

Proteocephalid cestode infection in tucunaré *Cichla* sp. (Osteichthyes: Cichlidae) from Paraná River, São Paulo

[*Infecção por cestóides proteocefalídeos em tucunaré Cichla sp. (Osteichthyes: Cichlidae), no Rio Paraná, São Paulo*]

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ABSTRACT

The occurrence of proteocephalid cestodes in tucunaré *Cichla* sp., captured monthly, between August 2000 and August 2001, in Paraná River, Presidente Epitácio, SP, was evaluated. From 128 specimens, 71 (55.6%) were parasitized by *Proteocephalus macrophallus* (Diesing, 1850) and/or *P. microscopicus* (Woodland, 1935). Total mean abundance and intensity were 157.08 and 223.41, respectively. The highest prevalence (90%) mean abundance (1,122.4) and intensity indexes (1,247.11) occurred in February 2001, while in September 2000 there were no observed animals infected by cestodes. No relationship between the sex of the host and parasitological indexes was found.

Keywords: fish, tucunare, parasitism, Proteocephalidae, Paraná River

RESUMO

Avaliou-se a ocorrência de cestóides proteocefalídeos em tucunaré Cichla sp., capturados mensalmente, entre agosto de 2000 e agosto de 2001, no rio Paraná, em Presidente Epitácio, SP. Um total de 128 espécimes foram analisados, dos quais 71 (55,6%) estavam parasitados por Proteocephalus macrophallus (Diesing, 1850) e/ou P. microscopicus (Woodland, 1935). A abundância e intensidade média total foram de 157,08 e 223,41, respectivamente. A maior prevalência (90%), juntamente com os maiores índices de abundância (1122,4) e intensidade média (1247,11) ocorreram no mês de fevereiro 2001, enquanto no mês de setembro 2000 não foram observados animais parasitados por cestóides. Não houve relação entre o sexo do hospedeiro e os índices parasitológicos.

Palavras-chave: peixe, tucunaré, parasitismo Proteocephalidae, rio Paraná

INTRODUCTION

Tucunare, *Cichla* sp., is a typical piscivorous cichlid fish native from the Amazon basin, and of great importance as commercial fish and sport (Gomiero and Braga, 2003). It was introduced into the lakes of many tropical and subtropical regions, including Brazil, Panama, Puerto Rico and Hawaii and Florida in USA (Jepsen *et al.*,

1997). In Brazil, it was introduced in the hydrographic basins such as the Paraná-Paraguay, São Francisco and others in northeastern Brazil (Gomiero and Braga, 2004a) and showed rapid adaptation in the hydroelectric reservoirs (Gomiero and Braga, 2004b).

Stable environments are more susceptible to the introduction of new species, especially piscivorous, than those unstable ones (Zaret,

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1982). Introduced species affect native species through competition for available resources, predation, pathogen transfer, or simply by habitat alteration (Gomiero and Braga, 2004a). The negative results of introduced non-native species on the parasite populations are not known, and it is frequently blamed for severe damage, especially for native populations (Gomiero and Braga, 2004a).

According to Rego (2004), 120 cestode species belong to 6 orders in the neotropics. Ninety percent belong to Proteocephalidae and are found in teleosts. Proteocephalids are the most numerous and important helminthes in freshwater fish, having been described approximately 80 species in freshwater fishes from South America, mostly found in siluriform fish (Rego *et al.*, 1999).

This group of parasites has been studied in several fish species from the Paraná River: *Paulicea luetkeni* (de Chambrier *et al.*, 1999); *Parauchenipterus galeatus* (Pavanelli and Santos, 1991); *Pseudoplatystoma corruscans* (de Chambrier *et al.*, 2006); *Sorubim lima* (Pavanelli and Takemoto, 2000; de Chambrier, 2003); *Cichla monoculus* (Machado *et al.* 2000); *Cichla piquiti* (Martins *et al.*, 2009); *Parauchenipterus galeatus* (Pavanelli and Santos, 1990) and other teleost fish (Rego *et al.*, 1998, 1999).

The sex of the hosts is an important factor that influences the fish parasite composition and may be related to behavior, biological and physiological differences between male and female specimens (Machado *et al.*, 1994; Takemoto and Pavanelli, 1996). Changes in diet and feeding habitats of the hosts can also influence their parasitic fauna (Dogiel, 1970).

The objective of this work was to study the proteocephalid parasitological occurrence in *Cichla* sp. in the Paraná River, SP and the influence of sex on the parasitism.

MATERIAL AND METHODS

A total of 128 fish were collected in Paraná River, Presidente Epitácio, SP, between August 2000 and August 2001, being 10 fish per month, except in August 2000, when only 8 fish were collected. The rainfall and water quality data such as pH, turbidity, temperature, dissolved

oxygen, conductivity and alkalinity were performed in each collection, except in January 2001.

Fish were anesthetized, the sex identified and then submitted to parasitological examination. The cestodes found in the gut were isolated and fixed in hot 4% formaldehyde solution and stored in ethanol 75%. The helminthes were stained with Mayer hydrochloric carmine (modified from Langeron, 1949), differentiated in acid ethanol, dehydrated through a gradual ethanol series, cleared with Eugenol or beech wood creosote and mounted as Canada balsam. Thick cross hand-cutting sections of the proglottides were stained following the same procedure; fragments of the strobila were embedded in paraffin wax, transversely sectioned at 13-19µm, stained with Weigert's hematoxylin and counterstained with 1% eosin B and mounted in Canada balsam (de Chambrier and Pertierra, 2002).

The prevalence, mean intensity and mean abundance were calculated according to Bush *et al.* (1997). For comparison of mean abundance and intensity among the months, data was transformed into log₁₀ and log₁₀ (n+1), respectively, and subjected to ANOVA and subsequently by Student's t test (P<0.05) using adjustment for multiple testes by Bonferroni adjust. The existence of a relationship between the sex of the host and parasitological index was verified.

Specimens of *Proteocephalus macrophallus* (Diesing, 1850) Scholz, de Chambrier, Prouza and Royero 1996 and *Proteocephalus microscopicus* (Woodland, 1935) have been deposited at the Natural History Museum, Geneva, Switzerland (respectively MHNG Collection number INVE 34677, 35401, 35402 and INVE 34065, 35403) and at the Helminthological Collection of Instituto Oswaldo Cruz (CHIOC), number 36845a-b (*P. macrophallus*) and 36846a-b (*P. microscopicus*).

RESULTS

The highest temperatures were observed in May 2001 (28.8°C), November 2000 (28.7°C), February (28.4°C) and March 2001 (28.2°C), while the lowest were found in August 2001 (22.0°C) and September 2000 (22.6°C). The

lowest values of dissolved oxygen were related in March (3.7mg/l) and August 2001 (3.8mg/L). The water pH ranged between 6.4 and 7.3 and alkalinity between 27.4 and 41.0mg/l. The highest rates of rainfall were found in February 2001 and December 2000, respectively (291.6 and 215.1mm), while the lowest value was observed in July 2001 (31.8mm) (Table 1).

The fish examined consisted of 81 males (63.3%) and 28 females (36.7%). The length of the hosts varied between 21.0 and 53.0cm (mean length 36.5cm) and weight from 200 to 2,500g (mean weight 865.9) (Table 2). There was no relationship ($P>0.05$) between the sex of the hosts and parasitological indexes.

Table 1. Mean values of the rainfall (RF) and water quality measured in Paraná River, Presidente Epitácio, SP, between August 2000 and August 2001 during the fish collection

Month	RF (mm)	Transparency (m)	Alkalinity (mg/l)	pH	Conductivity (μ S/cm)	Temperature ($^{\circ}$ C)	Oxygen (mg/l)
Aug/2000	64.8	2.4	27.4	7.1	50.1	23.6	9.1
Sep/2000	195.4	2.4	27.4	7.3	81.8	22.6	8.0
Oct/2000	67.0	2.4	27.4	7.1	56.4	26.7	7.3
Nov/ 2000	142.4	2.4	27.4	7.3	49.0	28.7	6.4
Dec/2000	215.1	2.4	27.4	7.3	49.8	27.7	6.2
Feb/2001	291.6	2.4	27.4	6.4	55.1	28.4	6.3
Mar/2001	130.6	2.4	27.4	6.4	57.0	28.2	3.7
Apr/2001	57.2	2.4	27.4	6.4	56.5	27.1	6.5
May/2001	140.8	2.4	27.4	6.4	56.6	28.8	6.5
Jun/2001	52.9	2.4	27.4	6.4	56.5	27.6	6.7
Jul/2001	31.8	2.4	29.5	6.7	57.0	23.6	6.0
Aug/2001	50.0	2.4	41.0	6.4	56.7	22.0	3.8

Table 2. Parasitological indexes in *Cichla* sp. parasitized by proteocephalid cestodes in Paraná River, Presidente Epitácio, SP

Month	Fish weight (g)	Fish length (cm)	CF/IF	P (%)	MA	MI
Aug/2000	1112.5 (450-1725)	40.3 (30-47.5)	8/5	62.5	40.4	80.8 (0-182)
Sep/2000	590 (450-850)	32.9 (30-37)	10/0	0	0	0
Out/2000	650 (350-950)	34.6 (27-39)	10/3	30.0	16.6	55.33 (0-102)
Nov/2000	805 (350-950)	35.4 (29-45.5)	10/7	70.0	11.0	15.71 (0-26)
Dec/2000	1100 (550-2500)	39.3 (34-50)	10/6	60.0	155.5	259.17 (0-1224)
Jan/2001	992.5 (500-1600)	39.1 (33-46)	10/8	80.0	72.7	90.87 (0-438)
Feb/2001	850 (200-2500)	34.0 (23-50)	10/9	90.0	1122.4	1247.11 (0-8301)
Mar/2001	935 (200-1600)	36.4 (21-48)	10/5	50.0	242.5	485 (0-1543)
Apr/2001	925 (450-1400)	38.0 (30-46)	10/8	80.0	2.9	3.62 (0-6)
May/2001	825 (350-1550)	36.1 (27-46)	10/5	50.0	26.6	53.2 (0-211)
Jun/2001	1271 (600-2400)	42.0 (32.5-53)	10/6	60.0	4.5	7.5 (0-14)
Jul/2001	585 (200-1100)	31.6 (23-42)	10/6	60.0	330.4	550.67 (0-1064)
Aug/2001	665 (450-950)	35.2 (32-39)	10/3	30.0	16.6	55.33 (0-102)
Total	865.9 (200-2500)	36.5 (21-53)	128/71	55.58	157.08	223.41 (0-8301)

IF/CF: infected fish/collected fish; P: prevalence; MA: mean abundance; MI: mean intensity. Range in parentheses.

The species of cestodes identified as *P. macrophallus* and *P. microscopius* were found during the whole period, except in September 2000. The total prevalence was 55.6%, with higher rates in February 2001 (90%), followed by January and April 2001 (80%) and the lowest

rates in October 2000 and August 2001 (30%). (Table 2)

The total mean abundance was 157.08, with the highest index in February 2001 (1,122.4) and the lowest in April 2001 (2.9). In February 2001 the mean abundance was significantly higher than

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August (P<0.01), September (P<0.05) and October 2000 (P<0.05) and April (P<0.05), May (P<0.01) and June 2001 (P<0.05) (Table 3). On the other hand, in September 2000 the lowest mean abundance (P<0.01) was found when compared to July 2001 (Figure 1 and Table 3).

The mean intensity during the whole period was 223.41 and remained between 3.62 (April 2001) and 1,247.11 (February 2001). In February 2001, a significantly higher mean intensity than

November 2000 (P<0.01), April (P<0.05) and June 2001 (P<0.05) was observed, while April 2001 showed the lowest (P<0.05) value when compared to December 2000, March and July 2001 (Figure 2 and Table 3). In July 2001 the mean intensity was significantly higher than November 2000 (P<0.01) and June 2001 (P<0.05), while June 2001 showed significant lower mean intensity than March 2001 (P<0.05) (Table 3).

Table 3. Statistical analyses of proteocephalid cestodes in *Cichla* sp. from Paraná River, Presidente Epitácio, SP, from August 2000 through August 2001

Month	Aug/2000	Sep/2000	Oct/2000	Nov/2000	Dec/2000	Feb/2001	Mar/2001	Apr/2001	May/2001	Jun/2001	Jul/2001	Aug/2001
Aug 2000	-	ns										
Sep 2000	ns	-	ns									
Oct 2000	ns	ns	-	ns								
Nov 2000	ns	ns	ns	-	ns	*	ns	ns	ns	ns	*	ns
Dec 2000	ns	ns	ns	ns	-	ns	ns	**	ns	ns	ns	ns
Feb 2001	*	**	**	ns	ns	-	ns	**	ns	**	ns	ns
Mar 2001	ns	ns	ns	ns	ns	ns	-	**	ns	**	ns	ns
Apr 2001	ns	ns	ns	ns	ns	**	ns	-	ns	ns	**	ns
May 2001	ns	ns	ns	ns	ns	*	ns	ns	-	ns	ns	ns
Jun 2001	ns	ns	ns	ns	ns	**	ns	ns	ns	-	**	ns
Jul 2001	ns	*	ns	-	ns							
Aug 2001	ns	-										

The mean intensity is above the diagonal and the mean abundance is below the diagonal. * P<0.01; ** P<0.05; ns P>0.05

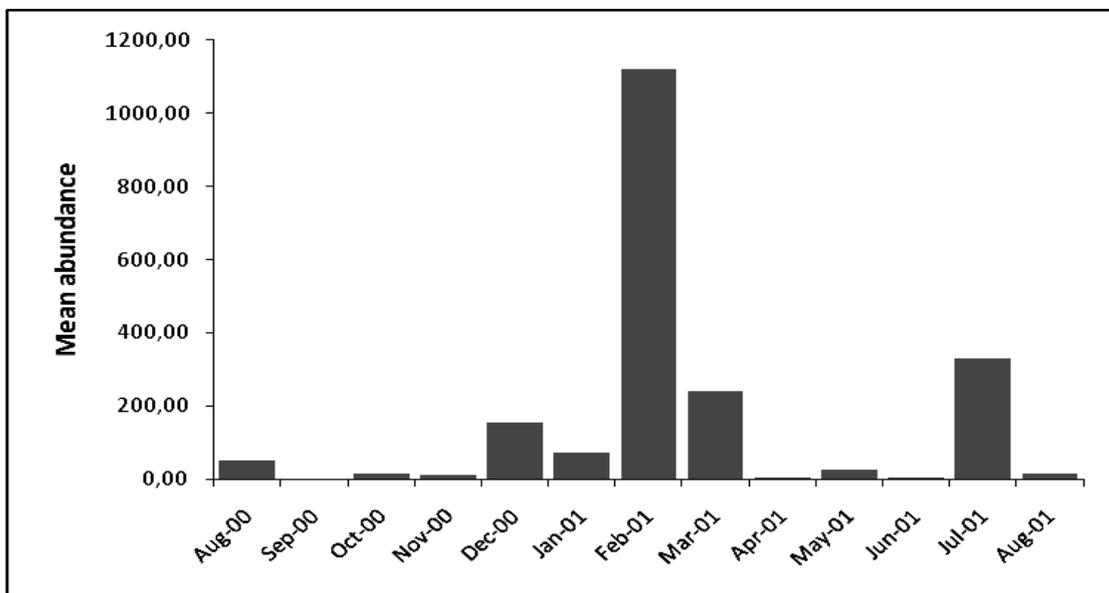


Figure 1. Mean abundance of proteocephalid cestode parasites in *Cichla* sp. in the Paraná River, Presidente Epitácio, SP, from August 2000 through August 2001.

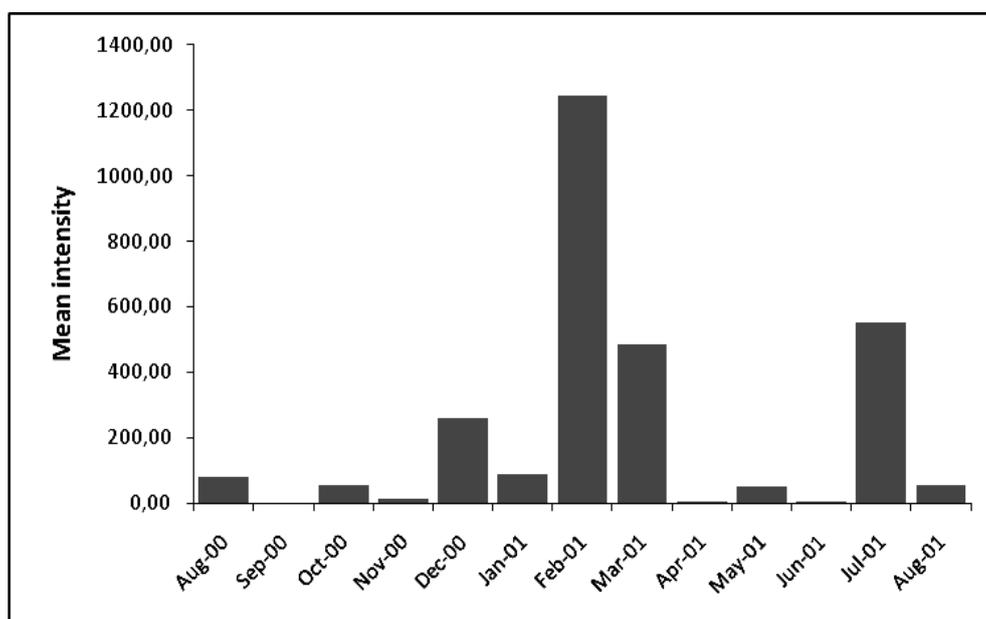


Figure 2. Mean intensity of proteocephalid cestode parasites in *Cichla* sp. in the Paraná river, Presidente Epitácio, SP, from August 2000 through August 2001.

DISCUSSION

It can be inferred that the highest mean abundance in December 2000, January, February and March 2001 were related to the rainfall values. But the unexplained fact was the isolated high mean abundance in July 2001. Such as observed by Martins *et al.* (2009) in a reservoir environment, this study cannot confirm this fact. These authors found that 100% prevalence and high mean intensity coincided with the highest biomass algae. This fact can be explained by the fact that a river is a very dynamic environment, very different from reservoirs.

Rego *et al.* (1999) referred 80 species of proteocephalid cestodes from freshwater fish in South America, most of them found in Siluriform fish. Proteocephalid cestodes constitute the most numerous and important helminthes of freshwater fish. These authors argued that the large concentration of proteocephalid genera occurs in South America, but the greatest species concentration, especially of the primitive genus *Proteocephalus* Weiwlawdi 1858 occurs in Eurasia and North America.

Proteocephalus macrophalus and *P. microscopicus* appear to be exclusive to the tucunaré, and have never been recorded in other species (Rego, 1994). Other studies with alien

fish *Cichla* sp. in Brazil identified the same species found in this work: *Cichla monoculus* in Paraná River, Paraná (Machado *et al.*, 2000) and *Cichla piquiti*, in Volta Grande Reservoir, Minas Gerais (Martins *et al.*, 2009). In both studies, the total prevalence rates were higher than that related in the present study, which were 83.3% and 97%, respectively.

In terms of total prevalence, in Paraná River four species of proteocephalid cestodes in *Sorubim lima* were found with higher prevalence rate (88.8%) than the present study and lower mean intensity (79.71 parasites per host) (Takemoto and Pavanelli, 2000). Similar proteocephalid prevalence rates during the whole studied period were also observed by these authors who have studied proteocephalid in another region of the Paraná River. De Chambrier and Rego (1994), Machado *et al.* (1994) and de Chambrier (2003) have found lower prevalence rates and mean intensities of proteocephalids in *Pseudoplatystoma corruscans*, *P. luetkeni* and *Sorubim lima*, respectively.

The feeding habit of tucunaré includes several species of fish that could act as intermediate or paratenic hosts for these parasites (Machado *et al.*, 2000). Furthermore, Gomiero and Braga (2004b) reported high rates of cannibalism in *Cichla* cf. *ocellaris* and *C. monoculus*, which

suggests a possible transmission of the cestodes by ingestion. Thomas *et al.* (2002) focused on the parasite strategies that can lead to their successful life or not. In fact, one of the strategies of *Cichla* sp. could be its feeding behavior constituted by small infected fish.

Parental care and predatory behavior are two ecological characteristics of the alien species, as tucunaré. The parental care may increase the competitiveness of these species and the predatory behavior put the alien fish on the top of the food web (Latini and Petrere Jr., 2004) which may explain the high number of parasites observed in this study.

In most of months the mean abundance was lower than that related by Martins *et al.* (2009). In fact, it must be emphasized that the reservoirs may play an important role on parasite dissemination especially to piscivorous fish that are on the top of the food web.

In the present work, no relationship between parasitological indexes and the sex of the hosts may be evidence that males and females have similar diets and feeding habits in agreement with the findings of Muzzal (1980) and Takemoto and Pavanelli (1994).

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