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Analysis of the phytoplankton community emphasizing cyanobacteria in four cascade reservoirs system of the Iguazu River, Paraná, Brazil

Análise da comunidade fitoplanctônica com ênfase em cianobactérias em quatro reservatórios em sistema de cascata do rio Iguaçu, Paraná, Brasil

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ABSTRACT

Knowing the ecological changes in a reservoir is of great relevance to study environmental impacts and assess water quality. Among these studies, the knowledge about the structure of the phytoplankton community is essential, once they represent a fundamental component of aquatic ecosystems, responding immediately to physical and chemical alterations in water. The objective of this work was to analyze the phytoplankton community and the influence of abiotic factors, along the longitudinal axis in four cascade reservoirs system of the Iguazu River, Paraná, Brazil, emphasizing the importance of cyanobacteria. Foz do Areia, Salto Segredo, Salto Santiago and Salto Caxias reservoirs were the objects of this study. The phytoplankton counting was made tubular sedimentation chamber using an inverted microscope. The results showed that the four reservoirs are under eutrophication processes, presenting intense cyanobacteria blooms, dominated mainly by Microcystis aeruginosa and Sphaerocavum brasiliense. There was no evident longitudinal gradient related to phytoplankton, as expected for reservoirs in a cascade system. The dominance of cyanobacteria shows that the Iguazu River is undergoing an intense process of environmental degradation, threatening the integrity of biological communities and causing serious damage to the ecosystem as a whole.

Keywords: Eutrophication; Water quality; Hydroelectric reservoirs; Limnology.

RESUMO

Conhecer as alterações ecológicas em um reservatório é de grande relevância para estudos de impacto ambiental e avaliação de qualidade da água. Dentro desses estudos, o conhecimento da estrutura da comunidade fitoplanctônica é necessário, pois esta comunidade tem uma importância fundamental nos ecossistemas aquáticos, respondendo de forma rápida às mudanças físicas e químicas da água. O objetivo deste trabalho foi analisar a comunidade fitoplanctônica e a influência das variáveis abióticas, considerando o eixo longitudinal em quatro reservatórios de hidrelétricas em cascata do rio Iguazu, Paraná, Brasil, destacando a importância das cianobactérias. Foram amostrados quatro dos reservatórios de hidrelétricas do rio Iguazu: Foz do Areia, Salto Segredo, Salto Santiago e Salto Caxias. A contagem dos indivíduos foi realizada utilizando câmara de sedimentação tubular em microscópio invertido. Os resultados mostraram que os quatros reservatórios estão sujeitos a processos de eutrofização, apresentando intensas florações de cianobactérias, com dominância principalmente de Microcystis aeruginosa e Sphaerocavum brasiliense. Não houve gradiente longitudinal evidente relacionado ao fitoplâncton, como é esperado para reservatórios em sistema de cascata. A dominância de cianobactérias indica que o rio Iguazu está passando por um intenso processo de degradação ambiental, ameaçando a integridade das comunidades biológicas e causando sérios danos ao ecossistema como um todo.

Palavras-chave: Eutrofização; Qualidade da água; Reservatórios de hidrelétricas; Limnologia.

INTRODUCTION

Reservoirs are lotic systems and as a lotic system, they present longitudinal gradients in channel morphology, flow velocity, water temperature, substrate type and also biological communities (SABATER et al., 2008). As result of river-lake hybrid features, reservoirs have vertical and horizontal gradients of abiotic factors, which control phytoplankton productivity in river-dam systems. In these systems, the river, intermediate (transition) and lacustrine zones can be distinguished (THORNTON; KIMMEL; PAYNE, 1990; WETZEL, 2001).

According to Barbosa et al. (1999) the concept of continuous cascade reservoirs represents reservoirs that have an interconnection between ecological processes, where water quality, structure and composition of phytoplankton are influenced by several processes of the system.

Phytoplankton is a main component of a limnic system biocenosis. They are the foundation of the trophic chain and perform an important role in nutrient cycling and energy transferring inside the aquatic ecosystem. The study of the structure of phytoplankton is a fundamental mechanism to indicate levels of water quality (CARDOSO et al., 2013). However, phytoplankton growth inside reservoirs may be affected mainly by phosphorus and nitrogen concentrations (CUNHA; CALIJURI; LAMPARELLI, 2013). High concentrations of these nutrients can cause uncontrolled phytoplankton growth and consequently the eutrophication of a reservoir.

The process of eutrophication leads to the growth of cyanobacteria populations in lakes and reservoirs (MANAGE; KAWABATA; NAKANO, 1999; CALIJURI; SANTOS; JATI, 2002; COSTA et al., 2006; COSTA et al., 2009; BECKER et al., 2010). Besides the important influence of elevated nutrients concentrations in cyanobacteria dominance, other environment factors can be associated, such as low turbulence, low light conditions, elevated temperature and low ratio between euphotic zone and mixing zone (WANG et al., 2011). Due to an excellent capacity to adapt and a high reproductive success, cyanobacteria proliferate in a fast rate in environments artificially enriched by nutrients (DOLMAN et al., 2012; HERNÁNDEZ-MORALES et al., 2016). Therefore, cyanobacteria identification and quantification are relevant to monitoring programs that intend to predict the beginning of toxic blooms based on these data (FERREIRA et al., 2005).

Researches regarding Brazilian eutrophic environments where there is dominance of cyanobacteria have been recurrent due to concern about fresh water ecosystems and the social and environmental consequences of such imbalance (SANT'ANNA et al., 2004; CHELLAPPA et al., 2009; FONSECA et al., 2010; SOARES et al., 2012). Several important hydroelectric reservoirs in Brazil are under intense eutrophication processes, which implicates in an increase of cyanobacteria blooms (SOARES et al., 2012).

Water quality monitoring programs have been performed in the Iguazu River reservoirs by the Environmental Institute of Paraná (Instituto Ambiental do Paraná – IAP), energy companies located in Paraná (Copel e Tractebel Energy) and by other institutes and research groups.

The main stream of Iguazu River and some of its tributaries are currently polluted and receive high man-induced loads of nutrients, substantially originated from domestic sewage (IDE et al., 2013). The source of the nutrients is confirmed by the presence of emerging compounds from domestic sewage in the Upper Iguazu watershed (IDE et al., 2013; MACHADO et al., 2014; KRAMER et al., 2015; OSAWA et al., 2015) which contributes to the increase in nitrogen and phosphorus concentrations, main contributors of eutrophication along cyanobacteria blooms.

Phytoplankton studies performed in cascade reservoirs system indicate that both construction and operation modify the composition and abundance of the biological community. For that reason, phytoplankton characteristics are considered sensitive indicators to assess the degradation of the aquatic ecosystem associated to cascade reservoirs (SILVA; TRAIN; RODRIGUES, 2005).

The hypothesis of this research is to assess if anthropic activities, associated to elevated water temperature and rainfall, may provoke blooming effects, mainly in cyanobacteria populations present in hydroelectric reservoirs.

The aim of this research is to analyze phytoplankton structure, emphasizing cyanobacteria and highlighting dominant species under influence of different abiotic features inside the longitudinal gradient of four cascade reservoirs in the Iguazu River.

METHODOLOGY

Study site

The Iguazu River is among the longest rivers in South Brazil and it is the longest river of Paraná State. The source is located in Curitiba, the capital of Paraná state, and the flow direction is towards west, flowing into the Paraná River, where the Iguazu falls are formed (Figure 1). Along the course of the Iguazu River, there are five reservoirs, and four out of the five reservoirs are objects of this research: Foz do Areia (26°00' S e 51°36' W), Salto Segredo (25°47' S, 52°07' W), Salto Santiago (25° 38' S, 52° 37' W) and Salto Caxias (25° 33' S, 53° 30' W). The description of the hydropower plants and the four reservoirs is in Table 1. The banks of Foz do Areia reservoir are constituted of natural vegetation and agricultural lands. In Salto Segredo reservoir there is an Environmental Protection Area (APA). The area that is not covered by the protection area is of agricultural use. In Salto Santiago and Salto Caxias reservoirs the banks are constituted manly of agricultural lands.

The Iguazu River watershed climate is classified as humid subtropical, with annual rainfall above 1000 mm and a temperature variation reaching 30 °C between winter and summer.

Sampling, abiotic and phytoplankton variables analysis

The limnological samples were collected in November of 2012, February and October of 2013 in 9 sampling points distributed in the riverine zones (3 sampling points), intermediate zones (3 sampling points) and lacustrine zones (3 sampling points) of each reservoir.

Abiotic water analysis were partially performed *in situ* with a Hanna multi-parameter probe, measuring water temperature (°C), pH, dissolved oxygen (DO) and electric conductivity ($\mu S\ cm^{-2}$).

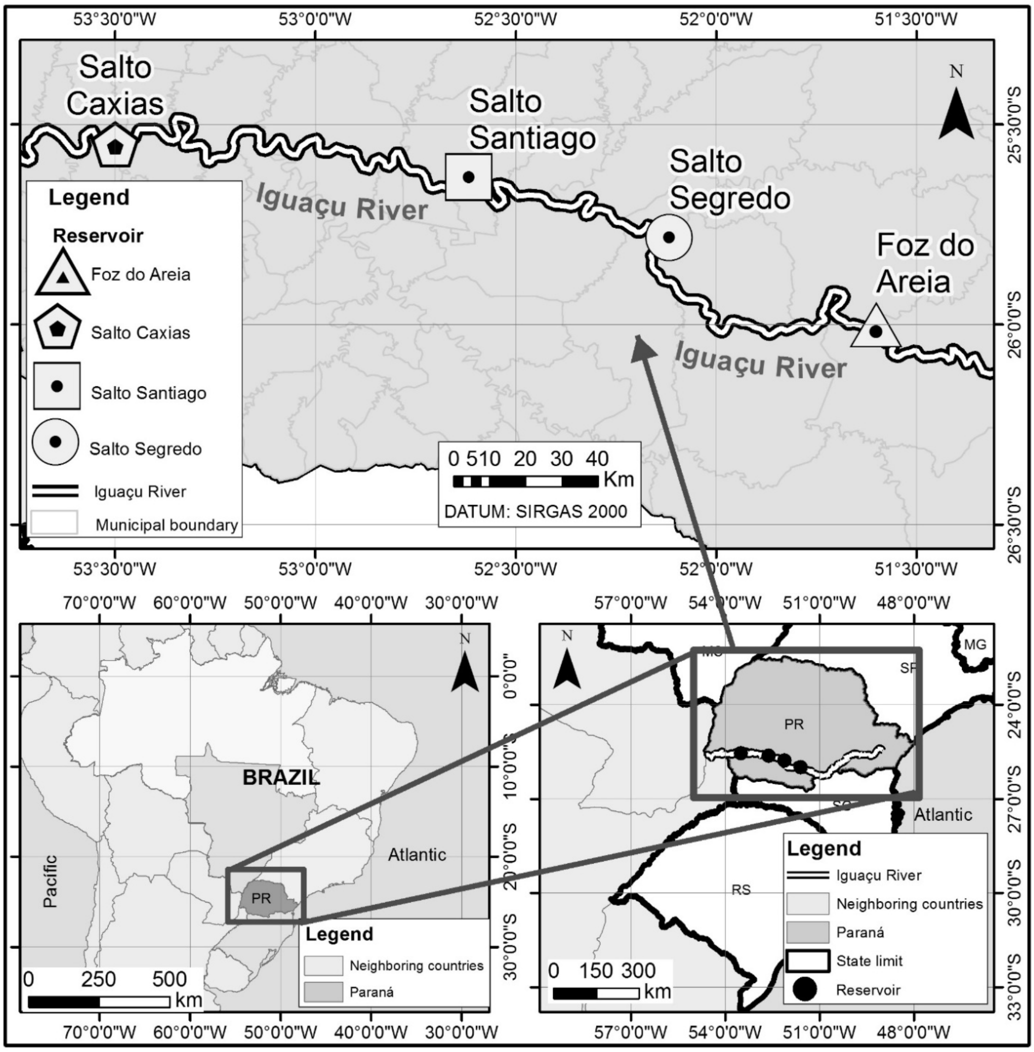

Figure 1. Localization map of Foz do Areia, Salto Segredo, Salto Santiago and Salto Caxias reservoirs in the Iguazu River, Paraná, Brazil.

Table 1. Characteristics of hydroelectric plants and studied reservoirs.

Reservoir	Installed capacity (MW)	Distance from Curitiba (km)	Flooded area (km^2)	Elevation (m)	Medium depth (m)	Flow (m^3 s^{-1})	Residence time (days)
Foz do Areia	1676	250	153	744	160	544	102.0
Segredo	1260	285	83	607	145	720	45.0
Salto Santiago	1420	410	208	510	80	902	50.8
Salto Caxias	1240	600	141	325	67	540	45.0

Adapted from: Mine and Tucci (2002); Tractebel Energia (2002) and LACTEC (2009).

Turbidity (NTU) was measured using a Hanna portable turbidimeter. Alkalinity values were obtained by Grahn method.

Dissolved organic carbon (DOC) analyses were performed in a Shimadzu (Total Organic Carbon) TOC equipment. Total phosphorus (TP), orthophosphate (P-PO_4^{3-}), nitrite (NO_2^-), ammoniacal nitrogen (N-NH_3) and nitrate (NO_3^-) analyses were performed in the laboratory, following the methods described in APHA (1999). IAP, Environmental and Water Resources Department of Paraná granted rainfall data, which are available in the Hydrological Information System (SIH).

Samples destined to phytoplankton quantification were collected in subsurface, at 0.5 meters deep and fixated in alkaline Lugol. Samples destined to qualification were collected in a conic plankton net, with apertures of 20 μm. The net was horizontally dragged during five minutes in a boat with a speed of two knots. The samples were fixed with a 50% Transeau solution. Every phytoplankton sample was preserved *in situ*. Phytoplankton identification was performed in an optical microscope and several authors were references to the identification: Hötzel and Croome (1999), Wehr and Sheath (2003), Bicudo and Menezes (2006), Sant'Anna et al. (2006) e Sant'Anna et al. (2012). The phytoplankton were ranked in Reynolds functional groups.

The quantification of the organisms was performed in sedimentation chambers in a Zeiss inverted microscope Axorvet 40C model, magnified 400x, employing the method of Utermöhl (1958). The count stopped at 100 cross fields or when 400 individuals of the most frequent species were identified. Cenobia, colonies and filaments were considered cell individuals. The cells, colonies and filaments were counted when cyanobacteria cells had a diameter $\geq$ 2,5 μm, that is until reach the minimal division of the micrometric ruler of the eyepiece, considering the counting method of Weber.

Phytoplankton species were classified as dominants when the density had values above 50% of the total value of the sample and were classified as abundant when the species density was superior to the value of the medium density of each sample (LOBO; LEIGHTON, 1986). The method of Padisák, Crossetti and Naselli-Flores (2009) was employed to classify the phytoplankton functional groups. The occurrence classification (%) was employed to define the most frequent species. The chlorophyll-*a* analysis was employed in the Trophic State Index (TSI) and was performed according to the descripted method in APHA (1999).

Ecological indexes and statistical analysis

In order to obtain the diversity level (H') of Shannon-Weaver the following Equation 1 was applied:

$$H' = -\Sigma Pi.Log2Pi \qquad (1)$$

Where Pi is the relative abundance of the species i in the sample; Pi = ni/N. The number ni represents the number of the individuals of the species i in the sample and N is the number of individuals in the sample. Since the equation uses logarithm in base 2, the measure unit is bit/individual. Criteria adopted to the classification were: high diversity to the values obtained between the scale of 2.0 to 3.0 bits ind^{-1}, and low diversity to values $<$ 2.0 bits ind^{-1}.

The Pielou (J') Index was used to determine the homogeneity of the abundance distribution between phytoplankton populations, obtained by the Equation 2 (MAGURRAN, 1988):

$$J' = H' / H\max \qquad (2)$$

Where Hmax is the maximum diversity occurring when all the species abundances are the same. Equitability assumes values between 0 and 1, with values below 0.5 considered low and above 0.7 considered high.

Trophic State Index (TSI) according Toledo Junior (1990) was employed to classify reservoirs according to their trophy status. Total phosphorus concentration and chlorophyll-*a* concentration were employed in the following Equations 3, 4 and 5:

$$IET\ (Cl-a)\ =\ 10\ .\ \{6-[2{,}04-0{,}695.\ln(Cl-a\ /\ \ln\ 2)]\}; \qquad (3)$$

$$IET(TP) = 10.\{6-[\ln(80{,}32\ /\ TP\ /\ \ln\ 2)]\}; \qquad (4)$$

$$IET = [IET(TP) + IET(Cl-a)/2] \qquad (5)$$

Where Cl-*a* = chlorophyll-*a* concentration (μg L^{-1}), TP = Total phosphorus concentration (μg L^{-1}) e ln = natural logarithm.

Statistical analysis was performed in Statistica 7.0 software, Copyright StatSoft Inc. The Principal Components and Classification Analysis (PCCA) was performed using two matrixes. The first matrix was set with abiotic data and the second with the biotic data. The abiotic and biotic data were log-transformed, Y = log (x+1), considering the correlations between parameters (abiotic and biotic) with the factor 1 and the factor 2. The correlation between biotic and abiotic factors, the diversity indexes and equitabilities were obtained using Pearson's linear correlation (r) with a significance level of $p<0.05$.

RESULTS E DISCUSSION

Abiotic factors

Results of the analysis of abiotic factors in the reservoirs are in Table 2. Water temperature was elevated in November/2012 and February/2013, corresponding to the end of spring and summer. In Salto Caxias reservoir was detected the highest temperature among the four reservoirs (26.7 °C), which occurred in November/2012. The lowest temperature was recorded in Foz do Areia reservoir (19.3 °C) in October/2013. The most important rainfall events occurred in February/2013 (in all of the four reservoirs) and the smallest rates of rainfall occurred in November/2012 (Table 2).

Dissolved oxygen concentrations (Table 2) were elevated in all the sampling campaigns, except for Salto Caxias reservoir in February/2013, the same month where turbidity had the lowest value of the analyzed period. However, turbidity was elevated in Foz do Areia reservoir in November/2012. Unlike other abiotic factor, alkalinity values didn´t indicate significant variations along the period.

Dissolved organic carbon (DOC) presented high concentration values in October/2013 in the four reservoirs. Orthophosphate ($P\text{-}PO_4^{3-}$) and Total Phosphorus (TP) concentrations were higher in Foz do Areia reservoir in November/2012. Nitrogen forms presented low concentrations, except for Nitrate ($N\text{-}NO_3^-$) in November/2012 in Salto Caxias reservoir (Table 2).

Composition of phytoplankton

The identification led to 230 different species identified in the four reservoirs of The Iguazu River. Cyanophyceae (85) and Chlorophyceae (76) classes corresponded to 70% of the individuals and the other 30% were represented by Bacillariophyceae (36), Cryptophyceae(3), Chrysophyceae (5), Dinophyceae (3), Conjugatophyceae (14), Euglenophyceae (4), Chlamydophyceae (3) and Xantophyceae (1).

Cyanophyceae and Chlorophyceae demonstrated higher diversity along the four cascade reservoirs in almost every analyzed period (Figure 2). A higher number of species was recorded in February/2013 in the four reservoirs. However, Foz do Areia reservoir had a greater species richness (129 identified species).

Table 2. Abiotic factors in November/2012 (C1), February/2013 (C2), and October/2013 (C3) in Foz do Areia, Salto Segredo, Salto Santiago and Salto Caxias reservoirs.

Parâmetros	**Foz do Areia**			**Salto Segredo**			**Salto Santiago**			**Salto Caxias**		
	C1	**C2**	**C3**	**C1**	**C2**	**C3**	**C1**	**C2**	**C3**	**C1**	**C2**	**C3**
T_{water} (°C)	23.7	26.0	19.3	24.9	26.7	20.8	27.2	26.3	20.0	28.9	23.9	21.6
Precipitation (mm)	138	252	164	44	211	136	105	201	141	46	361	125
pH	7.9	9.2	9.1	9.4	8.3	8.9	8.3	7.8	9.4	8.3	6.6	8.6
DO ($mg.L^{-1}$)	7.2	9.3	9.3	7.8	8.1	9.6	7.2	7.9	10.0	7.6	6.3	9.4
Conductivity ($\mu S/cm^2$)	34.5	33.6	7.4	13.3	27.8	3.7	14.1	17.0	1.6	18.8	13.1	3.4
Turbidity (UNT)	39.0	20.1	12.1	17.6	22.4	16.8	8.6	7.2	5.7	4.3	3.7	9.6
Alkalinity ($mEq.^{-1}$)	349	356	261	315	385	243	337	378	243	400	368	252
DOC ($mg\ L^{-1}$)	2.0	4.0	10.3	3.2	5.4	6.6	2.7	5.6	5.7	3.3	3.8	5.9
P-total ($\mu g\ L^{-1}$)	509	133	48	160	119	74	106	119	69	73	21	101
$P\text{-}PO_4^{3-}$ ($\mu g\ L^{-1}$)	51	15.5	41.3	6.1	0.1	35.4	8.0	13.5	20.7	0.0	3.6	39.5
$N\text{-}NO_2^-$ ($\mu g\ L^{-1}$)	10	54	11	2.4	10	16	2.5	10	6.7	0.0	12	12
$N\text{-}NH_3$ ($\mu g\ L^{-1}$)	8.5	91	34	5.7	76	152	3.1	69	229	0.0	87	123
$N\text{-}NO_3^-$ ($\mu g\ L^{-1}$)	57	26	174	41	46	99	243	94	117	250	62	124

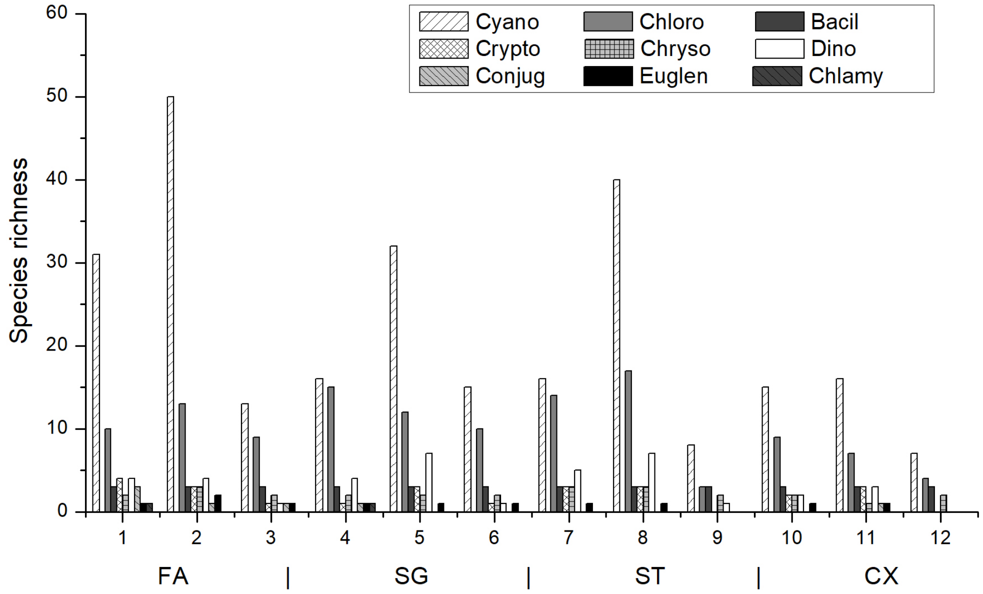

Figure 2. Richness of phytoplankton species distributed by taxonomic classes in Foz do Areia (FA), Salto Segredo (SG), Salto Santiago (ST) and Salto Caxias (CX) reservoirs located in Paraná, Brasil in the months of November/2012, February/2013 and October/2013. Legend: Cyano = Cyanophyceae; Chloro = Chlorophyceae; Bacil = Bacillariophyceae; Crypto = Cryptophyceae; Chryso = Chrysophyceae; Dino = Dinophyceae; Conjug = Conjugatophyceae; Euglen = Euglenophyceae; Chlamy = Chlamydophyceae; Xanto = Xantophyceae.

Density of phytoplankton

The density of the phytoplankton community (Figure 3A, 3B and 3C) was higher in November/2012 and February/2013, the same period where elevated temperatures occurred (Table 2). The higher density was registered in the riverine zones of Foz do Areia reservoir (273,977 ind mL^{-1}) in February/2013. In the first two sampling campaigns (Figure 3A and 3B) density peaks superior to 200,000 ind mL^{-1} were registered in sampling points with cyanobacteria blooms (Foz do Areia and Salto Segredo).

In November/2012, the higher density (Figure 3A) was registered in the lacustrine zone of the Foz do Areia reservoir (223,950 ind mL^{-1}). In February/2013, there was a decrease in the phytoplankton density in the river-dam direction in the first three reservoirs.

Cyanophyceae was dominant in months where the bloom was intense, along the longitudinal axis of the reservoir. In the first analyzed period (C1), cyanobacteria consisted of 80% of the phytoplankton community. In Salto Caxias reservoir the relation between Cyanophyceae and phytoplankton were not as relevant, but Cyanophyceae was still the dominant class (Figure 4A).

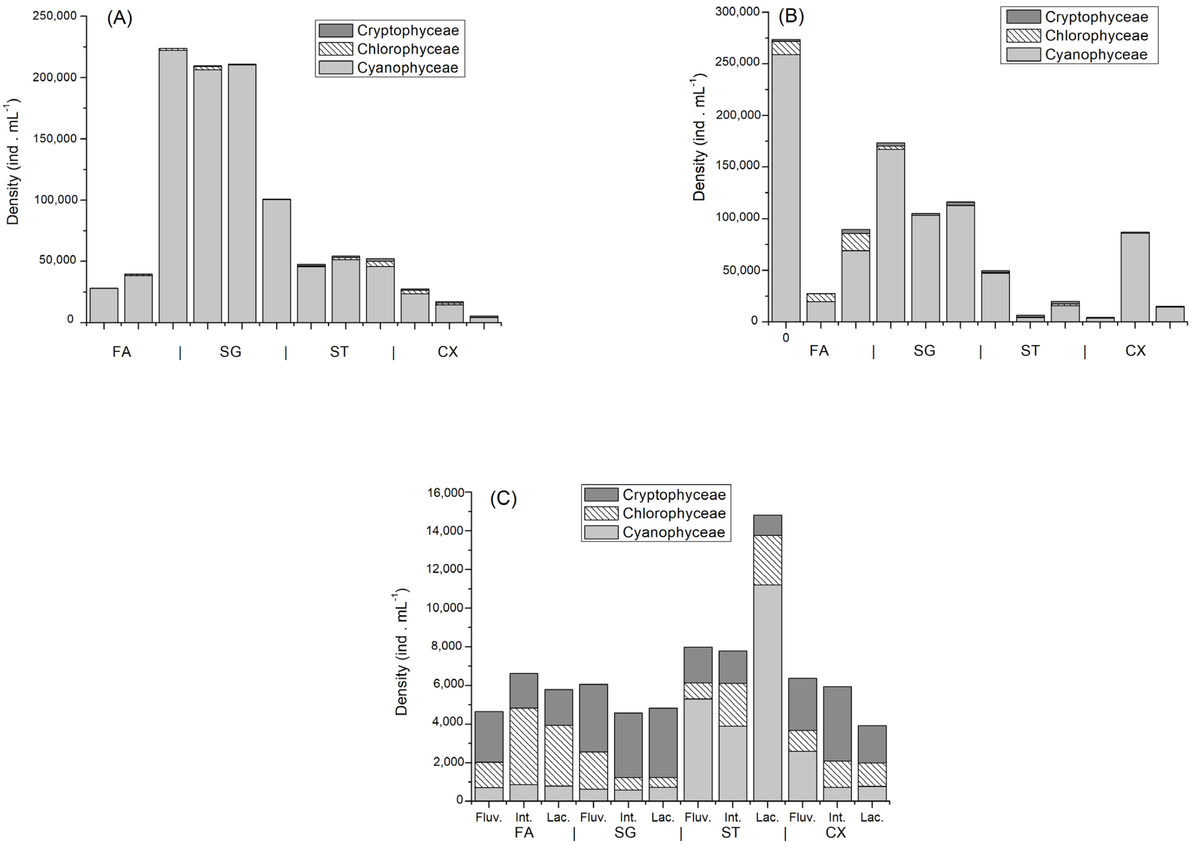

Figure 3. Total density of the main taxonomic classes of phytoplankton in the Foz do Areia (FA), Salto Segredo (SG), Salto Santiago (ST) and Salto Caxias (CX) reservoirs in the months of November/2012 (A), February/2013 (B) and October/2013 (C). Code: Fluv. = riverine zone; Int = intermediate zone; Lac = lacustrine zone.

Density was lower in October/2013, when compared to the previous analysis. However, the density peak was superior to 15,000 ind mL^{-1} in the lacustrine zone of Salto Santiago reservoir, consequence of the high number of cyanobacteria in the reservoir. Cyanophyceae and Cryptophyceae were the most representative classes.

Microcystis aeruginosa was the dominant species in November/2012 in Foz do Areia and Salto Segredo reservoirs. Besides, it was the dominant species in February/2013 in Foz do Areia reservoir, with the higher density (244,858 ind mL^{-1}) in the riverine zone of the reservoir, representing 86% of the total density of phytoplankton in this area

Sphaerocavum brasiliense species was dominant in Salto Segredo, Salto Santiago and Salto Caxias reservoirs in February/2014 (Figure 4B). The greater representativeness, in quantitative terms (124,452 ind mL^{-1}), was detected in the riverine zone of Salto Segredo reservoir (relative abundance of 95%) and in the intermediate zone of Salto Caxias reservoir. In October/2013 *Sphaerocavum brasiliense* had the higher relative abundance (78%) in the lacustrine zone of Salto Santiago reservoir, with a density of 27,580 ind mL^{-1}.

Rhodomonas minuta was the second most representative species in October/2013. The number of the individuals of the species was constant (in quantitative terms) in every analyzed zone in the reservoirs (Figure 4C). In October/2013, *Rhodomonas minuta* had a higher density (3,850 ind mL^{-1}) in the intermediate zone of Salto Segredo reservoir (62%). The taxa *Kirchneriella* sp. had a greater relative abundance in Foz do Areia reservoir in October/2013 and was classified as a dominant species in the period (Figure 4C).

Diversity indexes and equitability

Phytoplankton diversity (Figure 5A) had a large variation in February/2013 and had peaks (H'>2 bits ind^{-1}) in the intermediate zones of Foz do Areia and Salto Santiago reservoirs. Foz do Areia registered low diversity indexes in November/2012, however, the lowest diversity was registered in the intermediate zone of Salto Caxias reservoir in February/2013.

Equitability (J') was not elevated along the studied period (Figure 5B). The lowest index (0.06) was registered in the intermediate zone of Salto Caxias reservoir in February/2013, while the greater index (0.67) was registered in the riverine zone of Salto Santiago reservoir in October/2013. The equilibrium of the phytoplankton community was relatively low in Foz do Areia and Salto Segredo reservoirs in the three sampling campaigns.

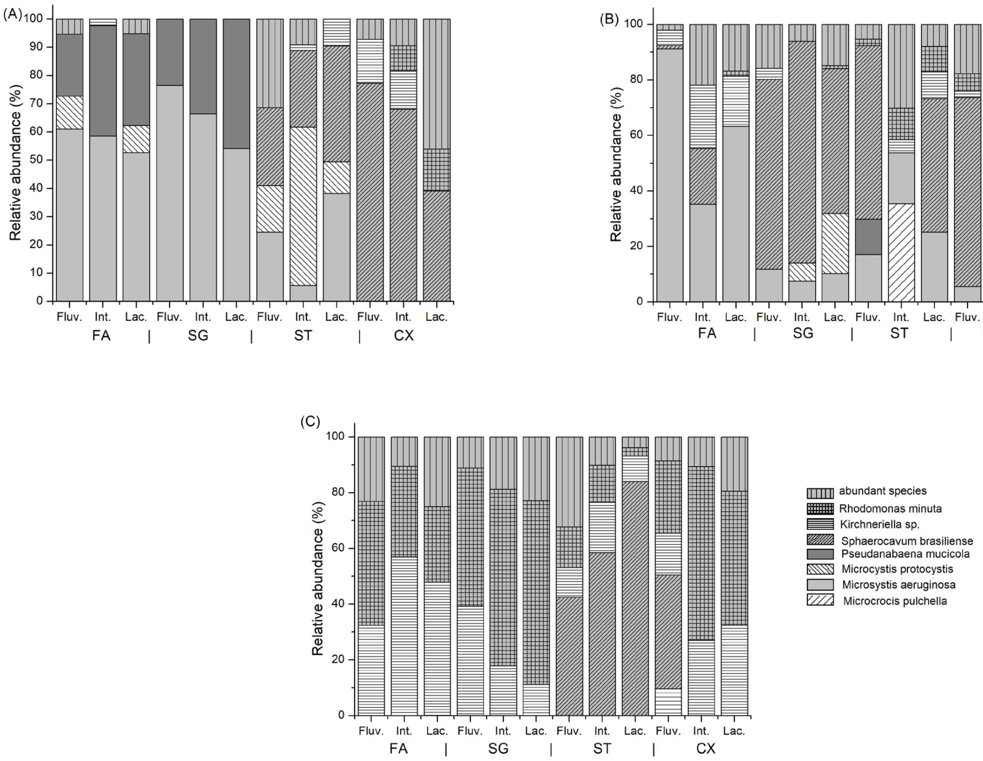

Figure 4. Relative abundance (%) of the dominant and abundant species registered in Foz do Areia (FA), Salto Segredo (SG), Salto Santiago (ST) and Salto Caxias (CX) reservoirs in the months of November/2012 (A), February/2013 (B) and October/2013. Legend: ab = abundant species; R. min. = Rhodomonas minuta; Kirch. = Kirchneriella sp.; S. Br. = Sphaerocavum brasiliense; P. muc. = Pseudanabaena mucicola; M. pro. = Microcystis protocystis; M. aer. = Microcystis aeruginosa; M. pulc. = Microcystis pulchella. Code: Riv. = riverine; Int. = intermediate; Lac. = lacustrine.

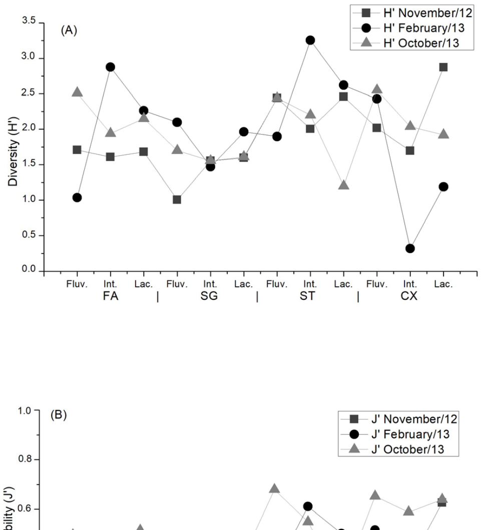

Figure 5. (A) Diversity medium values (H') and (B) equitability (J') of the three limnic zones of Foz do Areia, Salto Segredo, Salto Santiago and Salto Caxias reservoirs located in Paraná, Brazil. Fluv. = riverine; Int. = Intermediate; Lac. = Lacustrine.

Trophic State Index

Foz do Areia and Salto Segredo reservoirs showed Trophic State Indexes (TSI) above 54 and were classified as eutrophic environments. In November/2012 the lacustrine zone of Foz do Areia reservoir registered a TSI > 74 and was classified as hypereutrophic environment. In Salto Caxias reservoir, in February/2013, in the lacustrine and intermediate zones, TSI were below 24 and were classified as hyperoligotrophic (Table 3).

Relation between phytoplankton and abiotic factors

The factor 1 and 2 explained 50.9% of the total variability of data (Principal Components and Classification Analysis (PCCA)). Figure 6 (A and B) shows the result of the analysis, where Factor 1 (F1) separated data of November/2012 and February/2013 from October/2013 (Figure 6B), and also from Foz do Areia reservoir. There was not distinct clustering related to the limnic regions of the reservoirs.

F1 correlated (Figure 6A) with abiotic factors: positively with oxygen – DO (+0.7606) and with N-ammoniacal (+0.6537); negatively with temperature (-0.8244), conductivity (-0.8625) and alkalinity (-0.8422). F1 correlated biotic factors positively with R.min (0.4416); negatively with Cyanobacteria, highlighting *M. aeruginosa* (-0.6524) and *P. mucicola* (-0.5593) species.

Table 3. Trophic State Index (STI)* of the limnic zones in each reservoir in November/2012, February/2013 and October/2013.

Code	STI	Classification
FA-FL-C1	71.01	Eutrophic
FA-Int-C1	72.73	Eutrophic
FA-Lac-C1	74.07	Hypereutrophic
FA-FL-C2	64.63	Eutrophic
FA-Int-C2	59.31	Eutrophic
FA-Lac-C2	55.95	Eutrophic
FA-FL-C3	56.22	Eutrophic
FA-Int-C3	57.10	Eutrophic
FA-Lac-C3	55.60	Eutrophic
SG-FL-C1	63.50	Eutrophic
SG-Int-C1	62.75	Eutrophic
SG-Lac-C1	62.74	Eutrophic
SG-FL-C2	57.21	Eutrophic
SG-Int-C2	57.61	Eutrophic
SG-Lac-C2	56.02	Eutrophic
SG-FL-C3	54.40	Eutrophic
SG-Int-C3	54.49	Eutrophic
SG-Lac-C3	59.70	Eutrophic
ST-FL-C1	56.80	Eutrophic
ST-Int-C1	57.63	Eutrophic
ST-Lac-C1	53.95	Eutrophic
ST-FL-C2	47.92	Mesotrophic
ST-Int-C2	41.75	Oligotrophic
ST-Lac-C2	38.97	Oligotrophic
ST-FL-C3	61.54	Eutrophic
ST-Int-C3	50.35	Mesotrophic
ST-Lac-C3	56.68	Eutrophic
CX-FL-C1	52.16	Mesotrophic
CX-Int-C1	53.55	Mesotrophic
CX-Lac-C1	53.58	Mesotrophic
CX-FL-C2	40.20	Oligotrophic
CX-Int-C2	20.29	Hyperoligotrophic
CX-Lac-C2	21.71	Hyperoligotrophic
CX-FL-C3	63.24	Eutrophic
CX-Int-C3	54.78	Eutrophic
CX-Lac-C3	58.82	Eutrophic

*STI ≤ 24 (Hyperoligotrophic); 24 < STI ≤ 44 (Oligotrophic); 44 < STI ≤ 54 (Mesotrophic); 54 < STI ≤ 74 (Eutrophic) and STI> 74 (Hypereutrophic). Where FA = Foz do Areia; SG = Segredo; ST = Santiago; CX = Caxias; FL = riverine zone; Int = intermediate zone and Lac = lacustrine zone.

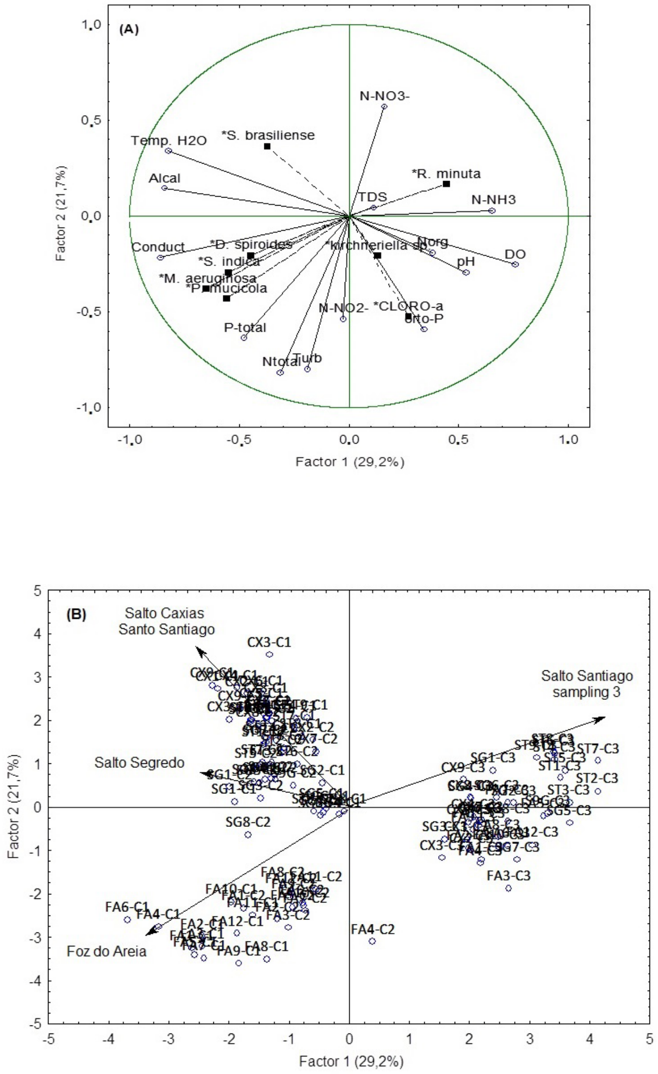

Figure 6. (A) Principal Components and Classification Analysis (PCCA) of biotic and abiotic factors, through the biplotting of the first two factors (F1 and F2), comparing the (B) three months in the reservoirs. Where: FA = Foz do Areia; SG = Segredo; ST = Santiago; CX = Caxias; C1 = sampling campaign 1; C2 = sampling campaign 2; C3 = sampling campaign 3.

The second fator (F2) correlated with abiotic factors: positively with nitrate (0.5726) and negatively with turbidity (-0.7963), P-total (-0.6359) and with orthophosphate (-0.5906). F2 correlated biotic factors negatively with *P. mucicola* (-0.4319) and with chlorophyll-a (-0.5239).

F1 negative was correlated with environments that presented intense cyanobacteria blooms, like Foz do Areia and Salto Segredo reservoirs, mainly in November/2012 and February/2013, where elevated temperatures, low water level and high concentrations of nutrients, like phosphorus, are possible causes. In February/2013 and October/2013 Salto Segredo, Salto Santiago and Salto Caxias reservoirs (positive F2) had a minor influence than Foz do Areia reservoir (negative F2) probably due to abiotic components originated from the Metropolitan Region of Curitiba (MRC) that reached Foz do Areia reservoir.

Biotic and abiotic factors are important water quality indicators, which directly influence the dynamic of limnic processes inside reservoirs. According to Dantas, Bittencourt-Oliveira and Moura (2012), the complex combination of abiotic and biotic factors interferes directly in the composition and biomass of phytoplankton inside reservoirs.

Rainfall rates were intense in February/2013 (one of the warmest months), coinciding with Silva and Costa (2015) previous work, where the rainy season occurred during elevated temperature months, affecting abiotic factors in Amando Ribeiro Gonçalves reservoir, located in Rio Grande do Norte.

Rainfall patterns may directly interfere in limnological characteristics of reservoirs, such as water temperature, DO, pH, conductivity, alkalinity, nutrients and others fundamental parameters to the hydrological system homeostasis. Rainfall volume contributed significantly ($p<0.05$), to the increase in NO_2^- ($r=0.79$) and $N\text{-}NH_3$ ($r=0.71$) concentrations.

Ammoniacal nitrogen promoted the development of Cryptophyceae $r=0.47$, $p<0.05$). Water oxygenation conditions (DO) were elevated in the fours reservoirs, what may indicate that phytoplankton contributed to the increase in DO concentrations. Elevated values of pH may relate with the photosynthetic activity of phytoplankton, which can be verified by the correlation between pH and DO ($r=0.79$, $p<0.05$). According to Concepción Tracanna et al (2014), elevated pH values in lacustrine zone of reservoirs are directly related to CO_2 consumption for photosynthetic processes and hydronium ion.

Turbidity was elevated during cyanobacteria blooms (Table 2, Figure 3A and 3B). Also, there is a strong positive correlation between turbidity and P-total ($r=0.68$, $p<0.05$). The number of cyanobacteria individuals increased when P-total concentrations were high, what in consequence increased turbidity. According to Silva and Costa (2015), high nutrients concentration and an elevated turbidity occur simultaneously along the dominance of cyanobacteria functional groups that are adapted to such environmental conditions.

DOC had significant correlation between DO ($r=0.52$, $p<0.05$), $N\text{-}NH_3$ ($r=0.58$, $p<0.05$) and R. *minuta* ($r=0.67$, $p<0.05$). According to Bovo-Scomparin and Train (2008), R. *minuta* is an opportunist species in elevated nutrient concentrations environment.

High concentrations of total phosphorus (TP) indicate an eutrophic environment in the river-dam axis of the four reservoirs, except in Salto Caxias reservoir in February/2012 ($21\ \mu g\ L^{-1}$). Foz do Areia reservoir had greater eutrophic rates in the lacustrine zone, in November/2012, with a TP concentration of ($525\ \mu g\ L^{-1}$). The high phosphorus concentration led to an increase in cyanobacteria populations.

Foz do Areia reservoir showed a longitudinal gradient pattern for turbidity, with decreasing values in the river-dam direction. Nevertheless, TP concentrations distribution was atypical in November/2012 and February/2013. The higher values were registered in the lacustrine zone, coinciding with bloom periods. In Salto Segredo reservoir turbidity didn´t present a typical longitudinal gradient pattern in November/2012 and February/2013. Total phosphorus distribution pattern in November/2012 and February/2013 was characteristic of longitudinal gradients in reservoirs.

Phytoplankton was mainly composed by cyanobacteria and chlorophycea in the four cascade reservoirs system, while cyanobacteria had the higher species richness. Previous studies of Borges, Train and Rodrigues (2008) and Cetto et. al. (2004), showed that Cyanophyceae and Chlorophyceae classes are expressively contributing to phytoplankton composition.

Great richness of Chlorophyceae is usually associated to absence of dominant species. However, it was verified that in strong cyanobacteria blooms where *M. aeruginosa* was dominant, this group was significantly rich. Train et al. (2005) observed such event in Iraí reservoir, also located in Paraná.

Colonial cyanobacteria have high occurrence frequency: *M. aeruginosa* (92%), *Aphanocapsa* sp. (92%), *Cyanodictyon* spp. (92%), *Pseudanabaena mucicola* (92%), *M. panniformis* (83%), *S. brasiliense* (83%), *Microcrocis pulchella* (83%), *M. protocystis* (75%), *M. wesenbergii* (75%), *Eucapsis densa* (75%), *Merismopedia tenuissima* (67%) and *Aphanocapsa delicatissima* (67%).

Beyond cyanobacteria and cyanotoxins quantitative studies, taxonomic studies of this group are fundamental to assess eutrophic environments. For example, taxonomic studies of coccoid cyanobacteria are usually performed, due to the presence of the group in eutrophic environments, like *Microcystis* sp. genus (SANT'ANNA et al., 2004).

Kirchneriella genus was documented in every sampling campaign in the four analyzed reservoirs. High frequency (100%) indicated great adaptability to the limiting conditions in the reservoirs, as observed by Tucci et al. (2006), Liu, Liu and Shen, (2010), Domingues and Torgan (2012). Other frequent group, chryptophyceae, was documented in every studied reservoir. According to Tundisi and Matsumura-Tundisi (2008), chryptophyceae are found in mesotrophic and eutrophic environments.

Cyanobacteria density increases when blooms occur, mainly in Foz do Areia and Salto Segredo reservoirs. The blooms may be associated to elevated water temperature and total phosphorus concentration, characteristics of eutrophic environments. Additionally, there was a slight tendency of biomass decrease from Foz do Areia to Salto Caxias reservoirs in bloom periods.

The greatest peak of cyanobacteria blooms was promoted by characteristics of a eutrophic environment, like water temperature and total phosphorus (Table 3 and Figure 3).

According to Brassac et al. (2009), a massive cyanobacteria bloom occurred in Foz do Areia reservoir, exceeding one million cells per milliliter. Frequent and intense cyanobacteria blooms indicate that Foz do Areia reservoir may suffer a serious process of environmental degradation.

Physical and chemical properties influenced in the *M. aeruginosa* intense bloom in Foz do Areia and Salto Segredo reservoirs. The number of individuals had significant correlation ($p<0.05$) between conductivity ($r=0.72$), turbidity (0.46), alkalinity ($r=0.56$) and total phosphorus ($r=0.47$). *M. aeruginosa* is frequently found in eutrophic environments (REYNOLDS et al., 2002; SANT'ANNA et al., 2004; CHELLAPPA et al., 2009; ROLLAND et al., 2009; WANG et al., 2011; MÜLLER et al., 2012; LIU et al., 2012) and can provoke sanitation issues, mainly due to toxicity (BLACK; YILMAZ; PHLIPS, 2011; REICHWALDT; GHADOUANI, 2012).

The dominance of *M. aeruginosa* was substituted by *S. brasiliense* along the cascade reservoirs system in November/2012 and February/2013. In February, *S. brasiliense* was registered as dominant in Salto Segredo, Salto Santiago and Salto Caxias reservoirs (Figure 6). The research of Gentil, Tucci and Sant'Anna (2008) also related the substitution of *M. aeruginosa* bloom by *S. brasiliense.* According to Reynolds et al. (2002), the relation between species from the functional group M creates extensive monospecific blooms; *M. aeruginosa* overlaps *S. brasiliense* promoting segregation through the control of nictimeral buoyancy and also are tolerant to intense radiation.

In the first two months (November/2012 and February/2013) intense blooms occurred, provoked by high density of *M. aeruginosa* and *P. mucicola*, what indicates co-dominance of both species. The last species belong to functional group S1 and had significant correlation ($p<0.05$) between conductivity ($r=0.63$), turbidity ($r=0.54$) and total phosphorus ($r=0.54$). The presence of *P. mucicola* is associated to elevated turbidity environments. Usually this species is associated to *M. aeruginosa,* and it is found adhered to the colonies. This interspecific relationship was also observed in Lagos's (2009) research.

The decrease in the density of *M. aeruginosa* enabled the coexistence with other cyanobacteria species that were considered dominant and abundant, according to Lobo and Leighton (1986) (Table 4). Vieira, Cardoso and Costa (2015) had similar results in their research: gradual density decrease of *M. aeruginosa* allowed an increase in the diversity and the coexistence with cyanobacteria species from functional groups S1, SN, M Lo and H1.

R. minuta, *Cryptomonas brasiliensis* e *Cryptomonas ovata* were relatively significant in quantitative terms. According to Padisák, Crossetti and Naselli-Flores (2009) there is coexistence between species that belong to functional group B and usually develop in mesotrophic environments. In October/2013 *R. minuta*, *Cryptomonas brasiliensis* e *Cryptomonas ovata* had a greater relative abundance as a result of the absence of dominance of *M. aeruginosa,* when DOC and TP concentrations were characteristics of a mesotrophic environment. *M. aeruginosa* was favored by DOC ($r=0.67$, $p<0.05$) and $N\text{-}NH_3$ ($r=0.50$, $p<0.05$). *R. minuta* is an opportunist species, living in autotrophy or heterotrophy conditions.

The association of species may indicate water quality, although nutrients concentrations (mostly phosphorus) and phytoplankton production are the main determinant factors of trophic conditions (SILVA; TRAIN; RODRIGUES, 2005).

Diversity and equitability had low values in the reservoirs, primarily during intense blooms. Other researches have been recording low diversity and equitability indexes in environments where cyanobacteria thrive (GENTIL; TUCCI; SANT'ANNA, 2008; BORGES; TRAIN; RODRIGUES, 2008).

Equitabilty (J') was low in most of the limnic zones of the reservoirs, evidencing an ecological imbalance in phytoplankton communities, what was corroborated by negative correlations ($p<0.05$) with *M. aeruginosa* ($r=-0.52$) and *P. mucicola* ($r= -0.48$).

The first two months of the study had intense cyanobacteria blooms. However, in February/2013, the intermediate zone of Foz do Areia and Salto Santiago reservoirs presented high diversity and certain balance between populations, due to decreased density of *M. aeruginosa*. The decrease in *M. aeruginosa* allowed the development of other phytoplankton populations, like cyanobacteria and chlorophyceae (Figure 5 and 6).

STI values showed that the first two reservoirs in the cascade, Foz do Areia and Salto Segredo, were eutrophic in the

Table 4. Phytoplankton functional groups and their taxonomic group, classified in dominant (dom.) and abundant (ab.) species.

Functional group	Taxon	Taxonomic group	Dominant and abundant species
H1	*Aphanizomenon gracile*	*Cyanobacteria*	ab.
H1	*Dolichospermum circinalis*	*Cyanobacteria*	ab.
H1	*Dolichospermum crassum*	*Cyanobacteria*	ab.
H1	*Dolichospermu solitarium*	*Cyanobacteria*	ab.
H1	*Dolichospermum spiroides*	*Cyanobacteria*	ab.
K	*Aphanocapsa annulata*	*Cyanobacteria*	ab.
K	*Aphanocapsa koordersii*	*Cyanobacteria*	ab.
K	*Aphanocapsa sp.*	*Cyanobacteria*	ab.
K	*Chroococcum minutus*	*Cyanobacteria*	ab.
K	*Cyanodictyon sp.*	*Cyanobacteria*	ab.
K	*Eucapsis densa*	*Cyanobacteria*	ab.
K	*Synechocystis sp.*	*Cyanobacteria*	ab.
S1	*Geitlerinema sp.*	*Cyanobacteria*	ab.
S1	*Microcrocis pulchella*	*Cyanobacteria*	dom. ab.
S1	*Pseudanabaena mucicola*	*Cyanobacteria*	dom. ab.
M	*Microcystis aeruginosa*	*Cyanobacteria*	dom. e ab.
M	*Microcystis panniformis*	*Cyanobacteria*	ab.
M	*Microcystis protocystis*	*Cyanobacteria*	dom. e ab.
M	*Microcystis robusta*	*Cyanobacteria*	ab.
M	*Microcystis wesenbergii*	*Cyanobacteria*	ab.
M	*Sphaerocavum brasiliense*	*Cyanobacteria*	dom. e ab.
Z	*Synechococcus nidulans*	*Cyanobacteria*	ab.
F	*Kirchneriella sp.*	*Chlorophyceae*	dom. e ab.
C	*Cyclotella meneghiniana*	*Bacillariophyceae*	ab.
B	*Cryptomonas brasiliensis*	*Cryptophyceae*	ab.
B	*Rhodomonas minuta*	*Cryptophyceae*	dom. e ab.
L_M	*Ceratium hirundinella*	*Dinophyceae*	ab.
L_O	*Merismopedia tenuissima*	*Cyanobacteria*	ab.

three sampling campaigns, coinciding with period of intense cyanobacteria bloom, mainly *M. aeruginosa* and *S. brasiliense* in the first two sampling campaigns (November/2012 and February/2013).

According to the results related to eutrophication, both natural and non-natural causes were responsible by the effects of cyanobacteria blooms. Non-natural secondary causes, such as nutrient carriage from agriculture and illegal effluent discharges, results in the increase of phosphorus concentration inside reservoirs. Non-natural causes associated with natural causes like rainfall and elevated water temperature caused cyanobacteria blooms, mainly of *M. aeruginosa* and *S. brasiliense* (Figure 2).

PCA analysis indicated that the cyanobacteria species responsible by blooms, *M. aeruginosa* and *P. mucicola*, were benefited by elevated temperatures. Their presence contributed to increase conductivity and alkalinity values in November/2012. The increase in conductivity values may occur as a consequence of the production of free ions in the water column, result of photosynthetic processes. Foz do Areia and Salto Segredo reservoirs, were related with strong eutrophication in the first two months, where elevated turbidity was promoted by cyanobacteria blooms. *S. brasiliense* was abundant in February/2013. DOC promoted the increase in *R. minuta* density in October/2013.

CONCLUSION

The results showed that the four reservoirs had water quality characteristics of eutrophic environments, with intense cyanobacteria blooms (*M. aeruginosa* and *S. brasiliense*). The blooms happened mainly in November/2012 and February/2013. *M. aeruginosa* was better adapted to the environment and dominated *S. brasiliense*. There was no evidence of longitudinal gradient related to phytoplankton. However, in February/2013 there was a slight tendency in the first three reservoirs. Intense bloom events, followed by elevated STI indexes, indicate that reservoirs in the Iguazu River suffer environmental degradation, with water quality alteration threatening biological communities, showing the influence of cascade reservoirs system.

Despite the natural vegetation in the banks of Foz do Areia reservoir and the environmental protection area in Salto Segredo reservoir, the banks of the reservoirs are mainly occupied by agricultural lands. Therefore, eutrophication occurrence, followed by cyanobacteria blooming, are consequence of the land use type in the watershed and by illegal effluent discharges upstream Foz do Areia reservoir, in the Metropolitan Region of Curitiba (MRC).

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Clarisse Teixeira Adloff: Carried out the qualitative and quantitative analysis of phytoplankton.

Carla Cristina Bem: Assisted in the sampling and chemical analyzes.

Gabriela Reichert: Assisted in the interpretation and in the final text.

Júlio César Rodrigues de Azevedo: Advisor, assistance in collecting, financial support and text.