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Eutrophication effects on fatty acid profiles of seston and omnivorous fish in tropical reservoirs

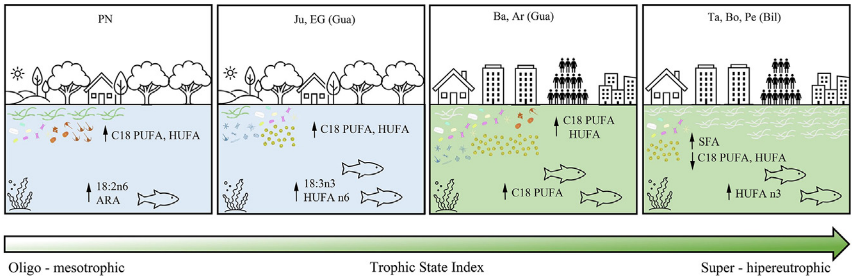
Aline Dal'Olio Gomes [a,*], Fernando Ribeiro Gomes [a], Björn Gücker [b], Carlos Eduardo Tolussi [c], Cleber Cunha Figueredo [d], Iola Gonçalves Boëchat [b], Lídia Sumile Maruyama [e], Lucas Chagas Oliveira [d], Marcela Muñoz-Peñuela [a], Marcelo Luiz Martins Pompêo [f], Rayssa de Lima Cardoso [g], Victor Hugo Marques [a], Renata Guimarães Moreira [a]

[a] *Departamento de Fisiologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, 101, SP 05508-090, Brazil*
[b] *Departamento de Geociências, Universidade Federal São João del-Rei, São João del-Rei, MG, Brazil*
[c] *Universidade Anhembi Morumbi, Campus Mooca, São Paulo, SP, Brazil*
[d] *Departamento de Botânica, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil*
[e] *Centro de Pesquisa de Recursos Hídricos, Instituto de Pesca, São Paulo, SP, Brazil*
[f] *Departamento de Ecologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, SP, Brazil*
[g] *Universidade Estadual Paulista (Unesp), Instituto de Ciência e Tecnologia, Sorocaba, Brazil*

HIGHLIGHTS

- Eutrophication reservoir affected partially seston and fish FA profiles.
- Sestonic FA profiles reflected phytoplankton composition.
- FA profiles in muscle from two fish species differed among studied sites.
- Fish from eutrophic sites had higher percentages of n3 HUFA.
- Both fish species had similar FA profiles within sites.

GRAPHICAL ABSTRACT



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ABSTRACT

It has been postulated that eutrophication causes replacement of n3 highly unsaturated fatty acids (n3 HUFA) rich taxa, such as Bacillariophyta, Cryptophyta and Dinophyta, with taxa poor in these fatty acids (FA), such as Chlorophyta and Cyanobacteria. Such a change in community composition at the basis of the food web may alter the FA composition of consumer tissues. Here, we investigated the effects of eutrophication on phytoplankton composition and FA profiles of seston and muscle of two omnivorous fish species (*Astyanax fasciatus* and *Astyanax altiparanae*) from reservoirs of different trophic status in Southeast Brazil. The phytoplankton composition and seston FA profiles reflected the degree of eutrophication at most of the studied sites. Three of the five most eutrophic sites were dominated by cyanobacteria and had the highest saturated fatty acid (SFA) and lowest polyunsaturated fatty acid (PUFA) relative contents among all sites. In contrast, the remaining two sites presented a higher phytoplankton diversity and higher relative contribution of sestonic PUFAs with 18 carbons (C18) and HUFAs than less eutrophic systems. However, there were no clear effects of sestonic FA profiles on the FA profiles of muscle of both fish species. A higher percentage of n3 HUFAs was found in the fish samples from a hypereutrophic and cyanobacteria dominated reservoir than in those from sites with a more diverse phytoplankton community in which fish mainly showed higher percentages of C18 PUFA. These results suggest a lack of a direct relationship between the degree of eutrophication and the percentage of n3 HUFAs in both fish species, which can be

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[*] Corresponding author.
E-mail address: eniladal@gmail.com (A. Dal'Olio Gomes).

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caused by specific characteristics of the reservoirs that may modulate eutrophication effects. Therefore, consumer FA biochemistry seemed to be dictated by their ability to select, accumulate, and modify dietary FAs, rather than by the eutrophication degree of the studied tropical reservoirs.

1. Introduction

Large urban reservoirs have become important ecosystems for the maintenance of biodiversity, besides providing a wide variety of services, such as power generation, water supply and recreation (Moggridge et al., 2014). However, these multiple uses are often accompanied by phosphorus and nitrogen pollution and consequently cultural eutrophication since these water bodies also serve as waste and sewage receptors (Carpenter, 2005; Azevedo et al., 2015). As a primary consequence of eutrophication, there is a breakdown in the balance between organic matter production, consumption and decomposition, altering phytoplankton community structure and composition through changes in species dominance and abundance (Moss et al., 2003; Thackeray et al., 2008). Phytoplankton organisms are the main suppliers of energy and nutrients for higher trophic levels in reservoirs, and anthropogenic changes in the phytoplankton community can affect energy and nutrient flow through the food chain, including several important biochemical compounds, such as fatty acids (FAs) (Müller-Navarra et al., 2004).

The species that compose the base of the freshwater food web are generally the main organisms capable of synthesizing omega 3 and 6 polyunsaturated fatty acids (n3 and n6 PUFAs) with 18 carbons, such as linolenic acid (ALA, C18:3n3) and linoleic acid (LA, C18:2n6), respectively (Arts et al., 2001; Müller-Navarra, 2008; Bell and Tocher, 2009). Those PUFAs act as precursors to long-chain n3 and n6 PUFAs with 20–22 carbons (highly unsaturated fatty acids, HUFAs), including eicosapentaenoic acid (EPA, C20:5n3), docosahexaenoic acid (DHA, C22:6n3) and arachidonic acid (ARA, C20:4n6). HUFAs are essential for consumers' growth, reproduction, regulation of membranes fluidity and eicosanoids synthesis (Hasting et al., 2001; Tocher, 2003; Arts and Kohler, 2009). Consumers can directly obtain HUFAs through feeding on nutritionally rich prey organisms, such as diatoms, dinoflagellates and cryptophytes (Taipale et al., 2013). Alternatively, consumers may synthesize HUFAs by converting dietary precursors (C18 PUFAs), which are abundant in green algae and land plants (Guo et al., 2016). Thus, the biochemical availability of important PUFAs in aquatic systems and their transfer efficiencies to higher trophic levels – including fish and humans – can be strongly influenced by the composition of the phytoplankton community.

It has been postulated that cultural eutrophication in reservoirs results in a replacement of taxa rich in n3 HUFAs (EPA and DHA), such as Bacillariophyta (diatoms), Cryptophyta and Dinophyta, with taxa poor in these FAs, such as Chlorophyta and Cyanobacteria (Müller-Navarra et al., 2004; Persson et al., 2007; Taipale et al., 2016). Such a change in community composition at the basis of the food web often alters the FA composition of the tissue of consumers, such as zooplankton organisms, aquatic insects and fish (Razavi et al., 2014; Gladyshev et al., 2012; Taipale et al., 2016; Williams et al., 2017; Mahbood et al., 2019), and may cause biochemical imbalances in these tissues. Reported physiological consequences of such biochemical imbalances due to dietary, biochemical limitation for animal consumers include deficient regulation of membrane fluidity, as well as compromised growth and reproduction rates (Ballantyne et al., 2003; Nalepa et al., 2006; Arts and Kohler, 2009). Changes in dietary FA availability can also affect animal immune function since FAs, such as EPA and ARA, act as precursors of prostaglandins, a class of eicosanoids directly involved in inflammatory and immune responses (Lall, 2000; Arts and Kohler, 2009).

However, some studies have shown that a decrease in primary producers' n3 HUFAs in eutrophic/polluted environments is not always accompanied by a decrease in consumer n3 HUFAs, even when primary producers, mostly Cyanobacteria, have very low EPA and DHA availabilities (Rocchetta et al., 2014; Gomes et al., 2016; Whorley et al., 2019; Marques et al., 2020). For instance, Gomes et al. (2016) investigated the FAs profile in two fish species (the omnivorous *Astyanax fasciatus* and the carnivorous *Hoplias malabaricus*) from an oligotrophic and a hypereutrophic reservoir. The hypereutrophic reservoir showed permanent cyanobacterial blooms (Moschini-Carlos et al., 2010; CETESB, 2011; 2012; 2013; Cortez, 2013) but also a high density of dinoflagellates (Matsumura-Tundisi et al., 2010; Cortez, 2013), resulting in high percentages of n3 HUFAs (mainly EPA) in tissue triacylglycerols and phospholipids (PLs) of both fish species. Similarly, no changes were observed in the relative contribution of n3 HUFAs to total PUFAs in the hepatic PLs of *Astyanax altiparanae* (omnivorous) from both a eutrophic and a mesotrophic reservoir in São Paulo (Brazil) (Marques et al., 2020).

These results suggest that trophic transference of FAs from primary producers to fish consumers may be affected by several factors related to system trophic state, but not exclusively by the FA composition of dominant phytoplankton. Those factors may include feeding selectivity for non-dominant phytoplankton species and the consumer's capacity to retain and convert dietary FAs into high energetic HUFAs (Gladyshev and Sushchik, 2019). Therefore, it is crucial to understand the complexity of changes in different aquatic ecosystems affected by eutrophication and as to how those changes may interact to affect the availability and assimilation of HUFAs by fish consumers. Artificial freshwater ecosystems are of particular interest in terms of their potential for HUFA transfer from aquatic to terrestrial organisms (Hixson et al., 2015), especially given the current trend to increase fish production in tropical reservoirs (Boëchat et al., 2021).

Thus, a detailed understanding of the transfer mechanisms of HUFAs through aquatic food webs is essential to identify and preserve the availability of HUFAs to consumers of higher trophic levels, including humans. Here we investigated the effects of eutrophication/urbanization of tropical reservoirs on their phytoplankton composition, sestonic FA profiles and FAs profiles of two fish species of the genus *Astyanax*. *Astyanax* is widely distributed in the neotropical region, occurring from the southern United States to northern Argentina (Ornelas-Garcia et al., 2008). Most species have omnivorous feeding habits, with highly variable diet composition, including animal and plant items of both autochthonous and allochthonous origins (Esteves, 1996, Vilella et al., 2002, Cassemiro et al., 2002, Bennemann et al., 2005; Alonso et al., 2019). In addition, some species of this genus present generalist feeding habits and high trophic plasticity in response to environmental changes and resource availability (Lobón-Cerviá and Bennemann, 2000; Carvalho et al., 2015). However, congeneric species may respond differently to changes in the aquatic environment and resource availability (Alonso et al., 2019).

Based on previous studies performed by Gomes et al. (2016) and Marques et al. (2020) in tropical reservoirs and the known ability of consumers to modify and retain FAs, we tested the hypothesis that the FA profiles of both fish species are not directly affected by phytoplankton composition, regardless of the eutrophication status of the system. Specifically, we hypothesized that 1) sestonic FA profiles reflect phytoplankton composition; therefore, the most eutrophic and cyanobacteria dominated systems exhibit the poorest sestonic n3 HUFAs content; 2) FA profiles in muscle tissue of both fish species differ among systems of different trophic state, but not as a direct effect of eutrophication, i.e., fish from the more eutrophic systems do not present consistently lower HUFA relative percentages, mainly n3. Lastly, as both fish species are omnivorous and share the same food resources, we expected that 3) they exhibit similar FA profiles when occurring together in the same system.

2. Material and methods

2.1. Study sites

The study area comprised four important reservoirs in the catchment of Upper Tietê River, located in the Metropolitan Region of São Paulo (MRSP, State of São Paulo, Brazil): Ponte Nova, Jundiaí, Billings and Guarapiranga reservoirs (Fig. 1). This region is legally protected due to its importance for water supply (FABHAT, Fundação Agência Bacia Hidrográfica do Alto Tietê, 2019). However, geographical characteristics and the process of territorial occupation led to a critical framework of water degradation over the years due to a disorderly urbanization process and the discharge of domestic and industrial effluents without adequate sanitary treatment, compromising water quality for different purposes (SMA, 2010). According to the Köppen-Geiger classification, the regional climate is humid subtropical (Cwa), with the rainy and warm season during summer and the dry and cold season during winter (IAG/USP, Instituto de Astronomia, Geofísica e Ciências Atmosféricas da Universidade de São Paulo, 2020).

The Ponte Nova (PN; 23°35′22.1″S 45°57′53.9″W) and Jundiaí reservoirs (Ju; 23°38′05.1″S 46°10′39.9″W) (Fig. 1) are in the catchment's headwater area and are located close to the upstream section of the Tietê River, 70 km from São Paulo city. The headwaters of the Upper Tietê are originally covered by Atlantic Forest, with a dominance of dense rainforest, riparian forest and floodplains (Marceniuk and Hilsdorf, 2010). Both reservoirs were built to control the floods of the Tietê River and, nowadays, together with other reservoirs in the region, constitute the Upper Tietê Producer System, which supplies water to around 4.2 million people in the east of São Paulo city (SABESP, 2020). Within the Upper Tietê catchment, only the headwater region exhibited water quality standards in accordance with the requirements of the Brazilian National Environmental Council (CONAMA) Resolution 357/05 for Class 2 water (Brasil, 2005), mainly dictated by total phosphorus, ammonium-nitrogen, dissolved oxygen and chlorophyll-a concentrations (CETESB, 2019).

Billings and Guarapiranga (Fig. 1) are urban reservoirs situated in the South of São Paulo city. Both reservoirs are characterized by intense anthropogenic activity along the shoreline, such as irregular land occupation, deforestation, soil erosion, and discharge of untreated domestic and industrial effluents (Whately and Cunha, 2006; Pompêo et al., 2013; Fontana et al., 2014). The Billings complex is the largest reservoir in the MRSP. It was built in 1927 to generate electricity but currently also provides public water supply and flood control services, receiving water from the highly polluted Pinheiros River, which has significantly contributed to the intense and accelerated eutrophication process in this reservoir (Capobianco and Whately, 2002). Due to its irregular shape, the Billings reservoir is divided into eight branches that differ in their biotic and abiotic characteristics (Cardoso-Silva et al., 2014). For this study, samplings were carried out (i) in the *Pedreira* branch (Pe; 23°43′37.1″S 46°40′12.0″W), considered the most polluted of the branches, due to its proximity to the water inlet of the Pinheiros River, and the (ii) *Bororé* (Bo; 23°46′12.3"S 46°38′33.1″W) and *Taquacetuba* branches (Ta; 23°47′56.1″S 46°37′21.5″W), the latter considered the

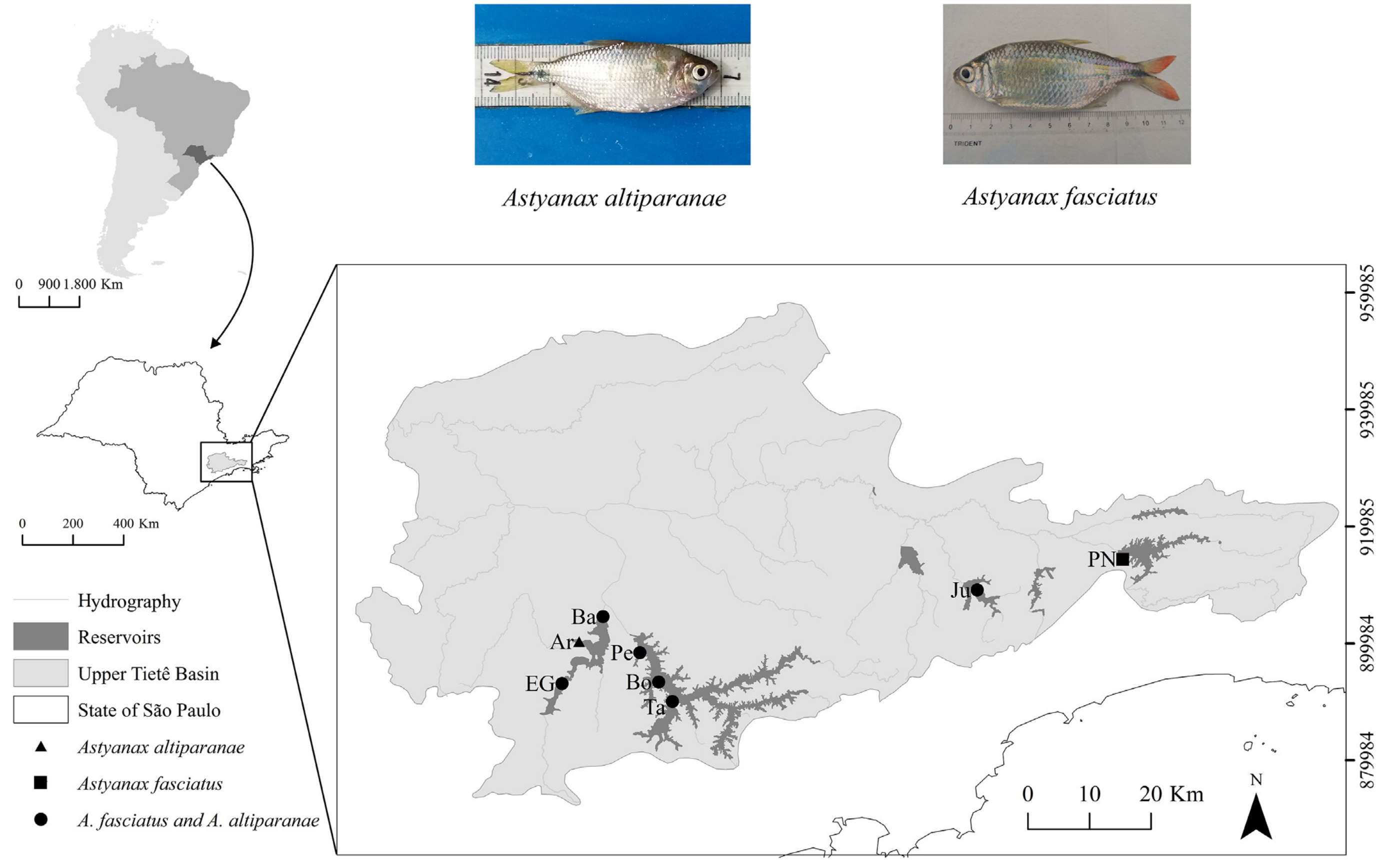

Fig. 1. Upper Tietê catchment in the São Paulo Metropolitan Region (São Paulo, Brazil), showing the geographical locations of the studied reservoirs and the species caught at each site: Ponte Nova reservoir (PN); B - Jundiaí reservoir (Ju); Pedreira (Pe), Bororé (Bo) and Taquacetuba (Ta) sites in the Billings reservoir, and Barragem (Ba), Aracati (Ar) and Embu-Guaçu (EG) sites in the Guarapiranga reservoir. See Table S1 for trophic state classifications of each sampling site.

least polluted among the studied branches of the Billings reservoir (Fig. 1).

The Guarapiranga reservoir is the second most important public water supply reservoir in the MRSP, providing water for over 2 million people (Fontana et al., 2014). This reservoir had been classified as eutrophic since 1960 but has recently been considered hypereutrophic due to intense urbanization processes (Beyruth, 2000; CETESB, 2015, 2016, 2017, 2018, 2019) that have impacted the reservoir's biotic community (CETESB, 2015). In the Guarapiranga reservoir, we also selected three sampling stations in order to cover differences in trophic state: one sample station at the reservoir dam, the *Barragem* station (Ba; 23°40′25.7″S 46°43′26.0″W), an intensively urbanized region both by regular and irregular settlements (*favelas*) as well as by yacht clubs; another sampling station in the *Aracati* branch, the most polluted area, due to intense irregular urban occupation (Ar; 23°42′38.7″S 46°45′31.7″W), and the last station at the *Embu-Guaçu* tributary (EG; 23°46′20.4″S 46°47′01.3″W), a region considered to be less polluted and well preserved (Schunck and Rodrigues, 2016) (Fig. 1).

2.2. Fish species

We studied the two commonly found fish species *Astyanax fasciatus* (Cuvier, 1819), popularly known as red-tailed lambari, and *Astyanax altiparanae* (Garutti and Britski, 2000), popularly known as yellow-tailed lambari (Fig. 1). The *Astyanax* genus belongs to the Characidae family and is one of the most diversified genera in South America (Reis et al., 2003). It is well known for its high adaptability to a broad range of environmental conditions, from well preserved and pristine to highly eutrophic systems (Souza and Lima-Junior, 2013; Carvalho et al., 2015; Alonso et al., 2019). Both studied species are small and omnivorous, with a tendency towards zooplanktivory and insectivory, but exhibit high trophic plasticity to environmental changes and food availability (Vilella et al., 2002; Bennemann et al., 2005; Foresti et al., 2005; Alonso et al., 2019). We selected these species due to their broad occurrence and convenient capture in the reservoirs of the Tietê catchment (Giamas et al., 2004). Further, their omnivorous feeding habit turns them into an essential link in the food chain and allows us to test for the hypothesis of effects of sestonic FAs on fish FA profiles.

2.3. Sampling

Three sampling campaigns were performed in March (rainy season) and July (dry season) 2017, and January 2018 (rainy season). Dissolved oxygen concentrations (mg L^{-1}), pH and water temperatures (°C) were recorded in situ using a portable multiparameter probe (Professional Plus, Yellow Springs Instruments) in the morning. Water transparency was measured using a Secchi disk, and the depth of the photic zone was estimated by multiplying Secchi disk measurements by 2.709 (Cole, 1979). Water samples were collected close to the margin and in the centre of the reservoirs, at three different depths of the photic zone (subsurface, middle and bottom), using a 5 L Van Dorn bottle and immediately pooled to create a composite sample of the three layers. From the integrated sample, four types of subsamples were taken: 1) water samples for total nutrient and metal analyses (the latter after acidification with nitric acid), stored in plastic bottles and kept on ice until transport to the laboratory; 2) water samples for phytoplankton identification, stored in plastic bottles protected from light, fixed with acetic Lugol's solution and kept at room temperature; 3) water samples for dissolved nutrient, total solids and chlorophyll-*a* analyses, filtered through GF/C glass fibre filters (Whatman), stored in plastic bottles and kept on ice until transport to the laboratory; 4) water samples for sestonic FA analysis, filtered through a 45 μm mesh size plankton net (to remove meso- and macrozooplankton), stored in plastic bottles and kept on ice until transport to the laboratory. Since algal species with cellular dimension higher than 45 μm represented only a small percentage (5–16%) of the total phytoplankton biomass in the analyzed samples, we do not expect them to cause large discrepancies between phytoplankton composition and sestonic FA analysis.

Concomitantly, adult males of the species *A. altiparanae* ($n = 150$; 11.6 ± 3.13 g, 9.0 ± 0.71 cm) and *A. fasciatus* ($n = 87$; 12.0 ± 4.11 g, 9.4 ± 1.10 cm) were caught at previously determined sites (Fig. 1) using gillnets (Environment Federal Agency, SISBIO Authorization 31,935–6) maintained for 12 h on the water surface and checked every 3 h. Both species present sexual dimorphism, with the males identified by the presence of spines in the anal fin that are absent in females (Chehade et al., 2015). After catching the animals, they were kept in plastic tanks of 200 L with constant aeration until tissue sampling procedures carried out on the same day of capture. First, males were anaesthetized with 1 g of benzocaine (0.1%) diluted in 10 mL of ethanol and then biometric data were recorded. Subsequently, the animals were euthanized by spinal cord section, as approved by the Animal Ethics Committee of the University of São Paulo (Protocols 282/2017). After dissection, fresh epaxial muscle samples were collected and kept at −80 °C until processing.

2.4. Sample processing

2.4.1. Water chemical analysis and TSI

Total nitrogen and phosphorus (Valderrama, 1981), nitrate-N and nitrite-N (Mackareth et al., 1978), ammonium-N (Koroleff, 1976) and chlorophyll-*a* (Wetzel and Likens, 1991) concentrations were analyzed by standard spectrophotometric procedures. Suspended solids concentration was determined by filtering water samples through pre-combusted and pre-weighed glass fibre filters (GF-F, Whatman), combusting the filters at 450 °C for 1 h, and weighing the filters again after drying at 110 °C for 4 h (Wetzel and Likens, 1991). The dissolved inorganic nitrogen (DIN) concentration was obtained from the sum of nitrate-N, nitrite-N and ammonium-N concentrations. The trophic state index (TSI) was calculated from chlorophyll-*a* and total phosphorus concentrations (Lamparelli, 2004).

To quantify copper concentrations, 100 mL water samples were digested with 5 mL nitric acid (HNO_3, Merck) at 90 °C to a final volume of 20 mL. The samples were then filtered through 0.45 μm glass fibre filters (Whatman) and stored at 4 °C until spectrophotometric analysis (Method 3005 A-EPA; APHA, 1998). The metal concentration in the water was analyzed by atomic emission spectroscopy with inductively coupled plasma (ICP-OES Model 720, Agilent). Copper concentration was analyzed in water samples because copper sulphate is an algaecide widely used in the studied region.

2.4.2. Phytoplankton quantification and identification

Phytoplankton identification was carried out to the lowest possible taxon (species or genus). Quantification of these microorganisms was performed using an inverted microscope (Primovert - Zeiss, Germany) according to the methodology established by Utermöhl (1958). Individuals were counted over a transect in sedimentation chambers with 400 x magnification until 100 individuals of the most abundant species were counted. When this number could be quickly reached, a minimum of 28 fields was evaluated for each sample to better estimate the species richness. Biomass was evaluated as biovolume (mm^3 L^{-1}) and was estimated from the number of cells and average cell volumes, calculated using geometric approximations (Rott, 1981; Sun and Liu, 2003). The phytoplankton dataset was analyzed on a higher taxonomic level (phylum) in this study since the literature generally mentions FA composition and content for phyla or classes. A synthesis of the data by species was obtained by calculating the Shannon-Wiener diversity index (H′), based on biovolume.

2.4.3. Fatty acid analysis

Total lipids from seston and fish wet muscle samples were extracted with chloroform:methanol:water (2:1:0.5) according to the method of Folch et al. (1957) adapted by Parrish (1999) for aquatic organisms.

Seston samples were homogenized for 5 min at 5000 cycles min^{-1} with ultrasonic sonication to break cell walls of bacteria and algae (Boëchat et al., 2011). The total extract was methylated with acetyl chloride (5% HCl in methanol) (Christie, 2003), and the composition of FA methyl-esters (FAMEs) was determined using a Varian gas chromatograph model 3900 equipped with a flame ionization detector (FID) and a CP8400 auto-sampler. FAMEs were separated in a CP Wax 52 CB capillary column (0.25 μm thickness, 0.25 mm internal diameter and 30 m length). Hydrogen was used as a carrier gas at a linear speed of 22 cm s^{-1}. The temperature program was: 170 °C for 1 min, followed by a temperature increase of 2.5 °C min^{-1} up to 240 °C, which was maintained for 5 min. The temperatures of the injector and FID were 250 and 260 °C, respectively. FAs were identified by comparing their retention times with the retention times of a known FAME standard solution (FAME-mix, Supelco, 37 components, Sigma-Aldrich and QUALMIX, PUFA *fish* M, Menhaden oil, Larodan). Data are presented as relative concentrations of total FAMEs based on peak area analyses. The main specific FAs and the ratios for biomarker FAs used in the present study are based on available literature data (Table 1).

2.5. Statistical analysis

Environmental variables, except for pH and the trophic state index (TSI), were $\log_{10}(x + 1)$ transformed before analyses to achieve data normality and homoscedasticity. Principal component analysis on environmental variables was performed with the function *prcomp* from the R package *stats*. Detailed environmental data are presented in the Supplementary Material for each site (Table S1).

Differences in total biovolume of phytoplankton organisms (absolute values), as well as the Shannon-Wiener index between sampling sites and campaigns, were tested with two-way ANOVAs, with a significance level of 95% ($P < 0.05$), with pooled margin and centre data. Besides that, differences in phytoplankton assemblage composition, based on square-root transformed biovolume data (absolute values), between sites, sampling campaigns, and sampling stations were tested with permutational multivariate analysis of variance using a Bray-Curtis distance matrix (PERMANOVA; R function *adonis*, package *vegan*). Multivariate homogeneity in group dispersions was confirmed prior to analysis (PERMDISP2 procedure; R function *betadisper*, $P > 0.05$, package *vegan*). To assess the effects of the environmental dataset on phytoplankton assemblage structure (absolute values), we performed distance-based redundancy analysis (dbRDA; R function *dbrda*, package *vegan*) based on Bray-Curtis dissimilarity matrices. Biovolume data of the phytoplankton assemblage composition were square-root transformed, and Wisconsin double standardized before analysis. A final model was elaborated by successively eliminating non-significant environmental variables using ANOVAs by terms and margins. As an alternative approach, we compared the dbRDA with a redundancy analysis (RDA; R function *rda*, package *vegan*) on a Hellinger distance-transformed phytoplankton community matrix based on relative phytoplankton community composition (proportional data, %) (Legendre and Gallagher, 2001). This approach was tested in order to use the Hellinger distance-transformed matrix for subsequent analyses of community effects on FA variables with Euclidian-based methods. As the Hellinger distance-transformed RDA approach performed very similar to the dbRDA approach, i.e., it selected the same dependent variables and showed a very similar correlation structure, we adopted the Hellinger distance-transformed RDA approach in this study. Here, RDAs were used to assess the effects of (1) environmental variables and the phytoplankton community on seston FAs, as well as the effects of (2) environmental variables, the phytoplankton community, and seston FAs on the two fish species' tissue FA compositions. To execute these analyses, we had to average data per sampling campaign and site, resulting in a total 24 samples (3 samplings × 8 sites) due to asymmetry in our dataset. Here, a larger number of fish FA profiles (up to 14 individuals per species, site and sampling) had to be matched to the composite water samples for water quality, phytoplankton, and seston FA analyses (2 samples per site and sampling).

Table 1
Individual fatty acids and fatty acid sums and ratios used as biomarkers in different food resources. Biomarkers (expressed as ratios) indicate the relative importance of one food source in relation to another.

Source	Biomarkers	Reference
Heterotrophic bacteria	$\sum$ Odd and branched fatty acids ↑SFA, 18:1n7	Kaneda, 1991; Navarrete et al., 2000
Cyanobacteria	↑ C18 PUFA ↓ HUFA	Müller-Navarra et al., 2004
Green Algae	18:3n3 $\sum$ C18 PUFA (18:2n6 + 18:3n6 + 18:3n3)	Léveillé et al., 1997; Volkman et al., 1998; Ahlgren et al., 1992
Bacillariophyta	20:5n3 16:1/16:0 > 1.0 20:5n3/22:6n3 > 1	Léveillé et al., 1997; Dunstan et al., 1994; Napolitano, 1999
Dinophyta	22:6n3 Specific ratio (16:0 + 18:4n3 + 20:5n3 + 22:3n6/18:3n3 + $\sum$ C16 PUFA)	Léveillé et al., 1997; Parrish et al., 2000
Zooplankton	20:1 + 22:1	Parrish et al., 2000; Falk-Petersen et al., 2002
Terrestrial vegetal	C18:2n6	Parrish et al., 2000
Urban discharge	18:1n9 + 18:1n7 18:1n9 + 18:2n6	Sakdullah and Tsuchiya, 2009

Around 30 FAs were identified in the samples, however, to achieve adequate variable:sample size ratios for multivariate analyses, FA sum parameters were selected: $\sum$ BFA – bacterial FAs; $\sum$ SFA – saturated FAs; $\sum$ MUFA – monounsaturated FAs; $\sum$ C18 PUFA – polyunsaturated FAs with 18 carbons (18:2n6 + 18:3n6 + 18:3n3); $\sum$ n3 HUFA – n3 highly unsaturated FAs; $\sum$ n6 HUFA – n6 highly unsaturated FAs; 18:1n9 + 18:2n6, 20:1 + 22:1, and specific Dinophyta ratio (16:0 + 18:4n3 + 20:5n3 + 22:3n6/18:3n3 + $\sum$ C16 PUFA). Since FA relative composition in the seston and muscle from both species from all studied reservoirs showed no significant temporal variation ($P > 0.05$), data from all sampling campaigns were pooled. Differences in individual FA profiles of seston and each fish species among reservoirs were tested by one-way ANOVAs or Kruskal-Wallis tests when data were not normality distributed and presented in the Supplementary Material (Tables S2-S4). The significance level adopted was 95% ($P < 0.05$). Finally, to identify differences in muscle FA profile between the two fish species and between these species at different sites, we performed linear discriminant analyses (LDA; R function *lda*, package *MASS*) with the factor "species" and the combination of the factors "species" and "site". The sample size, in this case, allowed for the use of individual FAs in the analyses: 16:0, 17:0, 18:0, 16:1, 18:1, 18:2n6, 18:3n3, 20:1n9, 20:4n6, 22:4n6, 22:5n6, 20:5n3, 22:5n3 and 22:6n3.

3. Results

3.1. Environmental variables

The sampled reservoirs spanned a gradient from oligo—/mesotrophic to hypereutrophic conditions (Fig. 2A, Table S1), and there were significant differences in several water chemical characteristics among sites. In general, the less eutrophic sites Ju, PN, and EG had significantly lower nutrient and chl-*a* concentrations than the eutrophic to hypereutrophic sites Ar, Ta, Bo, and Pe for all sampling campaigns. They were well separated from each other on the dominant environmental gradient detected by the PCA (i.e., PCA1) that explained 41.7% of the variability in environmental variables (Fig. 2A) and was positively related to DIN, TP, and TN concentrations.

The PCA detected a secondary environmental gradient related to water temperature and DO concentration that explained 17.2% of the variability in environmental variables. According to this gradient, the

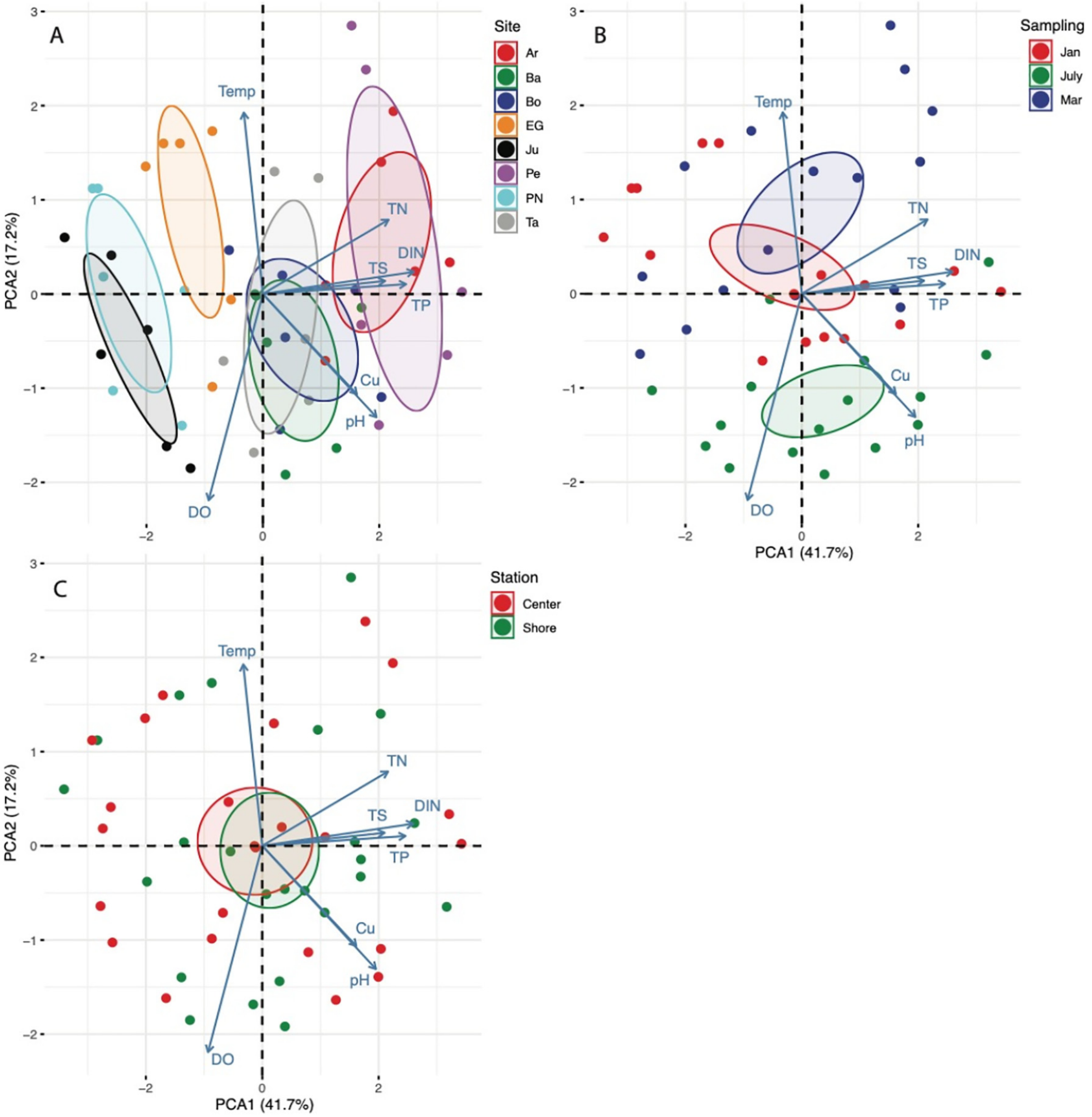

Fig. 2. Principal component analysis (PCA) correlation biplots of environmental variables (log-transformed) showing the three samplings at the eight sampled reservoir sites (circles), and the seven environmental variables (blue arrows). Samplings are grouped according to sampled sites (A), sampling campaigns (B), and stations sampled within each reservoir (C), and 95% confidence ellipses are shown for these groupings. The first two principal components of the PCA account for 58.9% of the total variation among samplings. The PCA identified a dominant environmental gradient related to nutrient concentrations (DIN - dissolved inorganic nitrogen, TP - total phosphorus, TS - total solids, and TN - total particulate nitrogen) and pH (PCA1), and a secondary gradient related to water temperature (Temp) and dissolved oxygen concentrations (DO) (PCA2). Ar – Aracati; Ba – Barragem; Bo – Bororé; EG – Embu-Guaçu; Ju – Jundiaí; Pe – Pedreira; PN – Ponte Nova, and Ta – Taquacetuba.

winter sampling (i.e., July) showed lower water temperatures and higher DO concentrations. In contrast, the summer sampling (i.e., January) showed higher water temperatures and lower DO concentrations (Fig. 2B). Moreover, water temperature and DO were the only variables showing differences between sampling campaigns (Fig. 2B). There were no significant differences (ANOVA, $P > 0.1$) between the shore and centre stations sampled at each site (Fig. 2C).

3.2. Phytoplankton

The phytoplankton community differed between sites (PERMANOVA, $P < 0.001$), but not between sampling campaigns, nor between shore and centre stations at each site (PERMANOVA, $P > 0.05$). There were also no significant interactions between the factors “site” and “sampling campaign” or “station” ($P > 0.05$). According to pairwise post-hoc tests, the PN community differed from those of all other sites, whereas Bo, Ta, and Pe communities differed from all other sites but not from each other.

A total of 9 phyla were recorded, and their absolute total biovolume varied among sites (two-way ANOVA, $F_{7,32} = 5.532$, $P = 0.003$; Fig. 3). Cyanobacteria were dominant in the PN site, followed by Dinophyta and Bacillariophyta in different sampling campaigns (Fig. 3). The Ju site presented a low total biovolume of phytoplankton organisms throughout the sampling campaign but with high seasonal variability and large contributions of the groups Chlorophyta, Cryptophyta, Bacillariophyta and Euglenophyta (Fig. 3). Likewise, the EG site showed low values of total biovolume and the groups Charophyta, Cryptophyta, Dinophyta, Euglenophyta, and Bacillariophyta were the most representative (Fig. 3).

Ba and Ar sites were the most diverse sites throughout the sampling period, presenting organisms of almost all groups found. On the other

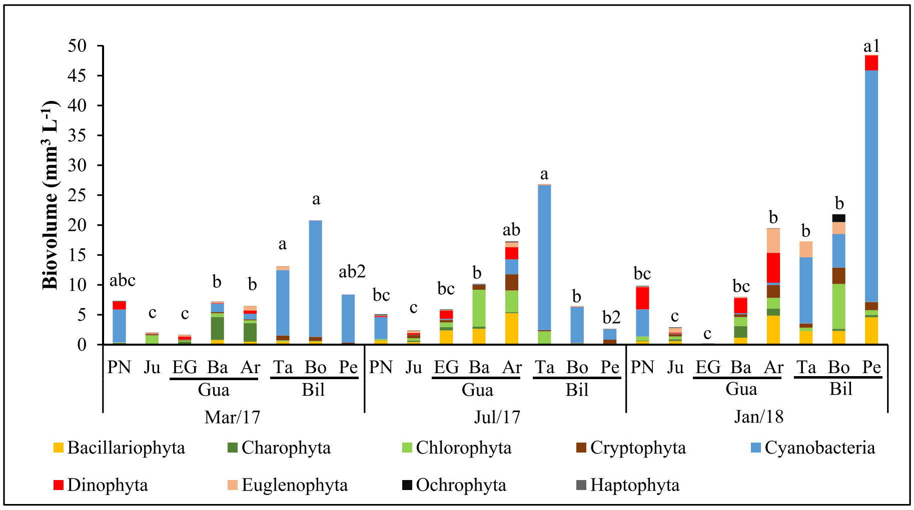

Fig. 3. Phytoplankton biovolume (mm^3 L^{-1}) total and by phyla (colored bars) of the Ponte Nova (PN) and Jundiaí (Ju) reservoirs and Embu-Guaçu (EG), Barragem (Ba) and Aracati (Ar) sites of the Guarapiranga reservoir and Taquacetuba (Ta), Bororé (Bo) and Pedreira (Pe) sites of Billings reservoir throughout the sampling campaign (March and July 2017 and January 2018). Mean values from samples collected at the margin and centre of each sampling site. Different letters indicate differences to total biovolume among sampling sites according to *two-way* ANOVA ($P < 0.05$). Different numbers indicate differences to total biovolume among sampling period according to *two-way* ANOVA ($P < 0.05$).

hand, in the Billings reservoir, Cyanobacteria dominated the phytoplankton composition at all studied sites (Ta, Bo and Pe) throughout the sampling campaigns (Fig. 3). However, in some campaigns, other taxa contributed, such as Bacillariophyta, Euglenophyta, Chlorophyta, Cryptophyta and Dinophyta (Fig. 3). The highest absolute values of total biovolume were detected at the Bo and the Pe sites (Fig. 3). In general, the highest H′ values were observed for the Ju site and the Guarapiranga reservoir (sites EG, Ba and Ar), and the lowest values for the PN site and Billings reservoir (sites Ta, Bo e Pe), despite the observed increase of this index for Billings branches in January (two-way ANOVA, $F_{7,32} = 38.284$, $P < 0.001$; Table 2).

The RDA on Hellinger transformed community data ($P < 0.001$) had a significant first ($P < 0.001$) and second axis ($P = 0.048$), and the terms Cu, TP, and TN (all $P < 0.05$) explained 23.2% of the variability in the phytoplankton community (Fig. 4). On the first axis of this RDA, scores of Cyanobacteria were positively correlated with TP and negatively with Cu, while the scores of all other groups were positively correlated with Cu and negatively with TP. High scores of Dinophyta and low scores of Chlorophyta were explained by high TN and TP concentrations on the second axis.

3.3. Fatty acid profiles

3.3.1. Seston

Seston FA metrics differed among sampling sites (PERMANOVA, $P < 0.05$) but not sampling campaigns (PERMANOVA, $P > 0.1$). There were no significant interactions between the factors "sampling site" and "sampling campaign" ($P > 0.1$). According to pairwise post-hoc tests ($P < 0.05$), the FA metrics of BA and EG differed from those of Ta, Bo and Pe, mainly due to differences in n3 and n6 HUFA, C18 PUFA, SFA and Dinophyta ratio. Further, FA metrics of Bo and Pe differed from those of PN, EG, Ba and Ar due to differences in C18 PUFA, n6 HUFA and Dinophyta ratio.

A total of 22 FAs were identified (Table S2). PUFAs contributed most to sestonic FA composition (42.9–45.5%), except for seston samples collected from the Billings reservoir, where SFAs were more important (40.3–50.7%). Among PUFAs, C18 (18:2n6, 18:3n3 and 18:4n3), followed by C20 (20:2n6 and 20:4n6) and C22 FAs (22:2n6 e 22:5n3) had the highest relative contributions to the total FA profiles. SFA accounted for 32.0–36.4% and MUFAs for 12.5–13.4% of the total FAs. The FA profiles of seston samples showed significant differences among the studied environments, with higher percentages of iso18:0 (Kruskal-Wallis test, $H = 20.763$, $P = 0.004$) in the seston samples from Ba and Bo (Table S2). In Ba, we also found the highest percentages of 18:2n6 (one-way ANOVA, $F_{7,54} = 3.287$, $P = 0.005$), 18:3n3 (Kruskal-Wallis test, $H = 25.630$, $P < 0.001$) and 20:2n6 (one-way ANOVA, $F_{7,54} = 12.956$, $P < 0.001$), especially compared to the sites in the Billings reservoir (Ta, Bo and Pe; Table S2). The site PN also showed high percentages of 18:3n3 (Table S2). The highest percentages of 16:0 (Kruskal-Wallis test, $H = 35.431$, $P < 0.001$), as well as the highest Dinophyta ratio (Kruskal-Wallis test, $H = 25.817$, $P < 0.001$), were found in the Billings reservoir sites (Ta, Bo and Pe), compared to sites PN, Ju, EG and Ba (Table S2).

The RDA between FA metrics and environmental variables was not significant ($P > 0.05$). However, the RDA between FA metrics and the phytoplankton community based on proportional data ($P < 0.01$) had a significant first ($P < 0.01$) and second axis ($P < 0.05$) and the phyla Chlorophyta, Cryptophyta, and Dinophyta (all $P < 0.05$) explained

Table 2
Shannon-Wiener index (H′ – bits μm^{-3}) for the phytoplankton communities from Ponte Nova (PN), Jundiaí (Ju), Guarapiranga reservoir (sites: Embu-Guaçu - EG, Barragem- Ba, and Aracati - Ar) and Billings reservoir (sites: Taquacetuba - Ta, Bororé – Bo, and Pedreira - Pe branches). Mean values obtained of samples collected at the margin and centre of each sampling site (Mean ± Standard deviation).

H′(bits μm^{-3})	PN	Ju	EG	Ba	Ar	Ta	Bo	Pe
Mar '17	1.6 ± 0.10^{B2}	3.1 ± 0.20^{A3}	3.1 ± 0.56^{A1}	2.8 ± 0.46^{A}	3.0 ± 0.76^{A2}	1.7 ± 0.25^{B3}	0.8 ± 0.04^{C2}	1.7 ± 0.17^{B12}
Jul '17	2.2 ± 0.25^{C1}	3.8 ± 0.10^{A2}	3.1 ± 0.33^{B1}	3.2 ± 0.19^{B}	3.7 ± 0.03^{A1}	1.0 ± 0.50^{D2}	1.3 ± 0.13^{D2}	1.3 ± 0.41^{D2}
Jan '18	2.5 ± 0.40^{C1}	$4.4 \pm 0.09\ ^{A1}$	2.5 ± 0.12^{C2}	3.2 ± 0.23^{B}	3.4 ± 0.25^{B12}	2.6 ± 0.33^{C1}	3.4 ± 0.42^{B1}	1.9 ± 0.10^{D1}

Different uppercase letters after mean values indicate differences among sampling sites according to *two-way* ANOVA ($P < 0.05$). Different number after mean values indicate differences among sampling period according to *two-way* ANOVA ($P < 0.05$).

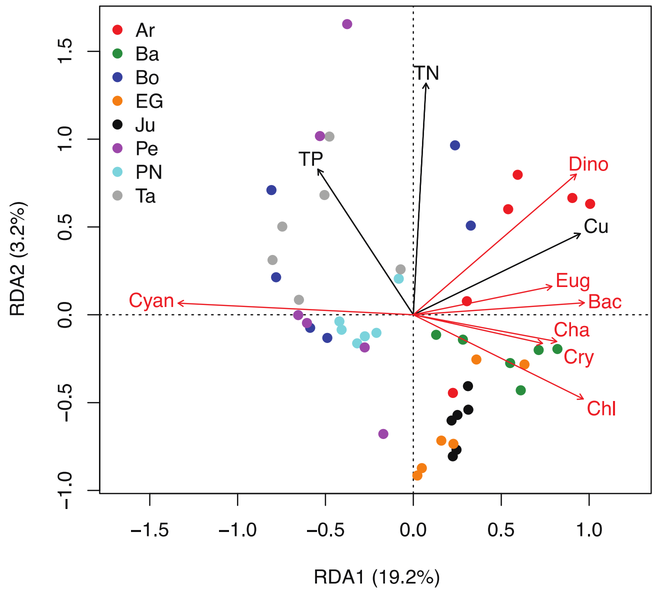

Fig. 4. Symmetrically scaled redundancy analysis (RDA; $P = 0.01$) tri-plot of phytoplankton phyla (red arrows) and explanatory environmental variables (black arrows; all with $P < 0.05$), showing samplings at the eight sampled sites (circles; Ar – Aracati branch; Ba – Barragem branch; Bo – Bororé branch; EG – Embu-Guaçu branch; Ju – Jundiaí reservoir; Pe – Pedreira branch; PN – Ponte Nova reservoir and Ta – Taquacetuba branch). RDA axis 1 (RDA1; $P < 0.001$) explains 19.2% of total variability. RDA axis 2 (RDA2; $P = 0.048$) explains 3.2% of total variability. Phytoplankton phyla are Bacillariophyta (Bac), Charophyta (Cha), Chlorophyta (Chl), Cryptophyta (Cryp), Cyanobacteria (Cyan), Dinophyta (Dino), and Euglenophyta (Eug). Environmental variables are total nitrogen (TN), copper (Cu), and total phosphorus (TP).

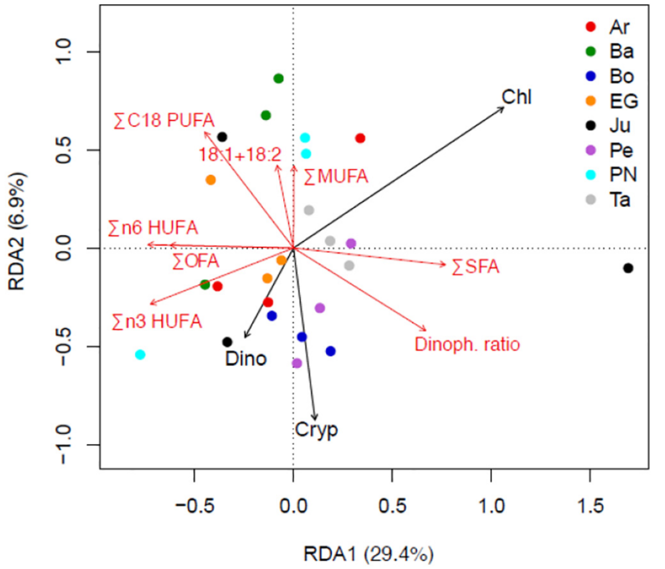

Fig. 5. Symmetrically scaled redundancy analysis (RDA; $P < 0.01$) tri-plot of seston fatty acid (FA) metrics (red arrows) and explanatory phytoplankton variables (black arrows; all with $P < 0.05$), showing samplings at the eight sampled sites (circles; Ar – Aracati; Ba – Barragem; Bo – Bororé; EG – Embu-Guaçu; Ju – Jundiaí; Pe – Pedreira; PN – Ponte Nova, and Ta – Taquacetuba). Phytoplankton phyla are Chlorophyta (Chl), Cryptophyta (Cryp), and Dinophyta (Dino). FA are odd *fatty acids (OFA), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA),* polyunsaturated *fatty acids with 18 carbons (C18 PUFA - 18:2n6 + 18:3n6 + 18:3n3), omega 3 highly unsaturated fatty acids (n3 HUFA), omega 6 highly unsaturated fatty acids (n6 HUFA); Dinophyta ratio - 16:0 + 18:4n3 + 20:5n3 + 22:3n6/18:3n3 + C16 PUFA, 18:1n9 + 18:2n6.*

36.3% of the variability in FA metrics (Fig. 5). On the first axis of this RDA, the scores of SFA and the Dinophyta ratio were positively correlated to Chlorophyta, while scores of most other seston FA metrics, especially n3 and n6 HUFA, were negatively correlated to Chlorophyta. On the second axis of this RDA, the scores of n3 HUFA and the Dinophyta ratio were positively correlated with Cryptophyta and Dinophyta, and negatively with Chlorophyta. The scores of C18 PUFA, 18:1 + 18:2 FAs and MUFA were positively correlated with Chlorophyta and negatively with Cryptophyta and Dinophyta on this axis (Fig. 5). Cyanobacteria were not selected as a significant additional determinant of seston FA composition in this RDA. However, Chlorophyta percentages in the dataset were negatively correlated with Cyanobacteria percentages (Pearson correlation, $P < 0.01$, $R^2 = 0.754$).

3.3.2. *Astyanax fasciatus*

Tissue FA metrics of *A. fasciatus* differed between reservoir sites (PERMANOVA, $P < 0.01$) but not between sampling campaigns (PERMANOVA, $P > 0.1$). There were no interactions between both factors ($P > 0.1$). According to pairwise post-hoc tests ($P < 0.05$), the FA metrics of fish tissue from sites Bo and Ta did not differ from each other but did differ from all other sites, except site Ju, due to differences in relative contributions of 18:1 + 18:2, C18 PUFA, n6 HUFA and, n3 HUFA (Fig. 6). Further, the animals from site EG differed from all other sites, except PN, due to differences in relative contributions of SFA, 18:1 + 18:2, and n6 HUFA.

Total FA profiles of *A. fasciatus* clearly differed among sites (Table S3). Fish muscle from PN showed higher percentages of 16:0 (Kruskal-Wallis test, $H = 21.306$, $P = 0.002$) and 18:2n6 (Kruskal-Wallis test, $H = 64.77$, $P < 0.001$) than fish from the sites Ta, Bo and Pe. 18:2n6 was also low in the fish from Ju, while high percentages of 18:3n3 were observed in these animals compared to fish from PN (Kruskal-Wallis test, $H = 26.485$, $P < 0.001$). Additionally, the SFA 16:0 and the PUFA 18:3n3 showed higher percentages in muscle samples from EG, compared to animals from the Billings reservoir (Ta, Bo and Pe) and PN, respectively. Higher percentages of 17:0 (Kruskal-Wallis test, $H = 18.284$, $P = 0.006$) and 16:1 (Kruskal-Wallis test, $H = 16.185$, $P = 0.013$) were found in fish from Ju and Ba than in fish samples from the Billings reservoir. Animals from Ju also had higher relative values of 20:2n6 (Kruskal-Wallis test, $H = 64.730$, $P < 0.001$) than the other study sites, and of 22:5n6 (Kruskal-Wallis test, $H = 39.034$, $P < 0.001$) than fish from the Guarapiranga sites (EG, Ba). In contrast, animals from Billings reservoir, mainly from Ta and Bo, exhibited higher relative contributions of 20:5n3 (one-way ANOVA, $F_{6,83} = 21.489$, $P < 0.001$), 22:5n3 (one-way ANOVA, $F_{6,83} = 15.042$, $P < 0.001$), 22:6n3 (one-way ANOVA, $F_{6,83} = 6.987$, $P < 0.001$), and 22:5n6 (Kruskal-Wallis test, $H = 39.034$, $P < 0.001$) in their muscle tissue than animals from less eutrophic sites.

For FA metrics of *A. fasciatus* muscle tissue, RDAs for environmental variables, phytoplankton (proportional data), and seston FA metrics were all significant ($P < 0.001$; Figs. 6A-C) and explained 75.3% of the variability in FA metrics, with considerable shared explained variability (see pRDA variance partitioning, Fig. 6D). The RDA between FA metrics and environmental variables had a significant first ($P < 0.001$) and second axis ($P < 0.05$). The terms Cu, DIN and TP (all $P < 0.05$) explained 45.8% of the variability in FA metrics (Fig. 6A). On the first axis of this RDA, scores of fish tissue 20:1 + 22:1, BFA and MUFA were positively correlated with Cu and negatively with DIN and TP, while tissue n3 and n6 HUFA and SFA were negatively correlated with Cu and positively with DIN and TP. On the second axis, mainly scores of tissue n6 and n3 HUFA were positively correlated with DIN, TP and Cu. Tissue 18:1 + 18:2, C18 PUFA, SFA, and the Dinophyta ratio were negatively correlated with DIN, TP and Cu (Fig. 6A).

The RDA between tissue FA metrics and the phytoplankton community (proportional data) had a significant first ($P < 0.01$) and a second

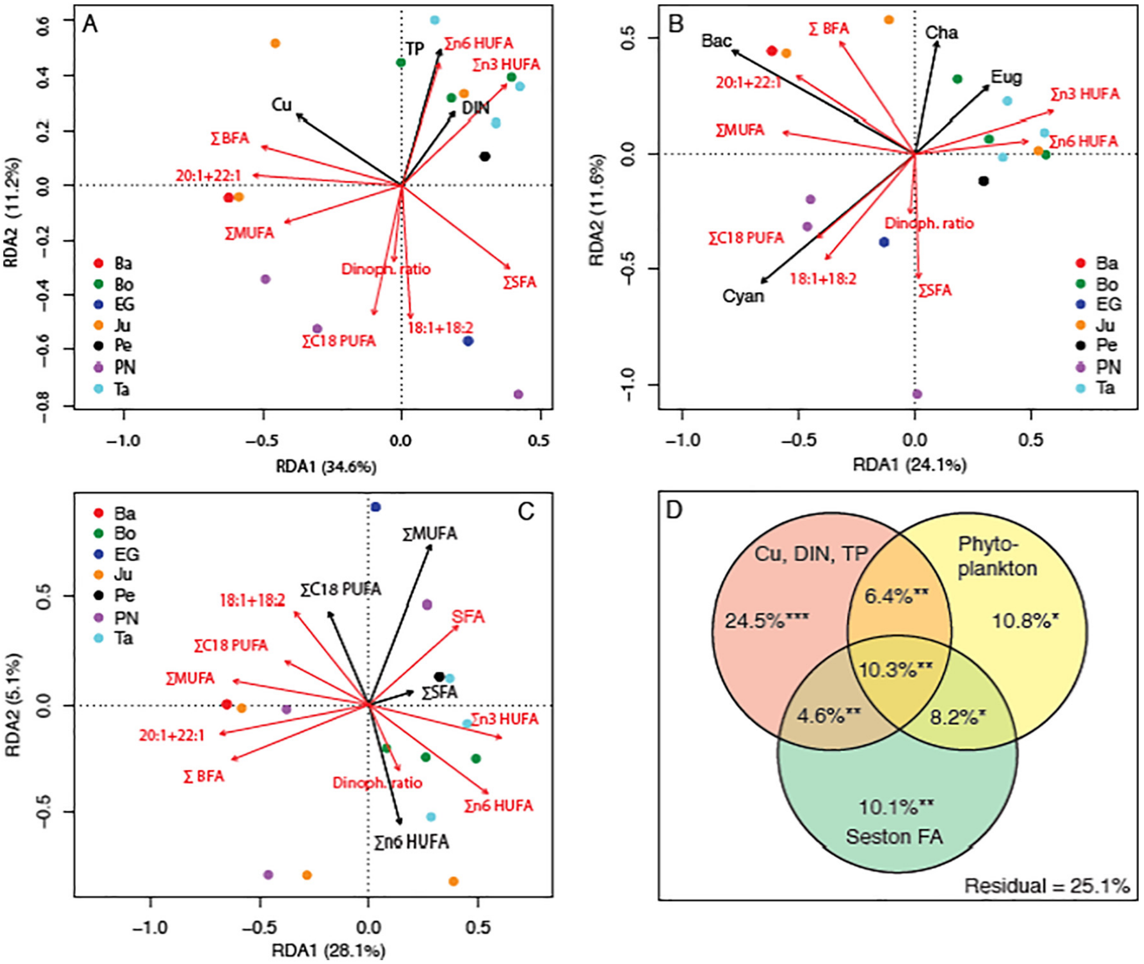

Fig. 6. Symmetrically scaled redundancy analyses (RDA; all $P < 0.01$) tri-plots of fatty acid (FA) metrics (red arrows) of *Astyanax fasciatus* and explanatory environmental (A), and phytoplankton (B) variables and seston FA metrics (C) (black arrows; all with $P < 0.05$), showing samplings at the seven sampled sites (circles; Ba – Barragem; Bo – Bororé; EG – Embu-Guaçu; Ju – Jundiaí; Pe – Pedreira; PN – Ponte Nova; Ta – Taquacetuba). Environmental variables are copper (Cu), dissolved organic nitrogen (DIN) and total phosphorus (TP). Phytoplankton phyla are Baccilariophyta (Bac), Charophyta (Cha), Cyanobacteria (Cyan) and Euglenophyta (Eug). FA are bacterial *fatty acids (BFA), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids with 18 carbons (C18 PUFA - 18:2n6 + 18:3n6 + 18:3n3), omega 3 highly unsaturated fatty acids (n3 HUFA), omega 6 highly unsaturated fatty acids (n6 HUFA), Dinophyta ratio - 16:0 + 18:4n3 + 20:5n3 + 22:3n6/18:3n3 + C16 PUFA, 18:1n9 + 18:2n6, 20:1 + 22:1*. A partial redundancy analysis (pRDA; D) is showing the variability explained (as adjusted R2 values, with * $P < 0.05$ and *** $P < 0.001$) by environmental variables, phytoplankton phyla, and seston FA metrics, as well as shared contributions.

axis ($P = 0.05$). The terms Baccilariophyta and Chlorophyta, Cyanobacteria, and Euglenophyta (all $P < 0.05$) explained 35.7% of the variability in FA metrics (Fig. 6B). On the first axis of this RDA, scores of tissue n3 and n6 HUFA were positively correlated with Euglenophyta and negatively with Baccilariophyta and Cyanobacteria, while MUFA, 20:1 + 22:1, C18 PUFA and 18:1 + 18:2 were positively correlated with Baccilariophyta and Cyanobacteria and negatively with Euglenophyta. On the second axis, mainly scores of BFA and 20:1 + 22:1 were positively correlated with Baccilariophyta, Chlorophyta and Euglenophyta, and negatively with Cyanobacteria. SFA, 18:1 + 18:2, and C18 PUFA were positively correlated with Cyanobacteria, and negatively with Bacillariophyta, Charophyta, and Euglenophyta (Fig. 6B).

The RDA between *A. fasciatus* and seston FA metrics had a significant first ($P < 0.01$) and second axis ($P = 0.05$). The terms seston MUFA, SFA, C18 PUFA and n6 HUFA (all $P < 0.05$) explained 33.2% of the variability in tissue FA metrics (Fig. 6C). On the first axis of this RDA, scores of fish tissue 20:1 + 22:1, BFA, MUFA, C18 PUFA, and 18:1 + 18:2 were positively correlated with seston C18 PUFA, and negatively with seston MUFA, SFA, and n6 HUFA. Tissue SFA, n3 and n6 HUFA were positively correlated with seston MUFA, SFA and n6 HUFA and negatively correlated with seston C18 PUFA. On the second axis, tissue 18:1 + 18:2, SFA, and C18 PUFA were positively correlated with seston MUFA and C18 PUFA, and negatively with seston n6 HUFA. Tissue n6 HUFA, Dinophyta ratio, and BFA were positively correlated with seston n6 HUFA, and negatively with seston MUFA and C18 PUFA (Fig. 6C).

3.3.3. *Astyanax altiparanae*

Tissue FA metrics of *A. altiparanae* differed among reservoir sites (PERMANOVA, $P < 0.01$) but not among sampling campaigns (PERMANOVA, $P > 0.1$). There were no interactions between both factors ($P > 0.1$). According to pairwise post-hoc tests ($P < 0.05$), the FA metrics of the animals from sites Ar and Ju differed from each other and the sites Bo, EG, and Ta, mainly due to differences in C18 PUFA, n6 and n3 HUFA and MUFA relative concentrations. Further, animals from sites Bo and EG differed from each other due to differences in the same metrics, whereas the fish from sites Ar and Ju differed from each other due to differences in C18 PUFA and n6 HUFA relative concentrations, as well as the Dinophyta ratio.

Total FA profiles of *A. altiparanae* muscle tissue showed differences among reservoir sites (Table S4), with higher percentages of 18:3n3 in fish samples at site EG compared to samples from Ju, Ba, Ta, Bo and Pe (Kruskal-Wallis test, H = 58.322, $P < 0.001$). Fish from EG and Ar also presented higher percentages of 18:2n6 than Ju, Bo and Pe (Kruskal-Wallis test, $H = 53.310$ $P < 0.001$). On the other hand, HUFAs, such as 22:6n3 (one-way ANOVA, $F_{6,121} = 4.565$, $P < 0.001$) and 22:5n6 (Kruskal-Wallis test, $H = 76.343$, $P < 0.001$), presented higher percentages in fish samples from Billings reservoir (Bo and Pe) than in the animals from Guarapiranga reservoir (EG, Ba and Ar). 20:5n3 also presented a higher percentage in fish samples from site Pe in comparison to Ba and Ar (one-way ANOVA, $F_{6,121} = 5.030$, $P < 0.001$). On the other hand, high values of n6 HUFA, such as 20:4n6 (one-way ANOVA, $F_{6,121} = 3.153$, $P = 0.006$), 22:4n6 (Kruskal-Wallis test, $H = 26.704$, $P < 0.001$) and 22:5n6 (Kruskal-Wallis test, $H = 76.343$, $P < 0.001$) were found in the animals from site Ju compared to those from Guarapiranga reservoir (EG, Ba and Ar; Table S4).

The RDA between tissue FA metrics of *A. altiparanae* and environmental variables was not significant ($P > 0.05$). The RDA between fish tissue FA and phytoplankton phyla based on proportional data ($P < 0.01$) had a significant first ($P < 0.01$) and second axis ($P < 0.05$) and Bacillariophyta, Euglenophyta, and Dinophyta (all $P < 0.05$) explained 29.5% of the variability in fish tissue FA metrics (Fig. 7A). On the first axis of this RDA, fish tissue n6 HUFA, Dinophyta ratio, and n3 HUFA were positively correlated with Dinophyta and negatively with Euglenophyta. Tissue C18 PUFA, 18:1 + 18:2, MUFA and SFA were negatively correlated with Dinophyta and positively with Euglenophyta. On the second axis, mainly tissue BFA and 20:1 + 22:1 were positively, and n3 HUFA and 18:1 + 18:2 negatively correlated with Bacillariophyta (Fig. 7A).

The RDA between fish tissue FA and seston FA metrics ($P < 0.01$) had a significant first ($P < 0.01$) and second axis ($P = 0.05$) and seston C18 PUFA, n3 and n6 HUFA, and 18:1 + 18:2 (all $P < 0.05$) explained 44.3% of the variability in fish tissue FA metrics (Fig. 7B). On the first axis of this RDA, fish tissue n6 and n6 HUFA, and the Dinophyta ratio were positively correlated with seston n6 HUFA and negatively with seston 18:1 + 18:2 and C18 PUFA. Fish tissue C18 PUFA, 18:1 + 18:2, and MUFA were positively correlated with seston 18:1 + 18:2 and C18 PUFA and negatively with seston n6 HUFA. On the second axis, fish tissue 20:1 + 22:1, BFA, MUFA, and the Dinophyta ratio were negatively and n3 HUFA and SFA positively correlated with seston n3 and n6 HUFA, and 18:1 + 18:2. Phytoplankton variables and seston FA metrics shared 16.1% of the explained variability and explained a total of 57.7% of the variability in fish tissue FA metrics (see pRDA results in Fig. 7C).

The LDA to identify differences in muscle tissue FA profiles between the two studied fish species (Fig. 8) explained 81.0% of the group separation ($P < 0.0001$). The FAs responsible for this separation were 20:4n6 (higher in *A. fasciatus* than in *A. altiparanae*) and 16:0, 18:0, and 20:1n9 relative concentrations (all higher in *A. altiparanae* than in *A. fasciatus*). The LDA to identify differences in muscle tissue FA profiles between the two studied fish species at different sites (Fig. 9) explained 71.8% of the group separation ($P < 0.001$). The first root LDA1 explained 46.3% of the group separation and separated Billings and Ju reservoir sites from all other sites (Fig. 9A). The FAs mainly responsible for this separation were 22:5n6, 18:2n6 and 20:1n9 relative percentage (Fig. 9B). The second root LDA2 explained 25.5% of the group separation

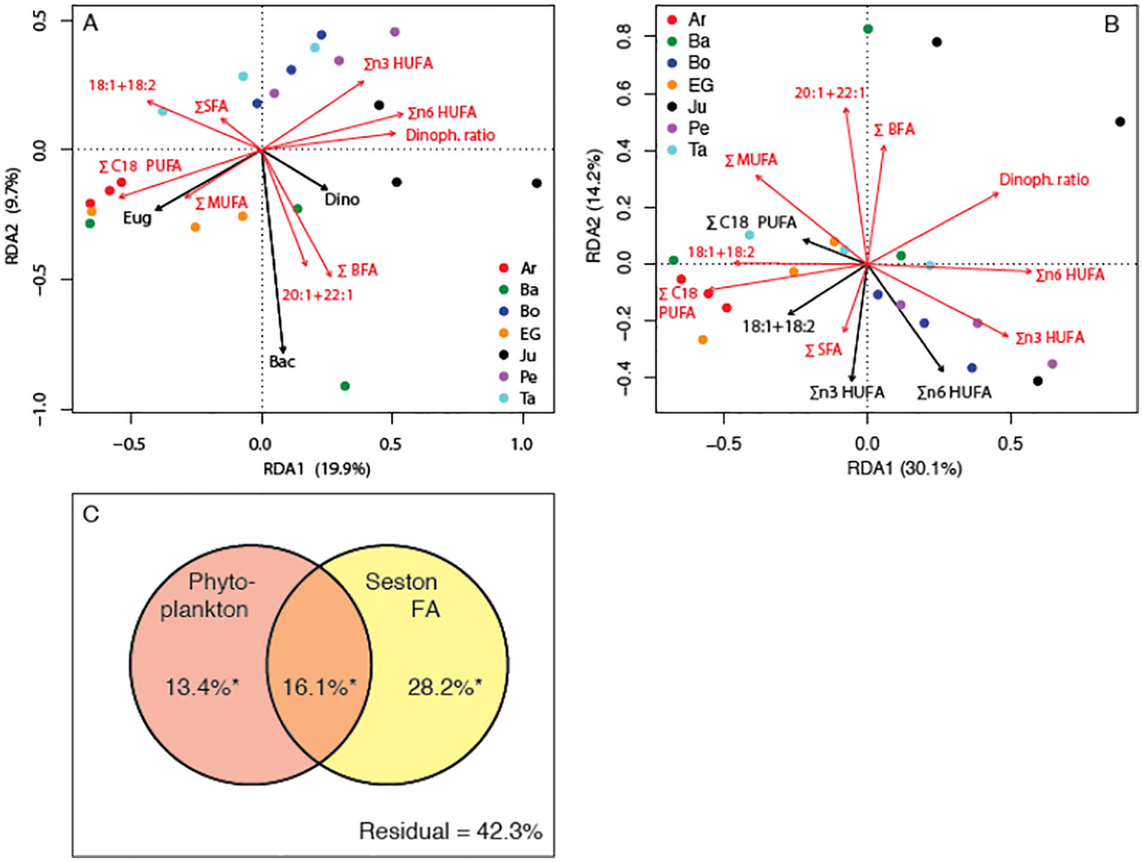

Fig. 7. Symmetrically scaled redundancy analysis (RDA; $P < 0.01$) tri-plots of tissue FA metrics (red arrows) of *A. altiparanae* and explanatory phytoplankton (A) variables and seston FA metrics (B) (black arrows; all with $P < 0.05$), showing samplings at the seven sampled sites (circles; Ar – Aracati; Ba – Barragem; Bo – Bororé; EG – Embu-Guaçu; Ju – Jundiaí; Pe – Pedreira; Ta – Taquacetuba). Phytoplankton phyla are Bacillariophyta (Bac), Dinophyta (Din), and Euglenophyta (Eug). FA are bacterial *fatty acids (BFA), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids with 18 carbons (C18 PUFA - 18:2n6 + 18:3n6 + 18:3n3), omega 3 highly unsaturated fatty acids (n3 HUFA), omega 6 highly unsaturated fatty acids (n6 HUFA), Dinophyta ratio - 16:0 + 18:4n3 + 20:5n3 + 22:3n6/18:3n3 + C16 PUFA, 18:1n9 + 18:2n6, 20:1 + 22:1.* A partial redundancy analysis (pRDA; C) is showing the variability explained (as adjusted R^2 values, with * $P < 0.05$) by phytoplankton phyla and seston FA metrics, as well as their shared contribution.

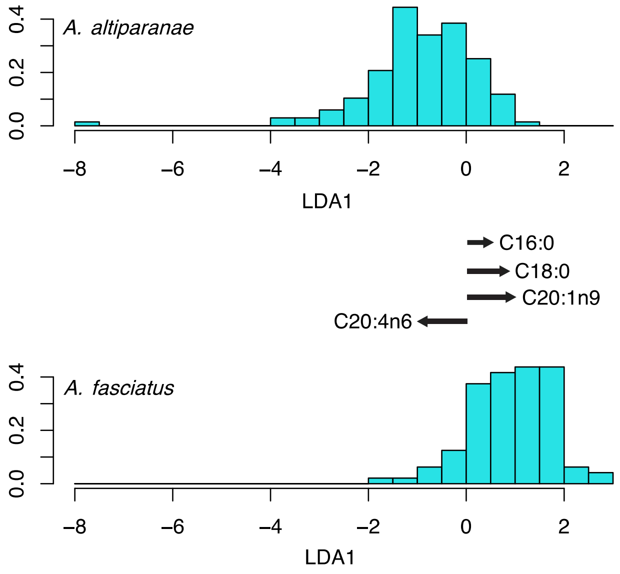

Fig. 8. Linear discriminant analysis (LDA; $P < 0.0001$) of tissue FA profiles of the two studied fish species, showing site score histograms of each species and standardized coefficients of the four significant ($P < 0.05$) linear discriminants C16:0, C18:0, C20:1n9, and C20:4n6, explaining 81.0% of the group separation between the two species.

among species and sites and mainly separated the site PN from all other sites (Fig. 9A). The FAs mainly responsible for this separation were 20:4n6, 18:0, 20:5n3 and 22:5n3 relative percentage (Fig. 9B). However, after removing the sites in which just one of the investigated species was present (PN and Ar) from the analysis, the LDA did not identify differences in muscle tissue FA profiles between the two studied fish species at different sites ($P = 0.34$).

4. Discussion

4.1. Trophic status, phytoplankton community and sestonic FA composition

Our first hypothesis, i.e., that sestonic FA profiles reflect phytoplankton composition in response to eutrophication, was only partially confirmed. As hypothesized, the most eutrophic sites of the Billings reservoir (Ta, Bo and Pe) were dominated by Cyanobacteria and showed the highest percentages of SFA and lowest percentages of PUFA among all studied sites. However, a similar pattern could not be found for the two hypereutrophic sites from the Guarapiranga reservoir (Ba and Ar). At both sites, the high phytoplankton diversity caused by the absence of Cyanobacteria dominance contributed to high percentages of HUFAs in the sestonic profile, similar to those observed at less eutrophic sites. Other system-specific factors mentioned below may modulate community responses to eutrophication and result in more diverse systems, with consequences for the FA composition of seston.

Significant correlations were detected between sestonic FAs, especially SFA, and Chlorophyta percentages in high TP sites from the Billings reservoir (Ta, Bo and Pe). SFA are the most common FAs found in all organisms. Accordingly, high SFA percentages in seston samples from sites with high algal biomasses is expected. Although Chlorophyta showed a positive correlation with SFAs in the seston at the Billings sites, both its percentage and biovolume were low. However, the positive relationship detected may result from an indirect effect of Cyanobacteria dominance in these environments since their biomass was strongly negatively correlated to Chlorophyta biomass. Cyanobacteria generally show smaller contributions of C18 PUFAs, such as 18:2n6 and 18:3n3, than other phytoplankton groups and lack HUFAs (Müller-Navarra et al., 2004). However, concentrations of SFAs, mainly C16:0, are normally high in Cyanobacterial cells (Vargas et al., 1998; Brett et al., 2006; Burns et al., 2011; Sharathchandra and Rajashekhar, 2011; Strandberg et al., 2015).

The high dominance of Cyanobacteria in the Billings sampling sites may have also been responsible for other unexpected significant correlations detected in our study. For example, the positive correlation detected between the Dinophyta ratio and sampling sites of the Billings reservoir was unexpected since no dinoflagellates were found at these sites during the studied period, except for the Pedreira branch in January 2018. Dinoflagellates are characterized by high amounts of 18:4n3, EPA, DHA, and 16:0 (Léveillé et al., 1997). Therefore, a ratio based on $16{:}0 + 18{:}4n3 + EPA + DHA/18{:}3n3 + \sum$ PUFA C16 has been proposed as an indicator of the presence of dinoflagellates (Léveillé et al., 1997). As the ratio considers the percentage of 16:0 in the calculation, the high values of this ratio were probably due to the high contributions of 16:0 detected at the Billings stations, probably due to the dominance

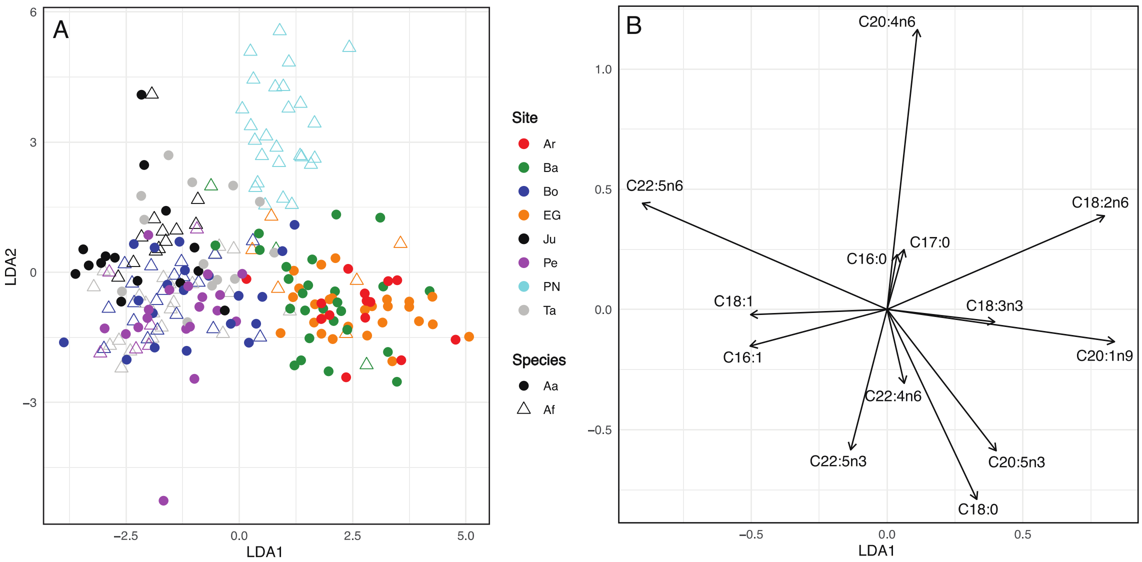

Fig. 9. Linear discriminant analysis (LDA; $P < 0.0001$) of the two studied fish species at eight different study sites. (A) site score plot and (B) standardized coefficients of the 13 significant ($P < 0.05$) linear discriminants. The first root LDA1 explained 46.3%, and the second root LDA2 explained 25.5% of the group separation among species and sites.

of Cyanobacteria. Although not observed during our study, there are occasional occurrences of Dinoflagellates blooms in this reservoir, mainly *Ceratium furcoides* (Matsumura-Tundisi et al., 2010; Cortez, 2013) which is a genus usually found in nutrient-rich waters (Lund, 1965). Their occasional presence would represent an additional source of n3 HUFAs to consumers (Taipale et al., 2013).

Unlike Cyanobacteria, Cryptophyta are characterized by high amounts of n3 HUFAs, mainly EPA (Brett et al., 2006; Burns et al., 2011; Taipale et al., 2013). In our study, Cryptophyta, besides Dinophyta, exhibited a positive correlation with the Dinophyta ratio at the highest TP stations. Although Cryptophyta were frequently found in the Billings reservoir, their biovolume was considerably lower than that of Cyanobacteria. However, many Cryptophyta species are known for their mixotrophic abilities, using heterotrophic carbon pathways to subsidize a deficient autotrophic growth under eutrophic, light-limiting conditions (Boëchat et al., 2013) and which can result in smaller contributions of PUFAs in algal cells than purely autotrophic growth (Boëchat et al., 2007). Thus, the observed positive relationships detected with SFA in the most eutrophic systems probably reflect the dominance of Cyanobacteria but may also suggest differential trophic strategies by other sub-dominant algal groups, such as mixotrophic Cryptophyta.

On the other hand, both the mesotrophic (Ju and EG) and the other eutrophic (Ba and Ar) sampling sites presented positive correlations found between sestonic MUFAs and C18 PUFAs and Chlorophyta, in addition to high percentages of HUFA, revealing the contribution of more diverse phytoplankton communities to the sestonic FA composition. These sites presented a variety of phytoplanktonic groups in their composition, from chlorophytes and charophytes, rich in 18:1n9, 18:2n6 and 18:3n3, to cryptophytes, diatoms and dinoflagellates, usually rich in n3 HUFAs, such as EPA and DHA, besides euglenophytes, characterized by a high percentage of n6 HUFAs (Léveillé et al., 1997; Brett et al., 2006; Lang et al., 2011; Taipale et al., 2013). The PN reservoir showed high overall phytoplankton biomass, despite its oligotrophic state and the dominance of Cyanobacteria. Other less representative groups include dinoflagellates (3–38%) and diatoms (1–13%), which may have contributed to the high percentage of HUFAs in the seston. Thus, we conclude that mesotrophic and eutrophic conditions can provide very diverse dietary sources of FAs, mainly PUFAs, to consumers in the studied tropical systems.

It is important to mention that the high phytoplankton variability observed at the most eutrophic sites of the Guarapiranga reservoir was probably induced by the use of copper sulphate, in addition to hydrogen peroxide, as an algaecide (Pompêo et al., 2013; Fontana et al., 2014; Beghelli et al., 2015; Silva et al., 2017; Cardoso-Silva et al., 2018; Leal et al., 2018; Rodrigues et al., 2019), corroborated even by a negative correlation between the copper concentration in the water and the presence of Cyanobacteria. After treatment, the species that remain in the water column are favoured by an abrupt increase in nutrient availability, in addition to decreased competition, caused by Cyanobacteria reduction (Beyruth, 2000). This could increase both PUFAs availability and diversity at the base of the food web in these sites. Copper sulphate affects the dominance sequence (Beyruth, 2000) and changes in the density of phytoplankton composition in Brazilian reservoirs (Padovesi-Fonseca and Philomeno, 2004). Therefore, this algaecide can partially mask the actual composition of the biomass (Nishimura et al., 2014).

The composition of freshwater seston is highly variable. In addition to phytoplankton cells, detritus, heterotrophic organisms, and microinvertebrates often contribute to the sestonic FA composition (Bec et al., 2010; Gladyshev et al., 2011; Taipale et al., 2013). The proportion of BFA (odd and branched FAs) in seston was relatively high in all systems (5.1–9.3%), suggesting that heterotrophic bacteria had an important contribution to sestonic FAs. The main source of lipids in raw domestic sludges is kitchen waste (mainly derived from vegetable oils) and human excreta, rich in 18:2n6 and 18:1n9, respectively (Jardé et al., 2005). In some cases, 22-24C SFA, 18:1n9 and 18:2n6 have also been found to be the main components of domestic sewage, in addition to BFA (Jardé et al., 2005; Boëchat et al., 2014; Dutto et al., 2014).

Moreover, the n3/n6 ratio has been suggested as a biomarker for the contribution of allochthonous versus autochthonous material in aquatic systems, corroborating urban influences, such as riparian clearcutting, in the most urbanized reservoirs (Boëchat et al., 2014). A high n3/n6 ratio (>1.0) is normally associated with a greater contribution of sestonic algae, whereas a low ratio (<1.0) is often reported as an indicator of allochthonous material from the surrounding terrestrial environment (Torres-Ruiz et al., 2007). In our study, all analyzed seston samples showed a low n3/n6 ratio, without any statistical differences among stations, indicating considerable contributions from allochthonous sources at all sites. The studied systems were surrounded by natural vegetation to some degree, which provided terrestrial plant material to the reservoirs, except for the Ba and Ar stations in the Guarapiranga reservoir. These sites were surrounded by very little natural area and receive massive sewage discharges, which may have been rich in n6 PUFAs.

In general, the results presented for the sestonic FA profiles are in accordance with the idea that the production of PUFAs by algae may be taxon-specific, with the composition and availability of FAs in the food chains determined mainly by the structure of the community of primary producers, even when spatial and seasonal variations are considered (Lau et al., 2012; Taipale et al., 2013; Strandberg et al., 2015). Although seasonal variations have not been observed, it is well known that the FA profile and lipid content in algae vary with temperature (Hu and Gao, 2006; Mangelsdorf et al., 2009; Teoh et al., 2013). For example, the production of HUFAs, such as EPA, increases with decreasing temperature (Jiang and Gao, 2004; Teoh et al., 2004; Hu and Gao, 2006). Lower temperatures also favour C18 PUFA and SFA production (Mühling et al., 2005; Hu et al., 2008). This trade-off occurs in response to the homeoviscous adaptation of membranes (Hochachka and Somero, 1984) and is very common in tropical and temperate environments (Teoh et al., 2013).

4.2. System trophic state and muscle FA composition of fish

Corroborating our second hypothesis, sestonic FA profiles did not show clear effects on the FA profiles of the muscle tissue of both studied fish species since high percentages of n3 and n6 HUFAs, mainly DHA and 22:5n6, were found in the fish samples from the hypereutrophic Billings reservoir, dominated by Cyanobacteria and with high sestonic SFA percentage. However, the same did not occur to the fish from the most eutrophic sites of the Guarapiranga reservoir, characterized by a high relative contribution of HUFAs in sestonic profiles. These results suggested that consumers' ability to selectively feed on more balanced and non-dominant prey, as well as their ability to accumulate and modify dietary FAs, may modulate the hypothesized limitation effect of biochemical imbalances observed in the dominant phytoplankton food resources.

FA profiles of fish, especially of *A. fasciatus,* showed relationships to the observed differences in sestonic FA profiles. For instance, fish muscle samples from the oligotrophic PN reservoir, dominated by Cyanobacteria and HUFA-rich Dinoflagellates, had high percentages of 18:2n6, one of the FAs used as biomarkers for Cyanobacteria (Olsen, 1999; Parrish et al., 2000; Hixson et al., 2015), indicating a possible direct transfer of these FAs to *A. fasciatus* with little or no modification. However, C18:2n6 is also considered a biomarker of terrestrial plants (Parrish et al., 2000). In previous studies, the stomach content of *A. fasciatus* collected from the PN reservoir showed a high contribution of vegetal material, evidencing high trophic opportunism of the species in response to food availability in the environment, as also demonstrated in other studies (Bennemann et al., 2005; Alonso et al., 2019).

Although the phytoplankton communities from the Billings reservoir were dominated by Cyanobacteria, the presence of n3 and n6 HUFAs in the fish samples may suggest a possible synthesis through assimilation, accumulation and transformation of dietary 18:2n6 and 18:3n3, both present in the sestonic samples analyzed. Synthesis of HUFAs from dietary precursors has been described for many aquatic organisms, including zooplankton species, aquatic insects and fish (Kainz et al., 2004; Boëchat and Adrian, 2005; Sushchik et al., 2006; Brett et al., 2006; Ravet et al., 2010; Koussoroplis et al., 2011; Gladyshev et al., 2011). Aquatic insects are known for the high contents of EPA in their tissues (Sushchik et al., 2013; Borisova et al., 2016; Popova et al., 2017; Gladyshev and Sushchik, 2019) and were an abundant food item in the stomach content of *A. fasciatus* from Ta branch (Gomes et al., 2016), corroborating its omnivorous feeding habits with a tendency to insectivory (Vilella et al., 2002; Bennemann et al., 2005; Foresti et al., 2005; Alonso et al., 2019). Moreover, despite their lower abundances in the seston, the presence of other microalgae taxa rich in n3 HUFAs, such as dinoflagellates, diatoms and cryptophytes, may have contributed to the high percentage of DHA in *A. fasciatus* muscle from the Billings reservoir via trophic transfer by the lower-level consumers. Thus, ARA, 22:5n6, EPA and DHA accumulation towards higher trophic levels may indicate that consumers can concentrate these FAs, either through selective feeding ((Koussoroplis et al., 2011; Hixson et al., 2015; Strandberg et al., 2015) or biochemical conversion performed by lower-level consumers and by the fish. Even despite low amounts of n3 HUFAs in producers, invertebrates and fish from higher trophic levels may be rich in EPA and DHA, suggesting that retention of HUFAs may be a pattern of consumers in poorer nutritional environments (Rochetta et al. 2014; Guo et al., 2017; Whorley et al., 2019).

On the other hand, the fish samples from mesotrophic (Ju and EG) and other eutrophic sites (Guarapiranga reservoir: Ba and Ar) were characterized by high phytoplankton variability and, consequently, more diverse availability of FAs to consumers. It has been suggested that the efficiency of PUFA retention may decrease exponentially with the intake of a PUFA-rich diet by zooplankton organisms (Koussoroplis et al., 2013). If this is also the case for fish consumers, it could explain why fish samples from mesotrophic reservoirs had low relative HUFA percentages, despite the high HUFA availability in the environment. Although algal biovolume was low in the Ju and Guarapiranga reservoirs, the availability of HUFAs may have been higher than that found for phytoplankton samples from the Billings stations, for instance. Thus, consumers did not have to selectively ingest and retain as much PUFAs. This is supported by the positive correlation found between sestonic and fish C18 PUFAs, suggesting that bioconversion of dietary PUFAs was unlikely. Therefore, the retention and conversion of FA performed by the animals may also depend on each animals' needs, so that they are not a direct reflection of sestonic FA profiles.

Moreover, fish can also use HUFAs in catabolic processes, without the need to accumulate or bioconvert precursors, once the availability of HUFAs is assured in the diet (Brett et al., 2006; Gladyshev et al., 2011). Marques et al. (2020) analyzed FA profiles of *A. altiparanae* liver PL from Guarapiranga (EG, Ba and Ar) and Ju reservoirs and did not observe a direct influence of eutrophication since the fish from Ju and Ba (meso- and eutrophic environments, respectively) exhibited higher percentages of DHA than fish from EG and Ar stations, meso- and supereutrophic, respectively. The authors concluded that eutrophication did not influence the FA profile of hepatic PL of *A. altiparanae*, which may be more related to the specific physiological requirements of HUFAs in different tissues and different populations.

Despite the vital contribution of n3 HUFAs for animal physiology, it is well known that the susceptibility of these molecules to peroxidation increases exponentially with the number of double bonds present in the carbon chain (Holman, 1954). Bivalves in Patagonia lakes showed a higher percentage of EPA and DHA in the PL of individuals from polluted sites (Rocchetta et al., 2014), which might favour the lipoperoxidation process in response to contaminant agents present in the water. In the present study, both the Ba and Ar sites from the Guarapiranga reservoir, as well as the Billings reservoir were characterized by the presence of metals and other contaminants (Moschini-Carlos et al., 2010; Gomes et al., 2015; CETESB, 2014, 2015, 2016, 2017; Cardoso-Silva et al., 2018; Escalante-Rojas et al., 2021), which can trigger lipoperoxidation process of the cell membranes, as already seen for *A. altiparanae* from the Aracati and Pedreira stations in previous studies (Muñoz-Peñuela, 2020). Therefore, the high proportion of n3 HUFAs found in fish from polluted environments may be a beneficial response. Still, it may also be affected by the adverse and well-described effects of habitat alteration, hydrology and other pollution-related stress factors that alter the organisms' physiology.

Corroborating our third hypothesis, both species showed very similar FA profiles, especially considering n3 and n6 HUFAs in samples from the Billings and Ju reservoirs. However, *A. fasciatus* and *A. altiparanae* from the Guarapiranga reservoir had more C18 PUFAs in their muscle than fish sampled from other reservoirs. Besides that, *A. fasciatus* from the oligotrophic reservoir presented a very distinct FA profile, also compared to the same species collected from other sites, characterized by high ARA percentages, probably as a result of elongation and desaturation of 18:2n6, found in high percentages in the seston samples. Although both species belong to the same genus and have similar feeding habits, they seem to fulfil their dietary requirements by feeding on different food items, according to their availability in the environment (Vilella et al., 2002; Peretti and Andrian, 2008; Hirt et al., 2011; Gomes et al., 2016). Studies from rivers, streams and reservoirs have shown that both *A. fasciatus* and *A. altiparanae* are omnivorous, with a high tendency to insectivory. However, considerable trophic plasticity has been reported for both species, which also feed on zooplankton, algae, macrophytes and terrestrial detritus, according to their availability in the environment (Arcifa et al., 1991; Andrian et al., 2001; Vilella et al., 2002; Casatti et al., 2003; Peretti and Andrian, 2008; Hirt et al., 2011; Bonato et al., 2012). High dietary flexibility, together with a possible capacity to modify dietary FAs, besides differences in physiological requirements, may result in different muscle FA profiles among environments.

In general, our data did not show a direct relationship between the degree of eutrophication and the percentage of n3 and n6 HUFAs, such as EPA, DHA and 22:5n6, for both species studied. However, the mechanisms through which eutrophication affects the linkage between dietary FAs and consumer FAs may be more intricate, involving the differential selection of feeding behaviour and physiological pathways in different aquatic systems. According to Gladyshev and Sushchik (2019), the deficiency of n3 HUFAs in eutrophic environments must be viewed with care for two main reasons: 1) even eutrophic environments do not have only Cyanobacteria throughout the year so that in some periods, consumers will have access to other phytoplankton organisms that produce HUFAs; 2) the need for single FAs by animals must be taken into account, as well as their capacity for bioconversion from C18 PUFAs precursors. Finally, the bottom-up effect of eutrophication associated with limitations caused by diet and by the characteristics of feeding and biochemical metabolism of fish need to be considered together with top-down changes (Von Rückert and Giani, 2008; Galbraith and Burns, 2010; Ger et al., 2016). In brief, the analysis of the availability of FAs in aquatic environments is multifactorial, taking into account the hydrological and ecological characteristics of the environments under study, including their peculiarities and the interactions between different trophic levels, in addition to the metabolism and requirement of the studied consumers.

5. Conclusion

Our study demonstrated a clear differentiation between reservoir sites according to the degree of eutrophication and urbanization, with direct influences on phytoplankton biomass and composition. Likewise, sestonic FA profiles reflected the phytoplankton composition, with higher relative percentages of SFA found at Cyanobacteria dominated sites and higher percentages of MUFAs, PUFAs and HUFAs at sites with higher phytoplankton diversity. However, the muscle FA profiles of fish did not reflect the seston profiles in the most eutrophic sites. Fish from cyanobacteria dominated sites did not present a low percentage of n3 HUFAs, probably due to their ability to select, retain and modify dietary FAs. On the other hand, fish from sites with the highest phytoplanktonic diversity, regardless of the eutrophication degree, presented a low relative percentage of n3 HUFAs, probably due to the availability of these FAs in the environment. In this latter case, the animals probably did not need to retain them in the same proportion as the animals from sites with low phytoplanktonic variability. Finally, both species exhibited a very similar FA profile, probably reflecting feeding habits, modification capacity and similar FA requirements.

CRediT authorship contribution statement

Aline Dal'Olio Gomes: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization, Project administration, Funding acquisition. **Fernando Ribeiro Gomes:** Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Björn Gücker:** Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Carlos Eduardo Tolussi:** Conceptualization, Investigation, Writing – review & editing. **Cleber Cunha Figueredo:** Methodology, Resources, Writing – review & editing. **Iola Gonçalves Boëchat:** Methodology, Writing – original draft, Writing – review & editing. **Lídia Sumile Maruyama:** Resources, Writing – review & editing. **Lucas Chagas Oliveira:** Methodology, Writing – review & editing. **Marcela Muñoz-Peñuela:** Investigation, Writing – review & editing. **Marcelo Luiz Martins Pompêo:** Methodology, Resources, Writing – review & editing. **Rayssa de Lima Cardoso:** Methodology, Writing – review & editing. **Victor Hugo Marques:** Methodology, Investigation, Writing – review & editing. **Renata Guimarães Moreira:** Investigation, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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