Parasitic infections in tambaqui from eight fish farms in Northern Brazil

[*Infecções parasitárias em tambaqui de oito psiculturas do Norte do Brasil*]

M.K.R. *Dias*[1], L.R. *Neves*[2], R.G.B. *Marinho*[1], M. *Tavares-Dias*[1,2]*

[1]Aluno de pós-graduação – Universidade Federal do Amapá –Unifap– Macapá, AP
[2]Embrapa Amapá – Macapá, AP

ABSTRACT

The aim of this study was to evaluate the parasites and parasite-host relationship in *Colossoma macropomum* from eight fish farms in Amapá State, Northern Brazil. A total of 12,242,559 parasites were recovered from hosts in the eight fish farms, and 87.4% of fish were infected by one or more parasite species, such as *Ichthyophthirius multifiliis*, *Piscinoodinium pillulare*, *Trichodina* sp. and *Tetrahymena* sp. (Protozoa), *Anacanthorus spathulatus*, *Linguadactyloides brinkmanni*, *Mymarothecium boegeri* and *Notozothecium janauachensis* (Monogenoidea), *Procamallanus* (*Spirocamallanus*) *inopinatus* (Nematoda) and *Perulernaea gamitanae* and *Braga patagonica* (Crustacea). *Ichthyophthirius multifiliis* presented dominance, but the greatest levels of parasitism were caused by *I. multifiliis*, *P. pillulare*, monogenoideans species and *P. gamitanae*, and all these parasites had an aggregate dispersion. The eight fish farms have differences in the parasitic infection levels, management and water quality, which are further discussed here.

Keywords: *Colossoma macropomum*, metazoans, parasites, protozoans, sanity

RESUMO

O presente estudo avaliou as infecções parasitárias e a relação parasito-hospedeiro em Colossoma macropomum *de oito psiculturas do estado do Amapá, norte do Brasil. Foi coletado um total de 12.242.559,0 parasitos em hospedeiros das oito psiculturas, e 87,4% dos peixes estavam infectados por uma ou mais espécies de parasitos, tais como* Ichthyophthirius multifiliis, Piscinoodinium pillulare, Trichodina *sp. e* Tetrahymena *sp. (Protozoa),* Anacanthorus spathulatus, Linguadactyloides brinkmanni, Mymarothecium boegeri *e* Notozothecium janauachensis *(Monogenoidea),* Procamallanus (Spirocamallanus) inopinatus *(Nematoda) e* Perulernaea gamitanae *e* Braga patagonica *(Crustacea). A dominância foi de* I. multifiliis, *mas os maiores níveis de parasitismo foram causados por* I. multifiliis, P. pilulare, *espécies de monogenoideas e* P. gamitanae, *e todos esses parasitos tiveram uma dispersão agregada. Nas oito psiculturas houve diferenças quanto aos níveis de infecções parasitárias, manejo e qualidade da água, que foram aqui discutidas.*

Palavras-chaves: Colossoma macropomum, *metazoários, parasitos, protozoários, sanidade*

INTRODUCTION

Colossoma macropomum (tambaqui) is a native Serrasalmidae of Amazon with a omnivorous natural diet (Soares *et al.*, 2008), a characteristic that enables intensive rearing, since this species has substantial acceptance to meal (Chagas *et al.*, 2005; Santos *et al.*, 2013). Due to these features favoring the intensive rearing, the tambaqui is the largest native fish produced in Brazilian aquaculture, primarily in the Amazon. In 2011, the national production of tambaqui by the fishery was of 4234.9 tons while the production of aquaculture was 111, 084.1 tons (MPA, 2013). However, the perspective is of unceasing growth for the next years.

In Amazon, the tambaqui has been reared in ponds (Tavares-Dias *et al*., 2006; Delgado *et al*., 2011; Godoi *et al*., 2012) and net-cage (Morais *et al*., 2009; Santos *et al*.; 2013). Due to the increasing intensive culture difficulties associated with infections by ectoparasites have been intensified (Tavares-Dias *et al*., 2006; Morais *et al*., 2009; Delgado *et al*., 2011, Godoi *et al*., 2012; Santos *et al*., 2013). The situation is even more problematic when management is deficient and the low levels of dissolved oxygen in the water are inappropriate for the cultivation of the species (Morais *et al*., 2009; Santos *et al*., 2013). Parasites may inversely influence the diet and development of the cultivated hosts, reduce body conditions and fish quality, affecting the productivity and commerce. Therefore, it is crucial to identify the agents responsible for parasite infections in farmed fish and evaluate sanitary conditions to implement and/or improve prophylactic management used in fish farms (Tavares-Dias *et al*., 2006; Delgado *et al*., 2011) to efficiently interfere in the process.

The aim of this study was to identify tambaqui parasites in eight fish farms in the municipality of Macapá, Amapá State. Furthermore, we evaluate host-parasites relationship and sanitary conditions of the fish farms investigated.

MATERIAL AND METHODS

Ten specimens of *Colossoma macropomum* were collected in each of the eight fish farm investigated in the municipality of Macapá, Amapá State. Sampling took place every three months, from September 2009 to April 2011 when we examined every collected fish and performed parasitological analysis in every farm in with differential management characteristics (Table 1).

The mouth, opercula, gills and gastrointestinal tract of each necropsied fish was examined. The gills removed were fixed in formalin 5% and used to collect ectoparasites. The gastrointestinal tract was removed in order to detect endoparasites. Methods of sampling, fixing, preparation of parasites for identification and counting followed previous recommendations (Eiras *et al*., 2006).

The ecological terms adopted were those recommended by Rohde *et al*. (1995) and Bush et al. (1997). The variance-mean ratio of parasite abundance (index of dispersion) and Poulin discrepancy index (D) were computed using the software Quantitative Parasitology 3.0 and were employed to detect distribution patterns of the parasite infracommunity (Rózsa *et al*., 2000) in species with prevalence ≥10%. Index of dispersion (ID) significance was tested using the statistic *d* according to Ludwig and Reynolds (1988) for each infracommunity.

Throughout fish sampling and in each fish farm we determined pH with a digital pH meter (pH-100, YSI), temperature and dissolved oxygen levels with a digital oximeter (DO-200, YSI). The mean water temperature of *C. macropomum* ponds in eight fish farms was similar, while the pH was lower only in one of the fish farms. Low oxygen levels were similar in all fish farms, and remained under 3 mg/L.

Table 1. Mean values ± standard deviation of weight and length, geographic localization, stocking density and total parasite prevalence (P) in *Colosssoma macropomum* from eight fish farms in the State of Amapá (Brazil). EF: Examined fish; PF: parasitized fish; P: Prevalence

Fish farms	Geographic coordinates	Weight (g)	Length (cm)	Density (fish/m^2)	EF/PF	P (%)
P1	00°08'42.3"N-051°06'04.2"W	527.3 ± 279.42	29.7± 6.29	2.5	54/54	100
P2	0°0'21.7"S-051°07'23.1"W	514.1 ± 385.1	26.6 ± 9.52	2.0	59/58	98.3
P3	0°00'13.5"S-051°06'12.8"W	884.7 ± 676.73	33.1±13.78	2.8	68/56	82.8
P4	00°01'48.3"S-051°07'52.9"W	332.9 ± 265.98	22.6 ± 9.73	2.0	68/61	89.7
P5	0°04'15.4"N-051°02'16.9"W	303.8 ± 145.53	26.4 ± 4.5	2.8	70/65	92.9
P6	0°02'31.4"S-051°07'34.4"W	747.4 ± 485.09	32.8 ± 8.48	2.3	71/70	98.6
P7	00°00'13.2"S-051°06'10.9"W	397.3 ± 168.92	27.9 ± 4.8	0.8	52/51	98.1
P8	0°00'04.5"N-051°05'52.1"W	384.3 ± 219.35	37.2 ± 43.9	1.5	31/23	74.2

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Colossoma macropomum of the fish farms were parasitized by *Ichthyophthirius multifiliis*, *Piscinoodinium pillulare*, *Trichodina* sp., *Tetrahymena* sp. (Ciliophora), *Anacanthorus spathulatus*, *Linguadactyloides brinkmanni*, *Mymarothecium boegeri*, *Notozothecium janauachensis* (Dactylogyridae), *Procamallanus* (*Spirocamallanus*) *inopinatus* (Camallanidae), *Perulernaea gamitanae* (Lerneidae) and *Braga patagonica* (Cymothoidae). High levels of infection by *I. multifiliis* were similar in the eight fish farms. However, infection levels by *P. pillulare* were greater only in 25% of the fish farms (Table 2-3). Only *A. Spathulatus* was collected from this fish in all of the eight fish farms. *Mymarothecium boegeri* did not occur solely in two fish farms (P2 and P8), *Linguadactyloides brinkmanni* was collected in fish from four fish farms (P1, P3, P4 and P6) and *N. janauachensis* in fish from three fish farms (P1, P3 and P6). However, the prevalence of monogenoidean species was similar among fish farms from the State of Amapá, while the intensity of these parasites was greater in three establishments (Table 3).

Table 2. Protozoans of *Colosssoma macropomum* gills in eight fish farms in the State of Amapá (Brazil). EF: Examined fish, PF: Parasitized fish, P: Prevalence, MI: Mean intensity, MA: Mean abundance, TNP: Total number of parasites

	Ichthyophthirius multifiliis					*Piscinoodinium pillulare*				
Fish farms	EF/PF	P (%)	MI	MA	TNP	EF/PF	P (%)	MI	MA	TNP
P1	54/53	98.1	174,955	171,715	9,272,628.0	54/21	38.9	73,203	28,468	1,537,255.0
P2	58/48	82.8	272,541	225,551	13,081,986.0	58/41	70.7	86,533	61,170	3,547,866.0
P3	68/56	82.4	357,589	294,485	20,025,001.0	68/34	50.0	75,458	37,729	2,565,573.0
P4	68/61	89.7	236,790	212,414	14,444,183.0	68/37	54.4	62,116	33,798	2,298,295.0
P5	70/63	90.0	194,314	174,881	12,241,708.0	70/21	30.0	17,909	5,373	376,090.0
P6	71/67	94.4	373,837	352,775	25,047,053.0	71/37	52.1	54,725	28,518	2,024,816.0
P7	52/51	98.1	136,917	134,283	6,982,743.0	52/38	73.1	25,265	18,463	960,077.0
P8	31/22	71.0	323,778	229,778	7,123,124.0	31/13	41.9	35,132	14,733	456,722.0

Table 3. Helminth of *Colosssoma macropomum* in eight fish farms in the State of Amapá (Brazil). EF: Examined fish, PF: Parasitized fish, P: Prevalence, MI: Mean intensity, MA: Mean abundance, TNP: Total number of parasites

	Monogenoidea					*Procamallanus* (*S.*) *inopinatus*				
Fish farms	EF/PF	P (%)	MI	MA	TNP	EF/PF	P (%)	MI	MA	TNP
P1	54/54	100	570.5	570.5	30,805	54/0	0	0	0	0
P2	58/57	98.3	108.6	106.7	6190	58/1	1.7	1.0	0.02	1
P3	68/56	82.4	492.5	405.6	27,579	68/0	0	0	0	0
P4	68/61	89.7	372.1	333.8	22,698	68/3	4.4	2.0	0.09	6
P5	70/65	92.9	164.1	152.4	10,667	70/18	25.7	1.0	0.2	18
P6	71/70	98.6	804.3	793.0	56,303	71/3	4.2	2.7	0.1	8
P7	52/51	98.1	168.1	164.9	8575	52/3	5.8	2.7	0.1	8
P8	31/23	74.2	166.2	123.3	3822	31/1	3.2	1.0	0.03	1

Procamallanus (*S.*) *inopinatus* was collected in the intestine of *C. macropomum* in some fish farms, but the highest prevalence occurred in one of the fish farms (Table 3). *Trichodina* sp. (13.2% of prevalence and mean intensity of 1.023±823) and *Tetrahymena* sp. (19.1% of prevalence and mean intensity of 6.249±6.513) occurred only in one of the fish farms (P4).

Lerneids *P. gamitanae* were found parasitizing *C. macropomum* from only two fish farms (P4 and P6). In the P4 fish farm, only the mouth of fish with low levels of infection was parasitized (prevalence = 10.3% and mean intensity = 1.8±1.07). In the other fish farm (P6), mouth (prevalence = 64.3% and mean intensity = 4.9±3.2) and gills (prevalence = 47.1% and mean intensity = 2.2± 1.4) were parasitized. Tambaqui

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from one fish farm (P6) of Amapá State was parasitized by isopod *B. patagonica*, but with low prevalence (16.0%) and a 2.0 parasite/host intensity. *Braga patagonica* was found in the dorsal region of hosts, close to the dorsal fin, causing the loss of scales and skin, triggering a critical inflammatory process in the region.

There was a dominance of infection by *I. multifiliis* followed by *P. pillulare* in *C.*

macropomum from eight fish farms. Camalanids *P. (S.) inopinatus* were the only endoparasites collected of *C. macropomum* and they presented low levels of parasitic infection. Overall, parasitism caused by *P. gamitanae* and *B. patagonica* in *C. macropomum* was low (Table 4). The infracommunities of *I. multifiliis*, *P. pillulare*, monogenoideans species and *P. gamitanae* had an aggregate dispersion pattern (Table 5).

Table 4. *Colosssoma macropomum parasites* in fish farms in the State of Amapá (Brazil). EF: Examined fish, PF: Parasitized fish, MI: Mean Intensity, SD: Standard deviation, MA: Mean abundance TNP: Total Number of Parasites, RD: Relative dominance, SI: Site of infection

Parasites	SI	EF/PF	P (%)	MI ± SD	MA	TNP	RD
Ichthyophthirius multifiliis	Gills	473/424	89.6	255,232.1±246,808.4	228,792.0	108,218,426	0.88528
Piscinoodinium pillulare	Gills	473/245	51.8	56,191.0±62,266.7	29,105.0	13,766,694	0.11262
Tetrahymena sp.	Gills	473/13	2.7	6,249.0±1,457.6	172.0	81,233	0.00066
Trichodina sp.	Gills	473/147	1.9	1,023.0±176.2	19.5	9,203	0.00007
Monogenoidea species	Gills	473/424	89.6	393.0±652.1	352.3	166,639	0.00136
Procamallanus (S.) inopinatus	Intestine	473/19	4.0	2.2±0.7	0.09	42	-
Perulernaea gamitanae	Gills	473/33	6.9	2.2±0.7	0.2	73	-
Perulernaea gamitanae	Mouth	473/53	11.2	4.5±1.7	0.5	237	-
Braga patagonica	Body	473/12	2.5	1.0±0.2	0.03	12	-

Table 5. Dispersion Index (ID), discrepancy index (D) and statistic *d* for the parasitic infracommunities in *Colossoma macropomum* from fish farms in the State of Amapá (Brazil)

Parasites	ID	*d*	D
Ichthyophthirius multifiliis	16.125	92.67	0.400
Piscinoodinium pillulare	22.114	113.93	0.665
Monogenoidea species	13.323	81.45	0.446
Perulernaea gamitanae	5.717	42.755	0.924

DISCUSSION

In the eight fish farms in this study, the fish collected presented different weight and length, because they were examined in two rearing stages – fingerling and fattening. In these fish farms the stocking densities and management also varied, but the high parasitic prevalence was similar, due to similar management used and the reduced expertise in the activity demonstrated by fish farmers. However, the high parasitic prevalence (100%) in *C. macropomum* in this study was similar to prevalence (96.5%) reported by Godoi *et al.* (2012) for this same fish when farmed in Rondônia State.

Four species of Protozoa, four species of Monogenoidea, one Nematoda and two species of Crustacea were found in *C. macropomum* in fish farms from Amapá State. Santos *et al.* (2013) described only two species of Protozoa, two species of Monogenoidea and one species of Hirudinea in tambaqui rearing in net-cage in the State of Amapá. For the same host of ponds in the State of Amazonas, Tavares-Dias *et al.* (2006) reported only one protozoan species, one monogenoidea and one myxosporean. Godoi *et al.* (2012) identified six species of Monogenoidea and one species of Crustacea in tambaqui reared in the State of Rondônia. Therefore, farmed tambaqui present high ectoparasites diversity, which have a direct life

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cycle. In fish farms the presence of endoparasites may be occasionally due to the absence or low occurrence of the infecting stages in intermediate hosts of endohelminths – parasites with a heteroxenic life cycle.

Colossoma macropomum in this study had high levels of infection by *I. multifiliis* in the eight fish farms investigated. *Trichodina* sp. occurred only in fish from one of the fish farms. However, infection levels by *P. pillulare* were greater in only 25% of the fish farms due to poor environmental quality, which benefits the proliferation of both protozoans that are transmitted directly from fish to fish (Colorni, 2008; Santos *et al.*, 2013). It occurs primarily in reared fish in high stocking densities and poor management conditions (Banu and Khan 2004), as occurred with the fish in the present study.

In *C. macropomum* gills only *A. Spathulatus* was collected from the host in the eight fish farms, while *M. boegeri*, *L. brinkmanni* and *N. janauachensis* were found in fish in a few fish farms. Even though it was not possible to determine the prevalence for each monogenoideans species, *L. brinkmanni* and *N. janauachensis* were the least prevalent species in samples. Morais et al. (2009) also reported such differences in the prevalence of these monogenoidean species in farmed *C. macropomum* in net-cage in the State of Amazonas. However, the prevalence of monogenoideans was similar among the fish farms investigated here, while the intensity of these parasites was greater only in three fish farms, due to differential management.

Nematode *P. (S.) inopinatus* occurred in *C. macropomum* in 75% of the fish farms investigated, but in low prevalence. However, only fish from one fish farm had higher prevalence, probably because of the presence of this nematode's infecting stages in ponds. In this farm, the ponds are supplied with water from a river and the floodplain, where it is possible to find arthropod species. Chironomids are intermediary hosts of *P. (S.) inopinatus* (Moreira *et al.*, 2009), a nematode with low parasitic specificity, which requires fish as paratenic or definite hosts (Silva *et al.*, 2011). Still, Camallanidae species may be damaging to reared fish, because when it is in the presence of a great quantity of intermediary hosts with infecting stages in this artificial environment, it may lead to high levels of parasitism.

Perulernaea gamitanae infested the mouth and gills of *C. macropomum* in fish farms investigated. However, fish from one fish farm were the source for the introduction of this ectoparasite in the other fish farm, when it acquired tambaqui for fattening. Such results highlight the importance of the quarantine period in order to avoid the transference of parasites among fish farms. In general, this procedure has not been used by most of the fish farms, including those in Amapá State, resulting in the use of chemotherapeutic treatments. To eliminate and control *P. gamitanae* in these fish, 100g of Diflubenzuron/1000m^3 of pond may be used (Tavares-Dias *et al.*, 2011), since copepodits are capable of surviving for seven days without the host.

The isopod *B. patagonica* occurred in low prevalence and intensity in *C. macropomum* in only one fish farm, and was found parasitizing the dorsal region of hosts, close to the dorsal fin. Wild fish (*Hoplias malabaricus*, Curimatidae species and *Ancestrorhynchus* spp.) were the source of the parasites for *C. macropomum* in this study, because the gills of these wild fish from the river that supplies the fish farm were also found with gills infection by *B. patagonica*. Isopod species are parasites that can also cause loss of blood, reduction of gill filaments and respiratory deficiencies, in addition to reducing the development of fish (Carvalho *et al.*, 2004), which are undesirable consequences in fish farms.

In this study there was a dominance of *I. multifiliis* followed by *P. pillulare*. High prevalence of both protozoans was similar to previous descriptions for the same host reared in net-cage (Santos *et al.,* 2013), while mean intensity and abundance were superior. We attribute this result to the low environmental quality in ponds in fish farms. Levels of infection by *Tetrahymena* sp. in the gills of *C. macropomum* were similar to the ones described for *Carnegiella strigata*, while parasitism by *Trichodina* sp. were similar to the levels of infection in *Carnegiella martae* (Tavares-Dias *et al.*, 2010), both hosts collected in the Negro River. However, *Tetrahymena* sp. is a ciliate with rare occurrence in Brazilian fish,

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contrasting with *Trichodina* sp., a species which parasites wild and farmed fish populations.

High levels of infection by monogenoideans were observed in the gills of *C. macropomum*, and the prevalence was similar to the one described for this host in net-cage from the Paru Lake-AM (Morais *et al.*, 2009) and Matapi River-AP (Santos *et al.*, 2013) and in fish farmed from the State of Rondônia (Godoi *et al.*, 2012). Despite that, mean intensity and mean abundance were higher than the ones found in those studies, due to the peculiar condition of the environment from each fish farm. Parasitism caused by monogenoideans species was due to low pH and oxygen levels in the rearing pond (Banu and Khan, 2004; Modu *et al.*, 2012), and also by high temperatures in the region.

Overall, parasitism caused by *P. gamitanae* and *B. patagonica* in *C. macropomum* farmed in the State of Amapá was low. The levels of infection by *P. gamitanae* were similar to the ones described for this same host farmed in the State of Rondônia (Godoi *et al.*, 2012). However, those levels were greater if compared to the corresponding host reared in the State of Amazonas (Morais *et al.*, 2011). High levels of infection by *P. gamitanae* in fingerlings of tambaqui farmed in the Peruvian Amazon resulted in 100% of mortality of the fish (Delgado *et al.*, 2011). This lerneid seem have parasitic specificity since it infected only *C. macropomum* and hybrids of C. *macropomum* x *Piaractus mesopotamicus* and C. *macropomum* x *Piaractus brachypomum* (Tavares-Dias *et al.*, 2011). In addition, *P. gamitanae* may cause inflammatory process, hyperplasia and gills anemia, which leads to the death of farmed fish, (Delgado *et al.*, 2011; Morais *et al.*, 2011; Tavares-Dias *et al.*, 2011), mainly of fingerlings with high parasitic infection levels.

In *C. macropomum*, infracommunities of *I. multifiliis*, *P. pillulare*, Monogenoidea and *P. gamitanae* had an aggregate dispersion, indicating stability in the dynamics of the host-parasite association. This pattern of aggregate dispersion is highly influenced by genetic, environmental and immunological factors of hosts (Moller, 2006). However, in the fish farm, the environment and the fish aggregation are the factors that primarily affect the pattern of dispersion of ectoparasites.

Finally, a great parasitic diversity occurred in *C. macropomum,* composed mainly by ectoparasites with a high level of infection. Qualitative and quantitative differences in ectoparasites of tambaqui were caused mainly by the management adopted (i.e, food quality and quantity, and sanitary quality of fish farms), as well as by low levels of dissolved oxygen and differences regarding the source of water used to supply the ponds. The results demonstrate the need for adopting adequate prophylactic actions in these fish farms in order to avoid economic loss by epizooties. This is the first report of *Tetrahymena* and *P. (S.) inopinatus* for farmed *C. macropomum* in Brazil, and extends the geographic distribution of this ciliate to eastern Amazon.

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