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ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

ECOLOGICAL ASPECTS OF MONOGENEAN GILL PARASITES (PLATYHELMINTHES) FROM *HOPLIAS* AFF. *MALABARICUS* (BLOCH, 1794) (PISCES, ERYTHRINIDAE) IN A NEOTROPICAL FLOODPLAIN

ASPECTOS ECOLÓGICOS DE MONOGENEOS PARÁSITOS BRANQUIALES (PLATYHELMINTHES) DE *HOPLIAS* AFF. *MALABARICUS* (BLOCH, 1794) (PISCES, ERYTHRINIDAE) EN UNA PLANICIE DE INUNDACIÓN NEOTROPICAL

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Abstract

We analyzed 54 specimens of *Hoplias* aff. *malabaricus* (Bloch, 1794) collected between March 2010 and March 2011 from the upper Paraná river Neotropical floodplain, Brazil. All hosts were parasitized by at least one species of Monogenea. The parasites species with higher prevalence were *Urocleidoides cuiabai* Rosim, Mendoza-Franco & Luque, 2011 (96.29 %) and *U. malabaricusi* Rosim, Mendoza-Franco & Luque, 2011 (92.59%). The community of gill ectoparasites examined showed an aggregated pattern. *Urocleidoides cuiabai* was the dominant species in the sample (C > 0.25). The parasitic abundance showed significant positive correlation with the standard length of the host for *U. malabaricusi* and *U. eremitus* Kritsky, Thatcher & Boeger, 1986. There were significant differences in the abundance values between male and female fish to the species of parasites *Urocleidoides brasiliensis* Rosim, Mendoza-Franco & Luque, 2011 and *U. cuiabai*. The gonad maturation stages of the host did not influence the levels of parasitism. Four species of parasites showed positive and significant correlation with parasitic abundance and the relative condition factor. The Ivinheima river presented higher abundance of parasitism in relation to other subsystems. The ecological study of monogeneans can provide a greater knowledge of the biology of these parasites and their hosts, the collection site, and even how the host responds to the presence of these parasites. We discuss the factors that were fundamental in the levels of parasitism in this important fish species in the floodplain of the upper Paraná river.

Keywords: Ancyrocephalinae - Brazil - Dactylogyridae - fish ectoparasites - "traíras" - upper Paraná River foodplain.

Resumen

Analizamos 54 especímenes de *Hoplias* aff. *malabaricus* colectados entre marzo de 2010 y marzo de 2011 en la planicie de inundación Neotropical del alto rio Paraná, Brasil. Todos los hospederos fueron parasitados por lo menos con una especie de Monogenea. Las especies de parásitos con las mayores prevalencias fueran *Urocleidoides cuiabai* (96,29 %) y *U. malabaricusi* (92,59%). La comunidad de ectoparásitos de branquias ha presentado patrón de distribución agregado. *Urocleidoides cuiabai* fue la especie dominante en la muestra (C > 0,25). La abundancia del parasitismo ha presentado correlación positiva y significativa con la longitud estándar del hospedero para las especies *U. malabaricusi* y *U. eremitus* Kritsky, Thacher & Boeger, 1986. Hubo diferencia significativa en la abundancia de parasitismo entre peces machos y hembras para los parásitos *U. brasiliensis* y *U. cuiabai*. La fase de maduración gonadal del hospedero no influyó los niveles de parasitismo. Cuatro especies de parásitos han presentado una correlación positiva y significativa con la abundancia de parasitismo y el factor de condición relativo. El rio Iviñeima ha presentado alta abundancia de parasitismo en relación a otros subsistemas. El estudio ecológico de monogeneas puede proveer un gran conocimiento de la biología de estos parásitos y sus hospederos, el sitio de colecta, y hasta como el hospedero responde a la presencia de estos parásitos. Este artículo presenta los factores que fueron fundamentales en los niveles de parasitismo en esta importante especie de pez en la planicie de inundación del alto rio Paraná.

Palabras clave: Ancyrocephalinae - Brasil - cuenca del alto rio Paraná - Dactylogyridae - ectoparásitos de pez -"traíras".

INTRODUCTION

Among the species of fish cataloged in the upper Paraná river floodplain is *Hoplias* aff. *malabaricus* (Bloch, 1794). This is a species widely distributed in rivers of South America and popularly known as "traíras" or "lobó" (Oyakawa, 2003). "Traíras" are carnivorous and sedentary, occur in fluvial and lakes environments (Nakatani *et al.*, 2001). In the larval stage these fish feed on plankton and when adult mainly feed on other fish, with low greed and high resistance to fasting periods (Paiva, 1974).

Along with the fish collected over the years in the upper Paraná river floodplain is the symbiotic fauna, composed of micro and macro organisms, between which the parasites are mainly represented by protozoa, helminths and microcrustaceans. Several studies on taxonomy and ecology of fish parasites have been conducted in the region, and according to Takemoto *et al.* (2009), 337 species of fish parasites have been cataloged between the years 2000 to 2007, of which 12 were new species. Monogenea was the group of parasites that presented more new species, describing eight new species.

The seasonal occurrence of monogeneans in fish seems to be influenced by several factors, biotic and abiotic. The biotic factors, such as age, size, sex and migratory behavior habits of the host can influence the prevalence and intensity of parasitic infection throughout the year. Among the abiotic factors, temperature is apparently what seems to have a greater influence on the seasonal distribution of monogeneans (Eiras, 1994). Although the aquatic environment facilitates access of parasites to their hosts. Lentic environments are more favorable to transmission the diseases than lotic (Thatcher, 1981). For monogeneans, this type of environment favors the ciliated larvae finding the host .

The relation parasite/host is part of a complex structural network ecosystems and particularly in aquatic systems, these relationships are key indicators of the structure of these networks (Silva-Souza *et al.*, 2006). Thus, the study of fish

parasites allows to obtain important information not only about the hosts, but also the environment in general (Takemoto *et al.*, 2005 a, b).

This paper presents a study about the component community of monogeneans gill parasites of *Hoplias* aff. *malabaricus* collected in the upper Paraná River Neotropical floodplain, Brazil, in order to evaluate the relationship of parasitism with biotic factors such as the standard length and sex of hosts, interspecific relationships, similarities and the possible influence of parasitism on the welfare of the host.

MATERIALS AND METHODS

The study area is part of the upper Paraná river floodplain in the Paraná and Mato Grosso do Sul States from Brazil (22º43'S, 53º10'W). It is located near to Porto Rico, Paraná, where are the Field Station of the Universidade Estadual de Maringá - Nupélia (Núcleo de pesquisas em Ictiologia, Limnologia e Aquicultura) (Fig. 1). The sampling points correspond to those used by the program "Long Term Ecological Research" (LTER site 6).

It were collected 54 "traíras" between the period of March 2010 to March 2011 in three different subsystems of the upper Paraná river foodplain; Paraná, Baia and Ivinheima rivers through gillnets of different mesh sizes (2,4; 3; 4; 5; 6; 7; 8; 10; 12; 14 e 16 cm). Were anesthetized with Benzocaine 10%, killed, identified, enumerated, measured (cm) and weighed (g), and also recorded the sex, stage of maturation, the total weight , total and standard length. After "traíras" had their gills removed and fixed in formalin solution 5%.

Gills arches were removed and examined individually in Petri dishes with water under a stereomicroscope for the collection of parasites. The parasites were fixed, prepared and assembled as Eiras *et al.* (2006).

To obtain the total number of parasites count was made under a dissecting microscope in a Petri dish. For identification, specimens were prepared in Hoyer medium, for observation under an optical microscope (Eiras *et al.*, 2006). The identification was made according to Kritsky *et al.* (1986), Thatcher (2006) and Rosim *et al.* (2011). The terminology of parasite ecology were used according to Bush *et al.* (1997).

The Brillouin's index of diversity and uniformity was used to determine the diversity of the infracommunities of ectoparasites on gill in the differents subsystems (Stone & Pence, 1978). The Berger Parker index of dominance was used to check if there was a dominance concentration among species. This index expressed the proportionate importance of the species more abundant of parasite in the sample (Magurran, 1988).

The dispersion index (DI) and Green index (GI) were used to examine the pattern of dispersion and aggregation of the species of ectoparasites. The dispersion index was tested using statistical d, being considered random distribution when d <1.96, uniform distribution when d < -1.96 and aggregate when d > 1.96. The degree of aggregation was measured by Green index which ranges from 0 (at random) and 1 (maximum aggregation) (Ludwig & Reynolds, 1988).

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

The test "G" log-likelihood, using the 2 x 2 contingency table was used to determine the effect of host sex on prevalence of each parasite species (Zar, 1996). The Mann-Whitney (U) was used to determine the effect of host sex in the

abundance of infestation (Siegel, 1975).
The Student "t" was used to determine if the standard length of the males and females hosts are similar. This test also verified the possible influence of the length of the host, in parasitism levels. The Kruskal-Wallis (H) test was used to verify that the different maturation stages are influencing levels of parasitism (Zar, 1996).

The relative condition factor (Kn) was calculated based on the weight and length of the fish (LeCren, 1951).

The gonadal maturation stages of the fish were determined according to Vazzoler *et al.* (1997). The tests mentioned above were applied only for parasite species with prevalence higher than 10%. Statistical analyzes were performed on 5.0 BioEstat and Past programs. Voucher specimens were deposited at Coleção Helmintológica do Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro, Rio de Janeiro, Brazil.

This project was analyzed and approved by committee of Ethics of the Universidade Estadual de Maringá (CEAE) number (123/2010).

RESULTS

All fishes analyzed were parasitized by at least one species of monogeneans. The total number of parasites found was 3640 specimens, the species most prevalent was *Urocleidoides cuiabai* Rosim, Mendoza-Franco & Luque, 2011 with 96.29% followed by *U. malabaricusi* Rosim, Mendoza-Franco & Luque, 2011 with 92.59%. The data obtained are listed in Table 1.

Of the 54 fish examined 16 were parasitized by six of parasites species and only three hosts were parasitized by one species (Fig. 2).

According with the index Berger-Parker there was a dominance of species *U. cuiabai* (42.08%). The dispersion of the parasite species presented itself aggregate (Table 2).

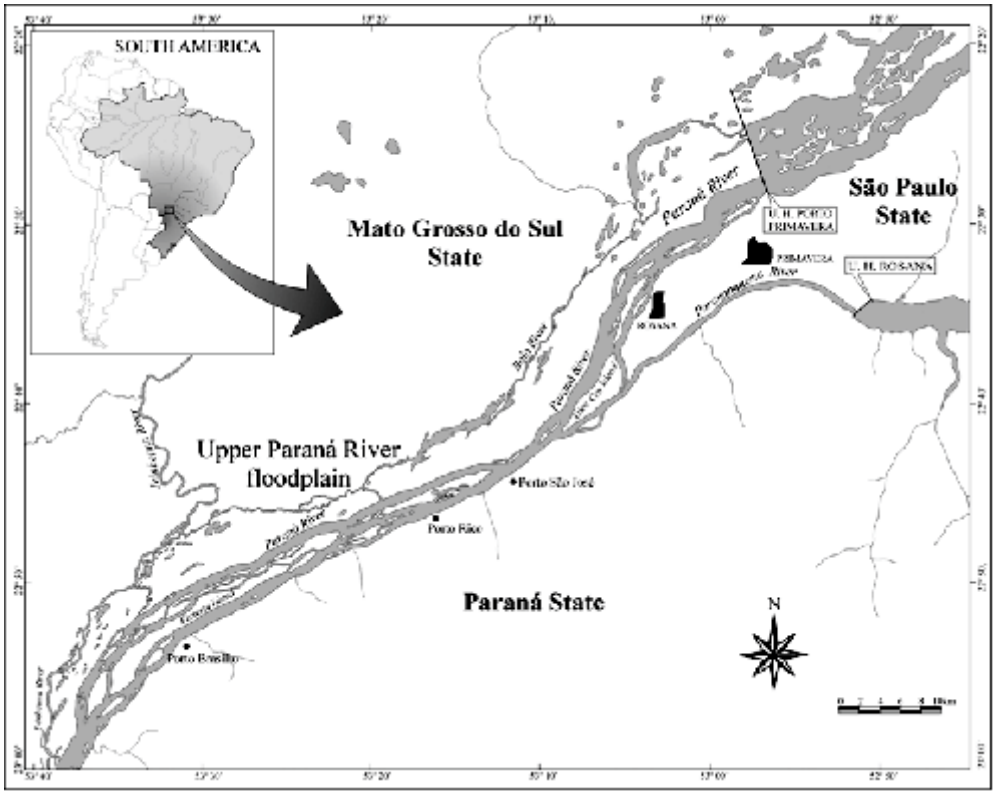

Figure 1. The upper Paraná river Neotropical floodplain, Brazil showing the Area of Long Term Ecological Research (ALTER).

Table 1. Acess Number CHIOC (AN); Number of fish infested (NI), Number of parasites collected (NP), prevalence (P%), mean intensity (MI) Mean Abundance (MA) Amplitude of parasitism (AP) of monogenean gill parasites of *Hoplias* aff. *malabaricus* in the upper Paraná river Neotropical floodplain, PR / MS, from March 2010 to March 2011.

Parasites	AN	NI	NP	P	MI	MA	AP
Urocleidoides malabaricusi	CHIOC 37791	50	691	92.59	13.82	12.79	1 - 117
Urocleidoides eremitus	CHIOC 37790	35	736	64.81	21.02	13.62	1 - 143
Urocleidoides cuiabai	CHIOC 37793	52	1532	96.29	29.46	28.37	1 - 193
Urocleidoides brasiliensis	CHIOC 37795	37	240	68.51	6.48	4.44	1 - 42
Anacanthorus sp.	CHIOC 37800	30	215	55.55	7.16	3.98	1 - 29
Vancleaveus janauacaensis	CHIOC 37797	2	2	3.7	1	0.037	1 - 2
Cosmetocleithrum bulbocirrus	CHIOC 37799	1	1	1.85	1	0.018	-
Dactylogyridae gen. 1 sp.	CHIOC 37802 a-b	26	222	48	8.53	4.11	1 - 47
Dactylogyridae gen. 2 sp.	CHIOC 37803	1	1	1,85	1	0.018	-

Table 2. The dispersion index (DI) and aggregation index of Green (IG) estimated for species of monogeneans of *Hoplias* aff. *malabaricus* in the upper Paraná river floodplain, PR / MS, from March 2010 to March 2011.

Parasites	DI	*D*	IG	Distribution
Urocleidoides malabaricusi	17.89	32.99	0.029	Aggregate
Urocleidoides eremitus	59.04	70.15	0.076	Aggregate
Urocleidoides cuiabai	4.41	75.0	0.044	Aggregate
Urocleidoides brasiliensis	12.8	26.58	0.049	Aggregate
Anacanthorus sp.	11.61	615.59	0.049	Aggregate
Dactylogyridae gen. 1. sp.	16.87	32.04	0.071	Aggregate

The average standart length of the fish analyzed was 24.35 cm (14.7 to 35.5). *Urocleidoides malabaricusi* showed significant and positive correlation between abundance and host standart length. *Urocleidoides eremitus* Kritsky, Thatcher & Boeger, 1986 showed significant and positive correlation between host standart length with prevalence (Table 3). There was no significant correlation between the hosts standart length with the results of diversity obtained by the Index Brillouin (rs = - 0.1320 p> 0.05).

Table 3. Values of the Spearman's rank correlation coefficient "rs" correlating host standard length and abundance of parasites, and the Pearson correlation coefficient "r" correlating the prevalence of parasitism and the standard length of *Hoplias* aff. *malabaricus* in the upper Paraná river Neotropical floodplain, PR / MS, from March 2010 to March 2011. p = significance level, * significant value.

Parasites	*rs*	*p*	*r*	*p*
Urocleidoides malabaricusi	0,244	0,050*	0,307	0,614
Urocleidoides eremitus	0,012	0,928	0,894	0,040*
Urocleidoides cuiabai	0,188	0,171	-0,000	0,999
Urocleidoides brasiliensis	0,048	0,728	0,720	0,169
Anacanthorus sp.	-0,131	0,343	-0,876	0,051
Dactylogyridae gen. 1. sp.	-0,158	0,251	-0,847	0,070

Of the 54 fish analyzed 31 were males and 23 females. According to the t test there was no significant difference in the standard length between males and females hosts (t = -0.805 $p >$ 0.05). *Urocleidoides cuiabai* and *U. brasiliensis* Rosim, Mendoza-Franco & Luque, 2011 showed a significant difference in abundance between male and female fish. Females were most affected by these species than the males. There were no significant differences in the prevalence of parasitism between males and females (Table 4).

Table 4. Test values "G" log-likelihood for detecting differences between the males and females in the prevalence of parasitism and the Mann-Whitney test with normal approximation "Z" to verify differences between the males and females in the abundance of parasitism of *Hoplias* aff. *malabaricus* in the upper Paraná river Neotropical floodplain, PR / MS, from March 2010 to March 2011. p = significance level, * significant value.

Parasites	Z	*P*	G	*P*
Urocleidoides malabaricusi	1,005	0,157	0,578	0,446
Urocleidoides eremitus	1,207	0,113	3,277	0,070
Urocleidoides cuiabai	1,670	0,047*	2,276	0,131
Urocleidoides brasiliensis	1,731	0,041*	0,546	0,459
Anacanthorus sp.	0,096	0,461	0,196	0,657
Dactylogyridae gen. 1. sp.	0,487	0,313	0,260	0,610

The fish studied were in different stages of maturation (rest, initial maturation, advanced maturation, partially spawn/spent, totally spawn/spent). The Kruskal-Wallis test was use to verify the possible influence of maturation stages in the levels of parasitism, but it was found no significant correlation (p>0.05).

Four of the six parasites species studied had abundance significantly and positively correlated with the relative condition factor (Table 5).

Table 5. Values of the Spearman's rank correlation coefficient "rs" correlating the relative condition factor and abundance of parasitism of *Hoplias* aff. *malabaricus* in the upper Paraná river Neotropical floodplain, PR / MS from March 2010 to March 2011. *p* = significance level, * significant value.

Parasites	*rs*	*p*
Urocleidoides malabaricusi	0.264	0.0530
Urocleidoides eremitus	0.567	0.0001*
Urocleidoides cuiabai	0.252	0.0650
Urocleidoides brasiliensis	0.475	0.0003*
Anacanthorus sp.	0.353	0.0088*
Dactylogyridae gen. 1. sp.	0.518	0.0001*

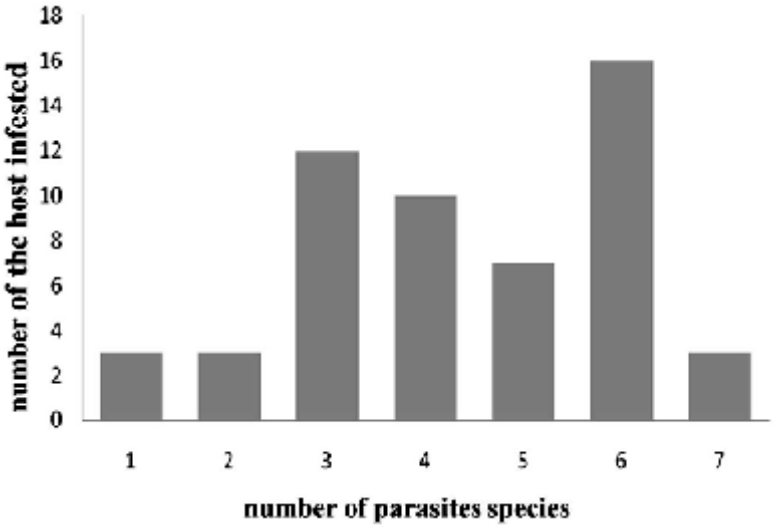

Figure 2. The gill parasites richness in *Hoplias* aff. *malabaricus* collected in the upper Paraná river floodplain, PR / MS, from March 2010 to March 2011.

DISCUSSION

Eiras (1994) stated that biotic factors such as host biology, behavior and migratory habits can interfere in the prevalence and intensity of infestation by monogeneans throughout the year. The fact that all "traíras" are parasitized of monogeneans, may be related to the behavior of these hosts, which according Graça & Pavanelli (2007) are sedentary, living in lentic environments such as lakes and canals. Pavanelli *et al.* (2008) mentioned that aquatic environments lentic or of confinement can favor the infestation by monogeneans this is because these parasites possess monoxenic cycle, finding facility to complete its life cycle, especially in environments with high densities of fish. Graça & Machado (2007) studying cichlids collected from an artificial lake in the city of Maringá in the Paraná State, Brazil, also observed that all fish were parasitized by at least one species of monogeneans, and justified this high prevalence by the fact that these fish feed on the lake bottom, which would facilitate the suspension of eggs and thus infestation by these parasites.

Another possible explanation for the high prevalence of monogeneans of "traíras" would be in the high density of fish species in the collection points, which according to Júlio Jr. *et al.* (2008) was a fish specie with higher abundance and biomass in the subsystems Baía and Ivinheima rivers in the upper Paraná river Neotropical floodplain. Takemoto *et al.* (2005 b) studied the influence of host density in the endoparasites diversity and found significant correlation as well as in the present study, possibly monogenetic showed increased levels of parasitism with the high population density of fish, as this may facilitate transmission of parasites.

Monogeneans, when present in fish farms, provokes high fish mortality rates, causing considerable damage (Eiras, 1994), however there are no records of high mortality caused by these parasites in fish of natural environments in Brazil (Thatcher, 1981). This probably occurs because of the difficulty in proving the cause of fish death and also by the fact that some fishes debilitated by parasites are easily preyed upon.

In the present study the distribution of species of monogeneans showed an aggregation pattern which, according to Abdallah *et al.* (2005) is characteristic of parasitic systems. Poulin (1998) argued that the distribution of parasites is due to processes that are not constant and

therefore is a dynamic event. There are several biotic and abiotic factors that may affect the distribution of parasites among hosts, these factors can increase the aggregation or even produce a more uniform distribution. Also according to this author, the composition of ectoparasite communities is strongly linked to the biology of the hosts. According to Zuben (1997) the aggregated distribution pattern acts to increase the density dependent regulation, the abundance of both host and parasite, and reduce the level of competition among parasite species.

Bagge *et al.* (2005) studying aggregation in carp monogenetic observed that by increasing the abundance parasitic there was a decrease in aggregation, and explained this result stating that with a greater abundance of parasites decreases the distance between these parasites increasing the chances of cross-fertilization between them.

The index of Berger - Parker indicated that *U. cuiabai* was dominant in relation to other species of parasites in the sample. Azevedo *et al.* (2007) when studying parasites of cichlids from Guandu river, observed that the monogeneans *Gussevia* sp. showed dominance in almost all studied specimens of fish, which the authors justified by the fact that these fish have preference for lentic environments, which facilitate infestation by this parasite. As the species of monogeneans found in "traíras" have similar biological characteristics and the hosts came from the same environments, the dominance of *U. cuiabai* studied in fish can be treated for greater efficiency of this species to colonize their host or because it presents features which causes it to be less susceptible to the host immune system. Another hypothesis is the competitive strength of this species and consequently a greater ecological niche than other species of Monogenea.

In our study the abundance of *U. malabaricusi* showed significant positive correlation with standard length of the host, *U. eremitus* its prevalence was also correlated with the host standard length. Lizama *et al*. (2007) when studying the monogeneans genus *Mymarothecium* Kritsky, Boeger & Jégu, 1996, observed a significant positive correlation between abundance and the standard length of their hosts, with increasing length of fish was increased abundance of this parasite species. However this same work for other species of monogeneans was found negative correlation between length and abundance of the monogeneans *Anacanthorus penilabiatus* Boeger, Husak & Martins, 1995, in this case the larger fish had a lower abundance of this parasite species. Lizama *et al.* (2007) justified that these variations in levels of parasitism in their study is possibly due to different characteristics of the sample points of their hosts.

Dogiel (1961) mentions that the length of the host is a major factor in the size range of parasitic infrapopulations. This is probably because the body surface and gill cavity can accommodate larger number of parasites, being more pronounced in monoxenic cycle of ectoparasites (Rohde, 1993). Luque *et al.* (1996) make a point of attention to avoid generalizations about the influence of the length of the host on the qualitative and quantitative composition of infracommunities of parasites. The fact that the hosts have a great length does not mean they will have a higher concentration of parasites, due to a longer exposure to infections. Still, according to Luque & Chaves (1999) in the case of ectoparasites, besides the size of the host the parasitism level may be influenced by the degree of specialization of the fasteners structure of the parasites and by the availability of infective forms to certain population groups of hosts.

In the present work, *U. brasiliensis* and *U. cuiabai* showed a significant difference in abundance correlated to host sex, but no significant differences in the prevalence of parasites between male and female fish. Luque & Cezar (2004) when working with ectoparasites of marine fish found no relation between levels of parasitism and host sex. This fact was also observed by Graça & Machado (2007). Similar results were observed by Rosim (2010) studying the parasite fauna of *H.* aff. *malabaricus* collected in different rivers of Brazil, noting that females had more parasites.

The fact that females of *H.* aff. *malabaricus* present a greater abundance of parasites may be related to differences in habits between males and females. Querol *et al.* (2003) stated that this fish species have parental care and, after breeding, the male cares for the eggs, getting closer to the nest. This characteristic of the male would leave them theoretically more exposed to monogeneans, but it was not what we found in our study. Thus, the factor that may have influenced the difference in parasitism between males and females is the reproductive period, which changes the physiology of the females, what may lower the immune defenses and thus increasing the susceptibility to parasites.

According to Le Cren (1951), the relative condition factor (Kn) is a quantitative indicator of the degree of welfare of fish, reflecting recent feeding conditions, given by the length / weight relation of the individual. The Kn takes into consideration the expected and observed weight, and the ideal value equal to one. Variations in Kn may be related to changes in the environment, lack of food or even by parasitism. By correlating Kn hosts with the abundance of parasitism, it is expected that most infected fish have a lower Kn, which is not necessarily true, because there are other factors that may influence the levels of parasitism.

Yamada *et al.* (2008) found significant and positive correlation to correlate the Kn of cichlids with the abundance of a species of monogeneans. Lizama *et al.* (2007) found negative and significant relation when relating the *Piaractus mesopotamicus* (Holmberg, 1887) Kn of fish farming with the levels of parasitism of two species of monogeneans. Tozato (2011) found no differences in Kn of *Corydoras aeneus* (Gill, 1858) parasitized and not parasitized by monogeneans, and concludes that these parasites did not affect the welfare of the host. The author also stated that the parasite community of fish studied has low pathogenicity to the host in the different environments of the basin, indicating the proper response of fish against parasitism.

In this study it was observed that the Kn of *H.* aff. *malabaricus* had significant and positive correlation with the abundance of parasitic species *U. eremitus*, *U. brasiliensis*, *Anacanthorus* sp., Dactylogyridae gen. 1 sp., or fish with a higher Kn were infected by these species of parasites. Cone (1995) stated that larger fish and a better Kn can withstand higher intensities of infection by monogeneans parasites despite being pathogenic. This is possibly the reason for the significant and positive correlation between Kn studied fish and abundance of parasitism by monogeneans.

The ecological study of monogeneans can provide a greater understanding of the biology of these parasites and their hosts, the collection site, and even how the host responds to the presence of these parasites. In this work we presented several factors that were crucial to the parasitism of monogeneans in *Hoplias* aff. *malabaricus* in the upper Paraná river floodplain, but we are far from understanding all the factors that influence the relation parasite / host. So we propose new ecological studies of fish parasites in the region, noting that there is no isolated factor favoring parasitism in fish, but rather a set of biotic and abiotic factors, and that generalizations can lead to erroneous interpretations that would undermine the studies on Ichthyoparasitology.

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