UNIVERSIDADE ESTADUAL PAULISTA “JÚLIO DE MESQUITA FILHO”
INSTITUTO DE BIOCIÊNCIAS DE BOTUCATU
DEPARTAMENTO DE BIODIVERSIDADE E BIOESTATÍSTICA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS
ÁREA DE CONCENTRAÇÃO: ZOOLOGIA

TESE DE DOUTORADO

Barreira química para a dispersão da ictiofauna: uma análise do rio Tietê e seus tributários

Bruna Quirici Urbanski

BOTUCATU
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Tese apresentada ao Instituto de Biociências da Universidade Estadual Paulista “Júlio de Mesquita Filho” – *campus* de Botucatu, como parte dos requisitos para obtenção do título de Doutor (a) em Ciências Biológicas (Zoologia).

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Coorientador: Dr. Fábio Fernandes Roxo

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Bruna Quirici Urbanski

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Prof. Dr. James Raúl García Ayala
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Sumário

Apresentação

A presente tese, intitulada "Barreira química para a dispersão da ictiofauna: uma análise do rio Tietê e seus tributários", foi desenvolvida com o objetivo de avaliar as limitações que ocorrem no processo de dispersão da ictiofauna da bacia do rio Tietê, em decorrência da poluição das águas. Para tanto, o estudo foi dividido em dois eixos distintos, porém complementares, organizados na forma de capítulos.

Em cada eixo, foram utilizadas diferentes estratégias metodológicas, que incluem análises ecológicas tradicionais e técnicas contemporâneas de genética molecular, de modo a ampliar as possibilidades de atingir o objetivo geral proposto. Como os temas dos capítulos estão razoavelmente conectados, algumas repetições são inevitáveis.

Para o desenvolvimento do presente estudo, selecionou-se uma área de trabalho que se localiza no trecho médio da bacia hidrográfica que inclui, além do rio Tietê, importantes tributários diretos como o rio Capivari (margem direita) e os rios Sorocaba e do Peixe (ambos da margem esquerda). Essa região é caracterizada pela existência de uma ampla conexão hidrográfica, ou seja, ausência de quaisquer barreiras físicas naturais (*e.g.* saltos e corredeiras) ou artificiais (*e.g.* barragens e reservatórios) que poderiam dificultar a potencial movimentação dos organismos entre os rios. Além disso, os ambientes considerados são contrastantes em termos de degradação das águas, com evidente deterioração do rio principal e melhor condição dos demais.

No primeiro capítulo, de mesmo nome da tese ("Barreira química para a dispersão da ictiofauna: uma análise do rio Tietê e seus tributários"), foram analisadas as diferenças na estrutura das assembleias de peixes por meio do levantamento da composição de espécies e demais atributos ecológicos, bem como sua correlação com as distintas condições limnológicas dos ambientes de estudo.

Já no segundo capítulo, intitulado "Análise preliminar da estrutura populacional do peixe migrador *Prochilodus lineatus* (Characiformes: Prochilodontidae) em ambientes contrastantes em qualidade da água da bacia do médio rio Tietê, Sudeste do Brasil", buscamos, por meio de técnicas de sequenciamento da região controle do DNA mitocondrial (D-loop), avaliar preliminarmente, em função do tamanho amostral restrito e dificuldades metodológicas enfrentadas, a possível existência de estruturação populacional de uma espécie-alvo de peixe amplamente distribuída na região e com grande capacidade de dispersão – o curimbatá, e relacionar as divergências genéticas, se encontradas, com as distintas condições ambientais dos rios analisados.

Por fim, análises biométricas e do conteúdo estomacal de exemplares capturados para a pesquisa resultaram em dois artigos publicados[1]. Nestes, reportamos a relação peso-comprimento e fator de condição para *Prochilodus lineatus* (Valenciennes, 1837), bem como a ingestão de plástico por essa espécie, comparando-se o rio Tietê com um dos seus tributários, menos poluído, rio do Peixe, evidenciando dessa forma mais um dos impactos da eutrofização/poluição sobre a ictiofauna.

Esperamos que os resultados apresentados nesse trabalho possam contribuir para o desenvolvimento de propostas de monitoramento e gestão ambiental, com foco na preservação dos recursos hídricos e da ictiofauna regional.

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[1]URBANSKI, B.Q., DENADAI, A.C., AZEVEDO-SANTOS, V.M., NOGUEIRA, M.G. First record of plastic ingestion by an important commercial native fish (*Prochilodus lineatus*) in the middle Tietê River basin, Southeast Brazil. Biota Neotropica 20(3): e20201005. https://doi.org/10.1590/1676-0611-BN-2020-1005

URBANSKI, B.Q., BRAMBILLA, E.M., NOGUEIRA, M.G. Length-weight relationship and condition factor for Prochilodus lineatus, an important commercial fish, in contrasting water-quality environments of the middle Tietê River basin, Southeast Brazil. Biota Neotropica 23(2): e20231467. https://doi.org/10.1590/1676-0611-BN-2023-1467

Barreira química para a dispersão da ictiofauna: uma análise do rio Tietê e seus tributários

Capítulo I

Chemical barrier for the dispersion of ichthyofauna: an analysis of the Tietê River and its tributaries

Resumo

Ao longo do tempo, barreiras artificiais para a dispersão das espécies surgiram como consequência do crescimento populacional e do desenvolvimento econômico da sociedade. No caso dos ambientes aquáticos, sabe-se que diferentes tipos de distúrbios antrópicos podem influenciar nesse processo, sendo a poluição um importante fator modificador da estruturação da biota regional, principalmente da ictiofauna. A bacia do rio Tietê, que possui um longo histórico de degradação ambiental, apresenta, em seu trecho médio, ambientes contrastantes em termos de qualidade de água. Contudo, estes sistemas fluviais estão amplamente interligados, sem quaisquer barreiras físicas naturais ou artificiais que possam interferir na livre circulação dos organismos. Nesse contexto, o objetivo deste trabalho foi avaliar as limitações que ocorrem no processo de dispersão da fauna de peixes dessa bacia, entre o rio Tietê e seus afluentes diretos (rio Sorocaba, rio Capivari e rio do Peixe), devido à poluição das águas do rio principal. O estudo se baseou em inventários da comunidade íctica por meio de redes de espera, seus atributos ecológicos e na determinação simultânea das variáveis limnológicas no final das estações chuvosa (abril de 2019) e seca (outubro de 2019). Para ambos os períodos de amostragem, a análise de componentes principais (ACP) mostrou uma clara diferença na qualidade da água do rio principal (pior condição) em comparação com os afluentes (melhor condição). Essa diferença também foi evidenciada através do nMDS baseado na composição da ictiofauna dos rios, com a diversidade de espécies muito maior nos tributários (11 a 14 spp.) em relação ao rio Tietê (3 a 4 spp.). A análise do DistLM mostrou que existe uma correlação significativa entre as variáveis limnológicas e a estrutura da ictiofauna apenas na estação chuvosa, o que pode ser explicado pela diferença marcante nas condições ambientais, especialmente neste período. Os resultados mostram que os efeitos da poluição atuam como uma barreira química para a dispersão de organismos sensíveis, ou menos tolerantes, e demonstram a urgência de implementar medidas de mitigação da poluição na bacia do Rio Tietê.

Palavras-chave: Barreira ecológica, Degradação ambiental, Dispersão dos peixes, Poluição das águas.

Abstract

Over time, artificial barriers to species dispersal have emerged as a consequence of population growth and the economic development of society. In the case of aquatic environments, it is known that different types of anthropic disturbances can influence this process, with pollution being an important modifying factor for the structuring of the regional biota, especially the ichthyofauna. The Tietê River basin, which has a long history of environmental degradation, exhibits, in its middle section, contrasting environments in terms of water quality. However, these river systems are widely interconnected, without any natural or artificial physical barriers that could interfere with the free movement of the organisms. In this context, the goal of this work was to evaluate the limitations that occur in the process of dispersal of the fish fauna of this basin, between the Tietê River and its direct tributaries (Sorocaba River, Capivari River, and Peixe River), due to the water pollution of the main river. The study was based on the fish community's inventories through gillnets, their ecological attributes, and the simultaneous determination of limnological variables, during late rainy (April 2019) and dry seasons (October 2019). For both sampling periods, the Principal Component Analysis (PCA) showed a clear difference in the water quality of the main river (worst condition) compared to the tributaries (better condition). This difference was also evidenced through the nMDS based on the composition of the rivers' ichthyofauna, with a much higher species diversity in the tributaries (11 to 14 spp.) compared to the Tietê River (3 to 4 spp.). The DistLM analysis showed that there is a significant correlation between the limnological variables and the structure of the ichthyofauna only in the rainy season, which can be explained by the marked difference in the environmental conditions, especially during this period. The results show that the pollution effects act as a chemical barrier to the dispersal of sensitive, or less tolerant, organisms and demonstrate the urgency of implementing pollution mitigation measures in the Tietê River basin.

Keywords: Ecological barrier, Environmental degradation, Fish dispersal, Water pollution.

Introduction

Dispersal plays a fundamental role in the maintenance of the structure and functioning of the populations and, consequently, of the species they represent, assuring the genetic flow over space and time (Ronce, 2007; Gibbs et al., 2010; Radinger & Wolter, 2014). This process is related to the ability of the biota to transpose barriers and move among distinct habitats in response to internal (e.g. genetic and physiological) and external signals (e.g. light, temperature, water quality, predation pressure) (Lucas & Baras, 2001), being one of the main explanatory models of the patterns of distribution of organisms on planet (Miranda & Marques, 2011).

In the current context of massive habitat degradation, understanding how, where, and why organisms disperse is extremely important (Gibbs et al., 2010; Radinger & Wolter, 2014). Numerous artificial barriers to dispersion have emerged as a result of the significant population growth and economic development of society. In the case of fluvial aquatic environments, the construction of hydroelectric dams, for instance, is a major interference for the free movement of the biota, especially fish (Vianna & Nogueira, 2008; Agostinho et al., 2008; Pelicice et al., 2014, Smith et al., 2018). However, other types of disturbances can also affect the distribution of organisms and other ecological processes related to the different components of these ecosystems (Jacquin et al., 2020).

Despite many studies, hypotheses, and theories that predict the structure of communities in this complex scenario of changes induced by man, we could not find detailed evaluations on the influence of the decline in water quality due to pollution on the dispersion of ichthyofauna.

The Tietê River is an emblematic example, worldwide, of excessive environmental degradation, particularly in its upper section that crosses the metropolitan region of São Paulo, the most populous in Brazil (about 21.9 million inhabitants) (IBGE, 2022). According to Tundisi (2018), the history of water quality deterioration in this river began with organic contamination in the mid-nineteenth century. Nevertheless, along the twentieth century, in addition to high loads of nutrients, it moved to a more complex scenario, with a considerable increase in inorganic contamination due to the introduction of a myriad of dissolved substances, such as pesticides, herbicides, medicines, and cosmetics, which constitute significant threats to water security due to the potential adverse effects on human health.

Recently, technical and scientific studies have tried to understand the complex degradation process of the Tietê River (Buckeridge & Ribeiro, 2018). However, the discussion is still restricted, predominantly focusing on the São Paulo metropolitan area. Nevertheless, due to the magnitude of the impact, the problem is relevant not only locally, transcending to a regional spatial scale, spreading vigorously to the interior of the São Paulo State.

In a long-term study, which included data from the end of the 1970s to the beginning of the 2000s, Tundisi et al. (2008) identified a clear trend of water quality deterioration in the Barra Bonita reservoir, more than 300 km downstream of São Paulo city. The electrical conductivity of water, for instance, increased from 103 to 370 μScm^{-1}; in terms of phosphorus concentration, from 15 to 40 μgL^{-1}; nitrate from 200 to 3,480 μgL^{-1} and ammonium from 16 to 232 μgL^{-1}. The authors also highlighted the increased potential for toxicity associated with recurrent cyanobacterial blooms.

The extraordinary increase of nutrients introduced in the Tietê River intensified the eutrophication process. This, in turn, leads to a rapid change in the basic levels of the aquatic food chains (magnification in the biomass of phyto- and zooplankton-tolerant species), which, through transference processes, directly and indirectly, affects 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 regional ichthyofauna (Barrella & Petrere, 2003; Dorgham, 2014). The consequences of this type of interference reflect in the composition and other structural characteristics of fish assemblages, leading to a reduction in the size of most populations and their reproductive performance, and in a decrease of species richness and diversity when compared to the original ecosystems (Agostinho et al., 1992; Barrella & Petrere, 2003; Dorgham, 2014).

In this context, the objective of this study was to evaluate the limitations that occur in the dispersion of ichthyofauna in the middle Tietê River basin (SE Brazil) due to the water pollution. The selected study area includes, in addition to this main river, the tributaries Capivari (right bank), Sorocaba, and Peixe (both on the left bank). Such environments have a wide physical connection with each other, without any natural (e.g. falls and rapids) or artificial (e.g. dams and reservoirs) barriers that could interfere in the free movement of the organisms. However, they exhibit contrasting environmental conditions, with evident deterioration of the main river and better condition of the others.

The main hypothesis of this work is that there is a difference in the composition and structure of the local ichthyofauna, determined by contrasting patterns of water quality that act as a chemical barrier for the dispersion of species on a regional scale.

Material and Methods

The study area is located in the middle Tietê River basin, between the municipalities of Laranjal Paulista and Anhembi, São Paulo State, Southeast Brazil. The fluvial system of this region are characterized by a wide physical connectivity, without any natural or artificial barriers (e.g. falls, dams), between the main river, Tietê, and its direct tributaries (Fig. 1).

Sampling was conducted at the end of the rainy period (April 2019) and the end of the dry period (October 2019) at six selected sites (Fig. 1; Tab. 1). Three sites were distributed in the Tietê River, one further downstream, below the mouth of the Peixe River (upstream of the Barra Bonita Reservoir); one further upstream, above the mouth of the Sorocaba and Capivari Rivers; and one site in the middle section. In addition, three sites corresponded to the lower reaches of the main tributaries, one in the Peixe River, one in the Sorocaba River, and one in the Capivari River. The length of the considered section of the Tietê River is approximately 40 km, and the positioning of the sampling points in this river was chosen to verify the possible influence of the tributary inputs.

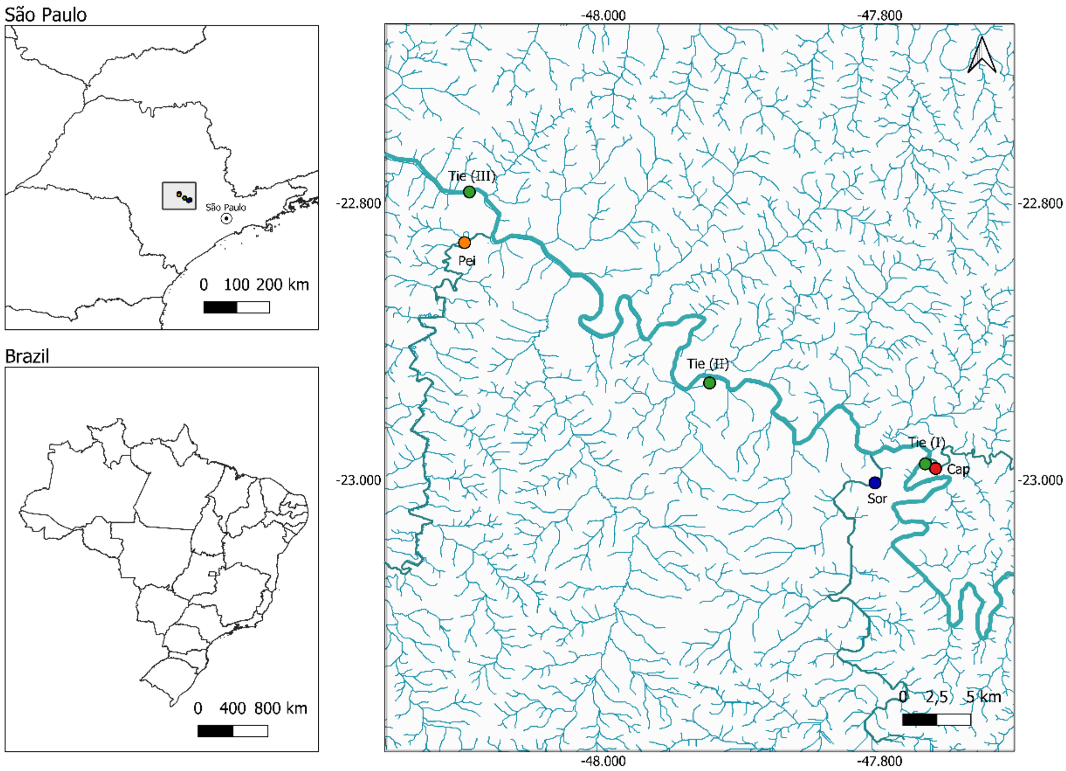

Fig. 1. Distribution of sampling sites (on the right) in the Tietê River (green circles), Sorocaba River (blue circle), Capivari River (red circle), and Peixe River (orange circle). The highlighted region (upper left), in gray, corresponds to the study area, in the center of São Paulo State, Brazil.

Tab. 1. Sampling sites denomination and location.

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

In each sampling site, the following environmental variables were measured *in situ*: local depth (m) (portable Speedtech sonar), water transparency (m) (Secchi disc), and vertical profiles (measurements at 0.5 to 1 m interval) (multi-parameter probe Horiba U-5000, previously calibrated) of water temperature (°C), electrical conductivity (µS.cm^{-1}), dissolved oxygen (DO) (mg.L^{-1}), turbidity (NTU), total dissolved solids (TDS) (g.L^{-1}), hydrogen ionic potential (pH) and oxide-reduction potential (ORP) (mV). Surface water samples were collected to analyze total phosphorus, total nitrogen, and chlorophyll *a* (APHA, 2017).

The trophic status index (TSI) was calculated using total phosphorus and chlorophyll data according to Lamparelli (2004), and includes six categories: Ultraoligotrophic (TSI ≤ 47), Oligotrophic (47 < TSI ≤ 52), Mesotrophic (52 < TSI ≤ 59), Eutrophic (59 < TSI ≤ 63), Supereutrophic (63 < TSI ≤ 67) and Hypereutrophic (TSI > 67).

The studied parameters were compared with the reference standards established by the Brazilian federal legislation (Tab. 2), following CONAMA Resolution nº 357/2005 (conditions for water quality categories for Brazilian aquatic ecosystems) for Class 2[2] waters – the present classification of the considered Tietê River stretch and its tributaries.

Tab.2. Standard references established by CONAMA Resolution 357/2005 (conditions for water quality categories for Brazilian aquatic ecosystems) for Class 2 waters.

Parameter	Reference
pH	6 up to 9
Turbidity (NTU)	< 100 NTU
DO (mg.L^{-1})	> 5 mgL^{-1}
TDS (g.L^{-1})	< 0.5 gL^{-1}
Total phosphorous (mg.L^{-1})*	< 0.10 mgL^{-1}
Total nitrogen (mg.L^{-1})	< 2.18 mgL^{-1}
Chlorophyll-*a* (µg.L^{-1})	< 30 µgL^{-1}

*Values for lotic environment.

The ichthyofauna was collected (IBAMA/SISBIO permanent sampling license to MGN: 13794-1) with gill nets exposed approximately 16 hours (overnight). Meshes of 30, 40, 50, 60, 70, 80, 100, 120, 140, 160 e 180 mm (between knots) were installed from the marginal habitats (smaller sizes) towards the river central channel (larger sizes).

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

[2]Class 2 may be intended for: a) supply for human consumption, after conventional treatment; b) the protection of aquatic communities; c) primary contact recreation, such as swimming, water skiing and diving (CONAMA Resolution n° 274/2000); d) irrigation of vegetables, fruit plants and parks, gardens, sports and leisure fields, with which the public may have direct contact and e) aquaculture and fishing activities.

(species) based on specialized literature (Britski, 1972; Graça & Pavanelli, 2007; Langeani & Rego, 2014; Ota et al., 2018).

The fish abundance was standardized in terms of CPUE (catch per unit effort) in numbers of individuals (CPUEn) and biomass (CPUEb), considering 1000 m^2 of gill nets and 16 hours of exposure (Carvalho & Silva, 2007). The assemblage's alpha (α) diversity was determined through the Shannon-Wiener indices and Pielou's equability. Beta diversity (β) was calculated based on the Whittaker index. Analyzes were performed in the software Primer v.6.1.12 & Permanova + v.1.0.2 (Clarke & Warwick, 2001) and PAST 1.48 (Hammer et al., 2001).

We also determined the trophic guilds of the fishes (indicative of functional diversity) based on "Diet" or "Food items" reported by Froese & Pauly (2023) and/or from published studies using the search tools Google Scholar (https://scholar.google.com.br/) and SciELO (http://www.scielo.org/php/index.php).

For the ordination of the studied rivers according to the limnological variables, we used a 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) (Anderson et al., 2008) was used to correlate the ichthyofauna structure with the environmental variables. All parameters (except pH) were log (x + 1) transformed for 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 (Clarke & Warwick, 2001). 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 (Anderson et al., 2008). These tests were performed using Primer v.6.1.12 & Permanova + v.1.0.2 (Clarke & Warwick, 2001) and R (R Core Team, 2023).

Results

Environmental conditions

The results (means and standard deviations of the vertical profiles) of the environmental variables for each site and sampling period are presented in Tab. 3. Detailed vertical distribution of selected variables, dissolved oxygen and temperature, are shown in Fig. 2.

All sampling sites exhibited shallow depths. A minimum of 0.90 m was measured in the Capivari River during the dry period, and a maximum of 7.80 m was measured at the Tie (III) site 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, was found at Sorocaba River during the dry period.

For turbidity, higher values occurred in the rainy period, exceeding the Brazilian legislation limits (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 were observed in the rain period, ranging from 6.21 to 6.80, compared to the dry period, ranging from 6.57 to 7.76. Total dissolved solids varied from 0.06 to 0.27 $g.L^{-1}$ in the rain period and from 0.08 to 0.44 $g.L^{-1}$ in the dry period. For both parameters, all measurements complied with the CONAMA nº 357/2005 resolution.

The average electrical conductivity values were high or relatively high in all sampling points, reaching 690 $\mu S.cm^{-1}$ at the Tie (I) site during the dry period. The values in this sampling campaign were higher for all sites than the measurements taken during the rainy period when the Peixe River had the lowest value, 92.29 $\mu S.cm^{-1}$. For ORP, the maximum value of 415.80 mV was measured at the Tie (II) site during the rainy period, and the lowest, 211.67 mV, was measured at the Capivari River during the dry period. The Tietê River had a higher salt concentration than its tributaries, especially in the dry period.

The results of nutrients showed high concentrations of total phosphorus and nitrogen in the Tietê River sites in both sampling periods. The maximum concentrations of 1.29 $mg.L^{-1}$ of phosphorus and 15.27 $mg.L^{-1}$ of nitrogen occurred in the dry period at site Tie (I), the most upstream located. During this period, higher concentrations of nutrients were observed, except for 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 Brazilian legislation.

Higher chlorophyll *a* concentrations were also observed in the dry period for all sites except the Peixe River. There was a significant decrease between the rainy and dry periods at this site, from 10.26 $\mu g.L^{-1}$ to 1.81 $\mu g.L^{-1}$. The Capivari was the only tributary that showed a high chlorophyll concentration, above the legal limits, with 92.03 $\mu g.L^{-1}$ in the dry period. At the Tie (III) site, the values exceeded the legal 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 were obtained in the Tietê River sites, which varied 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.

Concerning dissolved oxygen, the Tietê River sites showed extremely low concentrations, tending to 0 $mg.L^{-1}$ in the rainy period. A slight increase was observed in the dry period, especially at the Tie (II) site, where the mean values varied from 1.50 $mg.L^{-1}$ to 4.94 $mg.L^{-1}$ in the rainy and dry periods, respectively. For both periods, all values in the main river are below the legislative limits established for class 2 waters. For the tributaries, the values measured in Sorocaba River and Peixe River during the dry period were also below the limits. As expected for shallow rivers, the water column profiles for temperature and dissolved oxygen (Fig. 2) show a homogeneous condition in the vertical dimension.

Tab. 3. Mean values and standard deviation of the environmental variables measured at the sampling sites, in the rain (R) and dry (D) periods. Values in red are not in accordance with the limits established by CONAMA Resolution 357/2005 for Class 2 waters.

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
Water trasparence (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
Water temperature (ºC)	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)	0.08 (±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)

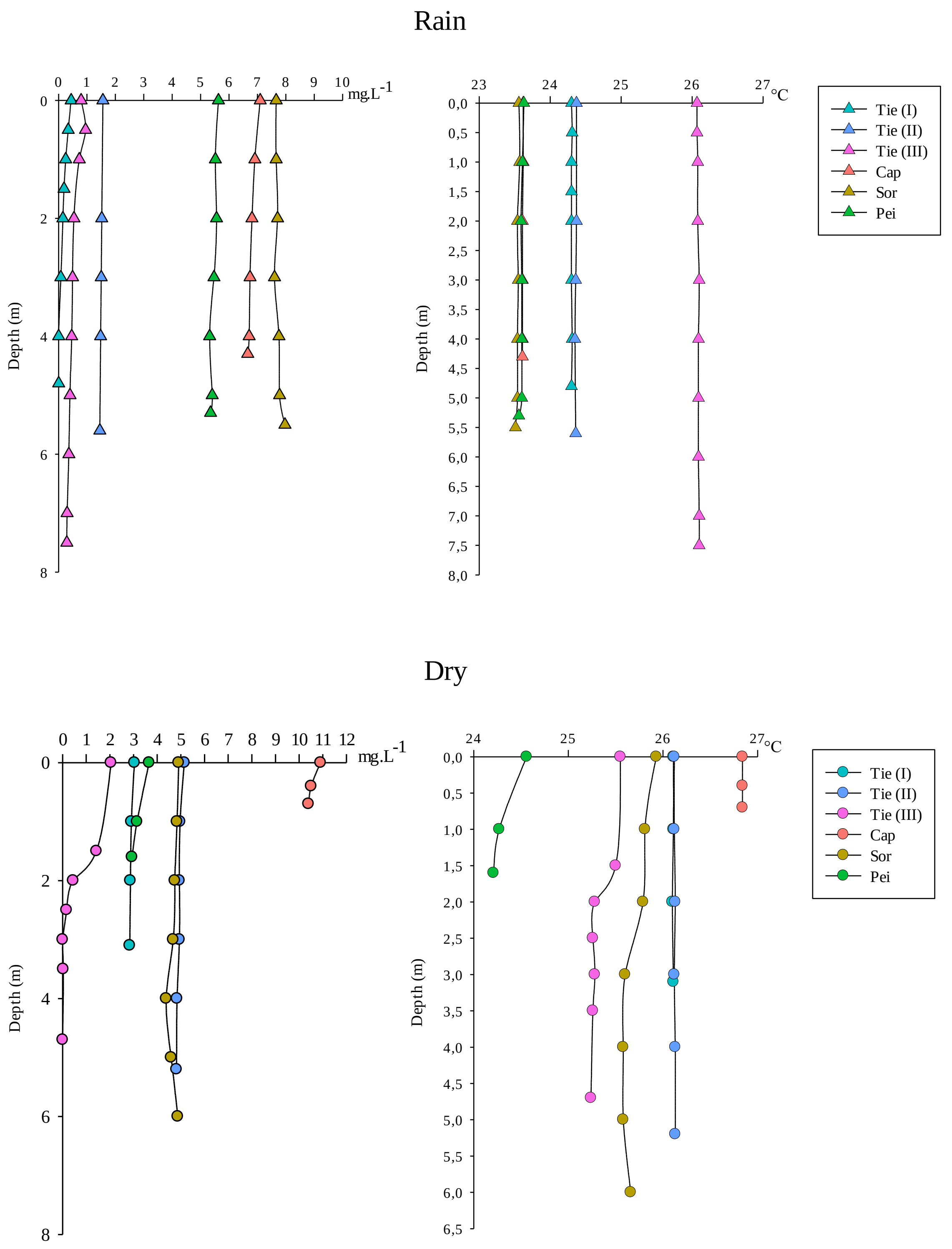

Fig.2. Vertical profiles of dissolved oxygen (left) and temperature (right) at sampling sites, in rain and dry periods.

The PCA explained 84.4% of data variance for the environmental data in the rain period and 84.7% in the dry period, considering the first two dimensions. The biplot result shows a clear difference between the main river s and the tributaries for both sampling periods (Fig. 3).

In the rainy season, the tributaries were positioned on the left side of the Dim1, negatively correlated with this dimension because of the higher concentrations of DO. In contrast, the sites of the Tietê River were placed on the right side of Dim1, determined by higher values of temperature, conductivity, total dissolved solids, chlorophyll and nutrients (phosphorus and nitrogen). A positive correlation with turbidity mainly determined 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 better correlated with conductivity, total dissolved solids and nutrients, Dim1, in opposition to higher dissolved oxygen values, Dim2.

The variable score values about the first and second PCA dimensions are shown in Tab. 4.

Tab. 4. Values of the coefficients of linear correlations between the environmental variables and the two first dimensions of the PCA, for rain and dry periods.

Variable	Rain		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 *a*	0.95351054	-0.09248894	Chlorophyll *a*	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
Water Trasparency	0.49713473	-0.81801113	Water Trasparency	-0.78653772	0.39283687

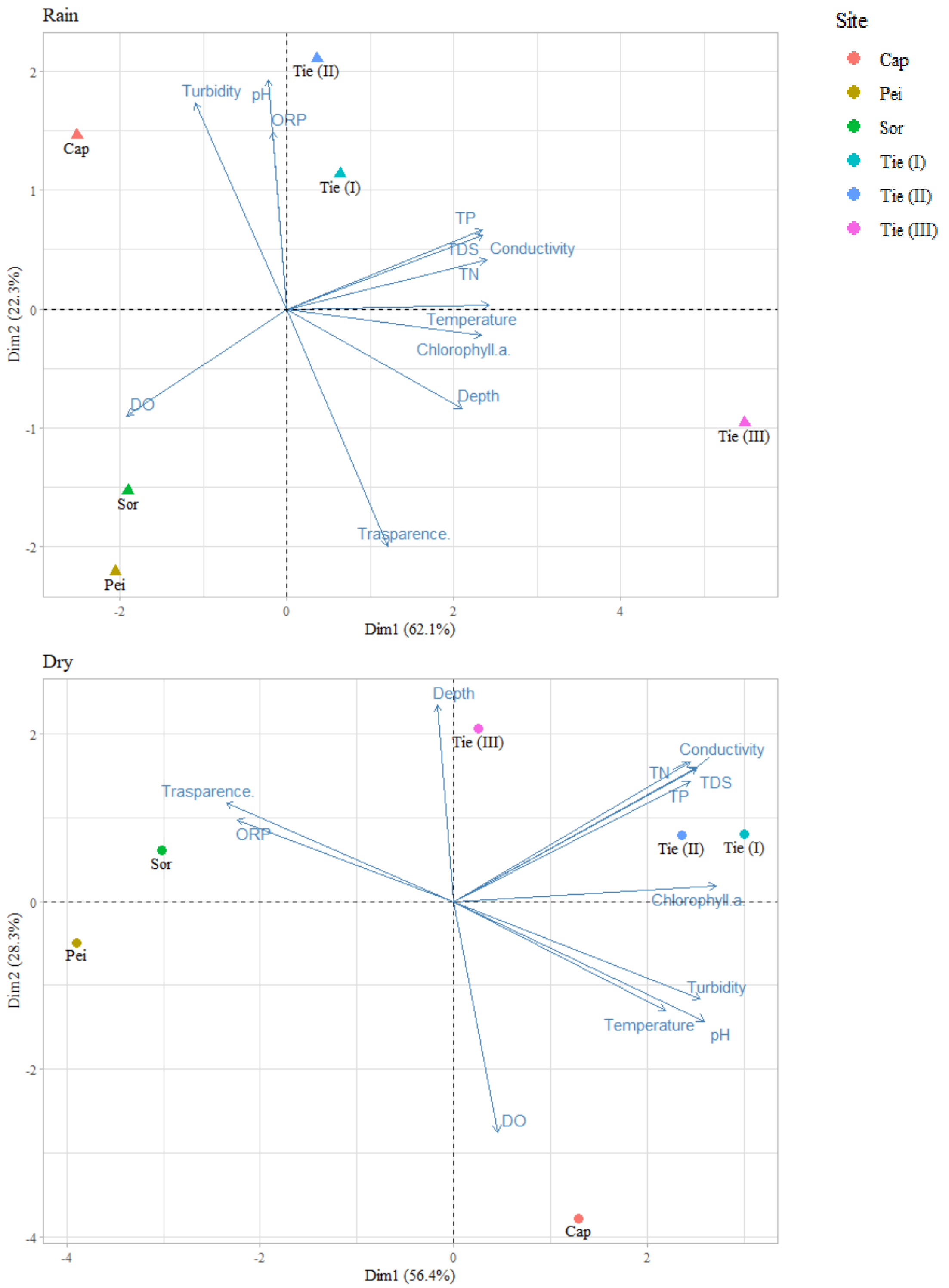

Fig. 3. Ordering of environmental parameters measured at sampling sites in rain and dry by PCA.

The trophic status index (TSI) (Tab. 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) sites were supereutrophic in the rainy season and hypereutrophic in the dry season. The tributaries Capivari and Sorocaba were considered mesotrophic in the rain period and hypereutrophic and eutrophic, respectively, in the dry period. The Peixe River exhibited the best trophic condition, classified as mesotrophic and ultraoligotrophic, in rain and dry periods, respectively.

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

Tab. 5. Trophic state classification of the Tietê River and tributaries sampling points during the rain and dry periods.

Sites	Rain		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

Ichthyofauna

Twenty-five fish species were collected in the Tietê River and its tributaries, belonging to 12 families and four orders (Tab. 6). In the rain period, only the orders Characiformes and Siluriformes were observed. Characiformes were relatively more abundant in tributaries, with a percentage of 100% of captures for the Capivari River, 95.45% for the Peixe River, and 71.43% for the Sorocaba River. Conversely, Siluriformes were more representative in the Tietê River, representing 100% of abundance except at Tie (II), due to capturing only a single specimen of Characiformes. In the dry period, in addition to the previously mentioned orders, Cichliformes and Gymnotiformes were also sampled. The predominance of Siluriformes occurred in all sampling sites, except Peixe River, where Characiformes predominated with 65.85% of representativeness. Cichliformes were found only in tributaries.

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

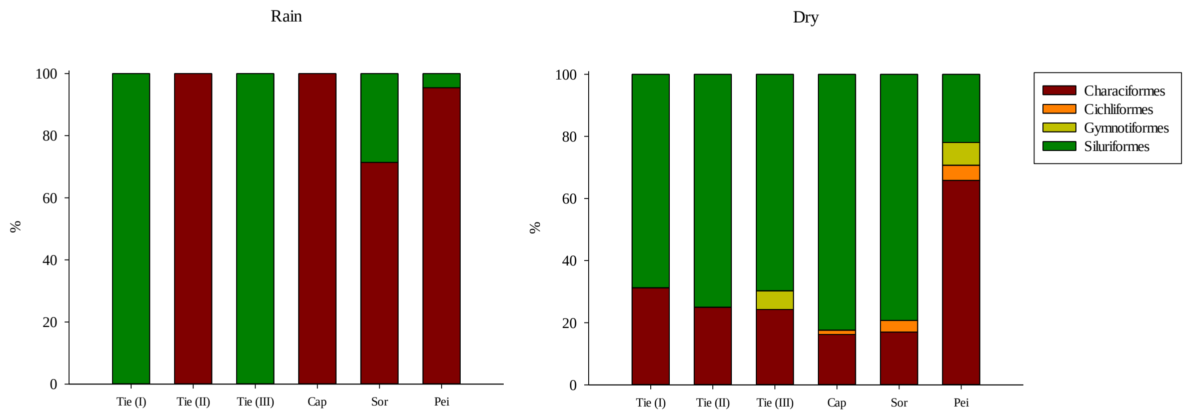

Fig. 4. Relative abundance per order of the ichthyofauna in the middle Tietê River basin, during the rain and dry periods.

Considering the taxonomical families, we observed the predominance of Callichthyidae in the Tietê River in both sampling periods, except in Tie (II) in the rain, explained once again by the capture of only a single representative of Prochilodontidae, *Prochilodus lineatus* (Valenciennes, 1837). In the rain period, this family was also predominant in the Capivari River, corresponding to 61.11% of fish abundance. Sorocaba River and Peixe River were better represented by Characidae (57.14%) and Anostomidae (36.36%), respectively. In the dry period, Loricariidae represented 79.25% abundance in the Sorocaba River and 81.69% in the Capivari River. In Peixe River, Erythrinidae (29.27%) was the dominant family. Prochilodontidae was the only family found in all rivers considering both sampling periods (Fig. 5).

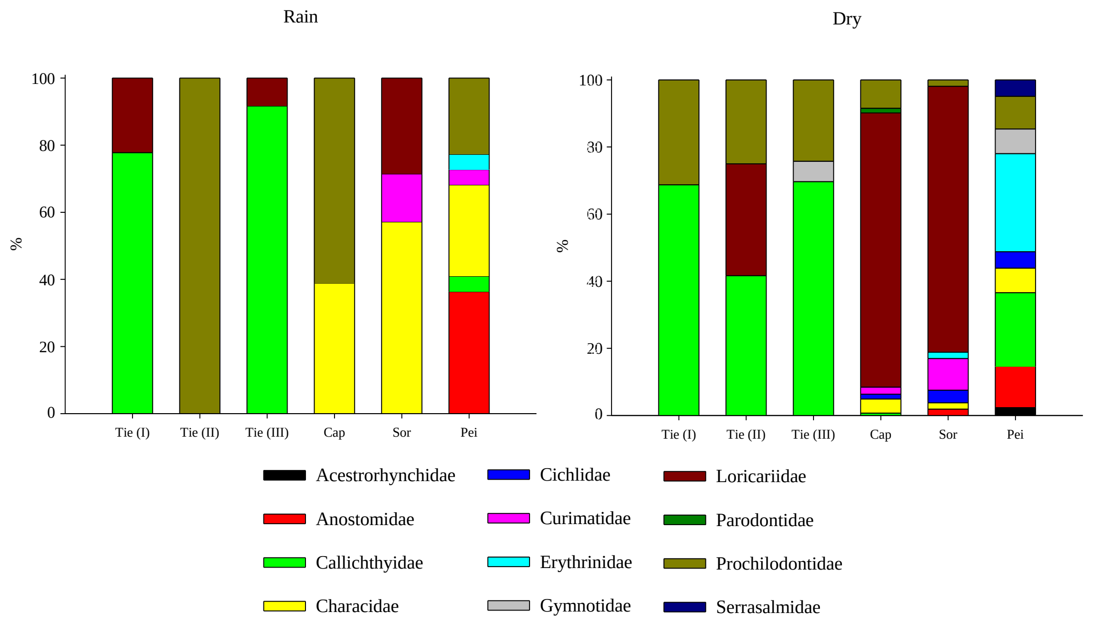

Fig. 5. Relative abundance per family of the ichthyofauna of the middle Tietê River basin during the rain and dry periods.

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

Tab. 6. List of ichthyofauna species captured in the middle Tietê River basin in rain (R) and dry (D) periods.

Species	**Tie (I)**	**Tie (II)**	**Tie (III)**	**Cap**	**Sor**	**Pei**
CHARACIFORMES						
Acestrorhynchidae						
Acestrorhynchus lacustris (Lütken, 1875)						D
Anostomidae						
Leporinus friderici (Bloch, 1794)					D	R/D
Leporinus lacustris Amaral Campos, 1945						R/D
Megaleporinus obtusidens (Valenciennes, 1837)						R
Schizodon intermedius Garavello & Britski, 1990						R/D
Characidae						
Astyanax lacustris (Lütken, 1875)				R/D	R/D	R/D
Psalidodon schubarti (Britski 1964)				R		
Roeboides descalvadensis Fowler, 1932				R/D	R	R/D
Curimatidae						
Cyphocharax nagelii (Steindachner, 1881)					D	R
Steindachnerina insculpta (Fernández-Yépez, 1948)				D	R	
Erythrinidae						
Hoplias malabaricus (Bloch, 1794)					D	R/D
Parodontidae						
Apareiodon sp.				D		
Prochilodontidae						
Prochilodus lineatus (Valenciennes, 1837)	D	R/D	D	R/D	D	R/D
Serrasalmidae						
Serrasalmus maculatus Kner, 1858						D
CICHLIFORMES						
Cichlidae						
Geophagus brasiliensis (Quoy & Gaimard, 1824)				D	D	
Oreochromis niloticus (Linnaeus, 1758)				D		
Saxatilia britskii (Kullander 1982)						D
GYMNOTIFORMES						
Gymnotidae						
Gymnotus cuia Craig, Malabarba, Crampton & Albert, 2018			D			D
SILURIFORMES						
Callichthyidae						
Hoplosternum littorale (Hancock, 1828)	R/D	D	R/D	D		R/D
Loricariidae						
Hypostomus ancistroides (Ihering, 1911)		D		D	D	
Hypostomus hermanni (Ihering, 1905)					R/D	
Hypostomus regani (Ihering, 1905)					D	
Hypostomus strigaticeps (Regan, 1908)				D		
Proloricaria prolixa (Isbrücker & Nijssen, 1978)					R/D	
Pterygoplichthys ambrosettii (Holmberg, 1893)	R		R			
Total richness	**3**	**3**	**4**	**11**	**12**	**14**

In terms of CPUE (individuals/1000 m^2/16 h), a total of 947.3 individuals were captured, totalizing a biomass of approximately 183.26 kg (Supplementary Material: Tab. A, B, C, D). In the rain period, the mean values in number of individuals (CPUEn) were higher at Tie (III), 2.62, and in biomass (CPUEb) at Cap, 805.66 g. The lowest mean value in the rain period for CPUEn was obtained at Tie (II), 0.10 and for CPUEb at Sor, 45.56 g. In the dry period, the Capivari River exhibited the highest values of CPUEn and CPUEb, with 14.34 individuals and 2928.07 g, respectively. At the same time, Tie (II) had the lowest values for both, with 1.30 (CPUEn) and 119.69 g (CPUEb) (Fig. 6).

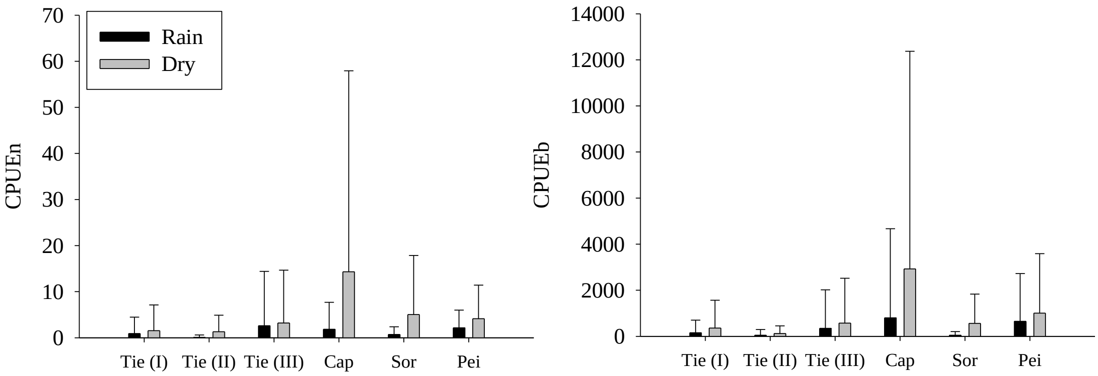

Fig. 6. Mean values of catch per unit effort in number of individuals (CPUEn) and in biomass (g) (CPUEb), for the sampled ichthyofauna in the middle Tietê River basin during the rain and dry period.

Diversity analysis

The Peixe River showed the highest Shannon-Wiener index (H') (bits $ind.^{-1}$) in both sampling campaigns, with values of 2.88 in the rain and 3.04 in the dry period. 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), in the rain, the Sorocaba River had the highest value, 0.92 and the Tie (III), the lowest value, 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, Tie (II) had the highest J value, 0.98, and Cap the lowest, 0.54 (Fig. 7).

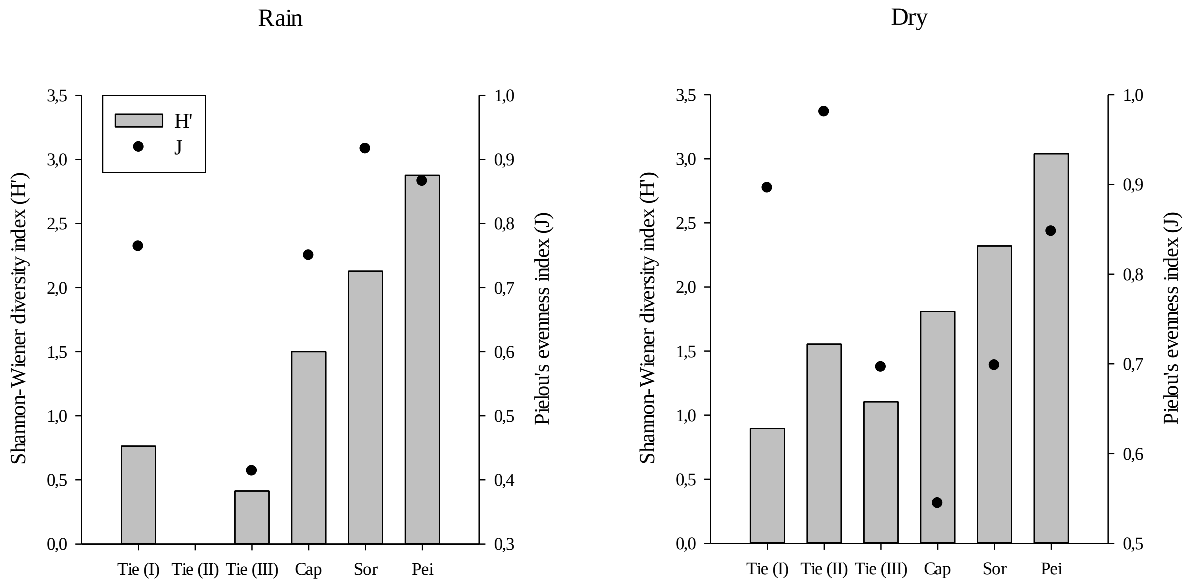

Fig. 7. Pielou equability values (J ') and Shannon-Wiener index (H') for the sampled ichthyofauna of the middle Tietê River basin during the rain and dry periods.

The beta diversity values for the rivers are summarized in Tab. 7. The composition of species in the rain, comparing the sites, did not present differences between Tie (I) and Tie (III), with a high difference between these environments and the other analyzed sites. Intermediate values of beta diversity were observed between the Sorocaba River and Capivari River (0.56) and between the Peixe River and Capivari River (0.57).

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, Sor/Cap and Pei/Cap) or relatively high (0.64, Sor/Pei) among themselves.

Tab.7. Ichthyofauna beta diversity (Whittaker) values among the sampling sites in the middle Tietê River basin during rain and dry periods.

		Dry					
	Site	Tie (I)	Tie (II)	Tie (III)	Cap	Sor	Pei
Rain	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	-

Regarding functional diversity, the fish captured were allocated into eight different trophic guilds (i.e. piscivore, omnivore, herbivore, invertivore, detritivore, algivore, insectivore and iliophagous) (Tab. 8). In the Tietê River, more than half of the species found are detritivores (50 – 67%). In the tributaries, eating habits are more diversified, especially in the Peixe River, where there is a predominance of omnivorous (37%) and piscivorous (21%) species. In the Sorocaba River, there was a greater quantity of detritivorous fish, however in a lower percentage than that found in the main river (42%), while in the Capivari River omnivorous species predominated (46%) (Fig. 8).

Tab.8. Trophic guilds of the fish species of the middle Tietê River basin.

Species	**Trophic guild**	**Reference (s)**
CHARACIFORMES		
Acestrorhynchidae		
Acestrorhynchus lacustris (Lütken, 1875)	Piscivore	Lopes et al. (2022); Carvalho et al. (2020)
Anostomidae		
Leporinus friderici (Bloch, 1794)	Omnivore	Brazil-Sousa et al. (2024); Barbosa et al. (2022)
Leporinus lacustris Amaral Campos, 1945	Omnivore	Barbosa et al. (2022)
Megaleporinus obtusidens (Valenciennes, 1837)	Omnivore	Carvalho et al. (2022)
Schizodon intermedius Garavello & Britski, 1990	Herbivore	Lima et al. (2018)
Characidae		
Astyanax lacustris (Lütken, 1875)	Omnivore	Carvalho et al. (2022)
Psalidodon schubarti (Britski 1964)	Omnivore	Froese & Pauly (2023)
Roeboides descalvadensis Fowler, 1932	Invertivore	Lopes et al. (2022)
Curimatidae		
Cyphocharax nagelii (Steindachner, 1881)	Iliophagous	Gandini et al. (2012)
Steindachnerina insculpta (Fernández-Yépez, 1948)	Detritivore	Lopes et al. (2022)
Erythrinidae		
Hoplias malabaricus (Bloch, 1794)	Piscivore	Brazil-Sousa et al. (2024); Ramos et al. (2022); Souza et al. (2022); Carvalho et al. (2022); Barbosa et al. (2022)
Parodontidae		
Apareiodon sp.*	Algivore	Carvalho et al. (2022)
Prochilodontidae		
Prochilodus lineatus (Valenciennes, 1837)	Detritivore	Lopes et al. (2022); Barbosa et al. (2022)
Serrasalmidae		
Serrasalmus maculatus Kner, 1858	Piscivore	Lopes et al. (2022); Ramos et al. (2022); Barbosa et al. (2022)
CICHLIFORMES		
Cichlidae		
Geophagus brasiliensis (Quoy & Gaimard, 1824)	Omnivore	Souza et al. (2022); Carvalho et al. (2020)

Oreochromis niloticus (Linnaeus, 1758)	Omnivore	Carvalho et al. (2022); Azevedo-Santos et al. (2019)
Saxatilia britskii (Kullander 1982)	Insectivore	Ganassin (2021)
GYMNOTIFORMES		
Gymnotidae		
Gymnotus cuia Craig, Malabarba, Crampton & Albert, 2018*	Invertivore	Ganassin (2021)
SILURIFORMES		
Callichthyidae		
Hoplosternum littorale (Hancock, 1828)	Omnivore	Azevedo-Santos et al. (2019)
Loricariidae		
Hypostomus ancistroides (Ihering, 1911)	Detritivore	Ganassin (2021); Bonato et al. (2012)
Hypostomus hermanni (Ihering, 1905)	Detritivore	Ganassin (2021)
Hypostomus regani (Ihering, 1905)	Detritivore	Ganassin (2021)
Hypostomus strigaticeps (Regan, 1908)	Detritivore	Ganassin (2021); Bonato et al. (2012)
Proloricaria prolixa (Isbrücker & Nijssen, 1978)	Herbivore	Ganassin (2021)
Pterygoplichthys ambrosettii (Holmberg, 1893)	Detritivore	Lopes et al. (2022)

*Trophic guild for the genus.

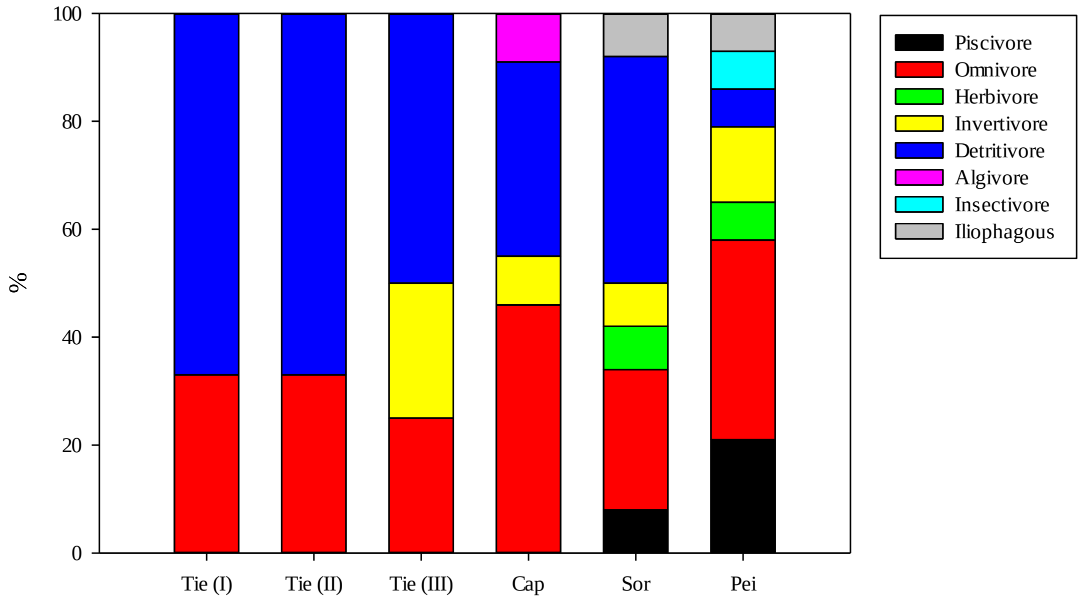

Fig. 8. Relative abundance of the trophic guilds of the fish species of the middle Tietê River basin.

The plotted result of the nMDS analysis shows a clear difference between the ichthyofauna of the sampling sites in the main river and the sampling sites in the tributaries (Fig. 9), with a very small richness of species directly associated with the Tietê River. The differences between the sampling periods are not very evident.

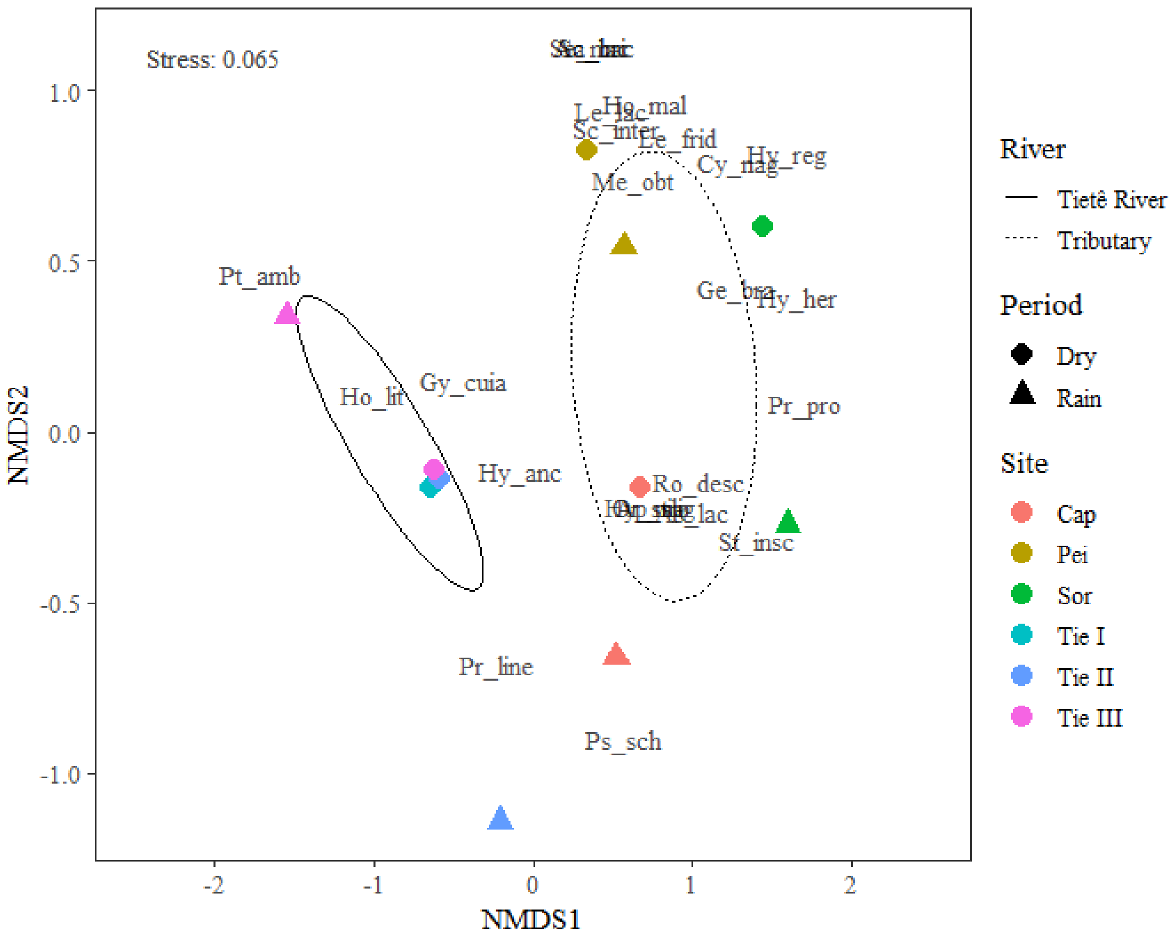

Fig. 9. Non-metric multidimensional scaling (nMDS) for the sampled ichthyofauna of the middle Tietê River basin during the rain and dry periods.

Influence of environmental variables on fish assemblage´s structure

The DistLM analysis indicated significant relationships ($p < 0.05$) between the structure of the fish assemblages and the environmental variables only in the rain period. Dissolved oxygen (DO) contributed with the highest percentage of explained variance (48.11%), followed by TDS (40.99%), conductivity (40.06%), total phosphorus (TP) (39.99%) chlorophyll a (36.28%) and temperature (33.89%) (Tab. 9).

Tab. 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 in the middle Tietê River basin, during rain and dry periods. In red, the significant variables ($p < 0.05$).

Variable	Rain			Dry		
	Pseudo-F	p	Prop. %	Pseudo-F	p	Prop. %
Temperature	20.507	0.026	33.89	11.573	0.399	22.44
pH	0.30005	0.927	6.98	13.611	0.248	25.39
ORP	15.062	0.15	27.35	0.90929	0.554	18.52
Condutivity	26.735	0.049	40.06	17.201	0.164	30.07
Turbidity	10.828	0.421	21.30	17.254	0.165	30.14
DO	37.083	0.003	48.11	15.284	0.226	27.65
TDS	27.779	0.023	40.99	20.837	0.122	34.25
Salinity	11.126	0.522	21.76	26.991	0.11	40.29
Chlorophyll *a*	22.769	0.031	36.28	15.465	0.148	27.88
TP	26.657	0.046	39.99	19.401	0.121	32.66
TN	24.214	0.059	37.71	15.972	0.193	28.54

Discussion

Environmental conditions

Our results showed a clear difference in the water quality of the studied rivers. This difference was demonstrated by the Principal Component Analysis (PCA), especially between the points of the Tietê River and the points of its tributaries, during both sampling periods. The main river receives a significant daily load of effluents from the largest Brazilian megalopolis, the city of São Paulo, which has a direct impact on the variables related to water quality (Abraham et al., 2007; Buckeridge & Ribeiro, 2018). The tributaries are less influenced by urban areas, especially in the case of the Peixe River, whose impact comes from other sources, mainly agricultural activities (Silva, 2022), and probably in a smaller magnitude. In contrast, the same analysis did not show great differences between the three points distributed along the Tietê River, demonstrating that it is a relatively homogeneous environment along the section of 40 km, with no relevant influence of the considered tributaries.

The high nutrient loads increase the primary productivity in rivers (Dorgham, 2014) and also explain the high levels of eutrophication indicated by the trophic state index, especially for the Tietê. In this river, biological oxidation is so intense that the oxygen concentration, even in the surface layer, is close to zero, creating an extremely unfavorable environment.

In all the rivers, eutrophication was higher during the dry period and lower during the rainy period, demonstrating the influence of rainfall as a dilution factor of the pollution. The exception was the River Peixe, which had a higher trophic state index during the rainy period, explained by the higher concentrations of chlorophyll *a* measured during this period compared to the dry period.

Based on the reference values established by CONAMA Resolution No. 357/2005, the concentrations of dissolved oxygen, total phosphorus, and total nitrogen in the Tietê River in both analyzed periods, turbidity in the rainy season and chlorophyll a in the dry season exceed the limits established by the legislation for Class 2 waters. For dissolved oxygen, the Tietê River sites had concentrations much lower than the minimum allowable limit of five $mg.L^{-1}$.

Ichthyofauna

Differences in the composition of the ichthyofauna between the considered rivers were verified. These differences are particularly evident when comparing the main river and the tributaries through nMDS analysis and Whittaker's beta diversity values.

Considering the two periods, the species richness was much higher in the tributaries (11 to 14) compared to the Tietê River (3 to 4). This trend was also corroborated by the values of the Shannon-Wiener index.

In the main river, fishes were mainly represented by specimens of the order Siluriformes (e.g. *Hypostomus* spp., *H. littorale* and *P. ambrosetti*), whose species are known for their high tolerance to poor environmental conditions (Gisbert et al., 2022), such as the anoxia or hypoxia verified in the Tietê River, possessing accessory breathing mechanisms (Hochachka & Lutz, 2001). The family Prochilodontidae, represented by the species *Prochilodus lineatus* (Valenciennes, 1837), was the only one found in all the rivers studied, showing a high resilience to environmental variations. These species are mostly detritivores. Prado et al. (2020) demonstrated that different trophic guilds changed their diets in degraded environments, but the detritivorous species showed greater trophic plasticity. This, associated with tolerating such inhospitable conditions, previously mentioned, makes these species benefit from the large amount of organic matter present in the substrate, the main food resource available in this environment. In the tributaries, feeding habits indicates the presence of a greater variety of food resources compared to the Tietê River.

Regarding the abundance of organisms, the highest values were found in the Capivari River during the dry period. This was due to the capture of more than 100 specimens of the species *Hypostomus strigaticeps* and *Hypostomus ancistroides*. This river was classified as hypereutrophic in that occasion, which may have contributed to the low diversity of the species caught.

Influence of environmental variables on fish assemblage´s structure

A significant correlation between the structure of fish communities and environmental variables was observed only in the rainy season. During this period, the differences between the rivers in terms of water quality and species composition were more pronounced. For example, the dissolved oxygen variable, which contributed to the highest percentage of variance explained by the DistLM analysis in this period (48.11%), was close to zero in the Tietê River points and greater than 5 mgL^{-1} in the tributaries. About the assemblage's composition, in the Tietê River, most fish belonged to only two families of the Siluriformes. In contrast, in its tributaries, species of Siluriformes and Characiformes belonging to 6 different families were observed.

Our results allow us to conclude that pollution acts as a chemical barrier to the dispersion of sensitive or less tolerant organisms to variations in environmental conditions. This concept of a chemical barrier for fish has been addressed in the literature in laboratory studies (Araújo et al., 2018) or in literary reviews (Noatch & Suski, 2012; Le Pichon et al, 2020). Our study address this problematic in the field, considering the effect of the dumping of numerous substances on the different variables related to water quality and consequently on the organisms present in the environment.

This work highlights the negative effects of pollution on biota. Results indicate the need to implement urgent mitigation measures to reduce degradation in the Tietê River basin. In this way, we encourage deepening these issues, not only for the fish community but also for all aquatic organisms living in these water bodies.

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Suplementary Material

Tab.A. CPUEn values at the sampling points in the rain period.

Species	Tie (I)	Tie (II)	Tie (III)	Cap	Sor	Pei	Total
CHARACIFORMES							
Acestrorhynchidae							
Acestrorhynchus lacustris (Lütken, 1875)	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**
Anostomidae							
Leporinus friderici (Bloch, 1794)	0.0	0.0	0.0	0.0	0.0	2.4	**2.4**
Leporinus lacustris Amaral Campos, 1945	0.0	0.0	0.0	0.0	0.0	2.4	**2.4**
Megaleporinus obtusidens (Valenciennes, 1837)	0.0	0.0	0.0	0.0	0.0	12.2	**12.2**
Schizodon intermedius Garavello & Britski, 1990	0.0	0.0	0.0	0.0	0.0	2.4	**2.4**
Characidae							
Astyanax lacustris (Lütken, 1875)	0.0	0.0	0.0	10.3	2.5	2.4	**15.3**
Psalidodon schubarti (Britski 1964)	0.0	0.0	0.0	2.6	0.0	0.0	**2.6**
Roeboides descalvadensis Fowler, 1932	0.0	0.0	0.0	5.1	7.6	12.2	**24.9**
Curimatidae							
Cyphocharax nagelii (Steindachner, 1881)	0.0	0.0	0.0	0.0	0.0	2.4	**2.4**
Steindachnerina insculpta (Fernández-Yépez, 1948)	0.0	0.0	0.0	0.0	2.5	0.0	**2.5**
Erythrinidae							
Hoplias malabaricus (Bloch, 1794)	0.0	0.0	0.0	0.0	0.0	2.4	**2.4**
Parodontidae							
Apareiodon sp.	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**
Prochilodontidae							
Prochilodus lineatus (Valenciennes, 1837)	0.0	2.5	0.0	28.3	0.0	12.2	**43.0**
Serrasalmidae							
Serrasalmus maculatus Kner, 1858	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**
CICHLIFORMES							
Cichlidae							
Geophagus brasiliensis (Quoy & Gaimard, 1824)	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**
Oreochromis niloticus (Linnaeus, 1758)	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**
Saxatilia britskii (Kullander 1982)	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**
GYMNOTIFORMES							
Gymnotidae							
Gymnotus cuia Craig, Malabarba, Crampton & Albert, 2018	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**
SILURIFORMES							
Callichthyidae							
Hoplosternum littorale (Hancock, 1828)	17.6	0.0	60.1	0.0	0.0	2.4	**80.2**
Loricariidae							
Hypostomus ancistroides (Ihering, 1911)	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**
Hypostomus hermanni (Ihering, 1905)	0.0	0.0	0.0	0.0	2.5	0.0	**2.5**
Hypostomus regani (Ihering, 1905)	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**
Hypostomus strigaticeps (Regan, 1908)	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**
Proloricaria prolixa (Isbrücker & Nijssen, 1978)	0.0	0.0	0.0	0.0	2.5	0.0	**2.5**
Pterygoplichthys ambrosettii (Holmberg, 1893)	5.0	0.0	5.5	0.0	0.0	0.0	**10.5**
Total	**22.7**	**2.5**	**65.5**	**46.3**	**17.7**	**53.7**	**208.5**

Tab.B. CPUEn values at the sampling points in the dry period.

Species	Tie (I)	Tie (II)	Tie (III)	Cap	Sor	Pei	Total
CHARACIFORMES							
Acestrorhynchidae							
Acestrorhynchus lacustris (Lütken, 1875)	0.0	0.0	0.0	0.0	0.0	2.5	**2.5**
Anostomidae							
Leporinus friderici (Bloch, 1794)	0.0	0.0	0.0	0.0	2.4	2.5	**4.9**
Leporinus lacustris Amaral Campos, 1945	0.0	0.0	0.0	0.0	0.0	7.6	**7.6**
Megaleporinus obtusidens (Valenciennes, 1837)	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**
Schizodon intermedius Garavello & Britski, 1990	0.0	0.0	0.0	0.0	0.0	2.5	**2.5**
Characidae							
Astyanax lacustris (Lütken, 1875)	0.0	0.0	0.0	2.5	2.4	2.5	**7.4**
Psalidodon schubarti (Britski 1964)	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**
Roeboides descalvadensis Fowler, 1932	0.0	0.0	0.0	12.6	0.0	5.1	**17.7**
Curimatidae							
Cyphocharax nagelii (Steindachner, 1881)	0.0	0.0	0.0	0.0	11.9	0.0	**11.9**
Steindachnerina insculpta (Fernández-Yépez, 1948)	0.0	0.0	0.0	7.6	0.0	0.0	**7.6**
Erythrinidae							
Hoplias malabaricus (Bloch, 1794)	0.0	0.0	0.0	0.0	2.4	30.3	**32.7**
Parodontidae							
Apareiodon sp.	0.0	0.0	0.0	5.0	0.0	0.0	**5.0**
Prochilodontidae							
Prochilodus lineatus (Valenciennes, 1837)	12.0	8.1	19.5	30.3	2.4	10.1	**82.3**
Serrasalmidae							
Serrasalmus maculatus Kner, 1858	0.0	0.0	0.0	0.0	0.0	5.1	**5.1**
CICHLIFORMES							
Cichlidae							
Geophagus brasiliensis (Quoy & Gaimard, 1824)	0.0	0.0	0.0	2.5	4.7	0.0	**7.3**
Oreochromis niloticus (Linnaeus, 1758)	0.0	0.0	0.0	2.5	0.0	0.0	**2.5**
Saxatilia britskii (Kullander 1982)	0.0	0.0	0.0	0.0	0.0	5.1	**5.1**
GYMNOTIFORMES							
Gymnotidae							
Gymnotus cuia Craig, Malabarba, Crampton & Albert, 2018	0.0	0.0	4.9	0.0	0.0	7.6	**12.4**
SILURIFORMES							
Callichthyidae							
Hoplosternum littorale (Hancock, 1828)	26.4	13.5	55.9	2.5	0.0	22.7	**121.1**
Loricariidae							
Hypostomus ancistroides (Ihering, 1911)	0.0	10.8	0.0	80.8	4.7	0.0	**96.3**
Hypostomus hermanni (Ihering, 1905)	0.0	0.0	0.0	0.0	61.7	0.0	**61.7**
Hypostomus regani (Ihering, 1905)	0.0	0.0	0.0	0.0	26.1	0.0	**26.1**
Hypostomus strigaticeps (Regan, 1908)	0.0	0.0	0.0	212.0	0.0	0.0	**212.0**
Proloricaria prolixa (Isbrücker & Nijssen, 1978)	0.0	0.0	0.0	0.0	7.1	0.0	**7.1**
Pterygoplichthys ambrosettii (Holmberg, 1893)	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**
Total	**38.4**	**32.5**	**80.2**	**358.4**	**125.7**	**103.6**	**738.8**

Tab.C. CPUEb values at the sampling points in the rain period.

Species	Tie (I)	Tie (II)	Tie (III)	Cap	Sor	Pei	Total
CHARACIFORMES							
Acestrorhynchidae							
Acestrorhynchus lacustris (Lütken, 1875)	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**
Anostomidae							
Leporinus friderici (Bloch, 1794)	0.0	0.0	0.0	0.0	0.0	439.3	**439.3**
Leporinus lacustris Amaral Campos, 1945	0.0	0.0	0.0	0.0	0.0	351.5	**351.5**
Megaleporinus obtusidens (Valenciennes, 1837)	0.0	0.0	0.0	0.0	0.0	4557.0	**4557.0**
Schizodon intermedius Garavello & Britski, 1990	0.0	0.0	0.0	0.0	0.0	522.3	**522.3**
Characidae							
Astyanax lacustris (Lütken, 1875)	0.0	0.0	0.0	298.6	35.4	63.5	**397.5**
Psalidodon schubarti (Britski 1964)	0.0	0.0	0.0	46.3	0.0	0.0	**46.3**
Roeboides descalvadensis Fowler, 1932	0.0	0.0	0.0	61.8	68.3	111.1	**241.2**
Curimatidae							
Cyphocharax nagelii (Steindachner, 1881)	0.0	0.0	0.0	0.0	0.0	90.3	**90.3**
Steindachnerina insculpta (Fernández-Yépez, 1948)	0.0	0.0	0.0	0.0	73.4	0.0	**73.4**
Erythrinidae							
Hoplias malabaricus (Bloch, 1794)	0.0	0.0	0.0	0.0	0.0	205.0	**205.0**
Parodontidae							
Apareiodon sp.	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**
Prochilodontidae							
Prochilodus lineatus (Valenciennes, 1837)	0.0	1259.5	0.0	19734.9	0.0	9802.3	**30796.7**
Serrasalmidae							
Serrasalmus maculatus Kner, 1858	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**
CICHLIFORMES							
Cichlidae							
Geophagus brasiliensis (Quoy & Gaimard, 1824)	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**
Oreochromis niloticus (Linnaeus, 1758)	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**
Saxatilia britskii (Kullander 1982)	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**
GYMNOTIFORMES							
Gymnotidae							
Gymnotus cuia Craig, Malabarba, Crampton & Albert, 2018	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**
SILURIFORMES							
Callichthyidae							
Hoplosternum littorale (Hancock, 1828)	1609.7	0.0	8537.6	0.0	0.0	214.8	**10362.0**
Loricariidae							
Hypostomus ancistroides (Ihering, 1911)	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**
Hypostomus hermanni (Ihering, 1905)	0.0	0.0	0.0	0.0	159.5	0.0	**159.5**
Hypostomus regani (Ihering, 1905)	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**
Hypostomus strigaticeps (Regan, 1908)	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**
Proloricaria prolixa (Isbrücker & Nijssen, 1978)	0.0	0.0	0.0	0.0	802.4	0.0	**802.4**
Pterygoplichthys ambrosettii (Holmberg, 1893)	2332.6	0.0	106.5	0.0	0.0	0.0	**2439.1**
Total	**3942.3**	**1259.5**	**8644.1**	**20141.6**	**1139.1**	**16357.2**	**51483.7**

Tab.D. CPUEb values at the sampling points in the dry period.

Species	Tie (I)	Tie (II)	Tie (III)	Cap	Sor	Pei	Total
CHARACIFORMES							
Acestrorhynchidae							
Acestrorhynchus lacustris (Lütken, 1875)	0.0	0.0	0.0	0.0	0.0	-	**0.0**
Anostomidae							
Leporinus friderici (Bloch, 1794)	0.0	0.0	0.0	0.0	341.5	-	**341.5**
Leporinus lacustris Amaral Campos, 1945	0.0	0.0	0.0	0.0	0.0	800.9	**800.9**
Megaleporinus obtusidens (Valenciennes, 1837)	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**
Schizodon intermedius Garavello & Britski, 1990	0.0	0.0	0.0	0.0	0.0	-	**0.0**
Characidae							
Astyanax lacustris (Lütken, 1875)	0.0	0.0	0.0	-	23.7	22.7	**46.5**
Psalidodon schubarti (Britski 1964)	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**
Roeboides descalvadensis Fowler, 1932	0.0	0.0	0.0	95.9	0.0	-	**95.9**
Curimatidae							
Cyphocharax nagelii (Steindachner, 1881)	0.0	0.0	0.0	0.0	796.9	0.0	**796.9**
Steindachnerina insculpta (Fernández-Yépez, 1948)	0.0	0.0	0.0	98.4	0.0	0.0	**98.4**
Erythrinidae							
Hoplias malabaricus (Bloch, 1794)	0.0	0.0	0.0	0.0	1083.8	11179.2	**12263.1**
Parodontidae							
Apareiodon sp.	0.0	0.0	0.0	128.7	0.0	0.0	**128.7**
Prochilodontidae							
Prochilodus lineatus (Valenciennes, 1837)	4137.9	768.4	5770.4	17147.5	457.7	5383.7	**33665.6**
Serrasalmidae							
Serrasalmus maculatus Kner, 1858	0.0	0.0	0.0	0.0	0.0	1063.6	**1063.6**
CICHLIFORMES							
Cichlidae							
Geophagus brasiliensis (Quoy & Gaimard, 1824)	0.0	0.0	0.0	63.1	645.1	0.0	**708.2**
Oreochromis niloticus (Linnaeus, 1758)	0.0	0.0	0.0	2478.5	0.0	0.0	**2478.5**
Saxatilia britskii (Kullander 1982)	0.0	0.0	0.0	0.0	0.0	202.1	**202.1**
GYMNOTIFORMES							
Gymnotidae							
Gymnotus cuia Craig, Malabarba, Crampton & Albert, 2018	0.0	0.0	177.5	0.0	0.0	252.6	**430.2**
SILURIFORMES							
Callichthyidae							
Hoplosternum littorale (Hancock, 1828)	4766.8	852.2	8352.8	600.7	0.0	2288.9	**16861.4**
Loricariidae							
Hypostomus ancistroides (Ihering, 1911)	0.0	1371.7	0.0	4669.3	441.1	0.0	**6482.1**
Hypostomus hermanni (Ihering, 1905)	0.0	0.0	0.0	0.0	4553.6	0.0	**4553.6**
Hypostomus regani (Ihering, 1905)	0.0	0.0	0.0	0.0	4878.5	0.0	**4878.5**
Hypostomus strigaticeps (Regan, 1908)	0.0	0.0	0.0	44991.5	0.0	0.0	**44991.5**
Proloricaria prolixa (Isbrücker & Nijssen, 1978)	0.0	0.0	0.0	0.0	884.6	0.0	**884.6**
Pterygoplichthys ambrosettii (Holmberg, 1893)	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**
Total	**8904.7**	**2992.3**	**14300.7**	**70273.6**	**14106.6**	**21193.8**	**131771.7**

Análise preliminar da estrutura populacional do peixe migrador *Prochilodus lineatus* (Characiformes: Prochilodontidae) em ambientes contrastantes em qualidade da água da bacia do médio rio Tietê, Sudeste do Brasil

Capítulo II

Preliminary analysis of the population structure of the migratory fish *Prochilodus lineatus* (Characiformes: Prochilodontidae) in contrasting environments in water quality of the middle Tietê River basin, Southeastern Brazil

Resumo

A estrutura populacional de *Prochilodus lineatus* em dois ambientes distintos em termos de qualidade de água na bacia do médio rio Tietê foi analisada preliminarmente através do marcador mitocondrial da região controle do DNA (D-loop). Foram identificados oito haplótipos para o rio principal altamente poluído, o rio Tietê, e cinco haplótipos para seu tributário mais preservado, o rio do Peixe. A diversidade haplotípica (h) foi de 1 (um) para ambas as populações e a diversidade de nucleotídeos (π) foi menor no rio do Peixe. Testes de neutralidade mostraram contração populacional tanto no tributário como no rio principal e o índice de fixação (Φ_{ST}) indicou alto fluxo gênico e ausência de estruturação genética entre os rios. O comportamento migratório de *P. lineatus* e sua elevada tolerância a condições adversas podem explicar o movimento irrestrito de indivíduos entre os ambientes estudados, apesar da elevada carga de poluição presente no rio Tietê. No entanto, a contração populacional em ambos os rios indica a influência negativa da degradação do habitat nas populações de curimbatá. Nosso estudo demonstra que a poluição do rio Tietê em seu trecho médio não parece impedir a dispersão desta espécie de peixe, embora haja que se considerar o número reduzido de indivíduos utilizados nas análises. Porém, o indicativo de contração populacional destaca a necessidade de esforços de conservação.

Palavras-chaves: Curimbatá, Dispersão dos peixes, Diversidade genética populacional, Poluição das águas.

Abstract

The population structure of *Prochilodus lineatus* in two distinct environments in terms of water quality in the middle Tietê River basin was preliminarily analyzed using the DNA control region mitochondrial marker (D-loop). Eight haplotypes were identified for the highly polluted main river, the Tietê River, and five haplotypes for its most preserved tributary, the Peixe River. Haplotype diversity (h) was 1 (one) for both populations and nucleotide diversity (π) was lower in the Peixe River. Neutrality tests showed population contraction in both the tributary and the main river, and the fixation index (Φ_{ST}) indicated high gene flow and a lack of genetic structure between the rivers. The migratory behavior of *P. lineatus* and its high tolerance to adverse conditions may explain the unrestricted movement of individuals between the environments studied, despite the high pollution load present in the Tietê River. However, population contraction in both rivers indicates the negative influence of habitat degradation on curimbatá populations. Our study demonstrates that pollution of the Tietê River in its middle section does not seem to prevent the dispersal of this fish species, although the small number of individuals used in the analysis must be taken into account. However, the indication of population contraction highlights the need for conservation efforts.

Keywords: Curimbatá, Fish dispersal, Population genetic diversity, Water pollution.

Introduction

Prochilodontidae is a family of freshwater fish in the Characiformes order that includes several species distributed throughout South American rivers and streams. One of these species, *Prochilodus lineatus* (Valenciennes, 1837), popularly known as "curimba" or "curimbatá", stands out for its significant economic importance and remarkable ability to perform extensive migrations (Castro & Vari, 2004). The migratory capacity of *P. lineatus*, as well as its large size, late maturation, and dependence on the external environment for the development of eggs and larvae, make it particularly vulnerable to human interventions in waterways, such as the construction of dams and pollution, which affect its breeding habitats and dispersal routes (Godinho et al., 2010).

Recent genetic studies, using mitochondrial markers and microsatellites, have improved our understanding of the population structure and phylogenetic relationships of *P. lineatus* in different Brazilian rivers, particularly in the face of anthropogenic interference. However, most of this research has focused on the Grande River basin (Garcez et al., 2011; Perini et al., 2021; Da Rosa et al., 2022), leaving a significant data gap in other river basins.

This gap is notable in the Tietê River basin, located in Southeastern Brazil, which stands out as an emblematic example of an ecosystem extensively degraded by pollution on a global scale, and which is particularly poorly studied, especially in its middle section, which crosses the interior of the State of São Paulo.

In this study, we conducted a preliminary analysis using mtDNA control region (D-loop) sequencing techniques to evaluate the potential occurrence of differentiated populations of *Prochilodus lineatus* in the middle Tietê River basin. The preliminary character of this investigation is due to the limited sample size we analyzed. The study area includes the main river (Tietê) and an important direct tributary, the Peixe River. These environments are widely connected, without any natural (e.g. waterfalls and rapids) or artificial (e.g. dams and reservoirs) physical barriers that impede the movement of organisms. However, they do differ in terms of degradation, with the Tietê River showing evident deterioration and the tributary showing improved conditions (Urbanski & Nogueira, in preparation; Urbanski et al., 2023). We hypothesize that there is a distinct population structure, which is determined by contrasting water quality patterns that act as a chemical barrier limiting the dispersal of this species on a regional scale.

Material and Methods

The study was carried out on the Tietê River (22°47'31.0" S 48°05'48.8" W) and on its tributary, Peixe River (22°49'42.8"S 48°06'01.5"W). Both sampling areas are located in the municipality of Anhembi, State of São Paulo, Southeast Brazil (Fig. 1).

Thirteen specimens of *Prochilodus lineatus* were collected (permanent sampling license from IBAMA/SISBIO for MGN: 13794-1) using gill nets or directly with professional fishermen in the region. Five specimens were prevenient from the tributary and eight specimens from the main river. Morphological identification was made based on a specialized bibliography (Britski, 1972; Graça & Pavanelli, 2007; Ota et al., 2018).

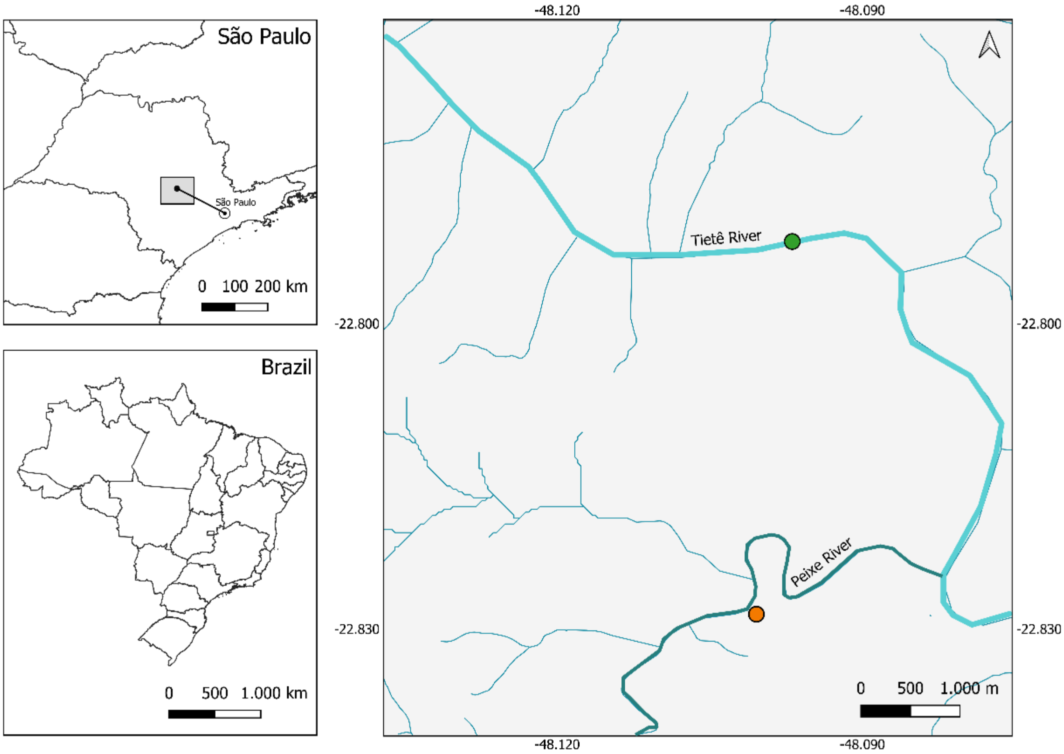

Fig. 1. Distribution of sampling sites in the Tietê River (green circle) and Peixe River (orange circle). The highlighted region, in gray, corresponds to the study area, in the center of São Paulo State.

All the individuals were euthanized with an overdose (more than 283.55 mgL-1) of anesthetic (eugenol) (Vidal et al. 2008). Then tissue samples from the gills were removed and stored in 95% ethanol to ensure the integrity and quality of the material. Finally, the fish were fixed in 10% formalin and transferred to 70% ethanol for conservation. Testimonial organisms used for genetic analysis and all tissues were identified and deposited in the ichthyological collection of the Laboratório de Biologia e Genética de Peixes (LBGP), at Universidade Estadual Paulista "Júlio de Mesquita Filho", Botucatu, São Paulo (lots: LBP 29184; LBP X).

DNA extraction and sequencing

Genomic DNA was extracted following the Fiberglass Plate DNA extraction protocol, according to the manufacturer's recommendations (Canadian Center for DNA Barcoding - CCDB). The mitochondrial DNA control region (D-loop) was amplified with the primers: F-TTF (GCCTAAGAGCATCGGTCTTGTAA); F-12R (GTCA-

GGACCATGCCTTTGTG) and PDR2-2 (GAGAGTGTATGCACCTGAT) describe by Sivasundar et al. (2001).

Polymerase chain reactions (PCR) were performed with approximately 2 µl of template DNA (30ng/µL), 0.25 µl of each primer (10 µM), 0.50 µl of dNTP (2 mM each), 1.25 µl of 10 X buffer (10 mM Tris-HCl), 0.25 µl of $MgCl_2$, 0.20 µl of Taq DNA Polymerase, and completing with 7.8 µl of water, thus totaling 12.5 µl of final solution. Thermal conditions were as follows: initial denaturation at 95°C for 3 min, 25 cycles of denaturation (94°C for 30s), annealing (56°C for 1min), and elongation (68°C for 1min), with final elongation at 68 °C for 7 min. PCR products were first identified on a 1% agarose gel and then purified with ExoSap-IT enzyme (USB Corporation) following the manufacturer's instructions.

Sequencing PCR reactions were performed using the BigDyeTM Terminator v3.1 Cycle Sequencing Ready Reaction Kit kit (Applied Biosystems). The product of this reaction was purified again by ethanol precipitation and sequenced on automatic sequencer, ABI 3130, produced by Applied Biosystems, present at the Instituto de Biotecnologia (IBTEC) of the Universidade Estadual Paulista "Júlio de Mesquita Filho", Botucatu, São Paulo. Consensus sequences were assembled and edited using Geneious Prime 2023.1.2 (Kearse et al., 2012) and aligned with Clustal Omega algorithm.

Genetic analysis of populations

For the characterization of molecular diversity, the relative composition of nucleotides, number of polymorphic sites, diversity of haplotypes (h), diversity of nucleotides (π), number of paired nucleotides, as well as the values of Theta S (Θ_S) and Theta pi (Θ_π) were calculated for both the Tietê and the Peixe river populations.

Levels of population genetic divergence for *Prochilodus lineatus* were estimated by calculating the Φ_{ST} fixation index using Molecular Variance Analysis (AMOVA) (Excoffier et al. 1992). The Φ_{ST} estimates were tested with 1,000 non-parametric bootstrap pseudo-replicas.

Evidence of population expansion was tested using Fu's FS (Fu, 1997) and Tajima's D (Tajima, 1989) neutrality tests. All these analyzes were performed using the ARLEQUIN 3.5.2.2 software (Excoffier & Lischer, 2010).

Finally, the ancestral haplotype relationships were graphically represented using a haplotype network elaborated in the NETWORK 10.2 software.

Results

Molecular diversity

Altogether, five haplotypes with 1.112bps each, for the Peixe River and eight haplotypes with 1.125bps each, for the Tietê River were analyzed. The relative nucleotide composition of the *Prochilodus lineatus* populations from these rivers is presented in Table 1.

Tab. 1. Relative nucleotide composition (%) for *Prochilodus lineatus* populations from the middle Tietê River basin.

Nucleotide	Peixe River	Tietê River
C	14.04	14.33
T	32.16	31.98
A	31.94	31.76
G	21.85	21.93

The observed haplotypic diversity (h) was 1 for both populations, with a standard deviation of ± 0.127 for the Peixe River, and ± 0.063 for the Tietê River. Values for the average number of paired differences were 25.800 (± 13.712) for the tributary and 53.143 (± 25.793) for the main river, and nucleotide diversity (π) was lower in the Peixe river (0.023 ± 0.014 vs. 0.047 ± 0.026). Theta (ϴ) indices values can be seen in Table 2.

Tab. 2. Theta (ϴ) indices values for *Prochilodus lineatus* populations from the middle Tietê River basin.

	Peixe River	Tietê River
Theta S (Θ_S)	23.52 (12.047)	57.08 (24.822)
Theta pi (Θ_π)	25.80 (16.030)	53.14 (29.382)

As for the number of polymorphic sites, 52 were observed in the tributary's population and 166 in the population of the main river, and, in relation to substitutions, the number of transitions was higher than the number of transversions in the Peixe River, observing the opposite on the river Tietê. Insertion-deletion (indel) substitution occurs in both rivers (Tab. 3).

Tab. 3. Rates of nucleotide substitutions for populations of *Prochilodus lineatus* from the middle Tietê river basin.

	Peixe River	**Tietê River**
Substitutions	52	162
Transitions	39	69
Transversions	10	73
Indel Sites	3	20

Neutrality Tests

Both applied neutrality tests showed population contraction occurring in the Peixe River. For the Tietê River, Tajima's D test showed population expansion and Fu's FS test showed population contraction. However, none of the values obtained was significant for both studied rivers. (Table 4).

Tab. 4. Neutrality tests for populations of *Prochilodus lineatus* from the middle Tietê river basin.

		Peixe River	**Tietê River**
Tajima's D test	Tajima's D	0.28226	-0.96127
	Tajima's D p-value	0.63100	0.20200
Fu's FS test	FS	0.81386	0.42337
	FS p-value	0.41500	0.37100

Population structure

The AMOVA results indicated that the observed variation occurs almost entirely within the populations (99.98%) and is practically absent among the studied populations (0.02%). The fixation index showed a value close to zero ($\Phi_{ST} = 0.00023$) revealing high gene flow and, consequently, absence of population structure. However, its value was not significant ($p > 0.05$).

The graphic representation of the haplotype network showed the absence of shared haplotypes and structure by study area (Fig. 2).

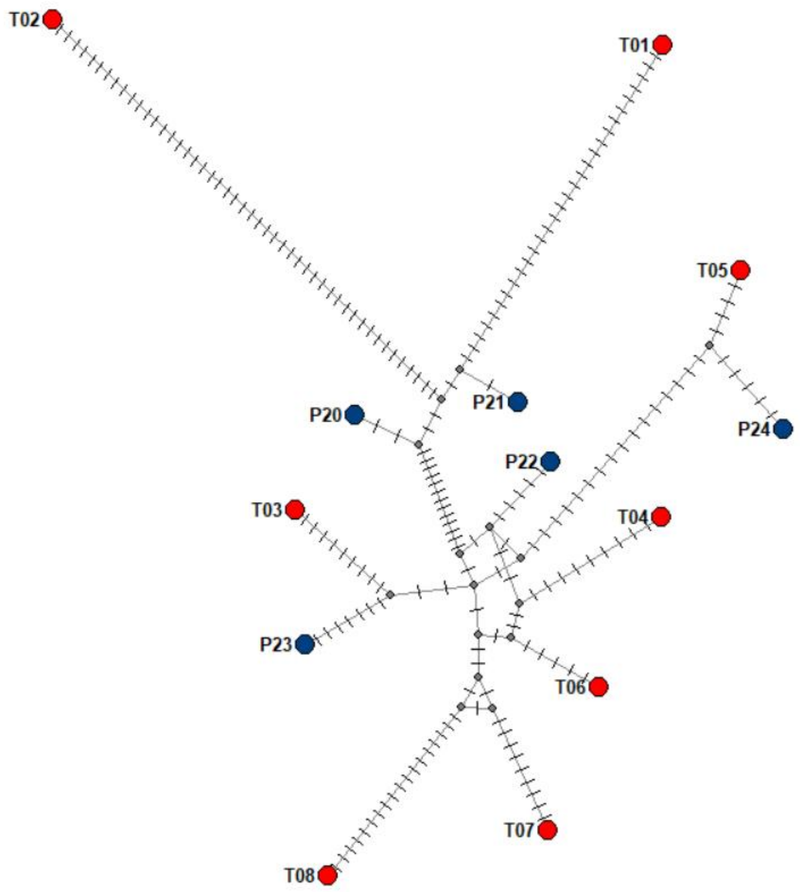

Fig. 2. Haplotype network for populations of *Prochilodus lineatus* from the middle Tietê river basin. The Peixe River individuals are in blue and the Tietê River individuals are in red.

Discussion

The haplotype diversity values obtained in our study are compatible with the values found in the literature for *Prochilodus lineatus* in analyses using the mitochondrial marker D-loop, which are close to 1 (one) (Garcez et al., 2011; Da Rosa et al., 2022). The combination of high haplotypic diversity and low nucleotide diversity observed in both Peixe and Tietê rivers may suggest that curimbatá populations are composed of closely related haplotypes (same origin), as demonstrated by the haplotypic network. As for the number of identified polymorphic sites, this can be considered high and explained by the rapid rate of evolution of the mtDNA control region.

Prochilodus lineatus is a fish species known for its high displacement capacity and is considered a long-distance migrator (Castro & Vari, 2004). The wide connectivity between the rivers studied, that is, the absence of natural and/or artificial physical barriers, and the short distance between the collection points, allows us to believe in the possibility of free movement of individuals between environments. This ist corroborated by our results, which demonstrated a high gene flow and lack of structure among the populations of curimbatás from the middle Tietê river basin (Φ_{ST} = 0.00023), being a panmictic population that is not significantly affected by the contrasting water qualities of the study sites (Urbanski & Nogueira, in elaboration, Urbanski et al., 2023), as hypothesized. This can be explained mainly by aspects of the biology of *Prochilodus lineatus*.

Studies show that migration affects species diversification, contributing to high gene mixing and lack of population structure (Burridge & Waters, 2020). Furthermore, this species is highly tolerant to unfavorable environmental conditions, being able to survive situations of hypoxia or even anoxia seen in the Tietê River (Urbanski & Nogueira, in elaboration; Urbanski et al., 2023). In this way, the movement of individuals between the main river and its tributary would not have impediments.

However, the population contraction evidenced by the neutrality tests in both the Peixe and Tietê rivers can demonstrate the negative influence on the populations of the degradation of habitats by pollution in the region.

Despite the low sample n used in this study, our results, although not significant, indicate slight mitochondrial divergence between populations of *Prochilodus lineatus*, so most of the variability found is within populations (99.98%, AMOVA). This is consistent with what has been found in other, more comprehensive studies, where even distant populations of this species often correspond to a single mitochondrial lineage (Henriques, 2014; Melo et al., 2018).

This study indicates that pollution does not act as a chemical barrier to the dispersal of the fish species *Prochilodus lineatus*. However, this conclusion cannot be extended to other species living in the middle Tietê river basin. Urbanski & Nogueira, in elaboration, demonstrated that the fish composition between these rivers is different, with few species in common. Species less tolerant to conditions of low concentration of dissolved oxygen in the water or non-migrants, for example, could avoid circulation through the Tietê River and be in the process of environmental isolation and genetic differentiation between these rivers, and future studies may find population structure among these other species.

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