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Primary Research Paper

Structural and functional phytoplankton responses to nutrient impoverishment in mesocosms placed in a shallow eutrophic reservoir (Garças Pond), São Paulo, Brazil

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Abstract

Experiments were performed in mesocosms placed in a shallow eutrophic reservoir for the purpose of testing a nutrient removal technique as an eutrophication reduction method. Garças Pond is in the *Parque Estadual das Fontes do Ipiranga* Biological Reserve, located in the southeastern part of the Municipality of São Paulo (São Paulo State). Three different treatments were designed and each was conducted within two enclosures (closed systems) containing 360 l of water. Mesocosms built with polyethylene bags and PVC pipes were attached to the reservoir bottom. Treatment dilutions were made by using different proportions of pond and oligotrophic tributary water, following Carlson's trophic state index modified by Toledo and collaborators. Dilutions constituted the oligo-, meso-, and eutrophic treatments. Ten abiotic and nine biological variables were simultaneously studied over 31 days. Species richness, diversity, evenness, and dominance are discussed. Dissolved oxygen concentration decrease was observed in all treatments during the entire study period, whereas ammonium and free CO_2 values increased, indicating decomposition process presence in all treatments. Nutrient impoverishment induced P limitation in all treatments during most of the study period, a fact that, considered together with the free CO_2 concentration increase, low alkalinity values, and pH reduction throughout the period clearly indicated a photosynthetic activity decrease. Confinement and nutrient dilution led to changes in floristic composition as well as in phytoplankton biomass in all treatments. The initial community mainly represented by R- and S-strategists (*Planktothrix*, *Cylindrospermopsis* and *Microcystis*) was gradually replaced by C-strategists (*Cryptomonas* spp., Chloroccoccales in general). Characteristics of the initial succession phases were observed in all treatments. Thus, the community was first inhabited by fast growing species but no important biomass contribution of size fractions was observed. Space liberated by blue-green species that did not adapt to the new nutrient-impoverished conditions was gradually occupied by other algal species, which together contributed to most of the total biomass registered in all treatments.

Introduction

Knowledge of phytoplankton community structure and dynamics, which is necessary to understand eutrophication effects, remains a research challenge. To understand phytoplankton composition and succession it is necessary to know both the ecological characteristics of certain species as well as those of particular taxonomical and ecological groups (Krienitz et al., 1996). Relatively little is known as yet about phytoplankton changes during restoration (reducing P-load) (Padisák & Reynolds, 1998), mainly in tropical systems. Examples of such works are those of Cronberg

(1982, 1999), Rip et al. (1992), Gaedke & Schweizer (1993), Sommer et al. (1993), Perrow et al. (1994), Pádisak & Reynolds (1998), Ruggiu et al. (1998), Annadotter et al. (1999), Cronberg et al. (1999), and Barbieri & Simona (2001).

According to Santos & Calijuri (1997), species composition and phytoplankton community dynamics are influenced by several factors, such as available nutrient quantities, physical variables (light, temperature, and water column mixing), and biological interactions (grazing and competition). Which factor really governs the community structure at each moment and place is the goal of phytoplankton ecologists.

Changes in both community composition and strategies can be understood as populational responses to environmental changes. For example, phytoplankton species are known to develop morphological and physiological adaptation strategies allowing survival in environments such as pelagic ones (Reynolds, 1988).

Many researchers in considering species composition biomass and variation have emphasized that most physiological and behavioral attributes of individual organisms or populations may influence the taxonomical composition variability of a community (Sandgren, 1988).

It is a well-known fact that phytoplankton use different survival strategies, and that many species can absorb phosphorus more efficiently than others. Populations with different attributes can make use of these strategies depending on the environmental conditions, and will remain in it while others may disappear. Any environmental condition modification might 'weight' other attributes and adaptations of a species towards alternative strategies, leading to alternative assemblages (Reynolds, 1998).

Nutrient reversion techniques have been continuously studied and applied to diminish the harmful effects of artificial eutrophication. As a part of this effort, experiments (Pearl & Bowles, 1987; Dodds & Randel, 1992; Dodds et al., 1993; Ortega-Mayagoitia et al., 2002, 2003) have been used to increase knowledge about nutrient dynamics and its effects on biological communities, as well as to test techniques for recovering impacted environments. In addition, such techniques can lead to important information about survival strategies and community relationships.

Scarcity of studies emphasizing the oligotrophication process or experimental simulation of phytoplankton community response to nutrient impoverishment in tropical and subtropical systems is considerable, mainly in the less developed countries. Experimental studies in environments that will be submitted to a recovery program, as will be the Garças Pond, allow to test, discuss and evaluate some techniques, so that scientific and financial efforts could be directly addressed.

Mesocosm studies designed to decrease nutritional concentration aim at simulating the oligotrophication process through nutrient input reduction, leading to new trophic conditions and, consequently, community structure changes. Understanding responses as well as phytoplankton community survival strategies will allow evaluation of the impoverishment technique as a method to decrease eutrophication content in shallow lakes.

The present paper aims at understanding the effects caused by chemical nutritional dilutions on floristic composition, dynamics, and phytoplankton community structure, thus contributing to the increase of information on this complex community in tropical and subtropical systems.

Study area

Garças Pond is located in the *Parque Estadual das Fontes do Ipiranga* Biological Reserve situated in the southeast region of the Municipality of São Paulo (23° 38′ 08″ S and 23° 40′ 18″ S; 46° 36′ 48″ W and 46° 38′ 00″ W). Its mean altitude is 798 m and the total area comprises 526.4 ha (Fernandes et al., 2002) (Fig. 1). The reserve is one of the few remaining patches of *Mata Atlântica* in the midst of the urbanized region of the city of São Paulo, and one of the largest metropolitan green areas in Latin America (Struffaldi-De-Vuono, 1985).

Although locally called Garças Pond, the system is, in fact, a reservoir recently classified as eutrophic (Bicudo et al., 2002). A shallow environment having a 4.7 m maximum depth, it is the most anthropically affected system in the reserve. The surface area is 88 156 m^2, with a 512 m maximum length, 319.5 m maximum width, and a 45-day mean residence time (Bicudo et al., 2002). Garças Pond has one exit and seven tributaries,

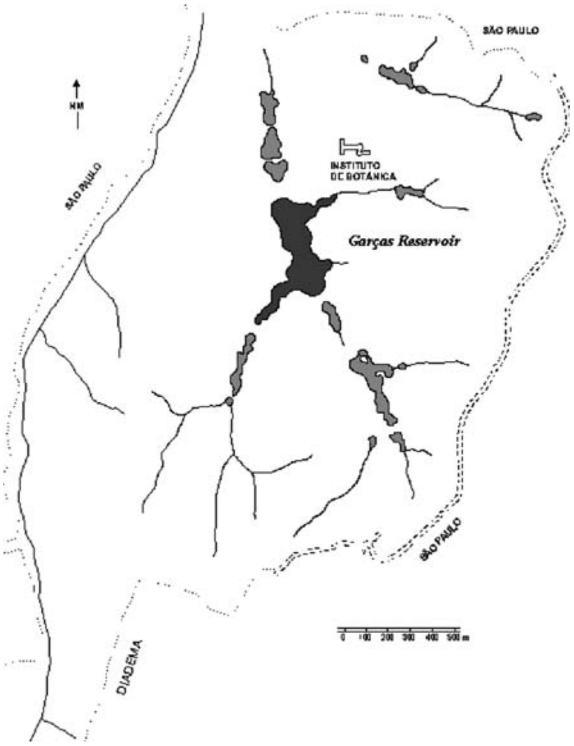

Figure 1. Parque Estadual das Fontes do Ipiranga Biological Reserve map showing the Garças Pond location (modified from Bicudo, 1984).

four of which carry sewage *in natura* and only one of which is considered oligotrophic (Carmo et al., 2002).

Material and methods

The study was carried out from 10 August to 9 September 2000. Three different treatments were designed, each one using two enclosures containing 360 l of water. Mesocosms were constructed of polyethylene bags and PVC pipes attached to the reservoir bottom 50 cm from one another to avoid mutual contamination. They were isolated from the sediment (closed systems). Mesocosms dimensions were 70 cm length and 45 cm width. Treatments had any interaction with the reservoir. Treatment dilutions were prepared by using different proportions of Garças Pond water and that from the reservoir's single oligotrophic tributary (Table 1), following Carlson's trophic state index modified by Toledo et al. (1983). Latter authors adapted the original Carlson's (1977) trophic index to tropical systems and proposed the use of weighed mean, using concentrations of orthophosphate (weight 2), total phosphorus (weight 2), chlorophyll *a* (weight 2), and water transparency (weight 1). Its has been used during many studies in Brazil, among which by Mercante & Tucci (1999) and Bicudo et al. (2002) who applied and discussed its use for the Garças Pond. Dilutions constituted oligotrophic (O1 and O2), mesotrophic (M1 and M2), and eutrophic (E1 and E2) treatments (Table 2). Then, treatments presented different trophic conditions and the initial community was constituted by the oligotrophic tributary and the eutrophic lake populations.

Ten abiotic and nine biological samplings were simultaneously carried out. The following physical and chemical water variables were analyzed: temperature, electric conductivity, dissolved oxygen (Golterman et al., 1978); pH, alkalinity (Golterman & Clymo, 1971); free CO_2, bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) (Mackeret et al., 1978); total nitrogen (TN) (Valderrama, 1981); ammonium (NH_4^+) (Solorzano, 1969); nitrate (NO_3^-) and nitrite (NO_2^-) (Mackeret et al., 1978); orthophosphate (PO_4^{3-}) and total dissolved phosphorus (TDP) (Strickland & Parsons, 1960); total phosphorus (TP) (Valderrama, 1981); and SiH_4O_4 (Golterman et al., 1978). Mesocosm's contents were continuously homogenized. Trophic state

Table 1. Water volume and dilution in each treatment, total phosphorus (TP) concentration, and conductivity in the lake and in the tributary water

	Pond water (l)	Tributary water (l)	Dilution	Lake TP and conductivity value	Tributary TP and conductivity value
Oligotrophic	30	330	12×		
Mesotrophic	60	300	6×	246 $\mu g\ l^{-1}$	5 $\mu g\ l^{-1}$
Eutrophic	360	–	–	239.3 $\mu S\ cm^{-1}$	31.3 $\mu S\ cm^{-1}$

Table 2. Total phosphorus concentration following Carlson's trophic state index modified and total phosphorus concentration in the mesocosms

	Carlson's trophic state index modified by Toledo et al. (1983) (μg P l^{-1})	TP concentration in the treatment (μg P l^{-1})
Oligotrophic	<44	<20
Mesotrophic	44–54	45–50
Eutrophic	>54	>58

index in each one was verified before sampling. Mesocosms were installed in the pelagic region of the lake to avoid human and shadow interference.

Samplings were always performed in the morning at 3-day intervals until day 20 and, after that, at 5-day intervals over 31 days. The samplings were drawn from the mesocosm water surface and samples were kept in cold polystyrene boxes until laboratory analysis. Chemical analyses were carried out immediately after collection at the Aquatic Ecology Laboratory of the Ecology Section of the *Instituto de Botânica*, located about 600 m from the reservoir.

Taxonomic samplings were preserved with 4% formaldehyde water solution (Bicudo, 1990).

Phytoplankton quantitative study was carried out according to Utermöhl (1958); sedimentation time followed Lund et al. (1958). Quantification procedure was performed according to Bicudo (1990), using the species rarefying curve. Biomass (mg l^{-1}) was estimated using the biovolume obtained by multiplying each species' density by the mean volume of its cells considering, whenever possible, the mean dimension of 30 individuals of each species following Edler (1979), Torgan et al. (1998), Hillebrand et al. (1999), and Tucci (2002), among others.

Taxonomic identifications followed the most recent possible literature (e.g. Komárek & Fott, 1983; Sant'Anna, 1984; Komárek & Anagnostidis, 1986, 1989, 1999; 2000; Sant'Anna et al., 1989; Castro et al., 1991; Xavier, 1994; Komárková-Legnerová & Cronberg, 1994; Azevedo et al., 1996; Komárek & Azevedo, 2000; Sant'Anna & Azevedo, 2000).

Size classification of species followed Sieburth et al. (1978), i.e., picoplankton included cells of between 0.2 and 2 μm, nannoplankton between 2 and 20 μm, microplankton between 20 and 200 μm, and macroplankton between 200 μm and 2 mm, and were obtained by the GALD (Greatest Axial Linear Dimension, in μm). Diversity index followed Shannon & Weaver (1963); evenness, Lloyd & Ghelardi (1964) and the dominance index, Simpson (1949).

Multivariate descriptive analysis was processed by applying the principal component analysis (PCA) to the abiotic data, to a covariance matrix with data transformed by ranging. For the canonical correspondence analysis (CCA), abiotic and biological data were transformed log ($x + 1$). For the transformed data, software used was FITOPAC (Shepherd, 1996); PC-ORD version 3.0 for Windows (McCune & Mefford, 1997) was used for the analysis.

Mean values comparisons between treatments were made by variance analysis (one-way ANOVA) using STATISTICA for Windows 5.5 (StatSoft, 1999).

Results

Abiotic variables

Water temperature and subaquatic solar radiation (Table 3) were not significantly different among treatments (one-way ANOVA: $F = 0.08$; $p > 0.0001$). Moreover, the Tukey test ($\alpha = 0.05$) considered all three systems similar considering these variables.

Conductivity was different between treatments after dilutions. Higher ammonium values registered at the end of the experiment period would have contributed to the gradual conductivity increase in the oligotrophic treatment. Dissolved oxygen decreased while the ammonium concentration increased. After nutritional impoverishment, increase of free CO_2 concentration and pH reduction were observed justifying the low alkalinity values (Table 3).

Table 3. Minimum and maximum values and, between parenthesis, mean and standard deviation values ($n = 20$) of water variables in the three treatments

	Eutrophic	Mesotrophic	Oligotrophic
Water temperature (°C)	14.9–19.0	15.7–19.6	14.8–19.4
	(17.3 ± 1.3)	(17.4 ± 1.2)	(17.2 ± 1.4)
Subsuperficial solar radiation (μmol s^{-1} m^{-2})	38.1–305.8	36.7–364.7	22.3–190.7
	(150.5 ± 98,1)	(151.0 ± 99.4)	(97.8 ± 56.2)
Conductivity (μS cm^{-1})	206.5–283.5	75.0–113.4	51.0–66.5
	(225.7 ± 7.4)	(89.9 ± 3.41)	(60.8 ± 1.4)
DO (mg l^{-1})	3.6–15.7	5.16–11.7	5.5–10.6
	(9.6 ± 1.4)	(9.17 ± 0.7)	(8.8 ± 0.5)
Free CO$_2$ (mg l^{-1})	0.001–8.7	1.1–8.5	3.1–13.7
	(2.0 ± 0.9)	(5.23 ± 0.8)	(8.3 ± 1.3)
HCO$_3^-$ (mg l^{-1})	15.5–60.9	11.6–18.9	7.4–10.6
	(38.7 ± 4.9)	(14.78 ± 0.7)	(8.5 ± 0.4)
CO$_3^{-2}$ (mg l^{-1})	0.04–13.4	0.002–0.013	0.0003–0.002
	(5.0 ± 1.6)	(0.005 ± 0.001)	(0.001 ± 0.0002)
PH	7.1–10.3	6.4–7.4	5.9–6.8
	(8.8 ± 0.4)	(6.75 ± 0.1)	(6.3 ± 0.08)
Alkalinity (mEq l^{-1})	0.7–1.0	0.2–0.3	0.12–0.17
	(0.8 ± 0.03)	(0.2 ± 0.01)	(0.14 ± 0.01)
PO$_4^{-3}$ (μg l^{-1})	1.0–9.1	1.0–5.1	1.0–3.2
	(3.7 ± 0.7)	(2.52 ± 0.4)	(1.9 ± 0.3)
TDP (μg l^{-1})	14.1–24.6	2.1–10.9	1.0–5.4
	(21.5 ± 1.1)	(6.51 ± 0.8)	(2.0 ± 0.5)
TP (μg l^{-1})	160.9–388.5	25.0–52.2	16.4–22.2
	(221.5 ± 22.1)	(38.8 ± 2.7)	(18.7 ± 0.6)
NO$_2^-$ (μg l^{-1})	5.0–390.2	5.0–87.1	5.0–35.4
	(77.2 ± 48.3)	(20.04 ± 9.8)	(10.6 ± 3.5)
NO$_3^-$ (μg l^{-1})	8.0–258.6	22.5–591.0	81.4–652.3
	(33.6 ± 25.0)	(239.42 ± 49.0)	(356.3 ± 53.9)
NH$_4^+$ (μg l^{-1})	4.0–659.9	4.0–336.8	4.0–181.9
	(90.8 ± 66.3)	(80.32 ± 37.1)	(39.2 ± 18.4)
TN (μg l^{-1})	1,244.5–6,819.5	253.9–1,367.3	108.7–965.6
	(3,420.3 ± 530.0)	(704.9 ± 109.0)	(587.5 ± 92.3)
Silicate (mg l^{-1})	1.1–3.1	1.0–3.7	1.0–3.8
	(2.2 ± 0.2)	(2.39 ± 0.2)	(2.5 ± 0.3)

Orthophosphate values were low during the entire experiment in all mesocosms. Total phosphorus concentration was unaltered during the experiment period, following the limits established by the Carlson trophic state index modified by Toledo et al. (1983), except for the end of the experiment period in the mesotrophic treatment.

In all mesocosms, a total nitrogen concentration rise at the beginning of the experiment was registered, probably stimulated by the higher nitrite and nitrate values registered also at the first period of present study. Correspondingly, at the end of the experiment, total nitrogen concentration declined.

PCA using 14 abiotic variables employed 76.4% of data variability in the first two ordination axes (Table 4, Fig. 2). The following seven variables were the most important ones ($r > 0.7$) for axis 1 ordination: electric conductivity, pH, total phosphorus, dissolved total phosphorus,

Table 4. Abiotic variables correlations (n = 30) with the principal components

Variable	Axis 1	Axis 2
NH_4^+	0.006	−0.655
NO_2^-	−0.427	0.350
NO_3	0.679	0.395
TN	−0.888	0.092
PO_4^{3-}	−0.577	0.440
TDP	−0.933	−0.223
TP	−0.942	−0.004
$SiOH_4O_4$	0.027	0.759
Conductivity	−0.948	−0.212
PH	−0.945	0.273
DO	−0.333	0.853
Free CO_2	0.719	−0.278
HCO_3^-	−0.670	−0.665
CO_3^-	−0.790	0.478
Explained variation	56.6%	19.8%

total nitrogen, carbonates, and free CO_2. However, other variables such as nitrates, bicarbonates, and orthophosphates were also important (r > 0.5). According to axis 2, dissolved oxygen and silicates were the variables contributing most (r > 0.7), followed by ammonium and bicarbonates (r > 0.5) (Table 4, Fig. 2). The first axis (56.6%) clearly separated the eutrophic from the oligotrophic treatment, the mesotrophic units remaining in an intermediate position. Consequently, axis 1 of the PCA represented the trophic gradient whereas axis 2 (19.8%) separated treatments according to temporal variation. This conclusion is possible because at the positive side of axis 2 higher values were observed during the beginning of the study in all mesocosms. At the negative side of axis 2, all treatment units of the end of the study period were grouped, associated by bicarbonate and ammonium concentrations, which presented their highest values at the end of the experiment.

Biological variables

One hundred and thirteen taxa including species, varieties, and taxonomic formae were identified in the treatments, which were grouped in 9 classes, 12 orders, 29 families, and 54 genera. Total biomass decline after dilutions was about four times in the

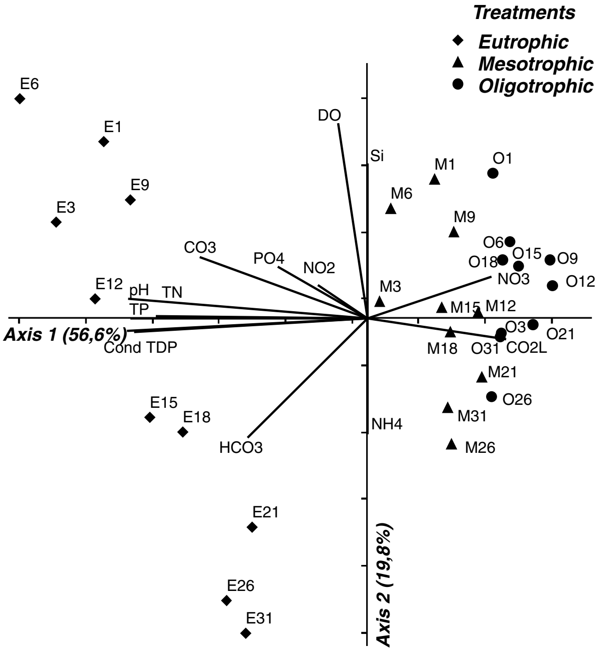

Figure 2. Biplot of PCA for the mean values of abiotic variables of the three treatments during the study period. Abbreviations: Cond.: conductivity; CO_2: free carbonic gas; DO: dissolved oxygen; TN: total nitrogen, pH: pH; TP: total phosphorus; TDP: total dissolved phosphorus; PO_4: orthophosphate; HCO_3: bicarbonate; CO_3: carbonate; NH_4: ammonium; NO_2: nitrite; NO_3: nitrate; Si: silicate; O_2: dissolved oxygen; E: eutrophic treatment; M: mesotrophic treatment; O: oligotrophic treatment. Numbers indicate experiment day.

mesotrophic and 13 times in the oligotrophic mesocosms, compared to the eutrophic one (Fig. 3), and differed significantly among treatments (one-way ANOVA: $F = 66.9$; $p = 0.0001$).

Richness increase was observed in the eutrophic treatment during the experiment period, the highest value (67 species) having occurred on day 31 (Fig. 4). In the diluted treatments, however,

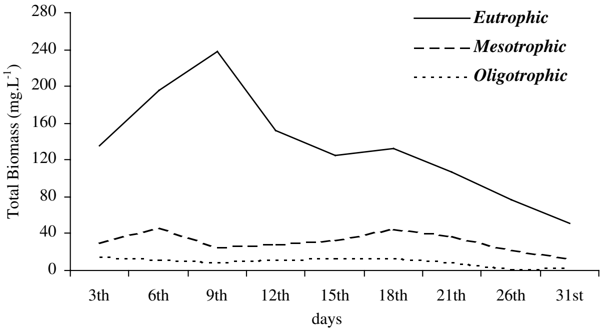

Figure 3. Total biomass (mg l^{-1}) in the three treatments during the experiment period ($n = 2$).

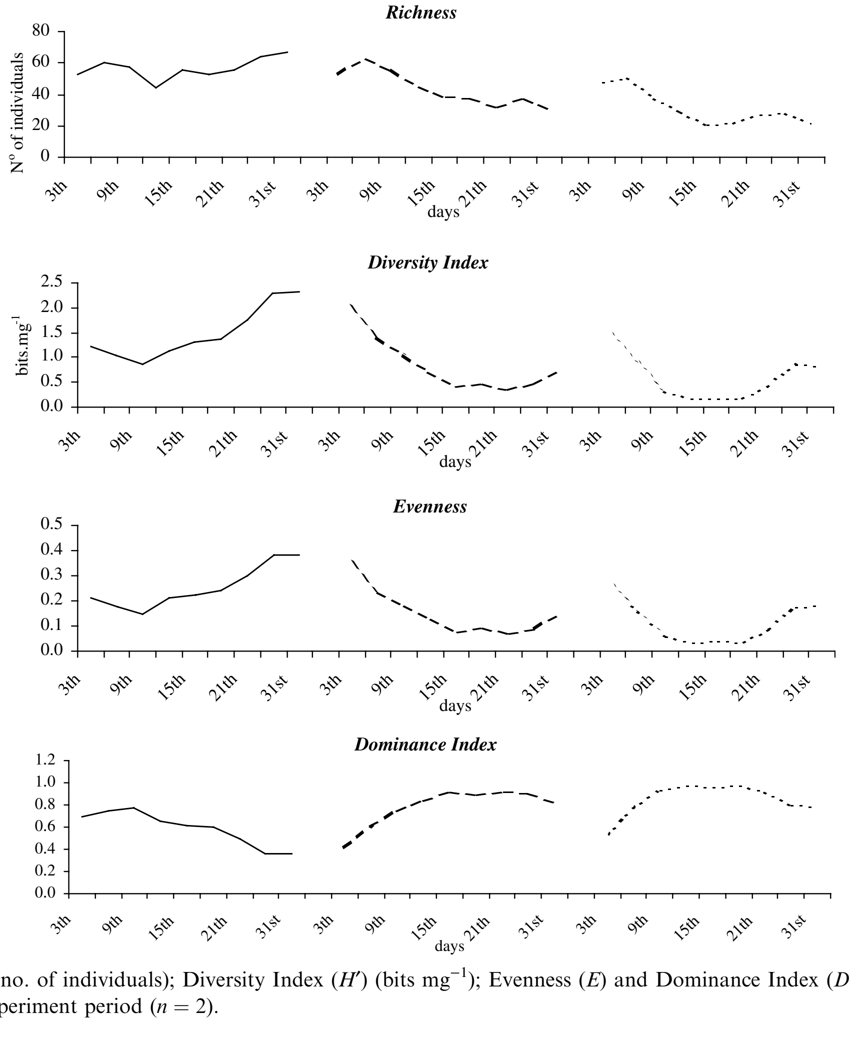

Figure 4. Richness (no. of individuals); Diversity Index (H') (bits mg^{-1}); Evenness (E) and Dominance Index (D) in the three treatments during the experiment period ($n = 2$).

richness declined from the very first day of study. In the latter systems, the impoverishment procedure did not equally favor the species initially living in the pond water used in the mesocosms. In other words, chemical stress generated by dilution did not favor some species that were adapted to eutrophic conditions.

Nano- and microplankton species were documented. In the eutrophic treatment, nannoplankton forms contributed 3.9% to the total biomass and microplankton forms accounted for 96.1%. In the meso- and oligotrophic treatments, microplankton contributed, respectively, 93.5 and 95.1%, followed by the nannoplankton algae (6.5 and 4.9%, respectively). A small rise in the percentage of contribution of nannoplankton in the eutrophic systems was registered from the beginning to the end of the experiment period (3rd day = 2.6%; 31st day = 8.5%), whereas microplankton declined (3rd day = 97.4%; 31st day = 91.4%). In the meso and oligotrophic mesocosms, nannoplankton contribution declined during the entire study period (3rd day = 18.1%; 31st = 6.7%; and 3rd day = 18.1%; 31st = 11.1%, respectively), and microplankton contribution increased (3rd day = 81.8%; 31st = 93.3%; and 3rd day = 81.8%; 31st = 88.8%, respectively).

Sommer (1981) and Taylor & Wetzel (1988) considered as the most important species in a phytoplankton community and the total biomass those that contribute with, respectively, more than 10 and 5% to the total biomass. During the present study, biological descriptors were considered those species contributing more than 1% to the total biomass. Therefore, 15 species were selected from all three treatments and contributed with more than 93% of total biomass (Fig. 5).

In all treatments, *Microcystis aeruginosa* presented the highest biomass values, which differed significantly among treatments (one-way ANOVA: $F = 38.9$; $p < 0.0001$). In the eutrophic mesocosms, *Planktothrix agardhii* also presented great relative biomass. In the diluted treatments *Trachelomonas sculpta* presented the highest biomass after *Microcystis aeruginosa* (Fig. 5).

Diversity index (H') increased in the eutrophic treatment from day 9 on, whereas in the diluted ones it declined since the beginning of the experiment period, increasing again in the very last days, probably influenced by some rare species contribution (Fig. 4). The highest diversity index value (2.32) in the eutrophic treatment was detected on day 31; meanwhile, in the meso- and oligotrophic ones it was detected on day 3 (2.02 and 1.46, respectively) (Fig. 4).

Evenness (E) values in the eutrophic mesocosms, as well as dominance index (D), indicated dominance reduction after isolation from allochthonous nutrient sources (Fig. 4). In the mesotrophic mesocosms, lowest evenness values were

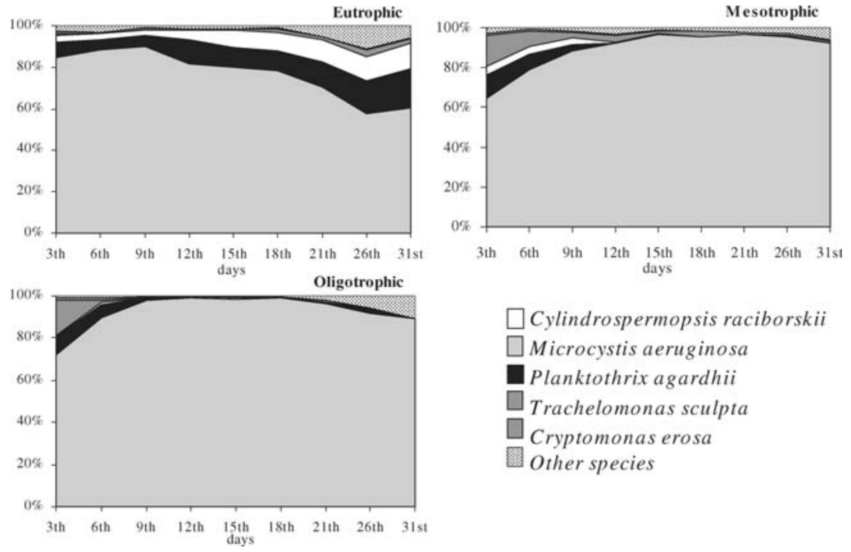

Figure 5. Relative biomass of phytoplankton descriptor species (% of mg l^{-1}) in the three treatments during the experiment period ($n = 2$).

registered from days 9 to 26, when the greatest dominance values were also measured. During this period, *M. aeruginosa* dominated. A similar situation was observed in the oligotrophic treatments, in which a decline of evenness values was registered until day 18 of the study, i.e., the period in which *M. aeruginosa* was dominant.

Integrated analysis of abiotic and biological parameters

Ordination of sample units of the eutrophic, mesotrophic, and oligotrophic treatments using canonical correspondence analysis (CCA) was performed using five abiotic (the ones presenting the highest correlation with the first two axis of the PCA, thus avoiding redundant variables) and 15 biological variables (species that contributed more than 1% of the total biomass). The eigenvalues for axes 1 and 2 were 0.171 and 0.102, respectively, which explained 55.6% of species variance in the first 2 axes (Table 5). The Pearson correlation of environment-species was high for both axes (0.919 and 0.937), indicating a strong correlation between abiotic variables and algal species distribution. The Monte Carlo test (99 permutations; $p \leq 0.05$) used to determine significance level of canonical axis 1 demonstrated that the ordination of axes 1 and 2 was statistically significant ($p \leq 0.05$), and did not occur at random (Table 5).

To explain data variability, the canonical coefficient that represents the importance of the contribution of each environmental variable to the ordination of the axis was used. Also used was the intra-set correlation representing possible correlations between the abiotic variables and their ordination with that axis, but retaining the dependence relation between biological and abiotic variables. To reinforce the latter, Pearson and Kendall's correlation (r) coefficient was also verified.

Canonical coefficient and intra-set correlation (Table 6) showed that conductivity was the most important variable related to axis 1 ordination. At the positive side of axis 1, most of the abiotic variables and sample units of the mesotrophic and oligotrophic treatments were associated with the highest free CO_2 values (Fig. 6). Species with highest correlation ($r \geq 0.7$) with this axis were: *Cylindrospermopsis raciborskii*, *Planktothrix agardhii*, *Microcystis aeruginosa*, *Cryptomonas tetrapyrenoidosa*, *C. erosa*, and *C. curvata*. At the other side of axis 1, eutrophic sample units were associated with the highest values of conductivity, pH, TP, and TN. Axis 1 represented, from right to left, the reduction of nutrients in the systems. Regarding axis 2, canonical coefficient (Table 6) showed pH as the most important variable to its ordination, whereas the intra-set correlation pointed free CO_2. Species with the highest correlation ($r \geq 0.5$) with axis 2 were: *Protocryptomonas ellipsoidea*, *Peridinium* cf. *volzii*, and *Coelosphaerium evidenter-marginatum*.

One functional group was formed related to the diluted mesocosms, which was composed by greens (*Scenedesmus bicaudatus*, *S. quadricauda*, and *S. ovalternus*), blue-greens (*Microcystis aeruginosa*), and euglenoids (*Trachelomonas sculpta* and *T. volvocina*). Biological descriptors of eutrophic treatment were *Cylindrospermopsis raciborskii*, *Planktothrix agardhii*, *Cryptomonas tetrapyrenoidosa*, *C. erosa*, and *C. curvata*.

Table 5. CCA synthesis for all three sets of treatment data

	Axis 1	Axis 2
Eigenvalue	0.171	0.102
% of variance explained	34.9	20.7
Cumulative% explained	34.9	55.6
Parsons correlation (species:environment)	0.919	0.937
Monte Carlo test (p) Eigenvalues	0.01	0.01
Correlation species–environment	0.01	0.01

Table 6. Canonical coefficient and intra-set correlation of environmental variables for axes 1 and 2 for five abiotic variables in the three treatments

Variable	Canonical coefficient		Correlation coefficient (intra-set)	
	Axis 1	Axis 2	Axis 1	Axis 2
TN	−0.313	−0.358	−0.888	0.261
TP	−0.352	−0.68	−0.947	−0.09
Conductivity	−0.554	−0.535	−0.986	−0.006
pH	0.057	1.915	−0.804	0.591
Free CO_2	−0.02	0.076	0.671	−0.648

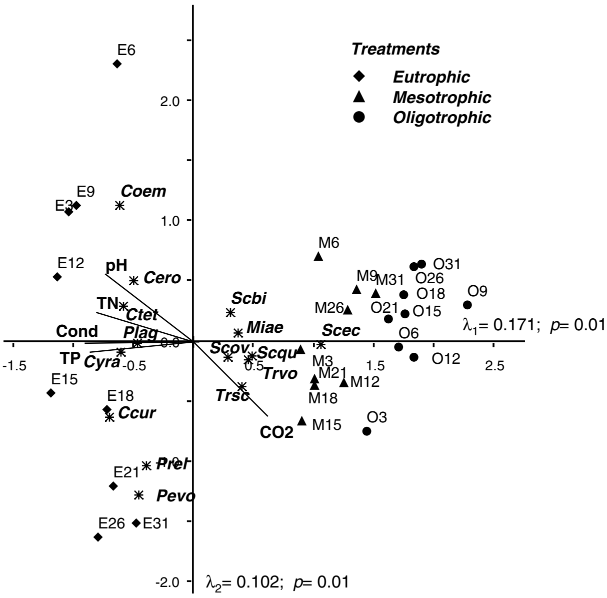

Figure 6. Biplot of CCA to mean values of five abiotic variables and 15 biological variables of the three treatments during the study period. Abbreviations: Scbi: *Scenedesmus bicaudatus*; Scov: *Scenedesmus ovalternus*; Scqu: *Scenedesmus quadricauda*; Scec: *Scenedesmus ecornis*; Ccur: *Cryptomonas curvata*; Cero: *Cryptomonas erosa*; Ctet: *Cryptomonas tetrapyrenoidosa*; Prel: *Protocryptomonas ellipsoidea*; Cyra: *Cylindrospermopsis raciborskii*; Miae: *Microcystis aeruginosa*; Plag: *Planktothrix agardhii*; Coem: *Coelosphaerium evidenter-marginatum*; Pevo: *Peridinium* cf. *volzii*; Trsc: *Trachelomonas sculpta*; Trvo: *Trachelomonas volvocina*; Cond.: conductivity; CO_2: free CO_2; TN: total nitrogen; pH: pH; TP: total phosphorous; E: eutrophic treatment; M: mesotrophic treatment; O: oligotrophic treatment. Numbers indicate experiment day.

Discussion

Dissolved oxygen decreased at the same time ammonium concentration increased suggesting some ongoing decomposition process in the mesocosms.

Phytoplankton community confinement in mesocosms led to changes in their floristic composition not only because of nutrient impoverishment, but also due to the isolation of mixing patterns. A similar observation was made by Santos & Calijuri (1998) after studying survival strategies of some species in microcosm experiments.

Community composition was very similar in all treatments. Mesocosms were built with eutrophic water from the lake and oligotrophic water from one tributary that also supply the pond. Not filtering the tributary water could have interfered in the floristic composition, but it was not observed since the populations registered at the beginning of the study period in all treatments were not so different.

Total biomass presented a significant decline from the beginning to the last day of the experiment period. Zooplankton was not quantified, but very few individuals were observed despite of an adequate sampling for representatives of this group has not been carried out. So, it was possible to infer that grazing should not be considered an important interference during the present research. Changes observed in the present study were not obvious enough to substantially increase specific richness in the diluted treatments. Rare species occurred in these systems, especially in the oligotrophic ones, however their relative density and biovolume did not contribute enough to the biomass increase. After some days, representatives of other algal classes began occupying the space left by cyanobacteria not adapting to the newly established conditions, such as *Microcystis aeruginosa*, *Cylindrospermopsis raciborskii*, and *Planktothrix agardhii*. However, this replacement was very subtle and perhaps the 31 days of the present study were not enough to detect their greater contribution to the total biomass.

Although it was observed a small rise of the nannoplankton contribution in the eutrophic mesocosms and their decline in the diluted systems, no important changes were observed in size fractions biomass contribution during the experiment.

Microcystis aeruginosa was the alga whose biomass contributed most in all treatments. However, its decline was verified along the entire experimental period. Even after homogenization of bags just before sampling, *M. aeruginosa's* buoyancy capacity may have favored their permanence in the mesocosms, mainly in the eutrophic ones. Santos et al. (1997, 1998) performed microcosm's experiments and registered the dominance of *M. aeruginosa* in very similar conditions to those of the eutrophic treatments of the present study.

In the diluted treatments, *Planktothrix agardhii* biomass quickly declined after the beginning of the experimental procedure, and totally disappeared from the mid until the last day of the experimental period.

Cylindrospermopsis raciborskii seemed to be ill adapted to nutrient impoverishment conditions, since its biomass in the diluted treatments dropped from the beginning to the end of the study period. This species is one of the most notorious among the blue-green algae due to its toxic potential and tendency to form blooms in eutrophic systems. *Cylindrospermopsis raciborskii* inhabits very different environments with wide habitat diversity in terms of both geomorphology and water chemistry, thus indicating a high level of ecophysiological adaptability of this species (Padisák, 1997). In the present study, biomass of *C. raciborskii* increased until day 18 in the eutrophic mesocosms. Its high capacity for quick phosphorus uptake, even from low concentrations, and for nitrogen fixing (Padisák, 1997), which extended its survival in a situation of nutritional stress, surely contributed to its success in eutrophic system during that period of the present experiment.

Among the green algae, *Scenedesmus quadricauda*, *S. ovalternus*, *S. bicaudatus*, and *S. ecornis* biomass in all systems were probably favored by *Microcystis aeruginosa* and *Planktothrix agardhii* decrease after enclosure. According to Shapiro (1973), low pH and low CO_2 availability may, among other variables, explain the density increase of the green after the blue-green algae density decline.

Cryptomonas are typical opportunist organisms. Studies in Brazilian reservoirs showed that their survival strategies lie between those of the colonizing (C-strategists) and tolerant species (R-strategists) (Santos & Calijuri, 1998). This fact would explain their permanence over long periods in the water column and their occurrence in environments under nutritional stress. According to Klaveness (1988), this alga increases its relative density closely following some disturbance that simultaneously causes the decline of another species. In the present study, this fact was conspicuous in the eutrophic treatment where the highest biomass contribution of *Cryptomonas* spp. was registered in the very first days right after enclosure, increasing eutrophic biomass values, followed by its decline until the end of the experiment period. From day 15 on, this decline was simultaneously accompanied by the appearance of *Protocryptomonas ellipsoidea*.

Trachelomonas sculpta and *T. volvocina* occurred in all treatments. In the diluted ones, these two species presented higher biomass at the beginning of the study period, which were followed by marked decline. The two euglenoids were also favored by the blue-green algae substitution in the enclosures.

Despite the blue-green algae having contributed to a greater extent to total phytoplankton biomass, some species in that class were not clearly favored by nutrient impoverishment, as was the case of *Coelosphaerium evidenter-marginatum*, *Chroococcus minutus*, *Cylindrospermopsis raciborskii*, *Microcystis aeruginosa*, *Planktothrix agardhii*, and *Merismopedia trolleri*.

Many studies had already discussed changes in phytoplankton in long-term oligotrofication process. In Lake Constance (Sommer et al., 1993; Gaedke & Schweizer, 1999) changes in phytoplankton species composition preceded the changes in biovolume. Ruggiu et al. (1998) showed that in Lake Maggiore those changes occurred probably at the same time, but biovolume never preceded species changes.

Cronberg et al. (1999) registered the occurrence of some toxic blue-green algae after the nutrient reduction program carried out for the Ringsjön Lake (Sweden). The results showed resistance of some species to nutritional impoverishment. In Lake Trummen (Cronberg, 1982), blue-green algae formed dense blooms before restoration, after what their biomass decreased drastically (85%). Some species disappeared completely while others increased their biomass.

Ortega-Mayagoitia et al. (2003) tested the controlling factors of phytoplankton assemblages in wetlands with microcosms experiments and suggested that alternate states of phytoplankton taxonomic structure occurred in those systems as a sequence of substitutions, thus showing their close relationship with the sediment and pointed out that trophic relationships or biotic factors were possibly irrelevant in the dynamics of phytoplankton assemblages when studying zooplankton and fish interactions.

According to the assemblages proposed in Reynolds (1997) and Reynolds et al. (2002), phytoplankton in the mesocosms was first composed by assemblages M, S_1 and Sn constituted of *M. aeruginosa, P. agardhii*, and *C. raciborskii.* These associations were progressively replaced, in response to mesocosms structure and new chemical conditions by assemblages Y and W_2 composed of *Cryptomonas* and *Trachelomonas*, and by assemblages L and J represented by *Peridinium* and *Scenedesmus* spp. Marinho & Huszar (2002) defined, after studying the Juturnaíba Pond in Rio de Janeiro State, its system as eutrophic and considered its assemblages as close to those in Reynold's (1997) assemblages first proposed for temperate region systems. Following these authors, Reynold's (1997) concepts would apply equally well to enriched tropical environments.

Mesocosm experiments are important consistent tools for aquatic community studies because they make it possible to estimate a specific variable and sometimes even eliminate the influence of some selected variables.

Nutritional impoverishment was attained by experimental nutrient concentration dilution and by keeping the selected trophic condition unaltered during the whole study period. Both enclosure and dilutions led to phosphorus limitation in the treatments during the greatest part of the study period. This fact, along with increasing free CO_2 concentrations, low alkalinity values, and pH reduction, also showed a fall in photosynthetic activity.

Dilutions led to changes in floristic composition in all three treatments. The phytoplankton community was mainly represented by R- and S-strategists (*Planktothrix, Cylindrospermopsis* and *Microcystis*) and subsequently by C-strategists (*Cryptomonas* spp. and Chloroccoccales in general).

Isolation from allochthonous nutrient sources and from physical characteristics, such as mixing and stratification patterns in Garças Pond water column, led to some features that recalled the beginning of the succession process when the systems were inhabited by fast-growing colonizing species. After some days, the blue-green algae monoculture was progressively replaced by some other well-adapted species. Although the sediment contribution and the mixing patterns are still being studied for the Garças Pond, phosphorus seems to be an important driving force.

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