

## ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

PARASITOLOGICAL ASSESSMENT AND HOST-PARASITE RELATIONSHIP IN FARMED CACHARA CATFISH FINGERLINGS (*PSEUDOPLATYSTOMA RETICULATUM* EIGENMANN & EIGENMANN 1889), MATO GROSSO DO SUL, BRAZIL

EVALUACIÓN PARASITOLÓGICA Y RELACIÓN HOSPEDERO-PARÁSITO EN ALEVINES DEL BAGRE CACHARA (*PSEUDOPLATYSTOMA RETICULATUM* EIGENMANN & EIGENMANN 1889), MATO GROSSO DO SUL, BRASIL

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### Abstract

Farmed fingerlings of South American catfish are frequently exposed to several parasites, but little information is available for implementing health programs for control and prevention of parasitic diseases in the hatcheries. This study evaluated the parasitic fauna of cachara catfish fingerlings (*Pseudoplatystoma reticulatum* Eigenmann & Eigenmann, 1889) and the host-parasite relationship during the early stages of rearing. A total of 302 cachara fingerlings were used for parasitic diagnosis and histopathological analysis. *Ichthyophthirius multifiliis* and *Trichodina heterodontata* were diagnosed as the most prevalent parasites, followed by *Cryptobia* sp., *Henneguya* sp., Monogenea and Nematoda. There was a positive correlation between the size of the fish and the mean intensity of parasitism by *I. multifiliis*. Proliferation of mucus cells, club cells, multifocal area of degeneration, epithelial necrosis on the body surface, skin ulceration, fusion of secondary gill lamellae and inflammatory infiltration were observed in *I. multifiliis*-infected fish. Ciliated protozoans were the main etiological agents diagnosed, and the cachara (*P. reticulatum*) is a new host for *T. heterodontata*. In addition, ichthyophthiriasis induced severe tissue damage thus making the fingerlings susceptible to opportunistic infections.

**Keywords:** Fish parasites – histopathology - *Ichthyophthirius multifiliis* - *Pseudoplatystoma reticulatum* - *Trichodina heterodontata*.

## Resumen

Los alevines de bagres del Sur de América en cautiverio están frecuentemente expuestos a muchos parásitos, pero poca información es evaluada para implementar programa de salud para control y prevención de enfermedades en criaderos. Este estudio evaluó la fauna de parásitos de alevines de cachara (*Pseudoplatystoma reticulatum* Eingenmann & Eingenmann, 1889) y la relación huésped-parásito durante las fases iniciales de cultivo. Un total de 302 alevines fueron sometidos a diagnóstico y análisis histopatológicos. *Ichthyophthirius multifiliis* y *Trichodina heterodontata* fueron diagnosticados como los parásitos más prevalentes, seguidos de *Cryptobia* sp., *Henneguya* sp., Monogenea y Nematoda. Ha sido observada una correlación positiva entre la longitud de los peces y la intensidad promedio de *I. multifiliis*. Proliferación de células de moco, células club, áreas multifocales de degeneración, necrosis epitelial en la superficie del cuerpo, ulceración de piel, fusión de laminillas branquiales secundarias e infiltración inflamatoria fueron observados en peces infectados por *I. multifiliis*. Los protozoarios ciliados fueron los agentes etiológicos más importantes y el cachara (*P. reticulatum*) es un nuevo huésped para *T. heterodontata*. En adición, la ictioftiriasis indujo un daño severo favoreciendo las infecciones oportunistas en los alevines.

**Palabras clave:** Histopatología - *Ichthyophthirius multifiliis* - Parasitos de peces - *Pseudoplatystoma reticulatum* - *Trichodina heterodontata*.

## INTRODUCTION

Production of native catfish is an expanding agribusiness in Brazil, in which production is concentrated in the central region. Interbreeding between species from two major river basins, the River Plate basin (which includes the Pantanal region) and the Amazon basin is a common practice. Among the fish used to perform this artificial interbreeding, carnivorous species of the genus *Pseudoplatystoma*, such as *P. corruscans*, *P. tigrinum* (*sensu lato*) and *P. fasciatum* (*sensu lato*), are one of the most appreciated fish (Carvalho-Costa *et al.*, 2011).

The state of Mato Grosso do Sul is responsible for the hybrid surubim catfish production (*P. corruscans* male x *P. reticulatum* female), while in the state of Mato Grosso the Amazonian hybrid pintado (*Leiarius marmoratus* male x *Pseudoplatystoma* spp. female) is the most important fish produced. However, production of hybrid fish with reproductive ability, as in the case of the hybrid surubim catfish, can cause an irreversible impact on natural stocks when they escape from fish farms to the natural environment, as has recently been demonstrated by Prado *et al.* (2012).

In order to minimize these problems and improve the conservation of pure species the production of native fish in captivity is a sustainable alternative. The cachara from the Pantanal (*P. reticulatum* Eigenmann & Eigenmann, 1889) has a great genetic similarity with the cachara from Amazonia (*P. fasciatum* Linnaeus, 1766). However, some doubts exist regarding the validity of the species (Carvalho-Costa *et al.*, 2011), but at the present time it is considered to be a valid species, according to the Fish Base (Froese & Pauly, 2013). This South American carnivorous catfish presents a real potential for exploitation in industrial scale, due to its easy reproduction (Leonardo *et al.*, 2004; Batlouni *et al.*, 2006) and the strategies for feeding training at the early stages are known (Inoue *et al.*, 2009). No data on the diagnosis of diseases of these fish in farmed settings have been reported. Most investigators have focused the studies on parasite diagnosis on adult fish from the natural environment (Campos *et al.*, 2008; Campos *et al.*, 2009; Naldoni *et al.*, 2011; Adriano *et al.* 2012).

Information on the physiology and nutritional requirements of cachara as well as parasitological assessment are needed to

optimize the fish production. According to the topic, the nutritional and operational management could be established for better development and preventive measures against diseases. This study presents new information on parasitological assessment in cachara fingerlings and the host-parasite relationship during the early stages of rearing.

## MATERIAL AND METHODS

### Study area and fish

This study was developed in a commercial hatchery located in the municipality of Terenos (20°25'57.7" S; 55°17'08.9" W), Mato Grosso do Sul, Brazil. In February 2010, artificial reproduction of cachara (*P. reticulatum*) was performed and the offspring were examined for 30 days. The larvae hatched approximately 16 h after fertilization, at a water temperature 26°C. The exogenous food was offered 48 h post-hatching, after the time required opening the mouth of the larvae. The food consisted primarily of nauplii of *Artemia salina* until the tenth day of rearing. Concurrent provision of wild zooplankton was then started, after prior fertilization of the earth ponds with poultry litter. After this period, the larvae were transferred to glass fiber tanks 1000 L of capacity and water renewal rate 14 L min<sup>-1</sup>, in which the fish were submitted to feed training (Inoue *et al.*, 2009). This process must be gradual starting from natural feeding to commercial extruded diet with 45% crude protein. Fish tanks were cleaned every day to reduce the levels of organic residues, especially coming from leftover feed. During the study, a total of 302 fingerlings of mean weight 0.2 ± 0.3 g and length 2.4 ± 1.1 cm were analyzed (Ethics Committee Approval 756/CEUA/UFSC).

### Water quality

The water supply was from natural source, the dissolved oxygen and temperature were measured daily using an oxygen meter (YSI 550®, YSI Inc., Yellow Springs, USA) and pH using a pHmeter (PH10®, YSI Inc., Yellow Springs, USA). Ammonia, nitrite and nitrate concentrations were measured using a

colorimetric kit (Alfakit®, Santa Catarina, Brazil). The mean values ± standard deviation were as follows: dissolved oxygen 5.9 ± 0.4 mg L<sup>-1</sup>, temperature 25.6 ± 0.4°C, pH 7.3 ± 0.2, total ammonia 0.3 ± 0.1 mg L<sup>-1</sup>.

### Biometry and parasitological assessment

The fingerlings were captured with nets, euthanized using clove oil (50 mg·L<sup>-1</sup>), measured with caliper and weighed on a semi-analytical balance (0.001) (Shimadzu®, Japan). The parasitological analysis consisted of examination of the fish under a stereomicroscope to search for ectoparasites. Fish larvae were evaluated on the whole on slides under a coverslip with saline solution (0.65%) during the first two weeks, because of the small size of the larvae. In the remaining days gills, body, liver and gastrointestinal tract of fingerlings were separated and mounted individually on slides under a coverslip with saline solution (0.65%), examined and parasite enumerated under a light microscope (Nikon® E100, Japan). The parasites were processed and identified in accordance with Lom & Dyková (1992) and Thatcher (2006). For the trichodinid identification, the material was dried out under dim light, and some smears were impregnated with silver nitrate using Klein's method (Klein, 1958) for the posterior examination of adhesive disc structures and denticles under a light microscope as suggested by Lom (1958), Arthur & Lom (1984) and Van As & Basson (1989). The parasitological indexes were calculated as proposed by Bush *et al.* (1997).

### Histopathological analysis

With occurrence of some deaths, ten fish with white spots on the skin were fixed in 10% buffered formalin solution for histopathological analysis. The skin and gills were embedded in paraffin and sliced into 5 µm thick sections for staining with hematoxylin and eosin (HE) and Periodic acid-Schiff stain (PAS). The histological sections were photographed using a photomicroscope (Nikon® E200, Japan) equipped with an image capture system (Motic® Moticam 2.300, Canada).

### Statistical analysis

Spearman's rank correlation was used between the parasite intensity and the weight and size of the fish.

## RESULTS

### Identification of parasites and parasitological index

From a total of 302 cachara fingerlings, 140 (46.4%) were parasitized by at least one parasite taxon. Ecto and endoparasites were diagnosed in cachara fingerlings (Table 1). However, in the skin, fins and gill, the most frequent parasites were the ciliated protozoan's *Ichthyophthirius multifiliis* Fouquet, 1876 and *Trichodina heterodontata* Duncan, 1977 (Figure 1a, b). In addition, the main infection sites by *I. multifiliis* in cachara fingerlings were the skin and fins, which showed greater infection than the gill tissue. It was not possible to identify the species of Monogenea and Nematoda because the numbers of parasites were too low for secure identification.

### Host-parasite relationship

No correlation was observed ( $p > 0.05$ ) between

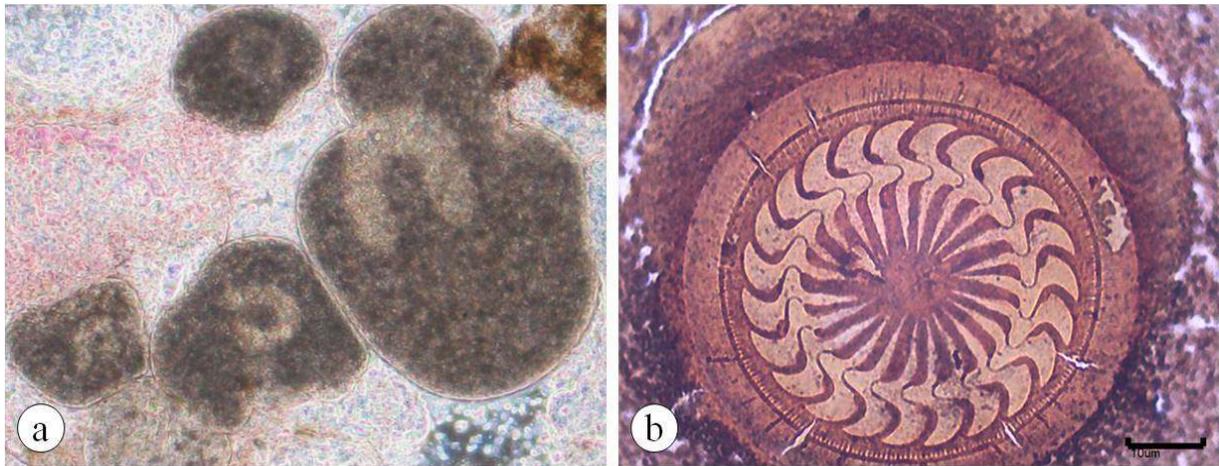
the weight and the mean intensity of infection by *Trichodina heterodontata* ( $rs = -0.09$ ) and *I. multifiliis* ( $rs = 0.31$ ). On the other hand, there was a positive correlation ( $p < 0.0001$ ) between the size of the fish and the mean intensity of infection by *I. multifiliis* ( $rs = 0.78$ ). This was not observed on *T. heterodontata* ( $rs = -0.07$ ) and the other parasites.

### Histopathological analysis

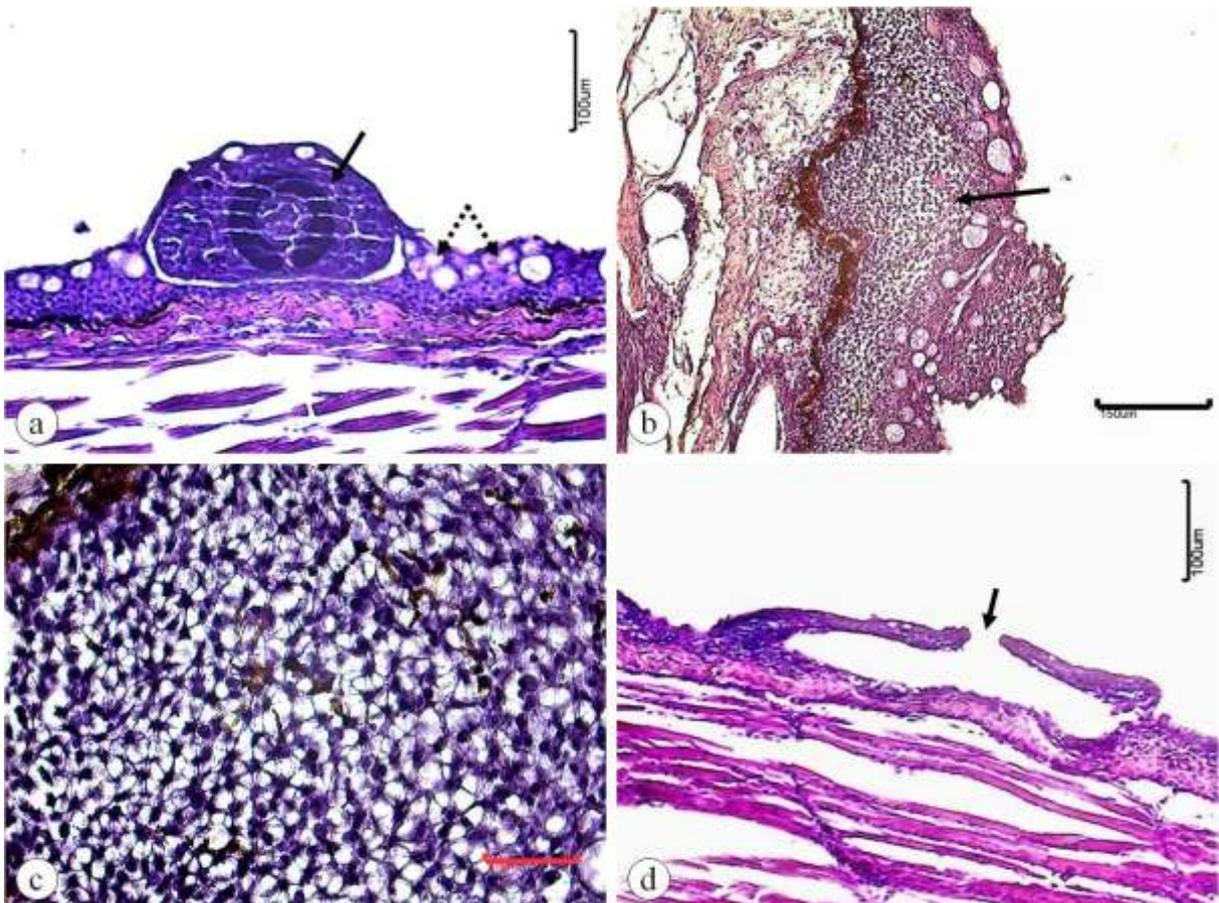
On the histological sections, the presence of *I. multifiliis* in the epithelium of the host, both in the skin (Figure 2a) and in the gill tissue, was responsible for epithelial proliferation (Fig. 2b), as well as proliferation of the club and mucous cells (Fig. 2a, 2a). Multifocal to coalescing areas of degeneration (Fig. 2b,c) and epithelial necrosis were also observed, with multifocal areas of ulceration (Fig. 2d) and inflammatory infiltration composed of PAS-positive granulocytes located in the epithelium (Fig. 3c-d) and around and within the parasite (Figure 3b, d). In the gill tissue, epithelial proliferation with fusion of the secondary lamellae, areas of necrosis, presence of mononuclear inflammatory infiltrate and mild congestion were found.

**Table 1.** Parasitological analysis in cachara catfish fingerlings (*Pseudoplatystoma reticulatum*). SI: Site of infection; EF: Examined fish; PF: Parasitized fish, P: Prevalence; MI: Mean intensity (minimum - maximum values).

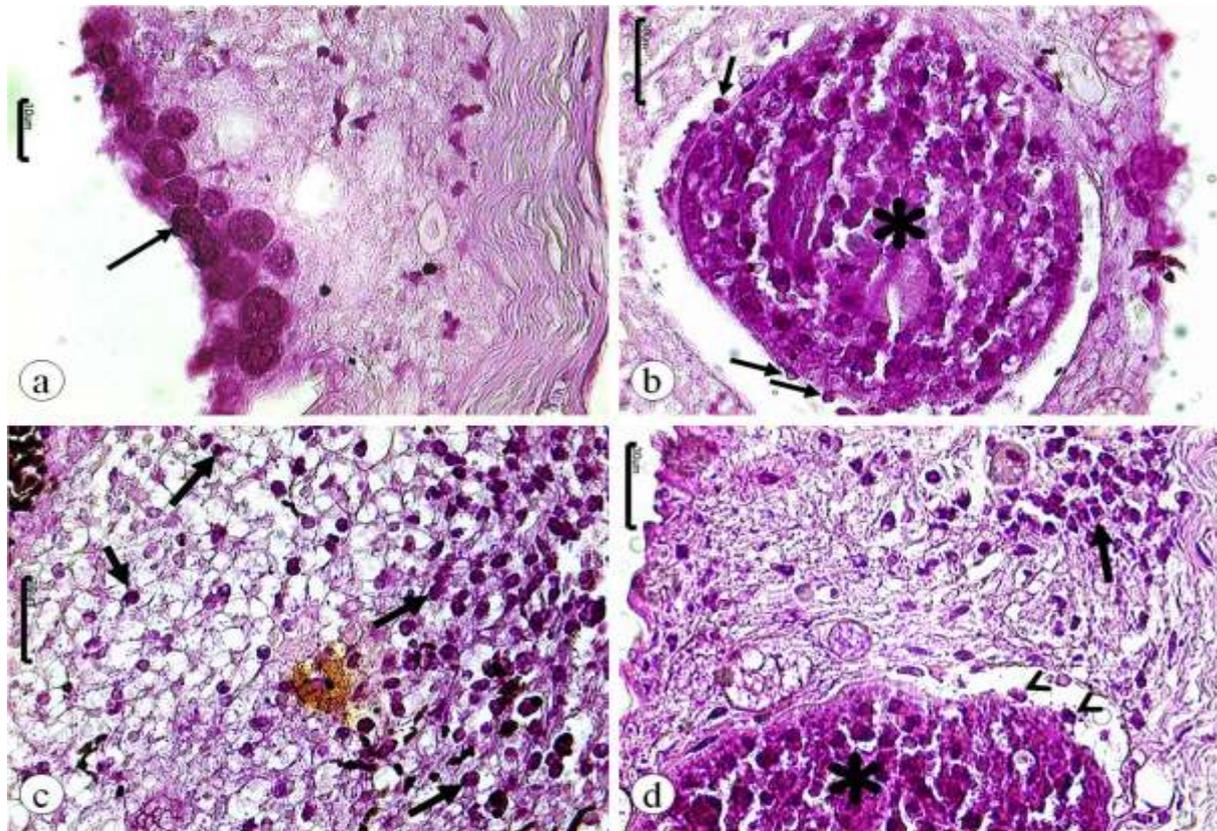
Parasites	SI	EF/PF	P (%)	MI
<b>Protozoa</b>				
<i>Ichthyophthirius multifiliis</i>	Skin, fins and gills	302/89	29.5	32.4 (1 – 154)
<i>I. multifiliis</i>	Skin and fins	302/86	28.5	23.7 (1 – 110)
<i>I. multifiliis</i>	Gills	302/64	21.2	13.2 (1 – 44)
<i>Trichodina heterodontata</i>	Skin, fins and gills	302/48	15.9	4.9 (1 – 52)
<i>Cryptobia</i> sp.	Gills	302/5	1.7	20.6 (4 – 50)
<b>Myxozoa</b>				
<i>Henneguya</i> sp.	Gills	302/1	0.3	–
<b>Platyhelminthes</b>				
Ancyrocephalinae gen. sp.	Gills	302/5	1.7	1 (1 – 1)
<b>Nemathelminthes</b>				
Nematoda larvae gen. sp.	Stomach	302/2	0.7	1 (1 – 1)



**Figure 1.** *Ichthyophthirius multifiliis* Fouquet, 1876 observed in gill fresh mount (a) and Klein's silver-impregnated *Trichodina heterodontata* Duncan, 1977, that show adhesive discs (b). Magnification 100 x (a) and bar scale 10 µm (b).



**Figure 2.** Histological alterations caused by *Ichthyophthirius multifiliis* in cachara fingerlings. Parasite in the epithelium showing the horseshoe-shaped nucleus (a – arrow) and in the adjacent tissue, mucous cell proliferation (a – dotted arrow). Hyperplasia and a multifocal to coalescing area with hydropic degeneration and necrosis can be seen (2b,c). Ulceration at the parasite site of attachment is shown in 2d. Hematoxylin-eosin staining. Bar = 100 µm (a,d), 150 µm (b) and 30 µm (d).



**Figure 3.** Histological alterations caused by *Ichthyophthirius multifiliis* in cachara fingerlings. Mucous cells marked with PAS (a), and parasite under the epithelium (b – asterisk), with several engulfed host cells and inflammatory cells adhering to the parasite surface (b - arrow). Inflammatory infiltrate composed of PAS-positive granular leukocyte (c and d – arrows); parasite inserted in the tissue (d – asterisk); and inflammatory cells engulfed and adhering to the surface (d – arrows). PAS staining. Bar = 10  $\mu$ m (a) and 30  $\mu$ m (b – d).

## DISCUSSION

In this study, the protozoan ciliates were the most prevalent parasites both *I. multifiliis* and *T. heterodontata*. In Brazil, *T. heterodontata* was first recorded in tadpoles of *Rhinella pombali* Baldissera, Caramaschi & Haddad, 2004 (Dias *et al.* 2009), and were subsequently diagnosed in channel catfish [*Ictalurus punctatus* (Rafinesque, 1818)] reared in southern Brazil (Martins *et al.* 2010a) and pacu fingerlings (*Piaractus mesopotamicus* Holmberg, 1887) farmed in the southeastern region (Pádua *et al.* 2012a). This study describes cachara (*P. reticulatum*) as a new host for *T. heterodontata*, in which more than 40 species of fish have already been described worldwide as hosts (Martins *et al.* 2010a), thus making this a

cosmopolitan parasite. It causes inflammatory and structural disturbance in the gills of the hosts with desquamated epithelial cells (Tang & Zhao, 2007), that can culminate to asphyxia and mortality.

*Ichthyophthirius multifiliis* was the main parasite found in this study, thereby confirming the findings of Pádua *et al.* (2012b) from the hybrid surubim catfish (*P. reticulatum* x *P. corruscans*) and Carneiro *et al.* (2005) from silver catfish [*Rhamdia quelen* (Quoy & Gaimard, 1824)] in central-western and southern Brazil, respectively. Pantoja *et al.* (2012) observed a positive correlation between the mean intensity of infection by *I. multifiliis* and the size and weight of juvenile tilapias. In the present study, it was observed only a positive correlation between the size of the fingerlings

and the mean intensity of infection by *I. multifiliis*. These differences might be related to different development stages of the fish, since fingerlings were used in our study, while Pantoja *et al.* (2012) used juveniles. Parasitism by *I. multifiliis* in cachara fingerlings was higher in the skin and fins than that observed in the gill tissue. We admit that greater intensity of infection in the skin and fins could be strongly associated to greater surface area for theront attachment.

The histopathological analysis showed severe tissue alterations due to *I. multifiliis* infection, that coincides with the findings of other investigators (Ventura & Paperna, 1985; Dickerson, 2006; Päck *et al.* 2011). Moreover, the ulcers formation on the skin after theront entrance might cause a portal of entry to secondary infections as demonstrated by Xu *et al.* (2012). In addition, the epithelial hyperplasia, mucous cell proliferation and necrosis in the gill tissue limit the osmoregulatory gas and ion exchanges in fish (Hines & Spira, 1974), leading to metabolic misregulation, being lethal to the host, mostly damaging the skin and fins represent a portal of entry to secondary infections.

The other parasites *Cryptobia*, myxosporeans, monogeneans and nematode larvae were found in prevalences less than 2%. In addition, the presence of Myxozoa, Monogenea and Nematoda in cachara fingerlings was lower than the studies on adult cachara from the natural environment (Campos *et al.* 2009; Naldoni *et al.* 2011; Adriano *et al.* 2012). As a result of rapid proliferation of parasites with monoxenic life-cycle in culture conditions an increase in the prevalence of *I. multifiliis* and *T. heterodontata* was found. However, adequate water quality conditions present a strong influence on the reproduction and maintenance of infection (Martins *et al.* 2010b; Jerônimo *et al.* 2011). In order to avoid the use of chemotherapics and consequently outbreaks the regular monitoring of fingerlings in nursery farms must be encouraged.

Important pathogenic parasites such as *I. multifiliis*, *T. heterodontata*, followed by *Crypyobia* sp., *Henneguya* sp., Ancyrocephalinae monogenean and Nematoda larvae were diagnosed in cachara fingerlings. Besides the ciliated protozoans *I. multifiliis* and *T. heterodontata* were the main etiological agents diagnosed, *T. heterodontata* was firstly reported on cachara. *Ichthyophthyrus multifiliis* infection induced severe tissue damage in cachara fingerlings, mostly damaging the skin and fins which can cause a portal of entry to secondary infections.

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