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Protozoan and metazoan parasites of juvenile tambaqui *Colossoma macropomum* farmed in the Lower São Francisco, Brazil

Protozoários e metazoários parasitos de juvenis de tambaqui *Colossoma macropomum* cultivados no Baixo São Francisco, Brasil

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Abstract Raising freshwater organisms currently represents a growing Market. However, animal confinement constitutes a factor that favors appearance of parasitic diseases, culminating in stressed fish and significant economic damage. In this context, monitoring fish health can ensure early diagnostic of pathogens. This study aims to verify the occurrence of parasites in juvenile tambaqui raised in the Integrated Center of Fish Resources and Aquaculture – Codevasf of Betume, Neópolis Sergipe State. Water quality of fish farms was assessed at the moment of fish collection. Fish were randomly collected and transported for parasitological analysis. The presence of protozoans, *Ichthyophthirius multifiliis* and *Epistylis* sp. were observed along with monogenean helminths and Digenean metacercaria in examined animals. The most prevalent parasites were: Monogenea (91%), Digenea (81.25%), *I. multifiliis* (59%) and *Epistylis* sp. (10%). The presence of parasites in analyzed fish shows the need for monitoring in order to avoid dissemination, proliferation and/or introduction of potential pathogens in fish farms and bodies of natural water.

Keywords: Serrasalmidae, pisciculture, restocking, parasitism and monitoring.

Resumo O cultivo de organismos de água doce representa, atualmente, um mercado crescente. No entanto, o confinamento de animais constitui um fator que favorece o aparecimento de doenças parasitárias, culminando em peixes estressados e danos econômicos significativos. Neste contexto, o monitoramento da saúde dos peixes pode garantir o diagnóstico precoce de patógenos. Este estudo tem como objetivo verificar a ocorrência de parasitas em juvenis tambaqui cultivados no Centro Integrado de Recursos de Peixe e Aquicultura - Codevasf de Betume, Neópolis estado de Sergipe. A qualidade da água dos viveiros foi avaliada no momento da coleta dos peixes. Os peixes foram coletados aleatoriamente e transportados para análise parasitológica. A presença de protozoários, *Ichthyophthirius multifiliis* e *Epistylis* sp. foi observada juntamente com helmintos monogênicos e metacercarias de Digenea nos animais examinados. Os parasitas mais prevalentes foram: Monogenea (91%), Digenea (81,25%), *I. multifiliis* (59%) e *Epistylis* sp. (10%). A presença de parasitas nos peixes analisados mostra a necessidade de monitoramento, a fim de evitar disseminação, proliferação e / ou introdução de patógenos potenciais em pisciculturas e corpos de água natural.

Palavras-cheve: Serrasalmidae, piscicultura, repovoamento, parasitismo e monitoramento.

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Introduction

Pisciculture is one of the animal production sectors that has been growing the most, currently around 86.6% of total national production (MPA, 2013). In Brazil, the raising of native species has increased, tambaqui *Colossoma macropomum* (Cuvier, 1816) being the second most cultivated species, with 28.1% of total raised fish (IBGE, 2015). This tropical fish is the most cultivated in the Amazon and frequently used on fish farms throughout the country (Lopera-Barrero et al. 2011), it's an omnivorous species that can measure up to 90 cm long and weigh up to 30 Kg (Gomes et al., 2010).

Due to optimum levels of water quality, good soil, privileged geographical localization and articulated institutions to encourage aquatic organism production, the lower São Francisco stands out because of its great pisciculture potential, (Rosa and Motta, 2001; Pacheco and Lira, 2009). According to data from the State Nucleus of Local Production Arrangements (2011), tambaqui is the main species cultivated in Sergipe, corresponding to 44.1% of the states. Pisciculture growth has increased considerably, relevance in studies with parasites and pathogens of other aquatic organisms, especially of those hosts with potential for cultivation and commercialization. (Luque *et al*., 2004).

Generally, appearance of parasitic diseases is related to imbalance in the host/parasite/environment relation (Martins *et al*., 2002). Situations involving animal confinement constitute a factor that favors the diseases (Diniz and Honorato, 2012). Thus, fish are submitted to chronic stress resulting in, among other causes, from inherent manipulation of farms, high density, transportation, degradation of water quality and artificial reproduction (Oba *et al*., 2009). This process provokes physiological alterations in fish, decreasing their capacity to react to pathogenic microorganisms (Lima *et al*., 2006; Vaz *et al*., 2007).

Tambaqui is the second most cultivated fish species in Brazil after tilapia and one of the main species used in São Francisco restocking programs promoted by São Francisco Valley Development Company (Codevasf). In this way, the study has the objective of analyzing parasitic fauna of tambaqui destined to restocking by Codevasf in lower São Francisco river.

Material and methods

Study location

In the months of September and December 2010 and August, October and November 2011, a total of 70 cultivated juvenile tambaqui (*Colossoma macropomum*) was collected at the Integrated Center of Fish and Aquaculture Resources of Betume, 4th Regional Superintendence of the São Francisco Valley Development Company/Codevasf (4th CIB/ SR Codevasf), located in the village of Betume, municipality of Neópolis-SE (10°19'29" S 36°34'46" W).

The farms are 2,000 m², with maximum and minimum depths of 1.20 m and 80 cm, respectively, were supplied through water capture in the São Francisco River by pumping. The property itself had a larviculture laboratory and the stocking density used on the dug farms was 25 juveniles/m².

Fish collection and Farm water analysis

Fish were randomly collected with a dragnet; then packaged live in plastic bags containing one-third water, with pure oxygen added before sealed. At the moment of sample collection, temperature, pH and concentration of dissolved oxygen were calibrated with the multiparameter Water Quality Meter (AK87, Akso, Rio Grande do Sul, Brasil). The average temperature was 27.35±1.3ºC, the dissolved oxygen was 5.3±0.7mg $L^{-1}$and pH was 7.0±1.7. Later, fish were transported to the laboratory at the Penedo teaching unit – UFAL (Federal University of Alagoas).

Parasitological Analysis

In laboratory, fish were euthanized by rapid cerebral concussion (Leite, 1999) to avoid detachment of parasites (Callahan and Noga, 2002) (procedure approved by Committee on Ethical Use of Animals CEUA/UFAL PP009976/2011-50 on 04/08/2011), followed by biometry. The standard length of fish was 20.65±11.87cm and average weight, 79.56±59.83g.

Microscopic observation was performed on the body surface of fish following a necropsy formula. The observation was made with the naked eye and under a stereomicroscope examining body surface, fins, gills, nostrils and opercula for the presence of possible lesions and pathogenic agents. The microscopic analysis

followed recommendations by Eiras *et al*. (2006) for future analysis on rate of prevalence, average intensity and average abundance (Bush *et al*.,1997).

Results

The most frequent parasites were protozoans, *Ichthyophthirius multifiliis* and *Epistylis* sp., Monogenean helminths and Digenea metacercaria. Parasitological indexes (Table 1) obtained throughout the collection period showed high prevalence of Monogenea (91%) and Digenea (81.25%), followed by *I. multifiliis* (59%) and even lower for *Epistylis* sp. (10%).

Table 1. Parasitic indexes of parasites *I. multifiliis*, *Epistylis* sp., Monogenea and Digenea in juveniles of tambaqui *C. macropomum*, cultivated at 4th CIB/ SR Codevasf, SE, Brazil. SII: infection/infestation site; P(%): Prevalence; IM±standard deviation: average intensity; AM±standard deviation: Average abundance; B: Gills; M: Body surface mucous and MU: Musculature.

Parasites	SII	P(%)	IM	AM
I. multifiliis	B	59	17.50±12.10	16.55±17.90
	M	53	48.15±45.00	14.45±12.20
Epistylis sp.	B	10	5.0±3.5	1.0±1.10
	M	-	-	-
Monogenea	B	91	7.2±65	72.4±18.70
	M	30	7.0±1,83	6.0±1.05
Digenea	B	81.25	61.9±79.65	41.45±72.47
	MU	25	4.0±1.00	3.0±1.45

Discussion

Ectoparasite *I. multifiliis*is common in cultivated fish and responsible for significant economic loss around the world (Pavanelli *et al*. 2008), meanwhile, there are few studies that relate the presence of this protozoan in fish cultivated in lower São Francisco. In this study, prevalence observed in gills and body surface mucous were greater than those described by Dias *et al*., (2015) (13.3%). However, those prevalences were less for tambaqui juveniles (96.7%) cultivated in the Matapi River - AP (Santos *et al*. 2013) and Paru Lake in the Amazon region (100%) (Morais *et al*. 2009). Similarly, Pinheiro and Santos (2015) report ich occurrence in hybrid gills of tambatinga (Hybrid of *C. macropomum* and *Piaractus brachypomus*) cultivated in tank-nets in Amapá. Proliferation of *I. multifiliis* can be favored by unfavorable conditions of water quality, low water temperatures, and high stocking density along with nutritional deficiencies (Piazza *et al*., 2006). Studies report that temperature is one of the main determining factors for success in parasites life-cycle (Ghiraldelli *et al*., 2007; Martins *et al*., 2015). This study points to sudden temperature oscillation and high population density as preponderant factors in ich proliferation, confirming Martins *et al*., (2015) and Melo *et al*., (2001).

In this study, the first record of *Epistylis* sp. was observed in gills of juvenile tambaqui. This parasite uses its hosts as a clamping and transportation substrate, which causes alterations in gas exchanges and worsens pre-existing lesions on the body surface and gills (Eiras *et al*., 2010). In Venezuela, Centeno *et al*. (2004) low prevalence (0.78%) was observed in the hybrid of *C. macropomum* x *Piaractus brachypomus*. Low parasitism by *Epistylis* sp.can be understood as a normal occurrence (Pavanelli *et al*., 2008), as registered in this study. Meanwhile, elevated levels of organic material constitute favorable conditions for dissemination of the sickness (Pritchett and Sanders; 2007; Pádua *et al*., 2013).

Studies report that, in commercial raising of tambaqui, there is large presence of Monogenoids which cause damage (Tavares-Dias *et al*., 2010; Eiras *et al*., 2011; Godoi *et al*., 2012; Murrieta-Morey and Malta, 2016). In this study, monogenoids helminths were the most pathogenic when compared to other parasites. Monogenoids were reported in juvenile tambaqui cultivated in Peru with 100% prevalence (Vagas *et al*., 2015). Differently, low prevalence for the same host were observed by Soberon (2014) (27,8%) and Murrieta-Morey and Malta (2016) (3.13%). Similar results were found in the gills of tambaqui kept in tank-nets in the Matapi River, state of Amapá (Santos *et al*., 2013). High prevalence of these ectoparasites can be associated to elevated stocking densities (Oba *et al*., 2009; Melo *et al*., 2001), a verified result in this study. Its reproduction can be favored by elevated temperature, as well as low oxygen levels in farm waters (Banu and Khan 2004; Modu *et al*. 2012). Another factor to be considered is the monoxene life-cycle which, in turn, eases transmission of parasite to cultivated fish (Eiras *et al*., 2010).

Metacercarias, as digenean larvae are called, are more pathogenic than the adult forms. Metacercaria prevalence is superior when compared to reports from Murrieta-Morey and Malta (2016) (3.13%) in tambaqui nostrils collected in Varzea Lakes from Central Amazon. Dealing with fish for consumption, digenea in metacercaria for mis worrying because along with cyst appearance that harms (Shoaibi *et al*., 2010), they can migrate through host tissue causing lesions and tissue alterations (Takemoto *et al.*, 2004). Moraes and Martins (2004) point to a potential zoonotic case since ingestion of fish by man, containing metacercaria with cysts can complete the life-cycle of this parasite. Parasitism by endoparasites observed in juvenile tambaqui cultivated in lower São Francisco can be related to their life-cycle. In addition, growth and weight gain can be negatively affected (Costa *et al*., 2015).

It's important to mention that, generally, youngest fish are less resistant, show clinical diseases and are more affected by pathogens, whereas adult fish are less affected due to their lower sensibility and greater immunocompetence (Figueiredo, 2010). Tavares-Dias *et al.* (2001) point to bad water conditions and inadequate handling in cultivation systems such as stress inductors, which act as elements which transmit sicknesses. This confirms the need for monitoring piscicultures to perform the diagnostic, prophylaxis and parasite control before introduction of fish to pisciculture stocks (Schalch and Moraes, 2005; Tavares-Dias *et al*., 2006).

Conclusion

Parasite presence in juvenile tambaqui cultivated for restocking the lower São Francisco river is registered for the first time in this study. The following pathogenic agents were observed: *Ichthyophthirius multifiliis*, *Epistylis* sp., Monogenea and Digenea metacercaria.

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