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Metazoan endoparasites of *Brycon orbignyanus* (Characidae: Bryconinae) in a neotropical floodplain

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ABSTRACT. Species richness and composition are central themes in community ecology of helminths because they improve the understanding of factors that determine community structure. Floodplain ecosystems and the environmental alterations induced by hydrological cycles are factors that maintain biodiversity over time, as observed in the Upper Paraná River floodplain, where the individuals of *Brycon orbignyanus* were collected. The objective of this study was to evaluate whether variables such as the richness and abundance of endoparasites are related to the fish size during different hydrological periods (high and low waters). A total of 163 endoparasites belonging to 13 species were collected in the 104 fish examined. Fish size had a positive correlation with mean diversity and abundance of the endoparasite infracommunity, supporting the idea that larger hosts are able to harbor a more abundant and diverse parasite infracommunity. The diversity of habitats provided by the floodplain can be considered the main factor explaining the differences between these attributes of parasite species. Variations in parasite richness between different hosts provide not only a good model for studies on community diversification but are also of great interest in species conservation. All parasite species found in *B. orbignyanus* were recorded for the first time in this host, especially in the Upper Paraná River floodplain.

Keywords: helminthes; Paraná River; hydrological phase; parasite richness.

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Introduction

Floodplains are a complex gradient between the river channel and uplands, within which a variety of secondary and tertiary ecotones is embedded (Ward, Tockner, & Schiemer, 1999). In floodplains of river systems there is a strong trend of biological communities to remain in synchronicity with the events of hydrological flooding and drought (Junk, Bayley, & Sparks, 1989). Regulation of the main river from the construction of reservoirs has changed the dynamics of the floodplain of the upper Paraná River, and consequently its biodiversity. Thus, to obtain a greater understanding of the patterns of organization of individuals in assemblages present in those ecosystems, compared with periodic disturbances, a holistic approach is necessary, as emphasized by Ward et al. (1999).

Floods can change the population dynamics of fish populations and their biological and physiological conditions, thus influencing the structure and composition of the fauna of fish parasites (Takemoto et al., 2009). Freshwater fish are vertebrates that can be utilized as a model to study hosts that are home to a large variety of parasite species, being ectoparasites or endoparasites belonging to different phyla (Eiras, Takemoto, & Pavanelli, 2006). Pavanelli, Machado, and Takemoto (1997) published a preliminary list of the helminth parasites of fishes from the floodplain of the Upper Paraná River with data collected from 81 host species.

Hydrological cycles are determinants in biological and ecological processes, and are important to the initial development of fish, especially migratory species that colonize the environment during times of flood (Lowe-McConnell, 1999). This is the case of *Brycon orbignyanus*, a species that performs reproductive migrations during the period from November to January, when food is more abundant. This study aimed at

studying the endoparasite fauna of *B. orbignyanus*, which has a large potential for pisciculture, increasing the range of information to make possible its cultivation. Furthermore, according to Reis et al. (2016) among the 3.130 known freshwater fish species in Brazil, 312 are found on official list of endangered species IN 445/2014, including *B. orbignyanus*. This is a neotropical fish found in several South American hydrographic basins. This species has high ecological importance and is included in the list of fish threatened with extinction. From the Paraná River basin, few species of parasites of *B. orbignyanus* were referred to this host.

Considering *B. orbignyanus* importance in economic and ecological scope, the hypothesis is that the size of *B. orbignyanus* is determinant for the composition and abundance of endoparasite fauna during periods of flood and drought. So, this study aimed to evaluate responses such as richness and abundance during different phases of the hydrological cycle. Moreover, the seasonal variation of the abundance of parasites is different during the sampling period, with an increase during the study period due to accumulation of parasites.

Material and Methods

Sampling site

The study area is part of the Upper Paraná River floodplain (Figure 1), in the State of Paraná (22°43'S and 53°10'W). Samplings were carried out in 36 environments from the Upper Paraná River floodplain (including connected and isolated floodplain lakes, backwaters, rivers and channels). This floodplain site is site six within the Brazilian network of Long Term Ecological Research (LTER) sites.

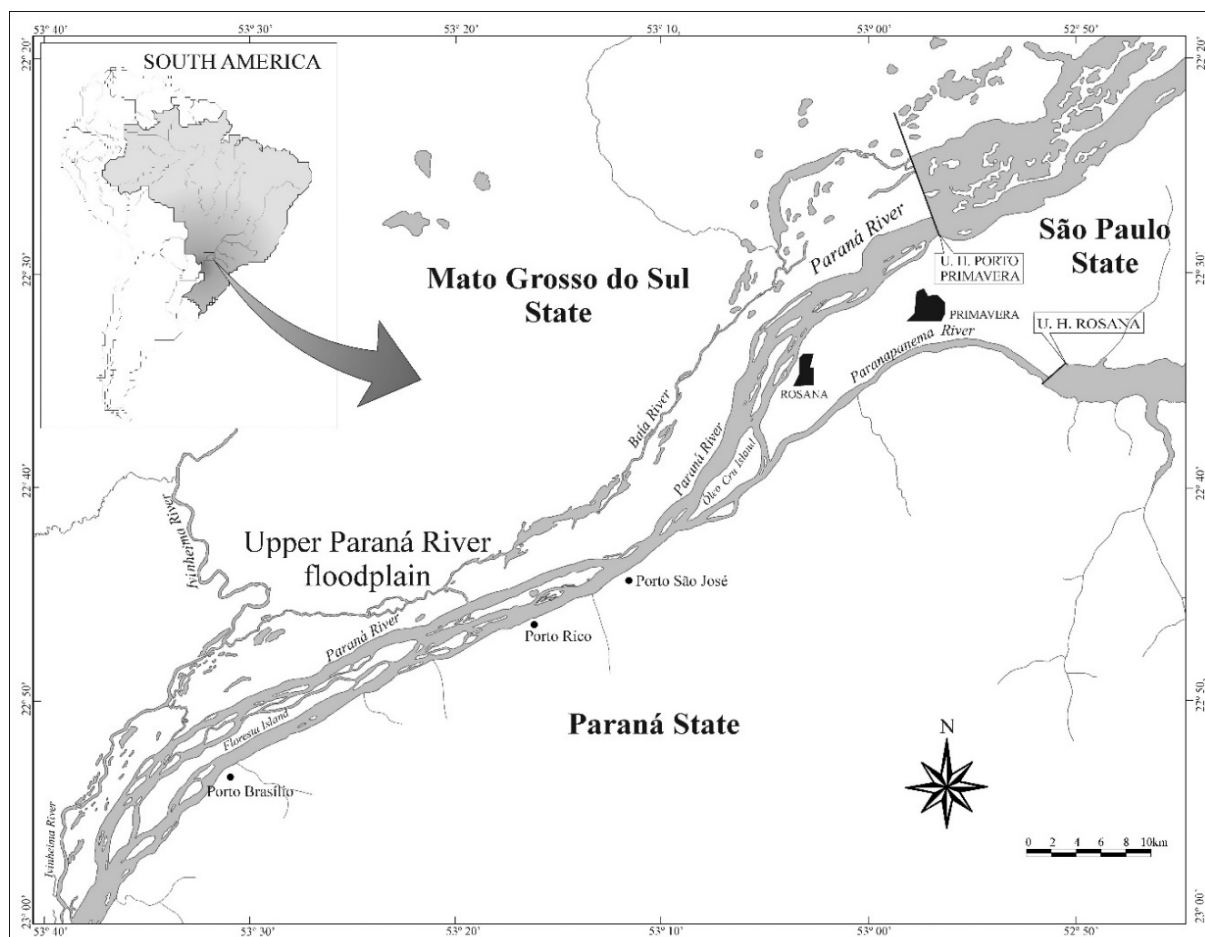


Figure 1. Study area in the Upper Paraná River floodplain located in the states of Paraná and Mato Grosso do Sul, Brazil.

Hydrological levels were obtained daily from the fluviometric station on the Paraná River, at the Advanced Research Base of Nupélia, in the municipality of Porto Rico, State of Paraná. Limnological data were provided by the Laboratory of Limnology, of the Research Group in Limnology, Ichthyology and Aquaculture - Nupélia, *Universidade Estadual de Maringá* (UEM). The hosts were caught during the sampling period, since the fish 'disappeared' from the environments sampled in the subsequent collections.

Collection of fish

Fish were caught quarterly in the period between March 2010 and September 2011. This collection was authorized by the Ethics Committee of the *Universidade Estadual de Maringá* (CEAE - Opinion 123/2010) and collection permission Ibama (22442-1). For sampling, gill nets of different mesh sizes were used over a period of 24 hours, with inspection every 8 hours. The sampling date, the standard length, total weight and sex of each fish were registered. The specimens are deposited in the Fish Collection of Nupélia (UEM).

Collection, fixation and conservation of endoparasites

After collecting the fish, taxonomic identification and determination of host biometric data were noted, then a longitudinal incision was made on the ventral surface of each individual fish and all organs were removed and separated. The visceral cavity and each organ were examined under a stereomicroscope to collect endoparasites. The methodology for setting endoparasites was different according to the parasite group, following the recommendations of Eiras et al. (2006). Species of endoparasites found in *B. orbignyanus* were identified according to Moravec (1998), Thatcher (2006), Kohn, Fernandes, and Cohen (2007), Eiras, Takemoto, and Pavanelli (2010), and specific literature.

Data analysis

Spearman's rank correlation coefficients (r_s) were calculated to determine possible associations between the standard length of hosts and the abundance of infection, the relative condition factor (K_n) and species diversity (Zar, 2010). Pearson's correlation coefficients (r) were used as an indication of the possible relationship between host total length and the prevalence of parasites, with previous arcsine transformation of prevalence data (Zar, 2010) and separation of samples of hosts into 11 standard length classes.

K_n was calculated using the following formula: $K_n = \frac{W}{L^b}$, where W is weight, L is total length and b is the slope of the weight: length ratio, which is estimated by the equation $y = ax^b$ (Le Cren, 1951).

The Mann-Whitney U test was applied to test differences in infracommunity diversity of parasites between K_n (Zar, 2010). Parasite prevalence, intensity and abundance were calculated according to Bush, Lafferty, Lotz, and Shostak (1997). The variance: mean ratio of parasite abundance (dispersion index) was used to determine spatial distribution patterns and was tested by the statistical index d . Over-dispersion or degree of aggregation was determined with Green's index (Ludwig & Reynolds, 1988). The Berger-Parker dominance index (total number of specimens of all species in the infracommunity) was calculated (Magurran, 2013). Finally, Brillouin's diversity index was calculated (Zar, 2010).

The analysis included only parasite species with a prevalence greater than 10% (Bush, Aho, & Kennedy, 1990). For a description of the structure and quantitative analysis of the parasites found, we used the parasitic indices described by Bush et al. (1997). The statistical significance level was evaluated at $p \leq 0.05$. Statistical analysis was conducted using the software Statistica 7.1 (Statsoft Inc., 2005).

Results and discussion

Out of the 104 fish examined, 72 (69.23%) were parasitized by at least one parasite species. A total of 163 endoparasites belonging to 13 species were collected (Table 1). The majority of parasite specimens collected were larvae of *Contracaecum* sp. (18.6%), followed by *Procamallanus* (*Spirocamallanus*) *inopinatus* (12.50%) and metacestodes of *Monticellia spinulifera* (57.69%); *Dadaytrema oxycephala* was the species that showed the highest mean intensity (Table 1).

Larvae of *Contracaecum* sp., *Hysterothylacium* sp., *Goezia* sp. and encysted *Octosporiniferoides* sp. were found in the mesentery of juvenile individuals of *B. orbignyanus*.

All species of endoparasites found in this host were recorded for the first time.

In an aquatic ecosystem, fish parasite communities reflect interactions with the aquatic environment, with their hosts and with communities of invertebrates. Parasite assemblages could therefore play a potential role as environmental indicators, decreasing or increasing in diversity, richness, abundance and prevalence according to changes in environmental conditions (Kadlec, Šimková, Jarkovsky, & Gelnar, 2003).

Increases in the abundance of endoparasites in fish of larger size are attributed to cumulative occurrences of the infection process. However, in order for an infection to be established, the niches of invasive parasite forms have to overlap with the host niches (Stewart et al., 2017). For endoparasites, these processes may be linked to cumulative behavioral or trophic changes along the life of the host (Holmes,

1990). Valtonen, Marcogliese, and Julkunen, (2010) observed that omnivores presented the highest diversity of endoparasite species; this could explain the richness of the parasite fauna in *B. orbignyanus*, omnivore fish that have a high feeding plasticity (Hahn, Agostinho, Gomes, & Bini, 1998).

The intermediate position that *B. orbignyanus* holds in the food web means that its trophic parasite community is composed of both autogenic species, for example, cestodes, as well as allogeneic species, such as *Contracaecum*. Thus, these fish can perform the role of intermediate and/or definitive hosts. The position of the hosts within a trophic network should determine whether their fauna consists principally of parasitic helminth larvae or adults, since vulnerability to predation determines the role of an animal's predator-prey relationship (Poulin & Leung, 2011). It is known that the main food resource for *B. orbignyanus* is of allochthonous origin (Sgnaulin, et al., 2018), as it is an omnivorous species that feeds on plants, small fish and insects (Vaz, Torquato, & Barbosa, 2000). Therefore, these fish can participate in the life cycle of different groups of parasites as second intermediate and/or paratenic hosts, which might explain the presence of larval *Octospiniferoides* sp. in juveniles and adults of *Octospiniferoides incognita* in adult fish. Nematoda was the most prevalent group and, it is also one of the groups of parasites with the highest species richness in the study region (Takemoto et al., 2009).

Table 1. Endoparasites of *Brycon orbignyanus* from the floodplain of the upper Paraná River, collected between March 2010 and September 2011.

Taxonomic group	Parasites	Prevalence (%)	Mean intensity	Mean abundance
Acanthocephala	<i>Echinorhynchus briconi</i> Machado Filho, 1959	2.88	2.33	0.06
	<i>Octospiniferoides incognita</i> Schmidt & Huggins, 1973	1.92	1.5	0.02
	<i>Octospiniferoides</i> sp. (larva) Bullock, 1957	8.65	1.77	0.15
Cestoda	<i>Monticellia spinulifera</i> (metacestode) Woodland, 1935	57.69*	–	–
Digenea	<i>Dadaytrema oxycephala</i> (Diesing, 1936)	5.76	3	0.17
Nematoda	<i>Contracaecum</i> sp. (larva) Railliet & Henry, 1912	18.26*	1.42	0.26
	<i>Goezia</i> (larva) Zeder, 1800	0.96	1	0.01
	<i>Hysterothylacium</i> (larva) Ward & Magath, 1917	2.88	1.33	0.03
	<i>Ichthyouris</i> sp. Inglis, 1962	0.96	2	0.02
	<i>Procamallanus</i> (<i>Spirocamallanus</i>) <i>hilarii</i> Vaz & Pereira, 1934	3.84	1.25	0.05
	<i>P. (S.) inopinatus</i> Travassos, Artigas & Pereira, 1928	12.50*	1.23	0.15
	<i>P. (S.) paraguayensis</i> Petter, 1990	1.92	1	0.02
	<i>Rhabdochona acuminata</i> Molin, 1860	1.92	1.5	0.03

The mean standard length of the fish analyzed was 17.10 ± 27.69 cm. Among the parasites that showed a prevalence exceeding 10%, a positive and significant correlation was detected between the standard length of *B. orbignyanus* and the abundance of *P. (S.) inopinatus* ($r = 0.25$, $p = 0.008$) (Figure 2). On the other hand, there was no significant correlation between the standard length of the fish and the prevalence of *P. (S.) inopinatus* ($r = 0.56$, $p = 0.07$). There was also no correlation between prevalence ($r = -0.08$, $p = 0.80$) and abundance ($r_s = 0.05$, $p = 0.558$) of *Contracaecum* sp. larvae and the standard length of hosts.

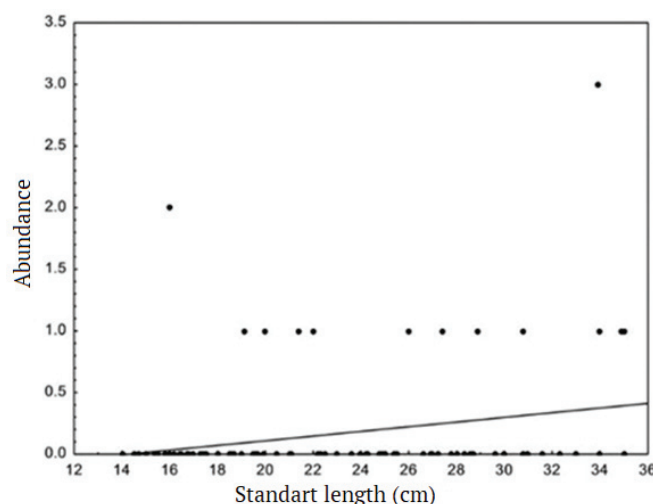


Figure 2. Correlation between abundance of *Procamallanus* (*Spirocamallanus*) *inopinatus* and standard length of *B. orbignyanus* from the floodplain of the upper Paraná River, collected between March 2010 and September 2011.

The mean total weight of fish was 110.8 ± 491.22 g. K_n was not different between fish parasitized and non-parasitized by *P. (S.) inopinatus* ($Z = 0.55$, $p = 0.30$) and *Contracaecum* sp. larvae ($Z = 0.72$, $p = 0.23$). K_n showed no significant correlation with the abundance of *P. (S.) inopinatus* ($r_s = 0.05$, $p = 0.55$) and *Contracaecum* sp. larvae ($r = 0.06$, $p = 0.49$), respectively. The lack of significant interaction between K_n and abundances of *P. (S.) inopinatus* and larval *Contracaecum* sp. indicates that the K_n of the fish is not affected by the parasites. Thus, we can affirm that the parasite community of *B. orbignyanus* causes low pathogenicity to the host. In the case of endoparasites where infection takes place via food, for example, *P. (S.) inopinatus*, the largest fish with a high K_n are also those most infected; this may be linked to the fact that fish that consume higher amounts of food can thus exhibit better health and also have more ingested infective forms of these parasites using the route of trophic transmission. This is more likely if the pathogenicity of the nematode in question is low. Acanthocephalans also acquired via food resources should also be considered because they are endowed with proboscis hooks that are used for fixing onto the intestinal wall of the host, causing reductions in the K_n of fish. However, the present study was not able to detect considerable damage due to low abundance.

The parasite *P. (S.) inopinatus* and *Contracaecum* sp. showed an aggregated distribution type, according to the dispersion index. It is probable that the low value of Green's index was caused by the low abundance of parasites, despite the aggregation (Table 2).

Table 2. Dispersion index (DI), d statistic, Green's index (GI) and pattern of distribution of endoparasite species of *Brycon orbignyanus* from the floodplain of the upper Paraná River, collected between March 2010 and September 2011.

Parasite species	DI	d	GI	Distribution
<i>Procamallanus (Spirocamallanus) inopinatus</i>	1.35	2.41	0.02	Aggregate
<i>Contracaecum</i> sp. (larvae)	1.64	4.12	0.02	Aggregate

The aggregate pattern observed for the endoparasite of *B. orbignyanus* is considered characteristic of parasitic systems and is a function of most hosts that are parasitized by parasitic species with a low intensity or are not infected. However, in only a few hosts parasite species are present at a high intensity (Poulin, 2007; Kennedy, 2009). According to Zuben (1997), the aggregate distribution pattern acts to increase the density-dependent regulation, the abundance of both hosts and parasites, and reduce the level of interspecific competition between the parasites. These factors make the present results of great significance in commercial species, such as *B. orbignyanus*.

Mean infracommunity diversity correlated positively and significantly with the standard length of hosts ($r_s = 0.30$, $p = 0.009$) (Figure 3A) and the abundance ($r_s = 0.20$, $p = 0.03$) (Figure 3B) of endoparasites.

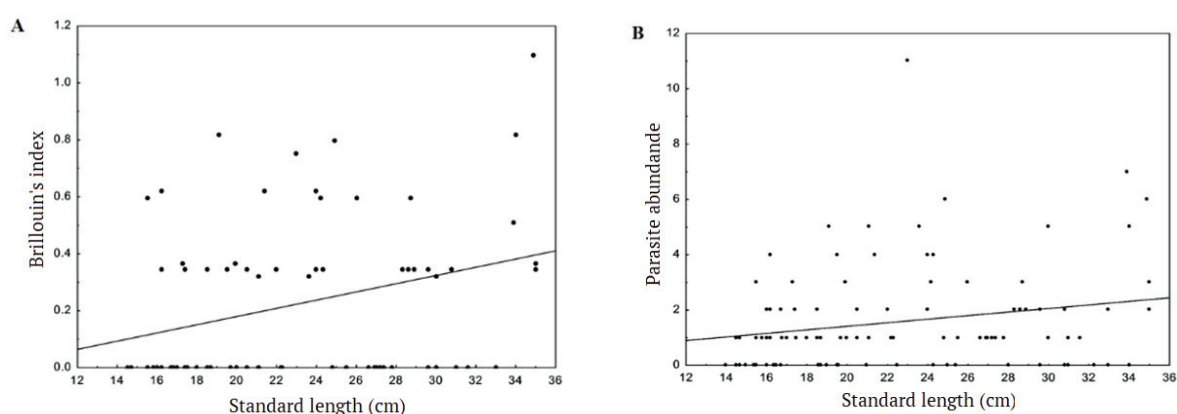


Figure 3. Correlation between infracommunity richness (A), and abundance of endoparasites (B) and standard length of *Brycon orbignyanus* from the floodplain of the upper Paraná River, collected between March 2010 and September 2011.

The nematodes found belong to three genera: *Contracaecum* sp., *Hysterothylacium* sp. and *Goezia* sp. The presence of these species in the larval stage may be associated with an intermediate position in the trophic web of the host. The predominant species of larvae occurring in *B. orbignyanus* were species with extremely low specificity, such as nematodes of the genera *Contracaecum*, *Goezia* and *Hysterothylacium*.

The number of species in an infracommunity reflects the number of species present in the locality, i.e. the richness of the community, as well as opportunities for infection and transmission within the locality

and thus the probability of being an infected host. When comparing endoparasite diversity among individuals of *B. orbignyanus*, a positive relationship was found between the body size of the host and parasite species richness, corroborating the hypothesis that larger hosts support a more diverse parasite community. Often the parasite composition may be an indicator of habitat, food type, and even migratory routes of the host.

The mean Berger–Parker dominance index was 3.85 ± 0.26 . *Contracaecum* sp. (larvae) was the predominant species, with 27 specimens (25.96% of total parasites), followed by the digenean *D. oxycephala*, nematodes of the species *P. (S.) inopinatus* and acanthocephalan *Octospiniferoides* sp. (larvae), resulting in high diversity (Figure 4).

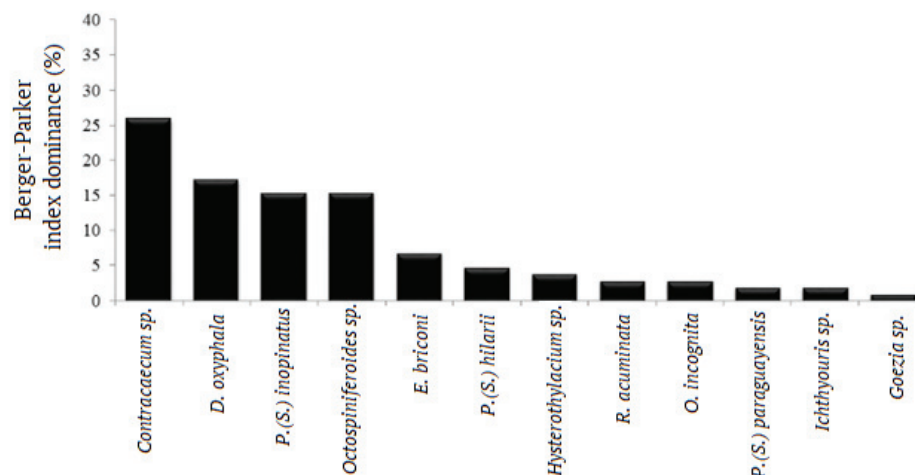


Figure 4. Berger–Parker dominance index for endoparasite species of *Brycon orbignyanus* from the floodplain of the upper Paraná River, collected between March 2010 and September 2011.

Data of monthly variation of water level in the Upper Paraná River floodplain with respect to the years 2010 and 2011 indicate very irregular annual cycles. The study highlights the remarkable absence of floods in the months from June to September 2010 and June 2011, with the lowest level registered in September 2011. The highest levels of the hydrometric occurred in March 2010, reaching the maximum value (approximately 5.5 m) in March 2011

Higher temperatures and hydrological levels were observed between December 2010 and March 2011, and also a greater abundance of endoparasites (Figure 5).

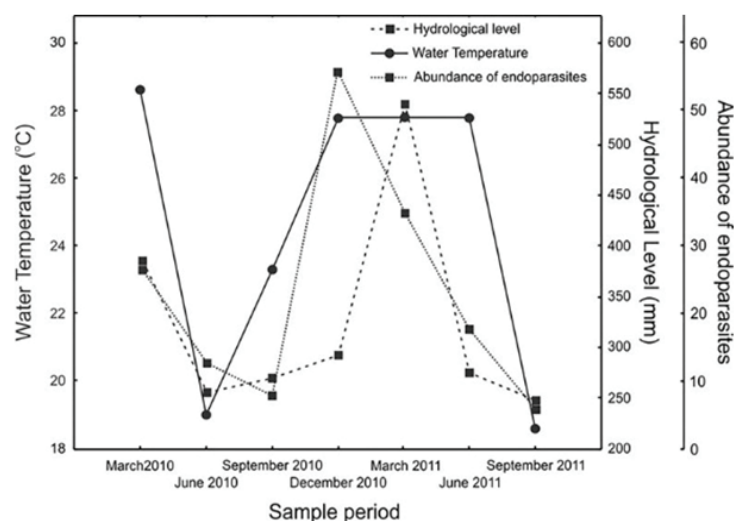


Figure 5. Temperature, water level and abundance of endoparasites during the sampling period (March 2010–September 2011).

The mean diversity was 1.60 ± 0.73 for the year 2010 and 1.77 ± 0.86 for 2011, obtaining thus a higher endoparasite richness despite the lower abundance during this period. The dynamics of fish assemblages

from of the upper Paraná River was quarterly analyzed and indicated that parasite assemblage structure was predictably linked to environmental characteristics that varied along temporal and spatial scales.

In the present study, we observed a higher endoparasite abundance during episodes of high water, indicating an increased availability of intermediate hosts in those periods of the hydrological cycle. In neotropical floodplains, temperature variations are not as pronounced as in regions that present more definite alterations, demonstrating seasonal influence on the occurrence of endoparasites. Even though endoparasite species, such as *Rhabdochona acuminata*, *P. (S.) inopinatus* and *Echinorhynchus briconi* are being reported for the first time in *B. orbignyanus*, endoparasites have been found in other species of *Brycon* such as *B. falcatus*, *B. melanopterus*, *B. cephalus*, *B. amazonicus* and *B. hilarii* (Moravec, 1998).

Therefore, between the phases of the hydrological cycle, increases in species richness and diversity of endoparasites were observed (mainly nematodes). In the present study, the species richness of parasites showed no association with various host characteristics, except for the size of the fish.

Conclusion

Variations in species richness of parasites between hosts not only provide a good model for studies of diversification of the community but are also of great interest in the context of disease risk prediction and conservation targets. The relationships between parasite species richness and host attributes were not established, probably because of the interconnections between the different microhabitats during the period of high water levels that minimize the heterogeneity of the ecosystem and its biota. All parasite species found in *B. orbignyanus* were recorded for the first time in this host, especially in the Upper Paraná River floodplain.

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