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ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

DIVERSITY OF NEMATODES OF RED-TAIL-LAMBARI *ASTYANAX* AFF. *PARANAE* (TELEOSTEI: CHARACIDAE) FROM POLLUTED SITES OF A TROPICAL RIVER SYSTEM

DIVERSIDAD DE LOS NEMATODOS DE TETRA COLA ROJA *ASTYANAX* AFF. *PARANAE* (TELEOSTEI: CHARACIDAE) DE SITIOS CONTAMINADOS DE UN SISTEMA DE RÍO TROPICAL

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Abstract

Aquatic systems are affected by a great variety of anthropogenic activities that alter the water quality through the introduction of organic and inorganic pollutants, consequently impacting all trophic levels of a biological community. The relationship between parasite species diversity and organic pollution in the high Paraná River basin, Paraná, Brazil was investigated during two seasons (Winter and Summer) of 2011. A total of 114 red-tail-lambari (*Astyanax* aff. *paranae* Eigenmann, 1914) were sampled from a non-impacted location (Perobas Reserve) and from impacted regions (agricultural and urban regions, upstream and downstream the city of Campo Mourão, respectively). The nematode *Spinitectus yorkei* Travassos, Artigas & Pereira, 1928 was found on the preserved area, while the nematode *Procamallanus (Spirocamallanus) inopinatus* Travassos, Artigas & Pereira, 1928 was found only on the severely polluted areas. The variability of the prevalence index suggests that the structure of the parasite community is affected at different ways by the same levels of pollutants on the water. The observed results assume that the alteration in parasite diversity can be related to increased organic pollution, and that the differences in the parasite community from different environments can be related to biotic stress.

Keywords: bioindicators - environmental alteration – fish - parasites.

Resumen

Los sistemas acuáticos se ven afectados por una gran variedad de actividades antropogénicas que alteran la calidad del agua a través de la introducción de contaminantes orgánicos e inorgánicos, por consiguiente, afectan a todos los niveles de la comunidad biológica. De esta forma, la relación entre la diversidad de especies de parásitos y la contaminación orgánica en la cuenca del alto Paraná, Paraná, Brasil, se investigó en dos temporadas (invierno y verano) de 2011. Un total de 114 tetra cola roja (*Astyanax aff. paranae* Eigenmann, 1914) se muestrearon en un sitio no impactado (Reserva Perobas) y en regiones impactadas (zonas agrícolas y urbanas, aguas arriba y aguas abajo de la ciudad de Campo Mourão, respectivamente). El nematodo *Spinitectus yorkei* Travassos, Artigas y Pereira, 1928 se encuentran en la zona protegida, mientras que el nematodo *Procamallanus (Spirocamallanus) inopinatus* Travassos, Artigas y Pereira, 1928 se encuentran sólo en las zonas gravemente contaminadas. La variabilidad del índice de prevalencia sugiere que la estructura de la comunidad de parásitos se ve afectada en diferentes formas por el mismo nivel de contaminantes en el agua. Los resultados observados asumen que la alteración en la diversidad parasitaria puede estar relacionada con el aumento de la contaminación orgánica, y que la respuesta de la comunidad de parásitos a las tensiones ambientales puede ser utilizada como bioindicadora.

Palabras clave: bioindicadoras - alteración del medio ambiente – pescado - parasitos.

INTRODUCTION

Most of the human impacts on the aquatic environment affects all the levels of a biological community, including the parasites populations; this have been confirmed with many observations about changes in parasite abundance or richness as a consequence of environmental impacts that interfere on free-living stages of the parasites and/or on the hosts populations (intermediate, definitive or paratenic) (Poulin, 1992; Marcogliese & Cone 1997; Sures, 2004; Costa *et al*., 2012).

The relative abundance of endo- and ectoparasites of fish in a particular aquatic system can be used as an indicator of environmental stress (Avenant-Oldewage, 2001). Ectoparasites, for having direct contact with water, present the same responses of free-living organisms, while protozoic endoparasites seems to be too small to provide sufficient material to analysis (Sures *et al*., 1999; Sures, 2001, 2003, 2008; Sures & Reimann, 2003; Vidal-Martínez et al., 2009). As a general pattern, the cestodes and nematodes seem to be good as sentinel species, specially the Acantocephala group as an indicator, by accumulation, of metal pollution (Sures, 2003, 2004).

The fact that parasites possess complex life cycles makes them extremely valuable information units about environmental conditions, because their presence/absence, as their ecological indexes, tell us a great deal not only about their host ecology but also about the ecosystem biodiversity and food web interactions, and about the environmental stress that may be occurring on the system (Overstreet, 1997; Marcogliese, 2003, 2004; Madanire-Moyo & Barson, 2010). Each parasite species reflects the presence of different organisms that participate in its life cycle; together, all these parasite species in a host reflect the presence of a plethora of host organisms and the trophic interactions in the environment (Marcogliese & Pietrcock, 2011). Given that pollution may have impacts on populations and communities, and thus on food web structure, parasites may be used as natural biological tags of ecosystem health. The species composition of parasite communities is clearly impacted by environmental stress, and species richness and abundance tends to change under degraded conditions (Sures, 2008).

The selected host, *Astyanax* aff. *paranae*, popular known as “red-tail-lambari”, is a common species of the Paraná River basin (Garutti & Britski 2000) and its occurrence was registered as very abundant on the Paraná river tributaries (Luiz *et al*., 2003). It is characterized by an omnivorous food habitat and sexual dimorphism, females being more robust than the males (Eigenmann, 1914; Godoy, 1975; Barbieri 1992; Ferreira, 2004). The characteristics used to its identification are described on Garutti & Britski (2000). The host selection was afforded by the studies of Overstreet (1997), Sures (2004) and Lewis & Hoole (2003). According to these researchers, the host must have a restricted home range, abundant populations of easy sampling and identification, wide geographic distribution and a great number of parasites – preferentially parasite species that present abundant numbers of hosts on their cycles.

This study intended to establish the relationship between the metazoan parasites and the pollution levels in the sampled places. The specific objectives of this study were to determine the biodiversity of metazoan parasite communities of *Astyanax* aff. *paranae* Eigenmann, 1914 in the upper Paraná River basin and to compare the diversity of metazoan parasite fauna along defined pollution gradients, assessing the suitability of using parasites as predictors of environmental change. Due to the great diversity of the stress effects on the hosts and on the parasites populations, it is believed that the metazoan community of *A.* aff. *paranae* of the affected areas, whether by agricultural or urban pollutants, will present lower levels of parasite richness, but an increase on the prevalence and abundance of the species resistant to the environmental stressors.

MATERIALS AND METHODS

Study area

The data used on the present work were collected in two hydric systems of the high Paraná River basin, Northwest of Paraná state, South of Brazil. On the Concórdia stream one sample point has been established and it was considered the positive control area owing to the absence of intensive agricultural, industrial and mining activities close to the river. The headspring of the Concórdia stream is located on the Perobas Biological Reserve damping area and heads to the referred conservation unity. The Perobas Biological Reserve (RE) (23°51'16"S, 52°45'2"O) belongs to the Tuneiras do Oeste and Cianorte counties, and it was established on 2005, representing, according to the Federal Law 9985 of 2000, a full protection unity, objectifying the biodiversity protection, with restricted use to scientific researches and management actions (Fig.1).

The other two collection points were placed on the Campo River (24°10' S, 52°31' O): 1- the Upstream Point (UP), that is surrounded only by agriculture areas, not crossing the urban area; 2- the Downstream Point (DP), that is located right after the urban area and is characterized by the influx of different effluents from the Campo Mourão city, including wastes from industries and from the sewer treatment. The collection sites are presented on Figure 1.

Fish and parasite collection

Fishes were collected over two seasons: the first one on July (Winter) 2011, which correspond to the plantation of corn and wheat; the second period was on November (Summer) 2011, which correspond to the plantation of soybean.

To evaluate the impact of the industrial, sewage and agricultural activity on the water quality, several analyses have been performed. Surface water temperature (^{o}C) and Dissolved Oxygen (DO, mg/L) content were measured using a HACH oxygen meter; the conductivity (µS/cm) was measured by a WTW conductivity meter and pH using a HACH pH meter. Subsurface water samples were collected in 1L plastic containers rinsed in deionized water. The samples were taken to the laboratory where they were analyzed for the following compounds: copper, zinc, aluminum and total phosphorus, according Standard Methods of APHA, AWWA, WEF (2005). On these same water samples analyses have been made to determine the glyphosate concentration, as this is the main compound of the fertilizers used on the geographic area, by gas chromatography/mass spectrometry performed the São Paulo University Chemistry Laboratory.

The RE collection was performed by the sieving methodology, owing to the few individuals observed on the reserve stream. The sieves nets had the dimensions of 0.60 X 1.2 m and 0.30 cm; the sieving was performed through active overhaul on the macrophyte banks and on the marginal micro-habitats. The fish collection on the UP and DP was made using a seine net with a dimension of 1,2 m x 5 m, which was installed parallel to the river, overhauled from hour to hour.

Right after the collection, the fishes were sacrificed through benzocaine hydrochloride overdose, and the standard and total lengths (mm) and mass (g) of each fish were recorded. The fishes were kept in alcohol 96% and were then transported to the laboratory, where the specific identification was confirmed and, after ventral incision, the sex was recorded and portions of the muscles, viscera and the gastrointestinal tract were dissected and examined under stereomicroscope for internal parasites. The nematodes and digeneans were fixed in formaldehyde and then preserved in 70% alcohol. They were then cleared and mounted according to Eiras *et al.* (2006), and identified according Moravec (1998).

Data analysis

To the evaluation of the interactions between the collected specimens and the biotic and abiotic factors, the allometric factor condition (K) was calculated, according the following equations: $W = a * L^b$; where: W = total weight, L = total weight, a = intercept e b = angular coefficient (Le-Cren, 1951). The "a" and "b" parameters were estimated after weight and length logarithmic transformation, and subsequent adjust through least mean square analysis technique (Vanzolini, 1993). The K was estimated by K=W/L*b, using the same parameters previously stipulated and analyzed on each collection (Vanzolini, 1993).

The bifactorial ANOVA was performed on the STATISTICA software (version 5.5), right before the Tukey test. The points and the seasons of each collection were the factors considered on these analyses. Parasite prevalence and abundance were measured and calculated as defined by Bush *et al.* (1997).

Voucher specimens of some collected individuals were accommodated on the Ichthyological Collection of the Limnology, Ichthyology and Aquiculture Research Center: the UP and DP fishes NUP 13381 and Field: NCG2011103101; the RE fishes NUP13382 and Field NCG2011071701.

RESULTS

Water quality

The glyphosate analysis on the water samples revealed that this compound concentrations were below the minimum detection levels commercially available and, for this reason, couldn`t be obtained. The results of the chemical analysis performed on the three locations water samples are on the Table 1.

The water temperature varied with the time of sampling, ranging from 17.0 to 21.25°C. The highest level of DO was at RE during the Winter (8.34 mg/l), and the lowest level was at DP during the Summer (5.78 mg/l). The highest level of conductivity readings was at the DP (42-86µS/cm). Water pH presented a light and continuous increase from the preserved area (RE) to the disturbed collection points (pH range: 6.67–7.5). All the nutrients analyzed were quite low on the control area (RE) and higher on the UP and DP, probably indicating an eutrophication process. All the water sampled on the UP and DP had higher levels of nutrients (Table 1). It was observed an increased level of heavy metals (aluminum, copper and zinc), phosphates, conductivity and a reduced DO observed on the urban area of Campo Mourão (DP), while the RE remained comparatively unimpacted; the agricultural local (UP) presented intermediary levels (Table 1).

Hosts` condition

The bifactorial ANOVA, performed with the K fish values, revealed an interaction among the period of the year and the collection points ($p<0.05$), demonstrating that season and locality did not act independently on the fishes` condition. Consequently, the group analyzes of these factors have been obligatorily performed.

The Tukey test revealed that the K values during the winter were significantly inferior than the Summer values. During the winter, significant differences between the collection points have not been visualized. The highest K values have been observed on the DP during the summer, but, during this same period, the fishes from UP and RE also presented high K values. Significant differences have been observed among all the collection points during the summer (Fig. 2).

Parasite composition, richness and distribution

A total of 22 "red-tail-lambari" out of the 114 fishes caught were infected with parasites. Five species of metazoan parasites were observed on the 22 infected fishes collected from the three collection points (Table 2). The endoparasites included four nematode species (larval *Contracaecum*, adult *Procamallanus (Spirocamallanus) inopinatus* Travassos, Artigas & Pereira, 1928, adult *Cucullanus (Cucullanus) brevispiculus* Moravec, Kohn & Fernandes, 1993 and adult *Spinitectus yorkei* Travassos, Artigas & Pereira, 1928). Parasite species richness increased as the chemical compounds levels increased (Table 2). The less altered location, the RE point collection, presented only one species of parasite (*S. yorkei*), while the location with higher levels of nutrients, the DP, had three species of parasites (*P. inopinatus*, *C. brevispiculus* and larval *Contracaecum*). On the UP, during the Winter, it has not been observed any species of parasites (Table 2).

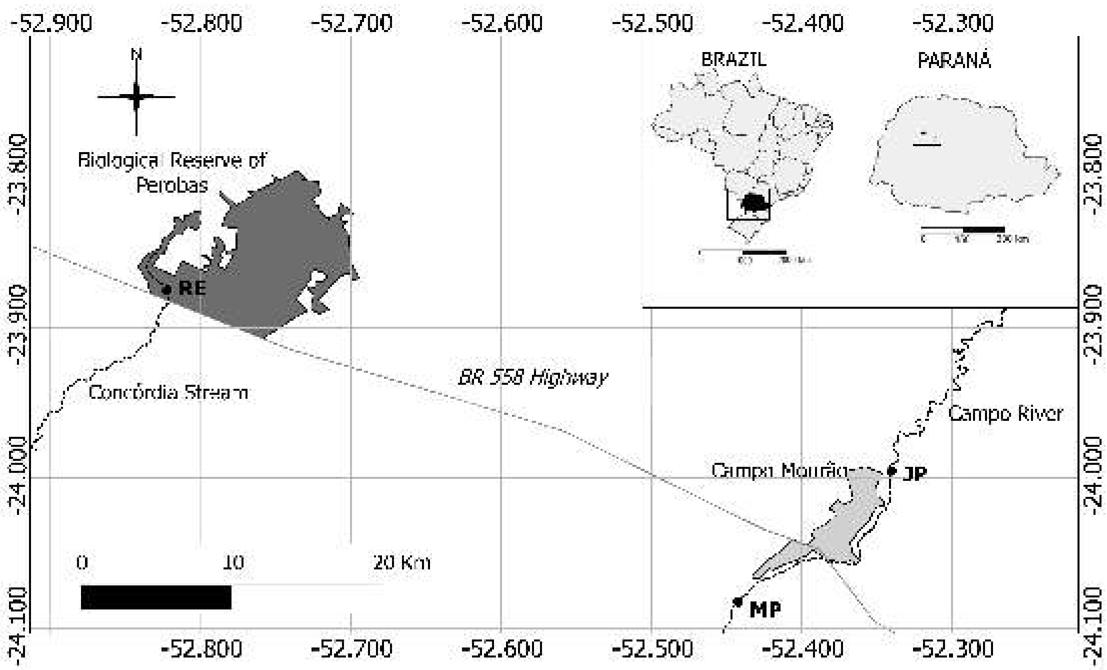

Figure 1. Localization of Campo Mourão county on the State of Paraná, Brazil. Visualization of the collection points (*) on the Biological Reserve of Perobas and on Upstream and on Downstream of Campo Mourão city - PR. Source: SEMA Campo Mourão.

The *S. yorkei* seems to be sensitive to eutrophication as its presence has not been recorded on the most altered site (DP), but more studies are necessary to confirm this punctual observation (Table 2). The other nematodes (*P. inopinatus*, *C. brevispiculus* and larval *Contracaecum*) seem to be very tolerant to organic pollution as they are associated with the sites with higher eutrophication levels (Table 2). The distribution of *P. inopinatus* and *C. brevispiculus* was limited to DP, the most altered collection site. The *C. brevispiculus* have been observed only on the UP collection point during the Summer. The severely altered site (DP) demonstrated close association with *P. inopinatus* and *C. brevispiculus*.

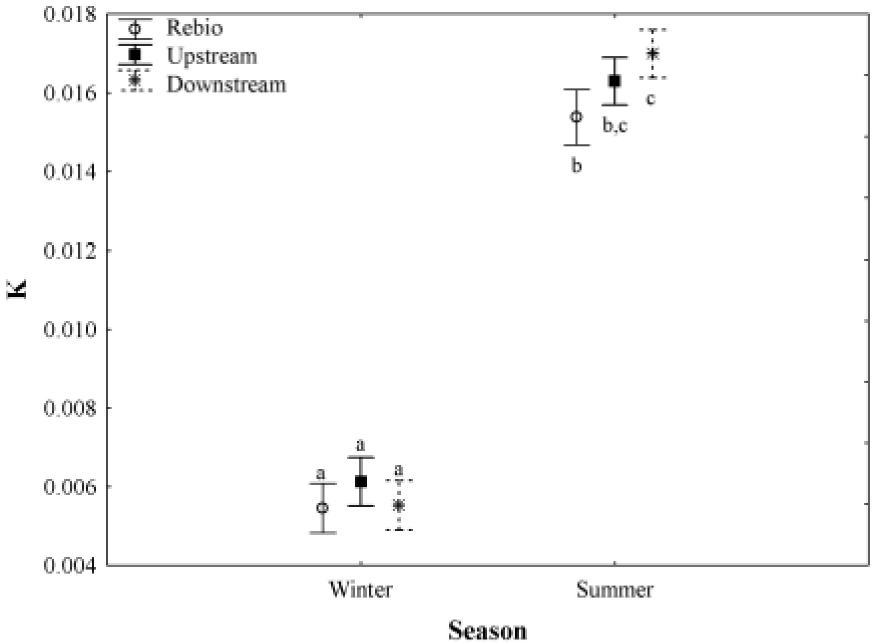

Figure 2. Mean and confidence interval of the hosts Condition Factor (K) values by collection point and by collection season. a,b,c = Tukey`s test results (different letters represent statistical differences p<0.05); Perobas Biological Reserve (RE), Upstream Point (UP), Downstream Point (DP).

Table 1.Chemical analyzes of the water samples collected on the Perobas Biological Reserve (RE), on the Upstream of the Campo river (UP) and on the Downstream of this same river (DP), on June (Winter) and on November (Summer). Aluminum (Al), Copper (Cu), Zinc (Zn), Phosphorus (P), Dissolved Oxygen (DO), Temperature (T).

Chemical Compounds analyzed	RE		UP		DP	
	Winter	Winter	Summer	Summer	Winter	Summer
Al (mg/L)	0.77	0.89	1.04	0.945	0.74	0.868
Cu (µg/L)	1.6	7.3	1.9	0.5	4	4.2
Zn (µg/L)	1.7	3.7	5.2	3.45	3.7	4.8
P (µg/L)	0.0124	< 0.01	0.091	0.945	0.0183	0.054
DO (mg/L)	8.34	7.16	6.36	6.09	7.99	5.78
pH	6.67	6.83	6.9	6.81	7.21	7.5
T (°C)	18.89	17	18.01	21.25	17	19.92
Conductivity (µS/cm)	25	40	20	29	86	42

Table 2. Parasite species found on the Perobas Biological Reserve (RE), on the Upstream of the Campo river (UP) and on the Downstream of this same river (DP), with the respective values of Prevalence (Prev.) and Mean Abundance (MA).

Collection sites	Pollution class	Collection period	Collected fishes	Parasitized fishes	Parasites	Prev.	MA
RE	Non-polluted	Winter	19	6	*S. yorkei* (n=9)	32%	0.18
		Summer	15	1	*S. yorkei* (n=2)	6,7%	0.33
UP	Polluted	Winter	20	1	*S. yorkei* (n=1)	5%	0.12
		Summer	22	0			
DP	Severely polluted	Winter	22	4	*P. inopinatus* (n=4)	18%	0.18
					Contracaecum sp. (n=1)	4%	0.2
		Summer	33	10	*P. inopinatus* (n=4)	21%	0.33
					C. brevispiculus (n=4)	9%	0.12

DISCUSSION

Water quality

Despite the fact that the RE water samples presented high concentrations of some of the evaluated compounds, the highest values of phosphorus, conductivity, pH, temperature, zinc and copper were observed on the Campo River, which confirms the assumption that the UP and DP would be the most altered locations. According to the National Council of Environment (CONAMA), the water from all the three collecting points is inappropriate to human consumption; however, the high aluminum concentrations in all the collection sites are explained by the characteristic soil of the region, which presents great quantity of this compound (Pavan *et al.*, 1985).

The higher conductivity at the UP and at the DP may be attributed to urban and agricultural effluent saturating these locations, while the slight decrease in conductivity during the Summer on both locations could be correlated to different plantations during this period. Different plant`s production leads to the different agrotoxic needs, what will unleash different compounds on the water, especially the ionic factors – the main elements responsible for the water conductivity (Kunze *et al*., 1968).

Increased urbanization and poorly treated effluent discharged into the Campo river are probably responsible for the high conductivity, especially on the DP collection point. During the Summer, there was a phosphorus concentration increase in all the three collection points, which is explained by the discharge of decomposition products by the summer rains. It is clear that the agricultural and urban wastes contribute to the nutrient loading to the Campo River.

Hosts` condition

The K values significantly inferior during the Winter could reflect a natural stress situation, when the temperature is lower and the system productivity is diminished. Probably the specimens are more influenced by these factors, independently the location they are collected. On the Summer, the availability of nutrients increases mainly because the high levels of temperature, rainfall and the higher activity of decomposers during this period (Jobling, 1995). However, local factors seems to also influence the fishes' condition, as observed to the specimens collected on DP, where the urban run-offs finds its way into the Campo river – what can explain the higher nutrient levels at the water samples and the better nutritional situation of the fishes.

Usually, organic polluted environments present higher levels of productivity (Colinvaux, 1993), what can be advantageous to species with more plasticity, making the accumulation of energetic reserve easier and, consequently, improving their Condition Factor (Alberto *et al*., 2005). On the present study it was not observed a negative relation between the inorganic pollutants observed on the water (aluminum, zinc and copper) and the K values of the hosts, what possibly indicates use of *Astyanax* aff. *paranae* as an accumulation bioindicator, but more studies and experiments are necessary to confirm the absence of morpho-physiological alterations after the exposure to high concentrations of the inorganic compounds. Therefore, it is possible to assume that the alterations observed on the parasite population indexes are not due to the diminished immunological hosts` capacity, as the K values did not reveal any condition loss by the hosts.

Analyzing the information obtained on this work, the responses to environmental alterations is not restrained to the ichthyologic community, but it is also observed on the parasite community. Despite the fact that the K values were not affected by the high inorganic compounds concentration, the hosts` parasite infracommunities from the RE e from DP were entirely different, a clear evidence that the parasite community responds to the environmental alterations and can be used as a bioindicator.

Parasite composition, richness and distribution

Two parasite species presented bioindicator capacity – *Spinitectus yorkei* and *Procamallanus (Spirocamallanus) inopinatus*. The first one has not been found in severely altered locations, so this sensitiveness probably indicates its importance as Effect Bioindicator. *P. inopinatus* may also represent a bioindicator species, specifically an Accumulation Bioindicator, for its toleration to the prejudicial effects that could occur after the exposure to the organic and inorganic compounds found on some of the collection points.

The presence of *S. yorkei* on UP during the winter could be explained by the similar water concentrations between this collection point on this period and the RE. Except for the copper concentration, all the other parameters analyzed on the UP are similar to the reserve values. Possibly, the copper concentration belatedly acted, which can explain the complete absence of parasites on UP during the summer.

The eutrophication process may also act directly on intermediate hosts and definitive hosts. The results are consistent with the general patterns found by Poulin (1992), Hartman and Numann (1977), Beer & German (1993), Lafferty & Kuris (1999), Lafferty (1997) and with the study performed by Valtonen *et al.* (1997), all suggesting a positive relationship between eutrophication and fish parasitism. Eutrophication often raises parasitism because the associated increased productivity can increase the abundance of intermediate hosts (Lafferty & Kuris, 1999).

The eutrophic nature of the DP contributed to the observed high prevalence values of the helminth community of *A.* aff. *paranae* at the polluted sites. The eutrophication probably increased the abundance of the intermediate hosts, a result of the sewage outflows; this eutrophic process is also reflected on organic enrichment of the sediments, providing excellent habitat for the alternative hosts (Weisberg *et al*., 1986; Sibley *et al.*, 2000). The presence of the nematodes in the polluted sites is also made possible by their possession of a cuticle, which enables them to withstand the harsh conditions, as the water pollution can influence aquatic endoparasites both directly and indirectly by acting on their intermediate hosts.

There is still missing a significant quantity of information about promisors bioindicative processes, such as biomarkers, and about the use of parasites as bioindicators (Williams & Mackenzie, 2003; Vidal-Martinez *et al.*, 2009; Sures, 2008) but the accumulation of data by serious researches performed along the years could reveal how each parasite species respond to environmental alterations. The initial hypothesis was not corroborated, as the affected areas presented higher species richness, but this could be related to the short collection period or to the few hosts analyzed. However, the results here presented possibly indicate that the parasite assemblages may be good indicators of environmental stress because they reflect the presence of many different types of organisms, based on the variety of complex life cycles displayed by the different parasite taxa.

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