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Matéria orgânica dissolvida como indicadora dos impactos de piscicultura de tanque-rede no reservatório da usina hidrelétrica de Furnas

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RESUMO

A piscicultura é uma atividade que pode liberar grande quantidade de matéria orgânica dissolvida (MOD) nos ecossistemas aquáticos. Sendo fonte de energia e nutrientes, a MOD desempenha importantes papéis na dinâmica de carbono, fósforo e nitrogênio de ambientes naturais e impactados. Para avaliar os efeitos da implantação recente de piscicultura em tanques-rede sobre as concentrações de carbono orgânico dissolvido (COD), índices de fluorescência da MOD, estequiometria elementar (carbono, nitrogênio e fósforo) na MOD, componentes do PARAFAC (parallel factor analysis) e frações isotópicas de $\delta^{13}C$ da MOD and $\delta^{15}N$ do nitrogênio total dissolvido (NTD), foram obtidas amostras de água ao longo de seis transectos que partiam de seis pisciculturas em direção a locais de referência em um grande reservatório tropical, entre abril de 2013 e dezembro de 2016. Embora não constatamos diferenças nas concentrações de COD ao longo dos transectos, foram detectadas mudanças significativas nos índices de fluorescência (FIs) a até 100 m de distância das pisciculturas. Valores mais altos de FI ocorreram próximo às pisciculturas e indicam aumentos na produção microbiana. Pequenas diferenças no índice freshness (β:α) e na relação pico T / pico C ao longo dos transectos indicaram que a contribuição relativa da MOD produzida recentemente e a demanda bioquímica de oxigênio aumentaram devido à piscicultura. O índice de humificação não respondeu à piscicultura. Foi observado, pela análise PARAFAC, um aumento da concentração de compostos lábeis e diminuição da concentração de compostos recalcitrantes. Foi constatado, por análise isotópica, um aumento de frações $\delta^{15}N$ do nitrogênio total dissolvido. O atual estágio inicial da piscicultura não parece causar grandes impactos na quantidade e qualidade da MOD no reservatório estudado, mas futuros aumentos na densidade de tanques-rede e tempos de operação mais longos podem alterar essa avaliação. Os índices de fluorescência (FI, β: α, a relação pico T / pico C), análise PARAFAC, estequiometria da MOD e análise de isótopos $\delta^{15}N$ do NTD, podem ser sinais de alerta precoces e úteis para monitorar os impactos das pisciculturas em reservatórios tropicais.

Palavras-chave: Aquicultura tropical; Piscicultura em tanques-rede; Índices de fluorescência; Tilápia do Nilo; Nitrogênio; Fósforo; Isótopos estáveis; Brasil

ABSTRACT

Dissolved organic matter as an indicator of the impacts of fish farm net cage in the reservoir of Furnas hydroelectric power plant

Fish farming is an activity that can release a large amount of dissolved organic matter (DOM) in aquatic ecosystems. DOM is a source of energy and nutrients, playing important roles in the dynamics of carbon, phosphorus and nitrogen in natural and impacted environments. To assess the effects of early-stage fish farming on dissolved organic carbon (DOC), DOM fluorescence indices, elemental stoichiometry (carbon, nitrogen and phosphorus) in DOM, PARAFAC (parallel factor analysis) components and δ13C isotopic fractions from DOM and δ^{15}N of the total dissolved nitrogen (TDN), we collected water samples along transects of six fish farms towards reference sites in a large tropical reservoir between April 2013 and December 2016. Although COD concentrations did not change over the transects, significant changes in fluorescence indices (FIs) were detected up to 100 m away from fish farms. Highest FI values near the fish farms indicated local increases in microbial production. Small differences in the freshness index (β:α) and in the peak T / peak C ratio across the transects indicated that the relative contribution of the recently produced DOM and the biochemical oxygen demand of the DOM increased due to aquaculture. The humification index did not respond to fish farming. It was observed, by analyzing PARAFAC, an increased concentration of labile compounds and decreasing concentration of recalcitrant compounds. The isotopic analyses showed an increase of δ^{15}N fractions in the total dissolved nitrogen (TDN). This study shows that there were some impacts of initial stages of fish farming on the quantity and quality of DOM, but future increases in the density of net cages and longer operating times may even result in more worrying situations. Further, it was showed that fluorescence indices (FI, β: α, peak T / peak C ratio), PARAFAC, DOM stoichiometry and δ^{15}N isotope analysis of TDN, can be early and useful warning signs for monitoring the impacts of fish farms in tropical reservoirs.

Keywords: Tropical aquaculture; Net cage fish farming; Fluorescence indices; Nile tilapia; Nitrogen; Phosphorus; Stable isotopes; Brazil

SUMÁRIO

INTRODUÇÃO GERAL

A aquicultura desempenha uma importante fração na produção de alimentos para a população humana, sendo que a produção mundial de animais aquáticos equivale a 80,1 milhões de toneladas ao ano. Essa produção continua em crescimento e já ultrapassou a oferta pesqueira (Fig. 1; FAO, 2018). Tamanho crescimento e perspectivas de ampliação para o setor, são alavancados por uma grande demanda por alimento de elevado teor proteico, principalmente como substituto para a carne bovina e suína, que possuem maior custo de produção (Goldburg e Naylor, 2005; FAO, 2018).

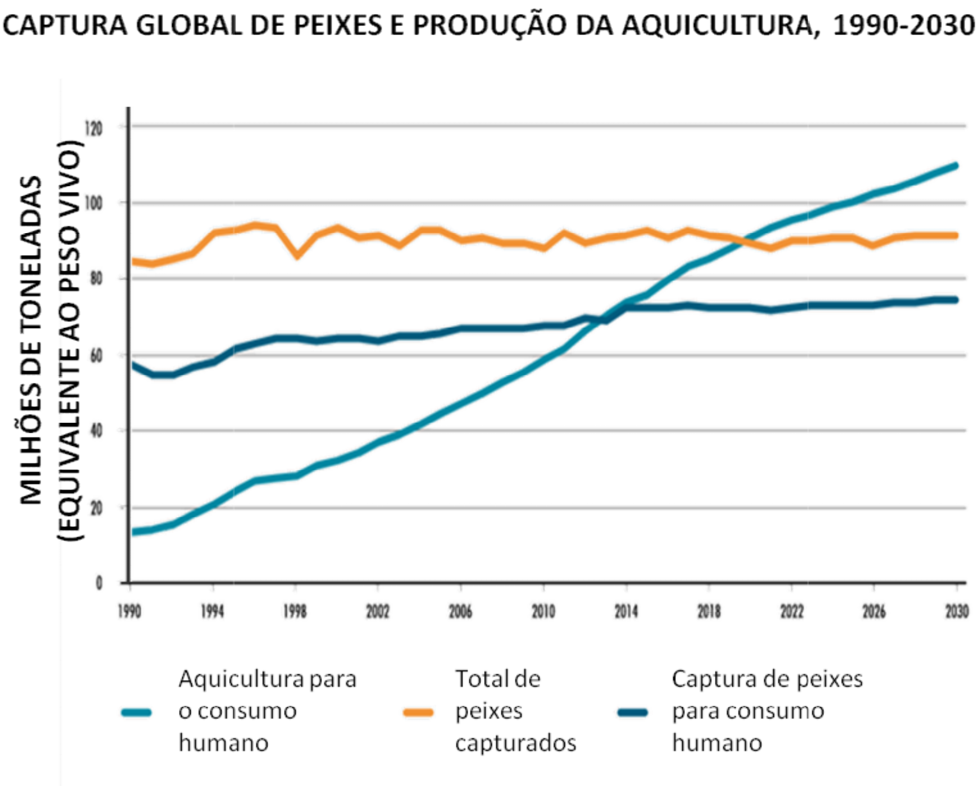

Figura 1: Produção global total da aquicultura e captura de peixes 1990-2030 (valores em milhões de toneladas; adaptado de FAO, 2018).

Comparado a outros países onde a aquicultura é praticada, o Brasil apresenta um histórico mais recente, uma vez que as práticas de produção foram implementadas somente no século XX (Azevedo, 1972). Apesar de uma tardia implementação, o Brasil teve um grande crescimento da aquicultura, com um aumento de aproximadamente 10 vezes somente na década de 1990 (IBAMA, 2005).

O desenvolvimento da aquicultura no Brasil, já com expressiva produção e perspectiva de crescimento, representa uma situação preocupante devido aos impactos comumente causados ao ambiente aquático. Desse modo, no caso específico de uma piscicultura sustentável, deve haver planejamento, uma vez que podem ocorrer alterações na qualidade da água e do sedimento (Wang et al., 2012) devido aos nutrientes liberados nas excretas dos peixes (Figueredo e Giani, 2005) e pela decomposição de ração não consumida. Sabe-se também que os ambientes lênticos são mais vulneráveis aos impactos da piscicultura se comparados aos ambientes lóticos (Alongi et al., 2003). Outro aspecto que vem sendo observado é que tanto a própria prática da piscicultura como outros serviços ecossistêmicos desempenhados pelos corpos d'água podem ser afetados pela criação de peixes de forma não sustentável (Figueredo e Giani, 2005). Dentre os serviços oferecidos pelos corpos d'água que podem ser afetados pela piscicultura, destacam-se o abastecimento de água para a população local, a pesca de peixes que crescem naturalmente, o provimento de condições de oxigenação compatíveis com a prática aquícola e o atrativo turístico.

Diante desse cenário de grande produção e crescente demanda de pescado, é importante estudar os possíveis efeitos da aquicultura, especialmente em relação à degradação dos ambientes aquáticos. A piscicultura em tanques-rede, uma técnica baseada no uso de gaiolas suspensas na coluna d'água, utiliza basicamente estocagem de peixes em alta densidade e abundância de alimentos. Como consequências, essa atividade pode liberar grandes quantidades de resíduos compostos por alimento não consumido, fezes e metabólitos dos peixes. Estes podem impactar diretamente na qualidade da água dos reservatórios pela liberação de nutrientes particulados e dissolvidos nas formas inorgânicas e orgânicas. As frações dissolvidas incluem amônia, fósforo e carbono orgânico dissolvido (que inclui nitrogênio orgânico dissolvido e fósforo orgânico dissolvido), dentre outros (Olsen et al., 2008; Wang et al., 2012). Por décadas, o foco das pesquisas sobre impactos em corpos d'água (Stepanauskas et al., 2002), principalmente em relação à aquicultura (Nimptsch et al., 2015), foi voltado para nutrientes inorgânicos dissolvidos. Entretanto, somente os impactos da exportação de nutrientes inorgânicos não explicam todos os efeitos de degradação que ocorrem devido à aquicultura. Uma importante lacuna de conhecimento está relacionada aos impactos provocados pela matéria orgânica dissolvida (MOD) e estes precisam ser melhor entendidos.

A MOD ocorre naturalmente no ambiente aquático, sendo proveniente de diversas fontes e ativamente consumida ou processada. Em riachos, ela é liberada, a partir da vegetação e do solo, para as correntes de cabeceira das bacias hidrográficas florestadas. Nesses ambientes, a MOD atua como uma importante fonte de energia e de nutrientes orgânicos para os ecossistemas aquáticos locais e a jusante (Hedin et al., 1995; Neff et al., 2003; Carpenter et al., 2005). Durante o transporte da MOD até rios de maior ordem, os ecossistemas fluviais processam parte dos seus componentes e também produzem nova matéria orgânica. Estes processos ocorrem de maneira dinâmica, sob influência da sazonalidade e de outras características do ambiente circundante. Assim, a MOD de regiões a jusante possui característica de ser mais processada, se comparada com a MOD encontrada mais a montante nos riachos. Como consequência, essa matéria apresentará características distintas quanto à sua forma estrutural e suas características nutricionais (Cole et al., 2007).

As atividades antrópicas podem diretamente modificar a qualidade da MOD, pela adição de compostos orgânicos diferentes ou em concentrações distintas daquelas originalmente presentes, ou ainda indiretamente, por meio de alterações do metabolismo do ecossistema aquático, disponibilidade de luz, modulação de atividades químicas e processos biológicos (Stanley et al., 2012; Jaffé et al., 2015). A estequiometria entre MOD e microrganismos heterotróficos consumidores podem controlar o metabolismo microbiano e, assim, a decomposição nos ecossistemas aquáticos (Hessen et al., 2004; Cross et al., 2005). Por exemplo, Gücker et al. (2016) verificou altas concentrações de nitrogênio na MOD em sistemas tropicais impactados, o que poderia contribuir consideravelmente para o metabolismo aquático, pois os organismos heterotróficos aquáticos são frequentemente limitados por N (Meyer e Johnson, 1983; White, 1993; Hall e Tank, 2003). É importante que se tenha um melhor entendimento dos processos que determinam a quantidade e a qualidade da MOD e seu potencial como fonte de nutrientes, a fim de que possamos compreender o seu papel nos processos ecossistêmicos e na dinâmica de impactos com a eutrofização (Glibert, et al., 2005).

Diante da importância do conhecimento acerca das características da MOD (concentração, composição e labilidade), existe uma necessidade urgente de métodos que permitam o monitoramento do comportamento da

MOD em ambientes aquáticos. Também é importante o desenvolvimento de instrumentos de avaliação mais rápida e precisa do impacto antrópico em ambientes aquáticos. Uma ferramenta amplamente utilizada para caracterizar a MOD é a espectroscopia de fluorescência, que gera resultados expressos em matrizes de excitação e emissão, a partir das quais podem ser inferidas importantes características da MOD. Por exemplo, estas podem ser decompostas por meio de uma análise fatorial paralela (PARAFAC), um procedimento que proporciona uma caracterização da MOD baseada na localização e intensidade de fluorescência liberada por seus componentes, identificados em modelos construídos a partir dessas matrizes (Murphy et al., 2013). A utilização dessa técnica era bastante restrita, mas os últimos anos vêm sendo caracterizados pela expansão de seu uso para identificar efeitos de intervenções antrópicas sobre a MOD (Coble, 1996; Baker, 2001; Stedmon et al., 2003; Boyer e Ishii, 2012; Jaffé et al., 2015).

Este trabalho tem por objetivo geral compreender os efeitos da presença de tanques-rede usados para a criação de peixes sobre a concentração e composição (análises das matrizes de excitação e emissão e razões estequiométricas elementares e isotópicas) da MOD em diferentes baías do Reservatório de Furnas. Em síntese, o estudo visa, por meio do uso de vários métodos, contribuir para o conhecimento da dinâmica dessa importante classe de compostos, bastante negligenciada até recentemente, em estudos de eutrofização antrópica, sobretudo em regiões tropicais. De modo geral, prevemos que a piscicultura em tanques-rede aumentará tanto a biodisponibilidade de MOD como a contribuição relativa de compostos lábeis, ou seja, de MOD recentemente produzida. Também prevemos um aumento nas concentrações de nitrogênio e carbono, nas formas orgânicas, nas proporções estequiométricas da MOD.

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CAPÍTULO 1 – Artigo científico

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Fluorescence indices of dissolved organic matter as early warning signals of fish farming impacts in a large tropical reservoir

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Abstract

Dissolved organic matter (DOM) can be an important source of energy and nutrients in aquatic ecosystems and play important roles in carbon and nutrient dynamics of natural and impacted environments. In order to assess the effects of early stage fish farming on dissolved organic carbon (DOC) concentrations as well as DOM fluorescence indices, we took water samples along transects from six fish farms towards reference sites in a large tropical reservoir (Furnas Reservoir, Southeast Brazil) between April 2013 and December 2016. While DOC concentrations did no change along transects, small but significant changes in fluorescence indices were detectable in up to 100 m from fish farms. Higher fluorescence index (FI) values near fish farms pointed to small increases in microbial production due to fish farming. Only in the more pristine of the two main reservoir branches, small differences in the freshness index (β:α) and the peak T/peak C ratio along transects indicated that the relative contribution of recently produced DOM and the biochemical oxygen demand of DOM increased due to aquaculture. The humification index did not respond to fish farming. In summary, current early-stage fish farming did not appear to cause major impacts on DOM quantity and quality, but future increases in net cage and fish farm densities, as well as longer operation times may change this assessment. The fluorescence indices FI, β:α and peak T/peak C ratio may be useful early warning signals for monitoring fish farm impacts in tropical reservoirs.

Keywords

Tropical aquaculture; Fluorescence indices; Net cage fish farming; Nile tilapia; Brazil

Abbreviations

DOC, dissolved organic carbon

DOM, dissolved organic matter

FI, fluorescence index

GLM, general linear model

GLMM, general linear mixed model

HIX, humification index

NH_4-N, ammonium-nitrogen

NO_3-N, nitrate-nitrogen

β:α, freshness index

1. Introduction

The annual growth rate of global aquaculture has dropped from 10.8 % to 5.8 % from 1980 to 2016 (FAO, 2018). Nonetheless, aquaculture continues to grow faster than other major sectors of animal protein production for human consumption. Among the major fish production zones, inland farming is the most important with 64.2% of the world's production (FAO, 2018). Similar to other tropical developing countries, freshwater aquaculture is expanding in Brazil, but basic principles of sustainability are often neglected, posing threats to the biodiversity, integrity and functioning of freshwater ecosystems (Lima Junior et al., 2018). Fish farming is often performed with high stocking densities and plentiful food addition, which may impact aquatic environments even when best management practices are followed (Cole et al., 2009). Such impacts include changes in water quality (Srithongouthai and Tada, 2017), alterations to the structure of the natural fish community (Macuiane et al., 2015) by the introduction of exotic species (Ortega et al., 2015), dissemination of diseases (Peeler et al., 2011), and pollution with toxic chemical elements, such as zinc, copper and cadmium (Dean et al., 2007).

Regarding water quality, one of the main aquaculture effects is nutrient pollution from fish excreta (McGhie et al., 2000; Figueredo and Giani, 2005) and surplus fish feed (McGhie et al., 2000). Differences in the nutritional value and the quantity of supplied fish feed determine specific responses of water and sediment quality to fish farming (McGhie et al., 2000; Lachi and Sipaúba-Tavares, 2008; Alongi et al., 2009) and thus, the degree to which increases in the biomass of autotrophs and primary production, i.e. eutrophication, occurs.

Eutrophication studies often focus on inorganic nutrient forms, such as ammonium, nitrate and phosphate (Stepanauskas et al., 2002). However, aquaculture can also contribute large quantities of dissolved organic matter (DOM) (Nimptsch et al., 2015) that can be directly assimilated by heterotrophic and autotrophic organisms (Granéli et al., 1999). In tropical environments, dissolved organic nitrogen and, to a lesser extent, dissolved organic phosphorus can comprise an important fraction of the nutrients exported by river basins impacted by anthropogenic activities (Graeber et al., 2015; Gücker et al., 2016). Since labile DOM fractions can be directly consumed by autotrophic and heterotrophic organisms, they may cause environmental impacts (Wang et al., 2012), such as increased trophic and saprobic state of aquatic systems, resulting in low dissolved oxygen concentrations or even hypoxia (Howarth et al., 2000; Schindler et al., 2008; Conley et al., 2009).

However, high concentrations of DOM can also occur naturally and are thus not always a result of environmental degradation (Stedmon and Markager, 2005; Boëchat et al., 2019). DOM can even be the main form of organic matter in most natural freshwater ecosystems (Findlay and Sinsabaugh, 2003). Its chemical heterogeneity is often due to a wide variety of DOM sources (Stedmon and Markager, 2005). Terrestrial vegetation and soils are important sources of DOM to aquatic environments determining the ecological functioning of these systems. Catchment terrestrial DOM can, for example, be an important source of energy and organic nutrients to forested headwater streams and rivers, and downstream aquatic ecosystems such as reservoirs (Hedin et al., 1995; Neff et al., 2003; Carpenter et al., 2005).

The DOM present in aquatic ecosystems is often divided into two major fractions. First, there is a more labile fraction that is preferentially consumed and mineralized by the biota. This fraction includes carbohydrates, peptides, amino acids, small carboxylic acids and alcohols. Second, there is a recalcitrant fraction that is more resistant to degradation and includes substances such as humic and fulvic acids, but also other organic acids with lower molecular weight (Coble et al., 1998; McKnight et al., 2001; Her et al., 2003). This fraction is less bioavailable and can remain for a long time in aquatic ecosystems prior do degradation (Sondergaard et al., 1995; Weiss and Simon, 1999).

Accordingly, the labile fraction can contribute to eutrophication due to its rapid assimilation by biota (Fiebig, 1997; Findlay and Sinsabaugh, 2003; Stepanauskas et al., 2002; Brookshire et al., 2005). With the aging of organic matter, the relative quantity of recalcitrant substances increases as a consequence of the consumption of more labile molecules. Thus, DOM from allochthonous sources, being already pre-processed in soils and along subsurface flowpaths, is more recalcitrant than the autochthonous freshwater DOM produced by algae (Fellman et al., 2010) and bacteria (Stepanauskas et al., 1999).

The characterization of the chemical composition of DOM by its optical properties, especially by UV-VIS and fluorescence spectroscopy, has been extensively utilized in freshwater ecology (Fellman et al., 2010). Optical assays allow to analyze a large number of samples with relatively low costs and high sensitivity (Findlay and Sinsabaugh, 2003; Jaffé et al., 2008) and to infer basic characteristics and sources of fresh and processed DOM (Hansen et al., 2016). The optical properties of colored and fluorescent DOM can be used to calculate several indices, often based on measurements of three-dimensional excitation-emission matrices (EEMs) (Coble et al., 1998; McKnight et al., 2001), which provide information to infer source, degree of humification, or relative contribution of recently produced to structurally complex and aromatic DOM (Fellman et al., 2010). The humification index (HIX), for example, correlates with the C:N ratio and aromaticity of DOM, with higher values indicating more polycondensation and lower C:N (Hansen et al., 2016). The freshness index (β:α) indicates the proportion of recently produced DOM (Huguet et al., 2009; Wilson and Xenopoulos, 2009). The fluorescence index (FI) indicates the relative contributions of plant/soil-derived and aquatic microbial material, i.e. if precursor material for DOM has a more aquatic microbial (FI ~1.8) nature or is mainly derived from terrestrial environments (FI ~1.2) (McKnight et al., 2001; Cory and McKnight, 2005). High peak T/peak C ratios indicate high biochemical oxygen demand relative to dissolved organic carbon (Baker, 2001).

The effects of aquaculture on reservoirs are generally investigated with a focus on inorganic nutrients and effects on DOM remain little studied. Since DOM is a fundamental component of organic matter cycling in aquatic ecosystems, detailed studies on its response to aquaculture, which permit the prediction, management and prevention of the ecological consequences of aquaculture, are necessary. Here, we assessed the effects of net cage fish farming on DOC concentrations and relative DOM composition in a large tropical reservoir (Furnas Reservoir, Southeast Brazil). We hypothesized that the relatively low-intensity and recently implemented fish farming in this reservoir increased absolute DOC concentration and DOM quality as indicated by fluorescence indices. We predicted that net cage fish farming increased both the bioavailability of DOM and the relative contribution of recently produced DOM, i.e. of labile DOM compounds in DOM. Thus, we predicted increases in the fluorescence index, the freshness index and the peak T/peak C ratio, and a decrease in the humification index, due to fish farming.

2. Material and methods

2.1. Study area

This study was performed between 2013 and 2016 in the Furnas reservoir, an artificial freshwater system created by the damming of the Grande river (20°40’S; 46°19’W, Minas Gerais, Southeast Brazil) to maintain a hydroelectric power plant that has been in operation since 1963. Upstream of the dam, the Sapucaí river is the major tributary to Grande river and their confluence gives to the reservoir its “V” shape. The two main branches of the reservoir, the Grande branch and the Sapucaí branch (Fig. 1), are both about 200 km long.

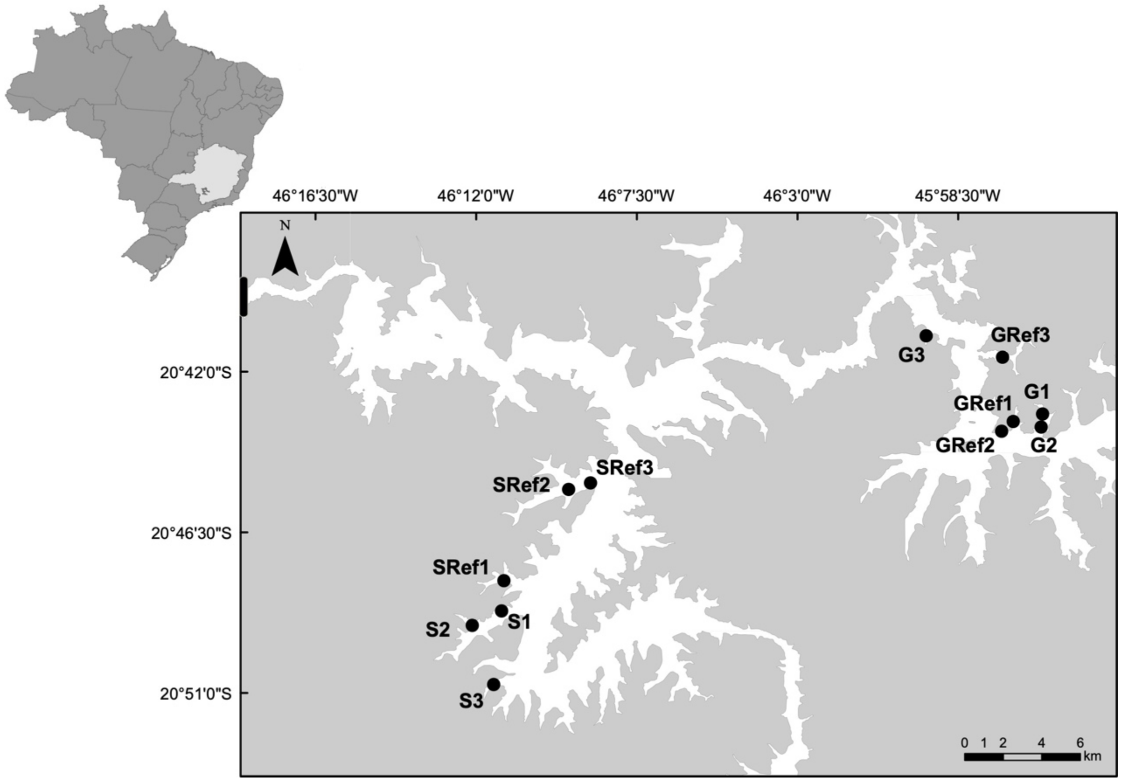

Figure 1: Sampling sites in the Furnas reservoir. G1, G2 and G3, and S1, S2, and S3 are sites potentially impacted by fish farms in the Grande and Sapucaí branches, respectively. G_{Ref1}, G_{Ref2}, G_{Ref3} and S_{Ref1}, S_{Ref2}, S_{Ref3} are local control sites in the Grande and Sapucaí branches, respectively. The crossbar indicates the dam.

Agriculture was the dominant land use in both branches during this study, with a higher contribution in the Sapucaí branch (65.3% agricultural area, 33.5% natural vegetation, 1.2% urban area in a 20 km radius of the study area, unpublished data) than in the Grande branch (52.8% agricultural area, 46.1% natural vegetation, 1.1% urban area, unpublished data). The reservoir has 1.459 km^2 of surface area, an average depth of 16 m and a maximum depth of 90 m (Figueredo and Giani, 2005). In 2007, the Special Secretariat for Aquaculture and Fisheries of the Presidency of the Republic of Brazil defined 16 areas for the future installation of aquaculture parks in the Furnas reservoir, with the aim of increasing fish production (Sampaio et al., 2008).

The tropical highland climate of the region (Cwa according to the Köppen-Geiger classification) is characterized by dry and mild winters (end of March–end of September) and warm, rainy summers (end of September–end of March), with an average annual temperature of 21°C and an average annual precipitation of 1444 mm (unpublished data, meteorological station São José da Barra).

2.2. Sampling

Samplings were carried out on nine dates covering different seasons (1: April 2013; 2: June 2014; 3: September 2014; 4: December 2014; 5: March 2015; 6: June 2015; 7: December 2015; 8: July 2016; 9: December 2016). Samples were collected at sites not impacted by aquaculture (henceforth referred to as reference sites) and sites impacted by fish farming in net cages (Fig. 1). Reference sites were among the locations recommended for the future installation of fish farms in the Furnas reservoir (Sampaio et al., 2008). At these sites, no fish farming had taken place prior to and during our study and our strategy was to generate a dataset that may also be useful for future comparisons, when net cages will be installed at these sites. The potentially impacted sites we investigated were in the lateral bays with the largest fish farms in the region, according to the number of net cages present. As these fish farms were rather small with average net cage numbers ranging between 23 and 121 and recently implemented (< 10 years), we refer to effects of early stage fish farming in the present study.

We studied three impacted sites and three reference sites in each of the two main branches of the reservoir, the Grande branch and the Sapucaí branch (Fig. 1). At each reference site, henceforth referred to as G_{Ref} (sites G_{Ref}1–3 in the Grande branch; Fig. 1) and S_{Ref} (sites S_{Ref}1–3 sites in the Sapucaí branch), water samples were obtained at two sampling stations, with one station closer to the distal end of the bay and another closer the main body of the reservoir. These stations were sampled in two depths, corresponding to the middle of the euphotic and the aphotic zone as determined by Secchi depth measurements.

In each site/bay containing fish farms (sites G1–3 and S1–3; Fig. 1), water sampling was performed at three sampling stations, henceforth referred to as G_A, G_B and G_C in the Grande branch and S_A, S_B, and S_C in the Sapucaí branch. These sampling stations spanned a spatial gradient from within the fish farms (G_A and S_A) in lateral bays towards the main body of the reservoir and were located in 100 m distance from each other, with G_B and S_B being the intermediate sampling stations, and G_C and S_C the stations closer to the reservoir outlet). These stations were also sampled in two depths corresponding to the middle of the euphotic and the aphotic zone, because there were no notable gradients or significant differences along 6 depths, starting from top to bottom of the water column, in initial samplings. To evaluate the effects of fish farms, we compared reference and impacted stations, and also impacted stations with different distances from fish farms.

The geographical coordinates of the sampled net cage fish farms in the Grande branch were G1 (20°43' 09,3"S 45°56' 06,4"W), G2 (20°43'31,1"S 45°56'08,2"W) and G3 (20° 40' 57,4"S 45° 59' 22"W). In the Sapucaí branch the fish farms were S1 (20°48'40,4" S46°11'15,1"W), S2 (20°49'04"S 46°12'04,5"W), and S3 (20°50'44,0"S 46°11'29,0"W). The average number of net cages per farm along the sampling period were 121 at G1, 30 at G2, 23 at G3, 39 at S1, 48 at S2 and 92 at S3.

2.3. Water Level

Variation in rainfall affects reservoir water level and water retention time, which in turn affects the dilution of organic matter released by fish farming. Therefore, the effects of temporal variation in water dilution due to rainfall in the reservoir were evaluated using average monthly water levels. Reservoir water level in relation to sea level (daily quota) were obtained from the Reservoir Monitoring System of the National Water Agency, and the average monthly level of the reservoir was calculated from January 2013 to January 2016.

2.4. Water sample collection and processing

Water Samples were obtained with a 5 L Van Dorn bottle, then transferred into clean polyethylene bottles and stored on ice and transported to the laboratory. In the laboratory, water samples were filtered through pre-washed glass-fibre filters (GF-5, 0.45 µm nominal pore size, Machery-Nagel), and transferred into clean, acid-washed polyethylene bottles (500 mL) prior to analysis.

2.5. Physical and chemical variables

Dissolved oxygen, pH and temperature were measured in the field using a multiparameter probe (YSI 556 MPS Yellow Springs Instruments, Yellow Springs, OR, USA) calibrated immediately prior to sampling. Water transparency was measured using a Secchi disk. The euphotic zone was calculated by multiplying the Secchi depth by 3 (Dokulil and Teubner, 2000).

Dissolved inorganic nutrients were analyzed with standard spectrophotometric methods on a continuous flow injection system (FIAlab 2500, WA, USA). The salicylate method was used for NH_4-N (Verdouw et al., 1978), the cadmium reduction-sulfanilamide method for nitrate+nitrite-N (henceforth referred to as NO_3-N; 4500-NO_3 E; APHA, 2005), and the ascorbic acid-molybdate method for SRP (4500-P F; APHA, 2005). Dissolved organic carbon (DOC) concentrations were measured by high temperature catalytic oxidation coupled to stable isotope ratio mass spectrometry (Thermo Flash EA, Thermo Delta V Advantage, Thermo Scientific, Germany) following sample concentration, acidification and preparation according to Gandhi et al. (2004).

2.6. DOM fluorescence

Fluorescence excitation emission matrices (EEMs) of DOM were measured with a combined scanning spectrofluorometer and photometer (Horiba, AQUALOG-UV-800, USA) in a 1 cm quartz cuvette. The cuvette was flushed once with ultrapure water (18.2 MΩ/cm, Milli-Q, Millipore) and three times with the sample before each measurement. Fluorescence intensity was measured during emission scans (250–800 nm every 4.65 nm, 8 pixel) at excitation wavelengths between 240 nm to 450 nm in 3 nm increments (2s integration time). Using the internal software of the equipment, correction of the fluorescence data (including blank subtraction) were performed before the correction of inner-filter effects, using quinine sulfate NIST and Raman water reference cells. Based on EEMs, the following fluorescence indices were calculated: (1) the freshness index (β:α), calculated as the intensity at a emission wavelength of 380 nm divided by the maximum intensity between emission wavelengths of 420–435 nm at an excitation wavelength of 310 nm (Huguet et al., 2009; Wilson and Xenopoulos, 2009); (2) the fluorescence index (FI), calculated as the intensity at an emission wavelength of 470 nm divided by the intensity at an emission wavelength of 520 nm, both at an excitation wavelength of 370 nm (Cory and

McKnight, 2005); (3) the peak T/peak C ratio, calculated as the ratio of peak C intensity (excitation 340nm, emission 440 nm) and peak T intensity (excitation 275 nm, emission 304 nm) (Baker, 2001); and (4) the humification index (HIX), calculated as the area under the emission spectrum 435–480 nm divided by the peak area 300–345 nm + 435–480 nm, at an excitation of 254 nm (Hansen et al., 2016).

2.7. Statistical Analyses

To test for the effects of fish farming on fluorescence indices and DOC, we used generalized linear mixed models (GLMM) in the package *lme4* in the R software (R Core Team, 2018). Models were calculated separately for the two branches of the reservoir. GLMMs included the fixed factor “station” with 4 factor levels (stations A, B, C, and Ref) and the random factors “sampling date” with 9 levels and/or “depth” with 2 levels (samples from the euphotic and aphotic zone) according to residual analyses. Distributions of residuals were analyzed using the *RT4Bio* package in R to check the adequacy of models and to select the most appropriate model. According to residual analysis, the random factor “site” (i.e. the sampled bays) was not included in any model. To tests for differences between stations, post-hoc tests were conducted in the R package *RT4Bio*. We also calculated simple generalized linear models (GLMs) with the fixed factors “reservoir branch” and “sampling date” and Pearson correlations between DOM characteristics and reservoir water level.

3. Results

3.1. Study site characterization

While there was no dominant seasonal trend in water depths of sampling sites due to large interannual variations in reservoir water level (Table 1, Fig. 2), mean seasonal water temperature tended to be higher in the warm, rainy season at all sites and ranged between 22.0 and 26.6°C (Table 1). Sampling sites in the Sapucaí branch of the reservoir, which had a higher percentage of agricultural land use, had in general higher mean seasonal NH_4-N concentrations than sites in the Grande branch (Table 2). NO_3-N was the dominant inorganic nitrogen form at all sampling sites, with the exception of site S3 (Table 2). At site S3 NH_4-N concentrations were generally higher than NO_3-N concentrations, probably due to low dissolved oxygen concentrations at this site (Table 1).

Table 1: Physical and chemical characteristics (mean ± 1SD) of impacted (A-C) and reference (Ref) stations in the Grande reservoir branch (G) and the Sapucaí branch (S) across all sampling dates in the dry and wet season. Water depths (D), euphotic zone depths (EZ), water temperature (T), pH and dissolved oxygen (DO).

Station	D (m)		EZ (m)		T (°C)		pH		DO ($mg.L^{-1}$)	
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
G_A	13.1±4.6	13.3±7.0	9.4±2.7	23.0±1.0	23.0±1.0	26.7±0.9	6.7±0.4	7.1±0.5	6.4±0.5	6.0±1.0
G_B	19.8±7.1	18.1±5.5	21.5±1.1	11.6±1.3	22.0±0.4	25.7±1.6	6.9±0.4	7.4±0.5	7.1±0.2	5.3±2.2
G_C	16.8±3.7	16.2±1.3	13.2±2.1	10.5±0.0	22.2±0.5	26.1±0.9	6.9±0.6	7.0±0.2	6.3±0.2	5.1±1.7
G_{Ref}	18.8±6.2	18.4±6.9	10.5±3.2	22.5±0.7	22.5±0.7	25.3±0.9	6.6±0.7	6.8±0.4	6.6±0.7	6.3±1.4
S_A	24.5±6.7	24.4±7.2	12.5±4.2	6.1±1.5	22.6±0.8	25.8±1.1	6.6±0.4	7.0±0.4	5.9±0.8	4.8±2.1
S_B	14.7±3.6	13.7±3.4	11.6±3.0	10.2±4.4	22.2±0.6	25.9±1.5	7.0±0.5	7.2±0.3	6.3±1.1	4.9±2.8
S_C	-	17.6±6.9	-	5.5±2.3	-	26.3±1.8	-	7.2±0.3	-	4.4±3.2
S_{Ref}	18.5±2.6	19.3±6.7	10.5±2.8	6.4±1.5	22.7±0.7	25.8±1.0	6.8±0.3	6.9±0.4	6.2±0.8	5.0±2.0

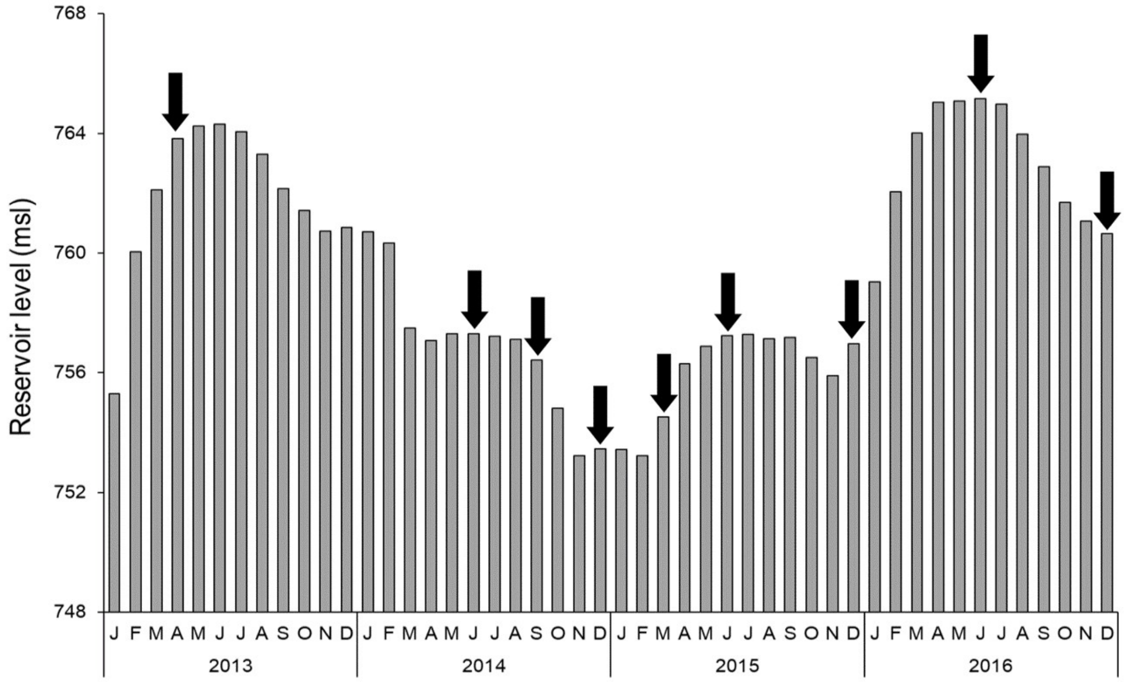

Figure 2: Reservoir level (average m.a.s.l. by month) between January 2013 and December 2016. The arrows indicate the months in which sampling campaigns were performed.

3.2. Spatial patterns in DOC concentrations

DOC concentrations were significantly higher in the Sapucaí branch (mean value: 7.2 mg.L^{-1}, range: 1.8–13.8 mg.L^{-1}) than in the Grande branch (4.9 mg.L^{-1}, 1.0–8.6 mg.L^{-1}) (GLM, *P*<0.001, see Fig. 3 and Table 2 for seasonal mean values per station). There were neither significant differences in DOC concentrations among stations A, B, C, and Ref in the Grande branch nor in the Sapucaí branch (GLMM, *P*>0.10).

Table 2: Inorganic nutrient and DOC concentrations (mean ± 1 SD) of for impacted (A-C) and reference (Ref) stations in the Grande reservoir branch (G) and the Sapucaí branch (S) across all sampling dates in the dry and wet season.

Station	NH_4-N (µg.L^{-1})		NO_3-N (µg.L^{-1})		SRP (µg.L^{-1})		DOC (mg.L^{-1})	
	Dry	Wet	Dry	Dry	Wet	Dry	Dry	Wet
G_A	44±24	34±28	211±45	378±129	20±7	32±7	5.1±1.0	4.7±1.3
G_B	32±13	38±17	208±23	378±243	20±5	26±4	4.5±0.8	3.6±1.1
G_C	35±18	22±3.4	238±45	197±20	20±8	30±2	4.9±0.4	3.8±0.2
G_{Ref}	14±8	44±37	215±29	315±104	20±7	25±6	5.7±1.0	5.0±2.1
S_A	70±61	69±65	236±43	161±68	18±7	25±6	7.2±0.9	7.3±2.2
S_B	42±18	61±69	232±45	109±94	21±4	29±2	7.4±1.3	6.5±1.7
S_C	-	132±131	-	20±18	-	30±2	-	7.1±0.9
S_{Ref}	71±59	100±86	210±55	179±69	19±8	25±6	6.9±0.8	7.2±2.6

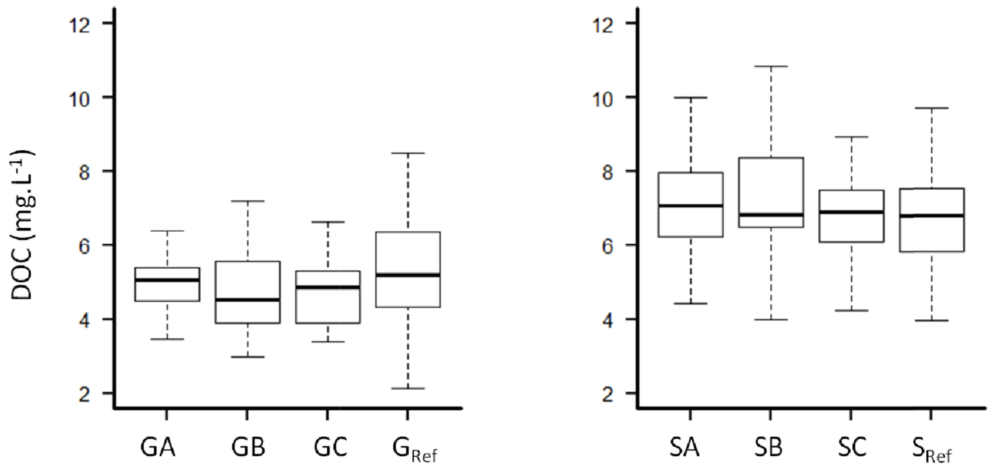

Figure 3: Dissolved organic carbon (DOC; mg.L^{-1}) along a gradient of potential fish farm impacts. Stations are A: fish farms in lateral bays, B: 100m distant towards the reservoir, C: 200 meters distant, and Ref: reference stations in the Grande branch (G) and the Sapucaí branch (S). Boxplots represent the interquartile ranges (25th and 75th percentile), horizontal bars the median, and whiskers extend to 1.5 times the interquartile range.

3.3. Spatial patterns in fluorescence indices

3.3.1. Peak T/peak C ratios

The peak T/peak C ratio ranged between 0.20 and 3.33 with a mean value of 0.97 in the Grande branch and between 0.00 and 7.79 with a mean value of 0.94 in the Sapucaí branch (see Table 3 for seasonal mean values per station). There were no significant differences between branches (GLM, P>0.1). There were significant differences among sampling stations in the Grande branch (GLMM, P<0.001; Fig. 4) with higher values near fish farms, but not in the Sapucaí branch (GLMM, P>0.1).

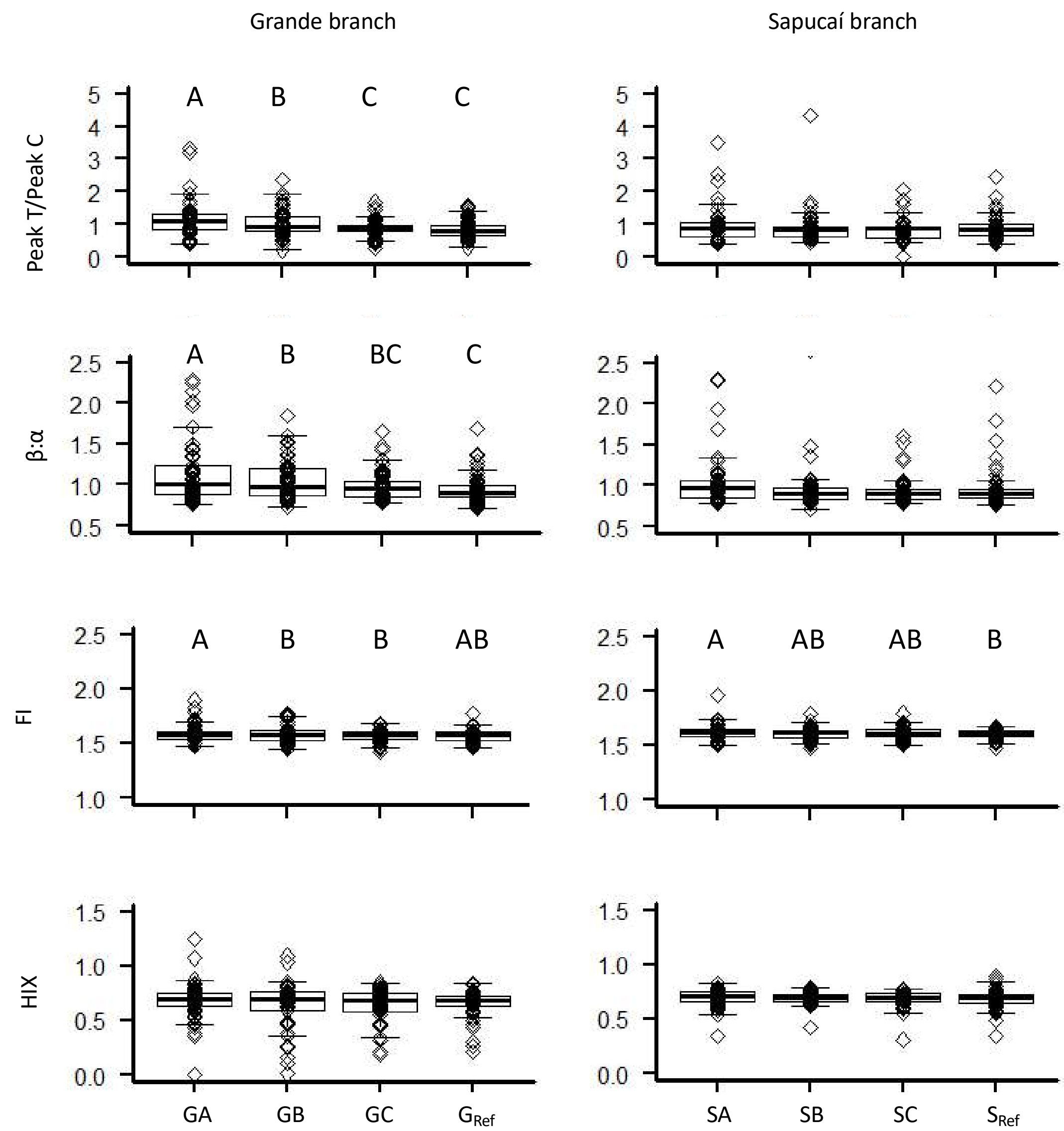

Figure 4: Fluorescence indices of DOM along a gradient of fish farm impacts. Stations are A: fish farms in lateral bays, B: 100m distant towards the reservoir, C: 200 meters distant, and Ref: reference stations in the Grande branch (G) and the Sapucaí branch (S). Boxplots represent the interquartile ranges (25th and 75th percentile), horizontal bars the median, and whiskers extend to 1.5 times the interquartile range. Diamonds represent original data. Different letters (A, B, C) above boxplots indicate significant differences between stations in decreasing order of index mean value (GLMM *P*<0.05 and post-hoc tests *P*<0.05).

Table 3: Fluorescence indices (mean ± 1 SD) of for impacted (A-C) and reference (Ref) stations in the Grande reservoir branch (G) and the Sapucaí branch (S) across all sampling dates in the dry and wet season.

Station	peakT/peakC		β:α		FI		HIX	
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
G_A	0.96±0.37	1.21±0.56	0.97±0.20	1.14±0.36	1.57±0.08	1.58±0.05	0.70±0.10	0.65±0.18
G_B	0.83±0.32	1.35±0.32	1.02±0.16	1.47±0.36	1.54±0.08	1.64±0.06	0.71±0.10	0.32±0.16
G_C	0.60±0.25	0.95±0.25	0.89±0.10	1.19±0.22	1.55±0.06	1.60±0.03	0.72±0.09	0.41±0.13
G_{Ref}	0.89±0.31	0.78±0.24	0.96±0.17	0.94±0.18	1.59±0.09	1.58±0.04	0.67±0.13	0.63±0.29
S_A	0.72±0.31	0.95±0.26	0.90±0.13	0.98±0.22	1.59±0.05	1.64±0.06	0.68±0.10	0.68±0.04
S_B	0.77±0.44	2.51±2.10	1.02±0.23	1.65±0.70	1.58±0.08	1.59±0.02	0.63±0.13	0.73±0.05
S_C	-	1.07±0.46	-	1.01±0.17	-	1.62±0.02		0.71±0.04
S_{Ref}	0.77±0.27	1.22±0.48	0.96±0.26	0.99±0.30	1.58±0.04	1.61±0.02	0.71±0.08	0.62±0.11

3.3.2. Freshness index

The freshness index (β:α) ranged between 0.71 and 2.29 with a mean value of 1.03 in the Grande branch and between 0.71 and 2.80 with mean value of 0.99 in the Sapucaí branch (see Table 3 for seasonal mean values per station). There were no significant differences between branches (GLM, P>0.1). There were significant differences in β:α among sampling stations in the Grande branch (GLMM, P<0.001; Fig. 4) with higher values near fish farms, but not in the Sapucaí branch (GLMM, P>0.1).

3.3.3. Fluorescence index

The fluorescence index (FI) was significantly higher in the Sapucaí branch (mean value: 1.61, range: 1.47–1.96) than in the Grande branch (1.58, 1.42–1.90) (GLM, P<0.001, see Table 3 for seasonal mean values per station). There were significant differences among sampling stations in both the Sapucaí and the Grande branch (GLMM, P<0.05; Fig. 4), generally with higher values near fish farms than at stations located 100 and 200 m towards the main reservoir. However, in the Grande branch, values at fish farm stations were not different from those at reference stations (Fig. 4).

3.3.4. Humification index

The humification index (HIX) ranged between 0.002 and 1.25 with a mean value of 0.65 in the Grande branch and between 0.31 and 0.89 with a mean value of 0.69 in the Sapucaí branch. There were no significant differences between branches (GLM, P>0.1). There were no significant differences among sampling stations along the studied gradients of fish farming impacts in both the Grande branch and the Sapucaí branch (GLMM, P>0.1).

3.4. Temporal patterns

Variability in DOM characteristics was clearly higher within sampling stations of the same reservoir branch than among stations (Figs 3 and 4). This was partially due to temporal effects and there were significant differences in DOM characteristics of reference stations among sampling campaigns (Fig. 5). For example, in two sampling campaigns (3: September 2014 and 9: December 2016) we found a high freshness and fluorescence index and peak T/peak C ratio (Fig. 5, GLMs, $P<0.05$). However, DOM characteristics in our study were not related to absolute reservoir water level in both branches (Pearson correlations, $P>0.1$; also see Fig. 2), with the exception of DOC concentration in the Sapucaí branch that was positively related to absolute reservoir water level (Pearson correlation, $P<0.001$, adj. $R^2=0.49$).

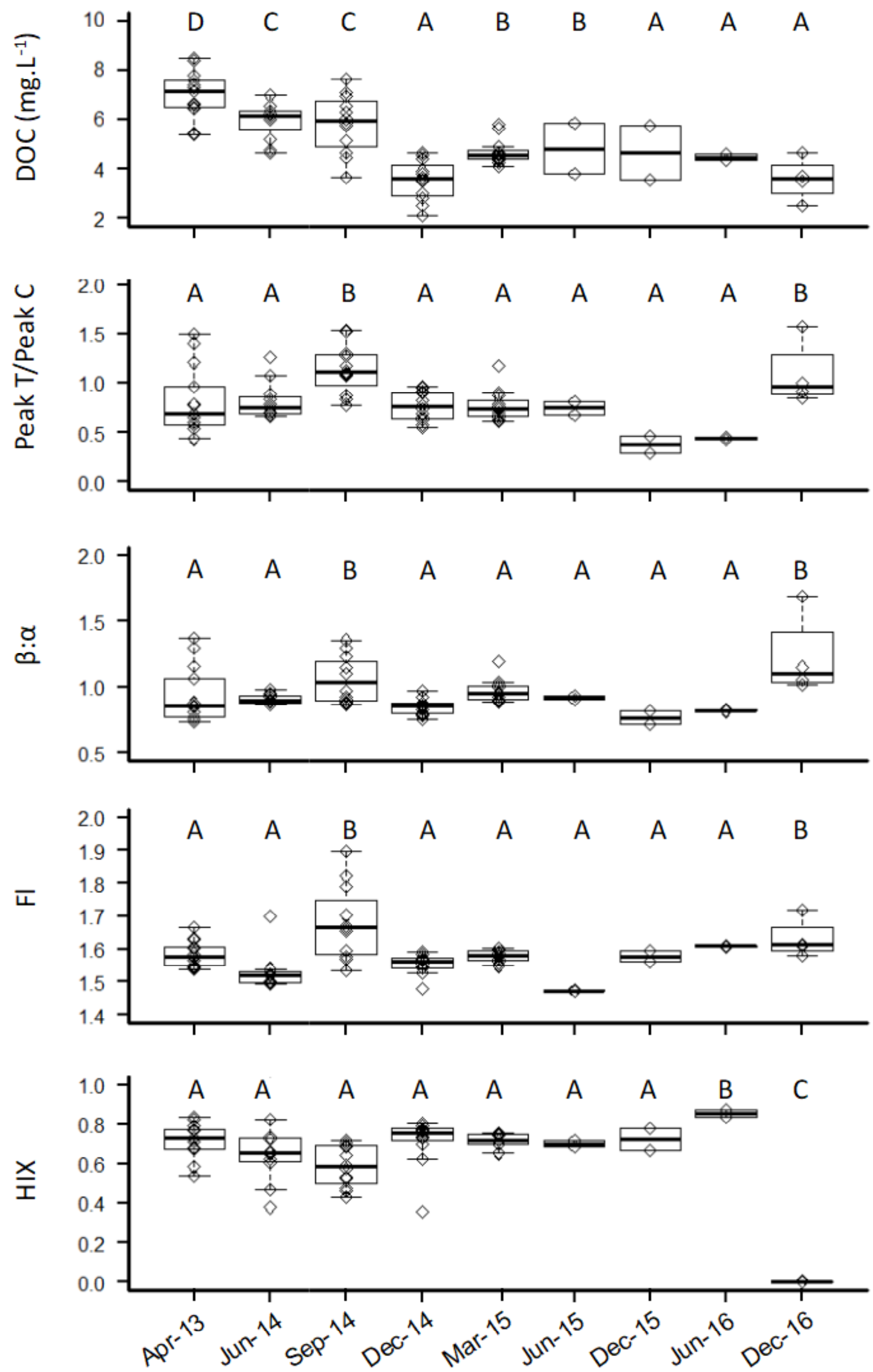

Figure 5: Fluorescence indices for the reference stations of the Grande branch from the 9 sampling campaigns. Boxplots represent the interquartile ranges (25th and 75th percentile), horizontal bars the median, and whiskers extend to 1.5 times the interquartile range. Diamonds represent original data. Different letters (A, B, C) above boxplots indicate significant differences between stations in increasing order of index mean value (GLM P<0.05 and post-hoc tests P<0.05).

4. Discussion

In addition to inorganic nutrients and particulate organic matter, fish farming can provide dissolved organic carbon, nitrogen and phosphorus subsidies to aquatic environments, deriving from surplus fish diet, accidental feed spillage and fish feces (Black, 2001; Read and Fernandes, 2003; Nimptsch et al., 2015). Without adequate management, aquaculture can therefore contribute to the deterioration of water quality and ecosystem integrity (Read and Fernandes, 2003; dos Santos Rosa et al., 2013). For example, 21% of the total carbon used in the first step of the production of salmonids in Patagonia, Chile, was exported to headwater streams as labile, protein-like DOC (Nimptsch et al., 2015). Similarly, tryptophan-like fluorescence of DOM was higher in the outflow of an aquaculture plant compared to its inflow (Stedmon et al., 2003). Kamjunke et al. (2017) found that land-based salmon aquaculture released large quantities of bioavailable metabolites, such as carbohydrates, peptides/proteins, and lipids to rivers and concluded that further research is needed to assess changes in the composition and structure of natural DOM due to land-based aquaculture, as well as its ecosystem-wide consequences.

Especially in tropical developing countries, freshwater aquaculture is expanding, but the effects of this development on ecosystem structure and functioning of rivers, lakes and reservoirs are poorly understood (Lima Junior et al., 2018). Here, we assessed early effects of fish farming on DOM concentrations and quality in a large tropical reservoir. While DOC concentrations were not affected by fish farming, higher values of the fluorescence index (FI) near fish farms suggested small, but significant increases in microbial production. Small increases in the freshness index (β:α) and the peak T/peak C ratio near fish farms indicated that the relative contribution of recently produced DOM and the biochemical oxygen demand of DOM increased due to aquaculture in the more pristine reservoir branch.

Increases in microbial productivity due to fish farming may be a consequence of the high nutritional quality of fish feces and waste fish feed. For example, the discharge of bioavailable DOM by salmon aquaculture resulted in an increase in bacteria in Patagonian streams (Kamjunke et al., 2017). The microbial community is generally the first to respond to chemical and physical changes and plays important biogeochemical roles in aquatic environments (Cotner and Biddanda, 2002; Fodelianakis et al., 2014). Thus, changes in microbial production as indicated by the FI may be an important early warning signal for upcoming aquaculture impacts.

FI values around 1.6 at reference stations in both the Grande and the Sapucaí reservoir branches indicated that DOM was derived from both terrestrial (FI~1.2) and aquatic microbial sources (FI~1.8), probably with a dominance of aquatic microbial sources (McKnight et al., 2001; Cory and McKnight, 2005). Interestingly, we found only small responses of the FI to early stage aquaculture, albeit the FI generally shows considerable responses to a wide range of human impacts, supporting the idea that the FI could be an important early warning signal for tropical fish farming. For example, the FI of fluvial DOM increases with increasing catchment cropland and decreasing wetland coverage (Wilson and Xenopoulos, 2009). Similarly, sewage discharge is usually accompanied by increases in FI values and microbially derived DOM components in rivers (Ghervase et al., 2010).

Due to a greater human occupation and land use, such as agriculture, industry and mining, the Sapucaí branch of the Furnas reservoir has a higher trophic state than the Grande branch (Corgosinho and Pinto-Coelho, 2006; COPASA, 2010). Land use related impacts can alter the quality and quantity of DOM in fresh waters (Williams et al., 2016). For example, agricultural land use can increase the concentration of labile DOM components, which in turn increases bacterial production (Wilson and Xenopoulos, 2009). Moreover, DOM produced by phytoplankton that is metabolically more active in eutrophic systems is mainly composed of labile, low-weight molecules, such as carbohydrates, proteins and amino acids (Fonte et al., 2013). In our study, the general response in DOM quality of the Sapucaí branch to fish farming may have been weaker than that of the Grande branch because it had a higher trophic state, as e.g. reflected in lower DO concentrations, higher DOC and NH_4-N concentrations, and higher FI values. Thus, absolute impacts of fish farming on DOM quality may have been greater in the Grande branch than in the Sapucaí branch due to different baseline pollution levels.

To a limited degree, our results also allowed for the evaluation of the spatial dimension of early fish farm impacts on DOM quality in the studied tropical reservoir. In general, effects on reservoir DOM quality were only detectable in up to 100m distance from the fish cages. Albeit relatively short in comparison to the reservoir dimensions, this distance is longer than the dispersion distance of feces and fish food residues in sediments below and around coastal net cage fish farms, which was estimated to extend to up to 30 m distance (McGhie et al., 2000; Srithongouthai and Tada, 2017). Detection ranges of only up to 100 m may suggest that the investigated fish farms did not cause major impacts on the DOM quality of the studied reservoir at current fish farm sizes and densities. However, this may change with the future installation of aquaculture parks in this reservoir (Sampaio et al., 2008).

In addition to providing nutrient and organic matter subsidies that affect biological communities and ecosystem primary production and respiration (dos Santos Rosa et al., 2013; Jean-Marc et al., 2018), fish farms affect the ecological integrity of aquatic ecosystems by introducing exotic species and diseases (Peeler et al., 2011; Ortega et al., 2015) and releasing toxic chemical elements (Dean et al., 2007). Further, aquaculture waste can progressively become a more important component of the aquatic food web and it may take many years of aquaculture operation until the full potential of its impact becomes evident (Kullman et al. 2009). Thus, the results of the present study do not suggest that aquaculture does not pose a risk to the studied reservoir. Both efficient aquaculture management (Read and Fernandes, 2003) and efficient natural waste dispersion (Gondwe et al., 2012) can help mitigating its ecological impact.

However, natural waste dispersion may become more inefficient with projected future climate change for the region and may already have affected the studied reservoir during the study period, i.e. the 2014 and 2015 draughts (Fig. 2). In these years, decreases in the reservoir level resulted in changes in the location of net cages and in the dilution capacity of the reservoir. Fish farmers moved net cages to formerly deeper locations of the reservoir, away from the reservoir margins.

Further, variation of rainfall in the catchment area of a reservoir may not only affect dilution of internal DOM and changes in biogeochemical processing within the reservoir (Buffam et al., 2001), but also directly affect DOM quantity and quality exported by river catchments to the reservoir via changes in catchment hydrological regimes (Lambert et al., 2016). In our study, we did not find relationships between reservoir level and fluorescence indices, but with DOC concentration. However, more refined analyses of catchment exports, riverine and within reservoir mineralization processes, and dilution as well as concentration processes may be necessary to adequately analyze the effect of the climatic regime.

5. Conclusion

In tropical countries, aquaculture is expanding, but the impacts of aquaculture development on water quality and the ecological integrity of aquatic ecosystems are poorly understood. Here, we showed that early stage aquaculture – characterized by relatively short operation times, farm size and density – in a large tropical reservoir had no detectable effects on DOC concentrations and small, but significant positive effects on DOM quality, as assessed by fluorescence indices. These effects were only detectable in up to 100 m distance from fish farms and were more pronounced in the more pristine branch of the reservoir. Future increases in fish farm and net cage densities in the reservoir and longer operation times in combination with projected climate and land use change may intensify aquaculture effects on reservoir DOM, underscoring the need for environmentally sound aquaculture practices and management in tropical reservoirs. For the monitoring of early aquaculture effects, the fluorescence indices FI, β:α and peak T/peak C prove to be useful. Our study also indicated complex interactions between land use, reservoir water level and aquaculture impacts, which should be investigated and included in management decisions in the future.

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CAPÍTULO 2– Manuscrito

Impacts of fish farming on elemental stoichiometry, fluorescence components, and stable isotope signatures of dissolved organic matter in a large tropical reservoir

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Keywords

Tropical aquaculture; Nitrogen; Phosphorus; Stable isotopes; Net cage fish farming; Nile tilapia

Abstract

The effects of fish farming on aquatic organic matter and ecosystem integrity are poorly understood, especially in tropical regions. Here, we investigated the impacts of Nile tilapia net cage farming on the elemental stoichiometry, fluorescence components, and stable isotope signatures of dissolved organic matter (DOM) in a large tropical reservoir (Furnas reservoir, SE Brazil). Early-stage fish farming, characterized by relatively short operation times and small farm size, already showed small, but significant effects on DOM characteristics and these effects differed between reservoir branches. In the less eutrophic Rio Grande branch of the reservoir, a decrease in natural humic-like DOM components and an increase in protein-like DOM components occurred in up to 100 m distance from fish farms. Further, a decrease in δ^{15}N-TDN was observed due to fish farming. In the more eutrophic Rio Sapucaí branch, there were only local decreases in C:N, and increases in C:P and N:P ratios of DOM due to fish farming. These results suggest that early-stage fish farming had local, but detectable effects on aquatic DOM that depended on previous eutrophication levels and highlight the need to combine different monitoring strategies to assess early impacts of fish farming on tropical reservoirs.

1. Introduction

Fish farming can release large amounts of organic and inorganic waste to aquatic ecosystems (Wang et al., 2012). Organic matter subsidies due to fish farming are mainly related to remnant food and fish feces, which can subsequently be released as high quality dissolved organic matter (DOM) (Olsen et al., 2008). In salmon farming, up to 22% and 26%, respectively, of the total carbon and nitrogen from fish feces leach within the first five minutes into the surrounding water (Chen et al., 2003). Moreover, 13.4 to 15.0 % of the phosphorus present in feces from trout production are in soluble form. Accordingly, fish farming waste can provide a variety of nutrients readily available to aquatic auto- and heterotrophs, potentially affecting ecosystem productivity. Moreover, aquatic DOM serves as a carbon source and its composition influences bacterial community dynamics and production, thereby controlling ecosystem carbon dynamics (Baines and Pace, 1991; Judd et al., 2006).

Fish farming with net cages is one of the most common types of intensive aquaculture, harnessing natural and artificial freshwaters for food production. However, excessive loads of organic wastes from net cage fish farms must be prevented and potential losses of water quality of rivers, reservoirs, lakes and coastal areas must be studied and managed (Islam and Tanaka, 2004). The impacts of this activity on aquatic DOM have to date been mainly investigated in marine systems (Olsen et al., 2008), but are poorly documented for large reservoirs. Large reservoirs provide various important ecosystem services to the adjacent population and the eutrophication of these systems may therefore have more extensive and economically important effects than e.g. the eutrophication of small lakes and streams (Tundisi et al., 2008).

DOM is one of the largest sources of nutrients in freshwater environments and its dynamics heavily affect, at local to global scales, the cycles of carbon (Battin et al., 2009), nitrogen (Berman and Bronk, 2003) and phosphorus (Baldwin, 2013). Changes in the quality of DOM can affect chemical, physical, and biological processes of aquatic systems (Fellman et al., 2009; Stanley et al., 2012). For example, Ávila et al. (2019) showed that autochthonous DOM production related to nutrient supply was the dominant driver of bacterioplankton community structure and succession processes in a tropical lake. The composition of DOM is poorly characterized for many tropical aquatic environments, both in terms of chemical composition and elemental stoichiometry (Jaffé et al., 2012; Lambert et al. 2016). DOM may originate from terrestrial environments and be transported to aquatic systems via surface and subsurface flow or be internally produced by aquatic plants and microorganisms (Aitkenhead-Peterson et al., 2003). Fluxes and composition of DOM from terrestrial environments depend on soil chemical and physical properties, and the biodegradation of catchment soil organic matter (Grieve and Marsden, 2001). DOM characteristics also depend on catchment vegetation, since leaf litter and detritus chemical quality differs among terrestrial plant taxonomic groups (Likens and Bormann 1970). Autochthonous DOM is produced by bacteria, algae, zooplankton, macrophytes, as well as the activities of sediment organisms (Bertilsson and Jones Jr., 2003). Autochthonous freshwater DOM is mainly derived from algae and macrophytes. In lentic environments, planktic algae typically dominate DOM production (Baines and Pace, 1991). Algal DOM is biologically labile and often an important source of carbon and nutrients for bacterioplankton (Baines and Pace, 1991; Del Giorgio and Cole, 1998). Macrophytes can be important sources of DOM in systems that have abundant macrophyte populations, such as lowland rivers and shallow lakes (Søndergaard,1983).

Organic matter is intensively processed in aquatic ecosystems (Maranger et al., 2018) and allochthonous DOM subsidies can change the dynamics of DOM decomposition and autochthonous production (Brandão et al., 2018; Ávila et al., 2019). Fish farms can export large quantities of DOM, rich in nitrogen and phosphorus (Olsen et al., 2008), which may lead to shifts in ambient DOM stoichiometry. DOM stoichiometry, in turn, affects the aquatic microbial community structure and thus the decomposition of DOM (Hessen et al., 2004; Cross et al., 2005).

Fluorescence spectroscopic characterizations and subsequent parallel factor analysis (PARAFAC) are important tools for tracing environmental impacts on aquatic DOM (Cory and McKnight, 2005; Ishii and Boyer, 2012). For example, PARAFAC components have provided important insights into the consequences of agricultural and urban land use (Williams et al., 2010). Agricultural land use is associated with increased exportations of structurally complex DOM with a low redox state (Graeber et al., 2012). Inland salmon aquaculture resulted in considerable exports of tyrosine- and tryptophan-like DOM to streams (Nimptsch et al., 2015; Kamjunke et al. 2017). The consequences of net cage fish farming on the DOM of tropical reservoirs, however, remain largely speculative.

Stable isotope signatures of carbon and nitrogen can be used to trace the impacts of aquaculture, when contaminant sources and impacted locations have distinct signatures (Yokoyama et al., 2002; Vizzini and Mazzola, 2004). For example, according to stable isotope analyses, aquaculture waste from a land-based fish farm in Sicily was mainly assimilated by benthic coastal organisms and pelagic and nekton-benthic organisms were less influences (Vizzine et al., 2005). Moreover, this study detected an impact gradient with benthic organisms being more enriched in $\delta^{15}N$ close to the outfall site compared to those collected at more distant sites. In a laboratory study, Redmond et al. (2010) showed that both $\delta^{13}C$ and $\delta^{15}N$ signatures can be used to trace salmon feed pellet assimilation into mussel populations. Jean-Marc et al. (2018) showed $\delta^{13}C$ enrichment in a stream food web due to the assimilation of fish feed from land-based salmon aquaculture. DOM $\delta^{13}C$ and $\delta^{15}N$ of total dissolved nitrogen (TDN) can also be used to infer DOM sources. In a large fluvial plain lake, urban sewage discharge caused a decrease of $\delta^{13}C$ -DOM and an increase of $\delta^{15}N$ -TDN (Zhou et al., 2018).

The objective of this work was to assess the effects of net cage fish farming on the elemental stoichiometry of aquatic DOM (DOC:DON, DOC:DOP and DON:DOP), as well as on PARAFAC components of DOM and stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) in a large tropical reservoir. We hypothesized that net cage fish farming decreased reservoir DOC:DON and DOC:DOP ratios, and thus increased DOM elemental quality, as well as enriched reservoir DOM in $\delta^{13}C$ and TDN in $\delta^{15}N$. Knowledge on fish farming impacts on reservoir DOM characteristics is important in order to predict, manage and mitigate its environmental consequences.

2. Methods

2.1. Study sites

With a total area of 1.459 km^2, the reservoir of the Furnas hydroelectric power plant (20°40'S; 46°19'W) we studied is the largest in the Brazilian state of Minas Gerais and the fifth largest reservoir in entire Brazil (Fig. 1). Its two branches, the Rio Grande and the Rio Sapucaí, have a thalweg extension of approximately 200 km and are relatively shallow (16 and 90 m mean and maximal depth; Figueredo and Giani, 2005). The local climate is a seasonal tropical highland climate. Winter ranges from April to September and is dry, with mean monthly rainfall ranging from 15 to 71 mm, and mild, with mean monthly temperatures between 17.3 and 21.2 °C (data from 1982 to 2012, S. José da Barra weather station). Summer ranges from October to March and is warm (mean monthly temperatures: 22.2 to 23.4 °C) and wet (mean monthly rainfall: 134 to 278 mm). Between 2013 and 2016, agricultural land use prevailed in the studied reservoir area. In a 20 km perimeter of study sites, the Sapucaí branch had a higher contribution of agriculture than the Grande branch (65% vs. 53%, respectively), and the Grande branch had more pristine vegetation than the Sapucaí (46% vs. 34%, respectively). Urban areas contributed 1.2% at Rio Sapucaí sites and 1.1% at Rio Grande sites. In the literature, there are reports of a higher trophic state in the Sapucaí than in the Grande branch (Corgosinho and Pinto-Coelho, 2006; COPASA, 2010). In a more recent study that occurred parallel to the present one, 124% (median; min: 15%, max: 11,822%) higher phytoplankton biovolumes were found in the Sapucaí than in the Grande branch in 7 out of 8 sampling campaigns (C. Figueredo; unpublished data), confirming previous reports.

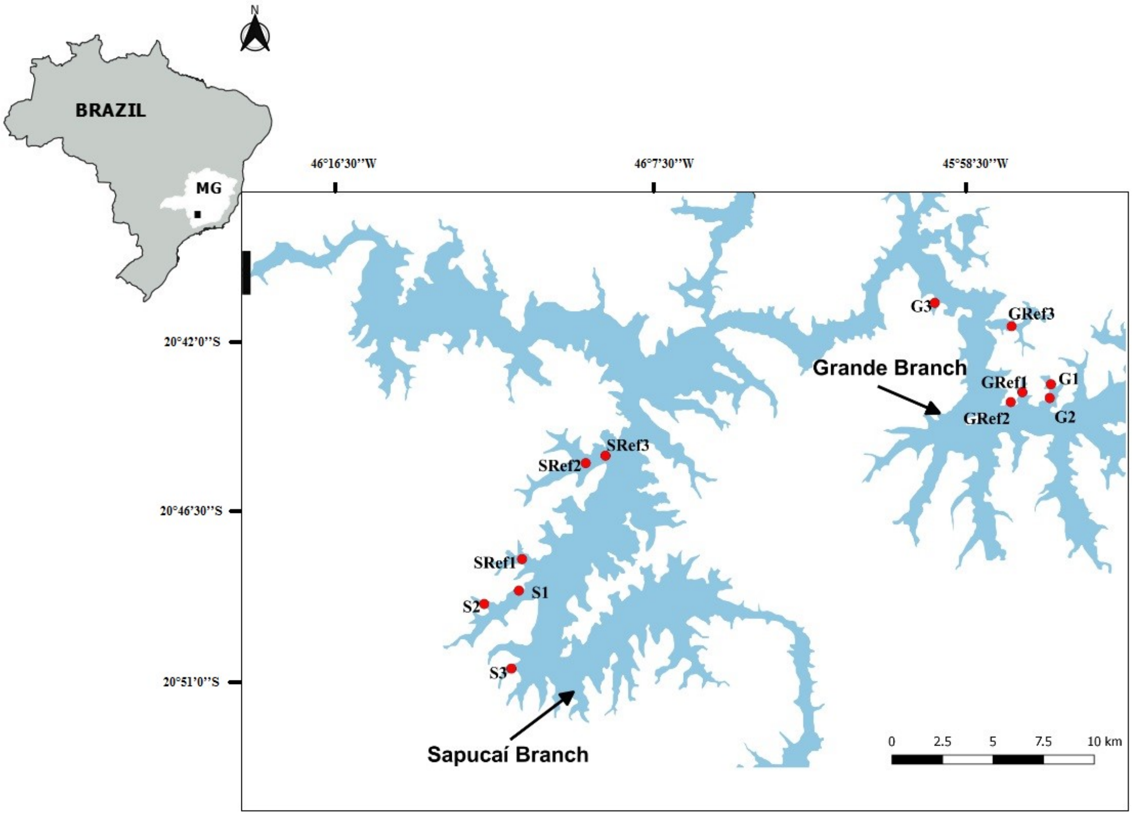

Figure 1: Sampling sites in the Furnas reservoir. G1, G2 and G3, and S1, S2, and S3 are sites with fish farms in the Rio Grande (G) and Sapucaí (S) branches, respectively. G_{Ref1}, G_{Ref2}, G_{Ref3} and S_{Ref1}, S_{Ref2}, S_{Ref3} are local control sites.

2.2. Sampling sites

Samples were collected in three sites/lateral bays with recently implemented fish farms (<10 years; 23 to 121 net cages per farm in the Rio Grande and 39 to 92 in the Rio Sapucaí) and another three sites/bays without aquaculture activity, representing local relative reference conditions, in both reservoir branches (Fig. 1). Nile tilapia (*Oreochromis niloticus L.*) was the main species cultivated. However, the cultivation of other species was observed in minimal quantities (less than 1% of production). In each fish farm sampling site/bay, we collected water samples along a hypothetical impact gradient spanning three stations. For the Rio Grande reservoir branch, these stations are henceforth denominated as G_0 to G_{200}, and for the Rio Sapucaí S_0 to S_{200}. This gradient included stations within fish farms (stations G_0 and $S_{0;}$ closest to the reservoir margin), 100 m away from the farms (intermediary stations; G_{100} and S_{100}) and 200 m away from farms (stations G_{200} and S_{200}; closest to the main body of the reservoir). At each station, we took water samples in the center of the euphotic zone and the center of the aphotic zone, which we estimated with a Secchi disk.

The three reference sites/bays without fish farms (henceforth referred to as G_{Ref} and S_{Ref}) in each reservoir branch were bays that Sampaio et al. (2008) suggested as suitable for the implementation of aquaculture in the future. In reference bays, we sampled one station near the reservoir margin and a second station nearer to the bays' outlets to the reservoir branch to account for potential gradients within bays. Here, we also took water samples in the center of the euphotic zone and the center of each station's aphotic zone. We assessed aquaculture impacts by both comparing impacted with reference bays and evaluating different distances (i.e., stations) towards bay outlets within fish farm bays.

We sampled both reservoir branches and all sampling bays within three days in each of nine sampling campaigns, covering both the rainy (December 2014, March 2015, December 2015, December 2016) and the dry season (April 2013, June 2014, September 2014, June 2015, July 2016). We analyzed DOM fluorescence and elemental stoichiometry in all nine sampling campaigns but stable isotopes for the December 2015 sampling only due to resource constraints.

2.3. Sampling

We used a Van Dorn sampler to retrieve water samples, which were then stored in acid-washed 5-liter PE bottles and shipped to the Furnas aquaculture lab on ice. Subsequently, we filtered these water samples through previously rinsed GF-5 filters with a nominal pore size of 0.45 µm (Machery-Nagel) and stored them in PE bottles (500 mL) previously cleaned and washed with hydrochloric acid before analysis.

2.4. Analyses of dissolved organic nutrients and carbon

We measured nitrogen (N) and phosphorus (P) spectrophotometrically using a FIAlab 2500 autoanalyzer (FIAlab, USA). Ammonium-N (NH_4-N) was analyzed with the indophenol method (Verdouw et al., 1978). The sum of nitrite- and nitrate-N (in the following denominated as NO_3-N for convenience) was determined by Cd reduction (APHA, 2005). We measured soluble reactive P (SRP) by ascorbic acid reduction (APHA, 2005). The absolute concentration of dissolved inorganic N (DIN) was computed as NO_3-N plus NH_4-N. We analyzed total dissolved N (TDN) and total dissolved P (TDP) as NO_3-N and SRP after persulfate oxidation (APHA, 2005). Concentrations of dissolved organic N (DON) and P (DOP) were obtained indirectly as DON = TDN – DIN and DOP = TDP – SRP.

We analyzed dissolved organic carbon (DOC) and stable isotopes with an elemental analyzer (Flash EA, Thermo Scientific, Germany) and an isotope ratio mass spectrometer (Delta V Advantage, Thermo Scientific, Germany). Preparation, concentration, and encapsulation of samples followed Gandhi et al. (2004).

2.5. DOM characterization

We obtained DOM absorbance and fluorescence data using an AQUALOG-UV-800 spectrofluorometer (Horiba, USA) in quartz cuvettes with a 10mm light path. Before measurements, we rinsed this cuvette with ultra-purified water and thrice with sample water. Fluorescence emission scans at 4.65 nm intervals, and 8 px resolution were performed from 250 nm to 800 nm with a 240-450 nm excitation range and integration times of 2 s at 3 nm intervals. Quinine Sulfate calibrations were performed, and inner-filter effects and Rayleigh and Raman scattering were corrected automatically using Raman water and NIST quinine sulfate fusion-sealed cuvettes in the Solo^{TM} program (Eigenvector Research Inc., USA).

We used parallel factor analysis (PARAFAC) to decompose excitation-emission matrices (EEMs) and identify underlying DOM fluorescence components (Stedmon et al., 2003). We performed the PARAFAC analysis with the staRdom package for R version 3 (R Core Team 2018). A stable three-component PARAFAC model (with the three components denominated as C1 to C3) was validated using split-half validation and component score correlation.

2.6. Statistical Analyses

We used the R software package *lme4* to test the effects of fish farming on the elemental stoichiometry of DOM (i.e., ratios of DOC:DON, DOC:DOP, DON:DOP), PARAFAC components (C1 to 3), and stable isotope signatures (δ^{13}C-DOC and δ^{15}N-TDN) with generalized linear mixed models (GLMMs) (R v3.6.3; R Core Team, 2018). Reservoir branches were analyzed separately due to their differences in trophic state and all samples taken entered separately into these analyses without pooling, e.g. of samples from different depths per station or samples from different stations in the same reservoir bay. In GLMMs, “station” was a fixed factor (factor levels: 0, 100, 200, and Ref), and “sampling date”, “sampling depth”, and “bay/site” were random factors. We analyzed distributions of residuals with the R package *RT4Bio* to verify model appropriateness and to choose the final model structure, which included the elimination of random factors that did not contribute to variance. Differences between stations (i.e., post-hoc tests) were tested with the function *difflsmeans* in the R package *lmerTest*.

3. Results

3.1. Elemental stoichiometry of DOM

The molar DOC:DON ratio ranged between 2.2 and 616.5 with a mean value of 68.0 and a median value of 53.7 in water samples from the Grande branch. In the Sapucaí branch, it ranged between 3.4 and 539.9 with a mean value of 63.8 and a median of 52.7. There were no significant differences between sampling stations in the Grande branch (GLMM, $p>0.1$), but there were significant differences in the Sapucaí branch (GLMM, $p<0.05$). According to post-hoc tests ($p<0.05$), station S_A had lower DOC:DON ratios than S_B, S_C and S_{Ref}, but there were no differences among stations S_B, S_C, and S_{Ref} (Fig. 2).

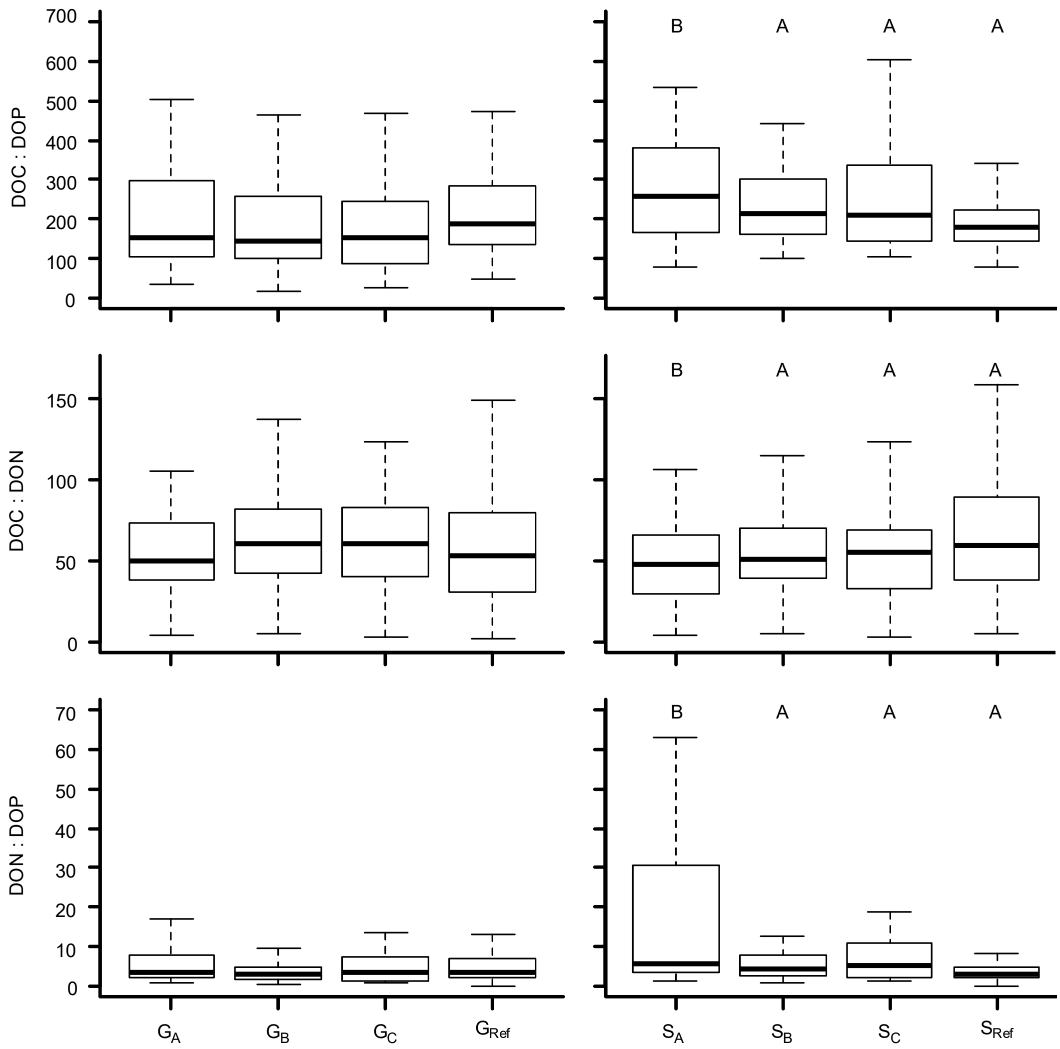

Figure 2. Elemental molar stoichiometry of DOM (DOC:DON, DOC:DOP and DON:DOP) along a gradient of fish farm impacts: net cage sites (A), 100 meters distant (B), 200 meters distant (C) and reference sites (Ref), in the Grande branch (G) and the Sapucaí branch (S). Different letters (A and B) above boxplots indicate significant differences between sites along the impact gradient in increasing order of ratio mean values according to GLMMs and post-hoc-tests ($p<0.05$). Boxes represent interquartile ranges (25[th] and 75[th] percentile), horizontal bars the median, and whiskers extend to 1.5 times the interquartile range.

DON:DOP ratios ranged between 0.14 and 194.8 with a mean value of 8.3 and a median value of 3.3 in water samples from the Grande branch and between 0.03 and 276.0 with a mean value of 15.5 and a median of 4.0 in samples from the Sapucaí branch. There were no significant differences between sampling stations in the Grande branch (GLMM, $p>0.1$), but there were significant differences between sampling stations in the Sapucaí branch (GLMM, $p=0.01$). Post-hoc tests showed that ratios at S_A were higher than those at S_B and S_C, but ratios at S_B did not differ from those at S_C (Fig. 2). According to post-hoc tests ($p<0.05$), station S_A had higher DON:DOP ratios than S_B, S_C and S_{Ref}, but there were no differences among stations S_B, S_C, and S_{Ref} (Fig. 2).

The DOC:DOP ratios ranged between 18.7 and 4868 with a mean value of 301.7 and a median value of 170.8 in water samples from the Grande branch and between 77.0 and 6415 with a mean value of 400.0 and a median of 205.7 for samples from the Sapucaí branch. There were no significant differences between sampling stations in the Grande branch (GLMM, $p>0.1$), but there were significant differences between sampling stations in the Sapucaí branch (GLMM, $p=0.01$). According to post-hoc tests ($p<0.05$), station S_A had higher DOC:DOP ratios than S_B, S_C and S_{Ref}, but there were no differences among stations S_B, S_C, and S_{Ref} (Fig. 2).

3.2. DOM components

PARAFAC analysis resulted in a three-component model (Fig. 3; Table 1) for the DOM samples from the Furnas reservoir. EEMs for C1 showed a main peak at an excitation of 246 nm and an emission of 436 nm and a secondary peak at excitation of 324 nm an emission of 436 nm. Fluorescence intensities of C1 (F_{max}, in Raman units [RU]; Fig. 4), ranged between 1.39 and 5.61 with a mean value of 4.74 and a median value of 5.03 in water samples from the Grande branch and between 0.68 and 5.68 with a mean value of 4.94 and a median of 5.16 for the Sapucaí branch (Fig. 4). In the Sapucaí branch, there were no significant differences between sampling stations (GLMM, $p>0.1$). However, there were significant differences between the sampling stations of the Grande branch (GLMM, $p<0.001$). According to post-hoc tests ($p<0.05$), stations G_A and G_B had lower F_{max} than stations G_C and G_{Ref} (Fig. 4).

Table 1: Query results from the Open Fluor database (www.openfluor.org, Murphy et al., 2014). Excitation end emission minimum similarity score 0.95.

Component, excitation (Ex), emission (Em) maxima (nm)	Characteristic	Component in reference	Reference
C1, Ex 246 (324), Em 436	Humic-like	C2 C3 C1 C1 C2	Shutova et al., 2014; Yamashita et al., 2010; Yamashita et al., 2011a; Yamashita et al., 2011b; Chen et al., 2018
C2, Ex <240 (294), Em 340	Protein-like	C5, C4 C3 C3 C5 C4	Murphy, et al., 2011; Catalá et al., 2015; Brogi et al., 2018; Yamashita et al., 2013; Chen et al., 2017
C3, Ex 270, Em 482	Humic-like	C2 C2 C4 C3 C1	Dainard et al., 2015; Yamashita et al., 2010; Chen et al., 2018; Murphy et al., 2008; Tanaka et al., 2014

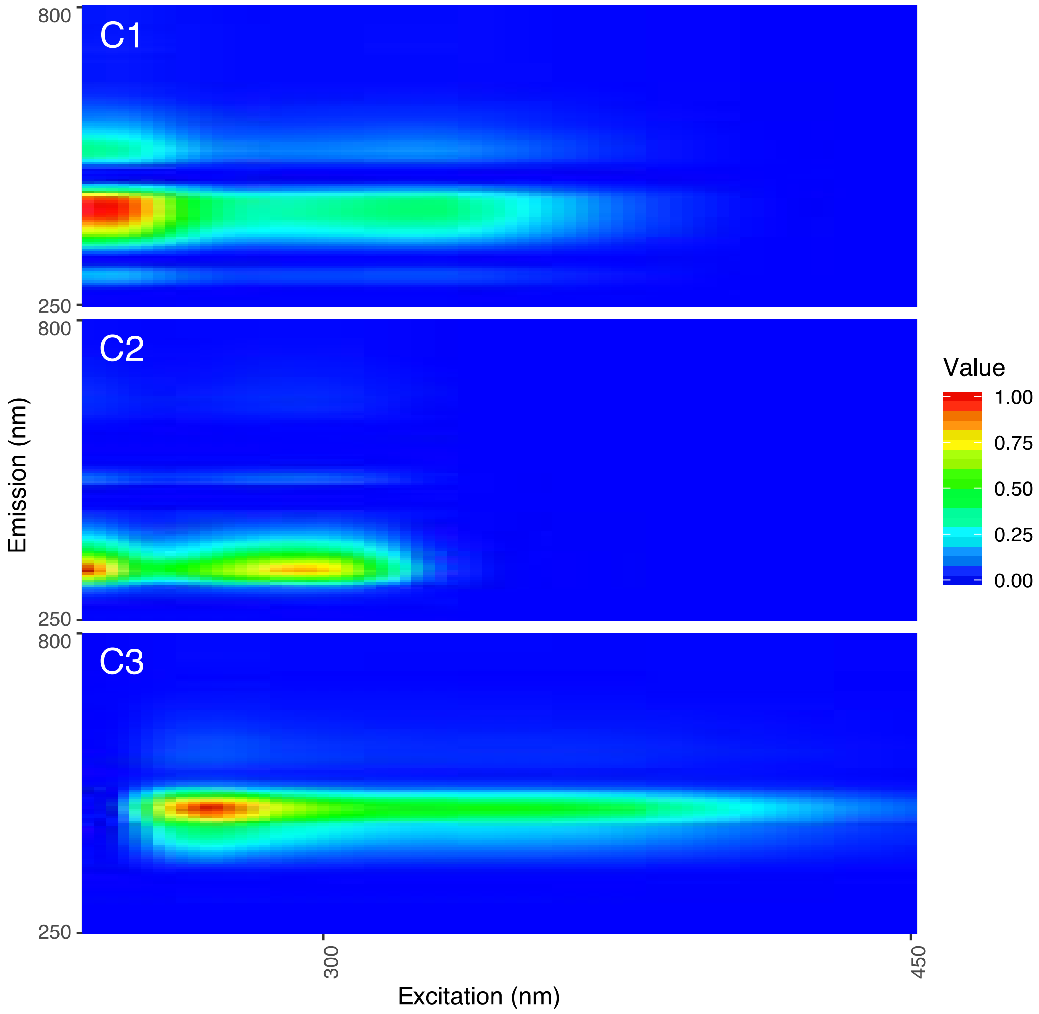

Figure 3. Component fluorescence intensities of the validated 3-component model.

EEMs for C2 showed a main peak at an excitation of <240 nm and an emission of 340 nm and a secondary peak at an excitation of 294 nm and an emission of 340 nm. Fluorescence intensities of C2 (F_{max}, in RU), ranged between 0.87 and 8.00 with a mean value of 3.74 and a median value of 3.30 in water samples from the Grande branch and between 1.49 and 8.26 with a mean value of 3.37 and a median of 3.09 for samples from the Sapucaí branch

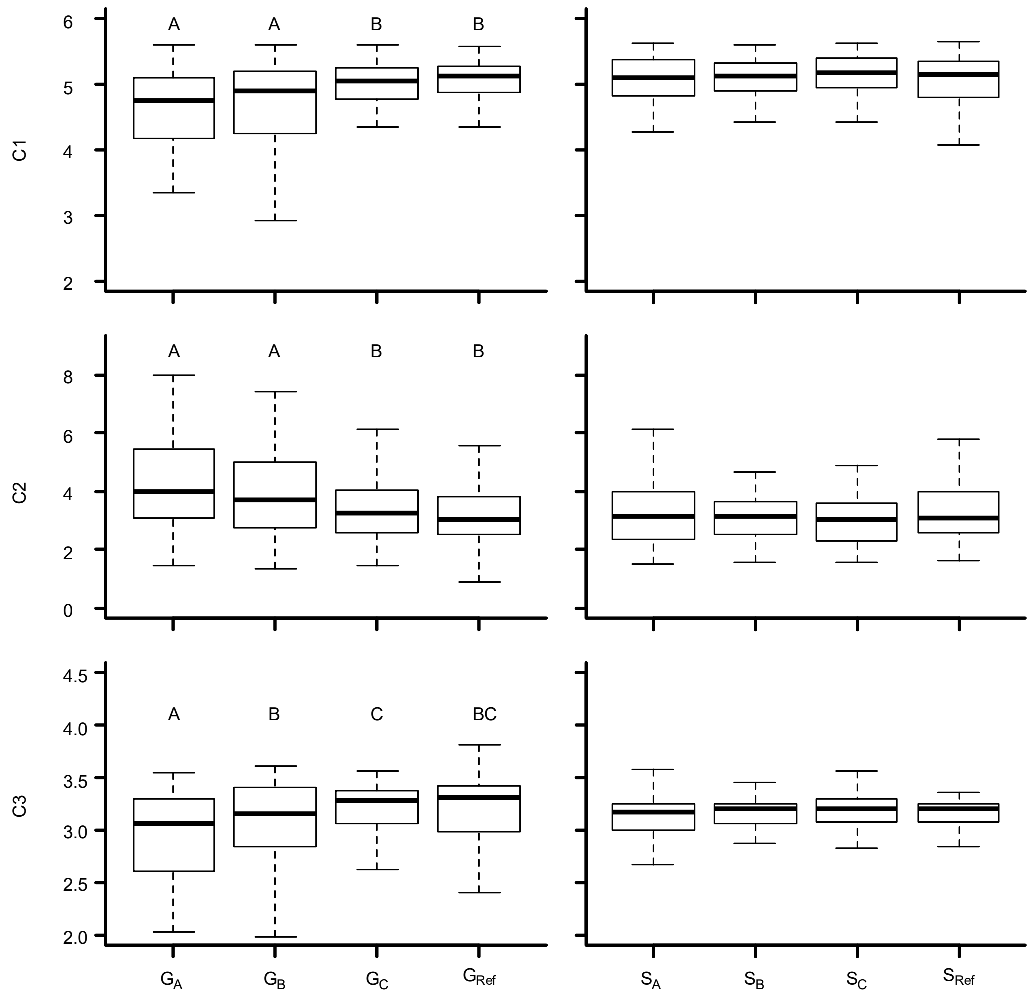

Figure 4. There were significant differences between sampling stations in the Grande branch (GLMM, p<0.001). According to post-hoc tests (p<0.05), stations G_A and G_B had higher F_{max} than stations G_C and G_{Ref} (Fig. 4). In the Sapucaí branch, there were no significant differences between sampling stations (GLMM, p=0.08).

EEMs for C3 showed a main peak at an excitation of 270 nm and an emission of 482 nm. Fluorescence intensities of component 3 (F_{max}, in RU) ranged between 1.04 and 3.81 with a mean value of 3.05 and a median value of 3.21 in water samples from the Grande branch and between 0.42 and 3.57 with a mean value of 3.06 and a median of 3.20 for samples from the Sapucaí branch (Fig. 4). There were significant differences between sampling stations in the Grande branch (GLMM, p<0.001). According to post-hoc tests (p<0.05), station G_A had lower F_{max} than station G_B, which in turn had lower F_{max} than station G_C. F_{max} values of G_{Ref} did not differ from those of G_B and G_C. In the Sapucaí branch, there were no significant differences between sampling stations (GLMM, $p>0.1$).

3.3. Stable isotopes

Values of $\delta^{13}C$ of DOM ranged between -26.78 and -23.68 with a mean value of -24.96 and a median value of -24.98 in water samples from the Grande branch and between -27.06 and -24.40 with a mean value of -25.16 and a median of -24.79 for samples from the Sapucaí branch. There were neither significant differences between sampling stations in the Grande branch (GLMM, $p>0.1$) nor in the Sapucaí branch (GLMM, $p>0.1$).

Values of $\delta^{15}N$ of TDN ranged between 11.03 and 17.93 with a mean value of 15.25 and a median value of 15.44 in water samples from the Grande branch and between 10.27 and 16.79 with a mean value of 12.12 and a median of 11.18 for samples from the Sapucaí branch. There were significant differences between sampling stations in the Grande branch (GLMM, $p<0.05$). According to post-hoc tests ($p<0.05$), station G_A had lower TDN $\delta^{15}N$ values than stations G_B and G_C (Fig. 5). In the Sapucaí branch, there were no significant differences between sampling stations (GLMM, $p>0.1$).

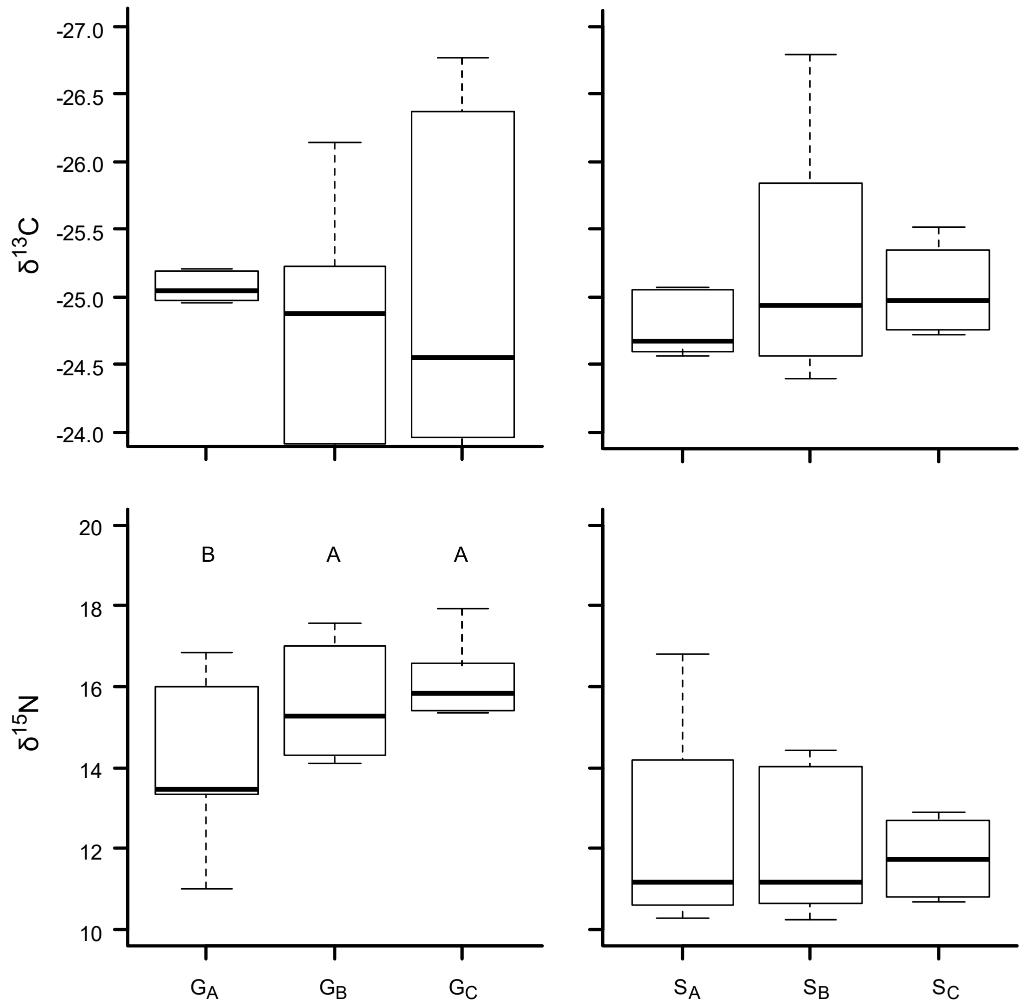

Figure 5. Stable isotope signatures (δ^{13}C-DOC and δ^{15}N-TDN) along a gradient of fish farm impacts: net cage sites (A), 100 meters distant (B), and 200 meters distant (C), in the Grande branch (G) and the Sapucaí branch (S). Different letters (A and B) above boxplots indicate significant differences between sites along the impact gradient in increasing order according to GLMMs and post-hoc-tests ($p<0.05$). Boxplots represent the interquartile ranges (25th and 75th percentile), horizontal bars the median, and whiskers extend to 1.5 times the interquartile range.

4. Discussion

Fish farming can release significant quantities of DOM rich in carbon, nitrogen and phosphorus, in addition to dissolved inorganic and particulate organic matter (Olsen et al. 2008). However, there is little information available about the characteristics of DOM released into freshwater ecosystems by fish farms, such as its elemental stoichiometry or fluorescence characteristics (but see Nimptsch et al., 2015; Kamjunke et al., 2017), especially regarding tropical systems with rapidly growing aquaculture. Even less information can be found in relation to the environmental impacts resulting from changes in the stoichiometric balance and characteristics of DOM.

Our data showed contrasting responses of the Grande and Sapucaí branches of the Furnas (Table 2). In the Grande branch, there were no effects of fish farming on the elemental stoichiometry of DOM, but there were effects on PARAFAC components. Conversely, in the Sapucaí branch there were no fish farm effects on PARAFAC components, but effects on DOM elemental stoichiometry. One potential explanation for these differences could be that the Grande branch of the Furnas reservoir was more oligotrophic than the Sapucaí branch (Corgosinho and Pinto-Coelho, 2006; COPASA, 2010) due to differences in catchment land use, but both reservoir branches were similar in relation to fish farm density. Moreover, the main cultivated species in both branches, the Nile tilapia *Oreochromis niloticus L.*, is omnivorous, and thus requires a lower amount of protein in its diet than carnivorous species. The impacts of its production are therefore often considered smaller than those of carnivorous fish production.

Table 2: Summary of aquaculture effects found in this study.

Variable	Grande branch	Sapucaí branch
DOC:DON	0	–
DOC:DOP	0	+
DON:DOP	0	+
C1 (humic-like)	–	0
C2 (protein-like)	+	0
C3 (humic-like)	–	0
δ^{13}C-DOC	0	0
δ^{15}N-TDN	–	0

0 = no effect, + = positive effect, and – = negative effect

The PARAFAC analysis of DOM fluorescence resulted in two humic-like components (C 1 and 3), which have previously been found in tropical rivers (Yamashita et al., 2010). Component 1 is a terrestrial and processed humic-like component (Bridgeman et al., 2011) and component 3 has been associated with recent biological activity (Yamashita et al., 2010; Coble, 1996). Component 2 probably resembles free and bound protein-like material (Coble et al., 1996; Cory and McKnight, 2005; Catalá et al., 2015). The significant, but small increase in the protein-like PARAFAC component 2 in up to 100m distance from the fish farms in the more oligotrophic Grande branch suggests that current fish farming has the potential to increase the bioavailability of DOM, especially in less impacted systems. Similarly, there were increases in labile, tryptophan- and tyrosine-like compounds in Patagonian streams due to land-based salmon farming (Nimptsch et al., 2015). In a detailed molecular-level characterization of DOM from Patagonian salmon farms, large quantities of readily bioavailable substances, such as carbohydrates, peptides and proteins, and lipids were found (Kamjunke et al., 2017).

While there was an increase in the protein-like PARAFAC component due to fish farming, we found an absolute reduction in the two humic-like components, C1 and C3, in up to 100m distance from fish farms in the same reservoir branch (Grande). This reduction was possibly related to a higher microbial activity at sites impacted by fish farming. Interestingly, the elemental stoichiometry of DOM in the Grande branch was not affected by fish farming, possibly because concomitantly with the increase in labile protein-like compounds there was a decrease in humic substances that are also rich in N. This conjecture is supported by the decrease in $\delta^{15}N$ of TDN due to fish farming we found in the Grande branch, indicating that the N in this system may be subsidized by another source, i.e. the fish feed.

In the Sapucaí branch, no effects of fish farming on $\delta^{15}N$ of TDN were observed, probably due to its higher trophic state caused by human occupation and land use (Corgosinho and Pinto-Coelho, 2006; COPASA, 2010). Urban sewage without proper treatment, agriculture, and industrial impacts potentially already altered the natural isotopic signature (Anderson and Cabana, 2005; Xu et al., 2005) of N in the Sapucaí branch, so that the signature of fish feed was not sufficiently different to cause an effect on the $\delta^{15}N$ of reservoir TDN. Another potential explanation for the absence of fish farm effects on reservoir TDN $\delta^{15}N$ in the Sapucaí branch would be the rapid assimilation of high-quality DON from fish feeds by the local microbiota. However, the negative effect of fish farming on DOC:DON ratios in the Sapucaí branch may not support this explanation.

Eutrophication, although often largely attributed to phosphorus, is greatly affected by N (Elser et al. 2007; Conley et al., 2009) and DON can directly or indirectly contribute to the nutrition of phytoplankton and bacteria and affect microbial community structure and function (Berman and Bronk, 2003; Attayde et al., 1999). Our results showed a higher relative contribution of DON to DOM, both in DOC:DON and DON:DOP ratios due to fish farming in the Sapucaí branch of the Furnas reservoir. Several components of DON can be directly or indirectly consumed by different species of algae and cyanobacteria as alternative N sources. The supply of DON can affect the composition of phytoplankton communities, which in turn affects general nutrient availability and stoichiometry due to the selective assimilation capacities of certain species (Berman and Chava, 1999).

Traditionally the main source of protein in fish feed comes from fish meal of marine origin, but recently tilapia feed formulations have replaced fishmeal with protein sources of terrestrial origin (Montoya-Camacho et al., 2019). Thus, it may be more difficult in the future to detect the impacts of fish farms with stable isotope techniques. In aquaculture production system using net cages, the main routes of dissolved nutrient pollution are related to direct nutrient release by fish feed (Phillips et al., 1993) and the feces produced (Chen et al., 2003). However, not only the C, N and P contents and biochemical composition of the feed, and the total amounts of feed and fish feces determine potential impacts, but a number of other factors, such as water depth, flow velocity, particle sinking velocity, disintegration and leaching must be considered (Olsen et al., 2008).

5. Conclusion

The impacts of aquaculture activity on the water quality and ecological integrity of aquatic ecosystems are poorly understood, especially in tropical regions, and there is a need to develop efficient monitoring methodologies. In our study, early-stage fish farming, characterized by relatively short operation times and farm size already showed small, but significant effects on DOM in a large tropical reservoir. Responses differed depending on reservoir trophic state and previous land use impact. In the less eutrophic reservoir branch, we found a decrease in natural humic-like DOM components and an increase in protein-like DOM components due to fish farming. Our results highlight the need to combine different monitoring techniques to detect early impacts of fish farming in tropical reservoirs.

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DISCUSSÃO GERAL

Dentre os impactos da aquicultura sobre o meio ambiente pode ser observada a liberação nutrientes inorgânicos e matéria orgânica particulada, sendo que grandes quantidades de carbono, nitrogênio e fósforo poderiam ser exportadas na forma de matéria orgânica dissolvida (Read e Fernandes, 2003; Olsen et al., 2008; Nimptsch et al., 2015). Entretanto, não existem estudos conclusivos quanto aos impactos da piscicultura sobre a MOD (Junior et al., 2018). Nossos resultados mostraram os efeitos iniciais de piscicultura em tanques-rede recentemente instalados (menos de 10 anos) nas características e na qualidade de MOD em um grande reservatório tropical. Embora as concentrações de carbono orgânico dissolvido não tenham sido afetadas pela atividade, houve aumento na qualidade da MOD através da contribuição relativa da MOD recentemente produzida, composta principalmente de compostos lábeis e também aumento de N e C nas proporções estequiométricas da MOD.

Com uso de diferentes técnicas, foram observados impactos da piscicultura em ambos os eixos do reservatório. Maiores valores do índice de fluorescência (FI) evidenciaram maior produção microbiana próximos às pisciculturas. Aumentos no índice (β:α) e na relação pico T / pico C perto de pisciculturas do eixo do rio Grande indicaram que a contribuição relativa do DOM recentemente produzida e a demanda bioquímica de oxigênio da DOM aumentaram devido à atividade aquícola. Nas relações estequiométricas de C, N e P da MOD, constatamos um aumento nas concentrações de nitrogênio e carbono no eixo do rio Sapucaí. Dentre os três componentes principais do

PARAFAC, houve aumento de um componente lábil, C2 (compostos de proteínas) e redução de dois componentes recalcitrantes, C1 e C3 (compostos húmicos), no eixo do rio Grande. Também para o rio Grande, houve aumento das relações isotópicas de $\delta^{15}N$ no nitrogênio total dissolvido e somente para um período amostral (dezembro de 2015).

As comunidades microbianas desempenham importantes papéis biogeoquímicos em ambientes aquáticos e são geralmente as primeiras a responderem às mudanças químicas e físicas (Cotner e Biddanda, 2002; Fodelianakis et al., 2014). O aumento da produtividade microbiana devido à piscicultura provavelmente resulta da alta qualidade nutricional das fezes e da ração não consumida (Olsen et al., 2008). Os índices de fluorescência permitiram detectar impactos e maior atividade bacteriana no eixo do rio Grande e menor resposta, ainda que também significativa, para o eixo do rio Sapucaí. O aumento da concentração do componente 2 da caracterização da MOD, indica que o incremento de compostos lábeis pode ter proporcionado maior atividade microbiana no eixo do rio Grande. Adicionalmente, nota-se que a modificação da qualidade estequiométrica da MOD pode estar relacionada com a maior atividade microbiana no eixo do rio Sapucaí. Portanto, mudanças na atividade microbiana, conforme indicado por FI, podem ser um importante sinal de alerta precoce para futuros impactos da aquicultura, que, como observado nesse estudo, possivelmente ocorreu devido à entrada de MOD lábil ou com maior proporção de N em termos estequiométricos.

Provavelmente devido a uma maior ocupação humana e uso do solo para atividades como agricultura, indústria e mineração, o eixo formado pelo rio Sapucaí possui um estado trófico maior que o eixo formado pelo rio Grande

(Corgosinho e Pinto-Coelho, 2006; COPASA, 2010). Em nosso estudo, a resposta geral na qualidade da MOD em função da piscicultura diferiu entre os eixos do reservatório de Furnas, possivelmente porque o eixo do rio Sapucaí apresentava um estado trófico mais elevado, refletido em concentrações mais baixas de oxigênio dissolvido, concentrações mais altas de DOC e NH_4-N e valores mais altos de FI. Assim, os impactos absolutos na MOD seriam influenciados pelas diferentes linhas de base para variáveis relacionadas à poluição. Essas diferenças de ocupação de solo também podem ter influenciando nas diferenças de respostas para os outros aspectos estudados.

Em certo grau, nossos resultados também permitiram avaliar a dimensão espacial dos impactos iniciais da piscicultura na qualidade da MOD em um reservatório tropical. Em geral, os efeitos na qualidade da MOD do reservatório foram detectados apenas em até 100m de distância das pisciculturas. Embora relativamente curta em comparação com as dimensões do reservatório, essa distância é maior que a distância de dispersão de resíduos de fezes e alimentos para peixes em sedimentos abaixo e ao redor de estações de pisciculturas em tanques-rede na zona costeira, que se estenderam apenas por até 30 m de distância (McGhie et al., 2000; Srithongouthai e Tada, 2017). Intervalos de detecção de até 100 m podem sugerir que as pisciculturas investigadas não causaram impactos em grande escala na qualidade MOD do reservatório estudado. No entanto, essa situação pode se tornar diferente com a futura instalação de parques de aquicultura neste reservatório (Sampaio et al., 2007), que resultaria em uma maior exportação de nutrientes e consequentemente proporcionaria um maior raio de impacto.

Assim como impactos nas comunidades biológicas de rios e na produção e respiração primárias do ecossistema (dos Santos Rosa et al., 2013; Jean-Marc et al., 2018), foi constatado que os nutrientes e matéria orgânica liberados pela piscicultura em tanques-rede podem afetar a qualidade da MOD e também ocasionar aumento de atividade microbiana. Além disso, os resíduos da aquicultura podem progressivamente se tornar um componente mais importante da cadeia alimentar aquática e também podem ser necessários vários anos de operação da aquicultura até que todo o potencial de seu impacto se torne evidente (Kullman et al. 2009). Os resultados do presente estudo, mostrando impactos até a 100 metros de distância das pisciculturas, sugerem que a aquicultura pode representar um risco para o reservatório estudado, sendo um importante sinal de alerta em relação a futuros impactos e que se deve ter um planejamento e gerenciamento eficiente da aquicultura (Read e Fernandes, 2003), incluindo a dispersão eficiente de resíduos produzidos (Gondwe et al., 2012) de modo a mitigar seu impacto ecológico.

Os resultados deste estudo forneceram uma primeira visão sobre evidências de impactos do cultivo de peixes em tanques-rede sobre a características da MOD em um grande reservatório tropical. As diferenças de valores nos índices de fluorescência, componentes (PARAFAC) e estequiometria da MOD mostraram efeitos da piscicultura e podem ser importantes ferramentas de monitoramento de impactos dessa atividade em tanques-rede.

Não foram encontradas relações entre o nível do reservatório e os índices de fluorescência. Por outro lado, o nível foi relacionado à concentração de DOC. Em um estudo realizado por Silva et al. (2018), avaliaram o efeito da piscicultura sobre a liberação de metano e demonstraram que as emissões de metano foram mais influenciadas pelas características do reservatório do que pela produção de peixes.. Tais resultados evidenciam, assim como parte de nossos resultados, que a dinâmica do reservatório tem importante papel na regulação de nutrientes. No entanto, análises mais refinadas das exportações de bacias hidrográficas, processos de mineralização de rios e reservatórios e diluição, bem como processos de concentração, podem ser necessários para analisar adequadamente o efeito do regime climático.

Em um cenário futuro, caso as previsões de mudanças climáticas sejam confirmadas, os níveis do reservatório de Furnas poderiam permanecer acentuadamente baixo com maior frequência (Ribeiro Júnior et al., 2016), causando concentração de nutrientes liberados pela piscicultura, devido a menor diluição desses compostos. Ambas, alteração no fluxo de água e uma diminuição no volume do reservatório, poderão potencializar os impactos das pisciculturas.

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CONCLUSÃO GERAL

A piscicultura em tanques-rede, ainda que em escala pequena com relação à dimensão de um grande reservatório tropical, pode proporcionar impactos sobre a MOD já nos estágios iniciais de implantação e com tempo de operação relativamente curto.

Foram detectados efeitos na qualidade da MOD, avaliados por índices de fluorescência, proporções estequiométricas de carbono, nitrogênio e fósforo da MOD, em componentes da MOD (PARAFAC) e efeitos sobre isótopo estável $\delta^{15}N$ do NT. Esses efeitos foram detectáveis em até 100m de distância dos tanques-rede e foram mais pronunciados no braço formado pelo rio Grande, exceto para as proporções estequiométricas para o eixo mais impactado. Não foram detectados efeitos nas concentrações de COD e relações isotópicas de $\delta^{13}C$ da MOD.

Futuros aumentos nas densidades das pisciculturas e nos tanques-rede e tempos de operação mais longos em combinação com as mudanças projetadas para o clima e para o uso do solo poderão intensificar os efeitos da piscicultura sobre a MOD do reservatório. Assim, deve ser ressaltada a necessidade de práticas e manejo ambientalmente saudáveis da piscicultura nesse ecossistema e em reservatórios tropicais de um modo geral.

Para o monitoramento dos efeitos iniciais da piscicultura, os índices de fluorescência FI, β: α e pico T / pico C, as relações estequiométricas, a análise PARAFAC e as proporções isotópicas de $\delta^{15}N$ do NT provam ser úteis. Nosso estudo também indicou interações complexas entre o uso do solo, o nível da água do reservatório e os impactos da piscicultura, que devem ser investigadas e incluídas nas futuras decisões de gestão.