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Endoparasitic fauna of *Serrasalmus* spp. (Characidae: Serrasalminae) in a neotropical floodplain

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ABSTRACT. Floodplains systems present complex biodiversity and upper Paraná river floodplain retains a wide variety of terrestrial and aquatic species. *Serrasalmus marginatus* (nonindigenous species) and *Serrasalmus maculatus* (native species) are part of this biodiversity component and can be infected by trophic transmission. In order to understand the ecological processes within the upper Paraná river floodplain, endoparasite fauna of those two hosts species were analyzed, considering prevalence, abundance, intensity and richness variables to determine this relationship. So, abundance of acantocephalan *Echinorhynchus* sp. was positively correlated to *S. marginatus* length, while *Kritskyia annakohnae* (Monogenea) prevalence and *S. marginatus* length presented a negative correlation. *S. marginatus* relative condition factor (Kn) was negatively and significantly associated to the abundance of *K. annakohnae*, and Kn of infected fish by that species is lower compared with the non- infected fish. Since hosts are in the context of the introducing of species, the research of endoparasites ecological variables allows to understansd infracommunity and component community as a way of analyzing the consequence of these parasites distribution in the native and non-native species.

Keywords: neotropical floodplain, parasite ecology, invasive species, piranha.

Fauna endoparasitária de *Serrasalmus* spp. (Characidae: Serrasalminae) de uma planície de inundação neotropical

RESUMO. Sistemas de inundação possuem uma complexa biodiversidade. Por causa desta característica, a planície de inundação do alto rio Paraná abriga uma gama muito grande de espécies aquáticas e terrestres. *Serrasalmus marginatus* (espécie introduzida) e *Serrasalmus maculatus* (espécie nativa) compõem a ictiofauna desta área, e estes hospedeiros podem ser infectados por endoparasitos pela transmissão trófica. A fauna endoparasitária das duas espécies de peixes foi analisada por meio da prevalência, abundância, intensidade e riqueza para averiguar a relação destas com as variáveis dos hospedeiros. Observou-se que a abundância do acantocéfalo *Echinorhynchus* sp. está positivamente correlacionada com o comprimento de *S. marginatus*, enquanto existe uma correlação negativa e significativa entre a prevalência de *Kritskyia annakohnae* (Monogenea) e o comprimento de *S. marginatus.* O fator de condição relativo (Kn) de *S. marginatus* está relacionado negativa e significativamente com a abundância de *K. annakohnae*, e o Kn dos peixes parasitados por esta mesma espécie é mais baixo se comparado com os peixes não parasitados. Como os hospedeiros estão no contexto de introdução de espécies, as investigações das variáveis ecológicas dos endoparasitos possibilitam o conhecimento de cada infracomunidade e comunidade componente, como forma de delimitar a atuação dos parasitos na mediação da relação ecológica entre a espécie nativa e não nativa.

Palavras-chave: planície de inundação neotropical, ecologia parasitária, espécie invasora, piranha.

Introduction

Floodplains are the most dynamic systems, because of a large and complex biodiversity existence and maintenance (Powers, Sun, Parker, Dietrich, & Wootton, 1995). Besides, floodplains form a wide aquatic habitats variety (as rivers, lagoons and canals) associated to the transition between aquatic and terrestrial environments (Junk, 1980). These complexity occurs because of flood pulses, which are considered the main force to regulates the community and ecosystem structure and activity (Junk, Bayley, & Sparks, 1989).

However, fish communities are adapted to the hydrological and geomorphological environment (Agostinho, Gomes, & Zalewski, 2001). So, structure changes in ecosystems can cause species introductions (Lockwood, Hoopes, & Marchetti, 2007). Agostinho, Julio Júnior, and Petrere Júnior (1994) reported, in the upper Paraná river floodplain, at least 17 exclusive species of the lower Paraná river that colonized the upper Paraná river because of the Itaipu reservoir, that was constructed 150 km downstream of the Sete Quedas falls, a geographical barrier for many fish species.

This introduction process happens to *S. marginatus* Valenciennes, 1837 and *S. maculatus* Kner, 1858[1]since the Itaipu reservoir construction, which caused the two population fauna mixing. Because of this process, *S. marginatus* achieved large population growing in the new habitat, demonstrated by the increasing number of specimens collected (Agostinho, 2003).

Regarding to the evolutionary time in the earth, fishes are the most parasitized vertebrates. Thus, they are exposure and adapted to parasitic organisms in a longer time and, besides, fishes live in aquatic environments which facilitate parasites transmission and spread (Malta, 1984). This transmission can happen in the food chain, where the hosts are infected by trophic transmission or by water (direct cycle)

Minchella and Scott (1991) assumed the parasites relevance to the ecosystem saying that they participate in the food chain, of the biomass of hosts (much of this is formed by the parasitic populations), of the imposition of energy demands to their hosts, increasing their mortality rate and of the outcome changes in interspecific competition. Furthermore, the parasites may help to increase host susceptibility to predation, influencing it in the choice of partner and still increasing the sex ratio of the host population. So, there is a several ways in which parasites act on the hosts population dynamics, influencing the abundance and diversity of species in the environment.

According to Bonsall and Hassell (1997) in a situation in which the parasite cause some damage to the native host, classical ecological theory predicts that simple interactions in which species share common natural enemies are unstable, leading to one species to be eliminated from the interaction. This effect arises through competitive interactions mediated by the natural enemies ‘apparent competition’.

Therefore, it is fundamental to study the host-parasite-environment relationship and analyze how fish and parasites population influences each other. Since hosts are in introduction species context, endoparasites ecological research allows to learn better these communities in order to determine parasites distribution in native and non-native host species as a way to analyze the influence of parasites in their inter and intraspecific competition (apparent competition).

[1]*S. maculatus* Kner, 1858 earlier identified as *S. spilopleura* Kner, 1860 (Jégu & Santos, 2001).

Material and methods

Sampling

Fishes were collected in the upper Paraná river floodplain (22°45’S and 53°16’W) in Mato Grosso do Sul State next to Porto Rico city - PR (Figure 1). Samples were made in many environments: canals, rivers, open and closed lagoons, performed by Ilter- CNPq project (International Long Term Ecological Research) - Site 6.

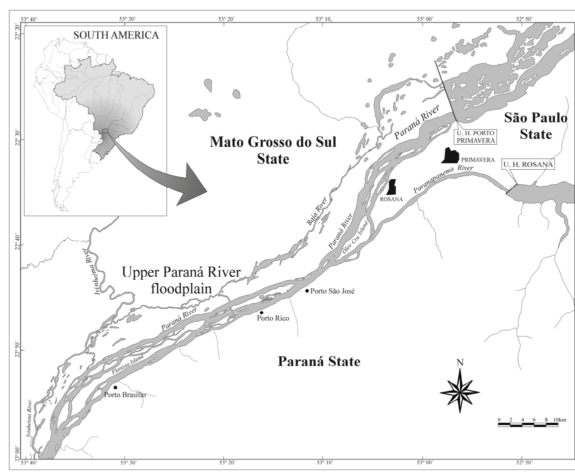

Figure 1. Paraná river floodplain, Paraná State/Mato Grosso do Sul State, Brazil.

Fishes were caught using different mesh sizes gill nets, from March 2013 until September 2014. This act was authorized by the Ethics Committee of the State University of Maringá (CEAE - Opinion 123/2010) and Ibama (22442-1). After identification, for each specimen captured, the following data were recorded: date, season and sampling point, total length (cm) and standard length (cm), total weight (g), sex, gonadal maturity stage and gonadal weight (g). Body condition was estimated by the relative condition factor (Kn), which is the relation between observed weight and weight provided by a regression of weight/length considering all fish sample (Le Cren, 1951).

Hosts autopsy and endoparasites collecting, fixing and preservation were made according to

Eiras, Takemoto, and Pavanelli (2006); ectoparasites were not considered. Parasitological variables analyzed were: infracommunity richness (parasite species number per host individual), prevalence (number of hosts infected per number of hosts examined given as percentage), abundance (number of individuals of a particular parasite in/on a single host regardless of whether or not the hosts is infected) and intensity (number of individuals of a particular parasite species in a single infected host (Bush, Lafferty, Lotz, & Shostak, 1997).

Data analysis

Only parasite species that presented over 10% prevalence were considered in ecological analyses (Bush, Aho, & Kennedy, 1990). To determine a possible correlation between the abundance/host size and abundance/relative condition factor (Kn), Spearman's rank correlation coefficient "rs" was used, obtained according to Zar (2010). Pearson correlation coefficient "r" was applied to detect correlations between parasite prevalence and host size. For this test, prevalence data was previously transformed angularly (arc sine) and samples of hosts were separated into standard length classes (Zar, 2010).

Nonparametric Mann-Whitney test (U) with normal approximation Z was used to determine differences between Kn of infected and non-infected individuals and to determine hosts sex influence in the infection abundance of each parasite species (Zar, 2010). The G loglikelihood test, using a 2 x 2 contingency table, was used to verify the host sex influence in endoparasites infection prevalence (Zar, 2010).

Subsequent analysis contemplated each specimen infracommunity to two fish species. Each infracommunity diversity was calculated using Brillouin diversity index (H) (Zar, 2010). Dominance was estimated by Berger-Parker index (Magurran, 2004), d = Nmax./Nt; Nmax. refers to number of individuals in most abundant parasite species and Nt refers to total number of individuals in the sample.

Spearman's rank correlation coefficient "rs" was used to evaluate correlations between host standard length and species diversity (Brillouin index) and between relative condition factor and diversity (Zar, 2010). In order to verify differences between hosts males and females due to parasitic infracommunities diversity, Mann-Whitney U test was used (Zar, 2010).

Statistical analyzes were made using BioEstat 5.0 (Ayres, Ayres, Ayres, & Santos, 2007) and Past (Hammer, Harper, & Yan, 2001) programs.

Results

In total, 58 fish were collected, 27 *S. marginatus* (standard length: 10.5 to 21.5) and 31 *S. maculatus* (standard length: 9.0 to 23.0). Endoparasites found in these two fish species totaled 897 individuals, with ten different species; five of those species present simultaneously in two hosts species (Table. 1). In total, eight endoparasite species were found in *S. marginatus* and seven species in *S. maculatus*.

Kritskyia annakohnae, *Echinorhynchus* sp. *Contracaecum* sp. *Contracaecum* sp. type 1 showed over 10% prevalence in *S. marginatus* and *S. maculatus*. Thus, following statistics analyzes contemplated such this species.

Table 1. Endorasites of *Serrasalmus marginatus* and *S. maculatus* from upper Paraná river Floodplain, collected from March 2013 to September 2014. P = prevalence; MA = mean abundance; MI = mean intensity.

Host species	Taxonomic group	Parasite species	Infection site	P (%)	MA	MI
S. marginatus	Monogenea	*K. annakohnae* Boeger, Tanaka & Pavanelli, 2001	Urinary bladder	71.42	4.53	6.35
	Acanthocephala	*Echinorhynchus* sp.	Intestine and Pyloric cecum	60.71	6.28	10.35
	Nematoda	*Procamallanus (Spirocamallanus) inopinatus* Travassos, Artigas &Pereira, 1928	Intestine	3.57	0.035	1
		Contracaecum sp. (larva) Railliet & Henry, 1912	Mesentery	17.85	0.32	1.80
		Contracaecum sp. type 1 of Moravec, Kohn & Fernandes, 1993	Mesentery	28.57	0.71	2.25
		Spiroxys sp.	Mesentery	3.57	0.035	1
		Hysterothylacium sp. (larva) Ward &Magath, 1917	Mesentery	3,57	0.035	1
		Goezia sp. (larva) Zeder, 1800	Mesentery	3.57	0.035	1
S. maculatus	Monogenea	*K. annakohnae* (Boeger, Tanaka & Pavanelli, 2001)	Urinary bladder	65.62	13.65	20.80
	Acanthocephala	*Echinorhynchus* sp.	Intestine and Estomach	50	2.65	5.31
	Nematoda	*Procamallanus (Spirocamallanus) inopinatus* Travassos, Artigas&Pereira, 1928	Pyloric cecum	3.57	0.035	1
		Procamallanus (Spirocamallanus) neocaballeroi (Caballero-Deloya, 1977)	Intestine	3.57	0.035	1
		Contracaecum sp. (larva) Railliet& Henry, 1912	Mesentery	34.37	0.84	2.45
		Contracaecum sp. type 1 of Moravec, Kohn & Fernandes, 1993	Mesentery	28.57	0.9	2.07
		Contracaecum sp. type 2 of Moravec, Kohn & Fernandes, 1993	Mesentery	9.37	0.09	1

Spearman's rank correlation coefficient "rs" showed positive and significant correlation between *S. marginatus* standard length and *Echinorhynchus* sp. abundance (Figure 2 and Table 2). Pearson correlation coefficient "r" showed a significant negative correlation only between *S. marginatus* standard length and *K. annakohnae* prevalence (Figure. 3 and Table. 2).

Fish relative condition factor (Kn) ranged from 0.89 to 1.42 for *S. marginatus* and 0.36 to 1.35 for *S. maculatus*. According to Spearman's rank correlation coefficient "rs", *S. marginatus* relative condition factor was correlated negatively and significantly with *Kritskyia annakohnae* abundance (Figure 4 and Table 3).

According to Mann-Whitney U tests, *S. marginatus* Kn differ between infected and non-infected fish to *K. annakohnae*, taking into account that the Kn of the infected fish was lower if compared to the Kn of non-infected fish. (Table 3).

Considering a total of 27 *S. marginatus* specimens, 15 were female and 12 were males. Those, 13 females (86.66%) and 11 male (91.66%) were infected by at least one endoparasite species. On the other hand, *S. maculatus* presented a total of 31 specimens, which 16 female and 15 male. Those, 15 (93.75%) females and 12 (80%) males were infected by at least one endoparasite species. Mann-Whitney U tests, and G loglikelihood showed that species abundance and parasites prevalence did not differ between males and females (Table. 4).

Spearman's rank correlation coefficient "rs" did not show significant correlation between infracommunities diversity (Brillouin Index) and hosts standard length for both: *S. marginatus* (rs = 0.302, p = 0.1613) and *S. maculates* (rs = 0.016, p = 0.937). Similarly, there was no significant correlation between relative condition factor and parasites mean diversity for *S. marginatus* (rs = -0.2273, p = 0.2969) or *S. maculatus* (rs = -0.0438; p = 0.8282).

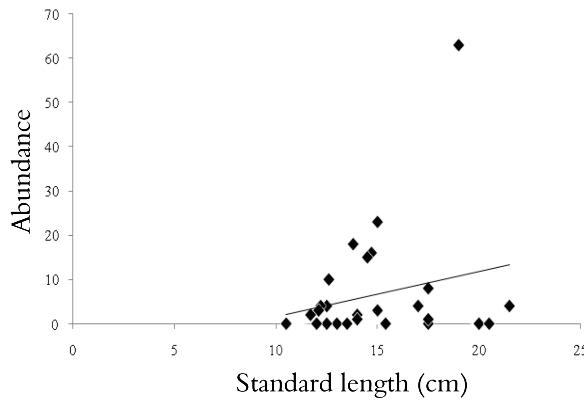

Figure 2. Correlation between *Echinorhynchus* sp. abundance and *S. marginatus* standard length, in the upper Paraná river floodplain, collected from March 2013 to September 2014.

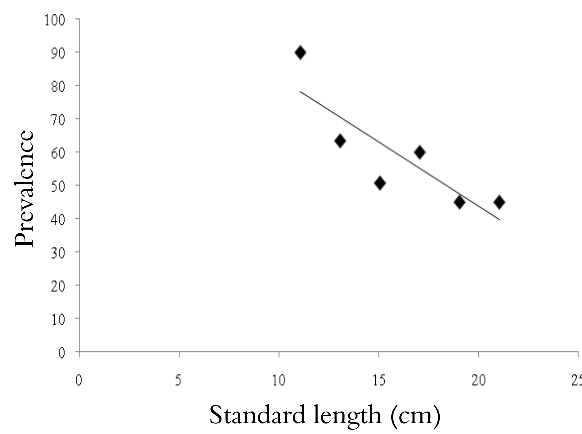

Figure 3. Correlation between *Kritskya annakohnae* prevalence and *Serrasalmus marginatus* standard length in upper Paraná river floodplain, collected from March 2013 to September 2014.

Berger-Parker dominance index presented mean around on 1 ± 0.36 for *S. marginatus* and 1 ± 0.40 for *S. maculatus*, being *K. annakohnae* the predominant species to *S. maculatus* with 429 specimens collected (76. 88%) and *Echinorhynchus* sp. predominant specie to *S. marginatus* with 181 specimens collected (53.07%).

Table 2. Correlations between hosts standard length and parasites abundance and prevalence values, obtained by Spearman's rank (rs) and Pearson correlation (r). *Serrasalmus margintaus* and *S. maculatus* collected in the upper Paraná river floodplain between March 2013 and September 2014 (p = significance level).

Host species	Parasite species	rs	p	r	p
Serrasalmus marginatus	*K. annakohnae*	-0.2219	0.2658	-0.8533	0,0307*
	Echinorhynchus sp.	0.4674	0.0090*	0.155	0.7693
	Contracaecum sp.	0.2984	0.1304	0.3513	0.4947
	Contracaecum sp. type 1	0.3039	0.1233	0.0831	0.8756
Serrasalmus maculatus	*K. annakohnae*	0.3074	0.0769	0.3561	0.3865
	Echinorhynchus sp.	-0.0877	0.5577	0.3691	0.3682
	Contracaecum sp.	0.0706	0.7058	0.0404	0.9243
	Contracaecum sp. type 1	0.0418	0.8235	-0.2789	0.5035

*Significant "p" values.

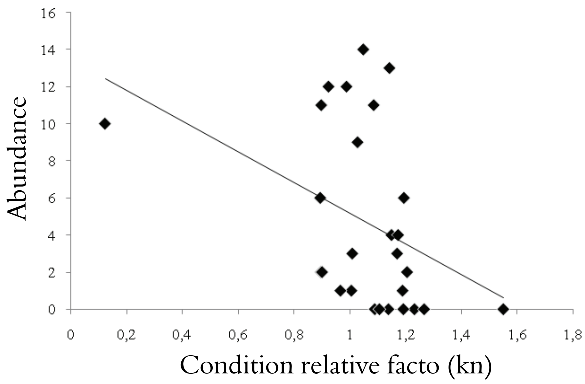

Figure 4. Correlation between *Kritskya annakohnae* abundance and *Serrasalmus marginatus* relative condition factor, from upper Paraná river floodplain, collected from March 2013 to September 2014.

Table 3. Correlation values between relative condition factor (Kn) and parasite abundance, obtained by Spearman's rank correlation coefficient (rs) and Mann-Whitney test. Values with normal approximation "Z" differentiating Kn of parasitized and non-parasitized fishes. *Serrasalmus marginatus* and *S. maculatus* data collected in the upper Paraná river floodplain between March 2013 and September 2014 (p = significance level).

Host species	Parasite species	rs	p	Z	p
S. marginatus	*Kritskyia annakohnae*	-0.4744	0.0124*	2.6004	0.0093*
	Echinorhynchus sp.	-0.1972	0.1838	0.7029	0.4821
	Contracaecum sp.	0.0581	0.7736	0.3433	0.7314
	Contracaecum sp. type 1	0.1765	0.3786	0.2196	0.8262
S. maculatus	*Kritskyia annakohnae*	-0.0136	0.9420	0.3096	0.7568
	Echinorhynchus sp.	0.0201	0.9143	0.1581	0.8744
	Contracaecum sp.	0.2828	0.1231	1.3418	0.1797
	Contracaecum sp. type 1	0.1569	0.3993	0.9924	0.3210

*Significant "p" values.

Table 4. Mann-Whitney U test values with normal approximation "Z", and G loglikelihood between hosts sex with infection prevalence and abundance, to *Serrasalmus marginatus* and *S. maculatus* collected in floodplain Paraná river, from March 2013 to September 2014. (p = significance level).

Hosts species	Parasites species	Z	p	G	p
S. marginatus	*K. annakohnae*	0.0244	0.9805	0.9943	0.3187
	Echinorhynchus sp.	0.2196	0.8262	0.1275	0.7210
	Contracaecum sp.	0.0976	0.9223	0.0494	0.8241
	Contracaecum sp. type 1	0.2196	0.8262	__*	__*
S. maculatus	*K. annakohnae*	1.4033	0.1605	1.6018	0.2056
	Echinorhynchus sp.	0.1779	0.8588	0.0345	0.8527
	Contracaecum sp.	0.6522	0.5143	0.9967	0.3181
	Contracaecum sp. type 1	0.8499	0.3954	0.3133	0.5757

*Data for which the analysis were not done.

In the total, the average diversity of endoparasites hasn't shown meaning ful difference when compared to the host sex of both species: *S. marginatus* (Z = 0.0615, p = 0.9509) and *S. maculatus* (Z = 0.2196, p = 0.8262); that is, parasite diversity has presented independency regarding to the host sex.

Discussion

Serrasalmus marginatus and *S. maculatus* were more parasitized by Monogenea. It can be related to monoxenic life cycle of these parasites, since they do not need an intermediate host to be transmitted from one host to another.

Considering the *K. annakohnae* monoxenic cycle, transmission occurs directly from one host to another, which can be explained by the behavior of *S. maculatus* and *S. marginatus* to form shoals (Sazima & Machado, 1990). Both species promote this parasite occurrence. On the other hand, the presence of this parasite in both fish species can also be explained by phylogenetic closely, since a parasite may have hierarchical preference for hosts, which occurs when the cost for the parasite access the same resource is explored between the host species (Sazima, Janz, & Nylin, 1998).

Monogeneans are mainly fish ectoparasites located in gills (Eiras, 1994) and records are rare in internal organs. Fischthal and Allison (1941), Kohn (1990) and Bilong Bilong, Birgi, & Euzet (1994) recorded these parasites in urinary bladder. Kearn (1987), in a study about stingrays internal monogeneans, proposed that swimmers parasite larvae can have direct access to sewer and, from there, they can go for their sites of infection such as rectal glands. So, it is possible that this happens with *K. annakohnae*, as urinary duct open up in fish cloaca, thus enabling infective forms access to the urinary bladder.

Because *S. marginatus* and *S. maculatus* eat mainly pieces of fins and other lower fish parts, it does not explain parasites transmission by trophic via, since a stomach contents analysis showed that crustaceans are also food content forthose fish species. Crustaceans are, in general, intermediate hosts for some *Echinorhynchus* (Acanthocephala) species and, because of this, crustaceans can carry larval forms of these parasites (Schmidt, 1985). As *S. marginatus* presents territoriality and aggression in its behavior (Agostinho, 2003), this allows a better foraging for this species and consequently a large amount of acanthocephalans. So, *S. marginatus* ingests a greater number of infected intermediate hosts than *S. maculatus* and consequently a large amount of acanthocephalans larvae.

Regarding to Nematoda, the results of the research indicate that the highest prevalences occurred to *Contracaecum* sp. and *Contracaecum* sp. type 1 in both hosts species, what suggest that two fishes species can be intermediate hosts for these nematodes, as these parasites uses those fishes as intermediate hosts (Eiras, 1994; Moravec, 1998).

In fish populations, intensity of infection by metazoan parasites increases with age or host size

(Dogiel, 1970). *Echinorhynchus* sp. abundance was correlated positively with *S. marginatus* standard length, since older fishes are bigger to accumulate parasites than younger. So, when fishes grows up, they can offer more internal spaces for parasites establishment (Poulin, 2000).

Significant negative correlation between *K. annakohnae* prevalence and *S. marginatus* standard length demonstrated that there is a higher number of infected fish in smaller size classes. It is possible considering direct cycle influence in parasitology variables as abundance (Zelmer & Arai,1998) and morphological and physiological factors that are involved in selection of microhabitats by parasites, especially the selection of smaller places as a facilitator for mating meetings (Rohde, 2005). Urinary bladder is this parasite site of infection and this organ is smaller in small fish, what suggested actually that the reduction of space facilitates mating and then a higher prevalence in smaller size classes fishes.

Kritskyia annakohnae abundance influenced in *S.* marginatus condition factor, which is explained by Yamada, Takemoto, and Pavanelli (2008): parasitized and non-parasitized fishes may show a difference in your composition, being Kn of infected fish lower if compared with the not infected fish. So parasites can change the hosts condition by direct action. *Echinorhynchus* sp. presented a high relatively abundance value, but this had not influenced the hosts relative condition factor. This is possible because of time adjustment in host-parasite relationship, that includes, in this case, a potential loss of parasites pathogenicity.

Parasites prevalence and abundance in congeneric host species did not show any differences between the sex of the hosts. According to Agostinho (2003), in a study about those species condition factor variation pattern in the Paraná river floodplain, it indicated that the most important is food availability and not reproductive events, since in food shortages periods have shown reduced condition factor, especially in immature individuals. Besides, it can be assumed that males and females have similar diets, what did not influence these fishes parasitism. Similar results were found by Machado, Pavanelli, and Takemoto (1994); Machado Almeida, Pavanelli, and Takemoto (2000) for different host species.

Richness and diversity communities are often related parasites to hosts body length. Different length class fishes differ in their life way and, as a consequence, differs in their exposure to parasites degree (Guégan & Hugueny, 1994). Besides that, some parasites species may occur in some hosts length classes and be absent in others, because of diet habits, behavior and fish microhabitats.

Relationship between host variables (body size, relative condition factor and host sex) and parasite diversity was not found. Although parasitic composition may be a indicator of habitat and hosts migration routes, these pattern may be related to fish immune system, or influenced by food habits (smaller fishes can use features that are not accessible to adults). Another explanation consists in environments, with higher intermediate hosts concentration, frequented by young fish (Monteiro, Santos, Zuchi, & Brasil-Sato, 2009), that allows the parasite transmission via trophic in this case.

Kritskyia annakohnae predominance in *S. marginatus* urinary bladder can be related to the idea of apparent competition. In this case, when two hosts species share infective stages, tolerant to infection species can act as a parasite reservoir to less tolerant. So, more tolerant host species acts as a competitive top, and this competition is mediated by mechanism of sharing parasites (Greenman & Hudson, 2000). In this context, it was suggested that *K. annakohnae* reservoir could be *S. marginatus* and less tolerant species could be *S. maculatus*.

Conclusion

Knowledge of parasitological variables in the context of species introduction opens way for new research that explain occurrence of each parasite species in hosts, native or introduced. If the parasite is the inductor subject of host population changes (apparent competition), it is necessary to carry out experiments that verify this mechanism.

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