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Driving factors of phytoplankton Reynolds functional groups (RFGs) in a subtropical reservoir (São Paulo, Brazil)

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ABSTRACT

Driving factors of phytoplankton Reynolds functional groups (RFGs) in a subtropical reservoir (São Paulo, Brazil).

Subtropical reservoirs are susceptible to the eutrophication process, largely due to inappropriate land use and occupation in the watersheds, resulting in changes in the composition of the phytoplankton community, according to the sensitivity and environmental tolerances of the different species. Application of the Reynolds functional groups (RFGs) method to the multispecies phytoplankton community is a valuable approach that can be used to answer ecological questions at different spatial and temporal scales. The Itupararanga reservoir, in the upper Sorocaba River basin (São Paulo state, Brazil), is used to supply drinking water to approximately one million people, as well as for agricultural and recreational purposes. This study examines the relationships between environmental variables and the composition of the phytoplankton community, identifying the main functional groups (RFGs) along a spatial gradient in the reservoir (from upstream to downstream), during one year. Five samplings were performed at seven points in the reservoir, in the rainy and dry seasons (from 2016 to 2017). The effects of spatial heterogeneity influenced the formation of different biomass gradients for each functional group. The most abundant RFGs were Sn (warm mixed environments), H1 (eutrophic, both stratified and shallow lakes with low nitrogen content), and Lo (deep and shallow, oligotrophic to eutrophic, medium to large lakes), which corresponded to the spatial heterogeneity of the reservoir. The variables with the greatest influence on the phytoplankton composition in the reservoir were total nitrogen, nitrite, and the N/P ratio, with phosphorus as the limiting nutrient. The presence of Cyanobacteria (codons Sn and H1) was driven by the stability of the water column, due to the long residence time of the water in the reservoir. This research demonstrated the effectiveness of using RFGs as environmental descriptors, providing an essential tool in aquatic ecology studies.

KEY WORDS: reservoir, eutrophication, Cyanobacteria, functional groups.

RESUMEN

Fatores determinantes dos grupos funcionais fitoplanctônicos (RFG) em reservatórios subtropicais (SP, Brasil).

Os reservatórios subtropicais são suscetíveis ao processo de eutrofização, em grande parte devido aos usos e ocupações irregulares de sua bacia hidrográfica. As mudanças na composição da comunidade fitoplanctônica ocorrem de acordo com à sensibilidade e tolerâncias ambientais das espécies. De acordo com os Grupos Funcionais de Reynolds (RFGs), o fitoplâncton é um excelente modelo de sistema multiespécies para responder questões ecológicas em diferentes escalas no espaço e no tempo. O reservatório de Itupararanga está localizado na bacia do alto rio Sorocaba, (Sorocaba, SP, Brasil). É usado para abastecimento de água potável para aproximadamente um milhão de pessoas, bem como para fins agrícolas e recreativos. O objetivo desta pesquisa consistiu em analisar a relação entre as variáveis ambientais e a composição da comunidade fitoplanctônica, identificando os principais grupos funcionais (RFGs), em um gradiente espacial longitudinal (montante a jusante) no reservatório, durante o período de um ano. Foram realizadas cinco *amostragens em sete pontos no reservatório, nas estações chuvosa e seca (2016 a 2017). Os efeitos da heterogeneidade espacial influenciaram a formação de diferentes gradientes de biomassa para cada grupo funcional. Os RFGs mais abundantes foram Sn (camadas mistas quentes), H1 (lagos eutróficos, estratificados e rasos com baixo teor de nitrogênio) and Lo (lagos profundos e rasos, oligo a eutróficos, médios a grandes), que responderam a heterogeneidade espacial do reservatório. As variáveis que influenciaram a composição do fitoplâncton no reservatório foram o nitrogênio total, o nitrito e a relação N/P, tendo o fósforo como nutriente limitante. A presença de cianobactérias (códons Sn, H1) foi impulsionada pela estabilidade da coluna d'água, devido ao longo tempo de residência da água neste reservatório. Esta pesquisa mostrou a eficácia da utilização de RFGs como descritores ambientais, sendo uma ferramenta essencial em estudos de ecologia aquática.*

PALAVRAS CHAVE: *reservatório, eutrofização, cianobactérias, grupos funcionais.*

INTRODUCTION

Subtropical reservoirs are susceptible to eutrophication, mainly due to improper land use and occupation in the watersheds. The impacts of anthropic activities have had profound effects on the nitrogen (N) and phosphorus (P) cycles in many aquatic ecosystems (Cardoso-Silva et al., 2018). Furthermore, climatic changes influence precipitation regimes, affecting the hydrology of these ecosystems, altering flows and retention times, promoting stratification of the water column, and increasing inputs from effluent discharges and leaching processes (Whitehead et al., 2009). According to Le Moal et al. (2019), there is no single solution to eutrophication processes. One of the main problems related to eutrophication is the proliferation of Cyanobacteria, which is increasing in frequency, magnitude, and duration, due to climatic changes (Figueredo & Giani, 2001; Vasconcelos, 2015; Huisman et al., 2018; Bižić et al., 2020; Chorus & Welker, 2021). In southeastern Brazil, most of the reservoirs intended for public supply are eutrophic, due to inputs of domestic effluents (Moschini-Carlos et al., 2009, 2010; Cunha & Calijuri, 2011a, 2011b; Nishimura et al., 2014; Machado et al., 2016, 2022) and pollutants from diffuse sources such as agricultural activities (Taniwaki et al., 2013; Beghelli et al., 2016; Cardoso-Silva et al., 2017; Melo et al., 2019; Biamont-Rojas et al., 2022; Frascareli et al., 2022a, 2022b; Machado et al., 2022).

These inputs directly influence primary productivity, with changes in the composition of the phytoplankton community that depend on the sensitivity and environmental tolerances of the different species. The organisms belonging to the phytoplankton community have specific responses to environmental variations, which has led to the creation of the concept of Reynolds functional groups (RFGs) (Reynolds et al., 2002; Padisák et al., 2009). According to Kruk et al. (2021), the small size and short lifespan of these organisms, together with the possibility of assembling several species into groups consistent with clear ecological rules, in the form of RFGs, make phytoplankton an excellent multispecies system model to answer many ecological questions at different spatial and temporal scales. Using this approach, it is possible to differentiate phytoplankton organisms in terms of their adaptations and specific characteristics, such as high affinity for phosphorus or carbon, efficiency in light harvesting, and others (Huszar & Caraco, 1998; Reynolds et al., 2002, 2006; Salmaso et al., 2015). The application of freshwater RFGs is considered a very robust tool for understanding and predicting the dynamics of natural phytoplankton communities, due to their sensitivity to latitude, morphometrics, and trophic states (Brasil & Huszar, 2011). RFGs were designed to represent phytoplankton diversity across a suite of freshwater ecosystems and are among C. S. Reynolds' most enduring legacies for freshwater phytoplankton ecologists (Kruk et al., 2021). Several studies in Brazil have used the RFGs approach, providing holistic interpretations regarding aquatic ecosystems (Alves-de-Souza et al., 2006; Moura et al., 2007; Fonseca & Bicudo, 2008; Brasil & Huszar, 2011; Nishimura, 2012; Crossetti et al., 2018; Santos et al., 2018; Amorim & Moura, 2022).

RFGs enable a complementary analysis of the aquatic ecosystem, since they can highlight potential risks to which reservoirs may be exposed (Moschini-Carlos et al., 2017; Kruk et al., 2021). Considering this, the objective of the present research was to analyze the relationships between environmental variables and the composition of the phytoplankton community in a subtropical reservoir, identifying the main functional groups (RFGs), as well as to evaluate the possible existence of spatial (longitudinal) and temporal distribution gradients of the community.

MATERIAL AND METHODS

Study area

The Itupararanga reservoir is in the southeastern region of São Paulo state, Brazil (Fig. 1). The main channel of the reservoir is 26 km long and the average depth is 7.8 m, reaching 21 m. The climate in the region is typically subtropical and the average temperature varies between 18 and 22 °C (Melo et al., 2019). The reservoir has a maximum storage volume of 286 million m^3 and an average surface area of 25 km^2, and is currently used to supply drinking water for approximately one million people, as well as for agricultural and recreational purposes (Cunha & Calijuri, 2011a). The water residence time varies between 95 and 270 days (Melo et al., 2019). The average daily precipitation at the reservoir during the period from 2015 to 2017 was 7.2 ± 14.8 and 3.2 ± 11.4 mm in the wet (October to March) and dry (April to September) seasons, respectively (Frascareli et al., 2022a).

Recent studies have reported significant degradation of the reservoir water quality, due to inflows of domestic effluents in the fluvial region (Cunha & Calijuri, 2011a; Taniwaki et al., 2013; Beghelli et al., 2016; Melo et al., 2019; Frascareli et al., 2022a, 2022b; Biamont-Rojas et al., 2022; Machado et al., 2022). During the period of sample collection, the reservoir was characterized as supereutrophic and eutrophic (Melo et al., 2019).

The limnological characterization adopted in the present study was based on the data reported by Melo et al. (2019). Water samples were collected at seven sampling sites (P1, P2, P3, P4, P5, P6, and P7) during the period from 2016 to 2017 (December 2016 and March, August, October,

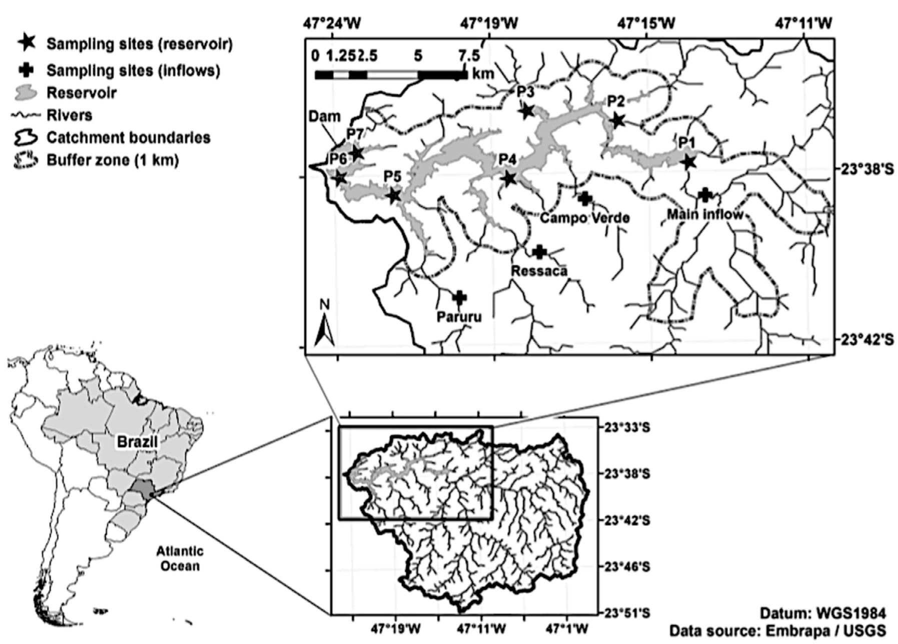

Figure 1. Sampling sites in the Itupararanga Reservoir (stars) and some inflows (+ signs). The maps also show the boundaries of the upper Sorocaba River catchment and the river network. Adapted from Melo et al. (2019). *Locais de amostragem no reservatório de Itupararanga (estrelas) e em alguns afluentes (sinais de mais). Os mapas também mostram os limites da bacia hidrográfica do alto rio Sorocaba e da rede fluvial. Melo et al. (2019).*

and December 2017) (Fig. 1). The environmental variables analyzed were as follows: total nitrogen (TN), nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+), total phosphorus (TP), orthophosphate (PO_4^{3-}), nitrogen to phosphorus ratio (N/P), chlorophyll-*a* (Chl-*a*), euphotic zone, depth, pH, dissolved oxygen (DO), electrical conductivity (EC), turbidity, and redox potential (Eh). Physical and chemical profiles were acquired at all the sampling sites using a portable multiparameter probe (Model 134 U-50, Horiba), which simultaneously measured temperature, pH, DO, EC, turbidity, and Eh. The methods used for analysis of the nutrients were those of Koroleff (1976) for NH_4^+, Strickland & Parsons (1968) for PO_4^{3-}, Valderrama (1981) for TN and TP, and Mackereth et al. (1978) for NO_2^- and NO_3^-. Chlorophyll-*a* in surface water samples was determined according to the method of Wetzel and Likens (2000), using 90% alkaline acetone solution for the extraction of Chl-*a* from 0.45 µm filters.

Samples for qualitative phytoplankton identification were collected by surface water trawls, using a 20 µm mesh size plankton net, followed by fixing with 4% formaldehyde. Species were identified using a Zeiss Axio Imager A1 light microscope equipped with a differential interference contrast (DIC) filter, as well as a Zeiss Axio Scope A2 microscope equipped with an HBO 50 illuminator and the corresponding filters for examinations performed under epifluorescence. Species were identified according to the specialized literature. Quantification was performed using an Zeiss Axiovert inverted microscope equipped with a micrometric reticle and operated in dark field mode, according to the methodologies of Lund (1958) and Utermöhl (1958). Cell measurements were performed using Zen Lite software and photographs acquired with a Zeiss Axiocam microscope camera. Biovolume was determined by geometric approximation, multiplying the density of each species by the average cell volume (Hillebrand,1999; Sun & Liu, 2003). Phytoplankton biomass was expressed in fresh weight units, where 1 mm^3/l = 1 mg/l (Wetzel & Likens, 2000). Total biomasses were calculated by summing the biomasses of taxa with relative abundance above 5%, at the points sampled on the different collection dates (in December 2016, March 2017, August 2017, October 2017, and December 2017). The classification into functional groups was carried out for representative taxa, considered to be those whose biomass was greater than 5% of the total biomass, according to the groups proposed by Reynolds (2002) and Pádisak et al. (2009). Hierarchical cluster analysis was performed using Euclidean distances, obtaining dendrograms of the clusters coupled to a heatmap produced using the Heatmap3 v. 3.5.3 tool (Zhao et al., 2014) of the R Project for Statistical Computing® v. 3.4.0 software. The analysis was performed to determine the correspondences between the RFGs and the environmental variables, as well as between the sampling locations, for the five sampling campaigns. For this, the RFGs that represented more than 5% of the total biomass in the campaigns were selected.

A canonical correspondence analysis (CCA) was also performed to evaluate the correlations between the biomass variations of the most representative RFGs and the limnological variables. The dataset was normalized to log10. To identify the environmental variables that had a significant effect in the CCA model, the scores were submitted to analysis of variance (ANOVA, $p < 0.05$). After selecting the most influential variables, redundancy analysis (RDA) was performed to evaluate the linear effects of the environmental variables on the biomasses of the RFG descriptors.

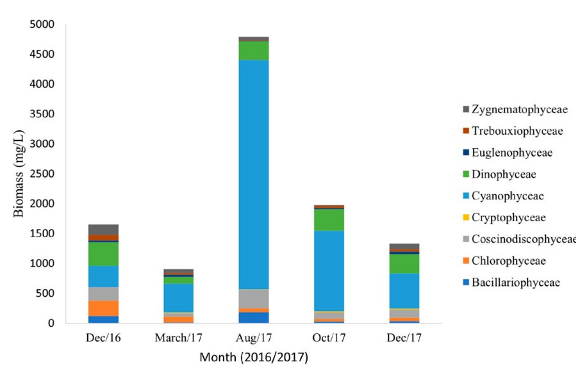

Figure 2. Total biomasses of phytoplankton classes (taxa with relative abundance above 5%) in the Itupararanga reservoir during the periods December 2016, March 2017, August 2017, October 2017, and December 2017. *Biomassa total das classes do fitoplâncton (táxons com abundância relativa acima de 5%) no reservatório de Itupararanga durante os períodos de dezembro de 2016, março de 2017, agosto de 2017, outubro de 2017 e dezembro de 2017.*

These and diversity analyses were performed using the vegan 2.5-2 package in R v. 3.4 (Oksanen et al., 2018).

RESULTS

Fig. 2 shows the total biomasses of the phytoplankton classes (taxa with relative abundance above 5%) during the study period. A total of 54 taxa belonging to 9 classes were identified.

The highest total phytoplankton biomass was recorded in August 2017, while the lowest was in March 2017. The Cyanobacteria biomass was much higher than for the other classes, ranging from 362.2 to 3838.6 mg/l (Fig. 2). There was also a substantial presence of Dinophyceae, ranging from 116.3 mg/l (March 2017) to 392.6 mg/l (December 2016), with an average of 298.7 ± 107.3 mg/l (Fig. 2). In all the sampling periods, except December 2016, the most prevalent phytoplankton class was the Cyanobacteria. No defined pattern was observed in the total biomass values between collections and sampling stations (Fig. 3). A marked change in the composition and biomass of phytoplankton was recorded upstream (P1 and P2), with alternation between dominance by *Raphidiopsis raciborskii* and by *Dolichospermum* spp. and *Aphanizomenon gracile* (Table S1, Supplementary Material. available at https://www.limnetica.net/en/limnetica).

The taxa with relative abundance above 5% were classified into 20 phytoplankton functional groups (RFGs), represented by the following codons (in descending order of importance): Sn, H1, Lo, P, J, S1, C, Mp, F, Tc, Td, W2, Tb, D, W1, Y, X1, Lm, G, and K (Fig. 4). The Sn codon was responsible for 42% of the total biomass, while H1 and Lo both had 14% contributions. The P and J codons represented 7% and 4% of the biomass, respectively, and the S1, C, and Mp codons each represented 3%. The F, Tc, and Td codons represented 2%, while the W2, Tb, D, W1, Y, X1, Lm, G, and K groups represented ≤ 1% of the total biomass (Fig. 4).

The species that most influenced the total biomass values was *R. raciborskii* (maximum 951.9 mg/l, average 127 ± 220.1 mg/l) (Table S1), belonging to the Sn codon representing the group of filamentous cyanobacteria, characterized by high adaptive capacity when faced with environmental variations, being able to tolerate warm mixed environments, or even acting as competing organisms in turbid environments. The species *D. solitarium* (maximum 621.5 mg/l, average 65.9 ± 157.47 mg/l), *D. planctonicum* (maximum 143.2 mg/l, average 36.5 mg/l), and *A. gracile* (maximum 80.8 mg/l, average 14.7 ± 24.05 mg/l) represented codon H1 (Fig. 4), whose habitat

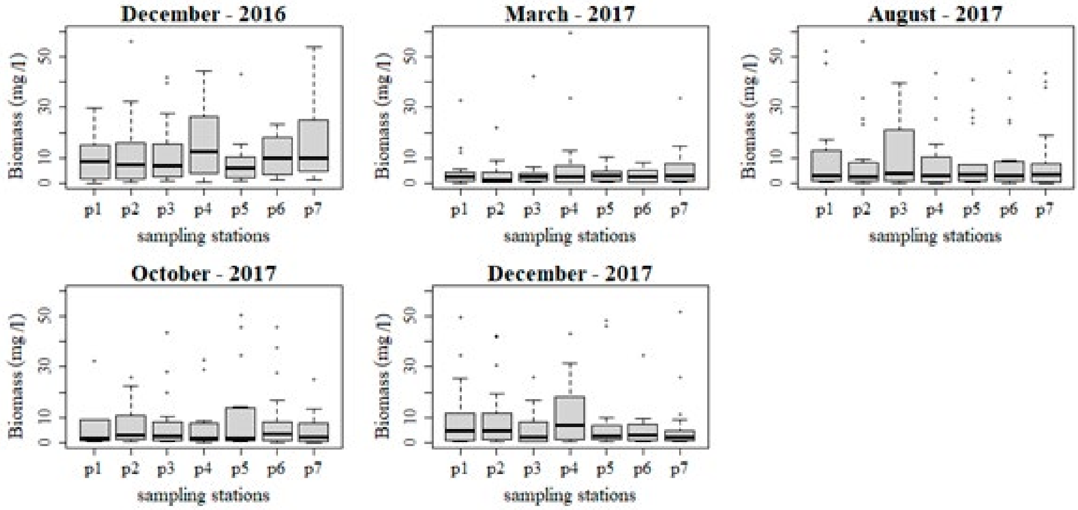

Figure 3. Boxplots of total biomass values (mg/l) for the different collection periods and sampling stations. *Boxplots da biomassa total (mg/l) nas estações e meses amostrados.*

characteristics consist of shallow environments, eutrophic conditions, and low nitrogen concentrations. The dinoflagellate *Ceratium furcoides* (maximum 92.3 mg/l, average 48.3 ± 18.8 mg/l) represented the Lo codon (Fig. 4), whose habitat is characterized by nutrient segregation. The specific biomass values for each species and the corresponding functional groups (RFGs) for the collection points and the sampling campaigns are provided in Table S1.

The groups represented by codons P and J, with similar habitat characteristics and belonging to chlorophyte taxa, made smaller contributions to the total biomass values. The P codon, which is characteristic of shallow eutrophic environments and normally inhabits the epilimnion of stratified lakes, was represented by *Staurastrum* sp. (maximum 18.3 mg/l, average 5 ± 5.1 mg/l) and *Closterium* sp. (maximum 9.5 mg/l, average 5.2 ± 2.1 mg/l) (Tables S1 and S3, Supplementary Material, available at https://www.limnetica.net/en/limnetica). Codon J is also associated with environments that are shallow and rich in nutrients, although its habitat is related to mixing conditions. In the Itupararanga reservoir, the most representative taxa in the biomass belonging to codon J were *Coelastrum reticulatum* (maximum 93.8 mg/l, average 63.2 ± 30.5 mg/l) and *C. microporum* (maximum 43.3 mg/l, average 10.8 ± 13.2 mg/l) (Table S1). The S1, C, and Mp codons represented a low contribution to the total biomass values (Fig. 4). The S1 codon, which only includes Cyanobacteria adapted to low light, was represented by *Planktothrix isothrix* (maximum 100.3 mg/l, average 25.8 ± 29.3 mg/l) and *Geitlerinema amphibium* (maximum 47.7 mg/l, average 16.1 ± 27.3 mg/l). The most representative organisms in codon C, whose habitat is eutrophic environments sensitive to the onset of stratification, were *Aulacoseira granulata* (maximum 148.9 mg/l, average 30.4 ± 32.3 mg/l) and *A. ambigua* (maximum 35.5 mg/l, average 11.7 ± 7.2 mg/l). Only the Mp codon, which is characteristic of inorganically turbid shallow lakes, occurred in almost all the samples, represented by *Fragilaria* sp. (maximum 43.8 mg/l, average 9.4 ± 10.9 mg/l) (Tables S1 and S3).

The patterns presented by the average phytoplankton biomass did not reflect the effects of the trophic state of the Itupararanga reservoir, which varied from supereutrophic to oligotrophic from upstream to downstream (P1→P7). According to Melo et al. (2019), the trophic classification ranged from eutrophic to supereutrophic at upstream locations (P1 and P2), and between mesotrophic and oligotrophic in the downstream region (P5, P6, and P7). *R. raciborskii* (Sn codon) showed high biomass and was dominant in most months, except October and December 2017, with high biomass observed in upstream and

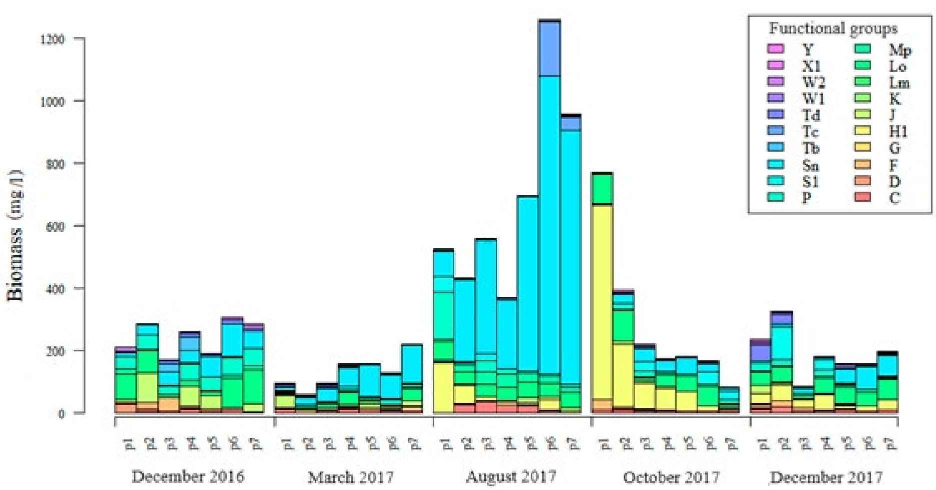

Figure 4. Barplot showing the biomasses of the different functional groups in the Itupararanga reservoir during the periods December 2016, March 2017, August 2017, October 2017, and December 2017. *Barplot com a biomassa dos GF's do reservatório de Itupararanga durante os períodos de dezembro de 2016, março de 2017, agosto de 2017, outubro de 2017 e dezembro de 2017.*

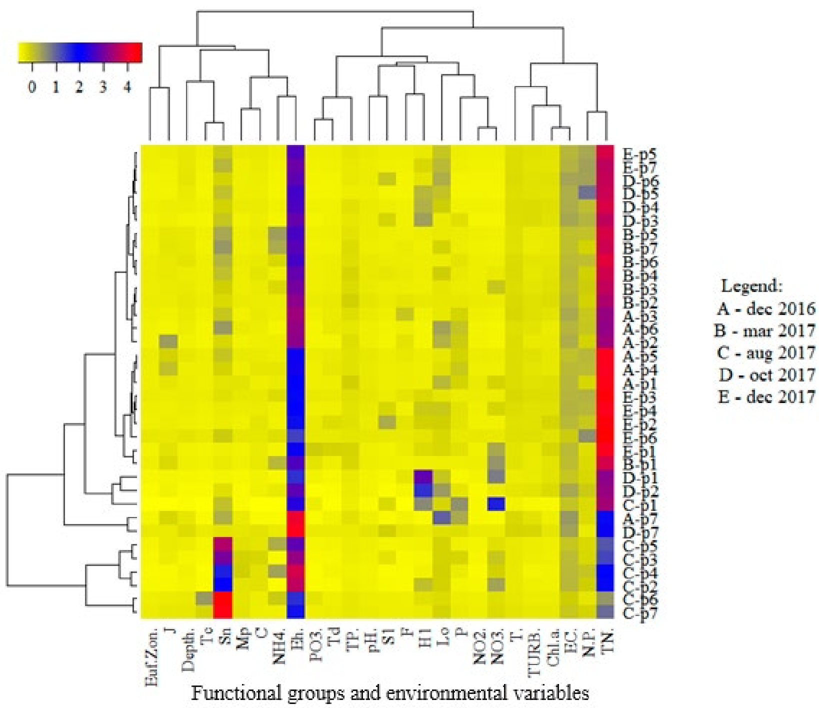

Figure 5. Heatmap and dendrograms of the clusters for the environmental variables and the functional groups that contributed more than 5% of the total biomass in the different collection periods (December 2016, March 2017, August 2017, October 2017, and December 2017). *Heatmap e dendrogramas dos clusters entre os grupos funcionais que contribuíram com mais de 1% da biomassa total entre todas as campanhas (dezembro de 2016, março de 2017, agosto de 2017, outubro de 2017 e dezembro de 2017) e as variáveis ambientais.*

downstream regions of the reservoir (Table S1).

In the cluster analysis, the heatmap and the dendrogram of the clusters obtained among the environmental variables (Table S2, Supplementary Material, available at https://www.limnetica.net/en/limnetica) and the functional groups showed the formation of three large groups, based on the distances between them. The first grouping included the environmental variables euphotic zone, depth, NH_4^+, and Eh, together with the RFGs J, Tc, Sn, Mp, and C. The second grouping comprised the environmental variables PO_4^{3-}, TP, pH, NO_2^-, and NO_3^-, together with the RFGs Td, S1, F, H1, Lo, and P. The third grouping consisted only of environmental variables (temperature, turbidity, Chl-*a*, EC, N/P, and TN) (Fig. 5).

The CCA analysis had high explanatory power, with the first two axes explaining 71.34% of the data variation (40.96% for axis 1 and 31.38% for axis 2) (Fig. 6). Correlation of the Sn codon with the variables euphotic zone and NH_4^+ (Fig. 6) confirmed the trend observed in the first grouping in the cluster analysis (Fig. 6). The H1 codon was correlated with the N/P ratio, while the Lo codon was correlated with TN, EC, and turbidity.

The groups that had a moderate contribution to the biomass values (F, P, J, and Td) were correlated with the variables Eh, TP, and NO_2^-, located opposite the H1 codon. The S1 codon had a positive correlation with Chl-*a* and its position in the CCA arrangement was precisely opposite to the euphotic zone variable. The Mp codon was positioned opposite to the Eh and TP variables, while the C codon was correlated with the N/P ratio.

Therefore, the CCA results validated the observed trends and enabled identification of the responses of the functional groups to the main environmental variables. The results of the ANOVA applied to the CCA scores for the environmental variables showed that the variables with significant influence ($p < 0.05$) were TN, NO_3^-, and N/P. The RDA analysis (Fig. 7) was even more robust

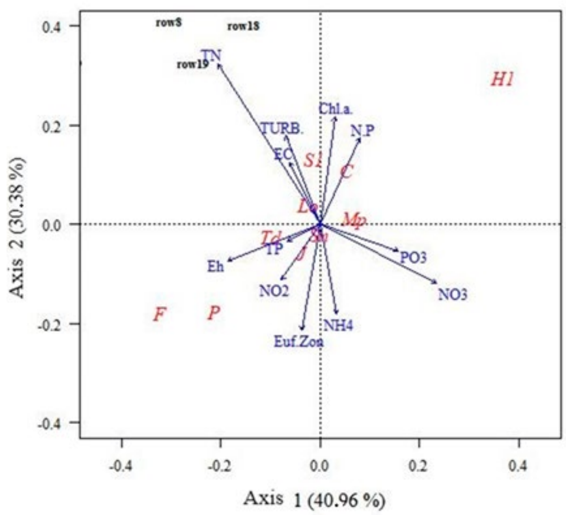

Figure 6. CCA plot for the most representative environmental variables and the functional groups with biomass >5%. *Plot da CCA entre as variáveis ambientais e grupos funcionais mais representativos em biomassa (> 1%).* TN: total nitrogen; TP: total phosphorus; NO_2: nitrite; NO_3: nitrate; NH_4: ammonium; PO_4: orthophosphate; N.P: nitrogen to phosphorus ratio; Chl.*a*: chlorophyll-*a*; Euf.Zon: euphotic zone; EC: electrical conductivity; TURB: turbidity; Eh: redox potential.

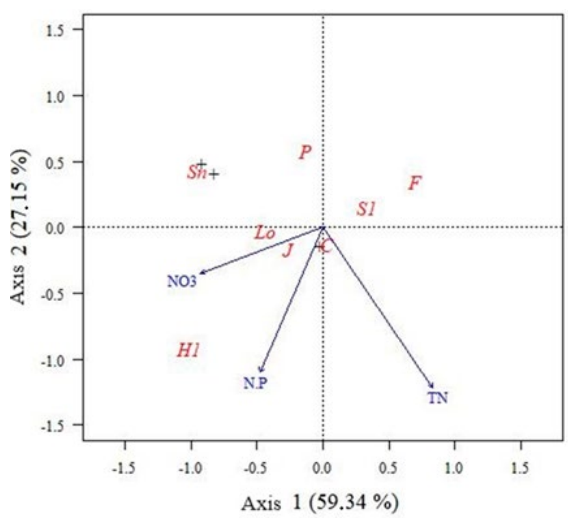

Figure 7. RDA plot for the most significant environmental variables and the most representative RFGs (with biomass >5%). *RDA entre variáveis ambientais com influência significativa na variação dos dados e os grupos funcionais (GFR) mais representativos na biomassa (> 1%).* TN: total nitrogen; NO_3: nitrate; N.P: nitrogen to phosphorus ratio.

than the CCA, explaining 86.5% of the effect of these variables on the occurrence of the RFGs (59.34% for axis 1 and 27.15% for axis 2).

In the RDA plot, the Sn codon (together with Tc and Mp) was located in the quadrant opposite the direction of the gradient of the TN variable, confirming the characteristic tolerance of *R. raciborskii* to low nitrogen availability (Fig. 7). This provided an explanation for its high biomass and the presence of heterocysts, which were mainly observed in August 2017, when TN levels were lower (Melo et al., 2019).

DISCUSSION

The high phytoplankton levels and the diversity of codons identified were consistent with the results reported by Cunha and Calijuri (2011b) for the main tributaries of the Itupararanga reservoir. In Brazilian reservoirs, Alves-de-Souza et al. (2006) described 8 RFGs in Lagoa Comprida (Rio de Janeiro state), Moura et al. (2007) reported 18 groups in the Mundaú reservoir (Pernambuco state), Santos et al. (2018) found 15 groups in a study of the Cantareira System reservoirs (São Paulo state), and Amorim & Moura (2022) described 20 RFGs in ten reservoirs in the semi-arid northeast region of the country. In the Itupararanga reservoir, the absence of significant variations in the water column may have been one of the factors related to the constant occurrence of groups Sn, H1, and Lo, since the lake environment provided stable conditions in the water column and a long water residence time. According to Gil-Guarín et al. (2022), the morphological, physiological, and ecological functional attributes of planktonic communities are sensitive to hydrological variation, although information is scarce regarding the functional dynamics of phytoplankton in tropical reservoirs with long water residence times. Barbosa et al. (2021), applied a regionalized climate model to the Itupararanga reservoir, where hydrological and hydrodynamic simulations projected a future increase in the thermal stability of the water column. This scenario would intensify the presence of Cyanobacteria (codons Sn and H1), as already observed in this study.

The occurrence of the Sn codon is favored when nutrient availability is restrictive, with its correlation with the euphotic zone and ammonium indicating tolerance to the effects of a decreasing trophic gradient. Furthermore, although the ability of these organisms to fix atmospheric nitrogen is an advantage, it is an energetically expensive process and many diazotrophic cyanobacteria suppress N_2 fixation when alternative forms of nitrogen (such as ammonium and nitrate) are available (Huisman et al., 2018). Similar results were reported by Rodrigues et al. (2019) for the occurrence of this species in oligo-mesotrophic reservoirs. Casali et al. (2017) identified the dominance of *R. raciborskii* in the Itupararanga reservoir dam region in the dry season, while *Monoraphidium contortum* was dominant in the rainy season. Other studies of Brazilian reservoirs have also identified the dominance of *R. raciborskii* (Figueredo & Giani, 2009; Moschini-Carlos et al., 2009; Bittencourt-Oliveira et al., 2014; Brentano et al., 2016; Machado et al., 2016, 2022; Santos et al., 2018; Vicentin et al., 2018; Rodrigues et al., 2019; Amorim & Moura, 2022).

In the RDA arrangement, the occurrence of functional groups was correlated with the Sn group. The Tc group (*Phormidium aerugineo-caeruleum*) was only observed at points P6 and P7. The fact that this group only occurred in the dam region could be explained by the type of habitat that favors it, such as stationary waters or slow-flowing rivers (Reynolds et al., 2002; Padisák et al., 2009). Hence, since the dam region was representative of a lake environment (Thorton et al., 1990), the maintenance of this habitat in the dry season favored the presence of this codon (August 2017).

The Mp group, with lower biomass values, presented a distribution following the same gradient as the Sn codon, in the direction of the dam, suggesting its tolerance to low concentrations of nutrients (phosphorus). The CCA plot supported this hypothesis, since the Mp codon was positioned opposite to the gradient of the TP variable. According to Visser et al. (2016), there may sometimes be alternation between the occurrence of diatoms and certain Cyanobacteria taxa.

In both the RDA and the CCA, the H1 codon was correlated with the N/P ratio, indicating its relationship with phosphorus limitation. The H1 codon occurred mainly in the upstream region of the reservoir, at P1 and P2, classified as eutrophic and supereutrophic, respectively (Melo et al. 2019), confirming the functional characteristics of this group in terms of sensitivity to low phosphorus levels. Therefore, the H1 functional group has a good response to trophic variation and its occurrence corroborated the decreasing gradient of the trophic state of the reservoir, from supereutrophic in the upstream region to meso-oligotrophic in the region close to the dam (Melo et al., 2019). According to Melo et al. (2019), the reservoir is an environment compartmentalized into three main regions, with similar environmental characteristics at P1 and P2 (upstream), P3, P4, and P5 (intermediate zone), and P6 and P7 (downstream).

In the Itupararanga reservoir, the H1 codon was represented by *Dolichospermum* spp. and *A. gracile*, which are often found in highly eutrophic environments with high nutrient loads and are capable of withstanding large fluctuations in environmental variables (Moschini-Carlos et al., 2009; Machado et al., 2016; Vicentin et al., 2018; Rodrigues et al., 2019). This capacity is due to the adaptive advantages of these organisms, such as the presence of akinetes, aerotopes, and heterocysts, which allow them to be mobile in the water column in stratified environments, fix N_2, and produce germ cells under restrictive environmental conditions (Huisman et al., 2018).

The CCA also showed a weak correlation of the Lo codon (represented by *C. furcoides*) with the variables TN, turbidity, and EC, which are directly related to the characteristics of eutrophic environments. In the RDA, the correlation with the NO_3^- variable was more evident and the position of the Lo group was intermediate to that of the H1 and Sn groups, suggesting similarity of the relationships between these groups. The Lo group is tolerant to stratification of the water column and the consequent segregation of nutrients (Reynolds et al., 2002; Padisák et al., 2009), as well as to light and nitrogen limitations (Amorim & Moura, 2022), since dinoflagellates can move through different layers of the water column. This adaptability allowed its occurrence, regardless of period and nutrient segregation. On the other hand, Rodrigues et al. (2019) observed the dominance of *C. furcoides* associated with lower levels of trophy in the Igaratá and Atibainha reservoirs, which are part of the Cantareira System. In this case, it was suggested that changes in the reservoir level during the atypical drought of 2013-

2015 might also have affected the structure of phytoplankton in that environment.

The S1 codon, represented by Cyanobacteria species, showed a positive correlation with Chl-*a*, while its position in the CCA arrangement was opposite to the euphotic zone variable, corroborating its occurrence in habitats with high turbidity and its tolerance of low nutrient availability (Reynolds et al., 2002; Padisák et al., 2009). The F codon, represented by colonial chlorophytes, was mainly correlated with the oxidative-reductive potential (Eh) in the CCA. In the case of the S1 group, the consequences may be worsened by the fact that this codon constitutes a group of potentially toxic Cyanobacteria, as with Sn and H1 (O'Farrell et al., 2015).

In all the samples, there was co-occurrence of the Sn and H1 groups. The alternation observed between the dominance of these codons was related to the spatial heterogeneity of the reservoir, with no pattern according to the period of the year. Figueredo & Giani (2009) commented that the absence of correlation between the biomass of dominant Cyanobacteria and the availability of phosphorus may suggest the influence of other environmental variables, or synergism between variables that favor the constant dominance of *R. raciborskii*.

The dominance of Cyanobacteria has been found to be common in several Brazilian reservoirs, under different environmental conditions (Fiore et al., 2005; Figueredo & Giani, 2009; Moschini-Carlos et al., 2009; Cunha & Calijuri, 2011b; Machado et al., 2016; Casali et al., 2017; Santos et al., 2018; Rodrigues et al., 2019; Amorim & Moura, 2021; Moraes et al., 2023). *R. raciborskii* (Sn codon) presents adaptations related to low nutrient availability. Therefore, it could be concluded that the abundance of the Sn codon was also driven by the stability of the water column, due to the long residence time of the water in this reservoir. In a study of the temporal and spatial variation of phytoplankton functional groups in a tropical reservoir, Gil-Guarín et al. (2022) showed that the biomass distribution of some functional groups responded to variables associated with the hydrodynamics of the reservoir, especially the light gradient and suspended and dissolved substances. It was also found that a long water residence time was a key factor influencing phytoplankton dynamics.

According to Barbosa et al. (2021), climatic changes could affect water levels and the thermal regime of the Itupararanga reservoir by the end of the 2020s. Hydrodynamic modeling suggests that falling water levels could threaten the multiple uses of the reservoir, especially the supply of drinking water and energy generation. Furthermore, the warming of surface water layers and increase of the number of days with stratification and thermal stability could have negative impacts on water quality, directly affecting the phytoplankton community. Beguelli et al. (2016) highlights the dominance of Cyanobacteria in the Itupararanga reservoir over a period of 20 years, while more recent research by Machado et al. (2021) revealed the dominance of *Raphidiopsis raciborskii* in this reservoir, associated with the presence of saxitoxin, low availability of NO_3^-, and phosphorus limitation. It was suggested that the occurrence of saxitoxin and *R. raciborskii* could become permanent with decrease of the trophic status index (upstream-downstream).

Application of the RFGs methodology provides support in ecological studies and requires knowledge of phytoplankton autecology, since species belonging to the same genus can be allocated to different codons (Padisák et al., 2009). The appropriate use of this approach has furnished important data concerning Brazilian aquatic ecosystems, demonstrating that the management of water resources can benefit from assessments performed using functional groupings (Gemelgo et al., 2009; Cunha & Calijuri, 2011b; Nishimura et al., 2014, 2015; Santos et al., 2018).

CONCLUSIONS

This study of the Itupararanga reservoir showed that the most representative Reynolds functional groups (RFGs), in terms of biomass, were Sn, followed by the H1 and Lo groups.

Longitudinal spatial heterogeneity (upstream-downstream) in the reservoir influenced the formation of different biomass gradients for the functional groups.

The variables that most influenced the composition of phytoplankton in the reservoir were total nitrogen, nitrite, and the N/P ratio, with phosphorus as the limiting nutrient. The H1 group showed a good response to phosphorus and the trophic gradient.

The presence of Cyanobacteria (codons Sn and H1) was driven by the stability of the water column, due to the long residence time of the water in the reservoir.

The use of Reynolds functional groups proved to be an important tool for understanding the seasonal and spatial dynamics of this subtropical reservoir.

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AUTHORS' CONTRIBUTIONS

All authors contributed to this study, as follows: L.M. and V. M.C.: writing original draft, formal analysis. A.R.: formal analysis, funding acquisition. D.F.: sample collection and analysis. M. P.: formal analysis, funding acquisition. V.L.A.: formal analysis. V.M.C.: formal analysis, funding acquisition. All authors commented on previous versions of the manuscript and read and approved the final manuscript.

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