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Contribution to the knowledge of the biology of *Prochilodus lineatus* (Valenciennes, 1837) in a neotropical river basin

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ABSTRACT

This study provides information on the spatial distribution, population variables, and natural diet of the species *Prohilodus lineatus* in the Sorocaba River Basin, located in the state of São Paulo, SP, Brazil. The species is distributed mainly along the middle and lower Sorocaba River channel, in addition to its tributaries. A total of 74 individuals were captured, 46 females and 28 males of *P. lineatus*. The captured specimens ranged in length from 31.0 cm to 48.0 cm and weighed from 177 g to 4530 g. Females (n = 46) had a mean weight of 892.34 ± 782.34 g and a mean length of 44.65 ± 2.54 cm, while males (n = 28) had a mean weight of 752.25 ± 390.33 g and a mean length of 44.57 ± 3.25 cm. The weight-length relationship was not statistically different between sexes, and both sexes showed negative allometric growth, with a regression constant (b) value of 2.6889 for females and 2.7586 for males. The species' natural diet consisted mainly of items of autochthonous origin, mainly vegetables, seeds, muddy and sandy sediments, filamentous algae, Chironomid larvae, nematode worms, and insect eggs, in addition to microplastic particles detected in 5% of the specimens.

Keywords: Curimbatá; Dam; Iliophagous; Migrator.

Contribuição ao conhecimento da biologia de *Prochilodus lineatus* (Valenciennes, 1837) em uma bacia hidrográfica neotropical

RESUMO

O presente trabalho traz informações sobre a distribuição espacial, variáveis populacionais, e dieta natural da espécie *Prohilodus lineatus* na Bacia do Rio Sorocaba, situado no estado de São Paulo, SP, Brasil. A espécie se encontra distribuída principalmente pela calha do médio e baixo rio Sorocaba, além dos tributários. Foram capturados ao todo 74 indivíduos, sendo 46 fêmeas e 28 machos de *P. lineatus*. Os espécimes capturados apresentaram comprimento entre 31,0 cm e 48,0 cm e pesos entre 177 g e 4530 g. As fêmeas (n = 46) apresentaram peso médio de 892,34 ± 782,34 g e comprimento médio de 44,65 ± 2,54 cm, enquanto os machos (n = 28) peso médio de 752,25 ± 390,33 g e comprimento médio de 44,57 ± 3,25 cm. A relação peso-comprimento não foi estatisticamente diferente entre os sexos e ambos apresentaram um crescimento alométrico negativo, com valor da constante de regressão (b) igual a 2,6889 para fêmeas e 2,7586 para machos. A dieta natural da espécie foi composta majoritariamente por itens de origem autóctone, principalmente vegetal, sementes, sedimentos lodoso e arenoso, algas filamentosas, larvas de Chironomidae, verme nematoide e ovos de insetos, além de partículas de microplástico detectadas em 5% dos exemplares.

Palavras-chave: Barramento; Curimbatá; Iliófago; Migrador.

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INTRODUCTION

Neotropical freshwaters have the richest ichthyofauna in the world, with 4,225 species currently (Tonella et al., 2023). Knowledge of the biology of these species is still deficient, partly evidenced by the advancement of functional ecology studies in recent years in the neotropical region, which requires basic information, like on functional tracts in other environmental situations and river basins. Without information from basic research on species and their characteristics, we cannot understand the biological composition of local ecosystems or ecological function, and it limits our understanding of other biodiversity topics such as species life history and functional ecology (Gomes et al., 2023).

In recent decades, numerous studies have addressed the biology of tropical and subtropical fish in lotic and lentic environments (Barbieri & Barbieri, 1983; Barbieri, 1995; Barbieri et al., 2000; Basile-Martins et al., 1975; Capeleti & Petrere Jr., 2006; Godoy, 1975; Hartz & Barbieri, 1994; Lopes et al., 2018; Lowe-McConnell, 1987; Machado-Allison, 1990; Toledo Filho et al., 1986, 1987; Vazzoler, 1996).

Prochilodus lineatus (Valenciennes, 1837) is considered a long-distance migratory species in Brazilian river basins (Agostinho et al., 2007; Makrakis et al., 2012), being predominant in lotic environments, and it has an ascending reproductive and descending trophic cycle as described in literature (Castro & Vari, 2004; Rêgo et al., 2008). In this way, the analysis of the population structure allows the interpretation of energy allocation as a life strategy and provides important information for the management and protection of resources (Machado & Foresti, 2012; Rêgo et al., 2008).

The target species of this study presents deficiencies in relation to basic information, and in recent years few works have been published on reproductive, population, and trophic ecology aspects, such as the one by Santos (2020), that detected spatial differences in the sexual proportion, size, growth, and age composition of the species; and by Urbanski et al. (2023), relating the condition factor to environmental conditions.

Prochilodus lineatus is a detritivorous species commonly found in neotropical freshwater environments. Its diet primarily consists of organic matter associated with muddy and sandy sediments, including decomposing plant material, algae, and microorganisms (Bowen, 1983; Yossa & Araujo-Lima, 1998). This feeding behavior plays a crucial role in nutrient cycling, as *P. lineatus* processes and redistributes organic matter in the aquatic ecosystems (Novakowski et al., 2008). Although its diet is predominantly composed of autochthonous resources, studies have reported the ingestion of allochthonous particles, including microplastics, indicating potential anthropogenic influences on its feeding habits (Kawakami and Vazzoler, 1980; Vicentin et al., 2012).

The sex ratio represents the proportion of males to females in a population. According to Fisher's theory (1930), sexually reproducing populations tend to stabilize at a 1:1 sex ratio, as deviations from this ratio favor the selection of one sex until equilibrium is restored. However, factors such as differential mortality, sexual dimorphism, and social behavior can influence this ratio, affecting population dynamics and reproduction.

Length and weight are key indicators of population growth and health, while the condition factor (Kn), which relates weight to length, provides insights into the individuals' physical and nutritional state. Together, these biological parameters offer a comprehensive view of population health and environmental influences.

The reproductive process is a critical aspect of population dynamics, as successful recruitment is essential for maintaining viable populations and ecosystem balance (Honji et al., 2020). Fish reproduction is strongly influenced by environmental conditions, resource availability, suitable spawning sites, and changes in the hydrological regime, which can impact reproductive activity (Godinho et al., 2010; Portella et al., 2021).

The present study aimed to characterize some biological aspects of the species *P. lineatus*, one of the important migratory species of the Sorocaba River basin, still abundant in the basin's rivers. Information about its spatial distribution, diet, and population parameters, such as abundance of males and females, biometric data, and the weight-length relationship, are presented in this manuscript.

MATERIAL AND METHODS

Study area

The Sorocaba River basin (Fig. 1) is in the subarea known as Middle Tietê, in São Paulo state, Brazil. The basin has a drainage area of 5,269 km^2, covering 22 municipalities where around 1.2 million inhabitants live. The average slope is 0.28%, indicating low flow velocity (Smith, 2003). Along the way, it suffers two dams, the first in Votorantim, for the energy use of the surrounding cities, and the second in Cerquilho, in the old San Juan plant, where there is a ladder for the fish to climb (Smith et al., 2014).

Literature review

To inventory records of the species' capture in the basin, a systematic review was carried out using specific criteria to select and exclude articles. The scope of the analysis included

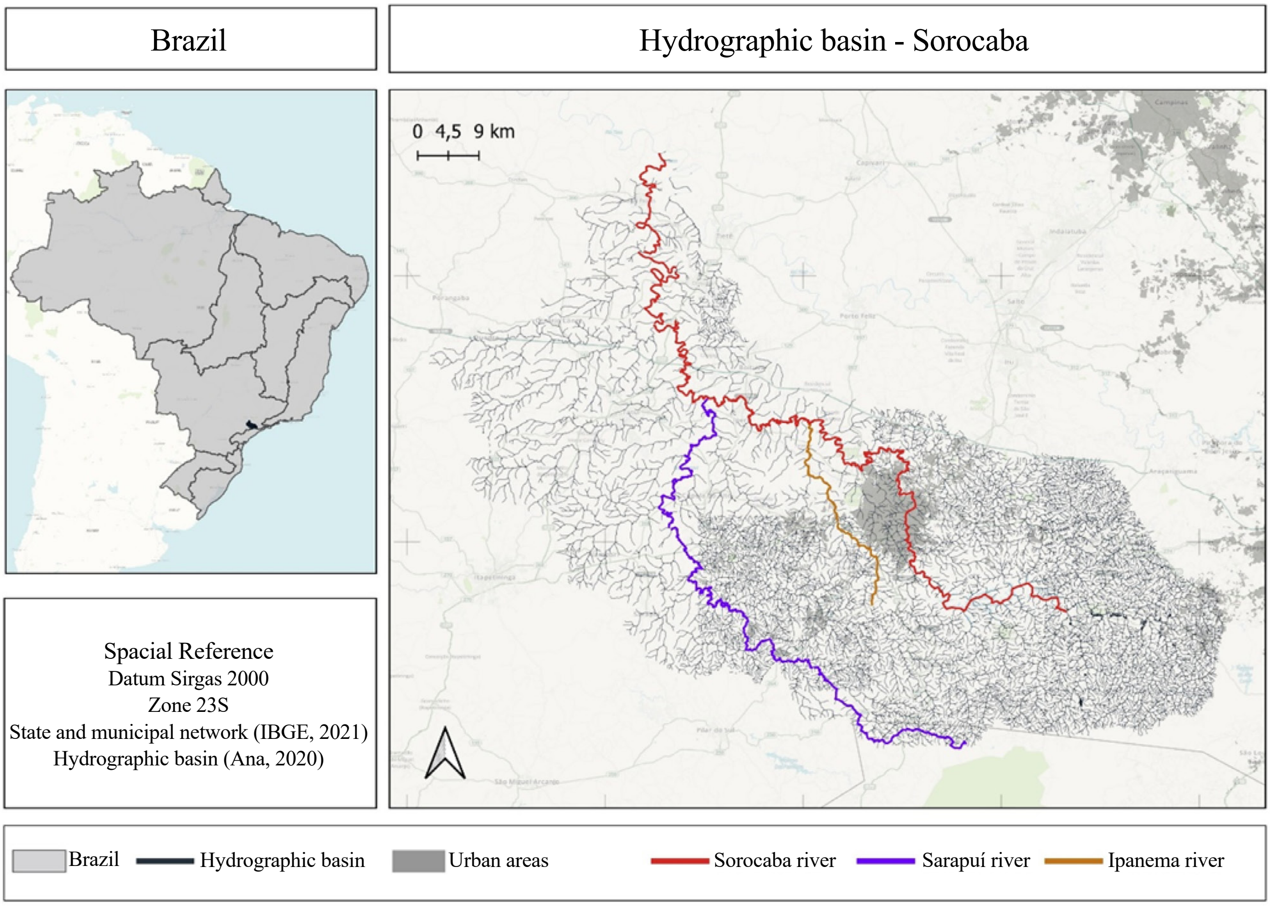

Figure 1. Study area, Sorocaba River basin, São Paulo state, Brazil.

publications covering the period from 1990 to 2023. To verify the spatial distribution of *P. lineatus* along the sampled stretches based on previous studies, a bibliographic search was performed for studies that addressed such species' distribution patterns, with the support of electronic databases such as Scopus, Web of Science, Scientific Electronic Library Online (SciELO), and Google Scholar. Based on the location of its occurrences, a map was constructed showing the distribution of the species in the studied basin.

Sampling

Primary data were obtained from collections carried out monthly, from September 2019 to September 2021, in sections of the Sorocaba, Sarapuí, and Ipanema rivers, under the Permanent License for Collection of Zoological Material no. 24,151-1 and the Ethics Committee on the Use of Animals' certificate no. 7268230523, from Universidade Paulista.

Two sets of 10-m-long gillnets with different heights and mesh sizes between 3 and 12 cm between opposite nodes were used. These gillnets were placed at each point for 12 hours, being placed at dusk on one day and removed at dawn the next day. The captured specimens were anesthetized with benzocaine, fixed in 10% formalin, and preserved in 70% alcohol. Vouchers of all species were deposited in the fish collection of the Laboratório de Ecologia Estrutural e Funcional de Ecossistemas, at Universidade Paulista, under no. 187.

After the capture, the specimens were taken to the laboratory, where their standard length, total weight, and sex were obtained. Through a ventral incision extending from the anus towards the cranial region, the gonads were exposed to identify the sex of the adult individuals, following the protocol described by Núñez and Duponchelle (2009) for determining reproductive activity. For analysis of the stomach contents, the individuals were dissected to remove the digestive tract (Abelha et al., 2001). To remove the gastrointestinal tract, the individuals were dissected through an incision that began at the anal opening and extended to the region close to the pectoral fins. The organs were stored in glass containers containing 10% formalin solution, properly labeled with information about the location, point, date, species, and structure of the collection. Subsequently, the stomach contents were analyzed under a stereoscopic microscope equipped with a digital camera. After these procedures, the specimens were fixed in 10% formalin and preserved in 70% alcohol.

Data analysis

Considering the low capture of the species in the Ipanema and Sarapuí rivers, it was decided to use only data from the Sorocaba River in the present study. To classify the collected *P. lineatus* specimens into juvenile and adult stages, we used standard length (SL) as the primary criterion. Individuals with an SL < 35 cm were categorized as juveniles, while those with an SL ≥ 35 cm were considered adults. This threshold was established based on previous studies on the species' growth patterns and sexual maturity (Vicentin et al., 2012). Morphological characteristics, such as body proportions and fin development, were also considered to confirm the classification.

To determine the weight-length relationship, using data only from fish caught, the measurements were plotted on the x and y axes, and a curve was subsequently generated, representing the dispersion of the intersection points between the x and y axes. From the weight-length relationship, the relative condition factor (Krelative) was verified using Eq. 1:

$$\text{Krelative} = (\text{observed weight} / \text{expected weight}) \quad (1)$$

The expected weight was calculated using Eq. 2:

$$y = a \cdot xb \quad (2)$$

where: y: the total weight; x: the total length; a and b: obtained through the logarithmic transformation of the length and weight data by linear regression (respectively, the regression constant and the angular coefficient according to the method defined by Le Cren, 1951).

After estimating individual values for Kn, the average value of the interval observed for treatment and statistical comparison to the centralizing value (Kn = 1.0) was calculated by the Student's t test (Ho: Kn = 1.0 and a = 0.05).

To calculate the 95% confidence interval (95%CI), a critical t-value of 1.9889 was used for both groups, corresponding to 45 degrees of freedom for females (n-1) and 27 degrees of freedom for males (n-1). First, the standard error (SE) of the mean was calculated using Eq. 3, for females, and Eq. 4, for males:

$$\text{SE} = \text{standard deviation} / \text{square root of n} \quad (3)$$

$$\text{SE} = 0.14 / \text{square root of } 28 \quad (4)$$

Then, the 95%CI was calculated by Eq. 5:

$$95\%\text{CI} = \text{mean} \pm (\text{critical t} \times \text{SE}) \quad (5)$$

To analyze the importance of the identified foods, the frequency of occurrence method was used, since it evaluates the percentage of stomachs in which a given food occurs, as well as frequency (F%) and relative volume (V%), which were combined to form the dietary index (IAi%) (Kawakami & Vazzoler, 1980). The trophic guild was identified based on the food index, checking the frequency of occurrence and volume of each food item. Among the various criteria used to classify trophic guilds (Delariva et al., 2013), the guild was determined considering an intermediate value (60% of the diet composed of a certain food group).

RESULTS

From the literature, 43 localities of occurrence of *P. lineatus* were mentioned for the Sorocaba River basin. The species was well distributed along the Sorocaba River channel, particularly in its middle and lower sections, as well as in the Sarapuí and Ipanema rivers, where it was also recorded in the middle and lower reaches (Fig. 2).

A total of 74 individuals of *P. lineatus* were sampled in the Sorocaba River, 46 females and 28 males, resulting in a sex ratio of 4:2. Females had an average weight of 892.34 ± 782.34 g and standard length of 44.65 ± 2.54 cm, while males had an average weight of 752.25 ± 390.33 g and standard length of 44.57 ± 3.25 cm. Weight values ranged from 177 to 4,530 g for females and 245 to 1,430 g for males, and standard length ranged from 20 to 59 cm for females and 22 to 43 cm for males. There was no significant difference in the weight-SL relationship between sexes, and both showed negative allometric growth, with regression coefficient (b) values of 2.6889 for females and 2.7586 for males, with a 95%CI (Fig. 3). The average growth rate for females was calculated as 1.02, with a standard deviation of 0.22, while for males the average was 1.01, with a standard error of 0.14. For females, this resulted in 95%CI = 1.02 ± (1.9889 × 0.0295), or 95%CI = 1.02 ± 0.0587, leading to a 95%CI 0.9613–1.0787. For males, the result was 95%CI = 1.01 ± (1.9889 × 0.0265), or 95%CI = 1.01 ± 0.0526, leading to a 95%CI of 0.9574–1.0626. This means that, with 95%CI, the true growth rate for females lies between 0.9613–1.0787, and for males, it lies between 0.9574–1.0626.

The diet of *P. lineatus* demonstrated a variety of food items consumed, consisting of nine food items of autochthonous

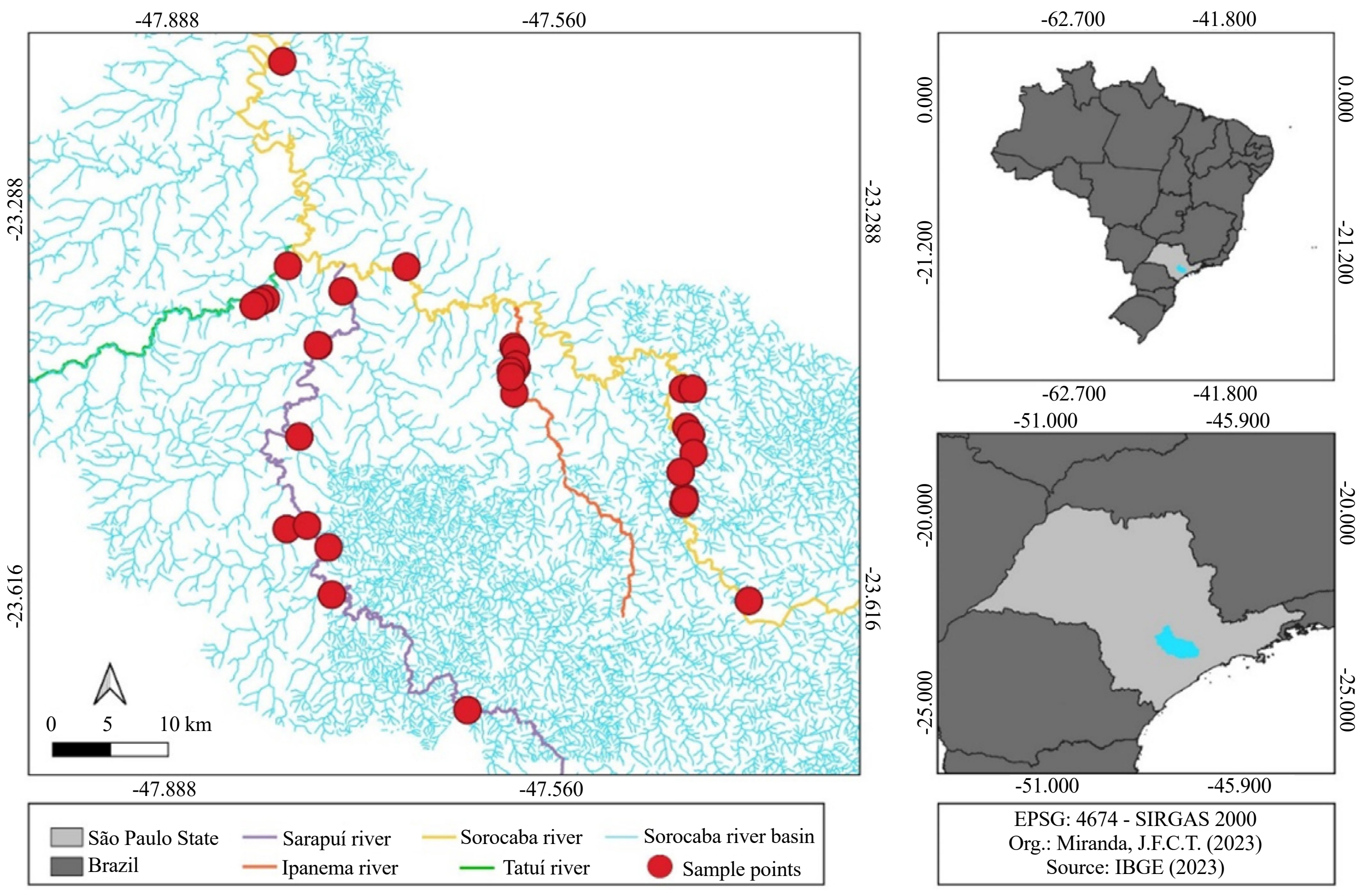

Figure 2. Occurrence of the migratory species *Prochilodus lineatus* in the Sorocaba River basin, according to publications and research carried out beyond the existing dams in the rivers studied.

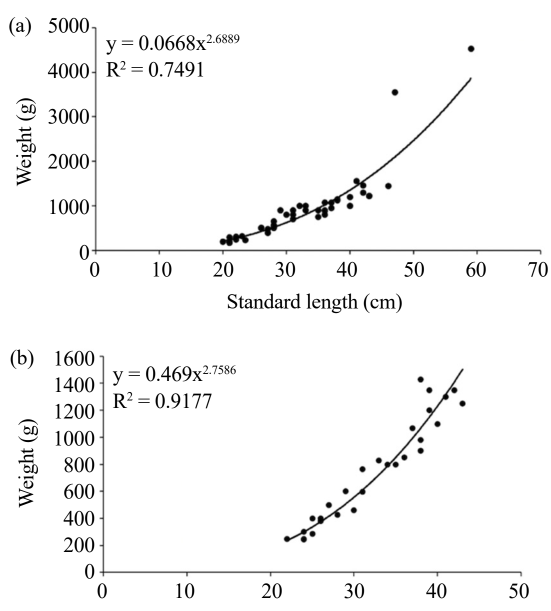

Figure 3. Length-weight relationship of *Prochilodus lineatus*: (a) females, and (b) males sampled in the Sorocaba River and tributaries.

origin (plant material, silt and sandy sediments, filamentous and fragmented algae, Chironomidae larvae, nematode worm, insect fragments, and eggs) and two particles of allochthonous origin (plastic fiber and possible paint), indicating the presence of microplastics in its diet.

Analysis of stomach contents revealed that the diet of *P. lineatus* was dominated by inorganic matter. Sandy sediment (IAi = 46.01%) and muddy sediment (IAi = 34.57%) were the most representative items, indicating a strong preference or functional feeding strategy linked to benthic environments. Plant material also appeared as a relevant component (IAi = 10.26%), while other food items, such as filamentous algae (IAi = 4.51%), insect fragments (IAi = 2.47%), seeds, chironomid larvae, nematode worms, and anthropogenic materials (paint and plastic fiber) contributed less than 1% of IAi. These findings reinforce the iliophagous feeding habit of the species (Fig. 4).

Based on SL, individuals were classified into developmental stages and grouped into size classes. A SL cutoff of 35 cm was used to distinguish adults from pre-adults or juveniles, following

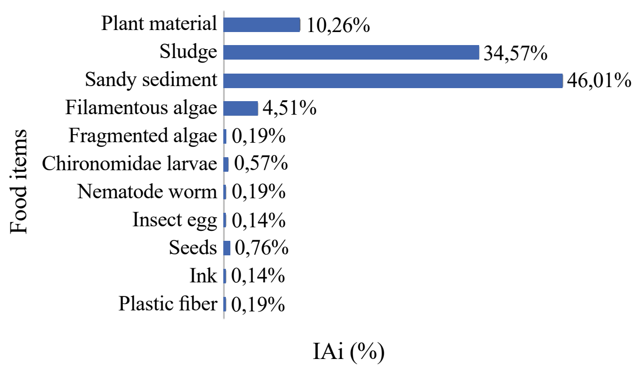

Figure 4. Feeding index (IAi) of food items found in *Prochilodus lineatus* sampled in the Sorocaba River and tributaries.

conservative ecological and ontogenetic criteria. Table 1 summarizes the biometric data and dietary composition by size class and developmental stage, revealing similar food items within the groups, and dietary analysis showed the predominance of autochthonous and allochthonous benthic items associated with low variation by size class. Juveniles with SL of 20.0 to 29.9 cm presented sandy and muddy sediments, and filamentous algae. The same occurred for the SL class of 35.0–39.9 cm, except for the presence of plant fragments and insect remains, indicating a detritivorous diet incorporating periphytic material. Similar patterns persisted in the SL class of 40.0–44.9 cm. In the largest class (SL 45.0–49.9 cm; n = 21; 705–4,530 g), the diet included fragmented algae, silt, nematodes, and insect fragments, suggesting an ontogenetic shift toward the consumption of more complex benthic organisms, likely reflecting growth-related adaptations or increased energy demand. Furthermore, it should be noted that plastic fibers were present in the stomach contents of immature adults with SL of 30.0–34.9 cm, indicating early exposure to anthropogenic debris. The species accumulation curve stabilized after analyzing 15 stomachs (Fig. 5), confirming the sample was adequate to represent dietary diversity.

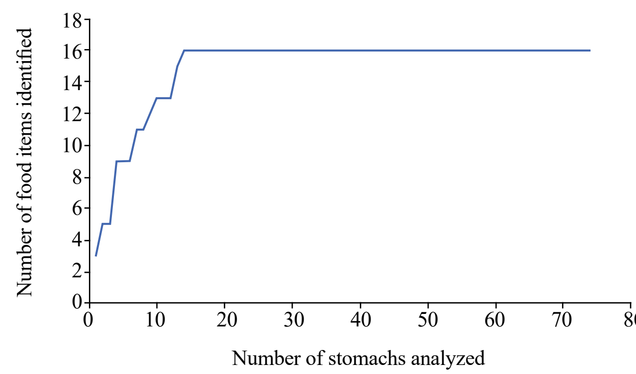

Figure 5. Accumulation curve of dietary items in *Prochilodus lineatus* in the Sorocaba River.

Table 1. Weight, standard length, and dietary composition of *Prochilodus lineatus* by size class and development stage.

Size class (cm)	Weight range (g)	Number of individuals	Dietary composition	Development stage
20.0–24.9	177–320	14	Sandy sediment, muddy sediment, filamentous algae	Juvenil
25.0–29.9	285–900	17	Sandy sediment, muddy sediment, filamentous algae	Juvenil
30.0–34.9	177–1,220	10	Sandy sediment, muddy sediment, filamentous algae, plant fragments, insect fragments, plastic fiber	Immature adults
35.0–39.9	229–1,560	16	Sandy sediment, muddy sediment, filamentous algae, plant fragments, insect fragments	Adult
40.0–44.9	300–1,450	12	Sandy sediment, muddy sediment, filamentous algae	Adult
45.0–49.9	705–4,530	2	Fragmented algae, silt nematode worm, insect fragments	Adult
55–59	4,530	1	Sandy sediment, muddy sediment, filamentous algae	Adult

DISCUSSION

The results indicated a wide distribution of *P. lineatus* in the studied basin, although possible constraints in this distribution are associated with the presence of dams in the upper and lower reaches of the Sorocaba River. The existence of free-flowing stretches in the main river channel and its tributaries allows for the movement of fish schools, maintaining the necessary connectivity between feeding and spawning habitats. However, a relatively small number of individuals were captured. This sampling limitation constrains the evaluation of ontogenetic shifts in feeding strategies, which could reveal variations in diet composition throughout the life stages of the species.

Thus, although *P. lineatus* appears to maintain connectivity between essential habitats in the Sorocaba basin, the available data do not support a clear prevalence of healthy nutritional or reproductive status, largely due to the limited number of individuals sampled. Expanded sampling efforts, including individuals in earlier life stages, are necessary to validate the findings presented and enhance the understanding of the species' dietary ecology and reproductive dynamics in the region.

Our results confirmed the wide distribution of *P. lineatus* in the basin. However, potential fragmentation due to dams remains a major concern. Free stretches in the river system keep facilitating migration between critical habitats, which is essential for the life cycle of this migratory species, as emphasized by Smith et al. (2024). The Sorocaba River supports diverse biotopes along its course (Portella et al., 2021), including rapids that are known to serve as reproductive habitats (Soinski et al., 2022). Furthermore, Smith and Barrella (2000) have shown that marginal lagoons of the Sorocaba River provide suitable conditions for fry development, including shelter and food, often surpassing those of the main river channel. However, these critical ecosystems are under increasing threat from urbanization (da Silva et al., 2019; da Silva et al., 2020; Stefani et al., 2025), reinforcing the urgency of conservation efforts. Dams and waterfalls, whether anthropogenic or natural, disrupt habitat connectivity (Agostinho et al., 2007, 2008), emphasizing the need to prevent further barriers to migration across the Sorocaba River system.

To define developmental stages, individuals of *P. lineatus* with a standard length (SL) equal to or greater than 28.2 cm were considered adults, based on the first maturation size (L_{50}) estimated for the species in the upper Miranda River, Paraguay basin (Vicentin et al., 2012). Individuals below this threshold were classified as juveniles, since they are still in development and have not reached full reproductive capacity. This criterion is consistent with reproductive studies in the species, which indicate that sexual maturity occurs from approximately 28 cm in total length, with females presenting mature gonads between August and February (Vicentin et al., 2012). Therefore, the classification adopted here provides a biologically and ecologically robust basis for distinguishing between juvenile and adult individuals, particularly in relation to feeding behavior and life-history strategies.

Given the species' widespread distribution and complex life cycle, understanding its ecological and developmental traits is essential. *Prochilodus lineatus* is broadly distributed across the basin and its tributaries (Smith et al., 2003, 2014, 2019), exhibiting a rheophilic reproductive cycle and early development in floodplain lagoons (Smith & Barrella, 2000). Maturation occurs at around 2 years old and ~18.9-cm SL in males, and 3 years old and ~27-cm SL in females, with individuals reaching up to 78 cm (Vicentin et al., 2012). In this study, specimens ranged from 21- to 43-cm SL, with about 50% presenting body mass above the expected values for their length. This may reflect the accumulation of energy reserves associated with migratory activity, supported by high gonadosomatic indices (K = 1.02 for females and 1.01 for males).

The uniform size range in the sample likely results from targeted collection in reproductive areas and possible mesh selectivity, excluding smaller specimens. Similar findings were reported by Machado and Foresti (2012), who observed morphometric homogeneity in migratory fish schools in the Mogi-Guaçu River, suggesting common origin populations in reproductive habitats. This pattern may also apply to the present study and warrants further investigation.

Differences between studies may be attributed to environmental, spatial, temporal, and methodological factors, including variation in habitat structure, water quality, food availability, and climate impacts. Additionally, *P. lineatus* is known for reproductive plasticity and may adjust its size and age at maturation based on environmental pressures (Barbieri et al., 2004), potentially explaining variations in size data across studies. The shorter sampling window in the present study may also have influenced the observed variability in specimen size.

Overall, the data suggested relevant population and ecological patterns, potentially driven by reproductive adaptations, fishing selectivity, and basin-specific environmental conditions. The increased average weight may indicate a productive environment or dietary shift, while high condition factor values reinforce possible reproductive activity. Conversely, the reduced average size could point to selective fishing pressure favoring earlier maturation.

Analysis of stomach contents revealed a predominance of inorganic materials, especially sandy and muddy sediments, followed by filamentous algae, plant fragments, and insect remains. This is consistent with the iliophagous feeding behavior described for *P. lineatus* (Bowen, 1983; Moraes et al., 1997; Novakowski et al., 2008; Oliveira et al., 2020; Urbanski et al., 2020; Yossa & Araujo-Lima, 1998), which involves the ingestion of sediments in search of organic material and associated microorganisms.

Although items such as plastic fibers and paint residue were identified in stomach contents, these represented a very low percentage compared to natural dietary components, reinforcing the limited incidence of anthropogenic material in the sampled population. This showed great similarity with the study by Oliveira et al. (2020), who found 2% in the Sorocaba River, also assessed by the present study, but very low values compared to what Urbanski et al. (2020) found in *P. lineatus* specimens in the Tietê River (71.88% of the individuals analyzed). These particles likely result from accidental ingestion during sediment foraging, as microplastics accumulate in riverbeds (Bernardo et al., 2022; Chubarenko et al., 2016; Jabeen, 2016).

Future studies should incorporate not only expanded qualitative and quantitative analyses of stomach contents, but also assessments of sediment composition in different habitats of the river system. Because microplastics tend to accumulate in benthic substrates, their availability in sediments may directly influence the dietary exposure and ecological risks faced by *P. lineatus* in the Sorocaba River basin. Future studies might provide more information to compare the results found in this study in terms of dietary items and population characteristics, further exploring the presence and effects of plastic microparticles on the species' biology. Furthermore, analysis of sediments along the river could be addressed, considering that plastic particles tend to settle on the riverbed and directly impact the species' diet and health, as it exploits the sediment as a food source.

CONCLUSION

In this study, we uncovered unprecedented information on the biology of *P. lineatus* in a major basin of São Paulo state. The species reaches sexual maturity in its first year of life and is considered iliophagous. This study was particularly interesting because it showed that the species is widely distributed in the basin, and that drainage maintains the essential conditions for its biology, including feeding and reproduction, which is vital for its conservation.

Additionally, microplastic particles were identified in the contents of the species' stomach, indicating that this may be an occasional ingestion likely related to the search for sediment. Assessing this species' biology is a crucial first step to understanding the ecological dynamics of its populations in the studied basin. Consequently, these findings highlight the need for caution when interpreting dietary data based on snapshot analyses, as stomach content analysis only reflects the feeding time at the time of capture. Furthermore, the limited sample size limits broader conclusions about the trophic variability of the species across all life stages and habitats. Given the detection of anthropogenic particles and the potential implications for the species' health and nutrition, further research is warranted.

CONFLICT OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

All data sets were generated or analyzed in the current study.

AUTHORS' CONTRIBUTION

Conceptualization: Silva, L.M.S., Smith, W.S.; **Investigation:** Silva, L.M.S., Martins, E.S.L., Oliveira, L.S.T., Juvenal, R.F.S., Silva, S.M., Porto, A.C., Smith, W.S., **Data curation:** Silva, L.M.S., Martins, E.S.L., Oliveira, L.S.T., Juvenal, R.F.S., Silva, S.M., Porto, A.C., Smith, W.S.; **Formal Analysis:** Silva, L.M.S., Smith, W.S.; **Resources:** Smith, W.S.; **Supervision:** Smith, W.S.; **Writing – orignal draft:** Silva, L.M.S., Martins, E.S.L., Oliveira, L.S.T., Juvenal, R.F.S., Silva, S.M., Porto, A.C., Smith, W.S.; **Writing – review & edition:** Silva, L.M.S., Martins, E.S.L., Smith, W.S.; **Final approval:** Smith, W.S.

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