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Helminthes and protozoan of farmed pirarucu (*Arapaima gigas*) in eastern Amazon and host-parasite relationship

[*Helmintos e protozoários de pirarucu* (Arapaima gigas) *cultivado na Amazônia oriental, e relação hospedeiro-parasito*]

R.G.B. *Marinho*[1,2], M. *Tavares-Dias*[1*], M.K.R. *Dias-Grigório*[1,2], L.R. *Neves*[1], E.T.O. *Yoshioka*[1], C.L. *Boijink*[3], R.M. *Takemoto*[4]

[1]Embrapa Amapá – Macapá, AP
[2]Pós-graduação – Universidade Federal do Amapá (Unifap) – Macapá, AP
[3]Embrapa Amazônia Ocidental – Manaus, AM
[4]Universidade Estadual de Maringá (UEM) – Maringá, PR

ABSTRACT

The parasitofauna in the giant Amazon basin, pirarucu (*Arapaima gigas* Schinz, 1822) cultured in fish farms from the state of Amapá, in eastern Amazonia (Brazil) was investigated. Of the 100 examined fish, 90.0% were parasitized by *Ichthyophthirius multifiliis* (Ciliophora), *Dawestrema cycloancistrium, Dawestrema cycloancistrioides* (Monogenoidea) and *Polyacanthorhynchus macrorhynchus* (Acanthocephala), which had an aggregated distribution pattern. The highest infection rates were caused by *I. multifiliis* and the lowest by *P. macrorhynchus.* Infection rates were different for each fish farm, due to different water quality and management characteristics. A negative correlation was found between the intensity of monogenoideans *D. cycloancistrium* and *D. cycloancistrioides* and the relative condition factor (Kn), but the welfare of fish was not affected by parasitism. The number of *I. multifiliis* was positively correlated with the weight and total length of hosts, while the intensity of monogenoideans was negatively correlated with body weight and total length. This study is the first to record the occurrence of *P. macrorhynchus* in *A. gigas* farmed in Amazon.

Keywords: Amazonia, fish farm, freshwater fish, parasites, sanity

RESUMO

*Investigou-se a parasitofauna no gigante da bacia amazônica, pirarucu (*Arapaima gigas *Schinz, 1822), cultivado em pisciculturas do estado do Amapá, na Amazônia oriental, Brasil. Dos peixes examinados, 90,0% estavam parasitados por* Ichthyophthirius multifiliis *(Ciliophora),* Dawestrema cycloancistrium, D. cycloancistrioides *(Monogenoidea) e* Polyacanthorhynchus macrorhynchus *(Acanthocephala), os quais tiveram um padrão de distribuição agregado. As maiores taxas de infecção foram causadas por* I. multifiliis, e *as menores por* P. macrorhynchus. *As pisciculturas examinadas apresentaram diferentes taxas de infecção devido às diferentes características de qualidade de água e de manejo. Houve correlação negativa entre a intensidade de monogenoideas e o fator de condição relativo (Kn), mas a saúde dos peixes não foi afetada pelo parasitismo. A intensidade de* I. multifiliis *foi positivamente correlacionada com o peso e o comprimento, enquanto a intensidade de monogenoideas* D. cycloancistrium *e* D. cycloancistrioides *mostrou correlação negativa com o peso e o comprimento total dos hospedeiros. Este estudo foi o primeiro registro da ocorrência de* P. macrorhynchus *em* A. gigas *cultivados na Amazônia.*

Palavras-chave: Amazônia, piscicultura, peixe de água doce, parasitos, sanidade

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*Autor para correspondência (*corresponding author*)
E-mail:marcos.tavares@embrapa.br

INTRODUCTION

In the giant Amazon basin, pirarucu (*Arapaima gigas*, Arapaimidae, Osteoglossiformes), is the largest scaled freshwater fish species in world; it can reach three meters of length (Araújo *et al*., 2009a; Pereira-Filho and Roubach, 2010; Núñez *et al*., 2011) and weight up to 200kg (Portes-Santos *et al*., 2008; Araújo *et al*., 2009a). This fish is a source of income for several riverine communities from Amazonia who live from extractive fishing. Hence, natural pirarucu populations have been subjected to excessive fishing for human food and ornamental fish trading. However, this fish very attractive for Amazon aquaculture due to its many advantages (Núñez *et al*., 2011).

Pirarucu *A. gigas* is a carnivorous fish that accepts an artificial diet when properly trained. Its aerial breathing facilitates cultivation in waters with low availability of dissolved oxygen, which is not possible for gill breathing fish. It has a high growth rate, reaching 7-10kg in 12 months of farming. In addition, this fish tolerates rough handling, high stocking densities and presents good zoo technical indexes when in captivity (Ono *et al*., 2003; Ono *et al*., 2004; Pereira-Filho and Roubach, 2010; Núñez *et al*., 2011). These positive factors have encouraged the culture of this Amazonian fish in Brazil, which closed 2010 with a production of 10.4 tons (MPA, 2012). There are few studies on the epidemiology of parasites for this fish cultured in other regions from Amazon, including the State of Amapá, in eastern Amazon. However, Brazilian aquaculture has reported parasitic infection data in other finfish.

A recent survey noticed that *A. gigas* has a high parasite diversity in the natural environment, including one Myxozoa specie, three Monogenoidea, nine Nematoda, two Cestoda, three Digenea, three Crustacea, two Acanthocephala and one Pentastomida species (Araújo *et al*., 2009a). Most of these studies are taxonomic descriptions, but there are few epidemiological reports for cultured *A. gigas*.

In fish farms from the State of Amazonas, in central Amazon, *A. gigas* infection by *Dawestrema cycloancistrium* and *Dawestrema cycloancistrioides* (Monogenoidea), and *Trichodina* sp. (Protozoa) were the most prevalent. In addition, infection by crustacean *Argulus* sp., protozoan *Ichthyobodo*, nematodes *Goezia spinulosa*, *Terranova serrata* and *Camallanus tridentatus* (Araújo *et al*., 2009a,b) were also reported. Therefore, fish farming is not without its problems, and these include disease outbreaks and the consequences of introducing parasites to new hosts and/or new locations with the transportation of live fish. Hence, severe epizootics can occur and cause economic losses to fish farmers.

The knowledge of the ecological relationships among parasites, their hosts and the environment in which they live is extremely important to avoid significant economic losses (Lizama *et al*., 2007a, b). Therefore, a concern for researchers has been to broaden the knowledge about the strategies used by different parasites and their hosts in such confined environments. This information, besides explaining the presence or absence of certain parasites and the rates of parasitism (Pavanelli *et al*., 2004; Takemoto *et al*., 2009; Eiras *et al*., 2010), may also assist in the proper use of management techniques leading to improvements in water quality. Thus, these techniques improve the quality of fish, the ultimate goal of all fish farms (Lizama *et al*., 2007b). For this reason, the aim of this study was to investigate the diversity of parasites in *A. gigas* from three fish farms in Macapá, State of Amapá, Brazil (in eastern Amazon), and their relationship to this host.

MATERIAL AND METHODS

In three fish farms from the municipality of Macapá, State of Amapá, in the eastern Amazon, *Arapaima gigas* fingerlings weighting on average 15g, were fed with a homogeneous mixture of zooplankton, minced fish meat, mineral and vitamin premix and extruded commercial ration with 46% crude protein (Ono, 2003; Ono, 2004) during the food training. After the training, the fish were fed from four to six times daily exclusively with a commercial ration containing 46% crude protein. During the growing period, these fish were fed twice daily with a commercial ration containing 40% crude protein. From October 2009 to September 2010, young fish (Table 1) were collected in three fish farms (named WR, PE and LM) and necropsied for parasitological analyses.

Table 1. Collection sites, pond sizes and *Arapaima gigas* age from three fish farms in eastern Amazon, Brazil

Fish farm	Geographical location	Pond size (m^3)	Age (months)
WR	S: 0º02'31.4"- W: 051º07'34.4"	10,000	24-33
LM	S: 0˚00'1.35'' - W: 051˚06'12,8''	1,800	18
PE	N: 0º00'04.5.4"- W: 051º05'52.1"	2,000	13-15

All fish were weighted (kg), measured in length (cm) and necropsied for parasite analyses. Their gills and gastrointestinal tract were examined. These organs were removed and analyzed with the aid of a common light microscope and a stereomicroscope, respectively. The methodology used for collection, fixation (Eiras *et al*., 2006; Thatcher, 2006) and quantification of parasites (Tavares-Dias *et al*., 2001a, b) followed previous recommendations. The identification of the collected parasites was according to Baylis (1927), Machado-Filho (1947), Kritsky *et al*. (1985) and Thatcher (2006). After these procedures, parasitological indexes were calculated for evaluation of the levels of infection according to the recommendations of Rohde *et al*. (1995) and Bush *et al*. (1997). The index of dispersion (ID) and discrepancy index (D) were employed to detect distribution patterns of the parasites infracommunity (Rózsa *et al.*, 2000), for species with prevalence ≥10%.

The length and weight of the hosts were used to calculate the relative condition factor (Kn) of parasitized and non-parasitized fish according to Le-Cren (1951). Spearman's rank correlation coefficient (*rs*) was used to determine the possible correlations between the total length of the host and the number of parasites, and also to verify correlations between the intensity of monogenoideans and the relative condition factor (Zar, 1999).

The potential of hydrogen (pH), temperature (T^oC) and levels of dissolved oxygen (DO) of the ponds were measured using digital devices suitable for each purpose

RESULTS

All the water quality parameters that were monitored showed similar values for the three fish farms, except the levels of dissolved oxygen which were lower in LM fish farm (Figure 1). In addition, the pond in this fish farm also showed high eutrophication levels.

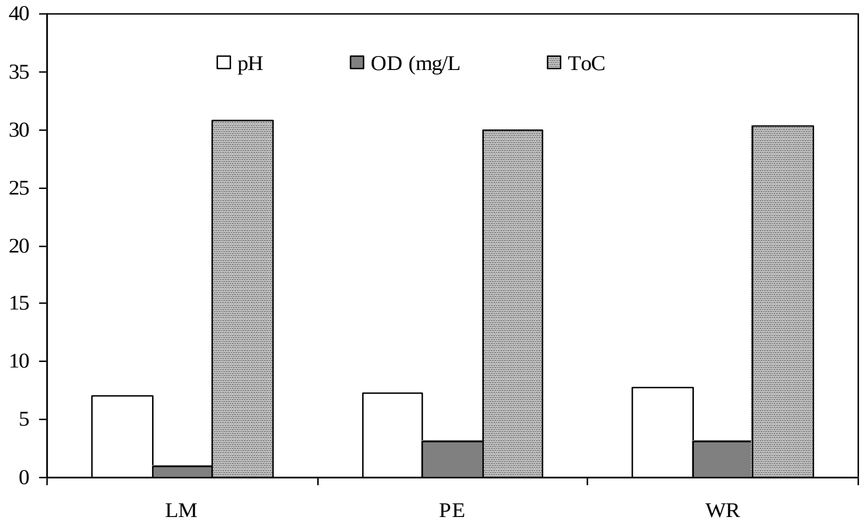


Figure 1. Mean values of water quality in ponds of farmed *Arapaima gigas* in eastern Amazon, Brazil. WR, PE and LM: Fish farm.

A total of 100 specimens of *A. gigas* were examined in the three fish farms, of which 95.0% were parasitized; there were no differences among the prevalence levels (Table 2). They showed one or more parasites such as *Ichthyophthirius multifiliis* Fouquet, 1876 (Protozoa: Ciliophora); *Dawestrema cycloancistrium* Price and Nowlin, 1967 and *D. cycloancistrioides* Kritsky, Boeger and Thatcher, 1985 (Monogenoidea: Dactylogyridae) and *Polyacanthorhynchus macrorhynchus* Diesing, 1856 (Acanthocephala: Polyacanthorhynchidae).

Table 2. Mean values ± standard deviation of body weight and total length for *Arapaima gig*as from three fish farms in eastern Amazon, Brazil

Fish farm	Weight (kg)	Total length (m)	EF	PF	P (%)
WR	24. 760 ± 3.49	1.44 ± 0.07	20	19	95.0
PE	14. 220 ± 2.59	1.17 ± 0.06	40	40	100
LM	12. 700 ± 2.17	1.10 ± 0.04	40	36	90.0
Total	-	-	100	95	95.0

EF: Examined fish; PF: Parasitized fish; P: Prevalence.

Table 3. Parasitological indexes of *Ichthyophthirius multifiliis* for *Arapaima gig*as from three fish farms in eastern Amazon, Brazil

	WR	PE	LM
Examined fish	20	40	40
Parasitized fish	14	40	36
Prevalence (%)	70.0	100	90.0
Mean intensity	482,728	221,999.3	6,841.051
Mean abundance	337,909.6	221,999.3	352,426.8
Range of intensity	36,557-1,034.796	39,889-393,120	41,300-373,670
Total number of parasites	6,758.192	8,879.973	14,097.072

WR, PE and LM: Fish farm.

The highest mean intensity by *I. multifiliis* on the gills of *A. gigas* occurred in the LM fish farm and the lowest one in PE fish farm; however, in last one the prevalence was of 100% (Table 3). In the gills, parasitism by monogenoideans *D. cycloancistrium* and *D. cycloancistrioides* was highest in PE fish farm and lowest in WR fish farm; however, these parasites were not found in fish from LM fish farm. In the intestines of *A. gigas*, infection by *P. macrorhynchus* occurred only in the WR fish farm (Table 4).

Table 4. Parasitological indexes of helminthes monogenoideans (*Dawestrema cycloancistrium* and *D. cycloancistrioides*) and acanthocephalans for *Arapaima gig*as from three fish farms in eastern Amazon, Brazil

Parasite	*Dawestrema* spp.		*Polyacanthorhynchus macrorhynchus*
	WR	PE	WR
Examined fish	20	40	20
Parasitized fish	14	40	19
Prevalence (%)	70.0	100	95.0
Mean intensity	57.3	214.7	28.2
Mean abundance	40.1	214.7	26.7
Range of intensity	26-131	110-371	3-118
Total number of parasites	802	8.589	535

WR, PE and LM: Fish farm.

I. multifiliis was the parasite with the highest mean relative dominance and *P. macrorhynchus* the one with the lowest mean relative dominance (Table 5). Parasites infracommunity showed a typical aggregated distribution pattern, with the higher values of aggregation in *Dawestrema* spp. and *P. macrorhynchus* (Table 6).

Table 5. Parasitological indexes for pirarucu *Arapaima gigas* (n=100) from three fish farms in eastern Amazon, Brazil

Parasite	P (%)	MI ± SD	MA	TNP	RD
Ichthyophthirius multifiliis	90.0	249,769.0±166,809.2	224,792.2	22,479.216	0.99955
Dawestrema spp.	54.0	173.9±89.0	93.9	9.391	0.00042
Polyacanthorhynchus macrorhynchus	19.0	28.1±32.6	53.5	535	0.00002

P: Prevalence; MI: Mean intensity; Standard Deviation; TNP: total number of parasites; MA: Mean abundance; RD: Relative dominance.

Table 6. Dispersion index (DI), *d* statistic and discrepancy index (D) for the parasites of *Arapaima gigas* in fish farms from the eastern Amazon, Brazil

Parasite	DI	*d*	D
Ichthyophthirius multifiliis	6.332	10164.3	0.379
Dawestrema spp.	8.556	263.8	0.641
Polyacanthorhynchus macrorhynchus	3.701	30.2	0.866

There was no significant (P=0.998) difference in the relative condition factor of parasitized (Kn=1.00±0.02) and non-parasitized fish (Kn= 1.00±0.002), but a significant negative correlation was found between the number of monogenoideans and the relative condition factor (Kn) of the hosts (Fig. 2). In addition, there was a significant positive correlation between the number of *I. multifilis* and the total length and body weight of the hosts (Fig. 3), as well as a negative correlation between the number of monogenoideans and the total length and body weight (Fig. 4). No correlation between the number of *P. macrorhynchus* and the total length (*rs*= -0.0776, P= 0.748) and body weight (*rs* = -0.0430, P = 0.854) of *A. gigas* was found.

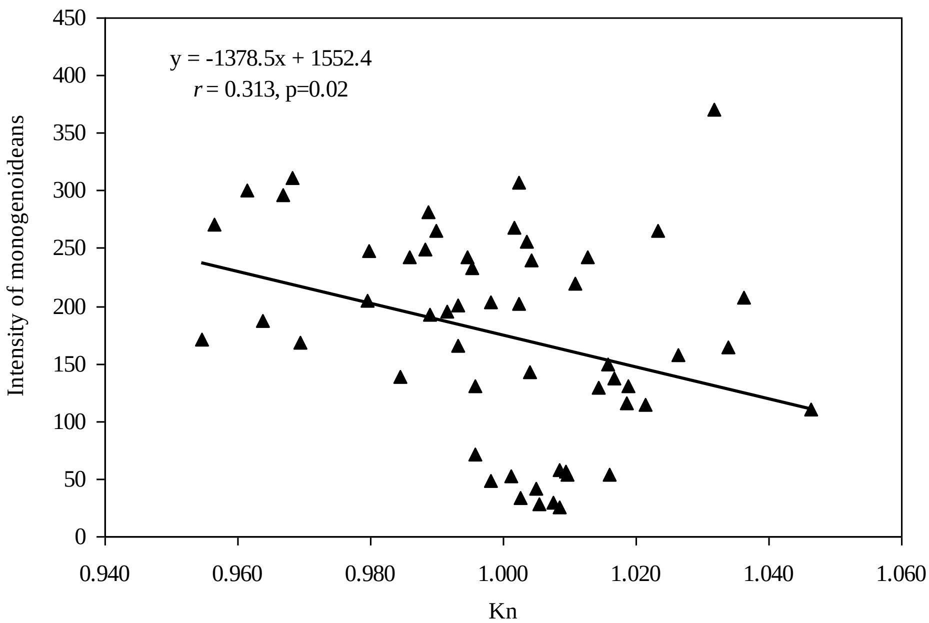
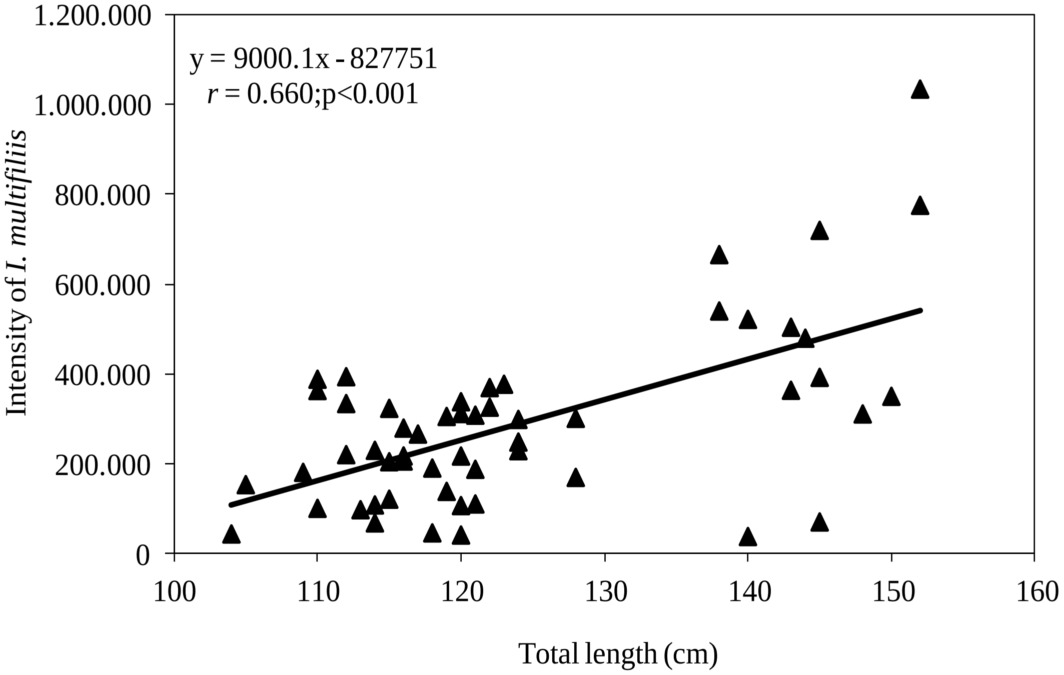


Figure 2. Correlation between Kn and intensity of monogenoideans (*Dawestrema cycloancistrium* and *D. cycloancistrioides*) in *Arapaima gigas* gills (n=54) from eastern Amazon, Brazil.



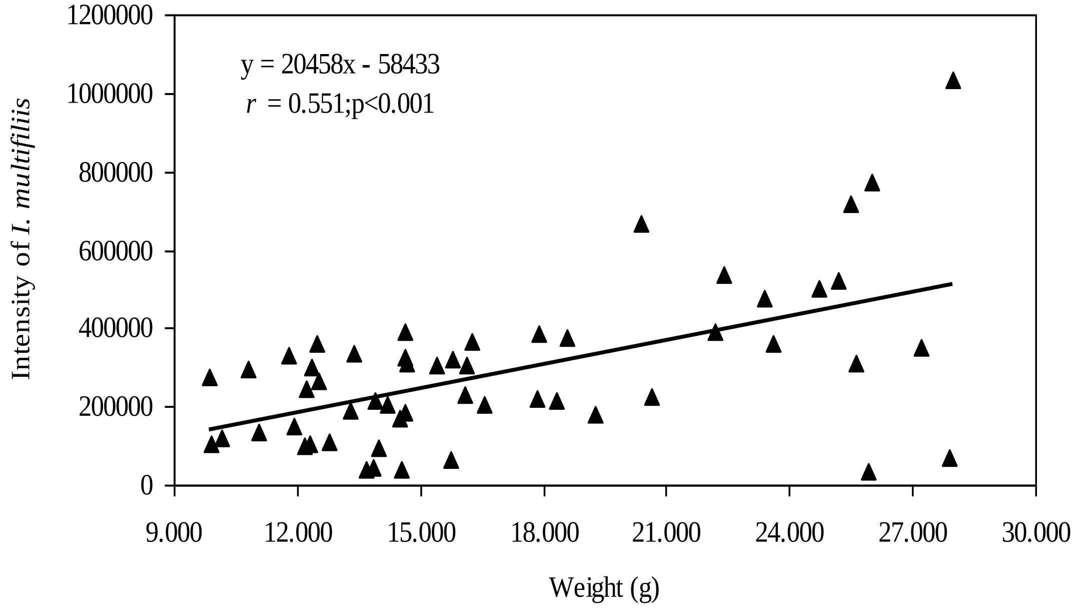


Figure 3. Correlation between the intensity of *Ichthyophthirius multifiliis* and body weight and length of *Arapaima gigas* (n=54) in fish farms from the eastern Amazon, Brazil.

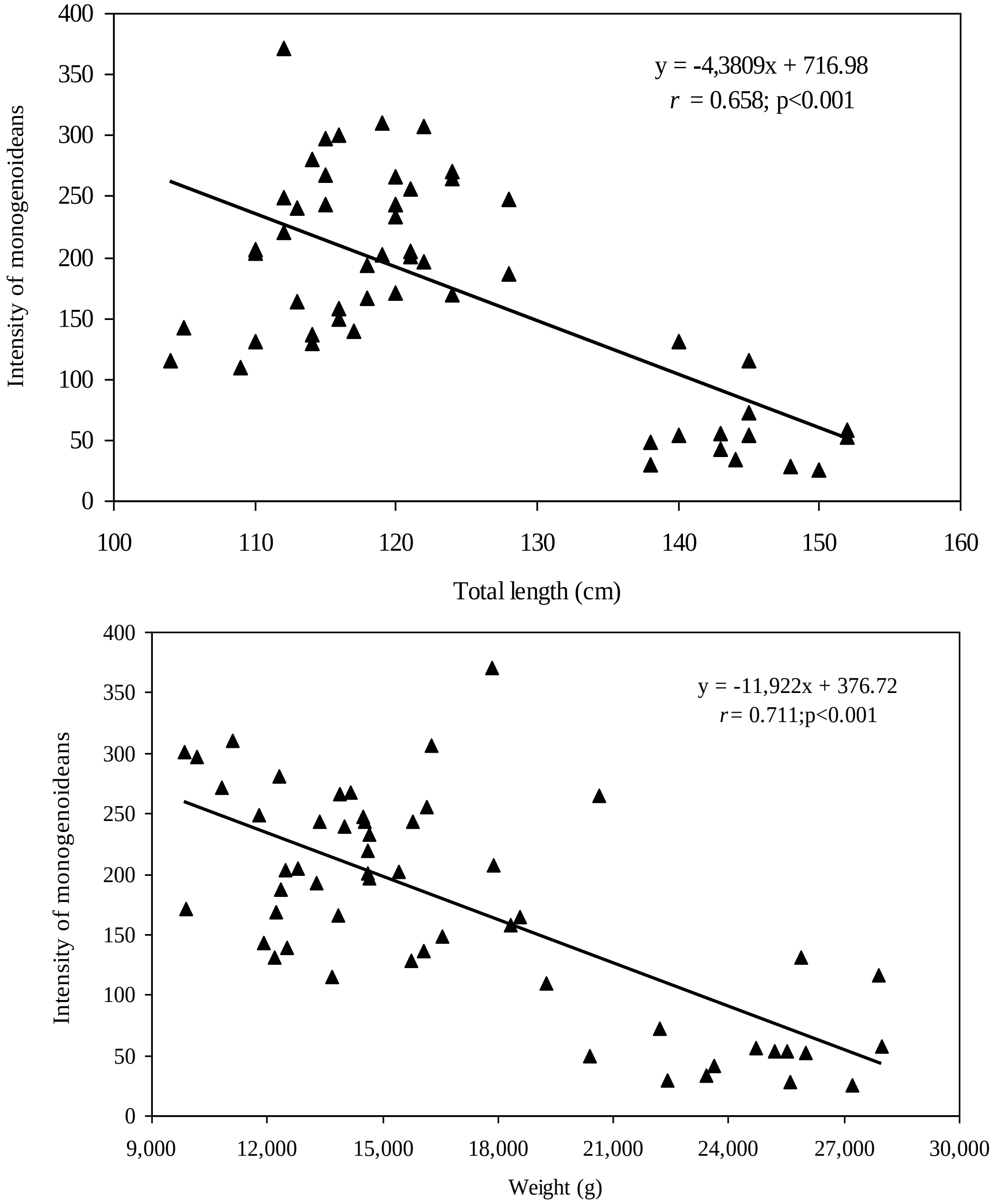


Figure 4. Correlation between the intensity of monogenoideans (*D. cycloancistrium* and *D. cycloancistrioides*) and body weight and length of *Arapaima gigas* (n=54) in fish farms from the eastern Amazon, Brazil.

DISCUSSION

The rapid expansion of aquaculture has provided opportunities for increased parasitic infections and additional exposure to emerging disease etiologies in fish. The *I. multifiliis* is one of the most pathogenic protozoa in fish because it has no host specificity. Therefore, it is a major problem for fish farming worldwide (Dickerson, 2006; Eiras *et al*., 2010). Ichthyophthiriasis occurs mainly when there are changes in the temperature (Eiras *et al*., 2010). In Amazon variation in the temperature hardly occurs. However, the mean temperature is above 30^{o}C, so any other factor that can stress fish in this temperature can favor this parasite. Usually, *I. multifiliis* appears mainly in fish kept under high stocking densities and stressful conditions, due to the low level of dissolved oxygen in the water (Martins *et al*., 2002; Dickerson, 2006). Thus, knowledge of the ecological relationships among the parasites, their hosts and the environment in which they live is extremely important to avoid economical losses (Lizama *et al*., 2007b).

Infection by *I. multifiliis* occurred in *A. gigas* from the three studied fish farms. However, higher levels of infection occurred in one of the fish farms which showed low levels of oxygen and excess organic matter in the water of the culture pond. In the *A. gigas* gills the number of *I. multifiliis* showed a highly positive correlation with the weight and total length of fish, which indicates an increase of parasitism with the growth of the fish. This increase may be due to the cumulative process, since the gills increase their surface area proportionally to the growth of fish (Alves *et al*., 2000; Lizama *et al*., 2007a, b). Thus, there is also a proportional increase in habitat for reproduction of this protozoan which has direct transmission. In addition, *I. multifiliis* showed an aggregated pattern of distribution in *A. gigas*. Parasite aggregation is common in fish (Rohde et al., 1995), and has important implications for the population and evolutionary dynamics of the parasite and its host.

Monogenoidea parasites are common both in fish from natural environment as in fish from cultures (Thatcher, 2006; Eiras *et al*., 2010) which serve as bioindicators (Madi and Ueta, 2009; Kaouachi *et al*., 2010). They are highly pathogenic parasites that attach to the gills, a vital organ in fish. Overall, they have a high degree of host specificity, as it is the case of the monogenoideans *Dawestrema* Price and Nowlin, 1967. In this study, *D. cycloancistrium* and *D. cycloancistrioides* were the monogenoideans found in the gills of the examined *A. gigas*. Similarly, these parasites have been described in the gills of this same host farmed in the State of Amazonas, but with higher levels of prevalence and intensity (Araújo *et al*., 2009b). Infection rates by *D. cycloancistrium* and *D. cycloancistrioides* differed among the three investigated fish farms. The lowest rates of parasitism were found in the fish farm where a lower number of *A. gigas* were examined. Marques and Cabral (2007) have demonstrated that even though a sampling smaller than 40 specimens does not influence the prevalence of parasites, it can lead to an underestimation of intensity and abundance.

In pirarucus of fish farms from the State of Amapá, the factor Kn correlated negatively with the number of *D. cycloancistrium* and *D. Cycloancistrioides*, which indicate a pathogenicity of these parasites in gills that had an aggregated pattern of distribution. However, an increase in body weight and length leads to a decrease in the intensity of these helminthes in the hosts' gills. Similarly, a negative correlation of Kn with the abundance of monogenoideans *Cichlidogyrus sclerosus* and *Cichlidogyrus* sp. in *Oreochromis niloticus* (Lizama *et al*., 2007a) and of Kn with the abundance of Monogenoidea *Anacanthorus penilabiatus* and *Mymarothecium* sp. in *Piaractus mesopotamicus* (Lizama *et al*., 2007b) has been reported. However, negative correlations for monogenoideans may result from host self-immunity to these helminthes over time (Lizama *et al*., 2007a). Therefore, the study of factors influencing the host-parasite relationships has been gaining the interest of fish parasitologists in recent years.

In this study, no monogenoideans were found in the gills of *A. gigas* from fish farms with a low level of oxygen and a high level of organic matter in the ponds. Although many monogenoideans species have a predilection for polluted environments (Galli *et al*., 2001; Buchmann and Brescani, 2006; Madi and Ueta, 2009), the occurrence of some is negatively influenced by pollution (Dzikowski *et al*., 2003; Kaouachi *et al*., 2010). Therefore, it is possible that monogenoideans *D. cycloancistrium* and *D. cycloancistrioides* are sensitive to polluted environments. Nevertheless, additional studies are needed for better conclusions.

Three species of Acanthocephala, genus *Polyacanthorhynchus* Travassos, 1926 are known in South America: *Polyacanthorhynchus macrorhynchus* Diesing, 1856, P. rhopalorhynchus Diesing, 1851 and P. caballeroi Diaz-Ungria and Rodrigo,1960 (Amin and Dezfuli, 1995; Aloo and Dezfuli, 1997). A fourth specie of parasite attacking several freshwater fish in Africa is P. kenyensis Schmidt and Canaris (1967), (Amin and Dezfuli, 1995; Aloo and Dezfuli, 1997; Aloo, 2002). In

Brazil, only *P. macrorhynchus* (Balis, 1927; Machado-Filho, 1947; Thatcher, 2006) and *P. rhopalorhynchus* (Thatcher, 2006; Santos *et al*., 2008) have been documented parasitizing freshwater fish, namely a natural population of *A. gigas.*

Usually the acanthocephalan species are endohelminthes with a heterogeneous life cycle involving two hosts. The intermediate host is usually a micro-crustacean (ostracod, copepod, amphipod or isopod) and the definitive host, a fish (Aloo, 2002; Thatcher, 2006; Eiras *et al*., 2010). In the definitive host, the abundance of endohelminthes depends on its eating habits and therefore, carnivorous fish have higher infection rates than omnivore fish **(**Pavanelli *et al*., 2004; Ahmed *et al*., 2007; Takemoto *et al*., 2009), since the transference of these adult endohelminthes among the host fish is also possible (Nickol, 2006). Reports of outbreaks in farmed fish due to acanthocephalan infection are rare **(**Eiras *et al*., 2010)**,** since these endohelminthes are more frequent in natural populations of fish, where there is a constant presence of intermediate hosts, and, consequently, of infectants forms.

Acanthocephalans *P. macrorhynchus* had an aggregated pattern of distribution, and until the present moment, these parasites were found only in *A. gigas* cultured in a fish farm in the State of Amapá, eastern Amazon. These helminthes had not been reported in *A. gigas* from other cultures in Brazil. The prevalence, mean intensity and abundance of *P. macrorhynchus* were similar to the ones described by Santos *et al*. (2008) for this same host from the Araguaia River (MT) parasitized by *P. rhopalorhynchus*. Even though the source of contamination by these endohelminthes in *A. gigas* has not been identified, it may be a result of the harvesting of the water supply, which facilitates the access of intermediate hosts, such as crustaceans living in the ponds. However, it is also possible that these fish were already parasitized by *P. macrorhynchus* since the fingerling stage.

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