face the nutrient availability, allowing a better understanding of the phytoplankton variation regarding the environmental changes. Based on the prediction potential of functional groups reported in the literature (e.g. Kruk et al., 2021; Crossetti et al., 2019) and in a previous study (Oliveira et al., 2020) we predict that functional groups may indicate signs of eutrophication in environments with low nutrient availability

2. Material and Methods

2.1. Study area

Phytoplankton of five reservoirs of the Mid Tietê/Sorocaba watershed, São Paulo, Brazil was evaluated (Figure 1). Selected reservoirs included a trophic gradient ranging from oligotrophic to hypertrophic, as follows: Barra Bonita (hypertrophic), Hedberg (eutrophic), Ipaneminha and Itupararanga (mesotrophic), and Santa Helena (oligotrophic). The trophic state of reservoirs was based on literature (Lucinda, 2003; Buzelli & Cunha-Santino, 2013; SAAE, 2013; CETESB, 2013; Oliveira et al., 2020). The summary of the main features of the five reservoirs studied is shown on table 1. Study area is in a region of tropical climate characterized by two seasonal periods, rainy (October to March) and dry (April to September) (Conti & Furlan, 2008).

2.2. Sampling and variables

Water and phytoplankton samples were gathered at different sampling stations of five reservoirs with different trophic states during the dry and rainy periods of 2014. Three or five sites were sampled in each reservoir (Barra Bonita 5 sites, Itupararanga 5, Hedberg 3, Ipaneminha 3 and Santa Helena 3 sites), totaling 19 sampling sites. Sampling sites were selected considering the main tributaries input, the reservoir deepest region, and the dam region.

Water samples for identification of physical and chemical characteristics were collected with a van

Figure 1. Location of the five reservoirs. Gray area represents the hydrographic basin presently studied (original by Oliveira et al., 2020).

Table 1. Morphometric, hydrological characteristics and classification of the trophic state of the studied reservoirs.

Reservoir	Construction year	Maximum volume (10 ⁶ m ³)	Area $(km2)$	Trophic state
Barra Bonita	1964	3160	310	Hypertrophic
Hedberg	1911	0.5	0.13	Eutrophic
Ipaneminha	1976	0.2	0.15	Mesotrophic
Itupararanga	1912	302	30	Mesotrophic
Santa Helena	1938	1.84	0.38	Oligotrophic

Modified from Oliveira et al. (2020).

Dorn bottle at the subsurface. Water temperature, pH, electric conductivity, and dissolved oxygen (DO) were measured '*in situ*' using a Horiba U50 multiparameter probe. Thermal profile was obtained at the deepest sampling station of each reservoir. Water transparency was measured with a Secchi disc (Cole, 1992). The following variables were also measured: nitrite, nitrate, ammonium, orthophosphate, total dissolved phosphorus (TDP), total nitrogen (TN), total phosphorus (TP) (APHA, 2012). Water samples for determination of dissolved nutrients were filtered under low pressure through glass fiber filters (GF/F Whatman). Chlorophyll-*a* concentration (phaeophytin corrected) was determined by the 90% ethanol method (Sartory & Grobbelaar, 1984). Chlorophyll-*a* and TP concentrations of the reservoir subsurface were used to calculate the Trophic State Index (TSI) proposed by Carlson (1977) modified by Lamparelli (2004).

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Phytoplankton was collected with a van Dorn bottle at different depths (subsurface, mean depth and \pm 1 m above the sediments), and the samples were integrated. Samples for identification of diatom species were oxidized using hydrogen peroxide (35-40%) heated (ECS, 2003) and mounted as permanent slides using Naphrax (RI = 1.74). All other phytoplankton groups were identified using binocular optical microscope (Zeiss Axioskop 2), and specialized literature for the species level identification. Quantitative analysis of phytoplankton was performed under the inverted microscope (Zeiss Axio Observer D1, 400x magnification) according to Utermöhl (1958). Phytoplankton biovolume was calculated (μ m³ ml⁻¹ \rightarrow mm⁻³ L⁻¹) according to Hillebrand et al. (1999). Assignment of species to functional groups followed Reynolds et al. (2002) and Padisák et al. (2009). Abundant functional

groups were the ones with above average community biomass, and dominant those with values exceeding 50% of total biomass and were calculated separately for each reservoir.

2.3. Data analysis

Two-way permutational multivariate variance analysis (two-way PERMANOVA; $\alpha = 0.05$) was used to investigate differences in the composition of phytoplankton functional groups between dry and rainy periods and between reservoirs with different trophic states. This analysis was performed using Bray-Curtis' similarity and PAST 3.01 statistical software (Hammer et al., 2001).

Considering the trophic state and seasonality as factors, the relationship between the phytoplankton total biomass (response variable) and environmental predictors (temperature, NH_{4} -N, TP, free CO₂, $HCO₃$, Secchi disk) was regressed by a Generalized Linear Model (GLM; MINITAB® Release 14.12.0). GLM residuals were examined. Redundancy analysis (RDA) was used to evaluate the relationships of environmental variables and phytoplankton functional groups. This analysis was chosen because the species ordination by Detrended Correspondence Analysis (DCA) showed that the gradient length was < 2.0, consequently indicating linearity in the relationship between environmental variables and phytoplankton functional groups. For the RDA environmental matrix, the six variables (Temperature, TN, TP, TDP, NH_{4} -N) were selected based on the correlation with axes 1 and 2 of the Principal Component Analysis (PCA). RDA was performed using covariance matrix with log transformed data $(x + 1)$ and accomplished using the PC-ORD 6.0 program (McCune & Mefford, 2006).

3. Results

3.1. Abiotic variables

Based on the Trophic State Index (TSI), reservoirs studied were classified oligotrophic to hypertrophic. Santa Helena reservoir was classified oligotrophic, Ipaneminha and Itupararanga mesotrophic, Hedberg eutrophic, and Barra Bonita hypertrophic. The oligo and mesotrophic reservoirs were characterized by low dissolved nutrients (nitrite, orthophosphate, and total dissolved phosphorus) and TP concentrations compared to the eutrophic and hypertrophic reservoirs. However, high TN and ammonium concentrations, as well as high conductivity were depicted. In opposition, the eutrophic and hypertrophic reservoirs were

characterized by high concentrations of dissolved and total nitrogen and phosphorus. Nutrient availability showed temporal variation in which the highest values were recorded in the dry period. Thermal stratification was identified only for the mesotrophic reservoir (Itupararanga) during the rainy period near by the dam. Environmental data for all five reservoirs are available in table 2.

3.2. Phytoplankton

The trophic state had significant effect on phytoplankton total biomass (GLM: $F = 3.01$; p = 0.040; Figures 2a-b). In the GLM, the variance explained was 81.21% (corrected $R^2 = 66.89\%$) and TP and $HCO₃$ ($p > 0.003$) were the main predictors of biomass changes. In the GLM, 66.89% of the variance in phytoplankton total biomass was explained by only $(P < 0.001)$. Considering all reservoirs, 154 phytoplankton taxa were identified and distributed in 24 functional groups. Eight out of the 24 functional groups were considered reservoir descriptors due to their total biomass contribution: **B**, **H1**, L_{M} , **M, MP**, **P**, **T** and **W1**. Group **M** (*Microcystis aeruginosa*) was dominant at the hypertrophic reservoir during the rainy period, and groups L_M (*Ceratium furcoides*) and **P** (*Aulacoseira granulata*) were abundant in the dry period. Functional group **H1** (*Dolichospermum solitarium*) was dominant at the eutrophic reservoir during the rainy period, and groups **MP** (*Oscillatoria tenuis*) and **L**_M (*Ceratium furcoides*) were abundant in the dry period. At the mesotrophic reservoir Ipaneminha, abundance of functional groups **B** (*Discostella stelligera*) and **W1** (*Euglena granulata* and *Lepocinclis acus* var. *longissima*) were observed during both the rainy and the dry periods. The other mesotrophic reservoir Itupararanga showed dominance of the functional group **T** (*Mougeotia* sp.) during the rainy period, whereas groups **T** (*Mougeotia* sp.) and **H1** (*Dolichospermum solitarium*) were abundant in the dry period. The oligotrophic reservoir Santa Helena exhibited dominance of the **H1** group (*Dolichospermum solitarium*) during both sampled climatic periods (Figure 2c-d).

Phytoplankton functional groups were significantly different among reservoirs (two-way PERMANOVA: $F = 3.39$; $p = 0.0001$) and climatic periods (two-way PERMANOVA: *F = 3.73; p = 0.0001*).

RDA was performed for 6 abiotic variables and 24 phytoplankton functional groups (Figure 3). The eigenvalues for axes 1 (λ = 0.17) and 2 ($λ = 0.08$) explained 41% of the

Figure 2. Total biomass (a: rainy period; b: dry period) and respective relative biomass of the abundant and dominant phytoplankton functional groups '*sensu*' Reynolds (c: rainy period; d: dry period).

Figure 3. RDA of the phytoplankton functional groups and five environmental variables in reservoirs of different trophic states. FG with a correlation greater than 0.5 was presented. Abbreviations: the first two characters indicate the reservoir studied, two numbers indicate the sampling point and the last letter indicates the climatic period (r, rainy;d, dry) Temp = water temperature; TN = total nitrogen; TP = total phosphorus; TDP = total dissolved phosphorus; NH4 = ammonium. Correlation of functional groups with axes 1 and 2 is shown in Table 3.

total variability. The high species-environment correlation for axes 1 ($r = 0.86$) and 2 ($r =$ 0.81) indicated a strong relationship between functional groups distribution and environmental variables. Monte Carlo randomization test showed that the first two axes were interpretable (*p* = 0.001). Correlation showed that TN and TDP were the most important variables for axis

Functional group	Axis 1	Axis 2
B	0.56	0.00
D	0.47	0.52
F	0.51	0.21
H1	0.70	0.11
LM	0.35	0.54
M	0.63	0.61
MP	0.70	0.21
т	0.74	0.62
W ₂	0.55	0.29

Table 3. Pearson's correlation of phytoplankton species with scores $(r > 0.5)$ of RDA axes 1 and 2.

1 ordering (*r* > 0.8). First ordering axis represents the trophic gradient, showing that reservoirs with higher trophic states were associated with higher nutrient concentrations. On the positive side of axis 1, oligo and mesotrophic reservoirs were correlated with low nutrient concentrations, mainly associated with functional groups **H1**, **T** and **W2** $(r > 0.5)$. On the negative side, eutrophic and hypertrophic reservoirs correlated with the highest nutrient concentrations. Last reservoirs were mainly associated with functional groups **B**, **M** and **MP** ($r > -0.5$). The second ordination axis represented seasonality, water temperature being the most important variable for the axis ordering $(r > 0.8)$, and there was a clear separation of dry and rainy periods for the hypertrophic and mesotrophic Itupararanga reservoirs. In contrast, eutrophic, mesotrophic Ipaneminha and oligotrophic reservoirs did not separate seasonally.

4. Discussion

Current results showed that phytoplankton functional groups were mainly driven by the reservoir trophic gradient, as demonstrated by the RDA ordination analysis. Differences in nutrient concentrations allowed the presence of distinct functional groups. Moreover, functional groups were also influenced by seasonality, and water temperature was the forcing function for the changes in abundance. Similar results were observed in several studies carried out for tropical reservoirs (Becker et al., 2010; Bortolini et al., 2014; Souza et al., 2018), indicating that nutrient availability and temporal dynamics are determinant factors for phytoplankton functional groups.

Abundant and dominant phytoplankton functional groups were associated with the trophic state of reservoirs studied. For the hypertrophic reservoir, functional group **M** was representative of the trophic condition in both climatic periods, as mentioned in the literature (Reynolds et al., 2002) and observed for tropical reservoirs (Gemelgo et al., 2009). Although **M** group may grow in a range of environmental conditions (Kruk & Segura, 2012), it seems related to enriched environments (Paerl & Otten, 2013). Functional group **M** is represented by species that have specialized traits (e.g. mucilage, aerotopes and heterocytes) related to competition for nutrients, light, and temperature (Kosten et al., 2012) as the dominant species in the present study, *Microcystis aeruginosa*. Furthermore, **MP** and **P** groups were also representative of the hypertrophic reservoir, as they are composed of species that tolerate high trophy (Lobo et al., 2018).

Functional groups present in the eutrophic reservoir were representative of the system trophic degree. In this reservoir, **H1** group was dominant during the high temperature period (rainy season), being a common group in the eutrophic environments (Souza et al., 2018), and that could be used as an environmental indicator in the tropical region (Gemelgo et al., 2009). In the dry period, \bf{MP} and \bf{L}_{M} groups contributed to biomass at the eutrophic reservoir and, according to the literature, both have broad trophic tolerance and represent environments with low to high nutrient concentration (Padisák et al., 2009). Current study representative species of L_M group, *Ceratium furcoides* is considered an invasive species in continental aquatic environments in South America (Silva et al., 2012; Crossetti et al., 2019) and shows easy adaptation to different ecological conditions. Due to its high dispersal and establishment capacity, the species has been recorded in several Brazilian reservoirs (Dias & Tucci 2020). In the reservoir in question, the species *C. furoides* greatly tolerated the nutrient limitation, since it may also be mixotrophic or be able to use its motility to avoid light restriction and search for nutrients in the deeper, usually more nutrient rich areas (Reynolds, 1998; Crossetti et al., 2019). Reynolds et al. (2002) included *C. furcoides* in the L_{M} group, the species being coexistent with *Microcystis aeruginosa*. In the present reservoir, such coexistence was detected. Therefore, phytoplankton in the hypertrophic and eutrophic reservoirs presented functional groups characteristic of their trophic conditions.

Mesotrophic reservoirs included functional groups that represented the system trophic state. Ipaneminha mesotrophic reservoir showed an abundance of group **B**, which consists of species that efficiently compete under nutrient enrichment

conditions, and are usually associated with mixed environments (Padisák et al., 2009). This group is most represented by centric diatoms (Padisák et al., 2009), as was currently observed during the isothermal period. Having silica-impregnated cell wall and high sedimentation rate, these organisms need water column mixing to maintain their biomass (Stević et al., 2013). Group **W1** was also abundant at the Ipaneminha mesotrophic reservoir, which was favored under conditions of high concentration of decaying organic matter commonly observed in shallow environments (Reynolds et al., 2002). At both climatic periods, the also mesotrophic Itupararanga reservoir exhibited dominance of functional group **T**. According to Reynolds et al. (2002), group **T** is sensitive to nutritional deficits, tolerant to low light intensity, and associated with mesotrophic condition. In the present study, the only representative of group **T** was *Mougeotia* sp., which presented high biomass to form bloom in the rainy season. *Mougeotia* species can grow in systems ranging from oligo to eutrophic, and at various pH levels (Graham et al., 1996). The occurrence of this genus was already related with trophic changes in temperate lakes, where the species development was associated with the first signs of eutrophication (Tapolczai et al., 2015). *Mougeotia* was already recorded thriving under meso-eutrophic conditions, such as at Lake Kinneret in Israel (Zohary et al., 2019). Furthermore, it is one of the most common bloom-forming algae in acidic waters of Europe and North America and considered an acidification indicator (Graham et al., 1996). Therefore, both current mesotrophic reservoirs included functional groups characteristic of the environment based on the literature. Nevertheless, presence of *Mougeotia* sp. in the Itupararanga reservoir may be indicative of some eutrophication process, as observed in a previous study (Oliveira et al., 2020).

Unlike previous reservoirs, the oligotrophic Santa Helena presented dominance of functional group **H1** in both sampling periods, a group typical of eutrophic environments. This group was the main representative of the eutrophic reservoir Hedberg, which is consistent with the most recent literature on functional classification (Padisák et al., 2009). The problem is the species *Dolichospermum solitarium*, that was classified in group **H2** associated, according to Reynolds et al. (2002), with oligo-mesotrophic environments and good light conditions, but due to its frequent occurrence in eutrophic-hypertrophic environments, it was classified in group **H1** by Padisák et al. (2009).

Studies reported the occurrence of *D. solitarium* in tropical and temperate lakes and reservoirs with high nutrient availability (Hu et al., 2013; Bortolini et al., 2014). Despite the reservoir is classified oligotrophic by the TSI, it has high concentrations of total nitrogen, ammonium, and high conductivity, suggesting a possible increase in the trophic state, which can explain the dominance of the **H1** group.

In summary, the high biomass of groups **T** (*Mougeotia*) at the Itupararanga mesotrophic reservoir and the **H1** group (*D. solitarium*) at the oligotrophic reservoir evidenced the systems' vulnerability, which may very quickly change their trophic states if under anthropogenic disturbance pressure. Studied reservoirs are inserted in a highly impacted watershed (IPT, 2008), however, still including oligo and mesotrophic reservoirs. Nevertheless, functional groups present results indicated that such reservoirs must have started some eutrophication process. In a previous study, this outcome was also suggested by the presence of a guild associated with high nutrient availability (Oliveira et al., 2020). We therefore, concluded evidenced that nutrient availability was the driving force for the phytoplankton functional groups in the trophic gradient. Our findings likewise evidenced that the functional groups were representative of the trophic status of the tropical reservoirs and showed signs of eutrophication onset. According to our prediction, the functional groups exhibited indications of the onset of the eutrophication process in environments with low nutrient availability, thereby highlighting the predictive capability of phytoplankton.

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