

Parasitism by Monogenoidea in *Piaractus mesopotamicus* (Characiformes, Characidae) cultivated in Paraná River (Brazil)

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Abstract

This study investigated the occurrence, prevalence, mean abundance and mean intensity of monogenoidean parasites in *Piaractus mesopotamicus* farmed in cages in the reservoir of the Itaipu Hydroelectric Power Station, Paraná River, Brazil. The parasite distribution pattern and the correlation of prevalence and abundance with the total length of hosts were also investigated. Four monogenoidean species were collected: *Anacanthorus penilabiatius*, *A. toledoensis*, *Mymarothecium ianwhittingtoni* and *M. viatorum*. All the parasites collected in *P. mesopotamicus* showed the typical aggregated distribution pattern, and the abundance and the prevalence did not shown any correlation with the total length of hosts.

Keywords: Monogenoidea, *Piaractus mesopotamicus*, Paraná River, *Anacanthorus*, *Mymarothecium*.

Parasitismo por Monogenoidea em *Piaractus mesopotamicus* (Characiformes, Characidae) cultivados no rio Paraná (Brasil)

Resumo

Neste estudo foi investigada a ocorrência, prevalência, abundância média e intensidade média de Monogenoidea parasitos de *Piaractus mesopotamicus*, cultivados em tanques-rede no reservatório da Usina Hidrelétrica de Itaipu, rio Paraná, Brasil. Foram investigados ainda o padrão de distribuição parasitária e a correlação entre a prevalência e abundância com o comprimento total dos hospedeiros. Quatro espécies de Monogenoidea foram coletadas: *Anacanthorus penilabiatius*, *A. toledoensis*, *Mymarothecium ianwhittingtoni* e *M. viatorum*. Todos os parasitos coletados em *P. mesopotamicus* apresentaram típico padrão de distribuição agregada e não foi observada correlação entre a abundância e a prevalência e o comprimento total dos hospedeiros.

Palavras-chave: Monogenoidea, *Piaractus mesopotamicus*, rio Paraná, *Anacanthorus*, *Mymarothecium*.

1. Introduction

Piaractus mesopotamicus (Holmberg, 1887) is a characiform fish commonly known as “pacu” in Brazil. This fish reaches maturity at a length of 40.5 cm and is one of the most commonly farmed species in Brazil, because of its importance for the food diet, ecology and sport fishery (Jégu, 2003). The significant increment in aquaculture around the world has increased the importance of studies of parasites and other pathogens of aquatic organisms, especially in those hosts that have the potential for production and sale (Luque, 2004). The monitoring of parasites and

other fish pathogens is an important part of prophylactic management both for production (Pavanelli et al., 2008). Parasitic infections are more relevant in the Neotropical region because of the climatic characteristics of this region, which facilitate the rapid and continuous spread of these pathogens (Thatcher and Brites-Neto, 1994). The Monogenoidea are one of the largest groups of parasitic flatworms, comprising about 60 families and more than 3000 described species. Some species have detrimental effects on both wild and farmed fish populations, causing

important economic damage (Fannes et al. 2015). Several authors have studied monogenoideans in *P. mesopotamicus*: to date, parasitological (Alexandrino et al., 1995; Eiras et al., 1995; Kohn and Cohen, 1998; Martins et al., 2002a; Pamplona-Basilio et al., 2001; Schalch et al., 2006; Lizama et al., 2007; Cohen and Kohn, 2005, 2009; Franceschini et al., 2013), histopathological (Martins and Romero, 1996; Campos et al., 2011; Tavares-Dias et al., 2002; Jerônimo et al., 2014), morphological (Boeger et al., 1995; Souza et al., 2000; Cohen and Kohn, 2008; Leão et al., 2015), pathological (Tavares-Dias et al., 2000), hemathological (Martins et al., 1995; Tavares-Dias et al., 2002, 2008) and therapeutic (Martins, 1998; Martins et al., 2000, 2002b; Tavares-Dias et al., 2002; Onaka et al., 2003; Belo et al., 2005; Schalch et al., 2006; Cruz et al., 2008; Fujimoto et al., 2010; Figueiredo et al., 2011; Carraschi et al., 2014) studies were conducted. The first monogenoidean found in “pacus” was *Anacanthorus penilabiatu* Boeger, Husack and Martins, 1995, described in the state of São Paulo and posteriorly reported by Martins et al. (2000) in the same region. One year later, Pamplona-Basilio et al. (2001) presented the descriptions of the egg and reported this species in another host, *Colossoma macropomum* (Cuvier, 1818). *Mymarothecium viatorum* Boeger, Piasecki and Sobeca, 2002 was originally described from *Piaractus brachypomus* (Cuvier, 1818) in Poland and later, it was reported by Cohen and Kohn (2005) parasitizing *P. mesopotamicus* in the state of Ceará, Brazil. Franceschini et al. (2013) reported findings of *A. penilabiatu* and *M. viatorum* in the same host. In 2007, Lizama et al. recorded *A. penilabiatu*, *Anacanthorus spatulathus* and *Mymarothecium* sp. from the same host. Recently, Leão et al. (2015) described two new species, *Anacanthorus toledoensis* and *Mymarothecium ianwhittingtoni*, increasing the diversity of known species of Monogenoidea in *P. mesopotamicus*. The aim of the present study was to investigate prevalence, mean abundance, mean intensity, parasite distribution pattern and the correlation of prevalence and abundance with the total length of hosts of monogenoidean parasites in *P. mesopotamicus* farmed in cages in the reservoir of the Itaipu Hydroelectric Power Station, Paraná, Brazil.

2. Material and Methods

In January 2011 (summer season), samples of gills were taken from forty-one *P. mesopotamicus* that were kept in cages upstream and downstream of the reservoir of the Itaipu Hydroelectric Power Station, Paraná River, state of Paraná, Brazil (24°44'29.0" S; 53°44'51.2" W).

During all the cultive period water physical and chemical parameters were measured with portable devices. Dissolved oxygen (Hanna Instruments, model HI9146) and pH (Hanna Instruments, model HI8314) were weekly monitored as the water temperature was monitored daily (mornings and evenings) with a mercury bulb thermometer. The fish were anesthetized with benzocaine at a concentration of 74mg/L and euthanized with benzocaine at a concentration of 250mg/L according to the recommendations of Gomes et al. (2001), weighed (g), measured (cm) and transported to the laboratory. The gills were removed and placed in vials containing 1:4000 formalin solution and shaken vigorously and the formalin solution of each vial was increased to about 5%. In the laboratory, parasites were collected with the aid of a stereoscopic microscope and were stored in the same fixative. Some specimens were mounted unstained in Hoyer's mounting medium (Humason, 1979) in order to study the sclerotized parts and the remaining were stained with Gomori's trichrome, cleared in beechwood creosote and mounted on slides in Canada balsam (Kritsky et al., 1978).

The prevalence (P), mean abundance (MA) and mean intensity (MI) of the parasites were calculated as described by Bush et al. (1997).

The index of dispersion (quotient between variance and mean of parasite abundance) and d test were used to determine distribution patterns (Ludwing and Reynolds, 1988). Spearman's rank correlation coefficient (*rs*) was used to determine possible correlations between host's total length and parasite abundance. Pearson's correlation coefficient (*r*) was used to determine possible correlations between the host's total length and the prevalence of parasites, with angular transformation of prevalence values (Zar, 1996). The statistical significance level of $p \leq 0.05$ was used.

3. Results and Discussion

The average values for the physical and chemical water parameters measured during the experimental period were 30.4 ± 0.7 °C for temperature; 6.40 ± 0.3 mg/L for dissolved oxygen; 7.1 ± 0.3 for pH; and 0.06 ± 0.03 mg/L for ammonia content (as shown in Table 1). These values are within the recommended range when rearing tropical species (Ostrensky and Boeger, 1998; Urbinati et al., 2010). Features of the habitat (local abiotic factors such as water temperature, pH and lakesize) and certain traits of the host population like size, could facilitate the transmission and establishment of fish parasites (Bagge et al., 2004; Takemoto et al., 2005; Poulin, 2006).

Table 1. Values for the physical and chemical water parameters measured during the experimental period.

Parameters	Values	References values
Temperature (°C)	30.4 ± 0.7	20-30*
Oxygen dissolved	6.40 ± 0.3 mg/L	3-10 †
pH	7.1 ± 0.3	6-8 *.†
Ammonia content	0.06 ± 0.03 mg/L	<0.6*

*Ostrensky and Boeger (1998); †Urbinati et al. (2010).

Forty-one specimens of *P. mesopotamicus* with mean total weight 1.13 Kg (0.690-1.726 ± 0.264 Kg) and mean total length 35.07 cm (30-41 ± 2.493 cm) were examined for monogenoideans. All the fish examined were parasitized with at least one species of Monogenoidea. Four species were collected: *Anacanthorus penilabiatus*, *Anacanthorus toledoensis*, *Mymarothecium ianwhittingtoni* and *Mymarothecium viatorum* (as shown in Table 2). *A. toledoensis* was the species with higher rates of prevalence, mean intensity and mean abundance. The other three species have high rates of prevalence, but low values of mean intensity and mean abundance: *A. penilabiatus*; *M. viatorum*; *M. ianwhittingtoni*.

Our results, compared with other studies, showed variations in the mean abundance and mean intensity of monogenoidean (as shown in Table 3). Lizama et al. (2007), Tavares-Dias et al. (2008), Franceschini et al. (2013) and Jerônimo et al. (2014) reported the prevalence indexes for *A. penilabiatus* in *P. mesopotamicus* in fish farming (P = 81.1%; 76%; 77.3%; 100%, respectively) that were similar to what was reported in this study (P = 97.5%), but higher from the studies of Martins and Romero (1996) and Cohen and Kohn (2009) (P = 33.3%; 50%, respectively). Considering mean intensity and mean abundance, Franceschini et al. (2013) (MI = 22.4; MA = 17.3) and Cohen and Kohn (2009) (MI = 23.6; MA = 11.8) demonstrated similar results for *A. penilabiatus*,

while other studies register higher values: (Lizama et al. (2007) MI = 166,3/ MA = 134,9; Tavares-Dias et al. (2008) MI = 2,287.6 and Jerônimo et al. (2014) MI=316.3 (cold season); 2,742.3 (hot season) ; MA = 316.3 (cold season); 2,742.3 (hot season).

The monogenoidean species reported in this study was also present in closely related fish species (as shown in Table 4). *A. penilabiatus* was reported in *P. brachypomus*, in hybrids *P. mesopotamicus* x *C. macropomum* and in *P. mesopotamicus* x *P. brachypomus* (Cohen and Kohn, 2009; Tavares-Dias et al., 2008; Franceschini et al., 2003). In the studies of Franceschini et al. (2013) (*P. mesopotamicus* x *P. brachypomus*) and Tavares-Dias et al. (2008) (*P. mesopotamicus* x *C. macropomum*) studies, the prevalence of *A. penilabiatus* was lower than in the present study. However, the prevalence presented by Cohen and Kohn (2009) for *P. brachypomus* was similar with our results for *P. mesopotamicus*. Considering the mean intensity, the hybrid examined by Tavares-Dias et al. (2008) showed high values than in the present study, while Cohen and Kohn (2009) and Franceschini et al. (2013) found similar values in mean intensity and mean abundance in *P. brachypomus* and in the hybrid *P. mesopotamicus* x *P. brachypomus*, respectively.

In the present investigation, *M. viatorum* showed values of mean intensity and mean abundance similar to those found by Franceschini et al. (2013) (MI = 27.5;

Table 2. Prevalence (P%), mean intensity (MI), mean abundance (MA) range (R), intensity (I) and confidence interval (CI) of monogenoideans parasites of farmed fish, *P. mesopotamicus* of the reservoir of the Itaipu Hydroelectric Power Station, Paraná River, state of Paraná, Brazil.

Parasites	P(%)	MI (CI)	MA (CI)	R
<i>Anacanthorus penilabiatus</i>	97.5 (86.8-9.9)	11.5 (8.1-16.3)	11.5 (7.9-16.2)	1-58
<i>Anacanthorus toledoensis</i>	100.0 (91.6-1.0)	43.1 (35.3-55.8)	43.1 (34.5-55.8)	1-175
<i>Mymarothecium ianwhittingtoni</i>	80 (64.3-90.9)	12.9 (8.5-23.0)	10.3 (6.5-18.5)	1-94
<i>Mymarothecium viatorum</i>	95 (83.0-99.3)	27.1 (18.8-42.7)	25.7 (17.8-40.9)	2-188

Table 3. Dispersion index (DI) and *d* statistical of the monogenoideans parasites of farmed fish, *P. mesopotamicus* of the reservoir of the Itaipu Hydroelectric Power Station, Paraná River, state of Paraná, Brazil.

Parasites	DI	<i>d</i>
<i>Anacanthorus penilabiatus</i>	14.2	24.5*
<i>Anacanthorus toledoensis</i>	26.8	35.9*
<i>Mymarothecium ianwhittingtoni</i>	29.5	39.2*
<i>Mymarothecium viatorum</i>	46.1	51.2*

*Significant values.

Table 4. Values of Spearman's rank correlation coefficient (*rs*) and Pearson's correlation coefficient (*r*) obtained in relations between total length of hosts and abundance and prevalence of monogenoideans parasites of farmed fish, *P. mesopotamicus* of the reservoir of the Itaipu Hydroelectric Power Station, Paraná River, state of Paraná, Brazil.

Parasites	<i>rs</i>	P	<i>r</i>	P
<i>Anacanthorus penilabiatus</i>	-0.1444	0.3739	-0.378	0.1003
<i>Anacanthorus toledoensis</i>	-0.1215	0.4553	0.2586	0.2709
<i>Mymarothecium ianwhittingtoni</i>	-0.1807	0.2645	0.4282	0.0595
<i>Mymarothecium viatorum</i>	-0.2069	0.2000	0.085	0.7216

MA = 19.9), and different from Cohen and Kohn (2009) (MI; MA = 277) from *P. mesopotamicus*.

Regarding the parasitism by *M. viatorum* on closely related fish of *P. mesopotamicus*, Cohen and Kohn (2009) presented similar prevalence values for parasitism in the host *P. brachyomus*, however, in the study of Vásques (2014) in the same host, these values were lower. Considering mean intensity and mean abundance, the studies of Cohen and Kohn (2009) and Vásques (2014) showed higher values. The prevalence of *M. viatorum* in hybrid (*P. mesopotamicus* x *P. brachyomus*) presented by Franceschini et al., (2013) showed similarity, while the mean intensity and mean abundance values were considerably higher than those from the present study. These differences are due to changes in the management of farming systems and susceptibility to infection of cultured individuals. The high indexes in farming mainly occurs due the Monogenoidea have a direct life cycle and their transmission could be facilitated by proximity between hosts (Bagge et al., 2004). These parasites in man-made environments, like aquaria and fish farms, multiply readily, sometimes killing their hosts (Rubio-Godoy, 2007).

All the parasites of *P. mesopotamicus* showed the typical aggregated pattern of distribution (as shown in Table 5). Our results show that these parasites present the typical aggregated distribution pattern, in conformity with the most common pattern for metazoan parasite populations. Poulin (2013) pointed that firstly, heterogeneity among host individuals in terms of exposure to parasites, resulting from the uneven distribution of infective stages in space and time relative to hosts, can lead to parasite aggregation. Secondly,

heterogeneity in numbers of parasites per host can result from either genetic or acquired variation in susceptibility to infection, arising from differences among hosts in behaviour or immune resistance. More knowledge about parasite and host biology is needed to improve the interpretation of these parasitological patterns, in an ecological framework incorporating pertinent environmental and biological information, as recommended by Marcogliese (2001).

There was no correlation between abundance and total length of the hosts, and between prevalence and total length of the hosts (as shown in Table 6).

According Luque et al. (2003), the size of the host is not always correlated with prevalence and abundance of parasites. As pointed by Poulin (2000), this pattern cannot be generalized, because in many host-parasite systems the correlation is positive, but weak and non-significant. In the present study, no correlation was observed between the abundance and prevalence of the parasites and the total length of the hosts. This lack of correlation may be related to the low amplitude of the host length classes observed among the specimens examined. In cages this fact could happen frequently because the standardization of fish sizes. Similar results were observed in *Plagiacion squamosissimus* by Tavernari et al. (2005), who reported that the non existence of correlation of the Monogenoidea number and their prevalence in relation to the size of the hosts might be due to uniformity of parasitism levels in relation to host length classes, along with the situation in which almost all the host samples were adult specimens.

Table 5. Comparison between the values of prevalence (P%), mean intensity (MI), mean abundance (MA), range (R), total number (n) of monogenoideans parasites of different localities and quality water.

Parasite	P (%)	MI	MA	R	Total (n)	Locality	Quality of water	Reference
<i>Anacanthorus penilabiatius</i>	33.3	-	-	-	37	São Paulo and Paraná State	-	Martins and Romero (1996)
	81.1	166.3	134.9	2-2387	-	São Paulo State	Unacceptable	Lizama et al. (2007)
	76	2,287.6	-	-	-	São Paulo State	Acceptable	Tavares-Dias et al. (2008)
	50	23.6	11.18	13-42	74	Ceará State	-	Cohen and Kohn (2009)
	77.3	22.4	17.3	-	-	São Paulo State.	Acceptable	Franceschini et al. (2013)
	100	316.31 2,742.3	16.3 2,742.3	24-1,113 1,979-4,269	-	Mato Grosso do Sul State	Acceptabl Unacceptable	Jerônimo et al. (2014)
97	11.3	11.5	1-58	439	ParanáState.	Acceptable	Present study	
<i>Mymarothecium viatorum</i>	100	277	277	54-854	1,662	Ceará State.	-	Cohen and Kohn (2009)
	72.7	27.5	19.9	-	-	São Paulo State	Acceptable	Franceschini et al. (2013)
	95	27.1	25.7	2-188	1,030	Paraná State.	-	Present study

Table 6. Prevalence (P), Mean intensity (MI), Mean abundance (MA) and Total number of *Anacanthorus penilabiatus* and *Mymarothecium viatorum* from different parental host species.

Host	<i>Anacanthorus penilabiatus</i>				
	P (%)	MI	MA	Total number	Reference
<i>Piaractus mesopotamicus</i>	50	23.6	11.8	74	Cohen and Kohn (2009)
<i>Piaractus brachypomus</i>	83.3	17.5	15	112	Cohen and Kohn (2009)
(<i>Piaractus mesopotamicus</i> x <i>Colossoma macropomum</i>)	11.7	433.8			Tavares-Dias et al. (2008)
(<i>Piaractus mesopotamicus</i> x <i>Piaractus brachypomus</i>)	77	7.5	5.8		Franceschini et al. (2013)
<i>Mymarothecium viatorum</i>					
<i>Piaractus mesopotamicus</i>	100	277	277	1,662	Cohen and Kohn (2009)
<i>Piaractus brachypomus</i>	100	105.7	105.7	740	Cohen and Kohn (2009)
	43.33	78.69	34.10	2,046	Vásques et al. (2014)
(<i>Piaractus mesopotamicus</i> x <i>Piaractus brachypomus</i>)	85	178.7	152.3		Franceschini et al. (2013)

4. Conclusions

This study showed high prevalence results in all the species, although the features of the cages were according recommended range when rearing tropical species. *A. toledoensis*, was the species with higher rates of prevalence, mean intensity and mean abundance. The other three species have high rates of prevalence, but low values of mean intensity and mean abundance. The Monogeneoidea collected in *P. mesopotamicus* showed the typical aggregated distribution pattern, and the abundance and the prevalence did not shown any correlation with the total length of hosts.

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