

Influence of N/P ratio on competitive abilities for nitrogen and phosphorus by *Microcystis aeruginosa* and *Aulacoseira distans*

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Abstract *Microcystis aeruginosa* and *Aulacoseira distans* strains were grown in batch cultures to investigate the consequences of N/P ratio on the growth of these species and on their abilities to take up nitrogen and phosphorus. N/P ratio did not influence the growth rates, which were similar under all the experimental conditions. However, exponential growth lasted longer in *Microcystis* than in *Aulacoseira*, especially under low N/P ratio conditions. Distinct patterns of nutrient uptake for *Aulacoseira* and *Microcystis* were observed. N-uptake was higher in *Microcystis*, but not influenced by N/P ratio. However, the amount absorbed was proportional to the concentration in the culture medium for both strains studied. Although *Microcystis* showed lower uptake of N per biomass unit, a greater yield of *Microcystis* growth relative to the diatom was observed. This could have resulted from its ability to produce biomass using less nitrogen per unit of

biomass. A variation of N/P ratio in the culture medium during the growth of both species was observed. This owed to the uptake of nutrients, with *Microcystis* showing greater potential than *Aulacoseira* to influence the N/P ratio. Thus, in contrast to what has been stated in the literature, our results indicated that a low N/P ratio could be a consequence of the capacities and rates of cyanobacterial uptake of nitrogen and phosphorus.

Keywords Competition · Cyanobacteria · Diatoms · Growth · N and P uptake · Batch cultures

Introduction

Increasing eutrophication of freshwater ecosystems has resulted in the dominance of cyanobacteria in nutrient-enriched environments, although these organisms could also be important components of phytoplankton in mesotrophic waters (Hecky and Kling 1987; Canfield et al. 1989; Blomqvist et al. 1994; Huszar and Caraco 1998).

The dominance of cyanobacteria in nutrient-rich environments has been associated with a variety of factors. Environmental factors such as low turbulence (Reynolds 1987, but see Ganf 1974), low light (Zevenboom and Mur 1980; Smith 1986), low ratio of euphotic zone to mixing zone (Jensen et al. 1994), high temperature (Shapiro 1990), low CO₂/high pH (King 1970; Shapiro 1990; Caraco and Miller 1998),

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high total-P (McQueen and Lean 1987; Trimbee and Prepas 1987; Watson et al. 1997), low total-N (Smith 1983), low-dissolved inorganic nitrogen (DIN) (Blomqvist et al. 1994) and phosphorus storage strategy (Petterson et al. 1993) have been referred to be able to promote or allow cyanobacterial dominance.

Most of the above-mentioned and other studies have focused on certain bloom-forming species, especially in eutrophic environments, and the findings have allowed to make these generalizations. However, cyanobacteria are an ancient and diverse group of species with many different and sometimes contrasting ecophysiological strategies. These generalizations cannot always be extrapolated to the whole group, but need testing. Caution is needed in applying such unsubstantiated statements to all cyanobacteria.

Based on resource-ratio hypothesis (Tilman 1982), cyanobacterial dominance had also been attributed to low N/P ratios (Smith 1983; Tilman et al. 1986; Bulgakov and Levich 1999), as these organisms are thought to be better nitrogen competitors than other phytoplankton taxa (Barica et al. 1980; Horne and Commins 1987; Blomqvist et al. 1994). However, Reynolds (1984) reviewed studies involving field observations on diatom communities and emphasized that the relationship between dominant algae and resource ratio is much less clear than reported by Tilman (1981).

The hypothesis that the N/P ratio can have strong effects on phytoplankton community structure involves interspecific competition to sequester these two nutrients. Hence, it is important to study the competitive abilities for phosphorus and nitrogen of the major phytoplankton species.

A review of studies on resource ratios and competition showed that many of these had used pairs of species grown in clonal cultures or multi-species assemblages taken directly from lakes. In general, the outcomes of these studies are consistent with the resource-ratio hypothesis (Bulgakov and Levich 1999).

Nevertheless, Reynolds (1999) presented an alternative explanation for these observations, based on three main criteria: (1) the success of N₂-fixing cyanobacteria on experiments with enclosures was determined by the availability of the individual nutrients at the beginning of the growth period,

rather than to the N/P ratio; (2) the application of Tilman's hypothesis by his followers did not consider that, at N and P concentrations above those necessary to satisfy growth, there would be no limitation and, consequently, the ratio would not exert a regulatory role; (3), and although phytoplankton cells are equipped to perceive variations in the availability of individual resources, no molecular mechanism has so far been demonstrated that enable the cells to perceive and react to a resource ratio. Therefore, whether or not the dominance of cyanobacteria is promoted by low N/P ratio remains unclear and the role of N/P ratio as an independent factor regulating composition of phytoplankton assemblage is still under discussion.

In a shallow, eutrophic, tropical reservoir a seasonal shift in the dominance of phytoplankton community was observed, from diatoms to cyanobacteria. Correlation and Canonical Correspondence Analysis has shown that the change coincides with a decreasing N/P ratio and a diminishing availability of DIN (Marinho and Huszar 2002). In this article, we describe experiments conducted with two phytoplankton species (*Microcystis aeruginosa* and *Aulacoseira distans*) isolated from this reservoir. The aims of our study were to examine the consequences of different N/P ratios on growth, on the abilities of these species to use nitrogen and phosphorus, and to relate these findings to field observations.

Materials and methods

Strains of *Microcystis aeruginosa* and *Aulacoseira distans* were isolated from phytoplankton samples collected at Juturnaíba reservoir (22°33' S; 42°18' W) in 1997. The two strains were maintained under nonaxenic conditions, routinely grown at a temperature of $25 \pm 2^\circ\text{C}$ and irradiance of $80 \mu\text{E m}^{-2} \text{s}^{-1}$ provided by cool-white fluorescent light tubes, with a light-dark period of 12:12 h. *M. aeruginosa* was grown in ASM-1 medium (Gorham et al. 1964) and *A. distans* was cultured in WC medium (Guillard and Lorentzen 1972). In order to avoid potential effects of adaptation to a different culture medium, the experiments were conducted using the original medium in which each strain was isolated.

In all experiments, batch cultures were grown in 1.6-l liquid medium, in 2-l Erlenmeyer flasks. Temperature and light conditions were the same, as already described. Cultures were kept mixed by bubbling with sterile air. Prior to the start of the experiments, inocula were adapted in the respective experimental conditions for 7 days. Each experimental treatment was carried out in triplicate.

For the experiments, media with two different N/P ratios were prepared: 3 and 15 by moles. These molar ratios represent the range in which values for these ratios were observed during earlier research at Juturnaíba reservoir. During the annual cycle, phytoplankton community shifted, from diatoms (*Aulacoseira distans* (Ehrenberg) Simonsen) to cyanobacteria (especially *Microcystis aeruginosa* Kützing). *A. distans* dominance was associated with higher N/P ratio (mean value = 15) and DIN (>10 μM), while *M. aeruginosa* dominated the phytoplankton community at N/P ratio below 10 (mean value = 3) and DIN fell below 5 μM . These variations on the N/P ratio were due to decreasing concentrations of DIN (Marinho and Huszar 2002).

Experiments with *M. aeruginosa* were conducted in ASM-1 medium. The standard concentrations of nitrate and phosphate in ASM-1 medium (2000 μM of NO_3 and 200 μM of PO_4 —N/P = 10) were used as control condition (C). Experimental treatments varied the nitrate concentrations: 600 μM (NP3) and 3000 μM (NP15). Experiments with *A. distans* were conducted in WC medium. The standard concentrations of nitrate and phosphate in WC medium (1000 μM of NO_3 and 50 μM of PO_4 —N/P = 20) were used as control condition (C). The other conditions were obtained through variations on nitrate concentrations: 150 μM (NP3) and 750 μM (NP15). In both experiments, NP3 and NP15 mean culture medium N/P ratios of 3 and 15, respectively. Nitrate and phosphate were the only N and P forms used to prepare both the ASM-1 and WC media.

Each culture was sampled every 3 days. Dissolved nitrate and phosphate concentrations were determined by spectrophotometric standard methods (APHA 1995). The growth of *M. aeruginosa* and *A. distans* was followed by counting cell numbers, using a Fuchs-Rosenthal hemocytometer. Growth curves, relative growth rates (μ) and mean doubling time (G) were derived according to Fogg and Thake (1987) and calculated as:

$$\mu = (\ln N_2 - \ln N_1) (t_2 - t_1)^{-1}$$

$$G = \ln 2 \mu^{-1}$$

where N_1 and N_2 are the cell numbers at the begin and at the end of exponential growth phase; $(t_2 - t_1)$ —time interval, in days, during the exponential growth phase.

The maximum yield of the cultures (R) was calculated as the ratio between maximum cell densities reached by each culture and the number of cells in the inocula. The biomass was estimated based on the biovolume of the cells, and expressed in $\text{mm}^3 \text{ l}^{-1}$.

A one-way ANOVA, coupled with Student–Newman–Keul’s Test (SNK, $\alpha = 0.05$), was used to test differences among N/P ratio conditions.

Results

Growth rates (μ) and mean doubling time (G) of *M. aeruginosa* were similar for all conditions tested, but the biomass yield differed significantly and was proportional to the nitrate present in the culture medium (Table 1). Exponential growth was observed from 3rd day, and was longer in NP15 condition, while stationary phase was observed from 12th day (Fig. 1).

The μ and G for *A. distans*, were also similar among different conditions. However, maximum yield (R) significantly differed in cultures. Cells cultivated in NP3 had maximum yield R six times lower than cells grown in NP15 and control (Table 2). Exponential growth of *A. distans* also started from 3rd day and persisted longer in NP15 and control conditions. The culture grown on these conditions showed similar growth curves but differed from NP3 that reached stationary phase from 9th day (Fig. 1).

A rapid decrease of dissolved inorganic nutrients concentrations in culture medium of *M. aeruginosa*

Table 1 Mean values of relative growth rate (μ), mean doubling time (G) in days and maximum yield of cultures (R) of *M. aeruginosa*

Experimental condition	μ	G	R
NP3	0.30	2.3	20*
NP15	0.33	2.1	60*
Control	0.31	2.2	42*

* Mean values differed significantly at $P < 0.05$

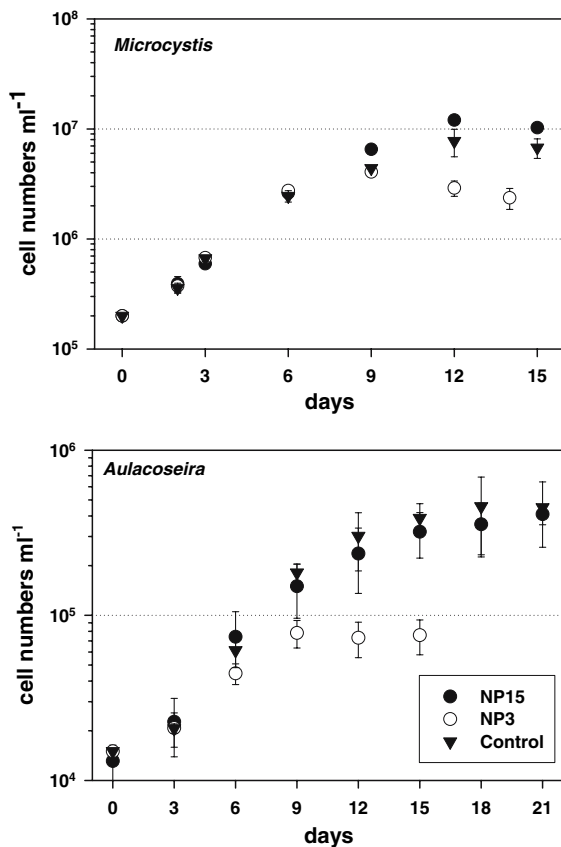


Fig. 1 Growth of *Microcystis aeruginosa* (upper panel) and *Aulacoseira distans* (lower panel). Each value is the mean of three replicates; bars represent standard deviation

Table 2 Mean values of relative growth rate (μ), mean doubling time (G) in days and maximum yield of the cultures (R) of *Aulacoseira distans*

Experimental condition	μ	G	R
NP3	0.28	3.2	5*
NP15	0.36	2.4	33
Control	0.39	2.6	30

* Mean values differed significantly at $P < 0.05$

was observed until the 3rd day, when on average 79% of nitrate and 76% of phosphate concentrations were absorbed (Fig. 2). By the 3rd day, the phosphate concentration was almost constant in NP3 condition, while other culture conditions presented a small uptake until the end of the experiment. Nevertheless, the phosphate was not exhausted in any of the experiments. Uptake of nitrate was proportional to concentration in culture medium until the end of

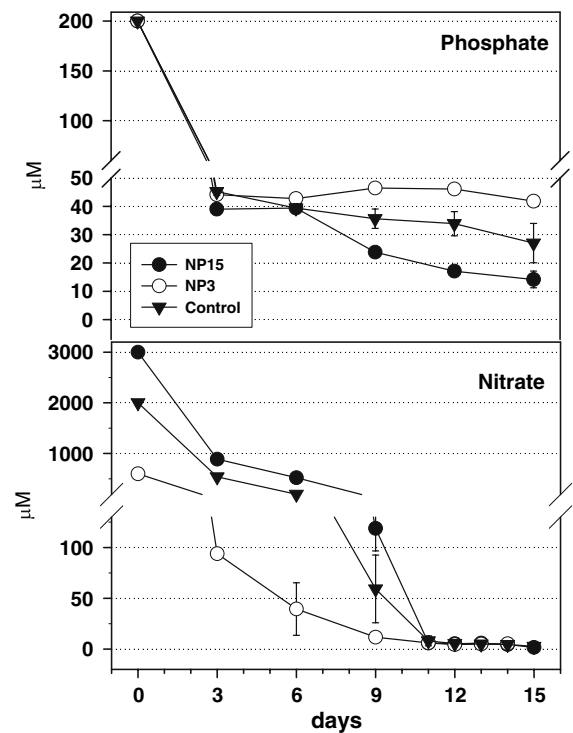


Fig. 2 Dissolved phosphate concentration (upper panel) and dissolved nitrate concentration (lower panel) in cultures of *Microcystis aeruginosa* cultivated at different N/P ratios. Each value is the mean of three replicates; bars represent standard deviation

exponential growth phase (Figs. 1, 2). Nitrate was not exhausted although the concentration observed at the end of the experiment was lower than 5 μM in all conditions.

The nutrient concentrations in the culture media of *A. distans* also rapidly decreased in all conditions. By the 3rd day, 63% of nitrate and 76% of phosphate had been absorbed (Fig. 3). Phosphate and nitrate in *A. distans* culture medium were not exhausted, but residual concentrations were lower than those in *M. aeruginosa* cultures. As observed for *M. aeruginosa*, the nitrate uptake differed among experimental conditions and was proportional to its concentration in the culture media.

The differences on biomass and nutrient concentrations in the time interval during the exponential growth phase are shown in Table 3. *Microcystis* showed greater increase in biomass than *Aulacoseira*. The increment was an order of magnitude higher for the cyanobacteria, which had also higher nutrient uptake than the diatom. The P uptake did not differ

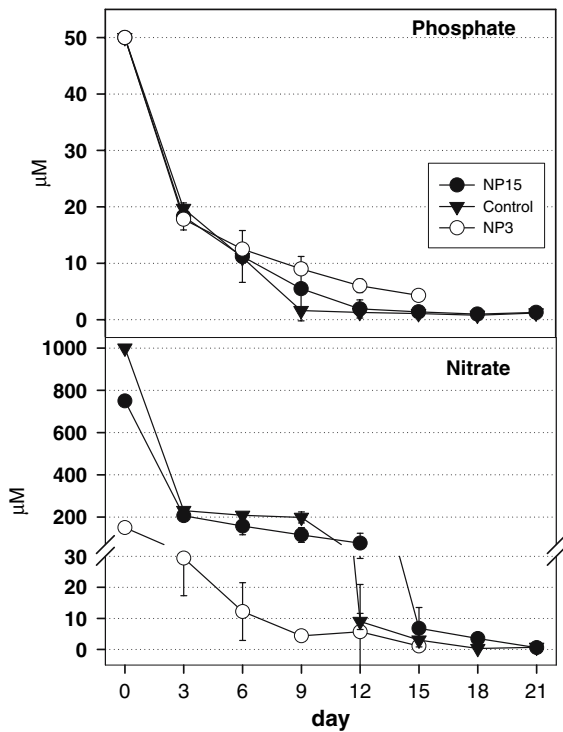


Fig. 3 Dissolved phosphate concentration (upper panel) and dissolved nitrate concentration (lower panel) in cultures of *Aulacoseira distans* at different N/P ratios. Each value is the mean of three replicates; bars represent standard deviation

among treatments, for either strain. However, the nitrate uptake was proportional to the concentration in the culture medium. The *Microcystis* growth in the NP15 treatment brought about a reduction of 2112 μM in nitrate concentration in the first 3 days, while only 506 μM was absorbed on NP3 condition. A similar effect was observed in *Aulacoseira*, with

the control treatment showing the highest ΔN, coinciding with its higher nitrate concentration. Although *Microcystis* showed higher overall-nutrient uptake, the observed uptake of nutrients per unit biomass (B) was higher in *Aulacoseira* than in *Microcystis*. The ratios ΔN/ΔB and ΔP/ΔB for *Microcystis* were always lower than those for *Aulacoseira*, and in same conditions the values were an order of magnitude higher for *Aulacoseira*.

N/P ratio variations in *M. aeruginosa* culture medium differed among the experimental treatments (Fig. 4a). During the exponential growth phase, an initial increment followed by accentuated decrease occurred in the control and NP15 treatments, while a more gradual decrease in N/P ratio was observed in NP3. The increase on 3rd day was higher in the NP15 treatment and reflects the higher phosphate uptake (see Fig. 3). N/P ratios <5 were recorded in all conditions during the stationary phase.

The variations of N/P ratios in *A. distans* cultures were also different among the experimental treatments (Fig. 4b). A gradual decrease occurred in NP3, and ratios <1 were observed from 6th day. However, the N/P ratios were maintained in NP15 and control conditions, until the 6th day. Sharp increases in NP15 occurred on 12th day and in the control on 9th day, due to the simultaneous decreases in N-uptake rate (see Figs. 3 and 4b).

Discussion

N/P ratio did not influence the growth rates of *M. aeruginosa* and *A. distans*, that were similar in the

Table 3 Mean variation on biomass (ΔB—mm³ l⁻¹), nitrate (ΔN—μM) and phosphate (ΔP—μM) concentrations and ΔN/ΔB (μM mm⁻³) and ΔP/ΔB (μM mm⁻³) ratios, during the exponential growth of *Microcystis* and *Aulacoseira*

Time interval	ΔB			ΔN			ΔP			ΔN/ΔB			ΔP/ΔB		
	NP3	NP15	C	NP3	NP15	C	NP3	NP15	C	NP3	NP15	C	NP3	NP15	C
<i>Microcystis</i>															
0–3	35	29	34	506	2112	1460	156	161	155	14	72	42	4	6	5
3–6	154	150	132	54	366	345	1	0	6	0	2	3	0	0	0
6–9	97	290	143	28	404	136	0	16	4	0	1	1	0	0	0
<i>Aulacoseira</i>															
0–3	2	4	2	121	541	769	32	31	30	60	135	385	16	8	15
3–6	9	19	15	17	43	22	5	6	9	2	2	1	1	0	1
6–9	12	28	45	8	51	10	4	5	9	1	2	0	0	0	0

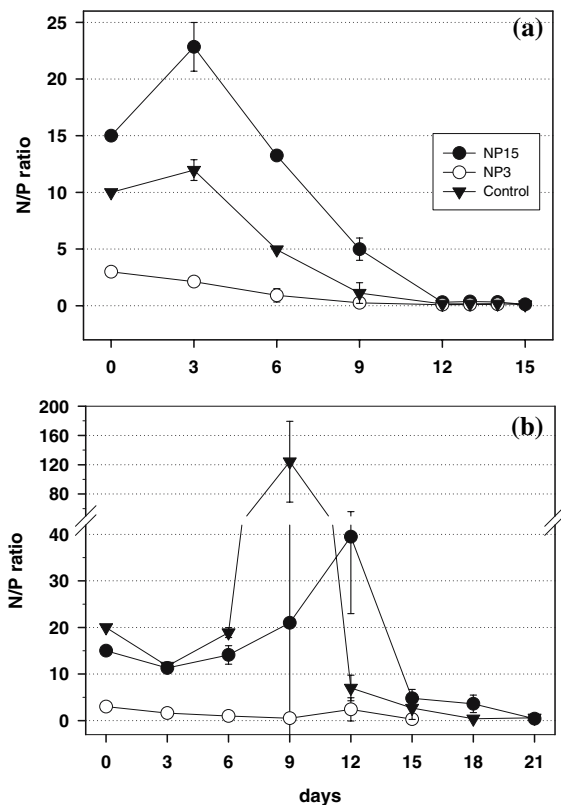


Fig. 4 N/P ratios in cultures of (a) *Microcystis aeruginosa* and (b) *Aulacoseira distans*. Each value is the mean of three replicates; bars represent standard deviation

exponential phases of all the experimental treatments but the maximum yield (R) presented differences in both *M. aeruginosa* and *A. distans* experiments. The highest maximum yields were obtained with *M. aeruginosa*. Moreover, the biomass produced by *M. aeruginosa* was lower, by a factor of 2, compared to either the Control or NP3 treatments, and was lower by a factor of 6 compared to *A. distans* in the NP15 and NP3 treatments (Tables 1, 2).

Aguiar and Azevedo (1998) also observed that a lower-nitrogen supply had strong effects on maximum yield of *M. aeruginosa* cultures, but it did not influence growth rates in experiments where the N/P ratio was kept at 10:1. However, the authors observed that growth rates of cultures in N/P ratio 20:1 were significantly lower than cultures with N/P ratio 10:1. Nevertheless, we did not find significant decrease in growth rates in cultures using NP15 compared with controls. A study with another cyanobacterial genus (*Synechocystis aquatilis* f. *salina*) also showed

similar growth rates when the strain was cultivated in N/P ratio 3:1 and 10:1 (Nascimento and Azevedo 1999). However, the authors observed that the maximum yield was higher in the highest N/P ratio condition, as observed in this study. This result could be related to the absolute concentrations of N originally supplied and not with the N/P ratio.

Both *Microcystis* and *Aulacoseira* showed rapid uptake of nutrients (N and P). Comparing phosphorus uptake of *A. distans* and *M. aeruginosa* cultures, distinct patterns were observed. Uptake was faster and higher in *M. aeruginosa*, but N/P ratio did not influence uptake rate. Lee et al. (2000) reported that even with a quite variation in the N/P ratios ranging from 1 to 100, the intracellular N/P atomic ratios of *M. aeruginosa* only changed about two-fold, while the cellular P content under N-fixed conditions increased substantially with an increased P medium. In contrast, the cellular N content did not change much. *M. aeruginosa* could be considered a phosphorus storage-adapted species, with a high capacity to absorb inorganic phosphorus (Olsen 1989). The storage of excessive P over the immediate needs has been found to be much higher in comparison with N (Oh and Rhee 1991). The elevated residual of dissolved phosphate observed in *M. aeruginosa* cultures, however, reflected a plentiful P supply in the culture medium and an absence of any significant phosphorus limitation (Fig. 2).

Although *A. distans* showed a lower rate of P absorption than *Microcystis*, the residual phosphate concentrations in the culture media were smaller at the end of experiments. This seems to be in agreement with the hypothesis that the diatoms are excellent competitors for phosphorus at low concentrations, having half-saturation constants for uptake that may be considered smaller ($K_m = 0.19\text{--}0.59\ \mu\text{M}$) than those observed for cyanobacteria ($K_m = 0.58\text{--}1.10\ \mu\text{M}$) (Tilman et al. 1982; Sommer 1989; Huszar and Caraco 1998; Arhonditsis and Brett 2005).

The uptake of N was proportional to the concentration in the culture medium. For *Microcystis*, the uptake of nitrate was four times higher in the NP15 than in the NP3 condition during the first 3 days. A similar pattern was observed for *Aulacoseira* (Table 3). Some studies have shown that centric diatoms (*Cyclotella* spp.) should compete well for nitrogen because their growth affinity is higher than cyanobacteria taxa (Grover et al. 1999). However,

M. aeruginosa possesses high nitrogen uptake capacity (Takamura et al. 1987; Fujimoto et al. 1997), and our data are consistent with this hypothesis.

Importantly, higher N uptake capacity of *Microcystis* was not due to differences in cell sizes or in inoculum size, but reflects the distinct nutritional requirements and strategies of the species. This could be inferred from the uptake per unit of biomass of the two genera investigated. *Microcystis* attained a lower uptake of N per unit biomass ($\Delta N/\Delta B$). However, the produced biomass by *Microcystis*, derived from the increase in its biovolume in the time interval (ΔB), was 1 order of magnitude greater than that of *Aulacoseira* (Table 3). The greater yield of *Microcystis* growth relative to the diatom could have resulted from its ability to produce more biomass per unit nitrogen assimilated.

We can thus infer that the dominance of *Microcystis* under conditions of nutrient abundance, such as those prevailing in culture media or those in eutrophic environments, could result from the ability of this cyanobacterium to produce greater biomass per unit N. This ability/strategy is not influenced by the N/P ratio.

The resource-ratio hypothesis, based on competition theory (Tilman 1982), argues that low N/P ratios imply nitrogen limitation, and high N/P ratio imply phosphorus limitation (Smith and Bennett 1999). So, considering a scenario of competition between *M. aeruginosa* and *A. distans*, it would be expected that N/P ratio influenced the relative growth rates. It is important to emphasize that nutrient competition occurs, by definition, whenever nutrient consumption by organisms leads to nutrient limitation of reproductive rates (Tilman 1981).

In Juturnaíba Reservoir, the replacement of *A. distans* by *M. aeruginosa* as dominant species in phytoplankton community is observed during course of the annual cycle (Marinho and Huszar 2002). This shift in phytoplankton structure and composition was correlated with a decrease in N/P ratio and nitrogen availability. The success of *M. aeruginosa* at low N/P ratio has been confirmed for both natural populations (Michard et al. 1996; Bulgakov and Levich 1999), and laboratory cultures (Fujimoto et al. 1997).

Nevertheless, in this study, the ratio of nutrients supplied in the medium has no influence on the rate of exponential growth and no significant differences between growth rates were observed at different N/P

ratios. The biomass yield in the cultures was proportional to the ambient concentration of nitrogen. We infer that it is actually related to the intracellular nitrogen content and the limit of biomass that this content can sustain. That being so, the N/P ratio that matters most is the intracellular one. The external ratio enables us to predict, whether it will be N or P, but before the onset of resource limitation, the rate of growth is not ratio-dependent and can have no instantaneous influence on the make up of the assemblage.

As expected, the variation of N/P ratio in culture medium was caused by the absorption of the nutrients by the cells of *M. aeruginosa* and *A. distans*. Even so, the dynamics was differentiated among the studied strains, resulting in distinct variation in the N/P ratio. The growth of *M. aeruginosa* resulted in a rapid reduction of this ratio, whereas in *A. distans*, the original ratio of culture medium was maintained in middle of the exponential growth phase, especially in NP15 and control conditions. Such results demonstrate that *M. aeruginosa* has greater potential than *A. distans* to influence the proportional availability of nutrients (N/P ratio). An enclosure experiment in a shallow subtropical lake showed that N/P ratio was not a deterministic factor, but that the low ratio was a result of *Microcystis* blooms (Xie et al. 2003).

Thus, contrary to what has been frequently stated in the literature (Smith 1983; Bulgakov and Levich 1999; Smith and Bennett 1999), our results do not attribute dominance of a cyanobacterium to low N/P ratios. Rather show that low N/P ratio can be the consequence of high uptake capacity and relatively greater uptake rates of N than P by cyanobacteria, causing a decrease in the ratio, confirming the results by Xie et al. (2003). Moreover, limitation by one or the other nutrient, in this case nitrogen could determine the competitive outcome (Reynolds 1999). In relation to the observations in Juturnaíba reservoir, our data pointed toward nitrogen availability as a major factor determining substitution of diatoms by cyanobacteria and not the N/P ratio variation. However, this is only one chapter of the book and other alternatives (biotic and abiotic factors) should not be neglected.

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