ECOTOX BRASIL
SOCIEDADE BRASILEIRA DE ECOTOXICOLOGIA

Ecotoxicol. Environ. Contam., v. 20, n. 1, 2025, 35 - 44
doi: 10.5132/eec.2025.01.04

EEC

Original Article

Melanomacrophage Centers as a Histological Indicator of the Influence of Land Use on the Health of *Prochilodus lineatus* (Vallenciennes, 1837): Comparison Between Two Neotropical Rivers

Maressa Ludgero[1,2]*, José Guilherme Xavier[2,3], Thiago Mündel Ribeiro dos Santos[2,3], Luís Gustavo Nogueira de Carvalho[2,4], Larissa Leandra Moro Silva[2,3], Welber Senteio Smith[2,3,4]

[1] Programa de Pós-Graduação de Neurologia e Neurociência, Universidade Federal de São Paulo (Unifesp), São Paulo, SP, Brazil
[2] Laboratório de Ecologia Estrutural e Funcional de Ecossistemas, Universidade Paulista (UNIP), Sorocaba, SP, Brazil
[3] Programa de Pós-Graduação em Patologia Ambiental e Experimental, Universidade Paulista (UNIP), São Paulo, SP, Brazil
[4] Programa de Pós-Graduação em Aquicultura e Pesca, Instituto de Pesca, Secretaria de Agricultura e Abastecimento, São Paulo, SP, Brazil

Received April 16, 2025; Accept July 02, 2025

Abstract

This study evaluated the hepatic structural integrity of *Prochilodus lineatus*, collected in two rivers with distinct environmental conditions in the Sorocaba River basin, SP, Brazil. The analysis focused on melanomacrophage centers (MMCs), hepatic structures essential for the immune system, and the response of xenobiotics. The specimens were captured in September 2023 in the Sorocaba River, characterized by high urbanization, and in the Ipanema River, more preserved, with protected riparian forest, and inserted in a conservation unit. Subsequently, the fish livers were subjected to histological procedures, followed by morphometric analysis of the MMCs using the Fiji/Image J software. Differences were observed between the specimens of *Prochilodus lineatus* collected in the Sorocaba and Ipanema rivers, evidencing an increase in the dimensions of the melanomacrophagous centers in the specimens from the Sorocaba river (respectively 15598 ± 3485.56 mm² and 563 ± 72.84 mm), compared to the specimens analyzed in the Ipanema river (4593.67 ± 631.37 mm² and 281.47 ± 24.55 mm), statistically proven, with p=0.0077 for area and p=0.0033 for perimeter. The analysis of land use and occupation revealed differences between the two rivers studied, showing that the Sorocaba River has a greater influence of urbanization (urbanized area – 87.5%) and less vegetation cover (4.4%), while the Ipanema River has a higher percentage of vegetation (vegetation cover – 53.8%) and less urbanized area (1.9%). Differences were observed between the specimens of *Prochilodus lineatus* from the Sorocaba and Ipanema rivers, both for the area of the MMCs ($t = 5.87$; $p = <0.001$) and for the perimeter ($t = 5.39$; $p = <0.001$). The GLM demonstrated that the increase in the dimensions of the MMCs occurs in the river where there is more urbanized land (or the absence of forest formation). These findings corroborate previous studies that identify the increase in MMCs as a biomarker of immunological stress due to exposure to pollutants. Although widely used as biomarkers in ecotoxicology, the presence and response of MMCs can vary between species, requiring careful application. This study emphasizes the relevance of biomonitoring in rivers. It highlights *P. lineatus* as a sensitive and efficient bioindicator of pollution, particularly in environments impacted by urbanization, such as the Sorocaba River.

Keywords: Biomarker; Fish; Histopathology; Prochilodontidae; Rural Influence; Urbanization.

*Corresponding author: maressa.ludgero@unifesp.br

INTRODUCTION

The use of histopathology to investigate the pollutant effects is a common practice, with some tissues being particularly suitable for this purpose, such as the investigation of the gills and liver, as they are sensitive to xenobiotics (Rubio-Vargas *et al*., 2024). Histopathological analyses function as indicators of aquatic pollution when they are more effective than other isolated biochemical parameters (Greenfield *et al.*, 2008; Kostić *et al*., 2017). Fish gills are frequently analyzed in ecotoxicological studies due to their direct interface with the aquatic environment. In both polluted rivers and controlled experimental models, gill histopathology serves as a sensitive biomarker for assessing the effects of contaminants (Macirella & Brunelli, 2017; Marinović *et al.*, 2021).

Melanomacrophages (MMCs) are components of phagocytic cells found in the spleen, liver, and kidneys of fish. Although it is a nonspecific marker, their increase may indicate that these specimens are exposed to stress, a common alteration found in aquatic animals in the presence of xenobiotics (Fasulo *et al.*, 2010; Goessling & Sadler, 2015; Simonato *et al*., 2016; Viana *et al*., 2021; Popovic *et al*., 2023). Although studies with fish, amphibians, and reptiles have been carried out using MMCs for the last 50 years, there are still gaps in the mechanisms of their physiological activity, and the response is not unanimous in all fish species (Alesci *et al*., 2022; Bjørgen & Koppang, 2024). The study of MMCs can be expanded to different fish species and new forms of environmental assessment. The relationship with landscape assessment can expand and refine the use of this biomarker. The approach using landscape ecology can help in understanding how environmental changes can affect fish species. Specifically, it indicates how land use in the studied area is carried out through the analysis of satellite images. Thus, the association of biomonitoring with landscape characteristics is an emerging trend (Sagan *et al.*, 2020).

Prochilodus lineatus is a native and abundant fish species in the Sorocaba and Ipanema rivers, located in the center-southeast of the State of São Paulo, commonly found along the main channel of the river in addition to the marginal lagoons, where they find favorable conditions for their initial development. Despite belonging to the same basin, these lagoons present distinct environmental conditions, including use and occupation (urbanization) that differ due to the predominance of urbanization in the Sorocaba River and forestry in the Ipanema River. It is a freshwater teleost widely distributed in the neotropical region, recognized for its life cycle that involves reproductive migrations upstream. During spring and summer, with rising water levels, these fish move to specific spawning areas, forming large aggregations that are often concentrated downstream of waterfalls and dams (Godoy, 1975; Lozano *et al.*, 2019). The species is detritivorous, feeding mainly on fine sediments from the bottom, rich in organic particles and periphyton, which plays an essential role in its diet (Castro and Vari, 2003).

Prochilodus lineatus is an iliophagous fish and has been recognized as an efficient environmental biomarker for several pollutants, including metals and pesticides, presenting responses at molecular, biochemical, and cellular levels (Vieira *et al*., 2016; Davico *et al.*, 2018; Lunardelli *et al.*, 2018; Santos *et al*., 2018, 2020; Deiú *et al*., 2021; Martinez *et al.*, 2004).

There are different methods of investigating biomarkers in aquatic biota, with the aim of evaluating river and fish health. *Ex situ* and *in situ* studies have been carried out with *P. lineatus* (Camargo & Martinez, 2006; Deiú *et al.*, 2021). However, no studies have correlated *P. lineatus* MMCs' histology with land use and occupation (Vieira *et al.*, 2016). Research with captured specimens of this species has never been carried out, and is difficult to perform because they travel long distances, are subject to stress in different locations, in addition to the stress generated by migration and reproduction.

Furthermore, landscape assessments related to biomarkers are innovative and extremely relevant because high rates of land use change are strongly related to human activities (Ghisi *et al.*, 2022). Biomarkers landscape details and ecological aspects of the aquatic environment can improve the consistency of results of negative effects attributed to environmental pollution (Yu *et al.*, 2019; Ghisi *et al*., 2022).

In the present study, we assume that the Sorocaba River is more disturbed due to urbanization than the better preserved and less urbanized Ipanema River, and that these conditions cause different levels of morphological and morphometric biomarkers in *P. linetus*. To test our hypothesis, these liver biomarkers were selected as indicators of stress in specimens captured in both rivers. We hypothesize that specimens from the river with greater urban influence will present more pronounced histopathological alterations than those from the river that are more preserved in terms of land use, i.e., more forested.

MATERIALS AND METHODS

Study Area

This study was conducted in the Sorocaba River Basin, located in the southeastern-central region of São Paulo State, Brazil. This basin covers an area of 5.269 km² and includes 18 cities. The Sorocaba River, approximately 230 km long and with approximate coordinates of 23°28'11.29"S, 47°27'5.60"O, is the main left-bank tributary of the Tietê River, formed by the Sorocamirim and Sorocabuçu watercourses, and empties into the town of Laranjal Paulista. In contrast, the Ipanema River, whose coordinates are approximately 23°25'18.22"S, 47°35'47.75"O, has significant environmental protection provided by the Ipanema National Forest and its buffer zone (Figure 1). The samples were obtained from a section of the Sorocaba River and the Ipanema River, according to ethics committees CEUA (no. 4721030821), SISBIO (no. 24151-1 and 85747-1), and SISGEN (no. ADAF4EF).

Sampling Procedure and Laboratory Analysis

Prochilodus lineatus adult specimens were collected in September 2023. Specimens of similar sizes were considered, using six specimens from the Sorocaba River (mean ± SD: 584.5 ± 515.1 g and 30.97 ± 8.09 cm) and six specimens from the Ipanema River (mean ± SD: 537.96 ± 320.69 g and 28.07 ± 8.84 cm). Collections were made using gillnets with mesh sizes ranging from 8, 10, and 12 cm between opposite nodes and lengths of 2 to 10 meters. The species is considered Least Concern (LC) in terms of conservation (ICMBio, 2023; IUCN, 2023).

The specimens were anesthetized and sacrificed by sectioning the spinal cord, and then a longitudinal incision was made in the ventral portion of the animal. The liver was removed and sent for macroscopic and histological analyses, where it was fixed in 10% buffered formalin for determination of MMCs. The livers were processed in an automatic tissue processor OMA DM40, being sectioned longitudinally and then dehydrated in a graded ethanol series, cleared with xylene, and embedded in paraffin. Paraffin blocks were serially sectioned at 5 µm thickness on a microtome Leica RM 2125RT (Leica, Germany), dewaxed and stained using a combination of haematoxylin and eosin (HE). Histological sections were evaluated by optical microscopy with a 40× objective, and digitized images were acquired with an OPTICAM® photomicroscope equipped with a 14.3 MP OPUHD digital camera. For each animal, 5 of the largest MMCs were visualized and manually outlined by tracing their margins with the help of a computer mouse, and the area and perimeter were measured in micrometers.

The analysis of land cover was conducted in two specific stretches: Ipanema River, covering the Ipanema National Forest to its mouth, and the Sorocaba River, from the border between the cities of Votorantim and Sorocaba to Parque das Águas, in Sorocaba. The data for these sections were extracted from the database of the National Water and Sanitation Agency (ANA) and subsequently processed and refined as needed.

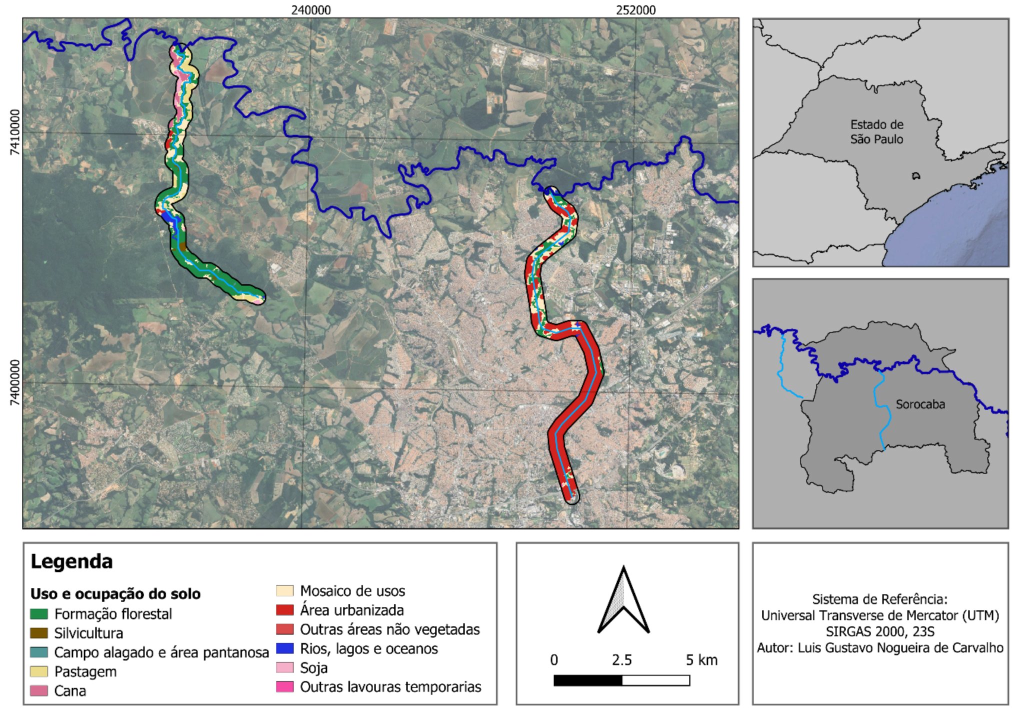

Figure 1. Map of land use and occupation along the studied stretches of the Ipanema River (orange point) and the Sorocaba River (yellow point), highlighting the main classes: forest formation (dark green), silviculture (brown), flooded field, and marshy area (light blue), pasture (yellow), sugar cane (pink), mosaic of uses (beige), urbanized areas (red), other non-vegetated areas (gray), bodies of water such as rivers and lakes (dark blue), soybeans (purple) and other temporary crops (pink).

The area of influence of the analyzed sections was defined as a 300-meter-wide strip from each riverbank along the entire length of the river. Based on this delimitation, the base map of land use and land cover for the year 2022 was clipped using data from Collection 8.0 of the MapBiomas Project. The data processing was carried out using the QGIS geoprocessing software, version 3.36.2, where clipping and spatial organization operations were performed. Subsequently, to quantify the area occupied by each land use and cover class within the defined area of influence, the r.report tool - part of the GRASS GIS module set - was employed. This tool enabled the precise extraction of the area corresponding to each physiognomy present in the analyzed image. Thus, it was possible to obtain a detailed characterization of the landscape surrounding the studied river sections.

Data analysis

Statistical analyses of the results were performed using GraphPad Prism 9® software and R language scripts (r.report). The data were subjected to the Shapiro-Wilk normality test, followed by an unpaired Student’s t-test, adopting a significance level of $p \leq 0.05$ to determine statistical significance. We used QGIS 3.36.2 software to identify the percentage of use and occupancy of the physiognomies present in the analyzed sections. To investigate the interaction between land use and occupancy with the area and perimeter of MMCs, a generalized linear model (GLM) with Gamma error distribution and log link function was applied.

RESULTS AND DISCUSSION

Differences were observed between the specimens of *Prochilodus lineatus* collected in the Sorocaba and Ipanema rivers, evidencing an increase in the dimensions of the melanomacrophagous centers in the specimens from the Sorocaba river (respectively 15598 ± 3485.56 mm^2 and 563 ± 72.84 mm), compared to the specimens analyzed from the Ipanema river (4593.67 ± 631.37 mm^2 and 281.47 ± 24.55 mm) (Figure 2). Statistically significant differences, with p=0.0077 for area and p=0.0033 for perimeter (Student's t-test) between the specimens from the rivers were verified (Figure 3). The results presented herein are corroborated by the findings of Camargo & Martinez (2007) with the same species in an experiment carried out in an urban river. Changes in melanomacrophagic centers have been considered reliable (Fournier *et al*., 2001; Balamurugan *et al*., 2012) and sensitive biomarkers for assessments of the influence of environmental conditions in fish (Balamurugan *et al*., 2012), as evidenced by Viana *et al*. (2021), who compared MMCs in different species from 3 other locations.

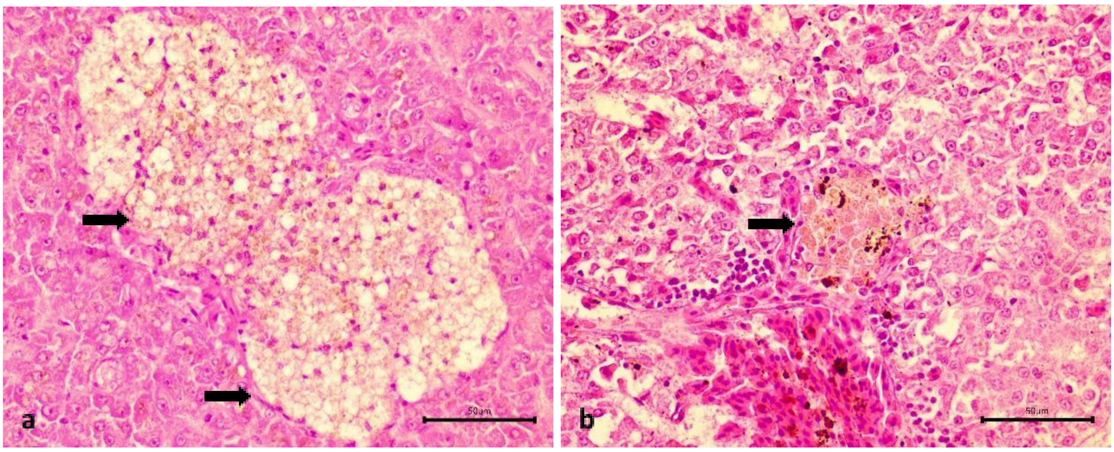

Figure 2. (A) Photomicrograph of the liver of *Prochilodus lineatus* from the Sorocaba river showing a large center of melanomacrophages, with a predominance of vacuolated, weakly acidophilic population, with occasional melanic pigmentation (arrows). Hematoxylin-eosin, 40x. (B) Photomicrograph of the liver of *Prochilodus lineatus* from the Ipanema river showing a discrete, poorly delimited melanomacrophage center, with occasional melanin pigmentation (arrow). Hematoxylin-eosin, 40x. Scale bar=50µm.

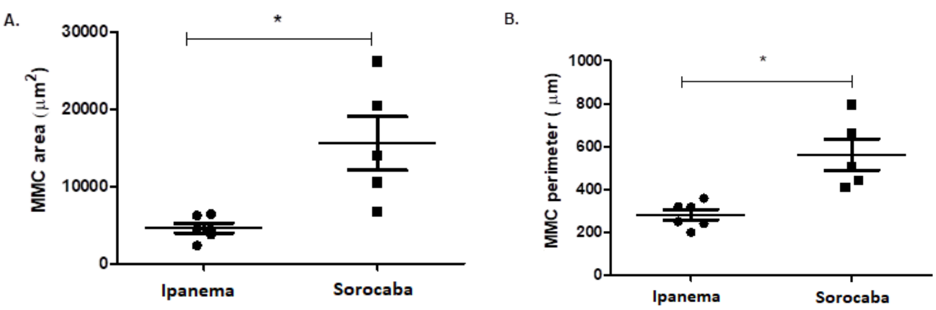

Figure 3. (A) Graph showing the mean area and standard deviation of the melanomacrophage centers present in the liver territory of *Prochilodus lineatus* from the Sorocaba river and Ipanema river, respectively, with p=0.0077 (Student's t test). (B) Mean perimeter and standard deviation of the melanomacrophage centers present in the liver territory of *Prochilodus lineatus* from the Sorocaba river and Ipanema river, respectively, with p=0.0033 (Student's t test).

The GLM demonstrated that the increase in the dimensions of the MMCs occurs in the river where there is more urbanized land (or lack of forest formation) (Figure 4). The results demonstrated that the effectiveness of the conservation of permanent preservation areas for the environmental quality of rivers is of paramount importance for maintaining fish health. Urbanization can result in significant changes in water quality due to increased surface runoff and the introduction of pollutants (Walsh *et al.*, 2005; Soinski *et al.*, 2022a). These changes can reduce the quality of aquatic habitats, negatively impacting fish health, as can be evidenced by biomarkers. Loss of riparian vegetation and increased sedimentation are other factors that can contribute to river degradation (Barrella *et al*., 2000).

According to Hartley *et al*. (1996), MMCs in fish are critical elements of the immune system, including responses to adverse environmental conditions such as pollution, and their number, size, and/or pigment content in fish, especially those exposed to environmental contaminants, may become greatly altered (Elston *et al.*, 1997; Nowak & Kingsford, 2003). According to Steinel & Bolnick (2017), MMCs are potentially practical biomarkers of immune function in laboratory studies and also in natural environments. Several studies evaluating metals (Simonato, *et al*., 2021; Alesci, *et al.*, 2022) and agricultural pesticides (Marteja and Modina, 2021) were carried out through *in situ* or *ex situ* exposure, but studies that use specimens collected in the river itself for evaluation are rare, among them we can mention Neves *et al.* (2018) who evaluated a species of detritivorous fish, whose feeding habits are the same as those of the species in the present study. These authors concluded that histological changes observed in fish from rivers with a higher percentage of natural vegetation cover were considered modest and indicated normal functioning of the organ, corroborating the results obtained in the present study.

As verified by Deiú *et al*. (2021), *P. lineatus* is an abundant fish and plays an important role in the fish community in rivers of the Upper Paraná, and can be used as a bioindicator, as it is a species sensitive to several classes of contaminants. In addition to the use of this species, the use of biomarkers in environmental assessment and monitoring has been used as an efficient tool, however this is not unanimous among the species, as stated by Cardoso *et al.* (2018) and Passantino *et al*. (2024), in their studies where in stressful conditions, the specimens in question presented almost no MMCs. These authors emphasized that it is a peculiarity of each species, which suggests that the use of this biomarker should be judicious. Therefore, in the present study, some criteria were adopted to minimize stress due to factors other than the environmental one. Adult specimens that were not in the reproductive phase were used, and as it is a migratory species, the stretches of the rivers where they were captured have similar environmental conditions. Environmental variables must be carefully considered in the analyses, and it is recommended that *in situ* studies using MMCs be interpreted with caution (Steinel & Bolnick, 2017).

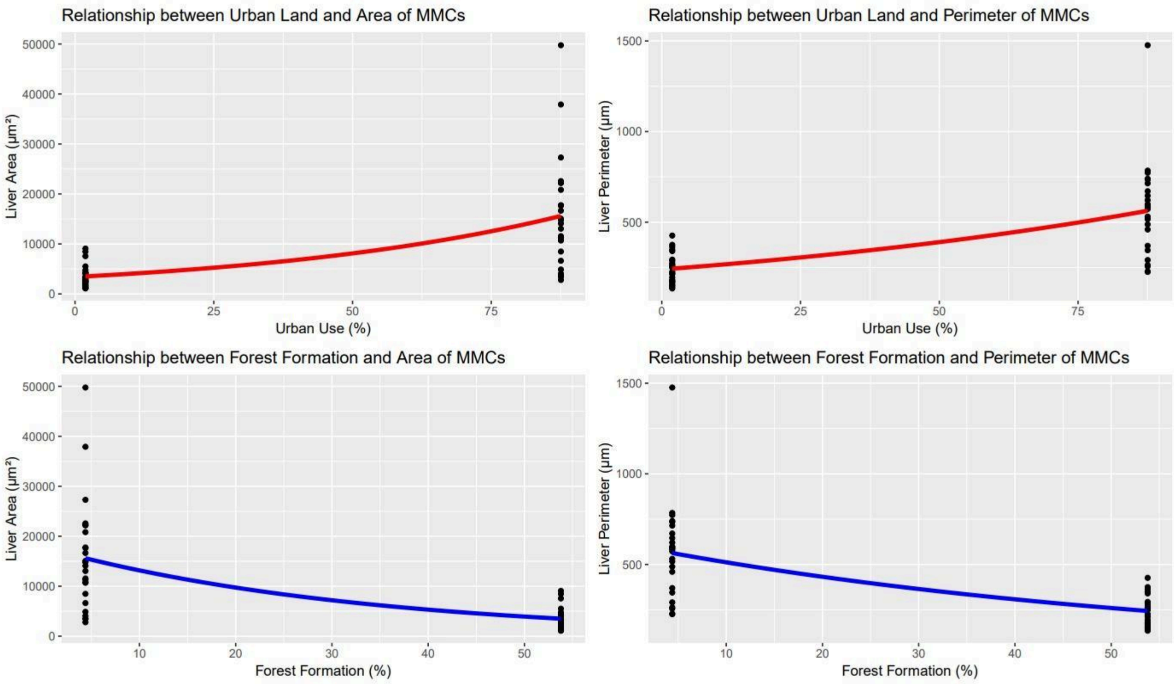

Figure 4. Interaction between land use and occupation with the area and perimeter of the MMCs.

Table 1. Land use and occupation variables for each river studied, including the description of the physiognomy, the corresponding area, and the relative percentage of each variable.

Physiognomy (Ipanema River)	Area	Corresponding percentage (%)
Other temporary crops	10.690	0.1309
Flooded field and marshy area	73.977	0.9057
Forestry	147.955	1.8114
Urbanized area	153.739	1.8822
Soybean	238.436	2.9191
River, lake, and ocean	316.470	3.8744
Sugarcane	464.601	5.6879
Pasture	760.552	9.3111
Mosaic of uses	1.608,087	19.6871
Forest formation	4.393,708	53.7903
Total	8.168,215	100
Physiognomy (Sorocaba River)	**Area**	**Corresponding percentage (%)**
Pasture	822	0.014959073
Other non-vegetated areas	4107	0.074740769
Other temporary crops	16.431	0.299017669
River, lake, and ocean	32.049	0.583240051
Forest formation	241.541	4.395656191
Mosaic of uses	389.458	7.087506754
Urbanized area	4.810,585	87.54487949
Total	5.494,993	100

The analysis of land use and occupation revealed differences between the two rivers studied, with potential implications for local fish species, including *Prochilodus lineatus* (Figure 1 and Table 1). The results showed that the Sorocaba River has a greater influence of urbanization (urbanized area – 87.5%) and less vegetation cover (4.4%), and the Ipanema River has a higher percentage of vegetation (vegetation cover – 53.8%) and a smaller urbanized area (1.9%). The Ipanema River is protected by a conservation unit, evidencing a mostly natural landscape (Smith *et al*., 2013, 2021). This situation indicates that it is a river with superior environmental quality to the Sorocaba River, presenting more preserved riparian vegetation and less influence of urbanization, which is a favorable condition for the target species of the study.

The Sorocaba River presents characteristics of the urban river syndrome (Walsh *et al.*, 2005), indicating contamination by raw or low-efficiency treated sewage, high nutrient concentrations, and silting (Soinski *et al.*, 2022a). According to Soinski *et al.* (2022b), urbanization directly affects rivers with numerous impacts, such as sewage discharge, dams, and pipelines, which cause profound changes in their characteristics and also in their ichthyofauna. According to Smith *et al*. (2019), the Sorocaba River suffers from poorly executed and planned dredging interventions that can also trigger severe stress in *Prochilodus lineatus*, which uses the studied stretch for reproduction.

The investigation presented in this manuscript highlights the low frequency of histopathological alterations in specimens collected in the most preserved river with the highest percentage of vegetation, indicating normal liver function, reinforcing the effectiveness of preservation areas for environmental quality and, consequently, for fish health. In addition, the results highlight the need to expand studies and use biomarkers to evaluate fish species in neotropical rivers and the adoption of this methodology by environmental agencies in monitoring programs (Ghisi *et al*., 2022). The use of biomarkers in fish provides rapid and sensitive results on the effects of a pollutant or other disturbances to which they are subjected and can anticipate harmful ecological effects (Neves *et al.*, 2018; Ghisi *et al*., 2022), assisting government agencies in decision-making.

CONCLUSION

In the present study, the data confirm the higher histopathological index in the liver of *P. lineatus* specimens in the most urbanized river compared to the less impacted river, with predominance of vegetation on its banks. This demonstrates that the morphometric characteristics and presence of MMCs in fish can be sensitive indicators for assessing environmental quality. The differences observed between specimens from the compared rivers reinforce the role of preserved riparian vegetation in maintaining them. Furthermore, these findings support the importance of incorporating histopathological markers into environmental biomonitoring strategies in aquatic ecosystems.

ACKNOWLEDGMENTS

We would like to thank the Coordination for the Improvement of Higher Education Personnel (CAPES), the Pro-Rectory of Graduate Studies and Research/Universidade Paulista, and all our colleagues for their help with the fieldwork and in the Laboratory of Structural and Functional Ecology of Ecosystems. Special thanks are extended to Paulo Ailton Vedovato for technical support.

AUTHOR CONTRIBUTIONS

ML: Conceptualization, Investigation and Review and Editing; **JGX**: Data Curation, Writing - Original Draft Preparation; **TMRS**: Data Analysis and Data Curation; **LGNC**: Data Analysis and Data Curation; **LLM**: Writing - Review and Editing; **WSS**: Supervision, Conceptualization, Methodology and Review and Editing.

REFERENCES

Alesci, A., Cicero, N., Fumia, A., Petrarca, C., Mangifesta, R., Nava, V., Lo Cascio, P., Gangemi, S., Di Gioacchino, M., & Lauriano, E. R. (2022). Histological and chemical analysis of heavy metals in kidney and gills of Boops boops: Melanomacrophages centers and rodlet cells as environmental biomarkers. Toxics, 10(218). https://doi.org/10.3390/toxics10050218

Balamurugan, S., Deivasigamani, B., Kumaran, S., Sakthivel, M., Rajsekar, T., & Priyadharsini, P. (2012). Melanomacrophage Centers Aggregation in *P. lineatus* Spleen as Bio-Indicator of Environmental Change. Asian Pacific Journal of Tropical Disease, 2(2), 635-638. https://doi.org/10.1016/S2222-1808(12)60235-7

Barrella, W.; Petrere Junior, M.; Smith, W. S.; Montag, L. F. A. (2000). As relações entre as matas ciliares os rios e os peixes. In: Rodrigues, R. R.; Leitão Filho; H. F. (Ed.). Matas ciliares: conservação e recuperação. São Paulo, SP: EDUSP, p. 187-207.

Bjørgen, H., & Koppang, E. O. (2024). The melano-macrophage: The black leukocyte of fish immunity. Fish & Shellfish Immunology, 148, 109523. https://doi.org/10.1016/j.fsi.2024.109523

Camargo, M. M. P., & Martinez, C. B. R. (2006). Biochemical and physiological biomarkers in *Prochilodus lineatus* submitted to in situ tests in an urban stream in southern Brazil. Environmental Toxicology and Pharmacology, 21(1), 61–69. https://doi.org/10.1016/j.etap.2005.07.016

Camargo, M. M. P., & Martinez, C. B. R. (2007). Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. Neotropical Ichthyology, 5(3):327-336. https://doi.org/10.1590/S1679-62252007000300013

Cardoso, R. Carvalho-Neta, R.N.F., de Castro, A.C.L., Ferreira, C.F.C., Silva, M.H.L., de Jesus Azevedo, J.W., Sobrinho, J.R.S.C., & Santos, D.M.S. (2018). Histological and Genotoxic Biomarkers in Prochilodus lacustris (Pisces, Prochilodontidae) for Environmental Assessment in a Protected Area in the Northeast of Brazil. Bull Environ Contam Toxicol. 101(5):570-579. doi: 10.1007/s00128-018-2464-8. Epub 2018 Oct 22. PMID: 30350121.

Castro, R.M.C. & Vari, R.P. (2004). Detritivores of the South American fish Family Prochilodontidae (Teleostei: Ostariophysi: Characiformes): a phylogenetic and revisionary study. Smith. Contrib. Zool. Washington, 1–189.

Davico, C.E., Loteste, A., Parma , M.J., Poletta, G. &

Simoniello, M.F. (2018). Stress oxidative and genotoxicity in *Prochilodus lineatus* (Valenciennes, 1836) exposed to commercial formulation of insecticide cypermethrin. Drug and Chemical Toxicology. 43(1):79–84. https://doi.org/10.1080/01480545.2018.1497643

Deiú, A.S., Miglioranza, K.S.B., Ondarza, P.M., de la Torre, F.R. (2021). Exposure to environmental concentrations of fipronil induces biochemical alterations in a neotropical freshwater fish. Environmental Science and Pollution Research International, 28(32), 43872–43884. https://doi.org/10.1007/s11356-021-13786-w

Elston, R. A., Drum, A. S., Pearson, W. H., & Parker, K. (1997). Health and condition of Pacific herring (Clupea pallasi) from Prince William Sound, Alaska, 1994. Diseases of Aquatic Organisms, 31 (2), 109-126. https://doi.org/10.3354/dao031109

Fasulo, S., Marino, S., Mauceri, A., Maisano, M., Giannetto, A., D´Agata, A., Parrino, V. Minutoli, R., y De Domenico, E. (2010). A multibiomarker approach in Coris julisliving in a natural environmental. Ecotoxicology and Environmental Safety, 73:1565-1573.

Fournier, J. W., Summers, J. K., Courtney, L. A., Engle, V. D., & Blazer, V. S. (2001). Utility of splenic macrophage aggregates as an indicator of fish exposure to degraded environments. Journal of Aquatic Animal Health, 13(2), 105-116. https://doi.org/10.1577/1548-8667(2001)013

Ghisi, N. C., Larentis, C., Oliveira, E. C., Neves, M. P., Zavaski, A. G., Roque, A. A., Wachtel, C. C., Silva, A. P., Lima, E. B. S., Costa, G. O. N., Amorim, J. P. A., Ribeiro, C. A. O., Delariva, R. L. (2022). Environmental assessment of neotropical streams using fish as bioindicators: a multibiomarker and integrated approach. Hydrobiologia, 849:4587–4604. https://doi.org/10.1007/s10750-020-04460-2(0123456789().,-volV() 0123458697().,-volV)

Godoy, M.P. (1975). Peixes do Brasil, sub-ordem Characoidei da bacia do rio Mogi-Guaçu. Vol. 4. Ed. Franciscana, Piracicaba, SP.

Goessling & Sadler, 2015; Goessling, W., & Sadler, K. C. (2015). Zebrafish: an important tool for liver disease research. Gastroenterology, 149(6), 1361-1377.

Greenfield, B.K., Swee, J., Teh, S.J., Ross, J.R., Hunt, J., Zhang, G.H., Davis, J.A., Ichikawa, G., Crane, D., Hung, S., Deng, D., Teh, F. & Green, P. 2008. Contaminant concentrations and histopathological effects in sacramento splittail (Pogonichthys macrolepidotus). Archives of Environmental Contamination and Toxicology 55: 270-281

Hartley, W. R., Thiyagarajah, A., & Treinies, A. M. (1996). Liver lesions in garfish (Lepisosteidae) as biomarkers of exposure. Marine Environmental Research, 42(1-4), 217-221. https://doi.org/10.1016/0141-1136(95)00037-2

ICMBio. Sistema de Avaliação do Risco de Extinção da Biodiversidade – SALVE. [Internet]. 2023. Disponível em: https://salve.icmbio.gov. br/ 4

International Union for Conservation of Nature (IUCN). Standards and petitions subcommittee. Guidelines for using the IUCN Red List categories and criteria. Version 14 [Internet]. Gland; 2023. Available from: https://www.iucnredlist. org/resources/redlistguidelines

Kostić, J., Kolarević, S., Kračun-Kolarević, M., Aborgiba, M., Gačić, Z., Paunović, M., Višnjić-Jeftić, Ž., Rašković, B., Poleksić, V., Lenhardt, M. & Vuković-Gačić, B. (2017). O impacto de múltiplos estressores na resposta dos biomarcadores nas guelras e no fígado de douradas durante diferentes estações. Sci Total Environ, 601:1670–1681. https://doi.org/10.1016/j.scitotenv.2017.05.273

Lozano, I. E., Llamazares Vegh, S., Gómez, M. I., Piazza, Y. G., Salva, J. L., & Fuentes, C. M. (2019). Episodic recruitment of young *Prochilodus lineatus* (Valenciennes, 1836) (Characiformes: Prochilodontidae) during high discharge in a floodplain lake of the River Paraná, Argentina. Fisheries Management and Ecology, 26(3), 260-268. https://doi: 10.1111/fme.12348

Lunardelli, B., Cabral, M. T., Vieira, C. E. D., Oliveira, L. F., Risso, W. E., Meletti, P. C., & Martinez, C. B. R. (2018). Chromium accumulation and biomarker responses in the Neotropical fish *Prochilodus lineatus* caged in a river under the influence of tannery activities. Ecotoxicology and Environmental Safety, 153, 188–194. https://doi.org/10.1016/j.ecoenv.2018.02.023

Macirella, R., & Brunelli, E. (2017). Alterações morfofuncionais em guelras de peixe-zebra (Danio rerio) após exposição ao cloreto de mercúrio. International Journal of Molecular Sciences, 18(4), 824. doi:10.3390/ijms18040824

Marinović, Z., Miljanović, B., Urbányi, B., & Lujić, J. (2021). Gill histopathology as a biomarker for discriminating seasonal variations in water quality. Applied Sciences, 11(21), 9504. https://doi.org/10.3390/app11209504

Marteja, J. C. & Modina, R. M. R. (2021). Melanomacrophage Centers in Nile Tilapia (Oreochromis niloticus L.) as Biomarker for Carbamate Exposure. Journal of Environmental Science and Management, 24-1: 25-35. https://doi.org/10.47125/jesam/2021_1/03

Martinez, C.B.R., Nagae, M.Y., Zaia, C.T.B.V. & Zaia, D.A.M. 2004. Morphological and physiological acute effects of lead in the neotropical fish *Prochilodus lineatus*. Braz. J. Biol. 64, 797–807.

Neves, M. P., Amorim, J. P. A., & Delariva, R. L. (2018). Influence of land use on the health of a detritivorous fish (Ancistrus mullerae) endemic to the Iguaçu ecoregion: Relationship between agricultural land use and severe histopathological alterations. Environmental Science and Pollution Research, 25(11), 11670–11682. https://doi.org/10.1007/s11356-018-1283-0

Nowak, B. F., & Kingsford, M. J. (2003). Exposure to endosulfan results in lipofuscin accumulation in hepatocytes of the freshwater catfish Tandanus tandanus. Diseases of Aquatic Organisms, 56(2), 135-143. https://doi.org/10.3354/dao056135

Passantino, L., Corriero, A., Pousis, C., Zupa, R., Perillo, A., Superio, J., Kumari Swain, J., Foss, A., Galindo-Villegas, J., & Ventriglia, G. (2024). Hepatic melanomacrophage centers in the arctic cultured fish, Cyclopterus lumpus are not indicative of its health state. Aquaculture, 581, 740417. https://doi.org/10.1016/j.aquaculture.2023.740417

Rubio-Vargas, D. A., Morais, T. P. D., Randi, M. A. F., & De Araújo, F. G. (2024). Multispecies and multibiomarker assessment of fish health from Iguaçu River Reservoir, Southern Brazil. Environmental Monitoring and Assessment, 196(564). https://doi.org/10.1007/s10661-024-12720-0

Sagan, V., K. T. Peterson, M. Maimaitijiang, P. Sidike, J. Sloan, B. A. Greeling, S. Maalouf & C. Adams. (2020). Monitoring inland water quality using remote sensing: potential and limitations of spectral indices, bio-optical simulations, machine learning, and cloud computing. Earth-Science Reviews. 205, 103187. https://doi.org/10.1016/j.earscirev.2020.103187.

Santos, C., de Oliveira, M. T., Cólus, I. M. de S., Sofia, S. H., & Martinez, C. B. dos R. (2018). Expression of cyp1a induced by benzo(A)pyrene and related biochemical and genotoxic biomarkers in the neotropical freshwater fish *Prochilodus lineatus*. Environmental Toxicology and Pharmacology, 61, 30–37. https://doi.org/10.1016/j.etap.2018.05.008

Santos, C. & Bueno dos Reis Martinez, C. (2020). Biotransformation in the fish *Prochilodus lineatus*: An organ-specific approach to cyp1a gene expression and biochemical activity. Environmental Toxicology and Pharmacology, 80, 103467. https://doi.org/10.1016/j.etap.2020.103467

Simonato, J. D., Mela, M., Doria, H. B., Guiloski, I. C., Randi, M. A. F., Carvalho, P. S. M., Meletti, P. C., Silva de Assis, H. C., Bianchini, A. & Martinez, C. B. R. (2016). Biomarkers of waterborne copper exposure in the neotropical fish *Prochilodus lineatus.* Aquatic Toxicology, 170, 31–41. https://doi.org/10.1016/j.aquatox.2015.11.012

Smith, W.S., Biagioni, R.C. & Halcsik, L. (2013). Fish fauna of Floresta Nacional de Ipanema, São Paulo State, Brazil. Biota Neotrop.,13(2): 175 - 181. https://doi.org/10.1590/S1676-06032013000200016

Smith, W.S., Halcsik, L., Biagioni, R.C., Pinheiro, L.A.S. & Stefani, M.S. (2021). An updated list of the ichthyofauna of Ipanema National Forest, São Paulo, Brazil. Check List., 17(3), 827-840. https://doi.org/10.15560/17.3.827

Smith, W.S., Silva F.L., Biagioni R.C. (2019). River dredging: when the public power ignores the causes, biodiversity and Science. Ambiente & Sociedade, 22 (3-4): e00571. https://doi.org/10.1590/1809-4422asoc0057r1vu19L1AO

Soinski, T. A., Gato, C. S., Matsumoto, A. A., Brazão, M. L., Smith, W. S. (2022a). Dredging on water bodies and margin interventions: effects on fish. Bol. Inst. Pesca, 48: e641. https://doi.org/10.20950/1678-2305/bip.2022.48.e641

Soinski, T. A., Gato, C. S., Matsumoto, A. A., Brazão, M. L., Smith, W. S. (2022b). Influence of different types of margins in the fish assemblage from an urban river. Bol. Inst. Pesca, 48: e651. https://doi.org/10.20950/1678-2305/bip.2022.48.e651

Steinel, N.C. & Bolnick, D.I. (2017). Melanomacrophage Centers As a Histological Indicator of Immune Function in Fish and Other Poikilotherms. Front. Immunol., 8:827. doi: 10.3389/fimmu.2017.00827

Popović, N. T., Čižmek, L., Babić, S., Strunjak-Perović, I., & Čož-Rakovac, R. (2023). Liver damage in fish related to wastewater treatment plant effluent. Environmental Science and Pollution Research International, 30(17), 48739–48768. https://doi.org/10.1007/s11356-023-26187-y

Viana, H. C., Jesus, W. B., Silva, S. K. L., Jorge, M. B., Santos, D. M. S., & Carvalho Neta, R. N. F. (2021). Hepatic melanomacrophage centers aggregation in Sciades herzbergii (Pisces, Ariidae) as indicators of environmental change and well-being. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 73(4), 868–876. https://doi.org/10.1590/1678-4162-12327

Vieira, C. E. D., Costa, P. G., Lunardelli, B., De Oliveira, L. F., Da Costa Cabrera, L., Risso, W. E., Primel, E. G., Meletti, P. C., Fillmann, G., & Bueno dos Reis Martinez, C. (2016). Multiple biomarker responses in *Prochilodus lineatus* subjected to short-term in situ exposure to streams from agricultural areas in Southern Brazil. Science of the Total Environment, 542, 44–56. https://doi.org/10.1016/j.scitotenv.2015.10.071

Walsh, C. J., Roy, A. H., Feminella, J. W., Cottingham, P. D., Groffman, P. M. & Morgan, R. P. (2005). The urban stream syndrome: Current knowledge and the search for a cure. Journal of the North American Benthological Society, 24:706–723.

Yu, H., X. Liu, B. Kong, R. Li & G. Wang, (2019). Landscape ecology development supported by geospatial technologies: a review. Ecological Informatics Elsevier 51: 185–192. https://doi.org/10.1016/j.ecoinf.2019.03.006.