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ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

ECOLOGICAL RELATIONSHIPS BETWEEN ENDOPARASITES AND THE FISH *SALMINUS BRASILIENSIS* (CHARACIDAE) IN A NEOTROPICAL FLOODPLAIN

RELACIONES ECOLÓGICAS ENTRE ENDOPARASITOS Y EL PEZ *SALMINUS BRASILIENSIS* (CHARACIDAE) EN UNA LLANURA DE INUNDACIÓN NEOTROPICAL

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Abstract

The present study was designed to characterize the community structure of parasites of *Salminus brasiliensis* in the upper Paraná River during different seasons, and to examine the levels of parasitism of this hosts. The community of parasites of *S. brasiliensis* did not have a dominant species. Four of the five most prevalent parasite species showed aggregate distribution. In relation to other aspects of the community, each group encompassed their particular parasite, which highlights the differences inherent in the biology and life cycle of each species.

Keywords: endoparasite - community of parasites - the upper Parana River floodplain - Brazil.

Resumen

El presente estudio tuvo como objetivo caracterizar la estructura de la comunidad de parásitos de *Salminus brasiliensis* en el curso superior del río Paraná a lo largo de las diferentes estaciones del año, entre los niveles de parasitismo y las diferentes características de los huéspedes. La comunidad de parásitos de *S. brasiliensis* no possuía ninguna especie dominante. Cuatro de las cinco especies de parásitos más prevalentes mostraron distribución agregada. En relación a otros aspectos de la comunidad, cada grupo presentó su parásito en particular, lo que destaca las diferencias inherentes al ciclo de la biología y de la vida de cada especie.

Palabras clave: endoparásitos - comunidad de parásitos – llanura de inundación de la parte superior del río Paraná - Brasil.

INTRODUCTION

The knowledge of the parasitic fauna of Brazilian fish has received increasing attention from researchers, in particular as regards the relationship of parasites and their hosts. Accordingly, the object of study was the freshwater fish *Salminus brasiliensis* (Cuvier, 1816) (Characiformes: Characidae), popularly known as dourado. This species of fish is found only in South America, the basins of the Paraná, Paraguay and Uruguay, in the drainages of the Patos Lagoon and the Mamore River in Bolivia (Lima *et al*., 2003). In the floodplain of the upper Paraná River until 2003 *Salminus brasiliensis* was identified as *S. maxillosus (*Valenciennes 1850) (Pavanelli & Grace, 2007).

Besides its ecological importance, the dourado fish is a much appreciated in sport fishing, presenting a high price in trade due to the excellent quality of its meat (Zaniboni-Filho *et al.*, 2003). Studies characterizing the relationship between helminth parasites and their hosts should consider the availability of related information: the environment (water quality, pH, ammonia concentration, dissolved oxygen availability, temperature, water level and seasonal effects), the host (habitat, feeding behavior, physiology, age and sex) and the parasite (availability of infective larvae of individual hosts, the host immune response to the establishment of the larva and the natural mortality of parasites) (Dogiel, 1961; Takemoto *et al.*, 2004).

Some work on *Salminus brasiliensis* parasites have been performed, however, mostly taxonomic (Boeger *et al.*, 1995; Petter, 1995; Kohn *et al.*, 1997; Molnar *et al.*, 1998). In addition, Rodrigues *et al.* (2002) studied the histopathological changes caused by the nematode *Neocucullanus neocucullanus* Travassos, Artigas et Pereira, 1928 in dourado in Mogi Guacu, SP.

For the floodplain of the upper Paraná River, only two articles were recorded referring to the ecological aspects *Prosthenhystera obesa* Diesing, 1850 (Isaac *et al*., 2000; Karling *et al*., 2013). The others are records of species, such as: Monogenea (*Jainus* sp., *Anacanthorus* sp. and *Rhinoxenus bulbovaginatus* Boeger, Domingues, Pavanelli, 1995), a species of Cestoda (*Monticellia coryphicephala* Monticelli,1892), two species of Digenea (*Cladocystis intestinalis* Vaz,1932 and *Prosthenhystera obesa* Diesing, 1850), Nematoda (*Eustrongylides ignotus* Jäegerskiold, 1909 in the larval stage and Anisakidae) and Branchiura (*Dolops longicauda* (Heller, 1857) and *Dolops* sp.) (Takemoto *et al*., 2009).

Thus, the present study aimed to characterize the community structure of parasites of *S. brasiliensis* in the upper Paraná River along the different seasons, as well as regarding parasitism levels and features of the hosts.

MATERIALS AND METHODS

The study area is part of the floodplain of the upper Paraná River. Located near the town of Porto Rico, State of Paraná (22 ° 43'S and 53 ° 10'W), where is the Laboratory for Advanced Research, State University of Maringa - Nupelia (Center for Research in Limnology, Ichthyology and Aquaculture). Samples were collected at several of the channels environments, such as rivers and lagoons as part of the project PELD-CNPq (Long Term Ecological Projects) - Site 6.

Specimens of *S. brasiliensis* were collected quarterly between March 2009 and August 2010. For its capture were used rods with reel and nets of different mesh sizes (2, 4, 3, 4, 5, 6, 7, 8, 10, 12, 14:16 cm between opposite knots), which were exposed during 24 h in each collection point (revised every 8 h). The fish were sacrificed by deep anesthesia with benzocaine: 3 g of benzocaine dissolved in 10 ml of ethanol to 30 l of water (Lizama *et al.*, 2007). Date of sampling, total and standard length, total weight, sex and stage of gonadal maturity of each fish were recorded.

After the taxonomic identification and decision of biometric data of the hosts, a longitudinal incision was made on the ventral surface of the individual and every organ and or structures were removed and separated. The visceral cavity and each organ were examined under a stereomicroscope to collect endoparasites. According to the methodology described by Eiras *et al.* (2006).

Data Analysis: the prevalence (P), mean intensity (IM) and mean abundance (AM) infection of parasites were obtained according to Bush *et al.* (1997). The Simpson Index was calculated to determine the dominance concentration, based on the proportion of infected fish is assumed for the dominance values greater than or equal to 0.25 (Stone & Pence, 1978). The dispersion index (ratio between the variance and mean abundance) and Green index were used to assess the pattern of dispersion and aggregation, and the dispersion index was tested by the statistic d, where the distribution is considered random when d <1.96, even when d <-1.96 and aggregate when d> 1.96 (Ludwig & Reynolds, 1988). The chi-square test with Yates correction was used to determine possible interspecific associations between pairs of co-occurring species (Ludwig & Reynolds, 1988). The covariation of abundance of parasites were tested using the correlation coefficient by Spearman ranks "rs".

The Pearson correlation coefficient "r" was calculated to determine the correlation between the prevalence and host standard length, prior to angular transformation of data on prevalence and separation of samples from the host at intervals of length classes. The correlation coefficient "rs" was used to determine possible correlations between host standard length and abundance of parasite species, and to investigate correlations of the abundances of parasites with the Kn of hosts (Zar, 1996).

The Kruskal-Wallis test was used to determine the seasonal abundance of infection and to determine the influence of gonadal maturity stages of fish (immature, maturing, advanced maturation, recovery, rest and semi exhausted) on the abundance of parasites (Zar, 1996).

The relative condition factor (Kn) was calculated for each host, which corresponds to the ratio between the observed weight (Wo) and the weight theoretically expected for a given length, that is, Kn = Wo / We (Le Cren, 1951). The constants a and b of length to weight ratios were used to estimate theoretically expected values of the body weight (We) by the formula a.Ls We = b, where Ls is the length standard.

The tests mentioned above were applied only to the parasite species with prevalence greater than 10%.

RESULTS

In the total sample, 56 specimens of *S. brasiliensis* were necropsied, and collected and processed 828 helminths belonging to four groups: Digenea, Nematoda, Acanthocephala and Cestoda. The most abundant group was Digenea, composed of a single species, *C. intestinales* with 623 specimens.

Species with prevalence greater than 10% in the total sample: *C. intestinales*, *M. coryphicephala*, *Octospiniferoides incognita* Schmidt & Hugghins; 1973, *Contracaecum* sp. larva, *Contracaecum* sp. larvae type 2 by Moravec, Kohn and Fernandes 1993 (found in the mesentery). It was not possible to quantify the Cestoda cysts present in the mesentery.

The prevalence values, mean intensity, mean abundance and average amplitude for each suprapopulation obtained are shown in Table 1.

The parasite community of *S. brasiliensis* showed no concentration for dominance (C = 0.22), but showed a typical pattern of aggregation, with the exception of Cestoda *M. coryphicephala*, which showed a random distribution. As the values of the Green Index parasite species that was more aggregate was *O. incognita* (Table 1).

Table 1. Parasites and parasitism levels of *Salminus brasiliensis* in the floodplain of the upper Paraná River (P%= prevalence, IM= mean intensity; AM = average abundance, AV = range of variation of abundance) and Index of dispersion (ID), Green Index (IG) estimated for the main species of parasites.

Species	P(%)	IM	AM	AV	ID	*d*	IG	Distribution
Cladocystis intestinales	67.85	16.39	11.12	1 – 161	53.8	65.88	0.08	Aggregate
Monticellia coryphicephala	10.71	1.16	0.12	1 – 2	1.18	0.978	0.03	Random
Octospiniferoides incognita	10.71	3.66	0.39	1 – 15	10.61	23.73	0.45	Aggregate
Acanthocephala larvae	7.14	1.75	0.12	1 – 3	-	-	-	-
Contracaecum spp. l arvae	16.07	2.33	0.37	1 – 6	3.44	9.036	0.12	Aggregate
Contracaecum sp. larvae type 2	39.28	6.72	2.64	1 – 56	24.77	41.76	0.16	Aggregate
Monticellia coryphicephala cyst	50	-	-	-	-	-	-	-

The five most prevalent species (above 10%) were divided into pairs to detect possible interspecific relationships. The pair *O. incognita* and *Contracaecum* sp. larvae was associated positively and significantly with their abundances also positively correlated (Table 2).

Table 2. Associations of species of the parasite *Salminus brasiliensis* collected in the floodplain of the upper Paraná River. (X^2 = chi-square test with Yates' correction; rs = correlation coefficient by Spearman posts between the abundances of species in each pair).

			X^2		
Species	1	2	3	4	5
(1) *Cladocystis intestinales*	-	(+) 0.23	(+) 0.55	(+) 0.76	(+) 0.03
(2) *Monticellia coryphicephala*	0.043	-	(+) 0.11	(-) 0.54	(+) 0.11
(3) *Octospiniferoides incognita*	0.21	0.07	-	(+) 4.67	(+) 2.84
(4) *Contracaecum spp.* larvae	0.17	-0.15	0.32 *	-	(+) 0.16
(5) *Contracaecum sp.* larvae type 2	0.01	0.05	0.33*	0.17	-
*Significant values			Rs		

The fish had standard length ranging from 18.3 to 68.5 cm (eight classes, with an interval of 7 cm each). There was no correlation between the standard length of hosts and the abundance and prevalence of parasite species found (Table 3).

Of the 56 fish analyzed (38 females, 15 males and three indeterminate), 34 females, 12 males and two with indeterminate sex were parasitized by one or more species of parasites.

There was no difference in the abundance of parasitism between the sexes of hosts except for *O. incognita* and *Contracaecum* sp. larvae, where females were more parasitized (Table 3).

Table 3. Values of the correlation coefficient "rs" for stations to determine Spearman correlations between the host standard length and abundance of parasite species; the Pearson correlation coefficient "r" to correlate the prevalence of parasitism and the standard length and test "G "Log-likelihood for detecting differences between hosts males and females in the prevalence of parasitism and with Z test to check for differences between males and females in hosts abundance of parasitism *Salminus brasiliensis*, collected in the floodplain of the upper Paraná River (p = significance level).

Species	rs	p	r	p	Z	p	G	p
Cladocystis intestinales	0.12	0.35	-0.49	0.21	0.07	0.94	0.09	0.75
Monticellia coryphicephala	-0.01	0.91	-0.04	0.91	0.18	0.85	0.19	0.65
Octospiniferoides incognita	0.25	0.06	0.41	0.32	0.88	0.38	4.28	0.04*
Contracaecum spp. larvae	-0.11	0.42	-0.31	0.45	1.19	0.23	5.86	0.01*
Contracaecum sp. larvae type 2	0	0.97	-0.35	0.38	0.55	0.58	0.35	0.55

*Significant values

Contracaecum sp. larvae type 2 showed significant differences in abundance values between seasons (Table 4), with the summer season with the highest mean abundance of this parasite (16 parasites per fish).

With regard to the stage of gonadal development, five fish were not identified. Among the others, 18 were in the immature stage, two in early-maturing, one in advanced maturation one in three in recoveries, 26 at rest and one semi exhausted. The stage of gonadal maturation did not affect the prevalence and abundance of groups of parasites, only *O. incognita* was found in fish in the resting stage. The values of the statistical tests are listed in Table 4.

Table 4. Values of the Kruskal-Wallis test to check the influence of gonadal maturity stages (immature, mature, breeding and resting) on the abundance of parasitism (H1) and values of the Kruskal-Wallis test to check the influence of seasonal variation (H2) in the abundance of parasites in *Salminus brasiliensis* collected in the floodplain of the upper Paraná River (p = significance level).

Species	H1	p1	H2	p2
Cladocystis intestinales	1.74	0.63	9.57	0.08
Monticellia coryphicephala	0.87	0.83	0.64	0.98
Octospiniferoides incognita	-	-	2.55	0.77
Contracaecum spp. larvae	0.11	0.75	0.68	0.98
Contracaecum sp. larvae type 2	1.61	0.65	14.92	0.010*

*Significant values

The relative condition factor differed between parasitized and non-parasitized hosts only for *Contracaecum* sp. larvae type 2, where the fish parasitized by this species showed Kn average of 0.86, lower than the non parasitized fish (Kn = 0.97) (Table 8). This same parasite species, showed significant negative correlation between their abundances and relative condition factor of hosts (Table 5).

Table 5. Values of the "U" test, Mann-Whitney, with normal approximation "Z", to check for differences between the relative condition factor of parasitized and non-parasitized individuals and analysis values of the correlation coefficient "rs", relative condition factor and abundance of parasitism of *Salminus brasiliensis* collected in the floodplain of the upper Paraná River (p = significance level).

Species	Z	p	rs	p
Cladocystis intestinales	0.81	0.41	0.04	0,75
Monticellia coryphicephala	0.64	0.52	-0.09	0.51
Octospiniferoides incognita	0.55	0.58	0.08	0.55
Contracaecum spp.larvae	1.24	0.21	-0.16	0.22
Contracaecum spp. larvae type 2	2.61	0.00*	-0.33	0.01*

*Significant values

DISCUSSION

The number of species of parasites in the infracommunities may vary between hosts according to the degree of the success of infection of the parasite (Poulin, 1996). The same author mentions that this probability is directly related to the parasite reproductive rates and their ability to disperse and settle. Even in rich component communities, the co-occurrence of species in a single infracommunity is unlikely, unless the prevalences of all species are very high. To *Salminus brasiliensis*, no parasite species showed concentration for dominance, probably because there is no competition in the infracommunity.

Parasites may or may not present a pattern of patchy distribution in their hosts. The aggregate distribution occurs when most hosts shelter no or few parasites and few hosts shelter many parasites (Poulin, 1998). The aggregate distribution pattern observed for endoparasites of *S. brasiliensis* was also found by other authors in the Floodplain of the Upper Paraná River working with other families of fish like cichlids, pimelodids and prochilodontids (Machado *et al.*, 2000 Guidelli *et al.*, 2003; Lizama *et al.*, 2005). This type of distribution tends to increase the stability of the host-parasite relationship, since regulatory mechanisms (host mortality, reduced fecundity and survival of the density dependent parasite) influence the proportion of their populations (Dobson, 1990). Only *M. coryphicephala* showed random distribution. This distribution may be due to reduced opportunity to colonize the host, or whether this parasite presents a major pathogenic effect.

Negative associations may be a result of competition between species in a host. Competition occurs when two or more species use the same resources and these are depleted (Piankas, 1983). In this study, there was a positive association between the species *O. incognita* and *Contracaecum* sp. larvae. This may be related to infection strategies and not direct interaction of these species since they have different infection sites: Acanthocephala inhabits the intestine, while Nematoda inhabits the mesentery. This hypothesis is consistent with Dobson (1990), which concluded that the abundance and distribution of parasites are a direct result of the characteristics of the life cycles of parasites and species interactions.

The hosts with larger body size are generally older and usually more heavily parasitized due to higher nutritional needs (Mccornmick & Nickol, 2004). Also, higher values of intensity of infection in larger specimens may be related to the accumulation of larvae in the host by repeated processes of infection (Luque *et al.*, 1996). Factors such as the quantity and type of food consumed, prey size, exposure time, increasing surface area of the body and habitat changes may be responsible for the increase in the prevalence and abundance of infection with increasing age of the host (Dogiel, 1961; Pennycuick, 1971; Hanek & Fernando, 1978). Thus, it is expected that the trophically acquired parasites are abundant in larger fish.

Studying the structure and diversity of the community of endoparasites of *Piaractus mesopotamicus* Holmberg 1887 and their interactions with the host, in the Pantanal (MS), Fields *et al.* (2009) observed that the species of Nematoda *Spectatus spectatus* Travassos, 1923 showed significant positive correlation between total length and parasite abundance. The same results were reported by Machado *et al.* (2000); Lizama *et al.* (2008) and Ceschini *et al*. (2010) in the floodplain of the upper Paraná River. In this study there was no correlation between the standard length and the prevalence and abundance of parasites of *S. brasiliensis.* Based on this result, we can infer that the host-parasite relationship is tightly adjusted.

Distinctions in parasitism in hosts males and females may be an important factor when energy needs unequal, different eating habits or physiological differences occur among both sexes (González-Acuña, 2000). Parasites tend to have more access to individuals whose gender have higher nutritional needs (Riffo & George-Nascimento, 1992).

Several studies relating the host sex and the infracommunity of endoparasites have been performed. Lizama *et al.* (2008), Albuquerque *et al.* (2008) and Moreira *et al.* (2009) observed no influence of sex on prevalence and intensity of parasitism. The lack of relationship between parasite indexes with host sex has been interpreted as a consequence of the similarity of the biological parameters of fish (Luque *et al.*, 1996).

According Guidelli *et al.* (2003) many species of endoparasites of *Hemisorubim platyrhynchos* (Valenciennes, 1840) presented positive correlation with the size and sex of the host, which may be due to the volume of food ingested and possible behavioral changes between the sexes, with females more parasitized. This same correlation was reported by Carvalho *et al.* (2003) for the parasites of *Acestrorhynchus lacustris* (Lütken, 1875), and Isaac *et al.* (2004) studying *Gymnotus* spp.

In this study only two species (*O. incognita* and *Contracaecum* sp. larvae) were influenced by host sex on the prevalence of parasitism, the females being the most affected, but there were no significant influences regarding abundance of parasitism. This indicates that differences may exist between behavioral and physiological hosts males and females.

During the development of a fish, several factors may interfere directly on the constitution of their parasite fauna. The maturation for example, hormonal conditions can trigger behavioral and physiological differences between male and female host, allowing different patterns of parasitic infection (Esch *et al*., 1988; Guégan & Hugueny, 1994; Takemoto *et al*., 2005a).

Studying isopods ectoparasites of *Scomberomorus brasiliensis* Collette, Russo & Zavala-Camin, 1978, Lima *et al.* (2005) observed that these parasites have a preference for younger fish and was attributed these results to the energy reserves, because mature fish have fewer energy resources due to mobilization for gonadal maturation and reproduction.

To *S. brasiliensis* there was no relationship between the level of parasitism and stage of gonadal maturation of the host, this probably occurred by the small number of specimens in each maturity stage.

Numerous factors affect the abundance and prevalence of parasites, and temperature is one of the most important in the host-parasite relationship and the environment (Kennedy, 1982). Seasonal events, like most ecological parameters, have varying effects that depend on the species involved and the combination of parameters in a particular area of study (Lawrence, 1970). The summer months generally have higher abundances of parasites since is the same period the fish feeds more actively (Dogiel, 1961).

Kadlec *et al.* (2003) studied the parasite community of three species of fish (*Rutilus rutilus* (Linnaeus, 1758), *Rhodeus sericeus amarus* (Bloch, 1782) and *Perca fluviatilis* Linnaeus,1758) during a major flood. The authors concluded that the three species respond differently to changes in water level with an increase of parasitism in *P. fluviatilis* in the summer.

In the present study, there was an increase in the abundance of *Contracaecum* sp. larvae type 2 in *S. brasiliensis* in the warmer months. Pertierra & Nuñes (1995) observed that the temperature triggers physiological processes for the development of the parasite. This result can also be related to the fact that breeding season of the species, between October and January (Suzuki *et al.*, 2004), which should be influencing the hormonal variation of the host, resulting in a greater abundance of this parasite.

In natural environments parasites usually do not cause significant damage to their hosts with rare records of high mortality rates. Under these conditions, the prospect is that over time there is an equilibrium between coevolutive host-parasite interactions, because there is no advantage to the parasite to damage the host to the point of being harmed himself (Chisholm & Whittington, 2008). This equilibrium can be destabilized by interference from natural or artificial processes such as atypical variations of physico-chemical parameters of the environment, polluting processes, health of the host and greater predation pressure and interspecific competition.

The welfare of fish can be measured by condition factor (K). According to Le Cren (1951), this factor is a quantitative indicator of the degree of healthiness and wellness of fish, reflecting recent dietary conditions, given by the length / weight relationship of the individual. But the relative condition factor (Kn) takes into account the expected and the observed weight. Variations are influenced by the environment, lack of food or even parasitism cause changes in this value, and under normal conditions the optimal value is one.

Studies on the condition factor of parasitized fish show that parasitism may be related to low values (Tavares-Dias *et al.*, 2000) or high values of the condition factor of hosts (Lizama *et al.*, 2006; Moreira *et al.*, 2010), and also may not provide correlation (Ranzani-Paiva *et al.*, 2000; Dias *et al.*, 2004; Moreira *et al.*, 2005; Tavernari *et al.*, 2005).

Analyzing the differences of Kn of parasitized and non-parasitized individuals of *S. brasiliensis*, we observed a significant difference just in *Contracaecum* sp. larvae type 2. This suggests that non parasitized fish have a better condition factor, since they are not influenced by parasitism.

The Kn of *S. brasiliensis* and abundance *Contracaecum* sp. larvae type 2 covaried negatively, demonstrating that most infected individuals have lower Kn. This negative covariance can be explained by the pathogenicity of the parasite.

In conclusion, the community of parasites of *S. brasiliensis* did not have a dominant species. Four of the five most prevalent parasite species showed aggregated distribution. In relation to other aspects of the community, each group presented their particular parasite, which highlights the differences inherent in the biology and life cycle of each species.

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