Chapter

Changes in the Fatty Acids Profile of the Zooplankton Community Reveals the Quality of Four Reservoirs in the Hydroelectric Power Plants Located in the Iguaçu River, Paraná, Brazil

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Abstract

Fatty acids are molecules with important physiological functions, proved to be good bioindicators of the presence of natural and chemical stressors and so used as early warning signals. Indeed, biochemical analyzes, such as fatty acids, are an important tool in water body management and water quality analysis, allowing detecting molecular changes in aquatic communities, related to the trophic status of the systems, before they are perceived in the environment. In this work was investigated the fatty acid composition on zooplankton community collected in four reservoirs of hydroelectric plants on the Iguaçu River, Brazil, and assessed the species distribution to assess and compare the water quality in these reservoirs. Results showed the trophic state index presented a wide variation among samples, ranging from oligotrophic (Salto Caxias) to hypereutrophic (Foz do Areia). The most abundant fatty acid was docosahexaenoic acid (DHA, C22:6n3) an essential fatty acid with health benefits, playing a pivotal role in biological functions. This study highlights the sensitiveness of the zooplankton community to environmental conditions and underlines the role of fatty acids as good bioindicators, being good endpoints to use in ecological studies. This supports the zooplankton contribution as a biological quality element in the assessment of reservoir quality elements.

Keywords: fatty acids, hydroelectric reservoirs, Iguaçu river, reservoir dynamics, zooplanktonic diversity

1. Introduction

The development of urban centers leads large cities in many regions (for example, as in South America and Asia) to discharges of industrial and domestic wastewater treated inadequately or depleted directly to the environment. The release of sewage from residential and industrial areas and the overuse of fertilizers and pesticides in aquatic environments, cause deoxygenation, increases the levels of toxic compounds and spread infectious diseases, with the degradation of water quality and significant negative impacts on health and mortality [1]. In addition, overexploitation of groundwater can damage wetlands, cause soil subsidence, and induce saltwater intrusion into coastal aquifers. In other regions, over-exploitation of surface water disrupts flow regimes, affecting aquatic ecosystems and the quantity and quality of water supply. Thus, extensive hydrological information and monitoring ecosystem plans are crucial for the development and protection of water resources. In studies of water quality assessment, a range of parameters are determined and assessed, which can be divided into three groups: (1) analysis of biological parameters (2) determination of physical, chemical, trophic, ecological and saprobicity indices (3) ecological aspects of community's biological processes [2, 3]. Several works were carried out based on the response of specific biological species to determine water quality [3–6]. With the implementation of the European Water Framework Directive (WFD), the ecological status of surface water is classified to standardize procedures based on the evaluation of a series of biological quality elements (BQEs). Different BQEs can act as pressure-respondents with complementary roles as proxies of structural and functional ecology [7]. In lakes, phytoplankton is the "fast responder" to eutrophication, while other BQEs are more sensitive to other pressures like hydromorphological or chemical ones [8–10]. However, zooplankton has not yet been included as a BQE, despite being considered as a key component of aquatic food chains, but the reason for this omission remains unclear.

According to Jeppesen et al. [11], the value of zooplankton as an indicator of ecological conditions stems from its position in the food chain, controlled by top-down regulators (fish) and bottom-up factors (phytoplankton), thus providing information on the relative importance of both main regulatory processes, as well as the impact of zooplankton on water quality. These authors concluded that the focus primarily on ecosystem structure and less on WFD should be reconsidered, and it should be demonstrated that zooplankton is a key element in understanding the function of lake ecosystems and perhaps also in large rivers and transitional waters [11].

The use of zooplankton for the environmental characterization of aquatic environments is potentially advantageous because of its key position in the food chain, and for wide geographic distributions [12, 13]. These organisms respond rapidly to acute and chronic stress factors, showing a high sensitiveness to chemical and environmental stressors, making them favorable candidates as indicators of ecosystem quality [2, 3].

The concentration of nutrients varies among the various aquatic systems influencing the chemical composition of the aquatic organisms. Some organisms are considered good bioindicators and can be used in studies of water quality, since their occurrence is related to the degree of pollution of the sampling site [3, 5]. Thus, observing the variations in the biochemical composition of the zooplankton organisms, can be correlated it with the eutrophication status of the system, and infer about the quality of the ecosystem. Indeed, lipids are very sensitive to environmental and chemical stressors [12, 14, 15]. In the last decades, the interest in the fatty composition of aquatic organisms has increased. The knowledge in biochemical composition of the main zooplankton groups has become important to understand the metabolism, physiological functions and nutritive value due to its relevance for the energy transfer in aquatic systems and secondary production.

Still, studies on the biochemical composition of zooplankton, more precisely on fatty acids, related to the trophic condition of the environment are scarce in the literature.

In Brazil, the reservoirs of the five hydroelectric plants located on the Iguaçu River—Foz do Areia, Salto Segredo, Salto Santiago, Salto Osório and Salto Caxias - are characterized by forming a cascade system. The mainstream of Iguaçu River and some of its tributaries are currently polluted and receive high man-induced loads of nutrients, substantially originating from domestic sewage. This study aims to determine the fatty acids composition of the zooplankton community collected from four reservoirs along the Iguaçu River, and to relate them to the ecological status of the aquatic system to assess its quality.

2. Material and methods

2.1 Study area

The Iguaçu River originates from the junction of the Iraí and Atuba rivers, in the metropolitan region of Curitiba, Paraná State. Its formations, originating at altitudes above 1000 m, constitute the Iguaçu River at an elevation of 908 m, from where it travels 1060 km, in the east-west direction, receiving water from various tributaries until reaching an altitude of 78 m and flowing into the Paraná River, near the city of Foz do Iguaçu [16]. From the Paraná rivers, it has the largest hydrographic basin, covering an area of approximately 72,000 km^2, of which 79.00% belong to the state of Paraná, 19.00% to the state of Santa Catarina and 2.0% to Argentina [17]. The Iguaçu River is the main river in the State of Paraná, runs from east to west, having its source located near the municipality of Curitiba and its mouth in the city of Foz do Iguaçu.

Due to the favorable conditions of uneven terrain, several hydrographic basins (among them that of the Iguaçu River) were used for the construction of reservoirs in sequence. The series of dams built in the same hydrographic basin forms what is known as a cascade of reservoirs [18], a condition that changed the physiography in many hydrographic basins in the country.

There are five large reservoirs for power generation along the Iguaçu River, located in the southern region of Brazil, in the Paraná state, all with more than 80 km^2 of surface area: Foz do Areia, Salto Segredo, Salto Santiago, Salto Osório and Salto Caxias (**Figure 1**). Together they have a surface area of 753.98 km^2 and an installed power generation capacity of 6644 megawatts, contributing to 6.54% of the national production. In general, they are dendritic and deep reservoirs, Foz do Areia has 180 m deep [16]. These reservoirs, built in a cascade system are usually operated as single units, so from the physical, chemical and biological point of view, each can behave as a unit with unique characteristics [19].

The Foz do Areia reservoir is the first of the large reservoirs of the Iguaçu River, it was formed in 1980 by a 160 m high and 820 m long dam, flooding an area of 139 km^2 on the border between the municipalities of Pinhão and Bituruna. The reservoir has its margins protected by natural vegetation and regions with secondary forests, mainly due to the relief of the region. The banks of Foz do Areia reservoir are constituted of natural vegetation and agricultural lands [20].

The Salto Segredo reservoir is located downstream of the Foz do Areia reservoir and upstream of the Salto Santiago reservoir, in the municipalities of Reserva do Iguaçu and Mangueirinha, was formed in 1992, with a flooded area of 82.5 km^2. It is

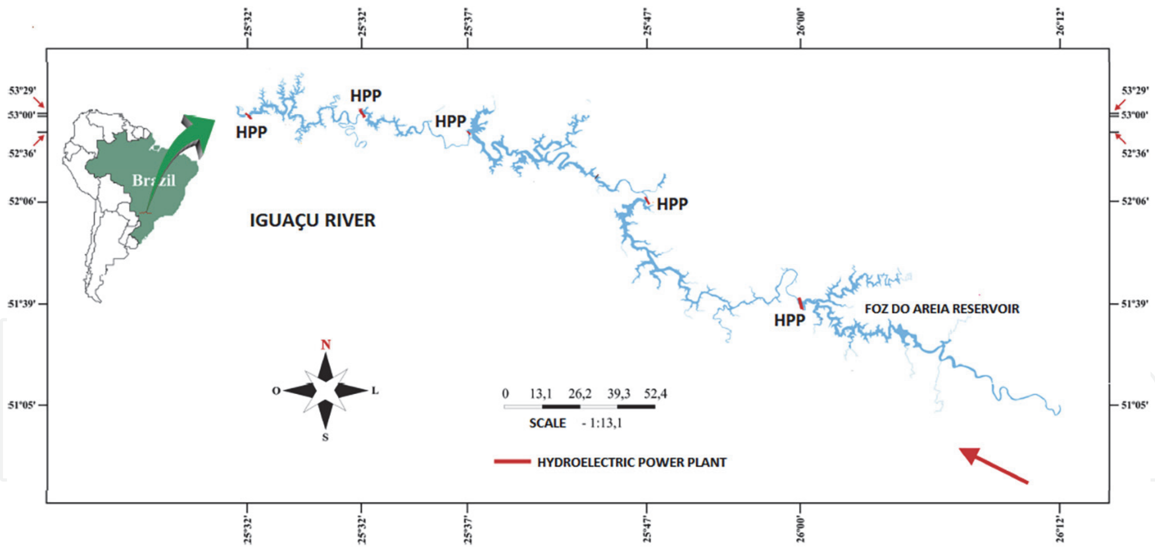

Figure 1.
Reservoirs along the Iguaçu River (modified from da Silva [19]).

a little dendritic reservoir with an average depth of 36.6 m (in some places it can reach up to 100 m) and a water residence time of 47 days. It receives numerous tributaries, both on its right bank (Forest, São Pedro, Verde and Touros) and on its left bank (Patos, Iratim, Butiá and Covó). In Salto Segredo reservoir there is an Environmental Protection Area (APA). The area that is not covered by the protection area is of agricultural use [20].

The closure of the Salto Santiago dam took place in 1979, flooding an area of 208 km^2, on the border between the municipalities of Rio Bonito do Iguaçu and Saudade do Iguaçu. The main dam is 80 m high and 1400 m long. This reservoir covers an area of 208 km^2 and covers part of the territory of eight municipalities, five on the right bank—Rio Bonito do Iguaçu, Porto Barreiro, Virmond, Candói and Foz do Jordão (central west region)—and three on the left bank—Saudade do Iguaçu, Chopinzinho and Mangueirinha (southwest region).

The Salto Santiago Lake differs from the others for its size, the great distance between its banks, the large meanders, the large number of inlets on the edges and, above all, the predominance of immense vertical walls on its banks [21].

Salto Caxias is the last of the large reservoirs on the Iguaçu River, it was closed in 1998, flooding an area of 124 km^2 on the border of the municipalities of Capitão Leônidas Marques and Nova Prata do Iguaçu. The relief of the area surrounding the reservoir is less accentuated than that of the Segredo region, with more intense agricultural occupation, being dominated by pastures with small areas of secondary forests [20]. In Salto Santiago and Salto Caxias reservoirs the banks are constituted mainly of agricultural lands. All these reservoirs are artificial, built by man, to generate energy.

It was not possible to carry out sampling in the Salto Osório reservoir due to logistical issues, with this reservoir not included in the study. According to the Paraná Reservoirs Water Quality Report (2005–2008), the water quality of the Foz do Areia reservoir is the worst among the plants on the Iguaçu River. This reservoir was considered moderately degraded. The other reservoirs showed better qualities, with Salto Segredo considered moderately degraded, and Salto Santiago, Salto Osório and Salto Caxias classified as little degraded. From the report, it is possible to observe a gradual improvement in reservoirs further away from the metropolitan region of Curitiba. When passing through this region, the Iguaçu River receives a large amount of polluting substances of domestic, industrial and diffused origin, most of which are untreated, as this is in a region with little access to basic sanitation, as well as the presence of industries. Thus, the pollution load from the metropolitan region of Curitiba (RMC) significantly influences the water quality of the Iguaçu River [22].

2.2 Collection of biotic and abiotic data

Four campaigns were carried out in the four reservoirs: the first in July 2012, the second in November 2012, the third in February 2013 and the last in October 2013. These campaigns were named according to the seasons, the first "W" (Winter), the second "Sp" (Spring 2012), the third "S" (Summer) and the latter "Sp2" (Spring 2013). For logistical reasons, it was not possible to perform sampling in autumn.

To study the spatial distribution and variation of the zooplankton community in the reservoirs of hydroelectric plants, nine collecting stations were chosen, three in the lake region, three in the intermediate region of the lake and three in the river region. In each region, sampling was carried out in three points: one in the center of the lake and at two points in the lake shores.

The collections were carried out during the period between 8:00 AM and 2:00 PM in all campaigns carried out, during high tide, maintaining the same conditions in all sampling collections. At each sampling site, two samples were collected, one of which was placed in a specific flask and preserved in 4% formaldehyde saturated with sucrose [23], to prevent distortion of the shells and loss of eggs in Cladocera. These samples were counted and analyzed using an optical microscope with a Sedgwick-Rafter counting chamber. The other sample was preserved on ice and transported to the laboratory, whereas much water as possible was removed, frozen and subsequently lyophilized.

For the qualitative and quantitative analysis of the zooplankton community were performed vertical hauls on the water surface to a 0.3 m depth, with zooplankton nets with 45 μm mesh opening and 0.3 m of mouth diameter. The tows were performed on the boat with an electric motor at a two-nodes speed, for 5 min. The total filtered water is calculated using the cylinder volume formula, and the final volume of each sampling was about 13 m^3.

In all collections, the following environmental variables were measured: water temperature (°C), dissolved oxygen concentration (mg L^{-1}) and pH, using a multiparameter probe. Water samples (1000 mL) were also collected at all collection points for further quantification of dissolved organic carbon (DOC), phosphorus forms (total phosphorus (P-total) and phosphate ($P\text{-}PO_4^{3-}$)), the forms of nitrogen (nitrite (NO_2^-), ammonia nitrogen ($N\text{-}NH_3$), nitrate (NO_3^-), organic nitrogen (N-org), total nitrogen (N-total)) were determined by the methods described in APHA [24].

2.3 Calculation of trophic state index (TSI)

To calculate the trophic state index of the reservoir, the trophic state index for chlorophyll (TSI_{CL}) and the trophic state index for phosphorus (TSI_P) were initially determined.

The trophic state indices (TSI_{CL} and TSI_P) were calculated according to Lamparelli [25]. In reservoirs, the calculation of the TSI from phosphorus values is performed by the equation (1),

$$\mathrm{TSI} = 10.\left(6 - \left(\frac{1,77 - 0,42.(\ln \mathrm{TP})}{\ln 2}\right)\right) \quad (1)$$

the concentration of total phosphorus (TP) is expressed in μg L^{-1}.

The calculation of the TSI from the chlorophyll values is performed by the equation (2),

$$TSI = 10.\left(6 - \left(\frac{0,92 - 0,34.(\ln Cl)}{\ln 2}\right)\right) \quad (2)$$

the concentration of chlorophyll (Cl) is expressed in μg L^{-1}. The chlorophyll concentration was quantified by the spectrophotometric method described in APHA [24].

The TSI is the result of the arithmetic mean between the TSI_{CL} and the TSI_P.

2.4 Determination of fatty acid profile

Zooplankton organisms collected in the field were previously frozen, lyophilized, placed in Eppendorf's and later maintained with silica gel in the freezer, to avoid lipid photooxidation [26] and subsequently frozen at −80°C. For each sampling site, three replicates were prepared and weighed.

The extraction of total lipids from the zooplankton community and the methylation of fatty acid methyl esters (FAMEs) for fatty acid analysis was performed as described by Gonçalves et al. [12]. Samples were incubated with methanol for the extraction of lipids. The fatty acid Methylnonadecanoate (C19:0) was added as an internal standard for quantification. The samples were centrifuged and dried under a vacuum. The FAMEs obtained were analyzed by a Trace 1300 ThermoScientific GC. The GC used a FAME biodiesel column (60 m × 0.250 mm × 0.20 μm). The column temperature was programmed to increase from 140 to 240°C, the analysis time was 45 min per sample, injecting 1.0 μl of the sample, and the carrier gas was Helium (20 cm/s, 175°C).

The FAMEs were identified by comparison with the retention times. The quantification of the individual FAMEs was performed by external standards, and the function of the quantification of each FAME was obtained by linear regression applied to the areas of the chromatographic peaks and corresponded with the known concentrations of the standards [12].

In fatty acid data analyses, an average of the sampling points to each region was calculated, because in some samples of some collection points it was not possible to extract the fatty acids, preventing the comparison with the results of other samplings. Region 1 (R1) corresponded to the region farthest from the dam, region 2 (R2), corresponded to the central region of the lake, and region 3 (R3) corresponded to the region near the dam of the reservoir.

2.5 Statistical analysis

Multivariate statistical analysis was carried out to examine the variation in fatty acid profiles, spatial and temporally, through multidimensional scaling (n-MDS) plots. Cluster analysis using the Bray-Curtis dissimilarity factor, using the group average was performed to assess the degree of similarity between the fatty acid samples. For these multivariate analyzes, the program PRIMER-E 6 was used. Data was not normalized, as the values are very similar, the Kruskal adjustment scheme was used, adopting 25 restarts and minimum stress of 0.01.

To assess the temporal and spatial changes of the physical, chemical and biological variables, data were processed from a matrix using Principal Component Analysis (PCA), which was based on eigenvalues greater than 1.0, which explained 70% of the total variability. To obtain greater reliability in the data analysis, greater importance was given to the correlations between factors and variables greater than or equal to 0.7.

3. Results

3.1 Abundance of zooplankton community

In the Foz do Areia reservoir, 35 species of rotifers, 11 species of cladocerans and 3 species of copepods were found throughout the study period (**Table 1**). Although, *Polyarthra dolichoptera* was the most abundant rotifer during the sampling in July 2012 (W) and spring 2013 (Sp2), the abundance averages were quite different, from 5978.58 to 537.48 ind m^{-3}, respectively. In addition, species' abundance was also differentiated, with 20 species in July 2012 (W) and 10 species in October 2013 (Sp2) collections. In spring 2012 (Sp—November 2012) and summer 2013 (S—February 2013) different abundances of rotifer species were registered, with 16 and 23 species respectively, with *Synchaeta jollyae* being the most abundant species in spring 2012 (average density of 1427.50 ind m^{-3}), and *Keratella cochlearis* the most abundant rotifer species in spring 2012 (average density: 1628.33 ind m^{-3}) (**Table 2**). The presence of several zooplankton species in the Foz do Areia reservoir may be related to the higher effluent load, as it is the first dam after the metropolitan region.

The abundance of cladocerans species in winter (W—July 2012) and in summer (S—February 2013) was the same, with *Ceriodaphnia cornuta* being the most abundant cladoceran (256.83 ind m^{-3} in winter and 106, 33 ind m^{-3} in the summer). In spring of 2012 (Sp—November 2012) and 2013 (Sp—October 2013) there was an equal abundance of species, with *Moina minuta* being the most abundant in spring 2012 (average density: 464.17 ind m^{-3}), and *Bosmina longirostris* the most abundant cladoceran species in spring 2013 (average density: 171.40 ind m^{-3}). Among copepods, naupliar stages were the most abundant (**Table 2**).

In the Salto Segredo reservoir 28 species of rotifers, 10 species of cladocerans and two species of copepods were found throughout the study period. The most abundant rotifer was *P. dolichoptera* in three of the four samples, November 2012 (Sp), February 2013 (S) and October 2013 (Sp2) with density values of 19.22, 1011.67 and 748.10 ind m^{-3}, respectively. Although, *P. dolichoptera* was the most abundant organism during these periods, the species abundance was different, with 9 (Sp), 13 (S) and 11 (Sp2) species (**Table 2**).

In July 2012 sampling (W) 17 species of rotifers were found, in which the most abundant was *K. cochlearis* with an average of 640.00 ind m^{-3}. Regarding cladocerans, *B. longirostris* was the most abundant species (156 ind m^{-3}) in July 2012 (W), *C. cornuta* was the most abundant cladoceran (120.22 ind.m^{-3}) in November 2012 (Sp), *Diaphanosoma spinulosum* was the most abundant cladoceran (210,44 ind m^{-3}) in February 2013 (S) and *Bosminopsis deitersi* the most abundant species in the October 2013 sampling (Sp2). In spring of 2012 (Sp—November 2012) nine cladoceran species were found, with *B. longirostris* being the most abundant, with an average of 156.00 ind m^{-3}, and in the summer of 2013 (S—February of 2013) six species of cladocerans were found, and the most abundant was *D. spinulosum* with a mean density of 210.44 ind m^{-3} (**Table 2**).

Regarding copepods, in all samples only two species of copepods were found, with the exception of the October 2013 sampling, where only one species was found. In all samples, the naupliar stages were the most abundant organisms.

In the Salto Santiago reservoir, in July 2012 sampling (W) 19 species of rotifers were identified, in which the most abundant was *Kellicottia longispina* with an average of 120.00 ind m^{-3}; in November 2012 sampling (Sp), were identified 13 species of

	Rotifera		Reservoirs			
Order		**Code**	**FA**	**SG**	**ST**	**CX**
Bdelloida (*)	*Bdelloida* (*)	Bdel	X	X	X	
Family	**Species/Genus**					
Asplanchnidae	*Asplanchna* sp.	Aspl	X	X	X	X
Brachionidae	*Anuraeopsis fissa*	Ap fiss	X			
	Brachionus caudatus	Brach cd	X			
	Brachionus dolabratus	Brach dl	X	X	X	X
	Brachionus falcatus	Brach fc	X		X	X
	Brachionus sp.	Brach	X			
	Brachionus urceolaris	Brach urc	X			
	Kellicottia longispina	K long	X	X	X	X
	Keratella americana	K amer	X	X		
	Keratella cochlearis	K coch	X	X	X	X
	Keratella sp.	K sp	X			
	Keratella tropica	K trop	X	X		X
	Keratella valga	K val	X			
	Notholca sp.	Noth	X	X		
	Platyas quadricornis	P quadr		X	X	
Collothecidae	*Collotheca ornata*	Cornt	X	X	X	X
	Collotheca sp.	Collot.	X	X	X	X
	Colurella sp.	Colur		X		
Conochilidae	*Conochiloides* sp.	Cchldes	X	X		
	Conochilus sp.	Cchilus	X	X	X	
	Conochilus coenobasis	Cchilus cb	X		X	X
Epiphanidae	*Epiphanes macrourus*	E macr	X		X	
Euchlanidae	*Euchlanis dilatata*	E dilat	X	X	X	X
Filinidae	*Filinia longiseta*	F long	X		X	
	Filinia opoliensis	F opo	X			
	Filinia terminalis	F term	X	X		
Flosculariidae	*Ptygura libera*	Pty lib	X		X	X
Gastropodidae	*Ascomorpha ovalis*	A ov	X	X	X	X
	Ascomorpha saltans	A salt			X	
Hexarthridae	*Hexarthra mira*	Hex m	X	X	X	X
Lecanidae	*Lecane bulla*	L bul			X	
	Lecane luna	L luna		X		
	Lecane sp.	L sp.	X	X	X	X
Philodinidae	*Philodina* sp.	Phil		X		
Synchaetidae	*Polyarthra dolichoptera*	P doli	X	X	X	X
	Synchaeta jollyae	Synch jo	X	X	X	X
	Synchaeta sp.	Synch sp.	X	X	X	X

	Rotifera		Reservoirs			
Testudinellidae	*Pompholyx sulcata*	Pom sul		X		X
Trichocercidae	*Trichocerca bicristata*	T bicr		X	X	
	Trichocerca bidens	T bid		X	X	X
	Trichocerca cylindrica	T cylin		X	X	X
	Trichocerca rattus	T rat			X	
	Cladocera					
Family	Species/Genus	Code				
Bosminidae	*Bosmina hagmanii*	Bn hag	X	X	X	X
	Bosmina longirostris	Bn long	X	X	X	X
	Bosminopsis deitersi	Bs deit	X	X	X	X
Chydoridae	*Alona* sp.	Al sp.	X		X	X
	Chydorus sp.	Chyd			X	
	Pseudochydorus globosus	Pschy glob		X	X	
Daphniidae	*Ceriodaphnia cornuta*	Cd corn	X	X	X	X
	Ceriodaphnia silvestrii	Cd silv	X	X	X	X
	Daphnia gessneri	Dp gess	X	X	X	X
	Daphnia laevis	Dp lvis	X	X	X	X
	Daphnia parvula	Dp par	X		X	
Moinidae	*Moina minuta*	Mn min	X	X	X	X
Sididae	*Diaphanosoma spinulosum*	Dph spin	X	X	X	X
	Copepoda					
Order	Species	Code				
Calanoida	Calanoida sp.	Calan sp	X			
	Notodiaptomus spinuliferus ♀	Nt spinF	X	X	X	X
	Notodiaptomus spinuliferus ♂	Nt spinM	X	X	X	X
	Calanoida copepodite	Cp Calan	X	X	X	X
Cyclopoida	Cyclopoida copepodite	Cp Cyclo	X	X	X	X
	Tropocyclops prasinus ♀	Tp prasF	X	X	X	X
	Tropocyclops prasinus ♂	Tp prasM	X	X	X	X
	Nauplius	Naup	X	X	X	X

Table 1.
List of zooplankton taxa found in the Iguaçu River reservoirs during the study period. (*) order. FA = Foz do Areia reservoir, SG = Salto Segredo reservoir, ST = Salto Santiago reservoir, CX = Salto Caxias reservoir.

rotifers, and the most abundant species was *Collotheca* sp. With an average density of 647.78 ind m^{-3}. In samples collected in February 2013 (S), 12 species of rotifers were identified. The most abundant among the rotifers species was *Colurella* sp. With an average of 1164.67 ind m^{-3}. In samples collected in Salto Santiago in October 2013 (Sp2), six species of rotifers were identified, and the most abundant was *Asplanchna* sp. With an average density of 4536.74 ind m^{-3} (**Table 2**).

During July 2012 sampling (W) nine cladoceran species were identified. The most abundant species was *Ceriodaphnia silvestrii* with an average of 140.00 ind m^{-3}, while in the November 2012 sampling (Sp) eight cladoceran species were

	W	Sp	S	Sp2
FOZ DO AREIA reservoir				
Polyarthra dolichoptera	**5978.58**	275.83	332.33	**537.48**
Synchaeta jollyae	0.00	**1427.50**	900.83	0.00
Keratella cochlearis	1083.50	340.00	**1628.33**	66.70
Ceriodaphnia cornuta	**256.83**	170.83	**106.33**	64.36
Diaphanosoma spinulosum	97.50	**464.17**	21.17	5.17
Bosmina longirostris	118.25	149.17	57.50	**171.40**
Nauplii	**8635.83**	**2338.33**	**3130.25**	**2779.07**
SALTO SEGREDO Reservoir				
Keratella cochlearis	**640.00**	9.33	192.33	66.70
Polyarthra dolichoptera	145.89	**19.22**	**1011.67**	**537.48**
Bosmina longirostris	**156.00**	22.00	60.67	107.32
Ceriodaphnia cornuta	36.67	**120.22**	23.89	3.77
Diaphanosoma spinulosum	21.56	3.22	**210.44**	66.53
Bosminopsis deitersi	115.56	1.11	0.00	**118.23**
Nauplii	**1582.22**	**138.11**	**2109.11**	**4356.47**
SALTO SANTIAGO Reservoir				
Kellicottia longispina	**120.00**	2.22	0.00	0.00
Polyarthra dolichoptera	112.22	**355.89**	128.78	217.90
Colurella sp.	0.00	0.00	**1164.67**	0.00
Asplanchna sp.	0.00	0.00	0.00	**4536.74**
Ceriodaphnia silvestrii	**140.00**	156.89	**78.44**	65.40
Ceriodaphnia cornuta	22.22	**804.78**	18.44	0.00
Bosmina longirostris	77.88	96.00	17.44	**171.44**
Nauplii	**254.44**	**948.67**	**703.33**	**2506.94**
SALTO CAXIAS Reservoir				
Polyarthra dolichoptera	**837.78**	406.44	84.86	116.06
Collotheca sp.	250.00	**808.00**	74.36	135.71
Synchaeta sp.	0.00	20.44	**1798.20**	0.00
Asplanchna sp.	0.00	0.00	1.24	**3018.71**
Ceriodaphnia silvestrii	**224.44**	**418.67**	18.06	**100.02**
Diaphanosoma spinulosum	48.56	74.89	**101.57**	18.34
Nauplii	**995.56**	**4473.56**	326.56	**2436.87**
Calanoid Copepodites	524.44	1646.89	**497.90**	91.02

Values in bold in the table indicate the most abundant species.

Table 2.
List of the most abundant taxa *in the Iguaçu River reservoirs.*

found and the most abundant was *C. cornuta*, with an average of 804.78 ind m^{-3}. Seven species of cladocerans were identified in the February 2013 sampling (S) and the most abundant species was *Ceriodaphnia silvestrii*, with a mean density of 78.44 ind m^{-3}. In October 2013 sampling (Sp2), five cladoceran species were identified, and the most abundant cladoceran was *B. Longirostris*, with a mean density of 171.44 ind m^{-3}. Regarding copepods, only two species were found in all samples, with naupliar stages being abundant throughout the study period (**Table 2**).

In the Salto Caxias reservoir, 20 species of rotifers, ten species of cladocerans and two species of copepods were found.

In the July 2012 sampling (W) eight species of rotifers were identified, in which *P. dolichoptera* was the most abundant species with an average density of 837.78 ind m^{-3}. Both in November 2012 (Sp) and February 2013 (S) samplings, the abundance of species was the same (14 species), being *Collotheca* sp. The most abundant in November 2012 (Sp), with an average density of 808.00 ind m^{-3}. In February 2013 (S) *Synchaeta* sp., was the most abundant with an average density of 1798.20 ind m^{-3} (**Table 2**). In October 2013 (Sp2), five species of rotifers were identified with *Asplanchna* sp. The most abundant, with an average density of 3018.71 ind m^{-3}. Except in the sampling of July 2012 (W), where eight cladoceran species were identified, the most abundant species was *Ceriodaphnia silvestrii*, with an average density of 224.44 ind m^{-3}. The other three samples had the same abundance of cladoceran species (eight species).

In November 2012 sampling (Sp), the most abundant species was *Ceriodaphnia silvestrii*, with an average density of 418.67 ind m^{-3}. In February 2013 (S), the most abundant was *D. spinulosum*, with an average density of 101.57 ind m^{-3}, and in the sampling of October 2013 (Sp2), the most abundant species was *Ceriodaphnia silvestrii*, with an average density of 100.02 ind m^{-3}. In all samplings carried out, the naupliar stages were the most abundant among the copepods, with the exception of the February 2013 sampling (S) where the calanoid copepodites were the most abundant, with an average density of 497.90 ind m^{-3} (**Table 2**).

3.2 Trophic state index (TSI)

The TSI showed a wide variation from sampling to sampling. In July 2012 (W) the environment was characterized as oligotrophic, while in the following sampling there was a sharp drop in water quality, and the environment was characterized as hypereutrophic and the following two as eutrophic. The eutrophication process that occurred in November 2012 (Sp), may have been possibly caused by the great drought that occurred at the time of the study, but just before this great drought, there was a large precipitation phase, which may have caused the entry of matter causing the concentration of nutrients that increased temporarily. When the calculated averages were observed in each sampling site, it was seen that the variation was low, with some collected areas with different trophic classifications (**Figure 2**).

3.3 Fatty acids composition of zooplankton community in the sampling sites

In Foz do Areia reservoir, 19 FA were determined during the entire study period, of which nine were saturated fatty acids (SFA), four monounsaturated fatty acids (MUFA), three polyunsaturated fatty acids (PUFA) and three highly unsaturated fatty acids (HUFA). The period in which a greater abundance of fatty acids was observed was in July 2012 (W), in which thirteen fatty acids were identified, mostly SFA, however, in the November 2012 (Sp2) sampling, only seven fatty acids were identified (**Table 3**).

The most abundant fatty acid was docosahexaenoic acid (DHA), with the exception of February 2013 sampling (S), where palmitic acid (C16: 0) was the only one that contributed to the total densities of fatty acids (100%). In the first sampling (W) there was an increase in the number of fatty acids from region 1 (R1) at the region 2 (R2), which covers the points furthest from the dam, and the

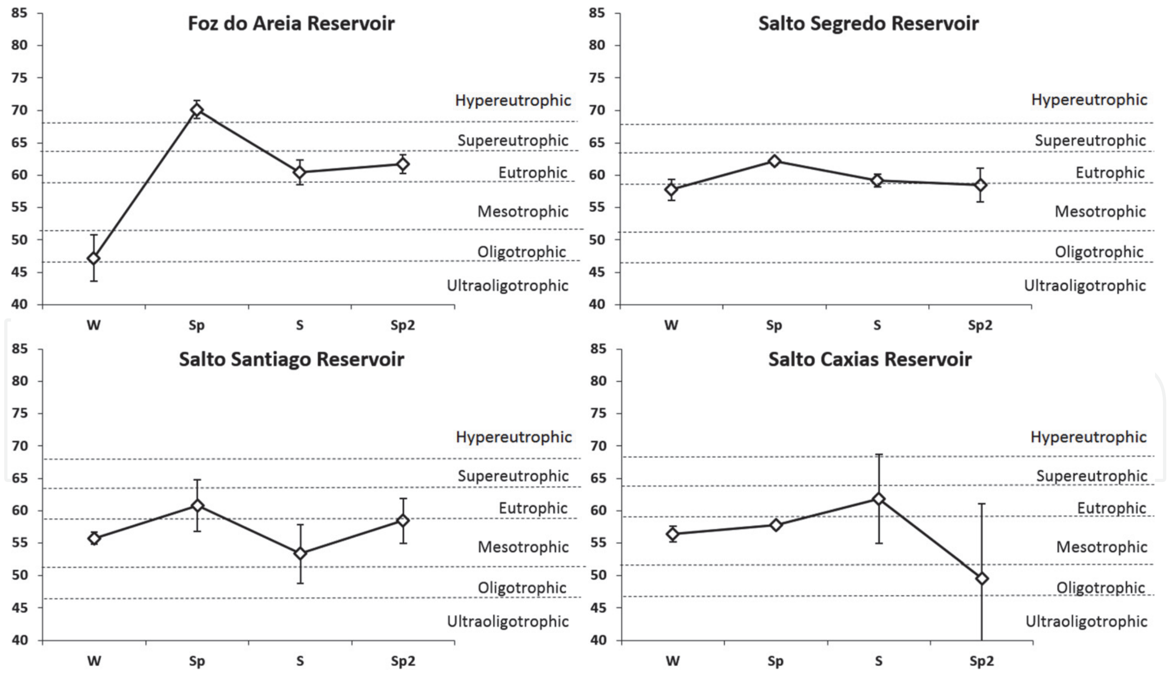

Figure 2.
Average of trophic state indexes for the Iguaçu River reservoirs. The bars indicate the standard deviation.

intermediate points of the reservoir respectively. At the other samples, region 3 (R3), which covers the points closest to the dam, was registered the highest fatty acid densities. Sampling in October 2013 registered the highest number of fatty acids. In region 2, sampling in November 2012 (Sp), DHA represented more than 91% of all identified fatty acids (**Figure 3**).

In Salto Segredo reservoir, 20 fatty acids were found, of which eight are saturated fatty acids (SFA), five monounsaturated fatty acids (MUFA), four polyunsaturated fatty acids (PUFA) and three highly unsaturated fatty acids (HUFA) (**Table 3**). In the two spring samplings (Sp and Sp2) more fatty acids were found, 15 and 16 respectively, of which eight are SFA found in November 2012 sampling (**Table 3**).

The most abundant fatty acid in the Salto Segredo reservoir was docosahexaenoic acid (DHA), representing 96% of the total concentration of fatty acids in the first sampling. The high concentration of this fatty acid appears to be due to the large presence of phytoplankton in the environment, which can be observed by the relatively high concentration of chlorophyll-*a* in the reservoir (on average 5.91 μg L^{-1}). However, in the February 2013 sampling (S) almost no fatty acids were found in the reservoir, and only palmitoleic acid (C16:1) was identified. EPA and C16:0 were also quite representatives (around 20% in both) (**Figure 3**). In a more general context, high C16:1 value indicates a high presence of diatoms [27].

In Salto Santiago reservoir, 18 fatty acids were identified, being six saturated fatty acids (SFA), six monounsaturated fatty acids (MUFA), three polyunsaturated fatty acids (PUFA) and three highly unsaturated fatty acids (HUFA) (**Table 3**). In October 2012 sampling (Sp2), 14 fatty acids were identified, being five SFA and five MUFA; in the February 2013 sampling (S), only three fatty acids were identified, two being SFA and one HUFA.

In Salto Santiago reservoir, DHA was the most abundant fatty acid in the three regions of the samplings of July 2012 (W), February 2013 (S) and October 2013 (Sp2). In the November 2012 sampling (Sp) the fatty acid with the highest density was C16:0, with more than 60% of the total densities (**Figure 3**). Looking at the results, C16:0 seems to be related to poorer water quality, which suggests the algae that is responsible for the production of this fatty acids is probably more abundant

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		FOZ DO AREIA				SALTO SEGREDO				SALTO SANTIAGO				SALTO CAXIAS			
									ReservoirS								
F. A	**C:D**	**W**	**Sp**	**S**	**Sp2**	**W**	**Sp**	**S**	**Sp2**	**W**	**Sp**	**S**	**Sp2**	**W**	**Sp**	**S**	**Sp2**
SFA	C8:0									9.24E-05							
	C12:0							8.46E-06									
	C13:0	1.69E-05															
	C14:0	1.75E-03	1.44E-03	9.79E-04	3.08E-03		9.73E-04		7.50E-03	6.09E-05	3.00E-05		5.51E-03	5.84E-03	7.63E-05	2.51E-04	1.57E-02
	C15:0	1.09E-04															
	C16:0	1.51E-02	7.76E-03	8.21E-03	2.16E-02	6.20E-04	8.63E-03		3.89E-02	4.66E-03	4.21E-03	1.99E-04	2.29E-02	4.30E-02	4.45E-03	7.77E-03	7.72E-02
	C17:0				1.94E-04		1.82E-04										
	C18:0	2.91E-03	7.19E-04	7.25E-04	2.46E-03	1.42E-04	1.62E-03		1.92E-03	3.63E-04		2.18E-06	6.66E-04	1.39E-02	5.37E-04	1.30E-03	7.20E-03
	C20:0	7.08E-05		1.75E-03	1.28E-03		6.60E-04						1.37E-04			8.37E-04	
	C21:0			1.45E-03	9.26E-03		4.76E-04		1.75E-02				8.82E-03				1.20E-02
	C22:0	8.22E-04			2.98E-03		2.54E-04		3.39E-03								
	Σ SFA	**2.08E-02**	**9.92E-03**	**1.31E-02**	**4.09E-02**	**7.62E-04**	**1.28E-02**		**6.92E-02**	**5.18E-03**	**4.24E-03**	**2.01E-04**	**3.80E-02**	**6.27E-02**	**5.06E-03**	**1.02E-02**	**1.12E-01**
MUFA	C15:1n5(cis-10)												4.17E-03				
	C16:1	2.07E-03	1.55E-03				1.45E-03	6.67E-04	2.85E-03				7.83E-03	2.70E-03			8.94E-03
	C18:1n9t				5.17E-03				6.05E-03	5.88E-06			3.94E-03	3.17E-03	5.62E-04	1.09E-03	1.30E-02
	C18:1n9c	1.01E-03		7.74E-05			4.28E-04		8.37E-03				5.52E-03	1.68E-05			1.33E-02
	C20:1n9								1.72E-03				9.39E-04				5.80E-03
	C22:1																2.68E-03
	C24:1n9		2.08E-04		3.56E-04	5.14E-04				1.26E-03				2.90E-03			3.60E-03
	Σ MUFA	**3.08E-03**	**1.76E-03**	**7.74E-05**	**5.53E-03**	**5.14E-04**	**1.88E-03**	**6.67E-04**	**1.90E-02**	**1.27E-03**			**2.24E-02**	**8.79E-03**	**5.62E-04**	**1.09E-03**	**4.73E-02**

		FOZ DO AREIA				SALTO SEGREDO				SALTO SANTIAGO				SALTO CAXIAS			
		ReservoirS															
F. A	**C:D**	**W**	**Sp**	**S**	**Sp2**	**W**	**Sp**	**S**	**Sp2**	**W**	**Sp**	**S**	**Sp2**	**W**	**Sp**	**S**	**Sp2**
PUFA	C18:2n6t								2.09E-03							4.68E-04	
	C18:2n6c				8.06E-04		2.93E-04		2.00E-03								4.69E-03
	C18:3	3.42E-05		9.65E-04							1.69E-04						
	C18:3n3						5.63E-04		2.78E-04				1.10E-03				5.99E-04
	C20:2(cis-11.14)		2.33E-05						9.05E-03	1.46E-03					5.90E-04		
	C22:2																1.41E-04
	Σ PUFA	**3.42E-05**	**2.33E-05**	**9.65E-04**	**8.06E-04**		**8.56E-04**		**1.34E-02**	**1.46E-03**	**1.69E-04**		**1.10E-03**		**5.90E-04**	**4.68E-04**	**5.43E-03**
HUFA	C20:4	1.53E-03					3.49E-04		3.49E-04				1.06E-03				4.74E-04
	EPA	9.09E-03		1.19E-03	1.50E-02	1.38E-04	2.08E-03		2.47E-02	1.17E-04			1.31E-02	9.17E-03	5.89E-04		1.89E-02
	DHA	9.62E-03	8.37E-02	4.01E-05	1.75E-02	2.95E-02	1.06E-03		3.53E-02	8.21E-02		1.88E-02	1.73E-02	1.11E-01		2.01E-02	2.38E-02
	Σ HUFA	**2.02E-02**	**8.37E-02**	**1.23E-03**	**3.25E-02**	**2.96E-02**	**3.49E-03**		**6.03E-02**	**8.22E-02**		**1.88E-02**	**3.15E-02**	**1.20E-01**	**5.89E-04**	**2.01E-02**	**4.32E-02**
	Σ FA	**4.41E-02**	**9.54E-02**	**1.54E-02**	**7.97E-02**	**3.09E-02**	**1.90E-02**	**6.67E-04**	**1.62E-01**	**9.01E-02**	**4.41E-03**	**1.90E-02**	**9.30E-02**	**1.92E-01**	**6.80E-03**	**3.18E-02**	**2.08E-01**
	N	**13**	**7**	**9**	**12**	**5**	**15**	**1**	**16**	**9**	**3**	**3**	**14**	**9**	**6**	**7**	**16**

Table 3.
List of fatty acids identified in the Iguaçu River reservoirs, units in mg of fatty acids per mg of zooplankton (mg.mg^{-1}). W = winter (July 2012), Sp 248 = spring (November 2012), S = summer (February 2013), Sp2 = spring (October 2013). C:D = carbons: saturations.

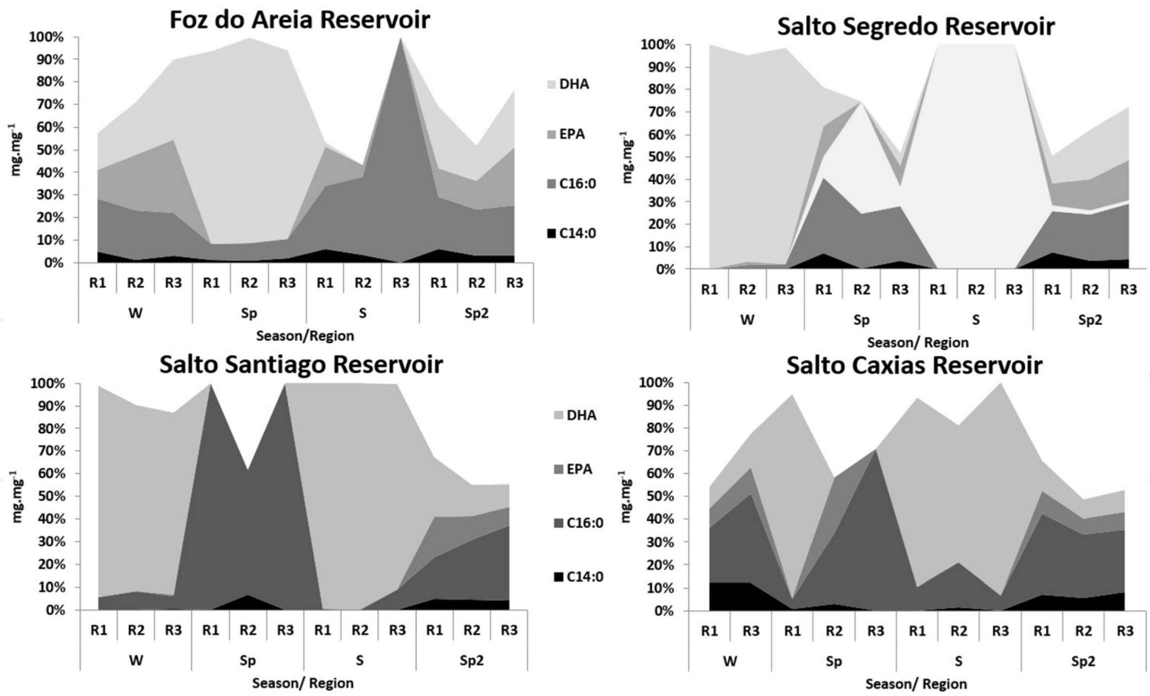

Figure 3.
Fatty acids densities of the Iguaçu River reservoirs.

in these waters. The bloom of the cyanobacterium *Microcystis aeruginosa* is a ubiquitous phenomenon in eutrophic lakes and reservoirs in many countries of the world. According to Cordeiro [28], *Microcystis* has a higher proportion of palmitic acid (C16:0). Adloff [29] in his work with phytoplankton, to the same studied area, found that the four reservoirs have water quality characteristics of eutrophic environments, with intense flowering of cyanobacteria, with a predominance of *M. aeruginosa* and *Sphaerocavum brasiliense*. Flowering occurred mainly in November 2012 and February 2013, and *M. aeruginosa* adapted to the best environment and dominated *S. brasiliense*.

In Salto Caxias reservoir, 19 fatty acids were found, being five saturated fatty acids (SFA), six monounsaturated fatty acids (MUFA), five polyunsaturated fatty acids (PUFA) and three highly unsaturated fatty acids (HUFA) (**Table 3**). In October 2013 sampling (Sp2) was found the most content in fatty acids, 16 in total, of which six are MUFA. In November 2012 sampling (Sp), only 6 fatty acids were identified, of which three were SFA, one PUFA, one MUFA and one HUFA.

In Salto Caxias reservoir, the most abundant fatty acid was DHA in the February 2013 sample (S), being quite representative in region 1 (R1) in November 2012 collection (Sp) with 89% of the total density, and more abundant in the February 2013 sampling (S) contributing more than 93% of all fatty acids (**Figure 3**). As it was not possible to extract the fatty acids from the samples in region 2 (R2) in July 2012 sampling (W), as there was not enough material to extract the fatty acids, only regions 1 (R1) and 3 (R3) were considered.

The collected stations were grouped into regions, with region 1 (R1) covering stations located in the lotic zone of the reservoir—stations P1, P2 and P3; region 2 (R2) includes stations located in the intermediate zone of the reservoir- P4, P5 and P6; and in region 3 (R3) were included the stations in the lake area of the reservoir, those close to the dam—P7, P8 and P9.

In the Cluster analysis, with a cut range of 50%, can clearly be observed the separation of the fatty acid composition according to the season. There a group was formed only with the samples from the collection carried out in spring 2012 (Sp) and the other clusters were composed of samples from other seasons, not being able to differentiate an isolated group.

In the Salto Segredo reservoir, a formation of groups can be well observed in the Cluster analysis. Four groups are distinguished: a group with the regions of the sampling of July 2012 (W), one with all the regions of the sampling of February 2013 (S), one with the samples from November 2012 (Sp) and the other with the samples from October 2013 (Sp2) (**Figure 4**). These results highlight seasonal differences in fatty acid content.

In the Cluster analysis of fatty acids in the Salto Santiago reservoir, it is possible to observe the separation of the fatty acid composition according to the seasons. Groups are formed only with the samples from the collection carried out in the spring of 2013 (Sp2) and spring 2012 (Sp) and the other clusters are composed of samples from other seasons, not being able to differentiate an isolated group (**Figure 4**).

In the Salto Caxias reservoir, the Cluster analysis clearly shows the separation of the fatty acid composition according to the season, where three groups are formed: one with the spring 2012 samples (Sp), another with the samples summer 2013 (S) and another that mostly has samples from spring 2013 (Sp2) (**Figure 4**).

In the multidimensional scaling analysis (n-MDS), in the Foz do Areia reservoir, it was observed the most similar regions according to the composition of fatty acids. Note that the composition of fatty acids was similar when observed the season (Season), showing similarities between the regions (Region), mainly observed in the samples collected in spring of 2012 (Sp). These similarities may indicate a greater homogeneity reservoir, possibly caused by the great drought that occurred there. It was also noted that the region with the greatest dissimilarity was region 3 in February 2013 sampling (S) (**Figure 5**).

In the Salto Segredo reservoir, it is noticed that the composition of fatty acids was similar when observed the season of the year and their respective collection stations, showing similarity mainly in the samples collected in February 2013 (S). This was also observed in the samples of October 2013 (Sp2) and July 2012 (W), with similarities around 60%, reaching up to 80%. In November 2012 (Sp) sampling is observed that the samples from region 2 (R2), show less similarity about the other

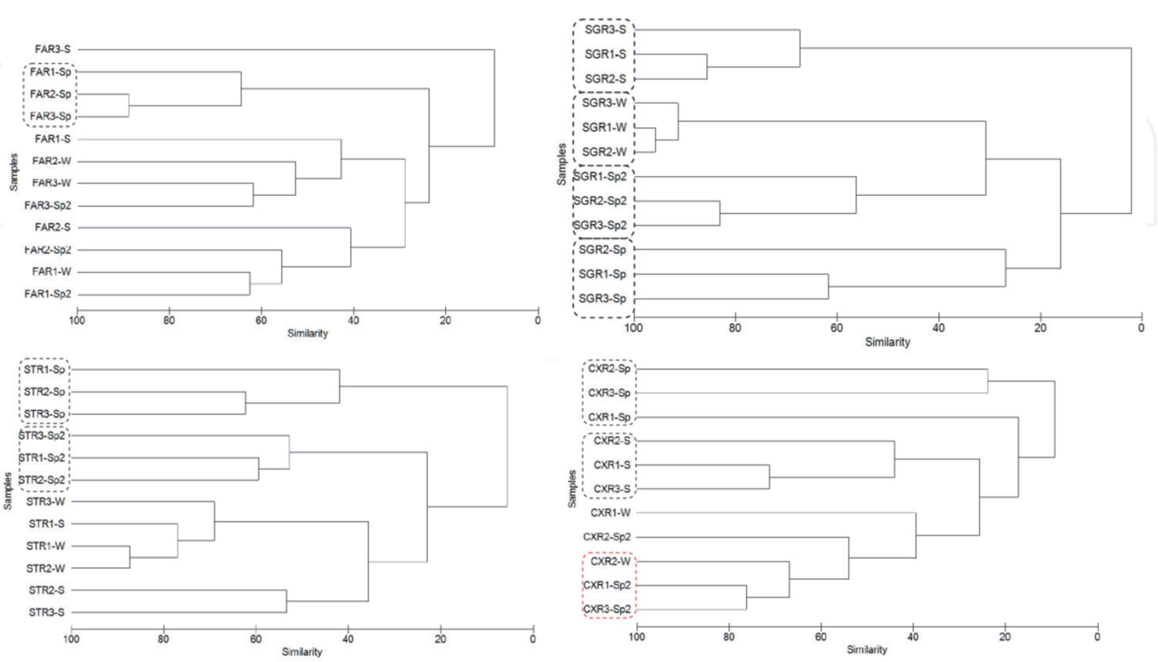

Figure 4.
Cluster analysis of the collection regions according to the seasons in the Iguaçu river reservoirs. W = winter of 2012, Sp = spring 2012, S = summer of 2013, Sp2 = spring 2013.

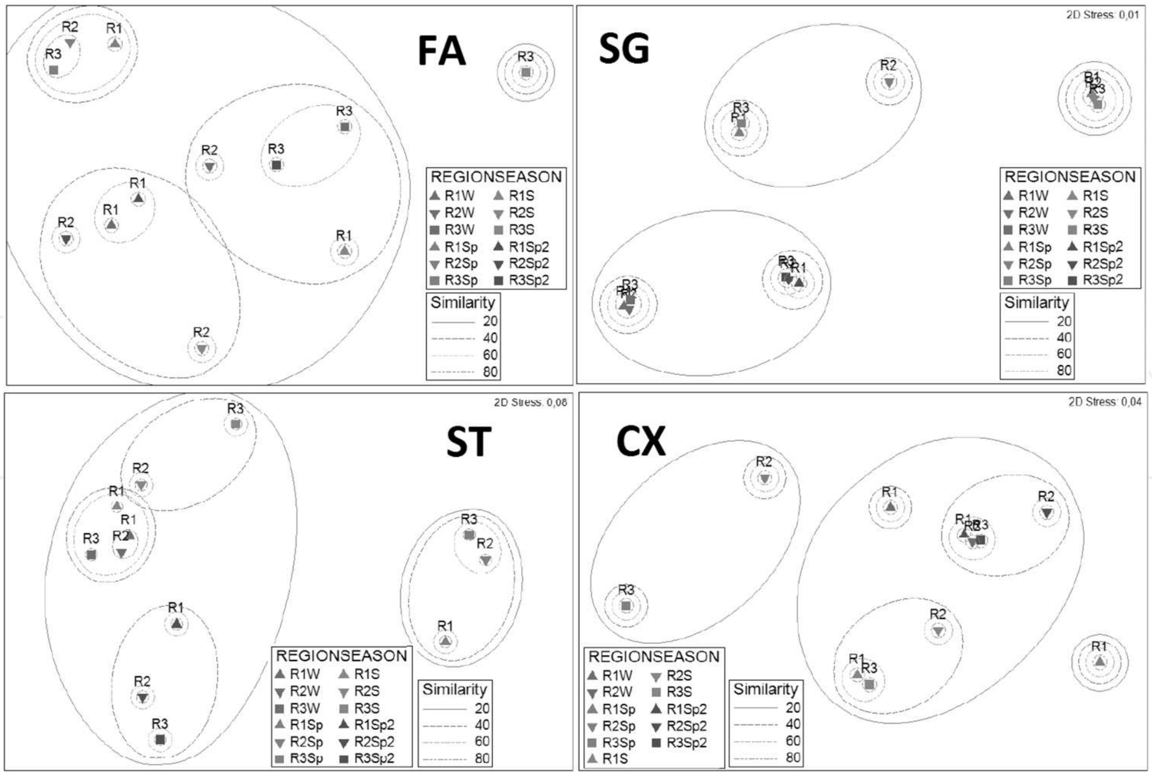

Figure 5.
n-MDS in the Iguaçu river reservoirs according to the fatty acids found. FA = Foz do Areia reservoir, SG = Salto Segredo reservoir, ST = Salto Santiago reservoir, CX = Salto Caxias reservoir.

regions (20%). As this is the intermediate region, that is under the fluvial influences, and also under the lake influences, may showing a different fatty acids content, that may also be related to the composition of the phytoplankton community, from which a large part of the zooplankton community feeds (**Figure 5**). During the sampling in February 2013 (S), in addition to a slight increase in diatom densities [30], there was a great drought in the region, which resulted in a decrease in the level of reservoirs, increasing the concentration of nutrients, which can become an indication of the sudden variation in the concentration of fatty acids.

In the Salto Santiago reservoir, it is clear that the composition of the fatty acids in the Sp and Sp2 samples were similar when observing their respective collected regions (Region), where the similarity is around 60% between the R2 and R3 in the two samples, and less similarity with the R1 region (**Figure 5**).

In the Salto Caxias reservoir, it is noticed that the composition of fatty acids was very different when observed in the collected region (Region). Regions 1 and 3 (R1 and R3) of October 2013 sampling (Sp2) were very similar to region 2 (R2) of the July 2012 sampling (W). Only the February 2013 (S) sample had similar regions (**Figure 5**).

Observing the n-MDS of fatty acids in the reservoirs, it can be noted that in Foz do Areia there is the formation of several groups, where EPA and C16:0 showed a similarity around 60% and DHA a similarity of 40%. The group formed by C14:0 (myristic acid), C16:1 (palmitoleic acid) and C18:0 (stearic acid) shows a similarity of 60% (**Figure 6**).

In Salto Segredo, it is possible to notice the formation of several groups in which EPA and C21:0 (Heneicosylic acid), and DHA and C16:0 (palmitic acid) show a similarity around 60%, one can also perceive a similarity around 80% in the group formed by C18: 1n9c (oleic acid) and C20: 2 (cis-11,14), and also in the group formed by C18: 3n3 (α-linolenic acid) and C20:4 (arachidonic acid).

In the Salto Santiago reservoir, observing the n-MDS of fatty acids, it is possible to notice the formation of two groups, one composed only by C8:0 (caprylic acid)

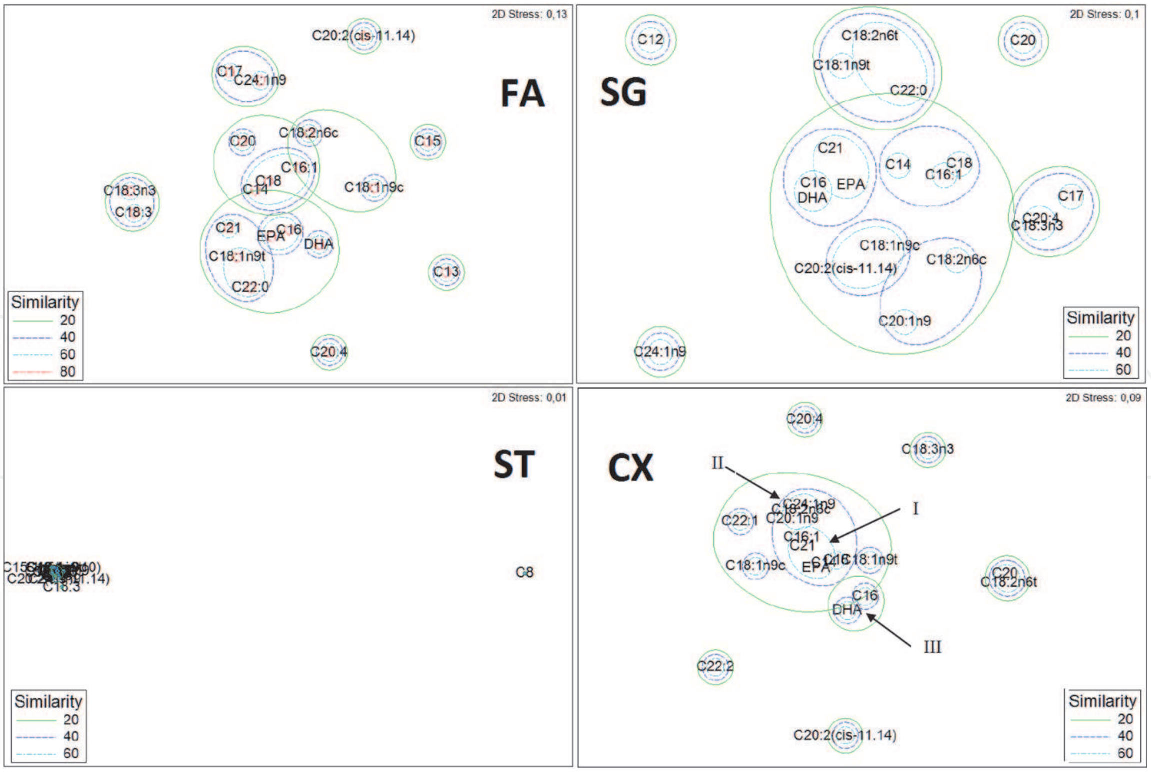

Figure 6.
n-MDS indicating the groups of fatty acids in the Iguaçu river reservoirs. FA = Foz do Areia reservoir, SG = Salto Segredo reservoir, ST = Salto Santiago reservoir, CX = Salto Caxias reservoir.

and the other covers all the other fatty acids in the reservoir. In the Salto Caxias reservoir, it is possible to notice the formation of several groups: group I composed by EPA, C14:0, C21:0 and C16:1, showing a similarity around 60%, as well as group II and group III composed by C20:1n9, C18:2n6, C24:1n9 and DHA, C16:0 respectively (**Figure 6**).

Comparing the fatty acid data with the TSI, it was observed that DHA had a higher concentration in sampling stations where the environment was hypereutrophic, whereas C16:0 was more abundant in sites classified as eutrophic, with other fatty acids like EPA and C20:0 also occurring in some specific sampling sites (**Table 4**).

At the response of zooplankton species and fatty acids behavior about the trophic environmental conditions, a Pearson correlation was made between taxa and fatty acids with the trophic state index (TSI). Regarding zooplankton species, the TSI presented a strong negative correlation with *P. dolichoptera* (r = −0.8232; p = 0.001), in the Foz do Areia reservoir, which may indicate that this species is more abundant in less eutrophic environments. However, it is clear that, despite the densities of *P. dolichoptera* decrease when the TSI increases, most samples accumulate in TSI values above 58, which means eutrophic environment (**Figure** 7).

In the Salto Segredo reservoir, about taxa, the TSI had a negative correlation with *K. cochlearis* ($r = -0.670$; $p = 0.017$), which may be an indication that this species may be an indicator of an intermediate trophic situation. However, it can be seen that regarding these values, most samples are between 59 and 60, which may mean that this species prefers eutrophic environments (**Figure** 7).

The TSI showed a positive correlation with *P. Dolichoptera* ($r = 0.765$; $p = 0.004$), which may be an indication of the contribution of this rate to the trophic state of the Salto Santiago reservoir. Most of the samples in which *P. dolichoptera* was more abundant were between the values of 55 and 60, showing a preference of these organisms for Mesotrophic to Eutrophic environments,

	TSI × fatty acids															
Collection stations	**FOZ DO AREIA**				**SALTO SEGREDO**				**SALTO SANTIAGO**				**SALTO CAXIAS**			
	W	**Sp**	**s**	**Sp2**	**W**	**Sp**	**s**	**Sp2**	**W**	**Sp**	**s**	**Sp2**	**W**	**Sp**	**s**	**Sp2**
P1	DHA[b]	DHA[f]	C16[d]	C16[d]	X[c]	C16[d]	C16:1[c]	C16[c]	C8[c]	C16[c]	X[b]	C16[c]	DHA[c]	DHA[c]	DHA[d]	C16[b]
P2	C16[b]	DHA[f]	C20[d]	C16[d]	DHA[c]	C16[d]	C16:1[d]	X[d]	DHA[c]	C16[e]	C16[c]	DHA[d]	C16[c]	C16[c]	C16[e]	C16[c]
P3	EPA[a]	DHA[f]	C16[e]	C16[d]	X[c]	C16[d]	C16:1[d]	C16[d]	DHA[c]	C16[f]	DHA[c]	C16[e]	C16[c]	C16[c]	DHA[c]	C16[a]
P4	DHA[a]	DHA[f]	C16[d]	C16[d]	X[c]	C16[d]	X[d]	DHA[c]	DHA[c]	X[c]	DHA[a]	C16[c]	C16[c]	C16[c]	X[c]	C16[b]
P5	EPA[b]	DHA[f]	C20[d]	C16[d]	X[c]	C16:1[d]	C16:1[d]	DHA[d]	DHA[c]	C16[d]	X[c]	C16[d]	C16[c]	C16[c]	C16[d]	C16[c]
P6	DHA[b]	DHA[f]	C16[d]	C16[d]	DHA[c]	C16[d]	C16:1[d]	X[c]	C16[c]	C16[d]	DHA[c]	C15:1n5	X[c]	C16[c]	DHA[d]	C16[c]
P7	DHA[b]	DHA[f]	C16[c]	DHA[d]	X[d]	C16[d]	C16:1[c]	X[d]	DHA[c]	C16[d]	DHA[c]	C15:1n5[d]	X[d]	C16[c]	X[c]	C16[c]
P8	C16[b]	DHA[f]	C16[d]	DHA[d]	C16[d]	C16[d]	C16:1[c]	C16[d]	DHA[c]	C16[c]	C16[b]	C16[c]	X[c]	C16[c]	C16[d]	C16[b]
P9	X[b]	DHA[f]	C16[d]	DHA[d]	DHA[d]	C18:1n9[d]	X[d]	X[c]	DHA[c]	X[c]	DHA[d]	C15:1n5[d]	X[c]	C16[c]	DHA[f]	C16[d]

[a]*Ultraoligotrophic*
[b]*Oligotrophic*
[c]*Mesotrophic*
[d]*Eutrophic*
[e]*Supereutrophic*
[f]*Hipereutrophic*

Table 4.
Trophic state index (TSI) compared to the most abundant fatty acid species in the four Iguaçu River reservoirs.

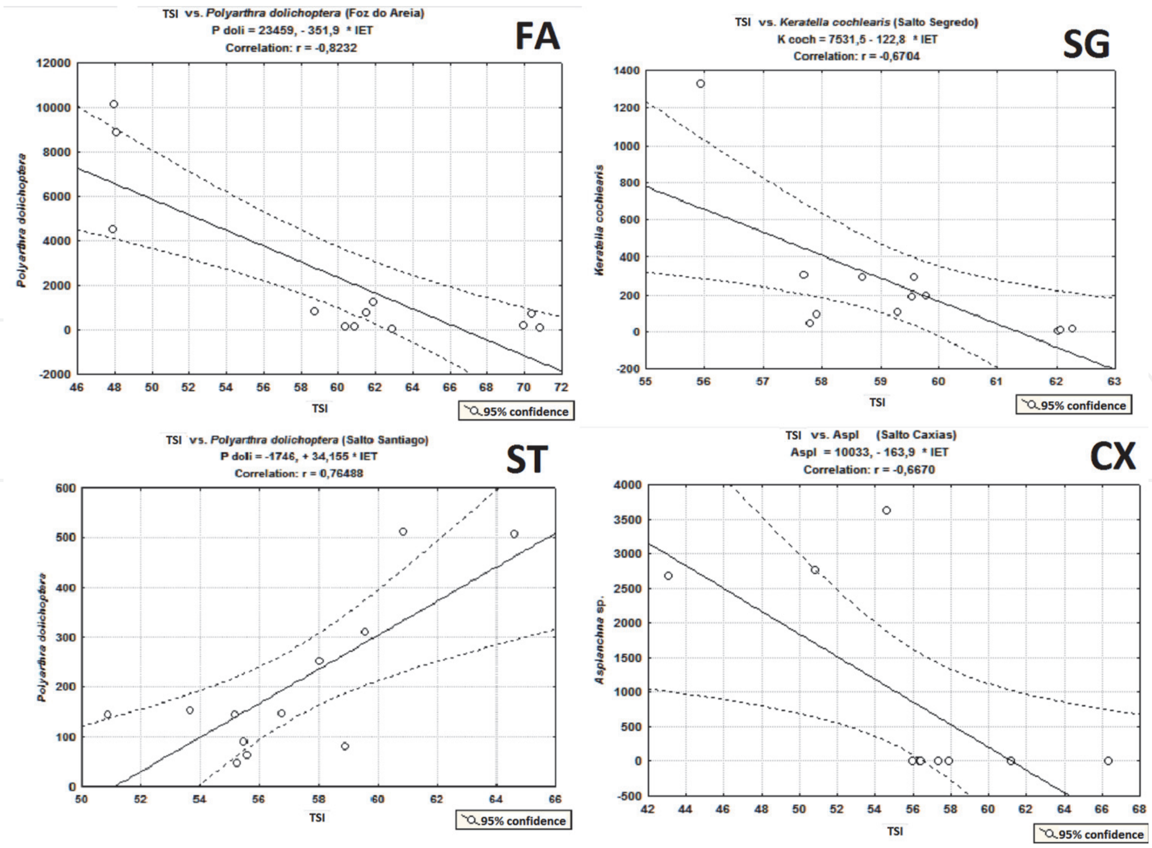

Figure 7.
Pearson correlation between TSI and most abundant zooplankton taxa in Iguaçu river reservoirs. B) Pearson correlation between TSI and DHA in the Foz do Areia reservoir.

although the highest density was found in the supereutophic range (63 < TSI <67) (**Figure 7**).

In the Salto Caxias reservoir, the TSI had a strong negative correlation with *Asplanchna* sp. ($r = -0.667$; $p = 0.025$) (**Figure 7**), which may be indicative of improved water quality over time, as this genus is a predator, and usually appears when there is a lot of food available, like herbivorous rotifers, and when food is scarce. Consequently, there is an improvement in the trophic state, since the photosynthetic organisms were consumed by the herbivores and there is a large amount of these small herbivores left, which are preyed upon by *Asplanchna* sp., and at this stage, the environment is already recovering from the large nutrient load caused by the activities changes and phytoplankton senescence.

Regarding fatty acids, the TSI had a strong negative correlation with oleic acid (C18:1n9c) ($r = -0.7392$; $p = 0.006$) and a strong positive correlation with docosahexaenoic acid (DHA) ($r = 0.666$; $p = 0.018$) (**Figure 8**), in the Foz do Areia reservoir. When the environment was hypereutrophic (TSI > 67), the highest concentrations of DHA appeared, however, most samples were in the range between 59 to 63, related to the eutrophic environment, although the concentrations were not very high.

In the Salto Segredo reservoir, regarding fatty acids, the TSI had a strong negative correlation with DHA ($r = -0.648$; $p = 0.023$), which may indicate that this fatty acid has its highest concentrations in less eutrophic environments. However, it can be seen that there are more samples of fatty acids in more eutrophic environments, however, with lower concentrations (**Figure 8**).

In the Salto Caxias reservoir, the TSI had a strong negative correlation with fatty acids C16:0 ($r = -0.610$; $p = 0.046$), C18:3n3 ($r = -0.748$; $p = 0.008$), C20:1n9 ($r = -0.663$; $p = 0.026$) and EPA ($r = -0.611$; $p = 0.046$) (**Figure 8**), which may

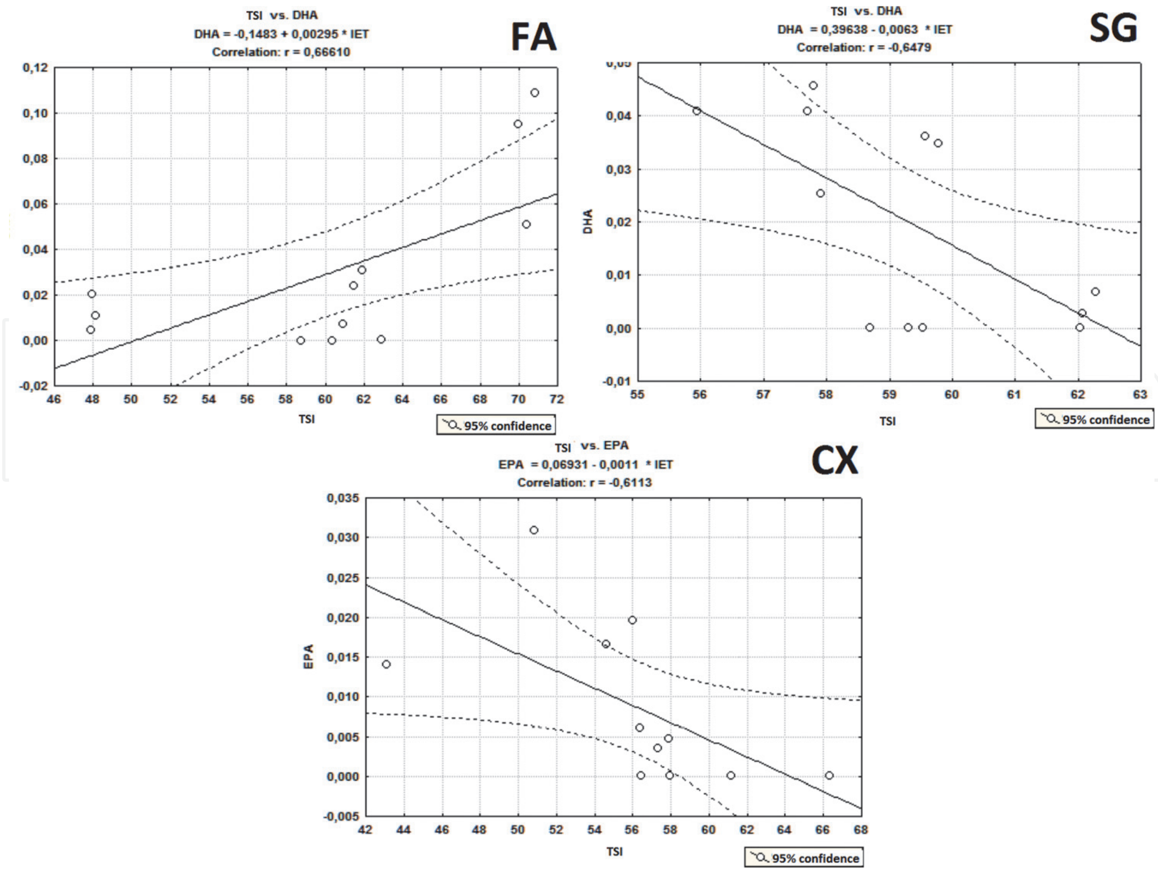

Figure 8.
Pearson correlation between TSI and most important fatty acids in the Iguaçu river reservoirs.

indicate that the reduction in water quality in relation to the TSI may result in a decrease in the concentrations of fatty acids above. In the Salto Santiago reservoir, the TSI did not show any significant correlation with fatty acids, which may be an indication that these fatty acids are not related to the trophic state of the reservoir.

The principal component analysis (PCA) between zooplankton species and fatty acids in Foz do Areia explained 75.45% of the total variability, with the first main component (PC1) explaining 44.22% and the second main component (PC2) 31.23%. PC1 (Factor 1) is separated into two distinct groups between the most abundant species and fatty acids. PC1 positively explained the following variables: Eicosapentaenoic acid (EPA) (0.96), *Docosahexaenoic* acid (DHA) (0.84), *B. longirostris* (0.82), as well as a large group of fatty acids. PC1 negatively indicated an association between DHA, α and γ-linolenic acids (C18:3n3 and C18:3, respectively) and the species *Synchaeta jollyae* (Synch jo) and *K. cochlearis* (K coch), which may indicate that these fatty acids can be associated with these species (**Figure 9**), since many organisms can synthesize DHA from α-linolenic acid (18,3n3) found in algae and plants.

Observing PC2 (Factor 2), it was noticed that there was a positive separation, mainly between *B. longirostris*, myristic acid (C14:0) and elaidic acid (C18:1n9t). That may indicate that this cladoceran species may be related to these fatty acids, as *Bosmina* species is a highly selective consumer [31], not absorbing some forms of fatty acids, preferentially feeding on EPA-producing algae. On the negative side of PC2, it was noticed that there is a great relationship between the copepod nauplii (Náup), *C. cornuta* (Cd corn) and *P. dolichoptera* (P doli) with oleic acid (C18:1n9c), which can be indicative that this fatty acid was important for the densities of these taxa (**Figure 9**). It has been suggested that myristic (C14:0), palmitic (16,0), and oleic (C18:1n9c) acids are derived from fatty acids from algae [32, 33].

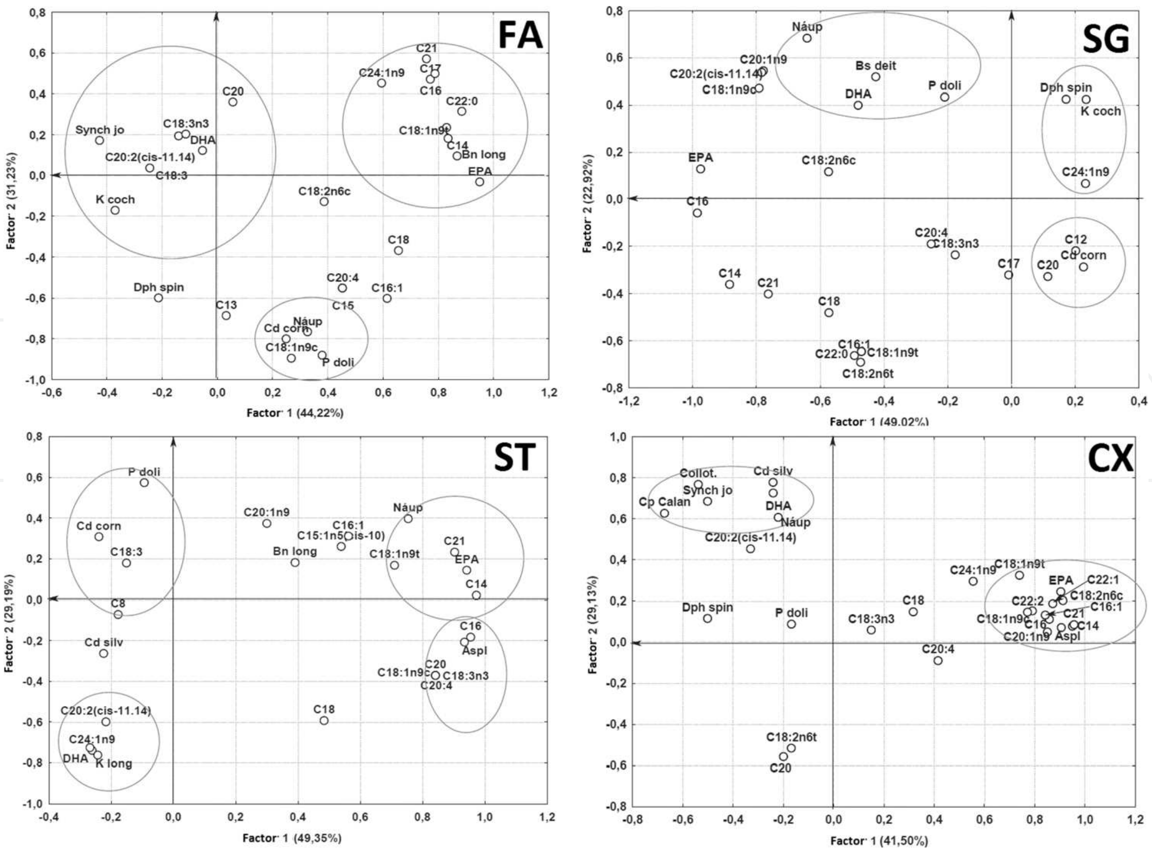

Figure 9.
Association between fatty acids and the most abundant taxa in the Iguaçu River reservoirs during the study period.

In the Salto Segredo reservoir, the principal component analysis (PCA) among the fatty acid data with the most abundant taxa explained 67% of the total variability, with the first main component (PC1) explaining 49.02% and the second main component (PC2) 22.92%. The first main component (PC1) separated two groups of distinct variables.

The positive side of the first component explained better the relationship between the following variables: the taxa *C. cornuta* (Cd corn), *K. cochlearis* (K coch) and *D. spinulosum* (Dph spin) and the fatty acids, lauric acid (C12: 0), arachidic acid (C20: 0) and nervous acid (C24: 1n9). These associations may indicate that these species may be feeding on phytoplankton that have these fatty acids. The negative side of the first component shows the association between DHA, *B. deitersi*, *P. dolichoptera* and copepod nauplii (Náup). These associations may indicate that certain environmental conditions provide the production of DHA and the zooplankton community is receiving these fatty acids through food. The second main component located on the positive side, associated taxa with some types of fatty acids indicating that these fatty acids may be contributing to the development of species. On the negative side, there is only a grouping of fatty acids (**Figure 9**).

The principal components analysis between fatty acids and the most abundant taxa in the Salto Santiago reservoir explained 78.54% of the total variability, with the first main component (PC1) explaining 49.35% and the second main component (PC2) 29.19%, and two distinct groups were formed, being explained by the first main component (PC1). The larger group located on the positive side indicates that the genus *Asplanchna* sp., influenced several fatty acids, possibly contributing to the increase of its densities, such as C16:0, C18:3n3, C18:1n9c, C20:0 and C20:4.

The variables contained in the other ellipse show a greater association between copepod nauplii and C14:0, C21:0 and EPA fatty acids, indicating that the concentrations of these fatty acids may have influenced nauplii densities. The second group formed by the ellipse on the negative side of factor 1 showed that the concentrations of DHA, C24:1n9, C20:2(cis-11,14), may have influenced the abundances of the rotifer *Kelicottia longispina* (K long), which was the most abundant rotifer in this reservoir. The second main component (factor 2) explained the relationship between *P.dolichoptera* and *C.cornuta* with C18:3 (**Figure 9**).

In the Salto Caxias reservoir, the principal component analysis, between fatty acids and taxa, explained 70.63% of the total variability, with the first main component (PC1) explaining 41.50% and the second main component (PC2) 29.13%. The positive side of factor 1 explained the relationship of *Asplanchna* sp. With several fatty acids, mainly with EPA, C14:0 and C16:0. These associations may indicate that a certain group of algae may be producing these fatty acids, which favored the abundance of rotifers, which are the food source of *Asplanchna* sp. The second main component, located on the positive side, associated DHA with several taxa, among them *S. jollyae*, *Collotheca* sp., *C. cornuta*, copepods and calanoid copepodites, indicating that these taxa may possibly be related to a diet rich in DHA (**Figure 9**).

4. Discussion

This study highlights the predominance of zooplankton species that are somehow adapted to eutrophic environments, corroborating, in part, with the observed trophic condition of the reservoir, with the aquatic system classified as oligotrophic, according to the TSI in winter of 2012 (W). In the four reservoirs, the dominant species were similar. *P. dolichoptera* was the most abundant species among the rotifers and *C. cornuta,* whereas in the cladoceran species *Ceriodaphnia silvestrii, D. spinulosum* and *B. longirostris* were the most abundant. In November 2012 (Sp) and February 2013 (S) collections, it may have a large phytoplankton density, and herbivorous rotifers such as *P. dolichoptera* become dominant over other species, and later can be controlled by small zooplanktonic carnivorous organisms [34], which may explain the dominance of *Asplanchna* sp. In upstream reservoirs, namely, Salto Santiago and Salto Caxias.

According to Hollowday [35], *P. dolichoptera* is found mainly in eutrophic environments, but also occur in water bodies with different trophic state degrees, but present higher densities in eutrophic water bodies in periods of low temperature [36]. This dominance of *P. dolichoptera* may indicates that environmental eutrophication could occur, which was confirmed in the Foz do Areia reservoir in the spring of 2012 (Sp). Regarding the TSI, the trophic environmental conditions were quite different, because in the spring of 2012 (Sp) the region was experiencing a great drought, and the reservoirs were below their normal level and present a probable "bloom" of cyanobacteria *M. aeruginosa*, according to Adloff *et al*. [29].

Due to this combination of factors, probably resulted in changes in trophic status in aquatic bodies. Another factor that may have been the cause of this eutrophic condition, mainly in the Foz do Areia reservoir, is the dumping of waste into the Iguaçu River, in the region of the city of Curitiba, as it is in a region with little access to basic sanitation, as well as, the presence of industries.

Over time, these wastes accumulate in the first reservoir, and combined with the drought, the high temperature and the low level of the reservoir, increases the concentration of nutrients, consequently making it hypereutrophic. This high concentration of nutrients in the Foz do Areia reservoir passes to the other reservoirs along the river, a fact that was possible to see in the following samples, arriving at the Salto Caxias Reservoir, in the last collection. In the last sampling, the reservoir floodgates were opened, which may have caused these nutrients to be carried along the river, however, as this study was carried out during this process, it is not possible to infer the impact caused.

Farage et al. [37] observed an opposite result, in which the trophic state of the studied aquatic environment increased during the rainy season, which is justified by the runoff mechanism, which often occurs during the rainy season, especially in soils without vegetation or predominance undergrowth.

Observing the results of the analysis of fatty acids superimposed with the TSI, it is noticed that the main compounds are DHA, C16:0. In lesser proportions occurred EPA and C15:1n5. Thant appears in certain trophic states, as in the spring sampling of 2012 (Sp), where in the Foz do Areia reservoir it was presented as hypereutrophic at all sampling sites, where the most abundant fatty acid was DHA. However, DHA was more abundant in places classified as mesotrophic in the 2012 winter sampling (W) in the Salto Santiago reservoir.

In the Salto Caxias reservoir, environments classified as mesotrophic, C16:0 fatty acid was the most abundant. This variation may have occurred due to changes in the climate and the water regime of the reservoirs, in the spring of 2012 (Sp), where there was a great drought, and the reservoir levels decreased considerably.

This variation may have occurred due to changes in the climate and the water regime of the reservoirs, in the spring of 2012 (Sp), where there was a great drought, and the reservoir levels decreased considerably. In Foz do Areia the environment was classified as hypereutrophic, and DHA was the most abundant fatty acid while the other fatty acids had a lower concentration. A dominance of DHA may indicate a diet based on flagellate organisms [38]. The seasonal variation of fatty acids, mainly DHA, indicates a temporal change in the availability of phytoplankton [39]. Observing the results obtained, DHA is more present in more eutrophic environments, where according to Nozaki [40], some flagellated algae are more abundant, while EPA, as well as C14:0 and C16:0 are more abundant in less eutrophic environments.

Palmitic acid (C16:0) is a fatty acid that is found in several groups of phytoplankton that are food sources for zooplankton [41] and also in some species of Cyanophyceae [42]. Also, according to Patil et al. [42], C16:0 is one of the main groups of saturated fatty acids (SFA) among freshwater phytoplankton, while marine phytoplankton is the main producer of DHA, and little is found in freshwater species, considering that DHA is the most important fatty acid for copepods and many fish species [43, 44]. Indeed, palmitic acid is one of the most common saturated fatty acids in organisms, in general. Generally, phytoplankton with high proportions of EPA or DHA, such as Cryptophyceae and Bacillariophyceae are excellent food sources for zooplankton. Cyanophytes have practically no EPA and DHA, while diatoms are rich in EPA, and dinoflagellates have high amounts of DHA [45].

In aquatic ecosystems, the level of essential fatty acids (EFAs), such as EPA and DHA, in algae is highly variable [46]. The HUFA content represents between 3 and 7% of the total fatty acids of phytoplankton during flowering, making the nutritional value of phytoplankton flowering questionable [46]. There is also evidence that the amount of EPA and DHA in algae varies significantly between the major taxonomic groups [47]. For example, Cryptophyceae have high proportions of EPA and DHA, while in Chlorophyceae it is nonexistent, scarce or has traces of these fatty acids. There is ample evidence that essential fatty acids (EFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are extremely important for several aquatic organisms [48]. Indeed, marine phytoplankton generally contains high amount of omega-3 (n-3) fatty acids, which are good for human health being one of the main foods sources the consumption in fish species. These essential fatty acids are related with the prevention and treatment of ocular, cardiovascular, autoimmune and cancer diseases [48–50].

The availability of PUFAs is a critical factor that influences trophic dynamics and biological production in the ecosystem. However, this PUFA pool is in turn influenced by the set of planktonic organisms present, as well as physical and chemical factors. To date, few studies have analyzed the transfer efficiencies of these fatty acids at various trophic levels.

The concentration of zooplanktonic fatty acids is a useful tool for defining the trophic state of the environment, especially EPA, DHA and C16:0. It is known that the zooplankton community is important for the flow of energy in the trophic chain. According to the concentration of nutrients in the environment, there may be a differentiation in the biochemical composition of this community. To understand how these differences can be related to water quality is an issue to be further studied.

Although, zooplankton is not included as BQE (Biological Quality Element) in the WFD (Water Frame Directive of the European Union Water Framework), some studies use this community to assess the water quality of aquatic systems [51, 52]. This work corroborates zooplankton is a good bioindicator to assess water quality and thus identified as a BQE by Water Framework Directive.

Biochemical analyzes of zooplankton, such as lipids (including fatty acids), showed to be an important tool for water body management and water quality analysis, and for detecting molecular changes in the zooplankton community, related to the trophic status of systems, before they are perceived in the environment.

Although, the use of fatty acid analysis is not a low-cost tool and can only be used with the help of a specialist, the results obtained are more accurate than other types of analysis, thus being able to generate faster conclusions, accelerating the assessment and evaluation process of the aquatic bodies.

Public policies and environmental campaigns should be adopted by the responsible environmental agencies to minimize the discharge of sewage into the river, in the region of Curitiba, or if possible, its treatment, so that this load of pollutants does not reach the first reservoir—Foz do Areia—as well as environmental education campaigns for the population.

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