BOLETIM DO INSTITUTO DE PESCA



ISSN 1678-2305 online version Scientific Article

CONDITION FACTOR AND ECOLOGY OF ENDOHELMINTHS IN *Metynnis lippincottianus* FROM THE CURIAÚ RIVER, IN EASTERN AMAZON (BRAZIL)

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Received: November 21, 2019 Accepted: July 22, 2020

ABSTRACT

Fish are among the vertebrate groups most susceptible to parasitic infections due to environmental characteristics that can favor the development of parasites; also, position of the host in food web has been associated with the parasitism. The aim of this study was to investigate the ecology of parasites endohelminth in *Metynnis lippincottianus* from the Curiaú River, in eastern Amazon (Brazil), as well as to evaluate the parasite-host interactions. From 110 specimens of *M. lippincottianus* examined, 76.3% were infected by one or more parasite species, and a total of 146 parasites such as *Procamallanus (Spirocamallanus) inopinatus* (Nematoda), larvae of *Contracaecum* sp. (Nematoda) and *Neoechinorhynchus pterodoridis* (Acanthocephala) were collected in intestine. However, the dominance was of nematode species, and the parasites had uniform or random dispersion. The parasitic infracommunities showed low Shannon-Wiener diversity (0.2 \pm 0.3), low evenness (0.2 \pm 0.3) and low species richness of parasites (1.1 \pm 0.8). The abundance of *Contracaecum* sp. presented a weak positive correlation with the weight of hosts, and relative condition factor was not affected by the parasitism. This first study about parasites of *M. lippincottianus* from Curiaú River basin is the first report of *N. pterodoridis* for this host.

Key words: infracommunity; parasites; Nematoda; Acanthocephala.

FATOR DE CONDIÇÃO E ECOLOGIA DE ENDOHELMINTOS EM *Metynnis lippincottianus* DO RIO CURIAÚ NA AMAZÔNIA ORIENTAL (BRASIL)

RESUMO

Os peixes estão entre os grupos de vertebrados mais suscetíveis as infecções parasitárias devido as características ambientais que podem favorecer o desenvolvimento de parasitos; além disso, a posição do hospedeiro na cadeia alimentar tem sido associada ao parasitismo. O objetivo deste estudo foi investigar a ecologia de parasitos endohelmintos em *Metynnis lippincottianus* do Rio Curiaú, na Amazônia oriental (Brasil), bem como avaliar as interações parasito-hospedeiro. De 110 espécimes de *M. lippincottianus* examinados, 76,3% estavam infectados por um ou mais espécies de parasitos, e um total de 146 parasitos como *Procamallanus* (*Spirocamallanus*) *inopinatus* (Nematoda), larvas de *Contracaecum* sp. (Nematoda) e *Neoechinorhynchus pterodoridis* (Acanthocephala) foram coletados no intestino. No entanto, a dominância foi de espécies de nematoides, e os parasitos apresentaram dispersão uniforme ou aleatória. As infracomunidades parasitárias apresentaram baixo índice de diversidade de Shannon-Wiener (0,2 ± 0,3), baixa uniformidade (0,2 ± 0,3) e baixa riqueza de espécies (1,1 ± 0,8). A abundância de *Contracaecum* sp. apresentou correlação positiva fraca com o peso dos hospedeiros e o fator de condição não foi afetado pelo parasitismo. Este primeiro estudo sobre parasitos de *M. lippincottianus* da bacia do Rio Curiaú é o primeiro relato de *N. pterodoridis* para este hospedeiro.

Palavras chave: infracomunidade; parasitos, Nematoda; Acanthocephala.

INTRODUCTION

The Neotropical region has the most diversified fish fauna in the world, presenting more than 9100 species, equivalent to 27% of the fish species in the entire planet (Reis et al., 2016). A great part of these fish is in Amazon, in regions as the state of Amapá, which has a diversity of fish species with importance for the fishing. Among

these species are the fishes from family Serrasalmidae, which are widely distributed throughout the rivers of Amazon system, inhabiting mainly floodplains, flooded forests, lakes and rivers (Froese and Pauly, 2019). *Metynnis lippincottianus* Cope, 1870 is a Serrasalmidae popularly known as "pacu" or "pratinha" and can be found in the Brazilian basins and rivers of French Guiana, bordering rivers and lakes. Its diet consists of aquatic plants, seeds, phytoplankton, mollusks, crustaceans and detritus (Moreira et al., 2009; Yamada et al., 2012; Hoshino and Tavares- Dias, 2014; Froese and Pauly, 2019).

Fish are among the vertebrate groups most susceptible to parasitic infections due to environmental characteristics that can favor the development of parasites. In addition, the position of the host fish in food web has also been a great influence in richness and communities of endohelminths (Silva et al., 2011; Neves et al., 2013; Gonçalves et al., 2014; Carvalho and Tavares-Dias, 2017). In freshwater fishes, more than 300 species of endohelminth parasites have been reported (Neves et al., 2013; Duarte et al., 2016; García-Lopez et al., 2016; Carvalho and Tavares-Dias, 2017; Oliveira et al., 2018). For M. lippincottianus from the upper Paraná River has been reported endohelminths such as digeneans Dadayus pacupeva Lacerda, Takemoto & Pavanelli, 2003; nematodes Spinoxyuris oxydoras Petter, 1994, Procamallanus (Spirocamallanus) inopinatus Travassos et al., 1928: larvae of Contracaecum sp. and Raphidascaris (Sprentascaris) mahnerti Petter & Cassone, 1984 (Moreira et al., 2009; Yamada et al., 2012). The endohelminths community in M. lippincottianus from the Igarapé Fortaleza basin (Macapá) was composed by nematodes *P.* (*S.*) *inopinatus*, *Contracaecum* sp., *Procamallanus* (*Spirocamallanus*) sp. and *S. oxydoras* and metacercariae of digeneans (Hoshino and Tavares-Dias, 2014). However, has not been studied before the endohelminth parasites fauna of *M. lippincottianus* from the Curiaú River, state of Amapá, in eastern Amazon (Brazil) has not been studied before.

Studies on fish parasitic ecology are important to improve the knowledge of the relationships between host and parasites. This knowledge may assist in plan of management in wild fish populations (Moreira et al., 2010; Silva et al., 2011). In fish populations, body length and weight can be used to estimate the relative condition factor (Kn), a tool qualitative of body conditions that can be associated with the parasites load (Moreira et al., 2010; Camara et al., 2011; Silva et al., 2011; Tavares-Dias and Oliveira, 2017). Thus, the aim of this study was to investigate the ecology of the endohelminth parasites in *Metynnis lippincottianus* from the Curiaú River, in eastern Amazon, as well as to evaluate parasites-host relationship.

MATERIAL AND METHODS

Fish and study area

From September 2017 to August 2018, 110 specimens of *Metynnis lippincottianus* were collected in the Curiaú River, region of Environmental Protection Area from the Curiaú basin, in the State of Amapá, located in the municipality of Macapá (Figure 1). Fish were collected using gillnets of 20-30 mesh sizes and immediately transported alive in containers containing water to the Laboratory



Figure 1. Collection sites of Metynnis lippincottianus in the Curiaú River basin, in eastern Amazon (Brazil).

of Morphophysiology and Animal Health (LABMORSA) of the State University of Amapá (UEAP), in Macapá (AP).

The Curiaú hydrographic basin is located in the estuarine coastal sector of the Amazon River. It is characterized as a river system with extensive floodplains that constitute physical systems connected via a silted-up river that forms a drain for freshwater. It is influenced by high rainfall and the daily tides of the Amazon River, to which it is connected. The main channel of the Curiaú basin transports rich organic matter that is brought in by the tides of the Amazon River and is carried to the floodplain, which is a protected environment. In this basin, during the rainy season, waters are rich in nutrients due to the fast decomposition of grass and animal remains and because the layer of humus produced by the forest is spread across the floodplain. This basin has dissolved oxygen levels of 2.5 mg L⁻¹ and pH of 5.6 (Takiyama et al., 2004; Torres and Oliveira, 2004). The region has a tropical climate; the rainy season occurs from December to May and the dry season, from June to November (Souza and Cunha, 2010).

Parasite sampling procedures

The fish were euthanized by medullary section, weighed (g) and measured for total length (cm) and then necropsied for collection and analysis of endoparasites. The viscera and gastrointestinal tract were examined for the presence of endoparasites and cysts. During the necropsy, small tissue fragments of organs were separated between slides and coverslips, where foci of parasite development were found for light microscope analysis. The collection, fixation, counting, preparation and staining of parasites for identification followed previous recommendations from Eiras et al. (2006). Nematodes were fixed in hot formalin (5%) and conserved in 70% alcohol, and then clarified in Faia's creosote. Acanthocephalans were fixed in formalin (5%), conserved in 70% alcohol and stained al alcoholic carmine of Langeron. The identification of parasites followed the recommendations of Moravec (1998) and Thatcher (2006). Ecological terms (prevalence, mean intensity and mean abundance) followed the recommendations of Bush et al. (1997).

The Ethics Committee for Animal Use from, Protocol N° 012-CEUA/CPAFAP and the System of Authorization and Information on Biodiversity (SISBIO) N° 50376-1 approved these procedures, besides the Secretary of the Environment of the State of Amapá (SEMA-AP), Letter N° 1014/2016, because it is an Environmental Protection Area from Curiaú River basin.

Data analyses

The following descriptors for the parasite component community were determined: species richness, diversity of Shannon-Wiener (*H*) and associated evenness (Magurran, 2004) using Diversity software (Pisces Conservation Ltda., UK). The frequency of dominance (percentage of infracommunities in which a species will be numerically dominant) was calculated according to Rohde et al. (1995). The dispersion index (ID) and the Poulin discrepancy index (D) calculated using Quantitative Parasitology 3.0 software, in order to detect the distribution pattern of each parasite infracommunity (Rózsa et al., 2000), in species with prevalence $\geq 10\%$. The significance of the ID, for each species of parasite, was tested by the statistical-*d* (Ludwig and Reynolds, 1988).

Body weight (g) and total length (cm) data were used to calculate the relative condition factor (Kn) of the fish (Le-Cren, 1951) that will be compared with the standard value (Kn = 1.0) using the test-*t*. The Spearman correlation coefficient (*rs*) was used to investigate correlation of parasites abundance with length and weight of hosts (Zar, 2010).

RESULTS

From 110 specimens of *M. lippincottianus* examined, 76.3% were infected by one or more parasites. A total of 146 parasites were collected, such as *P.* (S) *inopinatus* (Camallanidae: Nematoda), *Contracaecum* sp. larvae (Anisakidae: Nematoda) and *Neoechinorhynchus pterodoridis* Thatcher, 1981 (Neoechinorhynchidae: Acanthocephala) collected from the intestine. However, the dominance was of *P.* (S) *inopinatus* (Table 1).

Procamallanus (S.) inopinatus had a random pattern of distribution, while *Contracaecum* sp. and *N. pterodoridis* presented an uniform pattern of distribution (Table 2). The mean species richness was 1.1 ± 0.8 , diversity index was 0.2 ± 0.3 and evenness 0.2 ± 0.3 (Table 3), with predominance of individuals with one parasite (Figure 2).

In *M. lippincottianus*, only the abundance of *Contracaecum* sp. had a weak positive correlation with weight of hosts (Table 4).

Parasites	P (%)	$MI \pm SD$	$MA \pm SD$	DF (%)	SI
Procamallanus (Spirocamallanus) inopinatus	42.7	1.4 ± 0.8	0.6 ± 0.9	45.0	Intestine
Contracaecum sp. (larvae)	37.3	1.1 ± 0.5	0.4 ± 0.6	32.0	Intestine
Neoechinorhynchus pterodoridis	26.4	1.2 ± 0.6	0.3 ± 0.6	24.0	Intestine

P: Prevalence (P), MI: Mean intensity, MA: Mean abundance, SD: Standard deviation, DF: Dominance frequency, SI: Site of infection

Table 2. Dispersion index (DI), statistical index *d* and discrepancy index (D) of *Metynnis lippincottianus* parasites from the Curiaú River, eastern Amazon (Brazil).

Parasite species	DI	d	D	Dispersion type
Procamallanus (Spirocamallanus) inopinatus	1.09	0.68	0.65	Random
Contracaecum sp. (larvae)	0.78	-1.7	0.65	Uniform
Neoechinorhynchus pterodoridis	0.95	-0.6	0.76	Uniform

Table 3. Body parameters and diversity indexes for the community of endohelminths in *Metynnis lippincottianus* from the Curiaú River, in eastern Amazon (Brazil).

Parameters	Mean ± SD	Range
Length (cm)	7.7 ± 0.8	5.9-9.6
Weight (g)	9.1 ± 2.9	4.0-16.0
Condition factor (Kn)	1.00 ± 0.08	0.77-1.26
Species richness of parasites	1.1 ± 0.8	0-3
Shannon-Wiener (H)	0.2 ± 0.3	0-1.1
Evenness (E)	0.2 ± 0.3	0-1.0



Figure 2. Species richness of parasites in *Metynnis lippincottianus* from the Curiaú River basin, in eastern Amazon (Brazil).

Table 4. Spearman correlation coefficient (*rs*) of the parasite abundance with the length and weight of endohelminths in *Metynnis lippincottianus* from the Curiaú River, in eastern Amazon (Brazil).

Body parameters	Length		Weight	
Parasite species	rs	р	rs	р
Procamallanus (Spirocamallanus) inopinatus	0.04	0.66	-0.07	0.44
Contracaecum sp. (larvae)	0.13	0.18	0.22	0.02
Neoechinorhynchus pterodoridis	0.002	0.98	-0.12	0.23

DISCUSSION

The patterns among parasitic communities of host fish populations can be detected through quantitative and qualitative descriptors (Magurran, 2004). In *M. lippincottianus*, the endohelminth community was composed only by two species of nematodes *P.* (*S.*) *inopinatus* and *Contracaecum* sp. and one species of acanthocephalan *N. pterodoridis*, but with dominance of nematodes. In addition, the Shannon diversity, evenness and species richness of parasites were low. In contrast, for *M. lippincottianus* from the Fortaleza Igarapé basin (Macapá, AP) the endohelminths reported were *P.* (*S.*) *inopinatus*, *Procamallanus* (*S.*) sp., *Contracaecum* sp., *S.oxydoras* and metacercariae of digeneans (Hoshino and Tavares-Dias, 2014). In the Paraná River, *M. lippincottianus* was infected by *S.oxydoras*, *P.* (*S.*) *inopinatus*, *Contracaecum* sp., *Raphidascaris* (*Sprentascaris*) mahnerti and *D. pacupeva* (Moreira et al., 2009; Yamada et al., 2012). However, in wild fish populations, the parasite diversity and richness depend directly on the population dynamics, age and diet of the hosts, presence of infective forms, environment quality, seasonality and geographical factors, among other biotic and abiotic factors (Hoshino and Tavares-Dias, 2014; Tavares-Dias and Oliveira, 2017).

In *M. lippincottianus* from Curiaú River, we found random dispersion for *P.* (*S.*) *inopinatus* and uniform dispersion for *Contracaecum* sp. and *N. pterodoridis*. In contrast, for this same host from Igarapé Fortaleza River the dispersion of *P.* (*S.*) *inopinatus* and *Contracaecum* sp. was aggregated (Hoshino and Tavares-Dias, 2014), a typic pattern for freshwater fish (Guidelli et al., 2003; Poulin (2013); Hoshino and Tavares-Dias, 2014). Uniform distribution pattern is expected for parasites species. However, random dispersion pattern may be related to a reduced opportunity to colonize this host (Guidelli et al., 2003). Pathogenicity of parasite, individual differences in immunological reaction and susceptibility to infection might have also caused these patterns of parasite distribution.

In M. lippincottianus from the Curiaú River basin, the total prevalence of parasites (76.3%) was similar to that observed in the same host (72.7%) from the upper Paraná River (Moreira et al., 2009; Yamada et al., 2012), but this was lower (98.7%) than that reported by Hoshino and Tavares-Dias (2014). Therefore, these differenced results are related to differences in environmental characteristics. In addition, the infections by P. (S.) inopinatus were also similar to that found by Hoshino and Tavares-Dias (2014), for same host from the Igarapé Fortaleza basin. In contrast, infection levels of larvae of Contracaecum sp. were lower than that observed in *M. lippincottianus* from the Igarapé Fortaleza basin (Hoshino and Tavares-Dias, 2014). Both nematode species present low host specificity, but P. (S.) inopinatus uses fish species as definitive hosts, while larvae of Contracaecum sp. use fish species as intermediate or paratenic hosts, and piscivorous birds are definitive hosts (Gonçalves et al., 2014; Pinheiro et al., 2019). Therefore, the presence of *Contracaecum* sp. larvae is related to the low position of *M. lippincottianus* in food web and trophic level. Larvae of Contracaecum sp. belong to family Anisakidae, which has zoonotic potential to humans (Pinheiro et al., 2019), when there is ingestion of raw or under-cooked fish infected with this nematode.

Neoechinorhynchus pterodoridis, an acanthocephalan described of *Pterodoras granulosus* Valenciennes, 1821 (Siluriformes) from Amazon River system (Thatcher, 1981) whose cycle is unknown, was also reported in *Chaetobranchopsis orbicularis* Steindachner, 1875 from a tributary of the Amazon River system, a definite host (Tavares-Dias and Oliveira, 2017). However, in the present study *N. pterodoridis* is being reported for the first time in *M. lippincottianus*, representing a new host record.

Variation in the health status of host fish populations, such as alteration in body condition due to nutritional deficiency can lead to parasitic infections (Moreira et al., 2010; Hoshino and Tavares-Dias, 2014; Tavares-Dias and Oliveira, 2017). However, in *M. lippincottianus* infected by few endohelminth species, the condition factor indicated good body condition. The body size of

the fish can play an important role in determining the infection susceptibility of hosts, growth, and consequently has great implications in the dynamics of infection and the ecology of host-parasites interactions (Silva et al., 2011; Hoshino and Tavares-Dias, 2014; Tavares-Dias and Oliveira, 2017). In contrast, the body size of *M. lippincottianus*, had little influence in abundance of parasites, indicating that host body size was not an important determinant of variations in abundance of parasites species.

CONCLUSIONS

This first study on *M. lippincottianus* endohelminth parasites from the Curiaú River basin showed that the parasitic fauna was composed by a low diversity of endohelminth species, predominantly by nematodes and with low abundance and intensity. This low abundance of parasites did not influence the body conditions of host population. For *M. lippincottianus*, host size does not explain the species abundance of parasites. Lastly, other factors that were not analyzed herein are responsible for parasite richness, low diversity, intensity and abundance.

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