

Article

Excessive Eutrophication as a Chemical Barrier for Fish Fauna Dispersion: A Case Study in the Emblematic Tietê River (São Paulo, Brazil)

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Abstract: The Tietê River receives most of the effluents and diffuse wastes from the São Paulo metropolis (21.9 million inhabitants). The study aimed to assess the extent to which environmental changes affected the fish fauna. We compared, in rainy and dry seasons, three sites in Tietê and three in tributaries with much better water quality conditions. No physical barriers exist between the sites. Fish were sampled with gillnets (exposed overnight) and the assemblage's ecological attributes calculated. Water depth, transparency, temperature, electrical conductivity, dissolved oxygen, turbidity, total dissolved solids, pH, redox potential, total phosphorus, total nitrogen, and chlorophyll were simultaneously determined. Low oxygen concentrations (near zero in the rainy period), and the opposite for nutrients and total solids, separated the predominantly hypereutrophic Tietê from the tributaries (PCA). Differences in the fish assemblages were also evidenced (nMDS), including a higher richness per sample in tributaries (11 to 14 spp.) compared to Tietê (3 to 4 spp.). Siluriformes with accessory breathing dominated in Tietê and the highly tolerant detritivorous *Prochilodus lineatus* (the main commercial fish) was the only species found in all sites. The species correlated positively with oxygen in the tributaries and with turbidity, redox potential, and nutrients in Tietê (DistLM) (rainy season). Recovery measures are urgently required.

Keywords: ecological barrier; environmental degradation; fish tolerance; pollution



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1. Introduction

Dispersal plays a fundamental role in the maintenance of the structure and functioning of populations and, consequently, of the species they represent, assuring a continuous genetic flow, over space and time [1–3]. This ecological process is related to the capability of biota to transpose relative barriers and move among distinct habitats in response to internal (e.g., genetic and physiological) and external signals (e.g., light, temperature, water quality, and predation pressure) [4], this being one of the main explanatory models of the distribution patterns of organisms on planet [5].

In the current scenario of massive habitat degradation and climate uncertainties, understanding how, where, and why organisms disperse is extremely important [2,3]. Numerous artificial barriers to dispersion have emerged because of significant population growth and economic development. In the case of Brazilian fluvial environments, the construction of hydroelectric dams, for instance, is a major interference in the free movement of the biota, especially fish [6–11]. However, other types of environmental disturbances, such as pollution/eutrophication, can also affect the distribution of organisms and other ecological processes related to the different components of the ecosystems [12].

The Tietê River is a notorious example of severe environmental degradation, particularly in its upper section that crosses the metropolitan region of São Paulo, the most populous in Brazil (around 21.9 million inhabitants) [13]. The history of water quality

deterioration in this river began with organic contamination in the mid-nineteenth century. Nevertheless, across the twentieth century, in addition to high loads of nutrients, degradation became a more complex scenario. There was a considerable increase in inorganic contamination due to the introduction of a myriad dissolved substances, such as pesticides, herbicides, medicines, and cosmetics. The Tietê River represents a significant threat to society, in terms of to water security, due to the potential adverse effects on human health [14].

After a critical water crisis due to a deficit in precipitation, with an enormous impact in São Paulo State in the year 2014, technical and scientific studies tried to explain the complex degradation process of the Tietê River [15]. However, the discussion, especially in terms of governance, remained restricted to the São Paulo metropolitan area. The fact that this huge impact transcends to a much wider regional spatial scale, i.e., dramatically spreads towards the São Paulo State countryside, seems to be neglected.

In a long-term study, from the end of the 1970s to the beginning of the 2000s [16], a clear trend of water quality deterioration in the middle Tietê River was identified, more than 300 km downstream of São Paulo city. The electrical conductivity of water, for instance, increased from 103 to 370 $\mu\text{S cm}^{-1}$; phosphorus concentration from 15 to 40 $\mu\text{g L}^{-1}$; nitrate from 200 to 3480 $\mu\text{g L}^{-1}$; and ammonium from 16 to 232 $\mu\text{g L}^{-1}$. The authors also highlighted the increased potential for toxicity associated with the recurrent cyanobacterial blooms. After that, during the last two decades, there has been no signal of consistent improvement in the river water quality; maybe the contrary [17,18].

The extraordinary increase in nutrients introduced in the Tietê River resulted in a remarkable eutrophication, immediately changing the basic levels of the aquatic food chains (magnification in the biomass of phyto- and zooplankton-tolerant species). Through direct and indirect transference processes, such changes affected the upper trophic levels represented by the different species of fish. When trophic conditions become extreme, recurrent decreases in dissolved oxygen concentration occur, directly influencing the ichthyofauna [19]. The consequences of this type of interference are reflected in the composition and other structural characteristics of fish. Previous studies in the Tietê River have already pointed to a reduction in the size of native populations and their reproductive performance, and a general decrease in species richness and diversity [11,20].

In this context, the objective of this study was to assess the extent to which changes in environmental factors have affected the regional fish fauna in the middle Tietê River basin (SE Brazil). The selected study sites, in addition to the main river, include the lower stretches of the tributaries Capivari (right bank), Sorocaba, and Peixe (both on the left bank). These environments are widely connected, without any natural (e.g., falls and rapids) or artificial (e.g., dams and reservoirs) physical barriers that could interfere with the free movement of the organisms. However, they exhibit contrasting differences in terms of water quality, with the tributaries in a much better condition. Therefore, we hypothesized that water quality degradation acts as a chemical barrier to the dispersion of species, on a regional scale.

2. Material and Methods

The Tietê River is 1100 km long ($\sim 1300 \text{ m}^3 \text{ s}^{-1}$ in its final stretch) [20] and flows from east to west along almost the entire state of São Paulo, southeast Brazil. The study sites are located approximately 300 km downstream of São Paulo city.

Samplings occurred at the end of the rainy period (April 2019), early autumn, and at the end of the dry period (October 2019), spring, at six selected sites (Figure 1; Table 1). The study considered six sampling sites, without replicates, due to the difficulties found during the fieldwork—excessive amount of solid waste tapped into the nets and boat engine and health risks due to the inevitable direct contact with the Tietê contaminated water. Three sites were distributed in the Tietê River along a stretch of 40 km, one further upstream (TI), above the mouth of the Sorocaba (Sor) and Capivari (Cap) rivers; one site in the middle section (TII); and one further downstream (TIII), below the mouth of the Peixe (Pei) River

(upstream of the Barra Bonita Reservoir). Three additional sites corresponded to the lower reaches of the mentioned tributaries.

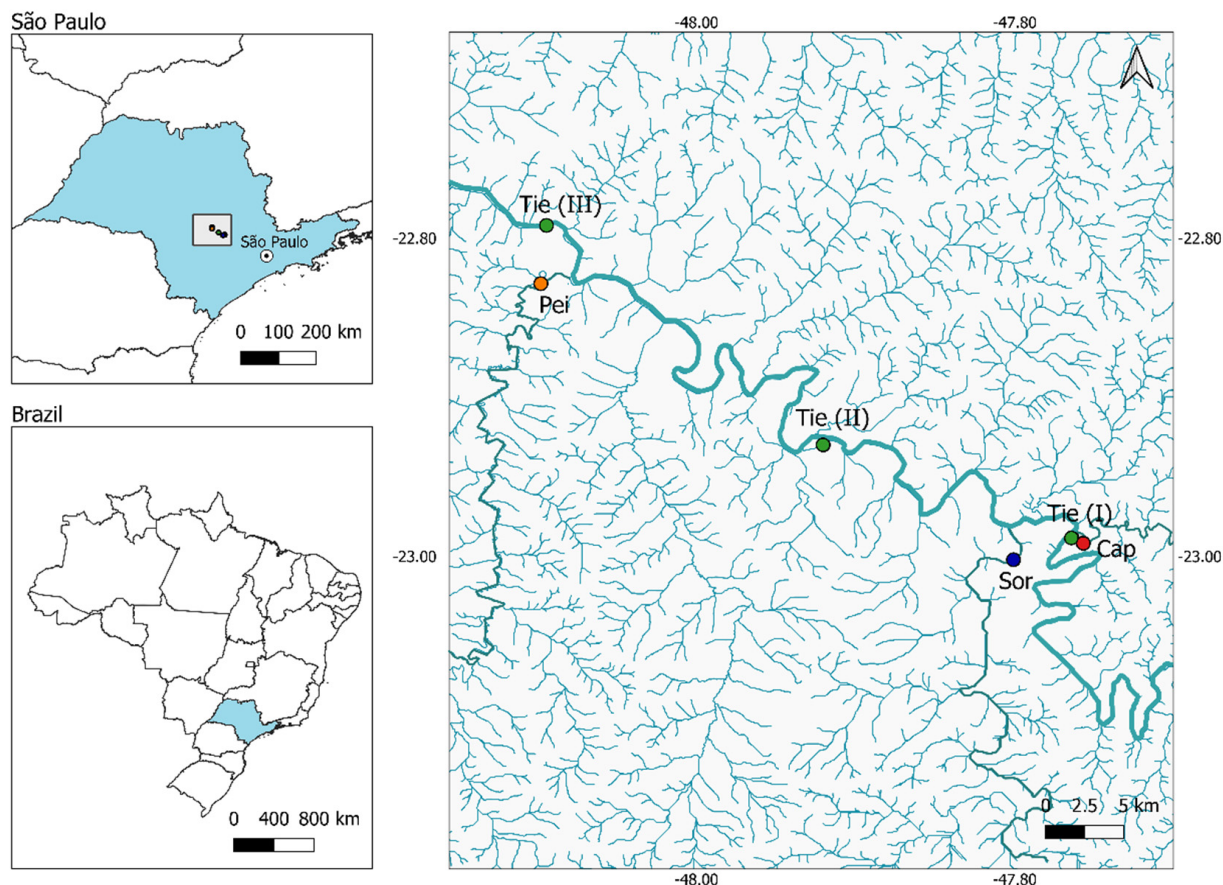


Figure 1. Distribution of sampling sites (on the right) in the Tietê River (green circles), Sorocaba River (blue circle), Capivari River (red circle), and Peixe River (orange circle). The highlighted region (upper left), in gray, corresponds to the study area, in the center of São Paulo State, SE Brazil.

Table 1. Sampling site denomination and respective locations.

Site	River	Municipality	Latitude	Longitude
Tie (I)	Tietê River	Laranjal Paulista	22° 59' 17.6" S	47° 46' 00.1" W
Tie (II)	Tietê River	Laranjal Paulista	22° 55' 47.6" S	47° 55' 22.8" W
Tie (III)	Tietê River	Anhembi	22° 47' 31.0" S	48° 05' 48.8" W
Cap	Capivari River	Laranjal Paulista	22° 59' 29.9" S	47° 45' 34.9" W
Sor	Sorocaba River	Laranjal Paulista	23° 00' 07.6" S	47° 48' 12.2" W
Pei	Peixe River	Anhembi	22° 49' 42.8" S	48° 06' 01.5" W

It is worth observing, through Figure 1 (detailed scale of 1:10,000), the extensive and complex net of small and middle-size tributaries distributed along the Tietê basin.

In each sampling site, the following environmental variables were measured in situ: local depth (m) (portable Speedtech sonar) (Great Falls, MT, USA); water transparency (m) (Secchi disc); and vertical profiles (measurements at 0.5 to 1 m intervals) (multi-parameter probe Horiba U-5000, previously calibrated) (Kyoto, Japan) of water temperature (°C); electrical conductivity ($\mu\text{S cm}^{-1}$); dissolved oxygen (DO) (mg L^{-1}); turbidity (NTU); total dissolved solids (TDS) (g L^{-1}); pH; and redox potential (ORP) (mV). Surface water samples were collected to analyze total phosphorus, total nitrogen, and chlorophyll a [21].

The trophic status index (modified TSI) [22] was calculated using the total phosphorus index and chlorophyll index, following the proposal of the Environmental Company of São

Paulo State (CETESB, in Portuguese) for river systems (https://cetesb.sp.gov.br/aguas-interiores/wp-content/uploads/sites/12/2019/10/Ap%C3%AAndice-D_%C3%8Dndices-de-Qualidade-das-%C3%81guas.pdf) (accessed on 15 April 2024):

$$\text{TSI (Chlorophyll)} = 10 \times (6 - ((-0.7 - 0.6 \times (\ln \text{CL}))/\ln 2)) - 20;$$

$$\text{TSI (Total Phosphorus)} = 10 \times (6 - ((0.42 - 0.36 \times (\ln \text{TP}))/\ln 2)) - 20;$$

$$\text{TSI} = [\text{TSI (TP)} + \text{IET (CHL)}]/2.$$

Values of the TSI include six categories: ultraoligotrophic ($\text{TSI} \leq 47$), oligotrophic ($47 < \text{TSI} \leq 52$), mesotrophic ($52 < \text{TSI} \leq 59$), eutrophic ($59 < \text{TSI} \leq 63$), supereutrophic ($63 < \text{TSI} \leq 67$), and hypereutrophic ($\text{TSI} > 67$).

The studied parameters were compared with the reference standards established by national legislation (Table 2), following CONAMA (National Environment Council, in Portuguese) Resolution n° 357/2005 [23] (conditions for water quality categories for Brazilian aquatic ecosystems) for Class 2 waters—the present classification of the considered Tietê River stretch and its tributaries. Class 2 waters are suitable (presently or intended in the future after recovery actions) for: (a) supply for human consumption, after conventional treatment; (b) the protection of aquatic communities; (c) primary contact recreation, such as swimming, skiing, and diving; (d) irrigation of vegetables, fruit plants, parks, gardens, and sports and leisure fields, with which the public may have direct contact; and (e) aquaculture and fishing activities.

Table 2. Standard references established by CONAMA (National Environment Council, in Portuguese) Resolution 357/2005 (conditions for water quality categories for Brazilian aquatic ecosystems) for Class 2 waters (see Material and Methods).

Parameter	Reference
pH	6 to 9
Turbidity (NTU)	<100 NTU
DO ($\text{mg} \cdot \text{L}^{-1}$)	>5 $\text{mg} \cdot \text{L}^{-1}$
TDS ($\text{g} \cdot \text{L}^{-1}$)	<0.5 $\text{g} \cdot \text{L}^{-1}$
Total phosphorous ($\text{mg} \cdot \text{L}^{-1}$) *	<0.10 $\text{mg} \cdot \text{L}^{-1}$
Total nitrogen ($\text{mg} \cdot \text{L}^{-1}$)	<2.18 $\text{mg} \cdot \text{L}^{-1}$
Chlorophyll-a ($\mu\text{g} \cdot \text{L}^{-1}$)	<30 $\mu\text{g} \cdot \text{L}^{-1}$

Note: * Values for lotic environment.

The ichthyofauna was collected (sampling license 13794-1 conceived to MGN by the National Institute of Biodiversity Conservation) (ICMBio, in Portuguese) with gillnets, exposed for approximately 16 h (overnight). Meshes of 30, 40, 50, 60, 70, 80, 100, 120, 140, 160, and 180 mm (between knots) were installed from the marginal habitats (smaller apertures) towards the river's central channel (larger apertures). Each net has a standard extension of 10 m and the heights vary between 1.5 m (smaller apertures) and 2.5 m (larger apertures).

Captured specimens were kept in buckets with water to be identified, photographed, counted, and measured (standard length and total weight) and then returned to the original water body. Morphological identification was performed at the lowest taxonomic level based on the specialized literature [24–27].

The fish abundance was standardized in terms of CPUE (catch per unit effort) in numbers of individuals (CPUE_n) and biomass (CPUE_b), considering 1000 m² of gillnets and 16 h of exposure. The assemblage's alpha (α) diversity was determined with the Shannon–Wiener index and Pielou's equability. Beta diversity (β) was calculated based on the Whittaker index. Analyses were performed with the software Primer v.6.1.12 and Permanova + v.1.0.2 [28] and PAST 1.48 [29].

We also determined the trophic guilds of the fish (indicative of functional diversity) based on “Diet” or “Food items” reported by Froese and Pauly [30] and/or from published studies using the search tools Google Scholar (<https://scholar.google.com.br/>) (accessed on 5 April 2024) and SciELO (<http://www.scielo.org/php/index.php>) (accessed on 5 April 2024).

For the ordination of the studied rivers, based on the environmental variables, we used principal component analysis (PCA). A non-metric multidimensional scaling (nMDS) analysis was performed to separate the species groups per similarity among sampling sites. Finally, a distance-based linear model (DistLM) [31] was used to correlate the ichthyofauna structure (abundances per species per site) with the environmental variables. All parameters (except pH) were $\log(x + 1)$ transformed prior the analyses and, posteriorly, normalized for PCA. For nMDS, the Bray–Curtis dissimilarity coefficient was used and the matrix was later transformed into the square root [28]. The DistLM analysis used the Best method with the Akaike Information Criterion (AIC) derived from the Bray–Curtis similarity matrix with a dummy effect of 0.01 [31]. These tests were performed using Primer v.6.1.12 and Permanova + v.1.0.2 [28] and R [32].

3. Results

3.1. Environmental Conditions

Means and standard deviations (vertical profiles) of the environmental variables for each site and sampling period are shown in Table 3. Figure 2 represents the vertical distribution of selected variables, dissolved oxygen and temperature.

Table 3. Mean values and standard deviation (water column) of the environmental variables measured at the sampling sites (Tietê River and tributaries), in the rainy (R) and dry (D) periods. Values in bold are not in accordance with the limits established by CONAMA (National Environment Council, in Portuguese) Resolution 357/2005 for Class 2 waters (see Material and Methods).

Variables	Tie (I)		Tie (II)		Tie (III)		Cap		Sor		Pei	
	R	D	R	D	R	D	R	D	R	D	R	D
Depth (m) *	5.00	3.40	6.00	5.40	7.80	5.00	4.50	0.90	5.70	6.40	5.50	2.00
Transparency (m) *	0.20	0.45	0.15	0.60	0.40	0.80	0.20	0.45	0.30	1.20	0.30	0.80
Temperature (°C)	24.30 (±0.00)	26.11 (±0.00)	24.36 (±0.01)	26.13 (±0.00)	26.09 (±0.01)	25.34 (±0.12)	23.61 (±0.00)	26.84 (±0.00)	23.54 (±0.02)	25.71 (±0.13)	23.60 (±0.02)	24.35 (±0.15)
pH	6.68 (±0.07)	7.45 (±0.07)	6.54 (±0.12)	7.28 (±0.19)	6.45 (±0.16)	7.05 (±0.20)	6.80 (±0.06)	7.76 (±0.01)	6.49 (±0.24)	6.57 (±0.23)	6.21 (±0.11)	6.61 (±0.23)
ORP (mV)	242.38 (±4.06)	224 (±3.54)	415.80 (±8.42)	254.67 (±16.01)	228.40 (±4.10)	283.86 (±16.18)	257.17 (±7.06)	211.67 (±2.62)	268.29 (±20.73)	260.14 (±15.49)	228.86 (±17.98)	344.67 (±14.08)
Conductivity (µScm ⁻¹)	274.75 (±0.43)	690 (±0.00)	249 (±0.00)	569.83 (±0.37)	409.80 (±0.40)	557.71 (±1.28)	149 (±0.00)	270.67 (±0.47)	121.86 (±0.35)	241.43 (±0.73)	92.29 (±0.45)	124 (±0.00)
Turbidity (NTU)	171.38 (±20.35)	25.98 (±1.69)	237.20 (±3.97)	26.47 (±1.07)	120.40 (±8.01)	16.33 (±0.72)	334.50 (±13.99)	27.13 (±0.40)	122.83 (±12.72)	8.97 (±0.64)	161.43 (±2.61)	15.83 (±0.96)
DO (mgL ⁻¹)	0.18 (±0.15)	2.92 (±0.08)	1.50 (±0.04)	4.94 (±0.11)	0.53 (±0.21)	0.59 (±0.76)	6.82 (±0.15)	10.61 (±0.22)	7.73 (±0.11)	4.72 (±0.17)	5.47 (±0.10)	3.25 (±0.30)
TDS (gL ⁻¹)	0.18 (±0.00)	0.44 (±0.00)	0.16 (±0.00)	0.36 (±0.00)	0.27 (±0.00)	0.36 (±0.00)	0.10 (±0.00)	0.18 (±0.00)	0.08 (±0.00)	0.16 (±0.00)	0.06 (±0.00)	0.03 (±0.00)
Total phosphorous (mgL ⁻¹)	0.16 (±0.01)	1.29 (±0.03)	0.16 (±0.01)	1.17 (±0.02)	0.26 (±0.01)	0.58 (±0.04)	0.08 (±0.01)	0.16 (±0.01)	0.06 (±0.00)	0.17 (±0.00)	0.04 (±0.00)	0.03 (±0.00)
Total nitrogen (mgL ⁻¹)	3.54 (±0.18)	15.27 (±0.33)	3.97 (±0.01)	13.40 (±0.18)	7.02 (±0.17)	14.49 (±0.53)	1.48 (±0.03)	4.90 (±0.15)	1.59 (±0.02)	3.57 (±0.10)	0.85 (±0.02)	0.67 (±0.04)
Chlorophyll-a (µgL ⁻¹)	17.95 (±4.66)	86.73 (±1.43)	14.29 (±1.55)	139.19 (±12.09)	50.27 (±0.00)	96.37 (±3.83)	12.09 (±3.89)	92.03 (±1.42)	6.78 (±0.26)	8.24 (±0.16)	10.26 (±4.14)	1.81 (±0.08)

Note: * Single measurements.

All sampling sites exhibited shallow depths. The minimum was 0.90 m in Capivari River, during the dry period, and the maximum was 7.80 m at Tie (III), during the rainy period. The lowest value of water transparency, 0.15 m, was found at site Tie (II) during the rainy period, and the highest, 1.20 m, at Sorocaba River during the dry period.

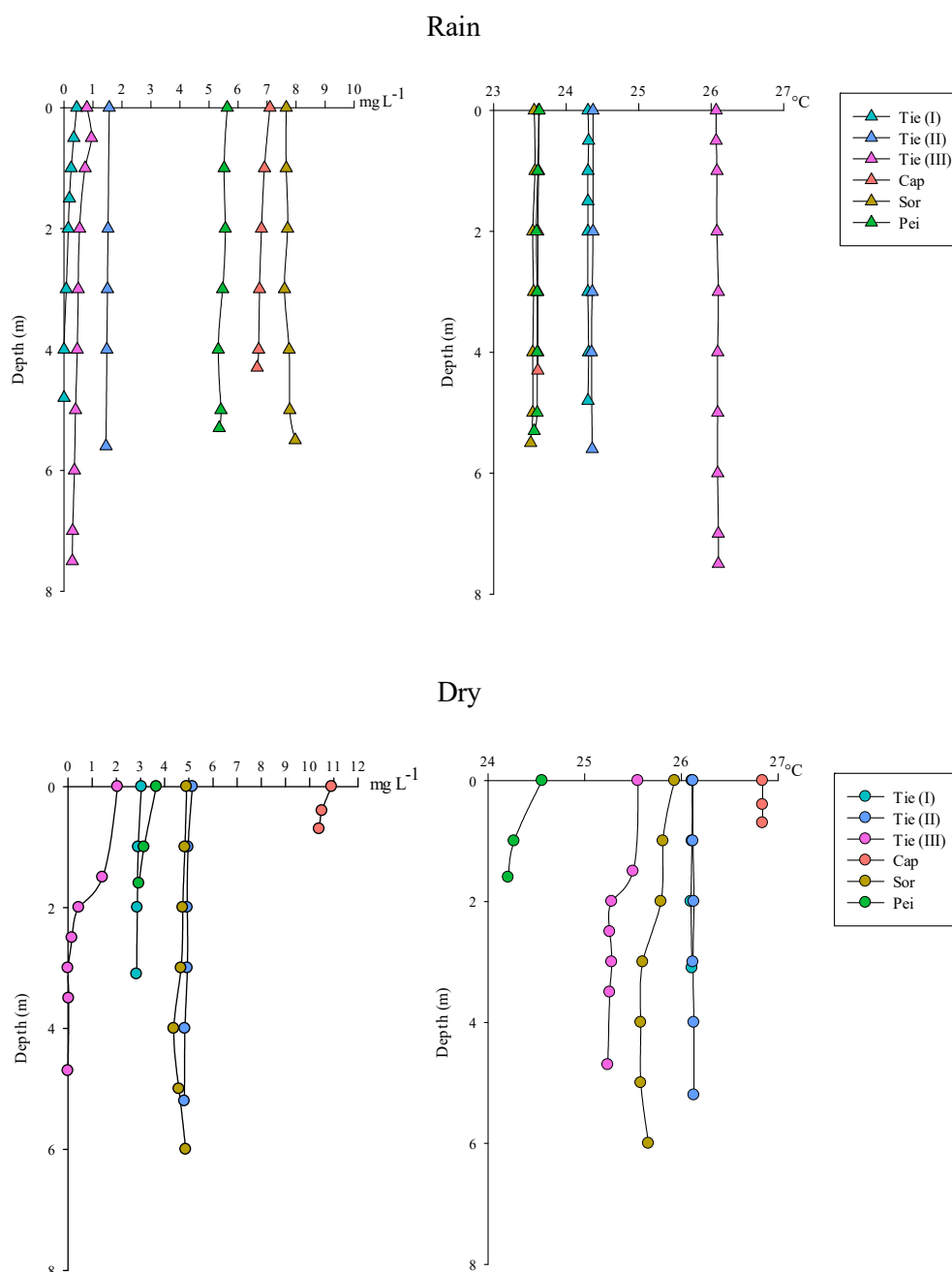


Figure 2. Vertical profiles of dissolved oxygen (**left**) and temperature (**right**) at sampling sites of the Tietê River and tributaries, during the rainy and dry periods.

For turbidity, higher values occurred in the rainy period, exceeding the national standards for Class 2 waters (see Material and Methods item) (maximum of 100 NTU) in all sites. The Capivari River had the highest averages for both periods analyzed, reaching 334.50 NTU and 27.13 NTU in the rainy and dry periods, respectively.

Lower mean pH values (water column) were observed in the rainy period, ranging from 6.21 to 6.80, compared to the dry period, from 6.57 to 7.76. Total dissolved solids varied from 0.06 to 0.27 g L⁻¹ in the rainy period and from 0.08 to 0.44 g L⁻¹ in the dry period. Both parameters were in conformity with the legislation.

The electrical conductivity values were high, or relatively high, in all sampling sites, reaching 690 $\mu\text{S cm}^{-1}$ at Tie (I) during the dry period. The values in this sampling campaign were higher for all sites, compared with measurements taken during the rainy period, when the Peixe River had the lowest value, 92 $\mu\text{S cm}^{-1}$. For ORP, the maximum value of 416 mV

was measured at Tie (II) during the rainy period, and the lowest, 212 mV, at the Capivari River during the dry period. The Tietê River had higher salt concentration than the tributaries, especially in the dry period.

Nutrients, total phosphorus and total nitrogen, exhibited high values in the Tietê River sites during both sampling periods. Maximum concentrations, 1.29 mg L⁻¹ of phosphorus and 15.27 mg L⁻¹ of nitrogen, occurred at Tie (I), the upstream Tietê River sampling site, in the dry period. During this period, higher concentrations of nutrients were observed, except for the Peixe River, which had the lowest values among the analyzed sites: 0.03 mg L⁻¹ of phosphorus and 0.67 mg L⁻¹ of nitrogen. The values for the Peixe River in both periods and for the other tributaries in the rainy period did not exceed the limits established by the legislation.

Higher chlorophyll *a* concentrations were observed in the dry period, except for the Peixe River (1.81 µg L⁻¹) where the value was considerably higher in the rainy season (10.26 µg L⁻¹). Capivari was the only tributary that showed a high chlorophyll concentration, above the reference limits, reaching 92.03 µg L⁻¹ in the dry period. At Tie (III), the values exceeded the limits in both sampling periods, which was also verified at the Tie (I) and Tie (II) sites in the dry period.

The highest average temperatures occurred in the Tietê River sites, from 24.30 °C to 26.09 °C during the rainy period and from 25.34 °C to 26.13 °C during the dry period. The tributaries showed a small variation in temperature during the rainy period, between 23.54 °C and 23.61 °C, compared to the dry period, from 24.35 °C to 26.84 °C.

The Tietê River sites showed extremely low concentrations of dissolved oxygen, tending to zero in the rainy period. A slight increase was observed in the dry period, especially at Tie (II), with a mean value (water column) of 4.94 mg L⁻¹. For both periods, all values in the main river were lower than the minimum established for Class 2 waters (5 mg L⁻¹). For the tributaries, the values measured in the Sorocaba River and Peixe River during the dry period were also below the limits. As expected for shallow rivers, the water column profiles (vertical dimension) for temperature and dissolved oxygen (Figure 2) show homogeneous conditions.

The PCA, based on the environmental variables explained (considering the first two dimensions), 84.4% and 84.7% for the rainy and dry periods, respectively, with a clear difference between the main river and the tributaries (Figure 3). The score values for the first and second PCA dimensions are shown in Table 4.

Table 4. Coefficients of linear correlations between the environmental variables and the first two dimensions of the PCA, for rainy and dry periods, based on the environmental variables measured in the Tietê Rivers and tributaries.

Variable	Rainy		Variable	Dry	
	Dim1	Dim2		Dim1	Dim2
Temperature	0.99286298	0.01413478	Temperature	0.73241485	−0.43456149
pH	−0.09129626	0.79169072	pH	0.86493133	−0.47645186
ORP	−0.06841088	0.61142919	ORP	−0.74714234	0.32708385
Conductivity	0.96290487	0.25353582	Conductivity	0.83829755	0.53407154
Turbidity	−0.45106079	0.71165828	Turbidity	0.85218413	−0.38708075
DO	−0.78753136	−0.36912688	DO	0.15311384	−0.91894482
TDS	0.96267064	0.25462677	TDS	0.83998185	0.53152241
Chlorophyll <i>a</i>	0.95351054	−0.09248894	Chlorophyll <i>a</i>	0.90682814	0.06351206
TP	0.96125535	0.27431054	TP	0.81519443	0.47951963
TN	0.98131456	0.16997972	TN	0.81519397	0.55900840
Depth	0.85914491	−0.34279742	Depth	−0.05614826	0.78493412
Transparency	0.49713473	−0.81801113	Transparency	−0.78653772	0.39283687

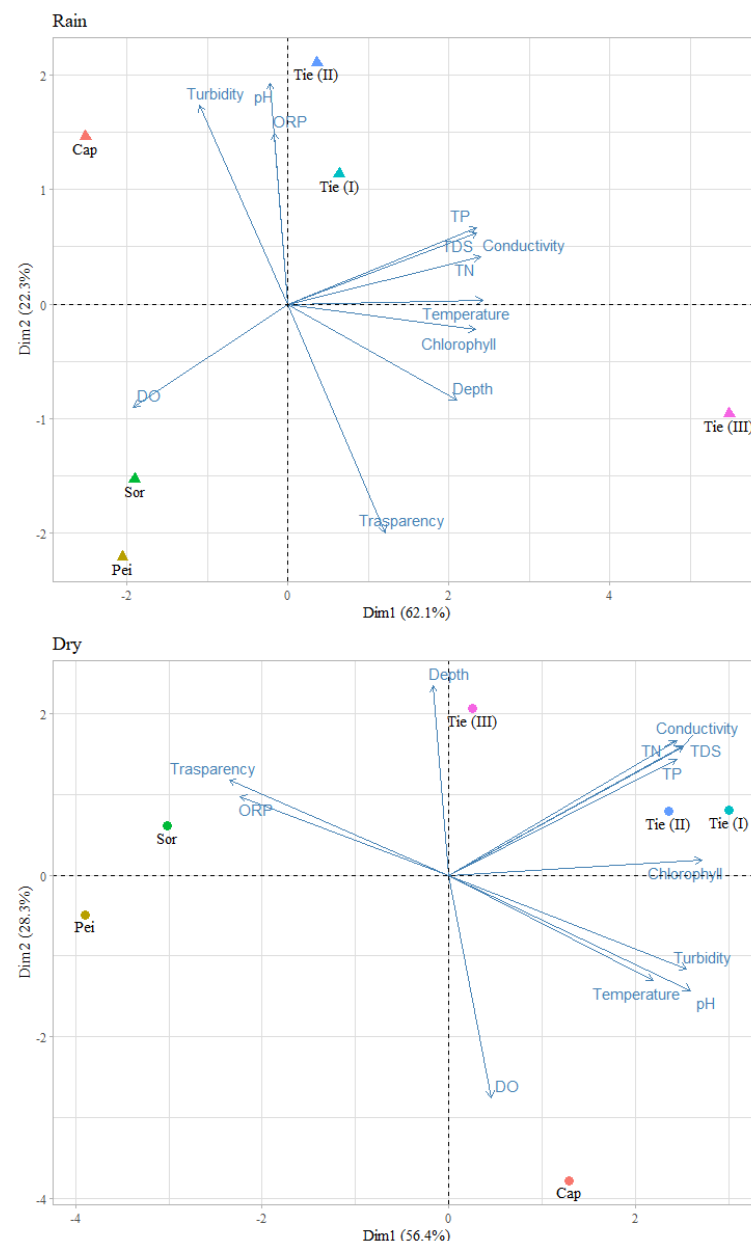


Figure 3. Biplot (Dimensions 1 and 2) representation of the PCA showing the distribution of the sampling sites of Tietê River and tributaries during the rainy and dry periods. DO: dissolved oxygen; TDS: total dissolved solids; ORP: redox potential; TP: total phosphorus; and TN: total nitrogen.

In the rainy season, the positioning of the tributaries, on the left side of Dimension 1, was determined by the higher concentrations of DO. In contrast, the sites of the Tietê River were placed on the right side of Dim1, determined by the lower values of oxygen and higher values of temperature, conductivity, total dissolved solids, chlorophyll, and nutrients (phosphorus and nitrogen). A positive correlation with turbidity explained the positioning of the Capivari River in the superior left quadrant.

This discrimination between the main river and tributaries also occurred in the dry period, with the Tietê sampling points ordinated in the superior right quadrant, positively correlated with conductivity, total dissolved solids, and nutrients, Dim1, in opposition to higher dissolved oxygen values, Dim2.

The trophic status index (TSI) (Table 5) showed a higher degree of eutrophication in the Tietê River compared to the tributaries. Tie (III) was classified as hypereutrophic in both periods. Tie (I) and Tie (II) were supereutrophic in the rainy season and hypereutrophic in

the dry season. The tributaries Capivari and Sorocaba were considered mesotrophic in the rainy period and hypereutrophic and eutrophic, respectively, in the dry period. The Peixe River exhibited the best trophic condition, classified as mesotrophic and ultraoligotrophic in the rainy and dry periods, respectively.

Table 5. Trophic state classification of the Tietê River and tributary sampling points during the rainy and dry periods.

Sites	Rainy		Dry	
	TSI	Classification	TSI	Classification
Tie (I)	64.9	Supereutrophic	87.7	Hypereutrophic
Tie (II)	63.8	Supereutrophic	89.4	Hypereutrophic
Tie (III)	73.5	Hypereutrophic	82.4	Hypereutrophic
Cap	57.5	Mesotrophic	72.9	Hypereutrophic
Sor	52.5	Mesotrophic	61.5	Eutrophic
Pei	52.4	Mesotrophic	40.2	Ultraoligotrophic

All sites, except the Peixe River, showed higher eutrophication in the dry compared to the rainy period.

3.2. Ichthyofauna

Twenty-five fish species were collected, considering the six sampling sites, belonging to 12 families and four orders (Table 6). In the rainy period, only the orders Characiformes and Siluriformes were observed. Characiformes fish were relatively more abundant in the tributaries, corresponding to 100% of the captures for Capivari, 95% for Peixe, and 71% for Sorocaba. Conversely, Siluriformes were more representative in the Tietê River, representing 100% of abundance, except at Tie (II) due to the capture of only a single specimen of Characiformes. In the dry period, in addition to the previously mentioned orders, Cichliformes and Gymnotiformes were also sampled, and the predominance of Siluriformes occurred in all sampling sites, except in the Peixe River, where Characiformes represented 66% of the individuals. Cichliformes were found only in the tributaries.

Table 6. List of ichthyofauna species captured in the Tietê River and tributaries during the rainy (R) and dry (D) periods.

Specie	Tie (I)	Tie (II)	Tie (III)	Cap	Sor	Pei
CHARACIFORMES						
Acestrotrichidae						
<i>Acestrotrichus lacustris</i> (Lütken, 1875)						D
Anostomidae						
<i>Leporinus friderici</i> (Bloch, 1794)					D	R/D
<i>Leporinus lacustris</i> (Amaral Campos, 1945)						R/D
<i>Megaleporinus obtusidens</i> (Valenciennes, 1837)						R
<i>Schizodon intermedius</i> (Garavito and Britski, 1990)						R/D
Characidae						
<i>Astyanax lacustris</i> (Lütken, 1875)				R/D	R/D	R/D
<i>Psilododon schubarti</i> (Britski 1964)				R		
<i>Roeboidea descavadensis</i> (Fowler, 1932)				R/D	R	R/D
Curimatidae						
<i>Cyphocharax nagelii</i> (Steindachner, 1881)					D	R
<i>Steindachnerina insculpta</i> (Fernández-Yépez, 1948)				D	R	
Erythrinidae						
<i>Hoplias malabaricus</i> (Bloch, 1794)					D	R/D
Parodontidae						
<i>Apareiodon</i> sp.				D		
Prochilodontidae						
<i>Prochilodus lineatus</i> (Valenciennes, 1837)	D	R/D	D	R/D	D	R/D
Serrasalminae						
<i>Serrasalmus maculatus</i> (Kner, 1858)						D

Table 6. Cont.

Specie	Tie (I)	Tie (II)	Tie (III)	Cap	Sor	Pei
CICHLIFORMES						
Cichlidae						
<i>Geophagus brasiliensis</i> (Quoy and Gaimard, 1824)				D	D	
<i>Oreochromis niloticus</i> (Linnaeus, 1758)				D		
<i>Saxatilia britskii</i> (Kullander 1982)						D
GYMNOTIFORMES						
Gymnotidae						
<i>Gymnotus cuia</i> (Craig, Malabarba, Crampton and Albert, 2018)			D			D
SILURIFORMES						
Callichthyidae						
<i>Hoplosternum littorale</i> (Hancock, 1828)	R/D	D	R/D	D		R/D
Loricariidae						
<i>Hypostomus ancistroides</i> (Ihering, 1911)		D		D	D	
<i>Hypostomus hermanni</i> (Ihering, 1905)					R/D	
<i>Hypostomus regani</i> (Ihering, 1905)					D	
<i>Hypostomus strigaticeps</i> (Regan, 1908)				D		
<i>Proloricaria prolixa</i> (Isbrücker and Nijssen, 1978)					R/D	/
<i>Pterygoplichthys ambrosettii</i> (Holmberg, 1893)	R		R			
Total richness	3	3	4	11	12	14

Figure 4 shows the relative numerical abundance per order in the distinct sampling sites and periods.

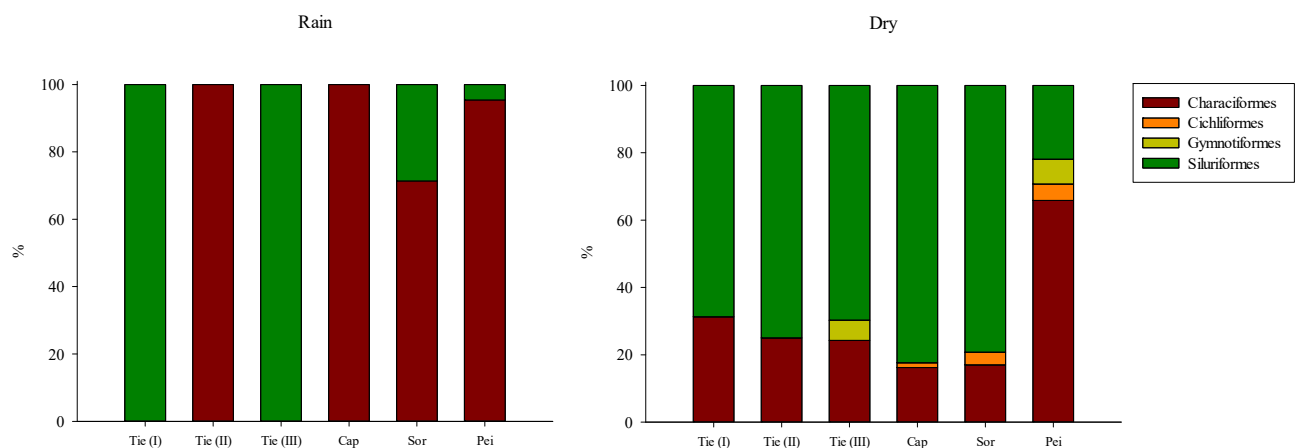


Figure 4. Relative abundance per order of the ichthyofauna in the Tietê River and tributaries, during the rainy and dry periods.

Considering the taxonomical families, we observed the predominance of Callichthyidae in the Tietê River, except at Tie (II) during the rainy period, explained by the capture of only a single representative of Prochilodontidae, *Prochilodus lineatus* (Valenciennes, 1837). In the rainy period, this family was also predominant in the tributary Capivari, corresponding to 61% of the fish abundance. The tributaries Sorocaba and Peixe were better represented by Characidae (57%) and Anostomidae (36%), respectively. In the dry period, Loricariidae represented 79% of the abundance in Sorocaba and 82% in Capivari. In Peixe River, Erythrinidae (29%) was the dominant family. Prochilodontidae was the only family found in all sampling sites, considering both sampling periods (Figure 5).

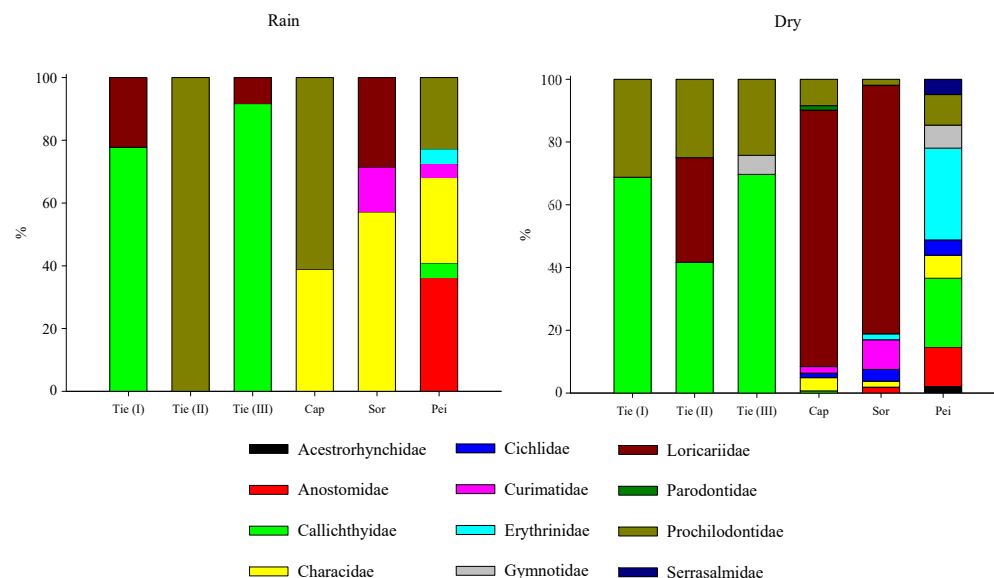


Figure 5. Relative abundance per family of the ichthyofauna in the Tietê River and tributaries during the rainy and dry periods.

The total species richness was higher in the tributary rivers, with 11 species in Capivari, 12 in Sorocaba, and 14 in Peixe, while in the Tietê River, only 3, Tie (I) and Tie (II), and 4 species were found, Tie (III). Three species were only captured in the rainy period, while ten were exclusively found in the dry period. *Prochilodus lineatus* was the only species common to all sampling sites and periods. Species richness was higher in the dry period for all sites, except for Tie (I), where the same richness was observed in both periods (Table 6).

In terms of CPUE (ind./1000 m²/16 h), 947.3 individuals were captured, totalizing a biomass of 183.3 kg (Supplementary Material: Tables SA–SD). In the rainy period, the CPUE_n mean values were higher at Tie (III), 2.6, and for CPUE_b at Capivari, 805.7 g. The lowest mean value in the rainy period, for CPUE_n, was obtained at Tie (II), 0.10, and for CPUE_b at Sorocaba, 45.6 g. In the dry period, the Capivari River exhibited the highest mean values of CPUE_n and CPUE_b, with 14.3 individuals and 2928.1 g, respectively. In the same period, Tie (II) exhibited the lowest mean values for both, with 1.30 (CPUE_n) and 119.69 g (CPUE_b) (Figure 6).

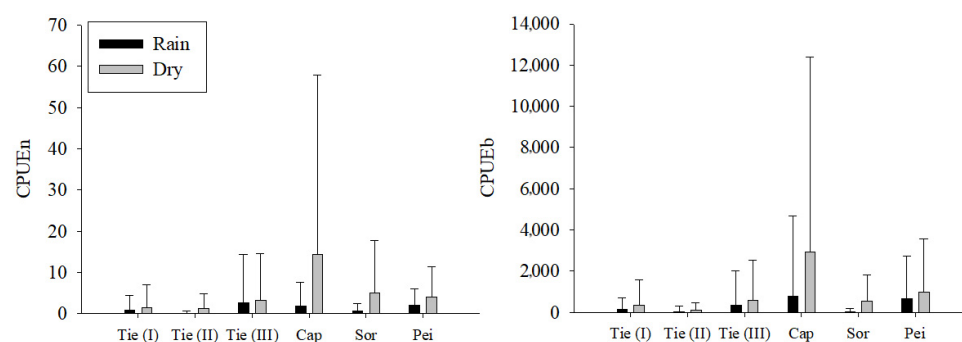


Figure 6. Values (means and standard deviation for the set of nets per site) of catch per unit effort in number of individuals (CPUE_n) and in biomass (g) (CPUE_b) for the sampled ichthyofauna in the Tietê River and tributaries during the rainy and dry period.

The Peixe River showed the highest diversity values with the Shannon-Wiener index (H') (bits ind.⁻¹), for both sampling campaigns, with values of 2.9 and 3.0 in the rainy and dry periods, respectively. The lowest value for the rainy period was found in Tie (II), 0, and for the dry period in Tie (I), 0.90. For Pielou's equitability (J), the Sorocaba River had the

highest value, 0.92, in the rainy period, and Tie (III) the lowest, 0.41. It was not possible to calculate the J value for Tie (II) in the rainy period due to the capture of only a single specimen of fish. In the dry period, Tie (II) had the highest J value, 0.98, and Capivari the lowest, 0.54 (Figure 7).

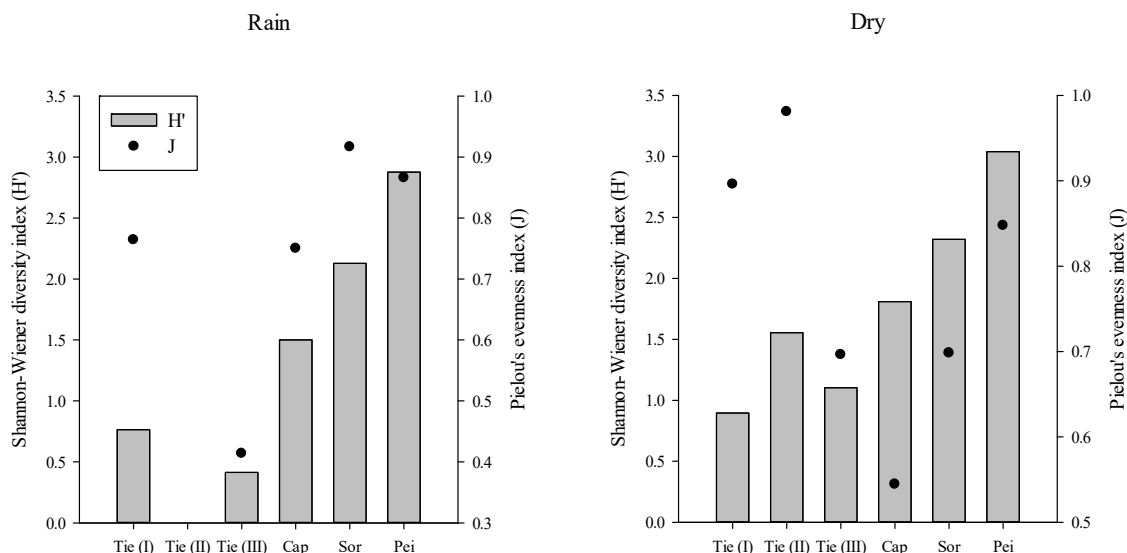


Figure 7. Pielou equability values (J') and Shannon–Wiener index (H') for the ichthyofauna of the Tietê River and tributaries during the rainy and dry periods.

The beta diversity values for the rivers are summarized in Table 7. The composition of species in the rainy period, comparing the sites, did not differ between Tie (I) and Tie (III), but there was a high difference between these environments and the other analyzed sites. Intermediate values of beta diversity were observed between the tributaries Sorocaba and Capivari, 0.56, and between the Peixe and Capivari, 0.57.

Table 7. Ichthyofauna beta diversity (Whittaker) values among the sampling sites of the Tietê River and tributaries during the rainy and dry periods.

		Dry					
	Site	Tie (I)	Tie (II)	Tie (III)	Cap	Sor	Pei
Rainy	Tie (I)	-	0.2	0.2	0.66667	0.83333	0.71429
	Tie (II)	1	-	0.3333	0.53846	0.69231	0.73333
	Tie (III)	0	1	-	0.69231	0.84615	0.6
	Cap	1	0.6	1	-	1	1
	Sor	1	1	1	0.55556	-	0.63636
	Pei	0.8333	0.81818	0.83333	0.57143	0.73333	-

In the dry period, the Tietê River sites showed low beta diversity, ranging from 0.20 to 0.33. The tributaries presented a maximum beta diversity, 1, Sorocaba/Capivari and Peixe/Capivari, or a relatively high beta diversity, 0.64, Sorocaba/Peixe.

Regarding functional diversity, fish were classified into eight different trophic guilds (i.e., piscivore, omnivore, herbivore, invertivore, detritivore, algivore, insectivore, and iliophagous) (Table 8). In the Tietê River, most species were detritivores (50–67%). In the tributaries, eating habits were more diversified, especially for the Peixe River, with a predominance of omnivorous (37%) and piscivorous (21%) species. In the Sorocaba River, there was a high percentage of detritivorous species (42%), but a lower percentage than that found in the main river, while in the Capivari River omnivorous species predominated (46%) (Figure 8).

Table 8. Trophic guilds of the fish species of the Tietê River and tributaries.

Specie	Trophic Guild	Reference(s)
CHARACIFORMES		
Acestrorhynchidae		
<i>Acestrorhynchus lacustris</i> (Lütken, 1875)	Piscivore	[33,34]
Anostomidae		
<i>Leporinus friderici</i> (Bloch, 1794)	Omnivore	[35,36]
<i>Leporinus lacustris</i> (Amaral Campos, 1945)	Omnivore	[36]
<i>Megaleporinus obtusidens</i> (Valenciennes, 1837)	Omnivore	[37]
<i>Schizodon intermedius</i> (Garavello and Britski, 1990)	Herbivore	[38]
Characidae		
<i>Astyanax lacustris</i> (Lütken, 1875)	Omnivore	[37]
<i>Psalidodon schubarti</i> (Britski 1964)	Omnivore	[30]
<i>Roeboides descalvadensis</i> (Fowler, 1932)	Invertivore	[33]
Curimatidae		
<i>Cyphocharax nagelii</i> (Steindachner, 1881)	Iliophagous	[39]
<i>Steindachnerina insculpta</i> (Fernández-Yépez, 1948)	Detritivore	[33]
Erythrinidae		
<i>Hoplias malabaricus</i> (Bloch, 1794)	Piscivore	[35–37,40,41]
Parodontidae		
<i>Apareiodon</i> sp. *	Algivore	[37]
Prochilodontidae		
<i>Prochilodus lineatus</i> (Valenciennes, 1837)	Detritivore	[33,36]
Serrasalminidae		
<i>Serrasalmus maculatus</i> (Kner, 1858)	Piscivore	[33,36,40]
CICHLIFORMES		
Cichlidae		
<i>Geophagus brasiliensis</i> (Quoy and Gaimard, 1824)	Omnivore	[34,41]
<i>Oreochromis niloticus</i> (Linnaeus, 1758)	Omnivore	[37,42]
<i>Saxatilia britskii</i> (Kullander 1982)	Insectivore	[43]
GYMNOTIFORMES		
Gymnotidae		
<i>Gymnotus cuia</i> (Craig, Malabarba, Crampton and Albert, 2018) *	Invertivore	[43]
SILURIFORMES		
Callichthyidae		
<i>Hoplosternum littorale</i> (Hancock, 1828)	Omnivore	[42]
Loricariidae		
<i>Hypostomus ancistroides</i> (Ihering, 1911)	Detritivore	[43,44]
<i>Hypostomus hermanni</i> (Ihering, 1905)	Detritivore	[43]
<i>Hypostomus regani</i> (Ihering, 1905)	Detritivore	[43]
<i>Hypostomus strigaticeps</i> (Regan, 1908)	Detritivore	[43,44]
<i>Proloricaria prolixa</i> (Isbrücker and Nijssen, 1978)	Herbivore	[43]
<i>Pterygoplichthys ambrosettii</i> (Holmberg, 1893)	Detritivore	[33]

Note: * Trophic guild for the genus.

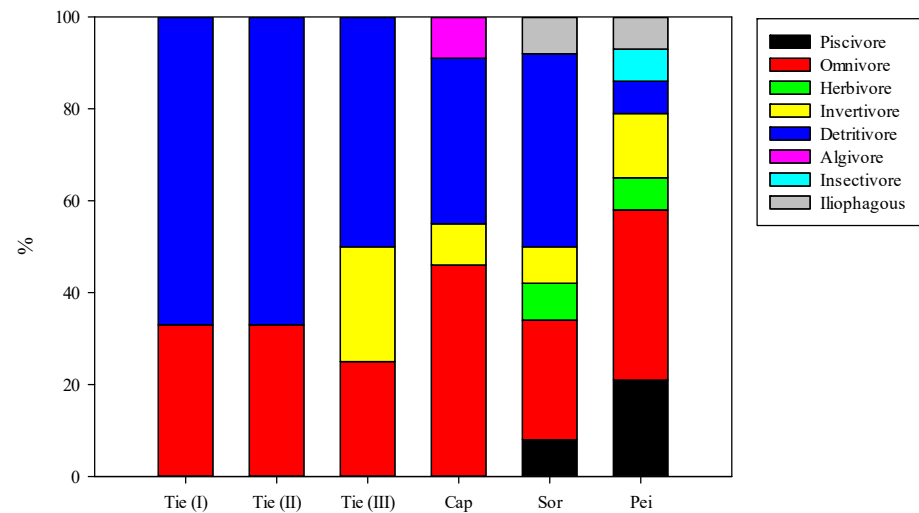


Figure 8. Relative abundance of the trophic guilds of the fish species of Tietê River and tributaries.

The plotted results of the nMDS analysis show a clear difference between the ichthyofauna of the main river compared to the tributaries (Figure 9), with very few species directly associated with the Tietê. Differences between the sampling periods were not evident.

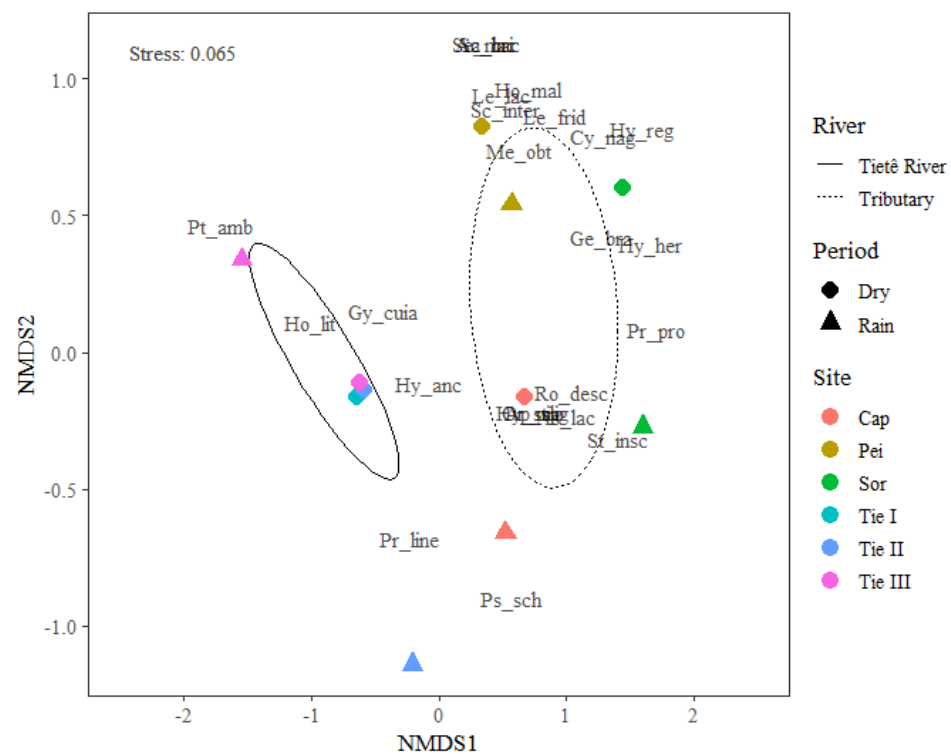


Figure 9. Non-metric multidimensional scaling (nMDS) for the ichthyofauna of the Tietê River and tributaries during the rainy and dry periods. *Ac_lac*: *Acestrorhynchus lacustris*; *Le_frid*: *Leporinus friderici*; *Le_lac*: *Leporinus lacustris* Amaral; *Me_obt*: *Megaleporinus obtusidens*; *Sc_inter*: *Schizodon intermedius*; *As_lac*: *Astyanax lacustris*; *Ps_sch*: *Psalidodon schubarti*; *Ro_desc*: *Roeboides descavadensis*; *Cy_nag*: *Cyphocharax nagelii*; *St_insc*: *Steindachnerina insculpta*; *Ho_mal*: *Hoplias malabaricus*; *Ap_sp*: *Apareiodon* sp.; *Pr_line*: *Prochilodus lineatus*; *Se_mac*: *Serrasalmus maculatus*; *Ge_bra*: *Geophagus brasiliensis*; *Or_nilo*: *Oreochromis niloticus*; *Sa_bri*: *Saxatilia britskii*; *Gy_cuia*: *Gymnotus cuia*; *Ho_lit*: *Hoplosternum littorale*; *Hy_anc*: *Hypostomus ancistroides*; *Hy_her*: *Hypostomus hermanni*; *Hy_reg*: *Hypostomus regani*; *Hy_strig*: *Hypostomus strigaticeps*; *Pr_pro*: *Proloricaria proluxa*; and *Pt_amb*: *Pterygoplichthys ambrosettii*.

3.3. Influence of Environmental Variables on Fish Assemblage Structure

The DistLM analysis indicated significant relationships ($p < 0.05$) between the structure of the fish assemblages and the environmental variables, but only for the rainy period. Dissolved oxygen (DO) contributed with the highest percentage of explained variance (48.1%), followed by TDS (41.0%), conductivity (40.1%), total phosphorus (TP) (40.0%) chlorophyll *a* (36.3%), and temperature (33.9%) (Table 9).

Table 9. Values of Pseudo-F, coefficient of significance (p), and proportion of explanation (Prop. %) obtained by DistLM analysis between environmental variables and fish assemblage structure (abundance per species per site) for the Tietê River and tributaries, during rainy and dry periods. In bold, the significant variables ($p < 0.05$).

Variable	Rainy			Dry		
	Pseudo-F	p	Prop. %	Pseudo-F	p	Prop. %
Temperature	2.0507	0.032	33.89	1.1573	0.391	22.44
pH	0.30005	0.937	6.98	1.3611	0.271	25.39
ORP	1.5062	0.124	27.35	0.90929	0.573	18.52
Conductivity	2.6735	0.039	40.06	1.7201	0.16	30.07
Turbidity	1.0828	0.401	21.30	1.7254	0.142	30.14
DO	3.7083	0.006	48.11	1.5284	0.23	27.65
TDS	2.7779	0.017	40.99	2.0837	0.118	34.25
Chlorophyll <i>a</i>	2.2769	0.017	36.28	1.5465	0.176	27.88
TP	2.6657	0.046	39.99	1.9401	0.096	32.66
TN	2.4214	0.071	37.71	1.5972	0.201	28.54

4. Discussion

4.1. Environmental Conditions

The Tietê River receives, daily, a significant amount of domestic and industrial effluents from the largest Brazilian megalopolis, the city of São Paulo, with an enormous and well-known negative impact on the water quality [15,45]. Our results demonstrated that even 300 km downstream from the major sources of contamination, a severe eutrophic condition still persists.

The principal component analysis (PCA) explained satisfactorily the environmental data variance (>84% for both seasons) and evidenced, graphically, the distinctiveness of the Tietê River in relation to the tributaries. The Tietê sites' positioning was determined by the higher values of temperature, conductivity, total dissolved solids, chlorophyll, and nutrients (phosphorus and nitrogen) found in the rainy season. In the dry season, these sites correlated with conductivity, total dissolved solids, and nutrients.

The analyzed tributaries are less impacted by urban centers, and their courses flow predominantly along typical countryside landscapes. Particularly, the Peixe River has no city located in its watershed.

An eventual longitudinal dilution process could be expected, due to the entrance of the tributaries. Despite their better water quality conditions, the volumes (tributary water flow) are probably insufficient to cause a significant positive effect on the Tietê River. However, this is not a reason to neglect the potential contribution of the small and middle-size tributaries widely distributed along the Tietê River basin [46,47]. This process is especially important for observation in the near future, due to the present trend of sanitation measures implementation (sewage treatments plants) all over São Paulo State, after decades without effective actions.

Concentrations of dissolved oxygen, total phosphorus, and total nitrogen in the Tietê River, during both rainy and dry periods, were not in conformity with the reference values of the Brazilian legislation. Turbidity, in the rainy season, and chlorophyll, in the dry season, also exceed the established limits. For the tributaries, turbidity was very high (above the limit) in the rainy season, indicating intensive soil erosion and a reduced riparian forest in the three sub-basins. In the dry season, oxygen was below the limit in

Sorocaba and Peixe and chlorophyll and nutrients (TP, TN) above the limits in Capivari and Sorocaba, respectively.

From a temporal series of ten years (2007–2017), as part of the environmental monitoring program carried out in the Barra Bonita hydropower reservoir, a high level of non-conformities for total phosphorus, dissolved oxygen, thermotolerant coliforms, and biochemical demand of oxygen was found [18]. This reservoir is located in the Tietê River, immediately downstream of our study area. Another study along the middle and lower stretches of the Tietê River also observed high concentrations of nutrients, as well as of heavy metals (cadmium, chromium, copper, and zinc) [17]. These authors performed tests (bioassays) with water and sediment samples that revealed acute toxicity for zooplankton and chronic toxicity for zooplankton and fish species.

Nutrient loads favor the primary productivity in rivers and this explains the high levels of eutrophication indicated by the trophic state index [19]. Using data of total phosphorus and chlorophyll, the Tietê River sampling sites varied from supereutrophic to hypereutrophic. The trophic conditions increased in the dry period, indicating that rainfall may act as a dilution factor for nutrients and phytoplankton biomass. A seasonal trend of trophy was unclear for the tributaries: all classified as mesotrophic in the rainy period and from ultraoligotrophic to hypertrophic in the dry period. It is worth mentioning that the downstream site of the Tietê River (Tie III) was classified as hypereutrophic in both seasons, demonstrating that an expected longitudinal improvement does not occur, which was corroborated by the DO values. In this case, the temporal variation was different, getting worse in the rainy season, when concentrations were even lower in the three Tietê sites (water column means from 0.18 to 1.50 mg L⁻¹). A considerable DO decrease in the rainy season may be associated with previous (a few days earlier) storm events on the São Paulo metropolis, resulting in the downstream displacement of a highly polluted water mass. In another emblematic case of contamination in South America, Riachuelo River in the Buenos Aires city area, a study using a statistical approach analysis on temporal variation (eight consecutive years) of six water quality variables observed that increasing storm water runoff may have determined the observed trends (e.g., non-biodegradable organic matter) [48].

4.2. Ichthyofauna

The Tietê River fish fauna has been deeply affected (e.g., disappearance of migrant carnivorous species) by human actions since the nineteenth century, with the incidental and deliberated introduction of non-native species, damming for hydropower generation, fisheries, and eutrophication [11].

Our study, focused on the effects of eutrophication, corroborated the initial hypothesis, showing considerable differences in the composition of the ichthyofauna when comparing the main river (Tietê) with the tributaries (Sorocaba, Capivari, and Peixe). This was clearly evidenced through analyses such as nMDS and Whittaker's beta diversity.

The number of captured species in the Tietê was very low. Considering the two seasonal periods (rainy and dry), the species richness was much higher in the tributaries, 11 to 14, compared to the Tietê River, only 3 to 4. A good correspondence occurred between the number of species and the values of the Shannon–Wiener index.

In a study carried out in the late 1990s [20], with sampling performed throughout the Tietê basin, the dominance of only one or two species was seen in the most polluted stretches; in fact, in stretches coincident with our study area. Probably, at that time the river condition was even worse in terms of water quality [49], as many sewage treatment plants have started to operate over the last three decades, or some other species have become further adapted to the ecosystem degradation.

An explanation for the very low number of species captured in the Tietê River main channel is that during episodes of poor water quality conditions, such as anoxia in the whole water column, fish may search for refuge in the marginal lagoons, which we did not sample. Local anglers (personal communication) frequently report this behavior. An

investigation in the Sorocaba River, next to the urban area of Sorocaba city during the early 1990s (at that time with ~500,000 inhabitants), when most industrial and domestic sewage was discharged into the river almost with no treatment, has already reported that the lagoons were an escape for fish from the river pollution [49]. The lagoons exhibited more stable conditions, including higher oxygen concentrations.

The capture method is also an important factor that influences the richness results. In our study, we employed gillnets, which are effective for sampling the fish fauna that explores the main river channels, but have limited efficacy for small species living in marginal habitats.

In terms of feeding habits, we observed that most species in the Tietê River were detritivores, while in the tributaries the fish have a higher variety of food resources. Evaluation through isotopic signatures demonstrated that different fish trophic guilds change their diets in degraded environments and the detritivores, particularly, exhibit even higher trophic plasticity [50].

It is worth mentioning that *P. lineatus*, a detritivorous fish, was the only species found in all studied sites. Its distribution evidences a wide resilience to the observed contrasting water quality conditions, including the ingestion of considerable amounts of microplastics [51]. *Prochilodus lineatus* is an abundant species in the middle Tietê River basin, representing the main fishery resource of the region, with a socio-economics relevance. As a detritivorous/iliophagous (bottom-dwelling) fish, it feeds on detritus and other organisms that live in the mud deposited on the riverbed [20]. Certainly, it benefits from the large amount of organic matter (including huge populations of tolerant macroinvertebrates) present in the substrate, originating from untreated or partially treated organic sewage. A comparative analysis of the condition factor based on the weight/length relationship showed a better performance (feeding efficiency) of *P. lineatus* in the highly eutrophic Tietê River than in the Peixe River [52]—as already mentioned, a tributary not affected by human settlements. It is also important to consider that as a long-distance migrator, i.e., an efficient swimmer, it can escape from sudden decreases in dissolved oxygen, moving into marginal lagoons and tributaries.

Other important detritivorous fish in the Tietê River were the Siluriformes *Hypostomus* spp., *H. littorale*, and *P. ambrosetti*. These fish are also known for their high tolerance to poor environmental conditions, such as anoxia or hypoxia, as they have accessory breathing mechanisms [49,53].

In terms of fish abundance, values in the tributary Capivari during the dry period, CPUEn and CPUEb, were remarkably high. This was due to the capture of more than 100 specimens of *Hypostomus strigaticeps* and *Hypostomus ancistroides*. On that occasion, when these two Loricariidae corresponded to approximately 80% of the fish dominance, this river was classified as hypereutrophic due to a very high value of chlorophyll concentration ($100 \mu\text{g L}^{-1}$).

Correlation between the structure of fish communities and environmental variables was significant for the rainy season. As previously discussed, during this period, the concentration of dissolved oxygen reached zero or close to zero in the Tietê River, while in the tributaries values were higher than 5 mg L^{-1} . In recurrent situations like that, only hypoxic-tolerant species survive (or remain) in the main river channel. Oxygen is a key variable, which contributed to the highest percentage of variance shown by the DistLM analysis (48.11%). In a long-term analysis carried out in a river with a long history of environmental degradation, followed by effective mitigation measures, the Seine (France), the level of tolerance to low oxygen values was considered a determinant factor for the recolonization of some fish species [54].

4.3. Final Considerations

The excessive eutrophication of the Tietê River acts as a chemical barrier to the dispersion of sensitive, or less tolerant, fish species. The concept of a chemical barrier applied to fish distribution has been previously considered, based on either laboratory studies [55] or literature reviews [54,56]. Our study addresses this problem directly in the field.

Our data evidence that the considered 40 km stretch of the Tietê River is highly degraded in terms of water quality, without any signals of resilience to the destructive impact caused by the huge loads of pollution it receives continuously. The official Class 2 classification is far from the river's present condition, with recurrent non-conformities in relation to the standard limits, and should be revised by the authorities.

Detritivorous feeding habits, especially when associated with accessory breathing or efficient swimming capability, is an essential fish characteristic that assures resilience and adaptability for living in degraded environments, such as the Tietê River.

Conservation of the tributaries is fundamental, given their higher diversity. That is the best guarantee of future recolonization processes in the basin, as long as restoration (urgently required) becomes effective in the main river.

Future studies are necessary for a better comprehension of the impact on the local and regional distribution of other biological communities. Temporal variability should also be addressed, considering both seasonality, alternation between rainy and dry periods, and the almost-instantaneous effects of random displacements of heavily polluted water masses (upstream storm events in the São Paulo metropolis).

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/w16101383/s1>.

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