# ENDOPARASITES OF *Rhaphiodon vulpinus* (Characiformes: Cynodontidae) AND THEIR RELATION TO THE STANDARD LENGTH AND SEX OF THEIR HOSTS

ENDOPARASITOS DE Rhaphiodon vulpinus (Characiformes: Cynodontidae) E SUAS RELAÇÕES COM O COMPRIMENTO PADRÃO E O SEXO DO HOSPEDEIRO

## Letícia Cucolo KARLING¹; Ana Carolina Figueiredo LACERDA¹; Filipe Mendonça Sella de ALVARENGA¹; Ricardo Massato TAKEMOTO¹,²; Gilberto Cezar PAVANELLI¹,2,3

1. Programa de Pós-graduação em Ecologia de Ambientes Aquáticos Continentais, Universidade Estadual de Maringá - UEM, Maringá, PR, Brasil. lekarling@hotmail.com; 2. Programa de Pós-graduação em e Biologia Comparada- UEM, Maringá, PR, Brasil; 3. Programa de Pós-graduação em Aquicultura e Desenvolvimento Sustentável, Universidade Federal do Paraná, Palotina, PR, Brasil.

**ABSTRACT:** Fish parasites are often related to some characteristics of their hosts, especially the length or age and the sex. The aims of the present study were to investigate the endoparasitic fauna of *Rhaphiodon vulpinus* and to test: (1) whether the parasitism is positively correlated with the standard length of the hosts; (2) parasitism levels in males and females, with the objective of detecting possible differences in the reproductive behaviour of the hosts. Samples of fish were taken from the floodplain of the upper Paraná River, from July 2004 to September 2008, quarterly; methodology for fish necropsy and fixation, conservation and preparation of parasites was based on specialized literature. The analysis of 39 hosts revealed that 15 fish were parasitized by at least one species of endoparasite. The species *Contracaecum* sp. (Nematoda) and *Quadrigyrus* sp. (Acanthocephala) were recorded, both at the larval stage. Considering both parasites, the prevalence and abundance of parasitism were not correlated with the standard length or the sex of hosts. From these results it is possible to infer that the length and the sex of the hosts had no influence on the immune response to infection, and that males and females present ecological similarities. This is the first record of the genera *Quadrigyrus* parasitizing *R. vulpinus*.

**KEYWORDS:** Ecology. Fish. *Dourado-fação*. Floodplains. Brazil.

## INTRODUCTION

The fish Rhaphiodon vulpinus Agassiz, (Cynodontidae), is popularly known as 'dourado-fação'. This species is widely distributed in South America, occurring in the basins of the Rivers Orinoco, Amazon, Paraná-Paraguay, Uruguay (TOLEDO-PIZA, 2000) and the middle and upper Tocantins River (NEUBERGER et al., 2007). It is a large, piscivorous species (GRAÇA and PAVANELLI, 2007), with significance for sport fishing and aquaculture (FROESE et al., 2008). It feeds on small to medium-sized fishes, mainly Prochilodus lineatus (Prochilodontidae) and Astyanax altiparanae (Characidae) (HAHN et al., 2004). Research that needs to be performed regarding this host are taxonomic and ecological studies on its parasites.

The influence exerted by parasites on their hosts is a widely studied theme in ichthyoparasitology (LEWIS et al., 2002). In an attempt to determine features of the hosts that could serve as an indirect indicator of possible negative effects of parasites on the host's physiology, some

studies have correlated parasitism levels to total length (ISAAC et al., 2004; TAVERNARI et al., 2005), condition factor (LIZAMA et al., 2006; LACERDA et al., 2009), sex (GUIDELLI et al., 2006; YAMADA et al., 2007), and hepatosomatic and splenosomatic relationships (LIZAMA et al., 2006; LACERDA et al., 2009) of the hosts.

According to Poulin (1998), the host's length is a good predictor of parasite richness because larger hosts provide a greater diversity of niches for parasites, consume more prey (which potentially harbour the larvae of parasites), and have greater longevity, thus providing a more stable habitat for parasites than ephemeral, small, shortlived hosts.

The aim of the present study was to identify the endoparasitic fauna of *R. vulpinus*, a freshwater fish, and to test the hypothesis that their parasites are positively correlated with the length of the hosts. In addition, parasitism levels were investigated in males and females, with the objective of detecting possible differences in the reproductive behaviour of the hosts.

#### MATERIAL AND METHODS

Samples of fish were taken from the floodplain of the upper Paraná River, the limit being between the States of Paraná and Mato Grosso do Sul, Brazil, near the City of Porto Rico (22°43' S and 53°10' W). Fish were caught from July 2004 to September 2008, quarterly, using gill nets exposed for 24 hours at different sampling points, as part of the Brazilian Long-Term Ecological Research, Programme (Programa Ecológico de Longa Duração (PELD)/Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) – site 6). For each analysed fish, standard length and sex were recorded.

Methodology for fish necropsy and fixation, conservation and preparation of endoparasites was based on EIRAS et al. (2006). For the collection of endoparasites, the fish were eviscerated through an incision in the ventral surface, from the anus to the pectoral fins. The organs were separated and placed in Petri dishes, the visceral cavity was washed with formalin, and all the contents were examined under a stereomicroscope. Cysts were fixated in formalin and conserved in 70% ethanol. For the identification of parasites, specimens were clarified using lactic carmine acid (nematodes) or acetic (acanthocephalans) and mounted in permanent slides using Canada balsam. The identification of parasites was in accordance with the studies of Yamaguti (1963), Moravec (1998), Rego (1999), Thatcher (1993) and Kohn and Fernandez (1994). The ecological terminology followed BUSH et al. (1997), the prevalence of infection meaning the number of hosts infected with one or more individuals of a particular species, divided by the number of hosts examined for that parasite species, and abundance meaning the number individuals of a particular parasite in/on a single host regardless of whether or not the host is infected. Data analyses used included: t tests, to verify significant differences between the standard length of male and female fish, to make sure that significant differences in parasitism between the sexes was not due to differences in length; Pearson correlation coefficient 'r', to determine the correlation between the host's standard length and the prevalence of infection; Spearman's rank correlation coefficient 'rs', to determine possible correlations between the host's standard length and the abundance of infection; the Mann-Whitney U test, to check the influence of the host's sex on the abundance of infection of each parasite species; and the statistic G log-likelihood, using a 2 x 2 contingency table to determine the effect of host sex on the prevalence of each parasite species (ZAR, 1996). Statistical analyses were applied to parasite species with a prevalence higher than 10% and the results were considered significant when  $p \le 0.05$ .

## **RESULTS**

The analyses of 39 hosts with length between 24.5 and 57.4 (37.9±6.9), being 14 females and 25 males, revealed that 15 fish were parasitized by at least one species of endoparasite, corresponding to a total prevalence of 38.46% and a mean intensity of 9.26 parasites per fish. All parasites were in the larval stage. Four parasites were recorded in the mesentery, but only *Contracecum* sp. nematodes and *Quadrigyrus* sp. acanthocephalans were present with a prevalence higher than 10%. In addition, two other non-identified species were found with low prevalence (5.12% and 3.9%). Parasites and parasitism indexes are presented in Tables 1 and 2.

**Table 1.** Levels of parasitism in the mesentery of 14 females and 25 males of *Rhaphiodon vulpinus* collected from the floodplain of the upper Paraná River from July 2004 to September 2008.

Parasites	Prevalence		Mean intensity		
	Females	Males	Females	Males	
Contracaecum sp.	29	40	12.8±13.8 (2-36)	6.6±7.1 (1–22)	
Quadrigyrus sp.	14	12	4.5±2.5 (2–7)	3.3±3.3 (1–8)	
Nematoda sp. 1	7	0	2	0	
Nematoda sp. 2	0	4	0	1	

**Table II.** Results of Pearson's correlation coefficient 'r' and Spearman's rank correlation coefficient 'rs' correlating the prevalence and abundance of parasites with the standard length of the host, and results of the G log-likelihood statistics and Mann–Whitney's U test with normal approximation of Z, to determine differences in prevalence and abundance of parasite species according to the sex of the hosts, regarding two larval parasites collected in the mesentery of *Rhaphiodon vulpinus* from the floodplain of the upper Paraná River from July 2004 to September 2008.

	Parasitism index		Parasites	
Tests and statistics			Contracaecum sp.	Quadrigyrus sp.
Pearson's correlation coefficient	Prevalence	r	-0.779	0.067
		p	0.446	0.374
Spearman's rank correlation coefficient	Abundance	rs	-0.136	-0.154
		p	0.426	0.368
G log-likelihood	Prevalence	G	0.518	0.041
		p	0.518	0.838
Mann–Whitney's $U$ test with normal approximation of $Z$	Abundance	Z	0.409	0.146
		p	0.681	0.883

No significant difference was observed between the standard length of males and females of R. vulpinus (t = 2.04, p = 0.83). Considering Contracecum sp. and Quadrigyrus sp., the prevalence and abundance of parasitism were not correlated with the standard length or the sex of the hosts (Table II).

#### **DISCUSSION**

Considering that only larval parasites were found in *R. vulpinus*, it is possible to consider that this fish acts as an intermediate and/or paratenic host in the upper Paraná River floodplain. Piscivorous fish often harbour adult parasites, acting as definitive hosts, since they ingest the larval forms of parasites with their food (intermediate hosts). In the present study, the presence of *Contracaecum* and *Quadrigyrus* larvae, and their location in the mesentery, places *R. vulpinus* as an intermediate host, providing the basis for the development of these organisms and intermediating the way to their definitive hosts, a fish-eating bird (MORAVEC, 1998).

Parasitism levels can be related to the standard length of the host (DOGIEL, 1958), as well as their age (SHOTTER, 1976). Thus, it is expected that with the increasing age of a host, parasitism levels rise due to the cumulative effect achieved by the increase in food consumption by the host and/or the increasing exposure to parasites. However, Bush et al. (2001) pointed that when the studied hosts are fish, extreme caution is necessary because these organisms often undergo ontogenetic changes that are followed by changes in diet. In *R. vulpinus* it was shown that the abundance and prevalence of parasites are not correlated with the standard length of the host.

According to Poulin (2000), the relationship between fish size and intensity of parasite infection

is usually positive, but varies from statistically nonsignificant to highly significant, and this variation may result from: the exact measure of, or the range of, fish sizes used; whether abundance or intensity is used as a measure of parasite numbers; or from biological differences between parasites. Considering the range of length used in the study, and the use of both prevalence and intensity of infection in the analyses, the absence of correlation in the case of *R. vulpinus* may reflect the low intensity of both species and the stage of development of the parasite.

For some fish species differences between the sexes become clearer during the reproductive period, due to physiological and behavioural changes (LIZAMA et al., 2008), as for Cicha monoculus (MACHADO et al., 2000) and Acestrorhynchus lacustris (CARVALHO et al., 2003). But other studies in the upper Paraná River have shown that host sex does not influence the level of parasitism (MACHADO et al., 1994; TAKEMOTO and PAVANELLI, 2000). In this study, no significant difference was observed between the prevalence and abundance of parasites in relation to the sex of the host. This absence of differences possibly occurred due to ecological similarities between males and females, e.g. habitat, behaviour and diet. It is noteworthy that this is the first record of the genera Quadrigyrus parasitizing R. vulpinus.

Rhaphiodon vulpinus is now widely distributed in the Upper Paraná, but came originally

from the middle and lower parts of the basin, colonizing the upper stretches after the flooding of the Sete Quedas Falls, a natural barrier, caused by flooding of the Itaipu Dam, in 1983 (AGOSTINHO et al., 2003). Low parasite burden in introduced species has been documented and is attributed to a series of causes: (1) introduced populations are often derived from a small subset (and sometimes non-infected) of hosts, and this reduces the probability of introducing parasites with a host species; (2) many heteroxenous parasites have life cycles that require more than one host, and not all the hosts are present in the new environment and; (3) the bottleneck effect, which occurs immediately after the introduction of a host species, can interrupt the transmission of parasites present in a founder population (TORCHIN et al., 2002). Considering that R. vulpinus is not native to the upper stretch of the Paraná River, the low parasite richness and parasitism levels may be explained by the hypothesis mentioned.

### **ACKNOWLEDGEMENTS**

The authors wish to thank Nupélia (Nucleo de Pesquisas em Limnologia, Ictiologia e Aquicultura)/State University of Maringá for logistic support and CNPq for financial support of the project PELD/site 6. L. C. Karling was supported by CNPq with a Masters scholarship; A. C. F. Lacerda was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) with a PhD scholarship; R. M. Takemoto and G. C. Pavanelli were supported with productivity grants from CNPq.

**RESUMO:** Os parasitas de peixes são frequentemente relacionados com algumas características de seus hospedeiros, especialmente o comprimento ou a idade e o sexo. O objetivo do presente estudo foi investigar a fauna endoparasitária de *Rhaphiodon vulpinus* e testar as hipóteses: (1) o parasitismo está correlacionado positivamente com o comprimento padrão dos hospedeiros, (2) detectar diferenças no nível de parasitismo entre hospedeiros machos e fêmeas. As amostras de peixes foram coletadas trimestralmente na planície de inundação do alto rio Paraná, entre 2004 à 2008; a metodologia para a necropsia de peixes e de fixação, conservação e preparação dos parasitas foi baseada na literatura especializada. A análise dos 39 hospedeiros revelou que 15 peixes estavam parasitados por pelo menos uma espécie de endoparasitas. As espécies *Contracaecum* sp. (Nematoda) e *Quadrigyrus* sp. (Acanthocephala) foram registradas, todas na fase larval. Considerando ambos os parasitas, a prevalência e a abundância de parasitismo não foram correlacionadas com o comprimento padrão ou o sexo dos hospedeiros. A partir desses resultados é possível inferir que o tamanho e o sexo dos hospedeiros não influenciaram na resposta imune à infecção, e que os peixes machos e fêmeas apresentam similaridades ecológicas. Este é o primeiro registro do gênero *Quadrigyrus* parasitando *R. vulpinus*.

PALAVRAS-CHAVE: Ecologia. Peixe. Dourado-facão. Planície de inundação. Brasil.

#### **REFERENCES**

AGOSTINHO, A. A.; GOMES, L. C.; SUZUKI, H. I.; JÚLIO, JR H. F. Migratory fish from the upper Paraná river basin, Brazil. In: CAROLSFELD, J.; HARVEY, B.; ROSS, C.; BAER, A. (Ed.). **Migratory Fishes of South America: Biology, Fisheries and Conservation Status.** Victoria, World Fisheries Trust, the World Bank and the International Development Research Centre. Washington, 2003. cap. 1, p. 19-99.

BUSH, A. O.; FERNANDEZ, J. C.; ESCH, G. W.; SEED, R. Parasitism: The Diversity and Ecology of Animal Parasites. Cambridge University Press, Cambridge, 2001. 531p.

BUSH, A. O.; LAFFERTY, K. D.; LOTZ, J. M.; SHOSTAK, A. W. Parasitology meets ecology on its own terms: Margolis et al. revisited. **Journal of Parasitology**, Lawrence, v. 83, n. 4, p. 575-583, 1997.

CARVALHO, S.; GUIDELLI, G. M.; TAKEMOTO, R. M.; PAVANELLI, G. C. Ecological aspects of endoparasite fauna of *Acestrorhynchus lacustris* (Lutken, 1875) (Characiformes, Acestrorhynchidae) on the Upper Paraná River floodplain, Brazil. **Acta Scientiarum. Biological Sciences**, Maringá, v. 25, p. 479-483, 2003.

- DOGIEL, V. A. (1958). Ecology of the parasites of freshwater fishes. In: DOGIEL, V.A.; PETRUSHEVSKI, G. K.; POLYANSKI, YU. I. (Eds.). **Parasitology of Fishes** (Engl. transl.). Oliver & Boyd, Edinburgh & London, 1958, p. 1–47.
- EIRAS, J. C.; TAKEMOTO, R. M.; PAVANELLI, G. C. **Métodos de estudo e técnicas laboratoriais em parasitologia de peixes.** Eduem, Maringá, 2006. 199p.
- FROESE, R.; PAULY, D. Editors. **FishBase.** World Wide Web electronic publication. http://www.fishbase.org, accessed on: 7 August 2008.
- GRAÇA, W. J.; PAVANELLI, C. G. Peixes da planície de Inundação do Alto Rio Paraná e áreas adjacentes. Eduem, Maringá, 2007. 241p.
- GUIDELLI, G. M.; TAVECHIO, W. L.; TAKEMOTO, R. M.; PAVANELLI, G. C. Fauna parasitária de *Leporinus lacustris* e *Leporinus friderici* (Characiformes, Anostomidae) da planície de inundação do alto rio Paraná, Brasil. **Acta Scientiarum. Biological Sciences**, Maringá, v. 28, p. 281-290, 2006.
- HAHN, N. S.; FUGI, R.; ANDRIAN, I. F. Trophic ecology of the fish assemblages. In: **The Upper Paraná River and its Floodplain.** THOMAZ, S. M.; AGOSTINHO, A. A.; HAHN, N. S. (Ed.). Eduem, Maringá, 2004. p. 247-270.
- ISAAC, A., GUIDELLI, G. M.; FRANÇA, J. G.; PAVANELLI, G. C. Composição e estrutura das infracomunidades endoparasitárias de *Gymnotus* spp. (Pisces: Gymnnotidae) do rio Baía, Mato Grosso do Sul, Brasil. **Acta Scientiarum. Biological Sciences**, Maringá, v. 26, p. 453-462, 2004.
- KOHN, A.; FERNANDES, B. M. M. *Rhipidocotyle gibsoni* n. sp. from a Brazilian fresh water fish and *Rhipidocotyle froesi* n. sp. for *R. baculum* (Linton, 1905) of Eckmann (1905) (Bucephalidae; Digenea). **Memórias do Instituto Oswaldo Cruz**, Rio de Janeiro, v. 89, p. 567-570, 1994.
- LACERDA, A. C. F.; TAKEMOTO, R. M.; PAVANELLI, G. C. Ecology of endoparasites of the fluvial stingray *Potamotrygon falkneri* (Chondrichthyes: Potamotrygonidae) from the upper Paraná river, Brazil. **Brazilian Journal of Biology**, São Carlos, v. 69, p. 297-303, 2009.
- LEWIS, E. E.; CAMPBELL, J. F.; SUKHDEO, M. V. K. **The Behavioural Ecology of Parasites.** CABI Publishing, Wallingford, Oxfordshire, 2002. 384p.
- LIZAMA, M. DE LOS A. P.; TAKEMOTO, R. M.; PAVANELLI, G. C. Parasitism influence on the hepato, splenosomatic and weight/length relation and relative condition factor of *Prochilodus lineatus* (Valenciennes, 1836) (Prochilodontidae) of the Upper Paraná River Floodplain, Brazil. **Revista Brasileira de Parasitologia Veterinária**, Jaboticabal, v.15, p. 116-122, 2006.
- LIZAMA, M. DE LOS A. P.; TAKEMOTO, R. M.; PAVANELLI, G. C. Ecological aspects of metazoan parasites of *Astyanax altiparanae* Garutti & Britski, 2000 (Characidae) of the Upper Paraná River Floodplain, Brazil. **Instituto de Pesca**, São Paulo, v. 34, p. 527-533, 2008.
- MACHADO, P. M.; ALMEIDA, S. C.; PAVANELLI, G. C.; TAKEMOTO, R. M. Ecological aspects of endohelminths parasitizing *Cichla monoculus* Spix, 1831 (Perciformes: Cichlidae) in the Paraná River near Porto Rico, State of Paraná, Brazil. **Comparative Parasitology**, Lawrence, v. 67, p. 210-217, 2000.
- MACHADO, M. H.; PAVANELLI, G. C.; TAKEMOTO, R. M. Influence of host's sex and size on endoparasitic infrapopulations of *Pseudoplatystoma corruscans* and *Schizodon borellii* (Osteichthyes) of the high Paraná River, Brazil. **Revista Brasileira de Parasitologia Veterinária**, Jaboticabal, v. 3, p. 143-148, 1994.
- MORAVEC, F. Nematodes of Freshwater Fishes of the Neotropical Region. Academia, Praha, 1998. 464p.

NEUBERGER, A. L.; MARQUES, E. E.; AGOSTINHO, C. S.; OLIVEIRA, R. J. Reproductive biology of *Rhaphiodon vulpinus* (Ostariophysi: Cynodontidae) in the Tocantins River Basin, Brazil. **Neotropical Ichthyology**, Porto Alegre, v. 5, p. 479-484, 2007.

POULIN, R. Evolutionary Ecology of Parasites: From Individuals to Communities. London, Chapman & Hall, 1998.

POULIN, R. Variation in the intraspecific relationship between fish length and intensity of parasitic infection: biological and statistical causes. **Journal of Fish Biology**, London, v. 56, p. 123–137, 2000.

REGO, A. A.; CHUBB, J. C.; PAVANELLI, G. C. Cestodes in South American freshwater teleost fishes: keys to genera and brief description of species. **Revista Brasileira de Zoologia**, Curitiba, v. 16, p. 299-367, 1999.

SHOTTER, R. A. The distribution of some helminth and copepod parasites in tissues of whiting, *Merlangus merlangus* L., from Manx waters. **Journal of Fish Biology**, London, v. 8, p. 101-117, 1976.

TAKEMOTO, R. M.; PAVANELLI, G. C. Aspects of the ecology of proteocephalid cestodes parasites of *Sorubim lima* (Pimelodidae) of the Upper Paraná River, Brazil: structure and influence of host's size and sex. **Brazilian Journal of Biology**, São Carlos, v. 60, p. 577-584, 2000.

TAVERNARI, F. C.; BELLAY, S.; TAKEMOTO, R. M.; GUIDELLI, G. M.; LIZAMA, M. DE A. P.; PAVANELLI, G. C. Ecological aspects of *Diplectanum piscinarius* (Platyhelminthes, Monogenea) parasite of gills of *Plagioscion squamosissimus* (Osteichthyes, Sciaenidae) in the Upper Paraná River floodplain, Brazil. **Acta Scientiarum. Biological Sciences**, Maringá, v. 27, p. 225-229, 2005.

THATCHER, V. E. **Trematódeos Neotropicais.** Instituto Nacional de Pesquisas da Amazônia, Manaus, 1993. 553p.

TOLEDO-PIZA, M. The Neotropical fish subfamily Cynodontinae (Teleostei: Ostariophysi: Characiformes): a phylogenetic study and a revision of *Cynodon* and *Rhaphiodon*. **American Museum Novitates**, New York, v. 88, 2000.

TORCHIN, M. E.; LAFFERTY, K. D.; KURIS, A. M. Parasites and marine invasions. **Parasitology**, Cambridge, v. 124, p. 137-151, 2002.

YAMADA, F. H.; TAKEMOTO, R. M.; PAVANELLI, G. C. Aspectos ecológicos dos ectoparasitos branquiais de *Satanoperca pappaterra* (Heckel, 1840) (Cichlidae) da planície de inundação do alto rio Paraná, Brasil. **Acta Scientiarum. Biological Sciences**, Maringá, v. 29, p. 331-336, 2007.

YAMAGUTI, S. **Systema Helminthum. Monogenea and Aspidocotylea.** Interscience Publishers, New York, 1963. 699p.

ZAR, J. H. Biostatistical Analysis. 2nd. Ed. Englewood Cliffs, Prentice-Hall, New Jersey, 1996. 662p.