Spatial and temporal variation of the phytoplankton structure in cascading oligotrophic reservoirs of southeast Brazil

Variação espacial e temporal da estrutura fitoplanctônica em reservatórios oligotróficos em cascata do sudeste do Brasil

Ana Margarita Loaiza-Restano, Gisele Carolina Marquardt, Carla Ferragut and Carlos Eduardo de Mattos Bicudo

Abstract: Aim: Structural changes in phytoplankton was evaluated in one ultraoligotrophic and two oligotrophic cascading reservoirs during two climatic periods (austral winter and summer) of 2014. Changes in phytoplankton biomass, species composition, and codons (functional groups) were evaluated along a vertical and a longitudinal profile. Methods: Four sampling sites were selected for the first reservoir in the cascade (Cachoeira do França) and three for each of the other two reservoirs (Cachoeira da Fumaça and Serraria). Samples were collected along a vertical and a longitudinal profile. Species biomass and codons ‘sensu’ Reynolds were identified and expressed in terms of biomass. Results: 18 codons were identified and associated to the reservoirs’ limnological conditions. Composition and biomass of descriptor species changed among seasons. Codon B consisting mainly of Discostella stelligera was the most frequent in all reservoirs in both climatic seasons. Conclusions: Our findings showed that seasonality was the key factor for the species composition and phytoplankton codons changes. However, the cascade effect on phytoplankton structure was shown by the continuous contribution of Ceratium furcoides (codon L_M) during the winter, and of Discostella stelligera (codon B) in both sampling periods in all three reservoirs. Keywords: codon; environmental monitoring; oligotrophic reservoir; phytoplankton; ultraoligotrophic reservoir

Resumo: Objetivo: Mudanças estruturais no fitoplâncton foram avaliadas em três reservatórios em cascata, um ultraoligotrófico e dois oligotróficos, no verão e no inverno de 2014. Avaliou-se a variação da biomassa fitoplanctônica, a composição de espécies e os códons (grupos funcionais) ao longo de um perfil vertical e outro longitudinal. Métodos: Foram selecionados quatro locais de amostragem na primeira represa em cascata (Cachoeira do França) e três em cada uma das demais duas (Cachoeira da Fumaça e Serraria). As amostras foram coletadas ao longo dos perfis vertical e longitudinal das represas. Foram determinados o biovolume das espécies e os códons que foram expressos em termos de biomassa. Resultados: 18 códons foram identificados e associados às condições limnológicas dos reservatórios. A composição e a biomassa das espécies descritoras mudaram conforme as estações do ano. Códon B constituiu principalmente por Discostella stelligera foi o mais frequente nas três represas e em ambos os períodos de amostragem. Conclusões: Os resultados mostraram que a sazonalidade foi um fator importante para as mudanças na composição de espécies e dos códons do fitoplâncton. No entanto, o efeito da cascata sobre a estrutura do fitoplâncton foi demonstrado pela expressiva e constante contribuição de Ceratium furcoides (côdon L_M) no inverno e de Discostella stelligera (côdon B) em ambos os períodos de amostragem nas três represas estudadas. Palavras-chave: côdon; fitoplâncton; monitoramento ambiental; reservatório oligotrófico; reservatório ultraoligotrófico.
1. Introduction

Reservoirs are usually considered synonymous with lakes, however, magnitude and phasing of the forcing functions or driving variables for lakes and reservoirs may be not identical (Thornton et al., 1990). A strong interaction with the hydrographic basin, the multiple uses and the functioning and operating mechanisms make reservoirs complex systems (Tundisi et al., 1999). Cascading reservoirs were first built in sizeable countries with large rivers to provide electric energy, irrigation, and drinking water. Their usual water abundance tends to decrease along the cascade (Straškraba, 1994). The great majority of reservoirs in Brazil are concentrated in the south and southeast regions (Tundisi, 1983), and several rivers were already organized in cascades. Cascading reservoirs are considered complex systems since the first reservoir may affect downriver until the very last one in the series, a fact amplified by the tributary rivers action (Nogueira et al., 2010). Effects and cumulative impacts could completely change the drainage basin biophysical, economic and social features (Tundisi et al., 1999; Nogueira et al., 2006). Such a configuration endorsed the concept of “continuum cascading reservoirs” that, from the ecological point of view states that reservoirs placed in a longitudinal sequence would have a significant interdependence, despite the river’s course interruption would affect the structure and functioning of the aquatic communities (Barbosa et al., 1999; Ferreira et al., 2005).

Composition and abundance of phytoplankton vary spatially and temporally in cascading reservoirs of different rivers (Bonilla, 1997; Padisák et al., 2000; Silva et al., 2005; Saldana-Fabela et al., 2014). In cascading reservoirs, phytoplankton structure may be affected by limnological factors such as nutrient availability and hydrodynamic changes. According to Saldana-Fabela et al. (2014), diatoms occur in places where connectivity is present, whereas there is a clear dominance of Cyanobacteria and chlorophyceans in the non-connected reservoirs related to nutrient enrichment and sewage discharge. In addition, environmental heterogeneity and rain precipitation are likely to impact phytoplankton density and richness, and codons in cascading reservoirs (Moura et al., 2013). Phytoplankton development takes place when the system water retention time is greater if compared to that of lakes, as opposed to its disappearance when water retention time is similar to that of the river (Riera & Morguí, 1990; Roldán, 1992), and its organization follows a vertical axis established by light attenuation, thermal gradient and algal adaptations (Reynolds, 1997; Wetzel, 2001; Mellard et al., 2011). Exploring such communities will help to understand the aquatic environmental changes, since they are considered fast water quality environmental sensors (Gemelgo et al., 2009) due to their intrinsic characteristics, as for instance their short-term generation time.

In Brazil, ecological research has successfully used phytoplankton as an indicator of changes in the environment quality of cascading reservoirs in the Tietê River Basin (Barbosa et al., 1999; Padisák et al., 2000; Silva et al., 2005; Ferrareze & Nogueira, 2006). Besides changes in species composition, studies showed that the use of codons ‘sensu’ Reynolds et al. (2002) may be very useful and effective for the evaluation of aquatic environmental changes in cascading reservoirs (Nogueira et al., 2010; Santana et al., 2017). Current assessment of different ecological assemblies in a series of cascading reservoirs emphasizes that the physical and chemical features of water are not the only causes for changes in the communities structure, given that connectivity may have a stronger effect (Santos et al., 2018). In fact, phytoplankton structure may change depending on the limnological and hydrodynamic conditions of cascading reservoirs (e.g. Padisák et al., 2000; Silva et al., 2005). Concerning the three reservoirs in the present study, Marquardt et al. (2018) reported that hydrological connectivity predictor proved to play an utmost role toward explaining the diatom metacommunity dynamics. In this sense, structural changes in phytoplankton was evaluated in three cascading reservoirs, one ultraoligotrophic and two oligotrophic, in two seasons (summer and winter). Changes in phytoplankton biomass, species composition and codons were evaluated along a vertical and a longitudinal profile. Our present main question was whether the taxonomic structure and the codons could be linked to the environmental conditions of each reservoir, or connectivity could have a homogenizing effect on the community.

2. Material and Methods

2.1. Study area

This study was carried out in three interconnected reservoirs of a cascading series, namely Cachoeira do França, Cachoeira da Fumaça and Serraria, located across the Juquiá Guaçu River, Ribeira do Iguape/Southern Litoral Basin, in the state of São Paulo (Figure 1). Cachoeira do França and Cachoeira...
da Fumaça are connected to Serraria forming a series of reservoirs across the Juquiá Guaçu River. Ribeira do Iguape/Southern Litoral Basin has a drainage area of 17,068 km² (SAO PAULO, 2006). Cachoeira do França (23°54′50.81″S, 47°15′31.98″W and 23°56′0.26″S, 47°09′42.99″W) and Cachoeira da Fumaça (23°59′27.79″S, 47°15′59.71″W and 24°00′50.43″S, 47°13′58.85″W) reservoirs border the Jurupará State Park, whereas Serraria (24°06′26.05″S, 47°34′4.14″W and 24°06′55.77″S, 47°31′9.3″W) lies in the Serra do Mar State Park. The three reservoirs are located in secondary Atlantic forest conservation areas. Climate of the region has two very well defined seasons, one warm and rainy (October to March) and the other one cold and dry (April to September) (CBH-RB, 2014). Information on morphometry, trophic state and hydrological characteristics of reservoirs are in Table 1.

2.2. Samplings

Water and phytoplankton samplings were performed during the winter (June and September 2014) and summer (January and February 2014). On the horizontal scale, samples were obtained from four sampling sites in the Cachoeira do França reservoir, and three each in the Cachoeira da Fumaça and Serraria reservoirs. Sampling sites were distributed upstream, central area of reservoir and close to the dam (reservoirs deepest region), thus prioritizing the tributaries entrances. In each sampling site, water column samples were obtained from the subsurface, middle, and bottom strata considering the column mixing and light regime, the surface being the euphotic zone identified by the Secchi disk transparency, middle (interphase), and 1 m above the bottom of reservoir, the aphotic zone.

Table 1. Main limnological characteristics of the connected reservoirs Cachoeira do França, Cachoeira da Fumaça and Serraria.

| Reservoir             | Construction (year) | Area (km²) | Volume (10⁶ m³) | Main reservoir use | TSI* (annual mean) | WRT* (days) (annual mean) |
|-----------------------|---------------------|------------|----------------|-------------------|--------------------|--------------------------|
| Cachoeira do França   | 1954                | 12.7       | 135.2          | Power generation  | Oligotrophic       | 76.4                     |
| Cachoeira da Fumaça   | 1964                | 6.92       | 53.5           | Power generation  | Ultraoligotrophic  | 42.5                     |
| Serraria              | 1978                | 2.2        | 37.6           | Power generation  | Oligotrophic       | 10                       |

*Abbreviations: TSI (Trophic State Index) and WRT (Water Retention Time).
2.3. Climate, hydrological and abiotic variables

Rainfall, air temperature and water flow rate data were obtained from the CBA, Brazilian Aluminum Company. Maximum depth ($Z_{\text{max}}$) was measured with a portable depth device. Water temperature, pH, turbidity and electric conductivity were measured 'in situ' with a multiparameter probe (Horiba U-53). Water transparency was obtained with a Secchi disk and the euphotic zone ($Z_{\text{eu}}$) according to Cole (1983). Mixing zone ($Z_{\text{mix}}$) was identified by the vertical temperature profile. To assess light availability, $Z_{\text{eu}}/Z_{\text{max}}$ and $Z_{\text{eu}}/Z_{\text{mix}}$ ratios were calculated.

The following variables were also measured: dissolved oxygen (DO) (Golterman et al., 1978), HCO$_3^-$, free CO$_2$, nitrite (NO$_2^-$-N) and nitrate (NO$_3^-$-N) (Mackereth et al., 1978), ammonium (NH$_4^+$-N) (Solorzano, 1969), soluble reactive phosphorus (PO$_4^{3-}$-P) (Strickland & Parsons, 1960), total phosphorus (TP) and total nitrogen (TN) (Valderrama, 1981), and soluble reactive silica (SRS) (Golterman et al., 1978). Water samples for identification of dissolved nutrients were filtered under low pressure using glass-fiber filters (GF/F Whatman). Chlorophyll-a corrected for phaeophytin was measured within at most one week from the sampling day, with 90% ethanol as the organic solvent (Sartory & Grobbelaar, 1984). Trophic State Index (TSI) was calculated based on the subsurface chlorophyll-a concentration and total phosphorus value (Lamparelli, 2004).

2.4. Biological variables

Phytoplankton samples were collected with a van Dorn bottle and fixed with 1% lugol acetic solution for the quantitative analyses. Cyanobacteria and algae were quantified using an inverted Zeiss Axio Observer D1 microscope at 400x, following Utermöhl (1958). The counting limit was determined by the species rarefaction curve. Algal biovolume was estimated by multiplying each species' density by the mean volume of its representative individuals, and whenever possible measuring the cell or coenobium dimensions of at least 10 specimens of each species according to its geometric formulae following Hillebrand et al. (1999) and Napiórkowska-Krzebietke & Kobos (2016). Biovolume was expressed as biomass (mg L$^{-1}$). Species were classified into codons 'sense' Reynolds (Reynolds et al., 2002; Padişak et al., 2009). Dominant species were selected upon 50% of the relative abundance in each sample.

2.5. Data treatment

Permutational multivariate analysis of variance (two-way PERMANOVA; $\alpha = 0.05$) was used to analyze the influence of seasonality (Past 4.02, Hammer et al. 2001). Redundancy Analysis (RDA) was used to evaluate the relationship between environmental variables, phytoplankton descriptive species ($\geq 5\%$ of total algal biomass) and codons. Environmental variables were chosen based on principal component analysis (PCA). Logarithm-transformed biological and abiotic data were used, except for pH. A randomization test was carried out to determine interpretable axes ($p < 0.05$). RDA and PCA analysis were run using PC-ORD 6.0 program (McCune & Mefford, 2011).

3. Results

3.1. Climate, abiotic variables and TSI

During the summer, precipitation ranged from 150 mm (Cachoeira do França) to 521.5 mm (Serraria), the highest values registered in February at all reservoirs’ sites. Mean air temperature for the period ranged from 20-38ºC (Serraria) to 16-46ºC (Cachoeira do França). During the winter, precipitation ranged from 49.5 mm to 144.25 mm (Cachoeira da Fumaça). Mean air temperature values ranged from 6-36ºC (Cachoeira do França) to 8-27ºC (Serraria).

Limnological data are shown in Table 2. During the summer, Cachoeira do França reservoir had neutral to alkaline pH, whereas Cachoeira da Fumaça and Serraria reservoirs had acidic to slightly alkaline pH. PO$_4^{3-}$-P concentrations dropped usually below the method detection limit in most sampling sites. NH$_4^+$-N, TN, TP, free CO$_2$ and NO$_3^-$-N concentrations, as well as electric conductivity vertical distribution pattern in the water column were more homogeneously distributed at the lower depths of reservoirs, especially during the summer, unlike the dissolved oxygen whose highest concentrations were detected near the reservoirs' subsurface. Secchi disk depth was greater during the winter than in the summer, especially at Serraria reservoir that also showed the lowest turbidity values. In the three reservoirs, the highest depth of the mixing ($Z_{\text{mix}}$) and euphotic ($Z_{\text{eu}}$) zones were found during the winter. $Z_{\text{eu}}/Z_{\text{mix}} > 1$ was detected during the summer and winter in the studied reservoirs.
3.2. Phytoplankton

Comparatively, the largest phytoplankton biomass was found in the upstream reservoir Cachoeira do França (Figure 2). The highest total phytoplankton biomass was found during the winter in the three studied reservoirs. During the summer, all three reservoirs showed greater biomass at their subsurface and middle layers, except for Cachoeira do França that site 2 bottom layer presented the greatest value.

Based on the descriptive species, no significant differences were found in the phytoplankton structure among reservoirs, but among seasons (two-way PERMANOVA: $F = 5.06$, $p = 0.0002$). The most constant species with higher relative biomass in all three reservoirs and all three layers of each reservoir were *Discostella stelligera* (Cleve & Grunow) Houk & Klee and *Aphanocapsa delicatissima* W. West & G.S. West with lower relative biomass (Figure 3). *Ceratium furcoides* (Levander) Langhans was more present during the winter in the entire water column, and *Cosmarium tinctum* Ralfs was exclusively found at the bottom layer of Cachoeira do França reservoir. Considering phytoplankton structure of each reservoir, based on biomass significant difference was found only between seasons (two-way PERMANOVA: França,

Table 2. Mean values of limnological variables and the standard deviation, in parenthesis, in Cachoeira do França, Cachoeira da Fumaça and Serraria reservoirs during summer and winter. Modified from Marquardt et al. (2018).

| Variable             | Cachoeira do França | Cachoeira da Fumaça | Serraria |
|----------------------|---------------------|----------------------|---------|
|                      | Summer | Winter | Summer | Winter | Summer | Winter |
| Chlorophyll-a (µg L⁻¹) | 1.8 (±1.1) | 10.4 (±14.7) | 1.5 (±1.4) | 1.5 (±1.1) | 2.1 (±2.6) | 2.1 (±2.4) |
| Conductivity (µS cm⁻¹) | 27.6 (±10.1) | 26.0 (±1.4) | 26.7 (±2) | 27.2 (±0.7) | 29.6 (±11.9) | 27.9 (±1.3) |
| DO (mg L⁻¹)           | 5.4 (±1.9) | 6.7 (±1.4) | 4.9 (±2.3) | 7.0 (±1.1) | 4.8 (±2.4) | 7.3 (±1.7) |
| Free CO₂ (mg L⁻¹)     | 0.5 (±0.9) | 10.1 (±13) | 26.8 (±42.2) | 30.0 (±30.1) | 31.5 (±37.9) | 27.0 (±44.7) |
| HCO₃⁻ (mg L⁻¹)        | 11.4 (±2.2) | 10.5 (±0.6) | 10.7 (±2.2) | 10.6 (±0.3) | 11.7 (±3.7) | 14.1 (±1.4) |
| NH₄⁺-N (µg L⁻¹)       | 33.7 (±24.0) | 27.2 (±14.8) | 73.7 (±57.9) | 23.7 (±16) | 22.3 (±21.4) | 15.9 (±17.8) |
| NO₃⁻-N (µg L⁻¹)       | 20.2 (±20.2) | 31.8 (±32.7) | 59.9 (±50.9) | 85.2 (±10.9) | 109.1 (±92.7) | 138.1 (±50.4) |
| pH                   | 7.6 (±0.5) | 6.5 (±0.9) | 6.0 (±0.6) | 6.0 (±0.4) | 6.0 (±0.8) | 6.3 (±0.9) |
| PO₄³⁻-P (µg L⁻¹)      | 5.3 (±1.0) | 4.1 (±0.3) | <4.0 | <4.0 | 5.2 (±3.7) | <4.0 |
| Turbidity (NTU)       | 15.5 (±13.4) | 15.2 (±27.2) | 10.7 (±9.3) | 4.1 (±2.5) | 4.8 (±8.5) | 2.5 (±2.9) |
| Secchi Disk (m)       | 1.7 (±0.1) | 1.8 (±0.2) | 1.7 (±0.2) | 3.4 (±0.7) | 2.9 (±0.2) | 5.0 (±0.3) |
| Silicate (mg L⁻¹)     | 1.9 (±0.6) | 3.5 (±0.3) | 1.5 (±0.3) | 3.2 (±0.1) | 2.0 (±0.9) | 4.8 (±0.5) |
| Total Phosphorus (µg L⁻¹) | 14.0 (±6.4) | 4.8 (±1.1) | 6.8 (±1.5) | 4.6 (±1.1) | 5.6 (±1.7) | 10.1 (±1.8) |
| Total Nitrogen (µg L⁻¹) | 307.2 (±176) | 258 (±65.1) | 407.1 (±138.6) | 136 (±23) | 186.7 (±192.3) | 243.1 (±47.5) |
| Temperature (°C)      | 25.6 (±3.0) | 16.9 (±12) | 25.2 (±3.6) | 18.0 (±1.4) | 24.7 (±5.1) | 19.2 (±1.5) |
| $Z_{eu}/Z_{max}$      | 0.34 (±0.09) | 0.41 (±0.1) | 0.20 (±0.06) | 0.39 (±0.05) | 0.26 (±0.4) | 0.56 (±0.1) |
| $Z_{eu}/Z_{mix}$      | 1.4 (±0.08) | 1.3 (±0.7) | 1.8 (±0.6) | 1.7 (±2.3) | 4.8 (±2.1) | 9.1 (±7.5) |
| $Z_{eu}$              | 4.6 (±0.1) | 4.9 (±0.7) | 4.4 (±0.7) | 9.0 (±2.1) | 7.8 (±0.6) | 13.5 (±0.8) |
| $Z_{max}$             | 13.6 (±2.1) | 13.7 (±2.0) | 27.0 (±6.1) | 29.3 (±6.5) | 30.0 (±6.6) | 26.9 (±5.8) |
| $Z_{mix}$             | 3.2 (±0.2) | 5.2 (±3.9) | 2.8 (±1.1) | 18.0 (±0.7) | 1.8 (±0.7) | 10.0 (±16) |

Figure 2. Phytoplankton total biomass (mg L⁻¹) in three strata (S subsurface, M middle, B bottom layers) and sampling sites (1, 2, 3, 4) during the winter and summer at the three cascading reservoirs.
F = 3.50, \( p = 0.0011 \); Fumaça, F = 6.62, \( p = 0.0001 \); Serraria: F = 2.25, \( p = 0.031 \).

Codon B was practically found throughout the three reservoirs and at all three strata in both seasons, but presented lower relative biomass during the winter and towards the sampling station FR4 of Cachoeira do França reservoir, which is the closest site to the entry of the Juquiá Guaçu river (Figure 4). On the contrary, codon LM presented the higher relative abundance at this site and both seasons. Codon LM was more present during the winter at the subsurface and medium layers in the Serraria reservoir, basically due to *Ceratium furcoides*. It was observed that during the stratification period (summer), the bottom layer showed dominance of one codon, whereas at both the subsurface and intermediate layers several codons dominated. On the other hand, during mixing period (winter) one codon tended to dominate the whole water column, mainly *Ceratium furcoides*.

RDA was performed using 37 phytoplankton species biomass and five environment variables (Figure 5, Table 3). Monte Carlo permutation test was significant for both axes 1 and 2 (\( p = 0.001 \)). Axes 1 (\( \lambda = 4.71 \)) and 2 (\( \lambda = 2.31 \)) eigenvalues explained 18.9% of the total variability of data in both axes. Pearson Correlation Coefficient (PCC) species-environment for axes 1 (0.850) and 2 (0.825) indicated a strong relationship.
Table 3. Descriptive species used to study community structure in the three reservoirs studied.

| Species                                | C  | Code | Axis 1 | Axis 2 |
|----------------------------------------|----|------|--------|--------|
| Aulacoseira tenella (Nygaard) Simonsen  | A  | Aten | 0.270  | -0.235 |
| Urosolenia eriensis (H.L.Smith) Round & R.M.Crawford | A  | Ueri | 0.562  | 0.034  |
| Discostella stelligera (Cleve & Grunow) Houk & Klee | B  | Dste | 0.271  | 0.414  |
| Fragilaria billingsii Wengrat, C.E.Wetzel & Morales | B  | Fbil | 0.608  | 0.068  |
| Bicosoeca petiolata (F.Stein) E.G.Pringsheim | E  | Bico | -0.141 | -0.141 |
| Mallomonas fresenii Kent                | E  | Mfre | 0.065  | -0.057 |
| Botryococcus braunii Kützing            | F  | Bbra | -0.384 | -0.362 |
| Keratococcus obtusus (Koršikov) Hindák  | F  | Kobt | -0.180 | 0.487  |
| Oocystis lacustris Chodat               | F  | Olac | 0.002  | 0.240  |
| Radiococcus planktonicus J.W.G.Lund    | F  | Rpla | -0.114 | 0.659  |
| Dolichospermum plantonicum (Brunnthalm) Wacklin et al. | H1 | Dpla | 0.375  | -0.017 |
| Hapalodiscus reticulata P.A.Dangeard   | J  | Hret | 0.035  | 0.163  |
| Tetraedriella jovetti (Bourrelly) Bourrelly | J  | Tjov | 0.532  | 0.110  |
| Aphanocapsa delicatissima W.West & G.S.West | K  | Egle | -0.277 | 0.320  |
| Epiploeoosphaera glebuleenta (Zalessky) Komárková-Legnerová | K  | Adel | -0.262 | 0.435  |
| Ceratium furcoides (Levander) Langhans | L  | Cfur | 0.242  | -0.054 |
| Microcystis aeruginosa (Kützing) Kützing | L  | Maer | -0.080 | 0.533  |
| Gymnodinium fuscum (Eyehnberg) Stein    | L  | Pinc | 0.389  | 0.040  |
| Parvovinum inconspicuum Lemmermann      | L  | Gfus | 0.152  | -0.074 |
| Cosmarium majae Strøm                   | N  | Cmaj | 0.454  | 0.130  |
| Cosmarium tinctum Rafs                  | N  | Ctin | -0.140 | 0.030  |
| Staurodesmus ralfsii (West) Tomaszewicz | N  | Staur | 0.059 | -0.057 |
| Eunotia waiminorum C.E.Wetzel           | N  | Ewai | 0.708  | 0.112  |
| Closterium acutum Brébiisson var variabile (Lemmermann) Willi Krieger | P  | Cacu | 0.751  | -0.143 |
| Stauroastrum tetracerum Rafs ex Rafs     | P  | Stet | 0.479  | 0.117  |

C: codon ‘sensu’ Reynolds, Code: 4 letters for species recognition.
between species distribution and environment variables. Most Cachoeira do França and Cachoeira da Fumaça scores during the summer and Serraria in both seasons were located on the negative side of axis 1. These sample units were correlated with the highest temperature ($r = -0.7$) and TP ($r = -0.6$) values. The opposite was observed during the winter at Cachoeira do França and Cachoeira da Fumaça reservoirs. Such ordination showed the temporal change in the phytoplankton taxonomic structure at both Cachoeira do França and Cachoeira da Fumaça reservoirs, but seasonality had a lower weight at the Serraria reservoir. Several species were associated with the winter environmental conditions at Cachoeira do França and Cachoeira da Fumaça reservoirs, mainly *Eunotia waimiriorum* Wetzel, *Closterium acutum* Brébisson var. *variabile* (Lemmermann) Krieger, and *Raphidiopsis raciborskii* (Woloszyńska) Aguilera et al. ($r > 0.7$).

RDA was performed using biomass of 18 codons and four environmental variables (Figure 6). Monte Carlo permutation test was significant for both axes 1 and 2 ($p = 0.001$). Eigenvalues of axes 1 ($\lambda = 3.08$) and 2 ($\lambda = 1.23$) explained 23.9%
of both axes total data set variability. Temperature and TP (r ≥ 0.7) were the highest weight variables in the ordination. Most Cachoeira do França and Cachoeira da Fumaça reservoir’s summer scores and those for both seasons at Serraria reservoir were ordered on the negative side of axis 1. However, Cachoeira do França and Cachoeira da Fumaça reservoirs were ordered in the winter on the positive side of axis 1 (r ≥ 0.66). Codons A, P, S_N and MP were associated with lower temperatures and TP in winter in both Cachoeira do França and Cachoeira da Fumaça reservoirs (r ≥ 0.66).

4. Discussion

Our findings showed little change in the phytoplankton structure among cascading reservoirs, the highest changes being detected among climatic seasons. The most abundant descriptive species Discostella stelligera and Ceratium furcoides, and codons B and L_M were predominant in all reservoirs. In addition, phytoplankton total biomass was not cumulative along the cascading reservoirs. In relation to the temporal scale, climatic periods were typical for the region with a rainy summer and high temperatures, the opposite occurring during the winter. According to the Trophic State Index, sampling sites ranged from ultraoligotrophic to oligotrophic. In a previous study, Marquardt et al. (2018) showed that hydrological connectivity played an utmost role in the dispersion processes of the community structure, playing an important part in explaining the variation of the diatom metacommunities. Therefore, our results suggest that connectivity was the determinant factor for phytoplankton structure during the winter and summer, consequently reinforcing the system spatial homogeneity.

Descriptive species did not change drastically with cascading both in the vertical and the longitudinal profiles, as well as among reservoirs. Descriptive species changed, however, among seasons. Thus, seasonality influenced the phytoplankton structure in the ultraoligotrophic and the two oligotrophic reservoirs. Influence of seasonality on phytoplankton structure was clearly evident in the first two cascading reservoirs (Cachoeira do França and Cachoeira da Fumaça), but it was not very noticeable in the third reservoir (Serraria). The most evident feature of Serraria reservoir was that during the summer, the subsurface layer behaved differently than the rest of the water column, when it was more affected by the high transparency, codon F being more associated to this condition. Consequently, although some studies have shown that changes in downstream reservoirs may be predicted from characteristics of changes upstream (Armengol, 1977; Straškraba, 1994), our findings show that other factors may also affect phytoplankton structure. For example, in cascading reservoir systems phytoplankton structure was noticed to be highly affected by hydrodynamic changes (Padisák et al., 2000; Silva et al., 2005).

In the vertical profile, the relative biomass of Ceratium furcoides was usually greater at the subsurface and intermediate layers during the winter, and its greatest relative biomass was detected at the Cachoeira do França reservoir. Ceratium furcoides was already recorded for several reservoirs in Brazil (Santos-Wisniewski et al., 2007; Matsumura-Tundisi et al., 2010; Crossetti et al., 2019) as well as in cascading lotic systems of the Paranapanema River Basin (Ferrareze & Nogueira, 2006). Currently, Ceratium furcoides is becoming a problem for the water quality of reservoirs in South America, and high temperature seems to promote its invasion (Meichtry-de-Zaburlin et al., 2016). Ceratium species was found throughout the year even at low densities in tropical reservoirs (Silva et al., 2012), though other studies reported winter dominance of Ceratium hirundinella (Müller) Dujardin in a temperate reservoir (Pérez-Martínez & Sánchez-Castillo, 2002). In a eutrophic tropical reservoir, establishment of Ceratium furcoides was associated with disturbance events, which changed the water column stability and transparency (Crossetti et al., 2019). Present study showed that cascading reservoir oligotrophic conditions favor dominance of Ceratium furcoides, especially during the winter, which is a warning sign. Ceratium furcoides is an invasive species in South American freshwater ecosystems, and its bloom can be harmful to several organisms due to oxygen depletion, besides causing serious problems in water treatment (Matsumura-Tundisi et al., 2010; Meichtry-de-Zaburlin et al., 2016).

Another species with high biomass in the present reservoirs was Discostella stelligera that showed greater total biomass at the subsurface and intermediate layers during the winter, probably because reservoirs were not stratified on the sampling day. In fact, depth of Cachoeira do França during the winter was lower than those of Cachoeira da Fumaça and Serraria reservoirs. Discostella stelligera, a common species in freshwater plankton communities (Spaulding & Edlund, 2009), like many other diatoms depends on the
turbulence to maintain its buoyancy in the upper layers of the water column. Besides being commonly present in tropical reservoirs, *Discostella stelligera* is also found in surface sediments and plankton (Zorzal-Almeida et al., 2017).

Considering the codons ‘sensu’ Reynolds, codons MP, P, and S₈, altogether reflected the conditions prevalent at the sampling sites of Cachoeira do França and Fumaça reservoirs during the winter due to mixing, with low TN, TP, temperature and transparency values. Codon MP, mainly *Eunotia wairimiorum* is a colonial planktonic diatom associated with shallow turbid waters, a typical species of the oligotrophic-pristine-high-sediment stage of Branco River in the Brazilian Amazon (Wetzel et al., 2010). In the current study, presence of that species matched with low values of transparency during the winter present in all sampling sites at Cachoeira do França reservoir, probably due to the turbulence induced by the entrance of tributary rivers, since this reservoir is shallower than the other two.

Codon S₈ represented by nitrogen fixing Cyanobacteria is sensitive to flushing and is typical from warm mixed environments. According to Soares et al. (2013), *Cylindropermopsis raciborskii* (=Raphidiopsis raciborskii) is a species related to systems with annual mixing. In the present study, one of the characteristics exhibited in the winter by Cachoeira do França and Cachoeira da Fumaça reservoirs was the thermal mixing, which implies that mixing is an important feature irrespective of the season. This process qualified typical co-dominance pointed out by Soares et al. (2013) of colonial Chroococcales with filamentous N₂-fixing Nostocales, codons H₁, S₈, and L₉ (partially *Microcystis aeruginosa* (Kützing) Kützing) during the winter season in these reservoirs.

Codon P, likewise, is typical of continuous or semi-continuous mixed layer of 2-3 m in thickness in higher trophic state reservoirs, which is another feature of Cachoeira do França in the winter, being eutrophic in the sub-surface layer, mainly at site 4. This sampling site is the corridor of the Juquiá Guaçu River. Although annual monitoring by Brazilian water agencies report the Juquiá Guaçu River as having good water quality, it seems that there are obscure sources of allochthonous material that could be the cause of the increased trophy in this sampling site, thus increasing P and N and development of several species, in the same way as for *Ceratium furcoides* (L₉).

5. Conclusions

While E, F, K, S₁, X₂ and Y were considered the summer codons, the winter codons were made up of A, B, H₁, J, L₀, MP, N₈, P, and W₂. It was evident that seasonality played a major influence in the codons’ composition in two reservoirs, Cachoeira do França and Cachoeira da Fumaça, and that Serraria reservoir could be influenced by other factors like hydrodynamic changes. We therefore conclude that seasonality was the determinant factor of the phytoplankton structure, and that connectivity acted as a homogenizing factor, mainly due to the continuous contribution of *Ceratium furcoides* (codon L₉) and *Discostella stelligera* (codon B) at all reservoirs studied.

Acknowledgements

We acknowledge CNPQ, Conselho Nacional de Desenvolvimento Científico e Tecnológico for a doctoral fellowship granted to AMLR (Process nº 155376/2014-4). This study was carried out within the framework of the AcquaSed project (Baseline diagnosis and reconstruction of anthropogenic impacts in the Guarapiranga Reservoir, focusing on sustainability in water supply and water quality management in reservoirs of the Upper Tietê and surrounding basins) financially supported by FAPESP, Fundação de Amparo à Pesquisa do Estado de São Paulo (Project nº 2009/53898-9). We also thank all students and technicians involved in the field and laboratory work at the Instituto de Botânica’s Ecology Department. We deeply appreciate the valuable assistance of the Votorantim Energia staff for the logistic support during the fieldwork. We are finally grateful to Prof. William de Queiróz (Geoprocessing Laboratory, Universidade de Guarulhos) for the study area illustration.

References

ARMENGOL, J. Tipología de los embalses españoles. In: *Seminario sobre medio ambiente y represas*. Barcelona: Universitat de Barcelona, 1977, vol. 1, pp. 3-13.

BARBOSA, F.A.R., PADISÁK, J., ESPÍNDOLA, E.L.G., BORICS G. and ROCHA, O. The cascading reservoir continuum concept (CRCC) and its application to the River Tietê-Basin, São Paulo State, Brazil. In: J.G. TUNDISI and M. STRASKRABA, eds. *Theoretical reservoir ecology and its applications*. Leiden: Backhuys Publishers, 1999, pp. 425-437.

BONILLA, S. *Composición y abundancia fitoplanctónicas de tres embalses en cadena sobre el río Negro, Uruguay*. Iheringia. *Série Botânica*, 1997, 49, 47-61.
Spatial and temporal variation…

COLE, G. *Textbook of limnology*. London: The C.V. Mosby Co, 1983.

COMITÊ DO RIBEIRA DE IGUAPE E LITORAL SUL – CBH-RB. *Elaboração do plano de bacias da UGRHI II*. Registro: CBH-RB, 2014, Relatório 2. Projeto FEHIDRO 2014-RB-278.

CROSSETTI, L.O., BICUDO, D.C., BINI, L.M., DALA-CORTE, R.B., FERRAGUT, C. and BICUDO, C.E.M. Phytoplankton species interactions and invasion by *Ceratium furcoides* are influenced by extreme drought and water-hyacinth removal in a shallow tropical reservoir. *Hydrobiologia*, 2019, 831(1), 71-85. http://dx.doi.org/10.1007/s10750-018-3607-y.

FERRAREZE, M. and NOGUEIRA, M.G. Phytoplankton assemblages in lotic systems of the Paranapanema Basin (Southeast Brazil). *Acta Limnológica Brasiliensis*, 2006, 18(4), 389-405.

FERREIRA, R.A.R., SANTOS, C.M. and HENRY, R. Estudo qualitativo da comunidade perifítica no complexo Canoas (Rio Paranapanema, SP/PR) durante as fases de pré e pós enchimento. In: M.G. NOGUEIRA, R. HENRY and A. JORCIN, orgs. *Ecoologia de represas: impactos potenciais, ações de manejo e sistemas em cascata*. São Carlos: RiMa Editora, 2005, pp. 205-234.

GEMELGO, M.C.P., MUCCI, J.L.N. and NAVAS-PEREIRA, D. Population dynamics: seasonal variation of phytoplankton functional codons in Brazilian reservoirs (Billings and Guarapiranga, São Paulo). *Brazilian Journal of Biology = Revista Brasileira de Biologia*, 2009, 69(4), 1001-1013. http://dx.doi.org/10.1590/S1519-69842009000500004. PMid:19967171.

GOLTERMAN, H.L., CLYMO, R.S. and OHMSTAD, M.A.M. *Methods for physical and chemical analysis of fresh waters*. Oxford: International Biological Programme, Blackwell Scientific Publications, 1978.

HAMMER, O., HARPER, D.A.T. and RYAN, P.D. PAST: paleontological statistics software package for education and data analysis. *Palaeontology Electronica*, 2001, 4, 1-9.

HILLEBRAND, H., DÜRSELEN, C.-D., KIRCHTEL, D., POLLINGER, U. and ZOHARY, T. Biovolume calculation for pelagic and benthic microalgae. *Journal of Phycology*, 1999, 35(2), 403-424. http://dx.doi.org/10.1046/j.1529-8817.1999.3520403.x.

LAMPARELLI, M.C. *Graus de trofa em corpos d’agua do Estado de São Paulo: avaliação dos métodos de monitoramento* [Tese de Doutorado em Ciências na Área de Ecossistemas Terrestres e Aquáticos ]. São Paulo: Universidade de São Paulo, 2004.

MACKERETH, F.J.H., HERON, J. and TALLING, J.F. Water analysis: some revised methods for limnologists. *Scientific Publication - Freshwater Biological Association*, 1978.

MARQUARDT, G.C., PADIAL, A.A. and BICUDO, C.E.M. Variance partitioning of deconstructed tropical diatom communities in reservoirs cascade. *Aquatic Sciences*, 2018, 80(2), 17. http://dx.doi.org/10.1007/s0027-018-0571-6.

MATSUMURA-TUNDISI, T., TUNDISI, J.G., LUZIA, A.P. and DEGANI, R.M. Occurrence of *Ceratium furcoides* (Levander) Langhans 1925 bloom at the Billings Reservoir, São Paulo State, Brazil. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, 2010, 70(3), 825-829. Supplement. http://dx.doi.org/10.1590/S1519-69842010000400013. PMid:21085787.

MCCUNE, B. and MEFFORD, M.J. *PC-ORD: multivariate analysis of ecological data*. Version 6. MJM Software. Oregon: Gleneden Beach, 2011.

MEICHTRY DE ZABURLÍN, N., VOGLER, R.E., MOLINA, M.J. and LLANO, V.M. Potential distribution of the invasive freshwater dinoflagellate *Ceratium furcoides* (Levander) Langhans (Dinophyta) in South America. *Journal of Phycology*, 2016, 52(2), 200-208. http://dx.doi.org/10.1111/jpy.12382. PMid:27037585.

MELLARD, J.P., YOSHIYAMA, K., LITCHMAN, E. and KLAUSMEIER, C.A. The vertical distribution of phytoplankton in stratified water columns. *Journal of Theoretical Biology*, 2011, 269(1), 16-30. http://dx.doi.org/10.1016/j.jtbi.2010.09.041. PMid:20932846.

MOURA, A.N., SEVERIANO, J.S., TAVARES, N.K.A. and DANTAS, E.W. The role of a cascade of reservoirs and seasonal variation in the phytoplankton structure in a tropical river. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, 2013, 73(2), 291-298. http://dx.doi.org/10.1590/S1519-69842013000200009. PMid:23917556.

NAPIÓRKOWSKA-KRZEBIETEK, A. and KOBOS, J. Assessment of the cell biovolume of phytoplankton widespread in coastal and inland water bodies. *Water Research*, 2016, 104, 532-546. http://dx.doi.org/10.1016/j.watres.2016.08.016. PMid:27611216.

NOGUEIRA, M.G., FERRAREZE, M., MOREIRA, M.L. and GOUVÉA, R.M. Phytoplankton assemblages in a reservoir cascade of a large tropical-subsropical river (SE, Brazil). *Brazilian Journal of Biology = Revista Brasileira de Biologia*, 2010, 70(3), 781-793. Supplement. http://dx.doi.org/10.1590/S1519-69842010000400009. PMid:21085783.

NOGUEIRA, M.G., JORCIN, A., VIANNA, N.C. and BRITTO, Y.C.T. Represas em cascata e os efeitos na limnologia e organização das comunidades bióticas (fitoplâncton, zooplâncton e zoobentos): um estudo de caso no rio Paranapanema (SP/PR). In: M.G. NOGUEIRA, R. HENRY and A. JORCIN, orgs. *Ecologia de represas: impactos potenciais, ações de...*
PADISÁK, J., BARBOSA, F.A.R., BORBÉLY, G., BORICS, G., CHORUS, I., ESPINDOLA, E.L.G., HEINZE, R., ROCHA, O., TÖRÖKNÉ, A.K. and VASAS, G. Phytoplankton composition, biodiversity and a pilot survey of toxic cyanoprobaryotes in a large cascading reservoir system (Tietê basin, Brazil). Verhandlungen des Internationalen Verein Limnologie, 2000, 27(5), 2734-2742. http://dx.doi.org/10.1080/03680770.1998.11898164.

PADISÁK, J., CROSSETTI, L. and NASELLI-FLORES, L. Use and misuse in the application of the phytoplankton functional classification: a critical review with updates. Hydrobiologia, 2009, 621(1), 1-19. http://dx.doi.org/10.1007/s10750-008-9645-0.

PÉREZ-MARTÍNEZ, C. and SÁNCHEZ-CASTILLO, P. Temporal occurrence of Ceratium hirundinella in Spanish reservoirs. Hydrobiologia, 2002, 452(1), 97-107.

REYNOLDS, C.S. Vegetation process in the pelagic: a model for ecosystem theory. In: O. KINNE, ed. Excellence in ecology. 9th ed. Oldendorf: Ecology Institute, 1997, vol. 9, 371 p.

REYNOLDS, C.S., HUSZAR, V., KRUK, C., NASELLI-FLORES, L. and MELO, S. Towards a functional classification of the freshwater phytoplankton. Journal of Plankton Research, 2002, 24(5), 417-428. http://dx.doi.org/10.1093/plankt/24.5.417.

RIERA, J.L.L. and MORGUÍ, J.A. Limnologia regional de embalses españoles. Mundo Científico, 1990, 104, 720-726.

ROLDÁN, G. Fundamentos de limnología neotropical. Medellín: Editorial Universidad de Antioquia, 1992.

SALDANA-FABELA, M.P., MARTINEZ-JIMENEZ, M. and GOMEZ-BALANDRA, M.A. Spatial and temporal changes in the phytoplankton community in a cascaded reservoir system: San Juan River, Queretaro, Mexico. International Journal of Environmental Monitoring and Analysis, 2014, 2(5), 244-251. http://dx.doi.org/10.11648/j.ijema.20140205.13.

SANTANA, L.M., CROSSETTI, L.O. and FERRAGUT, C. Ecological status assessment of tropical reservoirs through the assemblage index of phytoplankton functional codons. Brazilian Journal of Botany, 2017, 40(3), 695-704. http://dx.doi.org/10.1007/s40415-017-0373-4.

SANTOS, N.C.L., GARCÍA-BERTHOU, E., DIAS, J.D., LOPES, T.M., AFFONSO, I.P., SEVERI, W., GOMES, C.L. and AGOSTINHO, A.A. Cumulative ecological effects of a Neotropical reservoir cascade across multiple assemblages. Hydrobiologia, 2018, 819(1), 77-91. http://dx.doi.org/10.1007/s10750-018-3630-z.

SANTOS-WISNIEWSKI, M.J., SILVA, L.C., LEONE, I.C., LAUDARES-SILVA, R. and ROCHA, O. First record of the occurrence of Ceratium furcoides (Levander) Langhans 1925, an invasive species in the hydroelectricity power plant Furnas reservoir, MG, Brazil. Brazilian Journal of Biology - Revista Brasileira de Biologia, 2007, 67(4), 791-793. http://dx.doi.org/10.1590/S1519-69842007000400033. PMid:18278340.

SÃO PAULO. GOVERNO DO ESTADO. Plano Estadual de Recursos Hídricos de São Paulo – PERH. São Paulo: Secretaria de Saneamento e Recursos Hídricos, Coordenadoria de Recursos Hídricos, 2006.

SARTORY, D.P. and GROBBELAAR, J.E. Extraction of chlorophyll a from freshwater phytoplankton for spectrophotometric analysis. Hydrobiologia, 1984, 114, 177-187. http://dx.doi.org/10.1007/BF00031869.

SILVA, C.A., TRAIN, S. and RODRIGUES, L.C. Phytoplankton assemblages in a Brazilian Subtropical cascading reservoir system. Hydrobiologia, 2005, 537(1-3), 99-109. http://dx.doi.org/10.1007/s10750-004-2552-0.

SILVA, L.C., LEONE, I.C., SANTOS-WISNIEWSKI, M.J., PERET, A.C. and ROCHA, O. Invasão do dinoflagelado Ceratium furcoides (Levander) Langhans 1925 em uma represa tropical e sua relação com as variáveis ambientais. Biota Neotropica, 2012, 12(2), 93-100. http://dx.doi.org/10.1590/S1676-06032012000200010.

SOARES, M.C.S., HUSZAR, V.L.M., MIRANDA, M.N., MELLO, M.M., ROLAND, F. and LÜRLING, M. Cyanobacterial dominance in Brazil: distribution and environmental preferences. Hydrobiologia, 2013, 717(1), 1-12. http://dx.doi.org/10.1007/s10750-013-1562-1.

SOLORZANO, L. Determination of ammonia in natural waters by the phenol hypochlorite method. Limnology and Oceanography, 1969, 14, 799-801. http://dx.doi.org/10.4319/lo.1969.14.5.0799.

SPAULDING, S.A. and EDLUND, M.B. Diatoms of the United States: the source for diatom identification and ecology [online]. USA: Diatoms of North America, 2009 [viewed 16 Oct. 2018]. Available from: https://diatoms.org/genera/discostella.

STRASKRA, M. Vltava cascade as teaching grounds for reservoir limnology. Water Science and Technology, 1994, 30(10), 289-297. http://dx.doi.org/10.2166/wst.1994.0539.

STRICKLAND, J.D.H. and PARSONS, T.R. A manual of seawater analysis. Bulletin - Fisheries Research Board of Canada, 1960, 125, 1-185.
THORNTON, K.W., KIMMEL, B.L. and PAYNE, E.E., eds. Reservoir limnology: ecological perspectives. New York: John Wiley & Sons, 1990.

TUNDISI, J.G. A review of basic ecological processes interacting with production and standing stock of phytoplankton in lakes and reservoirs in Brazil. Hydrobiologia, 1983, 100(1), 223-243. http://dx.doi.org/10.1007/BF00027431.

TUNDISI, J.G., MATSUMURA-TUNDISI, T. and ROCHA, O. Theoretical basis for reservoir management. In: J.G. TUNDISI and M. STRAŠKRABA, eds. Theoretical reservoir ecology and its applications. Leiden: Brazilian Academy of Sciences, International Institute of Ecology and Backhuys Publishers, 1999, pp. 505-528.

UTERMÖHL, H. Zur Vervollkommnung der quantitativen Phytoplanktonmethodik. Internationale Vereinigung für Theoretische und Angewandte Limnologie, 1958, 9, 1-38.

VALDERRAMA, G.C. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. Marine Chemistry, 1981, 10(2), 109-122. http://dx.doi.org/10.1016/0304-4203(81)90027-X.

WETZEL, R.G. Limnologia. 3. ed. San Diego: Academic Press, 2001, 919 p.

WETZEL, C.E., ECTOR, L., HOFFMANN, L. and BICUDO, D.C. Colonial planktonic Eunotia (Bacillariophyceae) from Brazilian Amazon: taxonomy and biogeographical considerations on the E. asterionelloides species complex. Nova Hedwigia, 2010, 91(1-2), 49-86. http://dx.doi.org/10.1127/0029-5035/2010/0091-0049.

ZORZAL-ALMEIDA, S., BICUDO, D.C., LAMPARELLI, M.C., FAUSTINO, S.B., FERRAGUT, C. and BICUDO, C.E.M. Avaliação do índice de estado trófico e sua aplicação na Represa Guarapiranga em longa série temporal. In: C.E.M. BICUDO and D.C. BICUDO, orgs. 100 anos da Represa Guarapiranga: lições e desafios. Curitiba: CRV, 2017.

Received: 08 November 2018
Accepted: 30 April 2020

Associate Editors: Antonio Fernando Monteiro Camargo