

# Metazoan parasite communities of *Leporinus macrocephalus* (Characiformes: Anostomidae) in cultivation systems in the western Amazon, Brazil

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## ABSTRACT

In the Amazon, the growing demand for fish has been boosting the expansion of fish farms. However, the intensification of cultivation can generate disequilibrium in the parasite-host environment, predisposing fish to parasitic infections. The objective of this study was to evaluate the community structure of metazoan parasites in cultivation systems of piauçu, *Leporinus macrocephalus*, in the state of Acre, Brazil. We examined 100 specimens from a semi-intensive cultivation system (earth tanks) and 100 from an extensive system (dams). Overall 66.5% of the hosts were parasitized. We collected 1,240 parasite specimens, classified in 15 metazoan taxa (10 monogenean, one digenean and four nematodes). The parasite prevalence was higher by Monogenea in the dams, and by Nematoda in the earth tanks. The parasitic indexes were, in general, low and varied among species. Monogenea had higher values for quantitative and ecological descriptors of parasitism in the dams, while Nematoda had higher values in the earth tanks. A single species of Digenea was found in the dams, with low prevalence. No taxon was classified as central. In the dams, parasite abundance was correlated only with total fish length, while in the earth tanks, it was positively correlated with total length, weight and condition factor of fish. The endoparasite and ectoparasite infracommunities presented higher richness, dominance, diversity and evenness, respectively, in the earth tanks and in the dams. This is the first study of ecological descriptors of parasites of *L. macrocephalus* in the Amazon.

**KEYWORDS:** diversity, Digenea, Monogenea, Nematoda

## Comunidade de metazoários parasitos de *Leporinus macrocephalus* (Characiformes: Anostomidae) em sistemas de cultivo no oeste da Amazônia, Brasil

### RESUMO

Na Amazônia, a crescente demanda por pescado vem impulsionando a expansão da piscicultura. No entanto, a intensificação dos cultivos pode gerar desequilíbrio no sistema parasito-hospedeiro-ambiente, predispondo os peixes a infecções parasitárias. O objetivo deste estudo foi avaliar a estrutura das comunidades de metazoários parasitos de piauçu, *Leporinus macrocephalus*, em sistemas de cultivo no estado do Acre, Brasil. Foram coletados 200 peixes, sendo 100 espécimes de sistema de cultivo semi-intensivo em viveiro escavado e 100 de sistema extensivo em açude. Dos 200 hospedeiros analisados 66,5% estavam parasitados. Foram coletados 1.240 espécimes de metazoários, classificados em quinze espécies (10 de Monogenea, uma de Digenea e quatro de Nematoda). A prevalência de parasitismo por Monogenea foi maior em açude e por Nematoda em viveiro. De forma geral, os índices de parasitismo foram baixos e variaram entre as espécies, com maiores valores dos descritores quantitativos e ecológicos do parasitismo por Monogenea em açude e Nematoda em viveiro. A única espécie de Digenea foi encontrada em açude e com baixa prevalência. Nenhum táxon foi classificado como central. Nos açudes, a abundância parasitária foi correlacionada apenas com o comprimento total dos hospedeiros, e nos viveiros com o comprimento total, peso e fator de condição dos hospedeiros. Nos viveiros, a infracomunidade de endoparasitos apresentou os maiores índices de riqueza, dominância, diversidade e equitabilidade. Nos açudes, os ectoparasitos apresentaram os maiores índices. Este foi o primeiro registro de índices parasitários de *L. macrocephalus* em sistemas de cultivo na Amazônia.

**PALAVRAS-CHAVE:** diversidade, Digenea, Monogenea, Nematoda

## INTRODUCTION

*Leporinus macrocephalus* Garavello and Britisk 1988 (Anostomidae), known as piaçu, is native from the Prata and Paraguay river basins, and was introduced in fish farming in the 1990's in southeastern Brazil. The species has increasing commercial prospects due to its great productive capacity (Martins and Yoshitoshi 2003), being attractive for intensive and semi-intensive rearing in mono and polyculture. The species is omnivorous (Andrian *et al.* 1994) and adapts easily to artificial diets (Soares Júnior *et al.* 2013), presenting rapid growth and good weight gain (Takahashi *et al.* 2004).

Fish farming in the state of Acre, in the southwestern Brazilian Amazon, is diversified and increasing in importance as an economic alternative, using both extensive and semi-intensive cultivation systems. Usually, the extensive system is used by family fish farms, with limited use of feed, low stocking density and without water renewal. The semi-intensive system is more costly, using artificial rearing facilities with high stocking densities, balanced diet with intensive use of feed, water renewal and quality control, as well as other technologies.

Understanding the causal agents of parasitic diseases and the complex relationship between environmental factors and the hosts is important (Schalch and Moraes 2005). When fish cultivation systems with high stocking densities have inadequate water management, and substandard nutrition, parasitic diseases can emerge (Schalch and Moraes 2005; Zanoló and Yamamura 2006; Pavanelli *et al.* 2013; Zago *et al.* 2014), causing significant losses to production. Under intense infestations or infections, parasites can cause physiological damage to hosts, leading to death of fish in severe cases (Martins and Yoshitoshi 2003).

Studies on parasites of *L. macrocephalus* exist only for the southeastern region of Brazil. In the state of São Paulo, a prevalence of infection of 87.2% by Monogenoidea has been determined in *L. macrocephalus* (Tavares-Dias *et al.* 1999). High infection rates of *L. macrocephalus* by the nematode *Goezia leporini* caused symptoms such as lack of appetite, lethargy, pallidness and ascites (Martins and Yoshitoshi 2003). A reduction of hematological characteristics was also observed in *L. macrocephalus* parasitized by this species of Nematoda (Martins *et al.* 2004). In natural populations of *L. macrocephalus*, the presence of *Rhinoxenus* sp. and metacercariae of Digenea was reported in the upper Paraná River floodplain, although no quantitative or ecological parameters of parasitism were informed (Takemoto *et al.* 2009).

There is no information about parasites of *L. macrocephalus* in cultivation systems in the Amazon. Thus, the objective of the present study was to evaluate the communities and infracommunities of metazoan parasites of *L. macrocephalus* in cultivation systems in the state of Acre, in the Brazilian Amazon.

## MATERIALS AND METHODS

Fingerlings of *Leporinus macrocephalus* were obtained from a commercial fingerling producer in the region. They were reared in two fish farms in the municipality of Cruzeiro do Sul (07°37'52"S, 72°40'12"W), state of Acre, Brazil, each with a different cultivation system. In both cases the fish were fed with a commercial extruded ration with 32% gross protein, and, in the fattening phase, with an extruded ration containing 28% gross protein. Also in both systems, *L. macrocephalus* were reared in polyculture with *Prochilodus argenteus* and *Brycon cephalus*.

In one farm the fish were reared in a semi-intensive system. They were distributed in three excavated rectangular earth tanks, on firm ground, 1.20 m deep, each with an area of 200 m<sup>2</sup> and a volume of 240,000 liters, with water inlet and outlet control. Water renewal occurred gradually, with a supply of 5% of the total volume of the tanks weekly. Water color was dingy/greenish, being dominated by grasses on the margins. Stocking density was 1 fish/m<sup>2</sup> of water surface. The fish were fed twice a day.

The other farm used an extensive system. The fingerlings were distributed into two dams which formed through the accumulation of water from a stream. The dams were rectangular in shape, with irregular edges, a depth of 1.50 m, each one with an area of 300 m<sup>2</sup>, and a volume of 450,000 liters, without water inlet and outlet control. The color of the water was dark, with grass vegetation on the margins. Stocking density was approximately 1 fish/5m<sup>2</sup> of water surface. In addition to the natural food produced in the environment, the fish received food supplementation once a day.

From June 2014 to December 2015 a cumulative sample of 100 adult fish were collected from the earth tanks, and 100 from the dams. The collection was carried out by the local farmers. Collection always occurred at the same time in the morning. On each sampling occasion the dissolved oxygen (O<sub>2</sub>D), hydrogenic potential (pH), water temperature (T°C) and electric conductivity (EC) of the water at the collection point were measured with multiparameter equipment (Hanna Instruments, USA). Differences in the water characteristics between the two cultivation systems were analysed using ANOVA (p<0.05).

The collected fish were kept in thermal boxes and transported to the Fish Processing Laboratory (Laboratório de Processamento de Pescado) at the Instituto Federal do Acre, Cruzeiro do Sul Campus (Cruzeiro do Sul, Acre). The fish were weighed (g), and measured for total length (cm) and necropsied. The gills, operculum and fins were examined for the presence of ectoparasites, and the gastrointestinal tract for the presence of endoparasites.

For collection of Monogenoidea, the gills were removed and placed in bowls containing water at 65 °C and shaken; then, alcohol was added to reach a concentration of 70%. The parasites were collected under a stereoscopic microscope, fixed and stored in the same fixative. The Digenea were cold fixed in AFA (2% glacial acetic acid, 3% formaldehyde, and 95% of 70% alcohol), under light cover glass pressure. Nematodes were rinsed in 0.7% NaCl solution and fixed in hot AFA. The material was taken to the Fish Helminth Parasite Laboratory (Laboratório de Helminthos Parasitos de Peixes – LHPP) at the Oswaldo Cruz Institute (Rio de Janeiro, Brazil) where the helminthes were processed for light microscope studies. Some specimens of Monogenoidea were mounted unstained in Hoyer's medium for study of the sclerotized parts, and others were stained with Gomori's trichrome and mounted in Canada balsam. The Digenea were stained with Langeron's alcoholic acid carmine, dehydrated by means of ethyl alcohol series, cleared using beechwood creosote and mounted in Canada balsam as permanent slides. Nematodes were clarified and mounted on semi-permanent slides in phenol 50%. Specimens were studied under a light microscope Zeiss Axioscope 2 and the material was deposited at the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC).

To evaluate the effect of parasitism on the development and health of the fish we determined the relative condition factor (Kn), which is calculated from the relation between total length (Lt) and total weight (Wt) of the fish using the expression  $P = aCb$ , where  $P$  = total weight,  $C$  = total length,  $a$  = intercept and  $b$  = angular coefficient (Le Cren 1951). The coefficients  $a$  and  $b$  were estimated after logarithmic transformation of the weight and length data, and were used for the calculation of the theoretically expected values of weight. The condition factor was determined from the ratio of the observed total weight (Wt) and the estimated weight (We) as  $Kn = Wt/We$ . Differences in condition factor between parasitized and non parasitized fish were evaluated using ANOVA ( $p < 0.05$ ).

The dispersion index (ID) was calculated in order to detect the distribution pattern of the parasite communities in species with a prevalence of  $\geq 10\%$  (Rózsa *et al.* 2000). The significance of ID for each species was tested using the  $d$ -statistic. The dominance of each component of the communities was determined by relative dominance (number of specimens of a species / total number of specimens of all species in the community) (Rohde *et al.* 1995).

As descriptors for the parasite community we calculated richness of parasite species, the Brillouin diversity index ( $HB$ ); Evenness ( $E$ ) in association with the diversity index, and the Berger-Parker dominance index ( $d$ ) (Magurran 2004). We also calculated the parameters of infection as prevalence (P%), mean intensity and parasite abundance based on Bush *et al.* (1997). All

descriptors were compared between cultivation systems through the Mann-Whitney test (Zar 2010) at  $p < 0.05$ .

Parasite species were classified as central, secondary or satellite, according to Bush and Holmes (1986). The Spearman correlation coefficient ( $r_s$ ) was used to determine possible correlations of parasite abundance with length, weight and the relative condition factor of the hosts ( $p < 0.05$ ).

This study was authorized by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA, license nr. 396871-1/2013).

## RESULTS

Overall, 133 (66.5%) of the 200 specimens of *L. macrocephalus* were parasitized by at least one helminth species. Forty-six hosts were parasitized by monogenoideans, 49 by nematodes, and 38 by the association of monogenoideans, nematodes and digeneans. We identified 15 helminth species, 10 belonging to Monogenoidea, one to Digenea, and four to Nematoda, with a total of 1,040 collected specimens (Table 1), and a mean of  $9.3 \pm 16.7$  parasites/fish.

The prevalence of parasite infection was 73% in the earth tanks and 19% in the dams. Nine species were common in both systems (Table 2): *Urocleidoides paradoxus* Kritsky, Thatcher & Boeger, 1986; *Urocleidoides eremitus* Kritsky, Thatcher & Boeger, 1986; *Jainus leporini* Abdallah, Azevedo & Luque, 2012; *Tereancistrum parvum* Kritsky, Thatcher & Kayton, 1980; Dactylogyridae sp.1; Dactylogyridae sp.2. *Procamallanus (Spirocamallanus) inopinatus* Travassos, Artigas & Pereira, 1928; *Rhabdochona (Rhabdochona) acuminata* (Molin 1860); and *Goezia leporini* Martins & Yoshitoshi, 2003.

Two species of Monogenoidea were found only in the earth tanks (*Tereancistrum paranaensis* Karling, Lopes, Takemoto & Pavanelli, 2014 and *Kritskyia eirasi* Kritsky, Thatcher & Kayton, 1980), and four species occurred only in the dams (Dactylogyridae sp. 3; *Microcotyle* sp.; *Prosthenhystera obesa* (Diesing 1850) Travassos, 1922; and *Brevimulticaecum* sp., the latter in larval state).

Overall, Nematoda was the quantitatively predominant group, constituting 72.5% of the parasites collected, and also included the most prevalent species. Monogenoidea was the most diverse group, with ten species, yet all had prevalence lower than 10%, with the exception of *Urocleidoides paradoxus* (Table 1). Digenea was the least represented group, with only two specimens of *Prosthenhystera obesa*.

In the earth tanks *U. paradoxus*, *Procamallanus (Spirocamallanus) inopinatus*, *Goezia leporini* and *Rhabdochona (Rhabdochona) acuminata* had significantly higher abundance, prevalence and mean intensity of infection, and *Urocleidoides paradoxus* and *Urocleidoides eremitus* were significantly more prevalent. In the dams *Jainus leporini* had significantly higher

**Table 1.** Overall prevalence (P), mean intensity (MI), mean abundance (MA) and site of infection (SI) of parasites of *Leporinus macrocephalus* from cultivation systems in Acre State, Brazil. Values for MI and MA are means  $\pm$  standard deviation. Collection nr indicates the deposit code of specimens in the helminthological collection of Instituto Oswaldo Cruz.

Parasites	Collection nr	P (%)	MI	MA	SI
<b>MONOGENOIDEA</b>					
Dactylogyridae Bychowsky, 1933					
<i>Uroleidooides paradoxus</i> Kritsky, Thatcher & Boeger, 1986	38674a,b; 38675, 38676, 38677	13.5	3.67 $\pm$ 2.58	0.50 $\pm$ 0.76	Gills
<i>Uroleidooides eremitus</i> Kritsky, Thatcher & Boeger, 1986	38678, 38679 a-c, 38680	9.5	2.11 $\pm$ 1.24	0.20 $\pm$ 0.32	Gills
<i>Jainus leporini</i> Abdallah, Azevedo & Luque, 2012	38672 a-b, 38673 a-c	7.0	6.93 $\pm$ 5.12	0.49 $\pm$ 0.88	Gills
<i>Kritskyia eirasi</i> Kritsky, Thatcher & Kayton, 1980	38670 a,b, 38671 a,b	1.0	3.00 $\pm$ 1.00	0.03 $\pm$ 0.09	Kidney
<i>Tereancistrum parvus</i> Kritsky, Thatcher & Kayton, 1980	38681, 38682 a,b	9.0	2.61 $\pm$ 2.30	0.23 $\pm$ 0.41	Gills
<i>Tereancistrum paranaensis</i> Karling, Lopes, Takemoto & Pavanelli, 2014	38684a,b	1.5	4.33 $\pm$ 2.52	0.06 $\pm$ 0.19	Gills
Dactylogyridae sp.1		3.0	4.83 $\pm$ 2.99	0.14 $\pm$ 0.35	Gills
Dactylogyridae sp.2		1.0	1.50 $\pm$ 0.70	0.01 $\pm$ 0.05	Gills
Dactylogyridae sp.3		1.5	1.33 $\pm$ 0.57	0.02 $\pm$ 0.05	Gills
Microcotylidae Taschenberg, 1879					
<i>Microcotyle</i> sp. *		1.0	1.0	< 0.1	Gills
<b>DIGENEA</b>					
Callodistomidae Odhner, 1910					
<i>Prosthenthystera obesa</i> (Diesing, 1850) Travassos, 1922		1.0	2.0	< 0.1	Gall bladder
<b>NEMATODA</b>					
Camallanidae Railliet & Henry, 1915					
<i>Procamallanus</i> (Spirocamallanus) <i>inopinatus</i> Travassos, Artigas & Pereira, 1928	38,530-38,542	39.0	4.54 $\pm$ 3.51	1.77 $\pm$ 2.24	Intestine
Rhabdochoniidae Travassos, Artigas & Pereira, 1928					
<i>Rhabdochona</i> (Rhabdochona) <i>acuminata</i> (Molin, 1860)	38,543-38,544	12.0	3.42 $\pm$ 1.95	0.41 $\pm$ 0.62	Intestine
Anisakidae Railliet & Henry, 1912					
<i>Goezia leporini</i> Martins & Yoshitoshi, 2003	38,523-38,529	21.5	10.14 $\pm$ 6.05	2.18 $\pm$ 2.93	Stomach
Acanthocheilidae Wülker, 1929					
<i>Brevimulticaecum</i> sp. (Larva)	38,522	1.0	4.00 $\pm$ 1.41	0.04 $\pm$ 0.12	Intestine

(\*) Only one individual recorded.

abundance, prevalence and mean intensity of infection, and *Tereancistrum parvus* showed the highest values of mean intensity of infection (Table 2).

In the earth tanks, *P. (S.) inopinatus* was considered the central species (67% prevalence), while *G. leporini* was secondary (40% prevalence) and the other species were classified as satellites (Table 2). In the dams, all ecto and endoparasite species were classified as satellites (Table 2).

The components of the parasite community with prevalence  $\geq$  10% presented an aggregated dispersion pattern in both cultivation systems (Table 3). In the dams, the monogenoidean *J. leporini* was dominant, with 88 collected specimens (44.9% of the parasites collected), the highest value of relative dominance (0.328), and a higher index of dispersion (ID=31.13;  $d=64.52$ ). In the earth tanks, *Uroleidooides paradoxus* had the highest index of dispersion

(ID=8.13;  $d=26.11$ ) and *G. leporini* the highest value of relative dominance (0.443).

The dominance of endoparasites was high (Table 4), mainly owing to the relative dominance and prevalence of *P. (S.) inopinatus* and *G. leporini* (Tables 1 and 3). Endoparasites also had the highest index of dominance, diversity and total evenness, while ectoparasites had the highest total richness (Table 4). In the dams, ectoparasites were dominant, with highest values of richness and diversity, while in the earth tanks endoparasites had the highest indexes of richness, dominance, diversity and evenness, while the evenness indexes of ectoparasites were similar between the two systems (Table 4). Discrepancy in richness and abundance of nematode species was reflected in parasite diversity, since the more abundant community determined the greatest diversity ( $H=3.21\pm 0.35$ ) observed in the earth tanks (Table 4).

Total parasite abundance was significantly correlated with total length, weight and condition factor of the hosts in the earth tanks, while it correlated significantly only with total fish length in the dams (Figure 1). There was no significant difference between parasitized and non-parasitized fish for weight, total length and condition factor in both cultivation systems, except for a marginally higher weight of non-parasitized fish in the earth tanks (Table 5).

Water temperature during the time of collection was significantly higher in the earth tanks than in the dams ( $F_{3,92} = 7.06$ ;  $p < 0.001$ ) (dams:  $27.2 \pm 2.4$  °C; earth tanks:  $29.1 \pm 1.6$  °C). The other measured physicochemical water parameters did not differ between the cultivation systems [dissolved oxygen (dams:  $6.24 \pm 1.59$  mg/L; earth tanks:  $6.3 \pm 1.3$  mg/L); pH (dams:  $6.3 \pm 0.7$ ; earth tanks:  $6.3 \pm 0.6$ ); electrical conductivity (dams  $15.89 \pm 5.46$  mg/L; earth tanks:  $12.33 \pm 5.55$  mg/L)].

**Table 2.** Prevalence, parasite abundance and mean intensity of metazoan parasites of *Leporinus macrocephalus* in extensive (dams) and semi-intensive (earth tanks) cultivation systems in Acre State, Brazil. Values for abundance and intensity are means  $\pm$  standard deviation. Differences between the cultivation systems according to the Mann-Whitney test [Z(U)] were considered significant at  $P < 0.05$  (\*),  $P < 0.01$  (\*\*) and  $P < 0.001$  (\*\*\*).

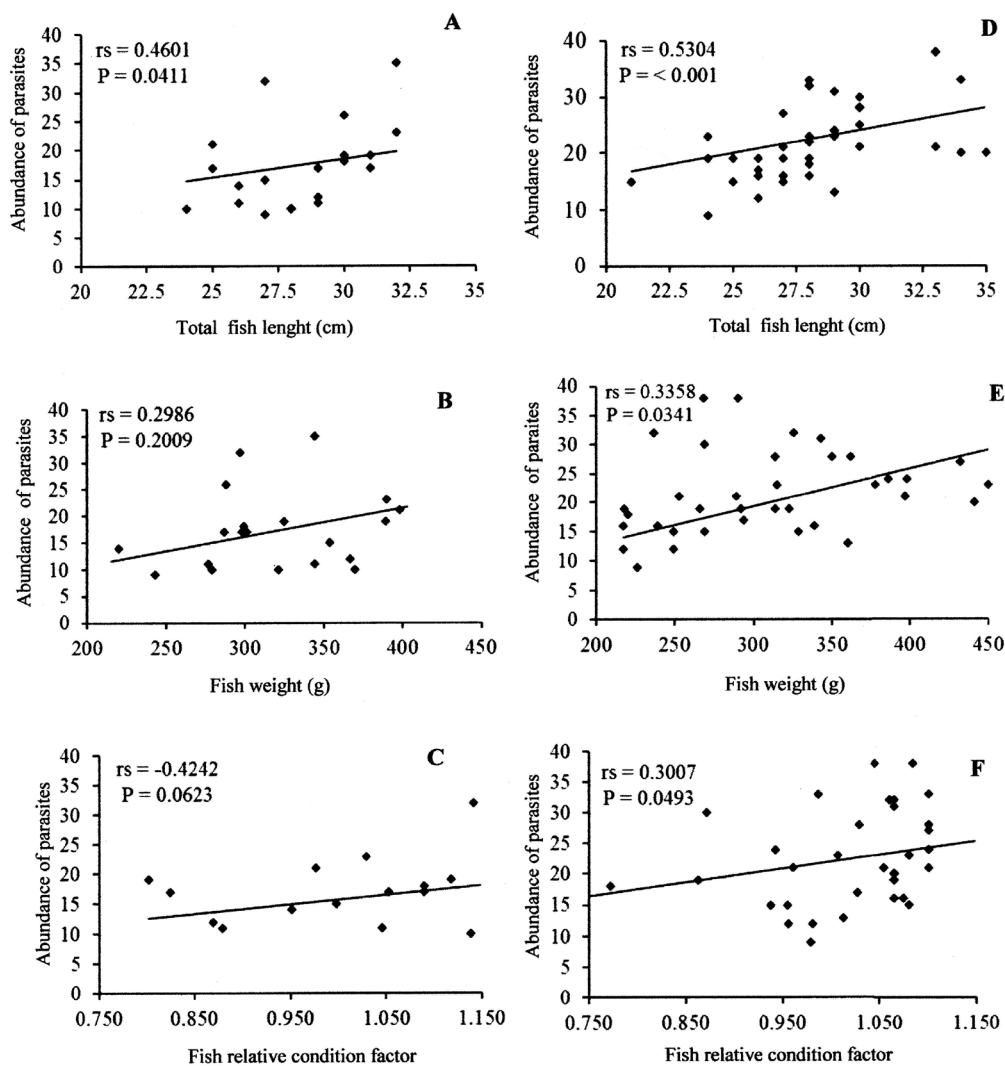
Parasites	Prevalence (%)			Parasite abundance			Mean intensity		
	Dams	Earth tanks	Z(U)	Dams	Earth tanks	Z(U)	Dams	Earth tanks	Z(U)
<i>Urocleidoides paradoxus</i>	11.0	16.0	1.538*	0.35 $\pm$ 0.03	0.64 $\pm$ 0.03	2.763***	2.91 $\pm$ 0.28	4.00 $\pm$ 0.14	0.491
<i>Urocleidoides eremitus</i>	8.0	12.0	2.237*	0.11 $\pm$ 0.06	0.12 $\pm$ 0.04	1.157	1.38 $\pm$ 0.09	2.64 $\pm$ 0.11	1.327
<i>Jainus leporini</i>	11.0	3.0	2.544***	0.88 $\pm$ 0.05	0.09 $\pm$ 0.21	2.088*	8.00 $\pm$ 0.47	3.00 $\pm$ 0.55	0.651*
<i>Tereancistrum parvum</i>	8.0	10.0	1.515	0.31 $\pm$ 0.09	0.16 $\pm$ 0.04	0.286	3.88 $\pm$ 0.37	1.60 $\pm$ 0.70	2.221*
Dactylogyridae sp.1	5.0	1.0	1.535	0.31 $\pm$ 0.09	0.04 $\pm$ 0.20	1.014	3.87 $\pm$ 0.38	1.33 $\pm$ 0.27	0.849
Dactylogyridae sp.2	4.0	3.0	0.437	0.07 $\pm$ 0.06	0.05 $\pm$ 0.01	0.966	0.80 $\pm$ 0.05	0.71 $\pm$ 0.04	0.480
Nematoda									
<i>Procamallanus (Spirocamallanus) inopinatus</i>	17.0	67.0	2.552***	0.17 $\pm$ 0.04	3.24 $\pm$ 0.03	1.862*	2.80 $\pm$ 0.11	5.00 $\pm$ 0.05	4.184***
<i>Rabdochona (Rabdochona) acuminata</i>	4.0	26.0	1.503*	0.04 $\pm$ 0.01	0.30 $\pm$ 0.02	3.192***	2.00 $\pm$ 0.25	2.75 $\pm$ 0.06	3.020**
<i>Goezia leporini</i>	8.0	40.0	3.265***	0.08 $\pm$ 0.03	0.39 $\pm$ 0.05	3.262***	3.25 $\pm$ 0.09	10.72 $\pm$ 0.12	0.221*

**Table 3.** Index of dispersion (ID), *d* statistic and relative dominance (RD) of metazoan parasites of *Leporinus macrocephalus* from extensive (dams) and semi-intensive (earth tanks) cultivation systems in Acre State, Brazil. Species with prevalence >10% are highlighted in bold. (\*) Observed only in earth tanks; (\*\*) observed only in dams.

Parasites	Dams			Earth tanks		
	ID	d	RD	ID	d	RD
Monogenoidea						
<b><i>Urocleidoides paradoxus</i></b>	27.89	60.32	0.130	8.13	26.11	0.066
<b><i>Urocleidoides eremitus</i></b>	-	-	0.041	6.67	22.33	0.030
<b><i>Jainus leporini</i></b>	31.13	64.52	0.328	-	-	0.009
<i>Kritskyia eirasi</i> *	-	-	-	-	-	0.006
<i>Tereancistrum parvum</i>	-	-	0.116	3.05	3.39	0.016
<i>Tereancistrum</i> sp.	-	-	-	-	-	0.013
Dactylogyridae sp.1	-	-	0.104	-	-	0.001
Dactylogyridae sp.2	-	-	0.007	-	-	0.001
Dactylogyridae sp.3**	-	-	0.015	-	-	-
Nematoda						
<b><i>Procamallanus (S.) inopinatus</i></b>	7.21	23.79	0.156	4.16	6.30	0.330
<b><i>Rabdochona (R.) acuminata</i></b>	-	-	0.011	4.97	17.37	0.082
<b><i>Goezia leporini</i></b>	-	-	0.078	8.04	25.91	0.443
<i>Brevimulticaecum</i> sp.(larva)**	-	-	0.029	-	-	-

**Table 4.** Richness, dominance, diversity and evenness of the parasite infracommunities of *Leporinus macrocephalus* from extensive (dams) and semi-intensive (earth tanks) cultivation systems in Acre State, Brazil. Values are means  $\pm$  standard deviation. Differences between cultivation systems according to the Mann-Whitney test [Z(U)] were considered significant at  $P < 0.05$  (\*),  $< 0.01$  (\*\*) and  $< 0.001$  (\*\*\*).

Ectoparasites	Overall	Dams	Earth tanks	Z(U)
Richness	2.97 $\pm$ 1.46	2.65 $\pm$ 0.31	2.36 $\pm$ 1.21	2.51**
Berger Parker dominance ( <i>d</i> )	0.31 $\pm$ 0.18	0.60 $\pm$ 0.05	0.41 $\pm$ 0.20	2.88*
Brillouin diversity ( <i>H</i> )	1.72 $\pm$ 0.69	1.69 $\pm$ 0.26	1.44 $\pm$ 0.60	4.73
Evenness ( <i>J</i> )	0.91 $\pm$ 0.02	0.90 $\pm$ 0.03	0.93 $\pm$ 0.04	3.02
Endoparasites				
Richness	2.73 $\pm$ 2.73	2.52 $\pm$ 0.81	7.83 $\pm$ 2.16	17.22*
Berger Parker dominance ( <i>d</i> )	0.73 $\pm$ 0.18	0.08 $\pm$ 0.13	0.92 $\pm$ 0.30	3.88***
Brillouin diversity ( <i>H</i> )	3.34 $\pm$ 0.37	1.43 $\pm$ 0.47	3.21 $\pm$ 0.35	9.31*
Evenness ( <i>J</i> )	0.94 $\pm$ 0.01	0.90 $\pm$ 0.04	0.95 $\pm$ 0.01	2.64*



**Figure 1.** Spearman correlation coefficient (*rs*) between the abundance of parasites and total length (cm), weight (g) and condition factor of *Leporinus macrocephalus* in dams (A, B, C) and earth tanks (D, E, F) in Acre State, Brazil.

**Table 5.** Weight (g), total length (cm) and condition factor of *Leporinus macrocephalus* in extensive (dams) and semi-intensive (earth tanks) cultivation systems in Acre State, Brazil. Values are means  $\pm$  standard deviation. Differences between the cultivation systems according to the *t*-test were considered significant at  $P < 0.05$  (\*).

Parameter	Dams		t	Earth tanks		t
	Parasitized	Non-parasitized		Parasitized	Non-parasitized	
Weight	286.70 $\pm$ 62.76	290.65 $\pm$ 64.27	1.48	339.80 $\pm$ 71.89	346.02 $\pm$ 82.27	3.54*
Total length	27.16 $\pm$ 1.66	28.59 $\pm$ 2.49	1.17	28.33 $\pm$ 2.27	29.26 $\pm$ 1.89	1.26
Condition factor	0.92 $\pm$ 0.13	0.95 $\pm$ 0.17	1.69	0.94 $\pm$ 0.15	0.96 $\pm$ 0.19	1.33

## DISCUSSION

Among the six species of Monogenoidea found parasitizing *L. macrocephalus* in this study, only *Microcotyle* sp. had not been already described as parasitizing other members of the Anostomidae (Kritsky *et al.* 1980; Kritsky *et al.* 1986; Guidelli *et al.* 2003; Schalch and Moraes 2005; Guidelli *et al.* 2006; Takemoto *et al.* 2009; Takemoto and Lizama 2010; Abdallah *et al.* 2012). Some ectoparasite species that presented higher prevalence and abundance may have specificity to members of Anostomidae, such as *U. paradoxus* and *T. parvus* (Cohen *et al.* 2013). In the upper Parana River floodplain, *U. paradoxus* parasitized *Leporinus lacustris* and *L. friderici* with respective prevalences of 32% and 46.1%, while *Jainus* spp. was the monogenoidean with higher prevalence, abundance and mean intensity in both host species (Guidelli *et al.* 2006). Similarly, in our study *J. leporini* was the monogenoid with the highest values of all parasitic indexes, except prevalence, in the dams.

The prevalence of parasites in the earth tanks in our study was similar to the prevalence reported for *L. macrocephalus* in fish farms in southeastern Brazil (65%, Martins and Yoshitoshi 2003, and 87%, Moraes 2005). The generally higher indices of parasite prevalence, abundance and mean infection intensity observed in the earth tanks in comparison to the dams, can be explained by the higher density of fish in the earth tanks, which favors the dissemination of infectious forms of parasites (Sanchez 2008).

The higher water temperature in the earth tanks, as compared to the dams, may also have contributed to the multiplication of parasites, reflecting in higher abundance and infection intensity. Water temperature is one of the key abiotic environmental factors controlling parasite dynamics in aquatic systems, which may directly influence the rates of parasite establishment, development and release of infective stages, as well as parasite transmission between hosts (Karvonen *et al.* 2013).

*Prosthenthystera obesa* was found parasitizing the gall bladder of the host. This parasite is relatively large in comparison with the size of the parasitized organ. The low prevalence of Digenea in the dams and their absence in the earth tanks was probably due to a reduced presence of their intermediate hosts. The application of calcium oxide in the surroundings of dams and earth tanks is common, which causes a reduction in the population of snails, which are intermediate hosts for these helminthes. In addition, fish farmers undertake the control of aquatic plants, thus minimizing the amount of organic waste that serves as mollusc feed.

Although the same nematode species were found in both cultivation systems (with the exception of *Brevimulticaecum* sp. larvae in the dams), only in the earth tanks they constituted the main component of the parasite community of *L. macrocephalus*. Among Nematoda, *P. (S.) inopinatus* had the highest prevalence and parasite abundance indexes. Several studies recorded an increase in the prevalence of this species in other neotropical fish, both in natural environments and in cultivation systems (Andrade and Malta 2006; Saraiva *et al.* 2006; Araújo *et al.* 2009; Gomiero *et al.* 2009). *Procamallanus (S.) inopinatus* was the most prevalent parasite species of *Leporinus lacustris* and *L. friderici* in the Nova Ponte Reservoir, in southeastern Brazil (Feltran *et al.* 2004). In the upper Parana River floodplain, in southern Brazil, the parasite had a prevalence of 20.6% in *L. lacustris* and 29.8% in *L. friderici* (Guidelli *et al.* 2006). The present study reports the first quantitative data for *P. (S.) inopinatus* parasitizing *L. macrocephalus*. *Goezia leporini* is known to parasitize *L. macrocephalus* in cultivation systems in São Paulo, in southeastern Brazil (Martins and Yoshitoshi 2003).

In this study, *G. leporini* did not show the clinical signs of disease reported by Martins and Yoshitoshi (2003). However, some specimens of *G. leporini* were attached to the gastric tract of the hosts, causing bleeding and gastric ulcers, which were reported as secondary lesions from this parasite's infection (Deardorff and Overstreet 1980). Although *G. leporini* in this study had the highest mean intensity of

infection in both systems (10.1), they had low prevalence (21.5%), while in southeastern Brazil prevalence was higher (65%), with lower mean infection intensity (4.1) (Martins and Yoshitoshi 2003).

The aggregated dispersion of metazoan parasites in *L. macrocephalus* found in our study is a common pattern in parasite communities of freshwater fish in different regions of Brazil (Machado *et al.* 1996; Abdallah *et al.* 2004; Moreira *et al.* 2005; Paraguassú and Luque 2007; Guidelli *et al.* 2009; Neves *et al.* 2013; Tavares-Dias *et al.* 2013). This mode of dispersion has been associated with the direct life cycle of Monogenoidea parasites, as well as the susceptibility and capacity of immunological response of the hosts (Paraguassú and Luque 2007; Tavares-Dias *et al.* 2013). Dispersion values were lower for species with high prevalence, because aggregation tends to decrease as the proportion of hosts that are infected increases, and parasites spread more evenly among hosts, leaving fewer hosts uninfected (Poulin 1993).

The significant positive correlation of parasite abundance with total host length was expected since fish length is positively correlated with age, and thus larger specimens had more contact time with the infecting forms and, consequently, a greater accumulation of parasites (Luque and Chaves 1999). However, the parasitism does not necessarily increase in larger fish as a function of mechanical accumulation over a longer exposure time. For example, in Monogenoidea the positive correlation of parasite abundance with fish weight and length is likely facilitated by larger gills in larger fish, which provides more space for parasite attachment (Luque and Chaves 1999; Azevedo *et al.* 2007).

## CONCLUSIONS

This is the first study of parasites in cultivated *Leporinus macrocephalus* in the state of Acre, Brazil, increasing the knowledge of the biodiversity and ecological descriptors of the parasite communities of this fish species in the Amazon region. Our results indicate that the parasite fauna of *L. macrocephalus* in extensive and semi-intensive cultivation systems in Acre does not differ very significantly. Parasitic indexes were low and varied among species, with the highest values in quantitative and ecological parasitism descriptors for Monogenoidea in the extensive, and Nematoda in the semi-intensive system. Although clinical signs of disease were not observed, parasite data suggest that prophylactic measures against future epizootic outbreaks may be indicated to avoid economic losses in fish farming due to parasitism. The occurrence of adult species of Nematoda indicated the availability of the intermediate hosts of these helminths and brings to attention the necessity of adequate sanitary control in fish farms.

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