

## PARASITE FAUNA OF TAMBAQUI REARED IN NET-CAGES AT TWO STOCKING DENSITIES

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### ABSTRACT

This study investigated the parasite fauna in *Colossoma macropomum* reared at two stocking densities in net-cages of a fish farm in the Matapi River, State of Amapá. Before stocking fish in the net-cages for fattening, fish were examined for parasites and also at the end of 90 and 180 days at densities of 50 and 100 fish m<sup>-3</sup>, respectively. All fish were parasitized by one or more species such as *Ichthyophthirius multifiliis*, *Piscinoodinium pillulare*, *Anacanthorus spathulatus*, *Mymarothecium boegeri*, *Notozothecium janauachensis*, *Procamallanus (Spirocamallanus) inopinatus* and Acarina gen. sp., but the dominance was of *I. multifiliis*. Parasites presented aggregated dispersion pattern, and there were no differences in the fish relative condition factor between the densities used. There was positive correlation of *I. multifiliis* abundance and monogenean abundance with length and weight of the hosts. Before fish stocking, the mean intensity and mean abundance of *I. multifiliis*, *A. spathulatus* and *M. boegeri* were lowest when comparing fish at densities of 50 and 100 fish m<sup>-3</sup>, which were similar to each other. The mean intensity and mean abundance of *N. janauachensis* was highest in the density of 50 fish m<sup>-3</sup> when comparing the fish before stocking and at a density of 100 fish m<sup>-3</sup>. The mean abundance of *P. (S.) inopinatus* was highest in fish kept at 100 fish m<sup>-3</sup> when compared to fish before stocking and 50 fish m<sup>-3</sup>. However, *P. pillulare* and mites occurred only in density of 50 fish m<sup>-3</sup>. There was a predominance of ectoparasites and few endoparasites, as expected. Therefore, it is necessary the constant monitoring of the parasites, to better implement control strategies aiming to avoid the occurrence of diseases.

**Key words:** aggregation; Amazon; fish farming; parasites.

### FAUNA PARASITÁRIA DE TAMBAQUIS CULTIVADOS EM TANQUES-REDE E EM DUAS DENSIDADES DE ESTOCAGEM

### RESUMO

Este estudo investigou os parasitos de *Colossoma macropomum* cultivados em duas diferentes densidades de estocagem em tanques-rede instalados no Rio Matapi, estado do Amapá. Antes da estocagem dos peixes nos tanques-rede para engorda, peixes foram examinados para parasitos e também ao final de 90 e 180 dias nas densidades 50 e 100 peixes m<sup>-3</sup>, respectivamente. Todos os peixes foram parasitados por uma ou mais espécies entre *Ichthyophthirius multifiliis*, *Piscinoodinium pillulare*, *Anacanthorus spathulatus*, *Mymarothecium boegeri*, *Notozothecium janauachensis*, *Procamallanus (Spirocamallanus) inopinatus* e Acarina gen. sp., mas a dominância foi de *I. multifiliis*. Os parasitos apresentaram padrão de dispersão agregada e não houve diferenças no fator de condição relativa dos peixes entre as densidades usadas. Houve correlação positiva da abundância de *I. multifiliis* e abundância de monogêneas com o comprimento e peso dos hospedeiros. Antes da estocagem dos peixes, foi menor intensidade média e abundância média de *I. multifiliis*, *A. spathulatus*, *M. boegeri* quando comparados os peixes das densidades de 50 e 100 peixes m<sup>-3</sup>, os quais foram similares entre si. A intensidade média e abundância média de *N. janauachensis* foi maior na densidade de 50 peixes m<sup>-3</sup> quando comparando os peixes antes da estocagem e 100 peixes m<sup>-3</sup>. A abundância média de *P. (S.) inopinatus* foi maior em peixes mantidos em 100 peixes m<sup>-3</sup>, quando comparados os peixes antes da estocagem e 50 peixes m<sup>-3</sup>. Porém, *P. pillulare* e ácaros ocorreram somente na densidade de 50 peixes m<sup>-3</sup>. Houve uma predominância de ectoparasitos e poucos endoparasitos, como esperado. Portanto, é necessário o monitoramento constante dos parasitos, para melhor implementar estratégias de controle que visem evitar a ocorrência de doenças.

**Palavras-chave:** agregação; Amazônia; piscicultura; parasitos.

### INTRODUCTION

Population growth and the consequent increased demand for high quality food have contributed to the global expansion of aquaculture in recent years. Aquaculture is the fastest growing productive sector and has great social and economic relevance because

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it is a great producer of animal protein for the world population (Ritter et al., 2014; FAO, 2018). In Brazil, the production of fish farming was 485,253.7 tons in 2017 (IBGE, 2018) and this includes the *Colossoma macropomum* Cuvier, 1818 (tambaqui).

Brazil is a country with great wealth of water resources, with emphasis for the Amazon basin that accounts for 20% of all freshwater on the planet (Roubach et al., 2003), presenting therefore enormous potential for expansion of fish farming, mainly for rearing in net-cages. Among the advantages of fish cultivation in net-cages are the reduction of physical space required for the activity, greater control and efficient utilization of feed, lower production cost, higher productivity without area increase and lower initial investment (Silva and Fujimoto, 2015). Thus, net-cage fish farming has shown to be a very promising intensive production modality in the country, and has been booming, mainly because it is aimed at sustainable use of the environment (Beveridge, 1996; Chagas et al., 2003; Ayroza et al., 2006; Chagas et al., 2007; Silva and Fujimoto, 2015). However, fish rearing in net-cages uses high densities of population stock that generally leads to increased levels of parasitic infections, mainly those parasites with direct life cycle (Mladineo, 2006; Nowak, 2007; Merella et al., 2009; Fernandez-Jover et al., 2010), due to stress.

Studies on the effects of stocking densities of fish in net cages are more frequent for marine fish (Nowak, 2007; Merella et al., 2009; Fernandez-Jover et al., 2010). However, in *C. macropomum* reared in net-cages in a varzea lake in the State of Amazonas (Brazil), although the intensity of monogeneans increased in the densities of 50 and 75 fish m<sup>-3</sup> after 60 days of cultivation, there was no pattern of the effects of stocking density on the parasitism up to 300 days of cultivation (Varella et al., 2003).

In Brazil, tambaqui is a species of native fish that stands out in the national production. Its cultivation has significant production mainly in the North and Northeast regions (Gomes et al., 2010; Santos et al., 2013), due to its zootechnical characteristics. The cultivation of tambaqui in net-cages can be a viable alternative for the production of this fish in different environments, mainly in the Amazon region. However, it is important to know the impact of the population density of tambaqui reared in net-cages on the levels of parasitism of fish. Thus, the aim of this study was to evaluate the parasite fauna of tambaqui grown at two stocking densities in net-cages in the Matapi River, State of Amapá, Brazil.

## MATERIAL AND METHODS

### Cultivation and study area

This study was conducted in a net-cage (11 m<sup>-3</sup>) fish farm located in the Matapi River (0°03'27.72"N and 51°14'10.54"W), municipality of Santana, State of Amapá (Brazil). Initially, *C. macropomum* fingerlings with weight of  $\pm 3$  g were stored during 30 days in net-cages and fed commercial rations containing 40% crude protein (CP). Subsequently, during the fattening phase, fish were transferred and stocked at densities of 50 and 100 fish

m<sup>-3</sup> using three replicates per treatment, and fed three times daily with commercial ration containing 36% CP for 30 days and then fed with 32 % CP up to 90 days of cultivation. At the end of this period, fish were given commercial ration containing 28% CP up to 180 days of cultivation.

### Collection and analysis of parasites

Fish samples were collected at the initial stage of rearing in the net cages (before stocking), when 30 fish (14.4  $\pm$  2.1 and 55.1  $\pm$  24.4) were examined; in the fattening phase with 30 fish examined in the density of 50 fish m<sup>-3</sup> (28.8  $\pm$  2.0 cm and 600.7  $\pm$  137.6 g) and with 30 fish examined in the density of 100 fish m<sup>-3</sup> (28.1  $\pm$  1.8 cm and 602.8  $\pm$  145.8 g). All the fish were transported in containers with ice, to the Aquaculture and Fishery Laboratory of Embrapa Amapá, Macapá (State of Amapá), for parasitological analysis. The Commission of Ethics in the Use of Animals of Embrapa Amapá (Protocol No 007/2016-CEUA/CPAFAP) approved this study.

The fishes were weighed (g) and measured in total length (cm) and then euthanized and necropsied for parasitological analysis. The mouth, gills, opercula and fins were examined for the presence of ectoparasites, and the viscera and gastrointestinal tract, for the presence of endoparasites. The collection, fixation, counting, preparation and staining of parasites for identification followed previous recommendations of Eiras et al. (2006). The ecological terms used followed previous recommendations of Bush et al. (1997).

### Data analyses

All data were evaluated for the assumptions of normality and homoscedasticity using Shapiro-Wilk and Bartlett tests, respectively. Since data had no normal distribution, these were analyzed by Kruskal-Wallis, followed by the Dunn test to compare the medians of abundance of parasites. The dispersion index (ID) and discrepancy index (D) were calculated using the software Quantitative Parasitology 3.0, in order to detect the distribution pattern of parasite infracommunities (Rózsa et al., 2000), for species with prevalence >10%. The significance of ID, for each infracommunity, was tested using the *d*-statistics (Ludwig and Reynolds, 1988). Spearman correlation coefficient (*r<sub>s</sub>*) was applied to determine possible correlations of parasite abundance with length and weight of hosts. The relative condition factor (Kn) was determined according to Le Cren (1951) and was compared using Kruskal-Wallis medians followed by the Dunn test for comparison between medians (Zar, 2010).

## RESULTS

The prevalence of *Ichthyophthirius multifiliis* Fouquet 1876, (Protozoa), *Anacanthorus spathulatus* Kritsky, Thatcher & Kayton, 1979, *Mymarothecium boegeri* Cohen & Kohn, 2005 and *Notozothecium janauachensis* Belmont-Jégu, Domingues

& Martins, 2004 (Monogenea) was high in all treatments. Before fish stocking, the mean intensity and mean abundance of *I. multifiliis*, *A. spathulatus* and *M. boegeri* were lower than in fish kept at a density of 50 and 100 fish m<sup>-3</sup>, which were similar to each other. The mean intensity and mean abundance of *N. janauachensis* were higher than in fish kept at a density of 50 fish m<sup>-3</sup> when compared to fish kept at a density of 100 fish m<sup>-3</sup>. The mean abundance of *Procamallanus (Spirocamallanus) inopinatus* Travassos, Artigas & Pereira, 1928 (Nematoda) was higher than in fish kept at a density of 100 fish m<sup>-3</sup>, when

compared to fish before stocking and fish kept at a density of 50 fish m<sup>-3</sup>. *Piscinodinium pillulare* (Schäperclaus, 1954) Lom, 1981 (Protozoa) and Acarina gen. sp. occurred only in fish kept at a density of 50 fish m<sup>-3</sup> (Table 1).

All fish examined were parasitized by one or more species. A total of 101,291 parasites were collected, but *I. multifiliis* was the dominant species (Table 2). The parasites found presented an aggregated dispersion pattern (Table 3), a common pattern for freshwater fish.

**Table 1.** Parasites of *Colossoma macropomum* reared in net-cages at two stocking densities.

Species of parasites	Before stocking			Density of 50 fish m <sup>-3</sup>			Density of 100 fish m <sup>-3</sup>		
	P (%)	MI	MA ± SD	P (%)	MI	MA ± SD	P (%)	MI	MA ± SD
<i>Ichthyophthirius multifiliis</i>	100	315.8±652.4 <sup>a</sup>	315.83±652.4 <sup>a</sup>	100	1487.3±765.4 <sup>b</sup>	1487.3±765.4 <sup>b</sup>	100	1340.7±1247.9 <sup>b</sup>	1340.7±1247.9 <sup>b</sup>
<i>Piscinodinium pillulare</i>	0	0	0	16.7	298.8±253.7	49.8±147.3	0	0	0
<i>Anacanthorus spathulatus</i>	60.0	5.9±5.4 <sup>a</sup>	3.53±5.2 <sup>a</sup>	93.3	43.4±38.0 <sup>b</sup>	40.5±38.3 <sup>b</sup>	100	51.3±45.9 <sup>b</sup>	51.3±45.9 <sup>b</sup>
<i>Notozothecium janauachensis</i>	3.3	1.0±0 <sup>a</sup>	0.03± 0.2 <sup>a</sup>	96.7	32.0±26.3 <sup>b</sup>	30.9±26.4 <sup>b</sup>	63.3	19.6±27.8 <sup>a</sup>	12.4±23.9 <sup>c</sup>
<i>Mymarothecium boegeri</i>	10.0	1.7±0.6 <sup>a</sup>	0.2±0.5 <sup>a</sup>	100	26.3±28.5 <sup>b</sup>	26.3±28.5 <sup>b</sup>	93.3	17.2±16.0 <sup>b</sup>	16.1±16.0 <sup>b</sup>
<i>Procamallanus (S.) inopinatus</i>	16.7	1.0±0.5 <sup>a</sup>	0.2±0.4 <sup>a</sup>	16.7	1.2±0.4 <sup>a</sup>	0.2±0.5 <sup>a</sup>	53.3	1.7±1.1 <sup>a</sup>	0.9±1.2 <sup>b</sup>
Acarina gen. sp.	0	0	0	13.3	1.3±0.5	0.2±0.5	0	0	0

Values followed by different letters indicate significant differences between treatments. P: Prevalence, MI: Mean intensity, MA: Mean abundance, SD: Standard deviation.

**Table 2.** Parasitological indices in *Colossoma macropomum* reared in net-cages.

Species of parasites	P (%)	MI	MA ± SD	FD (%)	TNP
<i>Ichthyophthirius multifiliis</i>	100	1048.0	1048.0 ± 1054.3	0.93	94,317
<i>Piscinodinium pillulare</i>	5.6	288.8	16.6 ± 87.3	0.01	1494
<i>Anacanthorus spathulatus</i>	84.4	37.6	31.8 ± 40.0	0.03	2860
<i>Mymarothecium boegeri</i>	67.8	20.9	14.2 ± 21.6	0.01	1276
<i>Notozothecium janauachensis</i>	54.4	26.6	14.5 ± 24.0	0.01	1301
<i>Procamallanus (S.) inopinatus</i>	28.9	1.5	0.4 ± 0.8	-	38
Acarina gen. sp.	4.4	1.3	0.1 ± 0.3	-	5

P: Prevalence, MI: Mean intensity, MA: Mean abundance, SD: Standard deviation, TNP: Total number of parasites, FD: Frequency of dominance

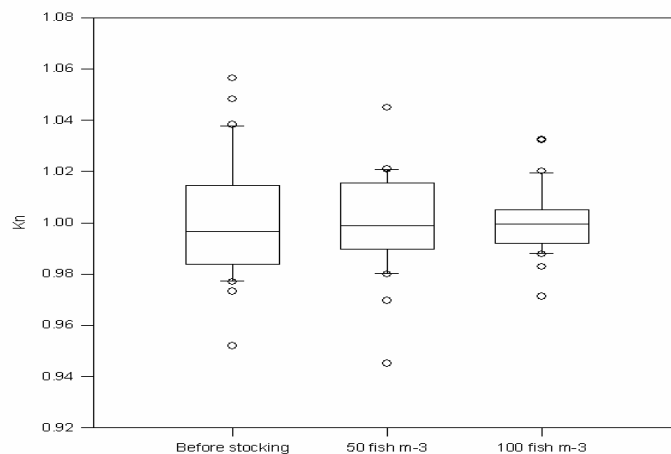
**Table 3.** Index of dispersion (ID), d-statistic and discrepancy index (D) for the parasite infracommunities of *Colossoma macropomum* reared in net-cages.

Species of parasites	ID	d	D	Type of dispersion
<i>Ichthyophthirius multifiliis</i>	3.272	16.3	0.310	Aggregated
<i>Anacanthorus spathulatus</i>	6.206	25.4	0.452	Aggregated
<i>Notozothecium janauachensis</i>	5.648	23.9	0.613	Aggregated
<i>Mymarothecium boegeri</i>	5.208	22.6	0.540	Aggregated
<i>Procamallanus (S.) inopinatus</i>	1.337	7.6	0.765	Aggregated

There was a positive correlation of *I. multifiliis* abundance with the length ( $rs = 0.59$ ,  $p = 0.001$ ) and weight of hosts ( $rs = 0.52$ ,  $p = 0.001$ ). The monogeneans abundance presented a positive correlation with the length ( $rs = 0.692$ ,  $p = 0.0001$ ) and weight of hosts ( $rs = 0.70$ ,  $p = 0.0001$ ). However, no correlation of *P. pillulare* abundance ( $rs = 0.06$ ,  $p = 0.79$ ) and *P. (S.)*

*inopinatus* ( $rs = 0.23$ ,  $p = 0.06$ ) with the length of hosts was detected, as well as between *P. pillulare* abundance ( $rs = 0.11$ ,  $p = 0.54$ ) and *P. (S.) inopinatus* abundance ( $rs = 0.19$ ,  $p = 0.07$ ) with the weight of hosts.

The relative condition factor (Kn) did not differ ( $H = 3.77$ ,  $p = 0.29$ ) between the stocking densities (Figure 1).



**Figure 1.** Relative condition factor (Kn) of *Colossoma macropomum* reared in net-cages (Box plots represent medians, interquartile ranges, minimum–maximum ranges and outliers). Similar values according to the Dunn test ( $p > 0.05$ ).

## DISCUSSION

Fish intensive production are threatened by diseases caused by monogeneans and *I. multifiliis*, resulting in significant economic losses (Fernandez-Jover et al., 2010; Santos et al., 2013; Tavares-Dias and Martins, 2017). Monogeneans are parasites with a direct and short life cycle, whose transmission facilitates in farmed fish (Fernandez-Jover et al., 2010; Morales-Serna et al., 2018). *Ichthyophthirius multifiliis* is a ciliated protozoan that causes a great problem to farmed freshwater fish worldwide. This ciliate is often found on the tegument and gills of infected fish, and disease is highly contagious and can spread rapidly from one fish to another, due to its direct life cycle (Wei et al., 2013; Wang et al., 2019).

All individuals of *C. macropomum* analyzed in this study (55.1–602.8 g) were parasitized and parasite community was composed of *I. multifiliis*, *P. pillulare*, *A. spathulatus*, *M. boegeri*, *N. janauchensis*, *P. (S.) inopinatus* and mites; but the dominance was of *I. multifiliis*, and there was an aggregated dispersion of parasites found. This dominance of *I. multifiliis* is related to the small size of this protozoan. However, in *C. macropomum* (21.9–772.1 g) reared in net-cages installed in a floodplain lake in the State of Amazonas, the prevalence was 76.6% and the parasite community was composed of *Myxobolus* sp., *Henneguya* sp., *A. spathulatus*, *Linguadactyloides brinkmanni* Thatcher & Krytsky, 1983, *Neoechinorhynchus buttnerae* Golvan, 1956, *Gamidactylus jaraquensis* Thatcher & Boeger, 1984, *Ergasilus* sp. and mites; but with dominance of monogeneans (Varella et al., 2003). In *C. macropomum* (2163.5 ± 324.6 g) reared in net-cages in the Matapi River, the prevalence was 96.7% and parasite community was composed of *I. multifiliis*, *P. pillulare*,

*A. spathulatus*, *M. boegeri* and leeches; with dominance of *I. multifiliis* and *P. pillulare* (Santos et al., 2013). Therefore, these differences in species diversity are related to the environment, fish age and stocking density used.

The stocking densities of *C. macropomum* in net-cage fish farms should be used efficiently for good production and productivity. Thus, adequate stocking density for the rearing phase of this fish has been investigated, and low densities do not cause stress in *C. macropomum* (Chagas et al., 2003; Brandão et al., 2004; Gomes et al., 2006; Chagas et al., 2007; Silva and Fujimoto, 2015). For the initial rearing phase of *C. macropomum*, density of up to 300 fish m<sup>-3</sup> has been recommended and 20 fish m<sup>-3</sup> for the finishing phase (Silva and Fujimoto, 2015). High stocking densities of fish may result in high parasitic infection levels of monogenean (Fernandez-Jover et al., 2010) and protozoan species, which have horizontal transmission, and at high population densities, there is a greater approximation between the cultivated fish (Santos et al., 2013). Costa et al. (2019) reported high rates of monogeneans in *C. macropomum* reared at higher stocking densities in earth ponds. In *C. macropomum* of this study, *P. pillulare* and mites occurred only in fish kept at a density of 50 fish m<sup>-3</sup>. Also, although the abundance of monogeneans and *I. multifiliis* increased in the densities of 50 and 100 fish m<sup>-3</sup> fish, when compared to the initial rearing in net-cages (before stocking), only the abundance of *P. (S.) inopinatus* increased with increasing stocking density, because *N. janauchensis* had lowest abundance in this higher stocking density. This increase of *P. (S.) inopinatus* with stocking density was due to high ingestion of infective stages of this endoparasite in the intermediate hosts, which are crustacean species. Similarly, in *C. macropomum*



reared in net-cages in the State of Amazonas, the intensity of monogeneas increased in the densities of 50 and 75 fish m<sup>-3</sup> after 60 days when compared to stocking before net-cages (Varella et al., 2003). These results suggests a relative rusticity of *C. macropomum* in these stocking densities.

The moderate parasitism levels, at the different stocking densities of *C. macropomum*, did not influence the condition factor of the hosts. Similar results have been reported for *C. macropomum* also reared in net-cages (Santos et al., 2013). The size of the host population may or may not be related to its age, since the parasitic abundance may increase, decrease or not be affected by its size or age (Poulin and Leung, 2011; Baia et al., 2018), due to factors related to parasites or host sampling. In this study, with *C. macropomum* at different ages, the abundance of monogeneas and *I. multifiliis* increased according to growth in weight and length of hosts. Santos et al. (2013) also reported increase in abundance of *P. pillulare* with increasing length of *C. macropomum* reared in net-cages.

## CONCLUSION

In *C. macropomum* reared in net-cages, there was 100% prevalence of parasites, with predominance of ectoparasites and presence of few endoparasites, as expected. The results showed little changes in the intensity and abundance of parasites influenced by the stocking density of this fish. The data and distribution pattern indicate the need for constant monitoring of the parasites, in order to better implement control that aim to avoid the occurrence of diseases in this fish.

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