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Ecology of parasites of *Metynnis lippincottianus* (Characiformes: Serrasalmidae) from the eastern Amazon region, Macapá, State of Amapá, Brazil

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ABSTRACT. The present study provides the first investigation on the ecological aspects of the parasites *M. lippincottianus* from the Amazonian basin, as well as the parasite-host relationship. 76 out of the examined fish (98.7%) were parasitized by at least one species of parasites. A total of 8,774 parasites were collected, being *Ichthyophthirius multifiliis*, *Anacanthorus jegui*, *Dadayius pacupeva*, Digenea gen. sp. (metacercariae), *Procamallanus* (*Spirocamallanus*) inopinatus, *Procamallanus* (*Spirocamallanus*) sp., *Spinoxyuris oxydoras*, *Contracaecum* sp. larvae, *Dolops longicauda* and Hirudinea gen. sp. *Ichthyophthirius multifiliis* was the dominant species, followed by *A. jegui*. Among the endoparasites, *S. oxydoras* and *D. pacupeva* were predominant. The mean diversity of parasites was $HB = 0.96 \pm 0.32$ and there was aggregate distribution pattern. A positive correlation of body weight with the abundance of *I. multifiliis* and *S. Oxydoras* was found. The relative condition factor (Kn) was not negatively affected by parasites, and a positive correlation between Kn and abundance of *I. multifiliis*, *S. oxydoras* and *D. pacupeva* was found. This study is the first one to record *I. multifiliis*, *D. longicauda* and *A. jegui* parasitizing *M. lippincottianus*, as well as the first record of *D. pacupeva* and *S. oxydoras* in the Amazonas river system.

Keywords: Amazon, Characiformes, Metynnis lippincottianus, parasites infracommunities.

Ecologia parasitária de *Metynnis lippincottianus* (Characiformes: Serrasalmidae) da região da Amazônia oriental, Macapá, Estado do Amapá, Brasil

RESUMO. O presente estudo constitui a primeira investigação sobre aspectos da ecologia parasitária e relação parasito-hospedeiro de *M. lippincottianus* da Amazônia. De 80 peixes examinados, 76 (98,7%) estavam parasitados por uma ou mais espécies. Foram coletados 8.774 parasitos, entre *Ichthyophthirius multifiliis, Anacanthorus jegui, Dadayius pacupeva,* metacercárias de Digenea gen. sp., *Procamallanus (Spirocamallanus) inopinatus, Procamallanus (Spirocamallanus) sp., Spinoxyuris oxydoras,* larvas de *Contracaecum sp., Dolops longicauda* e Hirudinea gen. sp. *Ichthyophthirius multifiliis* foi a espécie de parasito dominante, seguido por *A. jegui* e entre os endoparasitos houve predominância de *S. oxydoras* e *D. pacupeva*. A diversidade média de parasitosfoi $HB = 0.96 \pm 0.32$ e houve padrão de distribuição agregado. Houve correlação positivo do peso corporal com a abundância de *I. multifiliis e S. oxydoras* e correlação negativa da abundância de larvas de *Contracaecum* sp. Apesar do elevado parasitismo o fator de condição relativo (Kn) dos hospedeiros não foi comprometido e houve correlação positiva do Kn com a abundância de *I. multifiliis, S. oxydoras* e *D. pacupeva*. Este foi o primeiro relato de *I. multifiliis, D. longicauda* e *A. jegui* para *M. lippincottianus* e ampliou a ocorrência de *D. pacupeva* e *S. oxydoras* para a Amazônia.

Palavras-chave: Amazônia, Characiformes, Metynnis lippincottianus, comunidades de parasitos.

Introduction

Parasites are recognized as important components of ecosystems specially when considering the dynamics of food web (MORLEY, 2012). Consequently, there has been increased research of these components in fish species of diverse ecosystems (LACERDA et al., 2012; RAKAUSKAS; BLAZEVICIUS, 2009; ROHDE et al., 1995; TAKEMOTO et al., 2009). Parasites can use intermediary and definite hosts via trophic web, which allows them to infect the fish species, but most parasite species show a high specificity to their hosts. Thus, the knowledge of parasites infecting fish is of particular interest not only regarding the host health, but also considering the relationship between parasite and host within the aquatic environment (LACERDA et al., 2012; MOREIRA et al., 2009, 2010; NEVES

et al., 2013; RAKAUSKAS; BLAZEVICIUS, 2009; TAKEMOTO et al., 2009; YAMADA et al., 2012).

Parasites can cause alterations in population dynamics and behavior of their hosts. Parasites influence the competition capacity in the predator-prey relationship. They can hinder the ability to swim, influencing the mating choice, sexual behavior and corporeal health of the host fish (GOMIERO et al., 2012). This study concerns the parasite relationship of the freshwater fish, Metynnis lippincottianus Cope, 1870 (Serrasalmidae), also commonly known as pacu CD. It is native to South America and found in French Guyana and Brazil distributed through the Amazon Basin, rivers of French Guyana (JÉGU, 2003), Paraná river (FROESE; PAULY, 2013; MOREIRA et al., 2009; YAMADA et al., 2012) and Tocantins river (SANTOS et al., 2004). M. lippincottianus is primarily herbivore, feeding on vegetable sources, seeds and phytoplanktonic algae, but occasionally consumes arthropods and debris (FROESE; PAULY, 2013; SANTOS et al., 2004), thus occupying the second trophic level of the food chain. This pelagic fish inhabits along the shores of rivers and lakes (FROESE; PAULY, 2013; MOREIRA et al., 2010; SANTOS et al., 2004) and measures at a maximum of 20 cm in length becoming sexually mature when it reaches 10 cm in length (SANTOS et al., 2004). Commercially, it is more valuable as an ornamental fish, but it is important species in the diet of riverine populations (MOREIRA et al., 2009; YAMADA et al., 2012).

In the Paraná river basin (Brazil), M. lippincottianus has been parasitized by Dadayius pacupeva, Procamallanus (Spirocamallanus) inopnatus, Contracaecum larvae, Raphidascaris mahnerti Spinoxyuris and oxydoras (MOREIRA et al., 2009; TAKEMOTO et al., 2009; MOREIRA et al., 2010; YAMADA et al., 2012). Even though this characid occurs in other hydrographic basins, such as the Amazon Basin, no study has been conducted on its parasites in the region, or any research has not been performed on the ectoparasites harbored by this fish. Therefore, this study evaluated the ecological aspects of parasites and the parasite-host relationship in *M. lippincottianus* of a tributary from the Amazonas river, in Northern Brazil.

Material and methods

Study area, fishes and parasite sampling

The Igarapé Fortaleza basin (Figure 1), located in the county of Macapá in the State of Amapá (eastern Amazon region) is a tributary of the Amazon river and has a main canal and a floodplain.

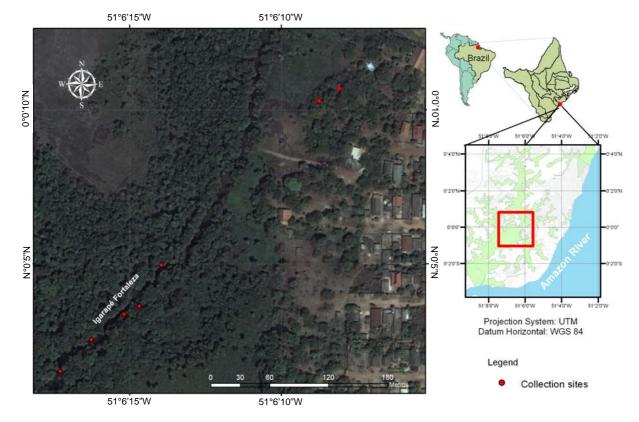


Figure 1. Collection sites of Metynnis lippincottianus along the Igarapé Fortaleza basin, eastern Amazon region, Brazil.

In the period from August to December of 2011, 80 specimens of *M. lippincottianus* were captured in the Fortaleza Igarapé basin (Figure 1), and analyzed for parasites. Fish were captured using nets with a 20-25 mm web (License ICMBio: 23276-1) placed into isothermal box with ice and taken to the Sanity Laboratory of Aquatic Organisms from Embrapa Amapá for parasitological analyses.

It exhibits unique characteristics: it is strongly influenced by the high pluviosity rates of the Amazon region and the diary strong tides of the Amazon delta (TAKYAMA et al., 2004), which create an ideal environment for fish inhabiting and feeding.

Collection and analysis of parasites

The captured fish where weighed (g) and measured for total length (cm). Each specimen was analyzed macroscopically and the observations were taken of the body surface, mouth, eyes, operculum, and gills. The gills were removed for ectoparasites collection while the gastro-intestinal tract was removed and examined for endoparasites collection. The parasites were collected, fixed, counted and stained using the method of Eiras et al. (2006). The ecological terms adopted follow the previous recommendations of Rohde et al. (1995) and Bush et al. (1997).

The index of Brillouin (*HB*), species richness, evenness index (*E*) and Berger-Parker dominance (*d*) were calculated for each infracommunity of parasites using the software Diversity (Pisces Conservation Ltda, UK). The index of dispersion (ID) and the index of discrepancy (D) were calculated using the software Quantitative Parasitology 3.0. All calculations were used to detect the distribution pattern of the infracommunities of parasites (RÓZSA et al., 2000) for species with prevalence >10%. ID significance was tested using the statistic *d* according to (LUDWIG; REYNOLDS, 1988) for each infracommunity.

The Pearson coefficient (r) was employed to test the relationship of total host length with prevalence of parasite infection and compared with previous studies on angular transformation of prevalence data (sinearc \sqrt{x}), after which the samples of hosts were separated into five length classes. The Pearson coefficient of correlation (r) was employed to investigate a possible correlation of *HB* between the prevalence of parasite infestation and the host length of in the sample collection (ZAR, 2010).

Body weight data (g) and total length (cm) were used for calculating the relative condition factor (Kn) of the sampled fish (LE-CREN, 1951), and then compared with the norm (Kn = 1.0) using the t-test. The Pearson coefficient (r) was also used to determine possible correlations in the abundance of parasites versus fish length, body weight, and Kn of the hosts (ZAR, 2010).

During three fish collections the pH (6.6 \pm 0.2), temperature (31.0 \pm 0.3°C) and dissolved oxygen levels (2.0 \pm 0.4 mg L⁻¹) were determined by using digital device for each purpose.

Results

80 specimens of M. lippincottianus with a total length of 7.1 \pm 0.8 cm and weight of 12.1 \pm 3.6 g were collected, 98.7% of the samples collected were infected with one or more species of parasites. Parasites were identified as Ichthyophthirius multifiliis Fouquet, 1876 (Ciliophora); Anacanthorus jegui Van Every and Kritsky, 1992 (Dactylogyridae); Dadayius pacupeva Lacerda et al. 2003 (Cladorchiidae), encapsulated metacercariae of Digenea gen. sp.; Procamallanus (Spirocamallanus) inopinatus Travassos et 1928. Procamallanus (Spirocamallanus) al. sp. (Camallanidae); Spinoxyuris oxydoras Petter, 1994 (Pharyngodonidae); Contracaecum Railliet & Henry, 1912 (Anisakidae), Dolops longicauda Heller, 1857 (Argulidae) and Glossiphoniidae gen. sp. (Hirudinea). Ichthyophthirius multifiliis was the dominant species, while nematode species were the most prevalent endoparasites (Table 1).

Table 1. Parasites of *M. lippincottianus* (n = 80) from the Igarapé Fortaleza basin, eastern Amazon, Brazil. MI: Mean intensity, MA: Mean abundance, TNP: Total Number of Parasites, SD: Standard deviation, RD: Relative dominance SI: Site of infection.

Parasites	Prevalence (%)	MI ± SD (Range)	MA	TNP	RD	SI
Ectoparasites						
Ichthyophthirius multifiliis	45.0	132.5 ± 157.2 (6-1133)	59.6	4.770	0.5437	Gills
Dolops longicauda	1.2	1.0 ± 0.1	0.01	1	0.0001	Gills
Anacanthorus jegui	95.0	$19.1 \pm 21.7 (1-138)$	18.1	1.450	0.1653	Gills
Digenea gen. sp. (metacercariae)	77.5	$11.1 \pm 10.2 (1-60)$	8.6	686	0.0782	Gills
Hirudinea gen. sp.	6.2	1.00 ± 0.2	0.06	5	0.0006	Gills
Endoparasites						
Dadayius pacupeva (adults)	82.5	$11.0 \pm 14.6 (1-77)$	9.1	727	0.0829	Intestine
Dadayius pacupeva (metacercariae)	22.5	$9.3 \pm 7.5 (2-60)$	2.1	168	0.0191	Intestine
Procamallanus (Spirocamallanus) inopinatus	36.2	$6.1 \pm 5.0 (1-32)$	2.2	176	0.0201	Intestine
Procamallanus (Spirocamallanus) sp.	8.7	$1.6 \pm 0.5 (1-4)$	0.1	11	0.0013	Intestine
Spinoxyuris oxydoras	65.0	12.5±17.9 (1-95)	8.1	652	0.0743	Intestine
Contracaecum sp. (larvae)	68.7	$2.3 \pm 2.2 (1-15)$	1.6	128	0.0146	Intestine

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The mean richness of species was 4.9 ± 1.3 per host, with predominance of parasites in parasitized individuals from 4 to 6 species (Figure 2). The mean diversity (*HB*) was 0.96 \pm 0.32), evenness (*E*) index was 0.48 \pm 0.15 and dominance (*d*) index was 0.55 \pm 0.18. The *HB* did not show a significant correlation (r = 0.17, p = 0.12) between parasite prevalence and total length of hosts.

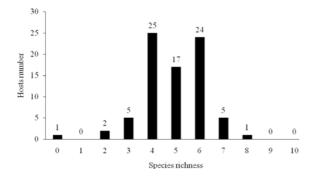


Figure 2. Species richness in parasites infracommunity of *M. lippinconttianus* from the Igarapé Fortaleza basin, eastern Amazon, Brazil.

Most of parasites species in *M. lippincottianus* showed aggregate distribution pattern, which is typical of parasites of freshwater fish. However, *I. multifiliis* and *P.* (*S.*) *inopinatus* showed a higher index of discrepancy (Table 2) indicating a greater level of parasitic aggregation

Table 2. Dispersion Index (ID), discrepancy Index (D) and statistic d for the parasites infracommunities of *Metynnis lippincottianus* from the Igarapé Fortaleza basin, eastern Amazon, Brazil.

Parasites	ID	d	D
Ichthyophthirius multifiliis	6.294	19.01	0.705
Anacanthorus jegui	4.218	13.28	0.374
Digenea gen. sp. (metacercariae)	4.761	14.89	0.478
Procamallanus (S.) inopinatus	4.278	13.46	0.764
Spinoxyuris oxydoras	4.573	14.34	0.590
Contracaecum sp. (larvae)	1.682	3.77	0.537
Dadayius pacupeva	4.283	13.48	0.486

The relative condition factor (Kn) for *M. lippincottianus* (Kn = 1.000 \pm 0.092, t = 0.023, p = 0.982) did not vary from the standard values of Kn, which indicates that the parasite infection did not harm the host's body condition. The prevalence of parasite infection showed negative correlation (r = -0.701, p = <0.0001) with the length of host indicating the higher prevalence of parasites in smaller hosts. However, there was a positive correlation in the abundance of *I. multifiliis* and *S. oxydoras* when weight and Kn of the fish were compared, as well as the abundance of *D. pacupeva*

with Kn relative condition factor. In contrast, the abundance of *Contracaecum* sp. larvae showed a negative correlation when compared with the length of the hosts (Table 3).

Table 3. Pearson coefficient (r) of correlation for parasite abundance compared with total length (cm), body weight (g), and relative condition factor (Kn) for *Metynnis lippincottianus* of the Igarapé Fortaleza basin, eastern Amazon, Brazil.

	Length (cm)		Weight (g)		Kn	
Parasites	r	р	r	р	r	р
Ichthyophthirius multifiliis	0.063	0.580	0.250	0.025	0.253	0.023
Anacanthorus jegui	-0.063	0.559	-0.116	0.304	-0.078	0.493
Digenea gen. sp. (metacercariae)	-0.066	0.559	-0.080	0.479	-0.097	0.389
Procamallanus (S.) inopinatus	-0.159	0.158	-0.055	0.628	0.110	0.332
Spinoxyuris oxydoras	-0.006	0.957	0.249	0.026	0.327	0.003
Contracaecum sp. (larvae)	-0.253	0.023	-0.132	0.240	0.073	0.518
Dadayius pacupeva	0.007	0.947	0.169	0.132	0.230	0.040

Discussion

Specimens of M. lippincottianus captured during the dry season in the Amazon showed the highest diversity of parasites (HB = 0.962 ± 0.32). Parasite samples were composed of one Protozoa, one Monogenoidea, two Digenea, four Nematoda, one Crustacea, and one Hirudinea. Ichthyophthirius multifiliis, A. jegui, D. pacupeva, metacercariae of Digenea, P. (S.) inopinatus, Procamallauns (S.) sp., S. oxydoras and Contracaecum larvae showed a distribution pattern of aggregation. Aggregate dispersion for D. pacupeva, S. oxydoras, P. (S.) inopinatus, Contracaecum sp. and Raphidascaris (Sprentascaris) mahnerti infesting M. lippincottianus in the upper Paraná river basin was also reported, where D. pacupeva and S. oxydoras were the dominant parasites with HB = 0.337 (MOREIRA et al., 2009). Such a distribution pattern could be related to a parasite strategy for survival, genetic and immunity environmental heterogeneity of the hosts, conditions (KNUDSEN et al., 2004; MOREIRA et al., 2009; NEVES et al., 2013) and the host's survival mechanism (KNUDSEN et al., 2004; NEVES et al., 2013).

Out of the parasites reported for *M. lippincottianus* from the Paraná river basin that were captured in different seasons of the year (MOREIRA et al., 2009, 2010; YAMADA et al., 2012), only *R.* (*S.*) *mahnerti* was not found in the present study. On the other hand, ectoparasites were not found in the gills (*I. multifiliis, A. jegui*, metacercariae of digeneas, *D. longicauda* and hirudineas), which were not studied for this host from the Paraná river basin. Diferences in richness and diversity of parasites for a host that inhabits different geographic regions could be associated with its ecology (size, age, heterogeneity of diet, and behavior), as well as the environmental

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factors, in particular, the physical and chemical parameters, the presence of intermediary hosts in the location, and seasonal differences (LACERDA et al., 2012; MOREIRA et al., 2009; NEVES et al., 2013; RAKAUSKAS; BLAZEVICIUS, 2009; SILVA et al., 2011; YAMADA et al., 2012), besides host density.

Ichthyophthirius multifiliis is a dominant parasite species infecting the pelagic fish, M. lippincottianus that showed higher levels of infection than those reported for the bentonic fish, Oxydoras niger of the Solimões river (SILVA et al., 2011). This ciliate fish parasite is common in natural habitats as well as in farmed fish (RAISSY et al., 2010; RAKAUSKAS; BLAZEVICIUS, 2009; WURTSBAUGH; TAPIA, 1998). However, the infection is higher in fish raised in tanks. This is due to horizontal transmission of this parasite, which causes lesions on the gills and cutaneous surfaces, thus hindering respiration of the host and facilitating the entrance of bacteria (RAISSY et al., 2010). However, epizooty caused by I. multifiliis also occurs in natural populations (RAISSY et al., 2010; WURTSBAUGH; TAPIA, 1998), but this occurrence has not been documented in wild fish in Brazil.

On the gills of *M. lippincottianus* captured along the tributaries of the Amazon river in the State of Amapá, high levels of infection by *A. jegui* were found. This is a species of monogenoidean originally described parasitizing another characid that inhabits the Amazonas River tributaries, *S. rhombeus* (VAN EVERY; KRITSKY, 1992). Monogenoideans are parasites with high specifity when compared with other taxa of helminths (SILVA et al., 2011; TAKEMOTO et al., 2009). For *M. lippincottianus* captured in any other location there is no record of *A. jegui*. Therefore, this is the first record of *A. jegui* for this host, in addition it has expanded its presence for the eastern Amazon region.

Leeches species of Glossiphoniidae family were observed on the gills of *M. lippincottianus* in low levels of infection, which is similar to the results described for *R. rutilus* from different ecosystems (RAKAUSKAS; BLAZEVICIUS, 2009). Glossiphoniidae are common parasites infecting freshwater fish worldwide, but they can occur in high density in some species of hosts (SKET; TRONTELJ, 2008). However, among freshwater fish inhabiting the Brazilian rivers and lakes, the species of the genera *Helobdella* and *Mymyzobdella* have been more common (TAKEMOTO et al., 2009).

Dolops longicauda occurred in low levels of infection on the gills of *M. lippincottianus*. This

argulid is distributed in fish throughout Uruguay, Argentina, and Brazil (States of Mato Grosso, São Paulo, Paraná, Rio Grande do Sul, and Amazonas) (MALTA, 1998). However, this study is the first one to relate the occurrence of *D. longicauda* in *M. lippincottianus*, and also expand the occurrence of this parasite species for the eastern Amazon region.

In M. lippincottianus of the eastern Amazon, low levels of infection by Digenea metacercarie were observed on the gills, and D. pacupeva metacercarie were collected in the intestine. However, these levels of infection by D. pacupeva were lesser than those described for this same host from the Paraná River basin (MOREIRA et al., 2009; YAMADA et al., 2012), which was probably due to a lower ingestion of the infective stages of this digenean. Differences in the abundance of D. pacupeva in M. lippincottianus were also reported by Moreira et al. (2009). Yamada et al. (2012) mentioned that ontogenetic alterations in the diet or habitat will make M. lippincottianus more susceptible to infections by D. pacupeva, a parasite that accumulates in the intestine. The species within Trematoda needs mollusks as first intermediate hosts (MOREIRA et al., 2009; MORLEY, 2012), and fish such as M. lippincottianus are the definite host of D. pacupeva. Although the main role of cercariae of these parasites, which are components of zooplankton (meroplankton), being to find and infect the target host; secondarily, they play an important role in the trophic web of aquatic environments. When they migrate in the water column searching for target hosts, they also become a food source for fish (MORLEY, 2012), including M. lippincottianus, a pelagic host, which showed high level of infection by D. pacupeva.

Metynnis lippincottianus is a omnivorous fish that feeds on aquatic plants, phytoplanktonic algae, and at times will eat microcrustaceans, and detritus (FROESE; PAULY, 2013; SANTOS et al., 2004), but along the Igarapé Fortaleza basin it was found that this fish is a frequent predator of microcrustaceans and other invertebrates. Consequently, it became infected by four species of nematodes: P. (S.) inopinatus, Procamallauns (S.) sp., S. oxydoras and Contracaecum sp. S. oxydoras was the predominant nematode; similar to that described for this same host from Paraná river basin (MOREIRA et al., 2009; YAMADA et al., 2012). On the other hand, the hosts of the present study showed higher levels of P. (S.) inopinatus, Procamallauns (S.) inopinatus and Contracaecum larvae, which have low parasitic specificity (MORAVEC, 1998; MOREIRA et al., 2009). Chironomids are intermediate hosts of P. (S.) inopinatus while microcrustaceans are

intermediate hosts of *Contracaecum* sp. (MOREIRA et al., 2009). Even though *S. oxydoras* is a nematode with a direct life cycle, it uses *Oxydoras kneri* (MORAVEC, 1998) and *M. lippincottianus* to reach its adult stage.

The Kn values of M. lippincottianus indicated that the body condition of the hosts was not affected by high infection of ectoparasites and endoparasites. The abundance of I. multifiliis, D. pacupeva and S. oxydoras was higher in fish with better relative conditions. Similarly, for M. lippincottianus from Paraná River basin was reported that the largest fish were infected by D. pacupeva and S. oxydoras and showed higher Kn values (MOREIRA et al., 2010). Larger hosts support a higher degree of infection by these parasites because such parasites are not pathogenic and cause little harm to their host. On the other hand, more pathogenic and abundant parasites may reduce the condition factor of hosts (LACERDA et al., 2012). This is because they have minor chance of reacting to the infections when their immunological system does not aptly respond to the parasite infection.

The total length (5.0 - 10.0 cm) of M. lippincottianus (juveniles and sub-adults), which may be an expression of their age, showed negative correlation with abundance of Contracaecum larvae and Kn, indicating that larger and older individuals ingested a smaller quantity of food containing infective stages of these nematodes. Only host body weight showed a positive correlation with abundance of I. multifiliis and S. oxydoras, which indicates that larger-sized hosts accumulate higher quantities of these parasites. For this same host in the Paraná river basin, positive correlation was found between total length and abundance of Contracaecum sp., D. pacupeva and S. oxydoras (YAMADA et al., 2012), whose fish examined had from 1.4 - 13.4 cm in total length and were at all stages of its life cycle (fingerlings, juveniles, and adults). However, such discrepancies may have been caused by differences in ecosystems and variations in size (age) of the hosts that were examined in both studies.

Conclusion

Metynis lippincottianus, a pelagic fish, showed diversity of ectoparasites and endoparasites. If one considers that this freshwater fish spends a greater part of its life cycle in areas with vegetation, it may be feeding on microcrustaceans and possibly, on cercariae of *D. pacupeva* and ostracods mollusks, which are constant items of their diet. Due to low parasites diversity, the *HB* was not correlated with

the length of the hosts, which indicates little ontogenic variation in the diet of this host in its juvenile and sub-adult stages. Our results indicated that juveniles and sub-adults *M. lippincottianus* are omnivore fish during these phases of its life cycle. This study records the first occurrence of *I. multifiliis* in *M. lippincottianus*, and expands the occurrence of *D. pacupeva* and *S. oxydoras* for the Amazon region.

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