UNIVERSIDADE FEDERAL DO RIO DE JANEIRO

DEPARTAMENTO DE ECOLOGIA

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

ECOLOGIA DO FITOPLÂNCTON DO RESERVATÓRIO DE JUTURNAÍBA, ARARUAMA- RJ, BRASIL: ESTRUTURA E DINÂMICA DA COMUNIDADE, ASPECTOS ECOFISIOLÓGICOS E METODOLÓGICOS.

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Tese apresentada ao Programa de Pós-Graduação em Ecologia do Departamento de Ecologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro, como parte integrante dos requisitos para obtenção do grau de Doutor em Ecologia.

Rio de Janeiro – RJ - Brasil

2000

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A vocês Flávia e Rodrigo
dedico este trabalho, com
minhas desculpas pelo
tempo que lhes foi
roubado.

FICHA CATALOGRÁFICA

MARINHO, Marcelo Manzi

Ecologia do fitoplâncton do reservatório de Juturnaíba, Araruama-RJ, Brasil: estrutura e dinâmica da comunidade, aspectos ecofisiológicos e metodológicos

102p: 27 figuras, 14 tabelas

Tese de Doutorado, Departamento de Ecologia, Universidade Federal do Rio de Janeiro, 2000.

Palavras-chave: 1.Fitoplâncton 2.Estrutura de comunidade 3.Ecofisiologia 4.razão N/P 5.Pigmentos 6. reservatório de Juturnaíba

Agradecimentos

À Profa. Dra. Sandra M. F. O. Azevedo, do Laboratório de Ecofisiologia e Toxicologia de Cianobactérias - Núcleo de Pesquisa de Produtos Naturais/UFRJ, pelo apoio, orientação, incentivo, e principalmente pelo carinho e amizade durante o desenvolvimento deste trabalho.

À Profa. Dra. Vera L. M. Huszar, Professora do Departamento de Botânica do Museu Nacional, Universidade Federal do Rio de Janeiro, pela amizade e parceria iniciada em longa data e pela co-orientação neste trabalho.

À Profa. Dra. Silvana V. Rodrigues, Professora do Departamento de Química Analítica, Universidade Federal Fluminense, pelo apoio e oportunidade de realizar este estudo e pela co-orientação neste trabalho.

Às Professoras Maria Célia Villac e Vera Lúcia de Moraes Huszar, membros da pré-banca, pela cuidadosa revisão deste manuscrito.

Aos Professores Drs. Bastian Adriaan Knoppers, Fábio Roland, Francisco Antônio Rodrigues Barbosa e Reinaldo Bozelli, membros da Banca Examinadora, pelas críticas e sugestões que contribuiram para aprimorar este trabalho.

Ao Flávio meu companheiro de trabalho de campo pela ajuda indispensável na realização das coletas.

Às amigas Izabel e Fabiana pela ajuda nas coletas, nas análises de pigmentos e nas análises de nutrientes do ambiente.

À José Luiz e Henrique, funcionários da Cia Nacional de Álcalis, pelo apoio na logística do trabalho de campo.

À Suzane pela ajuda no isolamento das cepas, manutenção das culturas e realização dos experimentos.

À Elisângela e Yuri pela ajuda na análise de nutrientes dos experimentos com cultivo.

À Valéria, amiga sempre presente através do carinho nas horas mais necessárias, pelo apoio e sugestões em todas as etapas deste trabalho.

À CAPES pela bolsa de Missão na Alemanha e pela bolsa de Doutorado, além do financiamento de parte do projeto através do Programa Probral.

Ao CNPq pelo financiamento de parte do projeto através do Pronex.

À toda equipe do Laboratório de Ecofisiologia e Toxicologia de Cianobactérias, da qual me orgulho em integrar, por todo apoio, carinho e amizade.

Às muito queridas Izabel, Graça, Lúcia e Mariângela, do Laboratório de Ficologia do Departamento de Botânica MN/UFRJ pelo apoio, carinho e amizade sempre demonstrados.

A todos aqueles que, eventualmente omitidos jamais por esquecimento, mas por verdadeira falta de memória neste momento, e que somado ao muito que aprendi, contribuíram para a concretização deste trabalho.

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Resumo

A dinâmica da comunidade fitoplanctônica de um reservatório de abastecimento (reservatório de Juturnaíba, RJ, Brasil - 22°33'S; 42°18'W) foi analisada relacionando-a com as variações de alguns fatores físicos e químicos, usualmente considerados como os principais reguladores da biomassa e composição do fitoplâncton. Estudos experimentais em laboratório, sobre a ecofisiologia das principais espécies fitoplanctônicas, foram realizados para verificação de algumas hipóteses levantadas a partir de observações no ambiente. Além dos aspectos ecológicos, também foram abordadas questões metodológicas sobre a aplicação da técnica de análise quantitativa de pigmentos fotossintéticos para avaliação do fitoplâncton. A avaliação do padrão geral de variação da composição e biomassa da fitoplâncton, através da análise de pigmentos fotossintéticos, foi consistente com os resultados obtidos pela metodologia de contagem de organismos em microscópio invertido, comprovando a aplicabilidade desta técnica para o estudo e monitoramento do fitoplâncton. A análise e comparação dos resultados sob a ótica de teorias ecológicas possibilitou um melhor entendimento dos processos ecológicos determinantes da estrutura e função do fitoplâncton. Nossos dados foram consistentes com a abordagem fitossociológica em relação a delimitação de associações de espécies do fitoplâncton em sistemas tropicais enriquecidos, a despeito da hipótese ter sido originalmente formulada para ecossistemas de regiões temperadas. Outro aspecto teórico também considerado foi a análise das relações empíricas entre os recursos e a abundância das espécies considerando a ecofisiologia das espécies. Tanto as descrições estatísticas quanto as baseadas nas observações demonstraram as fortes interações entre as condições de limitação em geral e a variabilidade relativa dos recursos, em particular na regulação da estrutura da comunidade fitoplanctônica. Com base nestas considerações, a disponibilidade de nitrogênio foi considerada como um dos principais fatores determinantes da sucessão sazonal observada no reservatório de Juturnaíba. Então, considerando um cenário de competição entre as principais espécies registradas (*Microcystis aeruginosa* e *Aulacoseira distans*) seria esperado que as taxas de crescimento destas algas fossem influenciadas pela razão N/P. Contudo, os resultados obtidos nos experimentos em laboratório não mostraram diferenças significativas entre as taxas de crescimento destas espécies. *M. aeruginosa*, entretanto, apresentou uma capacidade de produção de biomassa superior a de *A. distans*, especialmente em baixas razões N/P. Além disso, os experimentos demonstraram que *M. aeruginosa* tem um potencial maior que *A. distans* para influenciar a disponibilidade proporcional de nutrientes. Deste modo, a variação da razão N/P no reservatório de Juturnaíba pode ter sido conseqüência das elevadas capacidades de absorção de nitrogênio e fósforo das cianobactérias, o que sugere que o sucesso de *M. aeruginosa* e declínio de *A. distans* parece ter sido decorrente da maior capacidade das cianobactérias em crescer sob menor disponibilidade de nitrogênio. Os resultados deste estudo confirmam a importância de serem conciliadas observações de campo com estudos experimentais, para uma melhor compreensão dos processos ecológicos na comunidade fitoplanctônica.

Abstract

The phytoplankton community dynamics of a water supply reservoir (Juturnaíba reservoir, RJ, Brazil - 22°33'S; 42°18'W) was analysed relating it with the variations of some physical and chemical factors, usually considered as the main regulators of phytoplankton biomass and composition. Laboratory experimental studies on the ecophysiology of main phytoplankton species, were performed for verification of some hypotheses postulated from field observations. Besides the ecological aspects, methodological aspects were also approached about the application of quantitative analysis of photosynthetic pigment technique for phytoplankton evaluation. The general pattern of phytoplankton biomass and composition variability assessed through the photosynthetic pigment analysis, were consistent with the results obtained by the classic method of organisms counting by the inverted microscope and confirmed the applicability of this technique for the study and monitoring of the phytoplankton. Analysis and comparison of the results under ecological theories, made easy a better understanding of the ecological processes driving phytoplankton structure and function. Our data supports the view that the delimitation of assemblages applies reasonably to the one found in tropical enriched systems, despite the original formulation for temperate regions. Another theoretical aspect also considered was the analysis of the empirical relationships between the resources and species abundance considering species ecophysiology. Both statistical descriptions and observations demonstrated the strong interactions among limitation conditions, in general, and the relative resource variability, in particular, in the regulation phytoplankton community structure. Concerning these considerations, nitrogen availability was considered one of the main regulating factors of seasonal succession observed in the Juturnaíba reservoir. Then, considering a competition scenery among the main registered species (*Microcystis aeruginosa* and *Aulacoseira distans*) it would be expected that the growth rates of these algae were influenced by N/P ratio. However, the results obtained in the experiments did not show significant differences among growth rates. Even so, *M. aeruginosa* presented superior capacity of production biomass, especially in low N/P ratios. Besides, the experiments demonstrated that *M. aeruginosa* has a larger potential than *A. distans* to influence the proportional availability of nutrients. Thus, N/P ratio variation in the Juturnaíba reservoir can have been consequence of cyanobacteria high capacities for nitrogen and phosphorus uptake, that suggests *M. aeruginosa* success and *A. distans* decline, seem to have been due to the largest capacity of the cyanobacteria in growing under smaller nitrogen availability. Our results pointed that it is important to do experimental studies linked with field observations, for a better understanding of the ecological processes in the phytoplankton community.

1. Introdução

1.1. Considerações gerais

Um dos grandes desafios da humanidade no século XXI será, sem dúvida, o controle da qualidade e quantidade de água para abastecimento público. Nas últimas décadas, diversos estudos têm objetivado aprimorar as formas de gerenciamento dos recursos hídricos. As discussões sobre o tema têm sido amplas, e uma recente publicação sobre teoria ecológica em reservatórios serve como um exemplo do atual estado da arte sobre o assunto (Tundisi & Straškraba, 1999). Os autores destacam que o aperfeiçoamento do gerenciamento da qualidade da água necessita de conhecimento consistente sobre a limnologia dos reservatórios. As propriedades físicas, químicas e biológicas, bem como a previsibilidade dos mecanismos de seu funcionamento necessitam de sólida base científica e informação técnica aprofundada. Nesse sentido, o plâncton tem despertado interesse de muitos pesquisadores, por responder prontamente às mudanças do ambiente, funcionando como sensor refinado das variáveis ambientais. Sua estrutura e composição em diversos períodos de tempo refletem, melhor que qualquer artefato tecnológico, as flutuações dessas variáveis (Margalef, 1983).

Vários estudos têm abordado a comunidade fitoplanctônica em reservatórios no Brasil, especialmente na região Sudeste, onde as diatomáceas e cianobactérias são freqüentemente descritas como os grupos dominantes em sistemas eutrofizados (Calijuri & Tundisi, 1990; De Filipo, 1987, Marinho *et al.*, 1993; Sant'Anna *et al.*, 1997; Tundisi & Matsumura-Tundisi, 1990). Alterações físicas na estrutura da coluna d'água, associadas a fatores climatológicos, têm sido apontados como determinantes da variação sazonal na composição das comunidades fitoplanctônicas desses reservatórios (Tundisi, 1990). Além disso, outros estudos têm evidenciado os efeitos da eutrofização artificial que, em geral, resultam em alterações nessas comunidades (Tundisi & Matsumura-Tundisi, 1992, 1995).

As principais causas da eutrofização antropogênica são as descargas de efluentes domésticos, urbanos e/ou industriais, e o aporte de fertilizantes através da drenagem de áreas agrícolas. Este processo gera profundas mudanças qualitativas e quantitativas nas comunidades aquáticas, nas condições físicas e químicas do meio e na produção dos ecossistemas aquáticos (Esteves, 1988). O incremento da eutrofização leva, geralmente, à diminuição da diversidade e ao crescimento intenso de algumas espécies de algas

planctônicas, formando o que é conhecido como florações. Dentre estas algas, destacam-se as cianobactérias cujas florações vêm aumentando em intensidade e freqüência (Azevedo *et al*.,1994). Atualmente, é possível visualizar um cenário de dominância de cianobactérias em muitos ambientes lênticos brasileiros, durante os períodos de maior biomassa e/ou densidade (Huszar & Silva, 1999). Esta dominância é marcante sobretudo em reservatórios e em vários deles as cianobactérias predominam durante grande parte do ano (Bouvy *et al*., 1999; Branco & Senna, 1994; Huszar *et al.*, 2000; Sant'Anna *et al.*, 1997).

Além dos desequilíbrios ecológicos do ponto de vista de perda de diversidade e alterações ao longo da cadeia trófica, as florações de cianobactérias apresentam problemas ligados à saúde humana. Os gêneros mais freqüentemente observados em florações de cianobactérias no Brasil (*Microcystis* e *Cylindrospermopsis*) são descritos na literatura como potencialmente produtores de hepatotoxinas ou neurotoxinas (Carmichael, 1994; Lagos *et al*., 1999), já tendo sido registradas florações tóxicas em ecossistemas aquáticos brasileiros (Azevedo & Carmouze, 1994; Bouvy *et al*., 1999; Magalhães & Azevedo, 1999).

Apesar do conhecimento obtido através dessas pesquisas possibilitar a aplicação de técnicas de manejo e controle ambiental, novos estudos são necessários para que a implantação das ações de gerenciamento não só protejam e conservem, mas ampliem os usos múltiplos e a vida útil dos reservatórios. Para tanto, tornam-se necessárias observações de longa duração, com desenvolvimento de estudos experimentais e abordagens teóricas, com transferência de conhecimento básico para aplicação imediata e a longo prazo (Tundisi *et al*., 1999).

Neste contexto, o conhecimento de aspectos preponderantes da ecofisiologia das comunidades fitoplanctônicas possibilita, sob o ponto de vista econômico e social, uma melhor exploração dos ecossistemas aquáticos como fonte de alimento, além de aumentar a capacidade e habilidade de controle dos efeitos da poluição e monitoramento desses ambientes.

Em geral, o conhecimento ecológico sobre o crescimento dos organismos em seus habitats naturais somente é alcançado pela síntese dos resultados de investigações sobre fisiologia, bioquímica e estudos de campo. Deste modo, além de observações extensivas e detalhadas sobre o fitoplâncton e os fatores ambientais reguladores de sua composição e biomassa nos ecossistemas aquáticos, estudos em laboratório com culturas de algas

planctônicas sob condições controladas são também indispensáveis para a compreensão da dinâmica desta comunidade.

Este tipo de estudo é útil porque possibilita uma avaliação sob condições definidas e reproduzíveis e seus resultados são, freqüentemente, menos ambíguos do que os obtidos com amostras do ambiente natural. A vantagem do modelo experimental com culturas de laboratório é que o pesquisador pode manipular um fator a cada vez, enquanto que nos estudos em sistemas naturais muitos fatores estão condicionando as respostas simultaneamente, tornando difícil a identificação dos efeitos em particular (Darley, 1982). Entretanto, por tratarem-se de modelos simplificados, as culturas de laboratório apresentam respostas diferenciadas dos sistemas naturais. Portanto, as comparações e extrapolações de um sistema para o outro devem ser realizadas com cautela. A abordagem cuidadosa não anula as vantagens oferecidas pelos sistemas experimentais (Darley, 1982). Estudos sobre a ecofisiologia do fitoplâncton com culturas de laboratório são importantes por permitirem a obtenção do conhecimento sobre as estratégias e fatores que estimulam a resposta adaptativa das espécies, tornando as extrapolações para a natureza mais realistas (Zevenboom, 1987).

Apesar de sua importância, poucos estudos sobre a ecofisiologia de espécies fitoplanctônicas têm sido realizados no Brasil. Dentre estes destacam-se aqueles sobre ecofisiologia de cianobactérias (p.e. Aguiar & Azevedo, 1998 e Nascimento & Azevedo, 1999), fisiologia de espécies de água doce (p.e. Cáceres & Vieira, 1988; Cimbleris & Cáceres, 1991; Giroldo & Vieira, 1999 e Vieira *et al*., 1998) e ecofisiologia de espécies marinhas (p.e. Aidar *et al*., 1994; Baumann *et al*., 1994 e Lourenço *et al*., 1998).

Além do aspecto da ecofisiologia dos organismos, faz-se relevante o desenvolvimento e aplicação de novas metodologias que possibilitem o monitoramento do fitoplâncton, através de procedimentos automatizados e simplificados.

Atualmente, a composição e biomassa fitoplanctônica são usualmente analisadas pelo método de sedimentação em microscópio invertido, desenvolvido por Utermöhl (1958). Essa metodologia requer técnicos altamente especializados e treinados, sendo o processamento das amostras um procedimento muito moroso. Devido a esse fato, somente poucos ecossistemas têm sido monitorados continuamente.

Outra metodologia empregada para análise da biomassa fitoplanctônica é a quantificação do principal pigmento fotossintético, a clorofila *a*. Embora este método seja facilmente aplicado, ele somente permite uma estimativa da biomassa total, não possibilitando

a identificação taxonômica dos grupos fitoplanctônicos. Entretanto, não somente a biomassa, mas também a composição da comunidade é fundamental para o entendimento e monitoramento do fitoplâncton.

A análise quantitativa de pigmentos fotossintéticos do fitoplâncton através da técnica de cromatografia líquida de alta eficiência (High Performance Liquid Cromatography - HPLC), pode representar uma alternativa eficaz para estudos e monitoramento da comunidade fitoplanctônica dos ecossistemas aquáticos (Millie *et al.*, 1993). Alguns estudos têm demonstrado resultados com boa correlação entre os dados obtidos pela metodologia clássica de contagem de organismos (método de sedimentação) e a quantificação da biomassa fitoplanctônica através da análise de pigmentos por HPLC (Wilhelm *et al.*, 1991; Soma *et al.*, 1993; Tester *et al.*, 1995; Schmid *et al.*, 1998).

Esta metodologia vem sendo aplicada, ao longo dos últimos anos, especialmente em ambientes marinhos. Poucos estudos foram realizados com o fitoplâncton de ecossistemas continentais e não se tem conhecimento de estudo sobre fitoplâncton de ambientes tropicais através desta metodologia.

Segundo Wilhelm *et al.* (1991), este método apresenta três vantagens principais: 1) o tempo necessário para análise é três vezes menor que o método de sedimentação; 2) os procedimentos de análise podem ser automatizados e são de fácil execução; e 3) o método é capaz de quantificar todas as células fitoplanctônicas, independentemente de seu tamanho. Vários autores, contudo, ainda não recomendam a substituição do método de sedimentação e apontam para a necessidade de mais estudos antes que a quantificação de pigmentos por HPLC possa ser utilizada como procedimento padrão em estudos limnológicos (Wilhelm *et al.*, 1991; Roy *et al.*, 1996; Schlüter & Havskum, 1997).

1.2. O Reservatório de Juturnaíba como modelo de estudo

O reservatório de Juturnaíba, localizado na divisa dos Municípios de Silva Jardim e Araruama no Estado do Rio de Janeiro (22°33’S ; 42°18’W), é responsável pelo abastecimento hídrico das cidades da região dos lagos no Estado do Rio de Janeiro e dos Municípios de Rio Bonito e Silva Jardim. A economia da região apresenta intensa atividade turística com grande fluxo de população, sobretudo durante os meses de verão. Este fato torna

crítico o abastecimento de água, denotando a grande importância econômica e social desse reservatório. Além disso, apresenta problemas ambientais similares aos de muitos reservatórios do Brasil, especialmente os relacionados ao processo de eutrofização com ocorrência de florações de cianobactérias potencialmente tóxicas (Huszar & Silva, 1999). Assim, informações sobre a dinâmica desse ecossistema podem servir de modelo e constituir-se na base científica para adoção de técnicas de manejo, recuperação e controle ambiental de reservatórios.

Este reservatório foi formado a partir da construção de uma barragem no Rio São João, entre 1979 e 1984, localizada à jusante da Lagoa de Juturnaíba - uma lagoa costeira de águas doces originada no Pleistoceno Superior (Amador, 1986). A Lagoa de Juturnaíba, antes da construção da barragem, era formada pela contribuição dos rios Bacaxá e Capivari drenando, através do canal do Revólver, para o rio São João que, atualmente, deságua no reservatório cerca de 1,5 km a montante da barragem (Figura 1).

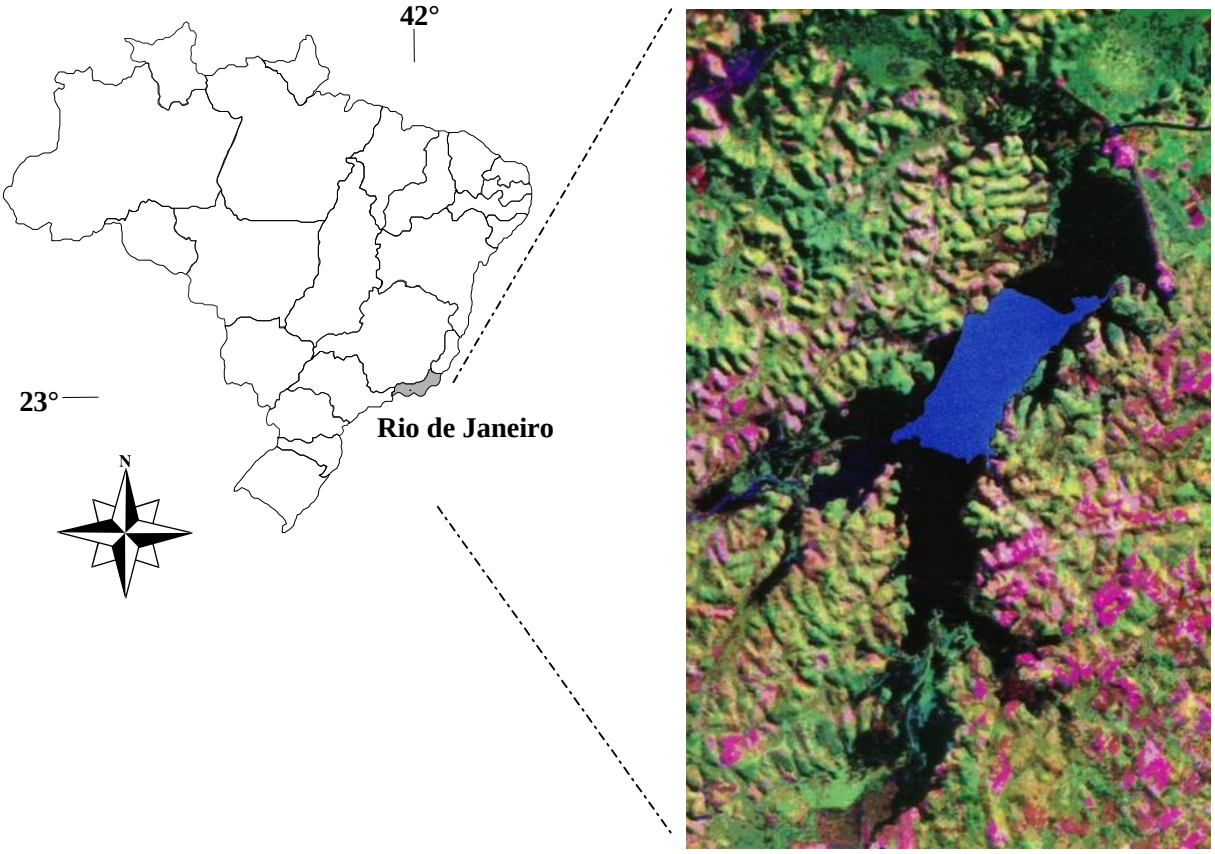

Figura 1. Mapa mostrando a localização do reservatório de Juturnaíba. Na imagem de satélite a antiga Lagoa de Juturnaíba (em azul).

A paisagem ao redor do reservatório é caracterizada por pequenos morros com alguns trechos planos próximos à desembocadura do rio São João. Os solos, predominantemente latossólicos, apresentam baixa taxa de cobertura vegetal resultante do processo de ocupação da região. À colonização, iniciada com extração de madeiras nobres como pau-brasil, guanandi, etc., seguiu-se a ocupação das terras por atividades agrícolas e pecuária. Este processo intenso de uso agrícola, associado à retirada de madeira para emprego como combustível em cerâmicas e olarias, provocou profundas alterações na fitofisionomia local. A base da agricultura local é a produção de laranjas, seguindo em menor escala pela produção de bananas, plantadas nas encostas, milho e feijão (FEEMA, 1987).

A bacia contribuinte ao reservatório abrange uma área de 1.290 km², com uma afluência média mensal de 29 m³/s (Tabela 1). Os rios contribuintes do ao reservatório percorrem uma área já bastante desmatada, onde predominam as atividades agrícolas e a pecuária. Estas atividades, associadas ao tipo de solos da região favorecem o transporte de sólidos e nutrientes para o corpo d'água. A bacia recebe ainda os despejos do Município de Silva Jardim e cidades circunvizinhas (Oliveira *et al*., 1978).

Tabela 1. Área e descarga média mensal dos rios da Bacia hidrográfica contribuinte ao reservatório de Juturnaíba (modificado de FEEMA, 1987).

Bacia	Área ($10^6 m^2$)	Descarga média mensal (m^3/s)
Rio São João	570	19
Rio Capivari	210	4,4
Rio Bacaxá	510	5,6

Na região prevalece o clima tropical, quente e úmido, com temperaturas médias anuais elevadas. A média das temperaturas máximas é de 30-32°C, chegando a atingir temperaturas máximas de 40-42°C. As mínimas, nunca inferiores a 8°C, ocorrem após a passagem de uma "frentes frias" de origem sub-polar e são de pouca duração. Em conseqüência do elevado grau de umidade relativa do ar, a evaporação apresenta níveis baixos, da ordem de 630mm/ano, com índices pluviométricos de 1.500mm/ano, concentrados nos meses de outubro a abril, sendo julho e agosto os meses mais secos (Bernardes, 1952).

Diversos estudos foram realizados na Lagoa de Juturnaíba quando ainda em condições

originais, anteriores ao represamento (Alvarenga, 1978; Alvarenga *et al.*, 1979; Alvarenga & Ricci, 1979a,b, 1981; Nunan & Cardoso, 1982; Sophia, 1984; Dias, 1985; Huszar, 1985, 1986, 1989; Marinho & Huszar, 1990). Após o barramento, entretanto, somente dois estudos foram realizados, evidenciando uma carência de informações à respeito das atuais condições do reservatório (Cunha, 1992; Marinho *et al.*, 1993). Em estudos realizados entre 1978 e 1981, período anterior à construção da barragem, foram registradas florações e predomínio de cianobactérias (Huszar, 1989; Marinho & Huszar, 1990). Após o enchimento do reservatório, em 1984, contudo, foi observada alteração na estrutura da comunidade fitoplanctônica incluindo acentuada redução na biomassa, sem ocorrência de florações de cianobactérias. Crisofíceas e criptofíceas foram então os organismos característicos da comunidade fitoplanctônica (Marinho *et al.*, 1993). Mais recentemente, porém, avaliações preliminares da comunidade fitoplanctônica, através de amostragens prévias realizadas no reservatório em dezembro de 1995, evidenciaram a ocorrência de florações de cianobactérias.

1.3. Objetivos

Neste contexto, três enfoques principais são abordados neste estudo. O primeiro envolve o aspecto metodológico, no qual objetivou-se a avaliação de uma nova tecnologia de análise da comunidade fitoplanctônica (Capítulo 2). Este estudo visou avaliar a aplicabilidade do método de análise de pigmentos por HPLC para a detecção das variações na biomassa e composição do fitoplâncton do reservatório de Juturnaíba, comparando com os dados obtidos por microscopia.

Esta parte do trabalho contou com a co-orientação da Dra. Silvana Viana Rodrigues, do Departamento de Química Analítica da Universidade Federal Fluminense, que foi responsável por todo o suporte analítico desta etapa.

O segundo aspecto abordado nesta pesquisa refere-se ao estudo da ecologia da comunidade fitoplanctônica do reservatório de Juturnaíba, com o objetivo de identificar os principais fatores determinantes das variações temporais da biomassa e composição da comunidade (Capítulo 3). Neste estudo é assinalada a hipótese de que a disponibilidade de nitrogênio inorgânico associada a razão N/P foi um dos principais fatores controladores da comunidade fitoplanctônica.

Este capítulo contou com a co-orientação da Dra. Vera Lúcia de Moraes Huszar, do Departamento de Botânica do Museu Nacional, Universidade Federal do Rio de Janeiro, onde foram analisadas as amostras do fitoplâncton.

A hipótese de que a razão N/P é um dos principais fatores reguladores do fitoplâncton foi avaliada através de estudos experimentais sobre a ecofisiologia das principais espécies registradas no reservatório de Juturnaíba (Capítulo 4). Estes visaram avaliar a influência da razão N/P sobre o crescimento destas espécies e relacionar os resultados observados com os dados obtidos no ambiente.

2. Phytoplankton of an eutrophic tropical reservoir: comparison of microscopy data with estimation from HPLC measurements of photosynthetic pigments using a matrix factorization program (CHEMTAX)

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Keywords: phytoplankton, chlorophyll, carotenoids, photosynthetic pigments, HPLC, CHEMTAX, cell microscopy

(ARTIGO SUBMETIDO PARA PUBLICAÇÃO NO JOURNAL OF APPLIED PHYCOLOGY)

Abstract

The seasonal variation of phytoplankton in an eutrophic tropical reservoir was evaluated through photosynthetic pigments analyzed by HPLC. The contributions of algal classes to total chlorophyll *a* (TChl-a) were estimated by two procedures. The first one used the marker pigment/chlorophyll *a* ratio available from culture studies of the major species of each class. In the second procedure, a matrix factorization program (CHEMTAX) was used to analyze the pigment data. These results were compared with estimated biomass (biovolume) from microscope analysis. High correlation was observed between total biovolume and TChl-a, suggesting that TChl-a can be used as a parameter for estimating total biovolume. The contribution of the major phytoplankton groups to TChl-a, based on pigment data, was significantly correlated with microscopy data, and variations in phytoplankton biomass and composition were detected. These results demonstrated that, in spite of some inherent limitations, the HPLC method is a valuable tool for monitoring and for ecological studies of phytoplankton. The CHEMTAX software satisfactorily calculated the contributions of the algal groups to TChl-a, and provided better agreement with microscopy data than the calculations based on marker pigment/chlorophyll *a* ratios.

2.1. Introduction

One of the greatest challenges for humanity in the 21st century will undoubtedly be the control of the quality and the quantity of water supplied to the population. In the last decades, several studies have aimed at improving water resource management. Limnological studies provide important information, which constitutes the scientific basis for the management and control of the environment. In this sense, the phytoplankton community can act as a refined sensor for the understanding of the dynamics of environmental variables (Margalef, 1983).

The traditional method for determining phytoplankton biomass and composition (Utermöhl, 1958) has the advantage of allowing sampling to be done without any special equipment, and storage to be possible for many years. However, this method requires highly specialized and trained technicians, and is a time consuming procedure. Therefore, only few ecosystems have been continuously monitored (Wilhelm et al., 1991).

In this context, it is relevant to develop and apply simplified and/or automated methodologies for the monitoring of phytoplankton. Although chlorophyll *a* – which can be easily estimated by existing methods – is a good indicator of the trophic state (Whilhem et al., 1995), in many cases the composition and the percentage contribution of the major algal classes to total chlorophyll reveal additional important information.

The quantitative analysis of phytoplankton pigments through the HPLC technique can represent an efficient alternative for studies and monitoring of the phytoplankton community (Wilhelm et al., 1995). The quantification of phytoplankton biomass by HPLC has been shown to correlate well with data obtained by microscopy count (Wilhelm et al., 1991; Tester et al., 1995; Roy et al., 1996; Schmid et al., 1998).

This method presents three main advantages: 1) the time needed for the analysis is three times less than that by microscopy; 2) the analytical procedures can be automated and are easily performed; 3) the method can quantify all phytoplankton cells, independently of their size (Wilhelm et al., 1991). However, different studies do not encourage the substitution of Utermöhl's method and point to the need for further studies before quantification by HPLC can be used as a standard procedure in limnological studies (Tester et al., 1995; Wilhelm et al., 1995; Roy et al., 1996; Schlüter and Havskum, 1997).

The aim of this work was to estimate the applicability of the method of pigment analysis by HPLC to the detection of biomass variations and phytoplankton composition in a tropical

freshwater reservoir, in comparison with microscopy data. To our knowledge, this is the first study involving this methodology in a tropical freshwater ecosystem. This data was used to evaluate the procedure most frequently used in the calculation of the contribution by different phytoplankton algal groups to total chlorophyll *a*, and to test the CHEMTAX software as a tool for the interpretation of pigment data obtained by HPLC.

2.2. Materials and Methods

Study site – The Juturnaíba reservoir is a water supply located at Rio de Janeiro State, Brazil (fig. 1). Originated by the damming of a natural freshwater coastal lagoon, it is a shallow water reservoir (8 m maximum depth), with an area of 43 km^2 and 100 x $10^6 m^3$ water volume. Its main tributaries (rivers São João, Capivari and Bacaxá) drain deforested areas with agricultural activity, and receive the waste of nearby towns (Oliveira et al. 1978). It is a warm polymictic eutrophic reservoir, with frequent occurrence of cyanobacteria blooms.

Phytoplankton – Phytoplankton was sampled fortnightly at the surface (0.1 m), at a site located in the center of the limnetic region (fig. 1), between June 12th 1996 and May 28th 1997. Samples were preserved with Lugol's solution and quantified with an inverted microscope (Lund et al., 1958). Specific biomass (biovolume) was estimated from the product of the population and the mean unit volume of each taxon (Edler, 1979), assuming a specific density for phytoplankton cells of 1 g mL^{-1}.

Pigment – Water samples (0.25 to 1.8 L) were filtered in the field under gentle vacuum (< 50 kPa), onto glass-fiber filters (Whatman GF/C), immediately frozen, and stored at – 20 °C until analysis (< 3 days). Pigments (chlorophyll *a* and xanthophylls) were identified and quantified by High Performance Liquid Chromatography (HPLC). Pigments were extracted with 100 % methanol from the frozen filters, using a cell homogenizer (Braun, Melsungen, Germany) under CO_2 snow cooling. The extracts were centrifuged and the supernatants filtered (0.45 µm) and directly injected (100 µl) in a Bischoff Analysentechnik Liquid Chromatograph. The system was equipped with two pumps, a high-pressure gradient mixer, a UV-Vis detector, and a C18 reversed phase column (Bischoff Chromatography – Nucleosil 300, 250 mm x 4 mm, 5 µm). Chromatography was carried out with a binary high-pressure gradient (Garrido and Zapata, 1993, modified). The flow rate was set to 0.8 mL/min. A linear gradient from 100% eluent A (methanol / 1 M ammonium acetate - 8:2 v/v) to 100% eluent B (acetonitrile / acetone - 7:3 v/v) within 25 min was used, followed by a 10 min isocratic hold

of 100% eluent B. Detection wavelength for integration was adjusted to 440 nm. The separation was carried out at room temperature.

Pigment peaks were identified and quantified through comparison of retention times with standards obtained from unialgal cultures. Pigment standards and calibration curves were obtained according to Wilhelm et al. (1995). The amounts of the following xanthophylls were used to estimate the abundance of different classes of algae: peridinin (Dinophyceae), alloxanthin (Cryptophyceae), fucoxanthin (Bacillariophyceae), lutein (Chlorophyceae) and zeaxanthin (Cyanobacteria).

The estimation of the phytoplankton community through marker pigments is based on the contribution of chlorophyll *a* (Chl-*a*) from each group or taxonomic class to total chlorophyll *a* (TChl-*a*). Two procedures were applied to calculate the abundance of classes from pigment concentration and the contribution of each class to TChl-*a*: 1) by means of the relative specific xanthophyll amount per chlorophyll *a* (Xan/Chl-a), known from a typical species of each group (Wilhelm et al., 1995); 2) with the aid of the CHEMTAX program, which uses the factor analysis approach to estimate the contribution of each specified phytoplankton class to the TChl-*a* in a water sample. The calculation is done through a steepest descent algorithm to determine the best fit based on an initial estimate of pigment ratios (Xan/Chl-*a*) for algal classes and a final phytoplankton class-composition matrix (Mackey et al., 1996).

The initial pigment ratio matrix represents an estimate based on data from cultures of species belonging to the main classes of algae present in the phytoplankton community of Juturnaíba reservoir. This data was obtained from the literature (Mackey et al., 1996; Soma et al., 1993; Wilhelm et al., 1995). Input for the program consists of a raw-data matrix of pigment concentration obtained by HPLC analyses and an initial pigment-ratio file. CHEMTAX Software is a product of CSIRO Division of Oceanography (Hobart, Australia) and available from D. J. Mackey (Denis.Mackey@marine.csiro.au) (Mackey et al., 1997).

The data matrix for pigment concentrations was subdivided in two, resulting in more accurate ratios than when a single matrix was used. The procedure of dividing the data as a function of the heterogeneity of the phytoplankton community is recommended by Mackey et al. (1996).

2.3. Results

Biovolume

The phytoplankton biomass estimated by the biovolume, expressed in wet weight, varied widely during the study (Table 1, fig. 2). Two periods were recognised by taking into account the variability in composition and phytoplankton biomass (Fig. 2a). Period 1 (June – November 96), was marked by reduced biomass, with the dominance of a small centric diatom, *Aulacoseira distans* (Ehrenberg) Simonsen, which contributed in average 24 – 51 % to total biomass. Important contributions of cryptomonads were also observed, especially in July, when three species [*Komma acuta* (Utermöhl) Hill, *Cryptomonas marsonii* Skuja e *Cryptomonas* sp.] contributed with approximately 50 % to total biomass (fig. 2b).

Period 2 (December 96 – May 97), of elevated biomass (Table 1, fig. 2a), was marked by an extensive (5 months) and intense cyanobacteria bloom (fig. 2b). These represented, at each sampling date, between 87 % and 98 % of total biomass. During this period, a dominance sequence was observed and *Microcystis aeruginosa* Kützing dominated between December 1996 and April 1997. Between April and May a co-dominance of *Anabaena spiroides* Klebahn and *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya et Subba Raju was observed.

Table 1 – Concentration range and mean values of the phytoplankton biomass estimated by biovolume (mg L^{-1}), in the different periods observed in the study.

	Range	Mean
Period 1	0.2 – 12.2	1.9
Period 2	17.4 – 83.5	37.5

Pigments

The five marker pigments analyzed were detected throughout the study. Alloxanthin and peridinin were present in higher concentrations during Period 1 (June – November 97), whereas concentration of lutein, fucoxanthin and zeaxanthin did not differ between the periods (fig. 3). The maximum values of fucoxanthin (4.57 µg L^{-1} – February) and zeaxanthin (2.89 µg L^{-1} – March) coincided with the highest Bacillariophyceae and Cyanobacteria biomass.

Considering the five marker pigments analyzed, only fucoxanthin and alloxanthin showed significant linear correlation with the biovolume of corresponding classes (Table 2). However, we observed a significantly high correlation between total biovolume and total chlorophyll *a*.

Table 2 – Correlation coefficients between marker pigments and chlorophyll *a* and biovolume of the corresponding classes of algae (Marked correlations are significant at $p < 0.05$; $n = 25$).

		r
Dinophyceae	X peridinin	0.31
Bacillariophyceae	X fucoxanthin	0.72*
Cryptophyceae	X alloxanthin	0.75*
Chlorophyceae	X lutein	- 0.20
Cyanobacteria	X zeaxanthin	0.25
Total biovolume	X total chlorophyll *a*	0.99*

The phytoplankton biomass estimated by the amount of chlorophyll *a* using CHEMTAX varied in a quite similar way as the total biovolume, characterizing the same periods described above (fig. 4a). During Period 1 (June – November 96) smaller concentrations were observed (3.7 – 36.4 µg L^{-1}), in which the groups with higher contributions were Chlorophyceae, Cyanobacteria and Cryptophyceae. During Period 2 (December 96 - May 97) high values of chlorophyll *a* were recorded (46.9 – 254.4 µg L^{-1}) with its maximum value occurring during the Cyanobacteria bloom. These organisms were dominant in this period, contributing with 81% to 99% of total chlorophyll *a* (fig. 4b).

Table 3 shows the initial values and those calculated by CHEMTAX and used to estimate the contribution of chlorophyll *a* to each group. The final pigment ratios were different in the two matrixes for all classes, except for the Dinophyceae (peridinin). The highest difference was observed for the ratio lutein/Chl-a (68%) during Period 1, and alloxanthin/Chl-a (90%) and zeaxanthin/Chl-a (83%) during Period 2.

The contribution of phytoplankton classes to total chlorophyll *a* (TChl-*a*), calculated through Xan/Chl-a ratio determined by unialgal cultures (fig. 5), showed different values from those calculated by CHEMTAX. By absolute values, two distinct periods and the cyanobacteria bloom in Period 2 were also observed (fig. 5a). Nevertheless, the relative contributions of the classes were quite different, showing the dominance of the Cyanobacteria

(50 –100%) throughout the study (fig. 5b), in contrast to the results obtained with CHEMTAX (fig. 4b).

Table 3 – Initial values of pigment ratio (input-matrix) and values of pigment ratio obtained (output-matrixes) for two subsets analyzed by CHEMTAX. (in parentheses - percentage variation in relation to initial values)

Algal classes	Pigments				
	Peridinin	Fucoxanthin	Alloxanthin	Lutein	Zeaxanthin
Initial values					
Cyanobacteria					0.117
Chlorophyceae				0.184	0.009
Bacillariophyceae		0.792			
Cryptophyceae			0.366		
Dinophyceae	0.988				
Period 1					
Cyanobacteria					0.151 (25%)
Chlorophyceae				0.053 (68%)	0.008 (10%)
Bacillariophyceae		1.257 (26%)			
Cryptophyceae			0.475 (20%)		
Dinophyceae	0.988				
Period 2					
Cyanobacteria					0.018 (83%)
Chlorophyceae				0.184	0.009
Bacillariophyceae		0.792			
Cryptophyceae			1.037 (90%)		
Dinophyceae	0.988				

Table 4 – Correlation coefficients between the contribution of each algal class to total chlorophyll *a* obtained by pigment data and the corresponding biovolume (Marked correlations are significant at *p < 0.05, **p < 0.01; n = 25).

	Ratio Xan/Chl-a		CHEMTAX	
	r	r^2	r	r^2
Dinophyceae	0.20	0.04	0.27	0.07
Bacillariophyceae	0.64*	0.41	0.76**	0.58
Cryptophyceae	0.39	0.15	0.73**	0.53
Chlorophyceae	0.39	0.15	-0.35	0.12
Cyanobacteria	0.89**	0.79	0.97**	0.95
Total biovolume	0.97**	0.93	0.97**	0.94

The contributions of marker pigments to total chlorophyll calculated with CHEMTAX presented higher correlation with biovolume of corresponding algal classes than that calculated based on the Xan/Chl-a ratios obtained from unialgal cultures (Table 4).

2.4. Discussion

The study of the phytoplankton community through marker pigments has already been performed using different approaches. The direct correlation between marker pigments and biovolume was proposed by Schmid et al. (1998). The authors found a better correlation between marker pigments and biovolume of the corresponding algal classes than between total chlorophyll *a* and total biovolume. They concluded that the concentrations of the marker pigments are better indicators of phytoplankton biomass than total chlorophyll *a*.

Nevertheless, in this study we observed a high correlation between total biovolume and total chlorophyll *a*. High correlation had also been observed by Woitke et al. (1996). Thus, total chlorophyll *a* can be used as a parameter for biovolume estimation. Further, of the five marker pigments analyzed, only fucoxanthin and alloxanthin showed significant correlation with the biovolume of the corresponding classes. A study on the pigment content in some species of planktonic algae has shown that, in some cases, the concentrations of marker pigments, and in others the ratios Xan/Chl-a, may be more useful to quantify the relative importance of different taxonomic groups of phytoplankton (Goerick and Montoya, 1998). Thus, the approach proposed by Schmid et al. (1998) can't be universally applied without further studies.

The determination of the contribution of different classes of algae to total chlorophyll *a* has already been estimated by regression analysis (Gieskes and Kraay, 1983; Tester et al., 1995; Woitke et al., 1996; Suzuki et al., 1997). The application of this procedure to our data resulted in a significant regression, but in a very low correlation ($r = 0.55$, $r^2 = 0.30$, $p < 0.05$). This procedure has been criticized by Mackey et al. (1996), since this interative method assumes that the pigment ratios within any group are constant over the domain encompassed by the data set and variations in the abundance of different algal groups are not correlated (Mackey et al., 1996). Regression analysis in this way result, many times, in inconsistent data and non-realistic ratios, because of violation of the above assumptions (Goerick and Montoya, 1998). Even having obtained a high value for the correlation coefficient in the multiple linear regression analysis of chlorophyll *a* as a function of marker pigments, Woitke et al. (1996)

observed large discrepancies for the most abundant classes in several samples. The authors ascribe such differences to variations in the species composition, which resulted in variations of the specific ratio marker pigment/Chl-a. The same fact could have been the cause of the low correlation observed with our data when this method was applied**.**

In most phytoplankton studies by HPLC analysis, the contribution of each class to total chlorophyll is based on the marker pigment/chlorophyll *a* ratios (for instance, Wilhelm et al., 1991; Soma et al., 1993; Roy et al., 1996; Woitke et al., 1996). Some studies have evaluated the accessory pigments as biomass indicators for specific groups of phytoplankton (Woitke et al., 1996; Goerick and Montoya, 1998; Nicklisch and Woitke, 1999). They concluded that the calculation of the contributions of the different phytoplankton groups to total Chl-a by the ratios Xan/Chl-a is a more conservative approach and, despite some limitations, it is a valuable tool in ecological studies about phytoplankton.

Regarding the procedures adopted in this study, cell count data was used as a basis for comparison, despite the difficulties many times pointed out in correlating pigment data, especially chlorophyll *a* to biovolume, mainly due to the variation of chlorophyll *a* content in the cell (Bidigare et al., 1990; Wilhelm et al., 1995). However, in this study, an excellent correlation between total chlorophyll *a* (measured by HPLC) and biomass estimated by the biovolume was obtained ($r^2 = 0.98$, $p < 0.05$), indicating only a slight variation in the content of algal chlorophyll *a* when compared to its fluctuations in biovolume. Thus, although displaying some differences, the general pattern of the phytoplankton community dynamics was described in a similar way by cell count and pigment analysis; two periods and the Cyanobacteria bloom were also recorded. To some degree, other studies have also verified agreement between cell count and HPLC results (for instance, Wilhelm et al., 1991; Soma et al., 1993; Roy et al., 1996; Schlüter and Havskum, 1997). Our results also prove the applicability of this technique to the study and monitoring of tropical freshwater phytoplankton.

The interpretation of pigment data with CHEMTAX resulted in a better correlation with biovolume than that based on the Xan/Chl-a ratios obtained from unialgal cultures. Only the contributions calculated for the Chlorophyceae and the Dinophyceae did not present significant correlation with cell count.

To present, only four other studies have used the CHEMTAX program as a tool for interpreting marker pigment data from phytoplankton. Wright et al. (1996) and Mackey et al.

(1998) tested the program with marine phytoplankton data and Pinckney et al. (1998) analyzed the phytoplankton community in an estuary. Schlüter et al. (2000) investigated the influence of light and nutrients on ratios of pigments/chl-*a*. They used the CHEMTAX program for calculating the abundance of phytoplankton groups. All these studies have also concluded that CHEMTAX is an excellent tool for the analysis of the phytoplankton community through marker pigment data.

The pigment ratios obtained by the final CHEMTAX solution did not vary significantly from the initial ones, except for three of them. Mackey et al. (1996) demonstrated that the calculated contributions of the groups of algae are not dependent on the initial matrix of pigment ratios, provided that these ratios are within reasonable limits with the correct values. The same reasoning could also be applied to other studies (Mackey et al., 1998; Pinckney et al., 1998).

However, the high variations observed for lutein/Chl-a, alloxanthin/Chl-a and zeaxanthin/Chl-a have to be mentioned. They can reflect diversity of species within classes, photo acclimation responses, nutritional status or different physiological states. For example, the Chlorophyceae biomass is estimated through the sum of lutein from individuals of this class and from other classes present in the reservoir that also have lutein (Zygnemaphyceae and Euglenophyceae). These classes were represented in this study by small contributions from 78 different species, of course, different populations find themselves in different physiological states. The same explanation can also be extended to the Cryptophyceae (alloxanthin).

Regarding zeaxanthin, another aspect is relevant: high variation was only observed during Period 2. During this period, it was observed that dominance (> 80% of the biomass) shifted from *M. aeruginosa* to *A. spiroides* and *C. raciborskii* during the Cyanobacteria bloom, which coincided with a reduction of the zeaxanthin/Chl-a ratio determined by the CHEMTAX solution. Experiments with strains of *M. aeruginosa* isolated from the reservoir demonstrated zeaxanthin to be the main xanthophyll produced by this organism, while some analysis performed with different strains of *Cylindrospermopsis* spp. showed the presence of a very small concentration of this pigment (data not shown). The zeaxanthin/Chl-a ratio in *A. spiroides* and *C. raciborskii* seems to be much lower than in *M. aeruginosa*, which would explain the results obtained by CHEMTAX. Another marker pigment (myxoxantophyll or echinenone) could perhaps be more suitable for the calculation of biomass related to these

species. However, studies with two other Cyanobacteria species found in eutrophic environments (*Limnothrix redekei* and *Planktothrix agardhii*), also showed inverse relations for the echinenone/Chl-a and myxoxantophyll/Chl-a ratios, demonstrating the difficulties inherent to the choice of a marker pigment (Nicklisch and Woitke, 1999).

2.5. Conclusions

In this study, a high correlation between biovolume and total chlorophyll *a* was observed. Thus, total chlorophyll *a* can be use as a parameter for estimation of biovolume.

The estimation of the contribution of each algal class to total chlorophyll based on the ratio marker pigment/Chl-a showed a significant correlation with the quantitative data obtained by microscopy. This procedure has been used in several phytoplankton studies by HPLC, and it constitutes, in spite of some limitations inherent to the method, a valuable tool for ecological studies and for the monitoring of phytoplankton.

The CHEMTAX software has satisfactorily calculated the contributions of the groups of algae to total chlorophyll, showing to be an efficient tool for the analysis of pigment data of phytoplankton organisms.

Acknowledgements

We would like to thank CAPES (Coordenação de Aperfeiçoamento de Ensino Superior) – Programa Probral and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) / Pronex for financial support and fellowship to M. M. Marinho. We also thank Izabel Crespo for help in field work and pigment analyses.

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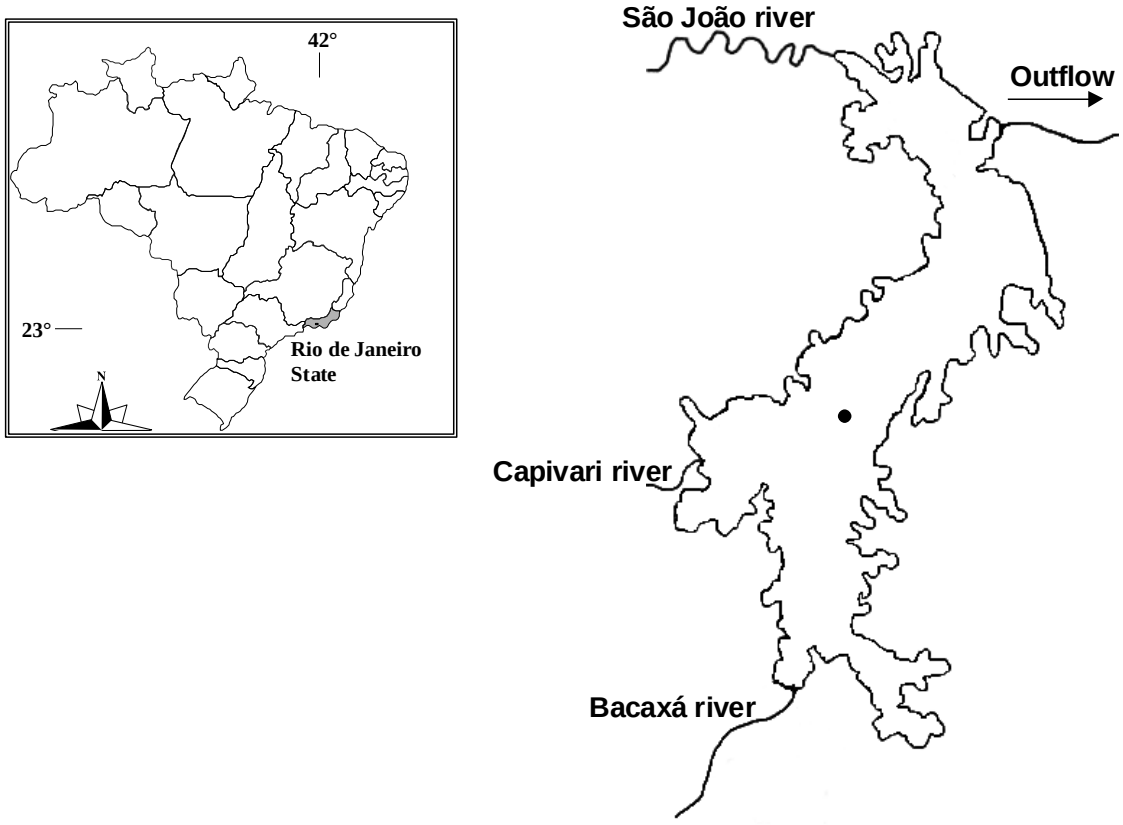

Figure 1 – Map showing location of sampling site at Juturnaíba reservoir (22°33'S e 42°18'W).

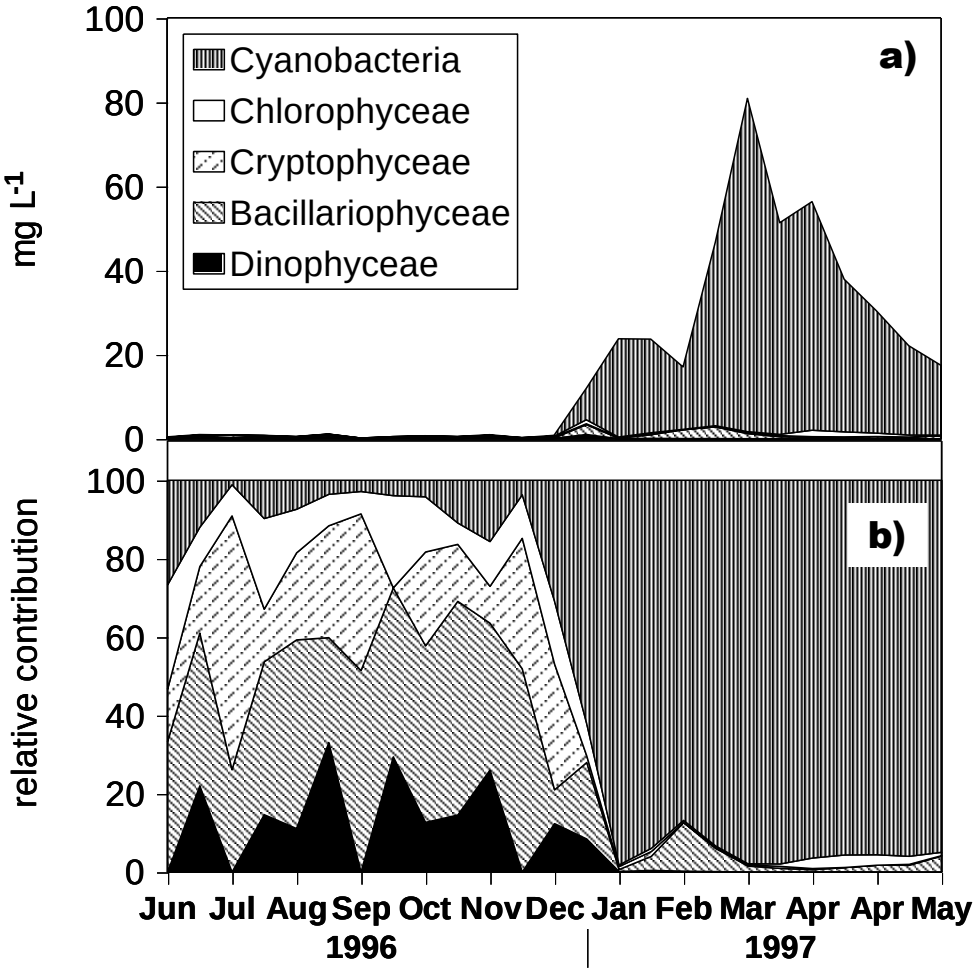

Figure 2 – Variation of the algal class biomass estimated by biovolume during the study. a) concentrations; b) relative contributions.

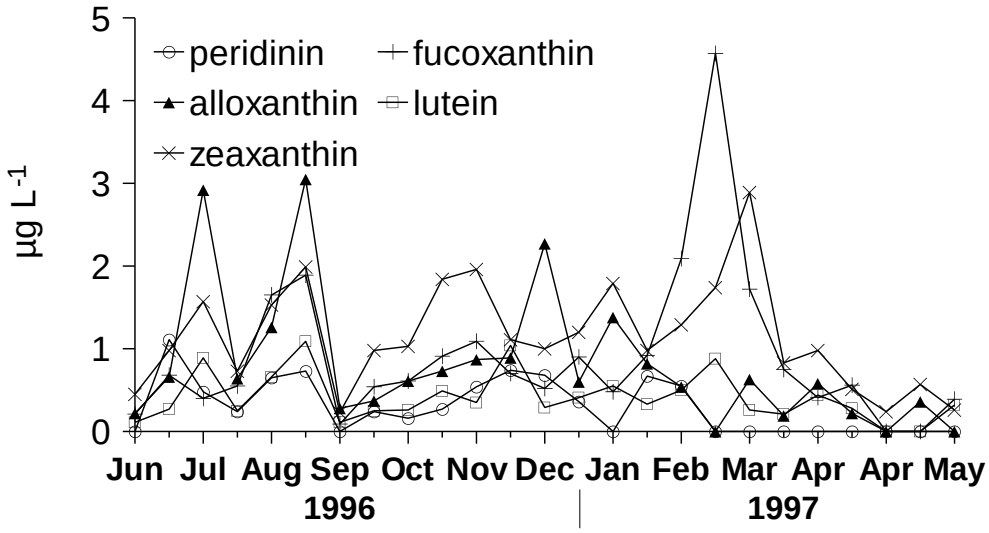

Figure 3 – Variation of the marker pigment concentration during the study.

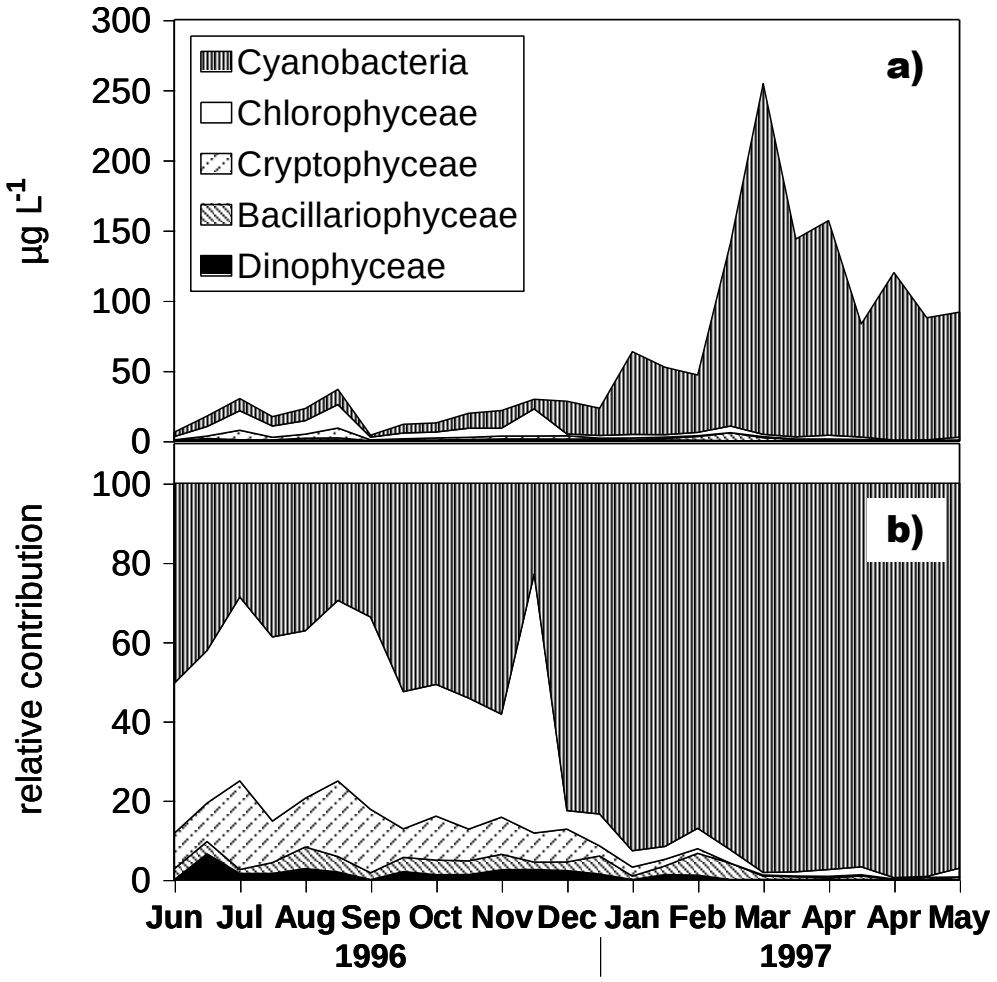

Figure 4 – CHEMTAX estimates of algal class contributions to total chlorophyll. a) concentrations; b) relative contributions.

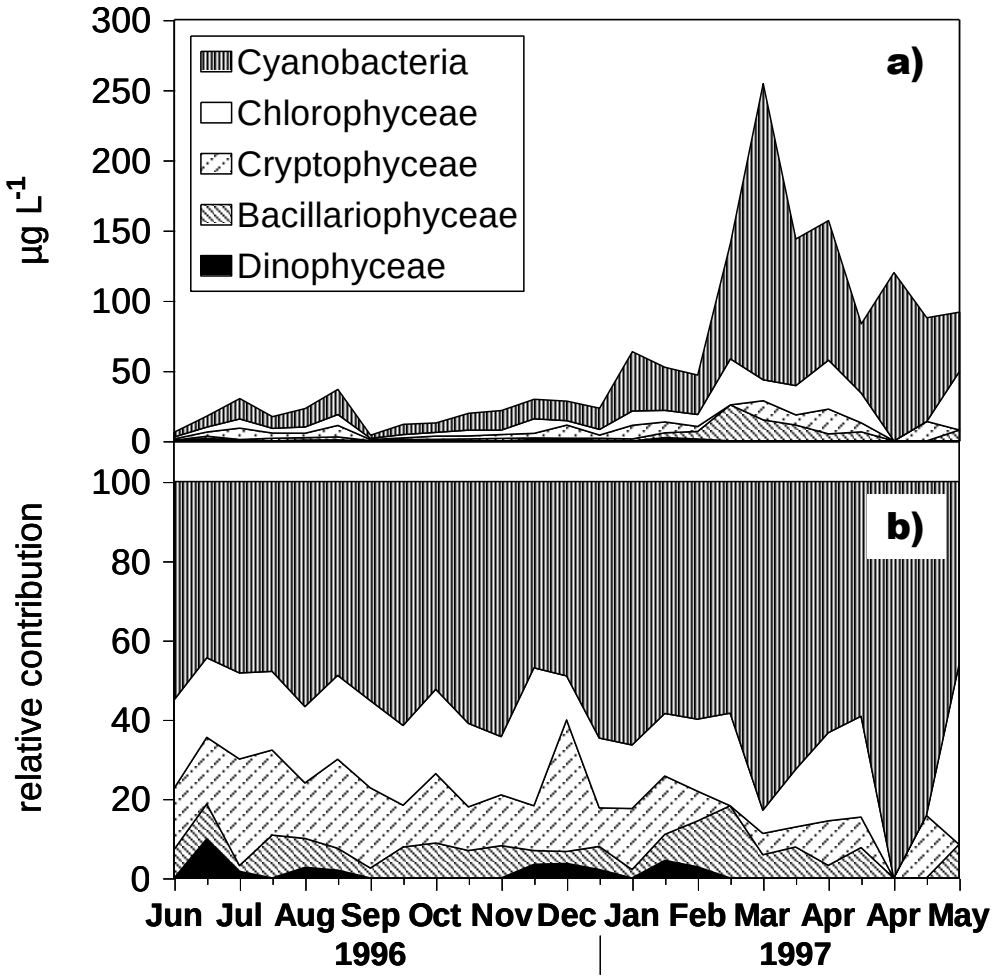

Figure 5 – Algal class contributions to total chlorophyll calculated through Xan/Chl-a ratios. a) concentrations; b) relative contributions.

3. Nitrogen availability and physical conditions as controlling factors of phytoplankton composition and biomass in a tropical reservoir (Southern Brasil)

Marcelo Manzi Marinho[1] & Vera Huszar[2]

Abstract

Phytoplankton species composition and abundance were recorded biweekly over a one-year period in an eutrophic drink water supply reservoir (Juturnaíba Reservoir, Brazil), together with physical and chemical variables. Based on the results of canonical correspondence analysis and variations on phytoplankton composition and biomass two distinct stages were recognised. The first was characterised by low phytoplankton biomass with dominance of *Aulacoseira distans* and cryptomonads (**D** and **Y** assemblages composed by C-strategists (invasive) small, relatively fast-growing species). In the second period *Microcystis aeruginosa* (**M** assemblage; an S-R strategist), *Anabaena spiroides* (**H** assemblage; R-strategist; ruderal) and *Cylindrospermopsis raciborskii* (**H-Sn** assemblage; R-strategist; ruderal) dominated with a high biomass. Abundances of *A. distans* and cryptomonads were positively associated with NO_3^- and N/P ratio and negatively with temperature and light. Cyanobacteria abundance was negatively associated with NO_3^- and N/P ratio, and positively associated with temperature and light. **D** and **Y** assemblages were selected by conditions of mixing and low light during the cold-dry season. Water column harder to mix but on daily scale and decreasing nitrogen availability, mainly DIN, favoured the **M** assemblage, and *M. aeruginosa* bloomed when DIN concentrations were < 5µM. With severe nitrogen limitation, **H** and **H-Sn** assemblages of N-fixing species were favoured and dominated the community.

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3.1. Introduction

Phytoplankton are the major primary producers in many ecosystems and the basis for the aquatic food web. Because of their importance, much work has been done to understand the structure and function of the phytoplankton community. Seasonal succession of phytoplankton in lakes is generally assumed to be a function of changing physical, chemical and biological variables (TILMAN et al. 1982, REYNOLDS, 1984, SOMMER et al., 1986). Although marked hydrological differences exist between lakes and reservoirs, the selective mechanisms of phytoplankton in reservoirs are, in fact, no different from those operating in lakes (REYNOLDS 1999a).

Models have been developed to understand the regulatory influence on phytoplankton of environmental factors acting upon phytoplankton assemblages (e.g. MOSS et al. 1996). Most of the predictive models of spatial and seasonal variation treat phytoplankton either as a single entity or by major taxonomic divisions. Far from being a uniform group, however, freshwater phytoplankton is composed of organisms drawn from eleven or so algal phyla, with high diverse morphometries. Although the traditional view, which examines taxonomic control at division level, has predicted relative abundance of phytoplankton with some success, the morphological-functional framework (REYNOLDS 1997) has performed better than the taxonomic approach (HUSZAR & CARACO 1998, HUSZAR et al. 2000), in simulating compositional changes.

Based on this approach, lakes can be characterised by groups of phytoplankton species that share common advantageous attributes. According to this model, C-strategists (invasive) are small phytoplankton, which grow quickly, selected by conditions of both high light and high nutrients; S-strategists (stress-tolerant) are slow-growing, large unicells or colonies, able to dominate under conditions of high light and low nutrients; and R-strategists (ruderals) are, generally, large elongated unicells and colonies or filaments, adapted to the low light incumbent upon frequent fluctuations of light in deep turbid layers, mixed by external physical forcing (REYNOLDS 1997). According to their growth strategies, the pelagic vegetation was classified in a number of assemblages, each one being more likely to occur in particular environmental conditions (REYNOLDS 1997, PADISÁK & REYNOLDS 1998, HUSZAR et al. 2000).

To improve the predictive value of the models and our knowledge about the phytoplankton regulatory factors, good data sets that relate ecophysiology to species-specific distribution patterns are needed. Some works have examined the correlation between environmental factors and individual species abundance (e.g. LUND 1954, TILMAN 1982, TALLING 1987, SOMMER 1993). However, few have attempted to determine whether species-specific distribution patterns are correlated to environmental factors and if these patterns are consistent with experimental observations about the physiology of particular species (INTERLANDI et al. 1999).

In this study, we have analysed the phytoplankton of Juturnaíba reservoir, a tropical eutrophic system, which is used for storage of drinking water. The reservoir is in a state of advanced eutrophication with frequent cyanobacteria blooms. (HUSZAR 1989, MARINHO & HUSZAR 1990, MARINHO et al. 1993),

In most tropical/subtropical eutrophic reservoirs, diatoms and cyanobacteria are often the two dominant phytoplankton groups (TUNDISI 1990, HARRIS & BAXTER 1996). In general, diatoms dominate these systems during cold-dry winter/spring periods, and cyanobacteria tend to become dominant in stable phases of hot-rainy summer periods (TUNDISI 1990). The control of the dominance of cyanobacteria (see SHAPIRO 1990) and diatoms (TILMAN et al. 1986, ZHANG & PREPAS 1996, WATSON et al 1997) has been attributed to many factors but none has been consistent under all conditions.

Many hypothesis and descriptive frameworks have been proposed in the literature to explain the patterns of distribution and succession of phytoplankton. Resource-ratio theory is prominent among these competing frameworks (TILMAN 1982, SOMMER 1993). Based on this hypothesis, a number of authors have proposed that nitrogen limitation (low N/P ratios) should favour the dominance of cyanobacteria (SMITH 1983, BULGAKOV & LEVICH, 1999), since these organisms are thought to be better nitrogen competitors than other phytoplankton taxa (TILMAN et al. 1982, BLOMQVIST et al. 1994). However, emphasis in absolute concentrations of dissolved nutrient has been pointed out as a driver of phytoplankton composition and N/P ratio as a consequence (REYNOLDS 1997, 1999b).

The study reported in this paper used the empirical relationship between resource availability and species abundance in the light of species ecophysiology. The approach taken here focused on the physical (light and mixing) and chemical (nutrients) controls of phytoplankton. Biological regulation by grazing or parasitism, which may be important in

controlling species composition and abundance, is not considered in this study. We also used the morphological/functional approach on basis of dominant species to examine the phytoplankton dynamic.

3.2. Materials and Methods

Study site

Juturnaíba Reservoir (22°33'S; 42°18'W) is a water supply system located at Rio de Janeiro State, southeastern Brazil (Fig. 1). Originated by the damming of a natural freshwater coastal lagoon, it is a shallow water reservoir (8 m maximum depth), with a 43 km^2 area and 100 million m^3 water volume. Main tributaries (São João, Capivari and Bacaxá rivers) drain deforested areas with agricultural activity and receive the waste of nearby towns (OLIVEIRA et al. 1978).

The reservoir is located in a region of hot and humid climate, bearing a rainy season in the summer (November/March) and a dry period in the winter (June/August); the latter season is not pronounced, displaying an average temperature for the coldest month above 18°C. NE winds prevail, coinciding with the longest reservoir axis. Stronger winds, with average monthly speeds varying between 8 and 10 m/s are generally observed between August and November (HUSZAR 1989). The Juturnaíba reservoir is currently subject to intense anthropic action, with frequent occurrence of cyanobacteria blooms.

Field Sampling

Phytoplankton was sampled every two weeks at the surface (0.1 m) at a central station (Fig.1) between June 1996 and May 1997. Samples for nutrients were collected with a Ruttner sampler at the surface and the bottom (0.2 – 0.3 m above sediment) at the sampling station. A vertical profile of temperature was measured at 0.5 m intervals on each occasion. Conductivity was measured with a conductivity meter / thermistor (WTW LF-196). Dissolved oxygen was measured with an oxymeter (WTW - Oxi 320), and pH with a pHmeter (WTW - pH 320). Water transparency was estimated by the Secchi disk extinction depth.

Sample analysis

Dissolved inorganic nutrients - soluble reactive phosphorus (SRP), nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+) and soluble reactive silicate (SRSi) were measured in filtered samples (Whatman GF/C) frozen in the field. Laboratory analysis was performed on melted water within 15 days, using standard techniques (APHA, 1992).

Phytoplankton samples were fixed with Lugol's solution and populations were enumerated in random fields (UHELINGER 1964), using the settling technique (UTERMÖHL 1958). The units (cells, colonies and filaments) were enumerated, at least to 100 specimens of the most frequent species ($p<0.05$, LUND et al. 1958). Specific biomass was estimated from the product of the population and mean unit volume of each species (EDLER 1979), assuming a specific density of phytoplankton cells of $1 g/cm^3$. In general, average cell size was based on measurements of at least 30 cells.

Data analysis

The five major taxonomic groups of algae considered (VAN DEN HOECK et al.1993, KOMÁREK & ANAGNOSTIDIS 1996) were: Cyanoprokaryota (cyanobacteria), Cryptophyta (cryptomonads), Dinophyta (dinoflagellates), Bacillariophyta (diatoms), and Chlorophyta (green algae). Result analysis was performed based on the study periods identified according to variations of phytoplankton composition and biomass.

The mixing zone (Z_{mix}) was identified through temperature profiles. The resilience of this structure was ascertained by reference to the Wedderburn number (IMBERGER & HAMBLIN 1982) when ever formation of water column was stratified (Z_{mix} < maximum depth). The Wedderburn number was considered by REYNOLDS (1992), among the various existing limnological expressions, as "most useful in providing a numerical value for the stability of a given structure and for quantifying its critical resilience to environmental conditions under variation".

The euphotic zone (Z_{eu}) was calculated as 2.7 times the Secchi disk extinction depth (COLE 1994). The ratio between the euphotic zone and mixing zone (Z_{eu}/Z_{mix}) was used as a measure for light availability in the mixing zone (JENSEN et al. 1994). The N/P ratio was calculated as the molar ratio between N ($NO_3^- + NH_4^+ + NO_2^-$) and P(SRP).

Nutrients may only be selective between phytoplankton groups if they are limiting to one of those groups. Approximations of nutrient absorption limitation by phytoplankton were made by comparing the half-saturation constants for absorption (K_m, see SOMMER 1989), presented in HUSZAR & CARACO (1998), to nutrient concentration in the reservoir.

Mann-Whitney's U test was used to evaluate the differences of mean environmental variables between the periods observed in the study. Relationships between major phytoplankton species and environmental variables were assessed using the Spearman rank correlation test.

We used detrended correspondence analyses (DCA) and canonical correspondence analyses (CCA) to explore the principal patterns in phytoplankton distribution during the study. CCA is a multivariate, direct-gradient analyses, in which the ordination axes are constrained to be linear combinations of environmental variables, whereas in DCA (an indirect gradient analysis), the species are displayed along ordination axes on the basis of only biological similarities (TER BRAAK & PRENTICE 1988). CCA was used to explore the relationship between phytoplankton and environmental variables, and DCA (with detrending segments and non-linear scaling) was used to ascertain whether the selected explanatory variables were sufficient to account for the major variance in phytoplankton data (TER BRAAK 1987). The environmental variables in the data matrix were temperature, Secchi depth, pH, SRP, NO_3^- , NH_4^+, SRSi and N/P ratio. Species matrix included those that were recorded in at least 20% of the samples (62 species in a total of 131). The significance of the eight variables was determined by forward selection with Monte Carlo permutation tests (99 unrestricted permutations, $p<0.05$). The biomass of each phytoplankton species was log(x+1) transformed. The ordinations were performed using the computer program CANOCO version 3.1 (TER BRAAK 1992).

3.3. Results

Physical and chemical regime

Two periods were identified during the study, based on the variation in biomass and species composition: Period 1 (June 1996 to November 1996), and Period 2 (December 1996 to May 1997). Average surface water temperatures were relatively high during the study and significantly higher ($p< 0.05$) in Period 2 with temperatures greater than 26 °C, except for May (Table 1). The temperature vertical profiles in the Juturnaíba reservoir were relatively homogeneous throughout the study period. The small depth, as well as the prevailing NE winds, coinciding with the reservoir's longitudinal axis, resulted in ephemeral stratification, probably on a daily basis, or for short periods of time (Fig. 2).

Water density gradients between 0.09 kg/m^3 (10/07/1996) and 0.76 kg/m^3 (10/01/1997), corresponding to temperature differences of 0.8 and 1.2 °C were observed in layers of 0.5 to 1.5 m and 3.5 to 4.5 m in depth, respectively. Considering these values and assuming 3.5 m/s winds, which is the minimum intensity for the ratio between wind-induced frictional speed and the winds above reservoir surface to be valid (see REYNOLDS 1992), it was possible to

approximate the degree of stability of the water column. The Wedderburn number was always lower than 1 (0.04-0.53), thus evidencing an unstable water column structure for these conditions, at least during sampling days.

From December to April, however, is reasonable to suppose that water column is harder to mix, because temperatures are higher. At that time higher chlorophyll concentrations were registered in the upper layers during the morning, despite vertical profiles of temperature measured after midday have shown homogeneous vertical patterns (data not shown). This fact confirms this diurnal stratification in Juturnaíba reservoir.

Table 1 – Mean values and variation intervals for the environmental variables and phytoplankton biomass between surface and bottom of the Juturnaíba reservoir (* significant difference between means according to Mann-Whitney's U-test; $p<0.05$).

	Period 1		**Period 2**	
	Mean	**Interval**	**Mean**	**Interval**
Temperature (°C)	24.4 *	20.9 - 30.1	27.5 *	23.5 - 30.4
Secchi depth (m)	0.45	0.3 - 0.5	0.60	0.5 - 0.7
Z_{max} (m)	4.5 *	4.0 - 6.0	5.6 *	5.1 - 6.5
Z_{eu}/Z_{max}	0.3	0.1 - 0.3	0.3	0.2 - 0.4
Conductivity (µS/cm)	74 *	57 – 86	54.2 *	59 - 63
OD (% saturation)	100 *	82 – 111	119 *	93 - 142
pH	7.3	6.6 - 8.1	7.1	5.5 - 8.4
SRP (µM)	1.0	0.3 - 1.8	0.7	0.4 - 1.5
NO_3^- (µM)	6.6 *	1.1 - 11.4	0.3 *	0 - 2.3
NH_4^+ (µM)	2.0	0 - 4.5	1.1	0.3 - 2.5
SRSi (µM)	28	9 – 69	33	6 - 97
N/P ratio	15 *	3 – 39	3 *	0.4 - 6
Biomass (mg/L)	1.9 *	0.2 - 12.2	37.5 *	17.4 - 83.5

The availability of light in the water column, expressed by the Z_{eu}/Z_{mix} ratio, may be an important determinant of phytoplankton composition and biomass. Considering that the whole water column was mixed, this ratio may be expressed by the Z_{eu}/Z_{max} ratio. Low light availability, with only 30 % of the water column illuminated, and moderate seasonal variability of the light regime characterised the reservoir (Fig. 3a). No significant differences for availability of light have been observed between the periods (Table 1).

The reservoir was characterised by circumneutral waters with pH values slightly lower at the bottom at different periods of the seasonal cycle (Fig. 3b) and generally over-saturated with dissolved oxygen (Fig. 3c). Significant differences between the two periods ($p< 0.05$) were observed for the dissolved oxygen saturation percentages, but not for pH (Table 1). Point values for pH greater than 8.0 occurred at the surface in higher temperature months.

The majority of chemical determinants were more seasonaly variable than the physical factors such as light and the mixing regime. A trend to greater concentrations in Period 1 was observed for SRP, NO_3^- , NH_4^+ and SRSi (Fig. 4 a,b,c,d, respectively). Significant differences ($p< 0.05$), however, were recorded only for NO_3^- (Table 1). SRP concentrations were relatively high in both periods (Fig. 4a); dissolved inorganic nitrogen (DIN) forms, however, were relatively low (Figs 4b and 4c). NO_3^- contents were not detected by the method used during April and May, and showed marked seasonal variability, especially in Period 1 (Fig. 4b). It is important to point out that the NO_2^- values have always been below the detection limit of the method. In contrast to DIN, SRSi concentration was in average high in both periods (Fig. 4d), not being significantly different ($p< 0.05$). However, except for the maxima found at the surface in May and June, Period 2 was characterised by lower SRSi values, like the other dissolved nutrients. The N/P ratio was relatively low during the study (Fig. 4e), and significantly lower ($p< 0.05$) in Period 2 (Table 1).

The percentage of limitation for nutrient absorption was calculated from the values of dissolved nutrients and from K_m (half-saturation constant) from the literature, as follows:

$$\% \text{ limitation} = 100 * [1 – (C_i/C_i+K_m)]$$

in which C_i is the concentration of dissolved inorganic nutrient in sample i.

Figure 5 shows the limitation percentages for Periods 1 and 2, considering the taxonomic divisions of phytoplankton at the limitation extremes, that is, for the division with lowest and highest K_m values. For example, for NO_3^-, the percentage of limitation is presented for the study, in which Chrysophyceae (Ch) and dinoflagellates (Dn) – which have, respectively, the lowest and the highest K_m – were limited by this nutrient. Exception is made for SRSi, when only diatoms need be considered, assuming that other groups have little demand for silica and no limitation by this nutrient. It was possible to ascertain the existence of three standards, by comparing nutrient limitation for the major phytoplankton groups. First, all groups could be severely limited. Second, there could be differences between groups, with only the one with the highest K_m being severely limited. Third, there could be little limitation for any group. For Juturnaíba reservoir, NO_3^- seems to have been severely limiting in Period 2 and, therefore, corresponding to the first standard, and may lead to severe selection of species. Yet, NO_3^- in Period 1 and NH_4^+ and SRP in both periods related more to the second standard and may lead, then, to the selection of different species. The SRSi showed the third standard for both periods, this nutrient not being expected to exert a substantial role in the selection of

phytoplankton groups. Our data have shown, therefore, a great potential for NO_3^-, but low for SRSi, in selecting different phytoplankton groups at the Juturnaíba reservoir.

Phytoplankton seasonal cycle

Among the 131 species recorded in the Juturnaíba reservoir, diatom species shared community dominance with cryptomonads in Period 1, and cyanobacteria species replaced one another along Period 2 (Figs 6 and 7). Total phytoplankton biomass, expressed in wet weight, varied from 0.2 to 84 mg/L, with a February peak with values 4.5 times greater than the annual average (Fig. 6). Two periods were recognised by taking into account the variability in composition and phytoplankton biomass (Table 2). Period 1, marked by reduced biomass (1.9 mg/L average), with dominance of a small centric diatom, *Aulacoseira distans* (Ehrenberg) Simonsen, belonging to Assemblage **D**, which contributed in average 24 % to total biomass, reaching between 20 and 51 % between August and November. Important contributions of cryptomonads belonging to Assemblage **Y** were also observed, especially in July, when three species [*Komma acuta* (Utermöhl) Hill, *Cryptomonas marsonii* Skuja e *Cryptomonas* sp.] contributed approximately 50 % to total biomass.

Table 2 - Percentages of phytoplankton assemblages (Ass.) as dominant groups of species, by period in Juturnaíba Reservoir. Labels according to REYNOLDS (1997), PADISÁK & REYNOLDS (1998) and HUSZAR *et al*. 2000).

Period 1	Ass.	Period 2[a]	Ass.	Period 2b	Ass.
12 Jun - 10 Dec		**26 Dec - 17 Apr**		**30 Apr - 28 May**	
24% *Aulacoseira distans*	**D**	72% *Microcystis aeruginosa*	**M**	46% *Cylindrospermopsis raciborskii*	**Sn**
21% *Cryptomonas* spp.	**Y**	11% *Anabaena spiroides*	**H**	42% *Anabaena spiroides*	**H**

Period 2, of elevated biomass (mean 37.5 mg/L), was marked by an extensive (5 months) and intense (maximum 84 mg/L) cyanobacteria bloom. These represented, at each sampling date, between 87 % and 98 % of total biomass. During this phase, a dominance sequence was observed (Fig. 6), dividing Period 2 in two subperiods (2a and 2b). *Microcystis aeruginosa* Kützing (Assemblage **M**) prevailed between December 1996 and April 1997 (Period 2a), in average with 75 % contribution to total biomass, followed by *Anabaena spiroides* Klebahn (Assemblage **H**), with 11 %. Between April and May (Period 2b) a codominance of *A. spiroides* (42%) and *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya et Subba Raju (46%) was observed (Assemblage **Sn**).

Correlation between phytoplankton populations and environmental variables

The CCA axes were quite close to those of the DCA axes (see eigenvalues in Table 3), suggesting the measured environmental variables accounted for a large proportion of the variation in species distribution. The slight difference between the CCA and DCA axes shows that other unidentified variables were also important.

Table 3 - Eigenvalues (proportion of variance of the phytoplankton data explained by the axis - λ) and species-environmental variable correlation (CC) of the first two axes of CCA and DCA, and the percentage of variance in the phytoplankton species-environment biplot (% variance) explained by the first two axes.

	CCA		**DCA**	
	Axis 1	**Axis 2**	**Axis 1**	**Axis 2**
λ	0.51	0.19	0.62	0.16
CC	0.91	0.77	0.93	0.36
% variance	65	24	53	3

Table 4 – Inter-set correlation of environmental variables with the first two axes, significance of unrestricted Monte Carlo permutation tests (p value; 99 permutations) of the eight environmental variables, and the variance explained by the selected variables.

	Inter-set correlation			
	Axis 1	**Axis 2**	**p**	**Variance**
Temperature	-0.32	-0.71	0.02	0.22
Secchi depth	-0.70	0.32	0.01	0.34
pH	0.04	-0.12	0.98	0.03
SRP	0.29	0.15	0.18	0.09
NO_3^-	0.89	0.09	0.01	0.49
NH_4^+	0.30	0.25	0.07	0.12
SRSi	-0.05	0.12	0.80	0.04
N/P ratio	0.66	0.12	0.01	0.30

The forward selection option of CCA with Monte Carlo permutation tests indicated that nitrate, N/P ratio, temperature and Secchi depth were significant in explaining the variation of the phytoplankton distribution pattern in the Juturnaíba reservoir (Table 4). The influence of these variables on the phytoplankton species is shown in the CCA biplot (Fig. 8). The distribution pattern of biomass and composition of the phytoplankton species obtained by CCA, which was better explained by the selected environmental variables, was the one displayed by axis 1, and could be interpreted as a gradient of nitrogen availability and light.

The same periods described by variations on phytoplankton composition and biomass could be recognised in the CCA ordination diagram (Fig. 8). Thus, samples of Period 1 presented higher values of NO_3^- and relatively higher values of N/P ratio. This period was characterised by low phytoplankton biomass (< 15mg/L; see Table 1) and dominance of *A.*

distans and cryptomonads. Period 2 presented an inverse situation with very low NO_3^- concentrations and relatively lower values of N/P ratio. High phytoplankton biomass with a maximum of 84 mg/L (Table 1) and dominance of *M. aeruginosa*, *A. spiroides* and *C. raciborskii* (Figs. 6 and 7) marked this period. The CCA also showed a gradient associated with temperature and light (second axis).

Fluctuations on these variables reflected shifts in phytoplankton composition and biomass, especially during Period 2, related with the two subgroups already identified (Fig. 8). Subgroup 2a (December – April 2nd) was characterised by temperatures greater than 26°C and dominance of *M. aeruginosa*. Subgroup 2b (April 17th – May) presented the dominance of *A. spiroides* and *C. raciborskii*, associated with higher light availability.

Table 5 – Subgroups characterised by CCA for Period 2. Mean values and variation intervals for the environmental variables and phytoplankton biomass (* significant difference between means according to Mann-Whitney's U-test; $p<0.05$).

	Period 2a		**Period 2b**	
	Mean	**Interval**	**Mean**	**Interval**
Temperature (°C)	28.9 *	27.2 - 30.4	25.8 *	23.5 - 28.1
Secchi depth (m)	0.57 *	0.5 - 0.6	0.61 *	0.6 - 0.7
Conductivity (µS/cm)	63	59 - 70	61	60 - 62
OD (% saturation)	117	93 - 142	116	93 - 139
pH	7.3	5.5 - 8.4	7.0	5.5 - 8.2
SRP (µM)	0.8	0.4 - 1.0	0.7	0.4 - 1.5
NO_3^- (µM)	1.0 *	0 – 2.7	0 *	-
NH_4^+(µM)	0.9	0.3 - 1.5	1.6	0.6 - 2.5
SRSi (µM)	21	6 - 41	47	14 - 97
N/P	3	1.3 - 6.6	3	0.44 - 6.1

The average of variables between Period 2 subgroups presented significant differences for NO_3^-, temperature and Secchi depth (Table 5). In relation to the variables prominent in the CCA, the dominant species in Period 1 displayed significant positive correlation with NO_3^- and the N/P ratio, and negative with temperature and Secchi depth (Table 6). Significant positive and negative correlation were also observed with SRP for *A. distans* and *C. marsonii*, respectively, although this variable was not significant in the CCA (Tables 4 and 6). The prevailing cyanobacteria in Period 2 displayed significant positive correlation with temperature and Secchi depth and negative with NO_3^- and N/P ratio (Table 6).

Table 6 – Spearman correlation coefficients between the biomass contributions percentage of main species and temperature (T), Secchi depth (SD), soluble reactive phosphorus (SRP),

soluble reactive silica (SRSi), ammonium (NH_4^+), nitrate (NO_3^-), SRSi/SRP ratio and N/P ratio (only significant correlations are presented – $p<0.05$).

Species	T	SD	SRP	SRSi	NH_4^+	NO_3^-	SRSi/SRP	N/P
Anabaena spiroides	0.43	0.78	-	-	-	-0.82	-	-0.70
Microcystis aeruginosa	0.74	0.51	-	-	-	-0.55	-	-0.59
Cylindrospermopsis raciborskii	-	0.65	-	-	-	-0.68	-	-0.43
Aulacoseira distans	-0.54	-0.76	0.48	-	-	0.78	-	0.62
Komma acuta	-0.42	-0.69	-	-	-	0.82	-	0.62
Cryptomonas sp.	-	-0.66	-	-	-	0.75	-	0.53
Cryptomonas marsonii	-	-	-0.39	-	-	-	-	-

3.4. Discussion

The seasonal pattern of phytoplankton dynamics at the Juturnaíba reservoir in 1996/97 presented substantial differences from those recorded in three previous studies. In the periods of 1978-80 (HUSZAR 1989) and 1980-81 (MARINHO & HUSZAR 1990) when it was still a freshwater coastal lagoon, phytoplankton composition and abundance was marked, in fall-winter, by an alternantion in dominance between cyanobacteria species (*Anabaena solitaria* f. *planctonica*) and green algae (*Koliella longiseta* f. *tenuis*), and in spring-summer by desmids (*Staurastrum lunatum* and *Staurodesmus dickiei*) and diatoms (*Melosira* = *Aulacoseira* spp.). Yet, in the study carried out three years after the filling of the reservoir (MARINHO et al. 1993), a remarkable change in phytoplankton community was observed, including a major reduction in biomass, with no cyanobacteria bloom. Chrysophytes and cryptomonads were then the characteristic organisms in the phytoplankton community.

In the present study, fifteen years later, the annual phytoplankton cycle of the Juturnaíba reservoir proved to be quite different, having moved from a more diversified community, with relevant diatom and cryptomonad contributions in the winter and spring months, to a community dominated by few cyanobacteria species, succeeding one another in high biomass for a long period, during summer and autumn. Blue-green dominance depends on a degree of environmental constancy (PEARL 1988). Relative constancy during warmer months led to this long-lasting blue-green dominance, especially in this period at the beginning the El Niño event (1997-1998). The interannual variability in the reservoir seems to have occurred due to changes from the natural to the artificial environment and also by other factors probably related to climatic conditions, eutrophication processes stemming from the agricultural and urban development of the region, and/or increasing of residence time, with consequent increasing in sedimentation rates. In fact, by comparing mean SRP and DIN values between

1980-81 (L.C. Alvarenga, pers. comm. 1998) with data acquired in the present study, was possible to ascertain an increase in dissolved P and N contents of around 18 and 3 times respectively.

Mixing in lakes and reservoirs has been pointed out as an important defining factor of phytoplankton composition and abundance (REYNOLDS 1999a), including tropical and subtropical reservoirs (SANTOS & CALIJURI 1998). The shallow reservoir depth and prevailing wind action along of its longitudinal axis favoured the mixing of the water column, which occurred daily. Estimates made through the Wedderburn number have confirmed the scarce resilience of the vertical structure of the Juturnaíba reservoir, even with increasing depth in the rainy season (November – March). Weak thermoclines, mostly on the top layers of the water column, prevented the existence of persistent thermal stratification in the reservoir. Daytime stratification, with the reduction of mixing depth during the day and erosion of daytime thermoclines, increasing mixing depth through night-time cooling, are common in shallow tropical environments (HENRY 1995). The Juturnaíba reservoir may then be classified as a warm continuous polimyctic system according to the criteria established by LEWIS (1983).

The occurrence of a daily mixture to the bottom implies in a frequent inflow of sediment material, thus resulting in low light availability, which is more severe in periods of high biomas. Indeed, water transparency was reduced throughout the entire study period ($Z_{eu}/Z_{max} < 0.4$), which warrants the assumption that light might have acted as a limiting factor for phytoplankton, in spite of the fact that continuous mixing allows, for short periods (minutes), organism access to the better-lighted surface layers (see REYNOLDS 1994). The deficiency of light in shallow reservoirs may be considered as a selective factor for phytoplankton (REYNOLDS 1999a). At the Juturnaíba reservoir, the main species selected both in Period 1 (*A. distans* and *Cryptomonas* spp.) and in Period 2 (*M. aeruginosa*, *A. spiroides* and *C. raciborskii*) may be considered as adapted to low light intensity (SOMMER 1988, KLAVENESS 1988, PADISÁK 1997, COLES & JONES 2000).

Another potentially regulating factor for the dynamic and structure of phytoplankton communities is nutrient availability. The concentration of dissolved P and N for all algae groups, and of Si for diatoms, are frequently thought to govern the distribution and variation in abundance of different groups of planktonic algae. Species selection is driven by the different requirements of each species, whenever nutrients are chronically low (REYNOLDS 1997). When nutrients fall to limiting concentrations, it may discriminate between species.

The data of this study, based on K_m values, have shown great potential for NO_3^-, especially in Period 2, albeit low for SRSi in both periods in selecting different phytoplankton groups in the Juturnaíba reservoir. Such data have also indicated that NH_4^+ and SRP displayed moderate selective potential, at least for phytoplankton groups with higher K_m (cyanobacteria and green algae, respectively). It is important to point out, however, that although SRP concentrations have fluctuated throughout the study, they have always been above the required levels for algae growth saturation (>0.1µM, see REYNOLDS 1997) which points towards the same direction of results on limitation estimated through K_m.

In spite of the relatively extensive literature on phytoplankton regulating factors at major classes level, little research on natural environments has been carried out towards understanding the relationship between the environmental factors and the abundance of individual species. High-resolution data on populations in nature is surprisingly rare in the literature (INTERLANDI et al. 1999). Recognised specific standards are presented in this study for the main species and their corresponding associations, in REYNOLDS's (1997) sense, which have taken place in the Juturnaíba reservoir. Diatom, cryptomonad and cyanobacteria representatives have occurred, with important contributions during the study.

The scarce data in the literature on the regulating factors for cryptomonads allow only for the statement that they are ubiquistic organisms occurring in great part of the trophic spectrum (WATSON et al. 1997). They display low light requirements (KLAVENESS 1988) and are intensely consumed by herbivores due to their adequate size and nutritive values (STERNER 1989). The three cryptomonad species (*K. acuta*, *C. marsonii* and *Cryptomonas* sp.), important in biomass in certain samplings during Period 1, were indeed associated to low light intensity as well as to lower temperatures, high NO_3^- concentrations, and high N/P ratios. In another polymictic eutrophic system, abundance of *Cryptomonas* species occurred in periods of low availability of light (SANTOS & CALIJURI 1998). Another factor associated to cryptomonad distribution may have been the availability of N, which was suggested by positive correlations with NO_3^- and the N/P ratio. However, as far as known, there are no data on the physiology of this species as to nitrogen, which hinders result interpretation.

Unlike the cryptomonads, the factors regulating diatom dominance have been relatively well analysed. Such dominance has been associated to turbulence (SOMMER 1988), high SRSi availability or high Si/P ratio (TILMAN et al. 1986), low temperatures (ZHANG & PREPAS 1996) and low availability of light (SOMMER 1988). At the division level, they contribute with

the greatest biomas in an increasing rate, in the meso-to-eutrophic interval of the trophic spectrum (WATSON et al. 1997). *A. distans* was the species of greatest importance to total biomass during Period 1. As far as it is known, there is no data in the literature on physiological aspects of this species. Its morphological features, however, allow for comparisons with other species of small centric diatoms, especially if we consider that only filaments with one, or rarely two, cells have been observed. This species was related to lower temperatures, lower availability of light and higher SRP and NO_3^- contents. Negative correlations with temperature and light agreed with the general data in the literature for diatoms as a group. However, the growth of *A. distans* has also been associated with high light availability (KILHAM et al. 1986). The lack of correlation with SRSi and with SRSi/SRP ratio is also noteworthy, which seems to reflect the relatively high concentration of this nutrient as to diatom requirements, as evidenced by the analysis of percentage of limitation. The study of KILHAM et al. (1986) on relationships between resources and African planktonic diatom species, associated *A. distans* to light/P ratio, and not to Si/P ratio, and suggested that *A. distans* has low P requirements. Our data, however, presented a positive correlation between *A. distans* and SRP. Physiological studies demonstrated that small centric diatoms have low Si requirement and a relatively high P requirement (VAN DONK & KILHAM 1990).

Another factor which may have favoured *A. distans* dominance during Period 1 was water turbulence. Due to the high density of their silicose frustules, *Aulacoseira* species are frequently associated to turbulent environments (KILHAM et al. 1986, HUSZAR et al. 1998). During Period 2, in spite of favourable conditions such as turbulence and good Si and P availability, *A. distans* was replaced by cyanobacteria indicating that other factors have determined the selection of this species. Although the knowledge of diatom physiology as to N is limited, recent studies have demonstrated that concentrations < 10µM of inorganic nitrogen are potentially limiting for diatoms (INTERLANDI et al. 1999). During Period 2, limitation by N was evidenced by the analysis of the percentage of limitation. Besides, *A. distans* associations with greater NO_3^- content indicate that this species may have been replaced by cyanobacteria due to low availability of dissolved nitrogen in the environment.

Cyanobacteria dominance has been related to high temperatures or high pH/low CO_2 (SHAPIRO 1990), high total phosphorus contents (TRIMBEE & PREPAS 1987), low light availability (SMITH 1986), water column stability (REYNOLDS 1987), low total-N content, low TN/TP ratio (SMITH 1983) and low herbivore pressure (HANEY 1987). *M. aeruginosa* was the

most important species in the reservoir in terms of reaching very high biomasses, which lasted for several months. As expected, it was associated with higher temperatures, low NO_3^- concentrations, and low N/P ratios. Although not expected, it was related to greater light availability (SMITH 1986). Despite a small light increase during the *M. aeruginosa* dominace, light intensity was reduced throughout the study period (Z_{eu}/Z_{max} 0.1 - 0.4m).

M. aeruginosa's success has frequently been ascribed to long-lasting vertical stratification of the water column, as those observed at the end of summer in eutrophic lakes in temperate regions (REYNOLDS 1984), and during the summer and autumn months in tropical reservoirs (GOMARA et al. 1997, SANTOS & CALIJURI 1998). In these conditions, its capacity to regulate position in the water column would allow for a vertical adjustment on account of light and nutrient availability (REYNOLDS 1987). However, this same species can accommodate to intense diel changes in vertical mixing better than non- or weakly-motile algae (GANF 1974).

Several studies have pointed to nutrient availability, with emphasis on the N/P ratio, as one of the major regulating factors for *Microcystis* dominance, independent of stratification stability (e.g. MICHARD et al. 1996). In Lake Kasumigaura (Japan), heavy blooms of *Microcystis*, observed from 1970's through 1986, were replaced by abundant growths of filamentous cyanobacteria and this shift was attributed to the increase in the N/P ratio (FUJIMOTO et al. 1997). On the contrary, REYNOLDS (1997, 1999) has pointed out that is the availability of a nutrient, not the ratio between it and another which is crucial: ratios are consequence of uptake, not its drivers. In Period 2, *M. aeruginosa* dominance was strongly associated with low NO_3^- availability.

As DIN decline took place, *A. spiroides* started its development, reaching maximum levels following the decline of *M. aeruginosa* upon NO_3^- exhaustion. It was also associated with higher temperatures, low N/P ratio, and higher light availability. In this period, it occurred with a high number of heterocytes per filament. The appearance of such structures in natural *Anabaena* populations points towards N deficiency, especially in DIN concentrations < 20µM (HORNE & GOLDMAN 1972).

By sharing total biomass with *A. spiroides* in Period 2b, *C. raciborskii* associated with the same variables as the other cyanobacteria. *Cylindrospermopsis* dominance has been attributed to its physiological adaptation to growth in low-light conditions – reduced I_k (PADISÁK 1997). In the present study, *C. raciborskii* appeared in the phytoplankton community only upon

severe limitation by N, in spite of reduced light availability observed throughout the study period.

Recent studies on the physiology of *C. raciborskii* point out that the species is extremely opportunistic as to P, displaying adaptation for storing and high affinity for this nutrient. Such adaptations would favour it upon chronic P deficiency or in environments with marked fluctuations (ISTVÁNOVICS et al. 2000). However, at the Juturnaíba reservoir, on account of the high SRP contents observed throughout the entire study and of the absence of potential limitation, these adaptations do not seem to have prevailed. *Cylindrospermopsis* dominance in tropical and subtropical reservoirs has also been explained as a consequence of the stability resulting from thermal stratification (BRANCO & SENNA 1994, HARRIS & BAXTER 1996, BOUVY et al. 1999). Initial increases in *C. raciborskii*, nevertheless, were associated with the first downward movement of the thermocline into the anoxia hypolimnium (FABBRO & DUIVENVOORDEN 1996). As already demonstrated, however, the Juturnaíba reservoir did not display thermal stratification during the period of *C. raciborskii* dominance.

Cylindrospermopsis ability to fix N_2 has been often used to account for its dominance in tropical or subtropical systems (HECKY & KLING 1987, HARRIS & BAXTER 1996). As N deficiency stimulates heterocyte formation, its appearance may be used as an N_2 fixing indicator (WOLK 1982). LEWIS (1986) has associated heterocyte presence in *Cylindrospermopsis* populations to reduced DIN availability. At Juturnaíba reservoir, *C. raciborskii* ability to fix atmospheric nitrogen was demonstrated by the relatively high incidence of heterocytes (40 % of filaments), if compared to other Brazilian systems, in which only around 10 % of filaments bore heterocytes (BRANCO & SENNA 1994, HUSZAR et al., 2000).

Studies on nitrogen fixation and nitrate and ammonium absorption during *C. raciborskii* blooms, however, have pointed toward ammonium absorption at low concentrations as being more important than the fixation process (PRÉSING et al. 1996). During the maximum growth period of *C. raciborskii*, low concentration of NH_4^+ was recorded, although there had been NO_3^- exhaustion. Thus, we can say that *C. raciborskii* success at the Juturnaíba reservoir has been associated with its low light requirements, N_2 fixing capacity, and the ability to absorb NH_4^+ at low concentrations.

Statistical analysis have shown the remarkable change in phytoplankton composition from diatoms and cryptomonads to cyanobacteria in Juturnaíba reservoir clearly associated

with low DIN availability and low N/P ratio. Ratios as a consequences of uptake and not as drivers of changes in phytoplankton composition have been postulated by REYNOLDS (1997, 1999b). A contrary view explaining those changes from the resource ratio hypothesis as an independent factor has been pointed out by SMITH (1983) and recently by BULGAKOV & LEVICH (1999). In this review the author present evidence from a wide variety of scales, ranging from laboratory cultures and mesocosmos to whole lakes. However, REYNOLDS (1999b) presented three objections for N/P as drivers of changes in composition: 1) based on experiments with enclosures, it was ascertained that the N/P ratio did not determine the success of fixing cyanobacteria but, the availability of nutrients at the beginning of the growth season instead; 2) the application of Tilman's hypothesis by his followers did not consider that, in N and P concentration above the necessary demand to keep growth, there would be no limitation and, consequently, the ratio would not exert a regulatory role; 3) phytoplankton cells are equipped to perceive variation in individual resource availability, but, no molecular mechanism has been demonstrated in which the cell would be able to perceive and react to resource ratio.

In the present study, changes in community composition from diatoms and cryptomonads dominace to cyanobacteria have shown a response time of few weeks in relation to changes in DIN concentrations, but also in N/P ratios. *M. aeruginosa*, for example, only became dominant around 6 weeks after DIN had reached values below 5 µM and N/P ratios below 10. Under these conditions, non-fixing cyanobacteria are favoured due to their superior competitive ability in relation to other phytoplankton groups (Blomqvist et al., 1994). The fact that changes in phytoplankton community composition at the Juturnaíba reservoir were followed by decreases both in DIN and in N/P ratio point out that, in addition to the proportion, the absolute concentrations may have also defined the changes in composition from Period 1 to Period 2. In order to clarify these aspects, Marinho & Azevedo (in prep.) performed laboratory experiments, and the results showed nitrogen availability and not N/P ratio as a major factor determining substitution from diatoms to cyanobacteria.

According to the comprehensive scheme of community assemblages and phytoplankton selection (REYNOLDS 1997), groups of species can be thought of as system descriptors. We are able to ascribe the phytoplankton species from Juturnaíba reservoir to six of the 28 assemblages proposed by REYNOLDS (1997) and complemented by PADISÁK & REYNOLDS (1998), independently of their respective major taxonomic groups. Three of them are made of

cyanobacteria, one of cryptomonads and one of diatoms. The environmental conditions allowed us to identify periods that are described by different assemblages.

The **M** assemblage, formed by *M. aeruginosa*, may be dominant in shallow lakes with daily mixing in low latitudes (GANF 1974). This species with spherical colonies is a very clear S-strategist as shown in REYNOLDS' (1997) diagram. The flattened colonies from Juturnaíba reservoir, however, can be placed on the transition between S and R strategists and consequently performing better in turbid conditions than spherical ones (Huszar et al. 2000). **H** assemblage of Nostocales group dominates in warm waters, with good nutrient supplies but low DIN conditions (REYNOLDS 1997). It was represented in Juturnaíba reservoir by *A. spiroides*. *Cylindrospermopsis* is a genus placed on **Sn** assemblage based upon physiological attributes, including low light requirements rather than N_2 fixing capacity (PADISÁK & REYNOLDS 1998). Because almost half the population in Juturnaíba reservoir carried heterocytes, it is reasonable to regard *Cylindrospermopsis* as a genus with alternative physiological adaptation between **S** (for Oscillatoriales) and **H** assemblages. Eventually, two other assemblages were recognised: **D**-assemblage comprises small centric diatoms of shallow, hypertrophic ponds, represented by *A. distans* (HUSZAR et al. 2000) and **Y**-assemblage for the ubiquitous cryptomonads, common in relatively enriched systems.

In short, our data supports the view that the delimitation of assemblages, originally formulated for temperate regions, applies reasonably well to a tropical enriched system. In the Juturnaíba reservoir, **D** and **Y** assemblages, composed of C-strategists (invasive) small, fast-growing species (*C. marsonii* and *A. distans*) were selected for conditions of high nutrients during winter and spring. As waters get warmer, biomass increases and nutrients get scarce, mainly DIN. Selection is then driven to a **M** assemblage composed of the S-R strategist *M. aeruginosa*, which bloomed when DIN concentrations was < 5µM. High *Microcystis* biomass resulted in NO_3^- depletion in water column with severe nitrogen limitation. As nitrogen availability decreases, assemblages of N-fixing species (*A. spiroides*, *C. raciborskii*) are favoured and dominate the community.

Acknowledgements

The authors would like to thank Dr. Colin S. Reynolds for helpful editorial changes to this manuscript. We also thank Izabel Crespo for help in fieldwork. This research was financially supported by Coordenação de Aperfeiçoamento de Ensino Superior, Conselho Nacional de

Desenvolvimento Científico e Tecnológico (CNPq / Pronex), Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro and Fundação José Bonifácio-UFRJ.

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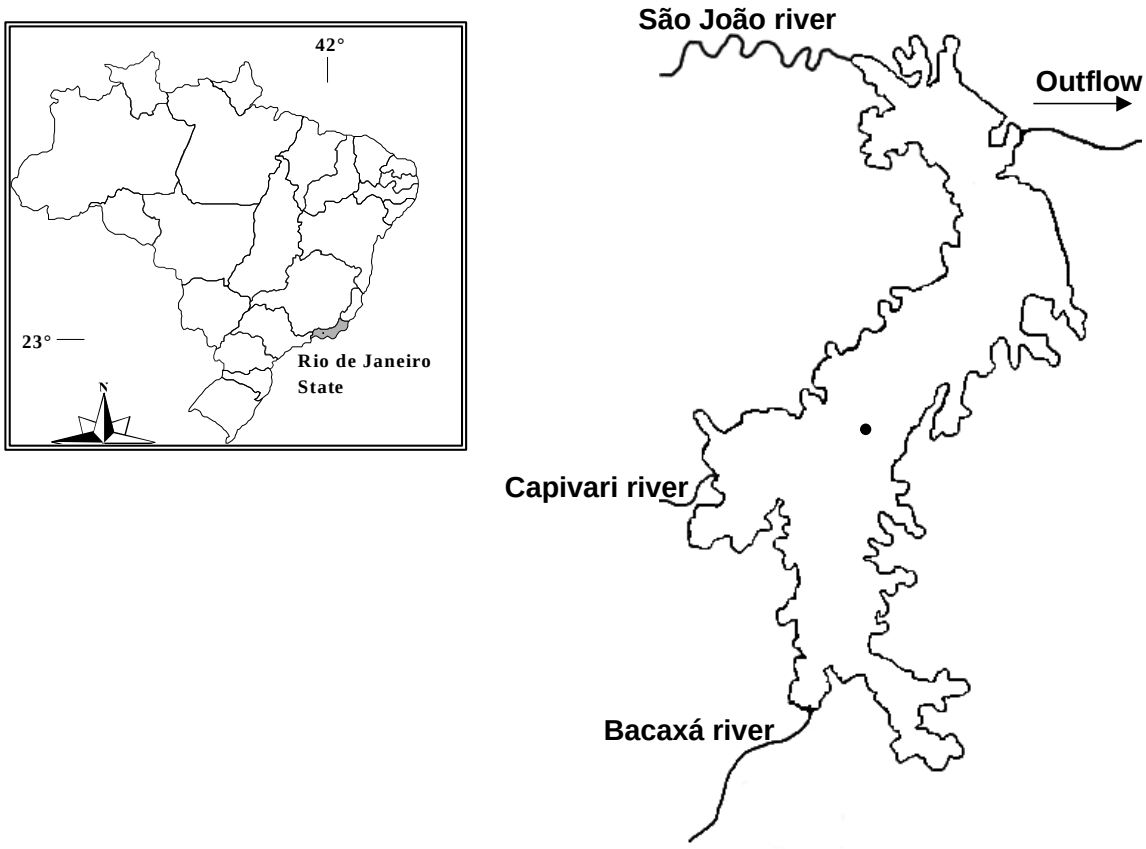

Figure 1 – Map of Juturnaíba reservoir showing the geographic location and sampling station.

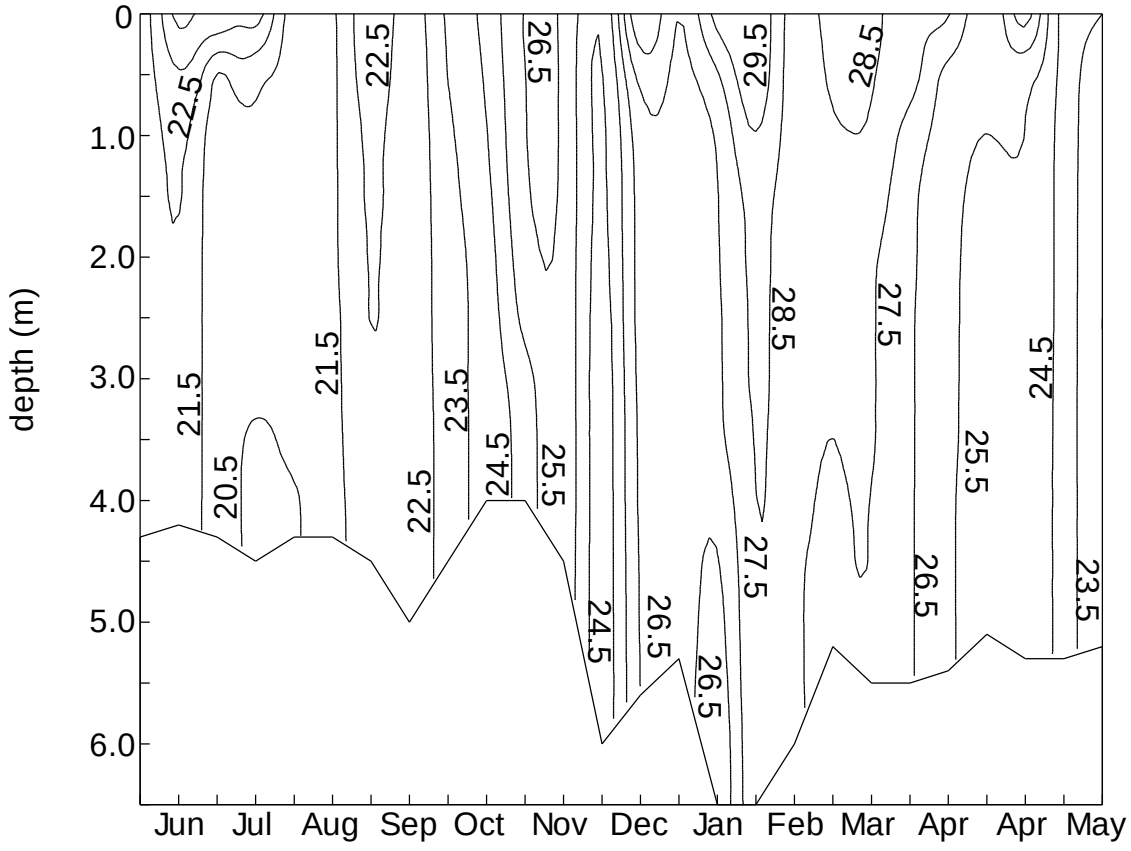

Figure 2. Depth-time temperature isopleths (°C) in Juturnaíba reservoir.

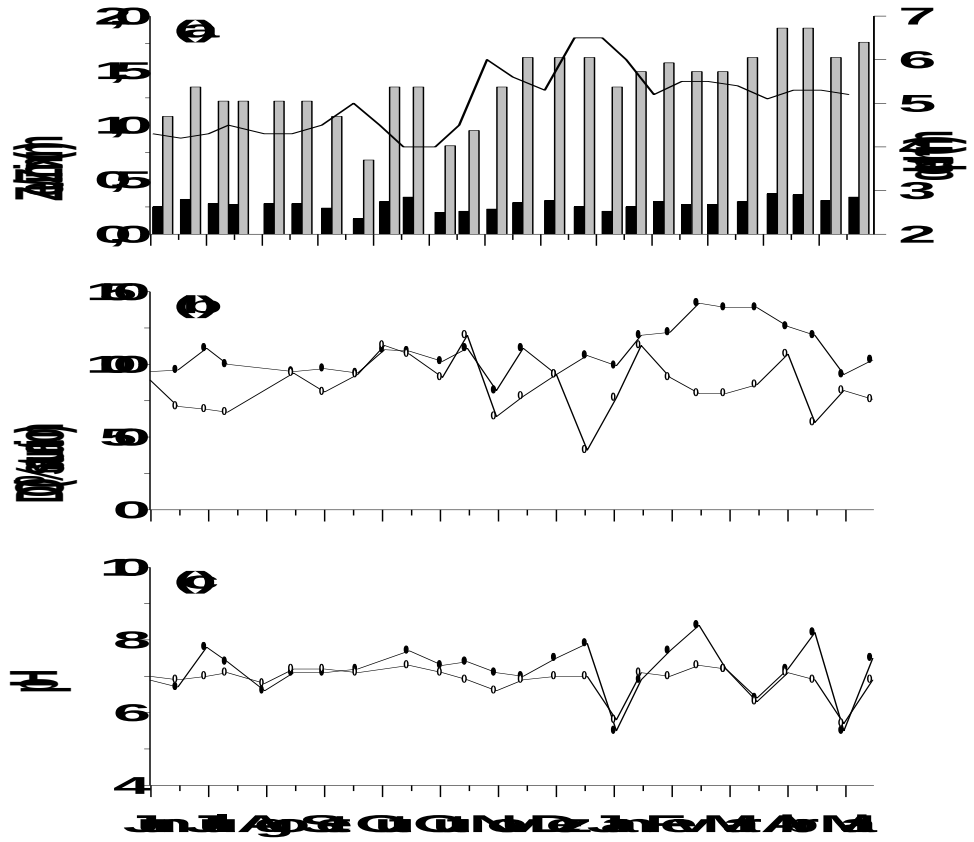

Figure 3. Seasonal variation of (a) depth (line), euphotic zone (dark shade bar) and Zeu/Zmix ratio (light shade bar); (b) dissolved oxygen; and (c) pH in Juturnaíba reservoir. Surface(●) and bottom (○)

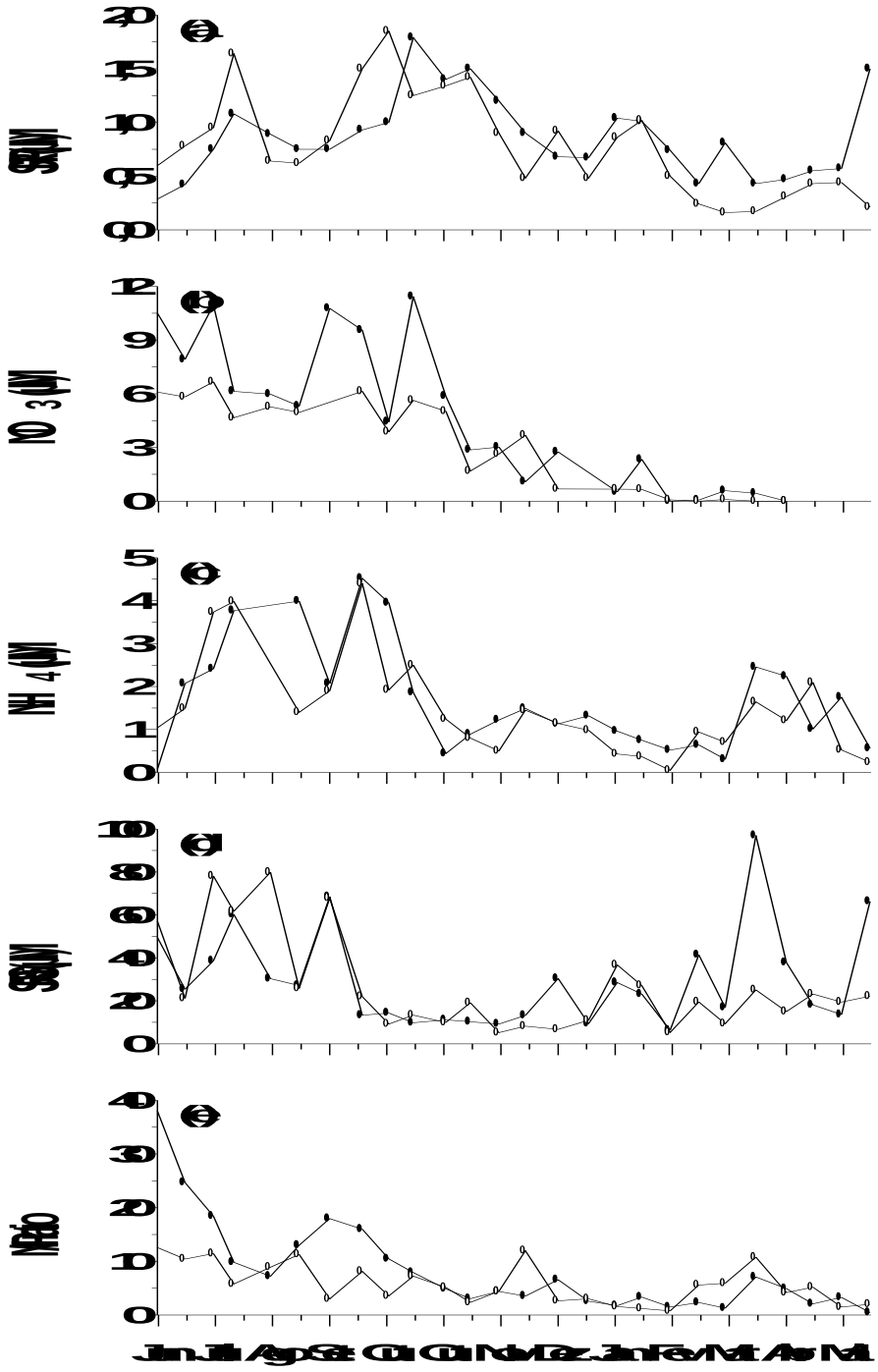

Figure 4. Seasonal variation of (a) SRP; (b) NO_3^-; (c) NH_4^+; (d) SRSi and (e) N/P ratio in Juturnaíba reservoir. Surface(●) and bottom (○)

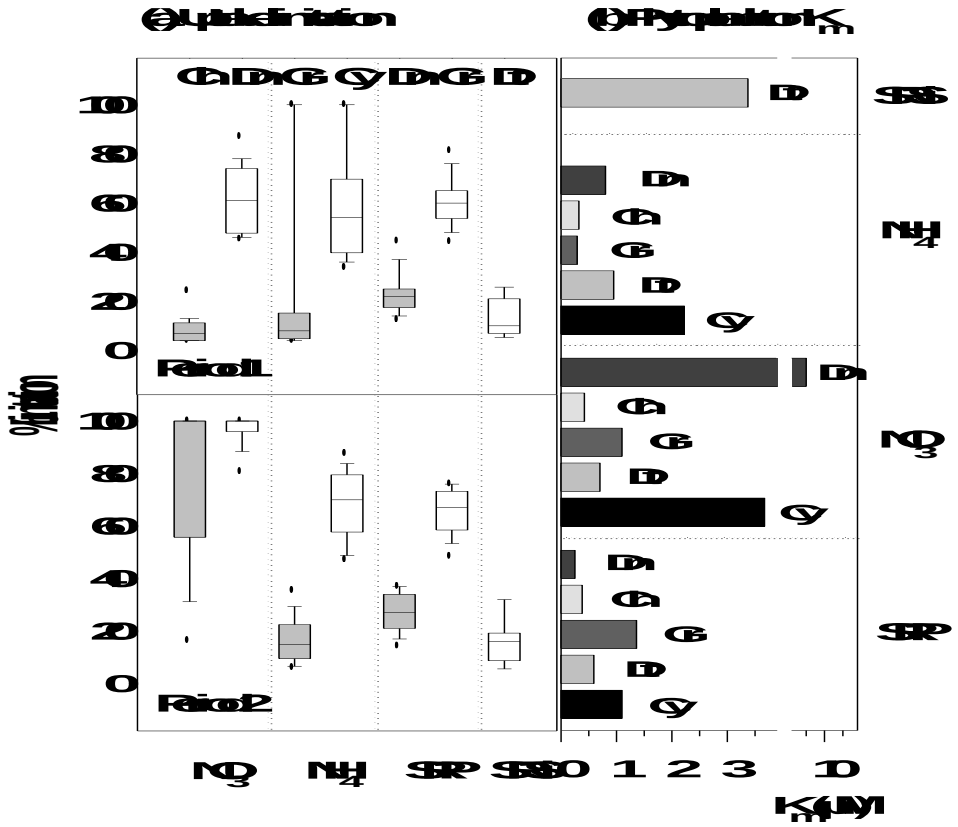

Figure 5. (a) Box-plots of the limitation percentage. For all nutrients, except SRSi, the groups (Cy, cyanobacteria; Dn, dinoflagellates; Dt, diatoms; Ch, chrysophytes; Gr, chlorophytes) with the lowest (shaded bars) and the highest (open bars) half-saturation (K_m) were considered. For SRSi other groups are assumed to have little Si demand and no limitation by this nutrient. The variation in response for a given phytoplankton group is caused by seasonal variation in nutrient concentrations. This variation is expressed by a box whisker plot, in which the line within boxes is the median, while the boxes, whiskers and dots encompass 75, 90 and 95% of the data, respectively. (b) The median literature based K_m for four nutrients as divided by five taxonomic divisions of phytoplankton (as summarised by HUSZAR & CARACO 1998).

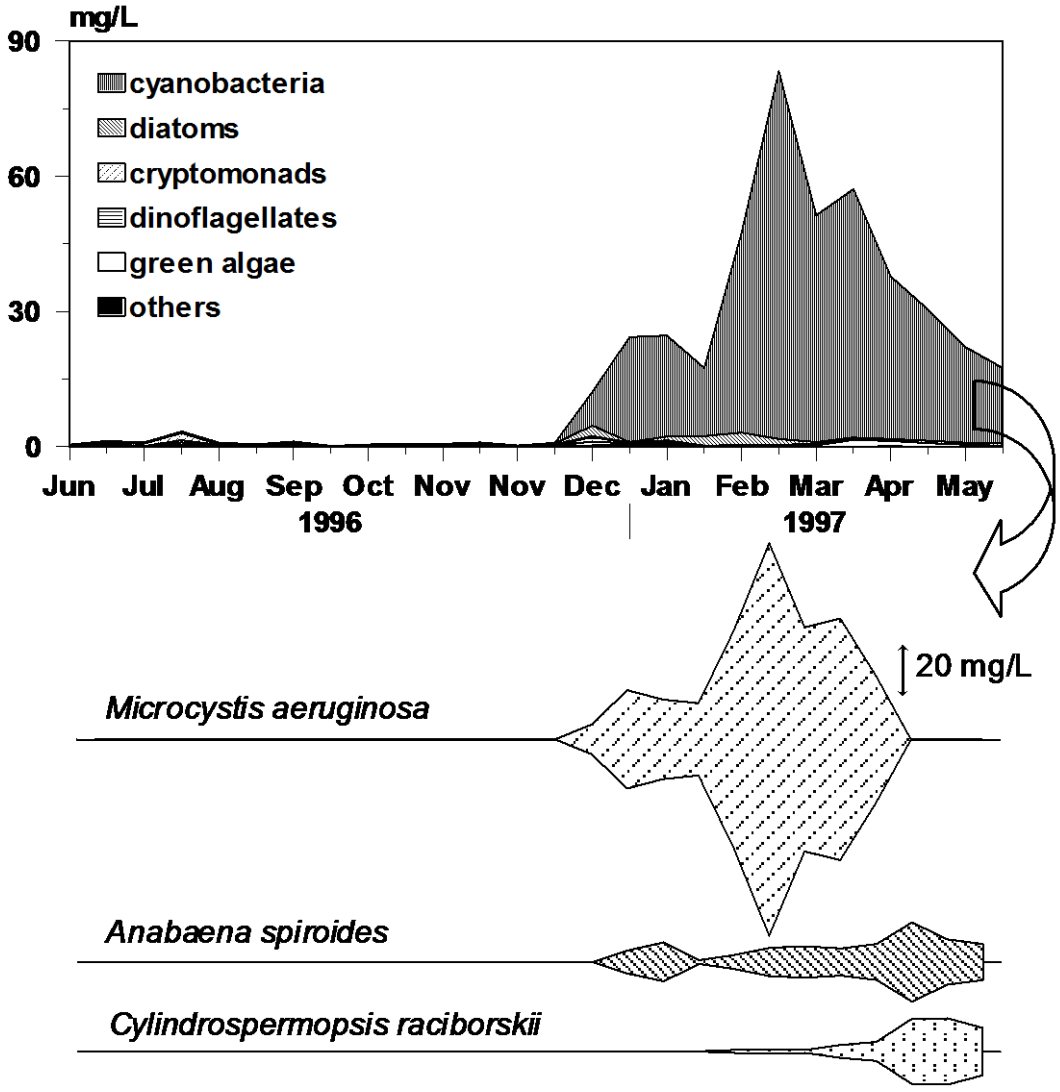

Figure 6. Seasonal variation of phytoplankton groups biomass (upper panel) and of major cyanobacteria species (lower panel) in Juturnaíba reservoir.

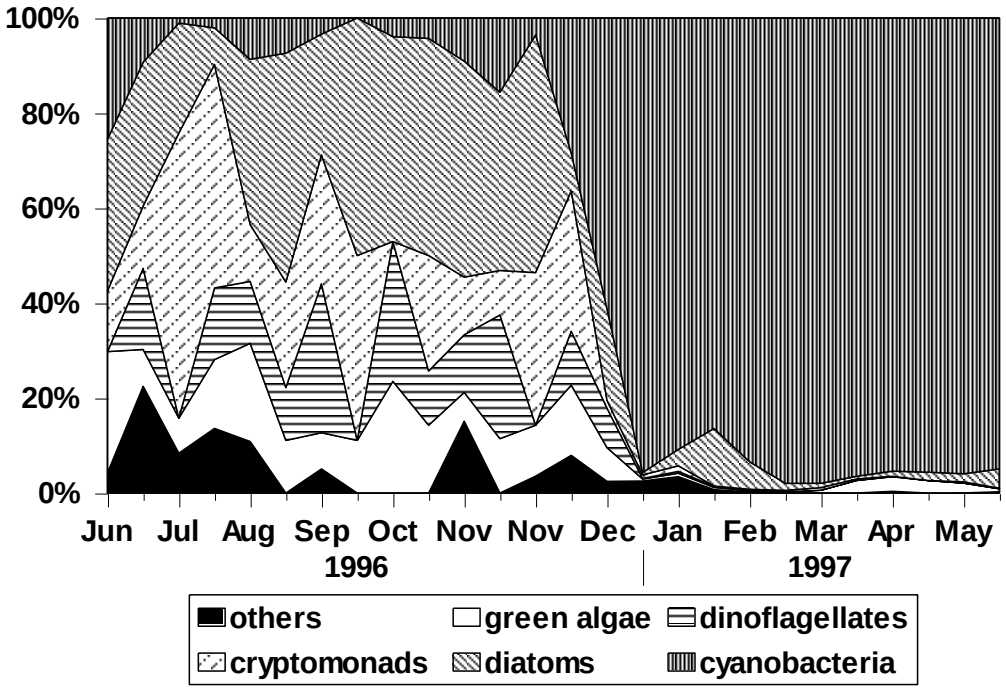

Figure 7. Seasonal variation of relative contributions of phytoplankton groups to total biomass in Juturnaíba reservoir.

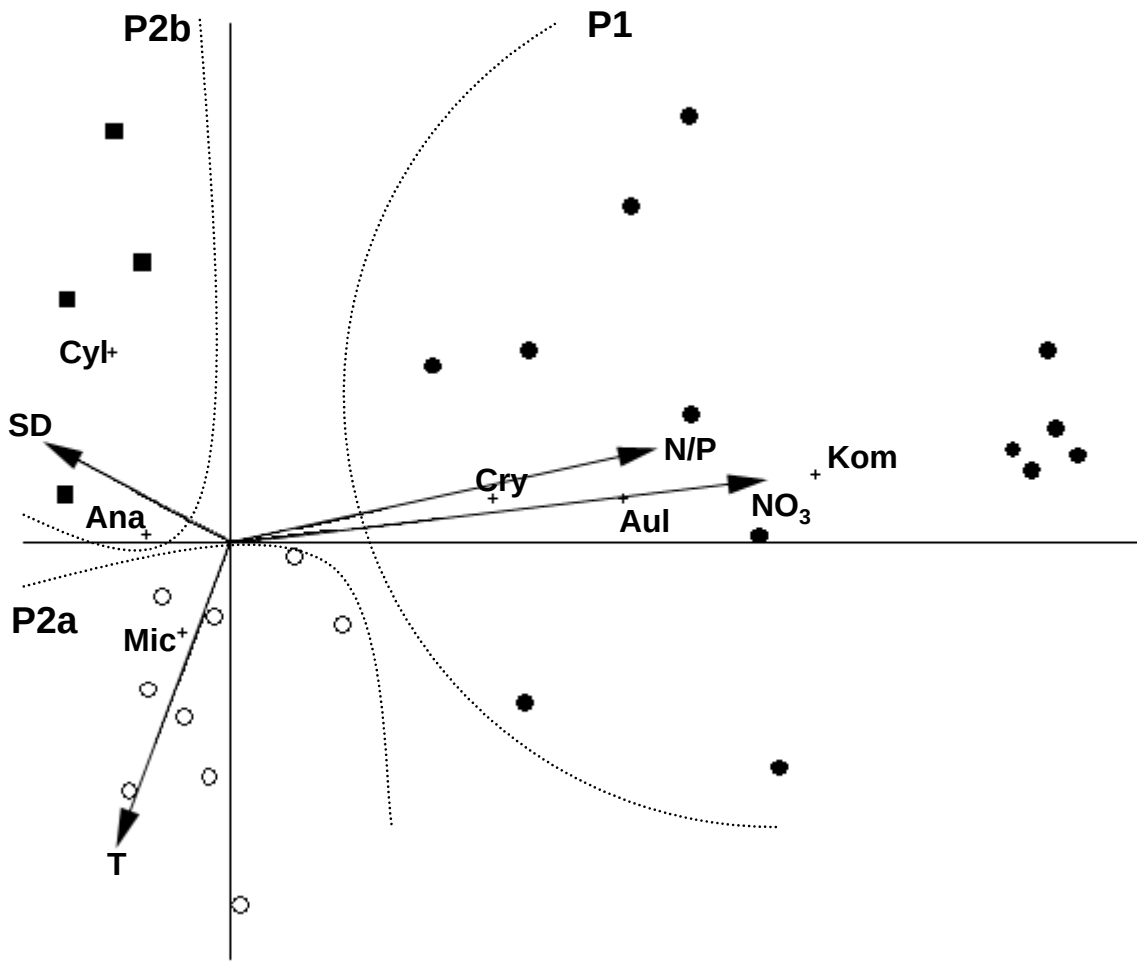

Figure 8. Canonical correspondence analysis (CCA) ordination diagram triplot showing the major phytoplankton species (Aul, *Aulacoseira distans*; Ana, *Anabaena spiroides*; Cry, *Cryptomonas marsonii*; Cyl, *Cylindrospermopsis raciborskii*; Kom, *Komma acuta*; Mic, *Microcystis aeruginosa*); environmental variables (arrows) that exert significant and independent influence on phytoplankton distributions, as detected by forward selection; and samples (circles and boxes) grouped by the identified periods (P1, Period 1; P2, Period 2; details see text).

4. N/P ratio and the dominance of cyanobacteria: cause or consequence?

Marcelo Manzi Marinho and Sandra Maria Feliciano de Oliveira e Azevedo

Abstract

Microcystis aeruginosa and *Aulacoseira distans* strains, isolated from Juturnaíba reservoir (Southeastern Brazil - 22°33'S, 42°18'W), were grown in batch cultures to investigate the consequences of different N/P ratios on growth, and nitrogen and phosphorous uptake abilities of these species, and to relate these findings to field observations. N/P ratio did not influence the growth rates and were similar for all experimental conditions. Exponential growth lasted longer in *M. aeruginosa* than in *A. distans*, especially at low N/P ratio conditions, thus *M. aeruginosa* cultures presented higher maximum yield. The growth rates observed for *A. distans* were in the lower range of values usually observed for diatoms and should be attributed in part to low light conditions in the experiments. Nevertheless, our data on *M. aeruginosa* growth rates were comparable to those from other studies. Distinct patterns of N and P uptake rates for *A. distans* and *M. aeruginosa* were observed. Uptake was faster and higher in *M. aeruginosa*, but N/P ratio did not influence uptake rate. However, the amount absorbed was proportional to N/P ratio in both strains studied. Our data on N-uptake were consistent with the hypothesis that *M. aeruginosa* possesses high nitrogen uptake capability. The variation of N/P ratio in culture medium was due to the absorption of the nutrients by the cells, and *M. aeruginosa* presented larger potential than *A. distans* to influence the proportional availability of nutrients (N/P ratio). Thus, in contrast to what has been pointed out in the literature, our results indicated that low N/P ratio could be a consequence of elevated cyanobacteria capacity and uptake rates of nitrogen and phosphorus. In relation to field observations, our data pointed towards nitrogen availability as a major factor determining substitution of diatoms by cyanobacteria and not the N/P ratio variation. So, the success of cyanobacteria and decline of diatoms observed in Juturnaíba reservoir could be due to the greater capacity of the former to grow under lower nitrogen availability, as showed in this study.

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4.1. Introduction

Increasing eutrophication of freshwater ecosystems has allowed cyanobacteria to become dominant in nutrient enriched environments, although these organisms are also important components of phytoplankton in oligo and mesotrophic waters (Hecky & Kling, 1987; Canfield *et al.*, 1989; Blomquist *et al.*, 1994; Huszar & Caraco, 1998). There is a growing record of harm caused to public health and to the environment by the development of cyanobacteria blooms. The most striking case, recently recorded in Northeast Brazil, led to human deaths during hemodialysis due to intoxication by cianotoxins (Jochimsen et al., 1998).

The dominance of filamentous and colonial species of cyanobacteria in nutrient-rich environments has been associated to a variety of causes. Environmental factors such as low turbulence (Reynolds, 1987, but see Ganf, 1974), low light (Zevenboon & Mur, 1980; Smith 1986), low euphotic zone to mixing zone ratio (Jensen *et al*, 1994), high temperature (Shapiro, 1990), low CO_2/high pH (King, 1970; Shapiro, 1990; Caraco & Miller, 1998), high total-P (McQueen & Lean, 1987; Trimbee & Prepas, 1987; Watson *et al.*, 1997), low total-N (Smith, 1983), low dissolved inorganic nitrogen (Blomquist *et al.*, 1994) and phosphorus storage strategy (Petterson *et al*.1993) have been refered to be able to promote or allow cyanobacteria dominance.

Based on the resource ratio hypothesis (Tilman, 1982), cyanobacteria dominance has also been attributed to low N/P ratios (Bulgakov & Levich, 1999; Smith, 1983, Tilman et al., 1986), since these organisms are thought to be better nitrogen competitors than other phytoplankton taxa (Barica et al., 1980; Blomqvist et al. 1994; Horne & Commins, 1987). Nevertheless, the role of N/P ratio as an independent factor that regulates phytoplankton assemblage composition and abundance is still under discussion.

The hypothesis that the N/P ratio can have strong effects on phytoplankton community structure involves competition for these nutrients. Hence, it is important to study the competitive ability for phosphorous and nitrogen of the major phytoplankton species. There are many studies on resource ratios and competition, recently reviewed by Bulgakov & Levich (1999), that used pairs of species from clonal cultures or multispecies taken directly from lakes. In general, these studies presented evidence that support resource ratio hypothesis. Rhee (1978) initiated an experimental program to examine the effects of N/P ratio and limiting concentrations of nitrogen and phosphorus on cultures of phytoplankton species. A major

publication (Rhee & Gothan, 1980) and a review (Rhee, 1982) also presented evidence that the concentrations of nitrogen and phosphorus limiting growth of the species of cyanobacteria and diatom tested were in lower molecular ratio than those of several chlorophytes.

Sommer (1989) reviewed studies on resource ratios and competition, in which taxonomic trends in nutrient competition were mentioned. Reynolds (1984), however, reviewed studies from field observations of diatom communities and emphasised that the relationship between dominant algae and resource ratio is much less clear than reported by Tilman (1981).

The recent revisions carried out by Bulgakov & Levich (1999) and Smith & Bennett (1999) present an extensive analysis of evidences, which support the contribution of the resource ratio hypothesis to the structuring of the phytoplankton community. Reynolds (1999) presented a counter view to this hypothesis, based on three main objections: 1) the success of fixing cyanobacteria on experiments with enclosures was determined by the availability of nutrients at the beginning of the growth season, instead the N/P ratio; 2) the application of Tilman's hypothesis by his followers did not consider that in N and P concentration above the necessary demand to keep growth there would be no limitation and, consequently, the ratio would not exert a regulatory role; 3) phytoplankton cells are equipped to perceive variation in individual resource availability, it being that so far, no molecular mechanism has been demonstrated in which the cell would be able to perceive and react to resource ratio. Therefore, whether the dominance of cyanobacteria is promoted by low N/P ratio is still unclear.

In a shallow eutrophic tropical reservoir (Juturnaíba reservoir, Southeastern Brazil - 22°33'S, 42°18'W) a shift in the dominance of phytoplankton community from diatoms (*Aulacoseira distans* (Ehrenberg) Simonsen) to cyanobacteria (especially *Microcystis aeruginosa* Kützing) during the seasonal cycle was observed (Marinho & Huszar, in prep.). Correlation analysis and Canonical Correspondence Analysis (CCA) has shown that this change was associated to a decrease in the availability of dissolved inorganic nitrogen (DIN) and N/P ratio. Dominance of *A. distans* was associated with DIN (>10µM), but also with higher N/P ratio (mean value = 15), while *M. aeruginosa* dominated the phytoplankton community after DIN fall below 5 µM, but N/P ratio had reached values below 10 (mean value = 3).

In this paper we describe experiments conducted with two phytoplankton species (*Microcystis aeruginosa* and *Aulacoseira distans*) isolated from Juturnaíba reservoir. The aims of our study were to examine the consequences of different N/P ratios on growth, and nitrogen and phosphorous uptake abilities of these species, and to relate these findings to field observations.

4.2. Materials and Methods

Algae and growth conditions

Strains of *Microcystis aeruginosa* and *Aulacoseira distans* were isolated from phytoplankton samples collected at the Juturnaíba reservoir (22°33'S e 42°18'W) in 1997. Both strains were maintained under nonaxenic conditions, routinely grown at a temperature of 25 ± 2 °C and irradiance of 80 µE m^{-2} s^{-1} provided by cool white fluorescent light, in a photoperiod of 12 h. *M aeruginosa* was grown in ASM-1 medium, considered by Gorham *et al*. (1964) as optimum for cyanobacteria, while *A. distans* was cultivated in WC medium (Guillard & Lorentzen, 1972). Previous tests showed that *A. distans* could not grow in ASM-1 medium.

M. aeruginosa experiments were conducted at three different N/P ratios: 3, 10 and 15. Phosphate concentrations were fixed at 200µM, original concentration of ASM-1 medium, whereas nitrate concentrations varied: 600µM (NP3), 2000µM (NP10) and 3000µM (NP15). *A. distans* experiments were also conducted at three different N/P ratios: 3, 15 and 20. Phosphate concentrations were fixed at 50µM, original concentration of WC medium, whereas nitrate concentrations varied: 150µM (NP3), 750µM (NP15) and 1000µM (NP20). The N/P ratios of 3 and 15 refer to the average values observed in a prior research at Juturnaíba reservoir during the annual cycle (Marinho & Huszar, in prep. This volume). The N/P ratios of 10 and 20 in the experiments with the cyanobacteria and the diatom, respectively, were used as controls, since these values represented the original N/P ratios of ASM-1 and WC medium. In all experiments, batch cultures were grown in 1.6L liquid medium, in 2L Erlenmeyer flasks. Temperature and light conditions were the same as already described, and the cultures were mixed by bubbling with sterile air. Prior to the start of the

experiments, inocula were adapted in the respective experimental condition during 7 days. Each experimental condition was assessed in triplicate.

The growth of *M. aeruginosa* and *A. distans* were followed by daily counts of cell numbers, using a Fucks-Rosenthal hemocytometer. Growth curves, relative growth rates (μ) and mean doubling time were calculated according to Fogg & Thake (1987). The maximum yield of the cultures (R), calculated as the ratio between maximum cell density reached by each culture and the number of cells in the inocula, were also compared.

Intra and extracellular components

Each culture was sampled every 3 days for chlorophyll a (Chl-a) and dissolved inorganic nutrient (nitrogen and phosphorus) determinations. Intracellular inorganic nutrients (nitrogen and phosphorus) were also determined only for *M. aeruginosa* cultures.

Different volumes of culture samples, according to biomass, were filtered over glass fiber filter and immediately frozen with liquid nitrogen, for Chl-a determinations. The pigments were extracted with methanol, and analysed by HPLC, according to methods describe by Garrido and Zapata (1993), and modified by Marinho & Rodrigues (in prep. – This volume).

For dissolved and intracellular nutrients, different volumes of culture samples, according to biomass, were centrifuged. The supernatants were filtered over Whatman DAWP filters and used for dissolved nitrate and phosphate determinations in the media. The pellet was extracted in deionized water at 100°C for 10 min, for intracellular dissolved inorganic nutrients. Prior to phosphate determinations, the extracts were subjected to acid digestion of the polyphosphates (Golterman et al., 1978). Nitrogen and phosphate concentrations were determined by spectrophotometric standard methods (APHA, 1992).

The nitrogen and phosphorus relative uptake rate (V) was estimated by:

$$V = (St_2 - St_1)/Bt_1$$

where, $St_2 - St_1$ is the nutrient variation in the culture medium between sampling days, and Bt_1 is the algal biomass (in volume units - mm^3) at the beginning of the time interval considered. Although the biomass could increase between t1 and t2, especially during the exponential growth phase, we considered only the biomass at time t1, since our aim was to compare the dynamics of relative uptake rates between the different experimental conditions.

Statistical analysis

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

4.3. Results

Growth

Growth rates (μ) and mean doubling time (G) of *M. aeruginosa* were similar for all conditions tested, but culture maximum yield differed significantly and was proportional to nitrate added to culture medium (Table 1). Exponential growth was observed from 2-3rd day, and was longer in the NP15 condition, while senescence was observed in NP3 on the 12th day (Fig.1).

Table 1. Mean values of relative growth rate (μ), mean doubling time (G) in days and maximum yield of cultures (R) of *M. aeruginosa*. (* mean values differed significantly at $p<0.05$)

Experimental condition	μ	G	R
NP3	0.30	2.3	20*
NP10	0.31	2.2	42*
NP15	0.33	2.1	60*

In *A. distans* experiments, μ and G were also similar at different N/P conditions. However, a significant difference was observed for culture maximum yield (R). Cells cultivated in NP3 presented an R 6 times lower than cells grown in NP15 and NP20 (Table 2).

Table 2. Mean values of relative growth rate (μ), mean doubling time (G) in days and maximum yield of the cultures (R) of *Aulaucoseira distans*. (* mean values differed significantly at $p < 0.05$)

Experimental condition	μ	G	R
NP3	0.28	3.2	5*
NP15	0.36	2.4	33
NP20	0.39	2.6	30

Exponential growth also started on the 3rd day for *A. distans*, and was longer in the NP15 and NP20 conditions. The cultures grown on these conditions presented similar growth curves, but differed from NP3 that reached stationary phase on the 9th day (fig. 2).

Chlorophyll

Chlorophyll *a* concentration per cell (Chl-a/cell) of *M. aeruginosa* was practically constant for the NP15 condition (fig. 3). An increment in Chl-a/cell was observed on the 6th day in NP10, followed by a gradual decrease. Initial concentration was significant higher ($p<0.05$) in the NP3 condition, nevertheless, between the 6th-9th days a significant decrease ($p<0.05$) was observed.

Chl-a/cell in *A. distans* initially increased, and then showed a decrease until the end of the experiments, for all experimental conditions (fig. 4). This increase occurred until the 3rd day in the NP3 and NP15 conditions, while it lasted until the 6th day in the NP20 condition. Although the variation was similar for all conditions, Chl-a/cell concentration was significantly lower ($p<0.05$) in the NP3 condition between the 6th-15th days.

Intracellular phosphate and nitrate

Initial intracellular phosphate concentrations were different from each of the inocula of *M. aeruginosa* (fig. 5) (NP3 – 0.42 µmoles 10^{-6} cells, NP10 – 0.63 µmoles 10^{-6} cells, NP15 – 0.29 µmoles 10^{-6} cells). However, by the 3rd day these differences were no longer significant ($p < 0.05$). The pattern of fluctuation was similar for the three conditions, with a decrease during the exponential growth. At the end of the experiment, intracellular phosphate concentrations increased in all conditions, but it was significantly higher ($p < 0.05$) in the NP3 condition.

Initial intracellular nitrate concentrations were similar in the inocula of *M. aeruginosa* in the NP10 (0.17 µmoles 10^{-6} cells) and NP15 (0.2 µmoles 10^{-6} cells) conditions (fig. 6), but lower in the NP3 condition (0,004 µmoles 10^{-6} cells). The variations of intracellular nitrate concentrations were similar for the three experimental conditions, but differed from the variation observed for phosphate. A sharp increase was recorded on the 3rd day, which coincided with an increase in total intracellular protein concentration (data not shown), and was followed by a sharp decrease on the 6th day. This increment of nitrate concentration in the

cells was significantly higher ($p < 0.05$) for the NP15 conditions, while the concentrations recorded at the end of the experiment (after the day 12-15) were significantly higher in the NP3 condition ($p < 0.05$).

The variation of intracellular N/P ratio in *M. aeruginosa* cells was similar to the intracellular nitrate concentration (fig. 7). A sharp increase was observed on the 3rd day, in all conditions, and the highest values were recorded for the NP15 condition. In general, the intracellular N/P ratio was very low (0.01 - 0.79) except for the values observed on the 3rd day.

Variation of phosphate and nitrate concentration in the culture medium

A rapid decrease of dissolved inorganic phosphate concentration in culture medium of *M. aeruginosa* was observed until the 3rd day, when 77 % - 80 % of initial phosphate were absorbed. Nevertheless, the phosphate was not exhausted in any experimental condition (fig. 8a). By the 3rd day, the phosphate concentration was practically constant in the NP3 condition, while other culture conditions presented a small uptake until the end of the experiment. The phosphate uptake rate of *M. aeruginosa* was similar for all experimental conditions, and 11 times higher during the first 3 days (fig. 8b).

The phosphate concentration in the culture medium of *A. distans* also presented a rapid decrease in all conditions, with 75 % - 78 % of the initial phosphate being absorbed until the 6th day (fig. 9a). The phosphate in *A. distans* cultures was also not exhausted, but residuals were lower than in *M. aeruginosa* cultures (figs 8a and 9a). Uptake rates were initially higher in the NP15 condition, but between the 3rd and 9th day, cultures in the NP20 condition presented higher uptake rates than in other conditions (fig. 9b). Phosphate uptake was 7 times higher during the first 3 days, in all experimental conditions.

Nitrate uptake was fast in *M. aeruginosa* cultures, with 70 - 84 % of initial concentration absorbed in the first 3 days, although not completely exhausted during the experiment (fig. 10a). Uptake rates were proportional to nitrate concentration in culture medium, and were different for experimental conditions until the 6th day (fig. 10b). By the 9th day, there was no nitrate uptake in the NP3 condition and a concentration around 5µM was observed until the end of the experiment. Low uptake rates were observed in the NP15 and NP20 conditions, between the 12th and 15th days of cultivation, and a residual around 1.5µM was observed from the 15th day on.

In *A. distans* cultures, 72 % - 80 % of initial nitrate concentration was absorbed in the first three days (fig. 11a). A residual around 1µM was recorded in the NP3 condition, while nitrate exhaustion occurred in the 21st and 18th days in NP15 and NP20, respectively. As observed for *M. aeruginosa*, nitrate uptake rates differed between experimental conditions and, initially was proportional to the N/P ratio (fig. 11b). Nitrate uptake rates decreased rapidly until the 6th day, in the NP3 condition, and then very low uptake rates were observed (<0.5µmol N mm^{-3}). A gradual decrease of uptake rate was observed in the NP15 condition during exponential growth (3rd – 15th days), while an accentuated decrease occurred in NP20 until the 9th day. It is noteworthy that only 3% of nitrate concentration were absorbed between the 3rd and 9th days in NP20 (fig.11a). However, an increment in the nitrate uptake rate occurred between the 9th and 12th days (fig. 11b), when almost all the available nitrate was absorbed.

N/P ratio variation in *M. aeruginosa* culture medium differed between experimental conditions (fig. 12). A initial increase, followed by the accentuated decrease until the 12th day, occurred in the NP10 and NP15 conditions, while a gradual decrease in N/P ratio was observed in NP3. The increase on the 3rd day was higher in the NP15 condition and reflects a higher phosphate uptake in this condition (fig. 8). N/P ratios < 5 were recorded in all conditions by the 9th day of cultivation.

The variation of N/P ratios in *A. distans* cultures were different for the experimental conditions (fig. 13). A gradual decrease occurred in NP3, and ratios < 1 were observed after the 6th day. The N/P ratios were maintained in the NP15 and NP20 conditions until the 6th and 9th days, respectively. Sharp increases occurred in NP15 on the 12th day and in NP20 on the 9th day, due to a decrease in the N-uptake rate (figs 11 e 13).

4.4. Discussion

Although the growth rates of *M. aeruginosa* and *A. distans* were similar, the cyanobacteria strain presented higher growth, especially at low N/P ratio conditions. In the NP3 condition, exponential growth lasted longer in *M. aeruginosa* than in *A. distans*, resulting in a maximum yield four times higher. Aguiar (1995) also observed that a lower nitrogen supply had strong effects on maximum yield of *M. aeruginosa* cultures, but did not influence

growth rates in experiments where the N/P ratio was preserved (10:1) but only when nitrogen supply varied. However, the author observed that growth rates of cultures in N/P ratio 20:1 were significantly lower in relation to cultures in N/P ratio 10:1. Nevertheless, in this study we did not find significant variation in growth rates between NP10 and NP15 conditions. Another cyanobacterium (*Synechocystis aquatilis* f. *salina*) also presented similar growth rates when cultivated in N/P ratio 3:1 and 10:1, however, the cultures maximum yield was higher in the highest N/P ratio condition (Nascimento & Azevedo, 1999), as observed in this study.

N/P ratio did not influence the growth rates of *M. aeruginosa* and *A. distans* that were similar for all the experimental conditions, but maximum yield was higher in *M. aeruginosa*. This fact could be explained as a result of previous adaptation of the inocula to the different conditions before the beginning of the experiments. Thus we can infer that *M. aeruginosa* strains can adapt better than *A. distans* strains to lower relative nitrogen availability.

The growth rates observed in this study for *A. distans* were lower than the range values usually observed for diatoms (Table 3). The lower growth rates for *A. distans* in our study should be in part attributed to low light conditions in the experiments. *A. distans* should be considered a species with high light requirements (Kilham *et al.*, 1986). Nevertheless, our data on *M. aeruginosa* growth rates were comparable to those from other studies (Table 3).

Table 3. Comparison of growth rate parameters, found by different authors for some diatom species and *M. aeruginosa*.

Species	Temperature (°C)	Irradiance ($\mu Em^{-2}s^{-1}$) (Light:Dark)	μ (d^{-1})	source
Aulacoseira subartica	16	Saturating (24:0)	0.74	(1)
A. granulata var. *angustissima*	25	40(16:8)	0.78	(2)
A. italica	22	Saturating (12:12)	1.6 – 3.1	(3)
Cyclotella meneghiniana	20	Saturating (14:10)	0.92	(4)
Stephanodiscus minutulus	20	Saturating (12:12)	1.02	(5)
Microcystis aeruginosa	23	38 (17:7)	0.6	(6)
Microcystis aeruginosa	20	29(24:0)	0.25	(7)
Microcystis aeruginosa	23	Saturating	0.48	(8)
Microcystis aeruginosa	21	22(12:12)	0.28	(9)
Microcystis aeruginosa	23	80-100(18:6)	0.81	(10)
Microcystis aeruginosa	25	40(16:8)	0.91	(2)

1- Foy and Gibson (1994); 2- Coles and Jones (2000); 3- Azevedo (1988); 4- Tilman and Kilham (1976); 5- Nicklisch and Woitke (1999); 6- Ward and Wetzel (1980); 7- Holm and Armstrong (1981); 8- Reynolds (1984); 9- Aguiar and Azevedo (1998); 10- Olsen (1989).

Selfshadding due to increasing cell numbers in cultures could raise chlorophyll *a* per cell concentration (Chl-a/cell), a known photoadaptative response (Prézelin, 1981; Rücker *et al*., 1995; Goerick & Montoya, 1998). In a study with three strains of *M. aeruginosa*, Woitke *et al*. (1997) observed that Chl-a/cell concentration was related to light intensity and, in general, the cellular concentration of Chl-a decreased with increasing irradiance.

However, we observed an increment in Chl-a/cell between the 3rd and 6th days followed by a gradual decrease during exponential growth phase. The decrease of Chl-a concentration during the exponential growth phase was higher in the lowest N/P ratio condition, and reflected the decrease in nitrogen availability. Nitrogen deficiency could cause a decrease in Chl-a/cell concentration in diatoms and cyanobacteria (Rosen & Lowe, 1984; Azevedo, 1988; Rücker *et al*., 1995). Thus, the reduction of chlorophyll a per cell observed in *A. distans* and *M. aeruginosa* cultures in NP3 and NP10 was caused, probably, by nitrogen exhaustion.

Moreover, the increment in Chl-a/cell observed at the begining of the exponential growth phase in *A. distans* cultures seems to be proportional to the relative growth rate, since Chl-*a*/cell shows linear relation to growth rate in diatoms, green algae and Prymnesiophyceae (Goerick & Montoya, 1998).

In regard to the strains studied, it is important to note that, apparently, *M. aeruginosa* has greater adaptation capacity to lower nitrogen supply. *M. aeruginosa* cells in the NP3 condition presented higher Chl-a/cell concentration at the beginning of the exponential growth phase, contrarily to what was observed for *A. distans*. The previous adaptation period of the inocula in a supply of low N/P ratio (NP3) resulted in higher Chl-a/cell concentration, possibly as an adaptative mechanism to preserve photosynthetic efficiency. This same trend was observed for *Synechocystis aquatilis* f. *salina* cultures in the same N/P condition (3:1), when the maintenance of Chl-a/cell concentration in low nitrogen availability had an important role in maintaining the photosynthetic process (Nascimento & Azevedo, 1999).

Although in this study we did not assess photosynthetic activity, we can speculate that *M. aeruginosa* has some mechanism that allows the maintenance of cell division in low N/P ratio, at least for a longer period than *A. distans*. Considering the *M. aeruginosa* biomass observed on the 9th day, when cells in the NP3 condition presented the lowest Chl-a concentration, the cell numbers produced were not significantly different ($p>0.05$) between the experimental conditions. In contrast, *A. distans* apparently did not present the same

capability. The lower Chl-a/cell concentrations observed in the NP3 condition resulted in significantly lower ($p<0.05$) biomass in relation to cultures in higher N/P ratio. Furthermore, *M. aeruginosa* cells in the NP15 condition presented constant Chl-a/cell concentration during the experiment, possibly due to the absence of nitrogen limitation. Although this fact did not reflect in an increase of growth rate, it could possibly maintain an extended exponential growth phase, raising cultures maximum yield. The same trend was reported for *Synechocystis aquatilis* f. *salina* (Nascimento & Azevedo, 1999). Thus Chl-a/cell concentration was possibly related to the different capacities of adaptation to nitrogen availability of the strains studied.

Intracellular phosphate concentration in *M. aeruginosa* was similar between experimental conditions, decreasing during exponential growth phase and with a minor increase at the beginning of stationary phase. This increment was significantly higher in the NP3 condition ($p<0.05$), possibly as a response to lower nitrogen availability, since phosphate concentration in culture medium was still high. However, intracellular phosphate concentration differed between inocula, although the values converged rapidly on the 3rd day of cultivation. It is noteworthy that the inocula were previously adapted rigorously under the same conditions, except for the N/P ratio of the culture medium.

According to Rhee & Gotham (1980), the optimum intracellular N/P ratio for this species is 9, very similar to the NP10 condition. This fact might have favoured the inocula in the NP10 condition, since it presented the highest initial intracellular phosphate concentration. However, more studies are necessary since our data did not allow good evaluations about this difference.

The intracellular phosphate analysed in this study is equivalent to the hot-water extractable fraction denominated surplus phosphorus (Rhee, 1973). The surplus phosphorus (SP) fraction is not well defined, but presumably includes cytoplasmic inorganic P in addition to inorganic poly-P (Rhee, 1973). Olsen (1989) observed that SP constituted 12 – 69 % of total cell P in *M. aeruginosa*. The author also noted that the SP fraction increased gradually with the growth rate. A similar trend was also recorded for *Cylindrospermopsis raciborskii*, another species known for their extreme storage adaptation (Istvánovics *et al.*, 2000). However, in this study we observed a gradual decrease of SP during the exponential growth phase. In the studies mentioned, the experiments were conducted in continuous cultures, so the difference could be attributed to the distinct experimental conditions and more studies are necessary to better assess this differences.

As noted for intracellular phosphate, during stationary phase, differences in intracellular nitrogen concentration were observed between experimental conditions, with significantly higher concentrations ($p<0.05$) in the NP3 condition. Studies in nutritive behaviour of *M. aeruginosa* showed that growth was rapidly affected by N-deficiency, but cyanobacteria strains stored nitrogen and phosphorus in quantities which maintained their growth after the 8th day of deficiency (Sbiyyaa *et al*., 1998). Nevertheless, the increment of intracellular N and P, especially in cells cultivated in NP3, could be due to decrease in growth, resulting in increased intracellular pools of N and P.

The intracellular N/P ratio variation was determined mainly by intracellular N variation. The increased protein concentration at the beginning of the exponential growth, which resulted in higher intracellular concentration, also influenced intracellular N/P ratio. Although our data did not consider structural nitrogen and phosphorus, it is noteworthy that the N/P ratio values observed were much bellow the Redfield proportion (Redfield, 1958), even considering the increment on the 3rd day, when maximum values were < 5.

The optimum intracellular N/P ratio found for *M. aeruginosa* varies between 7 (Olsen, 1989) and 9 (Rhee & Gotham, 1980). However, under nutrient saturating conditions, cellular N/P ratio can be much lower than optimum (Rhee & Gotham, 1980). This fact is due to different storage capacities for these nutrients because the pool size for P, relative to the total cell quota, is much larger than for N (Rhee, 1978).

Comparing phosphorus uptake rates, distinct patterns for *A. distans* and *M. aeruginosa* were observed. Uptake was faster and higher in *M. aeruginosa*, but N/P ratio did not influence uptake rate. However, the amount absorbed was proportional to N/P ratio in both strains studied. *M. aeruginosa* could be considered a species adapted to phosphorus storage with high capacity to absorb inorganic phosphorus (Olsen, 1989). The elevated residual of dissolved phosphate observed in the media of *M. aeruginosa* cultures, however, reflected plenty condition of culture medium.

Although *A. distans* presented slower uptake, the residue of phosphate in the culture medium was smaller at the end of the cultivation. This fact seems to be in agreement with the hypothesis that the diatoms are excellent competitors for phosphorus in low concentrations, mainly considering that their half-saturation constants can be smaller than those verified for cyanobacteria (Huszar & Caraco, 1998; Sommer, 1989).

The dynamics of absorption of N was different between *M. aeruginosa* and *A. distans*. *M. aeruginosa* absorbed N more quickly, and around 99% of initial N was absorbed in about 11 days in all the N/P ratios, while *A. distans* cells took around 15 days to absorb this same amount. Some studies showed that centric diatoms (*Cyclotella* spp.) should compete well for nitrogen because their growth affinity were higher than cyanobacteria taxa (Grover *et al*., 1999). However, *M aeruginosa* possess high nitrogen uptake capacity (Fujimoto *et al*., 1997; Takamura *et al.*, 1987), and our data were consistent with this fact.

The resource ratio hypothesis is based on competition theory (Tilman, 1982), where low N/P ratios imply in nitrogen limitation, while high values of N/P ratio would result in phosphorus limitation (Smith & Bennett, 1999). It is important to emphasise that nutrient competition occurs, by definition, whenever nutrient consumption by organisms leads to nutrient limitation of reproductive rates (Tilman, 1981). Some planktologists have adopted the view that algal biomass can be limited by nutrients without limitation of reproductive rates (Harris, 1986). But it is difficult to assume that nutrient can set a ceiling to the attainable biomass without limiting growth, the process by which biomass is attained (Reynolds, 1999; Sommer, 1999).

Therefore, considering a scenario of competition between *M. aeruginosa* and *A. distans*, it would be expected that relative growth rates were influenced by the N/P ratio, and significant differences between growth rates would be recorded in the cells cultivated at distinct N/P ratios.

In Juturnaíba reservoir, the substitution of *A. distans* by *M. aeruginosa* as dominant species in the community during the annual cycle was statistically associated to N/P ratio and DIN concentrations (Marinho & Huszar, in prep.). In addition, the authors also observed that the changes in community composition from diatom dominance to cyanobacteria have shown a response time of few weeks in relation to changes in DIN concentrations and in N/P ratios. The success of *M. aeruginosa* in low N/P ratio has been verified by data of natural populations (Michard et al. 1996; Levich et al., 1997 apud Bulgakov & Levich, 1999), as well as for laboratory experiments (Fujimoto et al., 1997). However, resource ratios can be thought as consequence of uptake and not as drivers of changes in phytoplankton composition (Reynolds 1997, 1999). The data of this study have shown no significant differences between the experimental conditions. So the N/P ratio did not influence the *M. aeruginosa* and *A. distans* growth rates.

As expected, the variation of N/P ratio in culture medium was due to the absorption of the nutrients by the cells of *M. aeruginosa* and *A. distans*. Even so, the dynamics was differentiated between the studied strains, resulting in distinct variation between the different N/P ratios. The growth of *M. aeruginosa* resulted in fast reduction of this ratio, whereas in *A. distans*, the original ratio of culture medium was maintained to middles of the exponential phase, especially in the NP15 and NP20 conditions. Such results demonstrate that *M. aeruginosa* has a larger potential than *A. distans* to influence the proportional availability of nutrients (N/P ratio).

Thus, in contrast to what has been pointed out in the literature, where dominance of cyanobacteria is determined by low N/P ratios (Bulgakov & Levich, 1999; Smith, 1983; Smith & Bennett, 1999), our results indicated that low N/P ratio could be a consequence of elevated cyanobacteria capacity and uptake rates of nitrogen and phosphorus. Moreover, limitation of one or another nutrient in particular, in this case nitrogen, could determine competition 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 as suggested by Reynolds (1999). So, the success of cyanobacteria and decline of diatoms in Juturnaíba reservoir could be due to the greater capacity of the former to grow under lower nitrogen availability.

Acknowledgements

The authors would like to thank Maria Elisângela Venâncio dos Santos and Yuri Coelho Pinheiro for help with nutrient analysis. This research was financially supported by Coordenação de Aperfeiçoamento de Ensino Superior and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq / Pronex).

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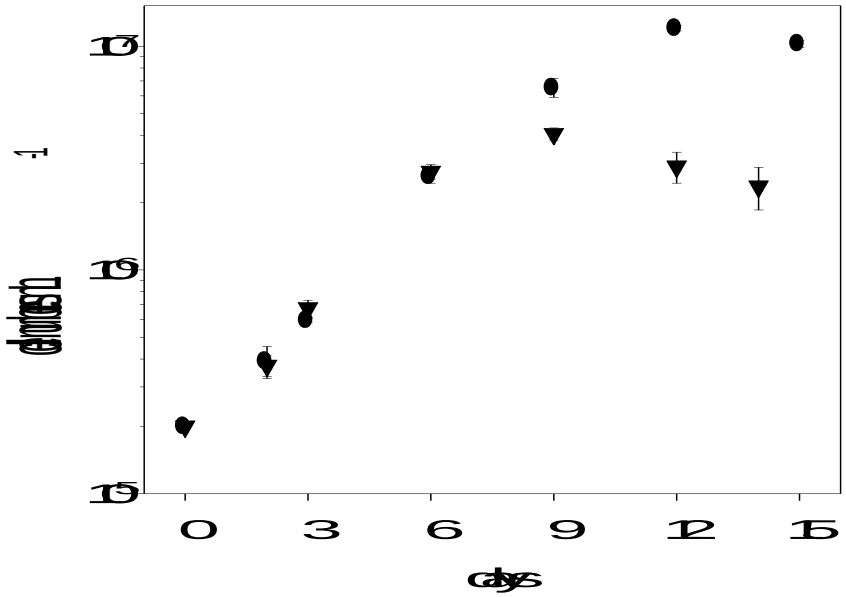

Figure 1. Growth curves of *Microcystis aeruginosa* cultivated in ASM-1 medium with N/P ratios of 3 (τ), 10 (ν) and 15 (λ). Each value is a mean of three replicates; bars represent standard deviation.

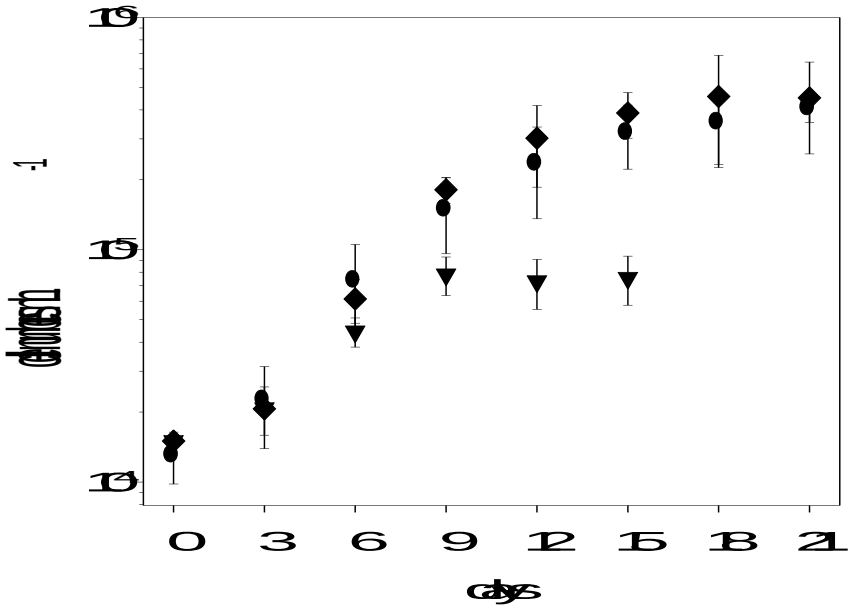

Figure 2. Growth curves of *Aulaucoseira distans* cultivated in WC medium with N/P ratios of 3 (τ), 15 (λ) and 20 (◆). Each value is a mean of three replicates; bars represent standard deviation.

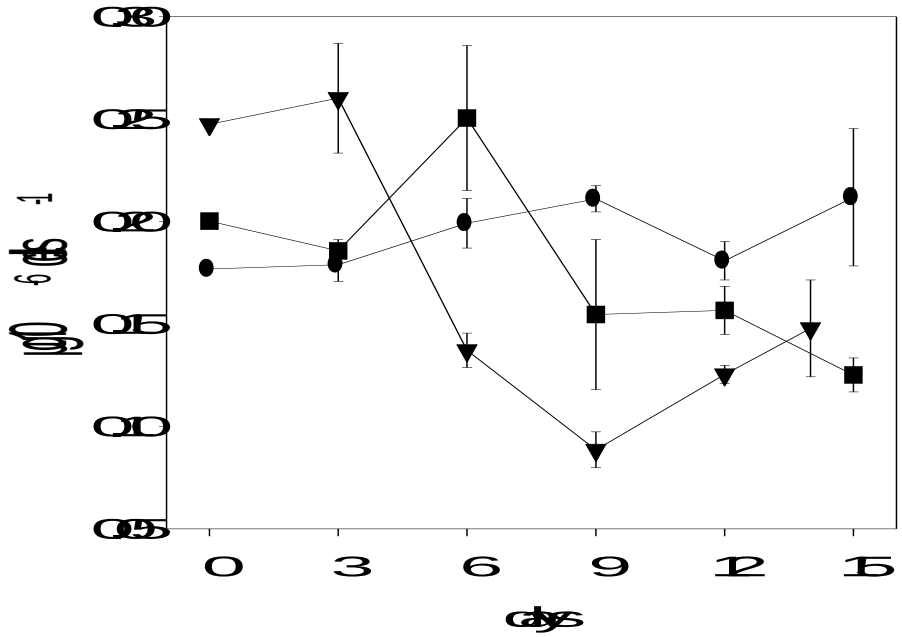

Figure 3. Chlorophyll *a* concentrations of *Microcystis* cultivated in ASM-1 medium with N/P ratios of 3 (τ), 10 (ν) and 15 (λ). Each value is a mean of three replicates; bars represent standard deviation.

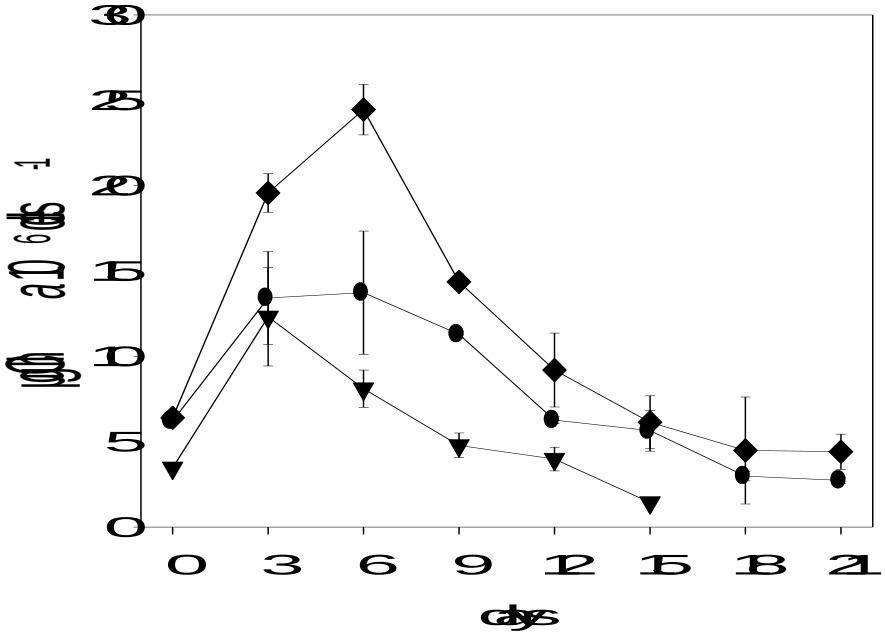

Figure 4. Chlorophyll *a* concentrations of *Aulaucoseira distans* cultivated in WC medium with N/P ratios of 3 (τ), 15 (λ) and 20 (◆). Each value is a mean of three replicates; bars represent standard deviation.

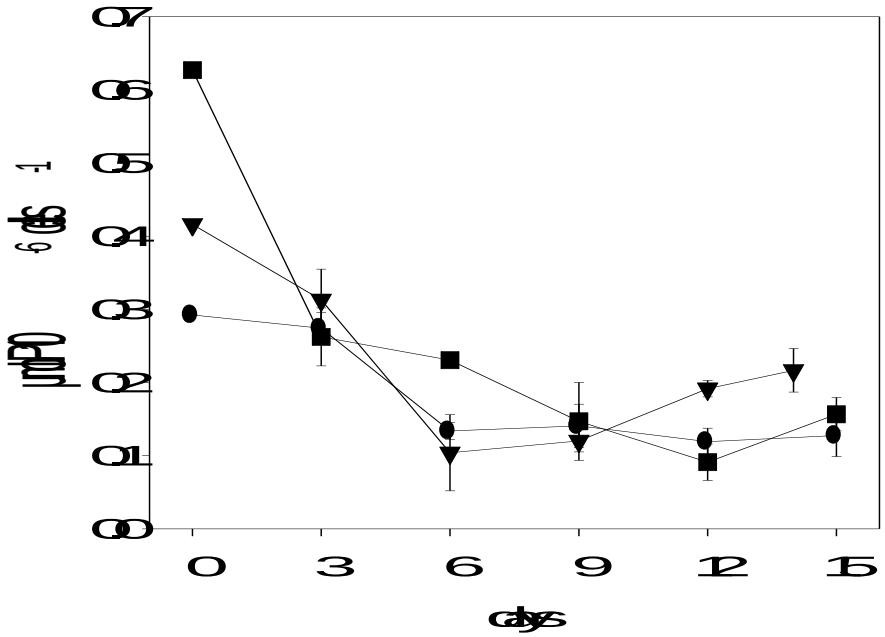

Figure 5. Phosphate intracellular concentrations in *Microcystis* cultivated in ASM-1 medium with N/P ratios of 3 (τ), 10 (ν) and 15 (λ). Each value is a mean of three replicates; bars represent standard deviation.

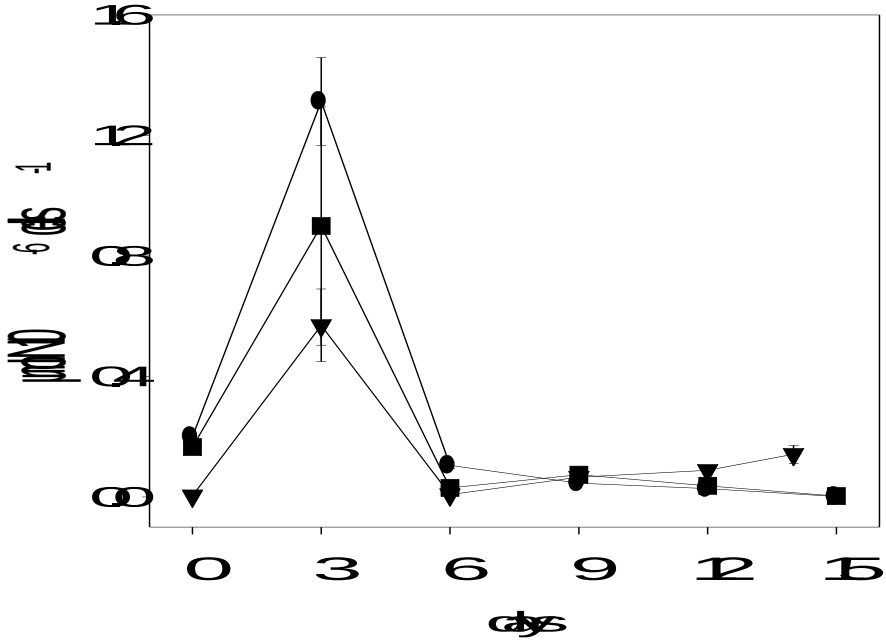

Figure 6. Nitrate intracellular concentrations in *Microcystis* cultivated in ASM-1 medium with N/P ratios of 3 (τ), 10 (ν) and 15 (λ). Each value is a mean of three replicates; bars represent standard deviation.

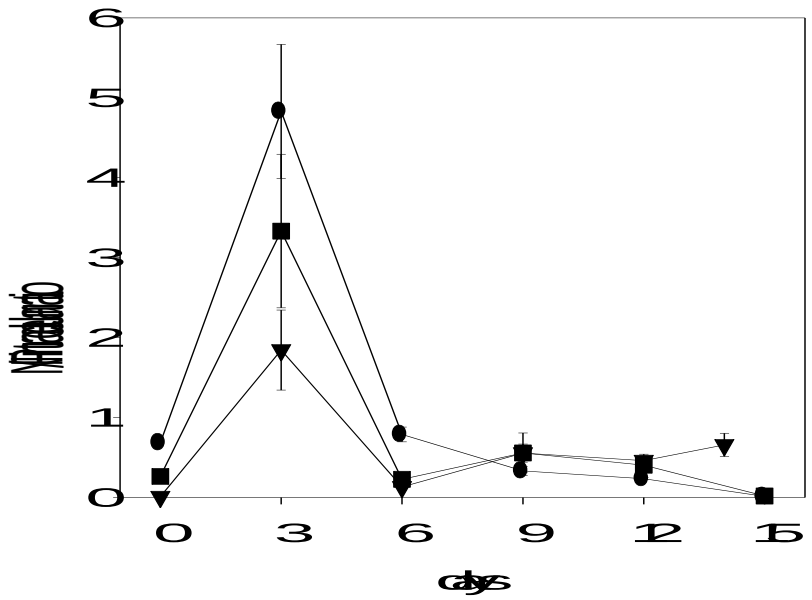

Figure 7. Intracellular N/P ratio in *Microcystis* cultivated in ASM-1 medium with N/P ratios of 3 (τ), 10 (ν) and 15 (λ). Each value is a mean of three replicates; bars represent standard deviation.

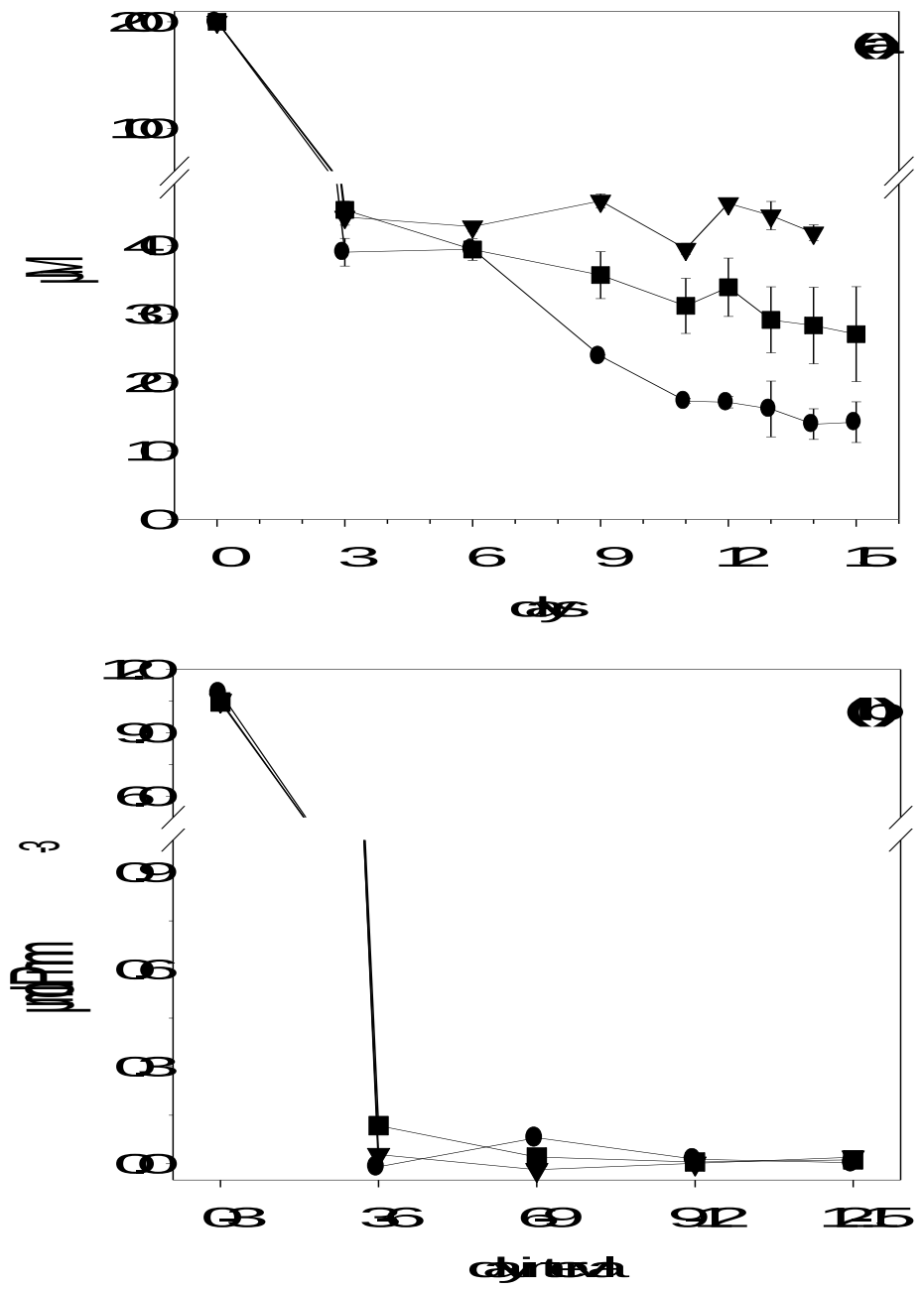

Figure 8. Dissolved phosphate concentration (a) and P-uptake (b) in cultures of *Microcystis aeruginosa* cultivated in ASM-1 medium with N/P ratios of 3 (τ), 10 (ν) and 15 (λ). Each value is a mean of three replicates; bars in (a) represent standard deviation.

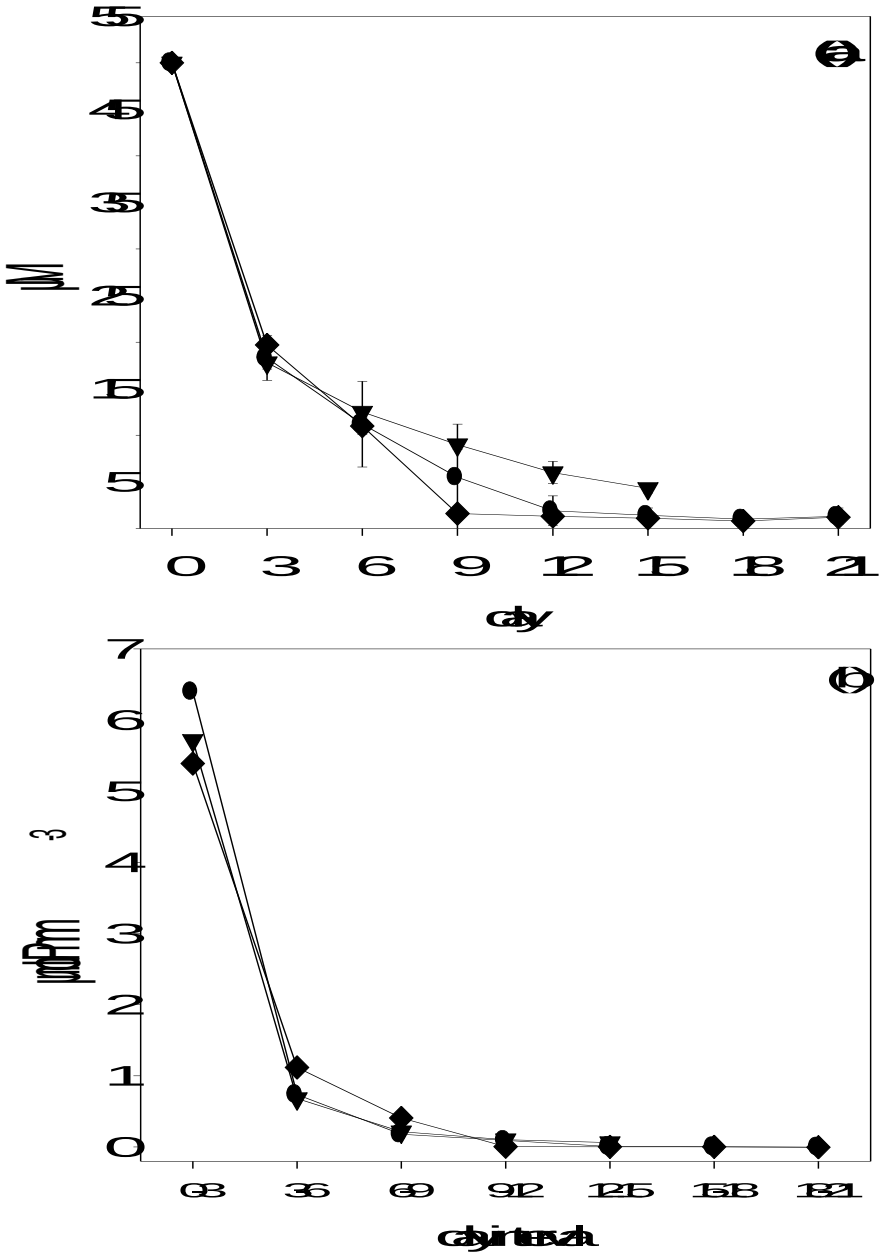

Figure 9. Dissolved phosphate concentration (a) and P-uptake (b) in cultures of *Aulaucoseira distans* cultivated in WC medium with N/P ratios of 3 (τ), 15 (λ) and 20 (◆). Each value is a mean of three replicates; bars in (a) represent standard deviation.

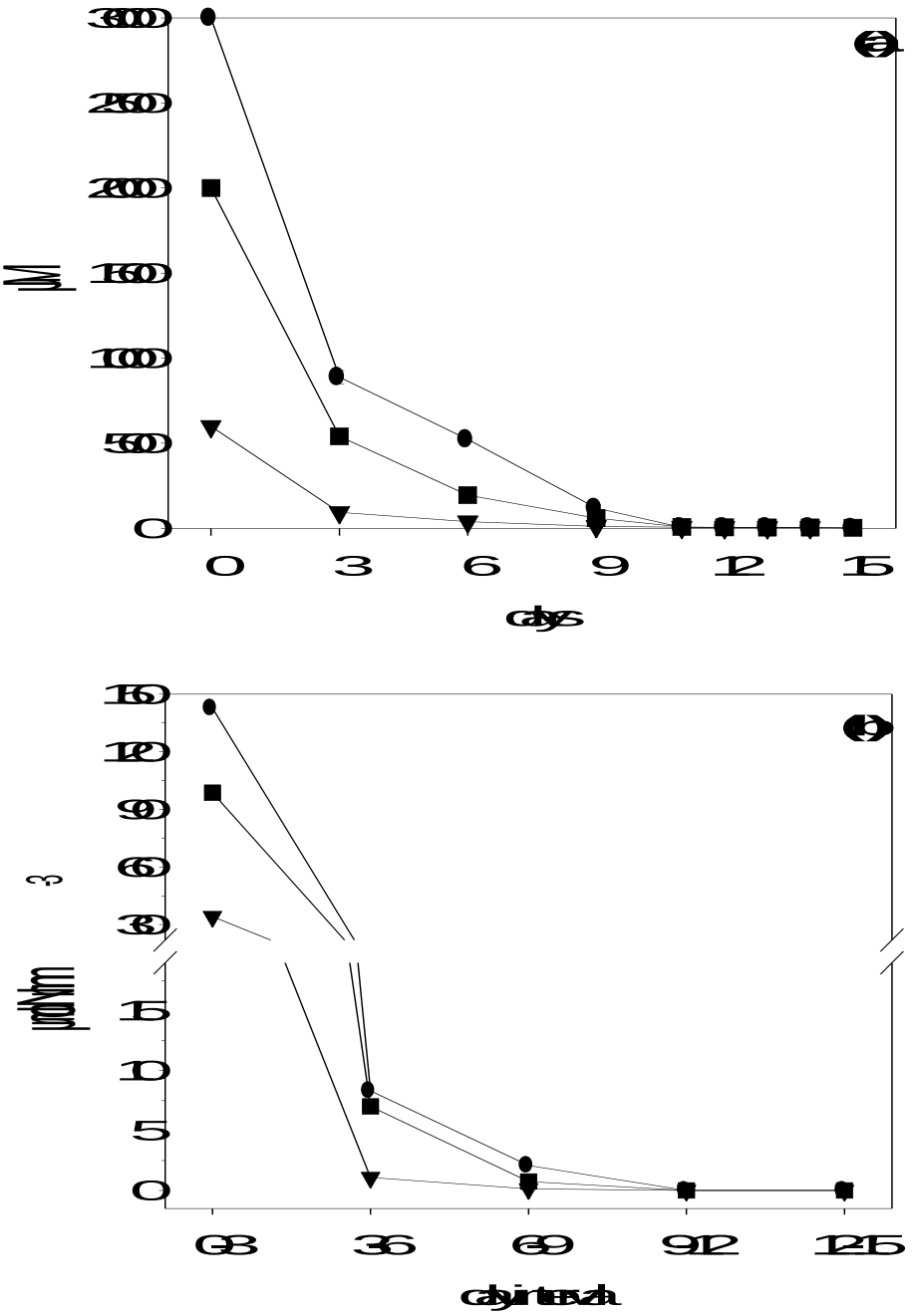

Figure 10. Dissoved nitrate concentration (a) and N-uptake (b) in cultures of *Microcystis aeruginosa* cultivated in ASM-1 medium with N/P ratios of 3 (τ), 10 (ν) and 15 (λ). Each value is a mean of three replicates; bars in (a) represent standard deviation.

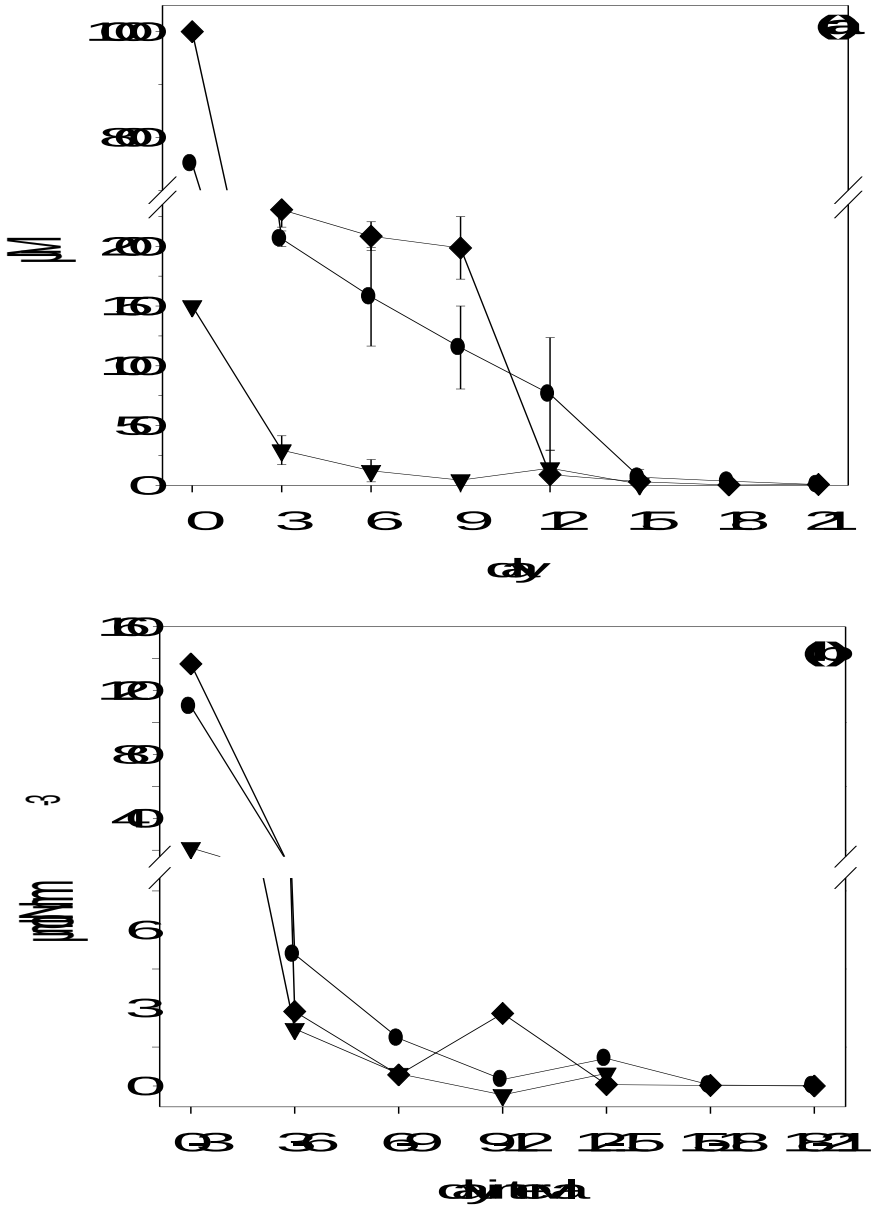

Figure 11. Dissolved nitrate concentration (a) and N-uptake (b) in cultures of *Aulaucoseira distans* cultivated in WC medium with N/P ratios of 3 (τ), 15 (λ) and 20 (◆). Each value is a mean of three replicates; bars in (a) represent standard deviation.

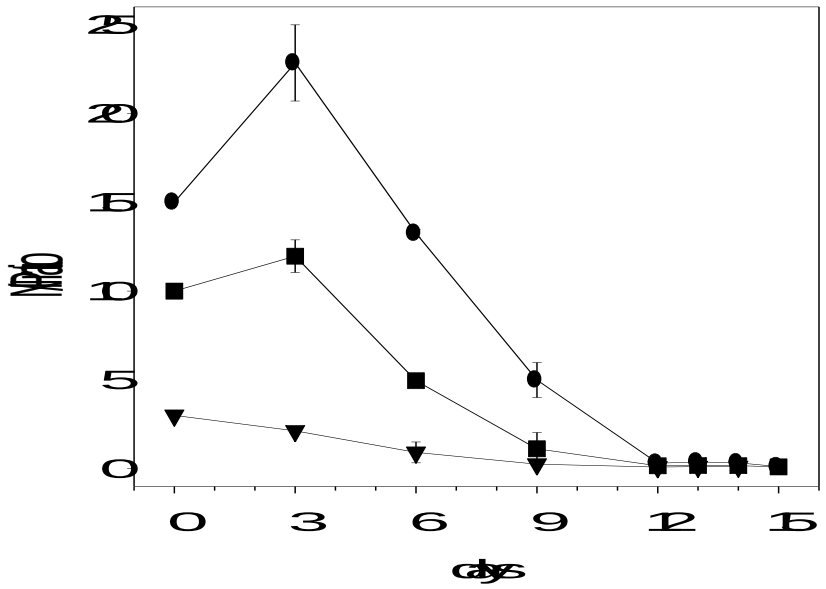

Figure 12. N/P ratios in cultures of *Microcystis aeruginosa* cultivated in ASM-1 medium with N/P ratios of 3 (τ), 10 (ν) and 15 (λ). Each value is a mean of three replicates; bars represent standard deviation.

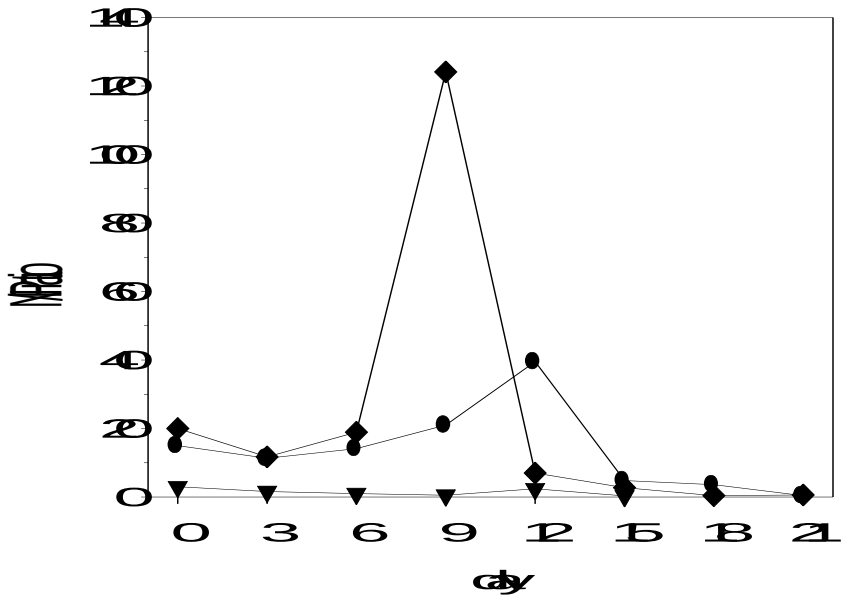

Figure 13. N/P ratio in cultures of *Aulaucoseira distans* cultivated in WC medium with N/P ratios of 3 (τ), 15 (λ) and 20 (◆). Each value is a mean of three replicates; bars represent standard deviation.

5. Discussão geral

Os resultados deste estudo confirmaram que o reservatório de Juturnaíba apresenta problemas ambientais similares aos de muitos reservatórios no Brasil, especialmente os relacionados ao processo de eutrofização e ocorrência de florações de cianobactérias potencialmente tóxicas.

O reaparecimento de florações de cianobactérias neste corpo d'água vem de encontro ao relatado na literatura em relação ao aumento da dominância de cianobactérias em reservatórios brasileiros (Azevedo *et al*.,1994; Huszar & Silva, 1999; Tundisi & Matsumura-Tundisi, 1992), e aponta para a necessidade urgente de monitoramento da comunidade fitoplanctônica, não somente neste ecossistema, mas nos reservatórios brasileiros em geral, especialmente aqueles utilizados para abastecimento público.

Deste modo, o desenvolvimento e aplicação de novas metodologias que possibilitem o monitoramento do fitoplâncton através de procedimentos automatizados e simplificados são de extrema importância. A avaliação do fitoplâncton através da análise quantitativa de pigmentos fotossintéticos pela técnica de HPLC pode representar uma alternativa eficaz para estudos e monitoramento da comunidade fitoplanctônica (Millie *et al.*, 1993).

O padrão de variação da composição e biomassa do fitoplâncton obtido neste estudo através da análise de pigmentos fotossintéticos foi semelhante ao descrito pelas contagens em microscópio, e caracterizou os mesmos períodos de dominância dos grupos fitoplanctônicos. Além disso, também foi registrada a ocorrência de floração de cianobactérias. Em maior ou menor grau, outros estudos também verificaram a concordância entre os dados obtidos pela metodologia clássica de contagem de organismos em microscópio invertido (Utermöhl, 1958), e a quantificação da biomassa fitoplanctônica através da análise de pigmentos por HPLC (Wilhelm *et al.*, 1991; Roy *et al.*, 1996; Tester *et al.*, 1995; Woitke *et al*., 1996). Nossos resultados comprovam a aplicabilidade desta técnica para o estudo e monitoramento do fitoplâncton continental.

É importante ressaltar também os resultados obtidos com a aplicação do software CHEMTAX. Nossos resultados demonstram o melhor ajuste obtido com o CHEMTAX em relação aos dados de microscopia, quando comparados com correlações baseadas em razões fixas pigmento marcador/Clorofila *a*. Além deste estudo, somente outros três utilizaram até o momento o programa CHEMTAX (Mackey *et al*., 1998; Pinckney *et al.*, 1998 e Wright *et al*.,

1996), porém todos demonstraram ser o CHEMTAX uma excelente ferramenta para análise da comunidade fitoplanctônica através de dados de pigmentos marcadores.

Contudo, a aplicação da metodologia de análise de pigmentos por HPLC implica que as relações entre as concentrações de carotenóides e clorofila *a* e a biomassa das algas sejam bem estabelecidas. Entretanto, tais relações ainda não são completamente conhecidas (Goerick & Montoya, 1998). As grandes variações observadas para as razões luteína/Chl-a, aloxantina/Chl-a e zeaxantina/Chl-a, quando da aplicação do CHEMTAX para interpretação dos dados de pigmentos marcadores, evidenciaram esta dificuldade.

Este fato torna-se relevante especialmente quando consideramos as variações observadas neste estudo, durante a floração de cianobactérias. Neste período foi observada a substituição de *Microcystis aeruginosa* por *Anabaena spiroides* e *Cylindrospermopsis raciborskii* como espécies dominantes da comunidade fitoplanctônica. Experimentos com cepas de *M. aeruginosa* isoladas da represa demonstraram ser zeaxantina a principal xantofila produzida por este organismo (Marinho & Rodrigues, em prep.), enquanto que em testes realizados com diversas cepas de *Cylindrospermopsis* spp. observou-se uma concentração muito pequena deste pigmento (Marinho, dados não publicados). A razão zeaxantina/Chl-a em *A. spiroides* e *C. raciborskii* parece ser muito menor do que a obtida para *M. aeruginosa*, o que explicaria a variação calculada pelo CHEMTAX. Nossos resultados estão de acordo com as observações de Nicklisch & Woitke (1999), sendo que os autores apontaram para a necessidade de mais estudos sobre a variabilidade na composição e conteúdo de pigmentos marcadores, especialmente de cianobactérias.

Deste modo, considerando as perspectivas de continuidade da avaliação da aplicação do método de análise de pigmentos por HPLC, serão relevantes estudos em laboratório com cepas das principais espécies, especialmente de cianobactérias, em relação a fatores ambientais. Cabe ressaltar que estudos sobre a variabilidade de pigmentos marcadores e clorofila *a* de cepas de *M. aeruginosa* e *A. distans* já estão em andamento.

Além do aspecto de monitoramento das variações na composição e biomassa do fitoplâncton, o conhecimento aprofundado dos fatores determinantes destas variações é fundamental para o desenvolvimento de modelos, onde a previsibilidade possibilite o aperfeiçoamento do gerenciamento da qualidade da água, especialmente considerando os ambientes sujeitos a freqüentes florações de cianobactérias potencialmente tóxicas.

Os resultados obtidos neste estudo, analisados e comparados sob a óptica de teorias ecológicas, possibilitaram um melhor entendimento dos processos ecológicos determinantes da estrutura e função do fitoplâncton no reservatório de Juturnaíba.

Dentre os aspectos teóricos analisados, merece destaque a abordagem fitosociológica que, baseada em um modelo de associações e seleção de espécies, considera as associações de espécies do fitoplâncton como eficazes descritores ambientais (Reynolds, 1997).

A variação das condições ambientais possibilitou a identificação de distintos períodos, que puderam ser descritos por diferentes associações de espécies. Dentre as associações propostas por Reynolds (1997) e Padisák & Reynolds (1998), cinco foram determinadas para as espécies do reservatório de Juturnaíba. Três constituídas por cianobactérias, uma de criptofíceas e uma de diatomáceas.

Então, no reservatório de Juturnaíba, as associações **D** e **Y**, compostas de espécies pequenas de rápido crescimento, C estrategistas (*C. marsonii* e *A. distans*), foram selecionadas por condições de alta disponibilidade de nutrientes, observadas durante o inverno e primavera. Com o aquecimento da água durante o verão, a biomassa aumentou e os nutrientes, principalmente o nitrogênio inorgânico dissolvido (NID), tornaram-se mais escassos e a seleção foi direcionada para a associação **M** com *M. aeruginosa*, uma S-R estrategista (senso Reynolds, 1997), que atingiu seu máximo quando as concentrações de NID foram menores que 5µM e a razão N/P menor que 10. Sob tais condições, cianobactérias não fixadoras foram favorecidas devido a superior habilidade competitiva, em relação aos demais grupos do fitoplâncton (Blomqvist et al., 1994). Contudo, a elevada biomassa de *M. aeruginosa* exauriu o NO_3^- resultando em severa limitação por nitrogênio. Consequentemente, associações de espécies fixadoras de nitrogênio (*A. spiroides*, *C. raciborskii*) foram favorecidas e passaram a dominar a comunidade.

Em síntese, os resultados deste estudo sustentam esta abordagem fitosociológica em relação a delimitação de associações de espécies do fitoplâncton em sistemas tropicais enriquecidos, a despeito da hipótese ter sido originalmente formulada para ecossistemas de regiões temperadas.

Outro aspecto teórico também considerado foi a análise das relações empíricas entre os recursos e a abundância das espécies considerando a ecofisiologia das espécies. As correlações observadas no campo foram explicadas, quando possível, através de argumentos da fisiologia das espécies.

Embora exista uma literatura relativamente extensa sobre os fatores reguladores das classes de algas planctônicas, pouco tem sido feito para verificar se os padrões de distribuição específicos das espécies são correlacionados com os fatores ambientais, e se esses padrões são consistentes com as observações experimentais sobre a fisiologia das espécies em particular (Interlandi *et al.*, 1999)

Neste estudo, a análise em escala detalhada da ocorrência e distribuição das principais espécies possibilitou uma melhor avaliação e entendimento das forças direcionadoras da estrutura e padrões sazonais de sucessão do fitoplâncton. Tanto as descrições estatísticas, quanto as baseadas nas observações, demonstraram as fortes interações entre as condições de limitação em geral e a variabilidade relativa dos recursos, em particular na regulação da estrutura da comunidade fitoplanctônica.

Assim, através da mudança na composição da comunidade fitoplanctônica, de diatomáceas e criptofíceas para cianobactérias, devida a variação dos fatores ambientais, ficou evidenciado um tempo de resposta de algumas semanas. *Microcystis aeruginosa* somente tornou-se dominante cerca de 10 semanas após a razão N/P decrescer para valores menores que 10 e as concentrações de NID alcançarem valores abaixo de 5µM.

As análises estatísticas evidenciaram que a substituição de diatomáceas e criptofíceas por cianobactérias esteve claramente associada com reduzidas disponibilidades de NID e baixas razões N/P. Então, o sucesso das cianobactérias no reservatório de Juturnaíba, durante o estudo, poderia ser atribuído a baixos valores da razão N/P, como considerado pela hipótese da razão de recursos (Smith, 1986; Bulgakov & Levich, 1999; Smith & Bennett, 1999). Contudo, o fato das mudanças na composição da comunidade fitoplanctônica no reservatório de Juturnaíba terem sido precedidas tanto por reduções na razão N/P, quanto nas concentrações de NID, aponta que, além das proporções, a disponibilidade de nitrogênio também podem ter definido as mudanças na composição do fitoplâncton. Com base nestas considerações, a disponibilidade de nitrogênio foi considerada como um dos principais fatores determinantes da sucessão sazonal observada no reservatório de Juturnaíba durante o estudo.

As respostas do fitoplâncton dependem, dentre outros fatores, das adaptações ecofisiológicas. Neste sentido, estudos experimentais sobre a ecofisiologia do fitoplâncton com culturas de laboratório são importantes, pois permitem o conhecimento sobre as estratégias e fatores que estimulam a resposta adaptativa das espécies, tornando as extrapolações para a natureza mais realistas (Zevenboom, 1987). Então, estudos sobre a

ecofisiologia das principais espécies registradas no reservatório de Juturnaíba são necessários para avaliação do real potencial regulador da razão N/P.

A hipótese da razão de recursos baseia-se na teoria da competição (Tilman, 1982), onde baixas razões N/P implicam em limitação por nitrogênio, enquanto que elevadas valores de razão N/P resultariam em limitação por fósforo (Smith & Bennett, 1999). Cabe ressaltar que, a competição por nutrientes ocorre, por definição, sempre que o consumo dos nutrientes pelos organismos resulta em limitação das taxas reprodutivas (Tilman, 1981). Então, considerando um cenário de competição entre *M. aeruginosa* e *A. distans*, seria esperado que as taxas de crescimento destas algas fossem influenciadas pela razão N/P.

Contudo, os resultados obtidos nos experimentos em laboratório não mostraram diferenças significativas entre as condições experimentais. Também não foram observadas diferenças significativas entre as taxas de crescimento de *M. aeruginosa* e *A. distans*. Porém, as culturas de *M. aeruginosa* apresentaram uma capacidade de produção de biomassa superior ao de *A. distans*, especialmente em baixas razões N/P. As células de *M. aeruginosa* mantiveram-se por mais tempo em crescimento exponencial. Como resultado, os cultivos de *M. aeruginosa*, mantidos na condição de menor disponibilidade de nitrogênio, apresentaram rendimento 4 vezes maior do que as culturas de *A. distans* mantidas nesta mesma condição.

Como esperado, a variação da razão N/P do meio de cultivo foi devida à absorção dos nutrientes pelas células de *M. aeruginosa* e *A. distans*. Neste caso as variações observadas durante os experimentos demonstraram que *M. aeruginosa* tem um potencial maior que *A. distans* para influenciar a disponibilidade proporcional de nutrientes.

Freqüentemente tem sido apontado na literatura que a dominância de cianobactérias é determinada, dentre outros fatores, por baixas razões N/P (Bulgakov & Levich, 1999; Smith, 1983; Smith & Bennett, 1999). Porém, contrariamente, nossos resultados indicam que a razão N/P no reservatório de Juturnaíba pode ter sido conseqüência das elevadas capacidades de absorção de nitrogênio e fósforo das cianobactérias. Além disso, a limitação por um ou outro nutriente em particular, neste caso o nitrogênio, poderia determinar o resultado da competição (Reynolds, 1999).

Então, em relação ao observado no reservatório, os resultados dos nossos experimentos sugerem que o sucesso de *Microcystis aeruginosa* e declínio de *Aulacoseira distans*, parece ter sido decorrente da maior capacidade das cianobactérias em crescer sob menor disponibilidade de nitrogênio.

Os resultados deste estudo confirmam a importância de conciliar-se observações de campo com estudos experimentais, para uma melhor compreensão dos processos ecológicos na comunidade fitoplanctônica.

6. Conclusões

1. Neste estudo, observamos uma elevada correlação entre biovolume e clorofila *a* total. Deste modo, em alguns casos, a Chl-a pode ser utilizada como parâmetro para estimar-se o biovolume.

2. O cálculo da contribuição de cada classe para a clorofila total com base na relação entre o pigmento marcador e a clorofila *a*, procedimento utilizado em muitos estudos sobre o fitoplâncton com HPLC, mostrou-se significativamente correlacionado com os dados de quantificação fitoplanctônica por microscopia constituindo-se, apesar de algumas limitações inerentes ao método, em ferramenta valiosa para estudos ecológicos e monitoramento do fitoplâncton.

3. O software CHEMTAX calculou de maneira satisfatória as contribuições dos grupos de algas para a clorofila total, mostrando-se uma ferramenta eficaz para análise de dados de pigmentos fitoplanctônicos. Esta informação é complementar aos dados obtidos através da microscopia.

4. A variabilidade interanual do fitoplâncton no reservatório de Juturnaíba parece ter sido determinada não somente pela mudança do ambiente natural para um sistema artificial, decorrente da construção da barragem, mas provavelmente também esteve relacionada a outros fatores como condições climáticas e eutrofização decorrente do desenvolvimento agrícola e urbano da região.

5. As mudanças na composição do fitoplâncton, de diatomáceas e criptofíceas para cianobactérias, estiveram associadas com baixa disponibilidade de NID e baixas razões N/P. A abundância de *A. distans* e criptofíceas foi positivamente correlacionada com NO_3^- e razão N/P e negativamente com temperatura e profundidade de Secchi. A abundância de cianobactérias apresentou associações inversas e foi negativamente correlacionada com NO_3^- e razão N/P, e positivamente com temperatura e profundidade de Secchi

6. As associações **D** e **Y**, compostas por espécies pequenas de rápido crescimento, C estrategistas (*C. marsonii* e *A. distans*) foram selecionadas por condições de alta disponibilidade de nutrientes, observadas durante o inverno e primavera. Com a escassez de nutrientes, principalmente NID, a seleção é direcionada para a associação **M** com *M. aeruginosa*, uma S-R estrategista, que atingiu seu máximo em NID < 5µM. Sob condições de severa limitação por nitrogênio, associações de espécies fixadoras de nitrogênio (**H**, *A. spiroides*, **H-Sn**, *C. raciborskii*) foram favorecidas e passaram a dominar a comunidade.

7. As taxas de crescimento de *M. aeruginosa* e *A. distans* foram semelhantes em todas as condições experimentais, não tendo a razão N/P influenciado a velocidade de crescimento. Os valores observados para *M. aeruginosa* foram comparáveis aos usualmente observados na literatura, enquanto que as taxas de crescimento de *A. distans* corresponderam aos menores valores citadas na literatura e, possivelmente, foram decorrentes da baixa luminosidade nos experimentos.

8. O rendimento máximo das culturas foi maior em *Microcystis aeruginosa*, especialmente nas condições de menor razão N/P. Portanto, podemos supor que cepas da espécie *M. aeruginosa* possuem melhores condições de adaptação à menores disponibilidades relativas de N do que cepas de *A. distans*.

9. As taxas de crescimento de *A. distans* observadas neste trabalho corresponderam aos menores valores citados na literatura e, possivelmente, foram decorrentes da baixa luminosidade nos experimentos. As taxas de crescimento de *Microcystis aeruginosa* foram comparáveis aos valores usualmente observados na literatura.

10. A razão N/P não influenciou a velocidade de absorção de fosfato nos cultivos de *A. distans* e *M. aeruginosa*. Porém, a absorção foi mais elevada e mais rápida na cianobactéria.

11. A variação da razão N/P do meio de cultivo foi devida à absorção dos nutrientes pelas células de *M. aeruginosa* e *A. distans*. Porém, a dinâmica dessa variação demonstrou que

M. aeruginosa tem um potencial maior que *A. distans* para influenciar a disponibilidade proporcional de nutrientes.

12. Em relação ao observado no reservatório, os resultados dos nossos experimentos sugerem que o sucesso de *M. aeruginosa* e declínio de *A. distans*, pode ter sido decorrente da maior capacidade das cianobactérias em crescer sob menor disponibilidade de nitrogênio.

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