

## ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

HELMINTH COMMUNITY STRUCTURE OF *ARAPAIMA GIGAS* IN SEMI-INTENSIVE AND INTENSIVE FISH FARMING SYSTEMS IN THE SOUTHWESTERN BRAZILIAN AMAZONESTRUCTURA DE LA COMUNIDAD DE HELMINTOS DE *ARAPAIMA GIGAS* EN SISTEMAS SEMI-INTENSIVOS E INTENSIVOS DE CULTIVO EN EL SUROESTE DE LA AMAZONIA BRASILEÑA

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**ABSTRACT**

In the Amazon, investments in new technology for fish farming have been made in recent years. *Arapaima gigas* is considered one of the species with greatest potential for fish farming in the region. The systems to farm this species in the Amazon are highly diversified, ranging from semi-intensive to intensive or industrial. The objective of this study was to analyze the helminth community structure of *A. gigas* comparing two farming systems. A total of 121 fish were obtained from a semi-intensive and an intensive systems in the state of Acre, Brazil. A total of nine species of parasites were identified, with only one species in common. There was a significant difference with respect to parasite prevalence levels between the two fish farms ( $\chi^2 = 44.99$   $p < 0.05$ ), and the fish from the semi-intensive system showed significantly higher levels of infection (90.63%) than those from the intensive system (31.57%). In the semi-intensive system, nine parasite species were collected and identified: *Dawestrema cycloancistrum*; *Capillostrongyloides arapaimae*; *Goezia spinulosa*; *Hysterothylacium* sp.; Camallanidae gen. sp.; Ascaridoidea gen. sp.; *Polyacanthorhynchus* sp. (juvenile), *Neoechinorhynchus* sp. and Acanthocephala gen. sp. In the intensive fish farm, only *D. cycloancistrum* was found, representing 100% of the specimens collected. The comparative analysis regarding the prevalence of *D. cycloancistrum* between the two fish farms showed a significant difference, with higher prevalence in the semi-intensive system ( $\chi^2 = 7.426$   $p = 0.006$ ). This is the first study of the helminth community of *A. gigas* in Acre and provides new information for fish farmers to enhance their production.

**Keywords:** Acanthocephala – aquaculture – *Arapaima gigas* – Brazil – Monogenea – Nematoda

## RESUMEN

En la Amazonia, las inversiones en nuevas tecnologías para el cultivo de peces se han hecho en los últimos años. *Arapaima gigas* es considerada una de las especies con mayor potencial para la piscicultura en la región. Los sistemas que cultivan esta especie en el Amazonas son muy diversos, que van desde semi-intensivo para intensivo o industrial. El objetivo de este estudio fue analizar la estructura de la comunidad de helmintos de *A. gigas* comparando dos sistemas de cultivo. Un total de 121 peces se obtuvieron de un sistema semi-intensivo y otro intensivo en el estado de Acre, Brasil. Se identificaron un total de nueve especies de parásitos, con una sola especie en común. Hubo una diferencia significativa con respecto a los niveles de prevalencia de parásitos entre los dos cultivos de peces ( $\chi^2 = 44,99$   $p < 0,05$ ), y el pescado desde el sistema de cultivo semi-intensivo mostraron niveles significativamente más altos de infección (90,63%) que los del cultivo intensivo (31,57%). En el sistema semi-intensivo, se recolectaron e identificaron nueve especies de parásitos: *Dawestrema cycloancistrum*; *Capillostrongyloides arapaimae*; *Goezia spinulosa*; *Hysterothylacium* sp.; Camallanidae gen. sp.; Ascaridoidea gen. sp.; *Polyacanthorhynchus* sp. (Juvenil), *Neoechinorhynchus* sp. y Acanthocephala gen. sp. En el cultivo intensivo, sólo se encontró *D. cycloancistrum*, que representa el 100% de las muestras recogidas. El análisis comparativo con respecto a la prevalencia de *D. cycloancistrum* entre los dos cultivos de pescado mostró una diferencia significativa, con mayor prevalencia en el sistema semi-intensivo ( $\chi^2 = 7,42$   $p = 0,006$ ). Este es el primer estudio de la comunidad de parásitos de *A. gigas* en Acre y proporciona nueva información para los acuicultores para aumentar su producción.

**Palabras clave:** Acanthocephala – acuicultura – *Arapaima gigas* – Brasil – Monogenea – Nematoda

## INTRODUCTION

The Amazon region has favorable environmental conditions for the development of fish farming, due to the high diversity of fish species, availability of water resources, stable temperatures throughout the year, large areas and strategic geographic position, enabling connection with Asian and American markets (Brown *et al.*, 2002).

In recent years, the Amazon region has received high investments to develop fish farming. Several studies have been conducted of fish species that have high market value and favorable traits for farming, such as high reproductive potential and high development and juvenile survival rates (Oliveira *et al.*,

2012). *Arapaima gigas* (Schinz, 1822) (Arapaimidae), known as arapaima or pirarucu, is considered the species with the greatest potential for fish farming in the region (Roubach *et al.*, 2003; Lima *et al.*, 2015).

The arapaima farming systems in the Amazon are highly diversified, ranging from semi-intensive to intensive or industrial. Semi-intensive breeding is usually performed by family farmers, with little use of feeding, moderate stocking densities and regular renewal of water. The intensive system is structured to provide a balanced diet, control physical and chemical parameters of water and assure constant water renewal with high densities of fish, through the use of technologies for increased productivity (Tacon & Silva, 1997; Naylor *et al.*, 2000).

Fish confinement, independent of the production system, favors the risk of parasitic infections (Malta & Varela, 2000; Araújo *et al.*, 2009; Santos & Moravec, 2009; Gaines *et al.*, 2012), which is one of the major limiting factors for the development, growth and maintenance of fish populations, causing considerable economic losses (Malta *et al.*, 2001).

Although *A. gigas* is a keystone species for science and has high economic value, there are few studies about the community structure of parasites, and they generally do not focus on the infra-communities. The objective of this work was to analyze the helminth community structure of *A. gigas* comparing two types of fish farming systems in the southwestern Amazon region, semi-intensive and intensive.

## MATERIALS AND METHODS

### *Ethics statement*

This study was authorized by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA, license no. 39106/2013) in accordance with the guidelines of the Brazilian College for Animal Experimentation (COBEA).

### *Study areas*

The fish were obtained from two fish farms, one using a semi-intensive system in the municipality of Bujari, (9°45'24.5"S 68°04'25.0"W) and the other an intensive system in Senador Guiomar Santos (10°05'00.7"S 67°32'06.8"W), both in Acre state, southwestern Amazon, Brazil (Fig. 1).

At the semi-intensive fish farm, the breeding stock fish were kept in earthen ponds receiving water *in natura* from a local stream. The level of the water from the ponds was completed monthly and supplied polyethylene tanks in

which fingerlings were grown after spawning. In this environment, the supply of feed of crude protein 40% was given twice a day. The stock density was 0.15 fish/m<sup>3</sup>.

In the intensive system, the breeding fish were placed in earthen ponds where the water supplied from local streams was renewed daily and filtered in a system with 400 microns mesh. The water temperature and oxygen were controlled daily. The fry were collected immediately after spawning and transported to the laboratory where they were first kept in aluminum vats, then transferred to circular concrete tanks where they lived until reaching about 15 cm, when they were taken to network tanks and then released into the growth ponds.

High quality balanced feed with 45% crude protein was provided 6 to 8 times a day and preventive control of parasites was carried out with anthelmintics. During all phases, fry were collected at random for analysis of possible parasites in the farm's laboratory. The stock density varied from 0.75 to 10 fish/m<sup>3</sup> depending on the size of the spawning from the breeding pair.

### *Collection of parasites*

A total of 121 fish were captured from October 2013 to June 2015 (Fig. 2), 64 from the semi-intensive system and 57 from the intensive fish farm. The specimens were weighed (W), measured for total length (TL) and examined in saline medium and under a stereomicroscope.

The parasites were fixed in 70% ethanol and a sample of each species was fixed in 4% formalin for scanning electron microscopic procedures (SEM). The formalin-fixed specimens were washed in 0.1 M cacodylate buffer pH 7.2, post-fixed in 1% OsO<sub>4</sub> and 0.8% potassium ferrocyanide for 1 h, dehydrated in a graded series of ethanol solutions (30–100%) for 1 h for each step, critical-point dried in CO<sub>2</sub>, sputter coated with gold and examined using a Jeol JSM 6390 microscope.

The Nematoda were cleared and examined on temporary slides mounted with glycerin while Monogenea were cleared in Berlese or Hoyer medium or were stained with Gomori's trichrome and examined as permanent mounts in Canada balsam. The Acanthocephala were cleared in glycerin or stained with paracarmine. Drawings were made with the aid of a drawing tube. Parasites were identified according to Kritsky *et al.* (1985), Costa *et al.* (1995), Moravec (1998), Santos *et al.* (2008 b, c), Santos & Moravec (2009), Thatcher (2006), Luque *et al.* (2011) and Cohen *et al.* (2013).

#### *Ecological data*

The ecological terms used for parasite populations and communities followed Bush *et al.* (1997). The prevalences from different fish farming systems were evaluated for common species using the chi-squared test using the Quantitative Parasitology 3.0 program (Rózsa *et al.*, 2000).

The helminth community was studied at infrapopulation and infracommunity levels (i.e., all parasites of a given species in an individual fish and all infrapopulations in an individual fish, respectively) using the data on the fish collected from each farm, representing a component community. The total abundance, Brillouin's diversity index, Pielou's evenness index, Margalef's richness index and the Berger-Parker dominance index of the infracommunities were also calculated using Past version 2.17c (Hammer *et al.*, 2001). Spearman's rank correlation coefficient ( $r_s$ ) was calculated to determine possible correlations between the total length of hosts and parasite abundance.

The variance-mean ratio of parasite abundance (dispersion index) and Poulin discrepancy index (D) were employed to detect distribution patterns of the parasite infracommunity (Rózsa *et al.*, 2000) in species with prevalence  $\geq 10\%$ . Index of dispersion (ID) significance was tested using the  $d$ -statistic according to

Ludwig & Reynolds (1988) for each infracommunity.

## RESULTS

#### *Fish samples*

A total of 121 specimens of *A. gigas* from the two fish farms were examined (Table 1). The 64 arapaimas from the semi-intensive farm (Fig. 3) measured 7-42 cm (TL), weighed 2-392 g (W) and harbored 14,822 individual parasites of nine species. The 57 arapaimas from the intensive fish farm (Fig. 4) measured 15.5-50 cm (TL) with 27-496 g (W) and contained 6,319 parasites of just one species (Table 1).

#### *Component community*

A total of nine species of parasites were identified in the fish from both farms, with just one species in common. There was a significant difference with respect to parasite prevalence levels between the farms ( $\chi^2 = 44.99$ ,  $p < 0.05$ ), and the specimens from the semi-intensive system showed significantly higher levels of infection (90.63%) than those from the intensive system (31.57%).

In the semi-intensive farm, a total of nine parasite species were collected, identified as *Goezia spinulosa* (Diesing, 1839) (Figs 5-6); the monogenean *Dawestrema cycloancistrum* Price and Nowlin, 1967 (Fig. 7); the nematodes *Capillostrongyloides arapaimae* Santos *et al.*, 2008c; *Hysterothylacium* sp. (Larva L3) (Figs 8-9); Camallanidae gen. sp. and Ascaridoidea gen. sp. (Larva); the acanthocephalans *Polyacanthorhynchus* sp. (juvenile) (Fig. 10), as well as *Neoechinorhynchus* sp. and Acanthocephala gen. sp. (Table 2). Monogenean parasites represented the majority of the collected specimens (95.64%), followed by Nematoda (4.04%) and Acanthocephala (0.32%). The Monogenea *D. cycloancistrum* was the predominant species, with 14,176 specimens

collected, and showed the highest values of mean abundance and mean intensity. At the intensive fish farm, only one species, the monogenean *D. cycloancistrum*, was found, representing 100% of the specimens collected.

The comparative analysis regarding the prevalence of *D. cycloancistrum* between the two farms showed a significant difference, with higher prevalence in the semi-intensive system ( $\chi^2 = 7.42$ ,  $p = 0.006$ ).

#### *Infracommunities*

From the 64 specimens of *A. gigas* examined from the semi-intensive fish farm, 58 (90.63%) were parasitized by at least one species. A total of 14,822 individual parasites were collected, with mean of 255.55 parasites/fish. Six fish hosts were not parasitized (9.38%); 22 fish were infected by only one parasite species (34.38%); 17 hosts harbored two parasite species (26.56%); and 14 fish contained three parasite species (21.88%). The remaining fish

**Table 1** Parasitological data of *Arapaima gigas* from two fish farms in Acre, Brazil

	Semi-intensive fish farm	Intensive fish farm	Total
N. examined fish	64	57	121
N. parasitized fish	58	18	76
Prevalence (%)	90.63	31.57	62.80
Total number of parasites	14,822	6,319	21,141

**Table 2** Quantitative descriptors, infection/infestation site and community status of parasites of *Arapaima gigas* from the two fish farms, Acre, Brazil

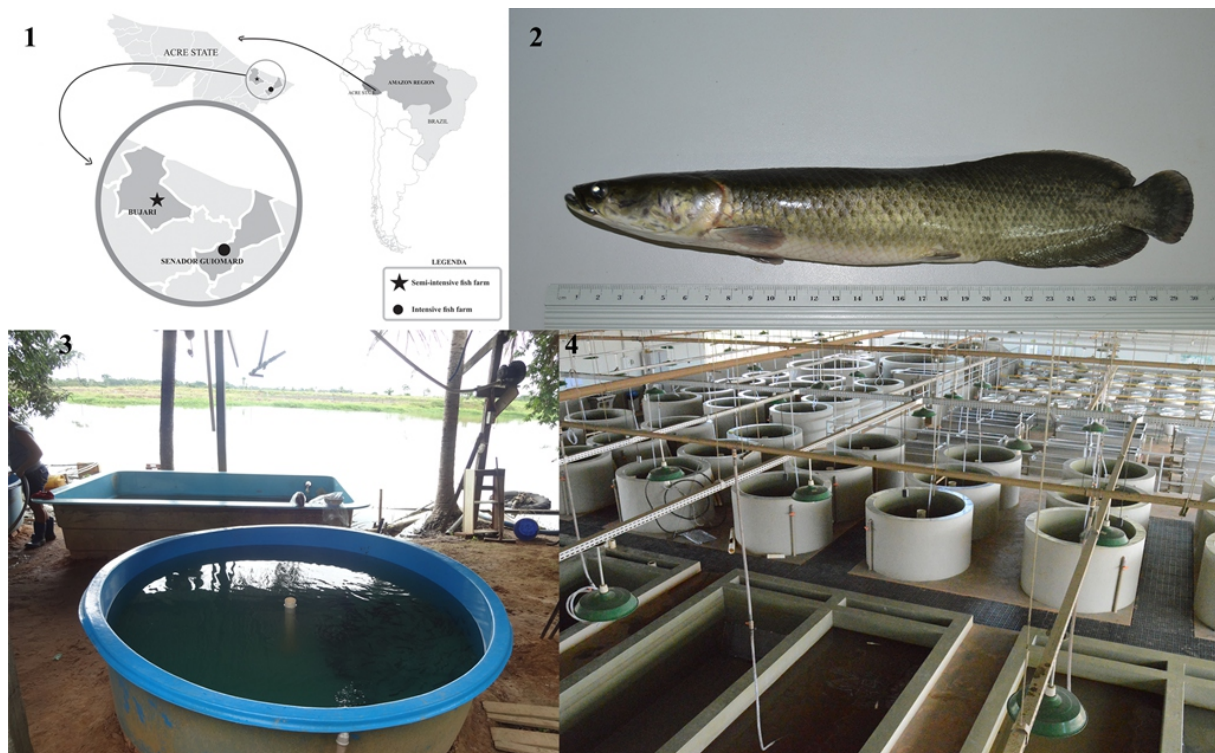
Parasites	Prevalence (%)	Mean Abundance $\pm$ s	Mean Intensity $\pm$ s	Total number of parasites	Infection / Infestation site
<b>Semi-intensive fish farm</b>					
<b>Monogenea</b>					
<i>Dawestrema cycloancistrum</i>	56.25	221.50 $\pm$ 293.03	393.78 $\pm$ 353.35	14,176	Gills
<b>Nematoda</b>					
Ascaridoidea gen. sp. (Larva)	3.13	0.13 $\pm$ 0.24	4.00 $\pm$ 1.00	08	Intestine
Camallanidae gen. sp.	1.56	0.02 $\pm$ 0.03	1.00 $\pm$ 0.00	01	Intestine
<i>Capillostrongyloides arapaimae</i>	20.31	2.03 $\pm$ 3.31	10.00 $\pm$ 11.08	130	Stomach, intestine and intestinal cecum
<i>Goezia spinulosa</i>	29.69	1.19 $\pm$ 1.73	4.00 $\pm$ 3.58	76	Stomach, intestine and intestinal cecum
<i>Hysterothylacium</i> sp. (Larva)	53.13	5.98 $\pm$ 7.76	11.26 $\pm$ 10.57	383	Stomach and intestine
<b>Acanthocephala</b>					
<i>Polyacanthorhynchus</i> sp.	21.88	0.72 $\pm$ 1.12	3.29 $\pm$ 2.08	46	Intestine
<i>Neoechinorhynchus</i> sp.	1.56	0.02 $\pm$ 0.03	1.00 $\pm$ 0.00	01	Intestine
Acanthocephala gen. sp.	1.56	0.02 $\pm$ 0.03	1.00 $\pm$ 0.00	01	Intestine
<b>Intensive fish farm</b>					
<b>Monogenea</b>					
<i>Dawestrema cycloancistrum</i>	31.57	110.86 $\pm$ 248.28	351.05 $\pm$ 248.27	6,319	Gills

s = standard deviation

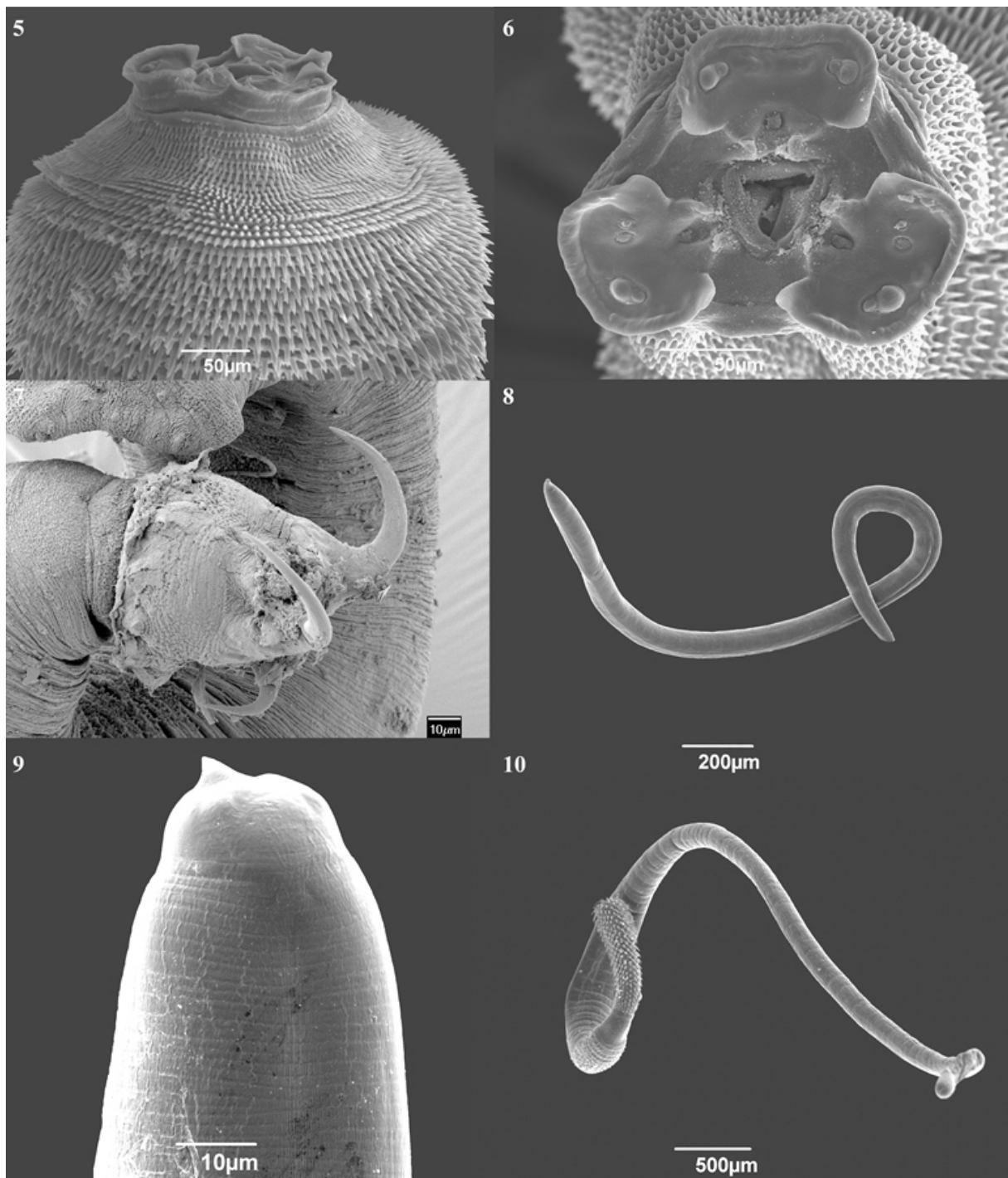
were parasitized by four, five and six parasite species. The parasites showed a typical pattern of aggregated distribution (Table 3). At the semi-intensive fish farm, the mean parasite richness index was  $d = 2.18$  and the mean Pielou evenness index was  $J = 0.54$ . The parasite infracommunities presented mean diversity of  $HB = 1.287 \pm 1.03$  and maximum diversity of 2.87. The Berger-Parker dominance index presented a mean of  $0.55 \pm 0.31$ . Significant correlation was observed

between total length of fish and parasite abundance (Spearman rank correlation coefficient,  $r_s = 0.54, p < 0.01$ ).

At the intensive fish farm, 18 fish (31.57%) were parasitized by a single species. The remaining hosts (68.43%) were free of parasites. The Spearman rank correlation coefficients between host length and parasite abundance were significant ( $r_s = 0.36, p = 0.005$ ).



**Figures 1-4.** 1. Study areas: Bujari ( $9^{\circ}45'24.5''S$   $68^{\circ}04'25.0''W$ ) and Senador Guiomard Santos ( $10^{\circ}05'00.7''S$   $67^{\circ}32'06.8''W$ ), Acre state, southwest Amazon, Brazil. Star = Semi-intensive fish farm, Circle = Intensive fish farm. 2. Specimen of *Arapaima gigas* collected from fish farm. 3. Polyethylene tanks at the semi-intensive fish farm where fingerlings are grown. 4. Laboratory with aluminum vats and circular concrete tanks where fingerlings are grown in the intensive fish farm.



**Figures 5-10.** 5. *Goezia spinulosa*: anterior end of body, lateral view. 6. *Goezia spinulosa*: cephalic end, apical view. 7. *Dawestrema cycloancistrum*: total. 8. *Hysterothylacium* sp. (L3): total. 9. *Hysterothylacium* sp. (L3): anterior end provided with ventral cephalic tooth, lateral view. 10. *Polyacanthorhynchus* sp. (juvenile): total.

**Table 3.** The Dispersion Index (DI), statistical test (*d*) and discrepancy index (D) of the parasites of *Arapaima gigas* from the Semi-intensive fish farm, Acre, Brazil\*

Parasites	DI	<i>d</i>	D
<i>Dawestrema cycloancistrum</i>	856.09	4876.83	0.768
<i>Capillostrongyloides arapaimae</i>	46.67	98.10	0.921
<i>Goezia spinulosa</i>	9.97	27.43	0.857
<i>Hysterothylacium</i> sp. (Larva)	23.44	121.76	0.777
<i>Polyacanthorhynchus</i> sp.	4 39	8.76	0.853

\*DI and D were employed in species with prevalence  $\geq 10\%$ .

## DISCUSSION

One of the barriers to the production of the arapaimas in fish farms is parasitic diseases, which influence the quantity and quality of fish produced (Gaines *et al.*, 2012). Study of the parasitic fish community structure provides a range of information on habits and habitat of the host and contributes to the understanding of the distribution, prevalence and specificity of parasites (Dias *et al.*, 2004). The literature reports 21 metazoan parasite species of *A. gigas* (Baylis, 1927; Thatcher, 2006). We identified nine species.

The high prevalence of fish parasites in the semi-intensive farm (90.63%) was similar to that reported by Marinho *et al.* (2013) for the same host species in fish farms in Amapá state (northeastern Amazon region) and by Araújo *et al.* (2009) in semi-intensive culture in Amazonas state (central Amazon). This pattern of infection, according to these authors, can be associated with the inadequate tank management. In the present study, this factor also explain the high prevalence of parasites in the semi-intensive farm, since the water used in nurseries, ponds and tanks does not pass through a filter system and can carry pathogenic organisms to the fish (Tavares-Dias, 2011). Allied to this, the monthly water level renewal with no filters, failure to control oxygen and temperature parameters and lack of disinfection of structures and devices can

facilitate the entrance and spread of parasites in the system (Lima *et al.*, 2015). In contrast, in the intensive fish farming system, where the water is renewed daily, filtered with a 400 mesh net, temperature and oxygen are controlled daily and anthelmintics are used, the parasitic infection was significantly lower, restricted to external monogenean parasites.

According to Lima *et al.* (2015), a factor that must be taken into consideration is the fact that the fingerlings are usually caught from the ponds more than 10 days after hatching, so an initial load of parasites is already present in these fish due to the contact with the environment and even with parents. In the intensive fish farming system, the fry are taken from nurseries soon after spawning, thus reducing the rates of parasitic infections.

*Dawestrema cycloancistrum* is a monogenean specific to arapaimas and was the only species found in both fish farms studied, with significantly higher abundance ( $14,176 \times 6,319$  specimens) and prevalence ( $56.25\% \times 31.57\%$ ) in the semi-intensive production system. This species has also been reported from the gills of farmed arapaimas in the states of Amazonas (Araújo *et al.*, 2009), Amapá (Marinho *et al.*, 2013) and Mato Grosso (Santos *et al.*, 2008a) in Brazil and in the state of Loreto in Peru (Mathews *et al.*, 2013; Serrano-Martínez *et al.*, 2015). It is highly pathogenic and can cause serious injury to the gills and cause death. High infestations of



species of *Dawestrema* were observed by Mathews *et al.* (2014) in a semi-intensive fish farming system in Peru. The authors related the high level of parasitism in *A. gigas* with an imbalance in the homeostasis of fish, caused by changes in the concentration of ammonia in the water. The occurrence of *D. cycloancistrum* in both farming systems and their occurrence in different geographical areas can be explained by their direct life cycle and small size and hermaphrodite reproduction, thus favoring their occurrence and transmission among different tanks, as occurs in general with monogenean species (Luque, 2004; Pavanelli *et al.*, 2008).

Nematodes also cause serious infections in farmed fish, and *G. spinulosa*, which occurred with prevalence of 29.69%, is a highly pathogenic parasite of arapaimas during the fingerling and juvenile stages, negatively influencing fish health (Freitas & Lent, 1946; Moravec *et al.*, 1994; Santos & Moravec, 2009). Cultured fingerlings are especially sensitive to this parasitosis, which contributes substantially to the high mortality of arapaima fingerlings in fish farms (Santos & Moravec, 2009). They become infected by consuming zooplankton (copepods) containing the L3 larvae, which are released into the digestive tract. Ensembles of multiple adults are then found attached to the stomach wall, producing ulcers. Santos & Moravec (2009) observed that fingerlings ranging in length from 6.5-15 cm only harbored larval *G. spinulosa* encysted on the surface of the stomach and pyloric caeca, in the mesentery, while sub-adults were found in the lumen of the stomach. The fish specimens examined here in both systems, ranging from 7-50 cm, harbored sub-adults and adults with eggs, without causing perforations, although some small ulcers were seen. It is possible that the water supply of the ponds and tanks in the semi-intensive system favored the *G. spinulosa* life cycle, given that lack of special barriers as filters in nurseries can allow infected plankton to enter these environments

with the water coming directly from nearby streams.

The prevalences of *C. arapaimae* (20.31%) and the juveniles of *Polyacanthorhynchus* sp. (21.88%) were considered important, since *C. arapaimae* and two species of *Polyacanthorhynchus* have previously been reported in arapaimas and have the ability to penetrate the intestine wall for attachment, where they cause tissue damages. *Hysterothylacium* sp. had a high prevalence (53.13%), but they were encysted L3 larvae with boring tooth, using the arapaimas as intermediate or paratenic hosts, and no visible damages were observed.

*Neoechinorhynchus* sp., Acanthocephala gen. sp. and Camallanidae gen. sp. were represented by a single individual each and Ascaridoidea gen. sp. by eight larvae, not representing important infrapopulations that could cause damage to the cultured fish.

The aggregate distribution pattern of the parasites of *A. gigas* is in accordance with that reported by Marinho *et al.* (2013) for the same species, in fish farms in Amapá state. Poulin (2007) called this common pattern of distribution the 'The First General Law of Parasite Ecology' and Gourbière *et al.* (2015) complemented that the aggregated distribution has significant implications both for hosts and parasites, because it can cause major consequences in production systems, also impacting public health. In this study, all the parasites had an aggregated distribution pattern. However, the Monogenea presented particularly high values, indicating that in farming systems where the density of hosts is high, these parasites with direct life cycle have greater dispersion capacity.

The ecological descriptors of diversity, evenness and dominance of the parasites of *A. gigas* were calculated only for the semi-intensive system, given that in the intensive

system just one type of parasite was found. According to Magurran (2004), these indices are very useful, but only when employed for comparisons, because the values alone are not informative. This is the first work that cites values of diversity, evenness and dominance indices of the parasites of *A. gigas*, and can serve as a basis of comparison for future studies.

In both cultivation systems, parasite abundance significantly increased with the host size. Poulin (2000) considers that this is a common feature in the host-parasite relationship, since larger fish have more varied diet, greater contact surface for parasites and higher number of niches, among other factors (Morand & Poulin, 1998; Timi & Poulin, 2003).

Finally, this study shows that good production conditions, including immediate collection of fingerlings after spawning, water filtering before entry into nurseries and use of disinfecting materials during breeding can drastically reduce infection by parasites. This is the first study of the parasite community of *A. gigas* in Acre state and provides new information for fish farmers to enhance their production.

#### Competing interests

The authors declare that they have no competing interests.

#### Authors' contributions

All authors contributed equally to the study. All authors read and approved the final manuscript.

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