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Ecological parameters of the endohelminths in relation to size and sex of *Prochilodus argenteus* (Actinopterygii: Prochilodontidae) from the Upper São Francisco River, Minas Gerais, Brazil

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ABSTRACT. This research aimed to investigate the ecological indexes of the helminths of the digestive system and coelom of *Prochilodus argenteus* Spix & Agassiz, 1829. A total of 150 specimens (53 males and 97 females) taken from the upper reaches of the São Francisco River in the municipality of Três Marias (18°12'32"S, 45°15'41"W), State of Minas Gerais, Brazil, were examined. The specimens were collected in July, 2003 and January, 2004. Ninety-eight fish (65.3%) were infected by at least one species of helminth. Five helminth species were found: one digenean, *Saccocoelioides nanii* Szidat, 1954; two Eucestoda, *Valipora* sp., and one undetermined metacestode; one nematode, *Spinitectus asperus* Travassos, Artigas & Pereira, 1928; and one acanthocephalan, *Neoechinorhynchus prochilodorum* Nickol & Thatcher, 1971. The sex of the host did not influence parasite indexes. The total length of the hosts influenced the abundance of *S. nanii* (r_s = -0.21, p = 0.01) and the prevalence of the metacestode (r = -0.91, p = 0.01). *Saccocoelioides nanii* was the dominant species in the parasite fauna of *P. argenteus*. *Saccocoelioides nanii*, *Valipora* sp., *S. asperus* and *N. prochilodorum* are reported here for the first time in *P. argenteus* and their known distribution is expanded to the São Francisco River.

KEY WORDS. Helminths; *Neoechinorhynchus*; *Saccocoelioides*; *Spinitectus*; *Valipora*.

Prochilodus argenteus Spix & Agassiz, 1829, popularly known as "curimatá-pacu" and *Prochilodus costatus* Valenciennes, 1850, known as "curimatá-pioa", are the only species of *Prochilodus* Agassiz, 1829 found in the São Francisco River and are both endemic to this basin. The curimatá-pacu, a detritivorous species (Alvim & Peret 2004), is the largest of the family, reaching up to 15 kg and is of great economic importance in the region of the Três Marias Reservoir, State of Minas Gerais, Brazil, accounting for roughly 50% of the total fish catch (Sato *et al.* 1996). It is a long-distance migratory fish and reproduces between November and January (Sato *et al.* 2005).

There are reports of various species of helminths in Prochilodontidae. In *P. argenteus* metacercariae of *Austrodiplostomum* sp. were found by Brasil-Sato (2003). The following helminths were reported in *Prochilodus lineatus* (Valenciennes, 1837): the digeneans *Sanguinicola argentinensis* Szidat, 1951, *Saccocoelioides elongatus* Szidat, 1954, *Saccocoelioides nanii* Szidat, 1954 and *Saccocoelioides* sp. according to Travassos *et al.* (1969), Kohn (1985) and Fernandes & Kohn (1994); the nematodes *Spinitectus asperus* Travassos, Artigas & Pereira, 1928 (senior synonym of *S. jamundensis* Thatcher & Padilha, 1977) by Travassos *et al.* (1928), Ramallo *et al.* (2000), larval forms of the nematodes *Procamallanus* sp. according to Kohn *et al.* (1985) and *Contracaecum* sp. following Moravec *et al.* (1993); and the acanthocephalans *Neoechinorhynchus variabilis* (Diesing, 1851) (see Travassos *et al.* 1928) and *Neoechinorhynchus curemai* Noronha, 1973 (see Kohn *et al.* 1985, Martins *et al.* 2000). Lizama *et al.* (2005) recorded the following endohelminths from *P. lineatus*: *Megacoelium* sp., *Colocladorchis* sp., *Lecithobotrioides* sp. (junior synonym of *Saccocoelioides* Szidat, 1954), *Saccocoelioides magnorchis* Thatcher, 1978, *S. nanii*, *S. saccodontis* Thatcher, 1976 (senior synonym of *Saccocoelioides leporinodus* Thatcher, 1978), *Saccocoelioides* sp., *Unicoelium prochilodorum* Thatcher & Dossman, 1975, and two indeterminate digeneans, plerocercoids of Proteocephalidea and *Valipora campylancristota* (Wedl, 1855), the nematode *Raphidascaris* sp., and juvenile acanthocephalans of *N. curemai* and *Quadrigyrus* sp. In *Prochilodus reticulatus* Valenciennes, 1850 the digeneans *Lecithobotrioides medicanoensis* Thatcher & Dossman, 1974 (junior synonym of *S. elongatus*) and *U. prochilodorum*, and the nematode *S. jamundensis* (junior synonym of *S. asperus*) were described. In *Prochilodus nigricans* Spix & Agassiz, 1829, an undetermined acanthocephalan species, *Neoechinorhynchus* sp. (see Tantaleán *et al.* 2005) was recorded.

This study introduces the helminths found in the digestive system of *P. argenteus*, one of the most important commercial fish in the upper São Francisco River (Sato *et al.* 2005), and evaluates the possible influence of the sex and total length of the fish on their parasite indexes. Additionally, the covariation between the helminth species and the dominant species of in the helminth community are presented.

MATERIAL AND METHODS

A total of 150 specimens of *P. argenteus* were collected in July, 2003 and January, 2004 from the Upper São Francisco River, downstream from the Três Marias Dam (18°12'32"S, 45°15' 41"W), State of Minas Gerais, by fishermen from the Estação de Hidrobiologia e Piscicultura da Companhia de Desenvolvimento dos Vales do São Francisco e do Parnaíba (EPT/CODEVASF). Fifty-three specimens were males varying from 24.8 to 40.2 cm total length (28.73 ± 4.37), and 97 specimens were females with total length varying from 23.3 to 40.8 cm (29.9 ± 7.7).

The host was identified following Britski *et al.* (1988) and the valid name of each host species follows Castro & Vari (2003). Author's name, valid scientific names and in some cases the citations for the respective synonyms follow the www.fishbase.org (Froese & Pauly 2009).

The organs of the digestive system and the coelom of each fish were separated and examined for parasites. The Endohelminths collected were fixed and processed following Amato *et al.* (1991) and Eiras *et al.* (2000).

Digeneans were identified according to Travassos *et al.* (1969) and classified following Jones (2005) and Overstreet & Curran (2005), and eucestodes were identified to genus according to Bona (1994), following the classification cited by Spassky (1995). Metacestodes were found encysted in large numbers along the viscera, and it was not possible to count them. Nematodes were identified and classified according to Travassos *et al.* (1928) and Moravec (1998), respectively, and acanthocephalans were identified following Nickol & Thatcher (1971) and classified according to Amin (1987). Ecological terms, prevalence, mean intensity, mean abundance and site of infection follows Bush *et al.* (1997).

The ecological analyses included only helminths with prevalence higher than 10%, in accordance with Bush *et al.* (1990). The Student's t-test (t) was used to compare total host length between males and females. Spearman's rank correlation coefficient (r_s) was used to calculate possible correlations between total host length and parasitic intensity and abundance. Pearson's coefficient correlation (r) was used to indicate the relationship between total host length and prevalence of helminths, with arcsene transformation of data (arc sen $\sqrt{\chi}$). The Chi-square test (χ^2) with Yate's correction and 2x2 contingency table was used to evaluate the prevalence of the parasites in relation to host sex. The Mann-Whitney test (U) was used to evaluate the parasitic intensity and abundance in relation to host sex. The possible covariation among the prevalence and abundance of parasite species was analyzed using Chi-square (χ^2) test and Spearman's rank correlation coefficient (r_s), respectively.

The statistical analysis followed Zar (1996) and the statistical significance was $p \leqslant 0.05$. The dominance frequency, the frequency of shared dominance and the relative dominance of each parasite species were calculated according to Rohde *et al.* (1995). Voucher specimens of *P. argenteus* were deposited in the Museum of Zoologia of the Universidade de São Paulo (USP), State of São Paulo, Brazil (MZUSP 95167). Voucher specimens of helminths were deposited in the Coleção Helmintológica do Instituto Oswaldo Cruz (CHIOC), State of Rio de Janeiro, Brazil (see respective deposit numbers below).

RESULTS

A total of 3,464 specimens were found (3,235 adults and 229 larvae) belonging to five species of helminths: *S. nanii* (Digenea, Haploporoidea, Chalcinotrematinae), CHIOC 36917, 36918a, b; *Valipora* sp. (Eucestoda, Cyclophyllidea, Gryporhynchidae) CHIOC 36914a, b, c; an unidentified metacestode (Eucestoda) CHIOC 36913a, b, c (these specimens were not included in the total sum of larvae); *S. asperus* (Nematoda, Spirurida, Cystidicolidae), CHIOC 36915a, 35916a, b and *Neoechinorhynchus prochilodorum* Nickol & Thatcher, 1971 (Acanthocephala, Eoacanthocephala, Neoechinorhynchidae), CHIOC 36919, 36920, and their ecological parameters are presented in table I. All these helminth species are reported here for the first time in *P. argenteus*, thus

Table I. Helminths and their parameters of infection of *P. argenteus* from the Upper São Francisco River, Minas Gerais. (SD) Standard deviation.

Helminths	Prevalence (%)	Range	Mean intensity ± SD	Mean abundance ± SD	Site of infection
Digenea					
Saccocoelioides nanii	64.0	1-177	32.60 ± 34.80	20.80 ± 31.90	Intestine
Eucestoda					
Metacestode	14.0	ND	ND	ND	Intestine serous
Valipora sp. (larvae)	2.00	1-4	2.300 ± 1.50	0.05 ± 0.40	Gall bladder
Nematoda					
Spinitectus asperus	33.3	1-10	2.40 ± 2.30	0.80 ± 1.80	Stomach
Acanthocephala					
Neoechinorhynchus prochilodorum	4.70	1-2	1.30 ± 0.50	0.06 ± 0.30	Intestine

(ND) Not determined.

expanding their geographic distribution to the São Francisco River. Of the total of 150 fish examined, 98 (65.3%) were parasitized, 63 (42.0%) of them with only one species of parasite, 24 (16.0%) with two, six (4.0%) with three, and five (3.3%) with four species. The total length of the female fish was significantly higher than that of the corresponding male (t = 2.02; p = 0.044).

Saccocoelioides nanii was the dominant species in the parasite community of *P. argenteus*, followed by *S. asperus* and the metacestodes (Tab. II). The parasitic parameters were not influenced by the sex of the host (Tab. III). *Saccocoelioides nanii* was significantly more abundant in smaller hosts (r_s = -0.21, p = 0.01). It was true for prevalence of the metacestodes found in the intestine (r = -0.91, p = 0.001) (Tab. IV). There was covariation in the prevalence (χ^2 = 11.103, p = 0.0009) and abundance (r_s = 0.30, p < 0.0001) between *S. nanii* and *S. asperus*.

DISCUSSION

According to the results of the present study, the helminth fauna of *P. argenteus* in the upper São Francisco River bears some similarity with that of *P. lineatus* from the Paraná River reported by Lizama *et al*. (2005). This resemblance is mostly due to shared species of *Saccocoelioides* Szidat, 1954, *Neoechinorhynchus* Hamann, 1892 (adult specimens) and *Valipora* Linston, 1927 (larvae). Adult digeneans stood out quantitatively, with *S. nanii* being the dominant species in *P. argenteus*. The significant covariation of the *S. nanii – S. asperus* pair in *P. argenteus* from the São Francisco River found in this study and the observation of helminths in *Prochilodus* spp. (besides digeneans, cestode larvae, nematodes – especially *Spinitectus* spp., and acanthocephalans – *Neoechinorhynchus* spp.) in other watersheds (Lizama *et al*. 2005) indicate that the transmission of these parasites to their definitive hosts may be through close intermediate hosts or through hosts that are more abundant in their habitats.

The presence of larvae of *Valipora* sp. in *P. argenteus* from the São Francisco River and in *P. lineatus* from the Paraná River (Lizama *et al*. 2005) is consistent with the hypothesis that the Prochilodontidae act as intermediate hosts in the life cycle of bird helminths, especially Ciconiiformes and Ardeidae (Bona 1994), in Brazil. Scholz *et al*. (2004) listed a wide spectrum of fish species used as hosts by these parasites, while stressing the absence of any definitive host records in various places where these metacestodes have been reported (e.g., South America). According to the authors, there are very few reports of larval pathogenesis in fish, but other studies will be necessary to clarify the subject.

Host total length as a reflection of age is an important factor in the variation of parasite infracommunities (Dogiel 1961). In the present work, the abundance of *S. nanii* was higher in smaller (presumably younger) fish, a fact that may result from peculiarities of the fish immune system, or influenced by the feeding habits (smaller fish can use resources that are not accessible to adults) or the environments frequented by young fish, with higher concentration of metacercariaes in vegeta-

Table II. Dominance analyses of helminths of *P. argenteus* from the Upper São Francisco River, Minas Gerais. (SD) Standard deviation.

Helminths	Frequency of dominance	Shared dominance	Mean relative dominance ± SD
Saccocoelioides nanii	95	2	0.62 ± 0.46
Spinitectus asperus	11	2	0.10 ± 0.26

Table III. Analysis of the influence of the sex of *P. argenteus* from the Upper São Francisco River, Minas Gerais, on the parasitic prevalence (χ^2), intensity (U) and abundance (U). (χ^2) Chi-square with Yate's correction, (U) Mann-Whitney test.

Helminths	Prevalence		Intensity		Abundance	
	χ^2	p	U	p	U	p
Saccocoelioides nanii	0.02	0.88	983.00	0.58	2553.00	0.96
Metacestode	0.28	0.59	ND	ND	ND	ND
Spinitectus asperus	1.32	0.25	218.00	0.751	2326.50	0.251

* Significant values, level of significance p < 0.05; (ND) not determined.

Table IV. Analysis of the influence of total length of *P. argenteus* from the Upper São Francisco River, Minas Gerais, on the parasitic prevalence (r), intensity (rs) and abundance (rs). (r) Pearson correlation, (rs) Spearman's rank correlation coefficient.

Helminths	Prevalence		Intensity		Abundance	
	r	p	rs	p	rs	p
Saccocoelioides nanii	- 0.65	0.08	- 0.11	0.28	-0.21	0.01*
Metacestode	-0.91	0.001*	ND	ND	ND	ND
Spinitectus asperus	- 0.43	0.30	0.23	0.11	-0.02	0.77

* Significant values, level of significance p < 0.05; (ND) not determined.

tion. One or all of these factors together can be implicated in the higher abundance of *S. nanii* in smaller fishes. Lizama *et al*. (2005), however, did not find a correlation between the parasitic parameters and host length.

Another important biotic factor determining the parasite communities of some fish species is host sex (Dogiel 1961). Even though sex was not found to affect parasite indexes in *P. argenteus,* a positive influence was reported for *P. lineatus* (Lizama *et al*. 2005). The lack of a correlation in our results may be explained by mininal behavioral and physiological differences between males and females of *P. argenteus*.

Among the helminths found, only *S. asperus*, which parasitizes the stomach of *P. argenteus*, is known to cause tissue lesions. Ramallo *et al*. (2000) reported macro- and microscopic lesions caused by this nematode in the cardiac and pyloric stomach regions of *P. lineatus*. Macroscopic lesions caused by *S. asperus* were not observed in the present research. This study and some others on the parasitic fauna of endemic fishes from the Upper São Francisco River (Brasil-Sato 2002, Brasil-Sato & Santos 2003, 2005, Santos & Brasil-Sato 2004, 2006) found that host sex did not influence parasite indexes. The relationship between standard lengths with parasitic indexes was reported to be variable, e.g.: a significant negative correlation between the prevalence of and a significant positive correlation between the intensity of *Neoechinorhynchus pimelodi* Brasil-Sato & Pavanelli, 1998 and the standard length of *Pimelodus maculatus* Lacépède, 1803 from the São Francisco River (Brasil-Sato & Pavanelli 1999); Santos & Brasil-Sato (2004) found that the prevalence of *N. pimelodi* also showed a negative correlation with the length of *Franciscodoras marmoratus* (Reinhardt, 1874). For the other parasites of *F. marmoratus* and for those of *Salminus brasiliensis* (Cuvier, 1817) and *Conorhynchos conirostris* (Valenciennes, 1840), no correlation was observed between the parasitic indexes and the total length of the hosts according to Santos & Brasil-Sato (2006), Brasil-Sato (2002) and Brasil-Sato & Santos (2005), respectively.

The influence of sex and age on the metazoan parasites of *P. lineatus* was analyzed by Lizama *et al*. (2005). In this host, a positive correlation between host length and parasitic abundance was found only for *S. nanii* and *S. magnorchis*. In *P. argenteus* the abundance of *S. nanii* was negatively correlated with the total host length. In *P. argenteus* the sex of the hosts did not influence the parasitic indexes.

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