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BIOLOGICAL SCIENCES

Biodiversity of parasites found in the trahira, *Hoplias malabaricus* (Bloch, 1794), collected in the Batalha River, Tietê-Batalha drainage basin, SP, Brazil

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Abstract: Eighty-one *Hoplias malabaricus* specimens were collected between February 2014 and June 2016. A total of 29 species of metazoan parasites were found, 13 of which were identified as monogeneans, seven were digenean species, seven of which were nematodes, and two of which were from the subclass Hirudinea. The highest prevalence values were presented by *Contracaecum* sp. and *Tylodelphys* sp. The highest mean abundance and mean intensity was recorded by *Tylodelphys* sp.; the values were 36.7 ± 61.8 and 55.65 ± 69.1, respectively. The abundance of the monogenean *Urocleidoides cuiabai* was found to be positively correlated with host weight. The abundance of Bucephalidae gen. sp. exhibited significant positive correlations with host weight and length. For *Contracaecum* sp., a significant negative correlation was found between its abundance and host length and weight. No significant differences between the diversity indexes (Margalef, Pielou and Shannon) of the parasites collected in the two points were found. The Sorensen similarity index, with a value of 0.82 between the two sampling points revealed that the parasitic diversity between them is similar. The findings from this study represent new records of occurrence of *H. malabaricus*, as well as of *Urocleidoides margolisi*, *Scleroductus* sp. and *Helobdella* sp.

Key words: Characidae, fish parasitology, freshwater parasites, Tietê-Batalha reservoir.

INTRODUCTION

Studies involving vertebrates in the Neotropical region have been carried out during the last four centuries (Rull 2011). However, considering the lack of studies on invertebrates, especially the parasite communities, several authors supported research on the systematics and diversity of this group (Poulin & Morand 2004).

Parasites are important for many of their hosts' biological processes, including diet, migration, recruitment, and phylogeny. Parasites may also be direct indicators of environmental quality (Galli et al. 2001). The composition of the parasitic fauna of fish in rivers depends on various factors inherent to each species' habitat, such as the characteristics of the water, as well as on factors inherent to the host, such as each fish's biological and physiological characteristics (Dogiel 1961).

The Tietê River and some of its tributaries, such as the Batalha River, from the Tietê-Batalha drainage basin, located in the state of São Paulo, Brazil. This basin possesses a drainage area of 3,149 km^2, and the Batalha River is 167 km in length, making it one of the most important tributaries of the Tietê River (Silva et al. 2009). In 2001, the drainage basin of the

Batalha River was turned into a state Área de Proteção Ambiental (APA). Despite the constant contamination of external agents that affect the river, data from Companhia Ambiental do Estado de São Paulo (CETESB) suggest that the river exhibits good water quality (Santos & Heubel 2008). The area surrounding the Batalha River and the drainage area suffered from poor use and land occupation by human activities (monocropping, reforestation, and livestock farming, as well as the deposition of sewage, trash, and industrial waste). These activities decreased the amount of native and riparian vegetation, triggering changes in water quality and continuous erosion of the land surrounding the river. The exposure of water sources to this erosion leads to silting on the riverbed, as well as to water pollution (Silva et al. 2009).

This study was performed using the fish species *Hoplias malabaricus* (Bloch 1794), the trahira. This species occurs in all drainage basins in South America, with the exception of those in the Trans-andine area and of the rivers of Patagonia (Fowler 1950). This species is well adapted to lentic environments, though it may also be found in other types of small and large rivers. It eats plankton in its larval phase, and it is typically piscivorous as an adult, with a limited appetite and large resistance to extended periods without food (Paiva 1974). It has also able to survive in low-oxygen environments, an adaptation which contributes to its wide range (Barbieri 1989). In several waters, *H. malabaricus* is the only fish that eats a larger prey. It is a lone hunter, and its prey includes species from the families Characidae, Curimatidae, and Cichlidae. It is an ambush predator that lives in benthic habitats and it may be found in rivers and lakes, particularly in shallow waters close to submersed or riparian vegetation. It captures its prey from surface water or midwater during the day (Oliveira 1994). Its reproductive behaviors include the formation of nests in waters with submersed vegetation and parental care provided by males (Prado et al. 2006).

Some parasitology studies considered *H. malabaricus* specimens collected from different sites around Brazil (Table I), in which a larger number of species belonging to the Monogenea and Digenea classes and to the phylum Nematoda are observed parasitizing this host. As exclusion criterion for the literature review, only species belonging to the groups of parasites identified in the present study were selected (Monogena, Digenea, Nematoda and Hirudinea).

There have been recent studies on fish parasites from the Batalha River; however, only one has compared lentic and lotic environments (Leite 2017). The current study therefore involves a survey of the parasitic fauna of this host, which is of substantial commercial importance. The aims of this study were: 1) to analyze the host's biotic factors and its main habitats with parasitic indexes; 2) to identify possible parasite species that may infect humans through the ingestion of raw or undercooked fish; 3) to determine similarities between the parasite species found at the two sampling points; 4) to add knowledge on parasite biodiversity in the state of São Paulo.

Table I. **Records of parasites identified in *Hoplias malabaricus* (Bloch, 1794) specimens from different locations in Brazil.**

Parasite	Location	Reference
Monogenea		
Anacanthorus sp.	Paraná River, Brazil	Graça et al. (2013)
	Eastern Amazon basin, Brazil	Ferreira et al. (2017)
	Paraná River, Brazil	Graça et al. (2018)
Cosmetocleithrum bulbocirrus Kristsky, Thatcher and Boeger, 1986	Paraná River, Brazil	Graça et al. (2013)
Dactilogyridae gen. sp.	Mogi-Guassu River, Brazil	Kohn et al. (1985)
	Brazil	Rosim et al. (2011)
	Paraná River, Brazil	Graça et al. (2013)
	Eastern Amazon basin, Brazil	Ferreira et al. (2017)
Dactylogyrus spp.	Paraíba State, Brazil	Bernadino et al. (2016)
Gyrodactilus trairae Boeger and Popazoglo 1995	Eastern Amazon basin, Brazil	Ferreira et al. (2017)
Monogenea gen. sp.	Paraná River, Brazil	Takemoto et al. (2009)
	Paraná, Brazil	Kohn et al. (2011)
	Amazônia Ocidental, Brazil	Malacarne & Godoi (2012)
	Maranhão State, Brazil	Rodrigues et al. (2017)
Vancleaveus janauacaensis Kristsky, Thatcher and Boeger, 1986	Paraná River, Brazil	Graça et al. (2013)
Urocleidoides brasiliensis Rosim, Mendoza-Franco and Luque, 2011	Brazil	Rosim et al. (2011)
	Paraná River, Brazil	Graça et al. (2013)
	Eastern Amazon basin, Brazil	Ferreira et al. (2017)
Urocleidoides cuiabai Rosim, Mendoza-Franco and Luque, 2011	Brazil	Rosim et al. (2011)
	Paraná River, Brazil	Graça et al. (2013)
	Paraná River, Brazil	Gasques et al. (2015)
	Eastern Amazon basin, Brazil	Ferreira et al. (2017)
Urocleidoides eremitus Kristsky, Thatcher and Boeger, 1986	Perú, Brazil	Iannacone & Luque (1993)
	Brazil	Rosim et al. (2011)
	Paraná River, Brazil	Graça et al. (2013)
	Amapá, Brazil	Gonçalvez et al. (2016)
	Eastern Amazon basin, Brazil	Ferreira et al. (2017)
	Amapá, Brazil	Alcântara & Tavares-Dias (2015)
Urocleidoides malabaricusi Rosim, Mendoza-Franco and Luque 2011	Brazil	Rosim et al. (2011)
	Paraná River, Brazil	Graça et al. (2013)
	Paraná River, Brazil	Gasques et al. (2015)
Urocleidoides naris Rosim, Mendoza-Franco and Luque, 2011	Brazil	Rosim et al. (2011)
	Eastern Amazon basin, Brazil	Ferreira et al. (2017)

Table I. Continuation

Parasite	Location	Reference
Digenea		
Austrodiplostomum compactum (Lutz, 1928)	Paraná River, Brazil	Machado et al. (2005)
	Tietê River, Brazil	Paes et al. (2010)
	Paraná River, Brazil	Santos et al. (2012)
	Doce River, Brazil	Belei et al. (2013)
	Paranapanema River, Brazil	Ramos et al. (2013)
Austrodiplostomum sp.	São Francisco River, Brazil	Corrêa et al. (2014)
	São Francisco River, Brazil	Costa et al. (2015)
Clinostomum complanatum Rudolphi, 1814	Paraná River, Brazil	Dias et al. (2003)
Clinostomum marginatum (Rudolphi, 1819)	Amapá, Brazil	Gonçalvez et al. (2016)
	Amapá, Brazil	Alcântara & Tavares-Dias (2015)
Clinostomatopsis sorbens (Braun, 1899) Dollfus1932	Marajó Island, Brazil	Benigno et al. (2014)
Dendrorchis neivai Travassos, 1926	Amapá, Brazil	Gonçalvez et al. (2016)
Dendrochis sp.	Santa Maria, Brazil	Weiblen & Brandão (1992)
Digenea gen. sp.	Paraná River, brazil	Takemoto et al. (2009)
Diplostomidae gen. sp.	Paraná, Brazil	Kohn et al. (2011)
Genarchella genarchella Kohn & Fernandes, 1988	Amapá, Brazil	Gonçalvez et al. (2016)
Genarchella sp.	Quebrada Juan Grande, Brazil	Choudhury et al. (2016)
Ithyoclinostomum dimorphum (Diesing,1850) Witenberg, 1926	Chaco Province, Argentina	Szidat (1969)
	Santa Maria, Brazil	Weiblen & Brandão (1992)
	Rio Grande do Sul State, Brazil	Gallio et al (2007)
	Lajes Reserve, Brazil	Paraguassú & Luque (2007)
	Doce River, Brazil	Belei et al (2013)
	Marajó Island, Brazil	Benigno et al. (2014)
Ithyoclinostomum sp.	São Francisco River, Brazil	Costa et al. (2015)
Phyllodistomum spatula Odhner, 1902	São Francisco River, Brazil	Costa et al. (2015)
Posthodiplostomum sp.	Amapá, Brazil	Gonçalvez et al. (2016)
Pseudosellacotyla lutzi (Freitas, 1941)	Mogi Guaçu River, Brazil	Kohn et al. (1985)
	Mogi Guaçu River, Brazil	Kohn & Fernandes (1987)
	Parana River, Brazil	Fernandes & Kohn (2001)
	Chocó, Colômbia	Perez-Caicedo et al. (2010)
	Paraná, Brazil	Kohn et al. (2011)
	São Francisco River, Brazil	Costa et al. (2015)
	Argentina	Núñez et al. (2017)

Table I. Continuation

Parasite	Location	Reference
Sphincterodiplostomum musculosum (Dubois, 1936)	Chaco Province, Argentina	Szidat (1969)
Thometrema overstreeti (Brooks et al., 1979)	Perú, Brazil	Iannacone & Luque (1993)
Tylodelphylus s Province1 (Szidat, 1969)	Chaco Province, Argentina	Szidat (1969)
	Santa Maria, Brazil	Weiblen & Brandão (1992)
Nematoda		
Amplicaecum sp.	Mogi Guaçu River, Brazil	Kohn & Fernandes (1987)
Brevimulticaecum sp.	Pantanal, Brazil	Vieira et al. (2010)
Contracaecum sp.	Uruguai	Lent & Teixeira de Freitas (1948)
	Iguaçú River, Brazil	Kohn et al. (1988)
	Santa Maria, Brazil	Weiblen & Brandão (1992)
	Barinas State, Venezuela	Moravec et al. (1997)
	Jaguari River, Brazil	Madi & Silva (2005)
	Maranhão State, Brazil	Martins et al. (2005)
	Lajes Reserve, Brazil	Paraguassú & Luque (2007)
	Córdoba	Pardo et al (2008)
	Cuiabá River, Brazil	Barros et al. (2008)
	Cuiabá River, Brazil	Barros et al. (2009)
	Paraná, Brazil	Kohn et al. (2011)
	Marajó Island, Brazil	Benigno et al. (2012)
	Pirassununga, Brazil	Corrêa et al. (2013)
	Dique Channel, Colombia	Olivero-Verbel & Caballero - Gallardo (2013)
	Argentina	Mancini et al. (2014)
	São Francisco River, Brazil	Corrêa et al. (2015)
	Trinidad	Suepaul et al. (2015)
	Amapá, Brazil	Gonçalvez et al. (2016)
	Dr. João Penido Dam, Brazil	Carvalho et al. (2017)
	Amapá, Brazil	Baia et al. (2018)
	Amapá, Brazil	Alcântara & Tavares-Dias (2015)
Contracaecum multipapillatum (von Drasche, 1882)	Cuiabá River, Brazil	Fontenelle et al. (2017)
Cystidicoloides sp.	Amapá, Brazil	Gonçalvez et al. (2016)
Eustrongylides ignotus Jäegerskiold, 1909	Dr. João Penido Dam, Brazil	Carvalho et al. (2017)
Eustrongylides sp.	Paraná River, Brazil	Martins et al. (2009)
	Rondonia, Brazil	Meneguetti et al. (2013)
	Trinidad	Suepaul et al. (2015)

Table I. Continuation

Parasite	Location	Reference
Guyanema baudi Petter & Dlouhy (1985)	Santa Maria, Brazil	Weiblen & Brandão (1992)
Guyanema raphiodoni Moravec, Kohn and Fernandes, 1993	Paraná, Brazil	Kohn et al. (2011)
Nematoda	Mato Grosso State, Brazil	Barros et al. (2007)
	Paraná River, Brazil	Takemoto et al. (2009)
Paracappilaria piscicola Travassos, Artigas & Pereria, 1928	Santa Maria, Brazil	Weiblen & Brandão (1992)
Parasseuratum soaresi	Santa Maria, Brazil	Weiblen & Brandão (1992)
Physalopteridae gen. sp.	Uruguai	Lent & Freitas (1948)
Procamallanus (Spirocamallanus) inopinatus Travassos, Artigas and Pereira, 1928	Mogi Guaçu River, Brazil	Kohn & Fernandes (1987)
	Amazonas River, Brazil	Baia et al. (2018)
	Amapá, Brazil	Gonçalvez et al. (2016)
	Amapa	Alcântara & Tavares-Dias (2015)
Procamallanus (Spirocamallanus) ihering Travassos, Artigas and Pereira, 1928	Mogi Guaçu River, Brazil	Kohn & Fernandes (1987)
Procamallanus (Spirocamallanus) hilarii Vaz and Pereira, 1934	Santa Maria, Brazil	Weiblen & Brandão (1992)
	Santiago del Estero, Argentina	Ramallo (1997)
	Dr. João Penido Dam, Brazil	Carvalho et al. (2017)
Procamallanus (Procamallanus) peraccuratus Pinto et al. 1976	Santa Maria, Brazil	Weiblen & Brandão (1992)
Hirudinea		
Glossiphonidae gen. sp.	Amapá, Brazil	Gonçalvez et al. (2016)
	Lajes Reserve, Brazil	Paraguassú & Luque (2007)
Mizobdella platensis (Cordero, 1933)	Santa Fé Province, Argentina	Cordero (1933)
		Lopretto (1995)

MATERIALS AND METHODS

Fish and study area

A total of 81 *H. malabaricus* specimens were collected between February 2014 and June 2016. Fish samples were collected from two sampling points along the Batalha River: the main channel, located in the city of Reginópolis, São Paulo; and the reservoir managed by the Departamento de Água e Esgoto (DAE) of the city of Bauru and located in the town of Piratininga, São Paulo. Fish were captured using simple gillnets of different mesh sizes.

During dissection, data on standard length (cm), weight (g), and sex were recorded. These collections followed the guidelines for scientific fish licensing and were authorized by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) through the Sistema de Autorização e Informação da Biodiversidade (SISBIO) under case number 40998-3.

The section of the Batalha River located in the city of Reginópolis, São Paulo State, Brazil (21º53’17”S, 49º13’31”W) presents largely lotic characteristics. Though a large part of the area is covered by native riparian forest, the area surrounding this stretch of the river is used for agriculture and livestock activities, such as cattle farming, sugarcane crops, cornfields, and eucalyptus groves. These activities result in a large quantity of organic matter and leached pollutants in the water. Another important factor is the lack of sewage treatment in the city. Local raw sewage is released into a tributary of the river (Santos & Heubel 2008, Sistema Nacional de Informações Sobre Saneamento 2016).

The reservoir managed by the DAE of Bauru is located in the town of Piratininga, São Paulo (22º24'46"S, 49º05'05"W) and it has the function to capture water for community supply. The area of the site is approximately 170,000 m^2, and water volume is approximately 1,256,40 m^3 per month (Departamento de Água e Esgoto de Bauru 2014). The site is characterized as a lentic and intensely anthropized environment (Leite 2017). Activities involving agriculture, livestock farming, and industrial plants occur close to the basin and produced environmental impacts on the basin, including silting, decreases in the already limited native vegetation, and a significant increase in pollutant levels (Leite et al. 2017). Despite these effects, the CETESB considers the water from the basin to be Class 2 water, which means that it is appropriate for human consumption, domestic water supplies, recreation, and protecting aquatic biodiversity (Brasil 2005, São Paulo 2010).

Parasite analysis procedures

The collection, fixation, conservation, and assembly procedures of parasites were performed based on the methodology provided by Eiras et al. (2006).

The accumulation curve obtained from each river sampling point represents species richness at each site. These curves were calculated using the Bootstrap estimator, which, according to Poulin (1998), is the best estimator for samples with fewer than 50 hosts. These results allow a comparison between the parasitic communities from each sampling point.

The prevalence, intensity, and abundance of each component of the parasite communities were calculated in accordance with Bush et al. (1997). The relationship between variance and mean parasite intensity (the dispersion index, DI) was calculated for each parasite species in order to determine its aggregation level and the type of distribution of each parasitic infrapopulation. The *d* statistical test was also performed to determine significance. The Green Index (GI) was calculated to verify the aggregation level of each parasite species present in the infracommunity (Ludwig & Reynolds 1988).

The dominance of each component of the parasitic infrapopulations was determined by the dominance frequency and relative dominance (the ratio of the specimens from a given species by the total number of specimens from all of the species in a given infracommunity) using the methodology provided by Rhode et al. (1995).

The Spearman rank correlation coefficient (*rs*) was used to detect possible correlations between parasite abundance and total host length and weight. The Mann-Whitney *U* test was applied to determine the effect of the sex of the fish on parasite abundance. Each species of parasite was tested separately (Zar 1999). Student's *t* test was used to determine differences in mean total length between male and female hosts.

The parasitic diversity of each infracommunity was determined using the Shannon Diversity Index, or H, and the Pielou's index was used to obtain the equitability values (J'). The richness was obtained according to the Margalef index (D) (Zar 1999). One-way ANOVA was performed to verify if there were significant differences between the values of the diversity, equitability and richness indexes obtained between the hosts of each river point (Magurran 2013).

The ratio between the sum of heteroxenous and monoxenous parasites species present in the host (H/M) was also calculated. The results were separated by sampling point in order to determine the ecological stability of each environment (Diamant et al. 1999). The similarities between these communities were calculated using the Sorensen Similarity index (Wolda 1981).

All statistical tests were applied only to parasites species with prevalence greater than 10%. The significance level adopted was $p < 0.05$. The fish anesthesia and euthanasia methodologies were made following the guidelines of the Conselho Nacional de Controle de Experimentação Animal (CONCEA), and the research project was submitted to the Comitê de Ética no Uso de Animais (CEUA) da Universidade do Sagrado Coração (USC) (authorization no 3295230615) before it could be performed.

RESULTS

A total of 81 fish were collected, 37 in the reservoir and 44 in the river channel. Only two specimens, in a total of 81 fishes, did not show the presence of parasites. A total of 3,107 parasite specimens were collected, 901 of which were collected in the reservoir (Piratininga) and 2,206 of which were collected from the river channel (Reginópolis). Mean total abundance values for the two sampling points were 24.33 ± 2.4 and 49.92 ± 6.8, respectively (Table II). Not all parasites species were found at both sampling points.

A total of 29 species of metazoan parasites were found. Of these, thirteen were monogeneans, six were digenean species in the metacercaria stage, one was a digenean species in the adult stage, seven were nematodes, and two were from the subclass Hirudinea (Table II).

The accumulation curves constructed using mean observed richness (S_{obs}) values and the values from the Bootstrap richness estimator did not reach an asymptote, but they exhibited a tendency to stabilize at approximately 25 species at both sampling points (Figures 1a, b).

In the reservoir managed by the DAE, the *Contracaecum* sp. larvae exhibited the highest prevalence and frequency of dominant values. In the river channel, the *Tylodelphys* sp. metacercarie exhibited the highest values (Table III). Regarding the Dispersion Index (DI) and the statistical test *d* in the DAE reservoir, most of the parasites presented an aggregate distribution pattern, confirmed by the results of the Green Index (GI). Exceptions were *Ithyoclinostomum dimorphum*, which presented a uniform distribution pattern, and *Austrodiplostomum compactum*, which presented a random distribution pattern. Meanwhile, in the river channel, all of the parasites presented an aggregate distribution pattern (Table IV).

In the case of *Urocleidoides cuiabai*, a significant positive correlation was found between its abundance and host length (rs = 0.37 and p = 0.02) and host weight (rs = 0.43 and p = 0.01). For *Contracaecum* sp., a significant negative correlation was found between its abundance and host length (rs = - 0.55 and p = < 0.0001) and host weight (rs = - 0.73 and p = <0.0001). Both correlations were determined based on samples from the DAE reservoir. In the river channel, only Bucephalidae gen. sp exhibited a significant positive correlation between its abundance and host weight (Table V). The host sex did not influence the parasite abundance at either sampling point (Table VI).

Although the river channel has presented higher average richness and average diversity

Table II. Prevalence (P), mean abundance (MA) and mean intensity (MI) of parasites of *Hoplias malabaricus* (Bloch, 1794) captured in the Batalha River, São Paulo State, Brazil, between February 2014 and June 2016.

Species	Point 1			Point 2		
	P (%)	MA ± SE	MI ± SE	P (%)	MA ± SE	MI ± SE
Monogenea						
Urocleidoides sp.	2.3	0.02 ± 0.02	1	-	-	-
Urocleidoides margolisi Molnar, Hanek and Fernando, 1974	4.5	0.1 ± 0.1	2.5 ± 2.1	-	-	-
Urocleidoides aimarai Moreira, Scholz and Luque, 2015	2.3	0.02 ± 0.02	1	2.7	0.03 ± 0.03	1
Urocleidoides cuiabai	38.6	3.0 ±1.05	7.7 ± 2.3	37.8	2.0 ± 0.5	5.3 ± 0.8
Urocleidoides malabaricusi	22.7	2.0 ± 0.7	8.7 ± 2.5	10.8	0.4 ± 0.2	3.5 ± 0.6
Urocleidoides eremitus	15.9	1.6 ± 0.7	9.8 ± 3.2	-	-	-
Jainus leporini Abdallah, Azevedo and Luque, 2012	2.3	0.02 ± 0.02	1	8.1	0.1 ± 0.3	1.7 ± 0.3
Sciadicleithrum sp.	2.3	0.02 ± 0.02	1	5.4	0.1 ± 0.1	1.5 ± 0.5
Scleroductus sp.	6.8	0.2 ± 0.14	3 ± 1.5	2.7	0.1 ± 0.1	3
Anacanthorus sp.	6.8	0.3 ± 0.2	4.3 ± 0.7	8.1	0.6 ± 0.5	7.3 ± 5.4
Dactylogyridae gen. sp.	6.8	0.8 ± 0.5	11.3 ± 5.5	-	-	-
Gyrodactilus trairae	2.3	0.1 ± 0.2	1	2.7	0.4 ± 0.4	15
Nothogyrodactylus sp.	2.3	0.04 ± 0.04	1	-	-	-
Digenea						
Ithyoclinostomum dimorphum	2.2	0.02 ± 0.03	1	27	0.3 ± 0.1	1.1 ± 0.1
Austrodiplostomum compactum	31.8	1 ± 0.40	3.1 ± 1	21.6	0.3 ± 0.1	1.4 ± 0.2
Austrodiplostomum mordax (Szidat and Nani, 1951)	-	-	-	5.4	0.1 ± 0.1	2.5 ± 0.5
Phyllodistomum rhandiae (Amato and Amato, 1993)	29.5	1.4 ± 0.5	4.8 ± 1.3	2.7	0.2 ± 0.2	6
Tylodelphys sp.	65.9	36.7 ± 9.3	55.6 ± 12.8	37.8	10.8 ± 5.7	28.6 ± 14.1
Clinostomum sp.	4.5	0.04 ± 0.03	1	-	-	-
Bucephalidae gen. sp.	27.2	1.2 ± 0.5	4.6 ± 1.3	8.1	0.2 ± 0.1	2.6 ± 1.2
Nematoda						
Goezia brasiliensis Moravec, Kohn and Fernandes, 1994	-	-	-	10.8	1.0 ± 0.6	9.0 ± 3.8

Table II. Continuation

Species	Point 1			Point 2		
	P (%)	MA ± SE	MI ± SE	P (%)	MA ± SE	MI ± SE
Contracaecum sp.	38.6	1.06 ± 0.4	2.6 ± 0.7	70.2	7.4 ± 1.5	10.6 ± 1.8
Spirox contortus (Rudolphi, 1819)	-	-	-	2.7	0.03 ± 0.03	1
Procamallanus (Spirocamallanus) inopinnatus Travassos, Artigas and Pereira, 1928	13.6	0.1 ± 0.07	1.3 ± 0.3	5.4	0.1 ± 0.1	1.5 ± 0.5
Guyanema baudi Petter and Dloughy, 1985	-	-	-	2.7	0.03 ± 0.03	1
Spinitectus rodolphihering Vaz and Pereira, 1934	4.5	0.1 ± 0.1	2.5 ± 1.5	2.7	0.05 ± 0.05	2
Eustrongylides sp.	-	-	-	2.7	0.03 ± 0.03	1
Hirudinea						
Placobdella sp.	2.2	0.06 ± 0.07	3	5.4	0.08 ± 0.5	1.5 ± 0.5
Helobdella sp.	4.5	0.09 ± 0.07	2 ± 1.5	-	-	-

Point 1 = Batalha River channel.
Point 2 = Detention basin managed by the Department of Water and Sewage (DAE) of Bauru.
SE = Standard error.

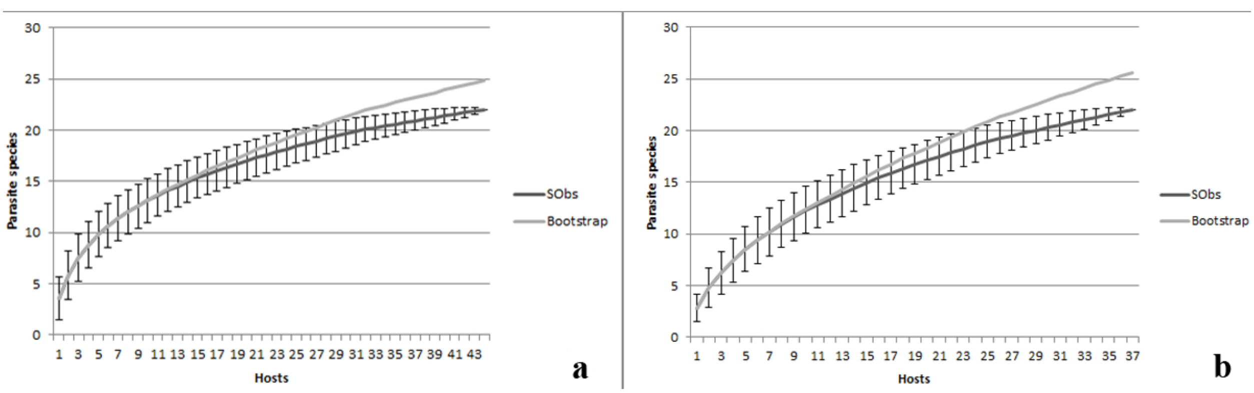

Figure 1. Accumulation curve of species richness observed (S_{Obs}) and Bootstrap richness estimates (Bootstrap) of the metazoan parasites in *Hoplias malabaricus* (Bloch, 1794) collected in the Batalha River in São Paulo State, Brazil. a) The river channel (Sampling Point 1); b) DAE reservoir (Sampling Point 2).

than the DAE reservoir, and the average uniformity has been higher at the river channel point, these values did not present significant differences according to the one-way ANOVA, i.e. the parasitic diversity in the two points is similar (Table VII). Another value that confirms this fact is the Sorensen Similarity Index, with a value of 0.82 between the two sampling points.

The result of the ratio between the sum of heteroxenous and monoxenous parasites found for river channel was 0.93 while in the DAE reservoir was 0.70. Although the values were different from each other, there were no significant differences between these values when verifying them through the ANOVA one-way test (p = 0.05).

DISCUSSION

At both sampling points, the parasitic community in *H. malabaricus* was characterized by high species richness but by relatively low diversity and uniformity. As mentioned previously, this fish species is typically piscivorous as an adult. Its appetite is limited, and it exhibits large resistance to extended periods without food (Paiva 1974), factors which may explain the high parasite richness but low uniformity.

Most of the parasites species showed an aggregate distribution pattern. The exceptions

Table III. Dominance indexes for each component of *Hoplias malabaricus* (Bloch, 1794) parasitic component community collected at two points on the Batalha River in São Paulo State, Brazil, between February 2014 and June 2016.

Species	Point 1		Point 2	
	DF (%)	RD	DF (%)	RD
Monogenea				
Urocleidoides sp.	0	0	0	0
Urocleidoides margolisi	0	0	0	0
Urocleidoides aimarai	0	0	0	0.001
Urocleidoides cuiabai	6.8	0.06	2.7	0.08
Urocleidoides malabaricusi	6.8	0.04	2.7	0.01
Urocleidoides eremitus	2.3	0	0	0
Jainus leporini	0	0.002	0	0.005
Sciadicleithrum sp.	0	0.03	0	0.003
Scleroductus sp.	0	0.004	0	0.003
Anacanthorus sp.	0	0.005	0	0.02
Dactylogyridae gen. sp.	0	0.01	0	0
Gyrodactilus trairae	0	0.003	0	0.02
Nothogyrodactylus sp.	0	0	0	0
Digenea				
Ithyoclinostomum dimorphum	0	0	0	0.01
Austrodiplostomum compactum	4.5	0.02	0	0.01
Austrodiplostomum mordax	0	0.03	0	0.005
Phyllodistomum rhandiae	4.5	0	0	0.006
Tylodelphys sp.	47.7	0.7	19	0.4
Clinostomum sp.	0	0	0	0
Bucephalidae gen. sp.	0	0.02	0	0.008
Nematoda				
Goezia brasiliensis	0	0	0	0.04
Contracaecum sp.	9.1	0.02	54	0.30
Spirox contortus	0	0	0	0.001
Procamallanus (Spirocamallanus) inopinnatus	0	0.003	0	0.003

Table III. Continuation

Species	Point 1		Point 2	
	DF (%)	RD	DF (%)	RD
Guyanema baudi	0	0	0	0.001
Spinitectus rodolphihering	0	0.002	0	0.002
Eustrongylides sp.	0	0	0	0.001
Hirudinea				
Placobdella sp.	0	0.001	0	0.003
Helobdella sp.	0	0.001	0	0

DF =Dominance frequency.
RD = Relative dominance.
Point 1 = Batalha River channel.
Point 2 = Detention basin managed by the Department of Water and Sewage (DAE) of Bauru.

Table IV. Dispersion index (DI), Green's index (GI) and statistical test d of each parasite species found in *Hoplias malabaricus* (Bloch, 1794) specimens collected at two points of the Batalha River in São Paulo State, Brazil, between February 2014 and June 2016.

Species	Point 1			Point 2		
	DI	*d*	GI	DI	*d*	GI
Urocleidoides cuiabai	16.31	37.32	0.35	5.11	10.78	0.11
Urocleidoides malabaricusi	12.19	32.2	0.26	3.57	7.63	0.07
Urocleidoides eremitus	15.01	35.81	0.32	-	-	-
Austrodiplostomum compactum	6.13	22.77	0.11	1.28	1.20	0.007
Phyllodistomum rhandiae	7.74	25.63	0.15	-	-	-
Tylodelphys sp.	104.09	94.56	2.39	111.41	22.63	3.06
Bucephalidae gen. sp.	7.85	25.8	0.15	-	-	-
Ithyoclinostomum dimorphum	-	-	-	0.90	-0.36	0
Contracaecum sp.	5.46	21.47	0.10	10.77	19.44	0.27
Goezia brasiliensis	-	-	-	13.38	22.63	0.34
Procamallanus (Spirocamallanus) inopinnatus	1.60	11.36	0.01	-	-	-

Point 1 = Batalha River channel.
Point 2 = Detention basin managed by the Department of Water and Sewage (DAE) of Bauru.

were *Ithyoclinostomum dimorphum*, which presented an uniform distribution pattern, and *Austrodiplostomum compactum*, which presented a random distribution pattern. Both species were collected from the same sampling point, but this finding is a result of the low collected number of individuals of these species. According to Zuben (1997), a uniform distribution pattern may be a result of factors such as parasite and host mortality, which would result in a more uniform distribution of parasitic metazoan, thus reducing their aggregation level. Environmental factors are those that have the largest impact on aggregate distribution. These factors include changes in the physical parameters of the environment, and differences

Table V. Correlation between parasitic abundance and standard length (SL) and weight (W) of *Hoplias malabaricus* Bloch, 1794 specimens collected at two points on the Batalha River in São Paulo State, Brazil, between February 2014 and June 2016.

Species	Point 1				Point 2			
	Abundance x SL		Abundance x W		Abundance x SL		Abundance x W	
	rs	*(p)*	*rs*	*(p)*	*rs*	*(p)*	*rs*	*(p)*
Urocleidoides cuiabai	-0.15	0.90	-0.21	0.18	0.37	0.02*	0.43	0.01*
Urocleidoides malabaricusi	-0.15	0.34	-0.21	0.18	0.10	0.54	0.13	0.45
Urocleidoides eremitus	-0.14	0.37	-0.22	0.15	-	-	-	-
Austrodiplostomum compactum	-0.01	0.96	0.11	0.47	0.18	0.28	0.25	0.13
Phyllodistomum rhandiae	0.25	0.09	0.25	0.11	-	-	-	-
Tylodelphys sp.	0.02	0.90	0.10	0.52	0.25	0.13	0.29	0.08
Bucephalidae gen. sp.	0.27	0.08	0.30	0.04*	-	-	-	-
Ithyoclinostomum dimorphum	-	-	-	-	0.00	0.99	0.01	0.97
Contracaecum sp.	0.04	0.78	0.02	0.90	-0.55	< 0.0001*	-0.73	< 0.0001*
Goezia brasiliensis	-	-	-	-	-0.20	0.23	-0.18	0.30
Procamallanus (Spirocamallanus) inopinnatus	-0.05	0.77	0.03	0.85	-	-	-	-

Point 1 = Batalha River channel.
Point 2 = Detention basin managed by the Department of Water and Sewage (DAE) of Bauru.
***rs* = Spearman rank correlation coefficient.**
*** = Value with significance levels of $p < 0.05$.**
***p* = significance level.**

in host susceptibility to infection, which may be a consequence of immunological differences (Zuben 1997).

A total of 29 species of parasites were collected, the majority of which (15) were ectoparasites from the class Monogenea. These are hermaphrodites and present a direct life cycle that contributes to parasitic reinfestation (Domingues 2004). Another characteristic that contributes to this result is the fact that the parasites of this class have morphological adaptations that allow their fixation in the substrate (Yamada et al. 2007), facilitating its infestation in the hosts, since this fish species presents benthic habits, being found in rivers and lagoons (Oliveira 1994). Fish of the order Characiformes present greater monogeneans abundance (Boeger & Vianna 2006), and besides being a representative of the Characiformes, it also feeds on prey included in this same order (Oliveira 1994). These facts may explain this result, since as they are ingesting the prey, its body surface come into contact with the oral cavity, gills and palate of the host, facilitating the parasite infestation.

The most frequent monogeneans in the host were *Urocleidoides eremitus* and *Urocleidoides malabaricusi*. Most of the specimens found from these species parasitized the hosts gills. The genus *Urocleidoides* does not exhibit host

Table VI. Effect of the sex of the *Hoplias malabaricus* (Bloch, 1794) specimens collected between February 2014 and June 2016 at two points on the Batalha River in São Paulo State, Brazil, on the parasite abundance of each parasite species.

Species	Point 1		Point 2	
	Z (U)	*p*	Z (U)	*p*
Urocleidoides cuiabai	0.73	0.22	13.4	0.09
Urocleidoides malabaricusi	0.46	0.31	0.51	0.31
Urocleidoides eremitus	0.15	0.43	-	-
Austrodiplostomum compactum	0.49	0.31	0.39	0.35
Phyllodistomum rhandiae	0.1	0.45	-	-
Ithyoclinostomum dimorphum	-	-	0.15	0.43
Tylodelphys sp.	0.12	0.44	0.39	0.35
Bucephalidae gen. sp.	0.55	0.29	-	-
Goezia brasiliensis	-	-	10.4	0.15
Contracaecum sp.	0.43	0.33	0.19	0.42
Procamallanus (Spirocamallanus) inopinnatus	10.44	0.14	-	-

Point 1 = Batalha River channel.
Point 2 = Detention basin managed by the Department of Water and Sewage (DAE) of Bauru.
***p* = Significance level.**
Z (U) = Mann-Whitney U test.

Table VII. Diversity indexes of the metazoan parasite communities in *Hoplias malabaricus* (Bloch, 1794) specimens collected between February 2014 and June 2016 at two points on the Batalha River in São Paulo State, Brazil (D = species richness according to the Margalef index; H = Brillouin diversity; J' = Pielou's evenness index; H/M = ratio between the sum of heteroxenous and of monoxenous parasites). All means followed by ± standard deviation.

	Point 1	Point 2
D	0.75 ± 0.59	0.68 ± 0.48
H	0.54 ± 0.45	0.50 ± 0.34
J'	0.51 ± 0.35	0.59 ± 0.33
H/M	0.93	0.70

Point 1 = Batalha River channel.
Point 2 = Detention basin managed by the Department of Water and Sewage (DAE) of Bauru.

specificity and may be found in fish of different orders, including Siluriformes, Characiformes, Gymnotiformes, and Cyprinodontiformes (Eiras et al. 2010). However, Graça et al. (2013) and Cohen et al. (2013) report that *U. cuiabai*, *U. malabaricusi*, *U. brasiliensis*, and *U. eremitus* have thus far been detected only in *H. malabaricus*, a finding which suggests a specificity for this species.

Contracaecum sp. was the most frequent species in the hosts collected from the river channel. It was found infecting four sites: the abdominal cavity, the stomach, the liver, and the intestine. Nematodes belonging to the family Anisakidae have been recognized for their zoonotic potential: when their larvae are ingested with raw or undercooked fish, they may cause a disease known as anisakiasis (Moreno-Ancillo et al. 1997). Shamsi & Butcher (2011) reported the occurrence of this disease in Australia, where a woman was infected by nematodes of the species *Contracaecum* sp. and presented symptoms such as vomiting, diarrhea and gastrointestinal pain. Another species of substantial zoonotic importance is *Eustrongylides* sp. Though there are no reports of its infection in humans in Brazil, it is a risk to human health since this species can also causes anisakiasis (Barros et al. 2007, 2009).

Tylodelphys sp. metacercariae presented a high value of frequency in the DAE reservoir. It was the second most prevalent species found in the hosts, and it exhibited three sites of infection: the eyes, the intestine, and the abdominal cavity of the fish. Larvae of this species have been found in *H. malabaricus*, as reported by Szidat (1969). Intense infection by diplostomid species in fish eyes can result in blindness and cataracts, impairing the host's vision and making it more vulnerable to being preyed by piscivorous birds (Owen et al. 1993, Pavanelli et al. 2008).

Significant positive correlations between parasite abundance and host weight and length were found in the cases of *U. cuiabai* and Bucephalidae gen. sp. According to Pavanelli et al. (2004), this relationship may be influenced by factors such as fish age, changes in their diet and in the food consumed by fish in each age group, and the life cycles of the parasites. The positive correlation between Bucephalidae gen. sp. abundance and host weight occurs due *H. malabaricus* can act as intermediary host for digeneans. This group of parasites present a heteroxenous life cycle: crustaceans are their first intermediate hosts, followed by fish, which are part of the host's diet, and their final hosts, which are birds and both aquatic and land mammals (Carnevia et al. 2005). Minhos et al. (2016) reported the same positive correlation between digenean parasite species and *H. malabaricus* specimens collected in riparian ponds established in the Miranda-Abobral subregion, located in the Pantanal region of Mato Grosso do Sul State, Brazil. The authors attribute this positive correlation to the fact that digeneans present a wide geographical distribution and have adaptation strategies involving asexual reproduction.

Urocleidoides cuiabai infestation was also found to be positively correlated with host length and weight; the weight host would be directly associated with host length in most cases, which means that the larger the host's body surface area and gills cavity are, the larger the site of monogenean infestation tends to be (Rohde et al. 1993). Alcântara & Tavares-Dias (2015) identified the same positive correlation between a Monogenea species and *H. malabaricus* from the Igarapé Fortaleza basin, located in the State of Amapá, in the Eastern Amazon region of Brazil.

Contracaecum sp. infection was found to share a significant negative correlation with host weight and length. This result may be explained by the diet changes that occur during fish development, causing to the fish in certain stages of development to stop feeding on some animals that act as first intermediate host in the *Contracaecum* sp. life cycle (Luque & Chaves 1999, Adams 1985). It can also be explained by increased immunity by the fish ages, since older individuals can eliminate the parasites through immune system mechanism (Iyaji et al. 2009). Duarte et al. (2016) reported this type of correlation with *Contracaecum* sp. infestation in a study on *Salminus hilarii* Valenciennes, 1850 collected from the São Francisco River in Brazil, as well as by Hoshino (2013) in a study on *Metynnis lippincottianus* Cope, 1870 collected from in the Igarapé Fortaleza basin in Amapá State, Brazil. Meanwhile, Carvalho et al. (2017) found a positive correlation between *H.*

malabaricus and *Contracaecum* sp. abundance in fish analyzed from the Dr. João Penido Dam, located in the city of Juiz de Fora, Minas Gerais State, Brazil. The authors also reported a high prevalence of *Contracaecum* sp. larvae in their study: larval prevalence was 77.5%, which is close to the 70.2% rate of abundance found in the DAE reservoir in the current study.

The sex of the hosts did not influence parasitism in the present study. According to Azevedo et al. (2007), this finding may mean that males and females' ecological relationships are similar. Though the male *H. malabaricus* cares for the offspring (Prado et al. 2006), this behavior did not result in differences in parasitism between the sexes. Madi & Silva (2005) found the same results in *H. malabaricus* specimens collected from the Jaguari Reservoir in Brazil. Benigno (2011) also reported no influence of sex on the parasitism of *H. malabaricus* collected from Lake Arari in Pará State, Brazil. In contrast to these findings, however, Corrêa (2014) performed a parasitological survey of *H. malabaricus* in the basins of the Grande River and Mogi Guaçu River in Brazil and found females to be more parasitized than males, contributing this result to the fact that females spend more energy in the reproductive period, feeding more and becoming more vulnerable to infection.

Communities may differ in their species diversity values due to differences in the distribution of relative abundance or to differences in the number of specimens collected (Denslow 1995). These differences may reflect biologically significant patterns resulting from resource availability or conditions for growth (Gotelli & Colwell 2001). For many taxa, the greater the number of individuals sampled is, the greater the number of species recorded will be (Bunge & Fitzpatrick 1993) because it will also contemplate the sampling of rare species. The influence of the sample effort will be related to other factors inherent to the host (such as age, weight, sex, eating habits, immunity, etc.), and all these factors will be responsible for the composition of the parasite community. However, a spatio-temporal pattern in studies with fish parasitology is scarcely observed (Yamada & Takemoto 2017), and most parasitic populations are dominated by stochastic events (Price 1980). Perhaps, this factor explains the lack of stabilization of the accumulation curves for the parasite species found in the current study.

Environmental conditions are of great importance for the survival and welfare of the host. However, they directly affect the parasites species that depend on it to complete its life cycle (Sures et al. 2017). Changes in their chemical composition and physiology, as well as their prevalence and intensity values, are consequences of changes in the habitat of these organisms, recognized as bioindicator species (Lafferty 1997, Vidal-Martinez et al. 2010). This type of interaction can be observed in relation to the value presented by the ratio between the sum of heteroxenous parasites and monoxenous parasites (Diamant et al. 1999). Significant differences were expected between the results presented by the ratio (H/M) of each point. However, when checking the values with one-way ANOVA, this difference was not significant. Although the two points suffers with anthropic actions, the reservoir of DAE presents characteristics of a lentic environment, which could increase the "damages" resulting from the modifications in the structure of the environment due to the damn present on the local. Environments located upstream of dams suffer from the intensification of processes such as sedimentation and eutrophication, affecting the local aquatic fauna (Agostinho et al. 2016, Affonso et al. 2016). According to Portella et al. (2017), reduction in environmental quality results in a decrease in the richness, equitability and diversity of species due to lack of resources and inter and intraspecific competition generated by it.

A relatively high rate of similarity was found between the parasite species collected from the two sampling points, due to the fact that this index varies between 0 (zero similarity) and 1 (maximum similarity), and a value of 0.82 was found. Although there is a considerable distance between the sampling points (135 km), the high similarity in the composition and abundance of the parasitic fauna at the two sampling points is likely due to the fact that they are both located along the Batalha River. This connection is responsible for the transport and transfer of aquatic organisms such as parasitic metazoa, organic matter, and energy between the sampling points (Freeman et al. 2007).

In general, the parasitic fauna of *H. malabaricus* was characterized by a high species richness with a predominance of ectoparasites, and there was no significant difference between the parasitic infracommunities sampled at the two collection points. Based on the results obtained herein, the metazoan species *U. margolisi*, *Scleroductus* sp., and *Helodbella* sp. are being reported in this host for the first time. The species *Anacanthorus* sp., *Nothogyrodactylus* sp., *G. trairae*, Bucephalidae gen. sp., *A. mordax*, *Tylodelphys* sp., and *Eustrongylides* are being reported in the Batalha River for the first time, a finding which expands the geographic distribution of these parasites and contributing to the global biodiversity inventory of fish parasites (Leite 2017, Negrelli et al. 2018, Pelegrini et al. 2018).

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