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CHAPTER 1

Parasitic fauna in tambaqui (*Colossoma macropomum*) from fish farms in the municipality of Rorainópolis-Roraima-Brazil

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ABSTRACT

Fish farming is a promising activity in the Western Amazon. The tambaqui (Colossoma macropomum) stands out for its good zootechnical performance, supply of fingerlings, rusticity and consumer market, being the most cultivated species (90% of all production) and consumed in Roraima. Like all confined animal production, there is a risk of pathogens and diseases emerging. The tambaqui can be affected by a wide variety of parasites, bringing irreversible damage both economically and healthily. The objective of this study was to identify and quantify parasites present in gills, intestine and tambaqui muscle from five fish farms in Rorainópolis-RR, as well as to correlate results with biometric variables and to estimate parasite prevalence and abundance. For this, 25 tambaqui specimens were acquired in five fish farms in the municipality of Rorainópolis-RR. Parasites of the class Monogenea (Girodactilideo sp.), Phylum Nematoda (Procamallanus (Spirocamallanus sp.) and Klossinemella sp.) and the class Myxozoa (Myxobolus sp.) were recorded . In two farms there was a 100% prevalence of infestation by Monogenea. The standard weight-length ratio curve (y=0.01x3.27 (R2=0.84)) was calculated, where the relative condition factor between parasitized and non-

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parasitized individuals was calculated, whose values did not differ statistically (p=1) by the Mann Whitney 'U' test. In this sense, it is important to properly manage and maintain the sanitary status of tambaqui production in the southern region of Roraima.

Keywords: Monogenea, Myxobolus, Procamallanus, Aquicultura.

INTRODUCTION

Fish farming has the potential to establish itself as a great example of sustainability in the Amazon, with a focus on the production of tambaqui, an Amazonian fish whose meat continues to appreciate in the domestic and foreign markets (Oliveira et al., 2023).

The Western Amazon has a great diversity of ichthyofauna, yet only 36 species are commercialized, of which only eighteen have significant production, among which the tambaqui, *Colossoma macropomum*, stands out for its acceptability and rusticity (BRASIL, 2003). Tambaqui is the main species of fish farming (90% of all production) and also the most consumed in Roraima, justified by its acceptability by consumers and mastery of technology in reproduction (EMBRAPA, 2009).

With the growth of production, the appearance of diseases is favored, as well as in any large concentration of animals, this factor is more explicit in super intensive fish farms where the animals are concentrated in high density. Due to the favoring of the development of several species of pathogens and the high stocking density and the problems caused by inadequate management and contamination of water, transmission and consequent infection/infestation becomes facilitated (Pavanelli & Takemoto, 2008; Onaka, 2009).

Naturally, fish live in equilibrium with parasites, but this balance can be disrupted by environmental disturbances, such as reduced dissolved oxygen, increased toxic ammonia and carbon dioxide, and these phenomena are more conducive in intensive fish farms where the reduction in water quality is associated with a poor feeding program (Kubiza & Kubiza, 2013). The excessive growth of the quantity and varieties of pathogens combined with poor water quality, contributes to the appearance of diseases and parasitosis, resulting in losses to the producer.

According to Jerônimo et al. (2012) and Luque (2004), the main fish parasites in fish farming are protozoa (*Ichthyophthirius multifilliis*, *Piscinodinium pilulare* and *Tricondina* spp), Myxoporides (*Myxobolus* sp, *Henneguya* ssp and *Kudoa* ssp), Monogenoids (main families Dactylogyridae and Gyrodactylidae), Digenetic (main families Diplostomidae and Clinostomidae), Cestodes (main families Diplostomidae and Clinostomidae), Cestodes (main families Parasites belong to the order Proteocephalidea), Nematodes (Species with zoonotic importance, Eustrongylides), Acanthocephalus and Crustaceans (*Argulus* sp*, Dolops* sp*, Perulernaea* sp *and Lernaea* sp), and most of these have the integument and gills as a preference for their fixation.

Different groups of parasites have been recorded in tambaqui from Brazilian fish farms, the main groups being: protozoa, myxoporids, crustaceans and helminths (Eiras et al., 2006; Valladão et al., 2014; Jerônimo et al., 2017).

Thus, the objective of this work was to identify and quantify parasites present in gills, intestine and tambaqui muscle from five fish farms in the municipality of Rorainópolis-Roraima-

Braisl and to correlate the results with the biometric variables, weight and length, as well as to estimate parasite prevalence and abundance.

METHODOLOGY

A total of 25 specimens of tambaqui (*Colossoma macropomum*) were acquired from five fish farms in the municipality of Rorainópolis-RR, with five specimens per farm. In each property, a semi-structured, complementary questionnaire of productive activity was carried out. The properties were named P1, P2, P3, P4 and P5. The aforementioned activities were carried out in April 2017.

After capture, each fish was immediately stunned/sacrificed through the sectioning of the marrow performed in the area of the first vertebra of the spine and placed in a plastic bag with the identification of a small pvc plate and accommodated in an isothermal box with ice.

The specimens were transported to the Federal Institute of Roraima- *Novo Paraiso Campus* (IFRR-CNP), where they were frozen and stored at -18º C for further analysis. The fish were transported in an isothermal box to the Laboratory of Applied Zoology (LABZOA) of the Federal University of Roraima (UFRR) - Cauamé Campus, where the necropsy was performed. A fish necropsy script for parasitology studies used at LABZOA was followed. After thawing at room temperature, the specimens were weighed (Economic® electronic scale, accuracy ± 5g) and the standard and total length were verified, and then the integument was scraped in the anteroposterior direction with the aid of a glass slide, and the scraping was collected in a beaker and analyzed under a stereomicroscope (Jerônimo, 2012). The muscles were cut to verify the presence of parasites. The cuts were made in the anteroposterior direction of the dorsal muscles. When a parasite was detected, it was immediately placed between the slide and coverslip, with a small drop of water for verification under an optical microscope (Eiras, 2006). The gills were individualized, placed in a glass container, 1:4000 formaldehyde was added and shaken for 10 seconds, leaving this material to be evaluated at the end of the necropsy (Eiras, 2006). To verify the internal organs, evisceration was performed, and the stomach, intestine, liver and swim bladder were individualized in Petri dishes and visualized under stereomicroscope. When nematodes were found, they were preserved in 70% alcohol (Eiras, 2006).

The identification for Monogenea and *Myxobolus* sp. was carried out according to Thatcher (2006), for nematode according to Moravec (1998).

The indices that estimated the size of the population were prevalence, mean intensity and abundance (Bush et al., 1997). Calculated as follows: Prevalence = number of hosts infected with 1 or more individuals of a parasite species (or taxonomic group) divided by the number of hosts examined multiplied by 100. Average intensity = total number of parasites of a given species divided by the number of hosts infected by that given species. Average abundance = is the total number of individuals of a parasite species in a sample of a host species divided by the total number of host species examined (infected and uninfected).

For the relative condition factor (Kn), standard length (Ls) and total weight (Wt) values of each host were adjusted to the Wt/Ls ratio curve (Wt = a.Lsb) and the values of the regression coefficients a and b were estimated. The values of a and b were used in the estimates of the expected weight values (We), using the equation: We = a.Lsb . The relative condition factor (Kn) was then calculated, which corresponds to the quotient between observed weight and expected weight for a given length (Kn = Wt/We) (Le Cren, 1951).

Statistical data were processed and graphs were generated with the free software BioEstat.5.3 and LibreOffice.calc. 24.2.3, respectively.

RESULTS AND DISCUSSION

The questionnaires of productive activity in the fish farms evaluated are presented in Table 1. This analysis is important for data comparison, since the presence of parasites may be associated with such variables.

Table 1 - Summary of the questionnaire on the productive activity of fish farms in the municipality of Rorainópolis.

Property	No. of nurseries	Origin of the water used	Sample nursery area (m2)	Sample nursery biomass (kg)	Age of the sample nursery	Date of fishing	Number of meals per day
Page 1	03	Igarapé	1500	750	02 years	11/2016	1
Page 2	06	Igarapé	2250	2000	02 years	03/2015	Sporadic
Page 3	03	Nonexistent	300	25	16 years	01/2017	Sporadic
Page 4	05	Nonexistent	2000	1000	03 years	10/2016	1
Page 5	03	nonexistent	2000	1200	02 years	11/2016	2

Source: Authors

The origin of the water is a relevant factor, as the producer may do prophylaxis in the tanks, but his water source is contaminated. Knowing the size of the pond area is necessary to correlate with the biomass present in the tank, and once this biomass is above the recommended level, there may be a drop in the immunity of the fish and they may be affected by diseases. The age of the nursery can be taken into account as to the emergence of diseases due to the substrate. The parasitological indices (prevalence, abundance and intensity) are described in Table 2.

Tabela 2- Índices parasitológicos (Mon.=Monogenea; Nen. = Nematóide; Myx. =Myxobolus;)

Prope rty	Prevalence (%)			Medium abundance			Medium Intensity		
	Itself	Nen	Myx	Itself	Nen	Myx	Itself	Nen	Myx
Page 1	100	0	0	76	0	0	76	0	0
Page 2	40	40	20	8.2	1.2	0.2	19	3	1
Page 3	80	0	0	25.4	0	0	31.75	0	0
Page 4	100	0	0	184	0	0	184	0	0
Page 5	0	0	0	0	0	0	0	0	0

Source: Authors

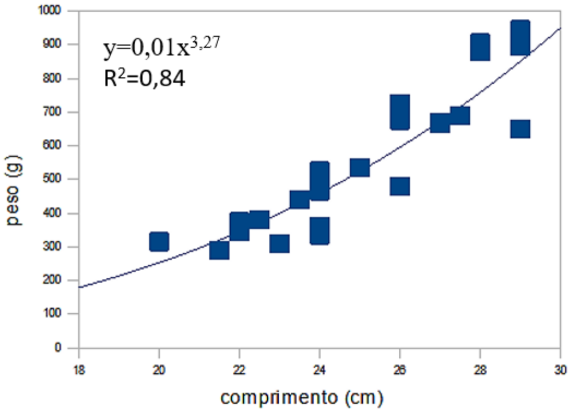
There was a 100% prevalence in P1 and P4 fish farms. Several factors can contribute to this occurring, including the disinfection of the tanks after each production cycle. Among the hypotheses of high prevalence, there is that the fingerlings may come infested from the places where they are purchased, because the cultivation sites did not have water renewal. If the presence of monogenea persists, breeding may become unfeasible due to these places not having water renewal. According to Martins (2004), monogenea are the first causes of mortality in fish farms in Brazil, so attention should be paid to the host-parasite interaction between tambaqui and monogenea. Santos et al. (2013) found an average intensity of 73.2 and an average abundance of 69.5 of monogena in tambaqui gills in a cage fish farm in the Matapi River, municipality of Santana, state of Amapá.

In the P5 property there was no record of parasites, and in this one there was feeding of the fish daily, twice a day and the water was being fertilized with cattle manure.

The parasite family found in all specimens evaluated was Girodactylids. Fujimoto et al. (2015) listed the main risk factors (Odds ratio-OR analysis) for tambaqui parasitism in the Lower São Francisco region, which are: Water scarcity (OR: 13.3, i.e. 13.3 times more risk of having monogenea), intercropping with other animals (OR: 17.9), communication of water from one nursery to another (OR: 5.64), nurseries without fertilization (OR:4.3), nurseries that did not disinfect and scrape the bottom (OR:70.0), high stocking density (>1kg/m3) (OR:4) and biometrics performed without adequate technique (OR:17.8). Table 2 shows that the biomass of the tanks of the two farms was adequate, as well as the age of the vivieros that are not old, so these factors may not be the cause of this high prevalence of monogeneous in these two farms. According to Paraguassú (2006), due to their monoxene life cycle, monogenetic fish reproduce very quickly and the confinement of fish of the same species can make these parasites a major problem in fish farming and losses due to parasitic diseases represent, in many cases, the determining factor for the success of the activity.

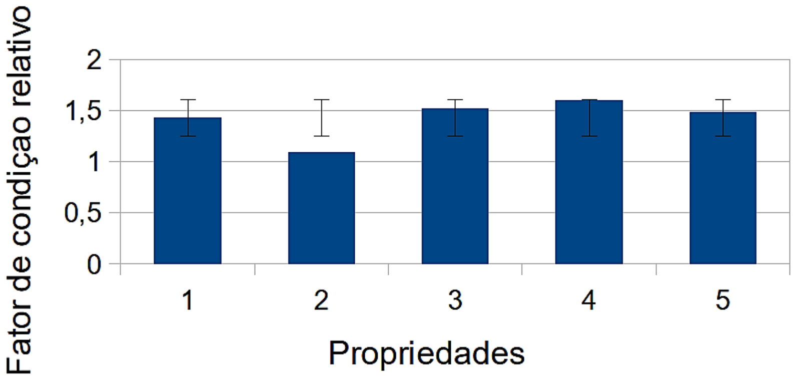
The weight-length ratio can be an indicator of zootechnical performance for fish, and under normal conditions it is expected that this correlation is high, otherwise some abnormality will be occurring and must be verified, one of them being the presence of parasites. Figure 1 shows the relationship between standard length and total weight.

Figure 1 - Standard length x total weight ratio for *Colossoma macropomum* in five fish farms in Rorainópolis-RR.



Due to the strong correlation presented, it is suggested that the fish did not have their performance affected. The relative condition factor (kn) is shown in Figure 2. There was no statistical difference (p=1) by the Mann-Whitney 'u' test between individuals parasitized and not parasitized by Monogenea. Similar results, where there were no statistical differences between parasitized and non-parasitized individuals, were also found by Yamada et al. (2008) and Zanolo et al. (2009). It is expected that parasitism brings imbalance in weight gain and growth, but the rusticity of the tambaqui may be responsible for the ability to resist these degrees of infestations.

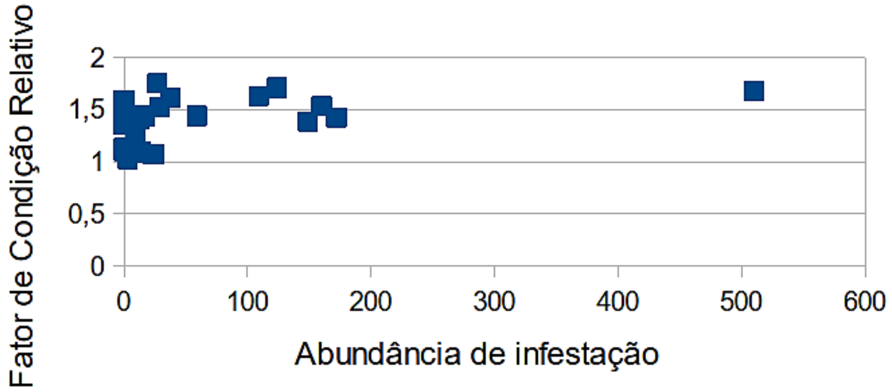
Figure 2 - Relative condition factor for the five properties analyzed.



Source: Authors

Graphically, the P2 farm presented a Kn below the others, probably due to the lack of adequate feeding of the animals, and even so they are above the expected weight curve, on average. In this property, the average weight was 0.427 kg, and age was two years, and at this age the fish should be more than 3.0 kg, but the standard length was proportional to this weight. Figure 3 shows the correlation between relative condition factor and abundance of Monogenea infestation.

Figure 3 - Correlation between relative condition factor and abundance of Monogenea infestation.



Source: Authors

It can be observed that the parasites may not be causing damage to the hosts, this indicates that the hosts are growing and gaining weight, or paralyzed growth independent of parasitism, and/or have a low pathogenicity, which did not influence the relative condition factor, as would happen if they were highly pathogenic parasites (Poulin, 1996).

Two genera of nematodes were found in the P2 estate, one of the genus *Klossinemella* sp. and another individual of the genus *Procamalanus* (*Spirocamallanus* sp.).

Fischer et al. (2003) studying the parasitic fauna in tambaqui and its potential as a biological indicator in the middle Solimões River (Amazonas) and in the lower Amazon River (Pará), virified the presence of *Procamallanus* sp. and *Spirocamallanus* sp., and none of these species showed statistical differences between the mean intensities compared by Student's 't' test. According to Pavanelli et al. (2013), species of *Procamallanus* sp. can cause lesions and inflammation in the intestinal mucosa due to fixation and feeding on the blood and can cause primary anemia, and in intentional infections in small fish there may be low growth rates and intestinal obstruction. According to Costa et al. (1968), species of the genus *Klossinemella* sp. have a monoxene evolutionary cycle, where reproduction can occur in the lumen of the host's intestine, and larvae may be released later. Infection by *klossinemella* sp. occurs through the eleimnation of larvae and adults through feces, which makes contamination very easy in a confined aquatic environment (Costa et al., 1968).

Only in the two farms were found spores of *Myxobolus* sp, in one specimen only, in the dorsal muscle. Feltran et al. (2017) made the first record for *Myxobolus* sp. found in *Colossoma macropomum* in the municipality of Cantá-RR, being the first record in Roraima. Maciel et al. (2011) have already found spores of *Myxobulus colossomatis* in the circulating blood of *Colossoma macropomum*, in the municipality of Manaus-AM. Capodifoglio et al. (2016) observed the presence of *Myxobolus* sp. in the operculum serosa of 15 tambaqui from the Amazon River basin. Senteno et al. (2004) found *Myxobolus* sp. in *Colossoma macropomum*, when they analyzed the ectoparasitophage associated with *Colosssoma macropomum* and the hybrid *Colossoma macropomum* x *Piaractus brachipomus*, cultivated in the State of Deuta Amacuro-Venezuela.

These records show the presence of this myxozoan in the State of Roraima and in the neighboring country (Venezuela), reporting the concern in preventive work and sanitary surveillance so that this parasite does not cause future damage to fish farms in the northern region.

CONCLUSION

It was possible to observe the presence of parasites in four of the five fish farms evaluated, most of which were of the Monogenea class, and the highest diversity of parasites (Monogenea, Nematoda, Myxosporea) was found in the P2 farm. It was observed a common practice of extensive fish farming with a low degree of technology, factors that can favor the appearance of diseases. The parasites that had the highest prevalence rates were the Monogenea, reaching 100% in farms one and four.

These parasites are already widely spread in rivers and fish farms throughout Brazil, and in the case evaluated they showed low pathogenicity demonstrated by the relative condition factor. In the P5 property there was no record of parasites.

This study listed some parasites in fish farms in the municipality of Rorainópolis-Roraima-Brazil, reporting the concern to maintain the sanitary stataus of tambaqui production in this region, requiring more studies, at a higher level of taxonomic depth.

The knowledge of the tambaqui parasitofauna enables decision-making in fish enterprises, and in prophylaxis and therapeutic programs, as well as in State and National health defense standards.

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