Paula Yuri Nishimura

A comunidade fitoplanctônica nas represas
Billings e Guarapiranga (Região
Metropolitana de São Paulo)

The phytoplankton community in Billings and Guarapiranga
reservoirs (São Paulo Metropolitan Area)

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reservoirs (São Paulo Metropolitan Area)

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Prof(a). Dr(a).

Prof(a). Dr(a).

Prof(a). Dr(a).

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Apresentação

A ideia do projeto “As cianobactérias e cianotoxinas na transposição Billings-Guarapiranga” surgiu a partir de resultados encontrados no meu projeto de mestrado “Ecologia do fitoplâncton em dois braços da Represa Billings (São Paulo - SP) com diferentes graus de trofia” (NISHIMURA, 2008), no qual foi constatada a frequente ocorrência de florações de cianobactérias tóxicas no braço Taquacetuba da represa Billings. A partir desta observação, passamos a indagar sobre o impacto da transposição de águas e, conseqüentemente, de cianobactérias e cianotoxinas, do braço Taquacetuba para a represa Guarapiranga. Esta transposição ocorre desde 2000 em períodos de estiagem, a fim de manter o nível da água da represa Guarapiranga elevada o suficiente para abastecer 3,8 milhões de pessoas na cidade de São Paulo com água potável.

Inicialmente, o objetivo principal deste projeto de doutorado era verificar o impacto da entrada da água do braço Taquacetuba (represa Billings) na represa Guarapiranga, como inóculo de cianobactérias e, conseqüentemente, de cianotoxinas. Este projeto também visava entender a dinâmica do caminho da água durante a transposição do sistema Taquacetuba para a represa Guarapiranga do ponto de vista físico, químico e biológico, em períodos anterior e posterior à operação do bombeamento. O projeto recebeu financiamento da FAPESP (Processo no. 2008/00784-3) e CNPq (Processo no. 471404/2010-1).

A fim de atingir os objetivos do projeto, pretendíamos realizar medidas de parâmetros importantes e amostragens de água no caminho das cianobactérias até o ponto de captação da água na represa Guarapiranga, em períodos anterior e posterior à operação do bombeamento do sistema de transposição Taquacetuba-Guarapiranga. Para tanto, uma primeira série de coletas foi realizada em abril de 2009, período em que a transposição estava inoperante. Porém, devido ao grande volume de chuvas em 2009 e 2010, a transposição continuou inoperante, impossibilitando a realização da segunda série de coletas em período em que a transposição estivesse funcionando. Outro contratempo encontrado durante a realização do projeto foi a ausência de florações de cianobactérias durante as coletas realizadas. Em contrapartida, foi encontrado o dinoflagelado invasor *Ceratium furcoides*.

Diante dos imprevistos encontrados durante a realização do projeto, nosso objetivo inicial não pôde ser alcançado. Portanto, ajustes e adequações foram feitos de forma a complementar e sanar novos questionamentos levantados durante a primeira série de

coletas, principalmente a respeito do dinoflagelado invasor encontrado nas represas Billings e Guarapiranga. Desta forma, uma segunda coleta foi realizada ao longo do eixo longitudinal da represa Guarapiranga, a fim de investigar a heterogeneidade espacial horizontal deste ambiente. Estudos anteriores do nosso grupo de pesquisa, já haviam evidenciado este fato e o presente trabalho focou na comunidade fitoplanctônica e seus grupos funcionais. Em 2011 surgiu a oportunidade de realizar um estágio no exterior (“sanduíche”) durante oito meses na Universidade de Veszprém (Hungria) sob supervisão da Profa. Dra. Judit Padisák, no qual pude aperfeiçoar meus conhecimentos em ecologia de fitoplâncton e na abordagem dos grupos funcionais.

A tese está estruturada em capítulos, sendo o capítulo 1 composto de uma introdução geral sobre o estudo do fitoplâncton e grupos funcionais, além da apresentação do local de estudo. Os capítulos seguintes, exceto o último, são apresentados os produtos do projeto, já em formato de artigo prontos para submissão em revistas científicas, em inglês. O capítulo 2 (“*Phytoplankton community and water quality in two linked tropical reservoirs: a functional groups approach*”) é fruto da primeira série de coletas nas represas Billings e Guarapiranga com o sistema de transposição fechado, na qual são feitas algumas inferências sobre os impactos deste sistema. O capítulo 3 (“*Invasive dinoflagellate Ceratium furcoides (Levander) Langhans in two linked tropical reservoirs*”) também é fruto desta primeira coleta, porém, focado na ocorrência do dinoflagelado invasor *Ceratium furcoides* nas duas represas. Diante da necessidade de aprofundar meus conhecimentos sobre a ocorrência, ecologia e biologia de *Ceratium furcoides* no Brasil e no mundo, surgiu o capítulo 4 (“*Ceratium furcoides (Levander) Langhans 1925, an invasive dinoflagellate in Brazilian freshwaters: worldwide distribution and review on its ecology*”), na qual é feita uma revisão sobre este dinoflagelado. Os capítulos 5 e 6 são fruto da coleta complementar realizada na represa Guarapiranga, visando o estudo da heterogeneidade do fitoplâncton nesta represa, sendo que o capítulo 5 (“*Does the plankton community follow the water quality heterogeneity in a tropical urban reservoir (Guarapiranga reservoir, São Paulo, Brazil)?*”) investiga a ocorrência de heterogeneidade horizontal das comunidades planctônicas e o capítulo 6 (“*Comparison of phytoplankton grouping methods on the example of a spatially heterogenic reservoir*”) compara as abordagens de grupos funcionais existentes na literatura na detecção de variações ambientais na represa. Um capítulo final (capítulo 7) contém as conclusões gerais e perspectivas futuras geradas ao fim deste projeto de doutorado.

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Abstract

Reservoirs exhibit horizontal and vertical gradients of abiotic factors that control phytoplankton dynamics, resulting in a marked spatial heterogeneity in phytoplankton productivity. Therefore, phytoplankton is an important object of study in reservoirs. Traditionally, ecological studies on phytoplankton community were based on species or taxonomic major divisions, such as classes. As science advanced, new approaches appeared, such as functional groups. Several authors have proposed methods of functional groups' classification based on different criteria, such as physiology, ecology and/or morphology. The general objective of this study was to investigate the structure of the phytoplankton community in relation to environmental characteristics in two water supply reservoirs in São Paulo Metropolitan Area, Billings and Guarapiranga reservoirs. To reach some specific aims, several aspects were investigated: the possible impacts of the water transfer from Billings to Guarapiranga reservoir, the use of functional groups in the detection of environmental patterns, comparison of different methodologies of phytoplankton functional groups' classification, the occurrence the invasive dinoflagellate *Ceratium furcoides* in Brazilian reservoirs and the environmental heterogeneity of Guarapiranga reservoir based on water quality and planktonic communities. Billings and Guarapiranga reservoirs exhibited distinct physical, chemical and biological characteristics, being both classified as meso/eutrophic. The phytoplankton community of Billings reservoir was dominated by *C. furcoides* (L₀), which is probably being transferred to Guarapiranga through the water transfer system. The poor water quality of the Parelheiros stream strongly influenced the water quality of Guarapiranga reservoir, mainly at the entrance of this tributary, with dominance of functional group L_M. Regions further away from Parelheiros stream in Guarapiranga reservoir exhibited different conditions, due to the influence of tributaries with better water quality. It was observed abundance of different functional groups, such as W_S, H₁ and A, demonstrating the presence of water quality heterogeneity in the longitudinal axis of Guarapiranga reservoir. Three main compartments were identified: 1) Embu Guaçu region, upstream, more protected and less eutrophic, dominated by phytoplankton R-strategists and rotifers; 2) Parelheiros region, a eutrophic branch of the reservoir, dominated by phytoplankton C-strategists and high density of copepods cyclopoids and 3) the lower part of the reservoir, with eutrophic and lacustrine features, co-dominance of phytoplankton C and S-strategists and higher contribution of copepods to the total density of zooplankton. The addition of the algacide copper sulfate in the reservoir's water influenced the formation of

compartments based on phytoplankton and zooplankton communities. Some final remarks were highlighted at the end of this study: 1) the importance of the preservation of Parelheiros stream's water quality in order to maintain Guarapiranga reservoir water quality, 2) the transfer of phytoplankton species from Billings to Guarapiranga reservoir must be closely monitored, 3) the occurrence and dispersion of *C. furcoides* in Brazilian waters must be closely monitored, 4) management programs in Guarapiranga reservoir must take into account the presence of water quality heterogeneity and 5) phytoplankton functional groups as a tool for identifying environmental patterns should be applied.

Resumo

Reservatórios apresentam gradientes horizontal e vertical de fatores abióticos que controlam a dinâmica fitoplanctônica, resultando em uma marcada heterogeneidade espacial na produtividade fitoplanctônica. Portanto, o fitoplâncton é um importante objeto de estudo em reservatórios. Tradicionalmente, os estudos ecológicos com fitoplâncton partiam da análise da comunidade a partir de espécies ou grandes divisões taxonômicas, como classes. Com o avanço do conhecimento, surgiram novas abordagens para o estudo desta comunidade, como os grupos funcionais. Diversos autores propuseram metodologias de classificação de grupos funcionais, baseados em diferentes critérios, como fisiologia, ecologia e/ou morfologia. O objetivo geral do presente estudo foi investigar a estrutura da comunidade fitoplanctônica em relação às características ambientais em duas represas de abastecimento de água da Região Metropolitana de São Paulo, as represas Billings e Guarapiranga. Para atender aos objetivos específicos, diversos aspectos foram abordados, como os possíveis impactos da transposição de águas da represa Billings para a Guarapiranga, a utilização de grupos funcionais na detecção de padrões ambientais, a comparação de diferentes metodologias de agrupamentos funcionais do fitoplâncton, a ocorrência do dinoflagelado invasor *Ceratium furcoides* em reservatórios brasileiros e a heterogeneidade ambiental da represa Guarapiranga com base na qualidade da água e nas comunidades planctônicas. As represas Billings e Guarapiranga discriminaram-se quanto às variáveis físicas, químicas e biológicas, sendo classificadas como meso/eutróficas. A comunidade fitoplanctônica da represa Billings foi dominada pelo dinoflagelado invasor *Ceratium furcoides* (L₀), que provavelmente está sendo transferido para a represa Guarapiranga através do sistema de transposição. A baixa qualidade da água do ribeirão Parelheiros influenciou fortemente a qualidade da água da represa Guarapiranga, principalmente na região de entrada deste tributário, com domínio do grupo funcional L_M. As regiões mais distantes da região de influência do ribeirão Parelheiros na represa Guarapiranga apresentaram condições distintas, devido à influência de tributários com melhor qualidade de água. Observou-se abundância de distintos grupos funcionais, como W_S, H₁ e A, evidenciando uma heterogeneidade da qualidade da água no eixo longitudinal da represa Guarapiranga. Três compartimentos principais foram identificados: 1) região do Embu-Guaçu, parte alta da represa, mais protegida e menos eutrófica dominada por fitoplâncton R-estrategista e rotíferos; 2) região do Parelheiros, um braço eutrófico da represa com domínio de fitoplâncton C-estrategista e elevada densidade de copépodes ciclopóidas e 3) parte baixa do

reservatório, com características eutróficas e lacustrinas, co-dominância de fitoplâncton C e S-estrategistas e maior contribuição de copépodes à densidade total do zooplâncton. A adição do algicida sulfato de cobre na água da represa Guarapiranga influenciou a formação de compartimentos com base nas comunidades fitoplanctônica e zooplanctônica. Ao fim deste trabalho, evidenciou-se a importância 1) da preservação do ribeirão Parelheiros e do monitoramento da transferência de espécies fitoplanctônicas da represa Billings para a Guarapiranga visando a preservação da qualidade da água represa Guarapiranga, 2) da necessidade do monitoramento da ocorrência e dispersão de *C. furcoides* no Brasil, 3) da implementação de programas de manejo na represa Guarapiranga que levem em consideração a presença de heterogeneidade ambiental neste reservatório e 4) da utilização dos grupos funcionais fitoplanctônicos como ferramenta na identificação de padrões ambientais.

Capítulo 1. Introdução geral

1. Introdução

1.1. Reservatórios

A maior parte do conhecimento em limnologia se baseia nos estudos clássicos em lagos. A estrutura, função e resposta dos sistemas lênticos estão muito bem descritos em livros clássicos como *Treatise on Limnology* (HUTCHINSON, 1957), *Fundamentals of Limnology* (RUTTNER, 1963), *Limnología* (MARGALEF, 1983) e *Limnology* (WETZEL, 2001). Com a intensificação da construção de reservatórios, a partir da década de 50 (STRASKRABA & TUNDISI, 2000), estes ecossistemas artificiais passaram a ser considerados um tipo de lago (HUTCHINSON, 1957). A abordagem de muitos estudos em limnologia de reservatórios era idêntica à abordagem dos tradicionais estudos em lagos e os padrões identificados nos reservatórios eram interpretados dentro do conhecimento convencional de limnologia de lagos (THORNTON, 1990).

Porém, lagos são ecossistemas naturais gerados ao longo do tempo geológico, enquanto reservatórios são ecossistemas criados pelo homem. Portanto, a hipótese inicial de que reservatórios e lagos são iguais, precisou ser revista. Processos como mescla, troca de gases na interface ar-água, reações de oxirredução, captação de nutrientes, interações presa-predador, produção primária e respiração ocorrem tanto em lagos quanto em reservatórios. Reservatórios distinguem-se de lagos naturais por apresentarem sistemas de circulação horizontal e vertical produzidos por forças naturais e antrópicas que atuam na operação da represa de forma significativa, alterando os mecanismos ecológicos (TUNDISI, 1990). Tempo de retenção, altura da tomada de água e sequência de operações em conjunto em cadeias dos reservatórios são exemplos de ações antrópicas que alteram significativamente o ecossistema de um reservatório. A Tabela 1 apresenta resumidamente as principais diferenças qualitativa e quantitativa entre lagos e reservatórios.

Tabela 1. Comparação entre reservatórios e lagos. Adaptado de Straskraba *et al.* (1993) e Wetzel (1990).

Característica	Lago	Reservatório
Origem	Natural	Antrópica
Idade	Velho ($\geq$ pleistoceno)	Novo ($\leq$ 70 anos)
Envelhecimento	Lento	Rápido
Local de formação	Depressões	Vales de rios
Bacia da drenagem	Circular, em posição central. Área pequena em comparação à área do lago ($\sim 10:1$)	Geralmente estreita, em posição central. Área grande em comparação à área do reservatório ($\sim 100:1 - 300:1$)
Posição em relação às bacias hidrográficas formadoras	Central	Marginal
Formato	Regular	Dendrítico
Razão de desenvolvimento	Lenta	Rápida
Profundidade máxima	Perto de centro	Perto do barramento
Sedimento de fundo	Autóctones	Importados
Gradiente longitudinal	Formação eólica	Formando corrente hídrica
Profundidade de descarga	Baixos gradientes	Gradientes mais pronunciados
Flutuações no nível d'água	Superficial	Profunda
Estratificação termal	Pequena, estável	Grande, irregular
	Regime natural, geralmente dimítico ou monomítico	Variável, irregular. Geralmente muito raso para estratificar nas zonas riverinas e transitórias. Geralmente estratifica temporariamente na zona lacustre.
Efluentes	Via tributários de pequena ordem. Fontes difusas.	Via tributários de grande ordem. Escoamento superficial.
Afluentes	Relativamente estável. Saída pela superfície (evaporação) ou fundo (escoamento).	Irregular (depende dos usos). Tomada d'água superficial ou hipolimnética.

Então, surgiu a necessidade de uma linha de pensamento específica, que ficou conhecida como “limnologia de reservatórios”, que leva em consideração os aspectos limnológicos específicos de reservatórios, ausentes nos ecossistemas lacustres, considerando aspectos qualitativos e quantitativos (STRASKRABA *et al.*, 1993). Particularmente no Brasil, a intensificação da construção de reservatórios para produção de energia hidroelétrica na década de 1960 impulsionou o desenvolvimento da pesquisa em limnologia voltada para a função e estrutura de reservatórios (TUNDISI & MATSUMURA-TUNDISI, 2003). Este período na limnologia de reservatórios brasileiros foi de extrema importância para entendimento da estrutura termal e padrões de circulação (HENRY & TUNDISI, 1988; ARCIFA *et al.*, 1990; HENRY, 1995, 1999), estudos temporais e espaciais (HENRY, 1992, 1993), ciclos biogeoquímicos e estudos de emissão de gases do efeito estufa (ROSA &

SCHAEFFER, 1994; FEARNSTIDE, 1995; ABE *et al.*, 2001), padrões de biodiversidade da comunidade de fitoplâncton, zooplâncton e peixes (AGOSTINHO *et al.*, 1994; ROCHA *et al.*, 1997; NOGUEIRA, 2000; AMARAL & PETRERE, 2001; NOGUEIRA, 2001; MATSUMURA-TUNDISI & TUNDISI, 2002), produção primária fitoplanctônica (MATSUMURA-TUNDISI & TUNDISI, 1997; CALIJURI *et al.*, 1999), funcionamento de reservatórios em cascata (BARBOSA *et al.*, 1999; ROCHA *et al.*, 1999), escoamento superficial e tempo de retenção como fator ecológico (TUNDISI *et al.*, 1993; BRAGA *et al.*, 1998; STRASKRABA, 1999), aplicação do sensoriamento remoto e GIS no manejo de reservatórios (NOVO *et al.*, 1995), envelhecimento e colonização de reservatórios (AGOSTINHO *et al.*, 1992; AGOSTINHO *et al.*, 1995; AGOSTINHO *et al.*, 1999), pesca (BECHARA *et al.*, 1999; BORGHETTI & OSTRENSKY, 1999; QUIROS, 1999) interações entre rio e reservatório (BONETTO, 1994).

1.2. O fitoplâncton em reservatórios

Reservatórios apresentam uma variada estrutura espacial, com sistemas de circulação horizontal e vertical produzidos em função das forças naturais e pela ação humana que atuam de forma significativa na operação hidráulica (TUNDISI, 1990). Essas condições físicas têm também consequências biogeoquímicas e influenciam a distribuição, a sucessão de organismos e a produtividade e biomassa das comunidades (TUNDISI & MATSUMURA-TUNDISI, 2008). A Figura 1 mostra a complexidade das principais inter-relações entre componentes da biota aquática e as condições físicas e químicas de um reservatório.

Figura 1. Processos internos de um reservatório. A, B, D, E, H e S representam processos físicos; F, G, K, L, C e R representam processos químicos e os retantes J, M, N, O e P, processos biológicos. Fonte: Tundisi & Matsumura-Tundisi (2008).

O fitoplâncton, em particular, vive em suspensão nas massas d'água e apenas condições ambientais muito particulares permitem movimentos ativos efetivos. Portanto, o fitoplâncton é uma comunidade totalmente dependente dos movimentos das massas de água (REYNOLDS, 2006). Desta forma, a seleção de espécies adaptadas a essas condições, bem como variações em composição e biomassa, é governada pelas inter-relações de fatores físicos (e.g. temperatura e circulação), fatores químicos (e.g. nutrientes e distribuição relativa dos íons dissolvidos na água) e fatores biológicos (e. g. interação das espécies, efeitos da predação e parasitismo) (TUNDISI & MATSUMURA-TUNDISI, 2008).

Reservatórios apresentam gradientes horizontais e verticais de fatores abióticos que controlam a dinâmica fitoplanctônica. Nestes sistemas, há uma marcada heterogeneidade espacial na produtividade fitoplanctônica devido aos gradientes longitudinais da morfologia da bacia, velocidade de fluxo, tempo de residência, sólidos em suspensão, luz e disponibilidade de nutrientes (KIMMEL *et al.*, 1990).

1.3. As estratégias adaptativas do fitoplâncton

Os fitoplanctólogos se apoiam no paradigma postulado pelo microbiologista Baas Becking: *"everything is everywhere, but the environment selects"*, traduzindo, tudo está em todo o lugar, mas o ambiente seleciona (QUISPEL, 1998). Parte-se do pressuposto que organismos microscópicos, dentre eles, o fitoplâncton, possuem propágulos viáveis em praticamente todos os ambientes, devido ao seu pequeno tamanho e fácil dispersão. Porém, os organismos só se estabelecem se o hábitat for adequado e apresentar condições de sustentar os requerimentos de crescimento e sobrevivência da espécie (REYNOLDS, 1984). O ambiente age como um filtro, segregando espécies menos adaptadas daquelas com adaptações e atributos que as permitem sobreviver. Consequentemente, as espécies mais adaptadas são mais propensas a crescer e contribuir para uma maior fração da biomassa da comunidade, i.e. serão dominantes (REYNOLDS, 2006).

Portanto, a composição de espécies no fitoplâncton é inicialmente aleatória e a sucessão autogênica é muitas vezes previsível ao conhecer o ambiente (STRASKRABA *et al.*, 1999). O curto tempo de geração das comunidades pelágicas permitiu a investigação do papel da seleção de atributos no desenvolvimento destas comunidades (ROJO & ALVAREZ-COBELAS, 2000), sendo bem estabelecido que as principais forças que agem sobre a composição de uma comunidade pelágica são as restrições de recursos e energia

(REYNOLDS, 2006). Desta forma, é intuitivo pensar que o fitoplâncton será formado por grupos de espécies com atributos específicos para superar estas restrições.

Para compreender o funcionamento dos ecossistemas, os ecologistas vêm tentando agrupar organismos com características similares em estrutura e função, como tamanho, forma, estratégias de vida e fisiologia (KÖRNER, 1993), na tentativa de encontrar padrões simplificados da complexidade ambiental (MACINTYRE *et al.*, 2002). O fitoplâncton, em particular, é capaz de desenvolver inúmeras estratégias para sobreviver às diferentes condições ambientais (REYNOLDS, 2006).

Tradicionalmente, os modelos preditivos de padrões de associações do fitoplâncton partem da análise da comunidade a partir de espécies ou grandes divisões taxonômicas, como classes (MOSS, 1973; MAULOOD & BONEY, 1981; SOMMER, 1986; WATSON *et al.*, 1997; YUNG *et al.*, 1997; FABBRO & DUIVENVOORDEN, 2000; BRETTUM & HALVORSEN, 2004; FIETZ *et al.*, 2005; HAJNAL & PADISÁK, 2008). Porém, o fitoplâncton não é um grupo uniforme, abrangendo organismos de filogenias, tamanhos, formas e estratégias adaptativas diversas. Portanto, o estudo da comunidade fitoplanctônica partindo de grandes divisões taxonômicas é problemático, necessitando de novas abordagens que levem em consideração não apenas a classificação filogenética, mas também a forma e a função destes organismos. Neste contexto, duas linhas teóricas principais foram desenvolvidas a partir dos atributos morfo-funcionais do fitoplâncton como alternativa para um grupamento não taxonômico: (1) o modelo de duas estratégias de vida e (2) o modelo de três estratégias de vida.

1.3.1. Modelo de duas estratégias de vida: r e K

O modelo de duas estratégias de vida aplica nos organismos fitoplanctônicos os conceitos de Pianka (1970) sobre a seleção r e K. Segundo Pianka (1970), os organismos r-selecionados possuem expectativa de vida curta e grande esforço reprodutivo. Os K-selecionados são organismos com expectativa de vida longa, cuja energia e recursos despendidos para a reprodução é pequena. Margalef (1978) foi pioneiro na utilização de uma abordagem de resposta dos grupos taxonômicos ou associações do fitoplâncton marinho ao estado nutricional e turbulência do ambiente na previsão da ocorrência destes grupos ao longo de gradientes ambientais. Margalef resumiu elegantemente suas idéias em seu famoso modelo de "mandala", no qual diferentes associações do fitoplâncton ocupam diferentes quadrantes em um gradiente nutricional e de turbulência (MARGALEF

et al., 1979) (Figura 2). Segundo a mandala, existem quatro estágios em um contínuo sucessional entre espécies r-selecionadas e K-selecionadas. A sucessão vai do estágio 1, dominado por diatomáceas em um ambiente desestratificado e rico em nutrientes, ao estágio 4, dominado por dinoflagelados capazes de explorar uma coluna d'água estratificada e com recursos nutricionais segregados, compensando a exaustão nutricional da superfície. Estudos utilizando este modelo discutem a alternância da seleção r e K nas associações da comunidade fitoplanctônica, propondo que a sucessão de espécies do fitoplâncton ocorre substituindo espécies r em um ambiente instável por espécies K em um ambiente estável (MARGALEF, 1978; HARRIS, 1986; ARAUZO & ALVAREZ-COBELAS, 1994; DOS SANTOS & CALIJURI, 1998).

Figura 2. Mandala de Margalef *et al.* (1978) relacionando mudanças sazonais com a seleção do ambiente pelas formas de vida (r e K). Esta mandala acomoda a seqüência principal de formas de vida, e não espécies, além uma seqüência paralela que favorece dinoflagelados formadores de maré vermelha. Modificado de Reynolds (2006).

Reynolds (1980) aplicou o modelo conceitual de Margalef (1978) no fitoplâncton de água doce, chamando atenção para a ocorrência frequente de condições de altas concentrações de nutrientes e estratificação em lagos rasos. Também foi observado que estas condições ambientais geralmente promovem rápido crescimento de organismos pequenos e competitivos, com atributos clássicos de r-selecionados. Porém, estes organismos tipicamente r-selecionados não se encaixavam no eixo sucessional r-K da mandala de Margalef. Diante destas observações, Reynolds (1980) concluiu que algumas espécies de diatomáceas consideradas exclusivamente r-selecionadas por Margalef (1978), precisavam ser reclassificadas. Reynolds *et al.* (1983) se referiram a estas diatomáceas

como w-selecionadas, com base nas adaptações morfológicas e fisiológicas necessárias para manter o crescimento em baixas intensidades luminosas devido à circulação da coluna d'água. Desta forma, foram inseridas na classificação dos organismos informações sobre adaptações do fitoplâncton para a captação de luz necessária nas diferentes condições ambientais.

1.3.2. *Modelo de três estratégias de vida: CRS*

Ao incluir uma terceira estratégia de vida, o modelo de Reynolds *et al.* (1983) baseado na seleção r e K, passou a ter uma notável semelhança com o modelo de três estratégias de vida proposto por Grime (1977) sobre os processos de sucessão ecológica para vegetação terrestre. Segundo este autor, existem três estratégias primárias em plantas terrestres, relacionadas com diversas características, como morfologia, alocação de recursos, fenologia e resposta ao estresse. A estratégia competitiva (C-estrategistas) prevalece na vegetação produtiva, relativamente sem distúrbios; a estratégia estresse-tolerante (S-estrategistas) está associada com condições continuamente não-produtivas; e a estratégia ruderal (R-estrategistas) é característica de vegetação severamente perturbada (Figura 3a). Reynolds (1988) adaptou as idéias de Grime (1977) às estratégias de sobrevivência do fitoplâncton, de acordo com as adaptações morfológicas e fisiológicas, classificando os organismos fitoplanctônicos nos três possíveis habitats pelágicos combinando circulação/turbulência e gradiente de recursos (refletindo, respectivamente, na “duração de habitat” e “produtividade do habitat” no modelo de Grime, ver Figura 3a e 3b). Em um ambiente estável, com radiação solar abundante, é esperado que o consumo do fitoplâncton esgote os nutrientes, fazendo com que os recursos disponíveis se tornem menos acessíveis e demande adaptações especializadas do fitoplâncton. Estes eventos representariam uma verdadeira sucessão autogênica, indo de organismos r-selecionados C-estrategistas para K-selecionados S-estrategistas. A luz disponível e a dependência da profundidade de mistura da coluna d'água compõem o eixo horizontal, uma vez que eventos de mistura interferem na seleção autogenética e selecionam organismos R-estrategistas (Figura 3b). As características morfológicas e fisiológicas dos estrategistas C, R e S do fitoplâncton estão resumidas na Tabela 2.

Figura 3. A) Modelo de Grime (1977) para vegetação terrestre sobre a sustentabilidade e insustentabilidade de habitats, mostrando as estratégias primárias de sobrevivência (C, R e S) necessárias para assegurar a sobrevivência no ambiente em relação à produtividade e duração do habitat. B) Modelo de Reynolds (1988) para o fitoplâncton sobre a seleção de espécies em um amplo espectro ecológico (nutrientes e circulação/luminosidade), de acordo com as estratégias primárias de ciclo de vida (C, R ou S), exceto onde os nutrientes e luz são continuamente deficientes ("vazio"). Ambos adaptados de Reynolds (2006)

O modelo C-R-S de Grime (1977) para vegetação terrestre nunca foi amplamente aceito pela comunidade científica, dando origem a debates calorosos (LOEHLE, 1988; TILMAN, 1988), até hoje em aberto (CRAINE, 2005). A aplicação do modelo C-R-S para o fitoplâncton não recebeu tantas críticas e o modelo foi amplamente aplicado (HUSZAR & CARACO, 1998; FABBRO & DUIVENVOORDEN, 2000; KRUK *et al.*, 2002; MORABITO *et al.*, 2002; CAPUTO *et al.*, 2008).

Tabela 2. Resumo das características morfológicas e fisiológicas dos estrategistas C, R e S na comunidade fitoplanctônica, segundo Reynolds (1988).

	C-estrategistas	R-estrategistas	S-estrategistas
Seleção	R	r ou K (w, segundo Reynolds <i>et al.</i> 1983)	K
Forma	Unicelular	Unicelular, cenobial ou colonial	Unicelular, cenobial ou colonial
Tamanho	Pequeno 10-1 – 103 μm^3	Médio 103 – 105 μm^3	Grande 104 – 107 μm^3
Suscetibilidade à predação pelo zooplâncton	Grande	Média	Pequena
Taxa de sedimentação	Baixa	Média	Alta
Crescimento favorecido em condições de	↑ luz ↑ nutrientes	↓ luz	↑ luz ↓ nutrientes
Gêneros representantes	<i>Chlorella</i> <i>Ankrya</i> <i>Chlamydomonas</i> <i>Coenocystis</i> <i>Rhodomonas</i>	<i>Asterionella</i> <i>Aulacoseira</i> <i>Limnothrix</i> <i>Planktothrix</i>	<i>Microcystis</i> <i>Anabaena</i> <i>Gloetrichia</i> <i>Ceratium</i> <i>Peridinium</i> <i>Uroglena</i>

1.4. Os grupos funcionais do fitoplâncton

Ao modelo de estratégias C-R-S se seguiu a abordagem de grupos funcionais de fitoplâncton, assembléias ou associações fitoplanctônicas (REYNOLDS, 1997), baseados em atributos morfo-funcionais. Reynolds *et al.* (2002) sugerem a utilização do termo “grupo funcional” em substituição ao termo “associação”, anteriormente utilizado. O termo “associação” foi adaptado da ecologia de vegetação terrestre e define um grupo de espécies que respondem de forma semelhante a uma certa condição ambiental. O termo “grupo funcional” é mais apropriado ao fitoplâncton por englobar espécies com morfologia e fisiologia semelhantes, além de semelhanças ecológicas, buscando diferenciar os organismos do fitoplâncton em relação às adaptações e requerimentos específicos (e.g. alta afinidade por fósforo ou carbono, necessidade de esqueleto silicoso, eficiência na

captação luminosa). A utilização dos grupos funcionais permite prever a ocorrência de certas espécies nos ambientes, contribuindo para o entendimento e previsão da distribuição e dinâmica de populações naturais do fitoplâncton (REYNOLDS *et al.* 2002)

1.4.1. Grupos funcionais de Reynolds *et al.* (2002)

Nesta abordagem, grupos de espécies do fitoplâncton, ou seja, os grupos funcionais (GF), foram definidos empiricamente com base na relação superfície/volume das espécies dominantes e na semelhança na resposta a um determinado conjunto de condições ambientais. Na década de 80, época sem os pacotes estatísticos disponíveis atualmente, Reynolds *et al.* (2002) se propôs ao árduo trabalho de identificar espécies que co-ocorriam frequentemente, raramente ou nunca, a partir de abordagem fitossociológica clássica de uma série de dados históricos da comunidade fitoplanctônica em cinco lagos contrastantes do noroeste da Inglaterra (REYNOLDS, 2006). Reynolds identificou 14 GFs que descreviam adequadamente a periodicidade do fitoplâncton ao longo das estações do ano. Desde então, estes GFs sofreram muitas modificações, principalmente pela adição de novos grupos que englobassem espécies de outros ambientes e outras regiões do globo. A maioria dos novos GFs foi delimitada utilizando métodos estatísticos, que, por sua vez, validaram a maioria dos grupos originais.

No livro “*Vegetation processes in the pelagic: a model for ecosystem theory*” (REYNOLDS, 1997) há uma descrição detalhada dos ambientes e dos GFs correspondentes. Em 2002, Reynolds e colaboradores compilaram as informações presentes no livro de Reynolds de 1997 na revisão “*Towards a functional classification of the freshwater phytoplankton*” Reynolds *et al.* (2002). Nesta revisão foram apresentados 31 GFs identificados através de códigos alfa-numéricos, denominados códons, formando grupos, em sua maioria, polifiléticos. Por exemplo, os primeiros GFs identificados receberam os códons A, B e C, originalmente aplicado às diatomáceas típicas de florações de primavera nos lagos temperados de diferentes estados tróficos. Os grupos D, N e P também envolvem diatomáceas, porém de ambientes diferentes dos originalmente estudados (lagos temperados). E assim, as espécies do fitoplâncton foram acomodadas em seus respectivos GFs, totalizando atualmente 33 GFs (PADISÁK *et al.*, 2009).

Reynolds *et al.* (2002) afirmam que a aplicação dos GFs de água doce é uma ferramenta muito robusta no entendimento e na predição da dinâmica de comunidades naturais do fitoplâncton, devido à sensibilidade às latitudes, morfometrias e estados tróficos. Além disso, os GFs levam em consideração apenas as preferências e sensibilidades dos

organismos fitoplactônicos, deixando de lado as relações filogenéticas. Diversos estudos independentes aplicaram e discutiram os GF *sensu* Reynolds *et al.* (2002), contribuindo para a confirmação de sua utilidade (KRUK *et al.*, 2002; DOKULIL & TEUBNER, 2003; LEITÃO *et al.*, 2003; NASELLI-FLORES & BARONE, 2003; NIXDORF *et al.*, 2003; PADISÁK *et al.*, 2006). O alto grau de refinamento dos GF leva à necessidade de informações sobre atributos que nem sempre são fáceis de se obter sobre a auto-ecologia de algumas espécies e, às vezes, baseadas no julgamento de um especialista. Portanto, a utilização dos GFs é de difícil aplicação e passível de erros por parte dos usuários (PADISÁK *et al.*, 2009).

1.4.2. *Grupos morfo-funcionais de Salmaso & Padisák (2007)*

Salmaso & Padisák (2007) se basearam em características morfo-funcionais simples de organismos fitoplânctônicos para desenvolver os grupos morfo-funcionais do fitoplâncton (GMF), através de análises multivariadas. Os critérios adotados para discriminar os grupos incluiu mobilidade (presença ou ausência de flagelo), capacidade potencial de obtenção de carbono (mixotrofia), requerimentos nutricionais específicos, tamanho, forma e presença ou ausência de envelope gelatinoso. Estes critérios, além da separação de Cyanobacteria dos demais grupos de algas, resultou em 31 GMF. O trabalho fornece uma tabela que se assemelha a uma chave de identificação, em que o usuário corre pelas características dicotômicas, até chegar ao GMF em que a espécie se encaixa. Desta forma, a classificação das espécies em seus respectivos GMF é simples e intuitiva. Ao fim do trabalho, os autores concluem que a aplicação dos GMF é uma ferramenta poderosa no estudo da dinâmica sazonal desta comunidade, principalmente ao comparar diferentes lagos. Além disso, por estar baseado em caracteres morfológicos e fisiológicos simples, há a superação de problemas relacionados à acurácia taxonômica e identificação dos organismos. Apesar da aparente simplicidade e fácil aplicação dos GMF, poucos trabalhos utilizaram esta ferramenta (TOLOTTI *et al.* 2010).

1.4.3. *Grupos funcionais baseados em morfologia de Kruk et al. (2010)*

Até então, as abordagens de grupos funcionais tentavam definir grupos de espécies que tipicamente eram encontradas juntas, através de uma visão de comunidade *sensu* Clements (1916), na qual a comunidade é vista como entidades funcionais. Análises estatísticas foram utilizadas para reconhecer e classificar tais agrupamentos de espécies. Utilizando uma linha de pensamento comunitária *sensu* Gleason (1926) assume-se que

espécies individuais respondem independentemente ao ambiente e, assim, é possível prever a composição da comunidade com base nas respostas das espécies individuais às condições ambientais. Tais condições devem favorecer grupos de espécies com habilidades competitivas similares (WEBB *et al.*, 2002). Partindo da premissa que habilidade competitivas (e.g. assimilação de nutrientes e luz, crescimento, mecanismos de flutuação) dos diferentes grupos de espécies são refletidas nos atributos morfológicos (e.g. tamanho, presença de flagelo ou mucilagem), Kruk *et al.* (2010) desenvolveram os grupos funcionais baseados em morfologia (GFBM) utilizando atributos apenas morfológicos. Neste contexto, apesar destes GFBMs basearem-se em características apenas morfológicas, os grupos refletem as características morfo-funcionais dos organismos.

Segundo os atributos morfológicos, os organismos fitoplanctônicos podem ser reunidos em sete GFBMs: Grupo I - organismos pequenos com elevada razão superfície/volume (e.g. *Chlorella*, *Synechocystis*, *Chroococcus*); Grupo II - organismos flagelados pequenos com estruturas silicosas (i.e. Chrysophyceae); Grupo III - filamentos grandes com aerótopos (e.g. *Planktothrix*, *Anabaena*, *Cylindrospermopsis*); Grupo IV - organismos de tamanho médio, sem estruturas especializadas (e.g. *Closterium*, *Monoraphidium*, *Pediastrum*); Grupo V - flagelados unicelulares ou de tamanho médio a grande (e.g. Cryptophyceae, Euglenophyceae, Dinophyceae); Grupo VI - organismos não flagelados com esqueleto silicoso (i.e. Bacillariophyceae); Grupo VII - colônias envoltas por mucilagem (e.g. *Botryococcus*, *Aphanocapsa*, *Microcystis*). Por serem baseados em atributos morfológicos de fácil observação, os GFBMs minimizam as falhas presentes na classificação baseada em atributos morfo-funcionais mais complexos: 1) reduz a necessidade de boa resolução taxonômica; 2) aplicação fácil e intuitiva, pode ser utilizada globalmente por usuários menos experientes e tomadores de decisão; 3) reduzido número de GFBMs (KRUK *et al.*, 2010). Apesar de ser uma abordagem relativamente recente, alguns trabalhos aplicaram e demonstraram a eficiência desta ferramenta em diversos ambientes (PACHECO *et al.*, 2010; KRUK *et al.*, 2011; CARONI *et al.*, 2012).

1.5. Perspectivas no estudo do fitoplâncton com base nos grupos funcionais

Atualmente existem diversas possibilidades de classificação ecológica para trabalhar com o fitoplâncton, além da abordagem clássica utilizando espécies individuais e agrupamentos taxonômicos (e.g. classes). Diante deste novo cenário, não houve um consenso em qual a melhor abordagem para agrupar as espécies visando prever os efeitos das mudanças ambientais na composição da comunidade. Portanto, surgiram alguns

trabalhos comparativos entre as diversas abordagens. Utilizando um banco de dados de 211 lagos abrangendo regiões subpolares até tropicais, Kruk *et al.* (2011) investigaram qual classificação (GF, GFBM, classes taxonômica ou espécies) explica melhor a variação ambiental, através de análises multivariadas e regressões lineares. Os autores concluíram que a composição da comunidade fitoplanctônica é melhor prevista em termos de GFBM. Izaguirre *et al.* (2012) comparou três classificações (GF, GMF e GFBM) em seis lagos rasos argentinos com diferentes condições de equilíbrio, através de análises multivariadas, explorando a força de cada classificação na discriminação de cada tipo de ambiente. Todas as abordagens separaram claramente os lagos turbidos com alto impacto humano dos lagos claros com macrófitas. Os autores concluíram que todas as classificações obtiveram resultados satisfatórios e que abordagem funcional é adequada para a análise do fitoplâncton em lagos com elevado impacto humano. Similarmente, Nishimura *et al.* (em preparação) compararam qual classificação do fitoplâncton (GF, GMF, GFBM, classes taxonômicas e espécies individuais) explica melhor a variação dos dados ambientais em 42 lagos húngaros de diferentes estados tróficos, através de análises multivariadas. Os autores concluíram que todas as abordagens refletem satisfatoriamente a variação do ambiente, não existindo, portanto, uma ferramenta melhor do que outra, apenas diferentes abordagens. Gallego *et al.* (2012) investigou a adequação de classificações taxonômicas (espécie, gênero, família) e ecológicas (GF e GFBM) como preditores da riqueza e composição da comunidade fitoplanctônica em 87 lagos artificiais estratificados da região da Andalúzia, escolhidos aleatoriamente. Gênero, família e GF predisseram satisfatoriamente tanto a riqueza quanto a composição da comunidade. GFBM, apesar de ser a ferramenta com utilização mais intuitiva, apresentou pior desempenho na determinação de padrões de riqueza e condições ambientais.

É importante ter em mente que o surgimento de novas abordagens não invalida abordagens anteriores. Cada classificação, seja taxonômico ou funcional, apresenta sua própria complexidade, refinamento, vantagens e desvantagens. Deve-se levar em conta o grau de conhecimento em taxonomia, o tempo e recursos disponíveis para análise e o grau de refinamento necessário para responder à sua pergunta. Cabe ao ecólogo compreender cada ferramenta e ponderar qual será mais vantajosa visando seus objetivos.

2. Objetivos

2.1. Objetivos gerais

Investigar a estrutura da comunidade fitoplanctônica em relação às características ambientais, baseado em diversas abordagens de grupos funcionais, em duas represas de abastecimento de água da Região Metropolitana de São Paulo.

2.2. Objetivos específicos

Cada objetivo específico descrito a seguir contempla um capítulo da tese:

Capítulo 2: Investigar os possíveis impactos na qualidade da água da represa Guarapiranga pela transposição de águas do Sistema Billing-Guarapiranga com base nas variáveis ambientais e na comunidade fitoplanctônica.

Capítulo 3: Investigar a ocorrência do dinoflagelado invasor *Ceratium furcoides* (Levander) Langhans nas represas Billings e Guarapiranga.

Capítulo 4: Revisar a ocorrência, biologia e ecologia do dinoflagelado invasor *Ceratium furcoides* (Levander) Langhans em ambientes tropicais.

Capítulo 5: Investigar a heterogeneidade espacial horizontal ao longo do eixo longitudinal da represa Guarapiranga com base na qualidade da água e comunidades planctônicas.

Capítulo 6: Comparar diferentes abordagens de grupos funcionais na detecção de gradientes ambientais na represa Guarapiranga.

3. Área de estudo

O presente estudo foi realizado em dois importantes mananciais da Região Metropolitana de São Paulo, as represas Billings e Guarapiranga. A Figura 4 apresenta a localização das bacias hidrográficas das represas Billings e Guarapirangas. A Tabela 3 apresenta as principais características dos sistemas e as seções seguintes discorrerão sobre cada um dos sistemas.

Figura 4. Região metropolitana de São Paulo e as bacias hidrográficas da Billings e Guarapiranga. Fonte: Whately & Cunha (2006).

Tabela 3. Principais características das represas Billings e Guarapiranga (São Paulo).

Característica	Represa Billings	Represa Guarapiranga
Bacia hidrográfica	Alto Tietê	Alto Tietê
Área da bacia (km ²)	582,8	639
Coordenada geográfica	23°47'S e 46°40'W	23°43'S e 46°32'W
Altitude (m)	746	742
Ano de construção	1927	
Finalidade inicial	Aproveitar as águas da bacia do Alto Tietê para gerar energia elétrica na Usina Hidrelétrica (UHE) de Henry Borden, em Cubatão	Regularizar as vazões contribuintes e ampliar a produção de energia elétrica em Santana do Parnaíba
Usos atuais	Abastecimento público (reservatório Rio Grande), pesca, esportes náuticos, lazer, fins paisagísticos.	Abastecimento público, esportes náuticos, pesca, fins paisagísticos.
Profundidade média (m)	10	7
Área do espelho d'água (km ²)	127	34
Volume (m ³)	1.200 x 10 ⁶	195 x 10 ⁶
Tempo de residência médio (dias)	720	120
Produção água (m ³ s ⁻¹)	4,7	14
População abastecida	1,6 x 10 ⁶	3,8 x 10 ⁶
População residente na bacia	865 x 10 ³	800 x 10 ³
Estado trófico	Eutrófico	Mesotrófico

3.1. O Complexo Billings

3.1.1. Caracterização geral

A bacia hidrográfica da Billings é uma sub-bacia da bacia do Alto Tietê e liga-se a esta através do canal do rio Pinheiros. Está localizada na porção sudeste da Região Metropolitana de São Paulo (RMSP) a 23°47'S e 46°40'W e a uma altitude de 746 m, ocupando um território de 582,8 km². A oeste faz limite com a bacia hidrográfica da

Guarapiranga e, ao sul, com a Serra do Mar. Sua área de drenagem abrange integralmente o município de Rio Grande da Serra e parcialmente os municípios de Diadema, Ribeirão Pires, Santo André, São Bernardo do Campo e São Paulo (Figura 4). Os principais tributários formadores da bacia hidrográfica da Billings são: Rio Grande ou Jurubatuba; Ribeirão Pires; Rio Pequeno; Rio Pedra Branca; Rio Taquacetuba; Ribeirão Bororé; Ribeirão Cocaia; Ribeirão Guacuri; Córrego Grota Funda e Córrego Alvarenga (Figura 5). A bacia hidrográfica da Billings está dividida em 11 sub-regiões: Corpo Central, Alvarenga, Bororé, Capivari, Cocaia, Grota Funda, Pedra Branca, Rio Grande (a jusante da Barragem Anchieta), Rio Grande (a montante da Barragem Anchieta), Rio Pequeno e Taquacetuba.

A bacia está inserida no Domínio da Mata Atlântica e a totalidade de sua área era, originalmente, recoberta por floresta umbrófila densa. Porém, o avanço da urbanização e de outras atividades antrópicas têm levado ao desmatamento acelerado. Desta forma, em alguns trechos da bacia, a vegetação restringe-se a manchas isoladas ao longo do reservatório. Existem sub-bacias com elevada urbanização, como é o caso da região do município de Diadema, porção norte da Bacia, onde a vegetação é praticamente inexistente (CAPOBIANCO & WHATELY, 2002).

A represa Billings é o maior reservatório de água da RMSP, ocupando área de 127 km² que corresponde a 18% da área total de sua bacia hidrográfica. Apresenta profundidade média de 10 m, apesar do nível d'água do reservatório ser bastante variável. Devido a seu formato peculiar, a represa está subdividida em oito unidades, denominadas braços, os quais correspondem às sub-regiões da bacia hidrográfica: braço Rio Grande ou Jurubatuba, separado do Corpo Central pela barragem da Rodovia Anchieta; braço Rio Pequeno; braço Capivari; braço Pedra Branca; braço Taquacetuba; braço Bororé; braço Cocaia e braço Alvarenga (Figura 5). Em função desta conformação dentrítica, o afluo de água ocorre preferencialmente ao longo do seu canal central, fazendo os braços apresentem características distintas do corpo central (CETESB, 2002a).

Figura 5. Bacia hidrográfica da Billings, municípios limítrofes, principais rios formadores e braços da represa Billings. Modificado de: Capobianco & Whately (2002).

3.1.2. *Histórico da represa Billings*

A área ocupada atualmente pela represa Billings foi inundada a partir de 1927, com a construção da Barragem de Pedreira, no curso do Rio Grande. O projeto foi implementado pela antiga Light (*"The São Paulo Tramway, Light and Power Company, Limited"*), hoje Eletropaulo, com o intuito de aproveitar as águas da bacia do Alto Tietê para gerar energia elétrica na Usina Hidrelétrica (UHE) de Henry Borden, em Cubatão, aproveitando-se do desnível da Serra do Mar (WHATELY, 2003).

No início dos anos 40, iniciou-se o desvio de parte da água do rio Tietê e seus afluentes para a Represa Billings, a fim de aumentar a vazão da represa e, consequentemente, ampliar a capacidade de geração de energia elétrica na UHE Henry Borden. Este processo foi viabilizado graças à reversão do curso do rio Pinheiros, através da construção das Usinas Elevatórias de Pedreira e Traição, ambas em seu leito. Tal operação que objetivava o aumento da produção de energia elétrica, também se mostrou útil para as ações de controle das enchentes e de afastamento dos efluentes industriais e do esgoto gerado pela cidade de São Paulo em crescimento.

O bombeamento das águas do rio Tietê para a represa Billings, no entanto, começou a mostrar suas graves consequências ambientais poucos anos depois. O crescimento da cidade de São Paulo e a falta de coleta e tratamento de esgotos levaram à intensificação da poluição do Tietê e seus afluentes que, por sua vez, passaram a comprometer a qualidade da água da represa Billings. Em 1981, devido à grande quantidade de esgotos que resultaram em sérios problemas de contaminação por cianobactérias, surgiu a necessidade de interceptação total do braço Rio Grande, através da construção da barragem Anchieta, para garantir o abastecimento de água da região do ABC paulista, iniciado em 1958. Atualmente, $4,5 \text{ m}^3 \text{ s}^{-1}$ de água são captadas do braço Rio Grande para atender a demanda por água potável.

Em 1992, a Secretaria Estadual do Meio Ambiente aprovou a Resolução restringindo o bombeamento das águas do rio Tietê a situações emergenciais, entre as quais ameaças de enchente na cidade de São Paulo e risco de colapso na produção de energia elétrica. O bombeamento das águas do Tietê para a Billings continua a ser utilizado até os dias de hoje como alternativa de controle de cheias em períodos de chuvas intensas. Estas operações, apesar de esporádicas, contribuem consideravelmente para o comprometimento da qualidade das águas da represa, dificultando a sua recuperação. Atualmente, a UHE Henry Borden tem capacidade de gerar cerca de 880 MW e está sendo

utilizada principalmente para suprir a falta de energia em horários de pico e situações emergenciais em São Paulo.

A população residente na bacia da Billings é de aproximadamente 865 mil habitantes, dos quais, 161 mil vivem em favelas (SMA, 2004). O uso e ocupação do solo da região ocorreram com a substituição da vegetação natural e da pequena atividade agrícola por um processo de intensa urbanização. Apesar do relevo acidentado, presença de áreas de várzeas e da existência da Lei de Proteção aos Mananciais, de 1976, a ocupação da área é intensa, com elevada densidade demográfica e necessidade de infra-estrutura e equipamentos urbanos diversos (CAPOBIANCO & WHATELY, 2002).

3.2. A represa Guarapiranga

3.2.1. Caracterização geral

A bacia hidrográfica da represa Guarapiranga é uma sub-bacia da bacia do Alto Tietê e liga-se a esta através do canal do rio Pinheiros. Está localizada na porção sudoeste da RMSP a 23°43'S e 46°32'W e a uma altitude de 742 m, ocupando um território de 639 km². Considerada um reservatório urbano, atualmente é o segundo maior manancial do sistema de abastecimento da RMSP, sendo responsável pelo abastecimento da parte do município de São Paulo, abrangendo mais de 3,7 milhões de habitantes (20% da população da RMSP), residentes nos bairros de Santo Amaro, Campo Limpo, Morumbi e Butantã. A produção média de 14 mil litros de água por segundo também é utilizada para abastecer uma parcela da população de Taboão da Serra (WHATELY & CUNHA, 2006). A bacia hidrográfica da represa Guarapiranga (Figura 4 e 6) abrange de forma parcial os municípios de Cotia, Embu, Juquitiba, São Lourenço da Serra e São Paulo, e a totalidade dos municípios de Embu-Guaçu e Itapequerica da Serra. O município de São Paulo contorna toda a margem direita e parte da margem esquerda, perfazendo 70% do perímetro da represa, o restante limita-se aos municípios de Embu-Guaçu (27%) e Itapequerica da Serra (3%).

A represa Guarapiranga apresenta morfologia dendrítica, estreita e alongada, o que acentua as influências do uso e ocupação do solo em sua bacia hidrográfica (BEYRUTH, 1996). Apresenta área de 33 km², profundidade média de 7 metros e volume aproximado de 194 milhões de m³ de água (CETESB, 2002a). Tem como principais tributários os rios Embu-Mirim, Embu-Guaçu e Parelheiros, além de diversos córregos e cursos d'água (Figura 6).

Figura 6. Bacia hidrográfica da Guarapiranga, municípios limítrofes e principais rios formadores. Fonte: Whately & Cunha (2006).

A bacia está inserida no Domínio da Mata Atlântica e a totalidade de sua área era, originalmente, recoberta por floresta umbrófila densa. Porém, o avanço da urbanização e de outras atividades antrópicas tem levado ao desmatamento acelerado, restando apenas 37% de vegetação remanescente de Mata Atlântica. Em 2003, 59% de seu território alterado por atividades humanas, divididas entre usos antrópicos e urbanos. Os usos antrópicos (42% da área total da bacia) incluem atividades agrícolas, campo antrópico, mineração, reflorestamento, solo exposto, indústrias e áreas de lazer. Os usos urbanos ocupam 17%, e compreendem áreas com ocupação urbana de alta e média densidade, ocupação dispersa e condomínios (WHATELY & CUNHA, 2006).

3.2.2. Histórico da represa Guarapiranga

A represa Guarapiranga foi construída entre 1906 e 1908, através da construção da barragem no rio Guarapiranga e seus afluentes, a fim de regularizar as vazões contribuintes e ampliar a produção de energia elétrica em Santana do Parnaíba (CETESB, 1991). O reservatório ficou com um perímetro de 85 km, inundando uma área de 34 km².

A partir de 1928, a represa tornou-se a principal fonte de água para abastecimento público de São Paulo, mediante fornecimento de 86,4 milhões de litros de água por dia (vazão média de mil litros por segundo). Além de servir ao abastecimento, a represa também tem como função o controle de enchentes, a geração de energia e a recreação (HELOU & SILVA, 1987).

A tendência de ocupação do entorno da represa foi marcada a partir da década de 1920 até 1950, por loteamentos residenciais, clubes, chpacaras e marinas, atraídos por ofertas de lazer e pela paisagem. A partir da década de 1970, núcleos clandestinos, precários e populosos, começaram a se instalar nas margens da represa. Desta forma, a bacia de drenagem do reservatório sofreu continuamente pressões intensas devido à expansão das áreas urbanas (BEYRUTH, 1996), sendo, em sua maioria, ocupada por população carente, residindo em favelas construídas em loteamentos clandestinos, sem infra-estrutura sanitária. Além disso, a extração mineral e mineração de areia, que ocasionam desmatamento, erosão e assoreamento nos tributários da represa, também contribuem para a redução da qualidade da água na Guarapiranga (COBRAPE, 1991).

Em 1960, o processo de eutrofização já era evidente, em função da descarga de esgoto urbano (ROCHA, 1976). No início da década de 1980, florações de algas e cianobactérias passaram a influenciar o processo de tratamento da água destinada ao abastecimento,

causando entupimento de filtros, demora na filtração, sabor e odor desagradáveis e comprometendo a qualidade do produto final. No final da década de 80, o custo de produtos químicos lançados na represa para controle das florações de algas era da ordem de US\$ 1.000.000,00 ao ano. O gasto aumentou para cerca de US\$ 8.400.000,00 (BEYRUTH, 1996), ou seja, 25% do custo total de produtos químicos aplicados para potabilizar a água.

Em agosto de 2000, a Companhia de Saneamento Básico do Estado de São Paulo (SABESP) iniciou a operação do sistema de adução de água bruta do braço do Taquacetuba para o reservatório Guarapiranga, com uma Licença de Operação para 2,0 m³/s. Este fato é outro agravante para qualidade da água na Guarapiranga devido ao longo histórico de eutrofização e florações de cianobactérias na represa Billings. A transposição entre as represas Billings e Guarapiranga (Sistema Billings-Guarapiranga) será descrita em maiores detalhes na próxima seção.

A população que vive ao redor da represa aumentou em quase 40% entre 1991 e 2000 e atualmente é estimada em aproximadamente 800 mil pessoas, sendo 67,5% desta população encontra-se no município de São Paulo (WHATELY & CUNHA, 2006). Dados do Censo 2000 demonstram que 30% ou 59 mil domicílios situados na bacia da Guarapiranga não possuem serviço de coleta de esgotos, despejando-os em valas, cursos d'água e demais afluentes da represa e mais de 22 mil domicílios (11% do total) não contam com serviço de abastecimento de água. O esgoto e a poluição difusa constituem as principais fontes de poluição da represa, com sérias conseqüências para o abastecimento público, e tem profunda ligação com a deficiência dos serviços de saneamento, em especial de rede de coleta, afastamento e tratamento do esgoto produzido pela população da bacia hidrográfica (WHATELY & CUNHA, 2006). Mesmo após a intervenção do Programa Guarapiranga (realizado durante a década de 1990) apenas parte do esgoto é coletado e, com exceção de parte dos domicílios dos municípios de São Paulo e Embu-Guaçu, os demais municípios da bacia não contam com qualquer tipo de tratamento de efluentes.

3.3. O Sistema Taquacetuba-Guarapiranga

O braço Taquacetuba é naturalmente ligado ao corpo central da represa Billings e possui aproximadamente 5 km de extensão entre os municípios de São Bernardo do Campo e São Paulo, com presença da Área de Proteção Ambiental Municipal Capivari/Monos e terras indígenas ao sul (WHATELY, 2003). A sub-bacia hidrográfica do Taquacetuba concentra atividades rurais (e.g. chácaras, agricultura, silvicultura, olarias, pesque-pagues) e uma

população aproximada de 33 mil habitantes, que se distribui entre o núcleo urbano de Colônia e duas aldeias indígenas. Inexistem favelas na região (SMA, 2004). O braço Taquacetuba possui dinâmica distinta do corpo central do Complexo Billings, para a comunidade fitoplancônica, devido a fatores meteorológicos desta bacia, que refletem em uma condição hidrodinâmica distinta entre o corpo central e o braço Taquacetuba (CETESB, 2006). Além disso, as densidades máximas registradas no braço do Taquacetuba são sensivelmente menores do que as encontradas no corpo central.

Em 1995, a SABESP iniciou o Programa Metropolitano da Água (PMA), cujo objetivo era regularizar o abastecimento de água na RMSP através de estudos, projetos e obras. O PMA visava, até o final de 1998, abastecer plena e continuamente com água potável a 100% da população da cidade de São Paulo, com investimentos previstos da ordem de US\$ 700 milhões (EDISON, sem data). Uma das obras prioritárias do PMA era a implantação do Sistema Produtor Taquacetuba-Guarapiranga que visava a transposição das águas do braço Taquacetuba na represa Billings para a Represa Guarapiranga a fim de aumentar o suprimento de água à população da cidade de São Paulo (Figura 7). Esta transposição de águas iniciou-se em 2000, com a concessão da Licença Ambiental de Operação pela Secretaria do Meio Ambiente, para a primeira fase do Sistema Produtor Taquacetuba-Guarapiranga, que correspondia à transferência de uma vazão máxima de $1,9 \text{ m}^3 \text{ s}^{-1}$ de água bruta. Em novembro de 2000, a SABESP solicitou, em caráter emergencial, o aumento de $2,0 \text{ m}^3 \text{ s}^{-1}$, totalizando $4,9 \text{ m}^3 \text{ s}^{-1}$ no bombeamento da Billings para a Guarapiranga.

Este empreendimento foi alvo de muitas críticas da comunidade científica e ambientalistas, devido aos potenciais impactos à biota aquática e à saúde pública associados à possibilidade de ressuspensão dos sedimentos contaminados da represa Billings e à introdução de organismos não existentes, como a cianobactéria tóxica, *Cylindrospermopsis raciborskii*, na represa Guarapiranga (CETESB, 1997).

Durante a transposição da água do sistema Taquacetuba para o reservatório de Guarapiranga, realizado pela SABESP, o caminho da água é o seguinte: a) a água é bombeada da elevatória flutuante para a chaminé de equilíbrio (braço Taquacetuba); b) da chaminé de equilíbrio a água é bombeada pela elevatória em terra até o “Stand Pipe” (13,9 km de adutora); c) do “Stand Pipe” a água vai por gravidade (80 m de diferença de cota) por 8,3 km até o sistema de dissipação de energia, que tem a função de diminuir energia da água; d) a água segue por canal aberto, passando pela várzea do ribeirão Parelheiros (93 ha); e) chegando a represa do Guarapiranga. Essa transposição é efetuada para regularização de nível da represa do Guarapiranga, e entra em funcionamento em

períodos críticos. A várzea do ribeirão Parelheiros apresenta importante papel na melhoria da qualidade da água que afluí à represa de Guarapiranga, amortecendo aporte de substâncias poluentes, bem como na redução de cianobactérias e microcistina (ANDRADE, 2005).

Figura 7. Caminho da água do Sistema Taquacetuba-Guarapiranga.

Capítulo 2. Phytoplankton community and water quality in two linked tropical reservoirs: a functional groups approach

Abstract

In August of 2000, the São Paulo State Basic Sanitation Company (SABESP) began to transfer raw water from Taquacetuba branch (Billings reservoir) to Guarapiranga reservoir (Parelheiros branch), in order to increase the water volume of Guarapiranga reservoir during the dry season. The objective of this study was to analyze the impacts of Billings-Guarapiranga water transfer system in Guarapiranga reservoir's water quality based on limnological variables and the phytoplankton functional groups. Three field works were performed in Billings (six superficial samples through Taquacetuba branch's longitudinal axis) and Guarapiranga (seven superficial samples through Parelheiros branch's longitudinal axis) reservoir and in Parelheiros stream that links both reservoirs (one sampling station in triplicate). In each sampling station we analyzed physical, chemical and biological variables. The phytoplanktonic community was also analyzed. Multiple discriminant analyses were used to test for differences among the three sampling locations (Billings reservoir, Guarapiranga reservoir and Parelheiros stream) based on physical, chemical and biological data. To explore the relationship between the limnological variables and the phytoplankton community in the sampling stations, multivariate analyses (CCA) were performed. Both reservoirs and Parelheiros stream exhibited differences in water quality and EC, TN and TS were the variables responsible for the groups' differences. Spatial heterogeneity was observed in Guarapiranga reservoir. The occurrence of nuisance phytoplankton species in Guarapiranga reservoir, previously absent (before the beginning of the transfer system), support the negative influence of Billings waters in Guarapiranga. In our study, we found the invasive dinoflagellate *Ceratium furcoides* in Guarapiranga reservoir, probably transferred from Billings. Our findings provide support to the argument that Guarapiranga reservoir water quality is being influenced by the water quality that enters in its tributaries, specifically, Parelheiros stream.

1. Introduction

The increasing demand by the growing populations and the irregular distribution of both human habitation and aquatic resources create a pressure to increase available water storage in many parts of the world (STRASKRABA & TUNDISI, 2000). For this reason, reservoirs have been built intensively in regions where natural water reserves are inadequate, but were also built in regions with an extreme abundance of water, like Brazil (STRASKRABA & TUNDISI, 2000). Built up primarily for hydroelectricity production, these artificial ecosystems now serve purposes such as water storage for public use, fisheries and aquaculture, recreation, tourism, irrigation and sewage receptor (TUNDISI & MATSUMURA-TUNDISI, 2003). The presence of reservoirs in Brazilian river basins altered profoundly not only their limnology and ecology, but also provided great economic and social changes, enhancing the regional development (TUNDISI, 1993). Thus, human activities around the reservoir are intensified, generating multiple impacts among which are eutrophication, a serious problem with various ecological, economic, and social consequences (TUNDISI & MATSUMURA-TUNDISI, 2003).

Some reservoirs from arid and semiarid regions require water transfer systems to fully satisfy the local human population demand. Some transfer systems possesses high water transfer capacity that can affect the water quality of the hydrographic basin from which the water is being captured and also to the basin to which the water is being transferred (STRASKRABA & TUNDISI, 2000). In the Brazilian semiarid region, the water transfer system in São Francisco river is one of the most important and controversial example (MIN, 2004). Another example is the Billings-Guarapiranga water transfer system, which is an important and strategic system to prevent failures in the water supply of the biggest South American city, São Paulo (CETESB, 1997).

In this context, the water quality in reservoirs and in the whole hydrographic basin affects the aquatic communities, resulting in the development of specific microbial, phytoplankton, zooplankton, fish and macrophytes assemblages in each particular combination of environmental characteristics (URABE, 1989; BRANCO *et al.*, 2007; CAPUTO *et al.*, 2008; THOMAZ *et al.*, 2009). Studies have suggested that phytoplankton assemblages change their composition and biomass in response to the environmental changes, like eutrophication (REYNOLDS *et al.*, 2002; NASELLI-FLORES *et al.*, 2003; PADISÁK *et al.*, 2003). The objective of this study was to investigate the possible impacts of Billings-Guarapiranga water transfer system in Guarapiranga reservoir's water quality based on limnological variables and the phytoplankton community in a period no transfer.

2. Methodology

Study site

This study was carried out in two linked Brazilian reservoirs in São Paulo metropolitan area: Billings (23°47'S/46°40'W) and Guarapiranga (23°43'S/46°32'W) (Figure 1). Billings reservoir was built in 1927 for electric power generation and it has 560 km² of watershed area and storage capacity of 1.2 billion m³ of water. Today, Billings' uses include electric power generation, leisure, fishery, navigation, flow control, domestic and industrial wastewater reception, and water supply (WHATELY, 2003). Billings' limnological characteristics changed substantially since 1940, when part of the polluted water from the Tietê and Pinheiros rivers (São Paulo city) started to flow into the Billings reservoir, aiming to increase the water flow and consequently, the electric power generation. This operation, along with the disorganized human occupation of the watershed, contributed to increase the eutrophication and consequently, the cyanobacterial blooms (SOUZA *et al.*, 1998). Due to its peculiar shape, Billings reservoir is divided into eight units called branches. Taquacetuba branch has a particular use. In August of 2000, the Companhia de Saneamento Básico do Estado de São (São Paulo State Basic Sanitation Company - SABESP) began to transfer raw water from Billings reservoir (Taquacetuba branch) to Guarapiranga reservoir (Parelheiros branch), in order to increase the water volume of the last during the dry season (CETESB, 2002b). This water transfer started with a license of 2.0 m³ s⁻¹; currently, it operates at a volume of 3.0 to 4.0 m³ s⁻¹, whenever necessary (CETESB, 2001). The water from Billings reservoir flows through a 13.9 km subterraneous pipe, reaching an area of 0.93 km² of wetland with great anthropic influence (ANDRADE, 2005). This wetland flows into Parelheiros stream, reaching Guarapiranga reservoir through Parelheiros branch. The water from Billings reservoir contribute with 29% of the total water produced in Guarapiranga reservoir (ANDRADE, 2005), which is mainly used to supply the southeastern part of São Paulo city at a rate of 1.2 billion L day⁻¹ (WHATELY & CUNHA, 2006). Guarapiranga reservoir was constructed in 1908 for hydroelectrical purposes; it has 36 km² of watershed area and storing capacity of 194 million m³ of water. Today, Guarapiranga's uses are water supply, flood control, electric power generation and recreation (HELOU & SILVA, 1987). According to the Companhia de Tecnologia de Saneamento Ambiental (São Paulo State Environmental Agency - CETESB), the current main problem of both reservoirs is the excess of organic matter from clandestine domestic sewage input (CETESB, 2009). Consequently, phytoplankton blooms, especially cyanobacteria, are frequent in both reservoirs (CARVALHO *et al.*, 2007; MOSCHINI-CARLOS *et al.*, 2009).

Sampling and data analyses

Samplings were performed in September 14, 16 and 18th, 2009 in Billings (six superficial sampling stations through Taquacetuba branch's longitudinal axis) and Guarapiranga (seven superficial sampling stations through Parelheiros branch's longitudinal axis) reservoirs, simultaneously. Additionally, Parelheiros stream that links both reservoirs was sampled in triplicate in only one station (Figure 1).

Figure 1. Location of São Paulo State and São Paulo metropolitan area. In detail, Guarapiranga, Billings reservoirs and Parelheiros stream and the respective sampling stations in Taquacetuba branch (Billings reservoir: B1-B6), Parelheiros stream (P1) and Parelheiros branch (Guarapiranga reservoir: G1-G7). Other important sites are indicated in the map.

Water temperature (T , °C), pH, electric conductivity (EC , $\mu S\ cm^{-1}$) and dissolved oxygen (DO , $mg\ l^{-1}$) were measured in the surface of each sampling station using standard electrodes (YSI 556). Additionally, vertical profiles of T , pH, EC and DO were taken in two sampling stations of each reservoir: one station close to the water intake/income area and another further from it (B1 and B4 in Billings reservoir and G1 and G3 in Guarapiranga

reservoir). In each sampling station, maximum depth ($Z_{\max}$) and Secchi disk depth (Z_{sd}) were measure. Due to the difficult access and lotic characteristics of Parelheiros stream, there are no data for $Z_{\max}$ and Z_{ds} . Superficial water was gathered to analyze the following variables in the laboratory: ammonium, nitrite and nitrate summed as total inorganic nitrogen (TIN, mg l^{-1}) (MACKERETH *et al.*, 1978), silicate (SRSi, mg l^{-1}) and soluble reactive phosphorus (SRP, $\mu\text{g l}^{-1}$) (STRICKLAND & PARSONS, 1960), total nitrogen (TN, mg l^{-1}) and total phosphorus (TP, $\mu\text{g l}^{-1}$) (VALDERRAMA, 1981), total solids (TS, mg l^{-1}) and total, organic and inorganic suspended material (TSM, OSM and ISM, respectively, mg l^{-1}) (WETZEL & LIKENS, 1991) and chlorophyll *a* (Chla, $\mu\text{g l}^{-1}$) corrected for phaeophytin using 90% acetone extraction (LORENZEN, 1967; WETZEL & LIKENS, 1991). Trophic State Index (TSI) was calculated based on TP and Chla according to Lamparelli (2004) for reservoirs.

Superficial water samples for phytoplankton community analysis were preserved with acetic lugol 4%. Phytoplankton species were identified based on specific bibliography and according to Van den Hoek (1997), except for Cyanobacteria (KOMÁREK & ANAGNOSTIDIS, 1999, 2005) and Bacillariophyceae (ROUND *et al.*, 1992) in a Carl Zeiss ScopeA1 microscope. Phytoplankton cells were counted using the settling technique (UTERMÖHL, 1958) in 2 ml settling chambers in a Carl Zeiss Axiovert40C inverted microscope. Sedimentation time followed Lund *et al.* (1958). A minimum of 400 individuals (cells, colonies or filaments) was counted in each sample giving a counting accuracy, expressed in terms of 95% confidence limits, of $< 10\%$ for the whole phytoplankton population (LUND *et al.*, 1958). Biovolume was obtained by geometric approximation, multiplying each species' density by the mean volume of its cells considering, whenever possible, the mean dimension of 30 individual specimens of each species (HILLEBRAND *et al.*, 1999). Algal biomass was estimated assuming a specific gravity for algal cells of 1 mg mm^3 . The phytoplankton species that contributed with more than 5% of the total biomass of the sample were considered a descriptors species of the community and included in the data analysis. Species that contributed with more than 50% of the total biomass of the sample were considered dominant (LOBO *et al.*, 2002). Phytoplankton descriptor species were classified according to Reynolds *et al.* (2002) functional groups.

Multiple discriminant analysis was performed to test for differences among the three sampling locations (Billings reservoir, Guarapiranga reservoir and Parelheiros stream) based on physical, chemical and biological superficial data (LEGENDRE & LEGENDRE, 1998). Variables included were: T, DO, pH, EC, TN, TP, TIN, SRP, SRSi, TS, TSM, OSM, ISM and Chla. To certify the independency of the variables, a tolerance level of 0.01 was

established in this analysis. All variables were transformed by range $[(x-x_{\min})/(x_{\max}-x_{\min})]$ in order to keep the same amplitude to all variables. Multiple discriminant analysis was performed with STATISTICA (version 7.0) Software.

To explore the relationship between the limnological variables and the phytoplankton community in the samplings stations, multivariate analyses were performed. Initially, to check the unimodal response of the phytoplankton data, a Detrended Correspondence Analyses (DCA) was carried out, resulting in long gradient length (4.06) proving true the unimodal response (LEPS & SMILAUER, 2003) and confirming the suitability of further canonical correspondence analyses (CCA). A first CCA was performed with all sampling stations from all sampling days to explore the relationship between the selected limnological variables and the phytoplankton community. A second CCA was performed excluding Parelheiros stream's sampling stations, due to its riverine characteristics and to better visualize the differences better Billings and Guarapiranga reservoirs. The variables included in all CCAs were selected by the forward procedure by a Monte Carlo test using simulations with 999 unrestricted permutations. Environmental variables data were standardized by $[(x-x_{\min})/(x_{\max}-x_{\min})]$ in order to keep the same amplitude to all variables and phytoplankton data were transformed by $[\log_{10}(x+1)]$ in order to reduce data amplitude. DCA and CCAs were performed with Canonical Community Ordination (CANOCO) for Windows (version 4.5) software and CCA plots were performed with CanoDraw for Windows (version 4.0) software.

3. Results

Billings and Guarapiranga reservoirs displayed distinct vertical water profiles (Figure 2 and Figure 3). Sampling stations near the water intake/income area in both reservoirs presented lower $Z_{\max}$ compared to the rest of the sampling stations within the same reservoir: 6.0 m in Billings (Figure 2a) 2.5 m in Guarapiranga (Figure 3a). In Billings, very similar vertical profiles were found in both sampling stations (Figure 2). Thermocline was observed in the first sampling day in B1 (between 2 and 2.5 m depth; Figure 2a) and in B4 (between 5 and 6 m depth; Figure 2d). In the other sampling days, a gradual vertical decrease in temperature was observed in both sampling stations (Figure 2b, Figure 2c, Figure 2e and Figure 2f). The other variables (DO, pH and EC) were homogenous along the water column in Billings reservoir (Figure 2). In Guarapiranga, the vertical profiles evidenced a dynamic environment in G1 and G3, especially concerning temperature in G3 and DO concentrations in G1 (Figure 3). No thermocline was observed in G1 (Figure 3a,

Figure 3b and Figure 3c), while in G3 a clear thermocline was observed in the first sampling day (Figure 3d). In the following days, only a gradual vertical decrease in temperature could be observed in G3 (Figure 3e and Figure 3f). DO concentrations were very low in G1, especially in the second sampling day (Figure 3b). Comparing changes in the vertical profiles throughout the sampling stations in Billings and Guarapiranga, it is possible to infer that Guarapiranga reservoir, exhibits lower stability, especially in G1.

According to the multiple discriminant analysis, both reservoirs and Parelheiros stream exhibited differences in water quality (Wilks' lambda = 0.00044, $p < 0.0000$). This analysis pointed out EC ($p < 0.0000$), TN ($p < 0.0000$) and TS ($p = 0.044$) as the variables responsible for the groups' differences.

Comparing both reservoirs, Billings displayed higher values for pH (Figure 4a), TN (Figure 4b), TIN (Figure 4b), EC (Figure 4c), TS (Figure 4c), TSM (Figure 4d) and OSM (Figure 4d). The remaining variables did not differ greatly between the two reservoirs. Parelheiros stream displayed very different chemical content, especially for TN (Figure 4b), SRSi (Figure 4b), TP (Figure 4c), SRP (Figure 4d) and TSM (Figure 4d). In Billings, only Chla showed a very clear horizontal pattern through the sampling stations, increasing towards the water intake area (B1) (Figure 4d). Guarapiranga showed a marked horizontal pattern: DO (Figure 4a) and Chla (Figure 4d) concentrations decreasing towards the water intake area and TP (Figure 4c) and SRP (Figure 4d) increasing in the same direction.

High trophic levels were observed in all sampling stations (Figure 5). Billings and Guarapiranga reservoirs were meso/eutrophic, while Parelheiros was supereutrophic.

Figure 2. Vertical profiles of temperature (T), dissolved oxygen (DO), pH, electric conductivity (EC) and Secchi disk depth (SD) of the water in sampling station B1 in day 1 (A), day 2 (B) and day 3 (C) and in sampling station B4 in day 1 (D), day 2 (E) and day 3 (F) in Billings reservoir.

Figure 3. Vertical profiles of temperature (T), dissolved oxygen (DO), pH, electric conductivity (EC) and Secchi disk depth (DS) of the water in sampling station G1 in day 1 (A), day 2 (B) and day 3 (C) and in sampling station G3 in day 1 (D), day 2 (E) and day 3 (F) in Guarapiranga reservoir.

Figure 4. Mean and standard deviation values in the three sampling days for A) temperature (T, °C), dissolved oxygen (DO, mg l⁻¹) and pH; B) total nitrogen (TN, mg l⁻¹), total inorganic nitrogen (TIN, mg l⁻¹) and silicate (SRSi, mg l⁻¹); C) electric conductivity (EC, $\mu\text{S cm}^{-1}$), total phosphorous (TP, $\mu\text{g l}^{-1}$) and total solids (TS, mg l⁻¹); D) phosphate (SRP, $\mu\text{g l}^{-1}$), chlorophyll-a (Chl-a, $\mu\text{g l}^{-1}$); total suspended material (TSM, mg l⁻¹) and organic suspended material proportion (OSM, %) in the samplings stations of Billings (B1 to B6) and Guarapiranga (G1 to G7) reservoirs and Parelheiros stream (P1).

Figure 5. Trophic State Index (TSI) according to Lamparelli (2004) based on the average of chlorophyll-a and total phosphorus for the three sampling days in all sampling stations from Billings and Guarapiranga reservoirs and Parelheiros stream. Each environment is represented by a box-whisker plot. The dot is the mean TSI for that environment. Boxes correspond to the standard error and the whiskers to the standard deviation. Letters a and b are the results of Kruskal-Wallis median test. Meso = mesotrophic; Eu = eutrophic; Supereu = supereutrophic.

From 127 phytoplankton species identified in all sampling stations, 30 were considered descriptors (Table 1). In Billing reservoir, 11 out of 58 phytoplankton species identified were considered descriptors. *Ceratium furcoides* was the most abundant species in Billings reservoir, comprising between 27.3% (1.33 mg l⁻¹; G4) and 44.2% (3.84 mg l⁻¹; G1) of the total phytoplankton community. In Guarapiranga reservoir, 21 out of 83 species were considered descriptors. In G1, *Sphaerocavum brasiliensis* was dominant, comprising 52.7% (7.15 mg l⁻¹) of the total phytoplankton biomass, and *Peridinium gatunense* was abundant, with 31.2% of the phytoplankton biomass (4.24 mg l⁻¹). *Peridinopsis unningtonii* was dominant in G2, comprising 70.1% (13.0 mg l⁻¹) of the total phytoplankton biomass. In Parelheiros stream, nine out of 66 species were considered descriptors. *Botryococcus braunii* was dominant, comprising 55.0% (0.6 mg l⁻¹) of the total phytoplankton biomass. *Synechocystis aquatilis* was abundant, comprising 28.0% (0.60 mg l⁻¹) of the total phytoplankton biomass.

Table 1. Biomass (mg l⁻¹; mean values for the three sampling days) and code of the phytoplankton descriptor species and the respective functional group (FG) (Reynolds *et al.* 2002) in the sampling stations of Billings and Guarapiranga reservoirs and Parelheiros stream.

Location <i>Species</i>	Code	FG	Biomass (mg l ⁻¹)						
			Sampling station						
			1	2	3	4	5	6	7
Billings									
<i>Botryococcus braunii</i> Kützing	Bobr	F	-	0.18	0.52	0.12	0.16	-	-
<i>Eremosphaera</i> sp.	Erem	F	0.69	0.89	-	0.06	0.10	0.13	-
<i>Synechocystis aquatilis</i> Sauvageau	Syaq	Lo	-	-	0.22	0.11	0.25	0.07	-
<i>Ceratium furcoides</i> (Levander) Langhans	Cefu	Lo	3.84	2.37	2.56	1.33	2.65	2.46	-
<i>Gymnodinium</i> sp.	Gymn	Lo	0.69	0.36	0.14	0.36	0.07	0.07	-
<i>Peridinium gatunense</i> Nygaard	Pega	Lo	0.43	0.20	0.81	0.61	0.61	0.81	-
<i>Peridinium umbonatum</i> F.Stein	Peum	Lo	-	0.12	-	0.54	0.12	0.36	-
<i>Staurastrum anatinum</i> Cooke & Wills var. <i>anatinum</i> f. <i>anatinum</i>	Stan	N	-	-	-	0.10	-	-	-
<i>Planktothrix agardhii</i> (Gomont) Anagnostidis & Komárek	Plag	S1	0.36	-	-	0.12	0.23	0.24	-
<i>Cryptomonas brasiliensis</i> A.Castro, C.Bicudo & D.Bicudo	Crbr	X2	1.44	0.27	0.65	0.57	0.38	0.19	-
<i>Cryptomonas ovata</i> Ehrenberg	Crov	Y	0.88	0.92	0.25	0.11	-	-	-
Guarapiranga									
<i>Nitzschia</i> sp.	Ntzs	C	-	-	-	0.16	-	-	-
<i>Urosolenia eriensis</i> (H.L.Smith) Round & R.M.Crawford	Urer	A/C	-	-	-	-	1.90	-	-
<i>Botryococcus braunii</i> Kützing	Bobr	F	-	-	-	-	-	-	0.60
<i>Anabaena spiroides</i> Klebahn	Ansp	H1	-	-	-	0.66	0.35	0.66	2.11
<i>Coelastrum microporum</i> Nägeli	Comi	J	-	-	0.10	0.07	-	-	-
<i>Franceia droescheri</i> (Lemmermann) G.S.Smith	Frdr	J	-	1.02	0.20	-	0.41	0.20	0.61
<i>Tetrastrum homoicanthum</i> (Huber-Pestalozzi) Comas	Teho	J	-	-	-	0.06	-	-	-
<i>Ceratium furcoides</i> (Levander) Langhans	Cefu	Lo	-	-	0.85	0.19	0.19	-	-
<i>Synechocystis aquatilis</i> Sauvageau	Syaq	Lo	-	-	0.32	0.11	0.14	0.88	-
<i>Coelomoron tropicale</i> P.A.C.Senna, A.C.Peres & J.Komárek	Cotr	Lo	-	0.23	-	-	-	-	-
<i>Peridiniopsis cunningtonii</i> (Lemmermann) Popovský & Pfister	Dino	Lo	0.11	13.0	0.11	0.32	0.21	0.11	0.11
<i>Peridinium gatunense</i> Nygaard	Pega	Lo	4.24	0.20	0.40	0.20	0.20	0.40	2.63

Cont. Table 1

Location <i>Species</i>	Code	FG	Biomass (mg l ⁻¹)						
			Sampling station						
			1	2	3	4	5	6	7
<i>Sphaerocavum brasiliensis</i> M.T.P. Azevedo & C.L. Sant'Anna	Spbr	M	7.15	1.59	1.42	0.43	0.11	0.04	0.12
<i>Staurostrum anatinum</i> Cooke & Wills var. <i>anatinum</i> f. <i>anatinum</i>	Stan	N	-	-	-	-	0.21	-	-
<i>Euglena agilis</i> H.J.Carter	Euag	W1	-	-	-	-	0.17	0.17	-
<i>Trachelomonas similis</i> var. <i>spinosa</i> Huber-Pestalozzi	Trsi	W2	0.14	0.18	0.14	0.11	0.07	0.07	0.14
<i>Trachelomonas volvocinopsis</i> Svirenko	Trvo	W2	-	-	-	-	-	0.04	-
<i>Synura</i> sp.	Synu	Ws	-	-	-	-	1.10	-	0.33
<i>Chlamydomonas</i> sp.	Chla	X2	0.09	-	-	0.23	0.07	0.04	-
<i>Cryptomonas brasiliensis</i> A.Castro, C.Bicudo & D.Bicudo	Crbr	X2	0.32	0.30	0.38	0.35	0.27	0.32	0.16
<i>Cryptomonas ovata</i> Ehrenberg	Crov	Y	0.20	0.16	-	0.06	0.15	0.37	0.38
Parelheiros									
<i>Aulacoseira ambigua</i> (Grunow) Simonsen	Auam	C	0.004						
Unidentified pennate diatom	Diat	C	0.01						
<i>Botryococcus braunii</i> Kützing	BoBr	F	0.60						
<i>Synechocystis aquatilis</i> Sauvageau	Cyco	Lo	0.30						
<i>Peridinium gatunense</i> Nygaard	Pega	Lo	0.01						
<i>Planktothrix agardhii</i> (Gomont) Anagnostidis & Komárek	Plag	S1	0.01						
<i>Cylindrospermopsis raciborskii</i> (Woloszynska) Seenayya & Subba Raju	Cyra	Sn	0.01						
<i>Euglena acus</i> (O.F.Müller) Ehrenberg	Euac	W1	0.01						
<i>Trachelomonas volvocinopsis</i> Svirenko	Trvo	W2	0.01						

In the CCA performed including all sampling stations, among all environmental variables initially included in the analysis (T, DO, pH, EC, TN, TP, TIN, SRP, SRSi, TS, TSM, OSM, ISM and Chla), eight were retained by the forward selection procedure: DO, EC, TP, SRP, TN, TIN and TSM. The CCA confirmed the differences among the two reservoirs and stream analyzed, segregating the sampling stations of each location (Figure 6a). The first two canonical axes explained 52.6% of the total variance of the phytoplankton community along the environmental gradient (Figure 6a). The first canonical axis can be interpreted as a gradient of EC values, while the second CCA axis can be interpreted as a gradient in nutrients, DO and Chla concentrations (Table 2). The CCA ordination evidenced the eutrophication process in Parelheiros stream with higher nitrogen and phosphorous content while Guarapiranga and Billings reservoirs displayed lower nutrient content. Billings reservoir exhibited higher EC, DO and Chla concentrations compared to the others sampling stations. It is also important to highlight that sampling stations from Guarapiranga reservoir were more spread along the second axis of the CCA ordination than the sampling stations from Billings reservoir and Parelheiros stream, indicating a higher degree of spatial heterogeneity in Guarapiranga reservoir. This ordination pattern is characterized by a modification in the composition of the dominant phytoplankton taxa along the environmental features.

In order to better visualize the differences among sampling stations within Guarapiranga and Billings reservoirs, a second CCA was performed excluding all sampling stations from Parelheiros stream. Among all environmental variables initially included in the analysis (T, DO, pH, EC, TN, TP, TIN, SRP, SRSi, TS, TSM, OSM, ISM, Chla, $Z_{\max}$ and Z_{ds}), seven were retained by the forward selection procedure: DO, EC, SRP, TN, TIN, TSM and $Z_{\max}$. The CCA confirmed the spatial heterogeneity within reservoirs, segregating the sampling stations of each reservoir along the second axis (Figure 6b). The first two canonical axes explained 57.8% of the total variance of the phytoplankton community along the environmental gradient (Figure 6b). The first canonical axis can be interpreted as a gradient of EC, DO and depth; while the second axis can be interpreted as a gradient of SRP and TN (Table 3). The CCA ordination clearly segregated Billings and Guarapiranga reservoirs' sampling stations in the first axis based mainly on EC values (positively correlated with Billings's sampling stations). Guarapiranga reservoir's sampling stations were spread along the second axis, indicating horizontal heterogeneity driven by nutrients, depth and DO. In general, sampling stations near the water intake (G1, G2 and G3) exhibited lower $Z_{\max}$, higher concentration of SRP, TN and DO. Moreover, they presented high abundance of some particular phytoplankton species, such as *Sphaerocavun brasiliensis* and *Peridinium gatunense* (Table 1). Sampling station G1 (especially in the second sampling day) was the

A

Plot A shows the relationship between environmental variables (red arrows) and species distribution (black dots). The x-axis ranges from -0.8 to 0.8, and the y-axis ranges from -1.0 to 1.0. Environmental variables include EC (Electrical Conductivity), DO (Dissolved Oxygen), Chla (Chlorophyll a), NIT (Nitrate), TN (Total Nitrogen), TP (Total Phosphorus), SRP (Soluble Reactive Phosphorus), TSM (Total Suspended Matter), and Frdr (Fragile Fragments). Species names are labeled near their respective points.

B

Plot B shows the relationship between environmental variables (red arrows) and species distribution (black dots). The x-axis ranges from -0.6 to 1.0, and the y-axis ranges from -1.0 to 1.0. Environmental variables include SRP (Soluble Reactive Phosphorus), TN (Total Nitrogen), EC (Electrical Conductivity), DO (Dissolved Oxygen), Zmax (Maximum Depth of Zero Light), NIT (Nitrate), TP (Total Phosphorus), TSM (Total Suspended Matter), and Frdr (Fragile Fragments). Species names are labeled near their respective points.

Table 2. Biplot scores of the selected environmental variables (DO=dissolved oxygen, EC=electric conductivity, SRP=phosphate, TIN=total inorganic nitrogen, TN=total nitrogen, TP=total phosphorous, TSM=total suspended material and Chla=chlorophyll-a) applied in the canonical correspondence analysis (CCA) with the phytoplankton descriptor species in Billings and Guarapiranga reservoirs and Parelheiros stream's sampling stations during the three sampling days.

Variable	1st axis	2nd axis
DO	-0.4758	-0.7222
EC	-0.7513	0.4109
SRP	0.4678	0.6707
TIN	0.2692	0.7152
TN	0.2708	0.7053
TP	0.4532	0.6513
TSM	0.2282	0.2691
Chla	-0.1899	-0.7930

Table 3. Biplot scores of the selected environmental variables (DO=dissolved oxygen, EC=electric conductivity, SRP=phosphate, TIN=total inorganic nitrogen, TN=total nitrogen, TSM=total suspended material and Z_{max}= maximum depth) applied in the canonical correspondence analysis (CCA) with the phytoplankton descriptor species in Billings and Guarapiranga reservoirs' sampling stations during the three sampling days.

Variable	1st axis	2nd axis
DO	0.8392	-0.3994
EC	0.8667	0.1884
SRP	-0.5474	0.7626
TIN	0.2334	0.0275
TN	0.3676	0.7860
TSM	0.3955	-0.1386
Z _{max}	0.5909	-0.5448

4. Discussion

Usually, the water transfer from Billings to Guarapiranga reservoir is activated during the dry season, as an emergency action to increase the water level in Guarapiranga and, consequently, supply part of São Paulo city population with potable water. Thus, the water transfer system does not have an operation schedule and the decision of turning it on and off is based on weather conditions. Since the beginning of the transfer operation (2000), it had happened very frequently, some years, non-stopping even during the rainy season (personal communication from SABESP employees). However, in 2009, during the sampling period, the water transfer was off due to an abnormal high precipitation during

the previous months, especially in July, August and September (Figure 7). High precipitation in 2009 led to an abnormal high quota in Guarapiranga reservoir in 2009 of almost 1.5 m higher than the mean quota from the previous years (Figure 8). Therefore, our results will not point out the direct influence of Billings' water on Guarapiranga reservoir. Nevertheless, important conclusions could be draw comparing both environments.

Figure 7. Average accumulated precipitation (1996-2008) and accumulated precipitation in 2009 in São Paulo Metropolitan Region, where Billings and Guarapiranga reservoirs are inserted. Source: CIIAGRO/Instituto Agrônômico.

Figure 8. Guarapiranga reservoir's quota from January 2006 to July 2010. Dotted line represents mean reservoir's quota until April 2009. Grey line represent mean reservoir quota from May 2009 on (during abnormal high precipitation). Source: EMAE.

Previous studies showed that Billings reservoir is in advanced stage of eutrophication (PEREIRA, 1987; CAPOBIANCO & WHATELY, 2002; MARIANI *et al.*, 2006; NISHIMURA *et al.*, 2008) that led to frequent blooms of cyanobacteria such as *Cylindrospermopsis raciborskii* (SOUZA *et al.*, 1998; YUNES *et al.*, 2003), *Planktothrix agardhii* (GEMELGO *et al.*, 2009), *Anabaena spiroides* (YUNES *et al.*, 2003), *Microcystis panniformis* (MOSCHINI-CARLOS *et al.*, 2009) and *M. aeruginosa* (BEYRUTH & PEREIRA, 2002). Because of this, during the implementation phase, the water transfer system from Billings to Guarapiranga reservoir was target of criticism from scientists and environmental activists worried about the possible consequences to Guarapiranga reservoir's water quality. Annual reports from the São Paulo State Environmental Agency (CETESB) affirm that the water quality in Guarapiranga reservoir have deteriorated after the beginning of the water transfer from Billings reservoir and that planktonic organisms are being transferred (CETESB, 2002a, 2006). Moreover, higher densities of cyanobacteria frequently found forming blooms in Billings reservoir, such as *Cylindrospermopsis raciborskii*, *Planktothrix agardhii*; *Pseudoanabaena* sp. and *Microcystis* spp., were observed in Guarapiranga reservoir after the beginning of the water transfer (CETESB, 2008). All these data reveal the influence of Billings' water in Guarapiranga reservoir's water quality. Our results showed distinct limnological features between Billings and Guarapiranga reservoirs in the water intake/income regions, probably due to the halt of the water transfer between the two reservoirs, leading to different water quality characteristics and phytoplankton communities.

Although recent studies showed a slight improvement in the water quality of the central body and the Taquacetuba branch in Billings reservoir since 2009, possibly associated with the Pinheiros river flotation pilot project (CETESB, 2008; WENGRAT & BICUDO, 2011), Billings reservoir is still under advanced eutrophication process, as our data showed. However, contradicting what was expected facing the historical cyanobacterial blooms trend in the reservoir, no cyanobacterial bloom was found. Instead, high biomass of the dinoflagellate *Ceratium furcoides* was observed. *C. furcoides* has been reported as an invasive species in Brazilian eutrophic reservoirs since 2007, when it was first detected in Furnas reservoir (SANTOS-WISNIEWSKI *et al.*, 2007). After that, *C. furcoides* was reported in semi-arid rivers (OLIVEIRA *et al.*, 2011) and in São Paulo state reservoirs: Billings (CETESB, 2009; MATSUMURA-TUNDISI *et al.*, 2010; CETESB, 2011), Guarapiranga (CETESB, 2011) and Itapararanga (CETESB, 2011; TANIWAKI, 2012). Matsumura-Tundisi *et al.* (2010) reported a *C. furcoides* bloom (535,209 - 21,455,060 org l⁻¹) in Billings reservoir and attributed its occurrence to a cold front, that caused turbulence and mixing of the water column suspending cysts and nutrients from the sediment and promoting

conditions for the development of *C. furcoides*. *Ceratium* is a natural inhabitant of temperate lakes and its dynamics is well known: it appears during the period of transition between mixing and stratification, reaching their maximum during the thermal stratification and nutrient impoverishment (POLLINGHER, 1988). However, unlike in temperate lakes, is most likely to find *Ceratium* in eutrophic environments in the tropics and subtropics (WHITTINGTON *et al.*, 2000; VAN GINKEL *et al.*, 2001; MACDONAGH *et al.*, 2005; HART, 2007; HART & WRAGG, 2009; LEONE *et al.*, 2009; SANTOS *et al.*, 2009). The occurrence of *Ceratium* species in tropical zones is recent and their dynamics is still not clear. The colonization and dispersion of *C. furcoides* populations in tropical environments should be closely monitored in order to better understand their dynamics and the possible negative impacts of this invasive species. At higher trophic states *Ceratium* species can occur together with large masses of *Microcystis* (PADISÁK *et al.*, 2009), consolidating the functional group L_M. However, *Microcystis* was not found in Billings reservoir as previous studies did (ANJOS *et al.*, 2006; MOSCHINI-CARLOS *et al.*, 2009). Hence, the occurrence of dinoflagellates (*C. furcoides* and *Peridiniopsis cunningtonii*) in the absence with species from codon M, comprised codon L₀ (Padisák *et al.*, 2009). As dinoflagellate population immobilize large amounts of nutrients and slow down the overall activity of the ecosystem (SERRUYA *et al.*, 1980), competition for nutrients can be one of the causes of the absence of cyanobacteria blooms. *C. furcoides* was also present in Guarapiranga reservoir, however, in much lower biomass, indicating that the colonization is still in early stages (probably coming from Billings reservoir) that or Guarapiranga reservoir's environment is not suitable for this species development. For example, the vertical profiles indicate that Guarapiranga's environment is more unstable than Billings'.

In Guarapiranga reservoir, the deterioration of the water quality and the eutrophication process began in the 70's due to the increase of irregular human settlements in the reservoir's surroundings (CETESB, 2002a). After 2000, the eutrophication process was intensified due to the entrance of Billings' water (CETESB, 2008). The water from Billings enters Guarapiranga reservoir through Parelheiros branch (G1), which main affluent is Parelheiros stream (P1). Although the water transfer was off, Guarapiranga's water quality deterioration goes on because Parelheiros stream has a very poor water quality, as our data showed. Human activities around the stream, such as agriculture and irregular human settlements, can be partly the causes of the impact (WHATELY & CUNHA, 2006). Parelheiros stream presented very low phytoplankton biomass, with dominant functional groups (L₀ and F) characteristics of eutrophic environments (REYNOLDS, 2006). The entrance of enriched water from Parelheiros stream is probably one of the causes of the spatial horizontal heterogeneity observed in Guarapiranga reservoir, which displayed

different characteristics in the sampling stations near the water intake area (G1, G2 and G3): low DO, high TP and SRP and dominance of the cyanobacteria *Sphaerocavum brasiliensis* and *Coelomorion tropicale* and the dinoflagellate *Peridiniopsis cunningtonii*. The presence of Dinophyceae species together with colonial mucilaginous Chroococcales cyanobacteria such as *Sphaerocavum brasiliensis* and *Coelomorion tropicale*, resulted in the dominance of codon L_M. Coda L₀ and L_M have generated confusion in the functional groups' users (PADISÁK *et al.*, 2009). *Ceratium hirundinella* and similar species, such as *C. furcoides*, should be placed in codon L_M only if occurs in association with *Microcystis aeruginosa* and/or overlapping subspecies and ecotypes, such as *Microcystis wesenbergii*, *Sphaerocavum brasiliensis* and *Woronichinia* (REYNOLDS *et al.*, 2002). When *Ceratium* spp. occurs alone, it should be placed in codon L₀. However, authors very often sorted *Ceratium* spp. into the codon L_M in ecosystems where *Microcystis* does not occur at all (PADISÁK *et al.*, 2009). For this reason, codon L₀ dominated in Billings reservoir, while codon L_M dominated in Guarapiranga reservoir. Overall, the dominance of coda L₀ or L_M indicates that both environments are under eutrophication process (PADISÁK *et al.*, 2009).

Spatial horizontal heterogeneity in Guarapiranga reservoir has been studied under many aspects, such as water quality and trophic status (CARDOSO-SILVA, 2008; NISHIMURA *et al.*, 2012), metals in water (CARDOSO-SILVA, 2008) and in sediments (PADIAL, 2008), aquatic macrophytes (RODRIGUES, 2011) and phytoplankton community (NISHIMURA *et al.*, 2012), indicating the presence of different compartments in the reservoir driven by the tributaries' water quality, human impact and management. All studies concluded that Embu-Guaçu branch has better water quality compared to the rest of the reservoir. The entrance of Embu-Guaçu's water (G4) led to changes in water quality and in phytoplankton composition biomass with no dominance of a single species in the dam direction (G5 → G6 → G7). *Synura* sp. (W_S) was especially abundant in G5. Codon W_S is a relative new codon, proposed by Padisák *et al.* (2003). It is composed by species that occurred in humic environments (PADISÁK *et al.*, 2009). The occurrence of this codon, particularly in G5, can be explained by the location of this sampling station, right beside the "Eucalyptus island", from where vegetal matter came from. *Anabaena spiroides* (H₁) first appeared in G5 and peaked in biomass near the dam (G7), where the water is taken for water supply. H₁ species usually occur in eutrophic environments with nitrogen deficiency, since this group species are nitrogen fixing cyanobacteria (REYNOLDS, 2006). However, in Guarapiranga reservoir, the most concerning fact is that *A. spiroides* is a potentially toxic cyanobacteria and its presence can increase the water treatment costs (Codd, 2000) and cause damages to the biota (Codd *et al.*, 2005). The proper classification of the diatom *Urosolenia eriensis* is dubious. Guarapiranga reservoir, in

general, is an eutrophic reservoir, placing *U. eriensis* in codon C. However, taking into consideration that *U. eriensis* occurred only in G4, under direct influence of Embu-guaçu branch, this species could be placed in codon A (diatoms from oligotrophic environments), in agreement with the literature (PADISÁK *et al.*, 2009).

Our findings provide support to the argument that Guarapiranga reservoir water quality is being influenced by the water quality that enters in its tributaries, specifically, Parelheiros stream. Thus, the preservation of Parelheiros stream and the wetland where it is inserted is crucial. Another important reason for the preservation of this area is that Parelheiros wetland plays major role in nutrients retention coming from Billings reservoir (ANDRADE, 2005; WHATELY & CUNHA, 2006). The occurrence of nuisance phytoplankton species in Guarapiranga reservoir, previously absent (before the beginning of the transfer system), support the negative influence of Billings waters in Guarapiranga. In our study, we found the invasive dinoflagellate *C. furcoides* in Guarapiranga reservoir, probably transferred from Billings.

Billings-Guarapiranga system is only a short term solution to the water shortage during the dry season in São Paulo city. Indeed, this system prevented the water supply system from the city to collapse and ensured enough water to the population. However, in medium-long term, this action will only deteriorate Guarapiranga's water quality, as our study showed that is happening right now, ten years after the beginning of the water transfer. In one hand, Sao Paulo city population needs water with good quality and in enough quantity. On the other hand, Billings-Guarapiranga system is clearly changing Guarapiranga environment and biota in a negative way. The most reasonable solution for the water scarcity in Sao Paulo city would be the conservation of its water sources, with more efficient sewage system and habitation policies to prevent irregular human settlements around the reservoir and, consequently, sewage water input into the reservoir, acceleration the eutrophication process. Another possible and less viable solution is to find other water source to supply Sao Paulo population.

Capítulo 3. Invasive dinoflagellate *Ceratium furcoides* (Levander) Langhans in two linked tropical reservoirs

Abstract

Phytoplankton blooms, especially cyanobacterial, are one of the main symptoms of the eutrophication process in tropical reservoirs. However, since 1999, reports of high biomass of the dinoflagellate *Ceratium* Schrank became more frequent in tropical and subtropical eutrophic waters. *Ceratium* is a large freshwater mixotrophic dinoflagellate protected by a rigid cellulose armor, characteristic summer inhabitant of temperate stratified lakes with low surface nutrient concentrations. Here we reported the occurrence of high biomass of *Ceratium furcoides* in Billings reservoir (São Paulo, Brazil), and we reported for the first time, the occurrence of *C. furcoides* in Guarapiranga reservoir (São Paulo, Brazil), however, in low biomass. Billings reservoir is used for electric power generation, leisure, fishery and navigation. Guarapiranga reservoir's main use is water supply. The main problem of both reservoirs is the excess of organic matter from clandestine domestic sewage input. Since 2000, water from Billings is pumped to Guarapiranga during the dry season, when the water level of the last is low. Probably, *C. furcoides* population was transferred to Guarapiranga during this pumping. The lower *C. furcoides* biomass in Guarapiranga reservoir suggests that the colonization of *C. furcoides* in Guarapiranga is still in early stages comparing with the colonization in Billings or that Guarapiranga's environment is not convenient for *C. furcoides*' establishment and growth as it is in Billings reservoir. The findings in this project point out the need for further studies on *C. furcoides* population in order to better understand its role in the ecosystem and, consequently, to prevent possible alterations in the ecosystem ecological properties and also, to prevent losses for human activities, especially water supply.

1. Introduction

Water storage is one the most ancient, important and efficient human intervention in natural systems (TUNDISI, 1996). These man-made reservoirs promote economic benefits due to hydroelectric power generation and due to water supply for irrigation and consumption, among others. Modern reservoirs can storage a large volume of water and also can have a very high capacity of water transfer between basins, affecting the water quality of the hydrographic basins involved (STRASKRABA & TUNDISI, 2000). In other words, reservoirs are inserted in a hydrographic basin and interact with it, capturing the human activities impacts along the basin (TUNDISI, 1996).

Eutrophication is a natural process that has been accelerated by human activities such as urbanization, industrialization and use of agricultural fertilizers (POMPÊO *et al.*, 2005). The high nutrients concentration in the water, that characterized the eutrophication process, leads to great alteration in the aquatic ecosystem, affecting the biological communities and the biogeochemical cycles (MOSS, 1998). Phytoplankton blooms are one of the main symptoms of the eutrophication process. Particularly in tropical regions, during the last three decades, cyanobacteria blooms have been more and more frequent in water supply reservoirs (DI BERNARDO *et al.*, 2002). However, very recently, blooms of an invasive dinoflagellate species are being frequently observed in tropical reservoirs.

Ceratium Schrank is a large freshwater mixotrophic dinoflagellate protected by a rigid cellulose armor (POPOVSKÝ & PFISTER, 1990). Because of these morphological characteristics, *Ceratium* is well protected from ingestion by herbivorous cladocerans (SOMMER *et al.*, 2003). Inorganic nutrients are often cited as factors that trigger blooms of *Ceratium* (WHITTINGTON *et al.*, 2000). Its occurrence can harm the environment since it can deplete resources (SANTOS-WISNIEWSKI *et al.*, 2007). The genus *Ceratium* is a characteristic summer inhabitant of temperate stratified lakes with low surface nutrient concentrations (DOKULIL & TEUBNER, 2003; GRIGORSZKY *et al.*, 2003). However, since 1999, reports of high densities of *Ceratium* in tropical and subtropical eutrophic waters became more frequent, such as Argentina (MACDONAGH *et al.*, 2005), Chile (SOTO & LEMBEYE, 1999), South Africa (VAN GINKEL *et al.*, 2001; HART, 2007), New Zealand (HART & WRAGG, 2009) and Australia (WHITTINGTON *et al.*, 2000; BALDWIN *et al.*, 2003). Since 2007, *Ceratium* is frequently found in Brazilian environments (SANTOS-WISNIEWSKI *et al.*, 2007; CETESB, 2009; LEONE *et al.*, 2009; MATSUMURA-TUNDISI *et al.*, 2010; OLIVEIRA *et al.*, 2011). Here is reported and discussed the occurrence of *Ceratium furcoides* (Levander) Langhans in two linked Brazilian reservoirs.

2. Methods

This study was carried out in September 14, 16 and 18th, 2009 in two linked Brazilian reservoirs in São Paulo metropolitan area: Billings (six superficial samples through Taquacetuba branch's longitudinal axis, 23°47'S/46°40'W) and Guarapiranga (six superficial samples through Parelheiros branch's longitudinal axis, 23°43'S/46°32'W) (Figure 1).

Figure 1. Location of São Paulo State and São Paulo metropolitan area. In detail, Guarapiranga and Billings reservoirs and the respective sampling spots in Parelheiros and Taquacetuba branches. The dotted arrow represents the water path when water is being transferred from Billings to Guarapiranga reservoir.

Billings reservoir was built in 1927 and its watershed covers an area of 560 km², storing 1.2 billion m³ of water. Billings' uses include electric power generation, leisure, fishery, navigation, flow control, domestic and industrial wastewater reception, and water supply. Billings' limnological characteristics changed substantially since 1940, when part of the polluted water from the Tietê River (São Paulo city) started to flow into the Billings

reservoir, aiming to increase the water flow and consequently, the electric power generation. This operation, along with the disorganized human occupation of the watershed, contributed to increase the eutrophication and consequently, the cyanobacterial blooms (SOUZA *et al.*, 1998). Due to its peculiar shape, Billings reservoir is divided into eight units called branches. Taquacetuba branch has a particular use. In August of 2000, the São Paulo State Basic Sanitation Company (SABESP) began the transfer of raw water from Taquacetuba branch to Guarapiranga reservoir (Parelheiros branch), in order to increase the water volume of Guarapiranga reservoir during the dry season. This water transfer started with a license for $2.0 \text{ m}^3 \text{ s}^{-1}$; currently, it operates at a volume of 3.0 to $4.0 \text{ m}^3 \text{ s}^{-1}$, contributing with 29% of the total water produced in Guarapiranga reservoir, which main use is water supply to southeastern São Paulo city at a rate of 1.2 billion L day^{-1} (WHATELY & CUNHA, 2006). Guarapiranga reservoir was constructed in 1908 and its watershed covers an area of 36 km^2 , storing 194 million m^3 of water. According to the São Paulo State Environmental Agency (CETESB), the current main problem of both reservoirs is the excess of organic matter from clandestine domestic sewage input (CETESB, 2009). Consequently, phytoplankton blooms, especially cyanobacteria, are frequent in both reservoirs (NISHIMURA *et al.*, 2008; MOSCHINI-CARLOS *et al.*, 2009).

Water temperature (T), pH, electric conductivity (EC) and dissolved oxygen (DO) were measured in each sampling station using standard electrodes (YSI 556). In each sampling station, maximum depth (Z_{max}) and Secchi disk depth (Z_{sd}) were measure and the euphotic depth (Z_{eu}) was estimated (AROCENA, 1999). Additionally, superficial water was gathered to analyze the following variables in the laboratory: ammonium, nitrite and nitrate (MACKERETH *et al.*, 1978), silicate and soluble reactive phosphorus (STRICKLAND & PARSONS, 1960), total nitrogen and total phosphorus (VALDERRAMA, 1981); chlorophyll *a* corrected for phaeophytin using 90% acetone extraction (LORENZEN, 1967; WETZEL & LIKENS, 1991).

Superficial water samples for phytoplankton community analysis were preserved with lugol 4%. Phytoplankton species were identified based on specific bibliography and according to Van Den Hoek (1997), except for Cyanobacteria (KOMÁREK & ANAGNOSTIDIS, 1999, 2005) and Bacillariophyceae (ROUND *et al.*, 1992) in a Carl Zeiss ScopeA1 microscope. Phytoplankton cells were counted using the settling technique (UTERMÖHL, 1958) in 2 ml settling chambers in a Carl Zeiss Axiovert40C inverted microscope. Sedimentation time followed Lund *et al.* (1958). A minimum of 400 individuals (cells, colonies or filaments) was counted in each sample giving a counting

accuracy, expressed in terms of 95% confidence limits, of < 10% for the whole phytoplankton population (LUND *et al.*, 1958). Biovolume was obtained by geometric approximation, multiplying each species' density by the mean volume of its cells considering, whenever possible, the mean dimension of 30 individual specimens of each species (HILLEBRAND *et al.*, 1999). The phytoplankton species that contributed with more than 5% of the total biomass of the sample were considered a descriptors species of the community. Species that contributed with more than 50% of the total biomass of the sample were considered dominant (LOBO *et al.*, 2002). To identify the species *Ceratium furcoides*, cells were clarified with NaClO 20% to see the plate tabulation (BOLTOVSKOY, 1995) and the specimens were observed in a Carl Zeiss ScopeA1 microscope. *Ceratium* species description was based on Popovský and Pfester (1990) and Lewis and Dodge (2002).

To explore the relationship between the limnological variables and the sampling stations, a Principal Components Analysis (PCA) was performed. To assess the contribution of each variable included in the PCA, the "equilibrium circle of descriptors" technique was applied (LEGENDRE & LEGENDRE, 1998). The variables inside the "equilibrium circle" were excluded from the analysis. Limnological data were standardized by ranging $[(x - x_{\min}) / (x_{\max} - x_{\min})]$ in order to keep the same amplitude to all variables and the PCA was carried out with the CANOCO for Windows 4.5 software and PCA plots were performed with CanoDraw for Windows (version 4.0) software.

3. Results

Table 1 and Table 2 show the main physical, chemical and biological variables measured in all sampling stations in Billings and Guarapiranga reservoirs, respectively. The first two axis of the PCA explained 86.2% of the data variance (64.7% by the first axe and 21.5% by the second, Figure 2). The PCA plot showed a clear segregation by the first axis of the sampling stations from Billings and Guarapiranga reservoirs (Figure 2). Billings' sampling stations were close from each other in the ordination, indicating homogeneity among the stations. Moreover, Billings sampling stations were positively correlated with the variables related with nitrogen, electric conductivity, pH, dissolved oxygen and maximum depth (Table 3). Guarapiranga's sampling stations were spread along the second axis, indicating heterogeneity among the stations. By the PCA ordination, it is possible detect a clear pattern of decreasing TN towards the central body of Guarapiranga reservoir (G1 →

G6, Table 3). G1 exhibited very distinct characteristics from the rest of the sampling spots, with very low depth and dissolved oxygen concentration, and high total phosphorous and ammonium concentrations. In G1 and G2 were observed the highest phytoplankton biomass (Figure 3). The high biomass in G1 was due to the dominance of the cyanobacteria *Sphaerocavum brasiliensis* ($7.15 \text{ mm}^3 \text{ l}^{-1}$) and in G2, due to the dominance of the Dinophyceae *Peridiniopsis cunningtonii* ($13 \text{ mm}^3 \text{ l}^{-1}$).

Altogether, 122 phytoplankton species were identified. Higher species richness was observed in Guarapiranga (81 species) compared to Billings (58 species). In Guarapiranga, 21 phytoplankton species were considered descriptors: four Chlorophyceae (*Botryococcus braunii* Kützing, *Coelastrum microporum* Nägeli, *Tetrastrum homoicanthum* (Huber-Pestalozzi) Comas and *Chlamydomonas* sp.), four Cyanophyceae (*Anabaena spiroides* Klebahn, *Synechocystis aquatilis* Sauvageau, *Coelomorion tropicale* P.A.C.Senna, A.C.Peres & J.Komárek and *Sphaerocavum brasiliensis* M.T.P. Azevedo & C.L. Sant'Anna), three Dinophyceae (*Ceratium furcoides* (Levander) Langhans, *Peridiniopsis cunningtonii* (Lemmermann) Popovský & Pfister and *Peridinium gatunense* Nygaard), three Euglenophyceae (*Euglena agilis* H.J.Carter, *Trachelomonas similis* var. *spinosa* Huber-Pestalozzi and *Trachelomonas volvocinopsis* Svirenko), two Bacillariophyceae (*Nitzschia* sp. and *Urosolenia eriensis* (H.L.Smith) Round & R.M.Crawford), two Cryptophyceae (*Cryptomonas brasiliensis* A.Castro, C.Bicudo & D.Bicudo and *Cryptomonas ovata* Ehrenberg), one Synurophyceae (*Synura* sp.), one Trebouxiophyceae (*Franceia droescheri* (Lemmermann) G.S.Smith) and one Zygnematophyceae (*Staurostrum anatinum* Cooke & Wills var. *anatinum* f. *anatinum*). In Billings 11 species were considered descriptors: four Dinophyceae (*Ceratium furcoides*, *Gymnodinium* sp., *Peridinium gatunense*, *Peridinium umbonatum* F.Stein), two Cryptophyceae (*Cryptomonas brasiliensis* and *Cryptomonas ovata*), two Cyanophyceae (*Synechocystis aquatilis* and *Planktothrix agardhii* (Gomont) Anagnostidis & Komárek), one Chlorophyceae (*Botryococcus braunii*), one Trebouxiophyceae (*Eremosphaera* sp.) and one Zygnematophyceae (*Staurostrum anatinum* Cooke & Wills var. *anatinum* f. *anatinum*). In general, phytoplankton biomass was higher in Guarapiranga reservoir compared to Billings (Figure 3).

Table 1. Mean values and standart deviation of physical, chemical and biological variables measured in all sampling stations in Billings reservoir (B1-B6; n = 3, each sampling day in each sampling station). [“Total average” referres to the mean value and standart deviation of all samples in Billings reservoir (n = 18); Zmax = maximun depth; Zsd = Secchi disk depth; Zeu = euphotic depth; N:P = nitrogen:phosphorous molar ratio]

Variable (unit)	Billings reservoir						
	B1	B2	B3	B4	B5	B6	Total average
Zmax (m)	5.9 ± 0.1	8.0 ± 0.1	8.7 ± 0.6	10.1 ± 0.3	10.5 ± 0.1	10.7 ± 0.3	9.0 ± 1.7
Zsd (m)	1.1 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.3 ± 0.2
Zeu (m)	3.0 ± 0.3	3.2 ± 0.3	3.3 ± 0.4	4.1 ± 0.4	3.8 ± 0.3	4.0 ± 0.3	3.6 ± 0.5
Zeu/Zmax	0.5 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.1
Temperature (°C)	21.0 ± 0.3	20.9 ± 0.3	21.0 ± 0.3	21.1 ± 0.2	21.3 ± 0.3	21.6 ± 0.8	21.1 ± 0.4
Dissolved oxygen (mg l ⁻¹)	8.4 ± 1.9	9.3 ± 0.5	8.9 ± 0.5	8.7 ± 0.5	8.1 ± 1.1	8.8 ± 0.7	8.7 ± 0.9
pH	7.6 ± 0.0	7.8 ± 0.5	7.9 ± 0.5	7.9 ± 0.3	7.9 ± 0.3	7.7 ± 0.4	7.8 ± 0.3
Conductivity (µS cm ⁻¹)	220 ± 9	221 ± 9	223 ± 6	226 ± 5	229 ± 5	231 ± 6	225 ± 7
Silicate (µg l ⁻¹)	2 ± 0	2 ± 0	3 ± 1	2 ± 0	2 ± 0	2 ± 0	2.0 ± 1
Phosphate (µg l ⁻¹)	< 10	< 10	< 10	< 10	< 10	< 10	-
Nitrite (µg l ⁻¹)	32 ± 15	35 ± 13	37 ± 13	41 ± 9	46 ± 13	45 ± 7	39 ± 11
Nitrate (µg l ⁻¹)	717 ± 138	812 ± 231	845 ± 250	902 ± 163	920 ± 91	942 ± 105	856 ± 161
Ammonium (µg l ⁻¹)	27 ± 7	44 ± 59	26 ± 28	38 ± 38	58 ± 65	60 ± 50	42 ± 40
Total nitrogen (µg l ⁻¹)	1371 ± 44	1380 ± 37	1363 ± 13	1415 ± 43	1511 ± 88	1506 ± 146	1424 ± 87
Total phosphorous (µg l ⁻¹)	42 ± 19	35 ± 19	33 ± 15	31 ± 16	33 ± 12	28 ± 8	34 ± 13
N:P	87 ± 49	117 ± 88	115 ± 72	133 ± 97	112 ± 51	130 ± 50	116 ± 60
Chlorophyll-a (µg l ⁻¹)	48 ± 7	42 ± 28	31 ± 12	24 ± 11	34 ± 18	27 ± 21	34 ± 14
Phaeophytin (µg l ⁻¹)	22 ± 1	57 ± 67	20 ± 15	15 ± 4	18 ± 5	15 ± 8	16 ± 4

Table 2. Mean values and standart deviation of physical, chemical and biological variables measured in all sampling stations in Guarapiranga reservoir (G1-G6; n = 3, each sampling day in each sampling spot). [“Total average” referres to the mean value and standart deviation of all samples in Guarapiranga reservoir (n = 18); Zmax = maximun depth; Zsd = Secchi disk depth; Zeu = euphotic depth; N:P = nitrogen:phosphorous molar ratio]

Variable (unit)	Guarapiranga reservoir						
	G1	G2	G3	G4	G5	G6	Total average
Zmax (m)	2.9 ± 0.2	4.7 ± 0.1	6.5 ± 0.2	8.0 ± 0.0	8.9 ± 0.6	8.5 ± 0.4	7.0 ± 2.2
Zsd (m)	1.3 ± 0.4	1.6 ± 0.1	1.5 ± 0.0	1.5 ± 0.0	1.3 ± 0.1	1.2 ± 0.2	1.4 ± 0.2
Zeu (m)	3.5 ± 1.0	4.4 ± 0.2	4.2 ± 0.1	4.0 ± 0.1	3.6 ± 0.3	3.3 ± 0.4	3.8 ± 0.5
Zeu/Zmax	1.2 ± 0.4	0.9 ± 0.1	0.6 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.6 ± 0.3
Temperature (°C)	20.5 ± 0.6	20.4 ± 0.6	20.6 ± 0.3	21.1 ± 0.6	21.4 ± 0.4	21.3 ± 0.5	20.9 ± 0.6
Dissolved oxygen (mg l ⁻¹)	0.6 ± 0.5	3.7 ± 1.9	5.1 ± 0.9	7.1 ± 1.6	7.3 ± 1.1	8.1 ± 0.5	5.5 ± 2.6
pH	7.0 ± 0.0	7.1 ± 0.1	7.2 ± 0.0	7.4 ± 0.2	7.5 ± 0.1	7.7 ± 0.2	7.4 ± 0.3
Conductivity (µS cm ⁻¹)	129 ± 22	117 ± 10	112 ± 6	87 ± 11	108 ± 13	110 ± 7	112 ± 15
Silicate (µg l ⁻¹)	2 ± 0	2 ± 0	2 ± 0	2 ± 0	2 ± 0	2 ± 0	2 ± 0
Phosphate (µg l ⁻¹)	46 ± 34	12 ± 11	< 10	< 10	< 10	< 10	-
Nitrite (µg l ⁻¹)	13 ± 9	33 ± 10	31 ± 9	13 ± 7	20 ± 6	24 ± 9	22 ± 10
Nitrate (µg l ⁻¹)	150 ± 175	384 ± 95	456 ± 46	242 ± 69	414 ± 64	472 ± 29	385 ± 152
Ammonium (µg l ⁻¹)	505 ± 194	372 ± 197	252 ± 55	69 ± 56	118 ± 79	80 ± 7	218 ± 180
Total nitrogen (µg l ⁻¹)	1392 ± 357	1100 ± 77	1003 ± 150	655 ± 69	820 ± 74	932 ± 89	999 ± 253
Total phosphorous (µg l ⁻¹)	117 ± 53	45 ± 4	36 ± 13	24 ± 4	32 ± 5	39 ± 12	49 ± 34
N:P	29 ± 9	54 ± 8	66 ± 20	63 ± 17	57 ± 9	56 ± 15	54 ± 17
Chlorophyll-a (µg l ⁻¹)	12.7 ± 6.8	15.9 ± 10.2	18.7 ± 11.3	20.4 ± 10.7	21.2 ± 6.0	36.9 ± 5.7	17 ± 13
Phaeophytin (µg l ⁻¹)	14.3 ± 6.4	29.5 ± 19.7	14.8 ± 1.0	19.4 ± 8.5	33.3 ± 21.4	32.6 ± 12.1	15 ± 7

Figure 2. Principal Components Analysis (PCA) biplot based on the mean values of some limnological variables (T=temperature; Zmax=maximum depth; DO=dissolved oxygen; pH; N:P=nitrogen and phosphorous molar ration; NO3=nitrate; NO2=nitrite; EC=conductivity; TN=total nitrogen; SRSi=silicate; TP=total phosphorous; SRP=phosphate; Zeu/Zmax=euphotic and maximum depth ratio; NH4=ammonium) standardized by ranging in Billings (B1-B6) and Guarapiranga reservoirs (G1-G6).

Table 3. Biplot scores of the selected environmental variables (Zmax=maximum depth; Zeu/Zmax=euphotic and maximum depth ratio; T=temperature; DO=dissolved oxygen; pH; EC=electric conductivity; SRSi=silicate; SRP=phosphate; NO2=nitrite; NO3=nitrate; NH4=ammonium; TN=total nitrogen; TP=total phosphorous; N:P=nitrogen and phosphorous molar ratio) applied in the principal components analysis (PCA) in Billings and Guarapiranga reservoirs' sampling stations.

	1st axis	2nd axis
Zmax	-0.8678	0.2929
Zeu/Zmax	0.8837	-0.4416
T	-0.7008	0.4215
DO	-0.9265	0.2988
pH	-0.9515	0.0512
EC	-0.7904	-0.5852
SRSi	0.3100	-0.4362
SRP	0.7619	-0.5431
NO2	-0.7973	-0.4074
NO3	-0.9400	-0.3256
NH4	0.8890	-0.3606
TN	-0.4460	-0.8754
TP	0.7198	-0.5624
N:P	-0.9283	-0.2689

Figure 3. Mean total phytoplankton biomass ($n = 3$, each sampling day per sampling station) per taxonomical class and *Ceratium furcoides* mean biomass ($n = 3$, each sampling day per sampling station) in each sampling station in (a) Billings and (b) Guarapiranga reservoirs.

Ceratium furcoides specimen was identified among the phytoplankton community. The cells were narrowly spindle-shaped, strongly dorsiventrally flattened, 42–54 μm wide, 114–154 μm long; epitheca formed into a narrow horn without shoulders; hypotheca broad and short, drawn out into two posterior horns of different lengths; plates smooth and with shallow net-like ornamentation. The apical plate's tabulation was crucial to

confirm the specimen as *C. furcoides*: the fourth apical plate does not reach apex of epitheca (Figure 4).

Figure 4. *Ceratium furcoides* from Billings and Guarapiranga reservoirs: a) ventral view; b) lateral view; c) ventral view with 4' plate in detail (white arrow) in phase contrast.

C. furcoides was found in all 18 samples from Billings and only in four out of 18 samples from Guarapiranga. Higher *C. furcoides* biomass was observed in Billings reservoir (Fig. 3). In Billings, *C. furcoides* biomass ranged from 0.2 to 5.7 mm³ l⁻¹, comprising up to 44% of the mean total biomass in B5. In Guarapiranga, *C. furcoides* biomass was lower, ranging from 0 to 2.6 mm³ l⁻¹ and comprising up to 15% of the mean total biomass in G3. *C. furcoides* was not found in the sampling spots G1 and G2, where low DO concentration, low maximum depth, high ammonium and total phosphorous concentrations were observed. Additionally, in these two sampling stations, dominance of other species was observed (*Sphaerocavum brasiliensis* and *Peridiniopsis cunningtonii*), as mentioned previously.

4. Discussion

Billings and Guarapiranga reservoirs were physically, chemically and biologically distinct, as shown by the PCA ordination. Billings exhibited a very homogenous environment, while Guarapiranga was spatially heterogeneous along the branch longitudinal axis, displaying higher nutrient content near the water inflow (sampling stations G1 and G2). Previous works have already evidenced the spatial heterogeneity in Guarapiranga reservoir in relation to water quality (CARDOSO-SILVA, 2008), metals in sediment (PADIAL, 2008) and in water (CARDOSO-SILVA, 2008), and aquatic macrophytes (RODRIGUES, 2011).

C. furcoides biomass in Billings were high compared to those recorded by Santos-Wisniewski *et al.* (2007) in the first report of *C. furcoides* in Furnas reservoir (Minas Gerais, Brazil) (maximum mean density of 12 cells ml⁻¹). *C. furcoides* was first recorded in Billings in 2008 (CETESB, 2009). The authors suggested that the appearance of *C. furcoides* caused the reduction of cyanobacteria density. Matsumura-Tundisi *et al.* (2010) reported a *C. furcoides* bloom (535-21455 cells ml⁻¹) in Taquacetuba branch (Billings reservoir) in 2008, period prior to this study. The authors, pointed out as the possible cause of the *C. furcoides* bloom the turbulence induced by wind, that caused the water column mixing, along with *Ceratium* cysts from the sediments and nutrients. In the present study, high *C. furcoides* biomass was observed in Billings reservoir, long with low cyanobacteria biomass and high nutrients concentrations. Further studies are required to explore *C. furcoides*-Cyanobacteria interaction in detail.

Here, we reported the first occurrence of *C. furcoides* in Guarapiranga. Since 2000, water from Billings (Taquacetuba branch) is pumped to Guarapiranga (Parelheiros branch) during the dry season, when the water level of the last is low. Probably, *C. furcoides* population was transferred to Guarapiranga during this pumping. The lower frequency and biomass of *C. furcoides* observed in Guarapiranga, suggest that the colonization of *C. furcoides* in Guarapiranga is still in early stages comparing with the colonization in Billings or that Guarapiranga's environment is not convenient for *C. furcoides*' establishment and growth as it is in Billings reservoir.

An important observation is that in the only two sampling stations where *C. furcoides* was not observed (G1 and G2), were the only two sampling spots where other phytoplankton species were dominant (*Sphaerocavum brasiliensis* and *Peridiniopsis cunningtonii*, respectively). All other sampling stations where *C. furcoides* was recorded, no dominance was observed. This fact can suggest that *C. furcoides* population is important for the

maintenance of the phytoplankton community non-equilibrium (ROJO & ÁLVAREZ-COBELAS, 2003). Further studies are required to investigate: (1) the interaction of this dinoflagellate with the phytoplankton and zooplankton community; (2) how this species is being dispersed; and (3) what are the consequences of the *C. furcoides* colonization for the water supply.

Invaders can alter fundamental ecological properties such as the dominant species in a community and an ecosystem's physical features, nutrient cycling, and primary productivity (MACK *et al.*, 2000). In this context, the presence of high densities of the invasive dinoflagellate *C. furcoides* in tropical waters is of great concern, especially in water supply reservoirs, such as Billings and Guarapiranga. The water in Billings reservoirs (Taquacetuba branch) is not treated nor managed before being transferred to Guarapiranga reservoir. Thus, monitoring should be intensified, and more effective measures should be taken by the agencies responsible in order to eliminate the causes of the eutrophication process, the consequent development of phytoplankton blooms, and the transference of potential harmful organisms. Furthermore, the occurrence and dispersal of *C. furcoides* needs be carefully monitored in tropical and subtropical reservoirs, especially those where the colonization process is still in early stages, such as Guarapiranga reservoir. The findings in this project point out the need for further studies on *C. furcoides* population in order to better understand its role in the ecosystem and, consequently, to prevent possible alterations in the ecosystem ecological properties and also, to prevent losses for human activities, especially water supply.

Capítulo 4. *Ceratium furcoides* (Levander) Langhans 1925, an invasive dinoflagellate in Brazilian freshwaters: worldwide distribution and review on its ecology

Abstract

Since 2007, the invasive dinoflagellate *Ceratium furcoides* is frequently found in Brazilian freshwaters. Because of the increasingly evident invasive behavior of *C. furcoides* and also because of the limited amount of information about this species, there is a growing interest in its biology and ecology. Though there is limited literature on this topic, this review intends to summarize the existing knowledge concerning taxonomical/morphological aspects, geographical distribution, population dynamics and physiological ecology of *C. furcoides*.

1. Introduction

Ceratium Schrank is a large free-living mixotrophic dinoflagellate that inhabits freshwater and marine environments worldwide (LEWIS & DODGE, 2002). Only six *Ceratium* species occur in freshwater environments (BICUDO & MENEZES, 2006). *Ceratium hirundinella* (O.F.Müller) Dujardin 1841 is the most prominent freshwater species from the genus, found in all temperate region (GUIRY & GUIRY, 2012). In fact, the genus *Ceratium* is a characteristic summer inhabitant of temperate stratified lakes with low surface nutrient concentrations (DOKULIL & TEUBNER, 2003; GRIGORSZKY *et al.*, 2003). Since 1999, reports of high densities of *C. hirundinella* in tropical and subtropical eutrophic waters became more frequent, such as Argentina (MACDONAGH *et al.*, 2005), Chile (SOTO & LEMBEYE, 1999), South Africa (VAN GINKEL *et al.*, 2001; HART, 2007), New Zealand (HART & WRAGG, 2009) and Australia (WHITTINGTON *et al.*, 2000; BALDWIN *et al.*, 2003).

Ceratium species are protected by a rigid cellulose armor (POPOVSKÝ & PFISTER, 1990). Because of *Ceratium*'s morphological characteristics, it is well protected from ingestion by herbivorous cladocerans (SOMMER *et al.*, 2003). Moreover, another feature that protects it from herbivores is its unpalatability. Sometimes it forms blooms triggered by inorganic nutrients (WHITTINGTON *et al.*, 2000). Its occurrence can harm the environment since it can deplete resources, such as oxygen, causing fish mortality (TAYLOR *et al.*, 1995) and can be responsible for taste and odor problem in drinking water (LALEZARY *et al.*, 1986). Conversely, *Ceratium* populations can control bloom forming species of cyanobacteria by nutrient competition (CETESB, 2009).

Ceratium furcoides (Levander) Langhans 1925 distribution is much more restricted than its counterpart *Ceratium hirundinella*. *C. furcoides* was first described as *C. hirundinella* var. *furcoides* by Levander in 1900 (GUIRY & GUIRY, 2012). After that, *C. furcoides* has been found in temperate regions (WHITTON *et al.*, 1998; LEWIS & DODGE, 2002; CARAUS, 2012). Recently, *C. furcoides* spread to subtropical and tropical environments. Since 2007, *Ceratium furcoides* is being frequently found in Brazilian eutrophic reservoirs (CETESB, 2009; LEONE *et al.*, 2009; SANTOS *et al.*, 2009; MATSUMURA-TUNDISI *et al.*, 2010; OLIVEIRA *et al.*, 2011; SILVA *et al.*, 2012; TANIWAKI, 2012).

Because of the increasingly evident invasive behavior of *C. furcoides* and also because of the limited amount of information about this species, there is a growing interest in its biology and ecology. This review intends to summarize the existing knowledge concerning taxonomical/morphological aspects, geographical distribution, population dynamics and physiological ecology of *C. furcoides*.

2. Taxonomy and morphological variability in *Ceratium furcoides*

The taxonomical classification of *C. furcoides* is the following:

Empire Eukaryota

Kingdom Protozoa

Subkingdom Biciliata

Infrakingdom Alveolata

Phylum Myzozoa

Class Dinophyceae

Subclass Peridiniphyceidae

Order Gonyaulacales

Family Ceratiaceae

Genus *Ceratium*

Species *Ceratium furcoides*

Heterotypic synonyms of *C. furcoides* in the literature are *C. hirundinella* var. *furcoides* Levander 1900 and *C. furcoides* f. *gracile* Entz 1927 (GUIRY & GUIRY, 2012). Although cells of *Ceratium* are conspicuous and readily recognizable, the genus as a whole consists of a complex of taxa often difficult to differentiate at the species level (HEANEY *et al.*, 1988). *C. furcoides* cells are narrowly spindle-shaped, strongly dorsiventrally flattened, 28–56 µm wide, 123–222 µm long; epitheca formed into a narrow horn without shoulders; hypotheca broad and short, drawn out into 2 (occasionally 1 or 3) posterior horns of different lengths; plates smooth and with shallow net-like ornamentation; plate formula: Po, 4', 6'', ?c, ?s, 6''', 2''''', with fourth apical plate not reaching the apex of epitheca; chloroplasts numerous and oval, pale yellow-brown; eyespot absent; nucleus prominent in epicone; vegetative reproduction by oblique fission of cell with theca shared between daughter cells; sexual reproduction anisogamous producing hypnozygotes smooth-walled, triangular, with one narrow horn extending from each corner (LEWIS & DODGE, 2002). Morphological descriptions and documentation (drawing and/or micrographs) of the species are available in some publications (POPOVSKÝ & PFISTER, 1990; LEWIS & DODGE, 2002; SANTOS-WISNIEWSKI *et al.*, 2007; MATSUMURA-TUNDISI *et al.*, 2010).

C. furcoides is often confused with *C. hirundinella*. At first, it was identified as a *C. hirundinella* variation, *C. hirundinella* var. *furcoides*. Though some morphological characteristics from the two species superpose, such as size and epivalve shape (SANTOS-WISNIEWSKI *et al.*, 2007), the main difference between the two species is the apical plates tabulation. *C. hirundinella* have 4' apical plates reaching the apex while in *C. furcoides* 3' apical plates reaches the apex and the 4' does not reach the apex.

For plate tabulation observation in optical microscope, cell clarification with NaClO 20% is recommended (BOLTOVSKOY, 1995). This procedure clarifies the chloroplast content, enabling better visualization of the plates. When epifluorescence microscope is available, optical brightener Calcofluor white can be used to stain cellulose-rich plates and posterior observation under near UV or blue excitation filter (BOLTOVSKOY, 1995).

Length and number of horns in *C. furcoides* can vary. In general, it is commonly found specimens with 2 posterior horns of different lengths. However, occasionally, it is found

only 1 or 3 posterior horns of different lengths. Some authors suggest that the development of horns in *Ceratium* depends on variations in water temperature, nutrient availability, light and turbulence (POPOVSKÝ & PFISTER, 1990; HAMLAOUI *et al.*, 1998). Though there was no study on horns number variation in *C. furcoides* specifically, some studies tried to elucidate the number and size variation of horns in *C. hirundinella*. In Lake Erken (Sweden), the size of a *C. hirundinella* decreased during the investigation period and the form with a long horn disappeared in early summer (LINDSTRÖM, 1992). In freshwater mesocosm experiment, Bertolo *et al.* (2010) explored the potential for direct and indirect effects of two planktivorous fishes with different feeding behaviors (*Rutilus rutilus* and *Perca fluviatilis*) on the morphology of *C. hirundinella*. The authors did not find relation between the morphological variation in *C. hirundinella* and fish mediated variations in turbidity or by predation pressure by the fish. In contrast, the proportion of three-horned cells was directly related to the biomass of filter-feeding cladocerans. A possible explanation is that the third horn might help these dinoflagellates avoid physical contact with the filtering apparatus of the cladocerans and the consequent potential damage caused by these herbivores, which were more abundant in the absence of planktivorous fish (BERTOLO *et al.*, 2010). Additional studies are required to elucidate wheater *C. furcoides* morphological variations mechanisms are the same as in *C. hirundinella*.

Taxonomic and genetic differences between Ceratium populations

Another aspect to consider is the taxonomic and genetic differences between *Ceratium* populations. Differences in seasonal distribution of *C. hirundinella* populations between systems might be related to presence of different *Ceratium* species or even of different genetic forms (ecotypes) within species. Differences between the freshwater species of *Ceratium* genera were barely discerned until the 1990s, with the result that *C. hirundinella* was considered to be widely distributed (PÉREZ-MARTÍNEZ & SÁNCHEZ-CASTILLO, 2001). For example, over the 41 year period (1945 – 1985) of study in three English lakes, though the genus was represented by two species, *C. hirundinella* and *C. furcoides*, the species have only been distinguished through later examination of preserved samples long after the study ended (HEANEY *et al.*, 1988). In this study, population changes of *Ceratium* spp. in three adjacent English lakes (four lake basins) of varying size and productivity was observed. From 1964 to 1973 *C. furcoides* was the dominant form, being replaced by *C. hirundinella* until 1982, the last year of large population densities of the genus in the lake.

Moreover, some other studies report the co-occurrence of these two species (SOMMER, 1993; GRIGORSZKY *et al.*, 2003; OLIVEIRA *et al.*, 2011), generally with *C. furcoides* density much lower than *C. hirundinella*. In Lake Erken (Sweden), populations of *C. hirundinella* and *C. furcoides* were also found co-existing. While *C. hirundinella* dominated the spring and summer populations, *C. furcoides* was rare in early summer, but it continuously increased and composed up to 18% of the *Ceratium* populations in the autumn (LINDSTRÖM, 1992). Although there are clearly large annual differences in the relative abundance of *C. furcoides* and *C. hirundinella*, their life histories are nevertheless similar in respect to the timing of the major growth phase, both being essentially “summer” forms (HEANEY *et al.*, 1988).

Identification difficulty can lead to misidentification of *C. furcoides*. For instance, *C. furcoides* in the English Lake District was long regarded as a form variant of *C. hirundinella* (Heaney *et al.*, 1988) and Hickel (1988) described *C. rhomvroides* as a new species that coexisted with the former two species in Lake Plußsee (PÉREZ-MARTÍNEZ & SÁNCHEZ-CASTILLO, 2001). Therefore, *C. furcoides* records can be underestimated in the literature.

3. Geographic distribution of *Ceratium furcoides*

C. furcoides was first described as *C. hirundinella* var. *furcoides* by Levander in 1900 (GUIRY & GUIRY, 2012). After that, there are records of *C. furcoides* in temperate regions of Britain (HEANEY *et al.*, 1988; WHITTON *et al.*, 1998; LEWIS & DODGE, 2002), Romania (CARAUS, 2012), Spain (De Hoyos, C. & Negro, A., 2004 *apud* GUIRY & GUIRY, 2012), Poland (STEPHANIAK *et al.*, 2007) and Germany (SOMMER, 1993). Recently, *C. furcoides* spread to subtropical and tropical environments. *C. furcoides* was observed in the Feitsui Reservoir (Taiwan, China) in 1991 (WU & CHOU, 1998) and in Brazil, as will be exposed in the next section (Figure 1).

Figure 1. Worldwide occurrence of *Ceratium furcoides*.

4. *Ceratium furcoides* in Brazilian freshwaters

According to Bicudo & Menezes (2006), the first and only citation of the occurrence of the genus *Ceratium* in Brazil until 2007 was Branco *et al.* (1963). However, Branco *et al.*'s (1963) publication merely cite *Ceratium* as an nuisance genera with particular interest for the water treatment process, without reporting its occurrence in Brazilian waters.

Thus, we believe that the first record of *Ceratium* in Brazilian waters was reported by Santos-Wisniewski (2007) in the mesotrophic Furnas reservoir (Minas Gerais, Brazil, Figure 2). A more detailed publication was released by Silva *et al.* (2012). Throughout the year of 2007 *C. furcoides* was observed in Furnas reservoir with densities up to 28,564 org l⁻¹. High densities of *C. furcoides* were related to the low temperatures and the increment of nutrients, especially nitrate and nitrite (SILVA *et al.*, 2012). Although Furnas reservoir was the first record of *C. furcoides* in Brazil in 2007, so far its presence apparently has not yet affected the environmental conditions or other communities, but eventually as it becomes fully established it could turn into a nuisance or spread out to other basins (SILVA *et al.*, 2012).

Matsumura-Tundisi *et al.* (2010) reported the occurrence of *C. furcoides* bloom in Billings reservoir (São Paulo, Brazil, Figure 2) in 2008, with density up to 21,455,060 org ml⁻¹. Over the past decades, Billings reservoir is characterized as highly eutrophic water body with frequent occurrence of cyanobacteria blooms, such as *Microcystis aeruginosa*, *Planktothrix agardhii* and *Cylindrospermopsis raciborskii*. During *C. furcoides* blooms events, cyanobacteria species exhibited low densities, maybe due to less competition strength for nutrients. The appearance of *C. furcoides* bloom was attributed to the mixing turbulence of the water column that removed cysts from the surface of the sediment and increased phosphorous concentration in the water column, promoting conditions for the growth of this species (MATSUMURA-TUNDISI *et al.*, 2010).

In 2009, *C. furcoides* was recorded in six freshwater ecosystems in the semiarid region of northeastern Brazil: Sobradinho reservoir (Bahia), Contas river (Bahia), Moxotó river (Pernambuco), Paulo Afonso reservoir (Bahia/Alagoas), Itaparica reservoir (Pernambuco) and Xingó reservoir (Sergipe/Alagoas) (Figure 2) (OLIVEIRA *et al.*, 2011). According to the authors, the morphological study of *Ceratium* specimens from these sites suggests that there are two populations co-occurring, probably *C. furcoides* and *C. cf. hirundinella*. It seems that the invasion of *Ceratium* species followed the course of the São Francisco river and their appearance in the semiarid region of northeastern Brazil is likely to climatic and hydrological changes (OLIVEIRA *et al.*, 2011).

Still in 2009, *C. furcoides* was found in Guarapiranga reservoir (São Paulo, SP) in density varying from 15 to 30 cells ml⁻¹ (Nishimura, 2012 – Chapter 3 from this thesis, Figure 2). Probably, *C. furcoides* arrived at Guarapiranga reservoir through the water transfer system that pumps water from Billings reservoir to Guarapiranga reservoir during the dry season, when the water level of the last is low. The lower frequency and biomass of *C. furcoides* observed in Guarapiranga suggest that the colonization of *C. furcoides* in Guarapiranga is still in early stages comparing with the colonization in Billings or that Guarapiranga's environment is not convenient for *C. furcoides*' establishment and growth as it is in Billings reservoir.

In 2010, *C. furcoides* was detected in the eutrophic Itapararanga reservoir (São Paulo) in density ranging from 40 to 346 cells ml⁻¹ (Figure 2) (TANIWAKI, 2012).

Figure 2. Occurrence of *Ceratium furcoides* in Brazil. The first report was in Furnas reservoir, Minas Gerais in 2007 (1, Santos-Wisniewski, 2007); the second report was in Billings reservoir, São Paulo in 2008 (2, Matsumura-Tundisi *et al.* 2010); the third report was in 2009 in six freshwater ecosystems in the semiarid region: Sobradinho reservoir (Bahia, 3a), Contas river (Bahia, 3b), Moxotó river (Pernambuco, 3c), Paulo Afonso reservoir (Bahia/Alagoas, 3d), Itaparica reservoir (Pernambuco, 3e) and Xingó reservoir (Sergipe/Alagoas, 3f, OLIVEIRA *et al.* 2011); the fourth report was in Guarapiranga reservoir, São Paulo, in 2009 (4, Nishimura, 2012); the fifth report was in Itupararanga reservoir, São Paulo (5, Taniwaki, 2012).

5. Biology and ecology of *Ceratium furcoides*

Cyst formation

The capacity of cyst formation is one important adaptative strategy in *Ceratium*. Cysts of *Ceratium* are found on the sediments surface (MOYA & RAMON, 1984) and their flourishing is seasonally restricted and dependent, in part, upon the size of the inoculum when the temperature threshold for growth is reached. For the establishment of new populations of *Ceratium* spp. an inoculum of ~ 0.1 cell ml^{-1} of cysts in the benthos is necessary (HEANEY *et al.*, 1988). The earlier the population is established, the greater is the time available for nutrient uptake and growth once sufficient temperature has been reached.

In small temperate lakes with short retention times, the inoculum for *Ceratium* populations is mainly derived from the excystment of overwintering cysts in the sediment (HEANEY *et al.*, 1988). Heaney *et al.* (1983) estimated that in years of high cyst formation and deposition, assuming no losses, only 0.03% would be required to excyst the following spring to give an inoculum of ~ 0.1 cells ml⁻¹. This may provide a reservoir of viable cysts in the sediment and may compensate for years of poor cyst production, assuming that buried cysts may be returned to the sediment surface (HEANEY *et al.*, 1988). Apart from years of poor cyst production, there are also losses. Some will be removed through the outflow. Other potential losses are grazing in the sediment and infections by parasitic fungi as will be discussed in more detail in the section *Parasitism*. If the reservoir of cysts in the sediment is greatly depleted it is probable that recruitment from excystment will decrease and the establishment of a subsequent “inoculum” will take longer (HEANEY *et al.*, 1988). Thus, gains or losses of *Ceratium* species cysts in the sediment may be important for changes in dominance of different species of *Ceratium*.

Mixing and light requirements

Mixing conditions can favor *Ceratium* cysts flourishing. In Billings reservoir, Matsumura-Tundisi *et al.* (2010) attributed *C. furcoides* bloom to a cold front that caused mixing turbulence of the water column, removing cysts from the surface of the sediment and increasing phosphorous concentration in the water column. This mixing condition favored *C. furcoides* cysts flourishing and development.

Weather induced changes are also important for dinoflagellates growth. Periods of strong mixing have been shown to inhibit their growth (REYNOLDS *et al.*, 1983). The stability of the epilimnion was considered by Heaney *et al.* (1988) as a factor regulating the size of *Ceratium* populations in British lakes. The authors found that strong stability correlated with large populations of *Ceratium* spp. and in environments with weak stability, growth was relatively poor and diatoms were more abundant.

Greater mixing and decrease in *Ceratium* growth may be related with reduction in light availability or lower temperatures due to entrainment of deeper colder water (HEANEY *et al.*, 1988). Increased mixing will lead to greater mixed depth and to an inability for depth-regulation by *Ceratium* (GEORGE & HEANEY, 1978). Thus, these organisms will not be able to position in preferred optical depths during day. Moreover, vertical migration into depth of potentially nutrient-rich layers at night will be hindered.

Heaney & Butterwick (1985) demonstrated that cultures of *C. furcoides* (at the time, incorrectly named as *C. hirundinella* according to Heaney *et al.* 1988) have higher light requirements for the onset and saturation of growth than the diatom *Asterionella formosa* and the cyanobacteria *Oscillatoria bourrellyi*. Consequently, *C. furcoides* will respond less favorably to mixing to greater depths.

Temperature

Temperature is important for the development of *Ceratium* populations. Heaney *et al.* (1983) found a threshold of 10 °C for vegetative growth. Newly formed cysts require a resting period for cellular reorganization (CHAPMAN *et al.*, 1982). Experimental studies (Huber & Nipkow, 1923 apud HEANEY *et al.*, 1988) and with natural population (HEANEY *et al.*, 1983) showed that cysts of *C. hirundinella* and *C. furcoides* are temperature dependent and do not exist at temperatures below 4°C. Besides the vegetative growth and cysts formation, temperature may determine the timing of excystment in relation to the period between formation of cysts and their temperature-dependent maturation (HEANEY *et al.*, 1988).

Nutrients and trophic status

Heaney *et al.* (1988) found depletions of cellular phosphorus during the development of large populations of *Ceratium* in English lakes, suggesting that this is the main element controlling summer production. The authors found a clear relationship between increasing nutrient enrichment and increasing potential to support large populations of *Ceratium* in some lakes (HEANEY *et al.*, 1988).

Relationships between the importance of Dinophyta taxa and trophic state has been controversial (GRIGORSZKY *et al.*, 2003). Some studies indicate a decline of species richness with increasing eutrophication (LEE *et al.*, 1991), and other have not recorded any trend (INTERLANDI & KILHAM, 1999).

In a cluster analysis for 23 Dinophyta species based on their distribution with respect to water temperature, chemical oxygen demand and total phosphorous in 86 Hungarian water bodies, *Ceratium* species (*C. hirundinella*, *C. furcoides* and *C. cornutum*) and the dinoflagellate *Diplopsalis acuta* were grouped together because they are commonly found at high temperature with mesotrophic conditions (GRIGORSZKY *et al.*, 2003). In a study in

Furnas reservoir (MG, Brasil), Silva *et al.* (2012) concluded that *C. furcoides* does proliferate successfully in oligotrophic waters, although it reached the highest densities, under mesotrophic conditions whereas bloom-forming cyanobacteria are more often dominant under eutrophic or hypereutrophic. *C. furcoides* was observed in the mesotrophic Feitsui Reservoir (Taiwan, China) in 1991 and its abundance was positively correlated with the concentrations of phosphorus, total organic carbon, bacterial number, and the biochemical oxygen demand in the water. This species did not appear in large numbers and its maximum density was 75 cells ml⁻¹ (WU & CHOU, 1998). In an eutrophic lake in Poland, Stephaniak *et al.* (2007) concluded that *Ceratium furcoides* can better compete for nutrients with cyanobacteria due to its vertical migration capacity.

Retention time

Comparing *Ceratium* populations in three English lakes, Heaney *et al.* (1988) found smaller populations in Blelham Tarn, where the retention time is lower (~10 days to 6 weeks). Therefore, in this lake, the probability of appreciable washout is greatest. Washout can be especially important for slow-growing species during the planktonic stages of their life cycles. However, the hydraulic regime of Spanish reservoirs did not impede the development of *C. hirundinella* population in winter time because in these environments washout does not operate as it does in lakes with a stable water level (PÉREZ-MARTÍNEZ & SÁNCHEZ-CASTILLO, 2001). Therefore, further studies are required in this topic.

Heterotrophy

Heterotrophy in dinoflagellates is widely known (CAREFOOT, 1968; LINDSTRÖM, 1985; GAINES & ELBRAECHTER, 1987). Temporal and spatial distributions of dinoflagellates, including *C. furcoides*, in the Feitsui Reservoir (Taiwan, China) clearly demonstrated close correlation between the outgrowth of dinoflagellates and the runoff from watershed (WU & CHOU, 1998). The authors suggested that the outgrowth of dinoflagellates was a consequence of water pollution and that dinoflagellates exhibited some degree of heterotrophy in growth.

Grazing

Dinoflagellates are believed to be hard to ingest and to have little nutritional value. For example, Williamson (1984) found that the dinoflagellate *Peridinium* was ingested by freshwater copepod *Mesocyclops edax*, but not digested.

Studies on grazing in *Ceratium* are contradictory. Brandl and Fernando (1979) reported that the predatory stages of the freshwater copepod *Mesocyclops edax* did not ingest *Ceratium* when fed with an almost pure fraction of this algae. In contrast, Karabin (1978) reported that *M. leuckarti* consumed *C. hirudinella* at low rates, when fed with phytoplankton containing 90% *Ceratium*. Santer (1996) found that that *C. furcoides* can be utilized by all three freshwater cyclopoids (*Mesocyclops leuckarti*, *Thermocyclops oithonoides* and *Cyclops abyssorum*).

Hungry copepods ingest low-quality food when no other food sources are available (SANTER, 1993). Under natural conditions, *Ceratium* might serve as an additional food source for copepods, when convenient food sources, such as soft-bodied flagellates, rotifers, calanoid and cyclopoid nauplii or cladoceran juveniles and eggs, are deficient (SANTER, 1996). *Ceratium* consumption by copepods might happen in early spring, when the density of predaceous copepods is high and suitable prey is scarce. The decrease in cyclopoid density in early summer due to the beginning of the summer diapause might favour *Ceratium* population increase (SANTER, 1996). *Ceratium* is not grazed by cladocerans or by most of the rotifers which dominate the zooplankton in summer (SOMMER *et al.*, 1986) enabling the development of high population densities during this season.

Parasitism

Both the vegetative cells and cysts of *Ceratium* are subject to parasitism by fungi. Canter & Heaney (1984) found the biflagellate fungus *Aphanomyces cryptica* within cells of *Ceratium* from an English lake. Infections of the fungus are normally associated with marked decreases in density of *Ceratium* populations (HEANEY *et al.*, 1988).

Cysts of *Ceratium* are known to be parasitized by the chytrid *Rhizophydium nobile* (CANTER, 1968). Evidences indicate that this fungus is restrict to cysts of *C. furcoides* (CANTER & HEANEY, 1984).

Other organisms found in the sediment can contribute to the depletion of viable cyst populations. In English lakes, *Ceratium* cysts were ingested by a multinucleate vampyrellid-like protozoan while many sporangia and resting spores of an as yet undescribed chytrid (HEANEY *et al.*, 1988). Canter & Heaney (1984) require further studies on organism responsible for the deaths of *Ceratium* cysts, affecting the potential inoculum in English lakes, such as *Lagenidium* sp. and a monad-type protozoan which ingests the chromatophore of *Ceratium* cells.

6. Biogeography and dispersal

There are many difficulties on biogeography of freshwater. For example, it is problematic to say that a species is non-existent in a given area. Is that species non-existent in that area or there is insufficiency of historical data? Species associated with extreme environments, such as hotspring species and snow algae are relatively easy to map. However, difficulties increase in case of species having “average” demands and wide distributions (PADISÁK, 1997), such as *C. furcoides*.

In the same way that *C. hirundinella* populations have spread to various water bodies in the Neotropical region, since 1990, as a regional phenomenon associated with specific dispersal mechanisms and local conditions that favor its growth (MACDONAGH *et al.*, 2005), *C. furcoides* seems to be spreading to this region as well.

The occurrence of so many common freshwater algae throughout the world is a reflection of easy transport. However, there is almost no information about transport mechanisms (ROUND, 1981). Freshwater algae are mainly passively dispersed, being the four main types of dispersal: by water, by organisms - from beetles, dragonflies and mammals to birds, the latter being the most important group, by man and airborne dispersal (KRISTIANSEN, 1996).

Water dispersal is the most natural way of dispersal of freshwater algae, taking place in running water and in connected water bodies. Atkinson (1988) studied the colonization of a newly constructed reservoir. The source of the inoculum was the inflow and in a short time, the same species appeared as in the catchment area. Only after several years did other species appear which were not present in the catchment area. The author concluded that these species must have been transported passively overland by other methods, and that had taken a longer time.

Cysts forming-species might have their dispersal favored during long internal or external transportation by birds. Cysts are more resistant to dissection during external transport or to digestive process that takes places in internal transport in birds.

It is impossible to ignore the effects of scientific activities for algae dispersal. Even the more careful scientist may be unconsciously responsible for extending the range of the species when cleaning the residues of his collection flask in some water body or stream or using a not so well washed plankton net (TALLING, 1951). During human activities in Antarctica, there was as increasing concern regarding the introduction of algae taxa. Thus, a study of algae carried on expedition equipment and boots, and in soil on imported vegetables, yielded that 50 taxa were dispersed by man, most of them soil algae (BROADY & SMITH, 1994). This study highlights the impacts of human activities in algae dispersal. It is not difficult to imagine a scientific expedition to investigate the colonization of *C. furcoides* in Brazilian freshwaters that unconsciously will disperse this species from reservoir to reservoir while transporting it attached to the expedition boat or to plankton nets.

The possibility for successful dispersal depends on the distance and of the tolerance of the algae to the transport conditions. Moreover, the algae need to find a favorable environment in the end transportation. It seems that *C. furcoides* found a way to transpose the distance barrier and transport conditions and reached a favorable environment in Brazilian freshwaters. Nevertheless, which are the mechanisms of dispersal and how *C. furcoides* first arrived in Brazilian freshwaters remains a mystery.

7. Final remarks

The success of *C. furcoides* in Brazilian environments can be attributed to multiple reasons, especially:

- Vertical mobility that allows on the one hand diurnal migration between nutrient rich lower strata and eutrophic upper layers and on the other hand to avoid surface accumulation during which many cells would be damaged physiologically;
- High affinity phosphorous uptake that allows *C. furcoides* to utilize this abundant nutrient in eutrophic reservoirs;
- Heterotrophy, when organic matter is available;
- Resistance to grazing.

Moreover, Brazilian reservoirs exhibit characteristics that may be advantageous to *C. furcoides* populations' success, such as:

- High energy inputs (light and nutrients);
- High retention time, leading to higher environmental stability.

In most environments, a combined action of some of the above features leads to *C. furcoides* dominance. Consequently, generalized prediction about its appearance is impossible to outline. Investigators have developed individual explanation with individually weighted reasons for different environments. The number of reports on *C. furcoides* in Brazil is still low, therefore generalized predictions about its occurrence is still difficult to make.

Capítulo 5. Does the plankton community follow the water quality heterogeneity in a tropical urban reservoir (Guarapiranga reservoir, São Paulo, Brazil)?

Este trabalho foi apresentado no XVI Congresso da Sociedade Ibérica de Limnologia (Guimarães, Portugal) que ocorreu de 2 a 6 de julho de 2012. Em 31 de outubro de 2012, este trabalho foi submetido à publicação como artigo original no periódico da Sociedade Ibérica de Limnologia, Limnética.

Paula Yuri Nishimura^{(1)*}, Patrícia do Amaral Meirinho⁽¹⁾, Viviane Moschini-Carlos⁽²⁾,
Marcelo Luiz Martins Pompêo⁽¹⁾

⁽¹⁾Departamento de Ecologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, 321, travessa 14, CEP: 05508-900, São Paulo-SP, Brazil. E-mail: nishimurapy@usp.br

⁽²⁾Departamento de Engenharia Ambiental, Universidade Estadual Paulista Júlio de Mesquita Filho, Campus Experimental de Sorocaba, Avenida 3 de Março, nº511, CEP: 18087-180, Sorocaba-SP, Brazil

*Corresponding author

Running title: Horizontal heterogeneity in reservoir plankton

Abstract

Reservoirs exhibit a marked degree of spatial heterogeneity and environmental heterogeneity affects directly the organism's composition and distribution. The objective of this study was to investigate the spatial horizontal heterogeneity of the planktonic communities along the longitudinal axis of Guarapiranga reservoir (São Paulo, Brazil). We

sampled nine stations along Guarapiranga reservoir longitudinal axis for physical, chemical and biological variables. Additionally, we analyzed phytoplankton and zooplankton communities. To explore the data, we applied multivariate techniques, such as cluster hierarchical analyses and discriminant analyses. Our results showed a clear tendency of compartmentalization of the epilimnetic layers in this reservoir, especially based on water quality. Three main compartments were evidenced: 1) Embu-Graçu region, the upper part of the reservoir, more protected and less eutrophic with phytoplankton R-strategists dominance; 2) Parelheiros region, a very eutrophic branch of the reservoir with phytoplankton C-strategists dominance; and 3) the down part of the reservoir, with eutrophic and lacustrine characteristics with co-dominance of C and S-strategists. The groups formed by the cluster analysis based on the plankton data did not coincided exactly with the ones based on water quality. These different outcomes indicate that water quality, phytoplankton and zooplankton communities captured different features from the epilimnetic layer in Guarapiranga reservoir's longitudinal axis. It is important to highlight the phytoplankton and zooplankton community in the dam region was altered, directly or indirectly, by the copper sulphate treatment. Therefore, phytoplankton biomass is being driven by other forces than the ones intrinsic to the reservoir, such as human induced losses. Consequently, zooplankton structure is being affected as well.

Key-words: phytoplankton, zooplankton, CRS-strategists, compartmentalization.

1. Introduction

Environmental heterogeneity, defined as spatial and temporal variation in the physical, chemical and biological environment, is a fundamental property of ecosystems (SCHEINER & WILLIG, 2008). Regarding the spatial heterogeneity, there are two main approaches in ecology: 1) the physical heterogeneity of the organisms or ecological entities in the space or 2) the heterogeneity of qualitative or quantitative values of parameters in a continuum space (DUTILLEUL, 1993). These two different approaches are closely related, because environmental heterogeneity affects directly organism's composition and distribution (MARGALEF, 1991). For example, as the environment gets more complex physically, the complexity of biological communities and diversity increase as well (DESHMUCK, 1986).

Reservoirs exhibit a marked degree of spatial heterogeneity (TUNDISI, 1996). The progressive physical, chemical and biological changes along the main axis of a reservoir

(river–lake transition) frequently result in strong spatial gradients (NOGUEIRA *et al.*, 1999). These gradients are not attributed only to the transformations of rivers into lakes, but also to the great influence that rivers continue to exert in the reservoir (MARCÉ, 2007). Additional heterogeneity can result from dendritic nature of reservoir basin (KIMMEL *et al.*, 1990) and from interactions with other ecosystems from the hydrographic basin, if the tributary rivers exhibit distinct characteristics due to geological origin or human interference, for example (TUNDISI, 1996).

The above mentioned abiotic factors that promote environmental heterogeneity are important to the structure and functioning of the biotic communities. In particular, phytoplankton productivity and biomass are highly influenced by spatial variation, such as longitudinal gradients in basin morphology, flow velocity, water residence time, suspended solids, and light and nutrient availability (KIMMEL *et al.*, 1990). Zooplankton organisms respond to changing quantity or quality of food resources by altering their reproductive performance, resulting in populations increase near the source of food (MARZOLF, 1990).

The objective of this study was to investigate the spatial horizontal heterogeneity of the planktonic communities along the longitudinal axis of Guarapiranga reservoir (São Paulo, Brazil). Thus, we hypothesized that:

- 1) the establishment of compartments with different water qualities will produce changes in phytoplankton community structure;
- 2) zooplankton community structure will follow similar pattern as shown by the phytoplankton community, as these two planktonic communities are closely related.

Therefore, we expect that water quality, phytoplankton and zooplankton data will evidence similar compartments in Guarapiranga reservoir.

2. Methodology

Study site

This study was carried out in Guarapiranga reservoir, a sub-basin of Alto do Tietê basin, located at 742 m of altitude in São Paulo city, Brazil (23°43'S/46°32'W, Figure 1). Guarapiranga's basin has 639 km² of watershed area and the reservoir itself can store 194

million m³ of water. The maximum depth is 13 m and the average depth is 6 m. It is considered an urban reservoir and it is surrounded mainly by the municipality of São Paulo (the biggest South American city) in its right shore and in part of the left shore, summing up 70% of the reservoir's perimeter. The main tributaries are Embu-Mirim river, Embu-Guaçu river and Parelheiros river, beside many others small streams and creeks. Guarapiranga's current uses are water supply, flood control, electric power generation and recreation. This reservoir supplies 3.7 million people in São Paulo metropolitan area (20% of São Paulo city population) (WHATELY & CUNHA, 2006).

Since the 60's, Guarapiranga reservoir is under eutrophication process due to urban sewage water input (ROCHA, 1976). As São Paulo city grew, Guarapiranga's watershed underwent through the impact of urban expansion. The reservoir's surroundings were occupied mainly by illegal human settlements, without proper sewage water catchment (BEYRUTH, 1996). Thus, sewage water was launched directly in the reservoir. Moreover, mineral and sand extraction contributed to the deforestation, erosion and sedimentation of the tributaries. Eutrophication problems remain until today (WHATELY & CUNHA, 2006).

Sampling and variables analyses

In October 27th, 2010, we sampled nine stations in Guarapiranga reservoir (Figure 1): "EG1", "EG2" and "EG3" at Embu-Guaçu branch, "P1" and "P2" at Parelheiros branch, "EM" at Embu-Mirim branch, "C1" and "C2" at the central body of the reservoir and one sampling station at the "Dam". Vertical profiles of temperature (T), dissolved oxygen (DO), pH and electric conductivity (EC) were measured at every sampling station, in every 50 cm from the surface to the bottom with standard electrodes (YSI 556). Thermocline was considered a drop of at least 1 °C in 1 m depth (WETZEL, 2001). Transparency was measured by Secchi disk (Z_{SD}) and euphotic depth (Z_{eu}) was estimated (AROCENA, 1999).

Integrated water samples (0–3 m) were collected with a plastic pipe in order to sample the whole euphotic zone in each sampling station. Sub-samples were isolated and kept in the dark and cold until arrival at the laboratory. The integrated water samples were analyzed for ammonium, nitrite and nitrate summed as total inorganic nitrogen (TIN, MACKERETH *et al.*, 1978), silicate (SRSi) and soluble reactive phosphorus (SRP, STRICKLAND & PARSONS, 1960), total nitrogen (TN) and total phosphorus (TP, VALDERRAMA, 1981), chlorophyll-a corrected for phaeophytin using 90% acetone extraction (LORENZEN, 1967;

WETZEL & LIKENS, 1991), and total solids (TS) and suspended solids (total: TSS, organic: OSS and inorganic: ISS) (WETZEL & LIKENS, 1991). Trophic State Index (TSI) was calculated based on TP and Chla according to Lamparelli (2004) for Brazilian reservoirs.

Figure 1. Guarapiranga reservoir, its main tributaries and sampling stations locations.

Plankton sampling and analyses

Sub-samples (100 ml) of the integrated water column were isolated and preserved with Lugol's iodine solution for phytoplankton community analysis in each sampling station. Phytoplankton species were identified based on specific bibliography and according to Van den Hoek (1997), except for Cyanobacteria (KOMÁREK & ANAGNOSTIDIS, 1999, 2005) and Bacillariophyceae (ROUND *et al.*, 1992) in a Carl Zeiss ScopeA1 microscope. Phytoplankton cells were counted using the settling technique (UTERMÖHL, 1958) in 2 ml settling chambers in a Carl Zeiss Axiovert40C inverted microscope. Sedimentation time followed Lund *et al.* (1958). A minimum of 400 individuals (cells, colonies or filaments) was counted in each sample giving a counting accuracy, expressed in terms of 95% confidence limits, of < 10% for the whole phytoplankton population (LUND *et al.*, 1958). Biovolume was obtained by geometric approximation, multiplying each species' density by

the mean volume of its cells considering, whenever possible, the mean dimension of 30 individual specimens of each species (HILLEBRAND *et al.*, 1999). Algal biomass was estimated assuming a specific gravity for algal cells of 1 mg/mm³. Phytoplankton species that contributed with more than 5% of the total biomass of the sample were considered a descriptors species of the community and included in the data analysis. Species that contributed with more than 50% of the total biomass of the sample were considered dominant (LOBO *et al.*, 2002).

Descriptor phytoplankton species were assigned to CRS life strategies according to their morphological and physiological traits (REYNOLDS, 1988): (1) C-strategists: competitive species with a short lifespan, characterized by small cells with a high surface area-to-volume ratio from environments with low disturbance levels; (2) R-strategists: ruderal species that are generally favored by high-resource and low-energy conditions; (3) S-strategists: stress tolerant species with large single-cells or colonies of small cells whose general motility allows them to regulate their position in the water column.

Zooplankton samples were collected in a 64 µm mesh net in vertical array 0-3 m depth in each sampling station. Filtered volumes were estimated by geometric approximation (sampled depth and aperture area of the net) for the quantitative analysis. Zooplankton organisms were narcotized with carbonated water and the samples were preserved with 4% formalin-sucrose solution. Zooplankton was identified according to specialized literature (e.g. KOSTE, 1978; REID, 1985; ELMOOR-LOUREIRO, 1997; NOGRADY & SEGERS, 2002). Sub-samples of the zooplankton sample were counted in a Sedgwick-Rafter chamber for rotifers and in a counting chamber for crustaceans until 100 individuals of the most abundant species were reached.

Data analyses

Cluster hierarchical analyses were performed to assess the compartmentalization of the sampling stations in Guarapiranga reservoir based on the limnological variables (water quality) and the plankton communities. A first cluster hierarchical analysis (Ward's clustering method and Euclidean distance) was performed with all physical, chemical and biological variables (all included variables, see Electronic table 1). A second set of cluster hierarchical analyses (Ward's clustering method and Manhattan distance) was performed with the plankton community data: 1) phytoplankton descriptor species' biomass, 2) phytoplankton CRS strategist's biomass and 3) zooplankton individual species' density.

To explore if the groups originated from each cluster analysis were statistically distinct from each other, the groups were tested with simple or multiple discriminant analysis (LEGENDRE & LEGENDRE, 1998). To certify the independency of the variables, tolerance level of 0.01 was established in the discriminant analyses. Correlation between phytoplankton and zooplankton communities was assessed by Spearman's rank correlation coefficient (between phytoplankton biomass and zooplankton density).

In all statistical analyses, water quality variables were transformed by range $[(x - x_{\min}) / (x_{\max} - x_{\min})]$ in order to keep the same amplitude to all variables. Phytoplankton and zooplankton data were transformed by $[\log(x+10)]$ in order to reduce the variance in the dataset. All analyses mentioned above were performed with STATISTICA (version 7.0) Software.

3. Results

Water quality and horizontal heterogeneity

Figure 2 shows the vertical profiles of the water column in the sampling stations followed by T, EC, pH and DO. Thermal stratification was only observed in P1, with a thermocline at 0.5 m deep. In the others sampling station, gradual decrease in temperature in the bottom direction was observed, without a thermocline *per se*. DO and pH followed T pattern. EC values were homogenous along the water column in all sampling stations, except in EG3, when it gradually increased in the bottom direction. EC values were much lower in Embu-Guaçu sampling stations, especially in EG1. As expected, Zmax increased toward the dam direction, ranging from 3.0 to 9.2 m (Electronic table 1). All analyzed variables in all sampling stations along Guarapiranga reservoir's longitudinal axis are indicated in Electronic table 1.

Electronic table 1. Physical, chemical and biological from the nine sampling stations along Guarapiranga reservoir's longitudinal axis. Zmax: maximum depth, Zeu: euphotic depth, T: temperature, DO: dissolved oxygen, Sat: oxygen saturation, EC: electric conductivity, SRSi: silicate, TIN: total inorganic nitrogen, SRP: phosphate, TN: total nitrogen, TP: total phosphorous, TS: total solids, TSS: total suspended solids, OSS: organic suspended solids, ISS: inorganic suspended solids, Chl a: chlorophyll-a, Phaeo: phaeophytin, TSI: trophic state index (M: mesotrophic, E: eutrophic), Phyto: phytoplankton biomass, Zoo: zooplankton density.

Variable (unit)	EG1	EG2	EG3	P1	P2	EM	C2	C1	Dam
Zmax (m)	4.9	3.0	6.7	3.4	5.4	4.7	8.0	9.2	7.8
Zeu (m)	2.2	3.0	3.4	2.3	2.8	2.3	2.7	2.8	2.9
T (°C)	21.4	21.4	21.3	21.2	20.9	22.2	21.3	21.6	21.9
DO (mg/l)	7.0	7.6	8.2	5.9	6.0	8.5	8.0	8.3	9.0
Sat (%)	79.8	86.5	93.5	65.6	67.2	98.1	90.7	92.9	103.7
pH	7.0	7.1	7.2	7.0	6.9	7.5	7.3	7.4	7.3
EC (µS/cm)	39	69	78	118	110	117	108	110	111
SRSi (µg/l)	2.0	1.3	0.7	0.7	0.6	0.9	0.8	0.7	0.7
TIN (µg/l)	201.6	423.3	629.9	1573.6	1359.0	1339.6	1336.8	1063.7	1399.1
SRP (µg/l)	<10	<10	<10	<10	<10	<10	<10	<10	<10
TN (µg/l)	599.5	910.7	971.6	2023.9	1846.2	1772.7	2042.9	1564.3	1482.5
TP (µg/l)	31.3	25.2	30.8	96.1	84.2	79.4	63.8	57.7	54.9
TS (mg/l)	50.5	43.0	69.5	71.5	81.5	76.5	71.5	66.5	71.5
TSS (mg/l)	6.4	4.1	4.8	6.8	9.4	6.2	6.5	6.0	4.8
OSS (mg/l)	3.4	3.2	3.2	5.2	4.7	5.2	5.7	5.2	4.5
ISS (mg/l)	3.0	0.9	1.5	1.6	4.7	1.0	0.8	0.8	0.3
Chl a (µg/l)	11.8	14.0	18.9	25.4	14.2	19.4	24.4	23.7	20.4
Phaeo (µg/l)	1.4	2.8	4.1	3.9	3.7	2.8	1.3	6.2	3.7
TSI	57 (M)	57 (M)	58 (M)	62 (E)	61 (E)	61 (E)	61 (E)	61 (E)	60 (E)
Phyto (mg/l)	23.6	15.3	56.0	53.3	65.5	49.1	37.2	31.1	105.1
Zoo (org/l)	77.5	150.1	281.5	581.4	550.3	229.6	253.8	259.9	1241.6

Figure 2. Vertical profiles of temperature (T), dissolved oxygen (DO) pH and electric conductivity (EC) of the water from the nine sampling stations along Guarapiranga reservoir's longitudinal axis.

Cluster analysis suggested the presence of heterogeneity along Guarapiranga reservoir's longitudinal axis based on water quality (Figure 3). Three clear compartments were formed: 1) Embu-Graçu region (EG1, EG2 and EG3); 2) Parelheiros region (P1 and P2); and 3) Down part of the reservoir, which will be called dam region (Dam, C1, C2 and EM). According to the multiple discriminant analysis, the three compartments formed by the cluster analysis based on water quality were different between each other ($p < 0.05$). The

most important variables to discriminate each compartment were OSS ($p=0.037$) and TP ($p=0.049$) in Embu-Guaçu and DO ($p=0.017$) and SRSi ($p=0.018$) in Parelheiros.

Figure 3. Hierarchical cluster dendrogram (Euclidean distance and Ward's linkage method) of the water quality variables from the nine sampling stations along Guarapiranga reservoir's longitudinal axis.

Figure 4 shows the variables that displayed clearer trend along the sampling station as well as among the compartments. Worsening in water quality (TSI) in the dam direction in the epilimnetic zone was observed (Figure 4a). EC, TIN and TP values in the epilimnetic layers showed clear gradients, with lower values in Embu-Guaçu region, intermediate values in the dam region and higher values in Parelheiros region (Figure 4b, Figure 4c and Figure 4d). DO concentrations were much lower in Parelheiros region compared to the rest of the reservoir (Figure 4e) and SRSi concentrations were higher in Embu-Guaçu region, especially in EG1 (Figure 4f).

Figure 4. Variability of a) Trophic State Index (TSI; O: oligotrophic; M: mesotrophic; E: eutrophic), b) electric conductivity (EC), c) total inorganic nitrogen (TIN), d) total phosphorous (TP), e) dissolved oxygen (DO) and (f) silicate (SRSi) along sampling stations in Guarapiranga reservoir. Sampling stations are grouped into the compartments provided by the hierarchical cluster analysis based on physical, chemical and biological data (Figure 3).

Phytoplankton community: composition, biomass and horizontal heterogeneity

Phytoplankton biomass ranged from 15.3 (EG2) to 105.1 mg/l (Dam) (Figure 5). Phytoplankton composition varied along the sampling stations (Table 1, Figure 6a). R-strategist centric diatoms *Urosolenia eriensis* and *Cyclotella meneghiniana* were most prominent in the mesotrophic Embu-Guaçu region, which displayed higher SRSi concentration. In particular, *U. eriensis* was present only in Embu-Guaçu region. C-strategists species such as *Aphanocapsa delicatissima*, *Cryptomonas curvata* and *Acanthosphaera zachariasii* were dominant in Parelheiros region, which was characterized by higher nutrients availability and lower DO concentration. Conversely, in the dam region, C-strategists (e.g. *Aphanocapsa delicatissima*) and S-strategists (e.g. *Dolichospermum spiroides*, *Eudorina illinoisensis* and *Gymnodinium fuscum*) tended to co-dominate, except in C1. In C1, S-strategists filamentous N₂-fixing cyanobacteria *Dolichospermum spiroides* and large ellipsoidal dinoflagellate *Peridinium gatunense* were the most prominent species.

Table 1. Phytoplankton descriptor species sorted by taxonomical class and the respective CRS-strategy classification (CRS) and biomass from the nine sampling stations in Guarapiranga reservoir's longitudinal axis.

Class	CRS	Biomass per sampling station (mg/l)								
Species		EG1	EG2	EG3	P1	P2	EM	C2	C1	Dam
Bacillariophyceae										
Urosolenia eriensis (H.L.Smith) Round & R.M.Crawford	R	6.5	15.2	8.1	-	-	-	-	-	-
Aulacoseira ambigua (Grunow) Simonsen	R	-	-	-	1.9	2.4	1.7	3.3	-	3.0
Cyclotella meneghiniana Kützing	R	-	-	11.6	-	-	-	-	-	1.9
Nitzschia fruticosa Hustedt	R	-	-	-	-	-	-	-	-	3.0
Chlorophyceae										
Botryococcus neglectus (West & G.S.West) J.Komárek & P.Marvan	S	-	-	-	-	-	-	-	1.8	-
Eudorina illinoisensis (Kofoid) Pascher	S	-	-	5.8	-	9.1	6.7	-	-	33.6
Acanthosphaera zachariasii Lemmermann	C	-	-	-	-	9.2	7.5	-	-	-
Actinastrum hantzschii var. subtile J.Woloszynska	C	-	-	1.4	-	-	-	-	1.3	4.0
Coelastrum indicum W.B.Turner	R	-	-	-	-	-	1.4	-	-	-
Desmodesmus denticulatus (Lagerheim) S.S.An, T.Friedl & E.Hegewald	C	-	-	-	-	-	-	-	-	1.2
Pediastrum duplex var. gracillimum West & G.S.West	R	-	-	4.6	1.8	1.6	-	-	2.7	1.5
Cryptophyceae										
Cryptomonas brasiliensis A.Castro, C.Bicudo & D.Bicudo	C	-	-	-	-	-	-	1.3	-	4.7
Cryptomonas curvata Ehrenberg	C	-	-	5.6	9.2	1.8	-	-	-	-
Chrysophyceae										
Dinobryon sertularia Ehrenberg	R	1.3	1.6	-	-	-	-	-	-	-
Cyanophyceae										
Dolichospermum spiroides (Kleb.) Wacklin, L.Hoffm. & Komárek	S	-	-	-	-	2.4	5.6	5.6	4.6	6.7
Aphanocapsa delicatissima West & G.S.West	C	-	3.2	5.6	12.1	19.4	11.3	11.8	-	21.4
Aphanocapsa incerta (Lemmermann) Cronberg & Komárek	C	-	-	-	-	-	-	-	1.2	-

Cont. Table 1

Class	CRS	Biomass per sampling station (mg/l)								
Species		EG1	EG2	EG3	P1	P2	EM	C2	C1	Dam
<i>Phormidium</i> sp.	R	-	-	3.8	-	-	-	-	-	-
Dinophyceae										
<i>Peridinium gatunense</i> Nygaard	S	-	-	-	13.4	3.1	3.4	-	2.6	-
<i>Peridinium</i> sp.	S	-	-	-	-	-	-	-	1.8	-
<i>Gymnodinium fuscum</i> (Ehrenberg) F.Stein	S	-	-	-	5.0	4.7	2.1	6.1	-	2.8
Euglenophyceae										
<i>Trachelomonas volvocinopsis</i> Svirenko	C	-	-	-	2.1	-	-	-	-	1.5
Xanthophyceae										
<i>Tetraedriella spinigera</i> Skuja	C	-	-	-	-	2.7	-	-	-	-
Zygnematophyceae										
<i>Cosmarium</i> sp.	R	-	-	-	-	-	-	-	-	4.9
<i>Mougeotia</i> sp.	R	-	-	-	-	-	1.5	-	1.5	4.6

The cluster analysis based on phytoplankton descriptor species' biomass resulted in three compartments: 1) EG1, EG2, EG3, C1; 2) P1, P2, C2, EM; and 3) Dam (Figure 7a). These compartments did not overlap exactly the compartments formed by the water quality variables (Figure 3). Moreover, members within each compartment neither were close geographically nor presented similar environmental characteristics. Still, cluster analysis based on phytoplankton CRS-strategists' biomass formed different three compartments: 1) Embu-Guaçu region (EG1, EG2, EG3); 2) P1, EM, P1, C1, C2; and 3) Dam (Figure 7b). CRS-strategists biomass segregated mesotrophic Embu-Guaçu region from the rest of the reservoir and isolated the Dam sampling station. Although visually CRS classification provided a clearer cluster arrangement of the sampling stations in comparison to phytoplankton individual species, according to the simple discriminant analysis, none of them formed compartments distinct from each other. For both individual species and CRS-strategists, the two main compartments (1 and 2) formed by the cluster analysis were not different between each other ($p>0.05$). In these cases, the third compartment (Dam) could not be included in the discriminant analyses because there was only one sampling station in this compartment. Accordingly, phytoplankton community did not exhibited horizontal heterogeneity along Guarapiranga reservoir's longitudinal axis.

Zooplankton community: composition, density and horizontal heterogeneity

Zooplankton density ranged from 78.5 (EG1) to 1730.9 org/l (Dam) and it exhibited similar trend as the phytoplankton community along the sampling stations (Figure 5). Positive correlation between phytoplankton biomass and zooplankton density was found by Spearman's rank correlation coefficient ($r_s=0.73$; $p<0.05$). This correlation is a good reflection of the close relationship between both communities.

Rotifers were dominant in the zooplankton community in all sampling stations, decreasing their relative abundance in the dam direction (Table 2, Figure 6b). In Embu-Guaçu region, rotifers *Conochilus unicornis* and *Polyarthra* aff. *vulgaris* were the most prominent and in Parelheiros region, these species were replaced by *Kellicottia bostoniensis* and *Filinia opoliensis*. *Polyarthra* aff. *vulgaris*, *Keratella tropica* and *K. cochlearis* were very important in the dam region, especially in the Dam sampling station. Copepod's contribution to the total density was very low in Embu-Guaçu region, increasing its contribution in the other sampling stations, especially in the dam region. In Particular, Cyclopoid copepods *Eucyclops subciliatus* and *Mycrocyclops anceps* were only found in Embu-guaçu region. Conversely, Cyclopoid copepods *Thermocyclops decipiens* was found in all sampling

stations, especially in Parelheiros region (P1 and P2). Cladoceran's abundance was very low in all sampling stations, which main representative species were *Bosminopsis deitersi*, *Bosmina longirostri* and *Moina minuta*.

The cluster analysis based on zooplankton species' density formed three groups: 1) EG1, EG2, EG3, C1, C2, EM; 2) P1, P2; and 3) Dam (Figure 7c). Although this arrangement segregated the Dam sampling station and the Parelheiros region from the rest of the reservoir, a large and heterogeneous group was formed with the remaining sampling stations, gathering locations with distinct environmental characteristics (e.g. Embu-Guaçu region and central sampling stations). However, based on the zooplankton community, the two main compartments (1 and 2) were different between each other, according to the discriminant analysis ($p=0.0006$). The third compartment (Dam) could not be included in the discriminant analysis because there was only one sampling station in this compartment. Accordingly, zooplankton community did exhibited horizontal heterogeneity along Guarapiranga reservoir's longitudinal axis. However, the pattern did not followed exactly the same compartments resulted by the water quality variables.

Figure 5. Phytoplankton biomass and zooplankton density in the nine sampling stations along Guarapiranga reservoir's longitudinal axis.

Figure 6. A) Relative biomass of phytoplankton CRS-strategists along the sampling stations in Guarapiranga reservoir. B) Relative density of the main zooplankton groups found in the nine sampling stations in Guarapiranga reservoir's longitudinal axis.

Figure 7. Hierarchical cluster dendrogram (Ward's method; Manhattan distance) A) of the phytoplankton descriptor species biomass, B) of the phytoplankton CRS-strategists biomass and C) of the zooplankton density from the nine sampling stations along Guarapiranga reservoir's longitudinal axis.

Table 2. Zooplankton species sorted by main groups and its density from the nine sampling stations in Guarapiranga reservoir's longitudinal axis.

Group Species	Density per sampling station (org/l)								
	EG1	EG2	EG3	P1	P2	EM	C2	C1	Dam
Copepoda Cyclopoida									
<i>Acanthocyclops robustus</i> (GO Sars, 1863)	-	-	-	-	-	-	-	0.03	0.02
<i>Eucyclops subciliatus</i> (Dussart, 1984)	-	0.01	-	-	-	-	-	-	-
<i>Mycrocyclops anceps</i> (Richard, 1897)	-	0.03	-	-	-	-	-	-	-
<i>Thermocyclops decipiens</i> (Kiefer, 1929)	0.01	0.01	-	26.14	1.16	0.03	0.26	1.11	0.07
<i>Thermocyclops inversus</i> (Kiefer, 1936)	-	-	-	0.26	0.10	-	-	-	-
Cladocera									
<i>Bosmina longirostri</i> (O.F. Muller, 1785)	0.01	-	0.62	0.40	0.72	0.03	0.74	-	0.07
<i>Bosminopsis deitersi</i> (Richard, 1895)	0.13	9.34	7.21	9.11	16.05	0.08	5.58	-	0.11
<i>Ceriodaphnia cornuta</i> (Sars, 1886)	-	0.37	-	-	-	-	-	-	0.02
<i>Daphnia gessneri</i> (Herbst, 1967)	-	0.01	0.04	0.07	0.06	-	-	-	-
<i>Diaphanosoma birgei</i> (Korinek, 1981)	-	0.03	-	-	0.11	0.11	0.11	-	0.09
<i>Iliocryptus spinifer</i> (Herrich, 1884)	-	0.04	-	-	-	-	-	-	0.02
<i>Moina minuta</i> (Hansen, 1899)	-	0.01	0.26	0.40	0.83	-	0.21	-	0.02
<i>Simocephalus serrulatus</i> (Koch, 1841)	-	0.06	-	-	-	-	-	-	-
Rotifera									
<i>Anuraeopsis navicula</i> (Rousselet, 1911)	0.33	-	2.28	5.55	4.64	0.88	0.95	1.39	5.49
<i>Asplanchna</i> sp.	-	-	0.57	0.79	-	8.33	0.47	-	-
<i>Brachionus angularis</i> (Gosse, 1851)	0.16	-	-	-	-	0.88	-	-	-
<i>Brachionus calyciclorus</i> (Bryce 1931)	-	0.34	-	3.17	6.19	3.95	5.69	5.11	17.84
<i>Brachionus caudatus</i> (Ahlstrom, 1940)	-	-	-	-	-	3.51	-	-	-
<i>Brachionus mirus</i> (Daday, 1905)	0.65	0.34	2.28	-	3.09	-	-	-	-
<i>Collotheca</i> sp1.	5.22	8.83	2.85	-	1.55	-	-	-	-

Cont. Table 2

Group Species	Density per sampling station (org/l)								
	EG1	EG2	EG3	P1	P2	EM	C2	C1	Dam
<i>Collotheca</i> sp3.	-	1.70	-	-	-	-	-	-	-
<i>Conochilus coenobasis</i> (Skorikov, 1914)	13.70	18.67	20.54	68.13	61.10	0.44	12.32	-	1.37
<i>Conochilus unicornis</i> Rousselet, 1892	18.75	31.24	38.80	38.03	70.38	-	11.37	0.46	2.06
<i>Filinia opoliensis</i> (Zacharias, 1898)	-	-	9.13	73.68	88.16	2.19	5.21	0.93	4.12
<i>Gastropus hyptopus</i> (Ehrenberg, 1838)	3.26	1.36	2.85	1.58	1.55	-	-	-	-
<i>Hexarthra</i> sp.	-	-	-	-	-	-	0.47	-	-
<i>Kellicottia bostoniensis</i> (Rousselet, 1908)	0.33	5.09	55.35	236.09	98.99	0.44	1.42	1.86	-
<i>Keratella americana</i> (Carlin, 1943)	0.33	9.17	16.55	7.13	20.88	18.86	9.95	17.19	41.85
<i>Keratella cochlearis</i> (Gosse, 1851)	4.73	6.79	20.54	33.27	75.79	29.82	28.91	34.37	283.37
<i>Keratella lenzi</i> (Hauer, 1953)	0.33	0.68	1.71	0.79	-	4.82	2.37	6.97	47.34
<i>Keratella tropica</i> (Apstein, 1907)	-	-	5.71	5.55	22.43	45.17	71.09	176.97	705.35
<i>Lecane bulla</i> (Gosse, 1851)	-	-	0.57	-	-	-	-	-	-
<i>Ploesoma</i> sp.	3.75	13.58	2.85	1.58	0.77	0.88	5.69	0.93	3.43
<i>Polyarthra vulgaris</i> (Carlin, 1943)	13.54	32.59	76.46	57.83	60.32	73.68	64.45	3.72	83.02
<i>Proales</i> sp.	-	-	-	-	-	4.82	-	0.46	1.37
<i>Synchaeta</i> sp.	3.10	4.07	10.27	10.30	10.05	19.74	22.27	2.32	19.90
<i>Trichocerca</i> sp.	1.14	1.36	1.14	1.58	1.55	3.07	0.47	0.46	7.55
<i>Trichocerca similis</i> (Wierzejski, 1893)	-	0.68	0.57	-	3.87	7.89	3.79	5.57	17.15

4. Discussion

Previous works revealed that Guarapiranga reservoir exhibits spatial heterogeneity along the longitudinal axis in several aspects, such as water quality and trophic status (CARDOSO-SILVA, 2008), metals in sediment (PADIAL, 2008) and aquatic macrophytes distribution (RODRIGUES, 2011). Except for Domingos (1993), who investigated the distribution of zooplanktonic community in Guarapiranga reservoir, little is known about planktonic organisms' distribution in this reservoir. Our study showed a clear tendency of compartmentalization of the epilimnetic layers in Guarapiranga reservoir, especially based on water quality. Guarapiranga reservoir's compartments were not well defined as proposed by Kimmel *et al.* (1990). Riverine, transition and lacustrine zones within a reservoir are not discrete and invariable entities, but result from the combined effects of a number of overlapping gradients (KIMMEL *et al.*, 1990), such as, nutrients input through sewage water and agriculture, tributaries water quality, algacide treatment and retention time as will be discussed further in this section.

One of the main factors influencing the process of temporal and spatial compartmentalization in reservoirs is water retention time (STRASKRABA *et al.*, 1993). Maximum environmental gradients are expected in reservoirs with high water retention time, high sedimentation rates, and flow that is controlled by advective processes (KENNEDY & WALKER, 1990). The long retention time of Guarapiranga reservoir (185 days, CARVALHO *et al.*, 2007) is one of the possible factors promoting the compartmentalization of the reservoir in zones exhibiting different characteristics on water quality. However, long retention time does not promote direct advective effects of water renewal on phytoplankton abundance (KIMMEL *et al.*, 1990), interfering on the formation of clear compartments based on phytoplankton biomass.

The process of vertical stratification has also a strong influence on the compartmentalization of reservoirs, because it influences the metabolism and structure of the ecosystem, not only in the vertical but also in the longitudinal axis (NOGUEIRA *et al.*, 1999). In São Paulo state, most reservoirs, including Guarapiranga, are polymictic due to their shallowness and continuous wind action (ARCIFA *et al.*, 1981; MATSUMURA-TUNDISI *et al.*, 1981; TAKINO & MAIER, 1981). In our study, mixing was more frequent than stratification in the sampling stations, indicating the polymictic pattern of Guarapiranga reservoir. The influence of thermal stratification on ecosystem metabolism,

especially in the lacustrine zone, can be demonstrated by the vertical distribution of some chemical variables, such as DO and pH, which followed the temperature pattern.

In general, reservoir branches, when present, contribute to the system heterogeneity (NOGUEIRA, 2001). Thus, reservoir branches may exhibit different features from the main axis of the reservoir, reflecting their tributaries' water quality. Embu-Guaçu and Parelheiros regions are Guarapiranga reservoir's branches, influenced mainly by their tributaries, Embu-Guaçu river and Parelheiros stream, respectively. Embu-Guaçu river is located in a well preserved area, without severe human impact. Accordingly, Embu-Guaçu region exhibited lower trophic status and lower nutrients associated with eutrophication process. Moreover, Embu-Guaçu branch has a semifluvial nature and relatively high levels of turbulence and SRSi loading into the reservoir, favoring the growth of large and non-motile R-strategists diatoms (WATSON & KALFF, 1981). In particular, centric diatom *Urosolenia eriensis* is characteristic of environments with low trophic status (PADISÁK *et al.*, 2009) and was found only in Embu-Guaçu region. This finding is a good reflection of the better water quality in the upper part of the reservoir under influence of Embu-Guaçu river.

Parelheiros branch's tributary is Parelheiros stream, which receives high loads of domestic sewage water from the irregular human settlements in the surroundings (WHATELY & CUNHA, 2006). The poor water quality that enters Parelheiros region is responsible for the characteristics of this compartment, such as eutrophication, high EC and nutrients and low DO. Small-celled, fast-growing C-phytoplankton species were dominant in this compartment. Phytoplankton C-strategists have generally low rates of sinking and are highly susceptible to grazing zooplankton (REYNOLDS *et al.* 2002). Phytoplankton C-strategists low rates of sinking could have been favoring their dominance in P1, the only sampling station where stratification was detected. Moreover, low abundance of grazing zooplankton could also have favored C-strategists growth.

While Embu-Guaçu and Parelheiros regions are located in the uplake riverine zone of the reservoir, the dam region gathered sampling stations with transitional and lacustrine characteristics, according to Kimmel (1990) classification. In Guarapiranga reservoir, it seems that allochthonous nutrient inputs become the predominant process in nutrient dynamics. Therefore, eutrophication process and high nutrient content are possibly consequences of the land uses in the surrounding of this compartment (human settlement without proper sewage water treatment and agriculture) (BEYRUTH, 1996). Moreover, the down part of reservoirs (lacustrine zone) usually presents higher retention time and

greater depth which contribute to the accumulation of elements (BEYRUTH, 2000), such as nutrients and suspended solids, leading to eutrophication, higher phytoplankton biomass and zooplankton density, as our data showed. The ability of flagellated and motile S-strategists phytoplankton (e.g. *Dolichospermum spiroides*, *Eudorina illinoisensis* and *Gymnodinium fuscum*) to adjust vertical position considerably enhances the opportunities to exploit the nutrient resources available in the dam region's water column (RAVEN & RICHARDSON, 1984), enabling their co-dominance with C-strategist phytoplankton species. During the field work, the environmental agency (SABESP) was adding copper sulphate in the down part of the reservoir, near sampling station C1. The algaecide application acts as an anthropogenic disturbance for the phytoplankton community, favoring invasive opportunist's fast-growing C-species.

Without reliable data from the environmental agency, it is impossible to know the total extent and the exactly frequency of the copper sulphate treatment in the reservoir's water body. Accordingly, it is impossible to know how much phytoplankton community is being influenced by the algaecide action. Studies on the copper sulphate effect on the phytoplankton community showed that this algaecide caused remarkable effects on the sequence of dominance (BEYRUTH, 2000) and abrupt phytoplankton changes in density (PADOVESI-FONSECA & PHILOMENO, 2004) in Brazilian reservoirs. Therefore, it is possible to infer that copper sulphate action is one of the main factors determining the phytoplankton distribution in Guarapiranga reservoir, explaining the absence of clear compartments based on phytoplankton biomass.

Phytoplankton consumption by heterotrophs is an important cause of phytoplankton loss (PADISÁK, 2004) and cannot be neglected in phytoplankton studies. Filter feeding efficiency is determined by the size of zooplankton population involved and the physical sizes (and other properties such as digestibility) of phytoplankton particles as compared with the abilities of grazers to take up the given size-spectrum (PADISÁK, 2004). Therefore, the close relation between phytoplankton and zooplankton communities found in our study might be attributed to ecological interactions, such as grazing or competition for nutrients. In a previous study in Guarapiranga reservoir, Caleffi (2000a) found that zooplankton community structure was being driven mainly by the phytoplankton community, especially by its potential as feeding resource by herbivorous zooplankton.

Rotifers were the most abundant organisms of zooplankton in all sampling stations. These small sized organisms react faster than other zoological groups of freshwater zooplankton to changes in water conditions, due to their short development cycle, being considered as

the group with more sensibility to physical and chemical changes that occur in the environment (GANNON & STEMBERGER, 1978). They can also develop numerous populations using low quality food resources, such as organic detritus (MANGAS & GARCIA, 1991). Among rotifers species, high density of *Keratella tropica* was associated with lentic characteristics of the environment and was found in high densities in the Dam region. Two eurytopic species *Polyarthra* aff. *vulgaris* and *Keratella cochlearis* (BIELANSKA-GRAJNER & GLADYSZ, 2010) were also important for the total density of the zooplankton community in the dam region. Due to the tolerance to a wide range of environmental conditions, *P. vulgaris* was also present in high densities in Embu-Guaçu region, a more preserved and less eutrophic area. The genus *Collotheca* was found only in Embu-Guaçu region and might be associated to less eutrophic conditions. *Conochilus unicornis* and *C. coenobasis* were present in high densities both in Embu-Guaçu and Parelheiros region. Therefore, the occurrence of these two species might be more related to lotic conditions than to trophic status of the environment. *Kellicottia bostoniensis* and *Filinia opoliensis* were found in Parelheiros region, confirming their preferences for eutrophic environments (LUCINDA *et al.*, 2004).

As a general rule, rotifers dominate zooplankton in Brazilian natural or artificial inland waters (ROCHA *et al.*, 1995; NOGUEIRA, 2001; SENDACZ & MONTEIRO JR., 2003). But it is important to consider that in terms of total zooplankton biomass, they can have only a minor contribution (MATSUMURA-TUNDISI *et al.*, 1989). However, if the same results were expressed in units of biomass, probably the importance of the copepods in the community structure would be even more prominent.

Copepod abundance increased towards the dam direction. Sartori *et al.* (2009) found association between copepod abundance and increase in the water retention time in Jurumirim reservoir (São Paulo). Copepod increase toward the dam could have been due to lacustrine characteristics in the dam region associated with food resources. Indeed, food resource is one of the main factors responsible for changes in population densities in zooplankton communities (FERGUSON *et al.*, 1982). Copepods cyclopoids capture alimentary particles, such as portions of filamentous and colonial algae (ESTEVEZ & SENDACZ, 1988). Colonial and filamentous cyanobacteria (e.g. *Aphanocapsa delicatissima* and *Dolichospermum spiroides*) and colonial chlorophyta (e.g. *Eudorina illinoisensis*) present in high biomass in the dam region could have served as food resource for cyclopoids populations in this region.

Copepod cyclopoid *Thermocyclops decipiens* is commonly found in environments with high human impact, especially mesotrophic and eutrophic environments (LANDA *et al.*, 2007), because this species can support a wide variety of environmental conditions. Accordingly, *T. decipiens* was found in high density in Parelheiros and Dam regions. In Embu-Guaçu region there was not a dominance of a single species probably due to less eutrophic conditions.

The presence of suitable phytoplankton species as food resource could have helped to sustain equilibrium among microcrustaceans species, especially cladocerans, in the dam region. Although cladocerans density did not increase in this region, in the sampling station Dam, there were several species co-occurring instead of the dominance of a few small sized species, such as *Bosminopsis deitersi* and *Bosmina longirostri*. In eutrophic environments with cyanobacteria dominance, rotifers and small sized microcrustaceans tend to be dominant due to their short life cycle that makes they spend more energy in reproduction than in growth (ESTEVEZ & SENDACZ, 1988; MATSUMURA-TUNDISI & TUNDISI, 2005). Matsumura-Tundisi & Tundisi (2005) observed larger zooplankton species and higher species richness associated with Chlorophyte dominance in an eutrophic Brazilian reservoir. Accordingly, zooplankton composition and density were possibly affecting phytoplankton community structure as our study showed.

Other parameters that were not included in our study, such as predation by planktivorous fishes, probably also affected the spatial and temporal distribution of zooplankton species, especially cladocerans (IGLESIAS *et al.*, 2008). As their population development is slower, cladocerans are more likely to be controlled by a top-down process while rotifer life histories are strongly influenced by bottom-up mechanisms (WALZ, 1997). Copepods populations lay in between as they display intermediate growth rate.

It is important to highlight that copper sulphate treatment can also affect zooplankton community, directly by the toxicity of the algacide (effects on reproduction) or indirectly by changes in the phytoplankton community. In Guarapiranga reservoir, Caleffi (2000b) observed lower zooplankton density and species richness associated with intense copper sulphate treatment. The author affirmed that cladocerans were the most affected group in terms of density. Gusmão (2004) confirmed the high sensitivity of cladocerans by copper sulphate toxicity in mesocosms experiments. According to this study, cladocerans are especially affected by indirect effects such as changes in food availability and competition, leading to small-sized species dominance. Moreover, rotifers seem to be less sensitive to copper sulphate effects and probably are favored by the competition reduction as

microcrustaceans have their density directly or indirectly reduced by the copper sulphate effects.

Though cluster analysis based on water quality resulted in three clear compartments with distinct characteristics, only the zooplankton density captured clear compartments. The phytoplankton biomass was not capable of capturing different compartments in the reservoir. These different outcomes indicate that water quality, phytoplankton and zooplankton communities captured different features from Guarapiranga reservoir's longitudinal axis. Phytoplankton and zooplankton communities in the dam region were altered, directly or indirectly, by the copper sulphate treatment. Therefore, this human intervention was probably the main force driving the phytoplankton composition and biomass in the reservoir. Probably for this reason, the phytoplankton community was not able to capture the reservoir compartmentalization. Consequently, zooplankton structure was affected as well, however, in lesser extent. Therefore, the zooplankton community was still being able to capture the reservoir compartmentalization.

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Capítulo 6. Comparison of phytoplankton grouping methods on the example of a spatially heterogenic reservoir

Abstract

As phytoplankton ecology developed, an urge for functional approaches emerged in the assessment of ecological status in aquatic environments, resulting in different concepts of phytoplankton grouping methods. In this study, we aimed to compare different classification methods (species, taxonomic classes, FG, MFG, FGMB) on the phytoplankton data from a horizontally heterogeneous Brazilian reservoir (Guarapiranga). Physical, chemical, biological and the phytoplankton data from nine sampling station in the epilimnetic zone through the longitudinal axis of Guarapiranga reservoir were analyzed. The data was analyzed by multivariate methods (PCA and CCA) and the degree of concordance between two multivariate configurations (sample scores) was tested with a Procrustes and Protests analyses. Guarapiranga reservoir was divided into three main compartments. Embu-Guaçu region is more protected and, consequently, less eutrophic. Parelheiros region is the most eutrophic due to a polluted tributary. Downstream region of the reservoir displays lentic characteristics. Conductivity, nutrient content and solids in the water seem to be the most important variables determining all phytoplankton groups. All tested classifications were proven to be good predictors of the phytoplankton community. In conclusion, there is no unique possibility for classifying phytoplankton species. There are particular aims and each grouping method will have advantages and disadvantages at reaching these aims. For example, grouping methods can be used in the assessment of horizontal heterogeneity in reservoirs and assist the establishment of management programs.

1. Introduction

Phytoplankton is a rich and diverse community and when attempting to find relationships between species compositions and environmental background the pattern is often

confused (LITCHMAN *et al.*, 2010). To describe and evaluate phytoplankton data, taxonomic grouping (Cyanobacteria, Chlorophyta, Chrysophyta, etc.) was intuitively used and became widespread (REYNOLDS, 1982; SOMMER, 1986). Moreover, water quality indices using these taxonomic groups were developed (THUNMARK, 1945; NYGAARD, 1949). As intuitive as it seems, taxonomic affiliation often allows to the prediction of functional behavior (see contributions in the excellent book by Sandgren, 1988). However, this approach failed in many cases because within the same taxonomic lineage morphological and physiological differences can be found. For example, *Synechococcus* and *Cylindrospermopsis* are both Cyanobacteria, though species within these genera behave quite differently and are morphologically distinct from each other. To overcome these issues associated with taxonomic affiliation, Weithoff (2003) proposed the concept of functional diversity (FD) and defined functional traits as a property of an organism that can be measured and the influences one or more essential process such as growth and reproduction.

Important functional traits of phytoplankton organisms are body size and shape (NASELLI-FLORES & BARONE, 2011). These features determine nutrient and energy acquisition capacities, extent and type of interspecific interactions, and therefore adaptive capacities of species with different characteristics (NASELLI-FLORES *et al.*, 2003). As phytoplankton ecology developed, an urge for functional approaches emerged in the assessment of ecological status in aquatic environments, resulting in three different concepts of phytoplankton grouping methods (REYNOLDS *et al.*, 2002; SALMASO & PADISÁK, 2007; KRUK *et al.*, 2010).

Reynolds' (1997; described later in detail by Reynolds *et al.*, 2002) functional group (FG) classification relies on two basic assumptions (NASELLI-FLORES & BARONE, 2011): 1) a functionally well-adapted species is likely to tolerate the constraining conditions offered by a given habitat more successfully than individuals of a less well-adapted species; and 2) a habitat characterized by a defined set of environmental constraints is more likely to be populated by species with the appropriate adaptations to be able to function there. Therefore, FG classification uses concept of habitat template and defines the phytoplankton groups in association with the habitat characteristics where they are more likely to compete successfully. Reynolds *et al.* (2002) labeled 31 groups with alpha-numeric coda. Later, Padisák *et al.* (2009) extended to 40 FG. The FG classification became widely used in case studies from different types of lakes and geographic latitudes (BECKER *et al.*, 2009; PADISÁK *et al.*, 2009; DEVERCELLI, 2010; CUNHA PEREIRA *et al.*, 2011; FONSECA & DE MATTOS BICUDO, 2011; WANG *et al.*, 2011). The FG method was

also tested successfully in estuaries (COSTA *et al.*, 2009) and rivers (ABONYI *et al.*, 2012; STANKOVIĆ *et al.*, 2012). Moreover, this grouping method was adapted for the requirements of the European Union Water Framework Directive (EC PARLIAMENT AND COUNCIL, 2000) for lakes (PADISÁK *et al.*, 2006) and rivers (BORICS *et al.*, 2007).

Salmaso & Padisák's (2007) morpho-functional group (MFG) classifies phytoplankton species based on their morphological characteristics. Thus, this method sorts species into groups with similar features such as ability to active buoyancy regulation (aerotops, flagella) and size, summing up 31 MFGs. The MFG method was first developed to describe seasonal succession of deep, temperate oligotrophic lakes (SALMASO & PADISÁK, 2007). This method was successfully applied in similar lakes (TOLOTTI *et al.*, 2010; CARONI *et al.*, 2012).

Recently, Kruk *et al.* (2010) proposed the morphology-based functional group (MBFG) that is simplified classification based only on morphology. This method defines even MBFG and was successfully applied in several studies cases (PACHECO *et al.*, 2010; TOLOTTI *et al.*, 2010; KRUK *et al.*, 2011).

Successful application of all the three proposed classification methods proved that they are widely accepted. Moreover, all of them were statistically tested with satisfactory results in several case studies (mentioned above). However, each grouping method differs in level of complexity and habitat template sensitivity. In this study, we aimed to compare different classification methods (species, taxonomic classes, FG, MFG, FGMB) on the phytoplankton data from a horizontally heterogeneous reservoir. More specifically, we will compare and evaluate the variance fractions explained by the environmental variables in relation with the use of the original data matrix (species) and the 4 classification criteria. The comparability of the different classifications will be quantitatively assessed using an analysis of congruence (PODANI, 1989). In addition, we will discuss the application of these phytoplankton classification methods on reservoir monitoring by environmental agencies.

2. Methods

Study site

This study was carried out in Guarapiranga reservoir, a sub-basin of Alto do Tietê basin, located at 742 m of altitude in São Paulo, Brazil (23°43'S/46°32'W). It is considered an

urban reservoir and is surrounded mainly by the municipality of São Paulo (the biggest South American city) in its right shore and in part of the left shore, summing up 70% of the reservoir's perimeter. The rest of the perimeter is surrounded by Embu-Guaçu (27%) and Itapecerica da Serra (3%) municipalities. The main tributaries are Embu-Mirim river, Embu-Guaçu river and Parelheiros river, beside many others small streams and creeks. Guarapiranga reservoir was constructed in 1908 for hydroelectrical purposes. Today, Guarapiranga's uses are water supply, flood control, electric power generation and recreation (HELOU & SILVA, 1987). Guarapiranga's basin has 639 km² of watershed area and the reservoir itself can store 194 million m³ of water. The maximum depth is 13 m and the average depth is 6 m. This reservoir supplies 3.7 million people in São Paulo metropolitan area (20% of São Paulo population) (WHATELY & CUNHA, 2006).

Since the 1960's, Guarapiranga reservoir is under eutrophication process due to urban sewage water input (ROCHA, 1976). As São Paulo city grew, Guarapiranga's watershed underwent through the impact of the urban expansion. The reservoir's surroundings were occupied mainly by illegal settlements, without proper sewage water catchment (BEYRUTH, 1996). Thus, sewage water was launched directly in the reservoir. Moreover, mineral and sand extraction contributed to the deforestation, erosion and sedimentation of the tributaries. Eutrophication problems remain until today (CETESB, 2009). Moreover, phytoplankton blooms, especially cyanobacteria, are frequent in the reservoir (CARVALHO *et al.*, 2007; MOSCHINI-CARLOS *et al.*, 2009). According to Whately & Cunha (2006), in 2003, 59% of Guarapiranga's territory was under anthropic or urban use (e.g. agriculture, mining, reforestation, exposed soil, industries, leisure areas, medium and high densities of human population). The remaining Atlantic forest surrounds 37% of the watershed, mainly in the Southern shore.

Sampling and variables analyses

In October 27th, 2010, we sampled nine points through Guarapiranga reservoir's longitudinal axis (Figure 1). Vertical profiles of temperature (T), dissolved oxygen concentration (DO), pH and electric conductivity (EC) were measured at every sampling station, in every 50 cm from the surface to the bottom with a standard electrodes (YSI 556). Transparency was measured by Secchi disk (ZSD) and euphotic depth (Zeu) (AROCENA, 1999) was estimated.

Figure 1. Guarapiranga reservoir, its main tributaries and sampling stations locations.

Integrated water samples (0–3 m) were collected with a plastic pipe in order to sample the whole euphotic zone in each sampling station. Sub-samples were isolated and kept in the dark and cold until arrival at the laboratory. The integrated water samples were analyzed for ammonium, nitrite and nitrate summed as total inorganic nitrogen (TIN, MACKERETH *et al.*, 1978), silicate (SRSi) and soluble reactive phosphorus (SRP, STRICKLAND & PARSONS, 1960), total nitrogen (TN) and total phosphorus (TP, VALDERRAMA, 1981), chlorophyll a corrected for phaeophytin using 90% acetone extraction (LORENZEN, 1967; WETZEL & LIKENS, 1991), total solids (TS, referencia), suspended solids (total, organic and inorganic) (WETZEL & LIKENS, 1991).

Phytoplankton sampling and analyses

Sub-samples (100 ml) of the integrated water column were isolated and preserved with Lugol's iodine solution for phytoplankton community analysis in each sampling station. Phytoplankton species were identified based on specific bibliography and according to Van den Hoek (1997), except for Cyanobacteria (KOMÁREK & ANAGNOSTIDIS, 1999, 2005) and Bacillariophyceae (ROUND *et al.*, 1992) in a Carl Zeiss ScopeA1 microscope. Phytoplankton cells were counted using the settling technique (UTERMÖHL, 1958) in 2 ml settling chambers in a Carl Zeiss Axiovert40C inverted microscope. Sedimentation time followed Lund *et al.* (1958). The number of settling units counted in each individual sample varied according to species accumulation curve. Biovolume was obtained by geometric approximation, multiplying each species' density by the mean volume of its cells considering, whenever possible, the mean dimension of 30 individual specimens of each species (HILLEBRAND *et al.*, 1999). Algal biomass was estimated assuming a specific gravity for algal cells of 1 mg mm³. The phytoplankton species that contributed with more than 5% of the total biomass of the sample were considered a descriptors species of the community and included in the data analysis. Species that contributed with more than 50% of the total biomass of the sample were considered dominant (LOBO *et al.*, 2002).

Data analyses

A Principal Components Analysis (PCA) was performed in order to determine the spatial variability within longitudinal axis of the reservoir. The variables used in this analysis were: Z_{max}, Zeu, Zeu/ Z_{max}, T, DO, pH, EC, SRSi, TIN, TN, TP, N:P, TS, TSS, OSS, ISS and Chla. In order to compare the explained variances of individual species and the four classifications (classes, FG, MFG and MBFG) by environmental variables, multivariate analyses were performed. To check the unimodal response of the phytoplankton data from individual species and the four classifications, five Detrend Correspondence Analyses (DCA) were carried out. All performed DCAs resulted in long gradient lengths (4.3, 2.5, 4.0, 3.8 and 3.1 for species, class, FG, MFG and MBFG, respectively) proving true the unimodal response of both data sets and confirming the suitability of further canonical correspondence analyses (CCA) (LEPS & SMILAUER, 2003). CCAs were performed to explore the explained variance of individual species and the four classifications by the selected environmental variables by forward selection procedure based on a Monte Carlo test using simulations with 499 unrestricted permutations. The explained variance was calculated as the sum of all canonical eigenvalues divided by total inertia. Significance of

the CCAs was evaluated by a Monte Carlo test using simulations with 499 unrestricted permutations. All multivariate analyses were performed with Canonical Community Ordination (CANOCO) for Windows (version 4.5) software and the respective plots were produced in CanoDraw for Windows (version 4.0) software. Environmental variables data were standardized by ranging $[(x-x_{\min})/(x_{\max}-x_{\min})]$ in order to fit all variables in the same interval (0-1). Phytoplankton data were transformed by $\log(x+1)$ in order to underweight the dominant species.

The quantitative degree of concordance between two multivariate configurations (sample scores) was tested by applying a Procrustes analysis, a procedure that minimizes the sum of squared differences between two configurations in a multivariate Euclidean space (in this case, an ordination defined by the first two PCA or CCA axes) (PODANI, 2000). First, to assess how close the spatial heterogeneity of the reservoir based on physical, chemical and biological data matches the spatial heterogeneity based on phytoplankton grouping methods paired Procrustes analyses were performed between the PCA sample scores and each of the five CCAs (sample scores for individual species, classes, FG, MFG and MBFG). The result was five paired-comparisons, obtaining 5 “Procrustes sum of squares” (m^2), which indicate how well two configurations match.

Then, to evaluate the variance fractions explained by the environmental variables in relation with the use of the original data matrix (species) and the four classification methods (classes, FG, MFG and MBFG), Procrusted analysis were performed between two CCA configurations (samples scores of individual species, classes, FG, MFG and MBFG). For every ordination obtained using species, classes, FG, MFG and MBFG, we performed 10 paired-comparisons, obtaining 10 “Procrustes sum of squares” (m^2), which indicate how well two configurations match. The correspondence among the ordinations obtained using the species and the 4 classification methods were represented graphically with a cluster analysis (average method) applied on a dissimilarity matrix including the Procrustes sum of squares.

The significance of all Procrustes analyses were assessed using a randomization test (Protest) to determine whether the sum of residual deviations is less than expected by chance (JACKSON, 1995). Procrustes and protest analyses were carried out with the package *vegan* (OKSANEN *et al.*, 2011) from R (R DEVELOPMENT CORE TEAM, 2011). The cluster analysis was carried out with Statistica software (version 7.0).

3. Results

The contrasting features of the sampling stations in Guarapiranga reservoir are indicated in Table 1. Principal Components Analysis (PCA) based on TSS, ISS, Zeu/Zmax, SRSi, Zeu, N:P, DO, T, pH, Zmax, Chla, EC, OSS, TIN, TN, TS and TP clearly reveals the main pattern of heterogeneity based on water quality that occurred in the reservoir during the study period (Figure 2). The first two canonical axes explained 72.4% of the total variance of the data. The first principal component axis can be interpreted as a gradient of progressive eutrophication, characterized by decreasing values of variables related with the eutrophication process, such as EC, TIN, TN, TP and OSS and increasing SRSi (Figure 2). The second PCA axis is being driven by some particular features of the reservoir, characterized by a downstream increase in Zmax, pH and DO and decrease in ISS and TSS in the epilimnetic layer of the reservoir (Figure 2). One group comprised stations EG1, EG2 and EG3, located upstream in Embu-guaçu region, characterized by lower trophic status: lower concentration of nutrients (except SRSi), EC and solids (total and suspended). Other two groups were represented by stations P1 and P2 (Parelherios region) and EM, C1, C1 and Dam (downstream region), characterized by more eutrophic waters: higher concentrations of nutrients associated with the eutrophication process (TP, TIN and TN) and higher values of other variables associated with the eutrophication process (EC and OSS). Although both Parelheiros and downstream regions were characterized by eutrophic waters, these two groups presented different characteristics that segregated them. Stations P1 and P2 displayed low DO concentrations and high TSS and ISS. Among the group of sampling stations from downstream region (Em, C1, C2 and Dan) is possible to identify two sub-groups: EM and C1, characterized by higher Chla concentration and C2 and Dam, with typical characteristics from downstream region of reservoirs, such as higher DO, Zmax and Zeu and lower TSS.

Table 1. Physical, chemical and biological characteristics from the nine sampling stations along Guarapiranga reservoir's longitudinal axis. Zmax=maximum depth, Zeu=euphotic depth, T=temperature, DO=dissolved oxygen, Sat=oxygen saturation, EC=electric conductivity, SRSi=silicate, TIN=total inorganic nitrogen, SRP=phosphate, TN=total nitrogen, TP=total phosphorous, N:P=nitrogen and phosphorous molar ratio, TS=total solids, TSS=total suspended solids, OSS=organic suspended solids, ISS=inorganic suspended solids, Chla=chlorophyll-a, Phea=pheophytin, TSI=trophic state index, Phyto=phytoplankton biomass.

Variable	EG1	EG2	EG3	P1	P2	EM	C2	C1	Dam
Zmax (m)	4.9	3.0	6.7	3.4	5.4	4.7	8.0	9.2	7.8
Zeu (m)	2.2	3.0	3.4	2.3	2.8	2.3	2.7	2.8	2.9
T (°C)	21.4	21.4	21.3	21.2	20.9	22.2	21.3	21.6	21.9
DO (mg l ⁻¹)	7.0	7.6	8.2	5.9	6.0	8.5	8.0	8.3	9.0
Sat (%)	79.8	86.5	93.5	65.6	67.2	98.1	90.7	92.9	103.7
pH	7.0	7.1	7.2	7.0	6.9	7.5	7.3	7.4	7.3
EC (μS cm ⁻¹)	39.0	69.0	78.0	118.0	110.0	117.0	108.0	110.0	111.0
SRSi (mg l ⁻¹)	2.0	1.3	0.7	0.7	0.6	0.9	0.8	0.7	0.7
TIN (μg l ⁻¹)	201.6	423.3	629.9	1573.6	1359.0	1339.6	1336.8	1063.7	1399.1
SRP (μg l ⁻¹)	<10	<10	<10	<10	<10	<10	<10	<10	<10
TN (μg l ⁻¹)	599.5	910.7	971.6	2023.9	1846.2	1772.7	2042.9	1564.3	1482.5
TP (μg l ⁻¹)	31.3	25.2	30.8	96.1	84.2	79.4	63.8	57.7	54.9
N:P	8.6	16.3	14.2	9.5	9.9	10.1	14.5	12.3	12.2
TS (mg l ⁻¹)	50.5	43.0	69.5	71.5	81.5	76.5	71.5	66.5	71.5
TSS (mg l ⁻¹)	6.4	4.1	4.8	6.8	9.4	6.2	6.5	6.0	4.8
OSS (mg l ⁻¹)	3.4	3.2	3.2	5.2	4.7	5.2	5.7	5.2	4.5
ISS (mg l ⁻¹)	3.0	0.9	1.5	1.6	4.7	1.0	0.8	0.8	0.3
Chl a (μg l ⁻¹)	11.8	14.0	18.9	25.4	14.2	19.4	24.4	23.7	20.4
Phea a (μg l ⁻¹)	1.4	2.8	4.1	3.9	3.7	2.8	1.3	6.2	3.7
TSI	57 (M)	57 (M)	58 (M)	62 (E)	61 (E)	61 (E)	61 (E)	61 (E)	60 (E)
Phyto (mg l ⁻¹)	23.6	15.3	56.0	53.3	65.5	49.1	37.2	31.1	105.1

Figure 2. Principal Components Analysis biplot of the physical, chemical and biological variables (TSS=total suspended solids; ISS=inorganic suspended solids; Zeu/Zmax=euphotic and maximum depths ratios; SRSi=silicate; Zeu=euphotic depth; N:P=nitrogen and phosphorous molar ratios; DO=dissolved oxygen; T=temperature; pH; Zmax=maximum depth; Cla=chlorophyll-a; EC=electric conductivity; OSS=organic suspended solids; TIN=total inorganic nitrogen; TN=total nitrogen; TS=total solids; TP=total phosphorous) and the nine sampling stations in Guarapiranga reservoir.

Canonical correspondence analyses (CCA) were performed using the physical, chemical and biological variables listed (Table 1) and the phytoplankton data grouped into five different classifications (individual species, taxonomical classes, FG, MFG and MBFG) (Supplementary material 1). Phytoplankton species composition and spatial variability are described in Nishimura (2012) (Chapter 5 of this thesis). Among all the environmental variables initially included in the CCA, the same seven were retained by the forward selection procedure in all five CCAs: EC, TIN, TS, SRSi, TN, OSS and TP. In all CCAs, environmental variables were significantly correlated with the first axis and also with all canonical axes (Table 2). In addition, all CCAs presented high explanatory power (Table 2). All CCAs exhibited similar configuration: the first canonical axis can be interpreted as a gradient of progressive eutrophication, characterized by decreasing values on the variables related with the eutrophication process (EC, TIN, TN, TP, OSS) and TS and increasing SRSi (Figure 3, Figure 4, Figure 5, Figure 6 and Figure 7).

Table 2. Cumulative variance of biomass data and explained variance between phytoplankton data [species, class, functional groups (FG), morpho-functional groups (MFG) and morphology-based functional groups (MBFG)] and the forward-selected environmental variables (EC, TIN, TS, SRSi, TN, OSS and TP) resulted from the CCAs.

	Cumulative variance of biomass data (%)		Explained variance (%)
	1st axis	2nd axis	
Species	34.3	22.5	0.93
Class	55.2	18.0	0.91
FG	46.8	20.4	0.94
MFG	42.5	17.8	0.92
MBFG	77.5	11.6	0.96

Procrustes and protest analyses results showed concordance between the sampling stations' ordination based on water quality and phytoplankton individual species ($m^2=0.50$, $p=0.02$), FG ($m^2=0.35$, $p=0.01$) and MFG ($m^2=0.55$, $p=0.03$). In contrast, Procrustes and Protest results showed a lower concordance between the sampling stations' ordination based on water quality and phytoplankton classes ($m^2=0.66$, $p=0.09$) and MBFG ($m^2=0.62$, $p=0.07$).

The ordination of sampling stations based on MFGs and, partly, FGs, were almost undistinguishable from that obtained with the individual species data (Figure 3, Figure 5 and Figure 6). Even from a first sight, the largest discrepancies were identifiable between the configuration based on MBFG and classes (Figure 4 and Figure 7). The graphical analysis of the "Procrustes sum of squares" (Supplementary material 2) provide an effective picture about the concordance among the five configurations, underlying the strong comparability of ordinations based on the species, MFG and FG (Figure 8). It is worth to underline that the ordination of lakes based on phytoplankton classes and MBFG showed a minor concordance with the group of configurations based on the species, MFG and FG (Figure 8). Nevertheless, despite the above differences, the sum of residual deviations among the five configurations was less than that expected by chance (PROTEST, $p < 0.05$), demonstrating how the different classifications were able to catch different properties of the original phytoplankton species matrix.

Figure 3. Canonical correspondence analysis (CCA) triplot of the selected physical, chemical and biological variables (EC=electric conductivity, TIN=total inorganic nitrogen, TS=total solids, SRSi=silicate, TN=total nitrogen, OSS=organic suspended solids and TP=total phosphorous) and phytoplankton individual species (species codes in Supplementary material 1) in the nine sampling stations along Guarapiranga reservoir's longitudinal axis (EG1, EG2, EG3, P1, P2, C1, C2, EM and Dam). In order to make the plot clearer, species fit range was set from 10-100% to display the species with the best fit (19 out of 26 species).

Figure 4. Canonical correspondence analysis (CCA) triplot of the selected physical, chemical and biological variables (EC=electric conductivity, TIN=total inorganic nitrogen, TS=total solids, SRSi=soluble reactive silica, TN=total nitrogen, OSS=organic suspended solids and TP=total phosphorous) and phytoplankton taxonomical classes (Baci=Bacillariophyceae, Chlo=Chlorophyceae, Cryp=Cryptophyceae, Chrys=Chrysophyceae, Cyan=Cyanophyceae, Dino=Dinophyceae, Eugl=Euglenophyceae, Xant=Xanthophyceae, Zygn=Zygnematophyceae) in the nine sampling stations along Guarapiranga reservoir's longitudinal axis (EG1, EG2, EG3, P1, P2, C1, C2, EM and Dam).

Figure 5. Canonical correspondence analysis (CCA) triplot of the selected physical, chemical and biological variables (EC=electric conductivity, TIN=total inorganic nitrogen, TS=total solids, SRSi=soluble reactive silica, TN=total nitrogen, OSS=organic suspended solids and TP=total phosphorous) and phytoplankton functional groups (Reynolds *et al.*, 2002) in the nine sampling stations along Guarapiranga reservoir's longitudinal axis (EG1, EG2, EG3, P1, P2, C1, C2, EM and Dam).

Figure 6. Canonical correspondence analysis (CCA) triplot of the selected physical, chemical and biological variables (EC=electric conductivity, TIN=total inorganic nitrogen, TS=total solids, SRSi=soluble reactive silica, TN=total nitrogen, OSS=organic suspended solids and TP=total phosphorous) and phytoplankton morpho-functional groups (Salmaso&Padisák, 2007) in the nine sampling stations along Guarapiranga reservoir's longitudinal axis (EG1, EG2, EG3, P1, P2, C1, C2, EM and Dam).

Figure 7. Canonical correspondence analyses triplot of the selected physical, chemical and biological variables (EC=electric conductivity, TIN=total inorganic nitrogen, TS=total solids, SRSi=soluble reactive silica, TN=total nitrogen, OSS=organic suspended solids and TP=total phosphorous) and phytoplankton morphology based functional groups (Kruk *et al.*, 2010) in the nine sampling stations along Guarapiranga reservoir's longitudinal axis (EG1, EG2, EG3, P1, P2, C1, C2, EM and Dam)..

Figure 8. Hierarchical cluster analysis' dendrogram based on the dissimilarity matrix of Procrustes sum of squares of species, classes, FG, MFG and MBFG data (UPGMA method and Euclidean distance).

Supplementary material 1. Phytoplankton descriptor species sorted by taxonomical classes and its respective code and classification according to functional groups (FG), morpho-functional group (MFG) and morphology-based functional group (MBFG) in the sampling stations through Guarapiranga reservoir's longitudinal axis.

Class		Grouping method			Biomass per ampling station (mg l ⁻¹)								
<i>Species</i>	Code	FG	MFG	MBFG	EG1	EG2	EG3	P1	P2	EM	C2	C1	Dam
Bacillariophyceae													
<i>Urosolenia eriensis</i> (H.L.Smith) Round & R.M.Crawford	Ueri	A	6a-LargeCent	VI	6.5	15.2	8.1	-	-	-	-	-	-
<i>Aulacoseira ambigua</i> (Grunow) Simonsen	Aamb	C	6a-LargeCent	VI	-	-	-	1.9	2.4	1.7	3.3	-	3.0
<i>Cyclotella meneghiniana</i> Kützing	Cmen	C	7a-SmallCent	VI	-	-	11.6	-	-	-	-	-	1.9
<i>Nitzschia fruticosa</i> Hustedt	Nfru	D	7b-SamllPenn	VI	-	-	-	-	-	-	-	-	3.0
Chlorophyceae													
<i>Botryococcus neglectus</i> (West & G.S.West) J.Komárek & P.Marvan	Bneg	F	11b-GelaChlor	VII	-	-	-	-	-	-	-	1.8	-
<i>Eudorina illinoisensis</i> (Kofoid) Pascher	Eill	G	3b-ColoPhyto	V	-	-	5.8	-	9.1	6.7	-	-	33.6
<i>Acanthosphaera zachariasii</i> Lemmermann	Azach	J	8a-LargeCoCh	IV	-	-	-	-	9.2	7.5	-	-	-
<i>Actinastrum hantzschii</i> var. <i>subtile</i> J.Woloszynska	Zhan	J	11a-NakedChlor	IV	-	-	1.4	-	-	-	-	1.3	4.0
<i>Coelastrum indicum</i> W.B.Turner	Cind	J	11a-NakedChlor	IV	-	-	-	-	-	1.4	-	-	-
<i>Desmodesmus denticulatus</i> (Lagerheim) S.S.An, T.Friedl & E.Hegewald	Dden	J	11a-NakedChlor	IV	-	-	-	-	-	-	-	-	1.2
<i>Pediastrum duplex</i> var. <i>gracillimum</i> West & G.S.West	Pdep	J	11a-NakedChlor	IV	-	-	4.6	1.8	1.6	-	-	2.7	1.5
Cryptophyceae													
<i>Cryptomonas brasiliensis</i> A.Castro, C.Bicudo & D.Bicudo	Cbra	Y	2d-Crypto	V	-	-	-	-	-	-	1.3	-	4.7
<i>Cryptomonas curvata</i> Ehrenberg	Ccur	Y	2d-Crypto	V	-	-	5.6	9.2	1.8	-	-	-	-
Chrysophyceae													
<i>Dinobryon sertularia</i> Ehrenberg	Dset	E	1a-LargeChry	II	1.3	1.6	-	-	-	-	-	-	-

Class		Grouping method			Biomass per ampling station (mg l ⁻¹)								
<i>Species</i>	Code	FG	MFG	MBFG	EG1	EG2	EG3	P1	P2	EM	C2	C1	Dam
Cyanophyceae													
<i>Dolichospermum spiroides</i> (Kleb.) Wacklin, L.Hoffm. & Komárek	Dspir	H1	5e-Nostocales	III	-	-	-	-	2.4	5.6	5.6	4.6	6.7
<i>Aphanocapsa delicatissima</i> West & G.S.West	Adel	K	5c-OtherChroo	VII	-	3.2	5.6	12.1	19.4	11.3	11.8	-	21.4
<i>Aphanocapsa incerta</i> (Lemmermann) Cronberg & Komárek	Ainc	K	5c-OtherChroo	VII	-	-	-	-	-	-	-	1.2	-
<i>Sphaerocavum brasiliense</i> Azevedo & Sant'Anna	Sbra	M	5b-LargVacC	VII	-	-	-	-	-	-	-	1.6	-
<i>Phormidium</i> sp.	Phor	S1	5a-FilaCyano	IV	-	-	3.8	-	-	-	-	-	-
Dinophyceae													
<i>Peridinium gatunense</i> Nygaard	Pgat	Lo	1b-LargeDino	V	-	-	-	13.4	3.1	3.4	-	2.6	-
<i>Peridinium</i> sp.	Peri	Lo	1b-LargeDino	V	-	-	-	-	-	-	-	1.8	-
<i>Gymnodinium fuscum</i> (Ehrenberg) F.Stein	Gfus	Y	1b-LargeDino	V	-	-	-	5.0	4.7	2.1	6.1	-	2.8
Euglenophyceae													
<i>Trachelomonas volvocinopsis</i> Svirenko	Tvol	W2	2c-SmallEugl	V	-	-	-	2.1	-	-	-	-	1.5
Xanthophyceae													
<i>Tetraedriella spinigera</i> Skuja	Tspi	J	9d-SmallUnic	VI	-	-	-	-	2.7	-	-	-	-
Zygnematophyceae													
<i>Cosmarium</i> sp.	Cosm	P	9a-SmallConj	IV	-	-	-	-	-	-	-	-	4.9
<i>Mougeotia</i> sp.	Moug	T	10b-FilaConj	IV	-	-	-	-	-	1.5	-	1.5	4.6

Supplementary material 2. Procrustes sum of squares (m²) performed with species, classes, FG, MFG and MBFG data.

	Species	Class	FG	MFG	MBFG
Species	0	0.28	0.07	0.06	0.49
Class	0.28	0	0.34	0.30	0.44
FG	0.07	0.34	0	0.04	0.39
MFG	0.06	0.30	0.04	0	0.32
MBFG	0.49	0.44	0.39	0.32	0

4. Discussion

Heterogeneity along the longitudinal axis of Guarapiranga reservoir has been evaluated previously under several aspects, such as water quality and trophic status (CARDOSO-SILVA, 2008; NISHIMURA *et al.*, 2012), metals in water (CARDOSO-SILVA, 2008) and sediment (PADIAL, 2008), aquatic macrophytes (RODRIGUES, 2011), phytoplankton community (NISHIMURA *et al.*, 2012) and zooplankton community (DOMINGOS, 1993). All of these works describe compartments in Guarapiranga reservoir similar to the ones revealed by our data (PCA), in which Embu-Guaçu region is the more protected one and, consequently, less eutrophic; Parelheiros region is the most eutrophic due to a very polluted tributary and downstream region of the reservoir displays lentic characteristics.

The CCAs results indicated that the biomass of the phytoplankton groups corresponding to individual species and the four classification approaches can be well predicted by the environmental variables. Conductivity, nutrient content and solids in the water seem to be the most important variables determining all phytoplankton grouping methods. In fact, most of these variables are related to the eutrophication process (OECD, 1982) and the trophic status was a constraining factor in the compartments segregation in the reservoir.

In all ordinations, sampling stations from Embu-Guaçu region (especially EG1 and EG2) were plotted separately from the rest of the sampling stations, indicating that this region displays very distinct characteristic. Indeed, as our data showed, lower trophic status and higher SRSi concentrations was observed in this region. These characteristics influenced the phytoplankton composition and biomass, as evidenced by the CCAs performed with all classification methods. Centric diatom *Urosolenia eriensis* and Chrysophyceae *Dinobryon sertularia*, both silica-dependent species, were closely related to sampling stations EG1,

EG2 and, partly, EG3. Accordingly, these sampling stations were closely related with Reynolds *et al.*'s (2002) FGs from codon A, which comprises diatoms from low trophic status environments, and codon E, that are mainly siliceous Chrysophyceae from low trophic status environments. Embu-Guaçu sampling stations were also related with Salmaso&Padisák's (2009) MFGs 1a and 6a, which gathers large Chrysophyceae and large centric diatoms, respectively, and with Kruk *et al.*'s (2010) MBFG II and VI, which comprises typically small silica scaled flagellates and diatoms, respectively. The strong correlation between these populations and Embu-Guaçu region emphasize the importance of SRSi for their metabolisms. In particular, the presence of species/groups characteristics from low trophic status environments points out the singularity of Embu-Guaçu region in comparison to the others compartments of the reservoir.

Sampling station C1 was segregated in CCAs configurations for species, FG and MFG. In these cases, C1 was correlated with phytoplankton species with different characteristics: large dinoflagellated *Peridinium* sp., large colonial Chlorophyceae *Botryococcus neglectus* and colonial mucilaginous cyanobacteria *Sphaerocavum brasiliensis* and *Aphanocapsa incerta*. FG classification captured well the environment conditions, correlating codon M (colonial mucilaginous cyanobacteria from eutrophic environments) and codon F (clear, deeply mixed meso-eutrophic environments) with sampling station C1. MFG classification captured the morphological characteristics of the most important species, correlating this sampling station with groups 5d (small Chroococcales colonies) and 11b (gelatinous Chlorococcales colonies). During the field work, a boat from the environmental agency (SABESP) was adding copper sulphate in the water in the sampling station C1. This is a routine action to control phytoplankton biomass, especially, cyanobacterial blooms. Although the real extent of the copper sulphate addition in the water was not assessed, and, consequently, the action of this algaecide on the phytoplankton community could not be measured, certainly the phytoplankton community biomass and structure were artificially changed. This may be one possible explanation for the segregation of sampling station C1 from the rest of the sampling stations. According to Beyruth (2000), after the addition of copper sulphate in the reservoir's water, the phytoplankton organisms' death, especially those mucilage-rich, leads to an increase in the availability of nutrients in the water column. The enriched environment favors efficient competitors, such as *Sphaerocavum brasiliensis* and *Aphanocapsa incerta*, attaining high biomass. During the chemical treatment, disturbance-dependent or disturbance-tolerant species such as *Peridinium* sp. increases its biomass as well (REYNOLDS, 1988).

The remaining sampling stations did not exhibit a clear distribution pattern among the grouping methods. This fact corroborates that all sampling stations present similar trophic status, except for sampling stations located at Embu-Guaçu regions (EG1, EG2 and EG3).

The concordance between multivariate solutions based on water quality and phytoplankton species, FG and MFG indicates that these datasets exhibit similar patterns. Thus, these grouping methods captured efficiently the environmental compartments in Guarapiranga reservoir. In contrast, classes and MBFG do not seem to be so efficient capturing the environmental variability in Guarapiranga reservoir, probably due to the small number of groups, resulting in highly heterogenic groups. The limitations of grouping methods by classes and MBFG were also evidenced by the minor concordance with these two classifications with the main group formed by species, FG and MFG. Comparing all classifications methods and individual species, all of them constitute a good approach to analyze the differences within Guarapiranga reservoir's longitudinal axis, catching different properties. It is valid to remark that the importance of taxonomy cannot be neglected. Taxonomy cannot be replaced by any kind of classification since species and its traits are closely related and its proper functional classification is dependent on this knowledge (PADISÁK *et al.*, 2009). However, the degree of knowledge required by each classification varies and the degree of information captured by each classification varies as well.

The functional classification proposed by Reynolds *et al.* (2002) provides much information about physiological requirements and environment template, allowing a more detailed description of the functional groups. However, seven years after Reynolds *et al.* (2002) functional groups being worldwide released, a revision performed by Padisák *et al.* (2009) revealed many misplacements and misunderstandings by the users while applying this classification: the authors revised 63 articles that used Reynolds *et al.* (2002) classification and analyzed 617 taxa classifications, detecting 37% of misplacements. 33% of the misplacements were due to incorrect classification and 4% due to low taxonomic resolution. This revision was crucial to elucidate some misunderstandings on the theoretical basis of Reynolds *et al.* (2002) method, like the necessity of deeper knowledge on the autoecology of species for its correct classification. Then, the proper application of Reynolds *et al.* (2002) method is restricted to expert knowledge and species information on its autoecology, impairing a worldwide usage.

The morpho-functional classification proposed by Salmaso & Padisák (2007) reflected the differences in algal assemblages among the longitudinal axis of the reservoir mainly based

on morphological characteristics. Because of that, this classification is relatively simple, requiring a basic knowledge on phytoplankton taxonomy and ecology. Thus, in a regional context, this classification seemed appropriate for monitoring horizontal heterogeneity in reservoirs, due to its relatively simplicity and good correlation with species classification.

The morphology-based functional classification proposed by Kruk *et al.* (2010) is very simple and intuitive, requiring a minimal knowledge on the main phytoplankton morphological features. Taxonomy knowledge is not necessary to properly classify the species into MBFG. Hence, this approach can be very valuable in the environmental biomonitoring, in particular, for long-term monitoring of aquatic systems or in the comparison of a great number of environments/sampling stations, due to its relatively simplicity and objectivity.

The variation in water quality in different compartments of a reservoir can result in different operation and treatment possibilities (STRASKRABA & TUNDISI, 1999). Thus, these grouping methods can be used in the assessment of horizontal heterogeneity in reservoirs and assist the establishment of management programs. For example, leisure areas for swimming and nautical sports can be established in the compartment with better water quality; the identification of water quality in the compartment where the water is taken for public supply enables the determination of the necessary treatments and strategies to make the water potable; the observation of compartments with higher biodiversity and/or endangered species, or conversely, with poor water quality, can guide the environmental agencies about the strategies to be followed.

All classifications aim at predicting the phytoplankton community, independently of the grouping method (SALMASO & PADISÁK, 2007). In this context, all tested classifications reached this goal and were proven to be good predictors of the phytoplankton community. In conclusion, there is no unique possibility for classifying phytoplankton species. There are particular aims and each grouping method will have advantages and disadvantages at reaching these aims.

Capítulo 7. Conclusões e perspectivas futuras

1. Conclusões

Após investigar a qualidade da água e a comunidade fitoplanctônica no sistema Billings-Guarapiranga em período de transposição inativa, surgiram as seguintes conclusões:

- ✓ Sobre a transposição Billings-Guarapiranga:
 - As represas Billings (região do braço Taquacetuba) e Guarapiranga e o ribeirão Parelheiros discriminaram-se entre si quanto às variáveis físicas, químicas e biológicas, sendo as duas represas meso/eutróficas e o ribeirão Parelheiros eutrófico.
 - Durante o período de estudo, a comunidade fitoplanctônica da represa Billings foi dominada pelo dinoflagelado invasor *Ceratium furcoides*, pertencente ao grupo funcional L₀ em praticamente todos os pontos estudados.
 - O ribeirão Parelheiros apresentou baixa biomassa fitoplanctônicas, com domínio dos grupos funcionais L₀ e F, característicos de ambientes eutróficos.
 - A baixa qualidade da água do ribeirão Parelheiros influenciou fortemente a qualidade da água da represa Guarapiranga, principalmente na região de entrada deste tributário, com domínio do grupo funcional L_M.
 - As regiões mais distantes do ribeirão Parelheiros na represa Guarapiranga apresentaram condições distintas, provavelmente devido à influência de tributários com melhor qualidade de água. Observou-se abundância de distintos grupos funcionais, como W_S, H₁ e A, evidenciando uma heterogeneidade da qualidade da água no eixo longitudinal da represa Guarapiranga.
 - O sistema de transposição Billings-Guarapiranga está influenciando a deterioração da qualidade da água da represa Guarapiranga, fato evidenciado pela pior qualidade da água na região do Parelheiros e transferência de espécies invasoras e/ou potencialmente tóxicas.
- ✓ Sobre o dinoflagelado invasor *Ceratium furcoides*:

- Contrariamente ao esperado, não houve floração de cianobactérias na represa Billings. Em contrapartida, foi encontrada dominância do dinofalagenado invasor *Ceratium furcoides*.
- *C. furcoides* provavelmente foi transferido para a represa Guarapiranga através do sistema Billings-Guarapiranga.
- A colonização do dinoflagelado invasor *Ceratium furcoides* está em estágio avançado na represa Billings e em estado inicial na represa Guarapiranga.

Diante da evidência de presença de heterogeneidade ambiental na represa Guarapiranga, estudo complementar foi realizado ao longo do eixo longitudinal desta represa e pode-se concluir:

- ✓ Sobre a heterogeneidade espacial horizontal na represa Guarapiranga:
 - Existe uma clara tendência a compartimentalização na camada epilimnética da água na represa Guarapiranga com base na qualidade da água: 1) região do Embu-Guaçu, parte alta da represa, mais protegida e menos eutrófica dominada por fitoplâncton R-estrategista e rotíferos; 2) região do Parelheiros, um braço eutrófico da represa com domínio de fitoplâncton C-estrategista e elevada densidade de copépodes ciclopóides e 3) parte baixa do reservatório, com características eutróficas e lacustrinas e co-dominância de fitoplâncton C e S-estrategistas e maior contribuição de copépodes à densidade total do zooplâncton.
 - Com base na comunidade fitoplanctônica, não foram formados compartimentos claros na camada epilimnética da água da represa Guarapiranga, provavelmente devido ao distúrbio antropogênico na forma do tratamento com o algicida sulfato de cobre.
 - Com base na comunidade zooplanctônica, foram formados compartimentos na camada epilimnética da água na represa Guarapiranga, porém, estes compartimentos não coincidiram exatamente com os compartimentos formados com base na qualidade da água. Tal diferença pode ter ocorrido pelos efeitos indiretos causados pelo algicida sulfato de cobre, que altera a disponibilidade alimentar dos organismos zooplanctônicos herbívoros.
- ✓ Sobre a aplicação de diferentes métodos de agrupamento funcional na comunidade fitoplanctônica:

- Espécies e as quatro classificações testadas conseguiram capturar os padrões ambientais na represa Guarapiranga.
- Variáveis relacionadas com o estado trófico, como condutividade e teor de nutrientes na água, foram as variáveis mais importantes identificação destes padrões ambientais.
- Espécies, grupos funcionais *sensu* Reynolds *et al.* (2002) e grupos morfo-funcionais *sensu* Salmaso&Padisák (2009) foram mais eficientes na captura dos padrões ambientais em comparação com classes taxonômicas e grupos funcionais baseados em morfologia *sensu* Kruk *et al.* (2010). Tal diferença provavelmente ocorreu pelo reduzido número de agrupamentos nas duas últimas classificações.
- O grau de conhecimento necessário em cada método de agrupamento varia, assim como o grau de informação capturado por cada método. Portanto, deve-se escolher o método de agrupamento a ser utilizado baseado nos objetivos particulares de cada pesquisa.

2. Perspectivas futuras

- ✓ Sobre a transposição Billings-Guarapiranga:
 - Preservação do ribeirão Parelheiros e o monitoramento da transferência de espécies fitoplanctônicas da represa Billings para a Guarapiranga são cruciais para a preservação da qualidade da água represa Guarapiranga.
 - Conforme inicialmente previsto neste projeto, a realização de um estudo comparativo do sistema em momento anterior e posterior à operação da transposição seria muito importante para o melhor entendimento da dinâmica da água e da comunidade fitoplanctônica ao longo deste sistema.
- ✓ Sobre o dinoflagelado invasor *C. furcoides*:
 - É necessário o intenso monitoramento da ocorrência e dispersão de *C. furcoides* no Brasil a fim de se elucidar as rotas de dispersão desta espécie, assim como os ambientes mais susceptíveis à colonização desta espécie.
 - Estudos em laboratório devem ser encorajados para investigar a interação deste dinoflagelado com cianobactérias.

- Elucidação dos possíveis malefícios deste dinoflagelado em mananciais para abastecimento humano, como encarecimento no tratamento e entupimento de filtros.

- ✓ Sobre a heterogeneidade horizontal na represa Guarapiranga:
 - O monitoramento e os programas de manejo na represa Guarapiranga devem levar em consideração a presença de heterogeneidade ambiental neste reservatório, assim como os compartimentos existentes.
 - Os grupos funcionais são uma ferramenta eficiente para identificar padrões de heterogeneidade ambiental e devem ser utilizadas no monitoramento e em programas de manejo de reservatórios. Para isso, deve escolher o método de agrupamento compatível com o nível de conhecimento das pessoas envolvidas e os objetivos almejados.

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