



Evaluation of the use of *Ancistrus brevipinnis* (Loricariidae) as a bioindicator of water quality in the Ilha river, RS, Brazil

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ABSTRACT. The use of fish as bioindicators of the effect of contaminants in the aquatic environment is usual. However, detritivorous species are less used for environmental monitoring. Thus, the aim of this study was to evaluate the use of *Ancistrus brevipinnis* as a potential bioindicator of water quality of the Ilha River, RS (southern Brazil) and compared to the result obtained for a routine species (*Bryconamericus iheringii*). For this purpose, the condition factor (CF), the presence of morphological changes in the gills and the quantification of metals in different tissues of the specimens collected in three sites of the Ilha River were evaluated. A homogeneity in the distribution of data was observed in the CF for the species *B. iheringii*, whereas the specimens of *A. brevipinnis* showed an oscillation in the distribution of data of the CF. Histological analysis showed greater sensitivity of *B. iheringii* in reproducing characteristics of the environment in its gill lamellae, which was not observed in *A. brevipinnis*. Conversely, Cr quantification was higher in the intestine samples of *A. brevipinnis* at all sites and in all collections due to its feeding behavior. The data reinforce the use of water column species as *Bryconamericus iheringii* for environmental monitoring, even if they do not reflect the whole aquatic ecosystem. Further studies evaluating other tissues and biomarkers, such as the gastrointestinal tract, may assist in describing the use of species more related to sediment.

Keywords: bioaccumulation; environmental impact; histopathology.

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Introduction

The aquatic environment plays a vital role in the development of the natural environment and, consequently, in the progress of societies. However, it is precisely the unplanned advancement of society that is being responsible for the degradation of aquatic environment and its ecosystem due to the release of high loads of untreated effluents directly into rivers (Tran, Reinhard, & Gin, 2018; Jiang et al., 2018).

These activities end up contributing to the increased contamination of the natural environment, given the high tenor of pollutants present in these effluents (Tran et al., 2018). Among these contaminants, it is important to point out pesticides (Slaby et al., 2022), pharmaceuticals (Sara, Galego-Rios, Peñuela, & Matinez-Lopes, 2021) and trace elements, such as chromium (Cr), aluminum (Al), and lead (Pb), which are not essential for the development of species and are toxic even at low concentrations (Vasanthi, Muthukumaravel, Sathick, & Sugumaran, 2019; Ali et al., 2021; Pinheiro et al., 2021; Ferro, Ferrari, & Eissa, 2021; Yin, Wang, Wang, Huang, & Zhang, 2021).

Fish have become good bioindicators for assessing the real damage that these activities cause to organisms that are present in this ecosystem, since they are directly exposed to contaminants and can occupy different trophic levels in the same river (Gentès et al., 2019). One of the main criteria in choosing a bioindicator is the use of a native species for a better perception of the evaluated environment (Rand & Petrocelli, 1985). However, the use of more than one bioindicator species is recommended for the evaluation of a natural environment, considering that they have distinct characteristics and sensitivity (Brigante & Espíndola, 2003). Thus, it is possible to achieve more accurate results with a greater number of species by evaluating different trophic levels within the same environment (Brigante & Espíndola, 2003).

The species *Bryconamericus iheringii* (Characidae) is found in different positions within the same river, preferably in the water column, which means it is exposed to different substances due to the great movement in the search for new territories and food (Bonato, Burres, & Fialho, 2017; Garnero, Monferran, González, Griboff, & Los Angeles, 2018; Kokubun, Bonato, Burres, & Fialho, 2018). These animals feed on insects, algae, plants, and seeds, and serve as food for many ichthyophagous fishes and piscivorous birds (Kokubun et al., 2018). Considering that *B. iheringii* is omnivorous, the literature shows that this species has a great potential for metal bioaccumulation, often in greater amounts compared to carnivorous fishes, for example (Yousafzai, Chivers, Khan, Ahmad, & Siraj, 2010). Additionally, several biomarkers have been evaluated in this species, such as histological (Dalzochio et al., 2019) and genotoxic (Marinowic, Mergener, Pollo, Maluf, & Silva, 2014; Caetano, Oliveira, & Zawadzki, 2016; Dalzochio et al., 2019).

In contrast, the literature shows that detritivorous and sediment-dwelling fishes are more likely to bioaccumulate metals compared to omnivorous and carnivorous fishes, due to the greater exposure and the tendency of metals to deposit in the sediment (Weber et al., 2013; Monroy, Maceda-Veiga, & Sostoa, 2014). Thus, the species *Ancistrus brevipinnis* (Loricariidae) represents this group of animals that prefers shallow and running water and stays between stones (Mattos, Carvalho, Brito, & Araújo, 2018), being characterized as a potential bioindicator. Even so, there is a predominance of studies that use species like *B. iheringii* for environmental monitoring and diagnosis, leaving aside the observation of settling contaminants. Faced with this situation, we aimed to evaluate the sensitivity of the species *A. brevipinnis* as a bioindicator of water quality. The chosen site for this evaluation was the Ilha River, which is considered one of the main tributaries of the Sinos River and is located in the municipality of Taquara, RS (middle section of the Sinos River Basin).

Aiming to assess the sensitivity of *A. brevipinnis* as a novel bioindicator of water quality in the Ilha River, we performed an analysis of the general status of the animals through the use of the condition factor (CF), the evaluation of the presence of morphological alterations in the gills and the quantification of metals in different tissues of the collected specimens in a 3-year monitoring (2017, 2018, and 2019).

Material and methods

Study area

Three sampling sites were determined along the Ilha River (Figure 1), being the first site (S1) (29°33'12.1" S - 50°37'38.8" W) located near the source of the river, with a predominance with shallow waters, strong currents, and stony substrate, with low population and having agricultural incidence in its surroundings and preserved riparian forest. Its vegetation is typical of the highest altitude areas in the basin, i.e., it has a dense vegetation cover with the occurrence of exotic invader species, such as the Japanese raisin tree (*Hovenia dulcis*) (Tomazelli et al., 2021). Some elements of Ombrophilous Forest are observed in this region, as it is in an ecotone between this plant formation and the Semideciduous Seasonal Forest (Comitê de Gerenciamento da Bacia Hidrográfica do Rio dos Sinos [Comitesinos], 2016). This site is also characterized by surrounding areas with plantations of exotic species like *Pinus* sp. and *Eucalyptus* sp., and there are also small farms and low population density. Site 2 (S2) (29°57'64.9" S - 50°69'19.7" W) is in the municipality of Padilha and has different characteristics from S1, including higher population and agricultural incidence, calmer and deeper waters in some stretches with stony substrate, and in some stretches sandy substrate begins to appear. The vegetation has been also altered due to greater anthropogenic action. Native vegetation at this sampling site is restricted to riparian vegetation, which is surrounded by cattle raising. Site 3 (S3) (29°43'05.2" S - 50°37'54.8" W) is located at the mouth of the Ilha River, has deeper and calmer water, and sandy substrate with little incidence of stones. This site is located on a private property in Taquara municipality, where the predominant economic activity is rice cultivation, and it is possible to observe the proximity of the crops to the riverbanks. Despite the presence of native species, the vegetation is altered due to human activities, such as the presence of houses, plantation areas for agriculture, cattle raising, etc.

Animal collection

All the experiments performed in this study were previously approved by the Institutional Committee for Animal Care and Use from *Universidade Feevale* (protocol No. 01.12.017 and SISBIO No. 54988). The collections took place in November of each year (2017, 2018, and 2019), being collected 10 specimens of each

species *B. iheringii* and *A. brevipinnis* per site. The specimens of *B. iheringii* were collected using traps at strategic stretches with deeper and calmer waters. The specimens of *A. brevipinnis* were collected using the dip method due the behavior of the species of staying between stones at the bottom of the river.

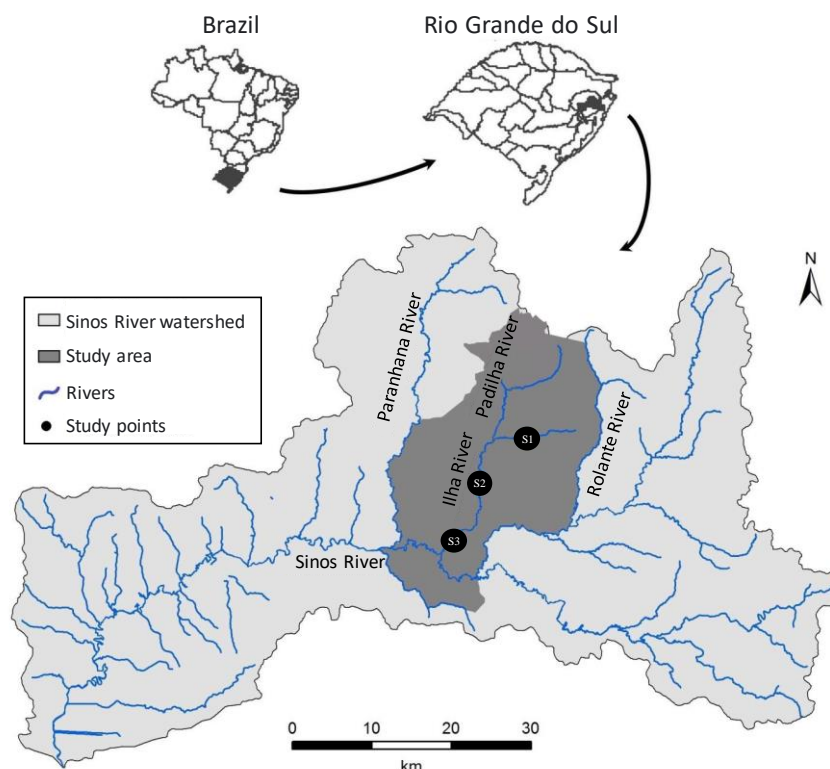


Figure 1: Map of the Sinos River Basin. The sites where the collections took place are highlighted in the Ilha River.

Soon after collection, animals were weighed and measured to obtain the CF. Subsequently, they were euthanized by medullar sectioning to remove the tissues to be analyzed. The collected gill samples were immersed in Bouin for fixation. Samples of muscles, intestines and livers were preserved in Styrofoam boxes with ice for transportation to the laboratory, to then be kept at -20°C for further analysis.

Condition factor

CF analysis was used for a preliminary view of the animal conditions, providing information regarding their general health, growth pattern, species habits, among other characteristics (Jirs, Younes, Sukhn, & El-Dakdouki, 2018). After collection, animals were measured and weighed to calculate the CF through the following formula: $\text{CF} = 100 \times (\text{body weight}/(\text{total body length})^3)$ (Ossana et al., 2016).

Morphological analysis of Gills

After 24 hours of fixation in Bouin, samples were dehydrated in a gradual series of ethanol to be embedded in paraffin. After that, the tissue was sectioned in a rotatory microtome (Leica®, Germany) in $5\text{ }\mu\text{m}$ of thickness and then stained with hematoxylin-eosin (H&E) (Merck, Darmstadt, Germany). The analysis followed that described by Dalzochio et al. (2018), which briefly consisted of evaluating the following morphological changes: hyperplasia, hypertrophy, edema, aneurysm, necrosis, fusion, lifting and also the percentage of normal lamellae. For each animal, 10 slides with 10 fields per slide (contain 8 secondary lamellae) were analyzed, totaling 100 fields per animal.

Quantification of trace elements

The quantification of trace elements was strategically defined, considering their natural presence in the environment and the preferred sites for metabolizing metals in organisms. Due to the natural occurrence of Al in large amounts in the region where the study was conducted (Bergamaschi et al., 2015), muscle was defined as the tissue of analysis, since bioaccumulation in this tissue predominantly occurs in environments with high metal concentrations (Mert, Alas, Bulut, & Ozcan, 2014; Rojo et al, 2019). Regarding the liver, an

organ rich in mitochondria (Shahid et al., 2021), Mn was quantified due to its preference for these cell organelles (Hamai, Campbell, & Bondy, 2001). Cr, in turn, was quantified in the intestine, aiming at observing the short-term exposure of animals to this metal, since there are records of this element at the study site (Dalzochio et al., 2017).

For the digestion of samples, the microwave digestion method was used. Samples of each tissue were put into pools of 3 to 5 animals to reach a minimum sample weight of 0.25 g. After weighing, samples were kept in nitric acid for 10 minutes and then introduced into the equipment, where they were heated at 220°C for 30 minutes. Subsequently, the volume of samples was adjusted to 100 mL with ultrapure water and Triton X-100.

The detection of trace elements in the tissues was performed through graphite furnace atomic absorption spectrometry (Perkin Elmer) in the Toxicology Laboratory of *Universidade Feevale*, following the standard procedure for each element. The detection and quantification limits are shown in Table 1 (Dalzochio et al., 2017).

Table 1. Limits of detection and quantification of elements in tissues.

Limits	Manganese	Aluminum	Chromium
Limit of detection	0.1 µg L ⁻¹	1.0 µg L ⁻¹	0.06 µg L ⁻¹
Limit of quantification	0.30 µg L ⁻¹	5.0 µg L ⁻¹	0.20 µg L ⁻¹

Statistical analysis

Statistical analysis was performed using the GraphPad Prism 9.0 software. All data were first submitted to the normality test using the Kolmogorov–Smirnov test. For the CF analysis, which refers to parametric data, the One-way ANOVA was used, followed by the Tukey's post-hoc. For the analysis of morphological changes in the gills, the Two-way ANOVA test was used, followed by the Tukey's post-hoc.

Regarding the trace element analysis between different periods in which the collections took place (2017, 2018, and 2019), the Kruskal-Wallis was used, followed by the Dunn's post-hoc. For the analysis between different sites (S1, S2, and S3), the same sequence of tests used in the analysis between periods was used. Results were considered significant when $p < 0.05$.

All analyses of the present study were performed in both collected species (*B. iheringii* and *A. brevipinnis*), always considering the results obtained for *B. iheringii* as standard due to the already proven sensitivity of the species (Dalzochio et al., 2019; Marinowic et al., 2014), and thus evaluating the linearity between responses provided by both species to determine the sensitivity of *A. brevipinnis*.

Results

Condition factor

Results obtained in the calculation of the CF can be seen in Figures 2A and 2B. Regarding *B. iheringii* specimens, a linearity (homogeneity) was observed between the sampling sites in the three collection periods, except in 2018, when a reduction in the CF was observed in the animals collected in the S2 ($p = 0.0051$). Comparing the same sampling sites, but from different periods, a reduction in the CF was observed in the animals of S1 (2017 x 2019 and 2018 x 2019) and S3 (2018 x 2019).

About *A. brevipinnis*, significantly lower CF values were observed in the S3 compared to S1 in 2017 ($p < 0.05$), 2018 ($p < 0.05$), and 2019 ($p < 0.05$) collections. Comparing the same sampling sites, but from different collection periods, S2 stood out with highest CF values in 2018 compared to 2017 ($p = 0.0021$) and 2019 ($p = 0.0021$) collections.

Morphological analysis of gills

Data from morphological analysis of the gills performed on the animals can be seen in Figure 2 (2C – 2H), whereas in Figure 3 we can observe the pattern of secondary lamellae visualized under optical microscopy. About *B. iheringii*, the highest percentage of normal lamellae was observed in the S1, except in 2019, when S3 had the highest percentage of normal lamellae ($p = 0.0416$). However, 2019 also stood out for the reduction in the percentage of normal lamellae in the three sampling sites, especially in comparison with 2018 results. Consequently, animals from the three sampling sites in the 2019 collection showed a higher percentage of lamellae with alterations, such as hyperplasia (Figure 2E) and hypertrophy (Figure 2G), in relation to 2017 and 2018 collections.

Despite presenting a lower percentage of normal lamellae in general compared to *B. iheringii*, *A. brevipinnis* specimens collected in the S3 stood out for having a greater percentage of normal lamellae compared to animals from S1 and S2 in all sampling periods. Consequently, a lower percentage of lamellae with hyperplasia was also observed in the three collections performed in the S3.

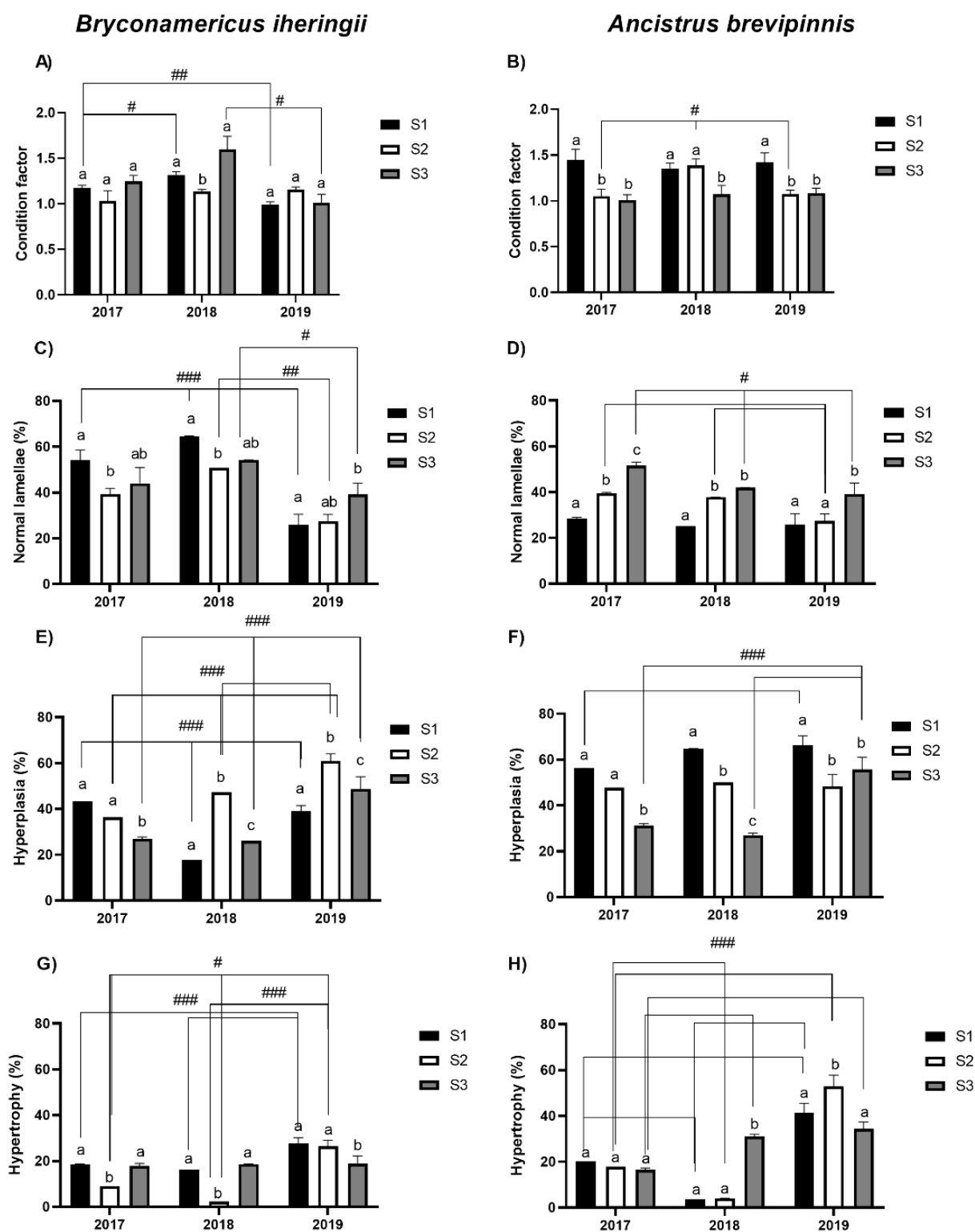


Figure 2. Histopathological and CF analyses performed in *Bryconamericus iheringii* and *Ancistrus brevipinnis* from the Ilha River. Data are expressed as mean and standard error. Different letters indicate significant differences between sampling sites in the same collection period. The pound (#) indicates significant difference at the same sampling site between different monitoring periods (# = $p < 0.01$, ## = $p < 0.001$, and ### = $p < 0.0001$).

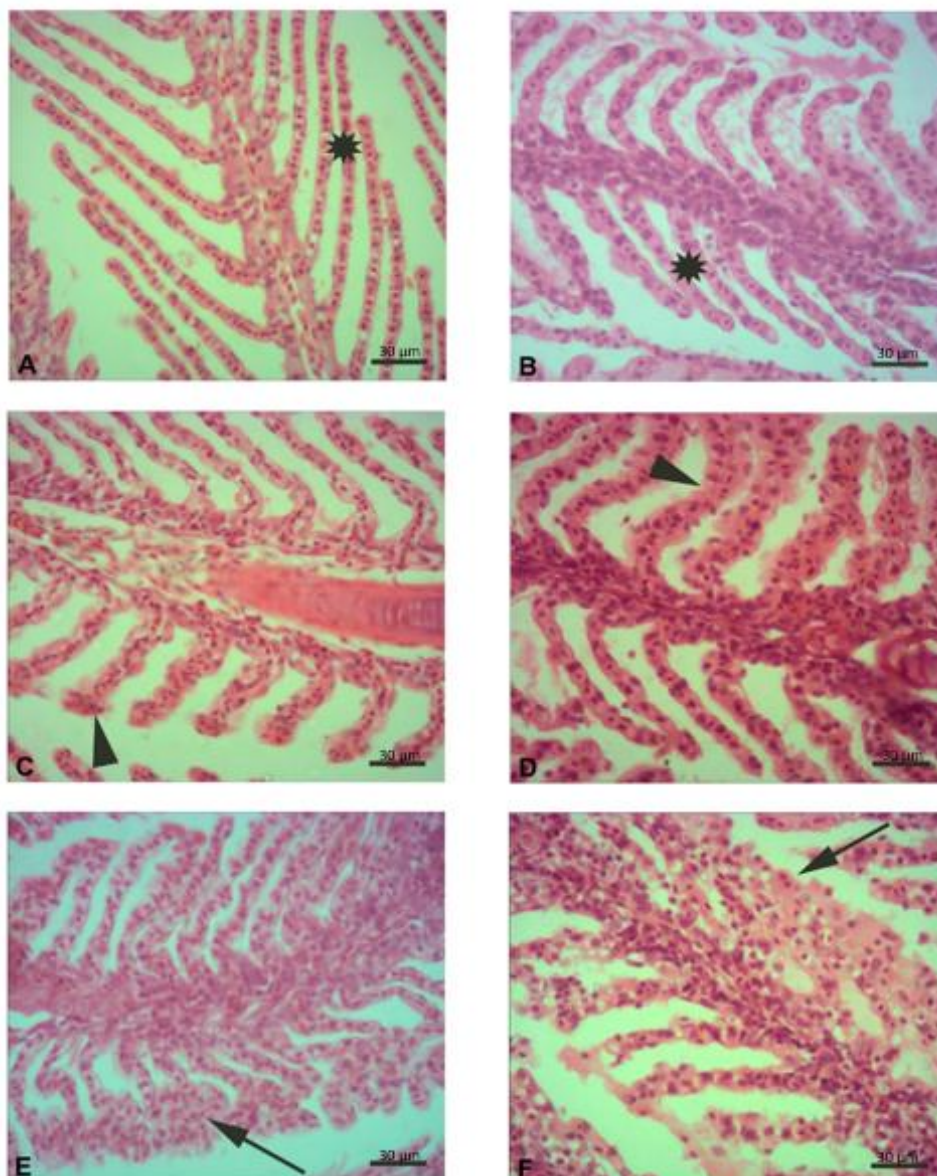


Figure 3. Photomicrographs on the left represent gills of *Bryconamericus iheringii*. Images on the right represent gills of *Ancistrus brevipinnis*. In A and B, asterisks indicate secondary lamellae without alteration. In C and D, arrowheads indicate secondary lamellae with hypertrophy. In E and F, arrows indicate secondary lamellae with hyperplasia.

Quantification of trace elements

The quantification of metals in different tissues of both collected species can be seen in Tables 2 and 3. We emphasize that due to the need of performing pools with tissues of animals destined for the detection of metals, which consequently reduced the sample size, the statistical analysis was performed only when possible.

From the results obtained for *B. iheringii*, we can highlight the increase of Al concentration in the muscle of animals from the S3 compared to animals from the S1 in the 2017 collection. Other comparisons were not statistically significant. Likewise, no significant differences were identified for the analysis performed on *A. brevipinnis*.

Discussion

The present study evaluated the sensitivity of the species *A. brevipinnis* as a bioindicator of environmental quality by comparing it to a species accepted in the literature (*B. iheringii*) for this type of evaluation (Dalzochio et al., 2017; Marinowicz et al., 2014) and having the Ilha River as study site, which is one of the main tributaries of the Sinos River.

The homogeneity of the CF observed between the sampling sites in animals of the species *B. iheringii* was not observed in the species *A. brevipinnis*. However, distinct characteristics of the sampling sites, such as volume and velocity of water, as well as type of substrate and depth, must be considered. Thus, highest CF values of *A. brevipinnis* recorded in the S1 can be attributed to local characteristics, which have less anthropic impact. Mattos et al. (2018) reported that species belonging to the Loricariidae family prefer clean water environments, stony bottoms, and with intense water flow, similar to what was observed in the S1 of the present study.

Table 2: Trace elements in tissues of *Bryconamericus iheringii*. Underlined values indicate results above those permitted by law (Instrução Normativa - IN n° 88, 2021). Values are expressed as mean and standard deviation or just as a single value when only 1 sample pool ($\mu\text{g g}^{-1}$) is available. Hyphen (-) represents absence of values. Different letters vertically indicate significant difference between sites in the same period.

Element	Site	Tissue	November 2017	November 2018	November 2019	P value
Mn	S1	Liver	158.16 ± 182.84	197.35 ± 196.24	24.48 ± 7.61	0.4250
	S2		47.22 ± 47.22	62.4 ± 21.78	80.81 ± 20.52	0.4333
	S3		288.81	-	-	-
P value			0.8000	0.6667	0.1000	
Al	S1	Muscle	10.63 ± 12.39 ^a	111.26 ± 65.12	225.54 ± 301.84	0.0500
	S2		50.27 ± 23.00 ^{ab}	91.48 ± 87.90	58.14 ± 18.75	0.5824
	S3		207.61 ± 205.14 ^b	29.32 ± 20.78	113.43 ± 113.43	0.1000
P value			0.0014	0.0857	0.5714	
Cr	S1	Intestine	<u>0.64 ± 0.23</u>	<u>1.74 ± 2.09</u>	<u>1.55 ± 0.38</u>	0.3393
	S2		<u>5.06 ± 1.37</u>	<u>5.55 ± 2.22</u>	<u>4.76 ± 1.99</u>	0.6571
	S3		<u>1.30 ± 1.30</u>	<u>0.55 ± 0.76</u>	<u>0.26 ± 0.26</u>	> 0.9999
P value			0.1000	0.2321	0.1351	

Table 3. Trace elements in tissues of *Ancistrus brevipinnis*. Underlined values indicate results above those permitted by law (Instrução Normativa - IN n° 88, 2021). Values are expressed as mean and standard deviation or just as a single value when only 1 sample pool ($\mu\text{g g}^{-1}$) is available. Hyphen (-) represents absence of values.

Element	Site	Tissue	November 2017	November 2018	November 2019	P value
Mn	S1	Liver	-	320.25 ± 43.47	38.74	-
	S2		-	136.99	879.89	-
	S3		16.23 ± 16.23	384.64	177.71	-
P value			-	0.4667	-	
Al	S1	Muscle	51.99 ± 26.76	58.33 ± 43.93	641.8 ± 1054.94	0.9222
	S2		491.37 ± 491.37	380.75 ± 359.36	105.4 ± 70.22	0.4857
	S3		176.48 ± 176.35	504.45 ± 622.53	338.23 ± 375.11	0.8686
P value			0.2667	0.3061	0.8286	
Cr	S1	Intestine	<u>20.23 ± 2.90</u>	<u>9.09 ± 5.35</u>	<u>19.42 ± 16.06</u>	0.2429
	S2		<u>6.84 ± 6.84</u>	<u>4.56 ± 0.91</u>	<u>10.09 ± 3.87</u>	0.1000
	S3		<u>6.03 ± 7.34</u>	<u>9.58 ± 13.08</u>	<u>11.39 ± 15.32</u>	0.9333
P value			0.3333	0.8964	0.6714	

This CF measure is used to compare the body condition and general well-being of fish, assuming that heavier fish would be in better conditions (Tavares-Dias, Monteiro, Affonso, & Amaral, 2011). However, this parameter is affected by body shape, physiological factors, sex, maturity, and spawning (Freitas & Salvador, 2022). In general, these factors are positively influenced by heat and the consequent increase in water temperature (Jirs et al., 2018), justifying the collection period of the present study. Many authors use this parameter as a tool to assess the status of the aquatic ecosystem (Lubich, Aguiar-Santos, Freitas, & Siqueira-Souza, 2021; Rotta & Yamamoto, 2021).

Another difference between the data obtained in the response of both species refers to the percentage of normal lamellae in their gills. While we observed a predominance of normal lamellae in the specimens of *B. iheringii* collected in the S1, the opposite was observed for *A. brevipinnis*, i.e., a predominance of normal lamellae in the S3. Considering that the gills represent the first organ in contact with external pollutants (Doria, Voigt, Campos, & Randi, 2017), and knowing that the S3 is located within a private property containing rice cultivation, we can consider that the results observed in *A. brevipinnis* do not seem to reflect the real impact of sampling sites.

The use of land for agricultural activities allows the entry of a wide range of pollutants in toxic concentrations to organisms, especially pesticides and metals, which are often included in the formulation of agrochemicals (Naveedullah et al., 2013). In studies performed near these areas of agricultural activity, changes in gill morphology are one of the numerous consequences observed in fish (Dane & Sisman, 2015) as an adaptive response. Previous research carried out under controlled laboratory conditions (Portruneli et al., 2021) also demonstrated morphological changes (hypertrophy and hyperplasia) in animals exposed to 2,4-dichlorophenoxyacetic acid (2,4-D), a widely used herbicide for rice cultivation (Yamashita, Zonta, & Machado, 2008).

Lamellar changes, such as hypertrophy and hyperplasia, are adaptations made by the branchial epithelium to change the area of contact and consequently increase the distance between the blood and the lamellar surface (Kumar, Krishnani, Gupta, & Singh, 2017). Therefore, these changes are directly related to the poor quality of the environment and are commonly associated with other systemic changes in the organisms (Dane & Sisman, 2015; Martins, Santos, Costa, & Costa, 2016; Santos, Monteiro, Cortes, Pacheco, & Sanches Fernandes, 2021).

A possible explanation for the predominance of normal lamellae in *A. brevipinnis* collected in the S3 is the characteristic of the site, which has a smaller area of stony bottom and a prevalence of sandy bottom, providing limited area of permanence for this species that prefers rocks. In addition, when these organisms are in the reproductive period, they tend to remain fixed in one place to prepare their nest for breeding (Sabaj, Armbruster, & Page, 1999; Ramos & Konrad, 1999), which may limit the contact of the studied specimens with possible contaminants.

It is known that turbulent conditions result in changes in the chemical properties of sediments, resulting in greater presence of metals and other pollutants in the water column (Sarkar, Favas, Rakshit, & Satpathy, 2014). S1 is characterized as the sampling site with the greatest water flow, a fact that puts in doubt the reduction of normal lamellae observed in *A. brevipinnis*, since the stony bottom of this site would have less accumulation of contaminants. However, it should be noted that the bioavailability of pollutants is influenced by many factors, such as water alkalinity, presence of organic matter, chemical form of the metal, and geochemical characteristics of the sediment (Zhang et al., 2014).

The behavior of organisms, including the feeding mode, also affects the exposure route of contaminants (Zhang et al., 2014; Lima et al., 2015). Considering the feeding habits of *A. brevipinnis*, it can be assumed that the intestinal exposure of animals of this species is superior to that of *B. iheringii*. Consequently, higher amounts of Cr were observed in the intestines of *A. brevipinnis* at all sampling sites and periods, although there was no statistical difference.

In a recent research carried out in the same study area, high levels of Cr were also detected in the muscle of *B. iheringii*, even though its presence have not been detected in water (Dalzochio et al., 2017). This confirms that physical-chemical analyses are momentary and do not accurately reflect the real impact of the biota (Dai et al., 2021), whereas bioindicators are more sensitive for environmental monitoring and diagnosis (Vieira, Almeida, Galindo, Pereira, & Martinez, 2014). It is known that exposures to Cr result in changes in behavior, physiology, and morphology of exposed animals (Bakshi & Panigrahi, 2018), while complex mixtures containing this and other pollutants are associated with increased hyperplasia and hypertrophy (Mishra & Mohanty, 2008; Macedo et al., 2020), as mentioned above. The main route of entry of these elements in the environment is through anthropic activities, which generate effluents that do not receive proper treatment before returning to the natural environment (Borges et al., 2022; Munner, Alobaid, Ullah, Relman, & Erinle, 2022). This justifies the impacts on aquatic biota observed in previous studies (Fontanella et al., 2009; Rodrigues, Dalzochio, & Gehlen, 2016).

In summary, the bioaccumulation of metals in fish tissues is a good bioindicator of exposure to metallic contaminants, although the difference in absorption depends on the type of metal, the physical and chemical properties of the water, and the species' characteristics themselves (Shah et al., 2020), a fact that may also have influenced our results. In the present study, we verified an increase in the concentration of Al in the muscle of fish of the species *B. iheringii* collected in the S3 in 2017. Since Al is a non-essential metal, it ends up easily bioaccumulating in tissues because it has no cellular function or pathways of metabolism and excretion (Exley & Mold, 2015). Therefore, after being absorbed by the gills, Al ends up being deposited in tissues, such as muscle (Sivakumar, Khayiwada, & Sivasubramanian, 2012), brain, and liver (García-Medina et al., 2022).

In view of the findings observed in the present study, we suggest the use of species that occupy the water column and are accepted in the literature for environmental monitoring and diagnosis, even if they do not

reflect the conditions of the whole aquatic ecosystem. For evaluations that include *A. brevipinnis* or other bottom-dwelling fish species, histopathological analysis of the intestine or liver may be more sensitive than the gills, given the greater exposure to contaminants via the gastrointestinal tract. Furthermore, further studies illustrating these conditions are necessary to rule out or direct the use of alternative species instead of those commonly used in environmental analyses.

Conclusion

The use of both species (*B. iheringii* and *A. brevipinnis*) highlighted the bioindicator potential of *B. iheringii*, being observed a standard behavior in different sampling sites for the CF and reflecting environmental conditions through the studied morphological changes. In contrast, the specimens of *A. brevipinnis* did not reproduce the environmental characteristics of the studied area in the evaluated biomarkers. The present study serves as a basis for further research that aim to evaluate other parameters in detritivorous species.

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