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Parasites in *Leporinus macrocephalus* (Anostomidae) of four fish farms from the western Amazon (Brazil)

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Abstract: This study evaluated the presence of metazoan parasites in *Leporinus macrocephalus* from four fish farms from the western Amazon (Brazil). In 160 fish examined, prevalence was 61.9%, and parasites found were: *Urocleidoides paradoxus*, *Urocleidoides eremitus*, *Tereancistrum parvus*, *Jainus leporini*, *Procamallanus* (*Spirocamallanus*) *inopinatus*, *Rhabdochona* (*Rhabdochona*) *acuminata*, *Dolops discoidalis* and *Ergasilus* sp., but *U. paradoxus* was the dominant parasite. *Jainus leporini* and *Ergasilus* sp. occurred only in *L. macrocephalus* from one fish farm, while *U. paradoxus*, *U. eremitus* and *T. parvus* were found in fish from three fish farms. *Dolops discoidalis*, *P.* (*S.*) *inopinatus* and *R.* (*R.*) *acuminata* occurred only in *L. macrocephalus* from two fish farms. Higher infection levels were caused by *U. paradoxus*, *U. eremitus* and *P.* (*S.*) *inopinatus*, which had an aggregated dispersion. There was positive correlation between abundance of parasites and the length of hosts. No difference in the condition factor of parasitized and non-parasitized fish were found. Such differences between fish farms were attributed to differences in management and quality of cultivation environments, and data indicate the need to adopt prophylactic measures in the fish farms to prevent diseases in the future. This was the first report of *D. discoidalis* and *Ergasilus* sp. for *L. macrocephalus*.

Key words: *Dolops discoidalis*, *Ergasilus* sp., freshwater fish, prevalence, *Urocleidoides paradoxus*.

INTRODUCTION

Leporinus macrocephalus Garavello & Britski, 1988; popularly known as piauçu or piavussu, is an endemic Anostomidae fish to the Paraguay River basin and can reach up to 60 cm in length; hence it is an important fishery resource and also valuable for aquaculture of some Brazilian regions. Among the species of the genus *Leporinus*, *L. macrocephalus* is the largest species. Thus, it had been cultivated mainly in the Southeast Brazilian region, once it presents high weight gain, high feed conversion, fast growth, tasty meat and rusticity to cultivation (Andrade et al. 2006, Capodifoglio et al. 2015). However, recently, *L. macrocephalus* has also been reared in the State of Acre, in northern Brazil (Martins et al. 2017a, b).

As the State of Acre has great potential for fish farming, in 2015 there was the creation of a state industrial complex to produce fish native to Amazon, to intending boost the activity to around 2,162 fish farmers. Thus, in 2017, the State of Acre produced 8,000 tons of farmed fish, mainly native species, including *L. macrocephalus*. In 2018, this production had an increase of 6.3% and reached 8,500 tons (PeixeBR 2019). Despite the economic importance of *L. macrocephalus* for the fish farming in the State of Acre, little is known about its parasite fauna and epidemiological indices.

For *L. macrocephalus* farmed in the northeastern region of the State of São Paulo, the following parasites have been reported: *Henneguya leporinicola* Martins, Souza, Moraes & Moraes, 1998 (Martins et al. 1999); *Goezia leporini* Martins & Yoshitoshi 2003 (Martins & Yoshitoshi 2003); *Ichthyophthirius multifiliis* Fouquet, 1876; *Piscinoodinium pillulare* (Schäperclaus, 1954) Lom, 1981; *Henneguya piaractus* Martins & Souza, 1997; *Myxobolus colossomatis* Molnar & Békési, 1993; *Lernaea cyprinacea* Linnaeus, 1758; *Dolops carvalhoi* Lemos de Castro, 1949; *Trichodina* sp., *Epistylus* sp., *Argulus* sp. and undetermined monogenean species (Martins et al. 2000, Tavares-Dias et al. 2001a, b, Martins et al. 2002, Schalch & Moraes 2005). For *L. macrocephalus* of two fish farms in the municipality of Cruzeiro do Sul, in the State of Acre, parasitic infections have been caused by *Jainus leporini* Abdallah, Azevedo & Luque, 2012; *Urocleidoides paradoxus* Kritsky, Thatcher & Boeger, 1986; *Urocleidoides eremitus* Kritsky, Thatcher & Boeger, 1986; *Tereancistrum parvus* Kritsky, Thatcher and Kayton, 1980; *Kritskyia eirasi* Guidelli, Takemoto & Pavanelli, 2003; *Tereancistrum paranaenses* Karling, Lopes, Takemoto & Pavanelli, 2014; Dactylogyridae spp., *Microcotyle* sp.; *Prosthenhystera obesa* Diesing, 1850; *Procamallanus* (*Spirocamallanus*) *inopinatus* Travassos, Artigas & Pereira, 1928; *Rhabdochona* (*Rhabdochona*) *acuminata* Molin, 1860 and *G. leporini* (Martins et al. 2017a, b). Therefore, as there is no study on the parasite fauna of *L. macrocephalus* in the municipality of Rio Branco, the aim of this study was to investigate the metazoan parasites for this fish reared in four fish farms from this municipality.

MATERIALS AND METHODS

Ethical disclosures

This study was developed in accordance with the principles adopted by the Brazilian College of Animal Experimentation (COBEA), and authorization from Ethics Committee in the Use of Animal of the Embrapa Amapá (#013/2018) was carried out.

From June 2015 to May 2017, 160 *L. macrocephalus* were collected in four fish farms (i.e., 40 specimens in each fish farm) in the municipality of Rio Branco, State of Acre (Brazil), for analysis of metazoan parasites. Each fish farm had different characteristics of management and structure (i.e., fish size, stocking density, sanitary quality, quality and source of water supply, etc.) (Table I). Fish from fish farm 1 were produced by the property, and the water supply of the tanks originates from the property. Fish from fish farms 2 and 3 were acquired from different suppliers of fingerlings, and the source of water supply for tanks in fish farm 2 is a river, but in fish farm 3 the source of water supply for tanks originates from the property. Fish from fish farm 4 were acquired from a supplier of fingerlings that made antiparasitic treatments using sodium chloride. The source of supply of the tanks of this fish farm originates from the property.

During fish collection, the pH was determined using a digital pH meter in each fish farm, as well as temperature (CDS107) and dissolved oxygen concentration, using a digital oximeter (HQ40D).

For each necropsied fish, we examined the mouth, opercula, gills, gastrointestinal tract and viscera. Gills were removed, fixed in 5% formalin and analyzed using a stereomicroscope (SMZ 800N, Nikon, Tokyo, Japan) and microscope (Eclipse E100, Nikon, Tokyo, Japan). The gastrointestinal tract and viscera were removed and examined under a stereomicroscope for

Table I. Localization, body parameters, stocking density and overall prevalence of parasites in *Leporinus macrocephalus* of four fish farms from Rio Branco, in State of Acre, western Amazon region (Brazil).

Fish farms	Geographical coordinates	N	Weight (g)	Length (cm)	Density (fish/m²)	Prevalence (%)
1	9°55'05.70"S – 67°47'01.46"W	40	29.3 ± 19.3	9.9 ± 2.3	2.0	92.5
2	10°03'11.28"S – 67°50'41.47"W	40	90.0 ± 27.9	14.7 ± 1.8	2.5	72.5
3	9°45'24.5"S – 68°04'25.0"W	40	14.7 ± 5.6	8.0 ± 0.9	3.2	82.5
4	9°02'53.71"S – 68°37'44.69"W	40	5.1 ± 207.8	3.5 ± 141.5	2.5	0

collection of endoparasites. The methodology used for collecting, fixing, counting and preparing the parasites for identification followed previous recommendations (Eiras et al. 2006).

The ecological terms used followed previous recommendations of Bush et al. (1997). The frequency of dominance (percentage of infracommunities in which a parasite species is numerically dominant) was determined (Rohde et al. 1995). The dispersion index (DI) and discrepancy index (D) were calculated using the Quantitative Parasitology 3.0 software, to detect the distribution pattern of parasite infracommunities (Rózsa et al. 2000), for species with prevalence >10%. The significance of DI, for each intracommunity, was tested using the *d*-statistics (Ludwig & Reynolds 1988).

Weight and total length of fish were used to calculate the relative condition factor (Kn) of parasitized and non-parasitized fish (Le Cren 1951), which were compared using the Mann-Whitney (*U*) test. The Spearman correlation coefficient (*rs*) was estimated to determine possible correlations between length and weight of host fish and the abundance of parasites (Zar 2010).

RESULTS

The specimens of *L. macrocephalus* examined had different body size due to the different stages of cultivation (fingerlings and fattening). Total parasite prevalence was high in fish farms 1-3 (Table I). In general, these fish farms had different management strategies and stocking density of *L. macrocephalus* and the size of this host.

In the four fish farms, pH and temperature were similar, but the levels of dissolved oxygen in water were low in fish farms 2-4 (Table II), which also had inadequate sanity conditions.

Monogeneans *J. leporini* occurred only in *L. macrocephalus* from fish farm 3, while *U. paradoxus*, *U. eremitus* only do not occurred in fish farm 4. However, *T. parvus* and *R.* (*R.*) *acuminata* occurred in fish from fish farms 1-3. *Procamallanus* (*S.*) *inopinatus* occurred in fish from fish farms 1 and 2, while *Dolops discoidalis* Bouvier, 1399 and *Ergasilus* sp. were found only in fish from fish farm 2 (Table III). In *L. macrocephalus*, the high infection levels were caused by *U. paradoxus*, *U. eremitus* and *P.* (S.) *inopinatus*, but the dominance was of *U. paradoxus*. The parasites presented an aggregated dispersion (Table IV).

There was a weak positive correlation between *U. paradoxus* abundance and the length (*rs* = 0.25, p= 0.001) and weight (*rs* = 0.24, p = 0.002) of hosts, but no correlation between *U. eremitus* abundance and the length (*rs* = 0.12, p = 0.13) and weight (*rs* = 0.12, p = 0.14) was detected.

Table II. Parameters of water quality in tanks of four fish farms of *Leporinus macrocephalus* from the Rio Branco, in State of Acre, western Amazon region (Brazil).

Fish farms	Dissolved oxygen (mg/L)	pH	Temperature (° C)
1	5.4 ± 1.1	7.7 ± 1.2.	28.4 ± 1.2
2	2.3 ± 1.3	5.6 ± 1.2	30.2 ± 1.1
3	1.4 ± 1.1	5.1 ± 1.1	29.2 ± 1.3
4	2.8 ± 2.0	5.9 ± 0.6	28.1 ± 1.0

Positive correlation between *P.* (*S*) *inopinatus* abundance and the length (*rs* = 0.55, p= <0.001) and the weight (*rs* = 0.55, p= <0.001) of hosts was found.

There was no significant difference (*U* = 2765.0, p = 0.37) in relative condition factor (Kn) between parasitized (Kn = 1.00 ± 0.13, n = 99) and non-parasitized fish (Kn = 1.03 ± 0.38, n = 61). The parasites abundance did not showed correlation (*rs* = - 0.045, p = 0.645) with relative condition factor of hosts.

DISCUSSION

In *L. macrocephalus* of four fish farms from Rio Branco, State of Acre, the parasitic prevalence was 61.9%. Similar prevalence was reported by Martins et al. (2017a) for this same fish cultured in thanks and dam in Cruzeiro do Sul, State of Acre. However, this was higher than the prevalence (21.3%) reported by Martins et al. (2002) for *L. macrocephalus* from fish farms in the State of São Paulo. Half of the fish farms investigated here presented low levels of dissolved oxygen in water and the majority had inadequate sanity conditions, which favored the prevalence of parasites found. However, in the four fish farms studied, fish did not present macroscopic signals of diseases, due to low to moderate levels of parasitism. In general, the parasitism rate in *L. macrocephalus* has been attributed to stocking density and poor water quality, which favors the dissemination of infectious stages of parasites (Tavares-Dias et al. 2001a, b, Martins et al. 2002, 2017a). Also, *U. paradoxus*, *U. eremitus* and *P.* (*S.*) *inopinatus* were the parasites with higher prevalence in *L. macrocephalus*, and they showed a high aggregated dispersion, a pattern also found by Martins et al. (2017a) for this same host species. Aggregated dispersion of parasites has been attributed to genetic heterogeneity, exposure and susceptibility of the host population and local environmental factors (Tavares-Dias et al. 2015, Martins et al. 2017a).

The condition factor is a quantitative indicator of fish welfare that can be used as a tool for studying the relationship between host health status and parasitism (Le Cren 1951, Santos et al. 2013, Morey & Arellano 2019). Thus, relative condition factor was used to evaluate the body condition in *L. macrocephalus* of the present study, and this was similar between parasitized and non-parasitized fish and no correlation between the parasitic load and relative condition factor was found, due to the moderate infection levels that not caused damages to the hosts. Similar results were reported for *L. macrocephalus* also infected by metazoan parasites found in the current study. Determining the factors that affect the presence of parasites is important to parasitology study. In fish populations, body size can influence parasite load (Santos et al. 2013, Martins et al. 2017a, Morey & Arellano 2019); however, it

Table III. Parasites in *Leporinus macrocephalus* of four fish farms from the Rio Branco, in State of Acre, western Amazon region (Brazil).

Fish farms	1			2			3			4		
Taxon/ species of parasites	P (%)	MI	MA	P (%)	MI	MA	P (%)	MI	MA	P (%)	MI	MA
Monogenea												
Jainus leporini	0	0	0	0	0	0	7.5	4.5	0.2	0	0	0
Urocleidoides paradoxus	87.5	27.0	23.7	35.0	11.4	4.0	75.0	13.3	9.7	0	0	0
Urocleidoides eremitus	25.0	12.2	3.0	22.5	8.2	1.8	52.5	10.2	5.1	0	0	0
Tereancistrum parvus	15.0	25.0	3.7	2.5	2.0	0.05	12.5	4.2	0.4	0	0	0
Nematoda												
Procamallanus (*Spirocamallanus*) *inopinatus*	32.5	2.0	0.67	62.5	2.4	1.5	0	0	0	0	0	0
Rhabdochona (*Rhabdochona*) *acuminata*	2.5	1.0	0.02	2.5	1.0	0.02	10.0	1	0.07	0	0	0
Crustacea												
Dolops discoidalis	0	0	0	22.5	5.2	1.2	0	0	0	0	0	0
Ergasilus sp.	0	0	0	2.5	1	0.02	0	0	0	0	0	0

P: Prevalence, MI: Mean intensity, MA: Mean abundance.

remains uncertain whether increased parasite presence is related to increased exposure time to parasites and their intermediate hosts due to the age of hosts, or due to greater surface area available for attachment due to the body size of hosts. However, distinguishing such effects can be very complicated since older fish are generally larger. There was a weak positive correlation between size (weight and length) of *L. macrocephalus* and the abundance of *U. paradoxus*, while the abundance of *P.* (*S.*) *inopinatus* presented positive correlation with the size of the host here examined. Martins et al. (2017a) also reported a positive correlation between the overall abundance of parasites and the weight and length of *L. macrocephalus* farmed in dam and tanks.

In *L. macrocepahalus* from four fish farms, only one fish farm did not have fish parasitized with monogeneans. *Urocleidoides paradoxus*, *U. eremitus*, *J. leporini* and *T. parvus* were found at similar infection levels among three fish farms. The infection levels by these monogeneans were similar to those reported by Martins et al. (2017a) for *L. macrocephalus* of intensive and extensive fish farm, but they were lower than the reported by Tavares-Dias et al. (2001b), for this same fish species from fish farms in the State of São Paulo. For *L. macrocephalus* from a fish farm in the State of Acre, 15 species of parasites

Table IV. Parasites in *Leporinus macrocephalus* (N= 160) of fish farms from the Rio Branco, in State of Acre, western Amazon region (Brazil).

Species of parasites	SI	EF/PF	P (%)	MI	MA	FD (%)	ID	*d*	D
Jainus leporini	Gills	160/02	1.25	4.5	0.05	0.4	-	-	-
Urocleidoides paradoxus	Gills	160/78	48.7	19.1	9.3	67.5	6.175	36.5	0.672
Urocleidoides eremitus	Gills	160/39	24.3	10.2	2.5	18.1	4.618	30.5	0.840
Tereancistrum parvus	Gills	160/11	6.8	15.3	1.0	7.6	-	-	-
Procamallanus (*S.*) *inopinatus*	Intestine	160/38	23.7	2.3	0.5	3.9	2.064	17.8	0.823
Rhabdochona (*R.*) *acuminata*	Intestine	160/5	3.1	1	0.03	0.2	-	-	-
Dolops discoidalis	Tegument	160/9	5.6	5.2	0.2	2.1	-	-	-
Ergasilus sp.	Gills	160/1	0.6	1	0.006	0.05	-	-	-

SI: Site of infection, EF: Examined fish, PF: Parasitized fish, ID: Index of dispersion, d: statistic-d, D: Discrepancy, P: Prevalence, MA: Mean abundance, MI: Mean intensity.

were reported (Martins et al. 2017a) and five of these parasite species were also found in the present study. Also, *L. macrocephalus* from fish farm 4 was represented by fingerlings recently purchased for fattening. Therefore, these fish did not have time to re-infect themselves in the fish farm and also acquired other parasite species.

Larvae of *P.* (*S.*) *inopinatus* have copepods as intermediate hosts and are ingested by fish, which are the definitive hosts of this nematode, an endoparasite frequent in wild fish that can also infect farmed fish (Hamann 1999, Martins et al. 2017a, Neves et al. 2020). Thus, this endoparasite occurred only in *L. macrocephalus* from two fish farms and at similar infection levels. However, the infection levels were lower than those reported for this same fish in tanks and dam in the State of Acre (Martins et al. 2017a). *Rhabdochona* (*R.*) *acuminata* occurred only in two fish farms, and at low and similar infection levels. As *Rhabdochona* species have as main intermediate hosts mayflies (Ephemeroptera), and less often some other aquatic insects, such as Trichoptera or Plecoptera (Moravec 2010), hence intermediate hosts containing infective stages of *R.* (*R.*) *acuminata* seem is present only in two fish farms. Therefore, the infection levels by both nematode species are highly dependent on the presence of intermediate hosts in the environment. Also, Martins et al. (2017b) reported that both nematode species have seasonal variation in *L. macrocephalus*.

Parasitic crustaceans such as Argulidae and Copepoda are components of the communities of ectoparasites in Brazilian freshwater fish and occur in several host species of several taxonomic groups (Tavares-Dias et al. 2015). However, details of the life history for most of the parasitic crustaceans of fish remain unknown. *Dolops discoidalis* is an argulid that infests diverse fish species, because it has low host specificity (Tavares-Dias et al. 2015, Morey & Arellano 2019). Among Ergasilidae, species of the genus *Ergasilus* have preference for the gills of hosts (Tavares-Dias et al. 2015), as occurred in *L. macrocephalus* in the current study. In

Brazil, around 17 species of *Ergasilus* are known and these occur most frequently in hosts from the Amazon region (Tavares-Dias et al. 2015). *Leporinus macrocephalus* was infested with *D. discoidalis* and *Ergasilus* sp. and at low parasitism levels. In contrast, Martins et al. (2017a) have not reported infestation with parasitic crustaceans in *L. macrocephalus* farmed in the State of Acre. In *L. macrocephalus* farmed in the State of São Paulo, the infestations by crustaceans were mainly caused by *L. cyprinacea*, a common lernaeid of farmed fish in the Southeast region of Brazil (Martins et al. 2000, 2002, Tavares-Dias et al. 2001b, Schalch & Moraes 2005). Therefore, these results indicate differences in the parasitic crustacean fauna for *L. macrocephalus* among Brazilian regions.

CONCLUSIONS

For *L. macrocephalus*, the parasite community was composed of species of monogeneans, nematodes and crustaceans, parasites with low to moderate infection levels and aggregated dispersion. The parasitism was influenced by the different management strategies of fish farms, mainly to the stocking density of *L. macrocephalus* and the size of this host, as well as by the oxygen levels in water, which varied among the fish farms. As expected, there was a low diversity of endoparasites, which depend on the presence of intermediate hosts with infective stages to maintain their complex life cycle. This was the first report of *D. discoidalis* and *Ergasilus* sp. for *L. macrocephalus*. Lastly, it was the second report of *P.* (*S.*) *inopinatus and R.* (*R.*) *acuminata* for *L. macrocephalus*.

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Author contributions

LUCIANO P. NEGREIROS contributed with the data collection and text elaboration, LIGIA R. NEVES contributed with the fish and data collection and MARCOS TAVARES-DIAS was the coordinator of this work.