UNIVERSIDADE FEDERAL DE SÃO CARLOS

CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA E RECURSOS NATURAIS

PRODUTIVIDADE BACTERIOPLANCTÔNICA E FITOPLANCTÔNICA NOS ECOSSISTEMAS AQUÁTICOS DO TRECHO MÉDIO DA BACIA DO RIO DOCE- MG.

MAURICIO MELLO PETRUCIO

Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ecologia e Recursos Naturais do Centro de Ciências Biológicas e da Saúde da Universidade Federal de São Carlos, como parte dos requisitos para a obtenção do título de Doutor em Ciências (Ciências Biológicas), área de concentração: Ecologia e Recursos Naturais.

SÃO CARLOS - SP
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Orientador

Prof. Dr. Francisco Antônio Rodrigues Barbosa

"À minha avó Maria Júlia, aos meus pais Elisa e João e a minha irmã Luciana, mesmo distantes, obrigado por todo apoio, carinho, ensinamentos e muita paciência para a concretização desse trabalho".

“Primeiro precisamos indagar se os elementos [terra, ar, fogo e água] são eternos ou sujeitos a geração e destruição. [...] É impossível que os elementos sejam gerados por algum tipo de corpo. Isso implicaria um corpo distinto dos elementos e anterior a eles”.

Aristóteles
Sobre o céu (*c*. 330 a.C)

"A crença de que as espécies eram produtos imutáveis era quase inevitável enquanto se considerou ser de curta duração a história do mundo [...] A principal causa de nossa [...] relutância a admitir que uma espécie originou espécies claras e distintas é que sempre somos lentos para admitir grandes mudanças das quais não vemos as etapas".

Charles Darwin
A origem das espécies (1859)

"It was a beautiful day
Don't let it get away
Beautiful day
Touch me, take me to that other place
Reach me, I know I'm not a hopeless case
What you don't have you don't need it now
What you don't know you can feel it somehow
What you don't have you don't need it now
You don't need it now"

Beautiful day
Letra: Bono
Música: U2

"Me abrace e me dê um beijo, faça um filho comigo, mas não me deixe sentar na poltrona no dia de domingo"

Minha Alma
Letra: Marcelo Yuka
Música: O Rappa

"Tudo que vai
Deixa o gosto
Deixam as fotos
Quanto tempo faz
Deixam os dedos
Deixa a memória
Eu nem me lembro mais"

Tudo que vai
Alvin L./ Dado Villa-Lobos/
Toni Platão

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ABSTRACT

This research had as major objective to quantify the existent relationships between bacterioplankton and phytoplankton production rates, determining the importance of these communities on the carbon fixation and organic matter transfer in rivers and lakes of the middle stretch of the Rio Doce basin in Minas Gerais state (southeast Brazil) during dry and rainy periods of the years 1999-2001. The concentrations of carbon, nitrogen and phosphorus and the N/P ratios were used to evaluate possible relationships between productivity and trophic status based in diel, seasonal, and annual patterns.

This thesis was divided into 5 chapters approaching mainly phytoplankton and bacterial production rates in rivers and lakes of Rio Doce basin. The testing hypothesis is that production rates varies seasonally (dry and rainy periods) thus resulting in changes of the BP/PP ratios, as a consequence of changes in the inputs of allochthonous matter (nutrients) being also affected by distinct internal loads during these periods. The study was conducted in 8 rivers of the middle stretch of Rio Doce basin and in 7 lakes of the Rio Doce State Park and surrounding area, during dry and rainy periods from 1999 to 2001. The ecosystems presented a wide trophic status (from oligo to eutrophic), as well as bacterioplantonic (0.005 – 5.7 mg $C.m^{-3}.h^{-1}$) and phytoplanctonic (0.01 – 747.5 mg $C.m^{-3}.h^{-1}$) production rates. For the lotic environments, seasonality and nutrient concentration were considered the most important factors for productivity. Significant positive correlations were obtained between phosphorus concentrations and bacterial production rates. For the lentic environments seasonality was only evident for bacterial production rates with high N/P ratios, suggesting phosphorus limitation in most of the lakes. Bacterial production in spite of presenting lower values than the ones recorded for phytoplankton could be an important carbon source mainly within deep layers and more eutrophic ecosystems.

RESUMO

Esta pesquisa teve como objetivo principal quantificar as relações existentes entre as taxas de produção bacterioplanctônica e fitoplanctônica, determinando a importância dessas comunidades na fixação de carbono e transferência de matéria orgânica em rios e lagos do trecho médio da bacia do Rio Doce-MG, nos períodos de chuva e seca. As concentrações de carbono, nitrogênio e fósforo e as razões N/P na água, foram usadas para avaliar possíveis relações entre produtividade e grau de trofia, com base em padrões diários, sazonais e anuais.

O trabalho foi dividido em 5 capítulos que abordam principalmente a produção primária e bacteriana em rios e lagos do médio Rio Doce. A hipótese central testada é que a produção primária varia sazonalmente resultando em alteração da relação PB/PF em função, principalmente, do aporte de matéria orgânica alóctone sendo também influenciada pela carga interna de nutrientes. Os estudos foram realizados em 8 rios do trecho médio da bacia do Rio Doce e em 7 lagos do Parque Estadual do Rio Doce e área de entorno, durante os períodos de seca e chuva de 1999 a 2001. Os ambientes apresentaram uma ampla variação trófica (oligo a eutróficos), assim como das taxas de produção bacterioplantônica (0,005 – 5,7 mg $C.m^{-3}.h^{-1}$) e fitoplanctônica (0,01 – 747,5 mg $C.m^{-3}.h^{-1}$). Para os ambientes lóticos, sazonalidade e a concentração de nutrientes foram considerados fatores importantes para a produtividade. Correlações positivas e estatisticamente significativas foram obtidas entre as concentrações de fósforo e os valores de produção bacteriana. Para os ambientes lênticos a sazonalidade ficou evidente apenas para a produção bacteriana com destaque para as elevadas razões N/P, sugerindo uma limitação por fósforo na maioria dos lagos. A produção bacteriana apesar de apresentar sempre menores valores pode ser considerada uma importante fonte de carbono principalmente nas maiores profundidades e nos ambientes mais eutrofizados.

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1- INTRODUÇÃO

As populações humanas têm usado rios e lagos para inúmeros propósitos por muitos anos. A água desses ambientes satisfaz necessidades domésticas e industriais, provê transporte, alimentação, energia hidroelétrica, destino para esgotos e dejetos, e ainda como importantes áreas de recreação e lazer. Como resultado dessas várias demandas, os habitats aquáticos naturais foram profundamente modificados, com isso, destruídos alguns compartimentos da biota aquática enquanto que em outros ecossistemas, espécies foram introduzidas inadvertidamente.

Os ambientes lóticos e lênticos são ecossistemas que possuem algumas características em comum, e outras peculiares, e são abordados pela ecologia aquática. A marcante zonação longitudinal de fatores físico-químicos e biológicos característico de um rio é contrastado com a distribuição vertical de lagos, sejam eles rasos ou profundos (COLE, 1994). Como característica em comum pode-se destacar que esses ambientes recebem sempre influencia de sua área de entorno, e a abordagem da bacia de drenagem como unidade de estudo, proposta por BARBOSA (1994) deve ser sempre adotada para qualquer tipo de estudo.

Nas últimas décadas podemos destacar, dentre vários avanços, dois fatos importantes. Primeiro a ocupação humana nas bacias de drenagem associada ao desenvolvimento desordenado das cidades, que têm alterado drasticamente os ecossistemas aquáticos, principalmente, acelerando o aporte de nutrientes para os corpos d’água. XU et al. (2001) ressaltam que durante as últimas décadas a frase “saúde dos ecossistemas” tem sido usada com maior freqüência na literatura, e que “gerentes ambientais” começaram a considerar a proteção dos ecossistemas aquáticos como uma das prioridades do manejo ambiental. Alterações nos ciclos de nutrientes e na estrutura biológica dos ambientes aquáticos definitivamente irão ocasionar alterações na saúde dos ecossistemas, e de acordo com ODUM (1985), o acompanhamento do balanço energético, do ciclo dos nutrientes, da estrutura e do funcionamento das comunidades nos ecossistemas em geral, são estratégias fundamentais para se detectar diferentes formas de impactos.

O segundo fato importante, anteriormente relatado, seria o grande impulso dado ao estudo de microorganismos com o advento de novas técnicas de microscopia de epifluorescência e de avaliação da produção bacteriana com o uso de radioisótopos. Os trabalhos de POMEROY (1974) e AZAM et al. (1983) evidenciaram a existência de importantes conexões entre matéria orgânica dissolvida, bactérias e protozoários, consolidando o "microbial loop". Este conceito, adicionado ao modelo convencional de redes tróficas, estabelece que parte do carbono fixado durante a fotossíntese é excretado pelo fitoplâncton na forma de matéria orgânica dissolvida e subseqüentemente absorvido por bactérias. Estas são predadas por protozoários (flagelados e ciliados), que por sua vez servirão de alimento ao zooplâncton. Parte da energia retorna então à rede alimentar clássica, para os níveis tróficos superiores. Embora o modelo do "microbial loop" esteja sendo recentemente reavaliado, ele é ainda o modelo conceitual mais utilizado nos estudos da cadeia de detritos e regeneração de nutrientes em ecossistemas aquáticos.

A incidência da radiação solar à superfície do ecossistema aquático é o inicio do processo de fotossíntese e do metabolismo como um todo, e estimativas da penetração da radiação fotossintética ativa (PAR) na água são essenciais para o conhecimento dos processos e mecanismos que controlam a transferência de energia e o ciclo da matéria orgânica em lagos. O estudo das interações tróficas entre fitoplâncton e bacterioplâncton, bem como das relações entre essas comunidades e a matriz abiótica alcançou grandes avanços com os trabalhos de POMEROY (1974) e AZAM *et al.* (1983). No entanto, VAN WAMBEKE *et al.* (2002) afirmam que estudos de produção primária e bacteriana são escassos e uma das razões básicas é que, assim como nas regiões temperadas, também nos ambientes aquáticos tropicais são pouco conhecidas as relações entre o fitoplâncton e o bacterioplâncton e a influência dos fatores abióticos na produção como um todo. Da mesma forma, carecem de estudos mais detalhados as relações entre taxas de produção primária (inclusive produção bacteriana) e o estado trófico de ambientes aquáticos.

Alguns estudos (e. g. LE et al., 1994; DI SIERVE et al., 1995) têm demonstrado que a abundância e a produtividade de bactérias heterotróficas estão positivamente relacionadas aos níveis de clorofila-*a* e produção fitoplanctônica. As relações numéricas entre bacterioplâncton, conteúdo de clorofila-*a* e taxas de produtividade fitoplanctônica têm sido interpretadas como o resultado de um acoplamento metabólico entre as duas comunidades, o qual é uma função da concentração de carbono dissolvido na água (VAN WAMBEKE et al, 2002). De acordo com KARLSSON et al. (2002) a produção fitoplanctônica é, na maioria dos lagos, limitada pelas concentrações de nitrogênio, assim como a produção bacteriana é limitada pelas concentrações de fósforo, no entanto, a quantidade de material alóctone é o principal fator que altera as razões N/P e BP/PP em lagos temperados.

Os ecossistemas aquáticos do trecho médio da bacia do Rio Doce (Minas Gerais), no qual está inserido o terceiro maior sistema de lagos do Brasil, apesar de estarem proximamente localizados apresentam características que os diferenciam ecologicamente, sendo os fatores específicos de cada ambiente os principais responsáveis por esta variação (BARBOSA et al., 1997; TUNDISI & SAIJO, 1997). Estudos prévios na região, salientam a importância e a influência da sazonalidade em diferentes processos ecológicos nos ecossistemas aquáticos. O período de chuvas no verão, conduz o maior aporte de material alóctone para os ambientes aquáticos, refletindo em alterações na ciclagem de nutrientes e no fluxo de energia. BARBOSA & TUNDISI (1980) e BARBOSA et al. (1989), relataram diferenças sazonais na produção primária do fitoplâncton, altas taxas de fixação de carbono no escuro e a existência de complexas interações a nível do bacterioplâncton nos ambientes aquáticos da região. Segundo estes autores, este trecho do médio Rio Doce, é muito privilegiado para estudos a cerca de interações entre lagos, rios e floresta, que compõem o ecossistema local.

Este trabalho foi dividido em 5 capítulos que abordam principalmente a produção primária e bacteriana em rios e lagos do trecho médio da bacia do Rio Doce. A hipótese testada é que a produção primária varia sazonalmente (períodos de chuva e seca) resultando em alteração da relação PB/PF em função, principalmente do aporte de matéria orgânica alóctone sendo também afetada pela carga interna de nutrientes. A expectativa é que este estudo contribua para o entendimento da fixação de carbono em sistemas lóticos e lênticos, possibilitando a definição de uma tipologia regional. Além disso, este estudo permitirá a ampliação e aprofundamento do conhecimento limnológico do sistema de rios e lagos do médio rio Doce e de suas interações com áreas naturais e alteradas (p. ex.: plantações de *Eucalyptus* spp), de forma a contribuir para a proposição de medidas de conservação dos ecossistemas aquáticos e uso sustentável de seus recursos.

1.1- Objetivos

O presente estudo pretende inferir sobre as relações existentes entre a produção bacterioplanctônica e fitoplanctônica, determinando a importância dessas comunidades na fixação de carbono e transferência de matéria orgânica em rios e lagos do trecho médio da bacia do Rio Doce-MG, nos períodos de chuva e seca. A partir das concentrações de carbono, nitrogênio e fósforo e das razões N/P na água, complementarmente, este projeto permitirá uma avaliação das possíveis relações entre produtividade e grau de trofia, com base em padrões diários, sazonais e anuais.

Especificamente, o trabalho pretende:

1- Caracterizar os teores de carbono orgânico e inorgânico dissolvido, nitrogênio, fósforo, e as razões N/P na água em 8 rios e 7 lagos do trecho médio do Rio Doce, durante dois anos consecutivos nos períodos de seca e chuva;

2- Estimar as taxas de produção bacterioplanctônica (PB) e de produção fitoplanctônica (PF), assim como as razões PB/PF em 8 rios e 7 lagos do trecho médio do Rio Doce, durante dois anos consecutivos nos períodos de seca e chuva, com isso, determinar a contribuição dessas comunidades na fixação de carbono para os níveis superiores da cadeia trófica;

3- Verificar as alterações nas taxas produção bacterioplanctônica e fitoplanctônica e nas concentrações de nutrientes, em períodos diurnos (manhã e tarde) e sua variação sazonal (chuva e seca), em 4 lagos selecionados do trecho do médio Rio Doce;

4- Correlacionar as taxas de produção do bacteroplâncton e do fitoplâncton com a ordem de grandeza e o grau de trofia de 8 rios do trecho médio do Rio Doce, durante dois anos nos períodos de seca e chuva; e

5- Determinar a relação entre o estado trófico dos ambientes estudados com as taxas de produção bacterioplanctônica e fitoplanctônica nos períodos de seca e de chuva;

2- MATERIAL E MÉTODOS

2.1- Ambientes Lóticos

Para os rios, este estudo foi desenvolvido no trecho médio da bacia do rio Doce, em Minas Gerais, sudeste do Brasil (figura 1), em oito estações de amostragem representativas das sub-bacias dos rios Caraça (20^{o}06’00”S - 43^{o}29’09”W), Barão de Cocais (19^{o}57’27”S - 43^{o}28’24”W), Santa Bárbara (19^{o}50’01”S - 43^{o}21’14”W), Peixe (19^{o}44’35”S - 43^{o}01’16”W), Severo(19^{o}36’57”S - 42^{o}50’50”W), Piracicaba (19^{o}29’25”S - 42^{o}31’08”W), Ipanema (19^{o}28’14”S - 42^{o}32’01”W) e Doce (19^{o}19’12”S - 42^{o}21’52”W). Este trecho possui regiões com diferentes características ambientais compreendendo desde áreas protegidas até áreas sujeitas a diferentes impactos antrópicos (PAULA et al., 1997), possibilitando estudos comparativos. Nos pontos de amostragem, o rio Caraça é um trecho de 2^{o} ordem; os rios Barão de Cocais, Severo e Ipanema são de 3^{o} ordem; o rio Peixe de 4^{o} ordem; o rio Santa Bárbara de 5^{o} ordem; o rio Piracicaba de 6^{o} ordem e o rio Doce de 7^{o} ordem, segundo o método de STRAHER (GORDON et al., 1992).

O ribeirão Caraça em grande parte localizado na área de proteção ambiental, Parque Natural do Caraça, é uma estação localizada num trecho de águas de cabeceira não sujeito às atividades antrópicas. Os demais rios apresentam influência antrópica variada, destacando-se pecuária extensiva, mineração, garimpo, efluentes domésticos e industriais, além de extensas áreas de reflorestamento com *Eucalyptus* *spp*. O ribeirão Ipanema apresenta grande parte de seu percurso canalizado e o rio Doce recebe descargas de todos os outros rios. Destaca-se que a amostragem neste rio foi feita à jusante da Cachoeira Escura, de fundamental importância na recuperação da qualidade das águas do rio Doce (BARBOSA et al., 1997).

2.2- Ambientes Lênticos

Em relação aos lagos, os estudos foram desenvolvidos em lagos do Parque Estadual do Rio Doce (PERD) e de seu entorno, incluindo áreas sujeitas a diferentes impactos antrópicos. O PERD (19º29’24”–19º48’18” S; 42º28'18”–42º38’30” W), com área *c*.36.000 ha, localiza-se no trecho médio da bacia do Rio Doce (MG, Brasil), constitui o maior remanescente da Mata Atlântica no Estado de Minas Gerais (Figura 2). Seu entorno é praticamente ocupado por extensas áreas plantadas com *Eucalyptus* spp e pastagens com distintos graus de degradação.

Foram selecionados dois lagos no PERD (Dom Helvécio e Carioca) e 5 lagos no seu entorno (Amarela, Águas Claras, Barra, Jacaré e Palmeirinha). O lago Dom Helvécio é o maior lago do sistema (6,87 km^2; 32,5 m de profundidade máxima) é aberto a turistas para pesca desportiva, banho e passeios de barco. A lagoa Carioca (0,13 km^2; 11,8 m de profundidade máxima) não é aberta a visitantes, apresentando-se mais preservada. Dos lagos no entorno do PERD, a lagoa Amarela é o menor e o mais raso (0,11 km^2; 2,0 m de profundidade máxima) e colonizada por uma densa comunidade de macrófitas emersas e submersas, sendo provavelmente o lago mais eutrófico do sistema; a lagoa Águas Claras, apesar de situada em área de plantações de *Eucalyptus* apresenta-se menos impactada e com características oligotróficas. A lagoa Palmeirinha sofre também a influência direta de uma carvoaria e as lagoas Barra e Jacaré (1,03 Km^2; 9,8 m de profundidade máxima), além de circundadas por plantações de *Eucalyptus* spp abrigam clubes de pesca, recebendo lançamentos intermitentes de efluentes domésticos não tratados.

2.3- Coleta e Amostragens

Durante os períodos de seca (agosto de 1999 e 2000) e chuva (janeiro de 2000 e 2001) foram realizadas as medidas de produção e amostragens de água nos rios e lagos. Para os lagos foi determinada uma estação central em cada ambiente e quatro profundidades (100%, 10%, 1% de penetração de luz e zona afótica) e nos rios um ponto de amostragem na sub-superfície de cada ambiente na margem esquerda. A temperatura, condutividade elétrica, pH e oxigênio dissolvido foram medidas "*in situ*" com auxílio de um multianalisador (Horiba, mod. U-22), assim como a radiação solar, estimada com um radiômetro portátil (LI-COR 196) e amostras de água foram coletadas, nos lagos com auxílio de uma garrafa de van Dorn e nos rios com auxílio de um balde, para posterior análise em laboratório dos teores de nutrientes.

2.4- Parâmetros Analisados da Coluna D'água

A transparência da água foi estimada através da profundidade de desaparecimento visual do disco de Secchi, assumindo-se que esta profundidade corresponde, em média, a 10% da luz incidente na superfície. A determinação da alcalinidade total foi feita de acordo com Mackereth et al. (1978), com ác. sulfúrico 0,01 N e os resultados expressos em m Eq CO_2 l^{-1}.

Nitrogênio total

Foi utilizada a metodologia descrita por Mackereth et al. (1978) com amostras não filtradas. Nesta determinação os compostos nitrogenados em água são oxidados a nitrato pelo aquecimento com solução alcalina de persulfato e pressão. O nitrato é determinado reduzindo-o a nitrito utilizando cádmio esponjoso. O complexo formado apresenta uma coloração rosa, a leitura da absorbância é feita no espectrofotômetro a 543nm, sendo sua concentração expressa em $\mu g\ l^{-1}$.

Nitrito

A partir de amostras filtradas foi utilizada a metodologia descrita por Mackereth et al. (1978). Em solução ácida, o nitito vai a ácido nitroso, que juntamente com a sulfanilamida resulta num sal diazônio, este é complexado com outra amina aromática, N-1-naftil etileno diamina dicloreto resultando um complexo de coloração rosa. A leitura da absorbância foi feita no espectrofotômetro a 543nm, sendo sua concentração expressa em $\mu g\ l^{-1}$.

Nitrato

A partir de amostras filtradas foi utilizada a metodologia descrita por Mackereth et al. (1978). O nitrato é reduzido a nirito usando-se cádmio esponjoso em meio a soluções de borax e cloreto de amônio. Após a redução utilizou-se as normas metodológicas de análise e leitura adotadas para nitrito, descritas acima e sua concentração expressa em $\mu g\ l^{-1}$.

Amônia

A partir de amostras filtradas foi utilizada a metodologia descrita por Grasshoff (1976). A amônia reage com o fenol e o hipoclorito em solução alcalina, formando o indofenol azul. A reação é catalisada pelo nitroprussiato, formando um complexo de coloração azul. A leitura da absorbância é feita no espectrofotômetro a 630nm, sendo sua concentração expressa em $\mu g\ l^{-1}$.

Fósforo total

Foi utilizada a metodologia descrita por Golterman et al. (1978) com amostras não filtradas. Os compostos fosfatados em água são submetidos à digestão ácida pelo aquecimento em presença de persulfato de potássio e solução de ácido perclórico a 15%. O fosfato resultante da digestão em meio ácido reage com molibdato, formando o ácido molibdofosfórico que, reduzido, forma um complexo de cor azul. A leitura da absorbância é feita no espectrofotômetro a 882nm, sendo sua concentração expressa em $\mu g\ l^{-1}$.

Fosfato solúvel reativo

A partir de amostras filtradas foi utilizada a metodologia descrita por Golterman et al. (1978). Em solução ácida, o fosfato reage com molibdato, formando o ácido molibdo fosfórico, que reduzido forma um complexo de coloração azul. A leitura da absorbância é feita no espectrofotômetro a 882nm, sendo sua concentração expressa em $\mu g\ l^{-1}$.

Silicato solúvel reativo

A partir de amostras filtradas foi utilizada a metodologia descrita por Mackereth et al. (1978). Em solução ácida o ácido silícico e seus derivados reagem com o íon molibdato, formando um complexo de cor amarelada, cuja absorbância é lida a 365nm. Quando reduzido forma um silicamolibdeto de cor azul. A leitura da absorbâcia após a redução é feita no espectrofotômetro a 810nm sendo sua concentração expressa em $\mu g\ l^{-1}$.

Clorofila –*a*.

Para a determinação da concentração de clorofila-a, um volume conhecido de cada amostra de água coletada foi filtrado em filtros Schleicher & Schuell GF 52-C, no laboratório, colocados em envelopes de papel e frascos âmbar contendo sílica-gel e mantidos no freezer até à extração. As determinações da concentração de clorofila-a a feofitina seguiram a técnica de extração com acetona 90%, de acordo com Lorenzen (1967) e os valores expressos em $\mu g\ l^{-1}$.

Carbono orgânico e inorgânico dissolvido

Amostras de água filtrada (filtros GF 52-C S&S) foram analisadas no aparelho TOC-5000A (Shimadzu), onde o carbono total dissolvido é determinado através da queima a alta temperatura (680 ^{o}C), de toda a matéria orgânica dissolvida na água. O carbono inorgânico dissolvido é determinado através do mesmo instrumento, com acidificação da amostra com ácido fosfórico e o carbono orgânico dissolvido determinado pela diferença entre o total e o inorgânico.

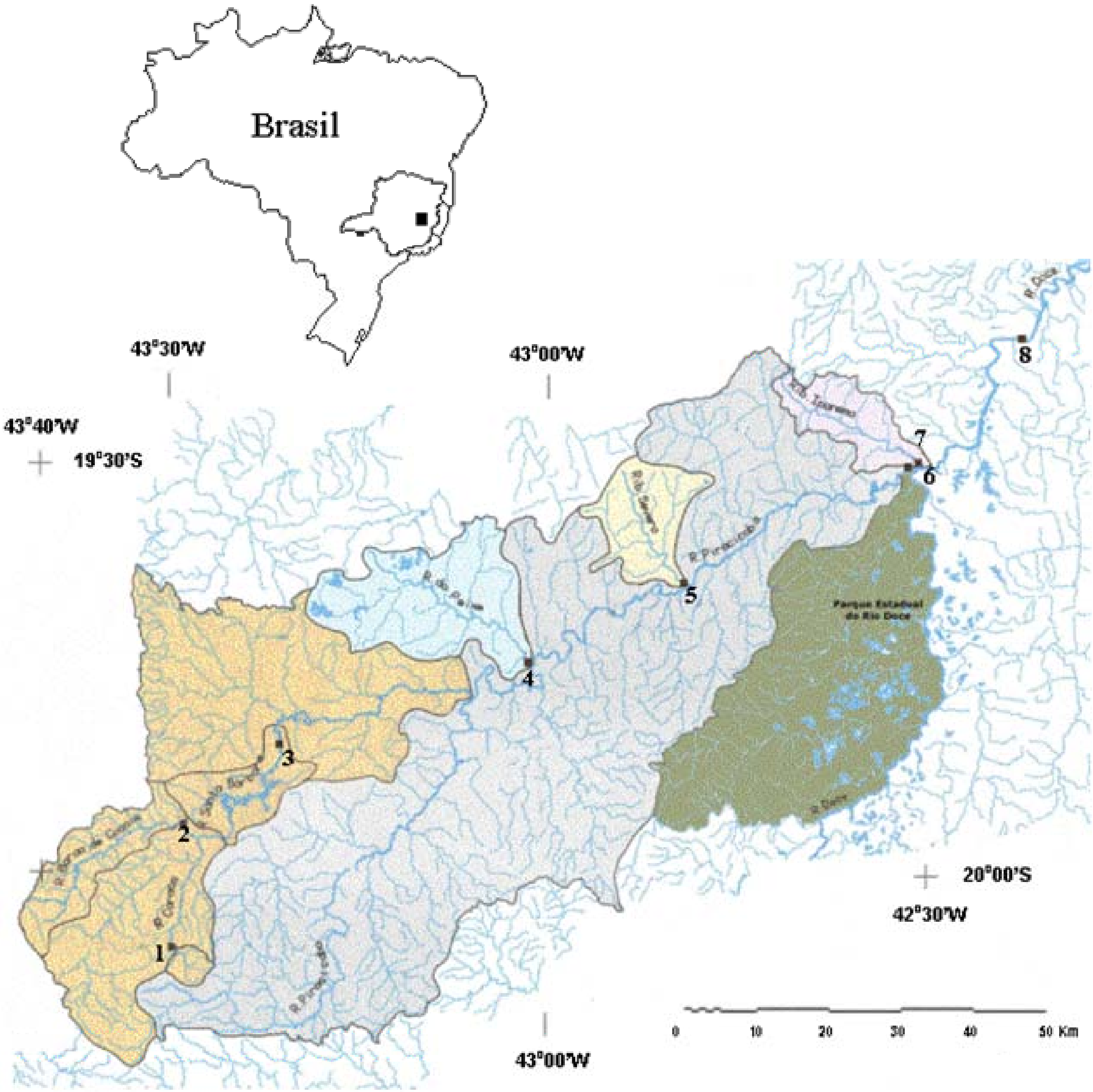
2.5- Estimativas da Produtividade Primária

Estimativas da produção bacterioplanctônica foram feitas em laboratório imediatamente após a coleta de amostras em campo. Em tubos de eppendorf, incubações foram feitas no escuro com 1,3 ml da amostra e 10 µl de leucina triciada (L-[4,5-^{3}H] Leucine, TRK 510, 142Ci/mnmol), na concentração final de 10nMolar, durante 40 minutos (SMITH & AZAM, 1992). Após este período a produção era interrompida com 90 µl de TCA 100% e todo material foi centrifugado a 13000 rpm para a formação de um "pellet". As leituras em DPM foram obtidas no Liquid Scintillation Analyzer (Packard, Tri-carb 2100TR) após adição de coquitel bray (Bray, 1960) e para o calculo final usou-se o fator de 0,86 para a correção de leucina incorporada transformada em carbono. Para cada amostra foram feitas tréplicas e um branco com adição prévia de TCA 100%, para não haver nenhuma reação.

Estimativas da produção fitoplanctônica foram realizadas em campo através da técnica de incorporação de carbono marcado ^{14}C em frascos claros e escuros (Steemann-Nielsen, 1952), descrita em Barbosa & Tundisi (1980). Foram utilizados um conjunto de três frascos de 70 ml (dois transparentes e um escuro) para cada profundidade de incubação. Inoculou-se 0,5 ml de $NaH^{14}CO_3$ (2 µCi) e as amostras ficaram durante um período de 3-4 horas de incubação. Após a incubação em laboratório, as amostras foram filtradas (20 ml) sobre vácuo e os filtros (Schleicher & Schuell, ME 25 com 0,45 µm de poro) imersos em "vials" contendo coquetel de cintilação Bray (Bray, 1960). As leituras em DPM foram obtidas no Liquid Scintillation Analyzer (Packard, Tri-carb 2100TR).

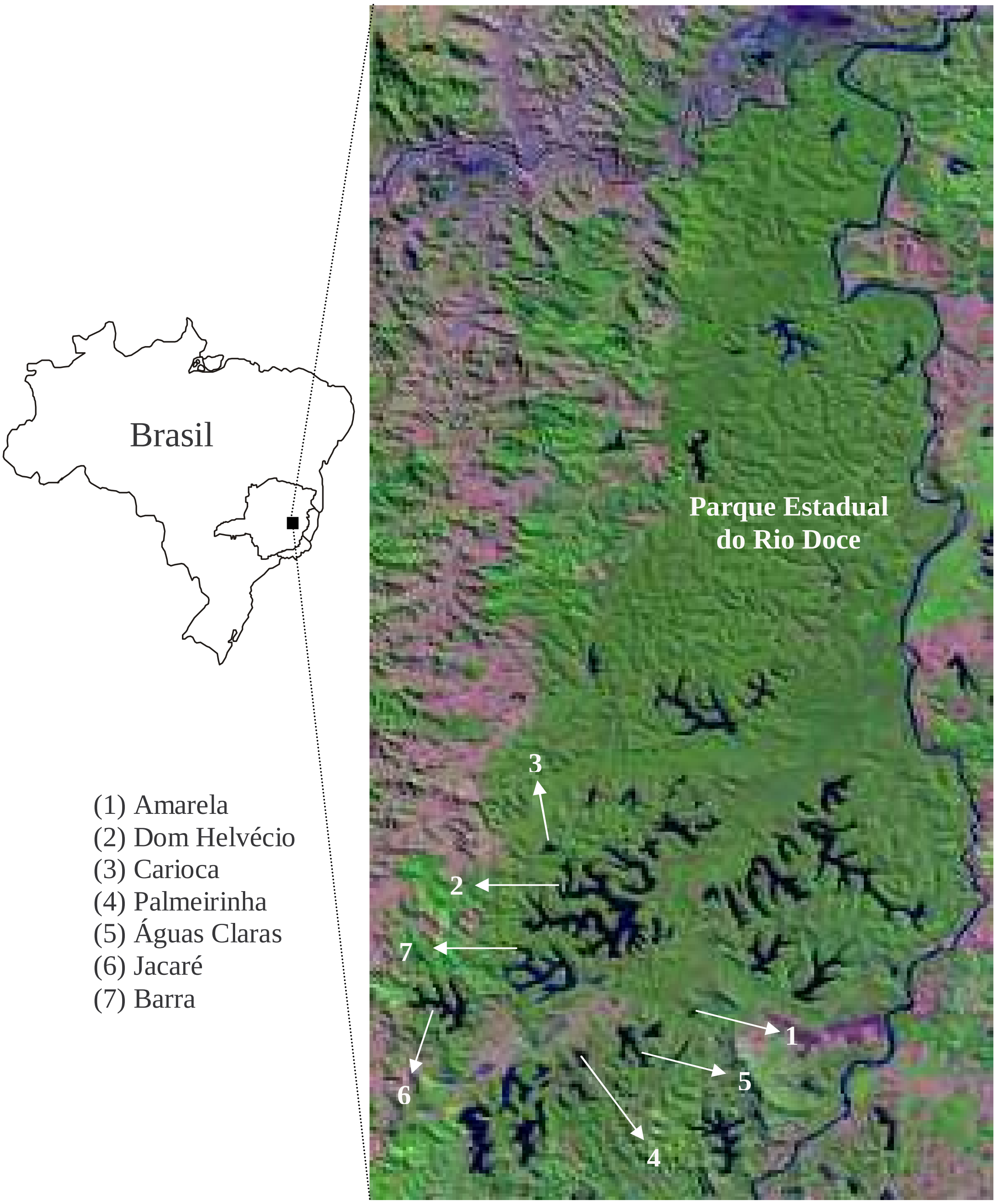
2.6- Tratamento dos dados

Para a maioria dos resultados obtidos foi aplicada a análise de componentes principais (Principal Component Analysis - PCA) para a identificação de fatores que poderiam influenciar as taxas de produção bacterio e fitoplanctônica e identificar similaridades/diferenças entre os ambientes estudados. Para a planilha de dados foram utilizados os seguintes parâmetros: temperatura, pH, condutividade, oxigênio, clorofila-a e as concentrações de nutrientes. Antes das análises um teste estatístico (ANOVA) foi utilizado para verificar se as diferenças encontradas entre os ambientes e entre os períodos (seca x chuva; manha x tarde) eram significativos. Correlações lineares de Pearson também foram testadas entre as concentrações de nutrientes e as taxas de produção.



Fonte: modificada de Paula et al. (1997).

Figura 1: Localização das sub-bacias e as estações de amostragens nos sistemas lóticos do trecho médio da bacia do Rio Doce, Minas Gerais, Brasil.



Fonte: modificada da EMBRAPA (http://www.embrapa.gov.br).

Figura 2: Localização das lagoas do Parque Estadual do Rio Doce e da área de entorno do PERD, Minas Gerais, Brasil.

Capítulo 1

Trophic state and microorganisms community of major sub-basins of the middle Doce river basin, southeast Brazil.

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Trophic State and Microorganisms Community of Major Sub-Basins of the Middle Doce River Basin, Southeast Brazil.

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ABSTRACT

Total phosphorus concentration was used to define the trophic state of the main sub-basins of the middle Doce river, in Minas Gerais state (southeast Brazil) and water physical, chemical, and microbiological variables were analised sazonaly durant 2000 to 2001 years. The study evaluated changes in water quality caused by seasonality and human activities. Water temperature, conductivity, pH, total alkalinity, dissolved oxygen, and concentrations of NH_4-N, NO_2-N, NO_3-N, total-N, PO_4-P, total-P, DOC, and chlorophyll-a were measured in seven rivers stretches (sampling stations). Total yeasts, faecal and total coliforms, and heterotrophic bacteria were also determined. The studied areas were considered to be under oligotrophic to eutrophic conditions. A high positive correlation between total-P and total-N and faecal coliforms was observed, and heterotrophic bacteria density was identified as a good parameter to differentiate the ecosystems These results suggest the inclusion of the trophic level and the distinct activities within a watershed as important elements when proposing conservation and restoration areas.

Key words: Microorganisms, phosphorus, rivers, trophic state

INTRODUCTION

The disordered human occupation of the drainage basins has drastically modified the aquatic ecosystems mainly by accelerating the deposition of nutrients in water bodies. Due to the increasing need to use water resources in the last decades, the evaluation of eutrophication impact in lotic ecosystems is imperative (Kelly & Whitton, 1998; Wetzel, 1991). Although lotic ecosystems are abundant in Brazil, they have not been studied enough and there are only a few data available that can support the definition for conservation and management policies (e. g. Barbosa *et al.*, 1997; Barbosa & Callisto, 2000). Water uses and socio-economic aspects of the drainage basin are fundamental elements for the understanding of the ongoing processes within aquatic ecosystems and for definition of strategies for conservation and management politicies of aquatic environments (Tundisi & Barbosa, 1995).

Inputs of allochthonous material from industrial and domestic effluents, associated with other human activities in densely occupied watersheds have been changing significantly the water quality as well as the ecological conditions of the rivers. This reflects considerably on the composition and structure of aquatic communities and their interactions, as demonstrated previously by Barbosa *et al.* (1997) for the major sub-basins of the middle Doce river, in the state of Minas Gerais. Thus, characterization of the major nutrients levels, trophic state, and composition and structure of aquatic communities constitutes essential tools to detect different types of impact as a way to propose mitigation and conservation measures.

Traditionally, the characterization of trophic status of an aquatic ecosystem involves determination of nutrient concentrations, mainly phosphorus and nitrogen, and can be complemented with information on variables such as chlorophyll-*a* concentration and water transparency (OECD, 1982; Salas & Martino, 1991). The quality of the waters has been routinely evaluated by measuring physical and chemical

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variables, without considering the important role played by the existing aquatic biota. The presence of bacteria of the coliform group is considered an indicative of potencial health risks and is a widely used microbial method for monitoring water quality (Greenberg, 1998). Several studies have focused on the occurrence of yeasts related to wastewater (Simard, 1971; Hagler & Mendonça, 1981) showing that yeast counts can be a monitoring method that can complement coliform counts also reflecting eutrophication (Hagler & Ahearn, 1997). Salas and Martino (1991) have used the concentration of total phosphorus to define the level of human impact on aquatic environments and consider it as the most appropriate index for tropical environments. However, its application to lotic environments still needs further and conclusive studies.

In an attempt to identify, among the components of the existing biota, potential indicators of the trophic status of the water, the present study had as the major objective to characterize the trophic status of the main sub-basins of the middle Doce river using physical, chemical and microbiological variables. This study also aimed at determining changes in water quality caused by human activities in order to propose the including of trophic indices for measuring the restoration of degraded areas.

STUDY AREA

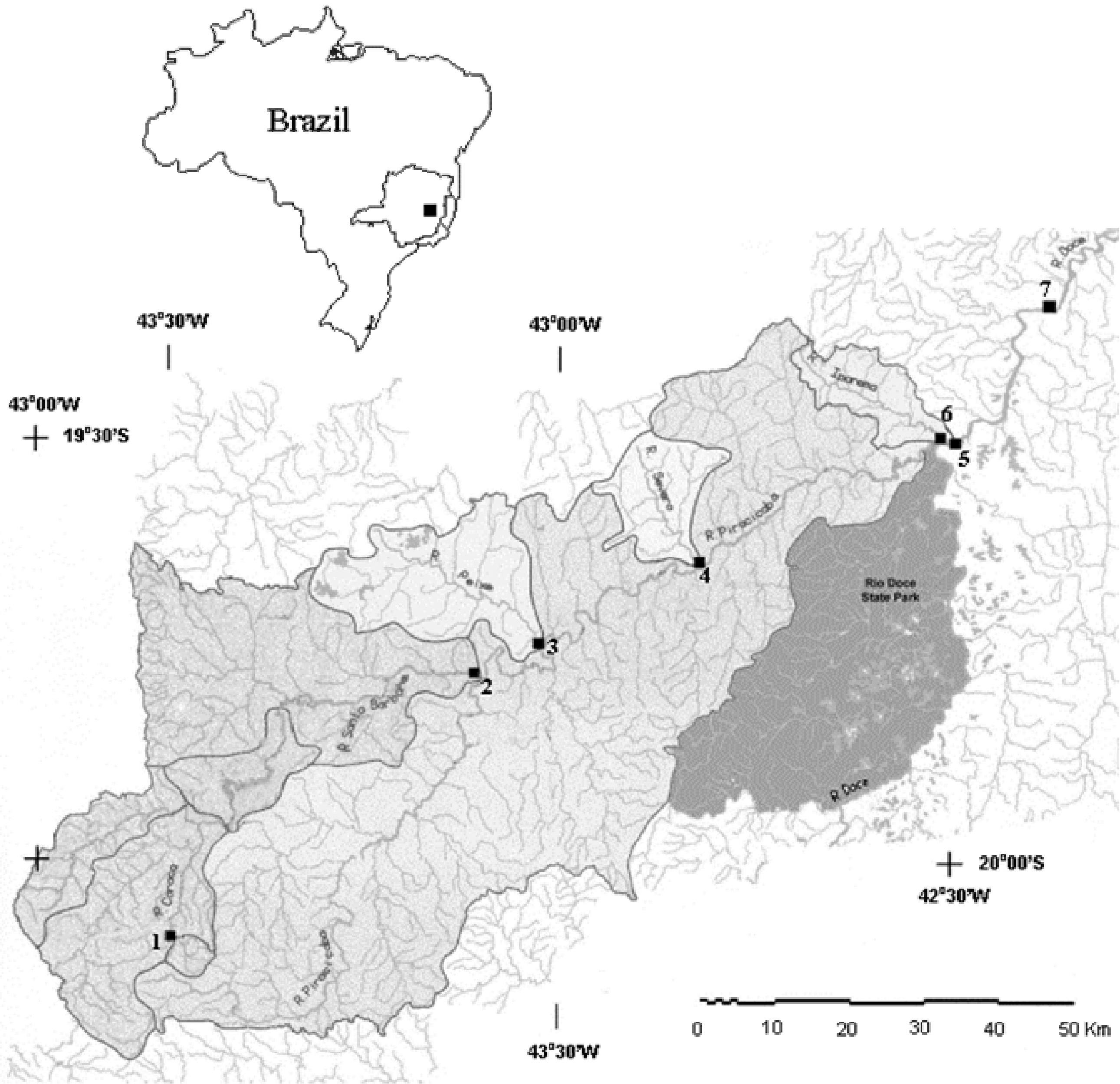
Traditionally, the characterization of trophic status of an aquatic ecosystem involves determination of nutrient concentrations, mainly phosphorus and nitrogen, and can be complemented with information on variables such as chlorophyll-*a* concentration and water transparency (OECD, 1982; Salas & Martino, 1991). The quality of the waters has been routinely evaluated by measuring physical and chemical variables, without considering the important role played by the existing aquatic biota. The presence of bacteria of the coliform group is considered an indicative of potencial health risks and is a widely used microbial method for monitoring water quality (Greenberg, 1998). Several studies have focused on the occurrence of yeasts related to wastewater (Simard, 1971; Hagler & Mendonça, 1981) showing that yeast counts can be a monitoring method that can complement coliform counts also reflecting eutrophication (Hagler & Ahearn, 1997). Salas and Martino (1991) have used the concentration of total phosphorus to define the level of human impact on aquatic environments and consider it as the most appropriate index for tropical environments. However, its application to lotic environments still needs further and conclusive studies.

In an attempt to identify, among the components of the existing biota, potential indicators of the trophic status of the water, the present study had as the major objective to characterize the trophic status of the main sub-basins of the middle Doce river using physical, chemical and microbiological variables. This study also aimed at determining changes in water quality caused by human activities in order to propose the including biological parameters in trophic indices for measuring the restoration of degraded areas.

METHODS

Measurements and samplings of the above described rivers were done during the rainy and dry seasons of 2000 and 2001 (January and August respectively). The water temperature, electric conductivity, pH and dissolved oxygen were measured "*in situ*", with the help of a multiprobe (Horiba, mod. U-22). At these places, water samples were collected to determine the contents of dissolved organic carbon (TOC-500 Shimadzu); total nitrogen, nitrate-nitrogen, nitrite-nitrogen and total alkalinity (Mackereth *et al.*, 1978); ammonium-nitrogen (Grasshoff, 1976); total phosphorus and soluble reactive phosphorus (Golterman *et al.*, 1978). Chlorophyll-*a* concentration was obtained after filtration of total samples in Schleicher & Schuell GF 50-A filters and determination according to Lorenzen (1967).

For microbiology analyses the surface water samples were taken directly into sterile glass bottles and returned to the lab on ice within 8 hr for processing. Total and faecal coliforms were determined using standard most probable number methods (MNP) and Heterotrophic bacteria counts were made using the pour plate method on NWRI agar (Heterothrophic plate count agar - HPCA) (Greenberg *et al.*, 1998). For total and faecal coliforms determination, the following dilutions 10^{-1}, 10^{-2} and 10^{-3} of the water sample, were used. For the yeasts counts (CFU)



Source: Paula et al. (1997) modified.

Figure 1: Location of the sub-basin and respective sampling stations in the middle Rio Doce basin, Minas Gerais State-Brazil.

aliquots of 0.1 ml of the samples were plated in triplicates on YM agar (1.0 % glucose, 0.5 % peptone, 0.3 % yeasts extract, 0.3 % malt extract, 2.0 % agar, 10 mg chloramphenicol). Plates were incubated at 25ºC and yeasts counts were obtained after 3 to 5 days as described in Kurtzman and Fell (1998).

Principal component analysis (PCA) was used in the treatment of the abiotic and biotic data. The following variables were used in the matrix: temperature, pH, electrical conductivity, dissolved oxygen, chlorophyll-*a*, total alkalinity, TP, $P\text{-}PO_4^{3-}$, TN, $N\text{-}NH_4^+$, $N\text{-}NO^{3-}$, $N\text{-}NO_2$, DOC, total and faecal coliforms, yeasts and heterotrophic bacteria counts.

RESULTS AND DISCUSSION

During the sampling periods precipitation varied between 0.0 mm (July 2000, 2001) and 59.2 mm (January 2000) (Data obtained from Instituto Mineiro de Gestão das Águas – IGAM/SIMGE).

A considerable seasonal temperature variation is shown (table 1), with the lowest value (12.7°C) registered during the dry season (winter), especiall in

Table 1: Abiotic variables (maximum and minimum values of temperature, pH, eletric conductivity, dissolved oxygen, alkalinity, chlorophyll-*a* and dissolved organic carbon) of the sub-basins of the middle Doce river basin, during the rainy and dry seasons of 2000 and 2001.

Rivers	Temp. ^0C	pH	Cond. µS/cm	D.O. mg/L	Alkal meq/L	Chlo. µg/L	DOC mg/L
Rainy	Min - Max	Min - Max	Min - Max	Min - Max	Min - Max	Min - Max	Min - Max
Caraça	16.0 - 19.3	4.0 - 5.2	9.0 - 16.0	6.5 - 9.9	0.0	2.9 - 3.7	3.9 - 12.4
Sta. Bárbara	23.1 - 25.6	6.6 - 7.0	39.0 - 58.3	5.0 - 9.8	0.1 - 0.4	6.4 - 8.6	2.2 - 3.6
Peixe	22.0 - 24.8	6.4 - 7.5	62.0 - 63.0	6.2 - 8.7	0.2 - 0.4	7.5 - 18.7	2.5 - 7.5
Severo	22.8 - 24.5	6.5 - 7.4	25.5 - 26.0	6.2 - 9.3	0.1 - 0.3	2.7 - 53.5	1.8 - 10.0
Piracicaba	26.4 - 27.5	7.1 - 7.6	75.0 - 120.4	3.8 - 7.6	0.2 - 0.5	1.6 - 20.3	2.7 - 4.3
Ipanema	27.1 - 29.0	7.4 - 7.5	283.0 -375.4	0.8 - 2.0	0.7 -2.1	2.7 - 4.3	9.3 - 13.7
Doce	26.6 - 28.3	6.8 - 7.6	54.0 - 107.8	5.4 - 9.0	0.2 - 0.5	6.4 - 15.5	3.2 - 4.0
Dry							
Caraça	12.7 - 17.9	5.2 - 6.6	1.0 - 9.2	7.7	0.0	5.1	1.9
Sta. Bárbara	19.3 - 22.1	6.8 - 6.9	6.0 - 61.5	5.6 - 7.6	0.4	3.7	0.9
Peixe	15.4 - 19.8	6.4 - 6.6	9.0 - 57.9	6.1 - 7.6	0.4	1.6	1.5
Severo	15.5 - 19.3	6.3 - 6.4	2.0 - 27.9	3.2 - 7.9	0.3	5.9	0.7
Piracicaba	21.6 - 26.1	6.8 - 6.9	121.5 - 148.0	7.3	0.4	27.5	1.9
Ipanema	25.9 - 27.0	6.8 - 7.3	322.0 - 356.0	0.3 - 0.4	1.7	0.7	9.3
Doce	21.9 - 23.9	6.6	88.8 - 101.0	3.2 - 8.2	0.4	8.6	1.7

Caraça river. This river also exhibited the lowest pH values (4.0) and the highest concentrations of dissolved oxygen (9.9 mg/L). Ipanema river showed the lowest concentration of oxygen (< 2.0mg/L) and the highest conductivity (375 µS/cm) and alkalinity values (2.1 meqCO_2/L). Chlorophyll-*a* concentrations were low in all rivers (0.7–27.5 µg/L), except in Severo river during the rainy season, when the highest value as observed (53.5µg/L).

Ipanema river showed the highest values of total-N (5,988µg/L), NH_4-N (3,320µg/L), total-P (843µg/L), PO_4-P (455µg/L), silica (5.5mg/L) and DOC (13.7mg/L) and among the sampled rivers, it showed the highest impact possibly due to the deposition of untreated domestic and industrial effluents (table 2). Caraça river, on the other hand, was considered the most preserved environment, presenting low values of total-N (48.6µg/L), NH_4-N (5.4µg/L), total-P (4.7µg/L) and PO_4-P (2.0µg/L). The stechiometrical ratios between nitrogen and phosphorus (N/P) during the rainy season, only in the first year, showed a limitation by nitrogen (N/P < 9, according to Vollenweider, 1983 in Salas & Martino, 1991), except in Caraça river, which showed high ratios in both sampling years (N/P = 97.7 and 52.8). During the two dry seasons, higher N/P ratios were observed in all of the rivers, showing a limitation by phosphorus, except in Ipanema river (N/P = 7.4 and 7.1 respectively) and in Caraça river (N/P = 4.4).

The countings of total and faecal coliforms were high during the rainy season (table 3). The highest densities of total coliforms were recorded in rivers Piracicaba and Ipanema (>160 x 10^3 MPN/100mL) and the lowest countings, in river Caraça (<2 x 10^3 MPN/100mL). For faecal coliforms the same variation was observed, with the highest values recorded in river Ipanema (> 160 x 10^3 MPN/100mL) and the lowest countings, in river Caraça (< 2 x 10^3 MPN/100mL). The highest value for heterotrophic bacteria was registered during the rainy season in rivers Santa Bárbara, Peixe e Severo (300 x 10^5 CFU/mL). The highest yeasts countings were recorded during the rainy season, except at Piracicaba river, which showed the highest counting during the dry season (460 CFU/mL).

The characterization of the trophic status according to the classification proposed by Salas and Martino (1991) is shown in table 4. The results show a clear seasonal variation in the trophic status for the majority of the studied rivers, except for Ipanema river, which is characteristically hyper-eutrophic and Caraça river, a typical oligotrophic environment, during both dry and wet seasons (table 4). Rivers Peixe, Piracicaba and Doce can be classified as eutrophic during the rainy season and meso-eutrophic during the dry season. Severo river can be considered eutrophic during the rainy season and

Table 2: Nutrient concentration (maximum and minimum values) of the sub-basins of the middle Doce river basins, during the rainy and dry seasons of 2000 and 2001.

Rivers	Tot-P µg/L	PO_4-P µg/L	Tot-N µg/L	NH_4-N µg/L	NO_3-N µg/L	NO_2-N µg/L	N/P
Rainy	Min - Max	Min - Max	Min - Max	Min - Max	Min - Max	Min - Max	Min - Max
Caraça	4.7 - 6.7	2.0 - 3.8	351.5 - 461.3	16.9 - 18.3	9.0 - 16.1	1.9 - 4.2	52.8 - 97.7
Sta. Bárbara	17.4 - 136.9	1.3 - 6.2	393.4 - 849.5	20.6 - 129.5	43.9 - 132.9	3.8 - 8.5	6.2 - 22.6
Peixe	52.9 - 170.8	11.1 - 19.3	1,181 – 1,358	26.3 - 473.3	87.5 - 337.5	13.6 - 171.0	8.0 - 22.3
Severo	14.7 - 469.7	1.2 - 7.8	234.1 – 1,836	9.4 - 32.6	23.3 - 68.3	2.9 - 3.5	3.9 - 15.8
Piracicaba	80.2 - 240.3	10.8 - 11.8	1,197 – 1,217	67.2 - 308.3	140.8 - 702.9	9.5 - 12.7	5.0 - 15.1
Ipanema	772.7 - 812.6	68.7 - 210.2	5,341 – 5,491	1,557 - 3,320	11.9 - 41.5	2.7 - 28.3	6.8 - 6.9
Doce	63.9 - 318.3	5.0 - 63.9	873.2 – 1,240	23.5 - 235.8	92.4 - 283.5	6.4 - 6.9	3.9 - 13.7
Dry							
Caraça	7.6 - 10.8	3.2	48.1 - 103.8	5.4 - 14.2	8.2 - 28.8	1.6 - 1.8	4.4 - 13.6
Sta. Bárbara	16.8 - 25.2	4.2 - 6.2	284.3 - 372.3	15.8 - 27.6	16.8 - 167.3	2.9 - 3.6	14.8 - 17.0
Peixe	95.8 - 125.5	62.1 - 73.5	1,894 – 1,957	192.0 - 369.1	43.5 - 902.9	76.8 - 193.2	15.9 - 19.8
Severo	22.8 - 25.9	3.6 - 9.7	226.0 - 277.7	5.5 - 30.1	15.3 - 122.4	2.0 - 2.4	9.9 - 10.7
Piracicaba	84.9 - 105.9	18.5 - 23.7	1,621 – 1,842	236.1 - 279.7	137.5 - 721.7	28.2 - 30.8	15.3 - 21.7
Ipanema	760.7 - 843.3	351.3 - 455.5	5,641 – 5,988	1,063 – 1,547	1.5 - 7.8	7.3 - 17.1	7.1 - 7.4
Doce	61.0 - 68.7	18.2 - 29.7	691.6 - 806.8	55.5 - 77.0	26.2 - 312.4	6.3 - 7.7	11.3 - 11.7

oligotrophic in the dry season when the quality of its waters is considerably improved. These variations demonstrate the importance of considering the contributions of allochthonous material and the consequent seasonal variations.
The first two axes of the PCA performed with only the significant variables accounted for 69.2 % of the total variance (Fig. 2A). The factor 1 (52.3 %) was negatively correlated with dissolved oxygen concentrations and positively correlated with total nitrogen and phosphorus, conductivity and total and faecal coliforms. Axis 2 was positively correlated with heterotrophic bacteria and pH. The first principal component scores contrasted the rich from poorer streams, in terms of organic matter, mainly TN and TP (Fig. 2B) and the second principal component scores contrasted the highest and the lowest value for heterotrophic bacteria. It is interesting to note the existence of an overlaping among streams sampled in winter and summer. In other words, different seasons were not clearly separated by both axis.

The quality of the waters in the middle River Doce

Due to the several human actions within the watershed (e. g. mining, siderurgy, Eucalyptus plantations, cellulose industry, and discharge of untreated sewage) most waters of the middle stretch of Doce river are heavily polluted. A particularly interesting example is provided by the sub-basin of Piracicaba river, where the major siderurgy and mining complexes of Minas Gerais state are located. According to CETEC reports (1998) this river received only in 1992 *ca*. 80,000 m^3 of untreated effluents and *ca*. 93,205 Kg/day of total suspended solids, which rendered *ca*. 71,855 Kg/day of chemical oxygen demand (COD), *ca*. 9,558 Kg/day of biochemical oxygen demand (BOD), and a toxicity of *ca*. 7,500 equitox/day.
In a previous study conducted in the same sampling areas Barbosa *et al*. (1997) found similar values for temperature, pH, electric conductivity, dissolved oxygen, total alkalinity, and soluble reactive silica. However, chlorophyll-*a* values during both wet and dry periods were lower. The concentrations of nitrogen and phosphorus reported during this previous study were lower than the ones recorded in the present study. The recorded increase in the concentrations of ammonium-nitrogen, soluble reactive phosphorus, and total phosphorus in Ipanema river during both sampled periods deserves attention since they reflect the high loads of untreated sewage of the municipality of Ipatinga. However, this situation shall be soon reverted due to the operation of a sewage treatment plant from September 2001.

Table 3: Countings (maximum and minimum values) of total and faecal coliforms, yeasts and heterotrophic bacteria of the sub-basins of the middle Doce river basins, during the rainy and dry seasons of 2000 and 2001.

Rivers	Total Coliforms MPN/100ml x 10^3		Faecal Coliforms MPN/100ml x 10^3		Yeasts CFU/ml		Heterotrophic Bacteria CFU/ml x 10^5	
	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry
	Min - Max	Min - Max	Min - Max	Min - Max	Min - Max	Min - Max	Min - Max	Min - Max
Caraça	N.D - 2	<2 - 4	N.D - <2	<2 - 2	N.D	3 - 15	N.D	N.D - 74
Sta. Bárbara	<2 - 28	<2 - 22	2 - 22	<2 - 2	N.D - 30	0 - 390	75 - >300	70 - 73
Peixe	<2 - ≥160	7	<2 - 30	1.1 - 2	N.D - 20	2 - 3	18 - >300	2.3 - 179
Severo	≥160	2 - 8	160	<2 - 7	33 - 190	10 - 26	48 - >300	2.3 - 50
Piracicaba	160	N.D - ≥160	3.4 - 50	N.D - 9	0 - 16	0 - 460	N.D - 49	N.D - 59
Ipanema	≥160	≥160	160	35 - 160	10 - 43	6 - 393	78 - 160	47.3 - >300
Doce	24 - 30	3.4 - 30	8 - 30	3.4 - 22	6 - 10	6 - 26	20 - 64	34 - 85

Table 4: Trophic state classification of the sub-basins of the middle Doce river (Southeast, Brazil), according to the total phosphorus concentration.

Rivers	Trophic State (Salas and Martino, 1991)					
	Oligotrophic (23,1)		Mesotrophic (39,6)		Eutrophic (118,7)	
	Rainy	Dry	Rainy	Dry	Rainy	Dry
Caraça	4.7 - 6.7	7.6 - 10.8				
Sta. Bárbara	17.4	16.8 - 25.2			136.9	
Peixe			52.9	95.8	170.8	125.5
Severo	14.7	22.8 - 25.9			469.7	
Piracicaba			80.2	84.9 - 105.9	240.3	
Ipanema					772.7 - 812.6	760.7 - 843.3
Doce			63.9	61.0 - 68.7	318.3	

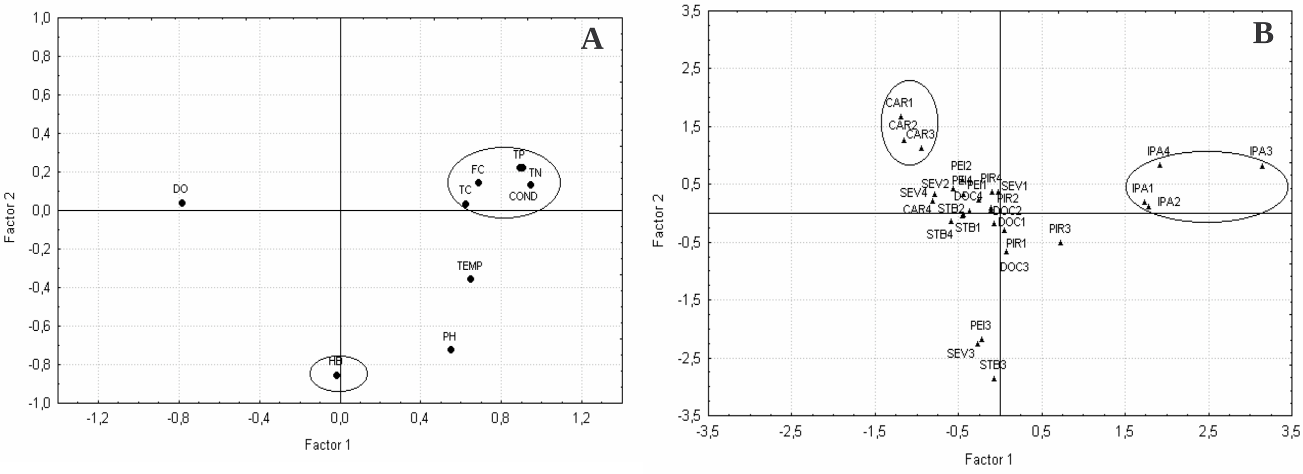


Figure 2: Correlations of biotic and abiotic parameters with the first two axes of principal component analysis (A) and score distributions of streams sampled in different seasons along the first two principal components axes (B). CAR=Caraça; STB=Santa Bárbara; PEI=Peixe;SEV=Severo; PIR=Piracicaba; IPA=Ipanema; DOC=Doce.
1= rainy2000; 2= dry2000; 3= rainy2001; 4= dry2001

Microorganisms and the trophic status of the waters in the middle Doce river

In an attempt to associate information on representative groups of the biota, and stress the use of organisms in the characterization of water quality, the presence of microorganisms (faecal and heterotrophic bacteria) in rivers could be consider as potential indicators of the trophic status of these aquatic ecosystems.

Although Huszar *et al.* (1998) suggested the use of phytoplankton species composition as a more sensitive trophic status definition in lakes, for lotic ecosystems this community is not probably the most appropriated, mainly due to the considerable washout during the rainy season. The recorded data clearly show the existence of significant correlations (Fig. 2A) between the levels of total phosphorus and nitrogen and faecal coliforms. The inclusion of these microorganisms, as well as heterotrophic bacteria, in the characterization of the trophic status is suggested here as a possibility for incorporating some biological information in the current classification of trophic status of stream and rivers.

High correlation between total phosphorus and faecal coliforms were also reported by Rosa *et al.* (1990; 1995) and Morais *et al*. (1996) in lentic environments in the Lagoa Santa plateau State of Minas Gerais. The observed correlation, suggests the possibility of using the variables faecal coliforms and heterotrophic bacteria as complementary parameters, which would allow for a better definition of the trophic state of an environment. These biological variables would be particularly important in aquatic systems receiving high organic loads, as demonstrated for the majority of the river stretches within the middle Doce river basin, which are environments showing high concentrations of total phosphorus and high densities of faecal coliforms and heterotrophic bacteria. An inverse correlation was observed for well-preserved environments that show lower values of phosphorus, coliforms, and bacteria, here represented by Caraça river.

The present results emphasizes the fact that propositions for restoration and conservation measures on river stretches under human impacts must consider the watershed as the unit for studies and interventions. In addition the definition of the trophic state is an essential tool for the implementation of any appropriate measure.

RESUMO

A concentração de fósforo total foi utilizada para determinar o grau de trofia das principais sub-bacias do trecho médio da bacia do Rio Doce, em Minas Gerais, e variáveis físicas, químicas e microbiológicas da água foram analisadas sazonalmente durante os anos de 2000 e 2001. O estudo visou determinar mudanças na qualidade da água causadas pela sazonalidade e por diferentes atividades antrópicas. As áreas estudadas variaram de oligotróficas a eutróficas. Foi verificada alta correlação positiva entre as concentrações de nitrogênio e fósforo total com as densidades de coliformes fecais e a densidade das bactérias heterotróficas mostrou-se capaz de diferenciar os ambientes. Estes resultados sugerem a inclusão do grau de trofia e da caracterização das atividades antrópicas na bacia, como ferramentas importantes para a proposição de medidas de recuperação e conservação de trechos de rios sujeitos a impactos antrópicos.

ACKNOWLEDGEMENTS

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Capítulo 2

Bacterioplanktonic and Phytoplanktonic Production Rates in 8 River Stretches of the Middle Rio Doce Hydrographic Basin (Southeast Brazil)

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Bacterioplanktonic and Phytoplanktonic Production Rates in 8 River Stretches of the Middle Rio Doce Hydrographic Basin (Southeast Brazil)

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ABSTRACT

Studies of the bacterial secondary production (BSP), together with primary phytoplanktonic production (PPP) in lotic ecosystems, and of the factors that affect these processes are very scarce. BSP and PPP measurements were taken during dry and rainy seasons, in 8 rivers of different orders submitted to different degrees of human impacts (different degree of trophy). We aimed to determine the importance of BSP and PPP in carbon fixation in these different lotic ecosystems. The results of our study support the view that nutrients inputs, probably from allochthonous material alter the degree of trophy of the streams and rivers considered, what is directly reflected by the BSP measurements.

Key words: Bacterioplankton, carbon, phytoplankton, rivers, trophic state

INTRODUCTION

Assessments of carbon incorporation by microorganisms (bacterial and phytoplanktonic algae) are very important to characterize and quantify the sources of authocthonous carbon to the lotic ecosystems, especially when bacterioplankton is considered together with phytoplankton. By using simultaneous measurements of bacterial secondary production (BSP), phytoplanktonic primary production (PPP) and the BSP/PPP ratios, it is possible to estimate the potential specific contribution of each community for carbon fixation, making it available to heterotrophic organisms (Cole *et al.*, 2002).

Studies of BSP, together with PPP in lotic ecosystems, and of the factors that affect these processes are very scarce (Hobbie, 1988; Currie, 1990; di Sierve *et al.*, 1995), especially in Brazil. For example, the importance of rains in determining seasonality in tropical aquatic ecosystems is well known (Esteves, 1998), but the effects of this functioning force upon BSP and PPP in tropical rivers are poorly understood. The same can be considered about the longitudinal patterns of BSP and PPP in lotic ecosystems. According to the River Continuum Concept (Vannotte *et al.*, 1980), an increase in the heterotrophic activity, relative to primary productivity, is expected in low order streams (1^{st} – 3^{rd} orders), whereas the opposite is expected in streams of intermediate order (4^{th} – 6^{th} orders). Hypothesis like this one are still to be tested in tropical lotic ecosystems.

In this study, BSP and PPP measurements were taken during dry and rainy seasons, in 8 rivers of different orders submitted to different degrees of human impacts, aiming to determine the importance of bacterial and phytoplanktonic algae in carbon fixation in these different conditions.

STUDY AREA

This investigation was carried out in the medium stretch of the Rio Doce basin, State of Minas Gerais, southeast Brazil. Water samples were collected in 8 stations, representative of the sub-basins of the rivers: 1- Caraça (20°06’00”S; 43°29’09”W), 2- Barão de Cocais (19°57’27”S; 43°28’24”W), 3- Santa Bárbara (19°50’01”S; 43°21’14”W), 4- Peixe (19°44’35”S; 43°01’16”W), 5- Severo(19°36’57”S;

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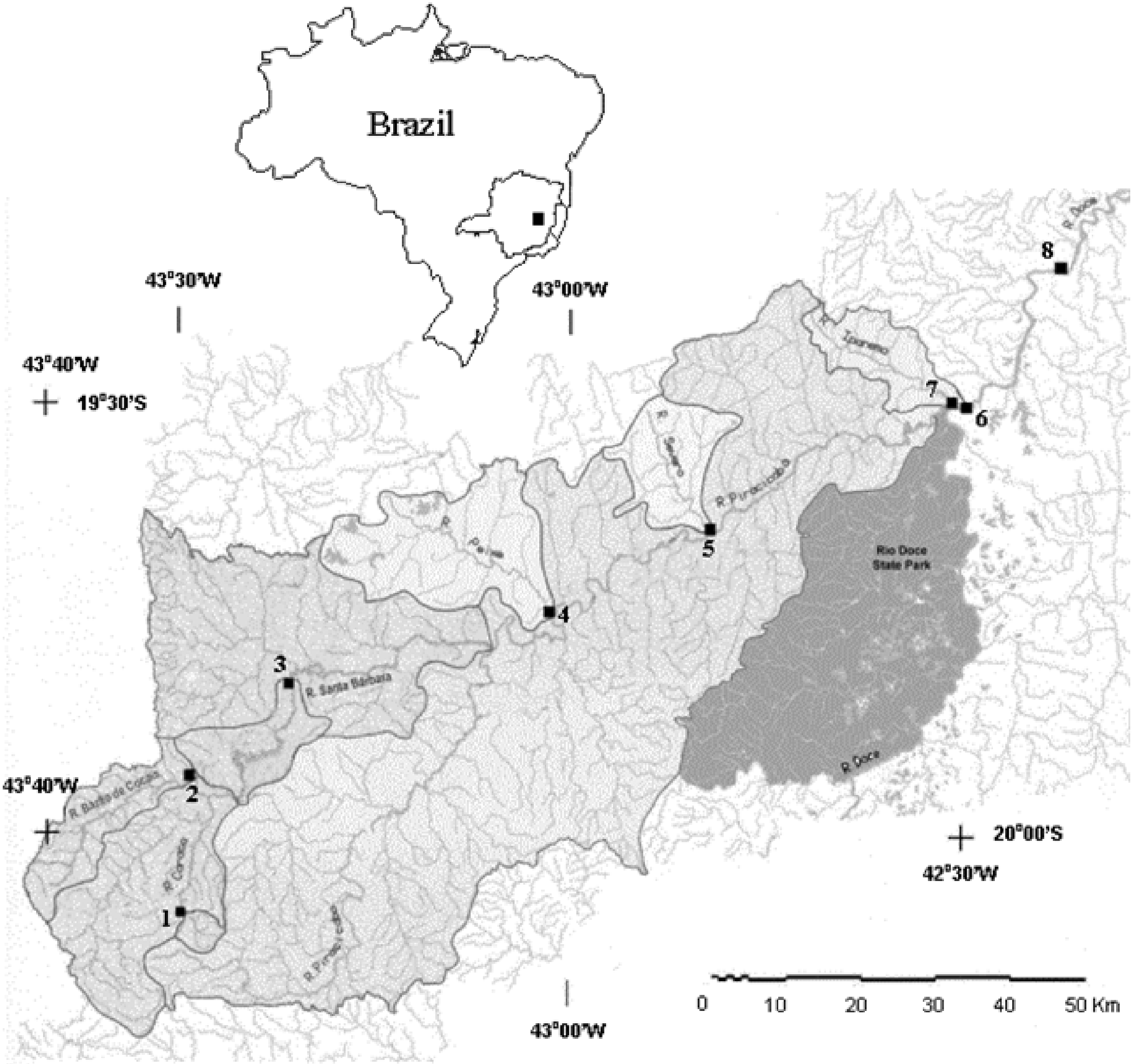

Source: Paula et al. (1997) modified.

Figure 1: Location of the sub-basin and respective sampling stations in the middle Rio Doce basin, Minas Gerais State-Brazil.

42°50'50"W), 6- Piracicaba (19°29'25"S; 42°31'08"W), 7- Ipanema (19°28'14"S; 42°32'01"W) and 8- Doce (19°19'12"S; 42°21'52"W).

These ecosystems (Fig. 1) were chosen because they permit comparative studies, given that strong differences are found in the environmental characteristics of the sampling stations, due to different degrees of human activities (Paula et al., 1997). Where the water samples were taken, Caraça is a second order river; Barão de Cocais, Severo and Ipanema are third order rivers; Peixe is a fourth order river; Santa Bárbara is fifth order river; Piracicaba is a sixth order river; and Doce is a seventh order river, according to the method of Strahler (Gordon *et al.*, 1992).

The Caraça river flows almost entirely inside an Environmental Protection Area (Caraça Natural Park) and it is considered as a "reference" to the basin, given it is submitted to minimum anthropogenic activity. The other rivers are submitted to different types of human influence, such as cattle rising, mining, domestic and industrial effluents. Extensive areas with *Eucalyptus* spp plantations are found in the basin of some environments. Ipanema river has great part of its course canalized and receive domestic and industrial effluents and Doce river receives discharges of all other rivers. The sampling station in this river was localized downstream the Escura falls, which according to Barbosa *et al.* (1997) is very important to the recovery its water quality, mainly through an efficient re-oxygenation of the water.

METHODS

Two samplings were carried out in dry (July 1999 and 2000) and two samplings in rainy (January 2000 and 2001) seasons. Water temperature, electrical conductivity, pH and dissolved oxygen were measured *in situ* with a multiprobe apparatus (Horiba, mod. U-22) and total alkalinity by titration (Mackereth *et al.*, 1978). Water samples were taken from sub-surface in the morning (8-12 am) and carried to the laboratory to determine dissolved organic carbon (TOC-5000 Schimadzu). Total nitrogen, nitrate-nitrogen, nitrite-nitrogen, reactive silicate (Mackereth *et al.*, 1978), ammonium-nitrogen (Grasshorf, 1976), total phosphorus and soluble reactive phosphorus (Golterman *et al.*, 1978) were also determined. Chlorophyll-a concentrations were measured in the material retained in membranes GF 52-C Schleicher & Schuell (∅ 47 mm), extracted with acetone 90% (Lorenzen, 1967).
The PPP was measured *in situ* using the ^{14}C incorporation method (Steemann-Nielsen, 1952). Incubations were carried out in dark and transparent 70 ml flasks, for 3-4 hours periods in the morning, with 0.5 ml of $NaH^{14}CO_3$ (2 µCi), after filtration in membranes ME 25 Schleicher & Schuell (∅ 25; 0.45 µm). BSP values were measured right after sampling by incubating 1.3 ml water samples in the dark with L-[4,5-^{3}H] Leucine (TRK 510, 142Ci/mmol), final concentration of 10 nM, for 40 minutes and C-incorporation was estimated after multiplying protein (estimated through leucine incorporation) by 0.86. The final concentration of 10 nM was chosen after a saturation x concentration curve and the incubation time (40 min) was fixed according to Smith & Azam (1992). Activity (DPM) for both BSP and PPP was measured in Bray cocktail (Bray, 1960) in a Liquid Scintillation Analyzer (Packard, Tri-carb 2100TR).
The effects of seasons (dry x rainy season) and river orders (2, 3, 4, 5, 6 and 7) upon BSP and PPP, as well as the interaction between both factors, were tested with a factorial ANOVA (General Linear Model of the package Statistica). Data were log-transformed before analyses to meet assumptions. Since two tests were performed, Bonferroni criteria for multiple tests were used to minimize possibilities of type I error and the accepted level of significance became 0.05/2 = 0.025 (Sokal & Rohlf, 1995).
Principal component analysis (PCA) was applied with purpose of reducing the dimensionality of the abiotic data (Manly, 1994) and to search for a gradient in trophic conditions. The following variables were used in the matrix: temperature, pH, electrical conductivity, dissolved oxygen, TP, P-PO_4^{3-}, TN, N-NH_4^+, N-NO_3^-, N-NO_2^-, silicate, DIC, DOC and chlorophyll-a. Before analyses, all data except pH, were log-transformed to get linear relationships. The Broken-Stick method (Jackson, 1993) was used as a stopping-rule in the PCA. Possible effects of trophic gradient (represented by first component principal – see Results) upon BSP and PPP were assessed using correlation analyses (package Statistica).

RESULTS

During the sampling periods precipitation varied between 0.0 mm (July 1999, 2000) and 59.2 mm (January 2000) (Data obtained from Instituto Mineiro de Gestão das Águas – IGAM/SIMGE).
The results of the physical and chemical variables analyzed in the 8 rivers are shown in Table 1. Considerable differences in water temperature were registered in the rivers, considering that they are in a tropical region, and the existing differences in altitude (Caraça river: 1,200 m and Doce river: 230 m). Values fluctuated between 14 and 23 ^{o}C during dry (winter) and between 19 and 29 ^{o}C during the rainy season (summer). pH values remained acid in Caraça river and varied from slightly acid to slightly alkaline (6.0 to 7.4) in the other sites. Higher values of the electrical conductivity (455 µS/cm) and alkalinity (1.0 meq CO_2/L) were measured in the Ipanema river, whereas in the others the values fluctuated from 17.7 to 164.1 µS/cm and 0 to 0.42 meq CO_2/L, respectively. Ipanema was the only river exhibiting low oxygen concentrations (0.4-2.0 mg/l).

Table 1: Minimum and maximum values of temperature, pH, electrical conductivity, dissolved oxygen, total alkalinity, chlorophyll-a and % of light penetration in the water in the eight rivers, during the dry (Jul/1999 and Jul/2000) and rainy (Jan/2000 and Jan/2001) periods.

Ecosystems	Temp °C	pH	Conduct. µS/cm	D.O mg/L	Alkal. meq/L	Chl.-a µg/L
	min-max	min-max	min-max	min-max	min-max	min-max
Caraça	12.7 - 19.3	4.0 - 6.4	9.2 - 17.7	6.5 - 9.9	0.003 - 0.02	2.9 - 5.1
Barão de Cocais	20.0 - 22.5	7.0 - 8.3	27.0 - 94.2	5.0 - 8.5	0.3 - 0.9	1.3 - 20.8
Santa Bárbara	18.0 - 24.5	6.1 - 7.1	37.0 - 58.3	5.0 - 8.7	0.1 - 0.4	6.7 - 28.3
Peixe	15.4 - 24.8	6.3 - 7.5	25.0 - 63.0	6.1 - 9.5	0.2 - 0.4	1.6 - 18.7
Severo	15.5 - 24.5	6.0 - 7.4	19.0 -27.9	3.2 - 10.0	0.1 - 0.3	2.7 - 53.5
Piracicaba	21.6 - 27.5	6.2 - 7.6	75.0 - 164	3.8 - 7.6	0.2 - 1.0	1.6 - 27.5
Ipanema	21.1 - 29.0	6.4 - 7.5	283 - 455	0.4 - 2.0	0.2 - 2.1	0.7 - 12.3
Doce	21.4 - 28.3	6.1 - 7.6	54.0 - 107	3.2 - 9.0	0.2 - 0.4	6.4 - 15.5

Despite being lotic systems, where low chlorophyll-a concentrations are expected, some sites had high concentrations and the values fluctuated from 1.6 and 63.5 µg/L.

Concerning nutrients, it was clear that Ipanema river exhibit high concentrations of dissolved organic carbon (28.5 mg/L), total nitrogen (6,762 µg/L) and total phosphorus (1,595 µg/L) during the sampling period (Tables 2 and 3). On the other hand, Caraça river exhibits low total nitrogen (103.0 µg/L) and total phosphorus (4.7 µg/L) during the same period.

Considering only the phosphorus concentrations, and following the typology presented by Salas & Martino (1991) for tropical lakes, the rivers considered in the present study present a wide range of trophy. Ipanema river can be considered eutrophic, Caraça oligotrophic, Barão de Cocais, Peixe, Piracicaba and Doce rivers from meso to eutrophic and the rivers Santa Bárbara and Severo, as oligotrophic during dry season and eutrophic during rainy season (Table 2).

The highest BSP value (5.7 mg $C.m^{-3}.h^{-1}$) was recorded in the Ipanema river and the lowest one in the Santa Bárbara river (0.004 mg $C.m^{-3}.h^{-1}$) (Fig. 2a). The BSP values were slightly higher during the rainy period, except in the Barão de Cocais and Ipanema rivers in the first year.

Results of the factorial ANOVA showed that, despite the apparent difference between periods, the effects of seasons (F = 0.081; p = 0.778) and river orders (F = 1.191; p = 0.348) upon BSP were not significant. The same was true for the interaction seasons/river order (F = 0.923; p = 0.486).

The highest PPP value (374.7 mg $C.m^{-3}.h^{-1}$) was recorded in the Piracicaba river, in the dry season of 2000, and the lowest one (0.01 mg $C.m^{-3}.h^{-1}$) in the Caraça river, in the dry season of 2000 (Fig. 2b).

Results of the factorial ANOVA showed that PPP values did not differ between seasons (F = 0.548; p = 0.468) and river order (F = 2.647; p = 0.054). The interaction seasons/river order was also not significant (F = 1.397; p = 0.268).

A wide range of the ratios BSP:PPP was found (from 0.0005 to 1.297) and in only two occasions PPP was smaller than BSP (Table 4). This ratio was higher in 5 rivers during the rainy period and only in the Caraça river the opposite result was found.

The first two axes of the PCA performed with abiotic variables accounted for 60.2 % of the total variance. The dissolved oxygen concentrations were negatively and the temperature, pH and nutrients (except $N\text{-}NO_3^-$) positively correlated with the first axis (45.6 %, eigenvalues = 6.382). $N\text{-}NO_3^-$ and $N\text{-}NO_2^-$ concentrations were positively correlated with axis 2 (14.6%, eigenvalues = 2.048) (Fig. 3a). The principal component 1 was the only significant according to the Broken-Stick model (eigenvalues = 3.252). If the broken-stick eigenvalue is less than the actual eigenvalue for an axis, then that axis contains more information than expected by chance and should be considered for interpretation (Jackson, 1993). Thus the principal component 1 was retained in the analyses to explain BSP and PPP.

The first principal component scores contrasted the rich, more eutrophic from poor rivers, in terms of nutrients (Fig. 3b). Chlorophyll-a, a variable indicating phytoplankton biomass was weakly correlated with both axes (Fig. 3a).

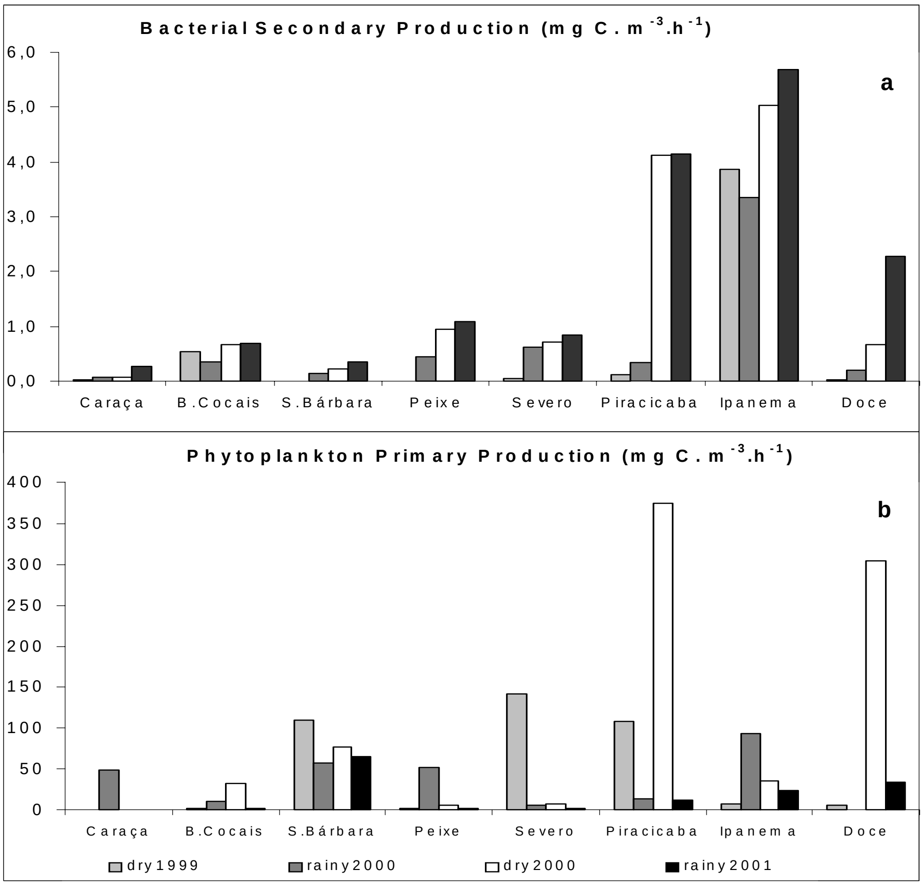

Figure 2. Bacterial secondary production (a) and phytoplankton primary production (b) values, during the dry (Jul/1999 and Jul/2000) and rainy (Jan/2000 and Jan/2001) seasons.

It is interesting to note the existence of an overlap among rivers sampled in winter and summer. In other words, different seasons were not clearly separated by both axes.

BSP was affected simultaneously by a group of factors, since this variable was significantly correlated with PC1 (R = 0.63; P < 0.001; N = 32) (Fig. 4a). On the other hand, the correlation between PPP and PC1 was not significant (R = 0.20; P = 0.259; N = 32) (Fig. 4b).

Table 2: Minimal and maximal concentrations of total and soluble reactive phosphorus; silicate and dissolved organic and inorganic carbon in the eight rivers, during the dry and rainy periods (1999-2001). The classification of trophic status considers only the total phosphorus concentrations.

Ecosystem	Tot-P µg/L	PO_4-P µg/L	Silicate mg/L	DIC mg/L	DOC mg/L	Tropic State (Salas & Martino, 1991)
Dry	min – max	min-max	min-max	min-max	min-max	
Caraça	4.7 - 14.5	2.0 - 4.4	0.3 - 0.8	0.2 - 0.7	1.2 - 12.4	Oligotrophic
Barão de Cocais	56.3 - 141	14.9 - 54.9	2.6 - 34.3	5.1 - 6.8	1.4 - 4.0	Meso-Eutrophic
Santa Bárbara	17.4 - 154	1.3 - 12.6	2.1 - 27.9	2.0 - 3.5	1.4 - 3.9	Oligo-Eutrophic
Peixe	52.9 - 170	11.1 - 62.1	1.8 - 38.7	1.0 - 2.5	1.1 - 7.5	Meso-Eutrophic
Severo	14.7 - 469	1.2 - 7.8	4.0 - 16.9	0.1 - 1.5	0.7 - 10.0	Oligo-Eutrophic
Piracicaba	78.0 - 240	10.8 - 23.7	3.3 - 46.7	0.4 - 3.2	1.9 - 4.8	Meso-Eutrophic
Ipanema	760 - 1,595	68.7 - 1,035	4.8 - 67.7	0.1 - 13.5	9.3 - 28.5	Eutrophic
Doce	45.7 - 318	5.0 - 33.1	3.5 - 44.0	0.1 - 3.8	1.7 - 5.1	Meso-Eutrophic

Table 3: Minimal and maximal concentrations of total nitrogen, ammonium-, nitrate-, nitrite-nitrogen, and N/P ratios in the eight rivers, during the dry and rainy periods (1999-2001).

Ecosystem	Total-N µg/L	NH_4-N µg/L	NO_3-N µg/L	NO_2-N µg/L	N/P
	min-max	min-max	min-max	min-max	min-max
Caraça	103 - 963	5.4 - 565	9.0 - 36.6	1.4 - 4.2	13.6 - 98.1
Barão de Cocais	627 - 852	42.1 - 697	2.9 - 400	5.8 - 42.1	5.5 - 15.3
Santa Bárbara	393 - 590	22.6 - 129	34.5 - 430	4.1 - 8.5	3.8 - 29.9
Peixe	1,181 - 2,019	26.3 - 473	87.5 - 852	13.6 - 171	7.9 - 22.3
Severo	234 - 1,836	5.5 - 32.6	23.3 - 229	2.0 - 11.8	3.9 - 15.9
Piracicaba	1,197 - 2,461	67.2 - 308	693 - 790	9.5 - 32.2	5.0 - 31.5
Ipanema	5,341 - 6,762	1,547 - 3,320	0.5 - 41.5	2.7 - 28.3	4.2 - 7.4
Doce	806 - 1,240	23.5 - 235	92.4 - 445	6.3 - 29.1	3.9 - 13.7

Table 4: Bacterial Secondary Production / Phytoplankton Primary Production (BSP/PPP) rates in the eight rivers, during the dry and rainy periods (1999-2001).

Ecosystem	BSP/PPP
	Minimal - Maximal
Caraça	0.001 - 1.297
Barão de Cocais	0.020 - 0.574
Santa Bárbara	0.000 - 0.005
Peixe	0.005 - 0.733
Severo	0.000 - 1.093
Piracicaba	0.001 - 0.348
Ipanema	0.036 - 0.514
Doce	0.002 - 0.382

DISCUSSION

A wide range of the BSP and PPP values were registered in the rivers investigated. In the Ipanema river, the most eutrophic habitat, BSP was considerably high compared with data from temperate lakes and rivers (Goosen *et al.*, 1999; Vicent *et al.*, 1996; Vrede, 1996; Le *et al.*, 1994). In tropical ecosystems, at coastal environments in the south of Brazil, Cesar and Abreu (2001) recorded wide range of variation with higher values than the ones recorded in the present study (0.11 to 6.55 mg C m^{-3} h^{-1}). On the other hand, PPP values were lower relative to other studies carried out in temperate rivers (Goosen *et al.*, 1999; Vicent *et al.*, 1996).

Despite the great variability in BSP and PPP results, seasons did not affect the values of both processes. The reasons leading to the absence of seasonal differences are not clear, but some possibilities can be considered. It is likely that the differences of trophic states among rivers investigated are so high that such differences overcome the effects of seasonality. This reason is even increased in the region given the high human impact observed in some of the rivers. Moreover, the differences in temperature and solar radiation, smaller in tropical than in temperate regions, may have not be sufficient to lead to metabolism

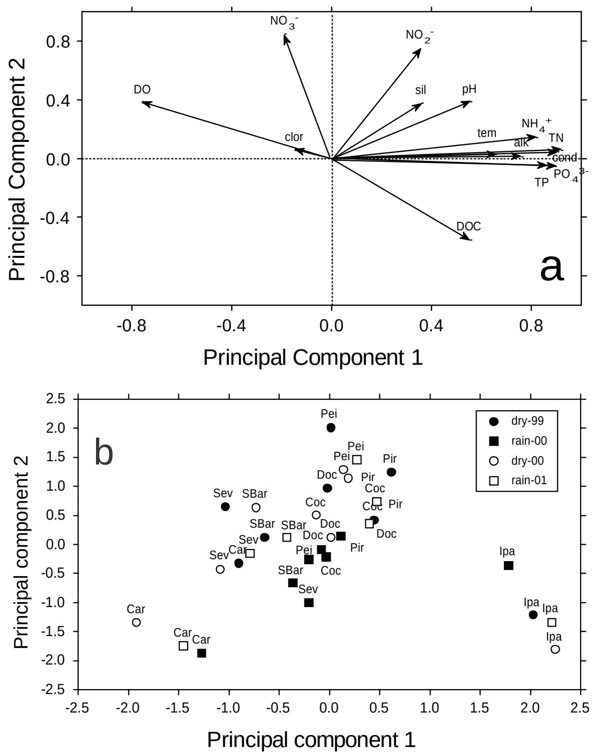

Figure 3: Correlations of physico-chemical parameters with the first two axes of principal component analysis (a) and score distributions of streams sampled in different seasons along the first two principal components axes (b). Car = Caraça; SBar = Santa Barbara; Coc = Barão de Cocais; Pei = Peixe; Sev = Severo; Pir = Piracicaba; Ipa = Ipanema; Doc = Doce.

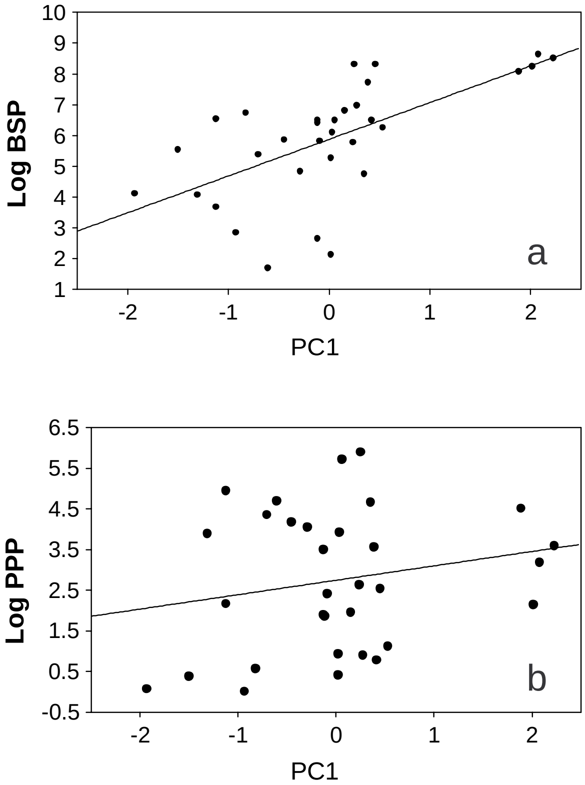

Figure 4: Effect of Principal Component 1 on Bacterial Secondary Production (BSP) (a) and on Phytoplanktonic Primary Production (PPP) (b).

differences in the investigated rivers and their microbiota. Finally it must be pointed out that the observed lack of seasonal difference may well be a consequence of the low number of samples.

The BSP was always lower than the PPP, except in the Caraça river, in the dry season of 1999 and in the Severo river, in the rainy season of 2001. The high PPP relative to BSP production was maintained even during the rainy season, when increased inputs of allochthonous material are expected. Inputs of allochthonous material in general increase the trophy of the studied habitats, especially those that are more subjected to human activities, leading these ecosystems to exhibit more eutrophic features.

BSP values were significantly correlated with the degree of trophy, as evidenced by the relationship between this variable and the scores of the first principal component. These results strongly suggest that BSP is very sensitive to changes in physical and chemical characteristics associated with trophy (N and P levels and conductivity) and thus it can be considered an alternative indicator of trophy states in tropical rivers and streams. This can be demonstrated through the higher BSP values (Fig. 2) and higher DOC, total-P, PO_4-P, NH_4-N and total-N values (Tabs. 2 and 3) recorded at the Ipanema river.

Phosphorus can be considered one of the main limiting factors to BSP in the set of environments investigated, except in the Ipanema river, where N:P ratio is smaller than 8. In the rainy period, with higher inputs from outside, or from sediments upwelling, the rivers had higher phosphorus concentrations and nitrogen probably became the main limiting factor (N:P < 8.0). The ratio N:P was considered at a weight basis and the limit 8:1 determined according to Salas & Martino (1991). Phosphorus limitation had also been demonstrated as a limiting factor for BSP in several temperate lakes (Le *et al.*, 1994; Vrede, 1996).

A lower degree, DOC seems to be also important to explain BSP, since this variable was positively correlated with the component principal 1. Nevertheless, it has to be emphasized that in the case of rivers, chlorophyll-a was poorly related with first principal component (see Fig. 2a) and thus with BSP, suggesting that DOC is basically allochthonous originated. In a similar investigation carried out in Amazonian lakes and rivers, DOC was also among the most important explanatory factors of BSP (Thomaz *et al.*, 1998).

Differently from the BSP, the PPP was not significantly correlated with degree of trophy, suggesting that other factors not considered here (e.g., turbidity, flow velocity) are more important than nutrients. Moreover, the lack of correlation can be due to the fact that PPP in this study refers only to the particulate one (retained on filters) not including the dissolved fraction which normally shows a good correlation.

In addition to seasons and degree of trophy, river order has been considered important in organizing rivers communities' structure and dynamic (Vannote *et al.*, 1980). According to the River Continuum Concept (RCC), proposed by these authors, we should expect an increase of PPP in medium size rivers and an increase of BSP (i.e., heterotrophic activity) in the smaller order rivers. For our data set, river orders were not significantly correlated with either PPP or BSP. The same explanation used to the absence of seasonality can be applied in this case, that is, the high human impact observed in some rivers may be obscuring a pattern of variation in response to river order. Ipanema river is a good example given that this is a third order river but exhibited the highest BSP values due to allochthonous material, originated from human activities.

It has to be emphasized that the values of BSP obtained simultaneously with the PPP are inedited to this stretch of the River Doce basin. Together with physical, chemical and other biological characteristics, these results are important elements to propose strategies aiming at restore and preserve such rivers. The present investigation can be considered a first tentative of using river community processes, such as heterotrophic and autotrophic activities, as a means of measuring the degree of impact derived from human activities and degree of trophy.

In conclusion, the results of our study support the view that nutrients inputs, probably from allochthonous material, alter the degree of trophy of the rivers considered, what is directly reflected by the BSP measurements. It also suggests that BSP can be considered a potential indicator of degree of trophy and as a consequence, an additional tool for rivers monitoring. On the other hand, the lack of a clear pattern concerning PPP variation prevents this variable of being used in this sense. Nevertheless, the inclusion of other explanatory variables not considered in the present research (e.g., flow velocity and underwater radiation), as suggested by Norris & Thoms (1999), would certainly improve the model proposed, and probably bring new conclusions concerning PPP and BSP measurements.

RESUMO

Estudos das taxas de produção secundária bacteriana (PSB), junto com as de produção primária fitoplanctônica (PPF) em ecossistemas lóticos são escassos na literatura. Estimativas de PSB e de PPF foram realizadas durante os períodos de chuva e seca em 8 rios de diferentes ordens e submetidos a diferentes graus de impactos antrópicos (diferentes graus de trofia). O objetivo foi quantificar PSB, PPF e as taxas PSB/PPF para inferir sobre a importância destas comunidades na fixação de carbono nestes ecossistemas lóticos. Os resultados deste estudo permitem concluir que o aporte de nutrientes, provavelmente de origem alóctone altera o grau de trofia dos ambientes, refletindo diretamente nas estimativas da PSB.

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Capítulo 3

Phytoplankton and Bacterioplankton Production Rates and Trophic State of 7 Lakes in the Middle Rio Doce Basin, South-East Brazil.

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Phytoplankton and Bacterioplankton Production Rates and Trophic State of 7 Lakes in the Middle Rio Doce Basin, South-East Brazil.

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"This paper has not been submitted elsewhere in identical or similar form, nor will it be during the first three months after its submission to *Hydrobiologia*.

Running title: Primary production and trophic state.

Abstract

Phytoplankton production (PP) and bacterial production (BP) were estimated simultaneously in protected and non protected lakes in the middle Rio Doce basin (south-east Brazil). The working hypothesis is that production rates varies seasonally (dray and rainy periods) thus resulting in changes of the BP/PP ratios, as a consequence of changes in the inputs of allochthonous matter (nutrients) being also affected by distinct internal loads during these periods. Both PP (carbon fixation) and BP (^{3}H-Leucine incorporation) were estimated in four depths (100%, 10%, 1% of incident radiation, and aphotic zone) along with measurements of carbon, nitrogen, and phosphorus concentrations in 7 natural lakes. The highest BP value (3.5 $mgCm^{-3}h^{-1}$) was recorded at lake Amarela while the highest PP value was recorded at lake Carioca (747.5 $mgCm^{-3}h^{-1}$). The recorded results showed that BP but not PP exhibited significant seasonal variations. However, the ratio BP/PP showed significant seasonal variations with highest values recorded at lake Amarela pointing out a high contribution of BP at this environment. Moreover, lakes Amarela and Carioca exhibited higher trophic states compared to the others. PP was always higher than BP in all studied lakes, but the importance of carbon fixation through the bacteria community shall not be underestimated particularly within deep layers and meso- to eutrophic lakes as represented in this study by lake Amarela.

Key-words: bacterioplankton, lakes, phytoplankton, primary production, bacterial secondary production, trophic state.

Introduction

Estimates of the penetration of photosynthetic active radiation (PAR) are essential to the knowledge of processes and mechanisms which control energy transfer and organic matter cycling in lakes. The understanding on phytoplankton needs in relation to light, temperature, nutrients to production processes as well as on the interactions between competition and predation are still among the major fields of interest of Limnology (Wetzel, 1993; Bicudo *et al.*, 1998; Roland, 1998). Besides PAR, nutrients concentration (mainly C, N and P) constitutes an important factor determining primary production. However, still there are questions without answer in relation to the several and complex mechanisms which regulate functioning and diversity of planktonic communities, making necessary studies such as the interactions between algae-bacteria (Le *et al.*, 1994; di Sierve *et al.*, 1995; Mariazzi *et al.*, 1998; Reche *et al.*, 1998).

The study of trophic interactions between phyto- and bacterioplankton, as well as the relations between these communities and the abiotic matrix have experienced considerable progress with the studies conducted by Pomeroy (1974) and Azam *et al.* (1983). These studies pointed out that planktonic bacteria form an important and complex trophic link between dissolved organic carbon (DOC) and the rest of trophic chain. According to Hobbie (1988) bacterioplankton community utilizes a considerable part of the DOC available within aquatic ecosystems, besides absorbing inorganic nutrients thus competing with phytoplankton community for these nutrients.

According to Van Wambeke *et al.* (2002) studies focusing simultaneously phytoplankton and bacteria remain scarce mainly due to the fact that, as in temperate regions, also in the tropical aquatic ecosystems both the relations between phyto- and bacterioplankton and the major influences of abiotic factors on production as a whole are scarcely known. Similarly, the relationship between primary productivity (including bacterial production) and trophic conditions of aquatic ecosystems need further detailed studies, since these relations constitute the result of the balance between processes such as commensalisms, competition, and predation (Le *et al.*, 1994; di Sierve *et al.* 1995).

In this study, phytoplankton production (PP) and bacterioplankton production (BP) were estimated simultaneously in preserved and impacted lakes in the middle Rio Doce basin (south-east Brazil), as well their trophic state, according to the model proposed by Salas and Martino (1991). The testing hypothesis is that primary production varies seasonally (dray and rainy periods) thus resulting in changes of the BP/PP ratios, as a consequence of changes in the inputs of allochthonous matter (nutrients) being also affected by distinct internal loads during these periods. Moreover, it is expected the study bring some contribution to the understanding of the energy flux and material cycling, characteristic of each ecosystem allowing a definition of a regional typology. Furthermore, this study shall allow a deepening on the existing limnological knowledge of the middle Rio Doce lake system and its interactions with the natural forest (Atlantic forest) and surrounding *Eucalyptus* spp plantations in order to allow for the proposal of conservation actions for the aquatic ecosystems and sustainable use of natural resources.

The study area

The studies were conducted in lakes of the State Park of Rio Doce (PERD) and its surroundings, including areas subjected to different anthropogenic impacts. The PERD (19º29’24” – 19º48’18” S; 42º28'18” – 42º38’30” W), with an area *c.* 36,000 ha, is located in the middle stretch of the Rio Doce basin (south-east Brazil) and constitutes the largest remnant of the original Atlantic forest in the State of Minas Gerais (Figure

1). Its surrounding is largely occupied by large plantations of *Eucalyptus* spp and degraded pastureland.

Two lakes were selected within the PERD (Dom Helvécio and Carioca) and 5 lakes in its surroundings (Amarela, Águas Claras, Barra, Jacaré, and Palmeirinha). Lake Dom Helvécio is the largest and deepest lake of the system (6.87 km^2; 32.5 m) open to tourism and offering fishing, swimming, and rowing. Lake Carioca (0.13 km^2; 11.8 m) is not open to the public, exhibiting more preserved conditions. Among the lakes of the surroundings lake Amarela is the smallest and shallowest (0.11 km^2; 2.0 m) and colonized by a dense community of floating and submerged macrophytes. Lake Águas Claras, despite situated within an area of eucalyptus plantation is not heavily impacted and exhibits oligotrophic features. Lake Palmeirinha suffers direct influence of a charcoal plant and lakes Barra and Jacaré, besides surrounded by eucaliptus plantations also hold fishing clubs thus receiving intermittent loads of untreated domestic sewage.

Materials and Methods

Samplings were performed in 1999-2001, during dry (July 1999 and 2000) and rainy periods (February 2000 and 2001), always during morning hours and at a central station in each lake. A light profile (radiometer Li-Cor, model Li-193SA) along the water column was characterized in each lake and determined four sampling depths (100%, 10%, 1% of incident light and aphotic zone) for production estimates. Water temperature, conductivity, pH and dissolved oxygen were measured *in situ* with a multiprobe apparatus (Horiba, model U-22) and total alkalinity by titration (Mackereth *et al.*, 1978). Water samples taken from the above mentioned depths were carried to the laboratory to determine dissolved organic carbon (TOC-5000 Shimadzu), total nitrogen, (Mackereth *et al.*, 1978) and total phosphorus (Golterman *et al.*, 1978). Chlorophyll-*a* concentrations were measured after filtration in Schleicher & Schuell GF 50-A membranes and extraction with 90% acetone (Lorenzen, 1967).

Phytoplankton productivity (PP) was measured *in situ* using the ^{14}C technique (Steemann-Nielsen, 1952). Incubations were carried out in dark and transparent 70 ml bottles, for 3-4 hours with $NaH^{14}CO_3$ (0.5 ml/2 µCi). Bacterial production (BP) was measured right after sampling by incubating 1.3 ml water samples in the dark with L-[4,5-^{3}H] Leucine (TRK 510, 142Ci/mmol), final concentration of 10 nM, for 40 minutes. Carbon incorporation was estimated after multiplying protein (estimated through leucine incorporation) by 0.86 (Smith & Azam, 1992). Activity (d p m) was measured in Bray cocktail (Bray, 1960) in a Liquid Scintillation Analyzer (Packard, Tri-carb 2100TR).

Principal component analysis (PCA) was applied and the following variables were used in the matrix: temperature, pH, conductivity, dissolved oxygen, total alkalinity, TP, TN, DOC and Chl.-a. The effects of seasons (dry x rainy season) upon BP and PP, as well as the interaction between both factors and nutrient concentrations, were tested with a factorial ANOVA (General Linear Model, package Statistica).

Results

Table 1 presents the abiotic characterization of the studied lakes. Water temperature and conductivity show a distinct seasonality with higher values in all lakes recorded during the rainy period (summer). The highest temperature was recorded at lake Jacaré (31.4 ^{o}C) and the lowest one at lake Amarela (18.5 ^{o}C). The highest conductivity (155.0 µS cm^{-1}) was recorded at lake Águas Claras and the lowest one at lake Carioca (25.0 µS

cm^{-1}). pH showed a considerable variation ranging from 5.2 (lake Palmeirinha) to 7.8 (lake Jacaré). Total alkalinity was higher at lake Amarela (1.1 meq. CO_2 l^{-1}) and lower values (0.1 meq.CO_2 l^{-1}) were recorded at lakes Carioca, Palmeirinha, and Dom Helvécio. The lowest oxygen concentrations (near to anoxy) were recorded during the rainy period in the deepest layers in all studied lakes that were stratified (e.g. lakes Carioca and Palmeirinha) and highest concentration (9.3 mg l^{-1}) was recorded at lake Águas Claras. The highest chlorophill-*a* concentration (260.9 µg l^{-1}) was recorded at lake Amarela and during the rainy period and the lowest one (2.9 µg l^{-1}) at lake Dom Helvécio during the dry season.

The recorded values of DOC, total N, and total P are low, except for lake Amarela where concentrations of 20.1 mg l^{-1}; 3,019 and 61.6 µg l^{-1}, respectively were registered. The lowest values of C and P were recorded at lake Dom Helvécio (0.6 mg l^{-1} and 2.1µg l^{-1}, respectively) and of nitrogen at lake Jacaré (841 µg l^{-1}). According to the model proposed by Salas & Martino (1991), lake Amarela show mesotrophic characteristics, lakes Carioca and Palmeirinha show oligotrophic (dry periods) and mesotrophic (rainy) characteristics, while the remaining lakes are characteristically oligotrophic in both periods (Table 2).

Depth profiles for phytoplankton and bacterial production are shown, respectively in figures 1 and 2. The highest value of phytoplankton productivity was recorded at lake Carioca (747.5 mg C m^{-3} h^{-1}) during the dry period of 2000 and the lowest (0.3 mg C m^{-3} h^{-1}) at lake Dom Helvécio during the rainy period of 2001. Moreover, the highest and lowest values of bacterial production (3.5 and 0.0005mg C m^{-3} h^{-1} respectively) were recorded in lake Amarela during the rainy period of 2000. At an area basis (Figure 4) lake Dom Helvécio presented the lowest (0.1 mg C m^{-2} h^{-1}) and the highest (9.3 mg C

m^{-2} h^{-1}) values of bacterial production rate, respectively during the rainy and dry periods of 2000. Phytoplankton production rate was highest in lake Carioca (2,247.7 mg C m^{-2} h^{-1}) and the lowest value was recorded in lake Amarela (18.6 mg C m^{-2} h^{-1}). Only bacterial production showed significant seasonal differences ($p < 0.05$). However, the ratio BP/PP showed significant seasonal differences with the highest values recorded in lake Amarela (Table 3) pointing out a greater contribution of bacterial production at this lake.

The result of PCA analysis is showed in figure 5, for this analysis the sampling periods were treated separately. For the first year (dry period 1999 and rainy period 2000) the first two axes of the PCA performed with abiotic variables accounted for 70 % of the total variance. Dissolved oxygen concentrations were negatively and conductivity, total alkalinity, chlorophyll-a, total phosphorus, and dissolved carbon, positively correlated with the first axis (43 %). Soluble reactive silica concentration was negatively, temperature, and BP were positively correlated with axis 2 (27 %) (Figure 5A). For the second year, the first two axes of the PCA accounted for 51 % of total variance. Total alkalinity, total nitrogen, and dissolved inorganic carbon were positively correlated with the first axis (28 %) while chlorophyll-a and PP were positively correlated with axis 2 (23 %) (Figure 5C). These results show that in the first year the effect of seasonality was well marked with the formation of two distinct groups: the first formed by the lakes during the dry period and the second one with the lakes in the rainy period (Figure 5B). However, in the second year such division was not recorded being all lakes close to the origin and exhibiting low percentage of explanation (Figure 5D).

Discussion

The trophic state of the lakes

The studied lakes are characteristically warm-monomictic with destratification beginning in May, exhibiting isothermal conditions in July/August and starting to stratify again late September (Barbosa *et al.*, 1989). Such stratification pattern affects the distribution of other environmental variables, particularly dissolved oxygen and nutrient concentrations as demonstrated previously by Barbosa et al. (1989) for lake Carioca. Despite well defined for the middle Rio Doce lakes, this pattern showed variations in January 2001 probably due to less amount of rain in this period (El Niño effect?), as demonstrated by the absence of the two identified groups showed in Figure 5D, which corresponds to the second year of samplings.

The highest N and P concentrations were consistently recorded in deeper layers and during the stratification period and all the lakes showed a clear phosphorus limitation ($N_{total}/P_{total} > 9$) except for a single value recorded in lake Barra during the rainy period of 2001 at depth corresponding to 1% of incident light.

The trophic model proposed by Salas and Martino (1991), based mainly in the geometric mean of total phosphorus concentrations ($\mu g\ l^{-1}$) recorded in *c.* 35 lakes and reservoirs in the tropics, defined the following limits for trophic categories: 21.3 = oligotrophic, 39.6 = mesotrophic, and 118.7 = eutrophic. Based on this classification lake Amarela is mesotrophic reflecting highest nutrient concentrations among the studied lakes, besides being a shallow environment (2.5 m maximum depth) and posses an abundant macrophytes' community (11 species identified so far). Lakes Carioca and Palmeirinha are oligotrohic during the dry period and mesotrophic during the rainy season, thus reflecting the importance of allochthonous contributions during this period, and lakes Dom Helvécio, Águas Claras, and Barra are oligotrophic ones, reflecting the prevailing low nutrient concentrations, particularly phosphorus.

The production of the lakes

Despite the same origin and age the evolution of the middle Rio Doce lakes reflects distinct processes within each lake resulting in different trophic states as demonstrated above. Similarly these individual lake differences are evident in phytoplankton production rates that varied between 0.29 and 747.5 mg C m^{-3} h^{-1}. Moreover, the occurrence of photo-inhibition at the surface layers was observed in most of the studied lakes, as demonstrated previously for lake Carioca by Barbosa and Tundisi (1980).

Maximum production values were recorded at the sub-surface although exceptions recorded in lakes Palmeirinha (rain/2000), Dom Helvécio (dry/2000) and Carioca, Palmeirinha, Águas Claras, and Barra (rain/2001). In general, the highest carbon fixation rates were recorded at depths corresponding to 10% and 1% surface irradiance and during the dry period, when the lakes are destratified and the availability of nutrients is greater along the water column. However, the recorded differences when comparing the values recorded during the rainy periods are not significant, not corroborating the findings of Barbosa and Tundisi (1980) for lake Carioca of values *c.* 3 times higher during dry periods.

Previous estimations of primary productivity in lake Carioca (Barbosa & Tundisi, 1980; Barbosa *et al.*, 1989) recorded values between 0.08 and 10.9 mg C m^{-3} h^{-1} for the dry period (winter) and between 0.03 and 1.25 mg C m^{-3} h^{-1} in the rainy season (Table 4). The present study besides corroborate such pattern (higher production rates during dry periods) also recorded carbon fixation rates considerably higher. Moreover, Tundisi *et al.* (1997) recorded, at the beginning of the 1980's, primary production rates in lakes Dom Helvécio, Carioca, Amarela, and Jacaré, during dry periods varying between 0.06-1.73; 0.42-14.59; 4.71-65.51 and 0.11-2.31 mg C m^{-3} h^{-1}, respectively. Similarly, Henry *et al.* (1997) recorded production values in lake Carioca varying between 16.2 and 17.5 mg C m^{-3} h^{-1} and in lake Dom Helvécio values between 18.3 and 187.5 mg C m^{-3} h^{-1}. Recent estimates (Table 4) show production rates considerably higher, thus indicating a considerable increase of primary production of these lakes in the last decades.

Bacterial production rates ranged between 0.005 and 3.5 mg C m^{-3} h^{-1} with both the lowest and highest values recorded in lake Amarela. The production profiles show higher values at depths corresponding to 1% of incident radiation and aphotic zone except for lakes Palmeirinha, Jacaré, and Barra where highest rates were recorded at the surface (0.1–1.12 mg C m^{-3} h^{-1}) thus evidencing the lack of pattern along the water column as recorded for phytoplankton production. Similarly, at coastal environments in the south of Brazil, Cesar and Abreu (2001) recorded wide range of variation with higher values than the ones recorded in the present study (0.11 to 6.55 mg C m^{-3} h^{-1}). Moreover, Gonzalez *et al.* (2000) recorded values between 0.5 and 3.86 mg C m^{-3} h^{-1} in distinct regions of Guanabara Bay with distinct trophic states. The values recorded in lake Amarela can be considered high when compared with the ones recorded in temperate lakes (Le *et al.*,1994; Vrede, 1996).

No positive correlation was observed between nutrient concentrations and bacterial production despite such correlation is more common than the one between nutrient concentration and phytoplankton production rates, according to Le *et al.* (1994) and Vrede (1996). Moreover, in the present study no correlation between phytoplankton production and nutrient concentrations was also recorded. The highest bacterial production values were recorded in lake Amarela that exhibits the highest DOC concentrations.

The ratio BP/PP is often used as an index to determine the amount of carbon fixed by phytoplankton that is processed by heterotrophic bacteria (Van Wambeke *et al.*, 2002). According to these authors the classical scheme during such a temporal evolution first implies a low BP/PP ratio at the time phytoplankton is more productive and not limited by nutrients. In that case, bacteria will essentially feed on phytoplankton exudates. Then bacteria take advantage of new sources of resource like detritus and products from grazing, a situation that explains the enhanced bacterial production, hence increased BP/PP ratios. Theoretically, uncoupling between phytoplankton and bacterial production should correspond to a situation where exportation processes by grazing, lateral advection or sedimentation would be maximised.

In the present study bacterial production and BP/PP ratio exhibited significant seasonal variations, with highest values recorded in lake Amarela pointing out a greater contribution of bacterial production within this lake and suggesting a possible correlation between bacterial production and the concentration of organic matter, as pointed out by Faria & Esteves (2001) and Farjalla *et al.*,(2001). Despite the fact that phytoplankton production was always higher than bacterial production in all the studied lakes, the importance of carbon fixed by bacteria shall not be underestimated, particularly within deeper layers and in lakes rich in organic matter such as lake Amarela which also exhibit a rich macrophyte's community.

Despite the fact no correlation between production values (phyto- and bacterioplankton) and nutrient concentrations has been recorded, phosphorus is likely to be the main limiting element to the primary production of the studied lakes as jugged by the low N/P ratios recorded. However, more detailed and long-term studies are necessary since it is possible that the periods considered in this study had been influenced by meteorological phenomena of large scale such as “El Ninõ” and/or “La Ninã” thus resulting in changes in the precipitation patterns of the region with significant consequences for the effects of seasonality as observed for the second year of sampling.

In conclusion, the recorded results demonstrated that only bacterial production exhibited significant seasonal differences and no correlation between production rates and nutrient concentration was registered despite a higher contribution of bacterial production was pointed out in lake Amarela. Moreover, it must be pointed out that despite phytoplankton production had been always higher than the bacterial one in all the studied lakes the amount of carbon fixation by this later community shall not be underestimated, particularly within deeper layers and environments rich in organic matter.

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Legends of Figures

Figure 1. Schematic map of the middle rio Doce basin showing the studied lakes within the Rio Doce State Park and Doce river.

Figure 2. Depth profiles of phytoplankton and bacterioplankton production measured during the dry period of 1999 and rainy period of 2000 in 7 lakes of the middle Rio Doce basin, State of Minas Gerais, Brazil.

Figure 3. Depth profiles of phytoplankton and bacterioplankton production measured during the dry period of 2000 and rainy period of 2001 in 7 lakes of the middle Rio Doce basin, State of Minas Gerais, Brazil

Figure 4. Phytoplankton and bacterial production per surface area unit ($mgC.m^2.h^{-1}$) measured during dry and rainy periods of 1999 to 2001.

Figure 5. Correlations of biotic and abiotic parameters with the first two axes of principal component analysis (A, C) and score distributions of lakes sampled in dry and rainy seasons along the first two principal components axes (B, D).
*AMA = Amarela; DH = Dom Helvécio; CA = Carioca; PAL = Palmeirinha; AG = Águas Claras; JA = Jacaré; BA = Barra.
** 1 = first sampling dry 1999; 2 = rainy 2000; 3 = dry 2000; 4 = rainy 2001

Table 1. Depth, temperature, pH, electrical conductivity, dissolved oxygen, chlorophyll-a, and nutrient concentrations in 7 lakes in the middle Rio Doce basin in dry and rainy periods from 1999 to 2001.

Lakes	Max Depth (m)	Temp. ºC	pH	Conduct. µS cm^{-1}	D.O mg l^{-1}	Alkal. meq l^{-1}	Chl-*a* µg l^{-1}	Tot-P µg l^{-1}	Tot-N µg l^{-1}	D.O.C mg l^{-1}	N/P
					Minimal – Maximal Values						
Dry											
Amarela	1.5	18.5–26.0	5.6-6.7	59-111	1.8-6.6	0.3-0.7	3.2-40.1	12.6-61.6	617-1.206	4.7-9.3	13.9-54.5
Dom Helvécio	25.0	22.5-25.2	5.4-6.0	34-55	0.9-6.5	0.1-0.4	2.9-7.2	6.5-16.7	674-1.181	3.5-4.9	62.3-107.5
Carioca	8.0	20.4-24.7	5.4-6.3	25-46	2.8-7.3	0.1-0.3	18.7-76.6	22.4-35.8	269-2.058	4.8-5.6	12.1-65.2
Palmeirinha	6.0	21.6-24.8	5.2-6.7	31-48	1.7-8.5	0.1-0.3	25.8-131.5	9.1-26.9	546-1.257	6.2-7.4	31.6-124.7
Águas Claras	8.0	21.8-24.5	5.5-6.7	33-64	4.7-9.0	0.2-0.3	16.8-33.9	11.6-29.5	363-957	5.9-7.3	24.8-47.7
Jacaré	9.0	21.3-24.1	5.4-7.2	31-63	5.0-9.3	0.2-0.3	6.4-24.9	18.7-29.7	312-841	5.3-6.2	14.6-36.7
Barra	8.0	21.6-24.5	5.6-6.6	46-73	2.4-8.1	0.2-0.5	1.9-25.9	18.6-25.6	660-1.194	3.6-5.8	25.8-59.8
Rainy											
Amarela	2.0	26.5-29.4	5.6-6.6	73-435	0.5-6.9	0.7-1.1	23.0-260.9	14.0-59.2	343-3.019	6.8-20.1	8.9-64.3
Dom Helvécio	30.0	23.0-30.9	5.9-7.4	36-93	0.6-9.0	0.3-0.5	8.0-78.1	2.1-12.7	270-2.015	0.6-3.9	31.3-200.1
Carioca	10.0	23.0-30.5	5.7-7.4	27-125	0.0-7.7	0.2-0.7	8.6-79.7	10.4-40.1	213-2.320	2.9-6.1	20.4-75.5
Palmeirinha	7.0	25.8-31.0	5.3-7.2	28-135	0.0-7.0	0.2-0.5	9.6-179.1	11.9-43.2	326-2.232	2.8-11.0	27.2-55.0
Águas Claras	9.0	25.6-30.5	5.5-6.9	39-155	2.4-8.4	0.3-0.6	9.8-137.4	3.0-19.6	337-1.688	5.5-7.0	31.8-111.6
Jacaré	9.0	26.4-31.4	5.8-7.8	35-151	0.5-8.9	0.3-0.5	9.4-63.6	12.6-25.2	395-1.333	3.8-6.9	20.8-81.0
Barra	8.0	26.0-30.9	5.7-7.7	41-105	0.7-6.6	0.3-0.8	10.4-39.8	13.4-34.8	171-1.928	3.9-12.0	6.9-55.4

Table 2.Trophic state of 7 lakes in the middle Rio Doce region (south-east Brazil) during the period July 1999- January 2001, according to the model proposed by Salas & Martino (1981).(in brackets, limits for the trophic categories)

Lakes	Trophic State Oligotrophic (23,1)		Mesotrophic (39,6)		Eutrophic (118,7)	
	Dry	Rainy	Dry	Rainy	Dry	Rainy
Amarela			12.6-61.6	14.0-59.2		
Dom Helvécio	6.5-16.7	2.1-12.7				
Carioca	22.4-35.8			10.4-40.1		
Palmeirinha	9.1-26.9			11.9-43.2		
Águas Claras	11.6-29.5	3.0-19.6				
Jacaré	18.7-29.7	12.6-25.2				
Barra	18.6-25.6	13.4-34.8				

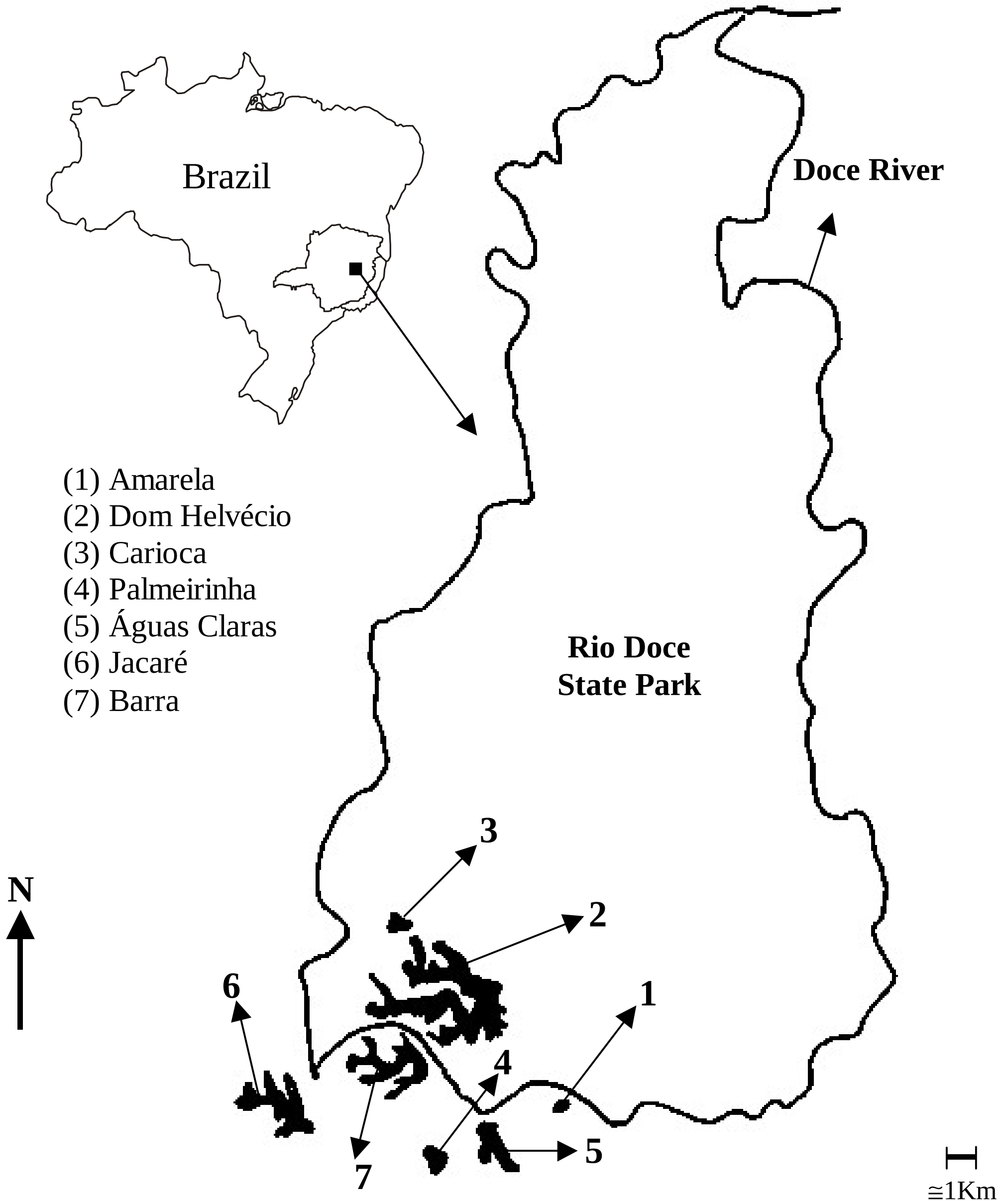
Table 3.Bacterial Production/Phytoplankton Production ratios (BP/PP) during dry and rainy periods in lakes of the middle Rio Doce basin, south-east Brazil.

Lakes	BP/PP ($mg\ C.m^{-2}.h^{-1}$)	
	Dry	Rainy
Amarela	0.008 – 0.073	0.004 – 0.099
Dom Helvécio	0.001 – 0.007	0.011 – 0.088
Carioca	0.002 – 0.004	0.005 – 0.029
Palmeirinha	0.001 – 0.002	0.005 – 0.018
Águas Claras	0.001 – 0.003	0.021 – 0.027
Jacaré	0.003 – 0.006	0.004 – 0.146
Barra	0.001	0.006 – 0.023

Table 4. Phytoplankton primary production rates recorded for dry and rainy periods in four lakes of the middle Rio Doce lakes, south-east Brazil.

Lakes	Period	**Phytoplankton Primary Production (mg C m^{-3} h^{-1})**				
		1977/78†	1979/80‡	1983§	1985¶	1999-2001
Carioca	Dry	0.1-10.9	8.4	0.4-14.6	_	15.6 - 747.4
	Rainy	0.03-1.3	2.7	_	16.2-17.5	2.1 - 218.5
Dom Helvécio	Dry	_	_	0.06-1.7	_	0.3-70.0
	Rainy	_	_	_	18.3-187.5	0.3-28.0
Amarela	Dry	_	_	4.7-65.5	_	0.8 - 151.7
	Rainy	_	_	_	_	_
Jacaré	Dry	_	_	0.1-2.3	_	1.0 - 108.9
	Rainy	_	_	_	_	_

†Barbosa & Tundisi (1980); ‡Barbosa et al. (1989); §Tundisi et al. (1997); ¶Henry et al. (1997)



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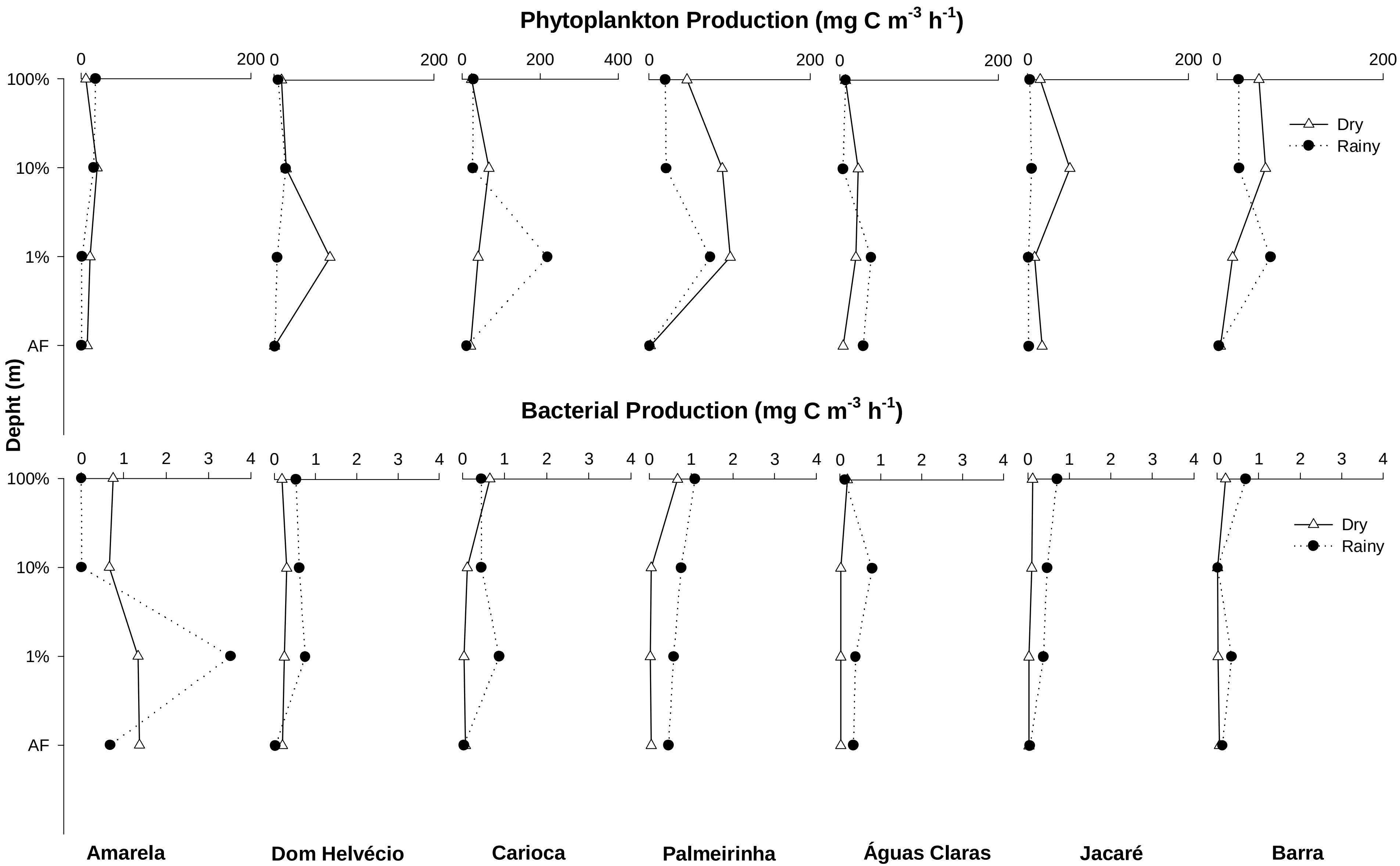
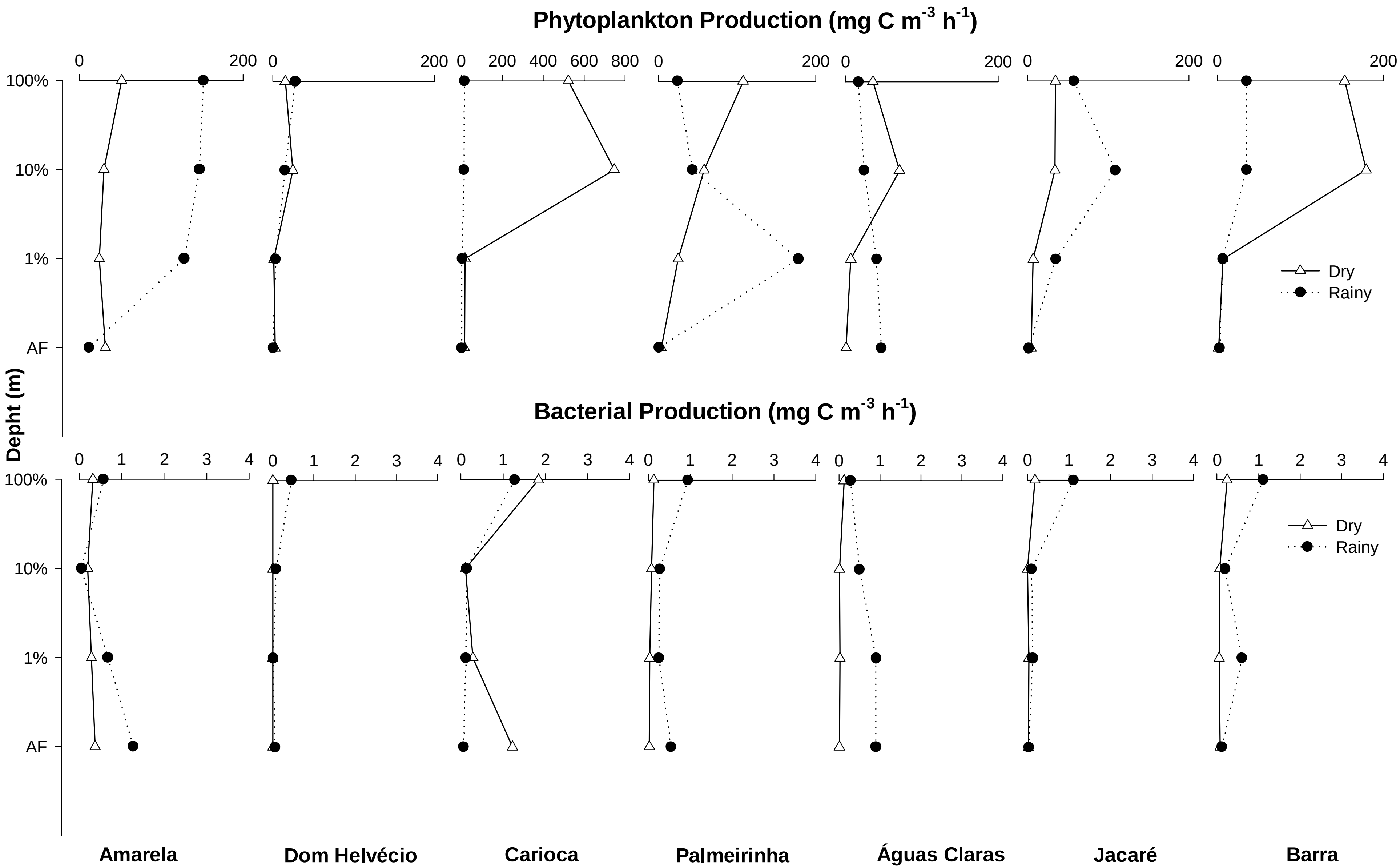
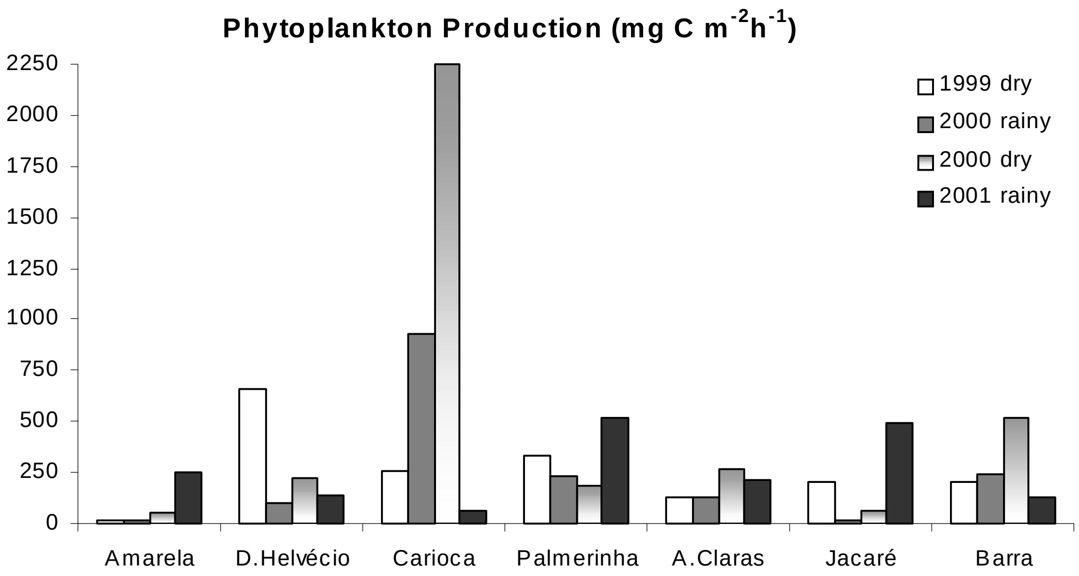


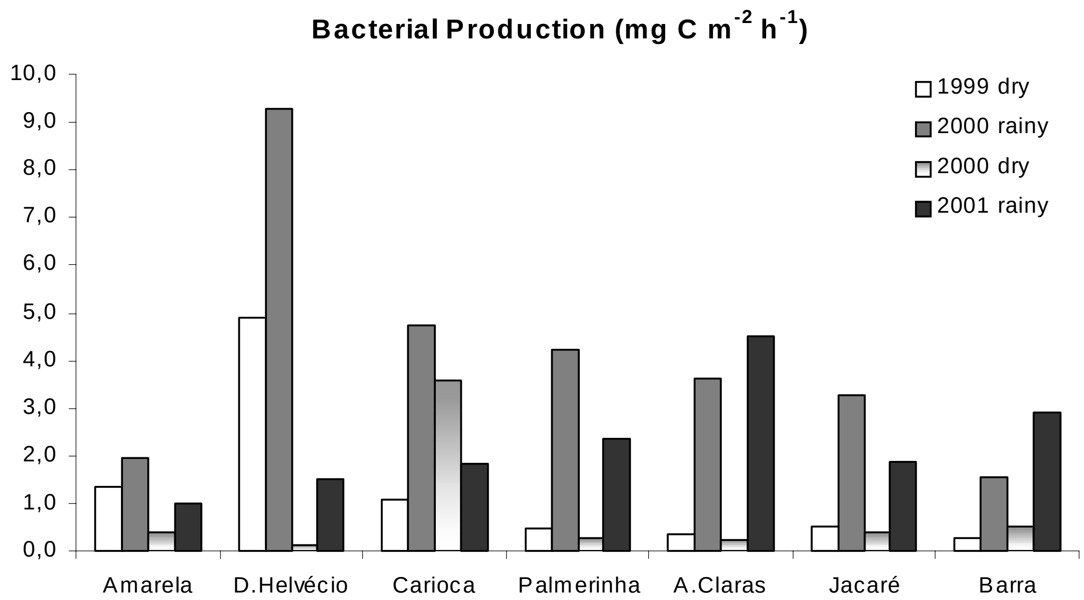
Phytoplankton Production (mg C m-3 h-1)
Bacterial Production (mg C m-3 h-1)
Depht (m)
100%
10%
1%
AF
0
200
400
1
2
3
4
Dry
Rainy
Amarela
Dom Helvécio
Carioca
Palmeirinha
Águas Claras
Jacaré
Barra



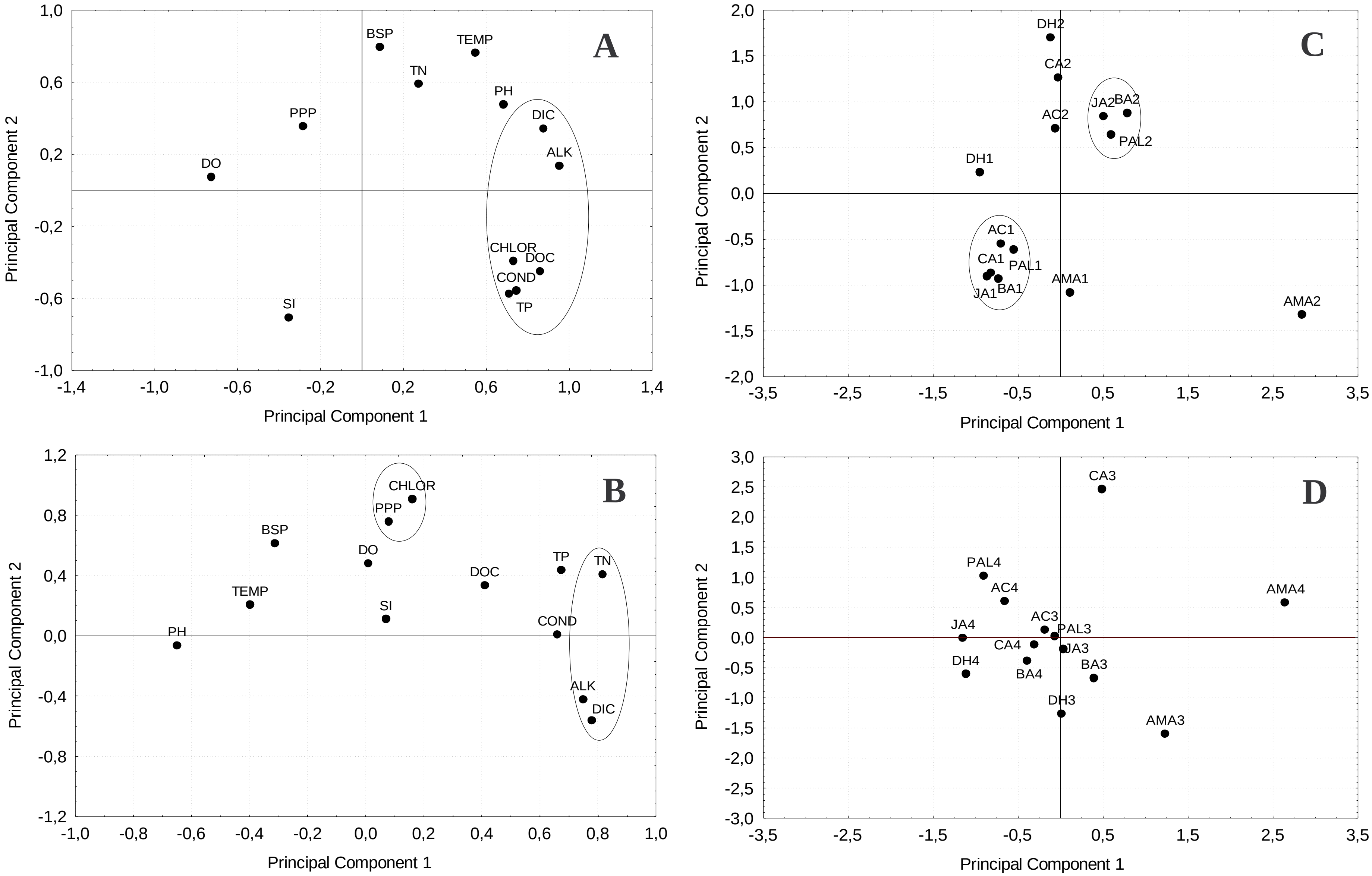
Phytoplankton Production (mg C m-3 h-1)
Bacterial Production (mg C m-3 h-1)
Depht (m)
100%
10%
1%
AF
Dry
Rainy
Amarela
Dom Helvécio
Carioca
Palmeirinha
Águas Claras
Jacaré
Barra



Phytoplankton Production (mg C m-2h-1)
2250
2000
1750
1500
1250
1000
750
500
250
0
1999 dry
2000 rainy
2000 dry
2001 rainy
Amarela
D.Helvécio
Carioca
Palmerinha
A.Claras
Jacaré
Barra



Bacterial Production (mg C m-2 h-1)
10,0
9,0
8,0
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
1999 dry
2000 rainy
2000 dry
2001 rainy
Amarela
D.Helvécio
Carioca
Palmerinha
A.Claras
Jacaré
Barra


A
Principal Component 1
Principal Component 2
1,0
0,6
0,2
-0,2
-0,6
-1,0
-1,4
-1,0
-0,6
-0,2
0,2
0,6
1,0
1,4
BSP
TEMP
TN
PH
PPP
DIC
ALK
DO
CHLOR
DOC
COND
TP
SI
B
1,2
0,8
0,4
0,0
-0,4
-0,8
-1,2
-1,0
-0,8
-0,6
-0,4
-0,2
0,0
0,2
0,4
0,6
0,8
1,0
CHLOR
PPP
BSP
DO
TP
TN
DOC
TEMP
SI
COND
PH
ALK
DIC
C
2,0
1,5
1,0
0,5
0,0
-0,5
-1,0
-1,5
-2,0
-3,5
-2,5
-1,5
-0,5
0,5
1,5
2,5
3,5
DH2
CA2
JA2
BA2
AC2
PAL2
DH1
AC1
CA1
PAL1
AMA1
JA1
BA1
AMA2
D
3,0
2,5
2,0
1,5
1,0
0,5
0,0
-0,5
-1,0
-1,5
-2,0
-2,5
-3,0
CA3
PAL4
AC4
AMA4
AC3
JA4
PAL3
CA4
JA3
DH4
BA3
BA4
DH3
AMA3

Capítulo 4

Diel variations of phytoplankton and bacterioplankton production rates in four tropical lakes in the middle Rio Doce basin (Southeastern Brazil).

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Diel variations of phytoplankton and bacterioplankton production rates in four tropical lakes in the middle Rio Doce basin (Southeastern Brazil).

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Key words: bacterioplankton, phytoplankton, diel variation, primary production, tropical lakes

"This paper has not been submitted elsewhere in identical or similar form, nor will it be during the first three months after its submission to *Hydrobiologia*."

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ABSTRACT

Studies on phytoplankton production conducted in lakes of the middle Rio Doce basin demonstrated significant diurnal and seasonal variations with high phytoplankton production rates recorded in the morning hours during the dry periods. Bacterial production data for these lakes had not been recorded until now. The present study had as working hypothesis that bacterial production may contribute significantly to carbon fixation, particularly in lakes rich in organic matter and that this production varies diurnal and seasonally thus altering BP/PP ratios. In order to test this hypothesis the study had as objective to estimate phytoplankton and bacterioplankton production rates along with the concentrations of carbon, nitrogen, and phosphorus in two periods of the day (morning and afternoon) and in two seasons (dry and rainy), in four lakes, being two lakes within the Rio Doce State Park and two at its surroundings. Among the selected lakes, Lake Amarela showed the highest nutrient concentrations and highest bacterial production. The results allow to conclude that despite exhibiting lower values when compared with the ones recorded for phytoplankton production, bacterioplankton production is essential to the general metabolism of the lakes, particularly for those rich in organic matter and exhibiting oligo-mesotrophic conditions.

Introduction

Some studies have shown that the abundance and productivity of heterotrophic bacteria are positively related to the levels of clorophyll-*a* and phytoplankton production (Le *et al*., 1994; Di Sierve *et al*., 1995). Studies of phytoplankton production in lakes of the middle stretch of the Rio Doce river basin have shown the existence of significant seasonal and diurnal differences, with higher phytoplankton production values during the dry period and in the morning (Barbosa & Tundisi, 1980; Barbosa *et al*, 1989; Tundisi *et al*, 1997). However, data concerning bacterioplankton production in these environments are practically nonexistent.

The ratio BP/PP (bacterioplankton/phytoplankton production) is often used as an index to determine the amount of carbon fixed by heterotrophic bacteria relative to that is processed by phytoplankton (Van Wambeke *et al.,* 2002). According to these authors the classical scheme during such a temporal evolution first implies a low BP/PP ratio at the time phytoplankton is more productive and not limited by nutrients. In that case, bacteria will essentially feed on phytoplankton exudates. Then bacteria take advantage of new sources of resources like detritus and products from grazing, a situation that explains the enhanced bacterial production, hence increased BP/PP ratios.

To test the hypothesis that bacterial production can represent a significant part of the carbon fixation, principally in environments rich in organic matter, and that this productivity exhibits diurnal and seasonal variation modifying the BP/PP ratio, the present study aimed to estimate the phytoplankton and bacterioplankton production rates and the concentrations of carbon, nitrogen, and phosphorus during the morning and the afternoon periods in the rainy and dry seasons in four lakes of the middle Rio Doce basin. These studies, apart from characterizing the contribution of each of these communities in carbon fixation, will also permit an estimate of their specific contributions to the transfer of organic matter in each of the studied environments.

Material and methods

The Study Area

Despite the disordered human occupation and intense process of urbanization, the middle Rio Doce basin, in the State of Minas Gerais, includes the largest remnant of the Atlantic Forest in the State, represented by the Rio Doce State Park - PERD (19º29’24” – 19º48’18” S; 42º28’18” – 42º38’30” W), c. 36,000 ha of which 9.8% are occupied by *c.* 42 lakes belonging to the middle Rio Doce lake system. The region, with altitudes varying between 340 and 680 meters, is occupied by extensive areas of degraded pastureland, heavy industries (iron and steel works, cellulose production) and extensive areas planted with *Eucalyptus* spp, with significant impact on lakes and rivers (Brito et al., 1997).

Among the lakes within the PERD, Lake Dom Helvécio, the largest (687 ha) and deepest (32.5 m) lake of the system, showing oligotrophic conditions and characteristically warm-monomictic, and Lake Carioca, a small (13.2 ha), shallow (11.8 m), warm-monomictic, oligo-mesotrophic environment, were selected. In the surroundings of the Park, the oligotrophic Lake Águas Claras (*c.* 130 ha; 8m max. depth), and the mesotrophic Lake Amarela (c. 12 ha; 2m max. depth), colonized by extensive and diverse macrophytes community, were also included in this investigation.

Trophic state of lakes were established according to total phosphorus scales published by Salas & Martino (1991) for tropical lakes.

Methods

Phytoplanctonic production (PP) and bacterioplankton production (BP) were measured during the day (morning and afternoon) in dry (July/2000) and rainy (January/2001) seasons, at a central station of each lake. Light penetration was measured *in situ* with a radiometer (Li-Cor, mod. Li-193SA) at four depths (100%, 10% and 1% of sub-surface irradiance and aphotic zone). PP was measured *in situ* using the ^{14}C incorporation method (Steemann-Nielsen, 1952). Incubations were carried out in dark and transparent 70 ml flasks, for 3-4 hours periods with 0.5 ml of $NaH^{14}CO_3$ (2 µCi), after filtration in membranes ME 25 Schleicher & Schuell (∅ 25 mm; pore size 0.45 µm).

BP values were obtained right after sampling by incubating 1.3 ml water samples in the dark with 0.1 ml of L-[4,5-^{3}H] Leucine (TRK 510, 142Ci/mmol), final concentration of 10 nM, for 40 minutes and C-incorporation was estimated after multiplying protein (estimated through leucine incorporation) by 0.86 (Smith & Azam, 1992). Activity (DPM) was measured in Bray cocktail (Bray, 1960) in a Liquid Scintillation Analyzer (Packard, Tri-carb 2100TR).

Water temperature, conductivity (25ºC), pH and dissolved oxygen were measured *in situ* with a multiprobe apparatus (Horiba, mod. U-22) and total alkalinity by titration (Mackereth et al., 1978). Water samples were taken and carried to the laboratory to determine dissolved organic and inorganic carbon (TOC-5000 Schimadzu). Total nitrogen, nitrate-nitrogen, nitrite-nitrogen (Mackereth et al., 1978), ammonium-nitrogen (Grasshorf, 1976), total phosphorus and soluble reactive phosphorus (Golterman et al., 1978) were also determined. Chlorophyll-*a* concentrations were measured after filtration in membranes GF 52-C Schleicher & Schuell (∅ 47 mm) and extraction with acetone 90% (Lorenzen, 1967).

Principal component analysis (PCA) was applied with purpose of reducing the dimensionality of the abiotic data (Manly, 1994) and to search for a gradient in trophic conditions. The following variables were used in the matrix: PP, BP, TP, TN, DOC, DIC and Chlorophyll-a. The effects of seasons (dry X rainy season) upon BP and PP, as well as the interaction between both factors and nutrient concentrations, were tested with a factorial ANOVA.

Results

In all the lakes the highest temperature values (29.1 to 33.0 ºC at the surface), dissolved oxygen (6.7 to 8.5 mg l^{-1} at the surface) and conductivity (93 to 435 µS cm^{-1} at the bottom) were found during the rainy period (summer), being evident a clear thermal/chemical stratification, with lower values of temperature and oxygen at the bottom (23.0 to 27.4 ºC; 0 to 6.5 mg l^{-1} respectively). During the dry period lower values of temperature and conductivity were found (18.5 to 22.7 ºC; 33 to 125 µS cm^{-1} respectively). The environments showed lightly acid water, with low values of pH (5.5 to 7.7) and alkalinity (0.2 to 0.8 meq CO_2 l^{-1}) variation.

Table 1 presents the average concentrations for morning and afternoon periods of organic and inorganic dissolved carbon, total nitrogen and phosphorus, and chlorophyll-*a* (no significant differences at $p < 0.05$).

Lake Amarela, the shallowest environment, showed the highest concentrations of organic and inorganic carbon, total nitrogen and phosphorus (8.0 and 6.1 mg l^{-1}; 3,613 and 64.2 µg l^{-1} respectively). A highest value of chlorophyll-*a* (186.0 µg l^{-1}) was registered at Lake Águas Claras and at Lake Dom Helvécio, the deepest environment a highest N/P ratio.

Lake Carioca presented the highest values of bacterial production (5,165 µg C m^{-3} h^{-1}) during the rainy period, in the morning; and phytoplankton production (747.4 mg C m^{-3} h^{-1}) during the dry period, in the morning. Lake Dom Helvécio showed the lowest values of bacterial production (4.3 µg C m^{-3} h^{-1}) during the dry period, in the afternoon; and phytoplankton production (0.4 mg C m^{-3} h^{-1}) during the rainy period in the morning. In relation to the BP/PP ratios, Lake Carioca presented the highest values (0.0289 and 0.0411, morning and afternoon, respectively) during the rainy period (table 2).

The highest values of bacterial and phytoplankton production per unit area were found at Lake Carioca during the dry period (5.7 and 2,247 mg C m^{-2} h^{-1} respectively, Fig. 1). In all of the lakes the diurnal variation of phytoplankton and bacterioplankton production was not significant ($p>0.05$) and, in seasonal terms, only Lake Carioca did not present significant seasonal differences ($p>0.05$) for bacterial production, as also observed regarding phytoplankton production in Dom Helvécio and Águas Claras lakes. Bacteria and phytoplankton productions were significantly different ($p>0.05$) among the 4 environments during the rainy and dry seasons.

Figure 2 presents the results of the PCA. The first two axes accounted for 58.2 % of total variance. Chlorophyll-*a*, total phosphorus and total nitrogen concentration are positively correlated with the first axis (39.4 %). Dissolved inorganic carbon concentration was positively correlated with axis 2 (18.8 %) (Fig. 2A). The first principal component scores could represent the trophic state of the lakes. Lakes Carioca (dry period) and Amarela (rainy period), which showed the highest concentrations of chlorophyll-a, phosphorus, and nitrogen are the farthest from origin in the PCA (Fig.2B).

Discussion

The studied lakes, despite possessing similar origin and age exhibit distinct physico-chemical and trophic conditions as shown for example through their oxygen and phosphorus levels. In this respect, Lake Dom Helvécio shows a clear oligotrophy while Lake Amarela exhibits, eutrophic conditions. This is reflected on their distinct production rates which depend on distinct conditions of light climate mainly for phytoplankton production and nutrient concentrations for bacterial one. Moreover, specific features of these lakes are also important to determine the contribution of phytoplankton or bacterioplankton in carbon fixation: Lake Amarela exhibits the highest total alkalinity and DIC values allowing for high phytoplankton production rates; however, due to likely high decomposition rates rendering high organic carbon values allowed for the highest bacterioplankton production rates.

The highest bacterial production values during the rainy period are probably directly related to the larger deposit of organic matter during this period, as also observed by Gurung et al. (2002) in Lake Biwa, in Japan. High bacterial production values in lakes are generally associated with high concentrations of organic matter and total phosphorus (Karlsson et al, 2002; Jansson et al., 2000). High values of phytoplankton production would mainly be related to greatest nitrogen availability, due to an increase in the circulation of nitrogen (especially ammonia) during the dry season, as suggested by

Barbosa & Tundisi (1980), for Lake Carioca. According to Karlsson et al. (2002) the phytoplankton productivity is, in most lakes, limited by the concentration of nitrogen.

Considering the possible seasonal effects, the results obtained showed that only Lake Carioca did not present significant differences in bacterial production and, in relation to phytoplankton production, the differences were significant only in the Carioca and Amarela lakes. The absence of significant diurnal variations observed in the 4 environments does not confirm the result obtained by Barbosa et al. (1989) in Lake Carioca, where high values of phytoplankton production were registered during the day period, especially during the dry season.

The results obtained in this study did not provide proof of differences in production among lakes situated in preserved areas (PERD) and impacted ones (surrounding PERD). However, the quantity and the quality of allochthonous material of the preserved and impacted areas can explain the differences in Carioca vs. Amarela; Dom Helvécio vs. Águas Claras lakes, that presented similar characteristics of nutrient concentration and production levels. The BP/PP and N/P ratios support this hypothesis, especially in relation to the high allochthonous deposit of organic matter during the rainy period and autochthonous**,** coming from the decomposition of aquatic macrophytes. Karlsson et al. (2002) pointed out that the quantity of allochthonous material is the main factor that alters the N/P and BP/PP ratios in temperate lakes.

In a previous study, and in the same lakes, Rahaingomanana et al. (2002) performing the fractionation of primary production, recorded the lowest values for Lake Dom Helvécio (5.0-6.3 mg C m^{-3} h^{-1}) and the highest ones for Lake Carioca, also showing that the primary production values of Lake Águas Claras are similar to the ones obtained in Lake Dom Helvécio. Besides this, previous studies have shown the importance of the contribution of smaller fractions (< 2-20 μm) for the total production of the environments (Barbosa & Tundisi, 1980; Barbosa et al., 1989; Rahaingomanana et al. 2002). The importance of these fractions was also emphasized particularly in environments with low phosphorus concentrations (Stockner, 1988).

The low values of total phosphorus and the high N/P ratio (N/P > 9.0) found in the present study suggest a limitation of phosphorus for the production of the environments studied. According to Hubble & Harper (2000), bottom-up control mechanisms of primary production are particularly important in oligotrophic lakes, though the presence of ciliate and flagellate in the trophic chain (top-down control) may assume greater importance. Besides that, according to Maia-Barbosa et al. (in press) the bacterial production, as well as the primary production of phytoplankton smaller fractions constitute important energy resources for the microzooplanktonic organisms of these ecosystems and, despite the lack of current knowledge about the ciliates and flagellates communities in the environments, studies in progress have shown that the richness and the density of filter feeding organisms (eg. rotifers and cladocerans) is high in Lake Amarela , followed by lakes Águas Claras, Dom Helvécio, and Carioca (Maia-Barbosa et al., unpublished data). This fact can also explain the differences in the production rates (especially bacterial and phytoplankton <10-20 µm) among the environments.

The present results allow us to conclude that Lake Amarela is the richest in nutrient concentrations among the studied lakes. The bacterial production, despite presenting lower values than the phytoplankton production is particularly important in oligo-mesotrophic lakes. Finally, for a better understanding of the mechanisms that regulate these communities it is necessary "*in situ*", experiments principally using the bottom-up approach as compared to the top-down, for which is essential studies on the ciliates and flagellates communities. Such studies, together with an estimate of the possible links between algae <20 µm and the bacteria are essential for the understanding of the transfer of organic matter to other links of the trophic chain in tropical lakes.

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Figure Legends

Figure 1. Bacterial (A) and phytoplankton (B) production (mg C m^{-2} h^{-1}) at a central station of 4 lakes during the morning and afternoon of dry (2000) and rainy (2001) periods.

Figure 2. Correlations of biotic and abiotic parameters with the first two axes of principal component analysis (A) and score distributions of lakes sampled in dry and rainy (morning and afternoon) seasons along the first two principal components axes (B).

*AM= Amarela; DH= Dom Helvécio; CA= Carioca; AC= Águas Claras

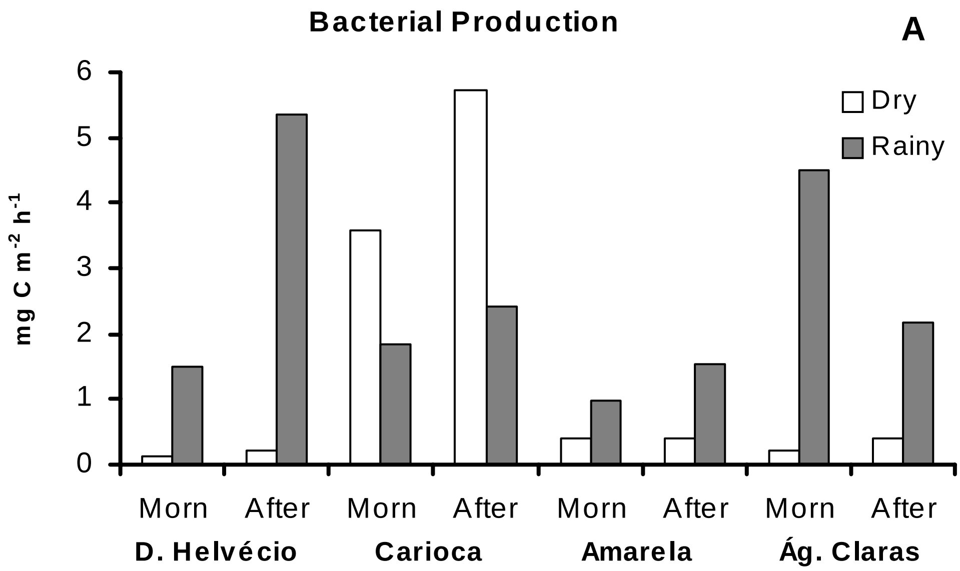
**M= Morning; A= Afternoon; D= Dry; R= Rainy

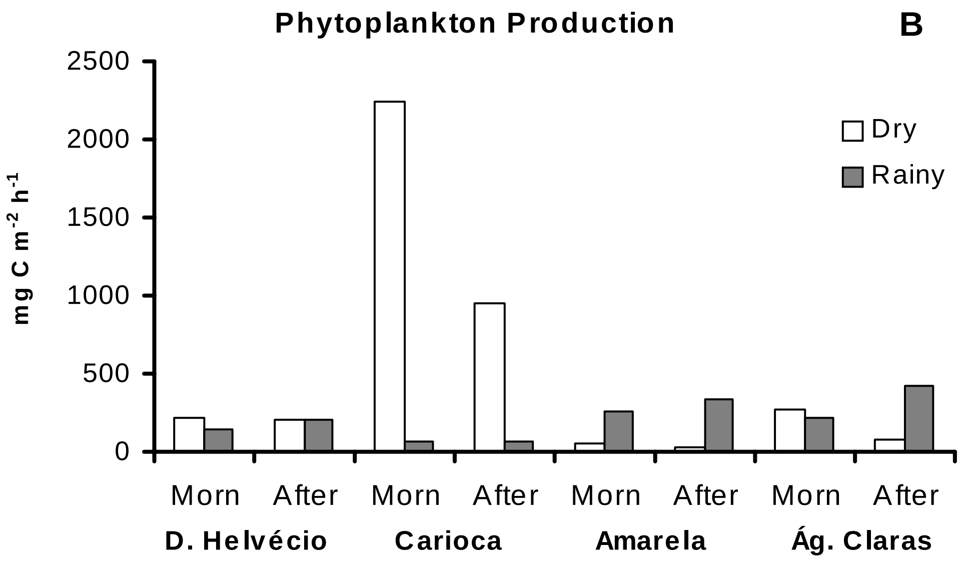
Table 1. Average concentrations for morning and afternoon periods of dissolved organic and inorganic carbon, chlorophyll-*a*, nitrogen, phosphorus and N/P ratios at a central station of 4 lakes during the morning and afternoon of the dry (2000) and rainy (2001) periods.

Lakes	Depth (m)		D.O.C mg l^{-1}		D.I.C mg l^{-1}		Chl-a µg l^{-1}		Tot-P µg l^{-1}		Tot-N µg l^{-1}		N/P	
	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy
Dom Helvécio	0.0	0.0	4.2	3.8	3.2	1.7	4.2	13.1	9.6	3.1	693.2	336.1	72.5	113.8
	2.0	4.0	3.9	3.8	3.2	1.6	4.9	5.8	12.7	3.0	983.0	371.0	77.3	152.3
	6.0	12.0	3.8	2.9	3.1	2.5	3.3	53.9	11.6	7.6	1,125.5	606.5	96.9	100.5
	15.0	20.0	4.5	3.0	3.2	2.8	4.0	11.6	14.3	6.3	1,124.0	699.0	78.1	111.0
Carioca	0.0	0.0	5.1	4.5	2.0	1.3	79.9	15.1	30.3	9.2	1,135.5	207.5	39.7	22.7
	1.5	2.5	4.9	4.6	2.0	1.3	81.9	16.9	31.6	11.6	1,520.5	392.5	48.3	33.9
	3.0	7.0	4.7	4.2	1.9	3.3	76.1	120.8	31.5	29.9	1,890.5	1,750.5	59.9	61.6
	7.0	9.0	4.7	4.4	2.1	4.0	71.7	55.6	28.5	30.0	1,634.5	2,143.5	51.8	70.7
Amarela	0.0	0.0	4.9	6.0	5.9	4.0	8.9	20.0	17.0	28.6	615.5	659.0	36.2	27.1
	0.7	1.0	4.8	6.6	5.8	4.8	16.0	27.4	20.3	24.3	643.0	924.5	31.1	46.1
	1.0	1.5	4.8	7.0	5.8	4.8	9.0	112.5	23.1	37.0	764.5	2,085.0	33.4	56.1
	1.5	2.0	4.9	7.2	5.8	5.0	21.2	71.7	23.6	61.1	886.5	3,316.0	36.8	54.1
Águas Claras	0.0	0.0	6.5	5.8	2.3	1.6	20.1	13.8	23.9	7.1	829.5	408.5	35.9	76.9
	1.5	3.0	6.9	5.2	2.3	1.8	26.9	10.4	23.3	8.6	688.5	529.0	29.4	59.6
	4.5	7.0	6.9	5.5	2.0	1.7	36.1	30.7	10.9	12.5	697.0	617.0	64.6	48.0
	6.5	9.0	6.5	5.2	2.3	3.1	32.2	161.5	25.5	23.9	801.0	2,184.5	31.3	90.4

Table 2. Bacterial Production/Phytoplankton Production (BP/PP) rates at a central station of 4 lakes during the morning and afternoon of the dry (2000) and rainy (2001) periods.

Lakes	Period	BP/PP	
		Dry	Rainy
Dom Helvécio	Morning	0.0005	0.0109
	Afternoon	0.0010	0.0266
Carioca	Morning	0.0016	0.0289
	Afternoon	0.0060	0.0411
Amarela	Morning	0.0079	0.0039
	Afternoon	0.0187	0.0045
Águas Claras	Morning	0.0008	0.0209
	Afternoon	0.0054	0.0052


Bacterial Production
A
mg C m-2 h-1
6
5
4
3
2
1
0
Dry
Rainy
Morn
After
Morn
After
Morn
After
Morn
After
D. Helvécio
Carioca
Amarela
Ág. Claras


Phytoplankton Production
B
mg C m-2 h-1
2500
2000
1500
1000
500
0
Dry
Rainy
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After
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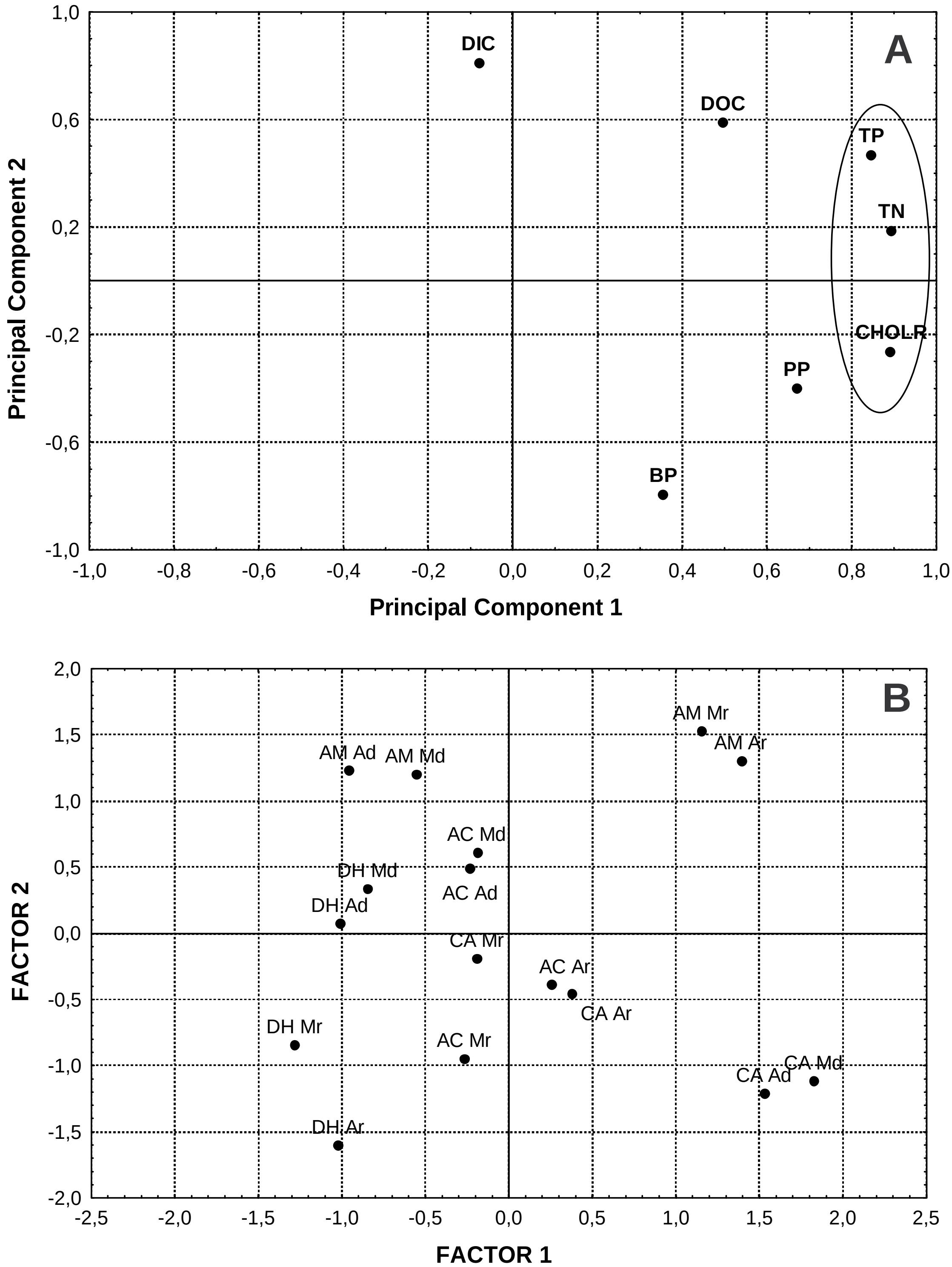
D. Helvécio
Carioca
Amarela
Ág. Claras


A
DIC
DOC
TP
TN
CHOLR
PP
BP
Principal Component 1
Principal Component 2
B
AM Mr
AM Ar
AM Ad
AM Md
AC Md
DH Md
AC Ad
DH Ad
CA Mr
AC Ar
CA Ar
DH Mr
AC Mr
CA Md
CA Ad
DH Ar
FACTOR 1
FACTOR 2

Capítulo 5

Fractionated primary production of phytoplankton in lakes of the rio doce valley (southeastern brazil).

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Fractionated Primary Production of Phytoplankton in Lakes of the Rio Doce Valley (Southeastern Brazil).

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Keywords: Lakes, phytoplankton, primary production,

Introduction

The lacustrine system of the middle Rio Doce valley in the State of Minas Gerais presents various limnological interests and uses to support an important biodiversity (Saijo & Tundisi, 1989).The system is comprised of c. 120 small (0.5-680 ha) and shallow lakes (1.5 to 15 m) lakes amidst the biggest remnant of the original Atlantic Forest, nowadays greatly surrounded by *Eucalyptus spp* plantations, agriculture, and posture land, together with iron/steel plants, mining activities, and cellulose production.

Ecological research have been conducted in order to understand the functioning of these ecosystem and to evaluate the impact of Atlantic Forest substitution by *Eucalyptus* spp monoculture and how it affects the existing lakes (De Meis, 1977). Among the aspects under studies in this system, phytoplankton production is of paramount importance and its evaluation began at least two decades ago when it was possible to demonstrate the occurrence of significant seasonal and diurnal differences (e.g. Barbosa & Tundisi, 1980) However, these studies considered mainly total phytoplankton production although there were some indication that the smaller fractions could play a central role in the production of new organic matter.

Since phytoplankton size structure influences the population dynamics and interactions in the food web, the determination of size-fractionated primary production is fundamental to know the processe of energy transfer in aquatic ecosystems (Munawar *et al*., 1978; Elser *et al*., 1986; Beaty & Parker, 1996).

Testing hypothesis:

1. Phytoplankton primary production varies among size fractions
2. Phytoplankton production is different between non impacted and impacted lakes

In order to test these hypothesis this study aims to determine the contributions of the micro-, nano- and picoplancton fractions to the primary production in four lakes of the middle Rio Doce region presenting different trophic states and located in impacted (Eucalyptus plantation area) and non-impacted (State Park of Rio Doce) areas.

The study lakes

Lakes Dom Helvécio and Carioca are located within the Rio Doce State Park (19º 29-42' S; 42-48º 28-38' W) which constitutes 36,000 ha of remaining Atlantic Forest. Lake Dom Helvécio is the largest (687 ha) and deepest (33 m) lake of the lake system, exhibiting oligotrophic conditions and characteristically a warm-monomictic lake. Lake Carioca, also a warm-monomictic lake, is a small (13.2 ha)and shallow (11.8 m) lake of mesotrophic conditions, and mostly well-protected from wind action by the surrounding secondary forest.

Lakes Águas Claras and Amarela are located in the surrounding *Eucalyptus* spp. plantation area. Lake Águas Claras is similar to Lake Dom Helvécio, also exhibiting oligotrophic conditions while Lake Amarela is shallow, mesotrophic and characterised by extense macrophyte's populations. The major characteristics of the four lakes are summarised in table 1.

Table 1. Major characteristics of the study lakes in the middle Rio Doce Valley

	Dom Helvécio	Carioca	Águas Claras	Amarela
Area	687 ha	13 ha	~130 ha	12 ha
Max. depth	30 m	11 m	8 m	2 m
Trophic state	Oligotr.	Mesotr.	Oligotr.	Mesotr.
Watershed	Natural Forest		*Eucalyptus spp.*	

Material and methods

The experiments were conducted in February (summer/rainy) and July (winter/dry) 2000. Phytoplankton primary production was measured by the ^{14}C technique (Steeman-Nielsen, 1952; Vollenweider, 1971). Incubations were conducted for 4 hours during the morning periods at a limnetic station at depths corresponding to 100%, 10%, 1% of surface irradiance estimated through the Secchi disk and another depth in the aphotic zone.

Fractionation was realised through differential filtration using 20 µm plankton net, 3 µm pore size cellulose ester or polycarbonate filters and 0.45 µm pore size cellulose ester filters. Countings were performed using Bray scintillation cocktail at 10 min counting intervals.

Results

Total phytoplankton primary production

Lake Dom Helvécio presented the lowest productivity with values ranging between 5.0-6.3 $mgC.m^{-3}.h^{-1}$ during the summer and below 10 $mgC.m^{-3}.h^{-1}$ during the winter.

The highest production levels were observed in Lake Carioca for the two periods. In summer, as the water column was stratified, primary production was around 25 mgC.m^{-3}.h^{-1} at depths corresponding to 100% and 18% of surface light, reaching a peak of 234 mgC.m^{-3}.h^{-}1 at 4% of light irradiance, a depth of 2.5 m above the metalimnion. During the mixing period (winter), the productivity increased markedly in the upper part of the euphotic zone, with a value of 175 mgC.m^{-3}.h^{-}1 recorded just below the surface, reaching the highest value of 370 mgC.m^{-3}.h^{-1} at the depth corresponding to 6% of surface light, and decreasing to 6.8 mgC.m^{-3}.h^{-1} at the bottom of the euphotic zone.

The values of primary production recorded for Águas Claras in summer were similar to those recorded at lake Dom Helvécio at depths corresponding to 100% and 10% of surface light. However, a peak of 60 mgC.m^{-3}.h^{-}1 was recorded at depth corresponding to 3% of surface light. In winter, the distribution of the primary production changed similarly to the one recorded at lake Carioca since it increased in the upper part of the water column (14-30 mgC.m^{-3}.h^{-1}) but decreased below 3.4-0.1 mgC.m^{-3}.h^{-1} in the lower layer of the euphotic zone.

Despite mesotrophic, primary production values recorded Lake Amarela during the winter remained between 10-20 mgC.m^{-3}.h^{-1}.

Fractionation

In summer, phytoplankton fractions below 20 µm (nano- + picoplankton) were the main contributors to primary production, with relative contributions above 80% observed in lakes Dom Helvécio, Carioca and Águas Claras. Estimate of nanoplankton contribution was above 75% in lake Dom Helvécio, where it reached 98% in the lower layer of the euphotic zone. In lake Carioca, this fraction contributed with *c*. 85% at depths corresponding to 100% and 18% of surface light, although it represented only 50% at depth corresponding to 4% of surface light where a peak was recorded. This resulted from an increase of the relative contribution of the micro- and picoplankton fractions that reached respectively 17% and 30% at this depth. In lake Águas Claras, nanoplankton contribution accounted for 60-80% of total production.

In winter, nanoplankton remained the major productive fraction in lakes Dom Helvécio, Carioca and Águas Claras. In lake Dom Helvécio, this fraction represented 73-76% of the total primary production at depths corresponding to 100% to 0% of surface light. and it represented 66-89% of total production in lake Carioca. On the other hand, its contribution was lower In lake Águas Claras, accounting for 45-60% of total production due to an increase of the relative contribution of the microplankton, which represented 31-44% of the total primary production. This increase of the microplankton contribution was also observed in lake Dom Helvécio where it reached 11-18% within the euphotic zone. The contribution of the picoplankton was marked in lakes Dom Hevécio and Carioca where it reached 25% in the euphotic zone. In lake Águas Claras this fraction accounted for *c*.10% of total production. As an exception, the microplankton fraction in the lake Amarela was the major contributor accounting for 56% of the total primary production. However, the nanoplankton remained an important contributor.

The percentage contribution of these fractions is summarised in Fig. 1

Discussion

Low to moderate phytoplankton productivity in the lakes of the Rio Doce Valley has been previously reported (Barbosa & Tundisi, 1980; Tundisi et al., 1997). This is mainly related to the nutrients deficiency in the water column, in particular for nitrate, that can be observed in the epilimnion of stratified lakes, as well as in mixing conditions (Tundisi et al., 1987). Stable thermal stratifications are usually characterised by an important gradient of ammonium-nitrogen concentrations, that may explain the peak of primary production at low light depth that occurred in the lakes during the stratification period, such as observed in lake Carioca (Barbosa & Tundisi, 1980; Reynolds, 1997).

The present results confirmed the importance of the small size fractions of phytoplankton for total primary production in the lakes of the Rio Doce that was suggested by previous observations (Barbosa, 1981; Tundisi et al., 1997). This is commonly observed in water with low nutrient levels and results the better competition of small cell organisms in nutrient uptake in such conditions (Stockner, 1988). Périn et al. (1996) and Pinel-Alloul et al. (1996) observed that the absolute and relative importance of picoplankton comparatively to nanoplankton decreased with the increase in phosphorus concentration. Concentrations of phosphorus reported for the lakes of the Rio Doce are low (5-20 $\mu g.l^{-1}$) but non-limiting for algal growth (Tundisi et al., 1987) and could explain the importance of nanoplankton observed in these lakes. Under conditions of nutrient availability, larger cells organisms are better competitors for nutrients assimilation while the grazing pressure increases on the small fractions (Agusti et al., 1990). The higher trophic state of the lake Amarela would explain the dominance of the microplankton contribution to primary production but Tundisi et al. (1997) observed that during some enrichment experiences in lakes of the Rio Doce Valley, the contribution to primary production of the fractions below 20 μm remained around 80%. However, various studies (Pick & Agbeti, 1991; Beaty & Parker, 1996; Carrick & Schelske, 1997) call attention that the relationship between the trophic state of lakes and the relative importance of the size fractions of phytoplankton to total biomass and/or productivity , is not well established.

During thermal stratification conditions, when strong nutrient depletion appears in the epilimnion, the adaptation of cyanobacteria to low light level often results in an increase of the relative contribution of picoplankton production near the metalimnion, where nutrients are available (Weisse, 1988; Nagata et al., 1994). This was apparently verified in lake Carioca, even if the low depth peaks of phytoplankton observed in the lakes of the Rio Doce Valley are mainly composed of cyanobacteria forming colonies such as *Lyngbia* (Reynolds, 1997), which may have bias the fractionation process since colonies can not be accurately separated. On the other hand, in mixing conditions, redistribution of nutrients in the water column allow higher production levels of microplankton within the upper layer of the euphotic zone, particularly in the oligotrophic lakes Dom Helvécio and Águas Claras.

The inputs of allochtonous material are of paramount importance for the metabolism of the lakes of the Rio Doce Valley (Tundisi et al., 1978; Barbosa & Coutinho, 1987) and changes resulting from *Eucalyptus* plantation and exploitation activities such as increase in sediments and nutrients loading has been reported (Saijo et al., 1991; Sabará, 1994). Differences in phytoplankton primary production were observed between impacted and non-impacted lakes of similar trophic state (Dom Helvécio/Águas Claras; Carioca/Amarela). However, the important heterogeneity of morphometry between lakes may also be considered to explain such differences. In particular, low deepness in lake Águas Claras compared to the deep lake Dom Helvécio would favour the nutrient cycling between water and sediment. In the case of lake Amarela, the extreme shallowness and the important development of macrophytes may limit the relevance of phytoplankton production very likely limited by light availability.

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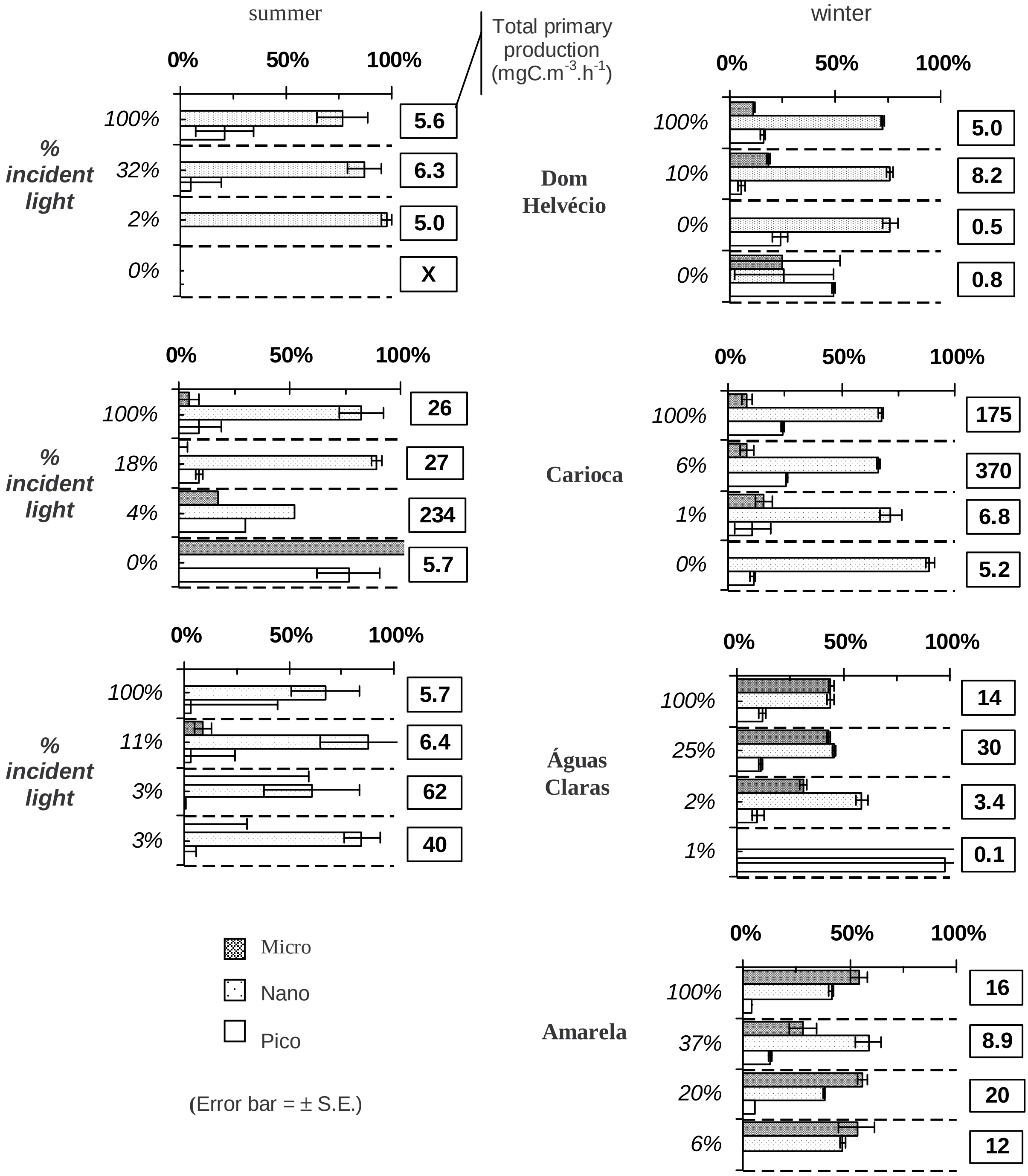
Figure Legend

Figure. 1. Relative contribution of micro-, nano- and picoplankton fractions to total phytoplankton production in four lakes of the Rio Doce Valley, south-east Brazil.

Contribution of phytoplankton fractions to Total Primary Production



8- DISCUSSÃO GERAL

8.1- Ambientes lóticos

A sazonalidade e a heterogeneidade dos 8 rios estudados no trecho médio da bacia do Rio Doce foram duas características marcantes evidenciadas durante os dois anos de amostragens. A presença de uma estação seca e outra chuvosa vai ocasionar alterações nas características físicas, químicas e biológicas dos ambientes estudados, assim como a influência da bacia de drenagem que resulta em padrões de estrutura e funcionamento diferenciados em cada rio.

De maneira geral os ambientes estudados variaram de oligotróficos (ribeirão Caraça) a eutróficos (ribeirão Ipanema), sendo que alguns ambientes, dependendo do aporte de material alóctone, apresentam grau de trofia variado dependendo dos períodos de seca ou chuva (rios Santa Bárbara, Peixe, Severo, Piracicaba e Doce). O ribeirão Caraça, pode ser considerado o ambiente mais preservado, apresentou a maior limitação por fósforo e o Ipanema, ambiente mais degradado, apresentou limitação por nitrogênio, devido principalmente ao maior aporte de efluentes domésticos e industriais. Os demais ambientes, mostraram limitações por fósforo durante o período de seca e no período de chuvas, com maior aporte de material alóctone, mostraram uma limitação por nitrogênio.

Outra característica importante verificada foi à alta correlação entre as concentrações de nitrogênio e fósforo total com as densidades de coliformes fecais, e a concentração de bactérias heterotróficas mostrou-se capaz de diferenciar os ambientes. Estes resultados sugerem a inclusão do grau de trofia e da caracterização das atividades antrópicas na bacia, como ferramentas importantes para a proposição de medidas de recuperação e conservação de trechos de rios sujeitos a impactos antrópicos.

Em relação à produtividade, novamente destacam-se os ribeirões Caraça e Ipanema. O primeiro por apresentar os menores valores de produção bacteriana e o segundo pelos maiores valores, demonstrando uma relação direta com os teores de matéria orgânica, em cada ambiente. Em relação à produção primária fitoplanctônica, baixos valores foram encontrados assim como, baixas correlações com os parâmetros analisados. A produção bacteriana apesar de apresentar menores valores não deve ser desconsiderada como fonte de carbono para os sistemas estudados, principalmente, nos ambientes mais impactados, onde os maiores valores refletem alterações no grau de trofia.

A influência do aporte de material alóctone e não o número de ordem dos rios é um fator determinante dos padrões obtidos neste estudo. Os resultados obtidos têm um caráter ímpar, pois pela primeira vez foi estimada, simultaneamente, a produtividade do fitoplâncton e de bactérias nestes trechos de rios. Estes resultados constituem ferramentas fundamentais para a proposição de medidas de recuperação e preservação destes rios principalmente considerando-se que já existem projetos de estações de tratamento de esgotos para alguns trechos da bacia (p. ex. Piracicaba) e que está em funcionamento desde 2001, uma estação de tratamento de esgotos no ribeirão Ipanema o que promoverá alterações significativas na qualidade das águas e na estrutura e funcionamento deste ambiente, como um todo.

8.2- Ambientes lênticos

O padrão térmico característico do tipo monomítico quente, iniciando o processo de desestratificação térmica em maio, estando completamente homogêneos em julho/agosto e iniciando a estratificação em setembro descrito na literatura para os lagos do médio Rio Doce, apresentou algumas variações. Apesar de bem definido para os lagos do médio rio Doce, este padrão apresentou variações em janeiro de 2001, provavelmente devido a menor quantidade de chuvas registrada neste período possivelmente influenciando os processos de ciclagem e produtividade desses lagos.

De maneira geral os ecossistemas são, com exceção das lagoas Amarela e Carioca, oligotróficos e com uma elevada razão $N_{total}l/P_{total}$, sugerindo que o fósforo é um elemento critico para estes lagos. Apenas a lagoa Amarela, ambiente raso, rico em macrófitas aquáticas e em estágio sucessional mais avançado, além do hipolímnio da lagoa Carioca, principalmente no verão, apresentam as menores razões N/P (8,9 e 12,1). O Lago Dom Helvécio destacou-se pelas maiores razões N/P (200,1) e por baixas concentrações de nutrientes.

Apesar de mesma origem e idade, a evolução dos lagos do sistema lacustre do médio rio Doce e a influência da área de entorno (mata preservada x área alterada) reflete processos distintos, com reflexos no grau de trofia. Tais reflexos são também evidentes nas taxas de produtividade do fito e bacterioplâncton. Os lagos do Parque Estadual do Rio Doce e áreas de entorno, apesar de proximamente localizados, apresentaram ampla variação de produção bacterioplantônica (0,0005 – 3,5 mg $C.m^{-3}.h^{-1}$) e fitoplanctônica (0,3 – 747,5 mg $C.m^{-3}.h^{-1}$).

De um modo geral, as maiores taxas de fixação de carbono pelas algas foram obtidas nas profundidades correspondentes a 10% e 1% de penetração de luz e no período de seca, quando os ambientes estão desestratificados havendo, portanto, uma maior disponibilidade de nutrientes na coluna d'água. Em relação aos perfis de produção bacteriana os maiores valores, geralmente estão localizados nas profundidades correspondentes a 1% de penetração de luz e na zona afótica embora nas lagoas Palmeirinha, Jacaré e Barra as maiores taxas foram registradas na superfície. Deve-se destacar os maiores valores de produção bacteriana da lagoa Amarela assim como os maiores valores de produção fitoplanctônica da lagoa Carioca. Num outro extremo temos os lagos Dom Helvécio e Águas Claras com menores taxas de produtividade.

A relação produção bacteriana/fitoplanctônica (PB/PF) é normalmente usada como um índice que determina quanto de carbono fixado pelas algas é processado pelas bactérias (VAN WAMBEKE *et al.* 2002). Teoricamente, a não existência de correlação entre a produção fito e bacteriana deve corresponder a uma situação onde o carbono utilizado pelas bactérias tem outra origem. No presente estudo a produção bacteriana e a relação PB/PF mostraram variações sazonais significativas, com os maiores valores obtidos na lagoa Amarela, evidenciando uma maior contribuição da produção bacteriana, na fixação de carbono, neste ambiente e sugerindo uma possível correlação da produção bacteriana com as concentrações de matéria orgânica.

Embora a produção primária fitoplanctônica foi sempre superior à bacteriana em todos os ambientes, a fixação de carbono por bactérias não deve ser desprezada, principalmente nas camadas mais profundas e em ambientes ricos em matéria orgânica como a lagoa Amarela, por exemplo, com uma rica comunidade de macrófitas aquáticas.

Apesar de nenhuma correlação significativa entre os valores de produção (fito e bacterioplanctônica) e as concentrações de nutrientes ter sido evidenciada, o fósforo é provavelmente o principal elemento limitante à produção primária dos lagos em estudo, a julgar pelas baixas razões N/P registradas. Segundo HUBBLE & HARPER (2000), mecanismos de controle da produção primária do tipo "bottom-up" são particularmente importantes em lagos oligotróficos, embora a presença de ciliados e flagelados na cadeia trófica pode assumir maior importância, exercendo um controle do tipo "top-down". Além disso, segundo MAIA-BARBOSA *et al.* (no prelo), a produção bacteriana, assim como a produção primária das menores frações do fitoplâncton (ex RAHAIGOMANANA et al, 2002) constituem importantes fontes de energia para os organismos microzooplanctônicos desses ecossistemas e, apesar do pequeno conhecimento existente sobre a comunidade de ciliados e flagelados nestes ambientes, estudos em andamento têm sugerido que a riqueza e a densidade de organismos filtradores (ex. rotíferos e cladóceros) é maior na lagoa Amarela, seguida pelas lagoas Águas Claras, Dom Helvécio e Carioca (MAIA-BARBOSA *et al.*, dados não publicados). Este fato pode também explicar diferenças nas taxas de produção (principalmente bactérias e fito <10-20 µm) entre os ambientes.

Apenas a produção bacteriana apresentou diferenças sazonais significativas. Apesar de apresentar menores valores comparados à produção fitoplanctônica, a fixação de carbono pelas bactérias é fundamental para o metabolismo dos lagos do PERD e seu entorno. No entanto, para um melhor entendimento dos mecanismos que regulam essas comunidades são necessários experimentos "*in situ*" utilizando tanto a abordagem "bottom-up" como "top-down" para os quais são essenciais estudos sobre a comunidade de ciliados e flagelados. Tais estudos, juntamente com uma avaliação das possíveis relações entre algas <20 µm e as bactérias são essenciais para o entendimento da transferência de matéria orgânica para os demais elos da cadeia trófica.

9- CONCLUSÕES

• Os ambientes do trecho médio do Rio Doce demonstraram ampla variação de trofia, apresentando desde ambientes oligotróficos (maioria dos lagos) até eutróficos (maioria dos rios no período de chuva);

• As elevadas razões N/P nos lagos estudados sugerem que o fósforo seria o principal elemento limitante à produtividade;

• Os rios e lagos estudados, apesar de proximamente localizados, apresentaram ampla variação da produção bacterioplantônica (0,005 – 5,7 mg $C.m^{-3}.h^{-1}$) e fitoplanctônica (0,01 – 747,5 mg $C.m^{-3}.h^{-1}$);

• A taxa de produção bacteriana mostrou-se altamente correlacionada à concentração de nutrientes (Caraça x Ipanema; Dom Helvécio x Carioca/Amarela);

• Os valores de produção bacteriana apresentaram correlações significativas com as concentrações de fósforo e a produção fitoplanctônica não apresentou correlações significativas com os nutrientes;

• A sazonalidade influenciou apenas as taxas de produção bacteriana;

• O aporte de matéria orgânica alóctone durante o período de chuvas altera o grau de trofia dos rios e tem reflexo nas maiores taxas de produção bacteriana;

• As menores frações do fitoplâncton (<10-20 µm) contribuem com o maior percentual da produtividade primária nos lagos Dom Helvécio, Carioca, Amarela e Águas Claras;

• As taxas de produção fitoplanctônica e bacterioplanctônica não apresentaram variações diurnas nos 4 lagos estudados; e

• As correlações entre N, P, coliformes fecais e bactérias heterotróficas, nos ambientes lóticos, sugerem inclusão do grau de trofia e da caracterização das atividades antrópicas na bacia, como ferramentas importantes para a proposição de medidas de recuperação e conservação de trechos de rios sujeitos a impactos antrópicos.

9.1- Perspectivas

Para um melhor entendimento dos mecanismos que controlam a produtividade primária dos lagos estudos com a estimativa da biomassa bacteriana em diferentes lagos assim como experimentos de campo e laboratório sobre os controles “bottom-up e top-down” são necessários. Testes com tratamentos diferenciados do tipo enriquecimento com fósforo e nitrogênio e presença e ausência de organismos do plâncton (fito e zooplâncton) seriam de suma importância para a definição de fatores que controlam a produtividade.

Para a definição de padrões de produtividade, séries anuais com medidas regulares (ex. amostragens mensais?) da produtividade são essenciais. Da mesma forma, o conhecimento de organismos ciliados e flagelados, com estimativas de densidade, riqueza e diversidade de espécies presentes em cada ambiente são necessárias para melhor compreensão da base das cadeias tróficas.

As relações da produtividade com as concentrações de nutrientes devem ser aprofundadas, principalmente no que se refere à qualidade e a quantidade do material alóctone que os rios e lagos recebem durante o período de chuvas. A influência de áreas de mata preservada x áreas com de plantios de *Eucalyptus* spp (ou área de regeneração) podem ser esclarecidas com estudos do teor nutricional do material alóctone assim como estudos de longa duração devem ser conduzidos com vistas a definição de padrões da produtividade.

Intensificar estudos sobre a concentração de nutrientes e as comunidades nos trechos de rios onde estão (e estarão) sendo implementadas as estações de tratamento de esgoto no trecho médio da bacia do Rio Doce constitui ação importante para se avaliar a recuperação da qualidade da água com profundas alterações na composição das comunidades, e no metabolismo desses ecossistemas lóticos.

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