













## Prevalence and risk factors of parasites in tambaqui *Colossoma macropomum* fingerling fish farming from São Francisco region AL/SE

[Prevalência e fatores de risco de parasitos na piscicultura de alevinos de tambaqui *Colossoma macropomum* da região do baixo São Francisco AL/SE]

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### ABSTRACT

This study identified the risk factors associated with parasite infestation in tambaqui fingerlings *Colossoma macropomum* from São Francisco region SE/AL. The fingerlings fish farms were and characterized about water quality, handling, feeding management and sanitary aspects. Parasitological indexes (prevalence, mean intensity and abundance) were obtained from 380 fish and correlated to the aspects of fish farms as well as biotic and abiotic factors to determine the risk factors. The fish rearing in earthen ponds increased the parasite infestations in tambaqui fingerlings, and the mainly parasites founded were monogenean, *Myxobolus* sp., *Ichthyophthirius multifiliis* and digenean metacercaria. The main risk factors identified were associated to irregular water supply and consequently low water quality (total ammonia); lack of biometric procedure which promoted an inadequate feeding rate; and absence of disinfection of tools and ponds. Thus, improvements on these specific aspects in fish farms from São Francisco region must be carried out to avoid disease outbreaks, dissemination, mortalities and economic losses.

Keywords: epidemiology, prophylaxis, illness, risk management

### RESUMO

Este estudo identificou os fatores de risco associados à ocorrência de parasitas em alevinos de tambaqui *Colossoma macropomum* da região do baixo São Francisco, SE/AL. As pisciculturas foram caracterizadas segundo parâmetros de qualidade da água, manejo, alimentação e dos aspectos sanitários. Os índices parasitológicos (prevalência, intensidade média e abundância) foram obtidos mediante a análise de 380 peixes e correlacionados com as características das pisciculturas, bem como com os fatores bióticos e abióticos, a fim de determinar os fatores de risco. A fase de crescimento dos alevinos realizada em tanques de terra aumentou as infestações de parasitas, e os principais parasitas encontrados foram monogenéticos, *Myxobolus* sp., *Ichthyophthirius multifiliis* e metacercária de digenético. Os principais fatores de risco identificados foram: abastecimento irregular de água e consequente baixa qualidade da água (amônia total); falta de procedimento biométrico, que promoveu uma taxa de alimentação inadequada; e ausência de desinfecção dos tanques e das ferramentas. Pelo exposto, melhorias, especificamente nesses aspectos, nas pisciculturas de alevinos da região do São Francisco, devem ser realizadas para evitar surtos e disseminação de doenças, assim como mortalidades e perdas econômicas.

Palavras-chave: epidemiologia, profilaxia, doença, gerenciamento de riscos

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## INTRODUCTION

The continental Brazilian fish farming has increased 4.9% from 2018 to 2019, producing 758 thousand tons (Anuário..., 2020). The states of Sergipe and Alagoas are the main fish producers from the lower São Francisco River region, producing tilapia (*Oreochromis niloticus*) and tambaqui (*Colossoma macropomum*) (Anuário..., 2020).

Tambaqui are the main native fish species farmed in Brazil, and commonly reared into earthen ponds at semi-intensive system (Melo *et al.*, 2001; Barroncas *et al.*, 2015). Tambaqui have some important characteristics to captivity rearing, such as rusticity, rapid growth, high productivity, resistance to hypoxia, and acceptance of industrial feeding (SainpPaul, 1986; Barçante and Sousa, 2015).

Nonetheless, disease outbreaks are frequently associated with increased tambaqui production. Although there is not official data about tambaqui mortality, economic losses in Brazilian fish farming reach US\$ 84 million annually due to fish diseases (Tavares-Dias and Martins, 2017). In addition, there is no national monitoring systems to establish the risk factors for diseases in tambaqui rearing.

Parasite infestation in tambaqui rearing can be associated to several stress factors, such as poor water quality, inadequate feeding, and high stocking density (Santos *et al.*, 2017). Furthermore, early stages as larvae ad fingerlings are more susceptible to parasite infestations (Dotta and Piazza, 2012; Tavares-Dias and Martins, 2017). Therefore, epidemiological studies have been used to determine the risk factors related to parasite infestations to apply adequate preventive measures (Querino *et al.*, 2003; Lovers and Bartholomew; 2003; Bebak *et al.*, 2015; Hoshino *et al.*, 2018; Fujimoto *et al.*, 2019).

Previous studies determined the risk factors for parasitic diseases in tambaqui during the fattening phase (Fujimoto *et al.*, 2019), however the risk factors related to rearing of larvae and fingerling

still unknown in the lower São Francisco River region.

The knowledge of risk factors is important to reduce the pathogen dissemination and the costs with ineffective therapeutic measures. Thus, the present study aimed to identify the associated risk factors with parasites of tambaqui fingerlings reared in fish farms from the lower São Francisco River region.

## MATERIAL AND METHOD

Fish farms (n=5) corresponding to 72% of all facilities were evaluated in the region between Sergipe (SE) and Alagoas (AL) following Development Corporation of Vale do São Francisco and Parnaíba (CODESVASF, personal information). The fish farms were divided in two categories: public fish farms (n=2) and private fish farms (n=3). The fish farms sampled were located in the Propriá -SE (n=2, two private fish farms, P1 and P5), Cedro de São João-SE (n=1, private fish farm, P2), Neópolis-SE (n=1, public fish farm, P3), and Porto Real do Colégio-AL (n=1, public fish farm, P4) (Fig. 1).

Characteristics of fish farms were collected *in loco*, aided by a semi-structured questionnaire about handling, feeding, water quality, and disease (Table 1).

Fish samplings (20 fish per sample) occurred in November 2018 through July/2019, at beginning and final of rearing period, repeated twice in each fish farm, totaling 80 *Colossoma macropomum* fingerlings per fish farm. In this region, the fingerlings are reared in earthen ponds lasted 30 to 60 days. The fish were placed in polyethylene bags (20 L water 1/3 and oxygen 2/3) and transported to the laboratory of aquaculture in Embrapa Tabuleiros Costeiros, Aracaju-SE to perform parasitological analysis.

The fish were anesthetized (1:10 eugenol: alcohol; sprinkled on gills), measured (length and weight), and euthanized by medullar section (Ethical committee – CEUA number 010219) for biometry and parasitological analysis.

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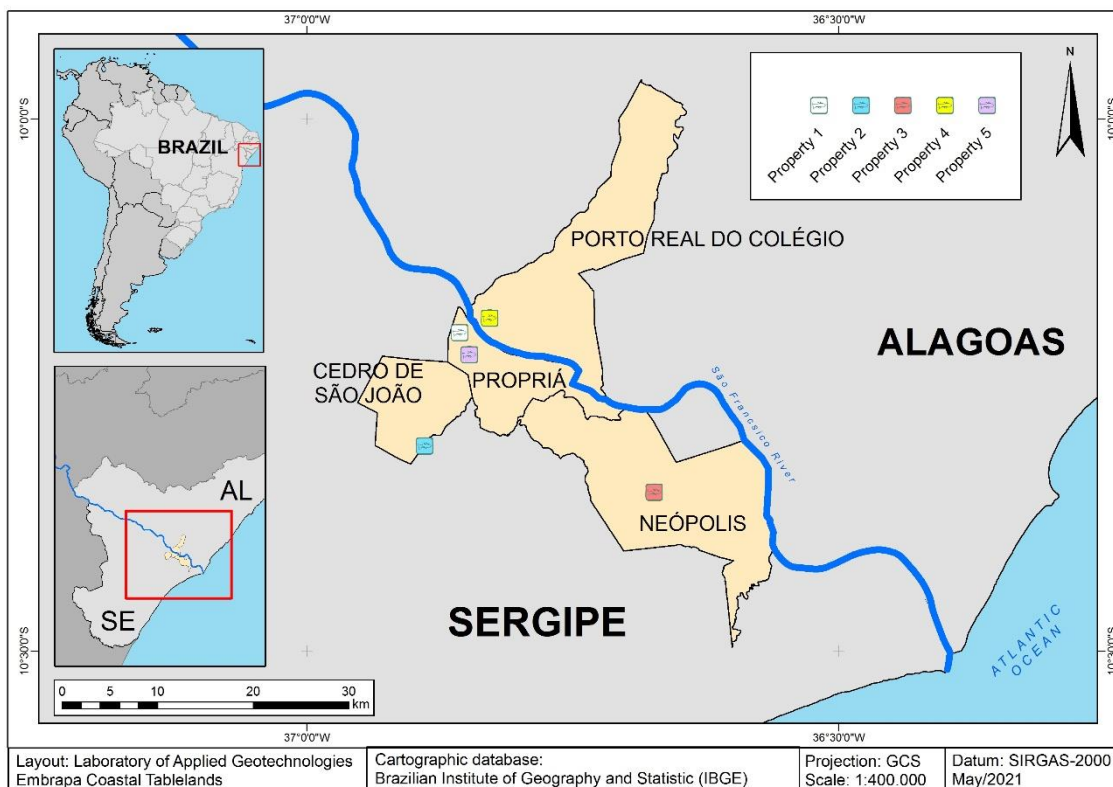


Figure 1. Map representing the location of *Colossoma macropomum* fish farms sampled from Sergipe and Alagoas state, Brazil.

Table 1. Fish farms aspects evaluated by semi-structured questionnaire applied in fingerlings fish farms from lower São Francisco River region AL/SE

Handling aspects	Feeding aspects
Fertilized pond	The feeding ratio and frequency is not monitored
Soil Physic-chemical analysis	The ration is adequately stored
The stocking density into incubator (eggs 1g/L)	Sanitary aspects
Adequate stocking density 200 juveniles/m <sup>3</sup>	Use of salt to prevention or therapeutic procedures
Water quality aspects	Pond Disinfection (scraping of bottom)
Water supply (pumping, river, frequency, etc)	Cleaning and disinfection of incubators
Water quality monitoring	Disinfection of tools (nets)
bovine manures used as fertilizer	Use of chemical products on water
Filters	Quarantine period

The fish underwent parasitological analysis in the mucus, gills, liver, kidney, pyloric cecum, intestine, stomach, gas bladder, and muscle aided by microscopy (Coleman N-120®). Procedures of preservation, quantification, and dye of the parasites (Protozoan, Myxozoan, Monogenea, Digenea, and Nematoda) followed methodology of Eiras *et al.* (2006) and Lom and Noble (1984) and were identified by specific literature (e.g.

Thatcher, 2006; Cohen *et al.*, 2013). Subsequently, parasitological indices (total and specific) of mean intensity (MI: total of parasite/total of infected host), prevalence (P: total of infected host/total host \* 100), and abundance (AB: total of parasite/total of host) were calculated according to Bush *et al.* (1997).

Water quality parameters, such as pH (AKROM KR20®), temperature (YSI 550A®), dissolved oxygen (YSI 550A®), electric conductivity (YSI 30®), and total ammonia (HANNA® HI93715) were measured *in loco* in each pond sampled.

All data were previously subjected to Shapiro-Wilk and Bartlett tests to determine normality and homoscedasticity, respectively. Water quality parameters were submitted to analysis of variance (ANOVA), followed by Tukey's test ( $p < 0.05$ ) to determine possible differences among fish farms. The total prevalence was analyzed by a paired t-test comparing the beginning and the final period of fingerling rearing.

Pearson correlation between parasitological indices and water quality parameters was also performed. The multivariate analysis of canonical correlation was used to determine any relationship

between parasitological indices (prevalence, mean intensity, and abundance) and characteristics of properties (water parameters, handling, feeding management, and sanitary aspects). Based on these analyses, the main risk factors were identified. All data were analyzed using the statistical software PAST3.0 and BioEstat5.0.

## RESULTS

A total of 1836 parasites (63% of analyzed fish) were found, and the highest mean prevalence was observed in fish farm P4 ( $44.15 \pm 9.15$ ) and the lowest value was found in fish farm P3 ( $10.80 \pm 2.50$ ). Furthermore, the highest abundance value ( $22.73 \pm 17.33$ ) was observed in fish farm P4 and the highest values of mean intensity were found in fish farms P1 and P5 ( $2.40 \pm 0.90$ ;  $1.82 \pm 0.32$ , respectively) (Table 2).

Table 2. Total parasitological indices on tambaqui *Colossoma macropomum*, at the final of fingerling rearing in earthen ponds in lower São Francisco region

Parasite index	P1*	P3	P4	P5
P	$18.65 \pm 15.35ab$	$10.80 \pm 2.50b$	$44.15 \pm 9.15a$	$21.45 \pm 9.25ab$
MI	$2.40 \pm 0.90a$	$1.15 \pm 0.15ab$	$0.56 \pm 0.44b$	$1.82 \pm 0.32ab$
A	$3.15 \pm 2.99a$	$0.46 \pm 0.13a$	$22.73 \pm 17.33a$	$2.28 \pm 1.68a$

P - Prevalence, M - Mean Intensity, A - Abundance. \* The parasitological index of fish farm P2 was not determined due to the absence of a second sample in the dry season.

The parasites identified were monogeneans (*Notozothecium janauachensis*, *Notozothecium nanayensis*, *Mymarothecium boegeri*, *Anacanthorus spathulatus*, *Linguadactyloides brinkmanni*), protozoans (tricodinids, *Piscinoodinium pillulare*, *Ichthyophthirius multifiliis*), myxosporidians (*Myxobolus* spp., *Henneguya* spp., *Thelohanellus* spp.), digenean metacercariae, and nematodes (*Procamallanus inopinatus*). The monogenean, *Ichthyophthirius multifiliis*, digenean metacercariae, and *Myxobolus* were the most prevalent parasites in all fish farms (Table 3).

The fingerling rearing in earthen pond increased the total prevalence in all fish farms ( $p < 0.05$ ) (Fig. 2). The increase in parasite infestation was related to biotic and abiotic factors. A negative correlation ( $p = 0.0384$ ) was found between standard length and *Ichthyophthirius multifiliis* infestation. However, a positive correlation

occurred between weight/ total ammonia for monogeneans ( $p = 0.0402$  and  $0.0136$ , respectively) and *Myxobolus* ( $p = 0.0054$  and  $0.0294$ , respectively) (Table 4).

The canonical correlation showed that *Piscinoodinium pillulare*, *Myxobolus* spp., and nematodes infestation was most related to lack of disinfection of bottom ponds, water supplied by pumping, inadequate feeding frequency, and lack of quarantine procedure. For ciliate protozoans *Ichthyophthirius multifiliis* and *Trichodina* spp., the presence of predatory fish, absence of filter on inflow water, use of salt for handling, and inadequate structure for the stocking ration influenced parasite infestation. *Thelohanellus* spp., monogeneans, and digeneans were related to an irregular water supply, lack of water monitoring, lack of tool disinfection, lack of biometric procedure, and incorrect feeding rate. For digenean metacercaria, a relationship was

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observed with the utilization of organic fertilizers in earthen ponds (Fig. 3).

In addition, the public fish farms showed similarities to each other, being clustered in one

group, however the private fish farms were divided in two groups, where one group was formed by P1 and P2, and another group formed by fish farm P5.

Table 3. Specific parasitological indices, weight, and length for tambaqui fingerlings *Colossoma macropomum* after rearing period in earthen ponds from five São Francisco fish farms

Fish farm		MON	ICT	MET	MYX	TRI	THE	DIG	NEM	PIS	WEIGHT	TL	GP
P1	P	18.00	26.00	22.00	6.00	6.00	-	-	-	-			
	MI	1.78	6.31	2.00	-	2.67	-	-	-	-	0.246±0.254	2.56±0.02	30
	Ab	0.32	1.64	0.44	-	0.16	-	-	-	-			
P2	P	24.0	8.0	2.0	22.0	2.0	2.0	-	-	-			
	MI	6.8	30.0	1.0	-	1.0	1.0	-	-	-	2.257±0.718	5.08±0.64	60
	Ab	1.6	2.4	0.2	-	0.2	0.2	-	-	-			
P3	P	4.0	2.0	20.0	2.0	-	-	2.0	-	-			
	MI	1.5	2.0	1.40	-	-	-	1.0	-	-	1.134±0.514	4.13±0.18	60
	Ab	0.6	0.4	0.3	-	-	-	0.2	-	-			
P4	P	86.0	4.0	6.0	85.0	-	28.0	-	4.0	-			
	MI	30.5	3.0	1.3	-	-	1.0	-	1.0	-	1.903±1.661	4.54±0.51	60
	Ab	26.2	0.1	0.1	-	-	0.3	-	0.4	-			
P5	P	28.0	20.0	15.0	46.0	-	15.0	2.0	5.0	22.0			
	MI	1.94	2.5	1.3	-	-	1.0	1.0	1.0	4.6	1.129±0.356	4.16±0.03	60
	Ab	0.5	0.5	0.2	-	-	0.1	0.1	0.1	1.0			

P - Prevalence; MI - Mean Intensity; A - Abundance; MON - monogenean; PIS - *Piscinoodinium pillulare*; TRI - *Trichodina* sp.; ICT - *Ichthyophthirius multifiliis*; MET - Metacercariae; MYX - *Myxobolus* sp, THE - *Thelohanellus* sp.; DIG - Digenea; NEM - Nematode; TL - Total length (cm); Weight - grams; GP- growth period in days.

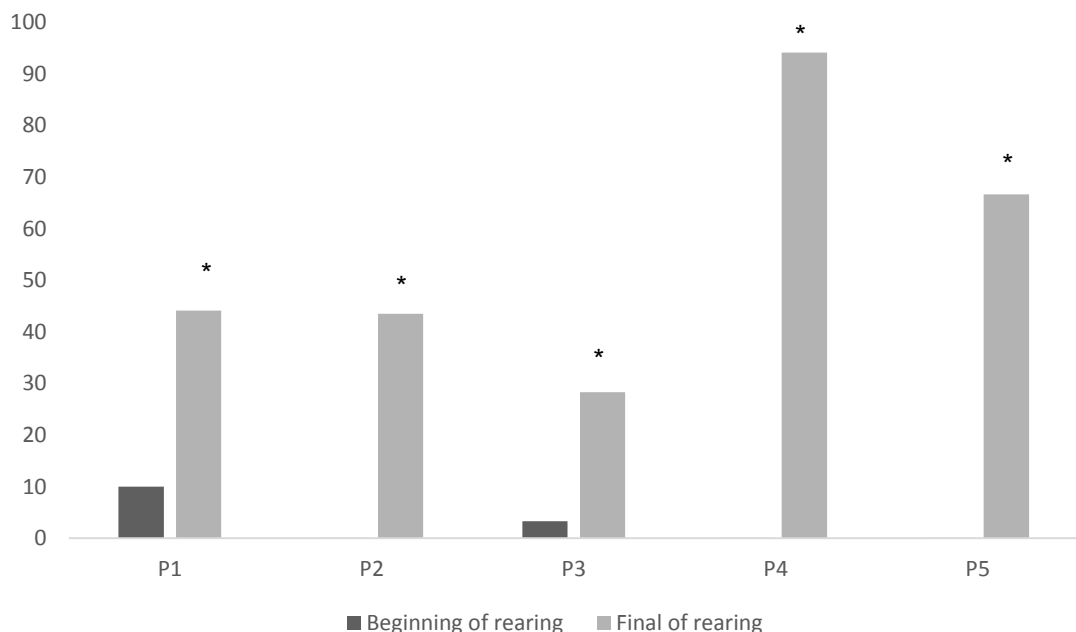


Figure 2. Total prevalence of parasites on tambaqui *Colossoma macropomum* from five fish farms lower São Francisco region, comparing beginning and final of fingerling rearing in earthen pond. \*Parasitological statistical difference between on initial and final fingerling rearing period by paired t-test (p<0.00009).

Table 4. Pearson correlation of parasite infestation to biotic and abiotic factors in tambaqui *Collossoma macropomum* from lower São Francisco River region

	Abiotic factors					Biotic factors		
	pH	Temp.	DO	EC	TA	Weight	TL	SL
MON	-0.7975	0.4925	-0.2376	-0.0658	0.9683*	0.9864*	-0.44	-0.435
ICT	-0.6988	-0.6120	-0.7799	0.6304	0.2754	0.3714	-0.9353	-0.9616*
MET	0.3418	-0.8337	-0.2819	0.4998	-0.8117	0.7638	-0.1074	-0.1349
MYX	-0.6033	0.3746	0.0246	-0.3423	0.9947*	0.9706*	-0.4821	-0.4470

MON – Monogenean, ICT – *Ichthyophthirius multifiliis*, MET – Metacercariae, MYX – Myxobolus, Temp – Temperature, DO - Dissolved oxygen; EC - Electric conductivity; TA – Total ammonia; TL - Total length (cm); SL - standard length. \* significant correlation p<0.05

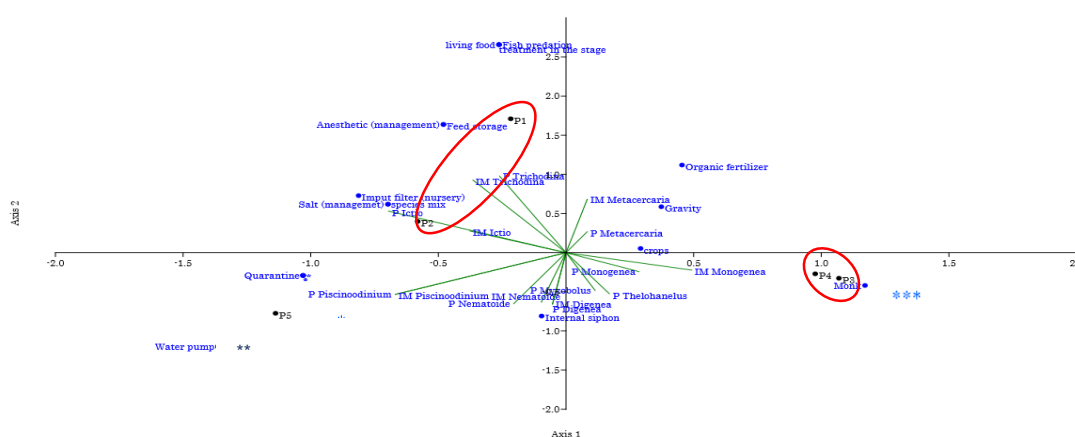


Figure 3. Canonical correlation analysis (CAA) between parasitological indexes (P - prevalence and IM - mean intensity) and characteristics of area (water parameters, handling, feeding management and sanitary aspects) from five fingerlings fish farms in the lower São Francisco river region. \* Not disinfection of bottom pond; \*\* Feed frequency, Larval mortality, Mortality in the winter, Lack of specialized technical assistance; \*\*\* Lack of Biometry, Irregular water supply, Lack of utensils disinfection, Uncontrolled feed rate, Fertilizing pond, Lack of water monitoring.

### DISCUSSION

The parasite fauna of tambaqui presents 20 taxa as reported by Eiras *et al.* (2010). In the present study, the fingerlings of tambaqui (0.2 to 2.2 g) presented nine parasite taxa, showing a greater diversity considering the short rearing time (30–60 days). Furthermore, in previous study, tambaqui (438 g) in the fattening phase, with at least 180 days of rearing, presented ten taxa (Fujimoto *et al.*, 2019), indicating the susceptibility of fingerlings to parasites, and possible dissemination to another growth phase. For these reasons, protocols of acclimation and quarantine must be carried out in fish farms to avoid outbreak and pathogen dissemination, specifically *Piscinoodinium pillulare*, *Myxobolus*

spp., and nematodes, which were related to the lack of quarantine by canonical correlation analysis.

The fingerling rearing in the earthen pond increased parasite infestation due to many influences, such as handling, infrastructure, water quality, and nutrition. The canonical and Pearson correlation identified the main relationship between parasites infestation and biotic/abiotic factors to tambaqui fingerling rearing. Then, the lack of a biometric procedure, which promoted an uncontrolled feeding rate; an irregular water supply and consequently low water quality (total ammonia); and absence of tools and pond disinfection were the main risk factors related to most prevalent parasites (monogenean,

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*Ichthyophthirius multifiliis*, digenean metacercaria, *Myxobolus* spp.).

The feeding management has been neglected in the lower São Francisco River region, reflected in the increased parasitism of *Piscinoodinium pillulare*, nematode, *Myxobolus* spp., and monogeneans demonstrated by canonical correlation. The risk factor related to inadequate feed management stand out as a problem, reducing water quality compromising zootechnical performance, health, and production costs. Adequate feeding rate and frequency plays an important role in maintaining good water quality and better nutrient absorption (Castelli and Barrela, 2005; Abe *et al.*, 2016; Brito *et al.*, 2017). Furthermore, the adequacy of feed rate and frequency, as well as protein levels and particle size for each stage of fish development prevent the parasite outbreaks (Abe *et al.*, 2016; Santos *et al.*, 2020).

The inadequate feeding had indirectly influenced monogenean parasitism by the increasing in total ammonia, as confirmed by the positive correlation between parasite infestation and total ammonia, for ammonia stimulates the hatching of monogenean eggs (Moraes and Martins, 2004).

Monogenean infection also showed a positive correlation with weight, indicating an accumulator effect (Vicente, 2018) through the fingerling rearing period. Contrary, older tambaquis (180 days old) showed a negative correlation between the weight and monogenean parasites (Fujimoto *et al.*, 2019). In older fish, due to better conditions of immunological system, high abundance of parasites did not cause any pathogenic effect or reduction of physical condition in fish as hybrid tambatinga (*Colossoma macropomum* X *Piaractus brachipomus*) (Dias *et al.*, 2015).

The accumulator effect for monogeneans can also be explained through its life cycle. The monogenean direct life cycle is not long (every 15 days) and four generations of monogeneans can infect the tambaqui fingerling within 60 days of rearing (Brazenor and Hutson, 2015).

Allied to feeding management, the total ammonia, unmonitored water parameter and pond fertilization are other important risk factor to parasite infestation. Pond fertilization can

determine a good water quality for fingerling rearing (Taque, 2014). However, 60% of producers do not perform such procedure and the other 40% apply untanned bovine fertilizer, an inadequate procedure that can worsen the water quality, increasing the total ammonia (Santos *et al.*, 2020).

The disinfection of tools and ponds is not carried out at 40% of properties, and this parameter is a risk factor to tambaqui fingerlings rearing from the São Francisco region. The *Piscinoodinium pillulare* and nematodes parasitism was influenced by the lack of pond disinfection/cleaning after each productive cycle. Scraping the bottom of the pond is an important procedure, since the accumulated organic matter contains larvae and parasite eggs that will infect the reared fish. Periodic scraping can aid in the maintenance of water quality, since removing the accumulated organic matter and disrupts the shelter used by intermediary hosts. The correct disinfection procedures for tools and ponds can prevent pathogen transmission among the ponds (Tavares-Dias; Fujimoto, 2014; Lima *et al.*, 2017).

Ciliate protozoa, such as *Ichthyophthirius multifiliis* and *Trichodina* spp., were influenced by the absence of a filter in the water catchment that allowed entrance of other fish in the ponds. Intruder fish could be a host of *Ichthyophthirius multifiliis* and *Trichodina* spp. and transmitted to tambaqui fingerlings. In the present study, abiotic factors, such as temperature and ammonia, were not the main causes of ciliate protozoa infestation (tab. 3) as previously reported (Tavares-Dias and Mariano, 2015).

We highlight the absence of acanthocephalans parasitizing the fingerlings in the São Francisco region, as previously reported in tambaquis reared in fattening phase (Fujimoto *et al.*, 2019). The acanthocephala is the most harmful parasite to tambaqui reared in captivity in northern of Brazil (Chagas *et al.*, 2019). Thus, the quarantine procedure is an important measure to prevent the dissemination between Brazilian regions.

### CONCLUSION

The fingerling rearing in earthen ponds have increased parasite infestations in a short time of rearing (30 to 60 days), mainly by monogeneans,

*Ichthyophthirius multifiliis*, digenean metacercaria, *Myxobolus* spp., and *Piscinoodinium pillulare*.

The inadequate feed management, lack of quarantine, unmonitored water quality, no disinfection of equipment and ponds are the main risk factors to tambaqui fingerling rearing in lower São Francisco River region. Improvements in these specific aspects are necessary to avoid disease outbreaks and pathogen dissemination to region.

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