



## First record of plastic ingestion by an important commercial native fish (*Prochilodus lineatus*) in the middle Tietê River basin, Southeast Brazil

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**Abstract:** This is the first study to report the plastic ingestion by *Prochilodus lineatus* in Brazilian fluvial ecosystems. We examined 32 individuals collected in two contrasting lotic environments: the highly polluted Tietê River and its much less degraded tributary, Peixe River. Most individuals, 71.88%, contained plastic in their digestive tract, with averages of 3.26 and 9.37 particles per individual in the tributary and main river, respectively. The blue color was predominant among the observed plastic particles and size ranged from 0.18 to 12.35 mm. Plastic ingestion must be accidental, predominantly, since the species has an iliofagous eating habit. As this species is regionally the most important fishery resource, potential adverse effects of this type of contamination may be transferred to human consuming populations. Mitigation measures against pollution are urgent in the Tietê River basin.

**Keywords:** Curimatá; Digestive tract content; Plastic contamination; River pollution.

## Primeiro registro de ingestão de plástico por um importante peixe comercial nativo (*Prochilodus lineatus*) na bacia do médio rio Tietê, sudeste do Brasil

**Resumo:** Este é o primeiro estudo a relatar a ingestão de plástico por *Prochilodus lineatus* em ecossistemas fluviais brasileiros. Nós examinamos 32 indivíduos coletados em dois ambientes lóticos contrastantes: o rio Tietê, altamente poluído, e seu tributário muito menos degradado, o rio do Peixe. A maioria dos indivíduos, 71,88%, continha plástico em seus tratos digestivos, com médias de 3,26 e 9,37 partículas por indivíduo no tributário e no rio principal, respectivamente. A cor azul foi predominante entre as partículas plásticas observadas e o tamanho variou de 0,18 a 12,35 mm. A ingestão de plástico deve ser predominantemente acidental, uma vez que a espécie possui um hábito alimentar iliofago. Como esta espécie constitui o recurso pesqueiro mais importante regionalmente, potenciais efeitos adversos desse tipo de contaminação podem ser transferidos para populações humanas consumidoras. Medidas de mitigação contra a poluição são urgentes na bacia do rio Tietê.

**Palavras-chave:** Contaminação plástica; Conteúdo do trato digestivo; Curimatá; Poluição dos rios.

### Introduction

Plastics are synthetic polymers that provide uncountable benefits to modern society. However, its massive consumption associated with improper disposal in nature has been the source of a serious and widespread contamination (Silva-Cavalcanti et al. 2017, Blettler et al. 2018, Olivatto et al. 2018).

The accumulation of plastic particles, especially in the aquatic ecosystems, has increased considerably over the last decades. Recent estimates suggest that about 4.9 billion tons of this material is found in natural environments (Geyer et al. 2017). In the oceans alone, millions of tons of plastic are dumped every year, mostly, approximately 80%, transported by rivers (Jambeck et al. 2015).

In the rivers, once fragmented into smaller particles, these polymers can be ingested, intentionally or accidentally, by many animals, especially the ichthyofauna (Lusher et al. 2013, 2015, Cardozo et al. 2018, Azevedo-Santos et al. 2019). In Brazil, the presence of plastic in the digestive tract of fish was first recorded in estuarine ecosystems (Possatto et al. 2011, Dantas et al. 2012, Ramos et al. 2012) and then in freshwater ones (Silva-Cavalcanti et al. 2017, Andrade et al. 2019).

The fish species *Prochilodus lineatus* (Valenciennes 1837), popularly known as “curimba” or “curimatá”, is widely distributed in freshwater environments of the Neotropical region (Castro & Vari 2003, Avigliano et al. 2017). In general, it is an important fishery resource in Southeast Brazil (Novaes & Carvalho 2009, Maruyama et al. 2010,

Novaes & Carvalho 2013), but catches have declined in the dammed (hydropower reservoirs) stretches of large rivers (David et al. 2016). The first report of plastic ingestion for this species was provided by Pazos et al. (2017), based on analyzes of individuals from La Plata River (Argentina), and more recently by Blettler et al. (2019), for the middle Paraná River, also in Argentina.

In this paper, we report the first case of plastic ingestion by *P. lineatus* in Brazilian rivers, from individuals of lotic (undammed) stretches of the middle Tietê River basin, where the species is regionally the main fishery resource. The aim of this study was to analyze and compare fish plastic ingestion from contrasting environments in terms of water pollution. Our hypothesis was to find a higher microplastic ingestion in individuals from the Tietê River, given by the strong environmental degradation of this river (which flows through the largest megalopolis of the Southern Hemisphere).

## Material and Methods

The study area is in the middle Tietê River basin. The region is located between the municipalities of Laranjal Paulista and Anhembi, São Paulo State, Southeastern Brazil, which includes the Tietê River and important tributaries such as the Peixe River and the Sorocaba River, on the left bank, and the Capivari River, on the right bank (Figure 1).

Thirty-two specimens of *Prochilodus lineatus*, 13 from the Tietê River and 19 from the Peixe River, were analyzed. Fish were collected in April 2019 (IBAMA/SISBIO permanent sampling license to MGN: 13794-1) using gill nets disposed for about 15 hours (overnight). Complementary, some individuals were also obtained from local professional fishermen. After being euthanized with an overdose (more than 283.55 mgL<sup>-1</sup>) of anesthetic (eugenol) (Vidal et al. 2008), the fish were fixed in 10% formalin and stored in 70% ethanol. All organisms used in the study are deposited in the ichthyological collection of the Laboratório de Biologia e Genética de Peixes (LBP), Universidade Estadual Paulista (UNESP), campus of Botucatu, São Paulo, Brazil (lots: LBP 29184; LBP 29185).

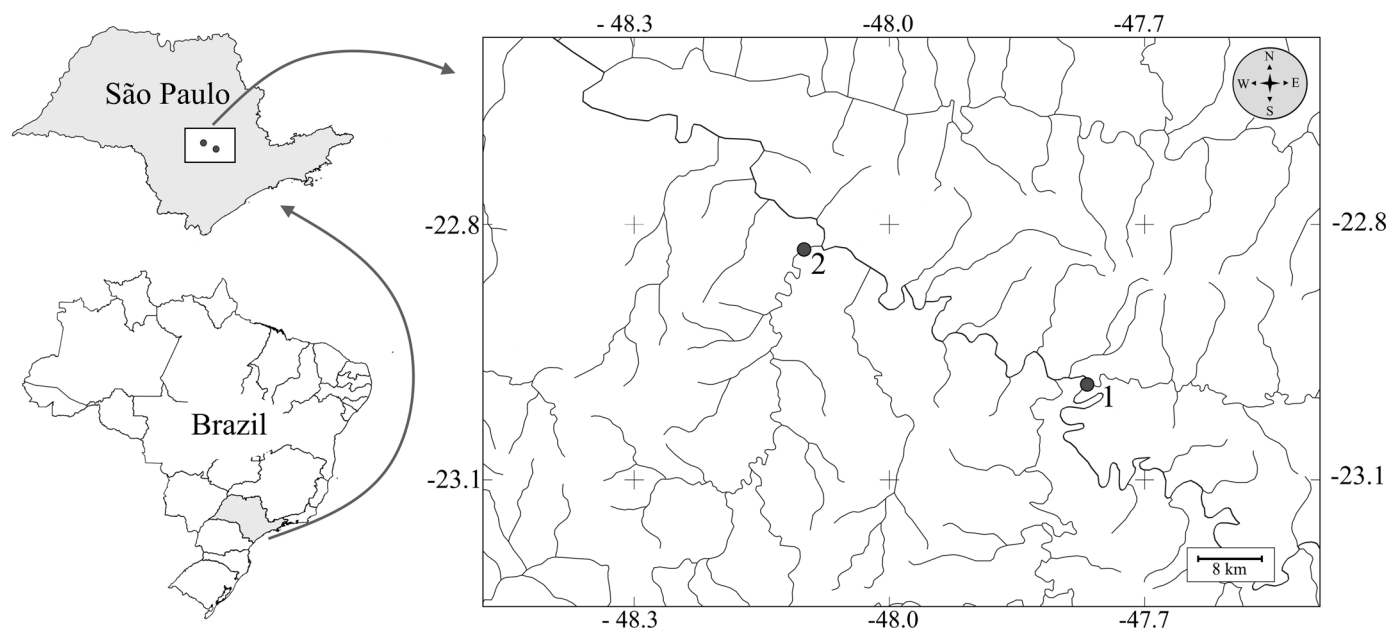
In the laboratory, individual digestive tracts were removed through a longitudinal incision in the abdominal region with the use of a scalpel. The entire digestive content of each animal was rinsed with 70% absolute ethylic ethanol (L. S. Chemicals and Pharmaceuticals) in a 50 µm mesh sieve and then transferred to Petri dishes. To avoid contamination during sample processing, we used cotton lab coats and chirurgical gloves, all work surfaces and utensils were alcohol sterilized, and Petri dishes were covered with glass during visual identification (protocols according to Silva-Cavalcanti et al. 2017). Additionally, a Petri dish filled with ethanol was left open over the laboratory bench, adjacent to the microscopy, and examined in the end of every analysis session, for quantification of eventual airborne contamination.

Under a stereomicroscope, plastic particles were separated from organic particles, counted, measured and classified by color. Identification followed visual criteria to determine if the particles were synthetic: absence of cellular or organic structures, clear and homogeneous colors, and fibers evenly thick throughout their entire length (Norén 2007, Hidalgo-Ruz et al. 2012). To confirm the plastic nature of the particles, we performed a peroxide hydrogen (also adding Fe II as catalyzer) digestion test, which is indicated to oxidize organic matter (Prata et al. 2019).

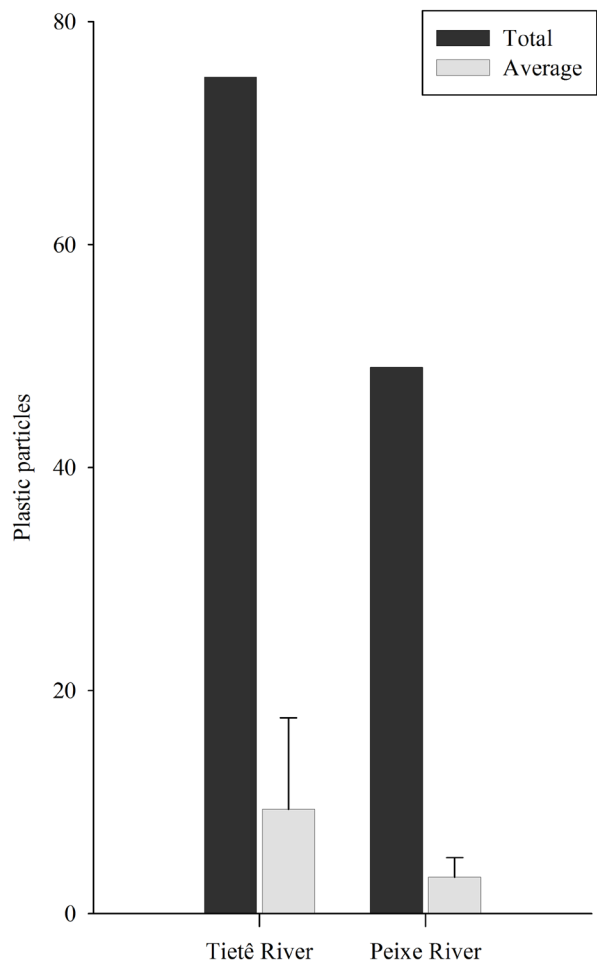
The largest longitudinal dimension was considered to determine the size of the plastic particles. Plastic intake was characterized per sampling location, number of individuals who ingested plastic and number of particles in the digestive tract of each animal.

## Results

Most analyzed fish (71.88%) ingested plastic, corresponding to 15 individuals from Peixe River and eight individuals from Tietê River. Forty-nine particles were found in specimens collected in the tributary and 75 in those from the main river, with respective mean values (and standard deviations) per individual of 3.26 ( $\pm$  1.75) and 9.37 ( $\pm$  8.19) (Figure 2).



**Figure 1.** Study area location – middle Tietê River basin, Southeast Brazil. Black circles indicate the sampling sites: (1) Tietê River; and (2) Peixe River.



**Figure 2.** Total and average per individual ( $\pm$  SD) of plastic particles found in *Prochilodus lineatus* from middle Tietê River basin.

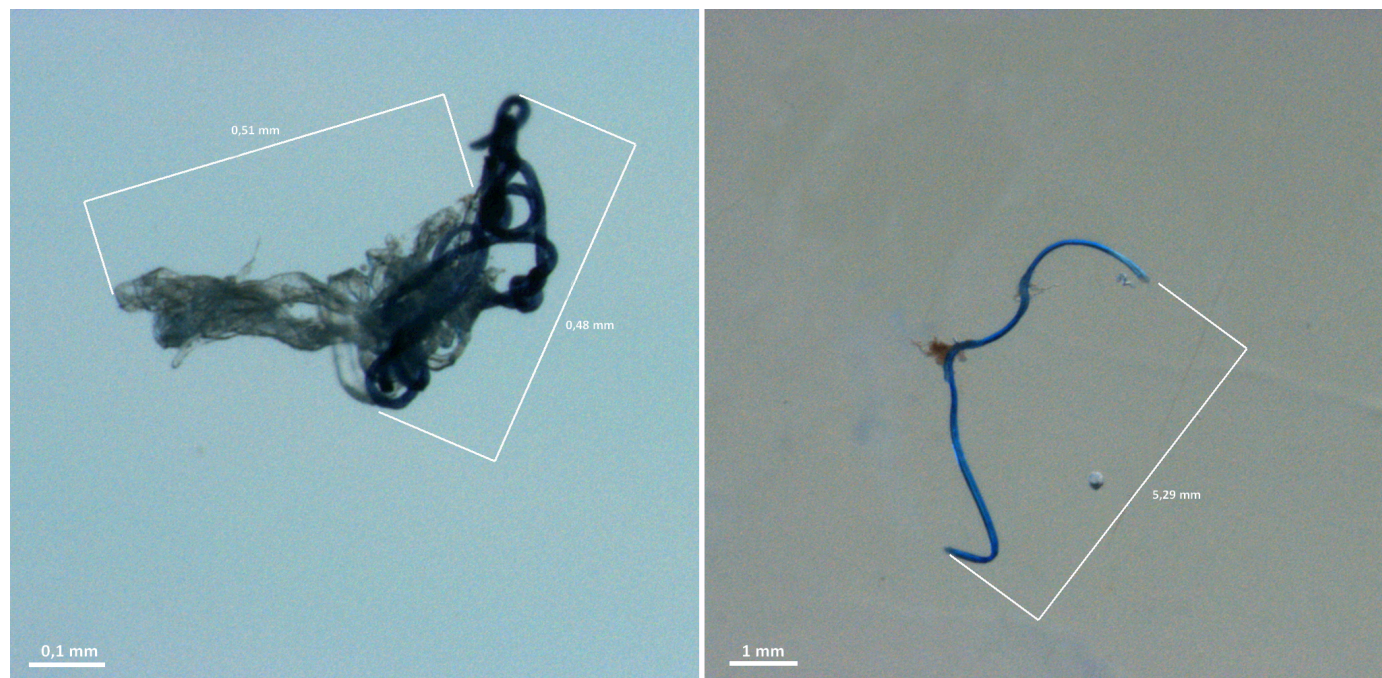
Particles presented eight distinct colors (yellow, blue, white, black, pink, transparent, green and red), with predominance of blue in both rivers (61.2% in Peixe River and 68% in Tietê River) (Figure 3, 4), and their sizes ranged from 0.18 mm to 12.35 mm, with 44.44% larger than 1 mm (Figure 3).

**Discussion**

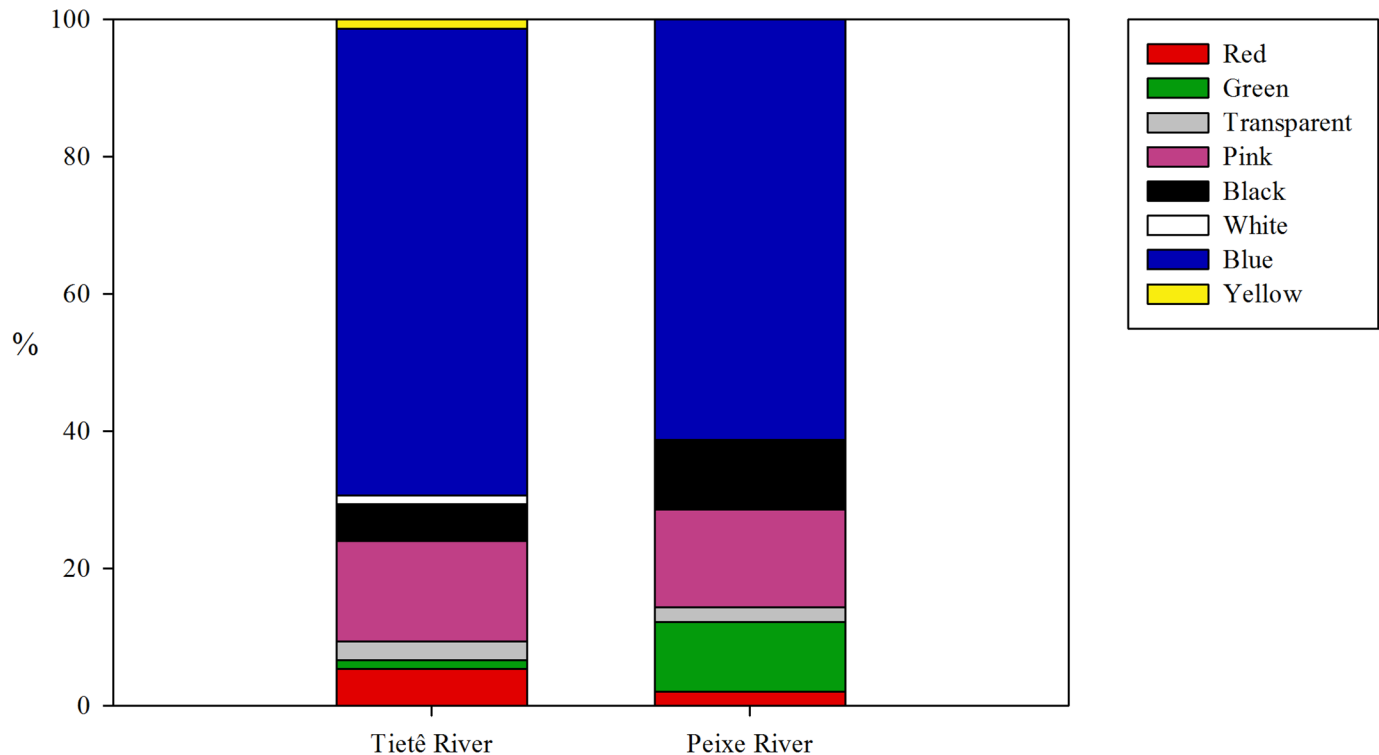
This work is the first to record plastic particles ingestion by *Prochilodus lineatus* in Brazil. Studies on plastic ingestion by freshwater fish are still scarce in Central and South America, including the Brazilian territory.

Most individuals we analyzed (71.88%) contained micro and mesoplastic in their digestive tracts. Studies show that the percentage of contaminated individuals is highly variable, depending on the considered ecosystem. In Brazil, moderate rates of plastic ingestion, 7.9% to 23%, were reported for estuarine environments (Possatto et al. 2011, Dantas et al. 2012, Ramos et al. 2012), in contrast with the remarkable value of 83%, for fish from an urban river (Silva-Cavalcanti et al. 2017). Andrade et al. (2019) observed a rate of ingestion of 25% for Xingu River, what we think is high, considering that this is an Amazonian river, a region scarcely occupied by human populations. Two other studies that also analyzed plastic ingestion by *P. lineatus*, both in highly polluted rivers in Argentina, observed that 100% of the analyzed individuals had ingested plastic particles (Pazos et al. 2017; Blettler et al. 2019).

It is noticeable in our results the fact that the degree of individual contamination (mean of plastic particles) was about three times higher in the Tietê River compared to its tributary, Peixe River. The number of particles per individual in Tietê River, 9.37, is very high, even when compared to the amount found by Silva-Cavalcanti et al. (2017), 3.6 particles per individual, for fish captured in a river crossing a city in northeast Brazil.



**Figure 3.** Examples of plastic particles, originated from irregularly dumped solid wastes, found in the stomachs of *Prochilodus lineatus* individuals from middle Tietê River basin.



**Figure 4.** Percentage variation of the different plastic particles in relation to the observed color pattern, found in the stomachs of *Prochilodus lineatus* individuals from middle Tietê River basin.

However, the mean value of ingested plastic particles by *P. lineatus* from the middle Paraná River in Argentina, 9.9 (Blettler et al. 2019), was even higher than in Tietê River.

Results corroborate our initial hypothesis, explained by the fact that the stretch of the Tietê River upstream the studied area is directly affected by the huge metropolis of São Paulo, with 19.6 million inhabitants (IBGE 2010) (last official census). The river receives, for more than a century, an enormous amount of solid waste, as well as domestic and industrial effluents (Tundisi et al. 2008, Tundisi 2018, Buckeridge & Ribeiro 2018). Other tributaries of the Tietê River, such as Pinheiros River, in the São Paulo city, are also heavily polluted and certainly contribute to the input of synthetic polymers into the main course.

The plastic particles size we observed ranged from 0.18 to 12.35 mm, which includes from micro to mesoplastic. Our minimum values are below the amplitude commonly found for other marine and freshwater ecosystems (Liboiron et al. 2019; Andrade et al. 2019), which could have influenced in the high rate of contamination we report. According to Song et al. (2015), visual identification of microplastics using a microscope is a reliable method for identifying particles > 1 mm. What could explain the small size of the plastic particles we found is the fact that this material may have been ingested from the sediments (see below). Plastic deposited for a long time in the bottom of rivers is probably highly fragmented.

The target species, *P. lineatus*, is well known to move dozens of kilometers, especially for reproductive migration (Agostinho et al. 2003, Capeleti & Petreire Jr. 2006, Stassen et al. 2010). The fact that the analyzed individuals from Peixe River were sampled only a few kilometers upstream the mouth, make us to consider the possibility that the plastic ingestion might have occurred in Tietê River, just before captures.

Another important characteristic of *P. lineatus* is the eating habit of the iliofagous type (Moraes et al. 1997), which consists in the behavior of searching for benthic invertebrates and organic detritus as food items in the rivers bottom. In this work, we did not evaluate the presence of plastic in the sediment, but much probably this was the source of contamination and certainly should be considered in future studies. Blettler et al. (2019) found an extremely high deposition of microplastic particles, reaching 12.687 per m<sup>2</sup>, in shoreline sediment samples from polluted Argentine river environments.

As many studies around the world we also verified the predominance of blue color among ingested plastics (e.g. Possatto et al. 2011, Ramos et al. 2012, Dantas et al. 2012, Alomar et al. 2017, Ory et al. 2017, Pazos et al. 2017, Bessa et al. 2018, Chagnon et al. 2018, Compa et al. 2018, Digka et al. 2018, Blettler et al. 2019). The different fish species may have a predilection for that color or most plastic pollution is composed by blue material. This second possibility seems to be more consistent to explain our results, once *P. lineatus* feeds on the bottom sediments were discrimination of the food items by color is difficult due to the limitation of light penetration. Again, the characterization of plastic particles deposition in the water column and sediments is a necessary investigation for a better understanding of this environmental problem.

The species *P. lineatus* constitutes an important resource for the regional professional fishery (Novaes & Carvalho 2009, Maruyama et al. 2010, Novaes & Carvalho 2013, David et al. 2016), which are commercialized both locally and in the metropolitan region of São Paulo. In addition to the effects of plastic contamination on fish, the health authorities should be concerned about potential adverse effects that may affect human consumers. Plastic particles can be vectors of microorganisms and also adsorb various chemical contaminants

(Paul-Pont et al. 2018), such as phthalates and bisphenol, endocrine disruptors commonly used in the manufacture of this kind of material, as well as heavy metals and persistent organic pollutants (POPs) (Rios et al. 2007, Massos & Turner 2017, Olivatto et al. 2018). However, the degree of biomagnification of plastics particles in the aquatic food chains and the adverse effects on other consumers such as birds and humans is currently poorly understood (Pegado et al. 2018).

The contamination we have demonstrated shows that additional studies should continue, such as the application of spectrometry techniques to discriminate the kinds of polymers. Other components of the regional biota (e.g. plankton and benthos) must be evaluated too, for a complete understanding of the problem. Control measures against solid waste pollution in the Tietê River basin are urgent. In fact, the government of São Paulo State has recently started an environmental recovery program for Pinheiros River, a tributary within the watershed (Folha 2019). That is an excellent initiative, but actions should be extended to the entire river basin.

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## Author Contributions

Bruna Q. Urbanski: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Ana C. Denadai: Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Valter M. Azevedo-Santos: Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Marcos G. Nogueira: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

## Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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