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Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre

CARACTERIZAÇÃO E IMPORTÂNCIA DA MATÉRIA ORGÂNICA DISSOLVIDA EM ECOSSISTEMAS AQUÁTICOS TROPICAIS

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SUMÁRIO

RESUMO

Embora seja reconhecida a diferença na ciclagem de carbono entre sistemas aquáticos temperados e tropicais, os estudos sobre a importância da matéria orgânica nos processos ecológicos e biogeoquímicos ainda são escassos em ambientes tropicais, principalmente no Brasil. Nosso objetivo foi caracterizar espacial e temporalmente a matéria orgânica dissolvida-DOM no lago Dom Helvécio (DH, MG) e comparar por meio de estudos também em outros sistemas aquáticos (reservatório da Pampulha-BH/MG e lago Carioca-MG) o efeito das diferentes fontes de DOM na transparência, disponibilidade de nutrientes e espectro de absorção de CDOM. Amostras de água subsuperficiais foram coletadas (2012-2014) para análise de Clor-a, sólidos em suspensão, DOC, CDOM e nutrientes. Foram obtidos dados de profundidade e pluviosidade, além de medidas in situ da radiação PAR e UV. $SUVA_{254}$, $S_{275-295}$, $S_{350-400}$, S_R, M, $a_{CDOM440}$ e $a_{CDOM254}$ foram utilizados para avaliar a qualidade de DOM. DH foi mais transparente nos períodos chuvosos estratificados e o estudo de partição de *Kd(PAR)* mostrou que embora 79% da atenuação PAR tenha sido devido ao tripton, a variação temporal de *Kd(PAR)* foi melhor explicada pelas variações de Clor-a. Para DOM as diferenças sazonais foram maiores que as espaciais e a pluviosidade afetou positivamente a quantidade de carbono aromático, sugerindo uma entrada de DOM alóctone com as chuvas. Houve uma redução no volume de chuvas nos anos de coleta em comparação com anos anteriores e considerando o efeito da pluviosidade observado, é possível estimar mudanças também nos padrões sazonais de entrada de DOM e nutrientes com consequências para o ecossistema. Em contraste, o eutrófico reservatório da Pampulha apresentou DOM predominantemente de origem algal. Comparando os dois sistemas, demonstramos que distintas fontes de DOM afetam a sazonalidade de nutrientes, transparência e qualidade de DOM. Por fim, avaliamos o efeito das diferentes fontes de DOM no espectro de absorção de CDOM através de experimento de mesocosmos no lago Carioca. DOM alóctone teve forte efeito na curva de absorção de CDOM entre 300 e 400 nm, enquanto DOM autóctone aumentou a absorção nos comprimentos de onda mais curtos. Dessa forma, as características ópticas (como transparência e susceptibilidade de DOM para foto e biodegradação) dependem das fontes de DOM, o que gera uma série de consequências para o ecossistema.

Palavras-chave: DOM, CDOM, matéria orgânica, coeficiente de atenuação, radiação

ABSTRACT

Although it is recognized the difference in carbon cycling between temperate and tropical aquatic systems, studies on the importance of organic matter in the ecological and biogeochemical processes are still scarce in tropical environments, especially in Brazil. Our aim was to characterize spatially and temporally the dissolved organic matter-DOM in Lake Dom Helvécio (DH, MG) and compare through studies in other aquatic systems (Pampulha-BH reservoir/MG and Lake Carioca/MG) the effect of different sources of DOM on lake transparency, availability of nutrients and CDOM absorption spectrum. Subsurface water samples were collected (2012-2014) for analysis of Chl-a, suspended solids, DOC, CDOM and nutrients. Rainfall and depth data were obtained, in addition to in situ measurements of PAR and UV radiation. $SUVA_{254}$, $S_{275-295}$, $S_{350-400}$, S_R, M and $a_{CDOM440}$ $a_{CDOM254}$ were used to evaluate the DOM quality. DH was more transparent in the stratified rainy periods and the *Kd(PAR)* partition study showed that although 79% of PAR attenuation was due to tripton, the temporal variation of *Kd(PAR)* was better explained by changes in Chl-a. Seasonal differences in DOM were greater than spatial, and rainfall positively affected the amount of aromatic carbon, suggesting an allochthonous DOM input with rains. There was a reduction in rainfall in the sampling years compared to previous years and considering the observed effect of rainfall, it is possible to estimate changes in seasonal patterns also in the DOM and nutrients inputs with consequences for the ecosystem. In contrast, the eutrophic Pampulha reservoir presented DOM predominantly from algal source. Comparing the two systems, we show that different DOM sources affect the seasonality of nutrients, lake transparency and DOM quality. Finally, we evaluated the effect of different DOM sources in CDOM spectra through mesocosms experiment in the Lake Carioca. Allochthonous DOM had a strong effect on the CDOM absorption curve between 300 and 400 nm, while autochthonous DOM increased absorption at shorter wavelengths. Thus, the optical characteristics (such as lake transparency and DOM susceptibility to photo and biodegradation) depend on DOM sources, which cause several consequences for the ecosystems.

Keywords: DOM, CDOM, organic matter, attenuation coefficient, radiation

APRESENTAÇÃO DA TESE

A estrutura da tese foi organizada conforme descrito nos seguintes tópicos:

- Introdução: abrange uma revisão bibliográfica sobre radiação, propriedades ópticas aparentes e inerentes e matéria orgânica em ecossistemas aquáticos. Os objetivos da tese estão descritos ao final da Introdução e os objetivos específicos se encontram em cada capítulo.

- Capítulo 1: manuscrito aceito com revisões no periódico *Anais da Academia Brasileira de Ciências*. Devido à notícia do aceite ter sido dada ao fim de Junho, não foi possível incluir as revisões sugeridas pela revista na versão do artigo nesta tese. No entanto, as sugestões e críticas feitas pelos revisores estão disponibilizadas no Anexo I da tese. Trata-se de um estudo realizado no lago Dom Helvécio (Médio Rio Doce, MG, Brasil) cujo objetivo foi avaliar a contribuição de cada componente opticamente ativo na atenuação da radiação fotossinteticamente ativa na coluna d’água.

- Capítulo 2: manuscrito formatado para ser submetido no periódico *Limnologica* após considerações da banca. Este estudo também foi realizado no lago Dom Helvécio e o objetivo foi avaliar as diferenças espaciais e temporais na qualidade e quantidade da matéria orgânica dissolvida considerando mudanças recentes na pluviosidade regional.

- Capítulo 3: manuscrito aceito no periódico *Journal of Limnology* e se encontra disponível em: http://dx.doi.org/10.4081/jlimnol.2016.1359 . Este trabalho foi realizado no lago Dom Helvécio e no reservatório da Pampulha (Belo Horizonte, MG, Brasil). O objetivo foi comparar os efeitos das diferentes fontes de matéria orgânica dissolvida na atenuação da radiação fotossinteticamente ativa e ultravioleta e na disponibilidade de nutrientes nos dois distintos sistemas tropicais.

- Capítulo 4: manuscrito formatado para ser submetido no periódico *Biogeochemistry* após considerações da banca, e originado a partir de um experimento de mesocosmos no lago Carioca (Médio Rio Doce, MG, Brasil), como parte do projeto Carbon Cycling in Lakes (COCLAKE). Este projeto foi desenvolvido em parceria com o pesquisador dinamarquês Peter Anton Staehr. O manuscrito foi escrito na Universidade de Aarhus (Roskilde, Dinamarca) durante o meu período de doutorado sanduíche (2015-2016) e contou com a colaboração de pesquisadores dinamarqueses e brasileiros. O objetivo foi avaliar sobre condições experimentais os efeitos do aumento das concentrações de matéria orgânica alóctone e autóctone na absorção espectral da matéria orgânica dissolvida colorida.

- Considerações finais: abrange a aplicabilidade, contextualização e importância dos resultados gerados com esta tese e as perguntas que surgiram com este trabalho.

INTRODUÇÃO

A radiação solar e as propriedades ópticas dos ecossistemas aquáticos

O fluxo de energia nos sistemas aquáticos, assim como nos terrestres, depende da incidência de radiação solar, a qual influencia fortemente as condições químicas e biológicas dos ecossistemas afetando o metabolismo e funcionamento aquático (Parsons et al., 1984; Kirk, 1994; Helbling e Zagareze, 2003; Loiselle et al., 2009).

A radiação solar é subdividida em três faixas segundo a Comissão Internacional de Iluminação (CIE): ultravioleta (UV – comprimentos de onda entre 100 e 400 nm), visível ou radiação fotossinteticamente ativa (PAR – entre 400 e 700 nm) e infravermelho (maior que 700 nm). A radiação UV ainda é dividida em UV-C (100-280 nm), UV-B (280-315 nm) e UV-A (315-400 nm). UV-C é completamente absorvida pela camada de ozônio, chegando à superfície terrestre apenas UV-B e UV-A e PAR.

Os ecossistemas aquáticos possuem a capacidade de refletir entre 2 a 100% da radiação incidente na superfície da água, sendo esta quantidade dependente das condições da superfície (plana ou ondulada) e do ângulo de incidência dos raios solares. Por isto, em estudos de radiação subaquática o horário de amostragem é importante e deve ser limitado aos horários de menores porcentagens de reflexão, entre 10:00 h e 14:00 h. A reflexão somada à refração, absorção e dispersão da radiação solar são denominadas propriedades ópticas dos ambientes aquáticos (Kirk, 1994; Esteves, 1998). A água, alguns materiais particulados bióticos e abióticos em suspensão na coluna d’água (como plâncton e detritos), e a matéria orgânica dissolvida são responsáveis por absorver e/ou dispersar a luz solar, e são denominados componentes opticamente ativos (Kirk, 1994; Pfannkuche, 2002; Branco e Kremer, 2005; Kelble et al., 2005). A fração dissolvida somente causa absorção, enquanto as partículas orgânicas e inorgânicas causam predominantemente dispersão (“scattering”), exceto os produtores primários (fitoplâncton, macrófitas aquáticas) que contribuem em ambos os processos (Figura 1).

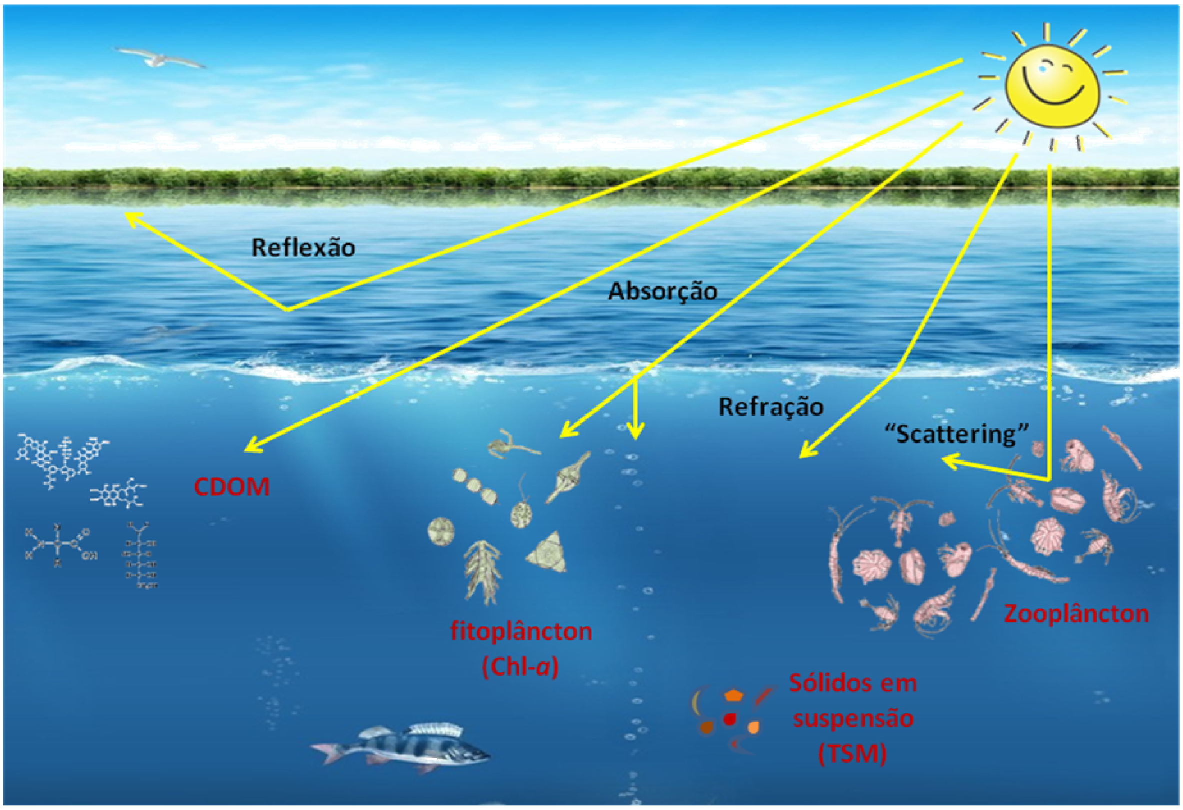

Figura 1 - A radiação solar na superfície aquática e na coluna d'água.

As propriedades ópticas são divididas em aparentes (AOP - apparent optical properties) e inerentes (IOP - inherent optical properties). AOP são aquelas que dependem da natureza angular do campo luminoso, como o coeficiente de atenuação difusa da radiação na coluna d'água (*Kd*) (obtida in situ através de radiômetros e disco de Secchi) e a reflectância para o sensoriamento remoto (Kirk, 1994; Morris et al., 1995; Effler et al., 2010). Em contrapartida, as IOP dependem somente das concentrações dos componentes opticamente ativos, e são representadas pelos coeficientes de absorção da radiação pela matéria particulada e dissolvida presentes na água (obtidos *in situ* com equipamentos modernos ou em laboratório através de amostras de água coletadas em conjunto com uma análise espectrofotométrica) (Morel, 1988, 1991; Kirk, 1994; Huovinen et al., 2003; Zhang et al., 2007a). A atenuação da luz decai exponencialmente com a profundidade e é dependente dos coeficientes de absorção e dispersão, que por sua vez dependem das concentrações dos componentes opticamente ativos na coluna d'água (Obrador e Pretus, 2008). Dessa forma, a camada fótica (limite inferior é definido como a profundidade onde a intensidade luminosa corresponde a 1% da radiação que atinge a superfície) pode variar de poucos centímetros a dezenas de metros de profundidade dependendo da capacidade do meio em atenuar a radiação subaquática (Esteves, 1998).

A quantidade de energia luminosa e o tamanho da zona fótica disponível na coluna d'água (principalmente a PAR) são fatores limitantes para a distribuição e abundância dos produtores primários e a produtividade do sistema, que por sua vez afetam todo o funcionamento do ecossistema (Parsons et al., 1984; McMahon et al., 1992; Gallegos e Moore, 2000; Zhang et al., 2007b). Nos últimos anos, o interesse também sobre o efeito da radiação UV e seu impacto subaquático tem aumentado devido a crescente incidência de raios ultravioleta por causa da redução da camada de ozônio estratosférico e também pela modificação dos ambientes aquáticos em função de poluição local e regional (Campbell et al., 2011).

A matéria orgânica: origem, características e importância para os ecossistemas aquáticos

A matéria orgânica (OM – organic matter) é constituída de uma fração particulada (POM – particulate organic matter) e uma fração dissolvida (DOM – dissolved organic matter). POM é representada pela biota aquática (por exemplo, bactérias, fitoplâncton, zooplâncton) e por detritos orgânicos e inorgânicos subprodutos de decomposição e metabolismo aquático, enquanto que a DOM por convenção representa os compostos orgânicos menores que 0,2-0,7 µm (Kalenak et al., 2013). A matéria orgânica dissolvida colorida (CDOM – colored dissolved organic matter) é a fração de DOM capaz de absorver radiação solar nas faixas do UV e PAR por possuir cromóforos em sua estrutura (Amon e Benner, 1996), o que influencia as propriedades ópticas dos ecossistemas aquáticos.

DOM é o principal reservatório de carbono orgânico em ecossistemas aquáticos (Bertilsson e Tranvik, 2000) e a sua dinâmica temporal e espacial tem recebido maior atenção nos últimos anos devido ao seu importante papel em processos ecológicos e biogeoquímicos (Cole et al., 2007; Tranvik et al., 2009). Também é atualmente reconhecido como o principal substrato para a comunidade bacteriana e representa a base da cadeia trófica planctônica (Farjalla et al., 2006). Através do fenômeno "microbial loop" o bacterioplâncton transforma DOM em POM, a qual pode ser assimilada pelo protozooplâncton (Azam et al., 1983). Além disso, a mineralização de DOM por bactérias heterotróficas proporciona a reutilização da forma inorgânica pelos produtores para a produção primária (Azam et al., 1994).

Além da degradação microbiana (biodegradação), outra importante forma de degradação de DOM é a fotodegradação, que devido à capacidade de CDOM em absorver radiação provoca a oxidação parcial ou total da molécula, sendo a oxidação total a transformação de CDOM até CO_2. Sendo assim, a fotodegradação pode ser de enorme importância na mineralização do

CDOM, podendo ser comparada a processos como a respiração fitoplanctônica (Granéli et al., 1996) e mineralização pelo bacterioplâncton (Amado et al., 2006).

A absorção da luz pelo CDOM ainda provoca uma série de consequências para os organismos e processos aquáticos, dentre elas a competição pela luz visível com os produtores primários (Kostoglidis et al., 2005) e proteção dos organismos aquáticos contra os raios nocivos UV limitando sua penetração à superfície da coluna d'água (Granéli et al., 1998). Em contrapartida provoca o fotobranqueamento da coluna d'água que consiste na perda de coloração da água devido à fotodegradação do CDOM, o que expõe os organismos às radiações nocivas (Del Vecchio e Blough, 2002). Sendo assim, as propriedades de absorção luminosa de CDOM torna-o um importante regulador da produtividade ecossistêmica, podendo favorecer a produção primária (através da proteção contra excesso de radiação PAR e UV) ou inibir (devido à competição por luz), além de afetar a estrutura termal e profundidade da camada de mistura da coluna d'água (Jones, 1988; Fee et al., 1996, Bezerra-Neto et al., submetido).

Existem duas fontes de DOM em ecossistemas aquáticos continentais: fonte alóctone, originada da degradação de vegetação e solo terrestre, onde DOM entra no sistema aquático carreada pelas chuvas (Kieber et al., 2006; Miller et al., 2009); e fonte autóctone, produzida dentro do sistema como resultado principalmente da degradação ou lise de células dos produtores primários (Kritzberg et al., 2004; Guillemette e Del Giorgio, 2012). A composição química e as características ópticas de DOM (qualidade de DOM) estão relacionadas com a sua fonte, e possuem susceptibilidades distintas aos processos de degradação por micro-organismos e fotodegradação (Wetzel et al., 1995; Bertilsson e Tranvik, 2000). A DOM autóctone é formada por compostos simples de baixo peso molecular como carboidratos, proteínas, lipídeos e aminoácidos (Wetzel, 2001), enquanto a DOM alóctone é constituída de uma mistura de compostos aromáticos de alto peso molecular que formam as substâncias húmicas (Thurman, 1985). As substâncias húmicas representam o principal componente de DOM em sistemas aquáticos naturais e são constituídas de ácidos fúlvicos (compostos hidrofílicos) e ácidos húmicos (compostos hidrofóbicos) (Piccolo, 2001).

O metabolismo bacteriano pode ser suportado por DOM alóctone e autóctone, mas existem estudos que mostram que apenas uma pequena fração de DOM alóctone é incorporada como biomassa pelas bactérias e transferida para a cadeia trófica (Cole et al., 2006; Roland et al., 2010). Nos trópicos, as substâncias húmicas são importantes fontes de energia para a comunidade microbiana, mas estudos demonstram que estão mais relacionadas com a respiração do que com a produção bacteriana (Amado et al., 2006; Farjalla et al., 2009a). Em contrapartida, as bactérias possuem a capacidade de assimilar rapidamente DOM autóctone de origem algal (Chen & Wangersky 1996). Portanto, atualmente é aceito o conceito de que DOM autóctone algal possui maior labilidade para a biodegradação (Farjalla et al., 2009b; Fonte et al., 2013), enquanto DOM alóctone é mais susceptível para a fotodegradação e absorve fortemente a radiação na faixa do UV (Amon e Benner, 1994; McKnight et al., 1994; Benner, 2002; Helms et al., 2008). Devido à complexidade e alto peso molecular as substâncias húmicas são recalcitrantes para a degradação microbiana, e por isso tendem a se acumular em lagos podendo representar 80% de DOM total (Wetzel et al., 1995). No entanto, após a fotodegradação parcial as substâncias húmicas podem se tornar foto-produtos biodisponíveis para a comunidade microbiana aumentando a produção do bacterioplâncton (Vähätalo et al., 2003). Nos trópicos, a fotodegradação e biodegradação são favorecidas devido à elevada radiação solar e altas temperaturas durante todo o ano, embora poucos ainda são os estudos nesses ecossistemas.

A concentração e qualidade de DOM em lagos podem ser heterogêneas verticalmente na coluna d'água ou horizontalmente, em função da proximidade de fontes de carbono, como tributários, margens com vegetação e outras características hidrológicas da bacia hidrográfica (Wetzel, 1992; Tao, 1998; Porcal et al., 2004; Kostoglidis et al., 2005). Temporalmente as concentrações de DOM também podem variar em função de aportes esporádicos ou sazonais, sendo estes últimos controlados pelo regime de chuvas ou pulso hidrológico (Farjalla et al., 2002, Suhett et al., 2006, Brandão et al., 2016). Embora a importância de DOM seja clara e bem demonstrada, a distribuição de DOM no espaço e tempo ainda foi pouco estudada, especialmente em lagos naturais tropicais e ambientes eutrofizados (Toming et al., 2009; Zhang et al., 2011, Brandão et al., 2016).

Atualmente as metodologias mais acessíveis que permitem o estudo da composição e origem de DOM são análises espectrofotométricas de CDOM, normalmente combinadas com estudos de fluorescência (Helms et al., 2008). Através do espectro de absorção de CDOM podemos extrair métricas que fornecem características sobre a qualidade das moléculas de carbono como: aromaticidade (absorbância específica para UV - $SUVA_{254}$; Weishaar et al., 2003); fonte, mudanças devido a fotodegradação e tamanho molecular ("spectral slope" - $S_{275-295}$; Helms et al., 2008); mudanças devido a biodegradação ("spectral slope" – $S_{350-400}$; Helms et al., 2008); relação entre fotodegradação e biodegradação ("slope ratio" - S_R; Helms et al., 2008); tamanho molecular estimado (“molecular weight = a250/a365”; De Haan and De Boer, 1987; Peuravuori e Pihlaja, 1997). Tais métricas têm sido largamente utilizadas para analisar as propriedades ópticas em ambientes aquáticos temperados (Zhang et al., 2007c, 2009; Helms et al., 2008; Bracchini et al., 2010), mas poucos são os estudos utilizando essa abordagem em sistemas tropicais, principalmente no Brasil (por exemplo Teixeira et al., 2011, 2013; Bittar et al., 2015).

Pretendo com esta tese contribuir com estudos de propriedades ópticas em ecossistemas aquáticos tropicais. Além disso, estudos que avaliam particularmente as mudanças no espectro de absorção de CDOM em função das fontes e transformações de DOM ainda são escassos inclusive em ambientes temperados (Helms et al., 2013; Reader et al., 2015) e, até onde pude verificar na literatura disponível, acredito ser um trabalho pioneiro nesta abordagem (capítulo 4) em sistemas tropicais.

Objetivos

Esta tese teve como objetivo inicial o estudo espacial e temporal da distribuição e qualidade de DOM no lago Dom Helvécio (Médio Rio Doce, MG, Brasil). Primeiramente avaliei qual a contribuição de CDOM na atenuação da radiação PAR neste lago (capítulo 1). Neste capítulo considerei quatro amostragens espaciais em 2013, sendo uma em cada estação do ano contemplando os períodos de estratificação e mistura, assim como os períodos intermediários. Além disso, incluí também amostragens mensais realizadas em um único ponto na zona pelágica entre 2011 e 2012.

O entendimento da variação espacial e temporal de DOM no lago Dom Helvécio foi o objetivo do capítulo 2. Para isso realizei seis amostragens entre 2012 e 2014, onde avaliei os períodos de entrada do DOM alóctone com as chuvas.

No capítulo 3 o objetivo foi comparar dois sistemas aquáticos com diferentes fontes de DOM quanto às características ópticas, qualidade de DOM e concentrações de nutrientes. Para isto, utilizei duas amostragens realizadas no lago Dom Helvécio (seca e chuva em 2013) e incluí duas amostragens realizadas no reservatório da Pampulha (Belo Horizonte, MG, Brasil) na mesma época.

Por fim, o capítulo 4 teve como objetivo avaliar o efeito de DOM alóctone e autóctone no espectro de absorção de CDOM. Para isto, realizei um experimento de mesocosmos no lago Carioca (Médio Rio Doce, MG, Brasil), um lago próximo ao lago Dom Helvécio dentro dos limites do Parque Estadual do Rio Doce. Foi manipulado neste experimento as concentrações de matéria orgânica alóctone e nutrientes (o que estimula crescimento fitoplanctônico com consequente aumento de DOM autóctone), assim como a disponibilidade de radiação solar.

Todas as amostragens foram realizadas em ecossistemas aquáticos em Minas Gerais. No entanto, os capítulos e artigos foram escritos completamente ou iniciados na Universidade de Aarhus, Dinamarca, durante meu período de doutorado sanduíche (entre abril de 2015 a fevereiro de 2016). Assim, todos os trabalhos contaram com a participação do coorientador Peter Staehr, e o capítulo 4 também com outros pesquisadores estrangeiros.

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CAPÍTULO 1

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Partitioning of the diffuse attenuation coefficient for photosynthetically available irradiance in a deep dendritic tropical lake

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Abstract

We studied the effects of particulate and dissolved optically active components on the attenuation of photosynthetic active radiation (PAR) in a tropical lake. The temporal and spatial distribution of tripton, Chl-*a* and $a_{CDOM}(440)$ and their relative contribution to the diffuse PAR attenuation coefficient (*Kd*) was investigated at 21 sites (dry and wet seasons and two intermediate periods) and at monthly interval at 1 pelagic site. Higher values of *Kd* were observed during the mixing period, characterized by a higher concentration of tripton and Chl-*a* compared to the stratified rainy season. In the spatial sampling PAR attenuation was dominated by tripton absorption/scattering (average relative contribution of 79%), followed by Chl-*a* (average 11.6%). In the monthly sampling tripton and Chl-*a* accounted for most of the *Kd* with relative contributions of 47.8% and 35.6%, respectively. Multiple linear regression analysis showed that Chl-*a* and tripton in combination explained 97% of the monthly variation in *Kd* ($p<0.001$), but Chl-a had more influence (higher regression coefficient). Thus, although most of light attenuation was due to tripton, seasonal variations in phytoplankton abundance were responsible for most of the temporal fluctuations in *Kd*.

Key words: CDOM, chlorophyll-*a*, *Kd* partition, light attenuation, tripton.

Resumo

Nós estudamos os efeitos dos componentes opticamente ativos particulados e dissolvidos na atenuação da radiação fotossinteticamente ativa (PAR) em um lago tropical. As distribuições temporal e espacial do tripton, Chl-*a* e $a_{CDOM}(440)$ e suas contribuições relativas no coeficiente de atenuação difusa PAR (*Kd*) foram investigadas em 21 pontos (estações seca e chuvosa e dois períodos intermediários) e em intervalo mensal em 1 ponto pelágico. Maiores valores de *Kd* foram observados durante período de mistura, caracterizado por maiores concentrações de tripton e Chl-*a* comparados com o período chuvoso estratificado. Na amostragem espacial, a atenuação PAR foi dominada pela absorção/dispersão por tripton (média contribuição relativa 79%), seguido por Chl-*a* (média 11.6%). Na amostragem mensal tripton e Chl-*a* contribuíram com a maioria do *Kd* com média das contribuições relativas de 47.8% e 35.6%, respectivamente. Análises de regressão linear múltipla mostraram que Chl-*a* e tripton explicaram 97% da variação mensal do *Kd* ($p<0.001$), mas Chl-a teve maior influência (maior coeficiente de regressão). Contudo, embora a maior atenuação da luz ocorreu devido ao tripton, variações sazonais na abundância do fitoplâncton foram responsáveis pela maioria da flutuação temporal no *Kd*.

Palavras-chave: atenuação da luz, CDOM, clorofila-*a*, partição de *Kd*, tripton.

Introduction

Availability of photosynthetically active radiation (PAR; 400 to 700 nm) in the water column provides the energy for primary producers and is a key determinant of the overall productivity of aquatic ecosystems (Parsons et al. 1984, Kirk 1994). While it is well known that PAR is attenuated exponentially with depth, little is known of the causes for temporal and spatial variability in the vertical diffuse attenuation coefficient for down-welling PAR (Kd; m^{-1}) in tropical lakes.

Light attenuation in aquatic ecosystems is basically the sum of light absorbing and scattering optically active components present in water. These components are phytoplankton pigments (of which chlorophyll-*a* dominates), colored dissolved organic matter (CDOM), pure water and tripton (defined in this type of studies as non pigmented particulate matter) (Kirk 1994, Pfannkuche 2002, Branco and Kremer 2005, Kelble et al. 2005).

Studies aiming to determine the relative contribution of optically active components to Kd have previously concentrated on shallow lakes (Somlyody and Koncsos 1991, Blom et al. 1994, James et al. 1997, Van Duin et al. 2001, Pierson et al.2003, Zhang et al.2007a, Zhang et al. 2007b, Balogh et al. 2009), estuaries and coastal lagoons (Phlips et al.1995, Christian and Sheng, 2003, Lund-Hansen 2004, Kelble et al. 2005, Yamaguchi et al. 2013), while little information about partition of Kd is available on deep (Effler et al. 2010) and tropical lakes (Loiselle et al. 2008). The partition of this optical property is based on the equation:

$$Kd = K_W + K_{CDOM} + K_{TRI} + K_{Chl\text{-}a} \qquad \text{(Equation 1)}$$

where K_W is the partial attenuation coefficient by pure water, K_{CDOM} by CDOM, K_{TRI} by tripton and $K_{Chl\text{-}a}$ by Chl-*a* (Kirk 1994, Phlips et al. 1995).

The concentrations of optically active components and how these attenuate light in the water column determine the amount of light available for photosynthesis. Therefore, the available light restricts production, abundance, and distribution of pelagic and benthic primary producers, with implications for higher trophic levels in the ecosystem (Parsons et al.1984, McMahon et al. 1992, Gallegos and Moore 2000). Studies about *Kd* partition provide important understanding of how each component is responsible for the attenuation of light and this fact also allows predict the underwater light climate from the concentrations of these components (Zhang et al. 2007).

Spatial variations in PAR light attenuation coefficients are expected in large and dendritic lakes because of the differences in depth and proximity to the border, which leads to less stability of the water column and increased input of allochthonous matter. The shallower regions of the lakes are susceptible to wind driven sediment resuspension which increases the concentration of suspended matter in the water column and reduces light penetration and thus primary production (Somlyody and Koncsos 1991). These variations also differ between seasons due to higher input via rainfall (rain period) and due to mixing of water column (dry period).

Lake Dom Helvécio (DH - Minas Gerais, Brazil) is a deep dendritic tropical lake located in a preserved area surrounded by Atlantic Forest. Although there is a greater input during rainy season, the water column stability in DH is large in this period (Brighenti et al. 2015) and the allochthonous matter coming with runoff from rains remains retained below the thermocline due to differences in water temperature (Reynolds 2009). Redistribution of particulate matter into the upper mixed layers accordingly occurs during the dryer and colder season which therefore is characterized by less transparent water. Following these observations, our hypotheses are that: 1) the total suspended matter - TSM (Chl-*a* and tripton) present greater relative contribution in the attenuation of PAR radiation at all the sampling stations and seasons, as CDOM occurs at low concentrations in Lake DH and absorbs more in the range of ultraviolet spectrum; 2) the spatial variability in PAR attenuation coefficient values among the 21 sites can be explained by the influence of the lake shore in the closest sites (direct input of allochthonous organic matter and shalower water column compared to deeper sites); 3) the PAR attenuation coefficient is higher during the dryer mixing period due to elevated levels of suspended matter in the water column. In order to test these hypothesis, our objectives were to document the spatial and temporal contribution of tripton, Chl-*a* and CDOM in total PAR attenuation through *Kd* partition study at Lake DH and determine how variations in optically attenuating conditions depend on the time and place of sampling.

Materials and Methods

Study site

Dom Helvécio lake is located inside the Rio Doce State Park – PERD Southeast Brazil (S19°46.94’, W42°35.48’). The lakes complex in the Middle Rio Doce is one of the most important districts of lakes in Brazil, with approximately 130 lakes, 42 of them located inside the PERD area, which is the largest remnant of the Atlantic Forest in Minas Gerais, totaling 36000 ha (Maia-Barbosa et al. 2010) (Fig. 1). This lake is one of the deepest natural lakes of Brazil (maximum depth: 39.5 m), surrounded by Atlantic forest. It is oligotrophic (total phosphorus: 3.0-22.0 $\mu g\ L^{-1}$, annual mean 11.2 $\mu g\ L^{-1}$; Chl-*a*: 0.5-11.0 $\mu g\ L^{-1}$, annual mean 4.5 $\mu g\ L^{-1}$), has a perimeter of 37.7 km, area equal to $5.27 \times 10^6\ m^2$ and volume equal to $5.94 \times 10^7\ m^3$ (Bezerra-Neto and Pinto-Coelho 2008). Dom Helvécio is a warm monomictic lake, with thermal stratification period beginning in September and lasting until April (rainy period) and a mixed period from May to August (dry period) (Barbosa and Tundisi 1980).

Field measurements

The spatial sampling was carried out in January (Summer), April (transition-Autumn), August (Winter) and December (transition-Spring) 2013 at 21 sampling stations in the lake (Fig. 1c). Although in tropical environments the four seasons are not well defined and then are commonly separated into dry and rainy periods in aquatic studies, we included in this study the samplings during the transitions periods (autumn and spring) because they are important intermediate periods between the rainy and dry seasons. Temporal samples were taken monthly at a single station in the pelagic part of the lake (station 8, Fig. 1), from June 2011 to November 2012. Six data of temporal sampling and one of the spatial sampling stations (station 1 in January 2013) were not included in the analysis because the Chl-*a* measurements were not analyzed in a timely manner and the values were of low accuracy.

Upon monthly sampling at the central deep region (station 8) of the lake, vertical profiles of water column temperature (°C) were measured every meter from surface to 28 m using a Hydrolab DS5 probe. The surface irradiance (E_0) was obtained by a solar radiation sensor (WE300, Global Water®) mounted just above lake surface on a buoy located at this station.

For spatial analysis, vertical profiles of photosynthetically active radiation (PAR) (between 10:00 and 14:00) were performed using a radiometer (BIC Biospherical Instruments). The profiles were made on the sun side of the boat avoiding shadows and measurements during days with great clouds were avoided. The radiometer was attached to a battery and notebook with the software LoggerLight ™ (Biospherical Instruments) while collecting radiation data PAR and depth during descent and ascent up of the probe. In the spatial sampling the maximum depth of measurement of radiation was defined on the field when PAR reached 1 µEinsteins m^2 s. For monthly analysis, light profiles were measured at depths 0.1, 1.0, 2.0 and 3.0 m using a model UA-002 light sensors (HOBO®).

Water samples (0.5 m from the surface) were collected using a van Dorn bottle (5L) and filtered immediately for Chl-*a* (0.47μm Millipore filter) and TSM (AP040 Millipore filter), and the filters were frozen until analysis. Water samples were also filtered for analysis of dissolved organic carbon (DOC) and colored dissolved organic matter (CDOM) (0.22 μm Millipore filter) and stored in amber glass bottles (pre-washed with distilled water and hydrochloric acid 10%) at 4°C in the dark.

Laboratory analysis and calculation of the optical properties

The diffuse PAR attenuation coefficient (*Kd*) was calculated as the linear slope between depth and the natural logarithm of the measured downwelling PAR radiation. From *Kd* we calculated the depth of the euphotic zone ($Z_{eu} = 4.6 / Kd$; m) where 1% of the radiation incident on the surface is reached (Kirk, 1994).

The measurement of concentration of Chl-*a* was obtained by acetone extraction (90%) measured in a spectrophotometer (UV-VIS Shimadzu) at 665 and 750 nm and calculated according to APHA (1998). Total suspended matter (TSM; mg L^{-1}) were determined by the gravimetric method, considering the difference between the dry weight of AP40 Millipore filters (105°C for 2 hours) before and after the filtration of water samples (APHA 1998). The tripton represents the non-living fraction of the suspension material, but in this study it represents the non-pigmented part of TSM (non-phytoplankton particulate matter). This was obtained subtracting the dry weight of the phytoplankton from the TSM. The ratio between concentration of Chl-*a* corrected by pheophytin and the dry weight of phytoplankton was assumed 1:100 (Phlips et al. 1995). Thus, the concentration of the Chl-*a* corrected by pheophytin (mg L^{-1}) was multiplied by 100 to obtain the dry weight of phytoplankton and then subtracted from TSM (mg L^{-1}) to obtain the concentration of tripton (mg L^{-1}) (Christian and Sheng 2003).

The DOC concentration (mg L^{-1}) was obtained by catalytic oxidation method of high temperature using TOC Analyzer (Shimadzu TOC – 5000).

The spectral absorption of CDOM was measured at 440 nm ($a_{CDOM}(440)$) in a spectrophotometer (UV-VIS Shimadzu) using 5 cm quartz cuvette and Milli-Q water as a reference. The absorption spectra of each sample was performed in triplicate and determined using the Spectrum Pack Program software (Shimadzu Inc.). The absorption coefficients were derived from the absorption spectra (A) at 440 nm (m^{-1}) according to the equation 2 (Kirk, 1994):

$$a_{CDOM}(440) = 2.303A(440)\, l^{-1} \qquad \text{(equation 2)}$$

Where *l* is the optical path of the cuvette (in meters). Absorption coefficients were corrected for backscattering by subtracting the value of the coefficient at 700 nm from the absorbance at 440 nm. The spectrophotometric absorption coefficient at 440 nm was used as an index of CDOM concentration (Williamson *et al.* 1996).

We used the software Lake Analyzer (Read et al. 2011) to determine the depth of the upper mixed layer (Z_{mix}) and the water column stability (Schmidt Stability-St) from monthly temperature profiles. Mean available light in the upper mixed layer (E_{mean}; mol photons m^{-2} d^{-1}) was estimated from surface light, light attenuation and mixing depth as described in Staehr and Sand-Jensen (2007). Accumulated rainfall recorded at every three hours was obtained from a meteorological station located less than 2 km from the lake (http://sinda.crn2.inpe.br/PCD/).

Unfiltered water samples were taken for analysis of total nitrogen (TOC Analyzer, Shimadzu TOC – 5000) and total phosphorus (according to Mackereth et al. 1978).

Partitioning of the difuse PAR attenuation coefficient

The partition of *Kd* was performed for the spatial (integrating the spatial data from four seasons sampling in 2013) and temporal data (monthly sampling at site 8 in 2011 and 2012) separately, but using the same equations.

The attenuation of the PAR radiation can be partitioned according to the following equation 3 (Yamaguchi et al. 2013):

$$Kd = K_W + K_{CDOM} + K_{TSM} \qquad \text{(equation 3)}$$

where K_W, K_{CDOM} and K_{TSM} are the partial attenuation coefficient due to respectively pure water, $a_{CDOM}(440)$ and TSM in the PAR range (400 to 700 nm).

The light attenuation by the pure water (K_W) was assumed to be constant at 0.027 m^{-1} (Smith and Baker 1978). The PAR attenuation by CDOM (K_{CDOM}) was estimated according to the equation 4 (Pfannkuche 2002, Lund-Hansen 2004, Zhang et al. 2007a):

$$K_{CDOM} = 0.221 * a_{CDOM}(440) \qquad \text{(equation 4)}$$

Where, $a_{CDOM}(440)$ is the value of the absorption coefficient measured at 440 nm and 0.221 is the specific absorption coefficient of CDOM at 440 nm.

The light attenuation of TSM can be subdivided according to the equation 5:

$$K_{TSM} = K_{Chl\text{-}a} + K_{TRI} \qquad \text{(equation 5)}$$

Where $K_{Chl\text{-}a}$ and K_{TRI} are the partial attenuation coefficients of phytoplankton (as measured by Chl-*a*) and tripton, respectively. To calculate the K_{TSM} we subtracted the sum $(K_W + K_{CDOM})$ from *Kd* (Yamaguchi et al. 2013).

A simple linear regression analysis was performed between the K_{TSM} (m^{-1}) and the Chl-*a* concentration (µg L^{-1}) to estimate the specific attenuation coefficient of the Chl-*a*, according to equation 6 (Yamaguchi et al. 2013):

$K_{TSM} = b * [Chl\text{-}a] + c$ (equation 6)

Where the slope *b* is the specific attenuation coefficient of Chl-*a* ($K*_{Chl\text{-}a}$) and *c* is the intercept. The Chl-*a* absorption coefficient ($K_{Chl\text{-}a}$; m^{-1}) was obtained by multiplying $K*_{Chl\text{-}a}$ with [Chl-*a*]. The K_{TRI} (light absorption by non-pigmented particulate matter) was finally obtained according to equation 5 by subtracting $K_{Chl\text{-}a}$ from K_{TSM} (Yamaguchi et al. 2013).

Following the model in equation 1 is possible to calculate the relative contributions (%) of each component in the total *Kd* (100%).

Statistical Analysis

Kruskal-Wallis (One Way Analysis of Variance) and pair wise test (Tukey Test) were performed to assess seasonal differences in *Kd* values. Simple and multiple linear regressions were made between concentrations of optically active components and partial and total coefficients of PAR attenuation. To demonstrate the spatial distribution of *Kd* and the optical components maps were made using a Kriging interpolation technique with Surfer 10 software.

Results

Overall patterns in lake physics and water chemistry

Periods with full mixing occurred between June and August in all three years. The water surface temperature ranged from 21 to 31 °C with lowest temperatures during the mixing period. The photic zone (Z_{eu}) ranged from 3.9 to 14.3 m, reaching greater depths during the stratified rainy periods. Greater stability of the water column (Schmidt) was accordingly observed in the rainy season (mean Schmidt stability = 386.4 J m^{-2}). The deep water temperature remained lower than the surface temperature during the entire rain period and was stable throughout the year (annual average 23.5ºC ± 1ºC standard deviation). The depth of the mixing layer was greater in dry and colder period reached a maximum of 27.9 m, while during the warmer rainy season Z_{mix} reached a maximum of 13.4 m (Table 1 and Fig. 2).

DOC occurred in higher concentrations during the onset of the rains (December, spatial average 6.5 mg L^{-1}) and mixing in winter (spatial average 6.5 and monthly average 5.9 mg L^{-1}). The DOC concentration in the deeper hypolimnetic waters was higher during thermal stratification (4.9 in November and 5.1 mg L^{-1} in December 2012) than in the upper mixed surface waters (2.1 mg L^{-1} in November and 1.5 mg L^{-1} in December 2012) and elevated DOC levels were accordingly stored in the hypolimnion until the onset of mixing in June. Total nitrogen (TN) occurred in higher concentrations during the mixed dry period (spatial average 357.6 and monthly average 446.6 µg L^{-1}) compared to conditions in the upper mixed layer during stratification (averages between 276.8 and 351.7 µg L^{-1}). The total phosphorus (TP) occurred in higher concentrations during winter, but only for the spatial sampling (average 12.8 µg L^{-1}), and no difference was observed between dry and rainy in the monthly sample (Table 1 and Fig. 2a-h).

Spatial variations in optical properties

The coefficients of variation were used to estimate the variability of *Kd* and optically active components at each site. The spatial distribution of these coefficients of variation and mean values for each parameter in the four sampling periods are shown in Fig. 3 and 4. The largest variation observed for *Kd* occurred at sites 7 (CV = 0.38), 11 (CV = 0.43) and 15 (CV = 0.35), while the lowest ocurred at sites 3 (CV = 0.10), 18 (CV = 0.14) and 21 (CV = 0.13). Chl-a presented coefficients of variation between 0.20 (site 12) and 0.83 (site 9), tripton presented between 0.08 (site 6) and 0.80 (site 1) and TSM ranged from 0.05 (site 7) to 0.56 (site 21). The highest variation occurred to $a_{CDOM}(440)$ ranging from 0.17 (site 21) to 2.00 (sites 5 and 7). There was no significant correlation ($p > 0.05$) among the coefficients of variation of all parameters measured or between the coefficients and the depth of the sites or proximity to shore.

The spatial variation of the *Kd* was also different for each sampling period, ranging from 15 to 18% (measured by the coefficient of variation - CV = standard deviation / average) among sites in the spring (December - CV = 0.15) and summer (January - CV = 0.18), and less than 8% in autumn and winter (April - CV = 0.08; August - CV = 0.06). The spatial variation for Chl-*a* was higher in summer (56%) and less than 30% in other sampling periods (April - CV = 0.26; August - CV = 0.30; December – CV = 0.29) , while the variation of tripton was higher in winter (45%) and close at other periods (January - CV = 0.36; April - CV = 0.31; December – CV = 0.30). $a_{CDOM}(440)$ presented larger variation than other optically active components with a minimum of 64% in summer and between 85 and 89% at other periods (April - CV = 0.85; August - CV = 0.89; December – CV = 0.88).

We found a significant variation in *Kd* between sampling seasons (Kruskal-Wallis One Way Analysis of Variance, $p < 0.001$), except between January (summer) and December 2013 (Tukey Test, $p > 0.05$). The *Kd* ranged from 0.32 m^{-1} (station P11 during the summer) to 0.91 m^{-1} (station P11 during the winter) during the entire period (average 0.60 m^{-1}). Higher values of *Kd* (average 0.78 m^{-1}) were observed during the dry winter period, characterized by a fully mixed water column, while lower values (average 0.46 m^{-1}) were observed during the warm, thermally stratified rainy season (January and December) (Table 1 and Fig. 3). The coefficients of determination (R^2) for the calculation of *Kd* from light vs depth were always higher than 0.9 which indicates a vertically homogeneous water column in relation to the optically active components (Zhang et al. 2007), even during the stratified period.

The Chl-*a* concentrations varied 20 fold between 0.5 (station P15 in the summer) and 11.0 µg L^{-1} (station 8 in the intermediate period in April) (average 4.5 µg L^{-1}). In comparison the TSM concentrations varied 8 fold and ranged from 0.4 (station P21 in the summer) to 2.8 mg.L^{-1} (station P21 in winter) (average 1.6 mg L^{-1}). Higher Chl-*a* and TSM concentrations were observed in April and winter. The tripton concentration ranged from 0.1 (station P8 in the winter) to 2.4 mg L^{-1} (station P21 in winter) (average 1.1 mg L^{-1}). Although tripton concentrations reached similar levels as TSM, the spatial variation in tripton was much higher (26 fold), with the highest tripton concentrations also observed during winter where water column was fully mixed. The $a_{CDOM}(440)$ varied from 0 to 0.4 m^{-1}, average 0.1 m^{-1}. Higher $a_{CDOM}(440)$ concentrations were observed in the summer rainy period (Table 1).

Using the additive model (equation 1*)* tripton was found to contribute most to the attenuation of visible light all year round and at all sampling stations during spatial sampling (minimum 50%, maximum 91% and average 79%). The relative contribution of phytoplankton pigments estimated from Chl-*a* showed a minimum of 2%, maximum 30.2% and average 11.6% for both seasonal and spatial variations. The $a_{CDOM}(440)$ varied from a minimum contribution of 0% to a maximum of 15.6%, and average of 4.8%. The pure water contribution ranged between 2.9% and 8.4%, with an average of 4.7% (Fig. 5). With increasing tripton concentration and its relative contribution to light attenuation, *Kd* also increased. In comparison the contributions of Chl-*a* and $a_{CDOM}(440)$ to PAR attenuation were lower and tended to decrease with higher *Kd* values (Fig. 6). Since the absorption of pure water is constant its relative contribution to *Kd* decreased with increasing attenuation by the other optical components. The specific attenuation coefficients for each optically active component are shown in Table 2.

Considering the four sampling events as replicates we did not observe significant relations among the spatial distribution of the concentration of optically active components (Pearson's correlations between Chl-*a* and tripton (R = -0.22, p = 0.33); Chl-*a* and $a_{CDOM}(440)$ (R = -0.10, p = 0.65); tripton and $a_{CDOM}(440)$ (R = -0.16, p = 0.47)) (Fig. 6). The spatial distribution of *Kd* was significantly correlated only with Chl-*a* (R = 0.31, $p < 0.01$) and we did not observe significant correlations between *Kd* and tripton (R = 0.13, p = 0.24) or $a_{CDOM}(440)$ (R = -0.04.22, p = 0.67).

Monthly trends in optical properties

From our monthly sampling we found that *Kd* ranged from a minimum of 0.4 m^{-1} (November 2011) to a maximum of 1.3 m^{-1} (July 2012) around an average of 0.8 m^{-1}. Highest values were as previously described observed in the dry period with full mixing of the water column (average 0.9 m^{-1}), while in the warm stratified rain period had a lower average of 0.7 m^{-1} (Table 1 and Fig. 2-h).

The Chl-*a* concentrations ranged 16 fold (min December 0.6; max August 8.9 μgL^{-1}) with an average of 4.5 μgL^{-1}. Higher TSM concentrations occurred during dry periods and the concentrations ranged only 3.5 fold from 0.9 (April 2012) to 3.2 (June 2011) (average of 1.9 mg L^{-1}). Higher tripton concentrations were observed in dry periods (June and July 2011 and August 2012) and in two months of rain period (January and November 2012), and ranged 8 fold (average 1.5 mg L^{-1}). The $a_{CDOM}(440)$ absorption ranged from 0.1 (November 2012) to 0.6 (July 2011) (average 0.3 m^{-1}). Overall higher concentration of Chl-*a*, TSM, tripton and $a_{CDOM}(440)$ occurred during the dry season of 2011 and 2012. The $a_{CDOM}(440)$ absorption ranged from 0.1 (November 2012) to 0.6 (July 2011) (average 0.3 m^{-1}). There was a noticeable fall in the concentration of DOC and $a_{CDOM}(440)$ from June 2011 to December 2012 (Table 1 and Fig. 2-e,f,g).

For the monthly and annual variations observed at the station P8 tripton was most predominant in June, July, November and December 2011, and January and December 2012. The contribution of tripton attenuation varied from a minimum of 18.3% (May 2012) to maximum of 77.2% (December 2011) and an overall average of 47.8 % throughout the sampling period. In October 2011 and March to August 2012, Chl-*a* was the primary contributor to *Kd*. Throughout the period the Chl-*a* showed a minimum contribution of 6.8%, maximum of 65.3% and an average of 35.6%. In June 2012, Chl-*a* and tripton contributed almost equally with 45.6% and 45.5%, respectively. We observed that the Chl-*a* contribution increased in the dry mixing periods. The $a_{CDOM}(440)$ contribution to *Kd* ranged between 3.4% and 22% with an average of 12%. Pure water contributed with only 4.6% on average (Table 2 and Fig. 7).

Using a multiple regression analysis we found that K_{Chl} and K_{TRIP} together explained 97% of *Kd* (F(2,9) = 139.832, $p < 0.001$, r = 0.98, $R^2 = 0.97$). The regression coefficient for Chl-*a* (0.96) was higher than for tripton (0.92), which means that while total PAR attenuation depended on a combination of optical conditions, the single most important component driving temporal variation in *Kd* was Chl-*a* (Fig. 8).

From the analysis of correlations between optical components and physical and chemical conditions characterizing changes at the central station in the lake we found significant correlations ($p < 0.05$) between: 1) *Kd* and Chl-*a* (R = 0.66) and rainfall (R = -0.58); 2) TSM and tripton (R = 0.92), Z_{mix} (R = 0.71) and Schmidt Stability (R= -0.61); 3) Schmidt Stability and E_0 (R= 0.74) and E_{mean} (R = 0.71); 4) E_0 and Z_{mix} (R = 0.61) and DOC (R = -0.49); 5) tripton and Z_{mix} (R = 0.62); 6) Z_{eu} and rainfall (R = 0.62); 7) $a_{CDOM}(440)$ and DOC (R = 0.60); and 8) Chl-*a* and rainfall (R = -0.59). All correlations of these parameters are shown in Table 3. These correlations indicate that the seasonal changes in optical components affecting PAR attenuation are strongly linked to meteorological conditions as these affect water column stability and mixing conditions. The combination of these couplings are finally shown as responses in mean light available and the depth of the photic zone.

Discussion

Spatial sampling at 21 stations

Considering the coefficients of variation, the optically active components and *Kd* varied among the 21 sampled sites, but these spatial variability were not significant related among them or explained by the sites depth or proximity with the lake shore as expected. Some deep stations (> 10 m) accordingly had variation in *Kd* values similar to stations located near the shore suggesting that other parameters such as complex internal movement of water driven by wind may control the spatial heterogeneity in PAR attenuation (Somlyody and Koncsos 1991). However, although there wasn't relation between the coefficients of variation of *Kd* and the coefficients of variation of the other parameters, we found a significant and positive correlation between *Kd* values and Chl-a concentrations analyzing all sites and sampling periods together. In this way, although tripton had contributed more with the total PAR attenuation in all sites and seasons, the Chl-*a* concentration was the only parameter correlated with the variation of *Kd*.

In addition to the spatial variability among sites, there were distinct spatial variability for Chl-*a*, tripton and $a_{CDOM}(440)$ also among sampling periods. During the dry and colder winter mixing period elevated levels of particulate matter (phytoplankton and tripton) caused overall higher PAR attenuation. However, the spatial variability of *Kd* in this period was lower (6%) than that observed in the summer (18%) and spring (15%), although the spatial variability of Chl-*a* and tripton in winter was 30% and 45%, respectively. It has previously been demonstrated that mixing of the water column causes resuspension of particulate matter increasing turbidity and tripton concentrations (Blom et al. 1994, James et al. 1997, 2004, Phlips et al. 1995, Zhang et al. 2007), which consequently would explain the higher values of *Kd* and the highest average contribution of tripton (83.9%) during the mixed dry season. Higher lake transparency (lower values of *Kd*) occurred in summer and spring, when concentrations of particulate matter were lower, but the $a_{CDOM}(440)$ higher and its contribution in *Kd* became more apparent in the sites (Fig. 5a,d). The higher spatial variability of *Kd* (15-18%) was observed in periods of more transparent waters (spring and summer), which coincided with the highest spatial variability of Chl-*a* during the summer (56%). Despite the increase in Chl-*a* and $a_{CDOM}(440)$ contribution to *Kd* during summer, tripton was largely responsible for the attenuation of visible light when comparing all sites and sampling periods. Moreover, tripton contribution to the attenuation increased with increasing *Kd* value (see Fig. 6). The relationship between reduced Chl-*a* and $a_{CDOM}(440)$ contributions and increasing tripton contribution with the increase of *Kd* was also demonstrated by Zhang et al. (2007). Other studies have similarly found a strong positive relationship between tripton and *Kd* and a great contribution of this component to PAR attenuation in lakes (Phlips et al. 1995, Christian and Sheng 2003, Yang et al. 2005, Zhang et al. 2007, Balogh et al. 2009) and estuaries and oceans (McMahon et al. 1992, Lin et al. 2009, Yamaguchi et al. 2013) (See Table 4). In this way, the main component responsible for the PAR attenuation was tripton, followed by Chl-*a*, which is in agreement with our first hypothesis since most of the variability in *Kd* was due to the particulate matter.

The low contribution of $a_{CDOM}(440)$ to *Kd* (maximum 15.6%) was expected since the $a_{CDOM}(440)$ occurred at low concentrations in this lake and its absorption decays with increasing wavelength (Green and Blough, 1994, Kitidis et al. 2006), implying that the overall contribution of $a_{CDOM}(440)$ absorption is higher in the ultraviolet part of the spectra (200-400 nm).

Monthly sampling at the deep central station

In the monthly sampling at the deep central station 8 (between 2011 and 2012), the contribution of Chl-*a* for the total light attenuation was more noticeable and higher than the tripton contribution in some periods (October-2011 and from March to August-2012). The majority of studies, predominantly in coastal waters, showed that the relative contribution of tripton to the PAR attenuation was always higher than that of Chl-*a*. This has been documented in Aarhus Bay, Kattegat (Lund-Hansen, 2004), Florida Bay (Phlips et al. 1995, Kelble et al. 2005), Tampa Bay and Charlotte Harbor (McPherson and Miller 1987, McPherson and Miller 1994) and in the Yellow Sea (Lin et al. 2009). While tripton is often dominate light attenuation, Zhang et al. (2007b) showed that increases in Chl-*a* during summer algal blooms makes light absorption by pigments a more dominant optical component than tripton in some regions of Taihu Lake (China). In hypereutrophic lakes Balogh et al. (2009) also observed higher Chl-*a* contribution to the PAR attenuation and Yang et al. (2005) in some regions of the Lake Donghu (China). Effler et al. (2010) in contrast, found that $a_{CDOM}(440)$ was the primarily factor responsible for PAR attenuation in Lake Superior (USA). In our study, Chl-*a* and tripton together explained 97% (R^2, multiple linear regression) of the temporal variation of *Kd* but the Chl-*a* was the most responsible. Similar results were found by Yamaguchi et al. (2013) where although tripton have been the predominant component responsible for the *Kd* in percentage of attenuation, Chl-a was the main component that influenced the *Kd* monthly variation in Harima Sound (R^2 = 75%) (Eastern Seto Inland Sea, Japan). Obrador and Pretus (2008) also demonstrated that the main driver for seasonal changes in *Kd* was phytoplankton (Chl-*a*) despite that DOC had a higher relative contribution. Reinforcing this conclusion, we found a smaller temporal variation in tripton concentration (coefficient of variation: CV = 0.51.) than in Chl-*a* (CV = 0.65), which suggests that although tripton contributed significantly to light attenuation, variations in Chl-*a* was the main driver for seasonal fluctuations in *Kd* values. This finding allows us to conclude that the main factor that attenuates light is not necessarily the same one that causes temporal variation of the light attenuation (Obrador and Pretus 2008) (see Table 4).

From our monthly sampling we found that higher irradiance and rainfall values in summer were positively related to the mean light available to primary production (E_{mean}) and Schmidt stability while negatively related to the mixed layer depth (Z_{mix}). In this period, the DOC and a_{CDOM}(440) occurred in low concentrations in the photic zone suggesting high photobleaching caused by the high incident radiation. It is known that with rain allochthonous carbon enters the deeper layers of the lake and remains in the hypolimnion until mixing period, when it becomes distributed in the water column (Reynolds 2009). Corroborating this finding we observed higher DOC concentration at the bottom of the lake than at the subsurface in the rainy period (November and December 2012). With a lower incidence of solar radiation and cooling of surface waters during winter the depth of the mixed layer increased, causing a more homogeneous distribution of nutrients and particulate matter in the water column (Barbosa and Tundisi 1980, Petrucio and Barbosa 2004, Brighenti et al. 2015). This would explain the observed increase in Chl-*a* concentrations in the full mixing period in 2012 and its higher relative contribution in PAR attenuation (maximum 65% in May 2012). Brighenti et al. (2015) investigating the patterns of primary production in Dom Helvécio lake during 2011 to 2012 demonstrated that during thermally stratified period, with higher surface light and lower light attenuation, coincided with a higher occurrence of photoinhibition of phytoplankton productivity. Also these authors found that the availability of nutrients and the highest phytoplankton biomass coincided with periods of lower photoinhibition (mixing periods). Thus, the increase of Chl-*a* combined with the increase of tripton concentrations in mixing period resulted in higher values of *Kd* at this time.

Conclusions

The present study demonstrated that is important to link temporal and spatial sampling in order to understand the role of the optical components influencing PAR attenuation, because the main component that attenuates light is not necessarily the same component responsible for the temporal variation of *Kd*. Optimally this should be combined with a high-frequency sampling of physical conditions characterizing water column mixing conditions. This combination allows better certainty of the importance of each optical component for the total attenuation of light and a better mechanistic understanding of the physical and chemical conditions driving temporal changes. Particularly, we found that there was a significant temporal and spatial variability in water transparency, and although most of PAR attenuation occured due to tripton concentrations, the variability of *Kd* was mainly associated to variation in the concentration of Chl-*a*. However, we did not find support for our expectation of higher variability in optical condition at near shore sites, suggesting that new studies on the variability in *Kd* must consider the hydrodynamic conditions in the distribution of optical components within the lake.

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Figures and Tables

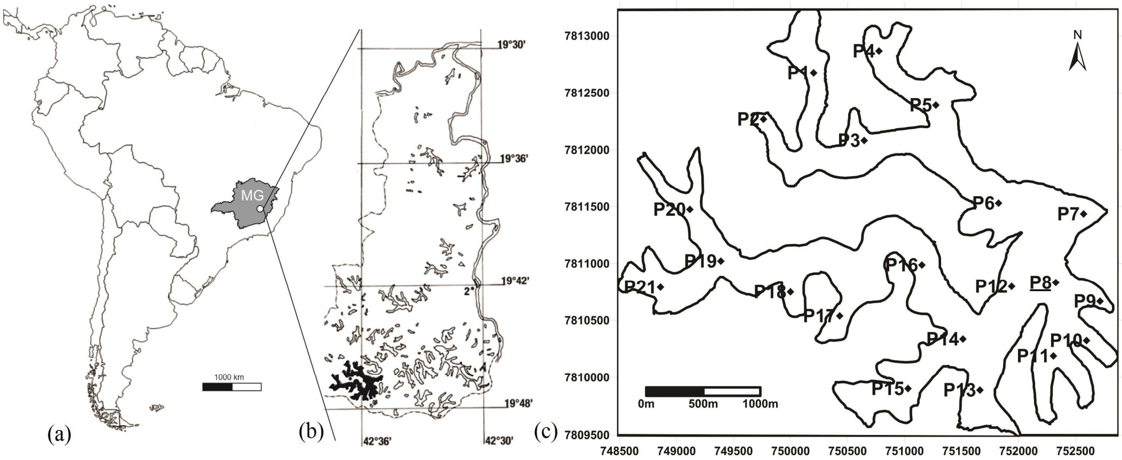

Figure 1 - Location of Dom Helvécio lake in Minas Gerais (a), inside of the Rio Doce State Park (b), Middle Rio Doce and the spatial sampling stations (P1-P21) (c). The station P8 (c; underlined) also represents the location of monthly sampling. The numbers on the axes correspond to geographic coordinates.

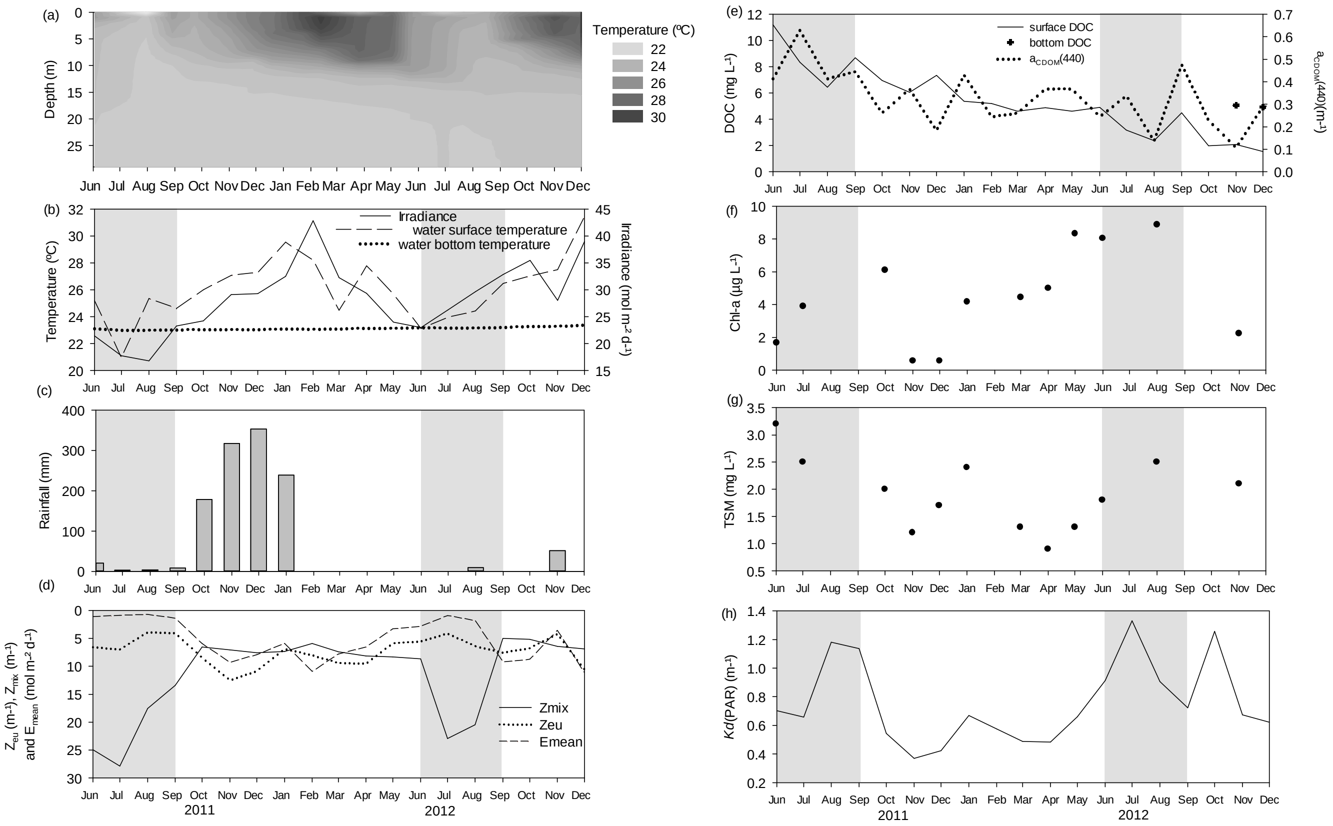

Figure 2 – Monthly variation of the (a) water temperature depth profile, (b) surface irradiance, (c) rainfall, (d) mixing layer depth (Z_{mix}), euphotic zone depth (Z_{eu}), mean light climate (E_{mean}), (e) dissolved organic carbon, colored dissolved organic matter, (f) chlorophyll-*a*, (g) total suspended matter and (h) PAR attenuation coefficient (*Kd(PAR)*) from June 2011 to December 2012.

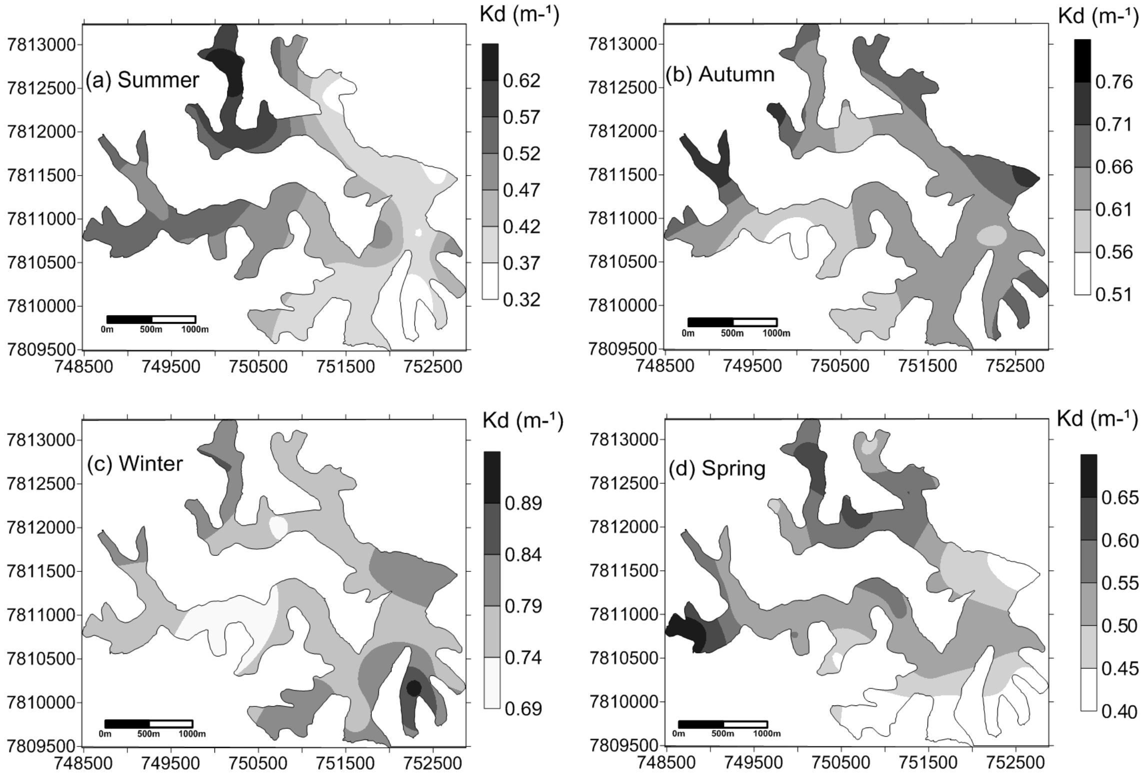

Figure 3 - Spatial variation of the attenuation coefficient of photosynthetically active radiation (m-¹) in the summer (January 2013 - a), autumn (April 2013 – b), winter (August 2013 – c) and spring (December 2013 – d). Notice that the scales are not similar. The numbers on the axes correspond to geographic coordinates.

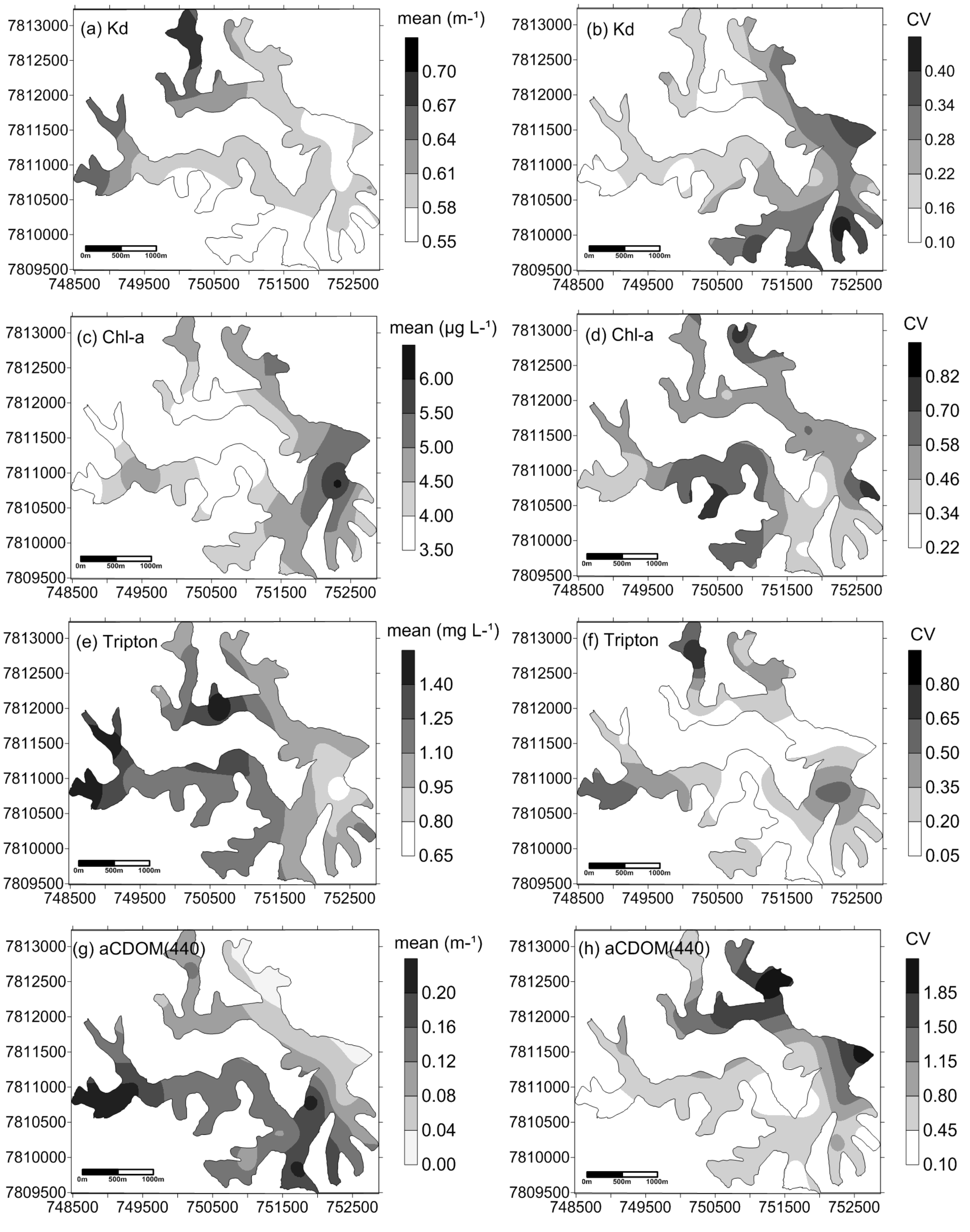

Figure 4 – Mean values (left panel) and coefficients of variation (CV; right panel) of each sampling site during the four seasons for *Kd* (a,b), Chl-*a* (µg L-^{1}) (c,d), tripton (mg L-^{1}) (e,f) and $a_{CDOM}(440)$ (m-^{1}) (g,h). The numbers on the axes correspond to geographic coordinates.

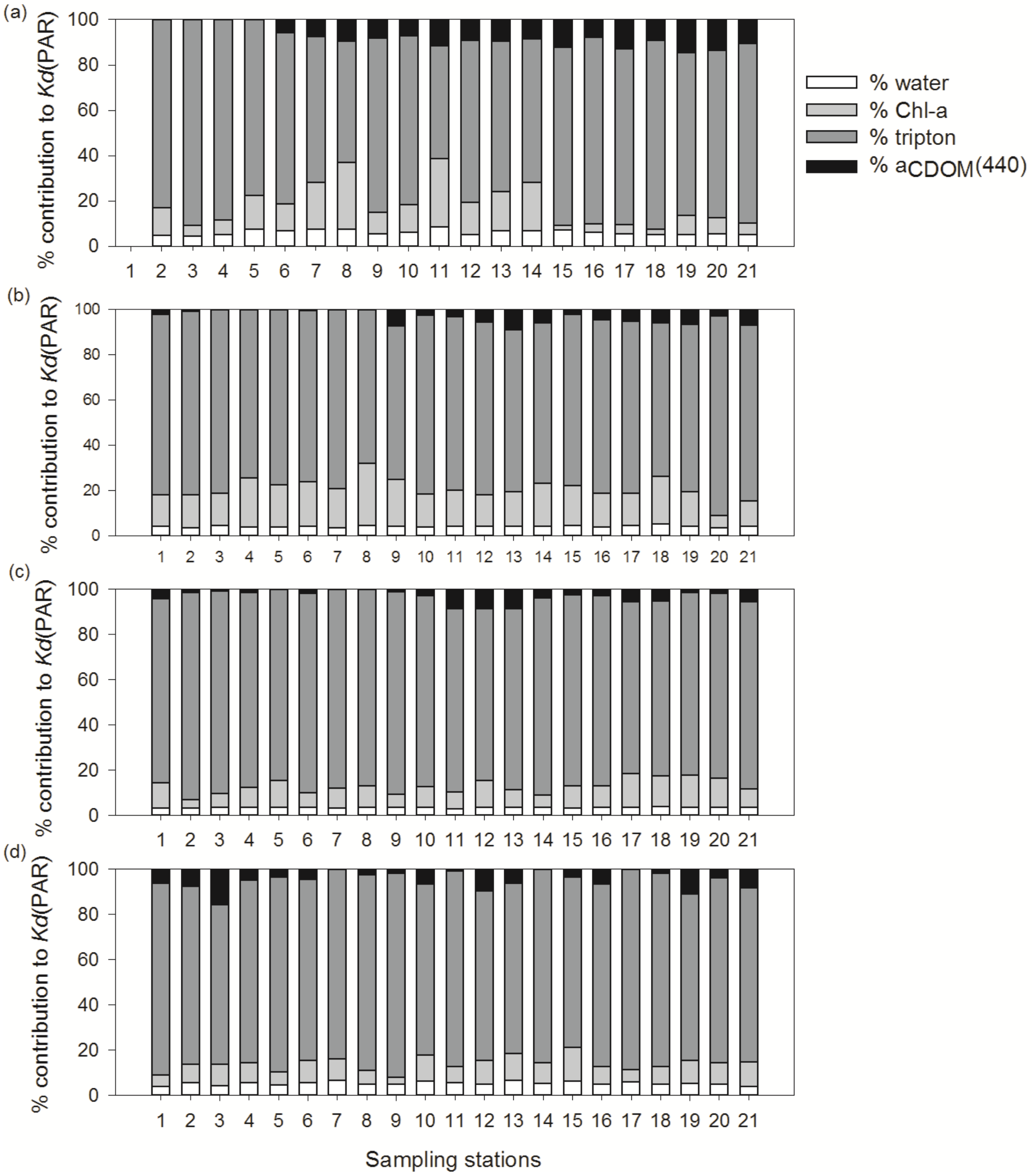

Figure 5 - The contributions of pure water, phytoplankton, tripton and colored dissolved organic matter to *Kd(PAR)* during (a) summer, (b) autumn, (c) winter and (d) spring.

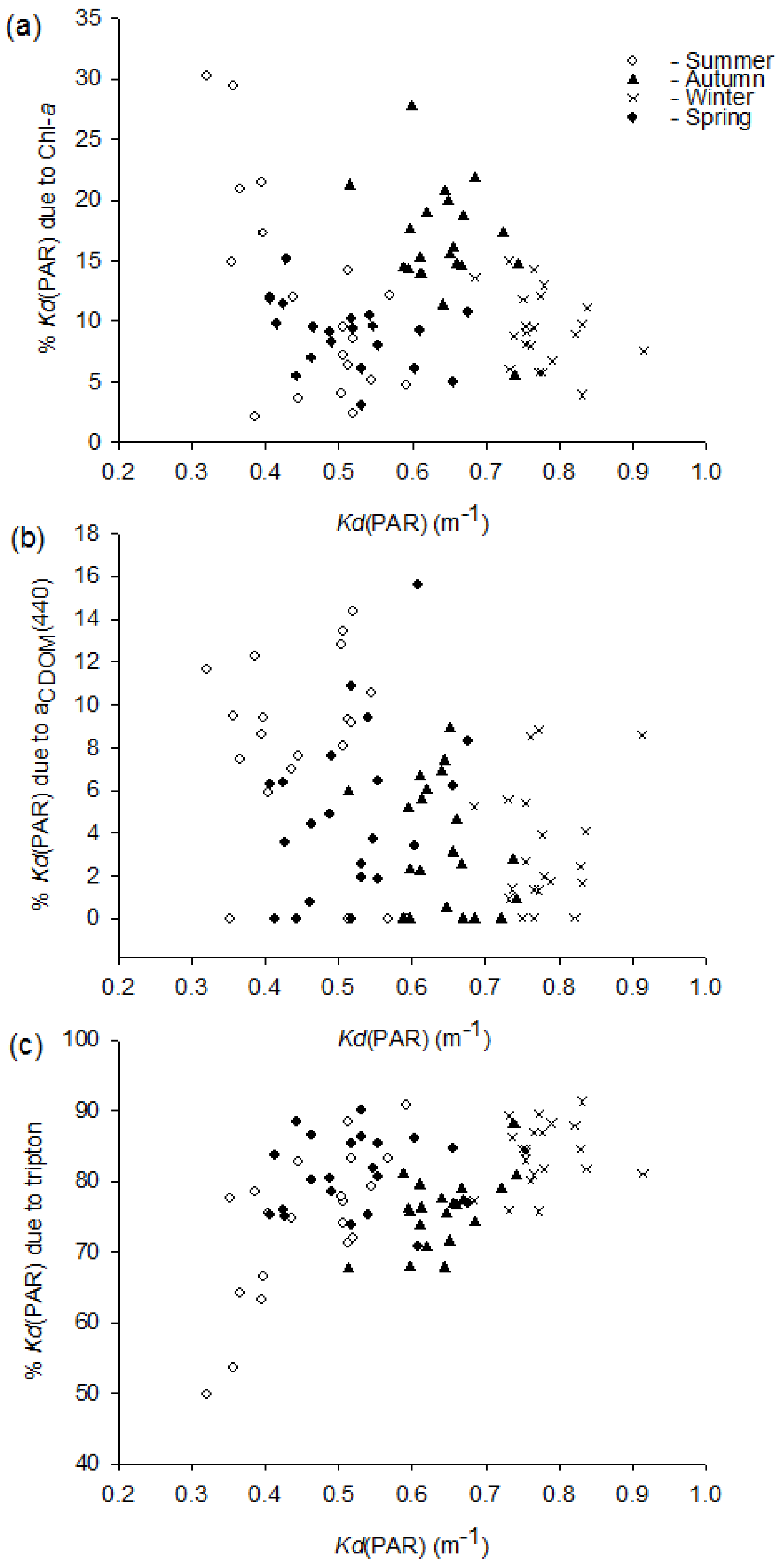

Figure 6 - The relative contributions of Chl-*a* (a), $a_{CDOM}(440)$ (b) and tripton (c) to *Kd(*PAR) during the spatial measurements.

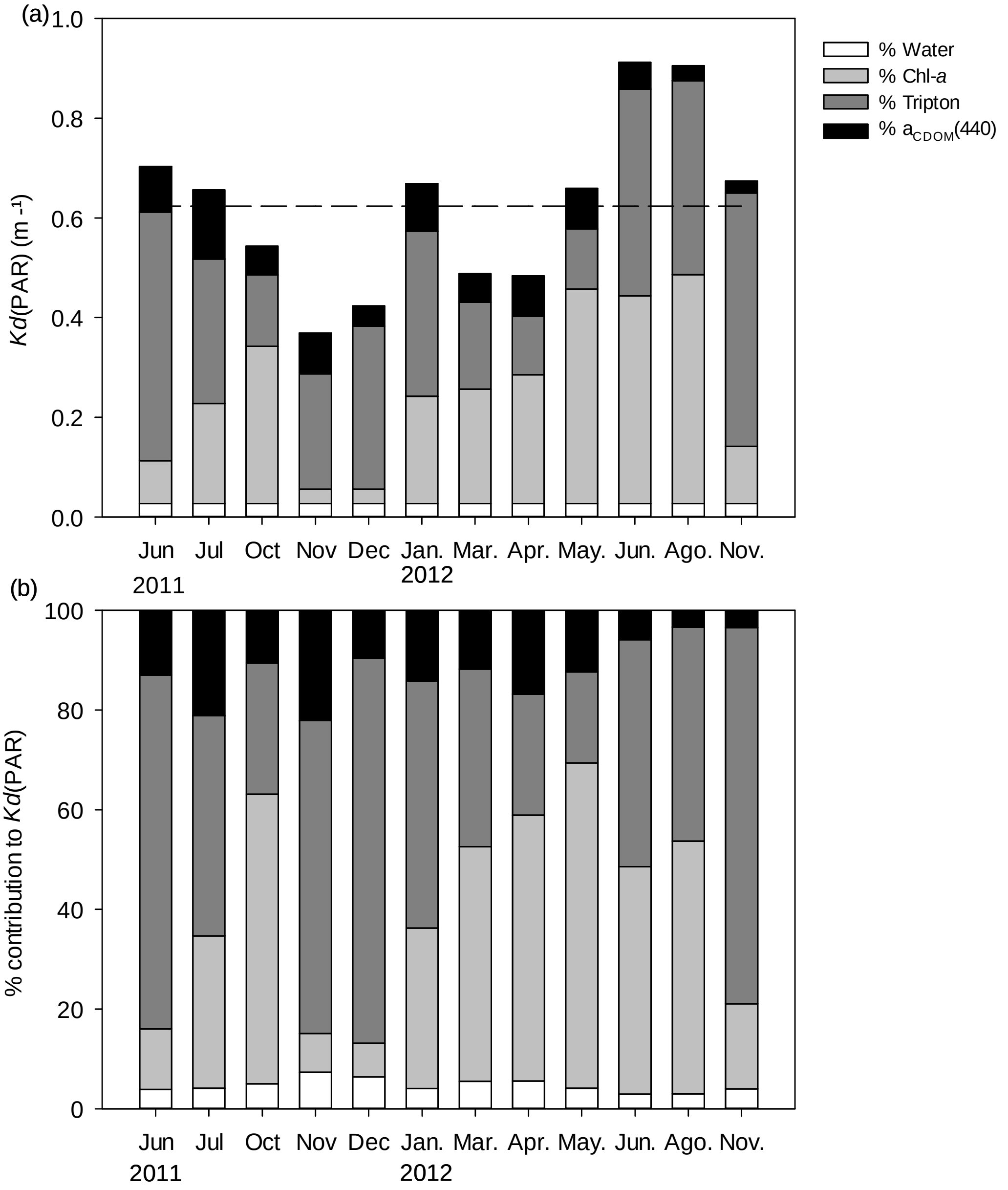

Figure 7– (a) Temporal variation of the PAR attenuation coefficient in the water column..White, light grey, dark grey and black bars show the partial attenuation coefficients due to pure water (K_w), phytoplankton ($K_{chl\text{-}a}$), tripton (K_{trip}) and $a_{CDOM}(440)$ (K_{CDOM}), respectively. The dashed line indicates the average of Kd_{PAR} through the study period (0.62 m^{-1}). (b) Percentage contributions of the K_w, K_{chla} , K_{trip} and K_{CDOM} to *Kd(*PAR) during the same period.

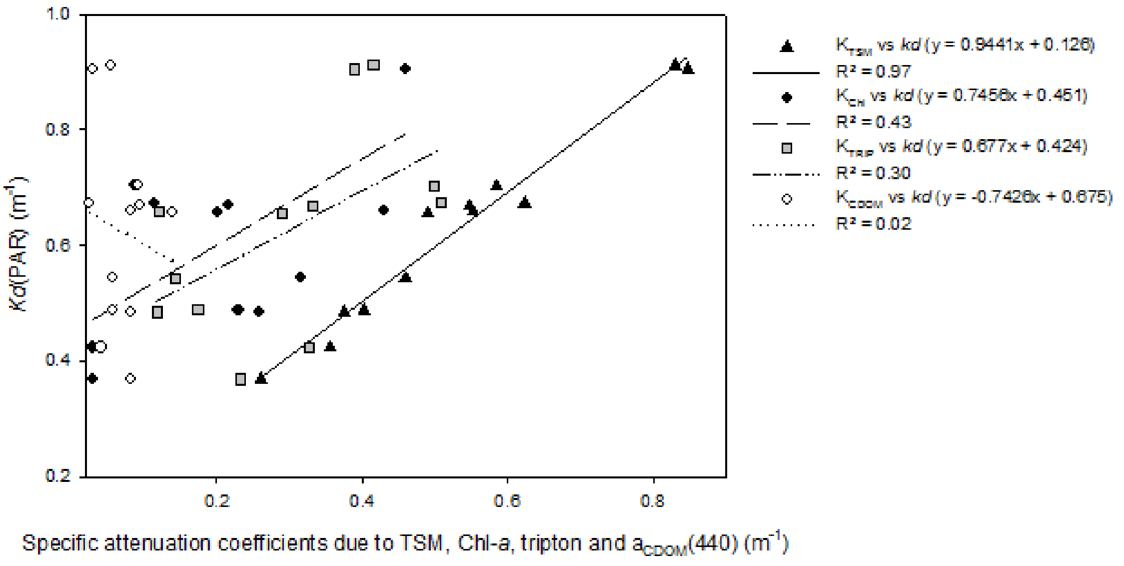

Figure 8 – Simple linear regressions between *Kd(*PAR) and partial attenuation coefficients of visible radiation due to TSM (triangles), tripton (crosses), Chl-*a* (black circles) and a_{CDOM}(440) (white circles) during monthly sampling.

Table 1- Summary of parameters measured during temporal and spatial analyzes in the lake between 2011 and 2013.

		Spatial sampling				**Temporal sampling**	
		Summer (Jan. 2013)	Autumn (Apr. 2013)	Winter (Aug. 2013)	Spring (Dec. 2013)	Dry season	Rainy season
Kd $_{(PAR)}$ (m^{-1})	Mean	0.46	0.64	0.78	0.52	0.91	0.66
	Min-Max	0.32-0.59	0.51-0.74	0.68-0.91	0.41-0.68	0.66-1.33	0.37-1.26
	std dev	0.08	0.05	0.05	0.08	0.26	0.26
Tripton (mg L^{-1})	Mean	1.03	1.09	1.19	1.14	1.65	1.33
	Min-Max	0.18-1.65	0.68-1.80	0.09-2.37	0.57-2.19	0.46-3.03	0.40-1.88
	std dev	0.37	0.34	0.53	0.34	0.99	0.57
Chl-*a* (µg L^{-1})	Mean	3.31	6.98	4.81	2.94	6.15	3.28
	Min-Max	0.47-6.90	2.68-10.91	2.10-7.22	1.13-4.80	1.69-8.91	0.59-6.11
	std dev	1.86	1.82	1.47	0.87	3.20	2.20
$a_{CDOM}(440)$ (m^{-1})	Mean	0.16	0.10	0.11	0.12	0.36	0.28
	Min-Max	0.00-0.30	0.00-0.30	0.00-0.40	0.00-0.40	0.10-0.60	0.10-0.40
	std dev	0.10	0.08	0.10	0.11	0.19	0.11
TSM (mg L^{-1})	Mean	1.36	1.79	1.67	1.43	2.26	1.66
	Min-Max	0.36-2.13	1.28-2.28	0.57-2.77	0.91-2.40	1.29-3.20	0.90-2.42
	std dev	0.39	0.24	0.55	0.35	0.73	0.54
DOC (mg L^{-1})	Mean	4.30	3.09	6.46	6.47	5.87	5.24
	Min-Max	3.31-5.05	2.49-3.46	5.25-8.32	4.10-8.47	2.38-11.21	1.92-8.73
	std dev	0.44	0.29	0.98	0.89	3.08	2.04
Mixing layer (m)	Mean	-	-	-	-	17.73	7.25
	Min-Max	-	-	-	-	8.40-27.90	5.00-13.40
	std dev	-	-	-	-	7.61	2.16
Euphotic zone (m)	Mean	10.37	7.22	5.94	9.08	5.65	8.22
	Min-Max	7.80-14.30	6.20-8.90	5.00-6.70	6.80-11.30	3.90-7.00	4.10-12.50
	std dev	1.91	0.62	0.36	1.31	1.12	2.55
Surface Temperature (ºC)	Mean	30.00	28.73	25.40	31.00	24.27	27.41
	Min-Max	-	-	-	-	20.90-25.70	24.50-31.40
	std dev	-	-	-	-	1.58	1.89
Water column depth (m)	Mean	14.03	-	13.04	-	28.50	28.50
	Min-Max	4.50-30.00	-	2.90-30.00	-	27.00-30.00	27.00-30.00
	std dev	8.52	-	8.58	-	0.84	0.84
Total nitrogen (µg L^{-1})	Mean	276.81	278.53	357.60	125.14	446.61	351.71
	Min-Max	215.49-424.10	242.62-342.10	12.73-519.71	3.68-431.47	292.21-688.20	229.27-695.29
	std dev	41.03	27.05	146.42	158.91	127.29	128.15
Total phosphorus (µg L^{-1})	Mean	3.78	-	12.75	8.27	8.06	8.22
	Min-Max	2.90-5.61	-	5.69-21.77	4.80-11.90	5.46-10.74	4.60-12.82
	std dev	0.97	-	4.10	1.83	2.09	2.42
Schmidt stability (J m^2)	Mean	-	-	-	-	124.16	386.43
	Min-Max	-	-	-	-	31.90-282.90	142.30-621.90
	std dev	-	-	-	-	82.72	167.89

Table 2- The specific attenuation coefficients of Chl-a, tripton, CDOM, pure water and TSM to the *Kd* and their relative contribution (%) to the total PAR attenuation.

			Specific attenuation coefficients (m-¹)					Contribution to PAR attenuation (%)			
Monthly sampling		***Kd(PAR)***	K_{Chl-a}	K_{TRI}	K_{CDOM}	K_{W}	K_{TSM}	K_{Chl-a}	K_{TRI}	K_{CDOM}	K_{W}
June-11		0.70	0.09	0.50	0.09	0.03	0.58	12	71	13	4
July-11		0.66	0.20	0.29	0.14	0.03	0.49	31	44	21	4
October-11		0.54	0.32	0.14	0.06	0.03	0.46	58	26	11	5
November-11		0.37	0.03	0.23	0.08	0.03	0.26	8	63	22	7
December-11		0.42	0.03	0.33	0.04	0.03	0.36	7	77	10	6
January-12		0.67	0.22	0.33	0.10	0.03	0.55	32	50	14	4
March-12		0.49	0.23	0.17	0.06	0.03	0.40	47	36	12	6
April-12		0.48	0.26	0.12	0.08	0.03	0.38	53	24	17	6
May-12		0.66	0.43	0.12	0.08	0.03	0.55	65	18	12	4
June-12		0.91	0.42	0.42	0.05	0.03	0.83	46	45	6	3
August-12		0.91	0.46	0.39	0.03	0.03	0.85	51	43	3	3
November-12		0.67	0.11	0.51	0.02	0.03	0.62	17	75	4	4
Spatial sampling		***Kd(PAR)***	K_{Chl-a}	K_{TRI}	K_{CDOM}	K_{W}	K_{TSM}	K_{Chl-a}	K_{TRI}	K_{CDOM}	K_{W}
January-2013	Mean	0.46	0.05	0.35	0.04	0.03	0.40	12	74	8	6
	min	0.32	0.01	0.16	0.00	0.03	0.26	2	50	0	5
	max	0.59	0.10	0.54	0.07	0.03	0.57	30	91	14	8
	SD	0.08	0.03	0.10	0.02	0.00	0.08	8	11	5	1
April-2013	Mean	0.64	0.11	0.49	0.02	0.03	0.59	17	76	3	4
	min	0.51	0.04	0.35	0.00	0.03	0.46	5	68	0	4
	max	0.74	0.17	0.65	0.06	0.03	0.71	28	88	9	5
	SD	0.05	0.03	0.07	0.02	0.00	0.06	4	5	3	0
August-2013	Mean	0.78	0.07	0.65	0.03	0.03	0.72	9	84	3	3
	min	0.68	0.03	0.53	0.00	0.03	0.62	4	76	0	3
	max	0.91	0.11	0.76	0.08	0.03	0.81	15	91	9	4
	SD	0.05	0.02	0.06	0.02	0.00	0.05	3	4	3	0
December-2013	Mean	0.52	0.04	0.42	0.03	0.03	0.46	9	81	5	5
	min	0.41	0.02	0.31	0.00	0.03	0.35	3	71	0	4
	max	0.68	0.07	0.56	0.10	0.03	0.59	15	90	16	7
	SD	0.08	0.01	0.07	0.02	0.00	0.07	3	5	4	1

Table 3 - Pearson correlations of the parameters obtained in the analysis of temporal changes at station P8 in 2011 and 2012. Significant correlations are highlighted in bold. (St = Schmidt stability; E0 = irradiance; Z_{eu} = euphotic zone depth).

	TSM (mg L-¹)	Chl-*a* (µg L-¹)	Tripton (mg L-¹)	aCDOM(440) (m-¹)	St (J m²)	E0 (mol photons m-² d-¹)	Emean (mol photons m-² d-¹)	Zeu (m)	Zmix (m)	DOC (mg L-¹)	Rainfall (mm)
kd **(PAR) (m-¹)**	0.5550	*0.656	0.2420	-0.1390	-0.4790	-0.3210	**-0.813	***-0.828	0.41	-0.238	*-0.579
TSM		-0.067	***0.922	0.153	*-0.614	-0.405	*-0.697	-0.517	**0.705	0.409	-0.122
Chl-*a*			-0.449	-0.13	-0.173	-0.121	-0.411	-0.485	0.0221	-0.451	*-0.586
Tripton				0.188	-0.482	-0.316	-0.464	-0.275	*0.623	0.542	0.119
a_{CDOM}(440)					-0.302	-0.493	-0.204	0.1	0.486	*0.604	-0.0948
St						***0.737	***0.712	0.404	***-0.714	-0.331	0.118
E0							***0.822	0.389	**-0.614	*-0.494	0.107
Emean								***0.718	***-0.784	-0.252	0.373
Zeu									-0.386	0.138	**0.624

*$p < 0.05$, **$p < 0.01$, *** $p < 0.001$

Table 4- Studies published since 2003 about the relative contribution of optically active components to the attenuation of PAR radiation in a range of aquatic systems.

Environment	Chlorophyll-*a %*	Tripton %	CDOM %	Water %	Type of Sampling	Reference
coastal lagoon, Florida	10.0-26.0% mean 10.0-26.0%	59-78% mean 61-59%	5-25% mean 25-11%		spatial in 6 sampling events, 2 different methods to partition	Christian, D. & Y. P. Sheng 2003
Aarhus Bay, Kattegat, North Sea-Baltic	mean 32%	mean 42%	mean 17%	mean 9%	time series study at one station	Lund-Hansen 2004
Florida Bay	mean 1-3%	mean 88-89%	mean 1-2%	mean 7-9%	spatial and monthly over 2 years	Kelble et al. 2005
subtropical Lake Donghu, China	49-65%	46-66%	18-24%	1-2%	spatial and monthly over 1 year	Yang et al. 2005
subtropical shallow Lake Taihu, China	2.8-34.6% mean 9.7%	58.1-95.9% mean 82.6%	0.8-22.8 mean 6.8%	0.2-3.1% mean 0.9%	spatial in 1 sampling event	Zhang et al. 2007
subtropical shallow Lake Taihu, China	*summer:* 8-70% mean 26%; *winter:* 1-11% mean 5%	*summer:* 21-65% mean 48%; *winter:* 38-91% mean 80%	*summer:* 7-37% mean 16%; *winter:* 3-50% mean 11%	*summer:* 2-25% mean 10%; *winter:* 2-8% mean 4%	spatial in summer and winter	Zhang et al. 2007b
Mediterranean coastal laggon, Albufera des Grau	mean 44%	mean 6%	mean 47%	mean 3%	spatial and monthly over almost 4 years	Obrador & Pretus, 2008
tropical Lake Victoria, Uganda and Kenya	<50%					Loiselle et al. 2008
30 shallow lakes and ponds, Hungary	56-70% in hypertrophic waters	55-88% in most water bodies	72-97% in highly coloured waters	-	comparison among lakes	Balogh et al. 2009
Sturgeon River, Keweenaw Bay and Lake Superior, USA	maximum 13%	7-13%	mean > 75%	maximum 3%	spatial in 2 sampling events	Effler et al. 2010
Harima Sound, Seto Inland Sea, Japan	mean 27%	mean 45%	9-18% mean 13%	8-21% mean 15%	monthly over 1 year at one point	Yamaguchi et al. 2013
Deep tropical lake, Brazil	*spatial:* 2-30% mean 12%; *monthly:* 7-65% mean 36%	*spatial:* 50-91% mean 79%; *monthly:* 18-77% mean 48%	*spatial:* 0-16% mean 5%; *monthly:* 3-22% mean 12.0%	*spatial:* 2.9-8.4% mean 4.7%; *monthly:* 3-7.3% mean 4.6%	spatial in 4 seasons and monthly	this present study

CAPÍTULO 2

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Temporal and spatial analysis of DOM quality and quantity in a dendritic tropical lake

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Abstract

Dissolved organic matter (DOM) is an important driver of biogeochemical and ecological processes in aquatic environments. In order to verify if there were spatial and temporal differences in DOM quantity and quality we analyzed at 21 sites during 6 campaigns the variability of DOM in a deep tropical natural lake (Lake Dom Helvécio, MG, Brazil). Surface water samples were taken to analyze DOC, Chl-a, suspended solids, nutrients and CDOM spectra (SUVA$_{254}$, $S_{275\text{-}295}$, $a_{CDOM440}$ and $a_{CDOM254}$). Depth of sites and rainfall were also used in the analysis. We found that seasonally the differences in DOM were higher than spatially, and there was no clear spatial pattern for DOC. Rainfall correlated negatively with DOC and $a_{CDOM440}$, and positively SUVA$_{254}$ and Chl-a. Aromatic carbon input increased with the increase of rainfall and the strong negative relation between SUVA$_{254}$ and DOC indicates that with the degradation of DOC the molecules became more aromatics, suggesting higher degradation in the PAR range. Negative correlations were observed between depth and $a_{CDOM254}$, probably because aromatic carbon remains restricted to the hypolimnion in deep sites. This study

showed that the change in rainfall pattern in the region in the last years has affected the seasonal patterns of input of DOM and nutrients in the lake, with possible impacts on the seasonality of phytoplankton and consequently the ecosystem functioning.

Key words: CDOM, DOC, SUVA, spectral slope, organic matter.

1 Introduction

The dissolved organic matter (DOM) is the largest fraction of organic carbon in aquatic ecosystems and easily exceeds the amount of carbon contained in particulate form (POM) (Hedges, 1992; Battin et al., 2008). Therefore, DOM is an important driver of biogeochemical and ecological processes in aquatic environments (Guo et al., 2014)

Although the quantity of DOM is important for the carbon cycle within the ecosystem, its role in biogeochemical cycles depends on its quality (source and composition) (Mueller et al., 2012). In turn, the DOM composition depends on its source, which can be allochthonous (terrestrial origin from the degradation of plant and soil) and autochthonous (production within the water column mainly from the excretion, decomposition and lysis of macrophytes and algae) (Thurman, 1985; Wetzel, 2001). The allochthonous DOM is in general more susceptible to photodegradation and absorbs solar radiation mainly in the UV range, because it is composed by aromatic compounds of higher molecular weights (Amon and Benner, 1994; McKnight et al., 1994; Benner, 2002; Helms et al., 2008). Autochthonous DOM can be further subdivided into two types with different characteristics: DOM from phytoplankton mainly consists of simple molecules (carbohydrates, proteins, amino acids) of low molecular weight and it is more labile for microbial community (Farjalla et al., 2009; Fonte et al., 2013); and DOM originating from macrophytes can be as aromatic and recalcitrant as DOM from terrestrial vegetation (Catalán et al., 2013). Nowadays, there are useful, rapid, sensitive and non-destructive tools to examine DOM quality, as absorbance and fluorescence spectroscopy (Weishaar et al., 2003; McKnight et al., 2001; Helms et al., 2008), but studies connecting DOM quantity and quality in tropical environments, especially in tropical lakes, are still scarce (Teixeira et al., 2011, 2013; Bittar et al., 2015; Brandão et al., 2016).

The quality and quantity of DOM in aquatic systems is dynamic and dependent on some characteristics such as regional climate, hydrologic regime, thermal stratification pattern, depth, environment morphology, land cover, and also dependent on seasonal events such as periods of algal bloom, and storms (Mattsson et al., 2005; Maie et al., 2006; Jaffé et al., 2008).

The input of allochthonous DOM coming from terrestrial plants and soils in freshwater systems is of great importance to the global carbon cycle and nutrients source as it represents a linkage between terrestrial and aquatic ecosystems (Jaffé et al., 2008). In addition, allochthonous DOM inputs influences the whole ecosystem functioning because it interferes in the quality and quantity of carbon. The increase of aromatic compounds changes the optical characteristics of water column as the transparency to visible and ultraviolet radiation affecting the light available to productivity of the system (Kieber et al., 2006; Miller et al., 2009 ; Brandão et al., 2016). In this way, allochthonous DOM also affects the microbial food webs because besides it is more recalcitrant to bacteria (Amon and Benner, 1994), it also influences the phytoplankton dynamic and consequently the production of autochthonous DOM which is more labile for microbial degradation (Lønborg et al., 2010). In addition, DOM quality also affects several chemical reactions (Fulton et al., 2004).

With the current pattern of climate change in recent decades, rainfall and temperature has been changed dramatically, with consequences for the inputs, production and degradation of DOM in aquatic ecosystems (Tian et al., 2013). Some regions of the world are facing reduction in annual rainfall, unseasonal rains and extremely warm summers, such as the Middle Rio Doce (MG, Brazil) where the studied Lake Dom Helvécio is located. These changes are expected to have affected allochthonous inputs of DOM. This will likely alter the concentration and quality of carbon and nutrients causing impacts on physical (thermal stratification patterns, temperature, light availability) and biogeochemical processes (metabolism, ecosystem productivity) of lakes in the region. However, studies on the dynamics of DOM in tropical aquatic ecosystems are still scarce (Teixeira et al., 2011, 2013; Bittar et al., 2015; Brandão et al., 2016) and to our knowledge none deals with effect of climate change. In this context, the aim of this study was investigate if there were differences in DOM quantity and quality in space and time in Lake Dom Helvécio through analysis of the optical properties of DOM, especially considering the changes in the rainfall observed for the last years in the region.

2 Material and Methods

2.1 Study site

Lake Dom Helvécio is located inside a protected area, the Rio Doce State Park – PERD Southeast Brazil (S19°46.94’, W42°35.48’). The lakes complex in the Middle Rio Doce is one of the most important districts of lakes in Brazil, with approximately 130 lakes, 42 of them located inside the PERD area, which is the largest remnant of the Atlantic Forest in Minas Gerais, totaling 36000 ha (Maia-Barbosa et al., 2010) (Fig. 1a-b). This lake is one of the deepest natural lakes of Brazil (maximum depth: 39.5 m), surrounded by Atlantic forest. It is oligotrophic (total phosphorus: 3.0-22.0 µg L^{-1}, annual mean 11.2 µg L^{-1}; Chl-*a*: 0.5-11.0 µg L^{-1}, annual mean 4.5 µg L^{-1}; Brighenti, 2014), has a perimeter of 37.7 km, area equal to 5.27×10^6 m^2 and volume equal to 5.94×10^7 m^3 (Bezerra-Neto and Pinto-Coelho, 2008). Dom Helvécio is a warm monomictic lake, with thermal stratification period beginning in September and lasting until April (rainy period) and a mixed period from June to August (dry period) (Barbosa and Tundisi, 1980; Brighenti, 2014). Since 2012 the region of the Middle Rio Doce has had its rainfall patterns changed, occurring unseasonal rains and lower volume of rainfall (mm) during the year. The sampling months had unusual volume of rainfall and between November of 2012 and April of 2013 the total volume of rainfall was 354 mm (in the sampling months were: November - 80.2, January - 0 and April - 103 mm), which was lower than that observed for the last years (Figure 1d; source: Climatological Station of Timóteo, MG, Brazil). The rains that usually begin in September did not happen in 2013 and it remained until January of 2014, which classified December of 2013 (sampling month) as an unusual dry period instead of expected rainy period. In contrast, during the dry period in 2014 there were more rains than in the last years (Figure 1d).

2.2 Field measurements

The 6 samplings were carried out in November of 2012, January, April (stratified periods), August (mixing period) and December (stratified period) of 2013 and in July (mixing period) of 2014. We collected samples at 21 sites covering all the lake (Fig. 1c). At the central deep region (station 8) of the lake, vertical profiles of water column temperature (°C) were measured every meter from surface to 28 m using a Hydrolab DS5 probe.

Water samples (0.5 m from the surface) were collected and filtered immediately for Chl-*a* (0.47μm Millipore filter) and TSM (AP040 Millipore filter), and the filters were frozen until analysis. Water samples were also filtered for analysis of dissolved organic carbon (DOC) and colored dissolved organic matter (CDOM) (0.22 μm Millipore filter) and stored in amber glass bottles (pre-washed with distilled water and hydrochloric acid 10%) at 4°C in the dark.

2.3 Laboratory analysis and calculation of the optical properties

The measurement of concentration of Chl-*a* (μg L^{-1}) was obtained by acetone extraction (90%) measured in a spectrophotometer (UV-VIS Shimadzu) at 665 and 750 nm and calculated according to APHA (1998). Total suspended matter (TSM; mg L^{-1}) were determined by the gravimetric method, considering the difference between the dry weight of AP40 Millipore filters (105°C for 2 hours) before and after the filtration of water samples (APHA, 1998).

The DOC concentration (mg L^{-1}) was obtained by catalytic oxidation method of high temperature using TOC Analyzer (Shimadzu TOC – 5000).

The spectral absorption of CDOM was measured between 250 and 700 nm (1 nm of interval) in a spectrophotometer (UV-VIS Shimadzu) using 5 cm quartz cuvette and Milli-Q water as a reference. The absorption spectra of each sample was performed in triplicate and determined using the Spectrum Pack Program software (Shimadzu Inc.). The absorption coefficients were derived from absorbance measurements (A) at λ (m^{-1}) according to the equation $a_{CDOM}(\lambda)= 2.303A(\lambda)\ l^{-1}$, where $a_{CDOM}(\lambda)$ is the absorption coefficient at λ and l is the optical path of the cuvette (in meters). Absorption coefficients were corrected for backscattering by subtracting the value of the coefficient at 700 nm from each absorption coefficient. The spectrophotometric absorption coefficient at 440 nm was used as an index of PAR-absorbing (Williamson et al., 1996) and at 254 nm as UV-absorbing of CDOM. The spectral slope S (nm^{-1}) was calculated for wavelengths between 275-295 nm and 350-400nm (Helms et al., 2008) and it was estimated using linear adjustment technique between log values of the absorption coefficients and the specific wavelength range. The specific UV absorbance ($SUVA_{254}$) (m^2 mg^{-1} C) was calculated dividing the value of the absorption coefficient at 254 nm by the concentration of DOC (mg L^{-1}), and the increase of SUVA indicates increase of compounds of higher aromaticity (Weishaar et al., 2003).

Accumulated rainfall recorded at every three hours was obtained from a meteorological station (Timóteo, MG) located near to the lake (http://www.inmet.gov.br).

Unfiltered water samples were taken for analysis of total nitrogen (TOC Analyzer, Shimadzu TOC – 5000) and total phosphorus (according to Mackereth et al., 1978).

2.4 Statistical analysis

Differences in DOM quality and quantity between seasons were assessed by Kruskal-Wallis One Way (Analysis of Variance on Ranks) and followed by Pairwise Multiple Comparison (Dunn's Method) analysis (significant $p < 0.05$).

Pearson correlations and regression analysis were made to examine the relationships between all the parameters measured (significant $p < 0.05$).

To demonstrate the spatial distribution of DOM average values from six temporal samplings of $S_{275-295}$, $SUVA_{254}$, and DOC were used to predict values for unsampled locations in a raster. The interpolation was made using ArcGIS's 10.3 – Geostatistical Analyst extension. Different surfaces were created using different interpolation methods (*i.e.* IDW and Kriging) or by using the same method with various parameters. These surfaces were systematically compared with each other, eliminating the worst of the two being compared, until only the best surface remained. The criteria for eliminating the surfaces was the standardized mean nearest to zero, the smallest root-mean-squared prediction error, the average standard error nearest the root-mean-squared prediction error, and the standardized root-mean-squared prediction error nearest to 1. Therefore, our model has simple kriging as its interpolation method, with Geostatistical Analyst's model optimization, standard neighbourhood, and four sectors.

A principal component analysis (PCA) was generated with correlation matrix (parameters with different units) using the parameters of quality and quantity of DOM (DOC, $a_{CDOM440}$, $a_{CDOM254}$, $SUVA_{254}$ and $S_{275-295}$), Chl-a, depth, sites, seasons and rainfall.

3 Results

3.1 Variation in the parameters measured

The lake was thermally stratified during November 2012 and April 2013 and not stratified (mixing periods) in August 2013 and July 2014. The water surface temperature ranged from 21 to 31 °C with lowest temperatures during the mixing periods.

Considering all sites and sampling periods, the $S_{275-295}$ values ranged from 0.019 (site 4 in July 2014) to 0.036 nm^{-1} (site 15 in January 2013), and there is no clear variation over time and seasons. $SUVA_{254}$ ranged from 0.880 (site 3 in August 2013) to 4.824 m^2 mg^{-1} C (site 4 in July 2014), while DOC concentrations ranged between 2.5 (site 3 in April 2013) and 8.5 mg L^{-1} (site 7 in December 2013).

The highest concentration of Chl-a occurred in the site 8 (11.0 µg L^{-1} during April 2013) and the lowest in the site 15 (0.5 µg L^{-1} during November 2012). Higher averages of Chl-a ocurred during mixing periods (August 2013 – 4.8 µg L^{-1}; July 2014 – 3.6 µg L^{-1}) and during thermal stratification in April 2013 (7.0 µg L^{-1}) (Table 1).

TN and TP did not have the same pattern. Higher averages of TN concentrations were observed during mixing periods (August 2013 – 0.4 mg L^{-1}; July 2014 – 0.7 mg L^{-1}). There was no seasonality for TP concentrations, and the highest averages occurred in the stratified November of 2012 (17.2 µg L^{-1}) and during the mixing period in August of 2013 (12.7 µg L^{-1}) (Table 1).

3.2 Spatial and temporal variation of DOM

Analyzing the average considering the 6 sampling, the west portion of the lake (sites 19, 20 and 21) had DOM more aromatic (higher $SUVA_{254}$) and compounds with higher molecular size (lower $S_{275-295}$) (Figure 2a-b). DOC concentrations did not have clear spatial differences (Figure 2c).

$S_{275-295}$ presented variation among sites about 0.002-0.003 nm^{-1} (Table 1, standard variation). $SUVA_{254}$ had higher standard variation among sites in November 2012 and July 2014 (Table 1), while for DOC it was observed in November 2012, August and December of 2013.

Although there were differences between the sampled sites, differences among seasons were higher than among sites for quantity and quality of DOM (Figure 3 and Table 1). There were differences among almost all the seasons to SUVA and DOC, but it was not the same to $S_{275\text{-}295}$ (Table 2). $S_{275\text{-}295}$ changed over time significantly, but differences were observed only between the months January-April 2013 and July 2014 (Figure 4a and Table 2). DOC and $SUVA_{254}$ were negative correlated ($R = -0.80$; $R^2 = 0.64$, $p < 0.000$), which indicates a strong connection between quality and quantity of DOM (Figure 5). We did not find defined seasonal pattern to DOM quality and quantity related to thermal stratification and mixing processes.

The PCA results showed that 4 principal components had eigenvalue higher than 1 and explained 76% of variance (PC1 – 29%; PC2 – 24%; PC3 – 12%; PC4 – 11%) of the parameters measured (DOC, $SUVA_{254}$, $S_{275\text{-}295}$, $a_{CDOM254}$, $a_{CDOM440}$, Chl-a, depth, seasons, sites and rainfall). With the plot of PC1 versus PC2 (Figure 6) and with the Pearson's correlations (Table 3) we see the opposite relation between DOC and $SUVA_{254}$, a positive effect of rainfall in $SUVA_{254}$ and Chl-a and negative in DOC concentration and $a_{CDOM440}$, and an effect of the increase of depth decreasing $a_{CDOM254}$. We also observed that the different sampling periods (seasons) had an effect in $a_{CDOM440}$ and going to the direction from site 1 to site 21 (going from north to west portion of the lake) the $a_{CDOM254}$ increased (Figure 6).

4 Discussion

There were spatial and temporal differences in the quantity (DOC) and quality of DOM ($SUVA_{254}$ and $S_{275\text{-}295}$) in Lake Dom Helvécio. Spatially the differences in DOM were lower than seasonally, and it was not identified a clear spatial pattern related to the variables investigated in this study (Chl-a, nutrients, TSM, depth). Only considering the seasonality is that we identified a relationship between DOM and Chl-a with rainfall and between $a_{CDOM254}$ and depth, while no direct relations were found with water column stratification and mixing periods.

Higher concentrations of Chl-a were observed during periods with full water column mixing (August 2013 and July 2014) but also during the stratified April of 2013. Phytoplankton community is known to be limited by nutrients (especially by phosphate) in the epilimnion during stratified periods in Dom Helvécio (Barros et al., 2006;

Brighenti et al., 2015). It happens because the input of terrestrial OM and nutrients with the rains remains in the hypolimnion due to differences in the temperature in the layers of water column (Reynolds, 2009) and they become distributed and available in the epilimnion only during mixing water between May and August as already demonstrated by some authors (Barros et al., 2006; Brighenti et al., 2015; Brandão et al., 2016). The highest Chl-a concentration observed in April of 2013 (stratified period) might have happened due to higher concentrations of TP at the same time and also as a consequence of smaller volume of rains since November of 2012.

The seasonal differences found for DOC, $a_{CDOM440}$ and $SUVA_{254}$ were related to cumulative rainfall for each sampling month (Table 3). This suggests that inputs of aromatic terrestrial DOM from the basin are coupled to rainfall events (Reynolds, 2009), increasing $SUVA_{254}$ values (Brandão et al., 2016). Although $SUVA_{254}$ has DOC concentrations in its formula ($a_{CDOM254}$/DOC) it is not guaranteed a strong negative correlation between them (Jaffé et al., 2008), but in this study they were strongly correlated ($R = -0.80$; $R^2 = 0.64$; $p < 0.000$). The relationship implies that there was a significant relation between quantity and quality of DOM (Jaffé et al., 2008). It means that with the degradation of DOC the molecules become more aromatics, in other words the compounds responsible for the absorbance in the PAR range (labile compounds) are more degraded than those responsible for the absorbance in the UV spectrum (aromatic compounds). It is known that photodegradation provides loss in PAR and UV-absorbing, but it has more impact mainly in the ultraviolet range (Markager and Vincent, 2000). In contrast, the loss by biodegradation is generally higher in PAR-absorbing due to preference of bacterial communities for more labile and bioavailable compounds (Asmala et al., 2014) and this results suggest that this is the main process of DOM degradation in this lake. It corroborates with the negative relation between $a_{CDOM440}$ and rainfall (Table 3), which means a loss of absorption in PAR range with the increase of rains e $SUVA_{254}$.

Although the seasonal differences for DOC, $SUVA_{254}$ and $S_{275-295}$ were statistically significant (Table 2, $p < 0.05$), there was only a smooth reduction of $S_{275-295}$ temporally (Figure 4a). The decline of $S_{275-295}$ may indicate an increase in molecular size (Helms et al., 2008), which corroborated with the increase of aromatic compounds viewed through the increase of $SUVA_{254}$ with DOC degradation.

The results obtained through the PCA indicated that the variation of $a_{CDOM254}$ was more related to the sampling sites (spatial variation) and of $a_{CDOM440}$ to the seasonallity (temporal variation) (negative Pearson correlations, Table 3). In deeper sites there may be a limitation in the depth of the mixed layer, and the aromatic carbon coming into the lake with the rains remain restricted in the hypolimnion and do not reach the surface layers even with the partial mixing of water column. This fact could be an explanation for the increase in depth of the sampling sites causing a decline in $a_{CDOM254}$. This is consistent with more aromatic carbon compounds (higher $SUVA_{254}$) with higher molecular size (lower $S_{275-295}$) observed in west region of the lake (points 19, 20 and 21; Figure 2a-b) in the sites of lowest depths (between 4.7 and 6.5 m). These points are close to the lakeshore and certainly accumulate much of the terrestrial carbon during the input by rains.

This study showed that the change in rainfall pattern in the region has affected the seasonal patterns of input of DOM and nutrients in the lake, reflecting in the seasonality of phytoplankton and consequently affecting other trophic levels and the metabolism of the ecosystem. In addition, the absence of spatial pattern to DOC proves the importance of including the quality of DOM in carbon dynamics studies on aquatic ecosystems in order to understand the lake functioning.

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Figures and Tables

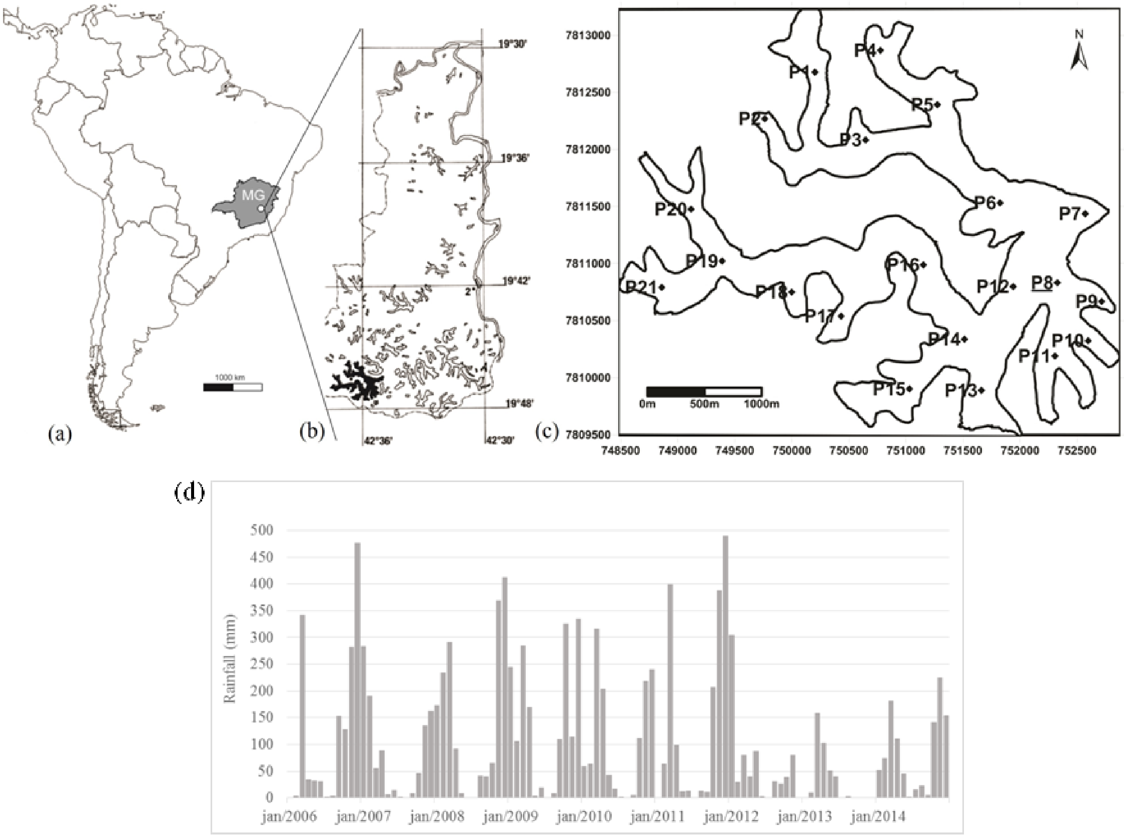

Figure 1 - Location of Dom Helvécio lake in Minas Gerais (a), inside of the Rio Doce State Park (b), Middle Rio Doce and the spatial sampling stations (P1-P21) (c). The numbers on the axes correspond to geographic coordinates. Rainfall from January 2006 to December 2014 (d).

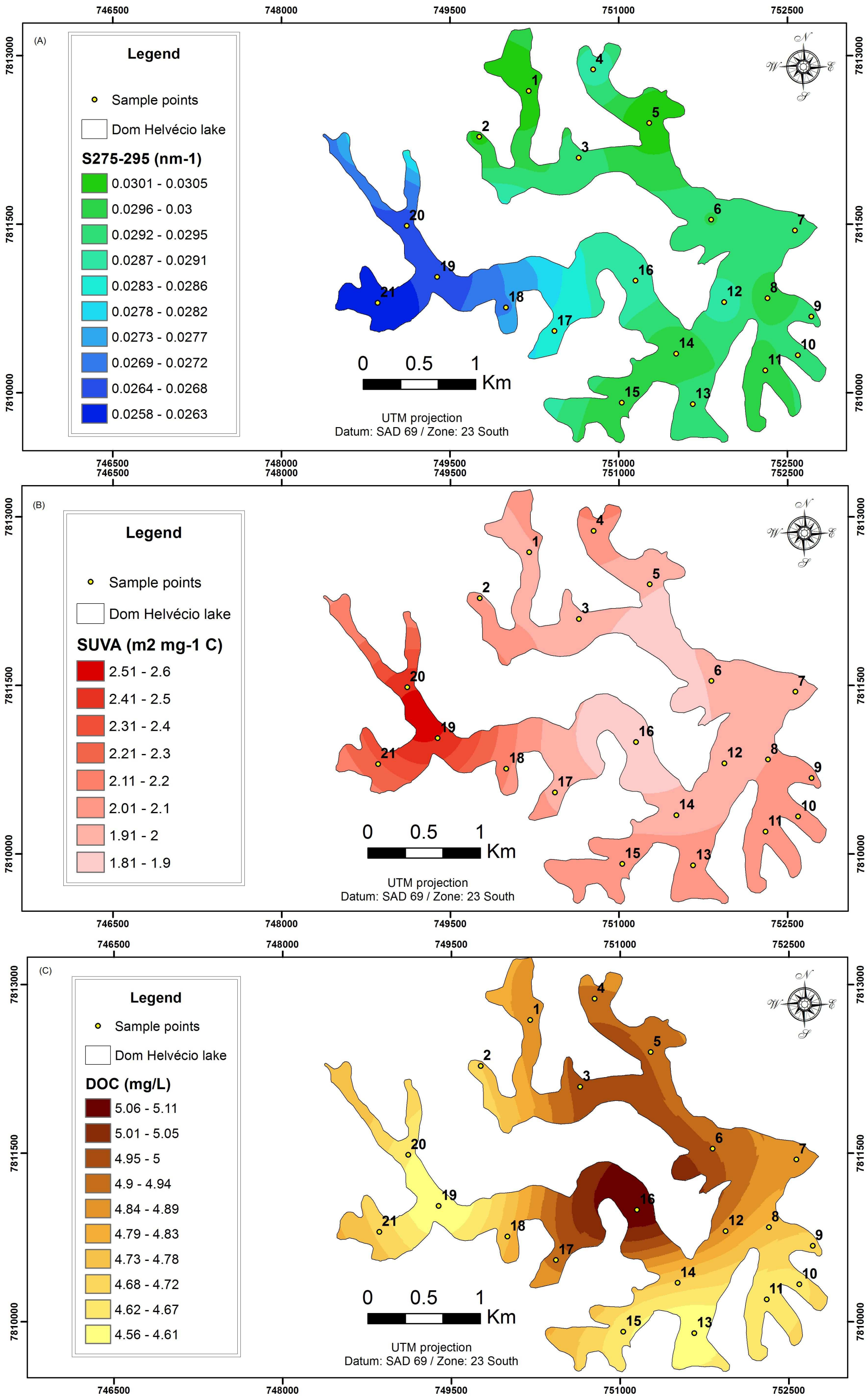

Figure 2 – Spatial maps of the average values from 6 temporal sampling of $S_{275\text{-}295}$ (A), $SUVA_{254}$ (B) and DOC (C).

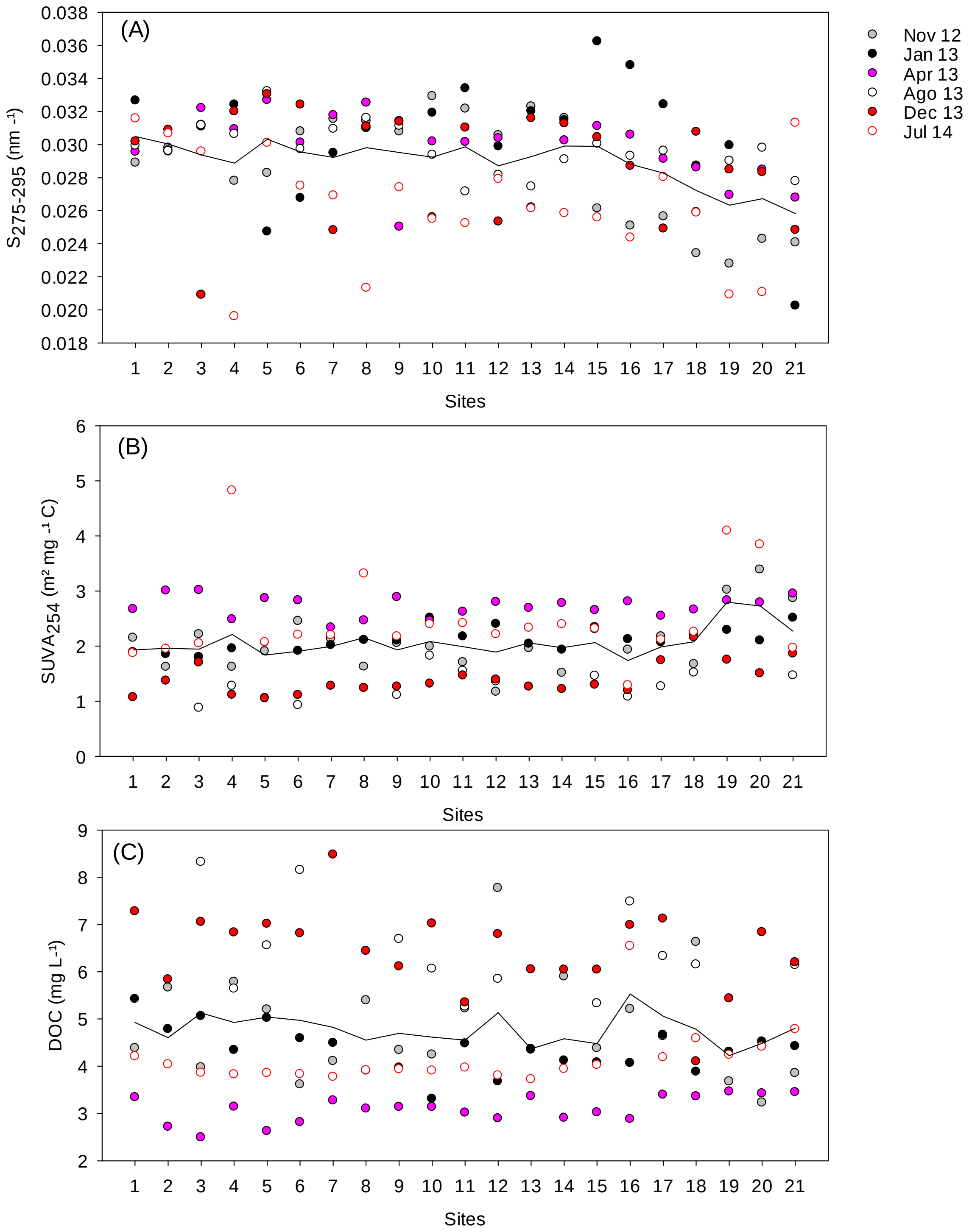

Figure 3 – Distribution and range of $S_{275-295}$ (A), $SUVA_{254}$ (B) and DOC (C) for all samples from studied sites. The black line represents the average of each site in the 6 sampling periods.

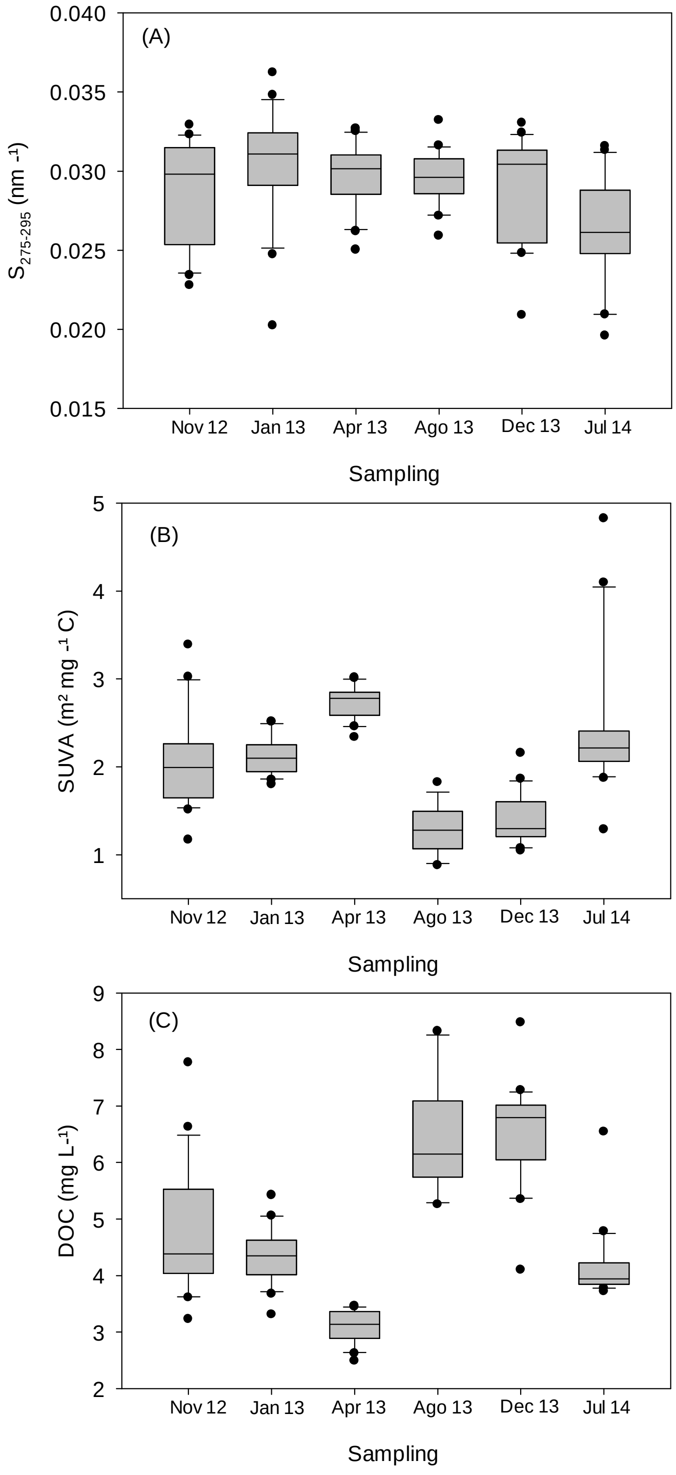

Figure 4 – Temporal variations (center line-median, outer grey-5th/95th percentiles, bars- 95% confidence level, black circles- outliers) of $S_{275-295}$ (A), $SUVA_{254}$ (B) and DOC (C) at 21 sites.

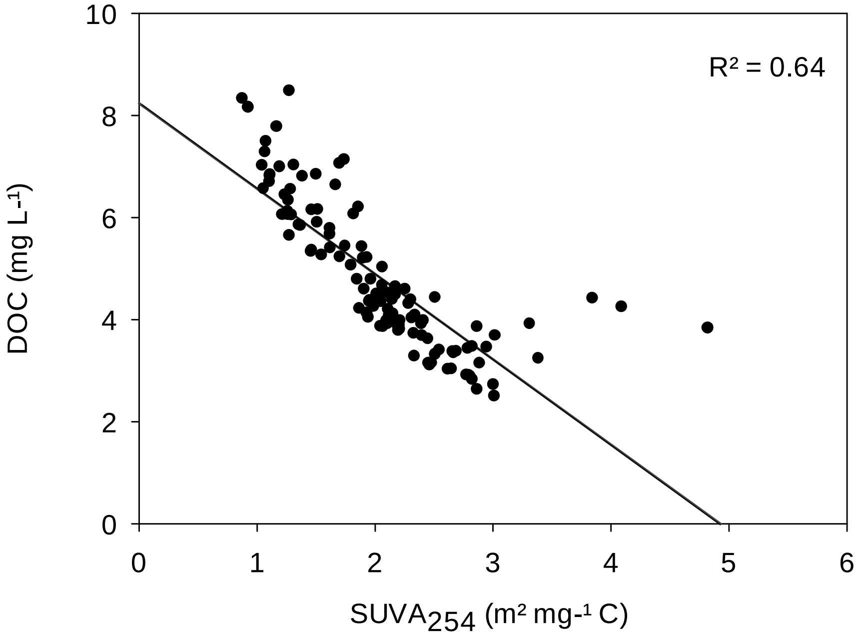

Figure 5 – Relation between DOC and SUVA$_{254}$ (Linear regression: R = -0.80, R^2 = 0.64, $p < 0.000$).

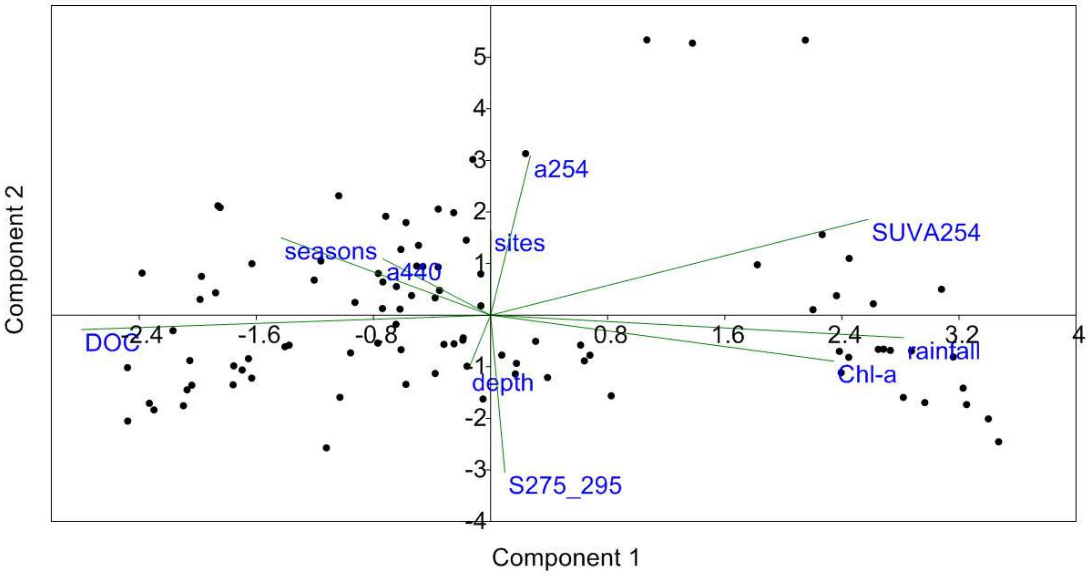

Figure 6 – Results of principal component analysis plotting component 1 (29% of explanation) versus component 2 (24%).

Table 1 - Summary (mean and standard variation-SD) of parameters measured during temporal and spatial analyzes in the six sampling campaigns.

		DOC (mg L^{-1})	SUVA254 (m^2 mg^{-1} C)	S275-295 (nm^{-1})	$a_{CDOM440}$ (m^{-1})	$a_{CDOM254}$ (m^{-1})	Chl-a (µg L^{-1})	TSM (mg L^{-1})	TN (mg L^{-1})	TP (µg L^{-1})	Depth (m)
Nov/12	mean	4.8	2.070	0.029	0.034	9.540	3.3	2.2	0.3	17.2	14.0
	SD	1.1	0.530	0.003	0.065	0.895	1.8	0.5	0.1	3.2	8.5
Jan/13	mean	4.4	2.115	0.030	0.152	9.148	3.3	1.4	0.3	6.0	14.0
	SD	0.5	0.204	0.003	0.105	0.805	1.8	0.4	0.0	3.4	8.5
Apr/13	mean	3.1	2.722	0.030	0.097	8.396	7.0	1.8	0.3	10.3	14.0
	SD	0.3	0.188	0.002	0.082	0.787	1.8	0.2	0.0	8.4	8.5
Aug/13	mean	6.5	1.291	0.030	0.115	7.979	4.8	1.7	0.4	12.7	14.0
	SD	1.0	0.274	0.002	0.102	0.913	1.5	0.6	0.1	4.1	8.5
Dec/13	mean	6.5	1.398	0.029	0.121	8.914	2.9	1.4	0.1	8.3	14.0
	SD	0.9	0.291	0.003	0.106	1.611	0.9	0.3	0.2	1.8	8.5
Jul/14	mean	4.2	2.490	0.026	0.269	10.216	3.6	1.2	0.7	3.5	14.0
	SD	0.6	0.835	0.003	0.164	3.293	1.0	0.3	0.2	1.1	8.5

Table 2 – Summary results of Kruskal-Wallis One Way (Analysis of Variance on Ranks) and Pairwise Multiple Comparison (Dunn's Method) analyzes.

	Differences among seasons by Dunn's Method ($p<0.05$)						Kruskal-Wallis results (n = 21)		
	S275-295						S275-295 ($p=0.003$)		
	Nov 12	Jan 13	Apr 13	Aug 13	Dec 13	Jul 14	Median	25%	75%
Nov 12	-	no	no	no	no	no	0.0298	0.0254	0.0315
Jan 13		-	no	no	no	yes	0.0311	0.0291	0.0324
Apr 13			-	no	no	yes	0.0302	0.0285	0.031
Aug 13				-	no	no	0.0296	0.0286	0.0308
Dec 13					-	no	0.0304	0.0255	0.0313
Jul 14						-	0.0261	0.0248	0.0288
	SUVA254						SUVA254 ($p<0.001$)		
	Nov 12	Jan 13	Apr 13	Aug 13	Dec 13	Jul 14	Median	25%	75%
Nov 12	-	no	yes	yes	yes	no	1.992	1.648	2.261
Jan 13		-	yes	yes	yes	no	2.1	1.944	2.251
Apr 13			-	yes	yes	no	2.779	2.585	2.848
Aug 13				-	no	yes	1.279	1.069	1.493
Dec 13					-	yes	1.296	1.208	1.604
Jul 14						-	2.214	2.06	2.406
	DOC						DOC ($p<0.001$)		
	Nov 12	Jan 13	Apr 13	Aug 13	Dec 13	Jul 14	Median	25%	75%
Nov 12	-	no	yes	no	yes	no	4.383	4.04	5.531
Jan 13		-	yes	yes	yes	no	4.351	4.017	4.627
Apr 13			-	yes	yes	yes	3.135	2.888	3.364
Aug 13				-	no	yes	6.152	5.745	7.09
Dec 13					-	yes	6.797	6.045	7.018
Jul 14						-	3.941	3.844	4.224

Table 3 - Pearson correlations of the parameters obtained in the temporal and spatial analysis (n = 126). Only significant correlations are shown.

	SUVA254 (m^2 mg^{-1} C)	Chl-a (µg L^{-1})	Depth (m)	Rainfall (mm)
DOC (mg L^{-1})	-0.80***	-0.43***		-0.53***
SUVA254 (m^2 mg^{-1} C)				0.46***
$a_{CDOM440}$ (m^{-1})		-0.19*		-0.29**
$a_{CDOM254}$ (m^{-1})			-0.218*	
Chl-a (µg L^{-1})				0.65***

* $p < 0.05$ ** $p < 0.01$ ***$p < 0.001$

CAPÍTULO 3

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Seasonal changes in optical properties of two contrasting tropical freshwater systems

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Abstract

We investigated how allochthonous and autochthonous sources of dissolved organic matter (DOM) affected the optical conditions and chemical characteristics of two contrasting tropical freshwater systems (Dom Helvécio-DH and Pampulha reservoir) in a dry and rainy period in 2013. We analyzed PAR (photosynthetically active radiation) and UV (ultraviolet) attenuation coefficients, nutrients, chlorophyll-a (Chl-a), dissolved organic matter (DOC) and spectral characteristics of CDOM (colored dissolved organic matter). Significant differences in CDOM sources and quantity were observed, with a dominantly terrestrial input in DH during the rainy period. The eutrophic Pampulha had several fold higher levels of DOC, Chl-a, TN and TP, with organic matter of mostly originating from phytoplankton in both seasons. Differences in source and quantity had strong implications on water transparency, DOC concentrations, CDOM quality and its susceptibility to photo- and biodegradation. DH was several fold clearer in both the UV and PAR spectrum. In DH transparency to both UV and PAR radiation was highest during the summer, suggesting elevated photo- and biodegradation during stratification.

Pampulha was most transparent in the dry period even during period of algal bloom. In both systems we observed seasonal variations in concentrations of nutrients and Chl-a, and in DH differences were also found in DOC concentrations as well as the specific UV absorbance ($SUVA_{254}$) and molecular size (M). Our results documents that different sources of DOM and seasonal inputs reflect in the seasonality of apparent and inherent optical properties and nutrients availability with implications for water quality and aquatic community.

Keywords: CDOM, DOM, optical properties, photodegradation, carbon source

1. INTRODUCTION

The dissolved form of organic matter (DOM) constitutes about 90% of the total pool of organic matter (Tranvik and Wachenfeldt, 2009), and understanding of the production and transformation of DOM is of key importance in studies of carbon cycling in lakes (Thurman, 1985; Bertilson and Tranvik, 2000). DOM represents a complex of compounds and derives from main sources: allochthonous material produced from degradation of humic substances coming from runoff of terrestrial origin (Kieber *et al.,* 2006; Miller *et al.,* 2009); and autochthonous material, produced by autotrophic and heterotrophic in-situ activities, but mainly by the bacterial degradation of phytoplankton and macrophytes (Kritzberg *et al.,* 2004; Hanamachi *et al.,* 2008; Henderson *et al.,* 2008; Guillenette and Del Giorgio, 2012). The two different sources of DOM can be distinguished from differences in their optical and chemical characteristics. The DOM from autochthonous source is furthermore more biologically labile than the DOM from allochthonous origin, which absorbs more solar radiation, has higher molecular weight due to the more complex aromatic molecules in its composition (McKnight *et al.*,, 1994; Benner, 2002).

CDOM is the chromophoric or colored part of the DOM and also an optically active component which plays an important role in freshwater systems. CDOM absorbs solar radiation reducing exponentially the penetration of light in the water column and the absorption increases from long to short wavelengths being higher in the ultraviolet (UV) range (Twardowski *et al.*, 2004). The CDOM absorption is due to photo-oxidation (known as photodegradation) of the molecules that can originate photo products or can be completely mineralized to CO_2, affecting the amount of CO_2 emission to the atmosphere (Arrigo *et al.*, 2011). This phenomenon restricts the available light for production, abundance and distribution of pelagic and benthic primary producers, with consequences for higher trophic levels in the ecosystem (McMahon et al. 1992, Gallegos and Moore 2000). Moreover, it significantly reduces harmful effects of UV radiation on aquatic organisms (Zhang *et al.*, 2011). Several studies in temperate aquatic systems have demonstrated the role of CDOM in the light attenuation (Christian and Sheng, 2003; Balogh *et al.*, 2009; Yamaguchi *et al.*, 2013), but the effect of CDOM in tropical systems still requires effort (Obrador and Pretus, 2008) especially in lakes (Brandão *et al.*, in review). DOM is furthermore an energy source for the bacterial production, which is responsible for the regeneration of nutrients in the water (Lønborg *et al.*, 2010). It also operates in the physical, chemical and biological processes of transport and availability of nutrients and the development of blooms of phytoplankton in eutrophic lakes (Qualls and Richardson, 2003; Coble, 2007; Stedmon *et al.*, 2007) and it is an important component to estimates and effective management of water quality and eutrophication control (Zhang *et al.*, 2011b). Understanding the environmental conditions and physical, chemical and biological processes involved in the production and mineralization of DOM, is therefore also of great importance to assess the role of lakes in carbon cycling at regional and global scale (Bertilsson and Tranvik, 2000; Johannessen *et al.*, 2007). Although the importance of distribution and cycling of DOM is well known, the origins, transport and transformations are not well understood (Zhang *et al.*, 2009), especially in tropical environments.

The composition and concentration of DOM in aquatic ecosystems depends mainly on the spatial and seasonal variability of its sources and the availability of nutrients and radiation, which also affects the rates of photo- and microbial degradation (biodegradation) of these compounds (Bracchini *et al.*, 2009; Zhang *et al.*, 2011b). The spatial variation is given by the heterogeneity of the environment, as the presence or absence of macrophytes, different depths and the proximity to the borders which increases the impact by the input of allochthonous matter and nutrients by runoff (Wetzel, 1992; Tao, 1998; Obrador and Pretus, 2013). The seasonal variation derives from changes in DOM sources, such as higher input of allochthonous organic matter in the lake during the rainy season and the highest incidence of radiation in spring and summer, which can increase photodegradation rates of CDOM and still favor the lability of the molecules through the breakage part, facilitating microbial degradation (Bertilsson and Tranvik, 1998; Neale *et al.*, 2007; Fasching and Battin, 2012; Catalán *et al.*, 2013). Furthermore, CDOM degradation is also affected by the availability of nutrients throughout the year, a limiting factor for microbial degradation. In eutrophic lakes, degradation of phytoplankton is an important source of CDOM and regeneration of nutrients for bacteria and also the phytoplankton community, especially after seasonal blooms of these organisms (Bracchini *et al.*, 2009; Zhang *et al.*, 2009; Zhang *et al.*, 2013b).

The CDOM spectrophotometry and fluorescence spectroscopy are important tools in studies of composition and origin of organic matter (Helms *et al.,* 2008). There are currently some metrics extracted from the CDOM absorbance spectrum that provide information about the chemical characteristics and quality of its composition. The increase of the specific UV absorbance ($SUVA_{254;}$ mg C L^{-1} m^{-1}) indicates an increase of compounds of higher aromaticity (Weishaar *et al.*, 2003). The spectral slope $S_{275-295}$ (nm^{-1}) indicates different sources of CDOM and changes related to photodegradation and molecular size, while $S_{350-400}$ (nm^{-1}) indicates changes in CDOM related to biodegradation (Helms *et al.,* 2008). S_R is a ratio between both spectral slopes ($S_{275-295}$:$S_{350-400}$) and indicates the relationship between photodegradation and biodegradation of CDOM (Helms *et al.,* 2008). The estimated apparent molecular size (M) is obtained by the ratio of two absorption coefficients (a250:a365), based on the fact that declining ratio indicates increasing of molecular size (De Haan, 1987; Peuravuori and Pihlaja, 1997). In recent decades researchers have used these spectral metrics to investigate the optical properties in temperate aquatic systems (Zhang *et al.,* 2007, 2007b, 2009; Helms *et al.,* 2008; Bracchini *et al.,* 2010), but studies using this approach in tropical environments are still scarce and little is known to Brazilian aquatic systems (Teixeira *et al.,* 2011, 2013; Bittar *et al.,* 2015).

The aim of the study was to investigate the effects of CDOM sources on the light environment and nutrient availability in the two contrasting tropical freshwater systems. Specifically we focus on DOM properties obtained from the CDOM spectral absorbance (absorbance coefficient (a_{350}), spectral slopes ($S_{275-295}$, $S_{350-400}$), slope ratio (S_R), molecular size (M)).

Dom Helvécio-DH (Middle Rio Doce, MG, Brazil) is a warm monomictic, deep (max 39.5 m), dendritic, oligotrophic and natural lake located in a natural Atlantic forest. The input of DOM and nutrients to DH occurs via runoff during the rainy season, when the environment is thermally stratified (Petrucio *et al.,* 2005). However, most of the DOM remains below the thermocline due to temperature differences (Reynolds, 2009) and becomes dispersed into the entire water column during the mixing of water in the dry season, increasing the DOC and CDOM concentrations in surface waters (Brighenti *et al.*, 2015; Brandão *et al.,* in review). In contrast, Pampulha (Belo Horizonte, MG, Brazil) is a shallower (max 17 m) and less dendritic eutrophic reservoir with reoccurring phytoplankton blooms throughout the year, especially in the summers during thermal stratification. As in other eutrophic lakes, concentrations and quality of the DOM are strongly influenced by the high phytoplankton abundance (Tôrres *et al.,* 1998; Bracchini *et al.,* 2009; Zhang *et al.,* 2009; Zhang *et al.*, 2013b). Considering that the main source of dissolved organic matter in the eutrophic Pampulha reservoir comes from degradation of phytoplankton, especially after an algal bloom, while the main source of DOM in DH Lake comes from allochthonous material from the forest in rainy season, we hypothesized that: a) The different sources of DOM (allochthonous in DH and autochthonous in Pampulha) provide different optical qualities of CDOM, which alters the under-water light climate and the availability of nutrients; b) The lakes have seasonal differences in their inherent and apparent optical properties. The autochthonous organic matter in the Pampulha reservoir is expected to increase with the increase of phytoplankton abundance during higher availability of nutrients, which will reduce the attenuation of visible and ultraviolet radiation by algae and CDOM absorption and also light dispersion by the phytoplankton community. In opposition to this we expect that DH Lake will show higher water transparency during the rainy season because of intense photobleaching in the epilimnion, and because most of the organic matter coming from the catchment remains in the hypolimnion until the mixing in winter.

2. METHODS

2.1 Study area

Lake Dom Helvécio (DH) is located inside the Rio Doce State Park - PERD (Middle Rio Doce, MG, Brazil). The lake complex in the Middle Rio Doce is one of the most important districts of lakes in Brazil, with approximately 130 lakes, 42 of them located inside the PERD area, which is the largest remnant of the Atlantic Forest in Minas Gerais, totaling 36000 ha (Maia-Barbosa *et al.*, 2010) (Fig. 1a). This lake is one of the deepest natural lakes in Brazil (maximum depth: 39.5 m), surrounded by Atlantic forest. It is oligotrophic (total phosphorus: 3.0-22.0 µg L^{-1}, annual mean 11.2 µg L^{-1}; Chlorophyll-a - Chl-a: 0.5-11.0 µg L^{-1}, annual mean 4.5 µg L^{-1}), has a perimeter of 37.7 km, area equal to $5.27x10^{6}$ m^{2} and volume equal to $5.94x10^{7}$ m^{3} (Bezerra-Neto and Pinto-Coelho, 2008). DH is a warm monomictic lake, with a stable thermal stratification period beginning in September and lasting until May (rainy period) and a mixed period from June to August (dry period) (Barbosa and Tundisi, 1980; Henry and Barbosa, 1989; Briguenti *et al.*, 2015).

The Pampulha reservoir was inaugurated in 1938 and rebuilt in 1957 following a dam rupture, and is together with its architectural set an important tourist area in the metropolitan region of Belo Horizonte, Minas Gerais, Brazil. This reservoir is eutrophic (total phosphorus: 135-819 µg L^{-1}, annual mean 6500 µg L^{-1}; Chl-a: 10-315 µg L^{-1}, annual mean 75 µg L^{-1}), has an original perimeter of 21 km, maximum depth of 17 m, and receives domestic and industrial sewage and other pollutants that contribute to poor water quality and exacerbate eutrophication (Champs, 1992; Novais, 1992, Pinto-

Coelho, 1998, Resck *et al.*, 2007) (Fig. 1b). Pampulha remains thermally stratified between September and April (rainy period) and the mixing water begins in May until August (dry period) (Figueredo and Giani, 2001; Pinto-Coelho *et al.*, 2003).

2.2 Field measurements and laboratory analysis

The sampling was carried out in January (rainy summer) and August (dry winter) 2013 in 21 sampling stations in DH Lake and in March (rainy autumn) and July (dry winter, during a bloom of cyanobacteria) 2013 in 16 sampling stations in Pampulha reservoir (Fig. 1a-b).

For characterization of the systems vertical profiles of water column temperature (°C) at the central point were measured every meter from surface to the bottom using a probe Hydrolab DS5 (Hach Inc.).

Vertical profiles of photosynthetically active radiation (PAR) and ultraviolet radiation (UV) (between 10:00 and 14:00) were performed using a radiometer BIC (Biospherical Instruments) in each site sampling. The profiles were made on the sun side of the boat avoiding shadows and measurements during days with great clouds were avoided. The radiometer was attached to a battery and notebook with the software LoggerLight ™ (Biospherical Intruments) while collecting radiation data PAR and UV (305, 340 nm) and depth during descent and ascent up of the probe.

Water samples (0.5 m from the surface) were collected using a van Dorn bottle (5L). These samples were filtered immediately after sampling for Chl-a (0.47μm filter) and TSM (AP040 filter) and the filters were frozen until analysis. Water samples were also filtered for analysis of dissolved organic carbon (DOC) and colored dissolved organic matter (CDOM) (0.22 μm Millipore filter) and stored in amber glass bottles (pre-washed with distilled water and hydrochloric acid 10%) at 4°C in the dark. The chlorophyll-a concentration corrected by pheophytin (Chl-a) was obtained by acetone extraction (90%) measured in a spectrophotometer (UV-VIS Shimadzu) at 665 and 750 nm and calculated according to APHA (1998). The DOC concentration (mg L^{-1}) was obtained by catalytic oxidation method of high temperature using TOC Analyzer (Shimadzu TOC – 5000). Total suspended solids (TSM) were determined by the gravimetric method, considering the difference between the dry weight of AP40 Millipore filters (105 °C for 2 hours) before and after the filtration of water samples (APHA, 1998).

Unfiltered water samples were taken for analysis of total nitrogen - TN (TOC Analyzer, Shimadzu TOC – 5000) and total phosphorus - TP (according to Mackereth *et al.*, 1978).

2.3 Calculation of the optical properties

The diffuse PAR and UV attenuation coefficients ($K_{d(PAR)}$ and $K_{d(UV)}$) were calculated as the linear slope between depth and the natural logarithm of the measured radiation.

Absorption spectra were obtained between 250 nm and 700 nm at 1 nm intervals in a spectrophotometer (UV-VIS Shimadzu) using 5 cm quartz cuvette and Milli-Q water as a reference. The absorption spectra of each sample was performed in triplicate and determined using the Spectrum Pack Program software (Shimadzu Inc.). The absorption coefficients (aλ) were derived from absorbance measurements (Aλ) according to the equation $a(\lambda)m^{-1} = 2{,}303A(\lambda)L^{-1}$, where L is the optical path of the cuvette (in meters). Absorption coefficients were corrected for backscattering by subtracting the value of the coefficient at 700 nm. The spectrophotometric absorption coefficient at 350 nm (m^{-1}) was used as an index of CDOM concentration (Zhang *et al.,* 2011b).

The spectral slope S (nm^{-1}) was calculated for wavelengths between 275-295 nm and 350-400nm (Helms *et al.,* 2008) and it was estimated using linear adjustment technique between log values of the absorption coefficients and the specific wavelength range. The slope ratio (S_R) was obtained by the equation $S_R = S_{275\text{-}295nm} / S_{350\text{-}400nm}$, where S is the spectral slope. The estimated apparent molecular size (M) was obtained by the ratio of the absorption coefficients at 250 and 365 nm (a250 / a365) (De Haan, 1987; Peuravuori and Pihlaja, 1997). The specific UV absorbance ($SUVA_{254}$) (mg C L^{-1} m^{-1}) was calculated dividing the value of the absorption coefficient at 254 nm by the concentration of DOC (mg L^{-1}) (Weishaar *et al.,* 2003).

2.4 Statistical Analysis

Differences in parameters between seasons were assessed by independent sample t-test (for parametric data) and Mann-Whitney (for non parametric data) (significant $p < 0.05$), and differences between freshwater systems were assessed with Two-way Analysis of Variance (significant $p < 0.05$). Pearson correlations were made to examine the relationships between all the parameters measured (significant $p < 0.05$).

3. RESULTS

3.1 Differences between the two contrasting freshwater systems

The hyper eutrophic Pampulha reservoir had significantly higher concentrations of nutrients (21 times higher for TN and 28 for TP), Chl-a (5.8 times) and DOC (1.4 times) compared to the oligotrophic DH Lake. As Pampulha also had higher $CDOM_{350}$ absorbance (3.3 fold higher), both PAR and UV attenuation was significantly higher than in DH. Indices of CDOM quality or composition furthermore indicated DOM of different origin as Pampulha had DOC of higher specific UV absorbance ($SUVA_{254}$), smaller spectral slopes ($S_{275\text{-}295}$, $S_{350\text{-}400}$), lower spectral slope ratio (S_R) and higher molecular size of CDOM (lower M) than DH (Tabs. 1 and 2, Figs. 2a-d and 3a-d). CDOM absorption was generally higher over the entire spectrum in Pampulha, especially in the UV range (Fig. 4a-b).

3.2 Seasonality in optical properties

Concentration of nutrients, Chl-a and DOC were significantly higher in the fully mixed dry season in DH (Fig. 2a-d). The PAR, UV-A and UV-B attenuation were also higher during dry period (Fig. 5a). In this lake, only $SUVA_{254}$ and M values (Fig. 3a) were higher in rainy period. In Pampulha we found that concentrations of TP (Fig. 2b), PAR and UV attenuation (Fig. 5b) were significantly higher during the rainy season. Only Chl-a and TN concentrations were higher in dry period (Fig. 2d) in this reservoir (Tabs.1 and 2).

Analyzing the CDOM absorption spectrum at 21 points in DH and at 16 in Pampulha, during a rainy and a dry period we noticed that the spatial variation in each environment was smaller during the rainy period compared with the dry period. For DH, the mean absorption spectrum (black line in Fig. 4a) was higher in the dry period for absorption at wavelengths shorter than 350 nm. For Pampulha the mean absorption (black line in Fig. 4b) was equal in both seasons.

Significant and positive Pearson correlation was observed to DH between $S_{350\text{-}400}$ and nutrients concentrations (TN; $r = 0.52$, $p < 0.015$; TP; $r = 0.82$, $p < 0.000$) only for rainy period data, which indicates that biodegradation might be limited by nutrients in this time. In the Pampulha reservoir, we found significant Pearson correlations between $CDOM_{350}$ and Chl-a in the dry period ($r = 0.88$, $p < 0.000$), when there was an algal bloom. The $CDOM_{350}$ was also positively correlated with the coefficients of attenuation of PAR only in the dry period ($K_{d(PAR)}$; $r = 0.80$, $p < 0.000$) and with UV radiation in both periods (dry period: $K_{d(305)}$; $r = 0.81$, $p < 0.000$; $K_{d(340)}$; $r = 0.90$, $p < 0.000$; rainy period: $K_{d(305)}$; $r = 0.52$, $p = 0.037$; $K_{d(340)}$; $r = 0.76$, $p < 0.000$). In addition, the TSM was positively correlated with Chl-a only in the dry period ($r = 0.91$, $p < 0.000$).

4. DISCUSSION

4.1 Sources of DOM and underwater light climate

The large difference between the quantity and quality of DOM in the two studied freshwater systems indicated a terrestrial input of organic matter in DH Lake occurring mostly during the rainy period in the summer, compared to a strong autochthonous phytoplankton contribution in Pampulha reservoir related to an algal bloom especially evident in the dryer winter period.

The differences in the $S_{275-295}$ values between the systems suggest that DOM originated from different sources (Helms *et al.,* 2008). The increase of aromatic DOC (higher SUVA) during rainy period in DH strongly suggests an allochthonous source of DOM from the forested catchment around DH primarily entering the lake during this time. In Pampulha, increases in CDOM absorbance was positively correlated with increasing of Chl-a during an algal bloom in dry winter indicating an autochthonous input of DOM. Other studies have linked DOM source from phytoplankton degradation considering the positive correlations between CDOM absorption and Chl-a (Kahru and Mitchell, 2001; Rochelle-Newall and Fisher, 2002; Zhang *et al.*, 2013b).

The DOM source affects some characteristics of lakes, as their transparency to UV and PAR radiation, and recently some studies have compared the differences in optical quality between DOM from autochthonous and allochthonous sources (Spencer *et al.*, 2009; Bracchini *et al.,* 2010; Shank *et al.,* 2010; Catalán *et al.,* 2013; Zhang *et al.,* 2013). The PAR and UV attenuation in Pampulha were much higher than in DH. In

Pampulha we observed that the predominantly autochthonous DOM was coupled to the attenuation of PAR (positive correlation between $CDOM_{350}$ and PAR attenuation coefficients only in dry period) and ultraviolet radiation (positive correlations in dry and rainy period). A similar relation was not found for the allochthonous DOM in DH, and it was already observed for this lake that PAR and UV attenuation were strongly dependent on light absorption by particulate matter (tripton and phytoplankton) while CDOM only contributed with less than 22% in total attenuation during monthly and spatial sampling in 2013 (Brandão *et al.,* in review). However, when the CDOM comes from phytoplankton degradation, as in eutrophic environments like Pampulha, it may greatly increase its contribution to the attenuation of radiation and CDOM absorbance ($CDOM_{350}$ higher in Pampulha) during blooms (Zhang *et al.,* 2007). The different DOM sources in these systems also alter the importance of DOM degradation processes, as allochthonous terrestrial carbon is known to be more humic consisting of heavier and more aromatic molecules being typically more photodegraded. In contrast, the autochthonous microbial/algal derived DOM consisted as expected by simpler molecules of low molecular weight, less affected by photodegradation but more susceptible to biodegradation (McKnight *et al.*, 1994; Benner, 2002; Catalán *et al.*, 2013, Zhang *et al.*, 2013b).

4.2 Seasonality of optical properties between the contrasting systems

Seasonal variations were observed for nutrients and Chl-a concentration and UV and PAR attenuation for both systems and also seasonal differences only to DH Lake in relation to the DOC concentration and the quality of CDOM ($SUVA_{254}$ and M). Seasonal changes in the quality and quantity of DOM in DH suggest linkages with rainfall events and water column mixing. In contrast, the reduced seasonality in Pampulha seems attributed to reoccurring algal blooms in this reservoir, which causes several events of autochthonous production of DOM throughout the year, although most prevailing during the well mixed dryer winter period.

As we expected, the water column in DH was most transparent (both PAR and UV radiation) during the rainy season when the water column was stratified and with DOM components of a more aromatic structure (higher SUVA) and more susceptible to photobleaching (Weishaar *et al.*, 2003; Helms *et al.*, 2008; Catalán *et al.*, 2013; Zhang *et al.*, 2013). Positive correlations between $S_{350\text{-}400}$ and nutrients (TP and TN) only during the rainy season suggest that microbial degradation in the epilimnion might be limited by nutrients during thermal stratification. Nutrient limitation of primary production during the rainy season in DH Lake was observed by Brighenti *et al.* (2015) in a two year study of lake metabolism. In agreement with this Chl-a concentrations were lower in the rainy period in DH. Furthermore, nutrient limitation of phytoplankton growth raises light transparency and is expected to explain elevated levels of photoinhibition during summer (Brighenti *et al.*, 2015). While our results showed that the increase of aromatic DOM originates from surface runoff during rainy period, DOC and nutrients concentrations were lower in the epilimnion in this period, indicating that much of the newly introduced DOM remained in the hypolimnion until later mixing of the water column in the dry and cooler winter period (Reynolds, 2009), when their concentrations increased in the water surface (Barbosa and Tundisi, 1980; Petrucio and Barbosa, 2004). Corroborating with this, we observed DOM characterized by higher molecular size compounds (lower M) during the mixed winter period (Peuravuori and Pihlaja, 1997; Helms *et al.*, 2008). Importantly, most of the sampling in DH Lake was made in areas where total depth exceeded 10 meters thus allowing a stable hypolimnion to develop over longer period of anoxia during thermal stratification from September to

May. Such conditions seem favorable for the changes in DOM quality observed after full mixing of the water column.

Contrary to what we expected, the Pampulha reservoir was most transparent (both UV and PAR radiation) in the dry mixing winter although it did experience a bloom of cyanobacteria. The bloom in this period can be explained by the higher concentration of nitrogen and the lower water level (approximately 1 meter), and also by the high incidence of solar radiation throughout the year. We expected that an increase of phytoplankton would increase the light attenuation coefficients (K_d), especially the $K_{d(PAR)}$, but the opposite occurred in Pampulha. Although at high concentrations, the autochthonous carbon from phytoplankton degradation tends to be less chromophoric and attenuates radiation less, especially in the UV range (McKnight *et al.*, 1994; Benner, 2002). The concentration of TSM during the dry-mixing period was however, lower than in the rainy season, during which TSM was uncorrelated with Chl-a and accordingly a greater amount of non-algal materials (tripton) occurred in the TSM. In agreement with other studies it therefore seems that tripton must have accounted for most of the PAR attenuation during the rainy stratified summer (Phlips *et al.*, 1995; Christian and Sheng, 2003; Zhang *et al.*, 2007b; Brandão *et al.*, in review).

5. CONCLUSIONS

As the presence of chromophoric carbon in the water protects the aquatic organisms from harmful ultraviolet radiation and from the excessive incidence of solar radiation (Zhang *et al.*, 2007), our results suggest that the photoinhibition of phytoplankton primary production observed in DH during summer by Brighenti *et al.* (2015) is directly linked with the seasonal pattern of photobleaching of CDOM and nutrients availability, where both dependent of the sources and quality of DOM. Thereby, we demonstrated that the main source of DOM in DH come from the terrestrial input during the rainy period, reinforcing the importance of preserving the original forest surrounding the lakes to the global carbon cycle. In Pampulha reservoir we observed that degradation of phytoplankton is an important source of DOM and most likely also to the regeneration of nutrients, which has already been demonstrated for other eutrophic systems (Zhang *et al.*, 2009; Zhang *et al.*, 2013; Zhang *et al.*, 2013b). We also found that autochthonous production of CDOM can increase the PAR and UV attenuation, as observed in the positive correlations between CDOM and attenuation coefficients only in Pampulha and during an algal bloom.

In conclusion, our results documents that tropical freshwater systems can have a clear seasonality in carbon quality and concentration related to trophic status and seasonal differences in DOM inputs, degradation and distribution in the water column. Our finding supports recent findings of a strong seasonality in primary production and organic matter decomposition in tropical lakes and has implications on our understanding of PAR and UV attenuation and water quality in such systems.

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Figures and Tables

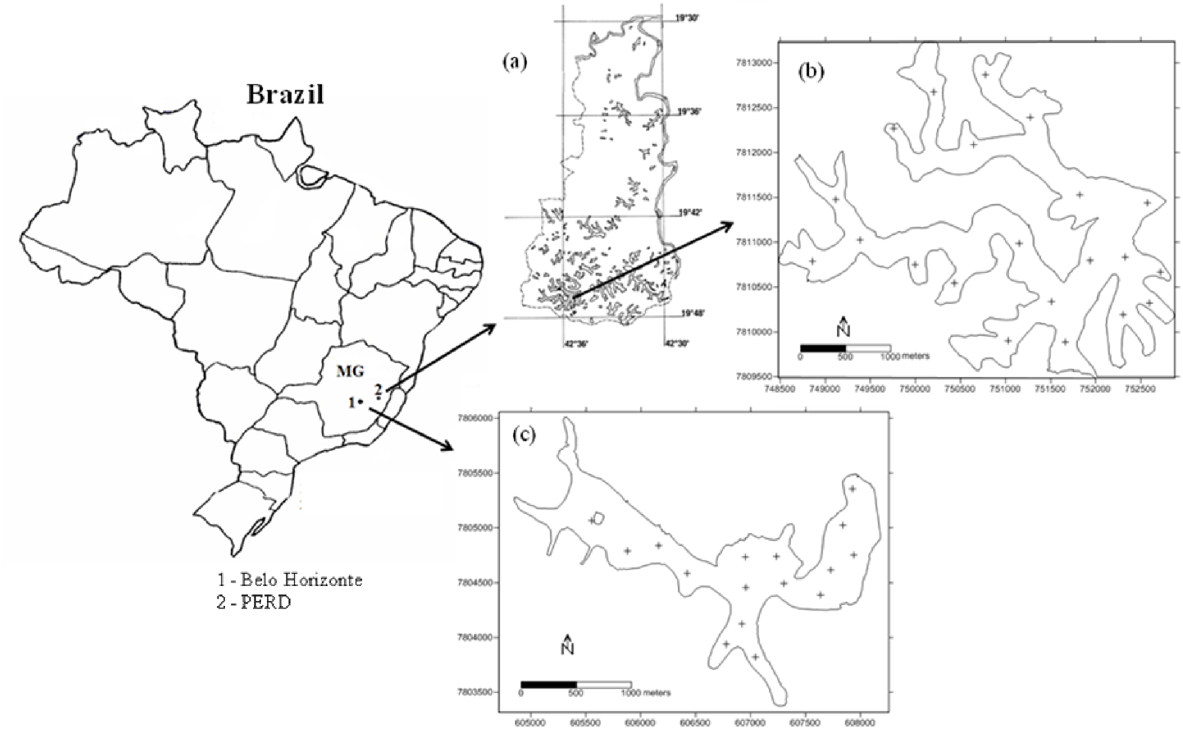

Fig. 1 Location at (a) Rio Doce State Park (PERD) and (b) Dom Helvécio lake, and (c) Pampulha reservoir in Belo Horizonte, Minas Gerais (MG), Brazil.

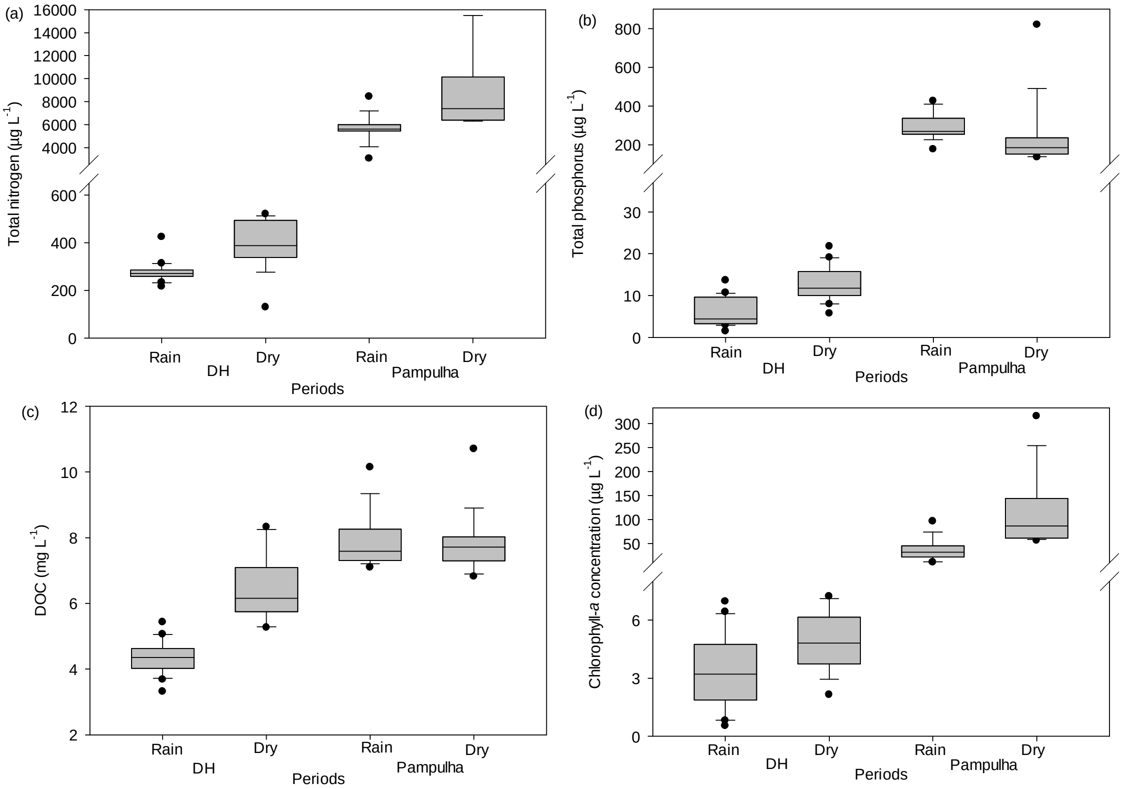

Fig. 2 Seasonal variations (center line-median, outer grey-5th/95th percentiles, bars-95% confidence level, black circles- outlier) in the concentrations of (a) total nitrogen, (b) total phosphorus, (c) dissolved organic carbon and (d) chlorophyll-*a* at 21 sites in Dom Helvécio lake and 16 in Pampulha reservoir collected in a dry and rainy period in 2013.

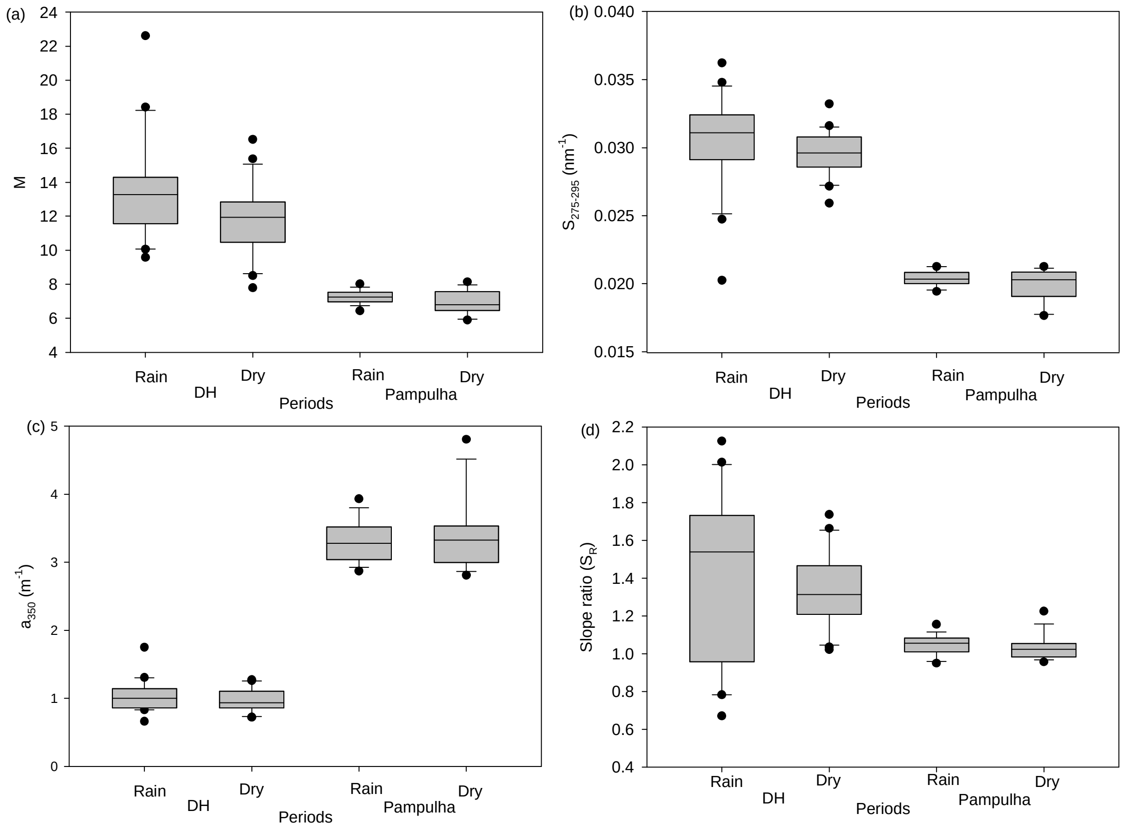

Fig. 3 Seasonal variations (center line-median, outer grey-5th/95th percentiles, bars-95% confidence level, black circles- outlier) in (a) M, (b) spectral slope $S_{275-295}$, (c) absorbance of colored dissolved organic matter at 350 nm (a_{350}) and (d) slope ratio S_R at 21 sites in Dom Helvécio lake and 16 in Pampulha reservoir collected in a dry and rainy period in 2013.

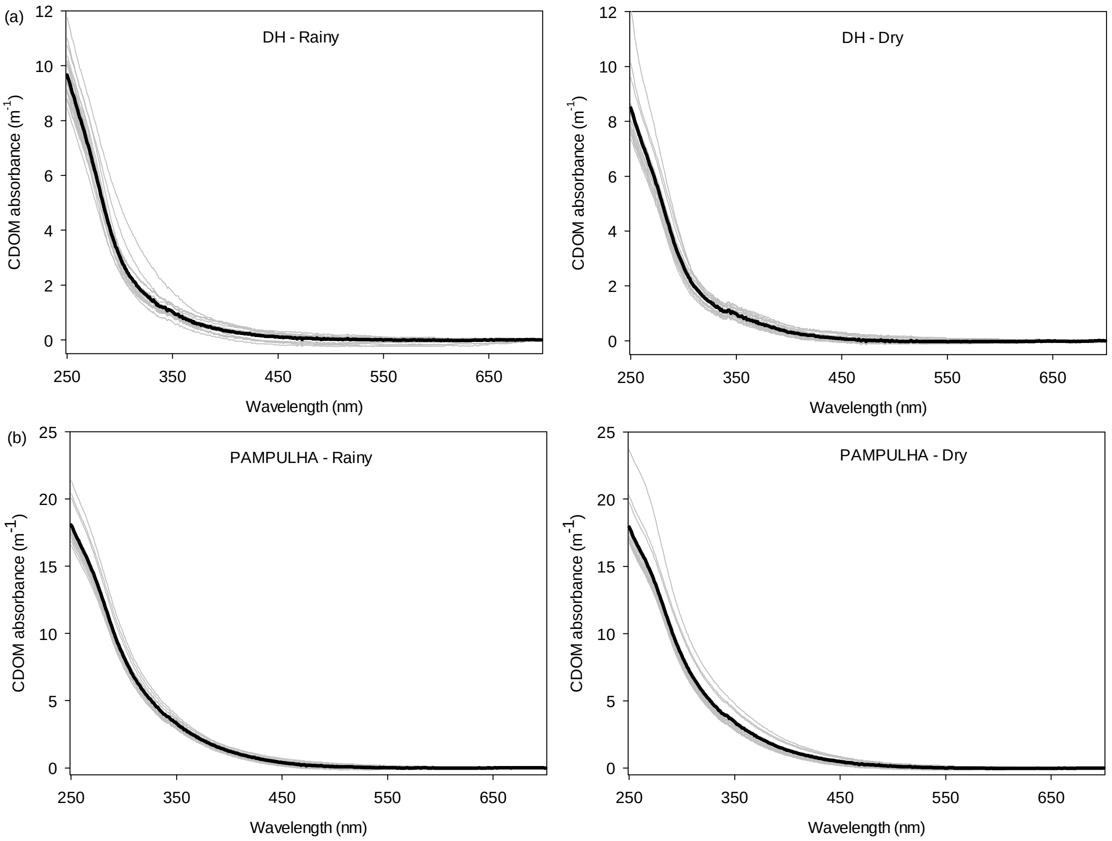

Fig. 4 – Spectral absorbance curves of CDOM (a) at 21 sampling sites (gray lines) in Dom Helvécio and the mean curve (black line) and (b) at 16 sampling sites (gray lines) in Pampulha and the mean curve (black line).

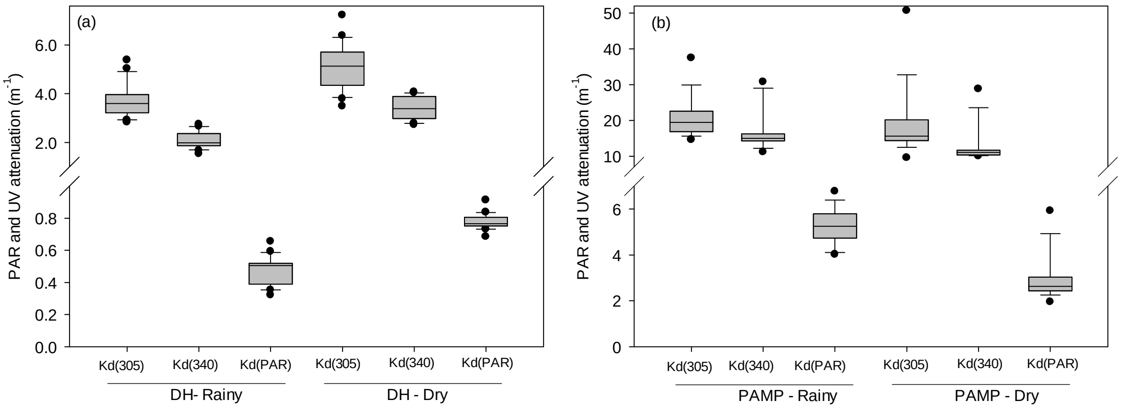

Fig. 5 - Seasonal variations (center line-median, outer grey-5th/95th percentiles, bars-95% confidence level, black circles- outlier) in the UV(305), UV (340) and PAR radiation at (a) 21 sites in Dom Helvécio lake and (b) at 16 in Pampulha reservoir collected in a dry and rainy period in 2013.

Table 1 - Summary of parameters measured during spatial-seasonal analyzes in Dom Helvécio lake and Pampulha reservoir, MG, Brazil. (n = number of sampling stations)

		Dom Helvécio lake		Pampulha reservoir	
		Rainy (n=21)	**Dry (n=21)**	**Rainy (n=16)**	**Dry (n=16)**
Surface Temperature (ºC)	Min-Max	30.0	25.4	26.0 - 28.3	22.5 - 23.7
	Mean ± SD	30 ± 0	25.4 ± 0	27.4 ± 0.8	23.3 ± 0.5
Water column depth (m)	Min-Max	4.5 - 30.0	2.9 - 30.0	1.0 - 10.0	0.6 - 9.5
Total suspended matter (mg L^{-1})	Min-Max	0.4 - 2.1	0.6 - 2.8	16.7 - 31.2	3.2 - 24.2
	Mean ± SD	1.4 ± 0.4	1.7 ± 0.6	24.7 ± 3.6	7.2 ± 5.6
Total nitrogen (µg L^{-1})	Min-Max	215.5 - 424.1	129.0 - 519.7	3000.0 - 8400.0	6300.0 - 15500.0
	Mean ± SD	276.8 ± 41.0	393.2 ± 98.9	5680.0 ± 1100.0	8615.0 ± 3300.0
Total phosphorus (µg L^{-1})	Min-Max	1.4.13.6	5.7-21.8	176.3 - 425.9	135.3 - 819.2
	Mean ± SD	6.0 ± 3.4	12.7 ± 4.1	292.3 ± 67.7	232.8 ± 165.5
Chlorophyll *a* (µg L^{-1})	Min-Max	0.5 - 6.9	2.1 - 7.2	10.7 - 96.2	56.1 - 315.4
	Mean ± SD	3.3 ± 1.9	4.8 ± 1.5	35.6 ± 21.4	111.6 ± 71.8
DOC (mg L^{-1})	Min-Max	3.3 - 5.4	5.3 - 8.3	7.1 - 10.1	6.8 - 10.7
	Mean ± SD	4.4 ± 0.5	6.5 ± 1.0	7.9 ± 0.8	7.8 ± 0.9
a(350) (m^{-1})	Min-Max	0.7 - 1.8	0.7 - 1.3	2.9 - 3.9	2.8 - 4.8
	Mean ± SD	1.0 ± 0.2	1.0 ± 0.2	3.3 ± 0.3	3.4 ± 0.6
$Kd_{(PAR)}$ (m^{-1})	Min-Max	0.3 - 0.7	0.7 - 0.9	4.0 - 6.8	1.9 - 5.9
	Mean ± SD	0.5 ± 0.1	0.8 ± 0	5.2 ± 0.8	3.0 ± 1.0
$Kd_{(UV\text{-}A\ 340)}$ (m^{-1})	Min-Max	1.5 - 2.7	2.7 - 4.1	11.1 - 30.7	10.0 - 28.8
	Mean ± SD	2.1 ± 0.3	3.4 ± 0.5	16.4 ± 5.3	13.1 ± 5.3
$Kd_{(UV\text{-}B\ 305)}$ (m^{-1})	Min-Max	2.8 - 5.4	3.5 - 7.2	14.6 - 37.5	9.5 - 50.7
	Mean ± SD	3.7 ± 0.7	5.1 - 0.9	20.7 ± 5.6	18.6 ± 9.4
$S_{275\text{-}295}$ (nm^{-1})	Min-Max	0.02 - 0.04	0.03 - 0.03	0.02 - 0.02	0.02 - 0.02
	Mean ± SD	0.03 ± 0	0.03 ± 0	0.02 ± 0	0.02 ± 0
$S_{350\text{-}400}$ (nm^{-1})	Min-Max	0.01 - 0.05	0.02 - 0.03	0.02 - 0.02	0.02 - 0.02
	Mean ± SD	0.02 ± 0.01	0.02 ± 0	0.02 ± 0	0.02 ± 0
S_R	Min-Max	0.7 - 2.1	1.0 - 1.7	1.0 - 1.2	1.0 - 1.2
	Mean ± SD	1.4 ± 0.4	1.3 ± 0.2	1.0 ± 0.1	1.0 ± 0.1
SUVA (mg C L^{-1} m^{-1})	Min-Max	1.8 - 2.5	0.9 - 1.8	2.0 - 2.4	1.6 - 3.0
	Mean ± SD	2.1 ± 0.2	1.3 ± 0.3	2.2 ± 0.1	2.2 ± 0.3
Molecular size (M)	Min-Max	9.6 - 22.6	7.8 - 16.5	6.4 - 8.0	5.9 - 8.1
	Mean ± SD	13.7 ± 3.1	11.8 ± 2.1	7.3 ± 0.4	7.0 ± 0.7

Table 2 – Results of the two-way analysis of variance comparing the seasons (rainy and dry) and the freshwater systems (DH = Dom Helvécio; PA = Pampulha) and results of the *t*-test and Mann-Whitney comparing the seasons within each system.

	Two-way Analysis of Variance (F value) df = 1			***t*-test / Mann-Whitney**	
	Systems (DH x PA)	**Seasons (Rainy x Dry)**	**Seasons and systems interactions**	**DH (Rainy x Dry)**	**PA (Rainy x Dry)**
Total nitrogen (µg.L^{-1})	425.45***	21.33***	18.20***	*** 2	** 2
Total phosphorus (µg.L^{-1})	169.67***	1.84	2.90	*** 1	*** 2
Chlorophyll *a* (µg L^{-1})	10.68**	5.25*	5.03*	** 1	*** 2
DOC (mg. L^{-1})	155.63***	25.76***	32.83***	*** 2	ns 2
a(350) (m^{-1})	887.87***	0.26	1.32	ns 2	ns 2
Kd$_{(PAR)}$ (m^{-1})	651.92***	48.44***	85.07***	*** 1	*** 2
Kd$_{(UV\text{-}A\ 340)}$ (m^{-1})	217.67***	1.53	8.26**	*** 2	** 2
Kd$_{(UV\text{-}B\ 305)}$ (m^{-1})	163.16***	0.08	2.16	*** 1	* 2
S$_{275\text{-}295}$ (nm^{-1})	383.27***	1.85	0.11	ns 2	ns 1
S$_{350\text{-}400}$ (nm^{-1})	9.86**	0.21	0.07	ns 2	ns 1
S$_R$	28.58***	0.63	0.35	ns 1	ns 1
SUVA (mg C L^{-1} m^{-1})	4.26***	2.55***	2.89***	*** 1	ns 2
Molecular size (M)	140.91***	5.54*	2.85	** 2	ns 1

*p < 0.05 **p < 0.01 ***p < 0.001 ns = not significant

1 = parametric data and *t*-test was used

2 = non-parametric data and Mann-Whitney was used

CAPÍTULO 4

Formatado para ser submetido em: *Biogeochemistry*

Distinctive effects of allochthonous and autochthonous organic matter on colored dissolved organic matter spectra

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Abstract

Despite the increasing understanding about differences in carbon cycling between temperate and tropical freshwater systems, our knowledge on the importance of organic matter (OM) pools on light absorption properties in tropical lakes is very scarce. We performed a mesocosm factorial experiment in a natural tropical lake (MG, Brazil) to evaluate the effects of increased concentrations of allochthonous and autochthonous OM on the light absorption of CDOM. We manipulated inorganic nutrients (to stimulate phytoplankton growth and OM autochthonous production), allochthonous OM and shading. DOC, CDOM, Chl-a, nutrients and total suspended solids (TSM) were measured every 3 days to evaluate how they responded to these treatments. CDOM quality was obtained by spectral indexes ($S_{250-450}$, $S_{275-295}$, $S_{350-450}$, slope ratio-S_R and $SUVA_{254}$). The effect of carbon sources on the CDOM spectra was investigated through principal component analysis (PCA) and a redundancy analysis (RDA). As expected, phytoplankton biomass (Chl-a as a proxy) was stimulated by addition of nutrients and the CDOM quality related to different sources was different between experimental units with addition of nutrients and with allochthonous OM. Spectral indexes ($S_{250\text{-}450}$ and S_R) were only affected by the addition of allochthonous OM. The PCA showed that enrichment by allochthonous carbon had a strong effect on the CDOM curve between 300 and 400 nm, while the increase of autochthonous carbon increased absorption at shorter wavelengths. In turn, the concentration of OM from different sources was linked to the availability of nutrients and allochthonous OM, which under natural conditions vary seasonally with rainfall and mixing conditions.

Keywords: DOC, DOM, mesocosm, carbon source, spectral indexes

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Introduction

Organic matter (OM) is a central theme in ecology and influence many processes from bacterial metabolism to primary production. In aquatic systems OM consists of particulate organic matter (POM; organic compounds represented by aquatic communities and detritus), and dissolved organic matter (DOM – in most of DOM studies it is the compounds smaller than 0.2 or 0.7 µm) which is far the largest pool of organic carbon in most of aquatic systems (Hedges 1992). A better understanding of carbon cycling in aquatic systems and their regional and global importance therefore requires knowledge of the physical, chemical and biological processes involved in the production and the mineralization of DOM (Bertilsson and Tranvik 2000; Johannessen et al. 2007; Tranvik et al. 2009).

The main sources of DOM in aquatic ecosystems can be divided into two main pools according to their sources. The allochthonous pool which comes from terrestrial vegetation and soil sources (Kieber et al. 2006; Miller et al. 2009) and the autochthonous pool which is produced mainly by aquatic primary producers (Kritzberg et al. 2004; Guillemette and Del Giorgio 2012). These two pools of DOM have fundamental differences in their optical and chemical characteristics which in turns influence the mechanisms by which DOM is degraded (Wetzel et al. 1995; Bertilsson and Tranvik 2000).

The allochthonous DOM is in general more susceptible to photodegradation because it contains aromatic compounds of higher molecular weights which strongly absorb UV light (Amon and Benner 1994; McKnight et al. 1994; Benner 2002; Helms et al. 2008). The autochthonous DOM originating from phytoplankton mainly consists of simple molecules (carbohydrates, proteins, amino acids) of low molecular weight and it is more labile for microbial community (Farjalla et al. 2009; Fonte et al. 2013). However, autochthonous DOM originating from macrophytes or macroalgae can be as aromatic and recalcitrant as DOM from terrestrial vegetation (Catalán et al. 2013).

Photochemical and microbial degradation are important processes driving the dynamics of DOM in aquatic ecosystems (Roland et al. 2010; Mopper et al. 2015). The rate of photodegradation depends on a combination of available sunlight and the chemical quality of DOM (Benner 2002) whereas the microbial degradation rate depends on DOM bioavailability and utilization efficiency of the bacterial community (Catalán et al. 2013; Asmala et al. 2014). Photodegradation is also an important process transforming DOM to ammonia and other highly bioavailable inorganic compounds (Aarnos et al. 2012) which can be an important nutrient supply for both phytoplankton (Hessen and Tranvik 1998) and heterotrophic bacterial communities (Kieber et al. 1989; Miller et al. 2002; Lønborg et al. 2010). Exposure to light transforms DOM into smaller molecules and depending on its origin the photoreactions can reduce (autochthonous DOM) or increase (allochthonous DOM) bioavailability (Pérez and Sommaruga 2007; Vahatalo and Wetzel 2008; Catalán et al. 2013). The mineralization of CDOM also causes direct CO_2 evasion to atmosphere influencing the global carbon cycle (Arrigo et al. 2011) and it is an important parameter to the management of water quality and eutrophication control (Zhang et al. 2011).

Microbial degradation is another pathway by which heterotrophic organisms convert DOM in to POM which can be assimilated by protozooplankton via the microbial loop (Azam et al. 1983). However, the ability of bacteria to degrade recalcitrant DOM is expected to increase with availability of labile molecules due to the “priming effect” phenomenon (Bianchi 2011), although some studies have contradicted this (Catalán et al. 2015). Additionally, biodegradation is stimulated by inorganic nutrients, mainly nitrogen and phosphorus, which increase the bacterial growth efficiency (Zweifel et al. 1995; Asmala et al. 2013) by reducing the energetic cost of nutrient acquisition (Hopkinson et al. 1998).

Some recent studies have demonstrated that the effect of photodegradation and biodegradation on the absorption properties of CDOM was not constant over the spectral range, thus influencing the shape of the absorption curve (Helms et al. 2013; Reader et al. 2015). As modifications in the spectral shape reflect underlying changes in the carbon compounds at the molecular level, studies on biological and chemical effects on CDOM spectra allows a better understanding of the DOM transformations and how this links to overall carbon cycling in aquatic ecosystems (Stubbins et al. 2014).

Tropical regions are subjected to intense solar radiation and high temperatures throughout the year, which accelerates the biodegradation and photodegradation processes. However, there are only few studies on the photochemical (Teixeira et al. 2013; Bittar et al. 2015) and bacterial (Farjalla et al. 2002; 2009; Roland et al. 2010) degradation of DOM in tropical environments compared to temperate freshwater systems and estuaries (Anesio and Granéli 2003; Bertilsson and Tranvik 2000; Boreen et al. 2008; Asmala et al. 2014; Attermeyer et al. 2015).

In this study, we performed a mesocosms experiment in the tropical lake, manipulating nutrients, OM and light conditions, in order to answer the following main question: what are the effects of increased inputs of allochthonous and autochthonous DOM on changes in lacustrine CDOM spectra? Also, we evaluated the effects of photodegradation on the different sources of DOM by manipulating the availability of sunlight. We expect that addition of nutrients causes algal growth, which increases the production of autochthonous DOM (Schindler 1977; Lean and Pick 1981) until nutrients become exhausted. In contrast, the addition of extracted organic matter from leaves of the native forest surrounding the lake is done to increase the allochthonous DOM concentration. Variable levels of OM with different origin are finally expected to change the CDOM absorption spectra and the associated indexes over time. Moreover, we expect that the light reduction will decrease the DOM degradation because of the reduction of photodegradation.

Methods

Study area

This study was conducted in Carioca Lake (19°45’26.0”S; 42°37’06.2”W) located in the southern part of the State Park of Rio Doce (PERD, Minas Gerais, Brazil) which is the largest remnant of the Atlantic Forest in Minas Gerais (36000 ha forest) with lakes occupying 9.8% (3530 ha) of its total area. This lake is surrounded by secondary Atlantic Forest and is a warm-monomictic lake with a mixing period during the dry winter (May to August) and thermal stratification during the rest of the year (September to April). It is a mesotrophic lake (total phosphorus: 5.6-21.4 $\mu g\ L^{-1}$, epilimnion annual average 13.9 $\mu g\ L^{-1}$; chlorophyll-a: 1.3-16.6 $\mu g\ L^{-1}$, epilimnion annual average 7.7 $\mu g\ L^{-1}$) (Petrucio et al. 2006; Brighenti 2014), with 1718.6 m of perimeter, 14.1 ha, $671x10^3$ m^3, and maximum depth of 11.8 and average depth of 4.8 m (Bezerra-Neto et al. 2010). Carioca is one of the lakes that have been monitored for water quality and aquatic biota since 2000 through the Brasil-LTER Programme (PELD-CNPq Proc. 403698/2012-0). It is known that the input of DOM and nutrients in Carioca Lake occurs via runoff during the rainy season, but most of them remain below the thermocline due to temperature differences (Reynolds 2009). During the mixing period, DOC, CDOM and nutrients become distributed in the water column, increasing their concentrations and availability in the surface layer, thus increasing primary production and respiration rates (Brighenti et al. 2015).

Experimental design and measurements

To test the effect of organic matter inputs, sunlight, and nutrients on DOM degradation, we conducted an *in situ* experiment using a total of 16 cylindrical mesocosms (diameter 1.3 m, height 1.5 m and volume 2 m^3) with eight different combinations (two replicates for each combination). The sampling has been carried out between January 20th and February 1st 2015 with daily measurements occurring between 10:00 am and 12:00 pm. Water samples (3L at 0.5 m from the surface) from mesocosms were collected every three days. The mesocosms setup was based on a 2^3 factorial design as follows: 1) with and without addition of organic matter extracted from leaves surrounding the lake (see next paragraph), 2) with and without addition of nutrients ($NaNO_3$, K_2HPO_4, NH_4Cl) and with and without 50% of shading of solar radiation (Figure 1).

The organic matter added in some mesocosms was obtained as follows: leaves of the native vegetation present in the soil around the lake were placed in buckets with distilled water for decomposition and disposed in the laboratory under room temperature (~ 25 °C). After a week this water was filtered at 20 µm, the concentration of DOC was measured using TOC analyzer and then a known concentration of DOC was added in the mesocosms in order to increase DOC concentrations with an organic matter from allochthonous origin.

Mesocosms were placed at the surface of the lake and filled with lake water. Mesocosms with light reduction (SH) were shaded with spectrally neutral shading screens (50%) and only opened for quick samplings and measurements. Every day, the mesocosms were gently stirred and measured for water temperature using a probe Hydrolab DS5 (Hach Inc.).

Water samples were filtered immediately after sampling for Chl-a and nutrients (0.47 μm filter) and TSM (AP040 filter). The filters were kept frozen until analysis. Water samples were also filtered for analysis of DOC and CDOM (0.22 μm Millipore glass microfiber filter) and stored in amber glass bottles (pre-washed with distilled water and hydrochloric acid 10%) at 4°C in the dark. The Chl-a concentration corrected by pheophytin (μg L^{-1}) was obtained by acetone extraction (90%) measured in a spectrophotometer (UV-VIS Shimadzu) at 665 and 750 nm and calculated using the protocol provided in APHA (1998). The TSM (mg L^{-1}) were determined by the gravimetric method, considering the difference between the dry weights of AP40 Millipore filters (105 °C for 2 hours) before and after the filtration of water samples (APHA 1998). The DOC concentration (mg L^{-1}) was obtained by catalytic oxidation method of high temperature using TOC Analyzer (Shimadzu TOC – 5000A). Filtered water samples were taken for dissolved nutrients (ammonia, nitrate, nitrite and phosphate; μg L^{-1}) and frozen until analyzes with an auto-analyzer (Metrohm 8000 IC-Plus).

CDOM optical properties

Absorption spectra of CDOM were obtained between 250 and 700 nm at 1 nm intervals with a spectrophotometer (UV-VIS Shimadzu) using 5 cm quartz cuvette and a Milli-Q water sample as blank reference. The absorption spectra of each sample were measured in replicate (standard deviation < 0.01). The absorption coefficients ($a_{\mathrm{CDOM}}(\lambda)$; m^{-1}) were derived from absorbance measurements according to the equation $a_{\mathrm{CDOM}}(\lambda) = 2.303A(\lambda)L^{-1}$, where $A(\lambda)$ is the absorbance measured at wavelength λ and L is the optical path of the cuvette (in meters). Absorption coefficients were corrected for backscattering by subtracting the value of the coefficient at 700 nm. The absorption coefficient at 254 nm (a_{CDOM254}) was used as an index of CDOM UV-absorption and at 440 nm (a_{CDOM440}) as a CDOM PAR-absorption.

We used a simple exponential curve to model the decrease in absorption with increasing wavelength using the equation (Jerlov 1968; Bricaud et al. 1981; Stedmon and Markager 2001): $a_{\mathrm{CDOM}}(\lambda) = a_{\mathrm{CDOM}}(\lambda 0)\ e^{-S(\lambda - \lambda 0)} + K$,where a_{CDOM} is the absorption coefficient (m^{-1}), λ is the wavelength (nm), λ0 is a reference wavelength (nm), K is a background constant (m^{-1}) accounting for scatter in the cuvette and drift of the instrument and S is the spectral slope (nm^{-1}) that describes the approximate exponential rate of decrease absorption with increasing wavelength. Furthermore, we calculated the spectral slope between 275-295 nm ($S_{275\text{-}295}$) and 350-400 nm ($S_{350\text{-}400}$). The slope ratio (S_R, Helms et al. 2008) was obtained by dividing $S_{275\text{-}295nm}$ by $S_{350\text{-}400nm}$. These metrics have been calculated using the *cdom* R package (Massicotte 2016). We also calculated $S_{250\text{-}450}$ as this wavelength to be used in the principal component analysis, and we created this new index to measure changes in the entire spectrum including UV and PAR-absorbing (we limit to 400 nm because after this wavelength the noise reading was high). The specific UV absorbance ($SUVA_{254}$) ($m^2\ mg^{-1}$ C) was calculated dividing the value of the absorption coefficient at 254 nm (m^{-1}) by the concentration of DOC (mg L^{-1}) (Weishaar et al. 2003).

Data analysis

Relative changes (%) of the parameters over time were calculated dividing the value at the end by the value at the beginning (day 0) of the experiment, after subtracting this result from 1 and multiplying by 100 [(1 - end/start) x 100]. Negative values of relative changes indicate decrease and positive values indicate increase compared to initial values.

To compare if there were differences in the quantity (a_{CDOM254}, a_{CDOM440} and DOC) and quality (SUVA_{254} and spectral slopes) of DOM between treatments with and without additions of OM we used *t*-test (for the parametric data) and Mann-Whitney (for non parametric data) (significant $p < 0.05$). To compare if there was difference in phytoplankton density (measured by Chl-a concentration) between treatments with and without nutrients we used *t*-test (significant $p < 0.05$). Differences between the treatments with and without shading were also tested by *t*-test for each parameter measured (significant $p < 0.05$).

A principal component analysis (PCA) was generated using absorption spectra on a $n \times p$ matrix where n is the number of observation in the dataset ($n = 80$) and p is the wavelength number ($250 \leq p \leq 450$). The PCA was performed on scaled data (0 mean and unit variance) as suggested by Borcard et al. (2011). Based on the Kaiser-Guttman criterion (Kaiser 1960), the first two principal components were kept for subsequent analyzes. PCA scores of first two axes (PC1 and PC2) were correlated against environmental variables (shade, nutrients, OM, Chl-a, TSM, DOC, S_R and SUVA_{254}) using a redundancy analysis (RDA) to help to understand their interpretation. All statistical analyzes were performed using R Software (R core development team 2011).

Results

Overall characteristics of the treatments

The water temperature of the mesocosms ranged between 28.4 and 31.3 ºC (average 30.8 ºC) throughout the experiment. As hypothesized Chl-a levels were significantly higher for treatments with addition of nutrients (Table 1). Concentrations of DOC,

$a_{CDOM254}$ and $a_{CDOM440}$ were significantly higher in all the treatments with addition of OM. Higher molecular size (lower $S_{275-295}$) and more aromatics compounds (higher $SUVA_{254}$) were observed also in the treatments with OM added as expected due to the addition of allochthonous DOM derived from the degradation of forest leaves surrounding the lake (Table 1). There were no significant differences between treatments with and without shading to any of the parameters above (*t*-test, $p > 0.05$).

Temporal changes in the mesocosms

Phytoplankton biomass (Chl-a) increased over time only in the treatments with addition of nutrients (from average of 3.9 in the day 0 to 19.1 µg L^{-1} in the 12th day) and OM (from minimum average 3.4 in day 0 to maximum of 12.1 µg L^{-1} in 9th day) (Figure 2A-B). DOC increased in the experimental units with added nutrients until day 9 (maximum 8.7 mg L^{-1}). In the treatments with addition of OM, DOC was higher as expected with a minimum of 8.3 mg L^{-1} in 3th day and maximum of 8.9 mg L^{-1} in 9th day, while in the treatments without addition of OM the DOC had a maximum of 8.2 mg L^{-1} in 6th day (Figure 2C-D). $S_{250-450}$ and slope ratio (S_R) had the same pattern in the treatments with and without nutrients addition, but decreased consistently in treatments with OM addition (Figure 2 E-H).

The relative changes in CDOM absorption along the spectral range were different for each sampling day (Figure 3A-E). On the initial day, only treatments with and without addition of OM had distinct absorption curves, especially in the UV range below 400 nm, and the absorption spectra for each treatment group in the day 0 are shown in Figure 3A. To evaluate treatment effects we determined the change in light absorption spectra for the other sampling days relative to the initial day (Figure 3B-E). On the 3rd day, treatments with and without nutrients added were quite similar, while those with and without OM and with and without shading showed opposing changes. Loss of absorption occurred only in treatments with full light (less than 5% between 300-420 nm) and in those without OM addition (the loss of absorption increased with the increase of wavelength) (Figure 3B). On the 6th day all treatments showed an increased absorption especially after 350 nm (higher increase with shade: ~ 40% at 450 nm), except the treatments with full light that still had a loss of absorption (Figure 3C). After the 9th day, all treatments had a loss of CDOM absorption with increasing wavelengths, especially for the full light treatment (Figure 3D-E).

The concentrations of DIP and DIN (µg L^{-1}) and the molar ratio between them (DIN:DIP) were higher in treatments with added nutrients as expected (Figure 4A-C). In the treatments without nutrients addition, DIP had concentrations between 3 and 9 µg L^{-1} and DIN between 1.7 and 100.4 µg L^{-1}, with DIN:DIP ratios ranging between 7.6 (12th day) and 21.3 (day 0) and below 13.9 after 3rd day indicating that phytoplankton community were mostly limited by nitrogen in these treatments (Redfield 1958; Reynolds 1999). All the dissolved nutrients decreased along the days in treatments with nutrients addition (DIP decreased from an average of 153 to 59 µg L^{-1} and DIN from 2600 to 400 µg L^{-1}). The DIN:DIP molar ratio ranged from 55.3 (3rd day) to 12.2 (12th day) in the units with addition of nutrients.

Principal component analysis results

The first principal component of the redundancy analysis (Figure 5A) was mostly associated with availability of OM. Samples presenting high score on the first principal component furthermore tend to have high values of DOC and $SUVA_{254}$ but low values of S_R. High scores on the second principal component were correlated positively with Chl-a, nutrients and TSM and negatively with shading.

Exploration of spectral PCA loadings (Figure 5B-C) reveled that principal component 1

(PC1) had the strongest effect on the shape of CDOM absorbance between 300 and 400 nm. Principal component 2 (PC2) loadings showed a quasi-linear decrease with increasing wavelength suggesting that phytoplankton enrichment had a stronger effect at lower wavelengths. Furthermore, loading values were negative after ~340 nm, indicating that phytoplankton was on average lowering CDOM absorption after this threshold. Based on RDA, PC1 was renamed “allochthonous carbon enrichment” whereas PC2 was renamed “autochthonous carbon enrichment”.

Discussion

Temporal responses in DOM and Chl-a

Our study supports previous studies showing that addition of allochthonous matter causes DOM to be dominated by more aromatic carbon and with higher molecular weight (Bertilsson and Tranvik 2000; Benner 2002) and lower spectral slopes (Helms et al. 2008; Figure 2F,H). Addition of nutrients also affected DOM quantity and quality related to autochthonous production of DOM by phytoplankton growth, and it is known that phytoplankton in high densities can be an important source of DOM (Zhang et al. 2009; Zhang et al. 2013; Brandão et al. 2016). In the treatments without addition of nutrients the phytoplankton community was limited by nitrogen since the beginning of the experiment (DIN concentrations below 100 µg L^{-1}; Reynolds 1999). The DIN:DIP molar ratio in these treatments indicates that nitrogen was consumed very fast and was limited after the 3rd day (ratio below 13.9; Redfield 1958). With the addition of nutrients, the concentrations of DIN and DIP were higher than that considered a limited condition proposed by Reynolds (1999), but the molar ratio values proposed by Redfield (1958) suggested limitation by phosphorus (molar ratios between 21.3 and 55.3) during the experiment and only in the 12th day became limited by nitrogen again (molar ratio 12.2). Moreover, these suggestive limitation indicated by molar ratio values did not restrict the phytoplankton growth in the treatments with addition of nutrients. In addition to increased nutrient availability (mainly nitrogen and phosphate) and phytoplankton growth (Schindler 1977; Lean and Pick 1981), the increase of allochthonous OM also caused an increase in Chl-a (Figure 2B). This suggest development and prevalence of mixotrophic phytoplankton species, present in Carioca Lake (Barros et al. 2010) which are known to be favored by organic matter (Cohen and Post 1993). Additionally, the light attenuation caused by enhanced light absorption from added allochthonous OM (Kirk 1994), may have favored growth of phytoplankton by reducing photoinhibition, known to occur in Lake Carioca (Brighenti et al. 2015). The increase of phytoplankton in these treatments therefore suggest that algal growth was stimulated by a combination of increases in nutrients availability due to degradation of the OM added (Hessen and Tranvik 1998) as well as a shift towards mixotrophic phytoplankton species.

Although additions of allochthonous OM and nutrients both contributed to increase DOC concentrations, divergent effects of these additions were evident in the quality of carbon assessed by optical indexes ($S_{250\text{-}450}$ and S_R). $S_{250\text{-}450}$ and S_R decreased significantly after addition of allochthonous OM (Figure 2F, H). The decrease in the slope $S_{250\text{-}450}$ occurred due to the increase of a higher molecular weight carbon, which lowered the values of $S_{275\text{-}295}$ and consequently of S_R (Helms et al. 2008). Addition of nutrients, however, had little effect on these metrics, which we interpret as a consequence of autochthonous production of DOM. Thus, both spectral indexes were more influenced by allochthonous contribution than by the produced autochthonous DOM. This is likely because these indices derive from slope intervals in the ultraviolet range (250-400 nm) known to be influenced by carbon with higher molecular weight and aromatic compounds capable of absorbing energy at shorter wavelengths (Bertilsson and Tranvik 2000; Benner 2002; Helms et al. 2008).

Manipulations with nutrients, allochthonous OM and shading caused visual changes in the spectral curves of CDOM over the sampling days (Figure 3). Several studies have shown that aromatic carbon, typically of terrestrial origin, has a higher absorption in the ultraviolet range (Bertilsson and Tranvik 2000; Benner 2002; Helms et al. 2008). This explains the initial (day 0) effects of allochthonous OM addition on elevated CDOM absorption primarily below 350 nm (Figure 3A). We interpret the following (day 3 and 6) increase in the CDOM absorption (especially above 350 nm) for most treatments to result from autochthonous DOM related to phytoplankton growth. Increases in absorption in the PAR range (Figure 3B-C), is known to be related to increases in carbon of algal origin, composed of labile molecules of simple structures and with low molecular weight (Amon and Benner 1994; McKnight et al. 1994; Benner 2002; Helms et al. 2008). The relative changes in the spectral curves shown in Figure 3 therefore reflect the final result of gains by autochthonous production and the loss of absorption by photodegradation and/or biodegradation, although these processes were not directly investigated in this work. We could only estimate the importance of photodegradation in this lake comparing the treatments exposed to full light (orange solid lines in Figure 3), which were the ones that lost absorption in all sampling days, and the shaded treatments (orange dashed lines), which were the ones that less had lost of absorption in the spectra. After the 9th day (Figure 3D-E) the absorption loss was larger than the gain by the autochthonous production in all treatments. Such spectral changes with loss of absorption with increasing wavelength, was described by Asmala et al. (2014) as a typical biodegradation curve. Thus, we believe that after the 9th experimental day the observed loss of DOM was mostly caused by biodegradation.

Effect of allochthonous and autochthonous DOM on CDOM spectra

The results obtained from the PCA and RDA demonstrated that the increase of allochthonous OM had greater effect between the wavelengths 300 and 400 nm, increasing the absorption in this range (PC1, Figure 5B). Several studies have shown that photodegradation is more pronounced at shorter wavelengths (300-400 nm) due to absorption of aromatic carbon compounds (Helms et al. 2008; Helms et al. 2013) typically related to degradation of either terrestrial vegetation (Bertilsson and Tranvik 2000; Benner 2002; Helms et al. 2008) or aquatic macrophytes (Catalán et al. 2013). It is possible assess the importance of photodegradation on CDOM absorption through the spectral slope between 275 and 295 nm ($S_{275\text{-}295}$) proposed by Helms et al. (2008). We noticed a decrease in the CDOM absorption below 300 nm, suggesting a greater degradation by photodegradation in these compounds from allochthonous origin affecting the absorption at shorter wavelengths and increasing the absorption between 300 and 400 nm.

In contrast, the increase of autochthonous OM coming from the high phytoplankton growth caused an increase of absorption in the UV range and a loss of absorption at wavelengths beyond 350 nm (PC2; Figure 5C). The loss of absorption above 350 nm indicates degradation by microorganisms which have greater impact on the PAR absorption. Substances that absorbs in this range are typically non-aromatic compounds originating from algal source with high lability for bacterial degradation (Baines and Pace 1990; Berggren et al. 2009).

Conclusions

Additions of terrestrial OM and inorganic nutrients to a tropical lake mesocosms caused fast changes in the production and transformation of OM pools as well as distinct changes in the absorption spectra of CDOM. The factorial design of the mesocosms experiment simulated seasonal changes commonly observed related to changes in primary production and water clarity caused by changes in temperature, stratification, and rains which affect the input of terrestrial OM and nutrients (Brighenti et al. 2015; Brandão et al. 2016). Increased production of autochthonous OM caused an increase of CDOM absorption in the UV range and a reduction in the PAR range, which we interpret to result primarily from bacterial degradation of highly labile algal material (Baines and Pace 1990; Berggren et al. 2009). In contrast, the additions of allochthonous OM caused increased absorption of CDOM, especially between 300 and 400 nm. The new index created $S_{250\text{-}450}$ was an effective tool to evaluate the spectral changes in general from short to long wavelengths, not restricting spectrum ranges where we can only perceive changes in some regions of UV-absorbing ($S_{275\text{-}295}$, $S_{350\text{-}400}$). Although the non-shaded treatments showed apparent effects of photodegradation, changes in CDOM absorption curves after the 9th day suggest that biodegradation was overall responsible for the largest percentage of OM degradation in these experiments. The 50% reduction of sunlight in some mesocosms accordingly had minor effects on overall changes in OM concentration and composition, likely due to the large amount of light still reaching the DOM in the shaded units.

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Figures and Tables

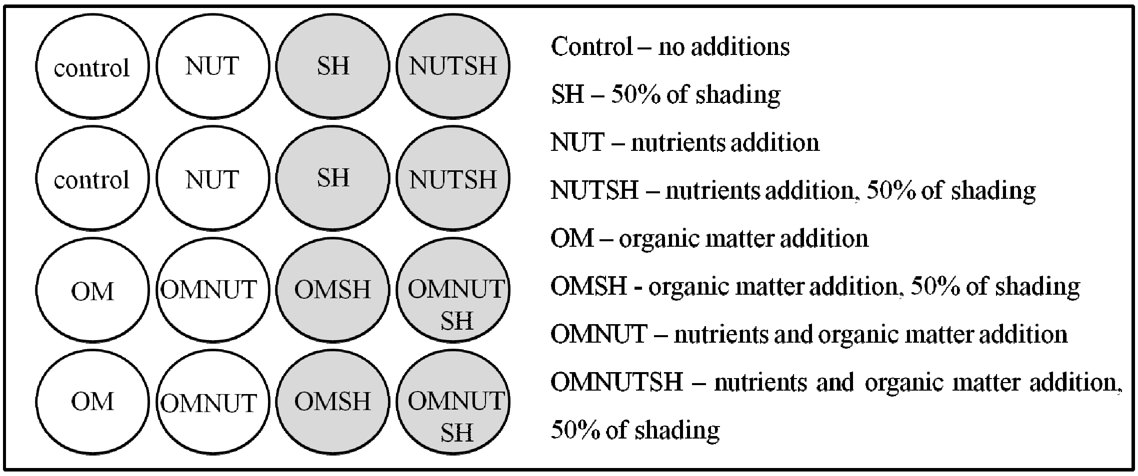

Fig. 1 Squematic figure of the factorial design of mesocosms experiments

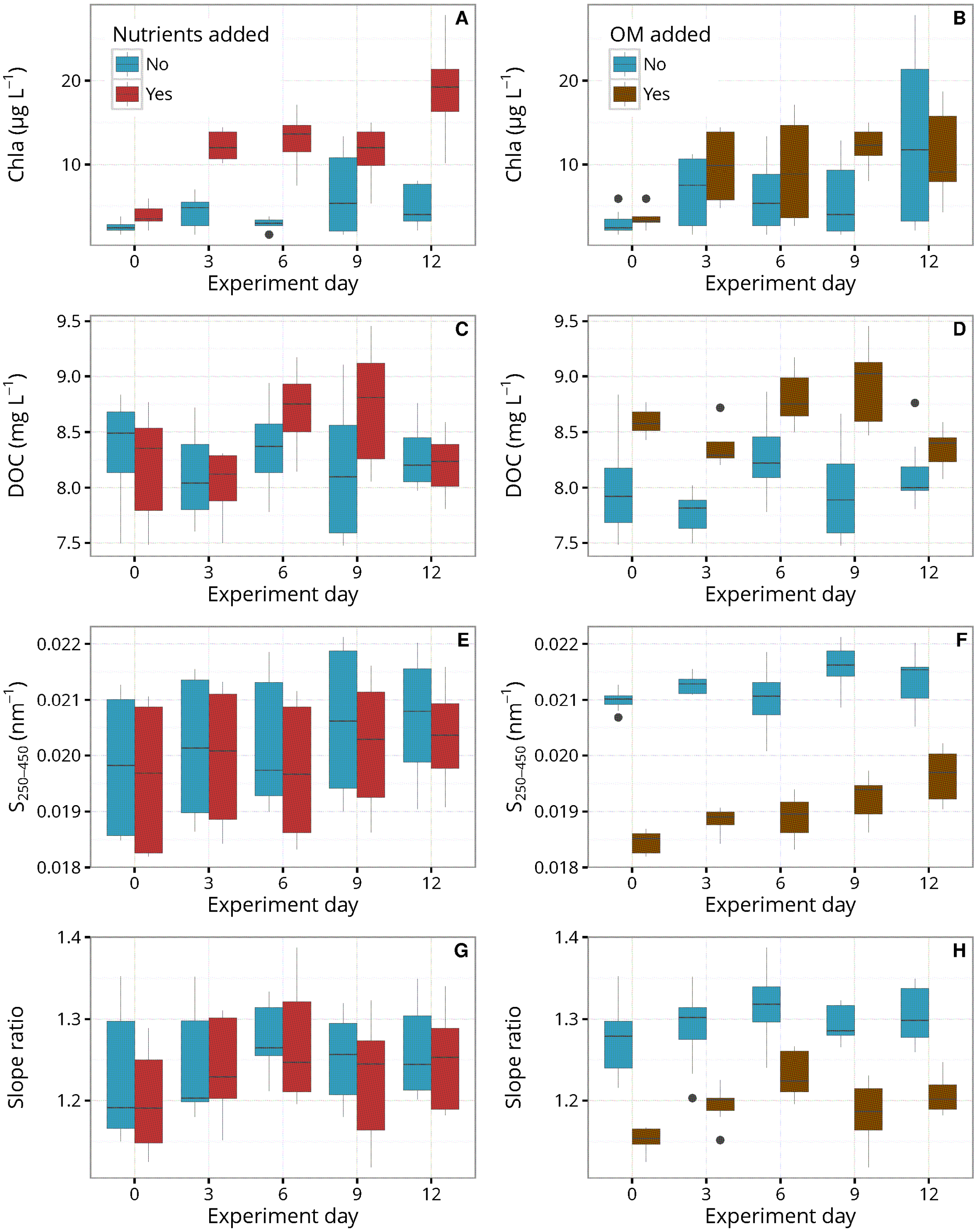

Fig. 2 Temporal variation in the mesocosms units with and without nutrients (left panel) and organic matter additions (right panel) for Chl-a (A,B), DOC (C,D), $S_{250\text{-}450}$ (E,F), S_R (G,H) (center line-median, 5th/95th percentiles, 95% confidence level, black circles-outlier)

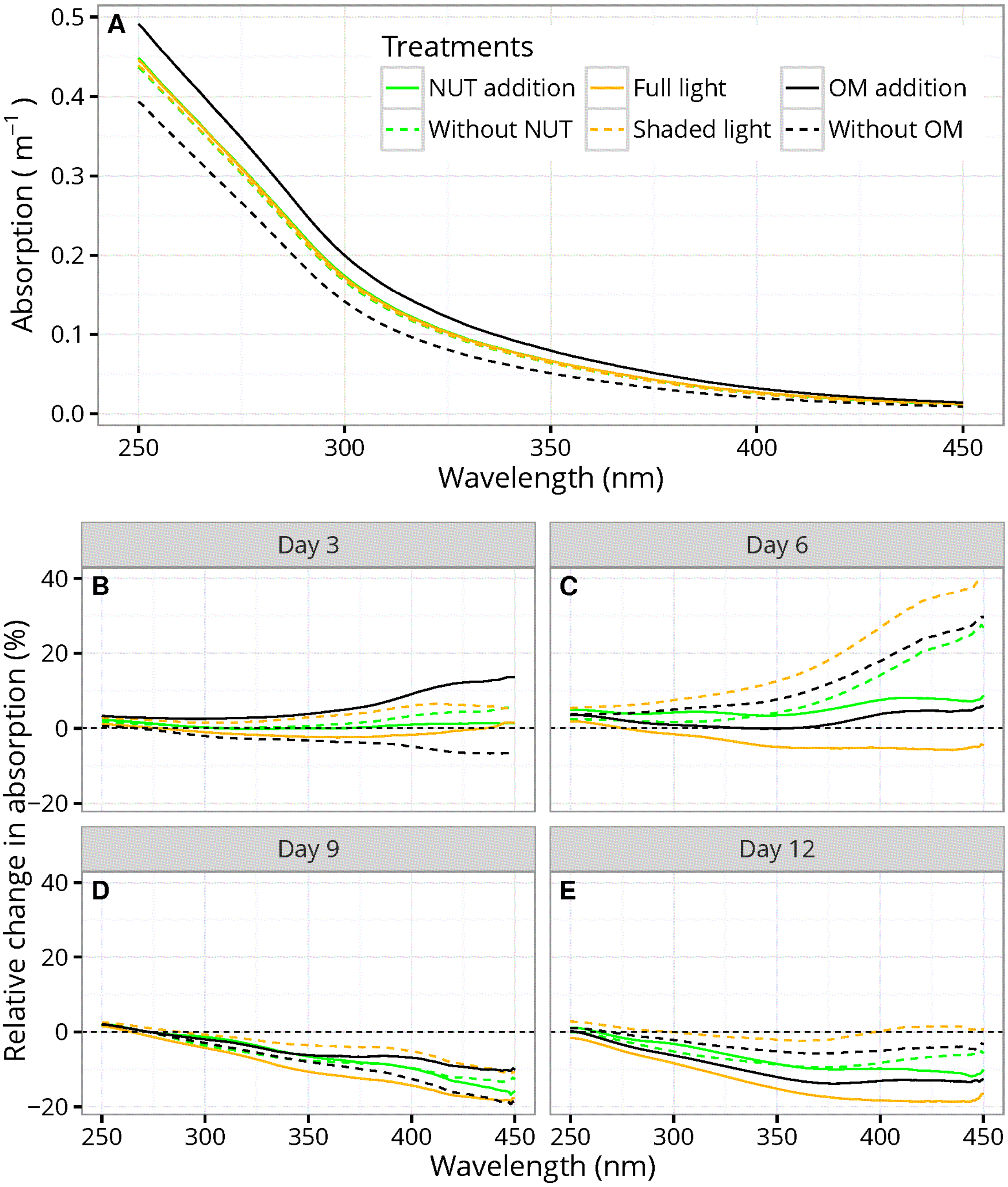

Fig. 3 Spectral absorption curves of CDOM in the different mesocosms units for the initial day (A) and the relative changes for each sampling day compared with the initial day (3rd day – B, 6th day – C, 9th day – D, 12th day – E)

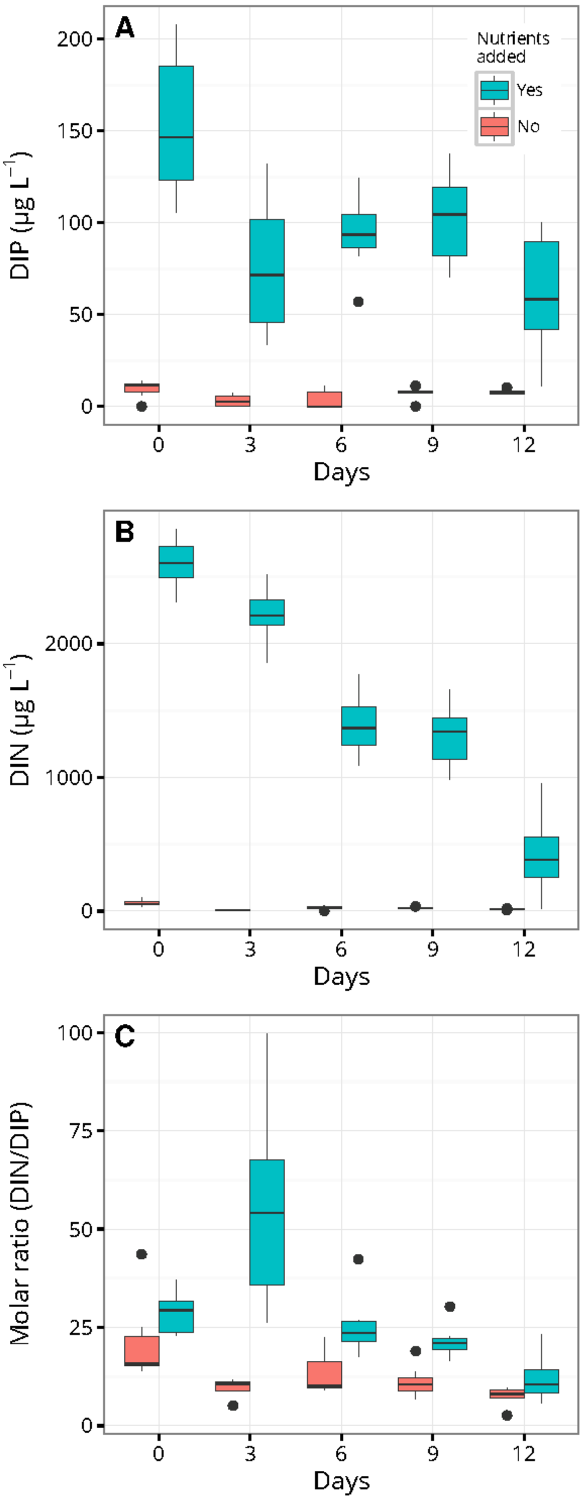

Fig. 4 Temporal variation in the mesocosms units with and without nutrients additions for dissolved inorganic phosphorus-DIP (A), dissolved inorganic nitrogen-DIN (B) and the ratio DIN/DIP (C) (center line-median, 5th/95th percentiles, 95% confidence level, black circles-outlier)

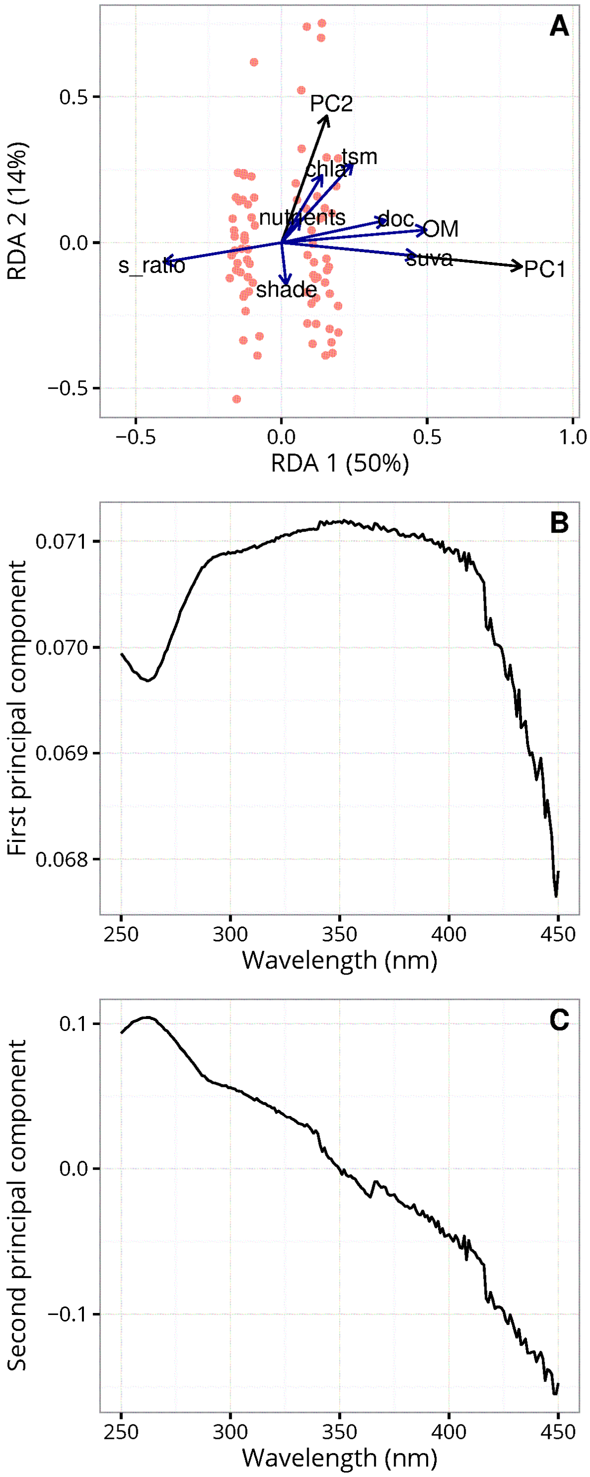

Fig. 5 Results of redundancy analysis-RDA (A) and the first two principal components obtained from PCA analysis, PC1 (B) and PC2 (C), plotted against wavelengths

Table 1 - Results of the *t*-test and Mann-Whitney (legend: OM = treatments with organic matter addition; no OM = treatments without addition of organic matter; NUT = treatments with nutrients addition; no NUT = treatments without nutrients addition).

Parameters	Analysis			significance $p<0.05$
a254 (m^{-1})	Mann-Whitney	Median	U	p
OM		21.857	0	<0.001
no OM		17.344		
a440 (m^{-1})	Mann-Whitney	Median	U	p
OM		0.616	0	<0.001
no OM		0.377		
DOC (mg L^{-1})	*t*-test	Mean (SD)	t (df)	p
OM		8.633(0.233)	6.797(22)	<0.001
no OM		7.955(0.255)		
SUVA$_{254}$ (m^2 mg^{-1} C)	*t*-test	Mean (SD)	t (df)	p
OM		2.502(0.120)		
no OM		2.167(0.059)	8.677(22)	<0.001
S$_{275-295}$ (nm^{-1})	*t*-test	Mean (SD)	t (df)	p
OM		0.023(0.001)	-7.431(26)	<0.001
no OM		0.026(0.000)		
Chl-a (µg L^{-1})	*t*-test	Mean (SD)	t (df)	p
NUT		9.628(3.192)	5.152(26)	<0.001
no NUT		4.697(1.625)		

Supplementary material

Table - Absolute values and relative changes of all the parameters measured during 12 days of experiment.

Treatment	Days	DOC (mg L-1)	a254 (m^{-1})	a440 (m^{-1})	SUVA$_{254}$ (m^2 mg^{-1} C)	S275-295 (nm^{-1})	S350-400 (nm^{-1})	S250-450 (nm^{-1})	S_R	Chl-*a* (µg L^{-1})	TSM (mg L^{-1})
control	0	8.44	17.38	0.46	2.064	0.025	0.019	0.021	1.336	1.87	1.27
	3	7.76	16.88	0.38	2.176	0.026	0.020	0.022	1.338	3.74	1.87
	6	8.01	17.24	0.38	2.152	0.026	0.020	0.022	1.318	2.67	1.41
	9	7.66	17.30	0.36	2.258	0.027	0.021	0.022	1.317	1.60	1.21
	12	8.37	17.25	0.39	2.064	0.027	0.020	0.022	1.343	2.67	1.61
	relative change (%)	*-0.81*	*-0.73*	*-15.00*	*-0.02*	*5.75*	*5.21*	*3.45*	*0.50*	*42.86*	*26.88*
NUT	0	7.98	17.38	0.45	2.180	0.025	0.019	0.021	1.279	4.54	1.12
	3	7.97	17.55	0.41	2.202	0.025	0.020	0.021	1.268	10.42	2.15
	6	8.69	17.64	0.40	2.029	0.026	0.019	0.021	1.348	10.16	2.73
	9	8.36	17.68	0.30	2.116	0.026	0.020	0.022	1.294	9.62	2.62
	12	7.90	17.46	0.37	2.211	0.027	0.021	0.021	1.300	23.79	3.60
	relative change (%)	*-1.00*	*0.46*	*-17.95*	*1.43*	*7.90*	*6.27*	*1.76*	*1.64*	*423.53*	*220.62*
SH	0	7.83	16.67	0.40	2.131	0.025	0.020	0.021	1.253	2.41	1.19
	3	7.73	17.00	0.36	2.200	0.026	0.021	0.021	1.246	2.14	0.78
	6	8.06	17.58	0.54	2.180	0.024	0.019	0.021	1.287	2.41	1.71
	9	7.53	17.18	0.37	2.283	0.026	0.021	0.022	1.286	2.41	1.61
	12	8.05	16.57	0.38	2.058	0.026	0.020	0.022	1.277	3.47	1.64
	relative change (%)	*2.79*	*-0.62*	*-5.71*	*-3.43*	*3.61*	*1.65*	*2.15*	*1.89*	*44.44*	*37.89*
NUTSH	0	7.65	17.01	0.40	2.226	0.025	0.020	0.021	1.235	3.21	1.42
	3	7.64	17.30	0.41	2.266	0.026	0.020	0.021	1.306	10.96	1.84
	6	8.29	17.91	0.47	2.161	0.025	0.019	0.021	1.308	10.42	2.65
	9	8.24	17.78	0.40	2.159	0.026	0.020	0.021	1.277	9.09	2.29
	12	8.20	17.46	0.45	2.132	0.026	0.020	0.021	1.294	22.45	2.54
	relative change (%)	*7.16*	*2.64*	*11.43*	*-4.25*	*3.21*	*-1.43*	*-0.63*	*4.71*	*600.00*	*79.25*
OM	0	8.56	21.25	0.64	2.482	0.022	0.019	0.019	1.167	2.67	1.26
	3	8.72	22.38	0.75	2.567	0.022	0.018	0.019	1.202	5.08	2.43
	6	8.72	21.42	0.66	2.457	0.023	0.019	0.019	1.238	3.47	1.87
	9	8.82	22.39	0.68	2.539	0.023	0.019	0.019	1.212	9.36	2.18
	12	8.38	20.84	0.55	2.487	0.024	0.020	0.020	1.222	5.88	2.45
	relative change (%)	*-2.12*	*-1.90*	*-14.29*	*0.20*	*10.58*	*5.54*	*7.95*	*4.77*	*120.00*	*93.89*
OMNUT	0	8.56	21.67	0.68	2.532	0.022	0.019	0.018	1.146	3.21	3.22
	3	8.30	22.19	0.74	2.675	0.022	0.018	0.019	1.213	13.90	3.00
	6	9.16	22.77	0.67	2.486	0.023	0.019	0.019	1.204	14.17	3.66
	9	9.33	22.23	0.58	2.383	0.023	0.020	0.020	1.196	12.56	4.14
	12	8.48	20.90	0.53	2.464	0.025	0.020	0.020	1.218	14.43	3.81
	relative change (%)	*-0.89*	*-3.56*	*-22.03*	*-2.70*	*12.85*	*6.22*	*8.74*	*6.33*	*350.00*	*18.36*
OMSH	0	8.61	20.95	0.63	2.432	0.022	0.019	0.019	1.158	3.21	1.30
	3	8.24	21.54	0.71	2.614	0.022	0.019	0.019	1.185	6.42	2.02
	6	8.61	21.46	0.71	2.494	0.023	0.018	0.019	1.263	3.21	2.15
	9	8.54	21.30	0.45	2.494	0.023	0.019	0.019	1.196	12.30	2.61
	12	8.26	21.73	0.63	2.616	0.023	0.019	0.019	1.202	8.02	2.32
	relative change (%)	*-4.12*	*3.74*	*0.00*	*7.58*	*5.16*	*1.29*	*3.59*	*3.82*	*150.00*	*78.98*
OMNUTSH	0	8.64	21.90	0.70	2.536	0.022	0.019	0.018	1.139	4.81	1.27
	3	8.26	22.93	0.77	2.775	0.022	0.018	0.019	1.177	13.63	2.68
	6	8.75	22.81	0.82	2.606	0.022	0.018	0.018	1.221	16.31	3.89
	9	9.02	22.26	0.68	2.467	0.022	0.019	0.019	1.134	14.43	3.86
	12	8.27	22.91	0.66	2.773	0.023	0.019	0.019	1.185	16.04	6.47
	relative change (%)	*-4.31*	*4.63*	*-6.56*	*9.32*	*7.03*	*2.86*	*5.16*	*4.06*	*233.33*	*408.49*

Negative values in relative changes indicate decrease and positive indicate increase compared to initial values [relative changes = -1*(1 - end/start) x 100]

CONSIDERAÇÕES FINAIS

Os estudos das propriedades ópticas aparentes e inerentes são importantes para a compreensão do funcionamento aquático em escala espacial e temporal. Através de simples análise da transparência das águas para a radiação PAR e UV, é possível estimar a distribuição dos produtores primários na coluna d’água, que somado a uma análise qualitativa destes, também se pode ter uma ideia da produtividade ecossistêmica. Avaliando também a análise da concentração e qualidade de DOM é possível compreender a dinâmica sazonal de entrada alóctone, produção autóctone e os principais meios de degradação de compostos de carbono nos ambientes.

A utilização de índices obtidos através de análises espectrais de CDOM para a sua caracterização possui a vantagem de ser relativamente de baixo custo e com resultados rápidos. Apesar da crescente utilização desta abordagem em ambientes temperados, principalmente após o trabalho de Helms et al. (2008), ainda são comuns estudos que avaliam apenas a concentração de DOC e CDOM, e bastante escassos nos trópicos e no Brasil aqueles que consideram também a qualidade dos compostos de carbono. Nesta tese, foi demonstrado que incluir a análise da qualidade espectral de CDOM fornece resultados extremamente importantes (quanto a fontes, tamanhos das moléculas, susceptibilidade aos processos de degradação, etc) para o entendimento dos processos biogeoquímicos e ecológicos, não obtidos apenas pela análise quantitativa. Inúmeras são as vantagens do estudo da qualidade de DOM através da análise espectral de CDOM, no entanto, é preciso cautela no uso e interpretação dos índices, pois alguns podem variar entre pesquisadores sem explicações bem definidas (por exemplo os intervalos de comprimentos de onda para cálculo dos “spectral slopes”).

As amostragens no lago Dom Helvécio (MG, Brasil) contemplaram um período de redução na pluviosidade regional do Médio Rio Doce, e alguns parâmetros (por exemplo clorofila-a, DOC, nutrientes) puderam ser comparados com o observado anteriormente durante 10 anos de monitoramento pelo programa PELD. Além disto, através da análise espectral do CDOM e seus índices também foi possível demonstrar como as recentes mudanças climáticas podem afetar a sazonalidade da entrada de matéria orgânica alóctone no lago e os potenciais efeitos disto para o ecossistema. Os resultados demonstrados nos capítulos 1 e 2 desta tese permitiram ainda estimar qual o efeito na atenuação da radiação PAR em função da redução do regime de chuvas, que por sua vez afeta as dinâmicas dos componentes opticamente ativos.

Compreender as fontes de DOM nos ecossistemas aquáticos, bem como a sazonalidade destas renovações de DOM e nutrientes, é de grande auxílio para o entendimento dos processos biogeoquímicos, ecológicos e metabólicos. Somente após esta compreensão é possível investigar como as mudanças climáticas, alterações do uso do solo, aportes esporádicos de esgotos, tempestades, dentre outros fenômenos, podem afetar a dinâmica de DOM e quais as consequências para os ambientes aquáticos.

A utilização de experimentos *in situ* com mesocosmos permitiu ainda avaliar como o aumento de DOM autóctone e DOM alóctone podem alterar a qualidade espectral dos compostos de carbono. Dessa forma, é possível extrapolar tais resultados para estimativas das consequências da eutrofização (por exemplo, o reservatório da Pampulha), desmatamento, substituição da vegetação do entorno por eucaliptos ou pastos (comuns no entorno do Parque Estadual do Rio Doce), e outros impactos que interferem na quantidade de DOM autóctone e alóctone disponível nos sistemas.

Os resultados apresentados nesta tese contribuem com o conhecimento sobre propriedades ópticas aparentes e inerentes, e qualidade e concentração de DOM em ecossistemas aquáticos tropicais naturais e artificiais, em escala espacial e temporal. Além disso, fornecem novas perspectivas no estudo do efeito das diferentes fontes de carbono na qualidade espectral de CDOM, o que reflete nos processos de degradação e características ópticas dos sistemas. Novos estudos que investiguem especificamente as taxas de fotodegradação e biodegradação nestes ambientes são necessários para melhor entendimento da dinâmica de DOM e do papel destes processos no ciclo global do carbono em ambientes aquáticos tropicais.