An Acad Bras Cienc (2023) 95(4): e20200307 DOI 10.1590/0001-3765202320200307
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

ECOSYSTEMS

Nematodes as indicators of environmental changes in a river with different levels of anthropogenic impact

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Abstract: Considering that changes in the biodiversity of parasite communities can be used as indicators of ecosystem health, the aim of this study was to investigate the potential use of *Geophagus brasiliensis* parasites as bioindicators of environmental changes. We established three sample points in the Iguaçu River, each presenting different degrees of environmental impact. Out of the 69 *G. brasiliensis* specimens analyzed, 32 (46.3%) were parasitized by at least one parasite. We collected a total of 56 specimens of endoparasites belonging to the phylum Nematoda. Fishes collected in point 3 presented a significantly higher abundance of nematode species (moderately degraded) (Kruskal-Wallis$_{2;69}$ = 8.62; p = 0.01) and species compositions between points were significantly different (F = 6.95, p = 0.002). No significant difference in relative condition factor (Kn) among the points ($F_{2;66}$ = 2.54; p = 0.08) or correlation in Kn and abundance of nematodes (rs = 0.1; p = 0.4) were indicated. The results presented in this study indicate that the parasitic community of *G. brasiliensis* is characterized by low diversity in polluted locations, which explains the absence of certain parasite species and the occurrence of nematode species with varied responses to the pollution gradient.

Key words: ecotoxicology, freshwater, parasites, fish.

INTRODUCTION

In the context of the serious, growing problem of pollution of aquatic ecosystems (Dalzochio et al. 2016), urbanization, industrialization, disordered use of fertilizers, pesticides and the transport of allochthonous substances to rivers are anthropic actions that contribute to higher pollution and degradation of water quality in aquatic ecosystems (Silva-Souza et al. 2006, Silva et al. 2022). In natural systems affected by such disturbance, the intensity of the impacts is directly proportional to the degree of diversity of the environment and the vulnerability of the species involved (Bastos & Abilhoa 2004, Alimba & Bakare 2016).

Aquatic organisms are often exposed to a variety of natural and artificial environmental stressors, such as variations and/or changes in physical and chemical parameters, alterations in diet and habitat availability (Adams & Greeley 2000, Reid et al. 20). Therefore, pollution, as well as the stress suffered by the aquatic environment, reflects directly in organisms, populations, communities, and food chain structure (Tundisi & Tundisi 2008). In this sense, the use of fish parasites as indicators of environmental changes or disturbances allows to assess the effect of stressors on hosts and aquatic ecosystems (Marcogliese 2005, Madi & Ueta 2009, Timi & Poulin 2020).

Studies have demonstrated the close relationship between parasitism and ecological conditions in a given environment for reflecting environmental impacts through their responses to changes in populational structure, such as changes in prevalence and intensity. In addition, their occurrence or abundance can describe the situation of the environment (Vidal-Martínez et al. 2010, Madi & Ueta 2012, Vidal-Martínez & Wunderlich 2017, Negreiros et al. 2018). Some groups of parasites are more sensitive to environmental disturbances than host species, which makes them efficient indicators for various contaminants and anthropic changes (Marcogliese 2005, Duarte et al. 2020).

Responses from hosts and parasitic communities vary according to the type and intensity of the stressor, parasite life cycle, and time of exposure, however, in general, pollution and stress are often associated with lower parasite species richness (Marcogliese 2004, Falkenberg et al. 2019). Parasite life cycle can include a definitive host and several intermediate hosts, and for the parasite to survive, all hosts must co-occur in a stable community structure (Marcogliese & Cone 1997). Changes in environmental conditions affect hosts, either directly or indirectly, and have a significant effect on the prevalence and intensity of infection and diversity of parasites present in fish (Marcogliese & Cone 1997, MacKenzie 1999). Therefore, such diversity of endoparasites can decrease since the stages of free living can be directly affected or certain intermediate hosts can be reduced, hampering the transmission of the parasite (MacKenzie 1999).

The endoparasitic fauna of *Geophagus brasiliensis* (Quoy & Gaimard 1824) includes digeneans, nematodes, and acanthocephalans (Fernandes & Kohn 2001, Azevedo et al. 2006, Bellay et al. 2008, 2012, Carvalho et al. 2010). Since parasites indicate environmental contaminants, their presence is an indication of environmental quality (Chubb 1980, 1982, Overstreet 1997). Good indicators can be exceptionally sensitive to pollution, and significant changes in the number of individuals in the populations can be considered an alert of changes in environmental conditions (Mackenzie et al. 1995, Sures & Streit 2001).

Considering the increasing urbanization and the presence or absence of these parasites, this study aimed at investigating the potential use of parasites of *G. brasiliensis* as indicators of environmental changes in a pollution gradient for the Iguaçu River.

MATERIALS AND METHODS

Sampling area

Iguaçu River basin is located mostly in the southern portion of the state of Paraná, Brazil, and presents geomorphological characteristics related to its hydrography. Thus, rivers and waterfalls influence its geographic distribution of species, and Iguazu falls act as an important geographical barrier generating a high endemism degree for ichthyofauna (Zawadzki et al. 1999, Baumgartner et al. 2012). However, due to the influence of several anthropic factors, such as the construction of hydroelectric plants, pollution, and species introduction, the risk of extinction of these fish species has increased significantly (Daga & Gubiani 2012). Currently, Iguaçu River is considered the second most polluted river in Brazil because of massive load of pollutants released at the source of the river, resulting from the anthropic activity in the Metropolitan Region of Curitiba (IBGE 2010).

We established three sampling points along the Iguaçu River (Figure 1). The first point is located in the upper Iguaçu (25°36'17.1"S 49°30'35.9"W), in the metropolitan region of Curitiba and Araucária and characterized by large population concentration, industrial, commercial and service activities, classified by the responsible environmental agency as critically degraded to polluted (Superintendência de Desenvolvimento

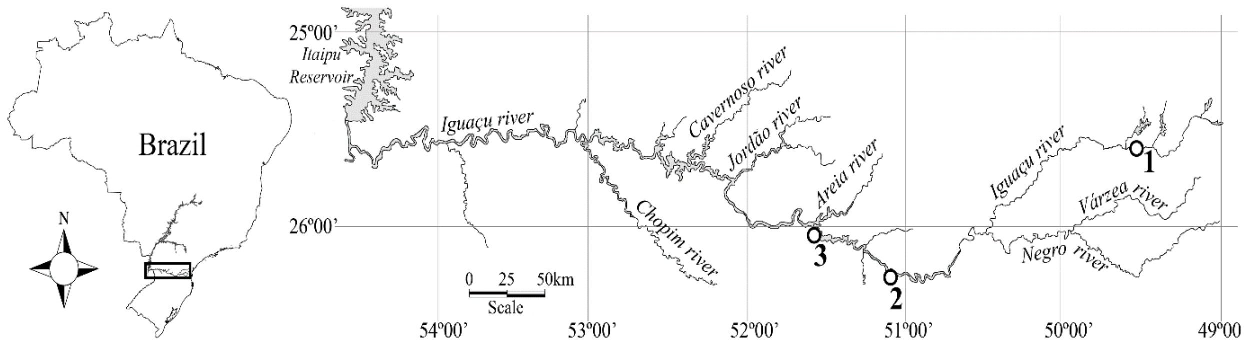

Figure 1. Map of the location of the Iguaçu River and the three sampling points. (Jaime Luiz Lopes Pereira - Nupélia/UEM).

e Recursos Hídricos e Saneamento Ambiental 1997, Carneiro et al. 2014, IAP 2017). Despite the degraded condition reported by the Instituto Ambiental do Paraná (2017), its water supplies the population of the city of Curitiba and its metropolitan region.

The second point is situated in the region of the middle Iguaçu (26°15'02.1"S 51°06'13.4"W), where agriculture predominates and the classification is moderately degraded (Superintendência de Desenvolvimento e Recursos Hídricos e Saneamento Ambiental 1997, IAP 2017). The third point is located in the region of the lower Iguaçu (26°02'51.9"S 51°36'04.1"W) and characterized by the beginning of the cascade of reservoirs in the river and use of water for public supply to cities. It is categorized as moderately degraded (Baumgartner et al. 2012, IAP 2017).

We collected specimens of *G. brasiliensis* in January 2019 by measuring the following limnological variables of water: pH, conductivity, temperature, and dissolved oxygen. Water samples were also collected and quantification of total phosphorus was carried out at the Basic Limnology laboratory of the Universidade Estadual de Maringá. The collections had been authorized by Ibama (SISBIO/64382-4) and Ethics Committee (CEUA Nº 2887071118).

Collection, fixation, and conservation of endoparasites

After collecting the fish, we conducted the taxonomic identification and determination of host biometric, followed by a longitudinal incision on the ventral surface of each fish, as well as removal and separation of all organs. The visceral cavity and each organ were examined under a stereomicroscope to collect endoparasites. The methodology for setting endoparasites differed according to the parasite group, following the recommendations of Eiras et al. (2006). Species of endoparasites were identified according Moravec (1998), Vicente & Pinto (1999), and Thatcher (2006).

Statistical analysis

To describe the structure and quantitative analysis of the parasites found, we used the parasitic indices described by Bush et al. (1997). Five descriptors based on the structure of the infracommunities were calculated: (1) abundance, (2) richness, (3) diversity, representing the average of the diversity of infracommunities in each fish calculated through Brillouin index, (4) equitability, representing the average species equitability in each fish, and (5) Simpson's dominance. We performed a non-parametric analysis of variance (Kruskal-Wallis) to verify significant differences in the abundance of parasites among the sampling points.

A (dis)similarity in parasites species composition appeared between the sampling points through a Principal Coordinate Analysis (PCoA) (Legendre & Legendre 1998) using a presence/absence matrix and the Jaccard index. A Multivariate Permutational Variance Analysis (PERMANOVA) assessed changes in parasites species composition according to the sampling point (Anderson 2005). We carried out 999 permutations to verify significance and applied a pair-wise PERMANOVA to assess significant differences between the sampling points.

The values of standard length (Ls) and weight (Wt) of each host were fitted to the curve of the Wt/Ls ratio ($Wt = a.Lt^b$) and the values of the regression coefficients a and b were estimated. The values of a and b were used to estimate the expected weight values (We) using the following equation: $We = a.Lt^b$. Therefore, we calculated the relative condition factor (Kn), which corresponds to the quotient between observed weight and expected weight for a given length (Kn = Wt/We) (Le Cren 1951).

We conducted an analysis of variance (ANOVA) to assess the differences between the relative condition factor of the hosts between the points. Spearman's rank correlation coefficients (rs) were calculated to determine possible association between the relative condition factor (Kn) of hosts and the abundance of infection for the hosts (Zar 2010).

Statistical analyses were performed using the R 3.2.4 software (R Development Core Team 2020) on the vegan (Oksanen et al. 2020) and barter (Simpson 2018) packages for PCoA and according to the "adonis2" function of the vegan package (Oksanen et al. 2016) for PERMANOVA. The level of statistical significance adopted was $p \leq 0.05$.

RESULTS

Table I presents the values for water quality parameters measured in the field and in the laboratory.

We collected 69 specimens of *G. brasiliensis* from the Iguaçu River: 20 at point 1, 24 at point 2, and 25 at point 3. The average fish length was 12.5 ± 3.35 cm (8.2–33.1 cm) and average weight was 41.37 ± 38.95 g (11.5–301.3 g). Among the fish examined, 32 (46.3%) were parasitized by at least one parasite species of parasite, with a total of 56 specimens of endoparasites belonging to the phylum Nematoda. The fish from point 3 had a higher prevalence of infection (Table II). Table II indicates the parasite species, sites of infection/infestation, and respective parasitological indices.

The values of the infracommunity descriptors were similar for points 2 and 3 (Table III). The abundance and richness of nematode species were slightly higher in fish collected in point 3. Point 1 showed the highest values for the indices (diversity and equitability), but its abundance was lower comparing with points 2 and 3.

Table I. Mean values of abiotic variables and nutrient concentration in water samples from the Iguaçu River, Paraná.

Point	WT	pH	DO	C	Total-p
Point 1	29.4±1.2	8.5±0.2	13.08±0.4	154.1±6.1	283.5
Point 2	27.3±1.1	7.84±0.03	15.5±0.7	65.6±6.1	96.5
Point 3	27.1±1	7.4±0.2	14.46±0.1	58±4	78.8

*** WT - Water temperature (°C); DO - dissolved oxygen (mg/l); C - conductivity (µS/cm); Total-p (µg/l). Point 1: critically degraded to polluted; Point 2: moderately degraded; and Point 3: moderately degraded.**

A comparison of the abundance of nematode species revealed a significant difference between the sampling points ($Kruskal\text{-}Wallis_{2;69} = 8.62$; $p = 0.01$) (Figure 2), swith points 2 and 3 presenting the highest mean abundance in relation to point 1.

The result of the Principal Coordinates Analysis (PCoA) applied to assess the (dis) similarity between sampling points (Figure 3) pointed out to significant differences in species composition ($F = 6.95$, $p = 0.002$). The pairwise PERMANOVA showed distinct nematode species compositions between point 1 and points 2 and 3 (Table IV).

The relative condition factor of the hosts demonstrated that the Kn values did not differ between the sampling points ($F_{2;66} = 2.54$; $p = 0.08$), but when analyzed separately, points 2 and 3 had a higher average comparing with point 1 (Figure 4).

When verifying the correlation between relative condition factor (Kn) and abundance of nematode infestation for the hosts, the results did not indicate any significant correlation ($rs = 0.102$; $p = 0.402$) (Figure 5).

Table II. Species of parasites, sites of infestation/infection, point of collection and their parasitological indices found in the host *Geophagus brasiliensis* in the Iguaçu River, Paraná.

Parasites	Prevalence (%)			Mean intensity (±SD)			Mean abundance (±SD)		
	Point 1	Point 2	Point 3	Point 1	Point 2	Point 3	Point 1	Point 2	Point 3
Contracaecum larvae type 2 described by Moravec, Kohn and Fernandes, 1993 Mesentery	5	0	0	1	-	-	0.15 ±0.37	-	-
Procamallanus (Procamallanus) peraccuratus Intestine	10	40	68	1	2.3 ±1.4	1.64 ±1.11	0.1	0.92 ±1.33	1.12 ±1.2
Procamallanus sp. 1 Intestine	0	4	0	-	1	-	-	0.04	-
Procamallanus sp. 2 Intestine	0	0	4	-	-	1	-	-	0.04
Procamallanus (Spirocamallanus) sp. Intestine	0	0	4	-	-	1	-	-	0.04

Table III. Attributes of the parasites infracommunities of *Geophagus brasiliensis*.

Descriptors	Point 1	Point 2	Point 3
Total abundance (N)	4	22	30
Richness (S)	2	2	4
Diversity	0.34	0.14	0.22
Equitability	0.81	0.26	0.26
Simpson's dominance	0.62	0.91	0.87
Dominant specie	*Contracaecum* larvae type 2	*Procamallanus (Procamallanus) peraccuratus*	*Procamallanus (Procamallanus) peraccuratus*

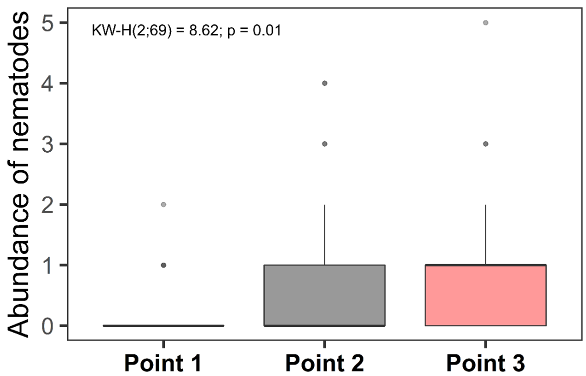

Figure 2. **Mean values of nematode abundance in *Geophagus brasiliensis* between the sampling points of the Iguaçu River, Paraná.**

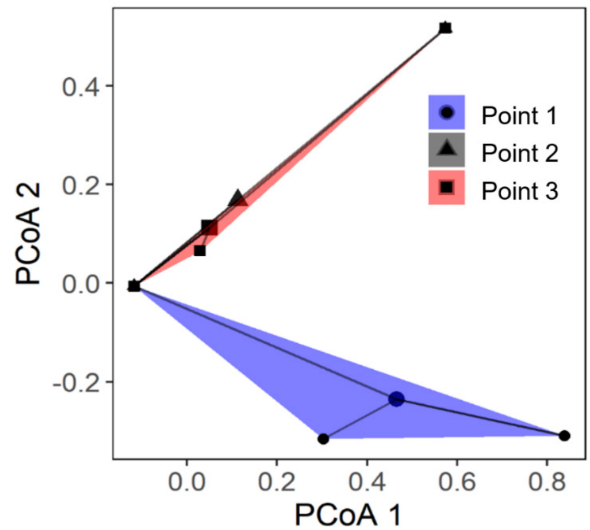

Figure 3. **Principal Coordinates Analysis (PCoA), showing the variability in the composition of nematode species in *Geophagus brasiliensis* between the sampling points of the Iguaçu River, Paraná. Some points are overlapping, as the values of the scores are the same.**

DISCUSSION

The results obtained in this study indicate that the parasitic community of *G. brasiliensis* is characterized by the absence of certain species of parasites registered for this host in Iguaçu River. The abundance of nematode species showed varied responses to the pollution gradient, with the lowest abundance for point 1 (critically degraded to polluted), and the highest abundance for point 3 (moderately degraded).

Water quality parameters, such as temperature, pH, and dissolved oxygen, did not vary in values between the sampling points; however, for point 1, the electrical conductivity and phosphorus showed relatively high values comparing with points 2 and 3. These results indicate that altered parameters of water quality may be related to the large supply of organic waste and domestic and industrial sewage released in the region (Carneiro et al. 2014, Mizukawa et al. 2017, De Andrade Brito et al. 2018, Lehun et al. 2021).

Electrical conductivity measurements can be used as a proxy to identify wastes as pollution (Ouedraogo et al. 2016) for its close relationship with the content of dissolved salts present in the water column, which is generally associated with organic matter supply, thus representing a well-established water quality parameter (Thompson et al. 2010, Chalupová et al. 2012). High conductivity values are associated with high contamination risks (Rahman 2008), therefore, rivers contaminated by industrial and domestic effluents have higher values of electrical conductivity, the case of point 1 in this study. In turn, environments with low amount of particles in suspension generally have low values of electrical conductivity, as observed for points 2 and 3.

Freshwater ecosystems receive phosphorus leached from the earth and anthropic discharges (Smil 2000, Vitousek et al. 2010), consequently generating high levels of phosphates to cause eutrophication (Esteves 2011). The factor of eutrophication has been found to influence the

Table IV. **PERMANOVA pairwise to compare the composition of nematodes between the sampling points. In bold: significant value (p <0.05).**

	Pseudo-F	*P*
Point 1 vs Point 2	8.74	**0.01**
Point 1 vs Point 3	14.23	**0.005**
Point 2 vs Point 3	0.60	1.0

composition of parasite species in fish (Valtonen et al. 1997, Zagar et al. 2012) and can increase or decrease the properties of the infection depending on the parasite and the taxon life history. This means that it depends on the presence, absence, and density of intermediate and definitive hosts, as well as the sensitivity of both hosts and parasites to environmental changes (Sures 2004).

Environments that were altered by pollution, as demonstrated in the phosphorus concentration and high level of conductivity, can present changes in the community structure and abundance of species in response to environmental and pollutant conditions. Environmental changes affect parasites in different manners: pollution can increase parasitism as contaminants can act as immunosuppressants of the host's immune system, or can be fatal to certain species, especially with parasites from complex life cycles, leading to lower parasitism (Lafferty & Kuris 2005). Generally, infections by ectoparasites tend to increase, while infections by endoparasites tend to decrease with higher levels of pollution (Lafferty 1997, Sures 2005, Gilbert & Avenant-Oldewage 2021).

The sampling points showed similar values of richness, diversity, and equitability, however, the abundance of species was relatively higher in points 2 and 3. The species of nematodes found in this study had been registered for this host in Iguaçu River, but due to the absence of species from other groups (for example, digeneans, and acanthocephalans), a low diversity of parasite species was recorded. The low values of diversity can be explained by the dominance of the larvae *Contracaecum* sp. at point 1 and the adult *Procamallanus (Procamallanus) peraccuratus* at points 2 and 3. In fact, the dominance of these species may be the most appropriate way to infer the possible effects of anthropogenic stress because of their higher infectious potential (in terms of prevalence), but it also suggests that in terms of population structure, they can change according to the environment.

The prevalence of larvae *Contracaecum* sp. in point 1 and the use of fish as intermediate host indicate that environmental conditions are somehow adequate to complete their life cycle through planktonic copepods and piscivorous birds (Szalai & Dick 1990). However, the record of low abundance suggests that levels of pollution or anthropogenic stress occurring in the location could have had negative impacts on its intermediate hosts, thus reducing the nematode infectious potential (Fajer-Ávila et al. 2006).

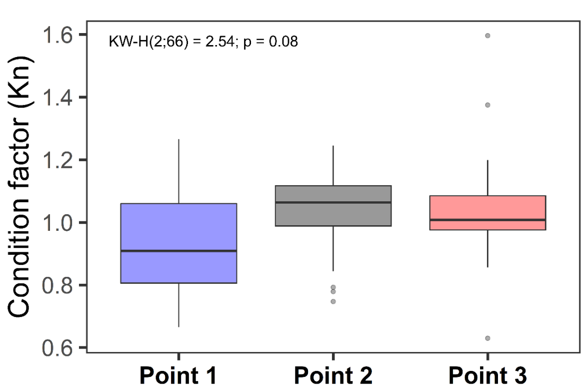

Figure 4. **Mean values of the relative condition factor (Kn) of *Geophagus brasiliensis* between the sampling points of the Iguaçu River, Paraná.**

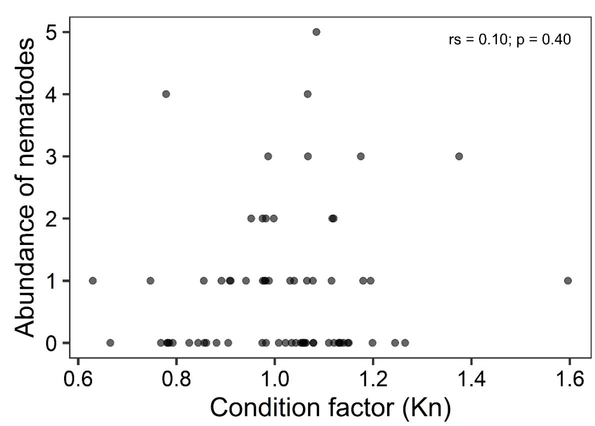

Figure 5. **Correlation between the relative condition factor (Kn) and the abundance of nematodes in *Geophagus brasiliensis*.**

Among other factors, the diversity of parasites results from interactions between the evolutionary history and ecology of hosts, also associated with the diversity of intermediate and definitive hosts (Von Zuben 1997). Thus, the low abundance of adult nematode species in point 1 can be explained by the absence of possible intermediate hosts, indicating that the environment directly affects the life cycle of these parasites. Landsberg et al. (1998) demonstrated that nematodes that typically use crustaceans as intermediate hosts are directly affected by contaminants, as these crustaceans are particularly sensitive to these compounds. Regarding the parasitic community, Nachev & Sures (2009), Chapman et al. (2015) and Blanar et al. (2016) reported higher diversity in less polluted sampling sites, while fauna composition and abundance of some parasites showed to be related to the pollution gradient.

At first, environmental conditions are important for the survival and well-being of the host, but the well-being of the host is also extremely important for the survival of the parasites, although their effects may differ depending on the life cycle (Sures 2008). The presence of larval stage at point 1 that parasitizing *G. brasiliensis* indicated that the fish occupies an intermediate position in the trophic web and must be consumed by a definitive host (other fish, piscivorous birds or mammals) (Lacerda et al. 2018). This abundance of nematodes may be related to the omnivorous host, which allows the ingestion of several organisms that act as intermediate hosts, facilitating the infection (Santos & Brasil-Sato 2006).

The lowest values of condition factor appeared in the hosts collected in point 1, despite the absence of statistically significant difference between the points. Changes in the condition factor values can occur due to the quality or the state of the environment in which the fish is inserted, in addition to parasitism on the hosts (Ranzani-Paiva et al. 2000). Although there was no correlation between Kn and the abundance of parasites, points 2 and 3, with the highest values in the condition factor, showed a higher prevalence of infection. Lizama et al. (2006) demonstrated that parasitized fish had higher values for the condition factor than non-parasitized fish, identifying that larger individuals who also had higher values for Kn tolerated higher levels of parasitism.

A healthy system that does not suffer from anthropogenic changes is rich in parasite species, therefore, parasites are essential to biodiversity and production of ecosystems (Hudson et al. 2006). Pollution induces a shift in community structure towards dominance by tolerant species (Holt & Miller 2011, Parmar et al. 2016), thus, richness decreases as a result of the disappearance of taxa as the level of pollution increases, as well as the abundance of sensitive species is reduced, while the abundance of tolerant species is unaffected or increase (Lafferty 1997, Sures 2005, Adewole et al. 2019). In this study, the absence of parasites already reported for the host *G. brasiliensis* in Iguaçu River demonstrates that the environment can alter species diversity, therefore, pollution of the aquatic environment reflects directly in the organisms. Thus, the use of fish parasites as indicators proved a relevant tool to identify the impact caused by changes in the environment. Thus, parasites can be used to indicate anthropogenic impacts in aquatic environments.

Acknowledgments

We would like to thank the Research Group in Limnology, Ichthyology and Aquaculture (Nupélia) of the Universidade Estadual de Maringá (UEM) for logistical support; and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the supply of scholarship during the course of postgraduate research. And we would thank Ana Carolina de Deus Bueno

Krawczyk and Universidade Estadual do Paraná for support in the sampling.

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How to cite

LEHUN AL, DUARTE GSC & TAKEMOTO RM. 2023. Nematodes as indicators of environmental changes in a river with different levels of anthropogenic impact. An Acad Bras Cienc 95: e20200307. DOI 10.1590/0001-3765202320200307.

Manuscript received on March 6, 2020;
accepted for publication on August 2, 2020

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