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Parasitological assessment in the hybrid surubim (*Pseudoplatystoma reticulatum* x *P. corruscans*), with uncommon occurrence of Monogenea parasites

Avaliação parasitológica no híbrido surubim (*Pseudoplatystoma reticulatum* x *P. corruscans*), com ocorrência incomum de parasitos Monogenea

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Abstract

This study evaluated the parasite fauna of farmed hybrid surubim (*Pseudoplatystoma reticulatum* x *P. corruscans*) and the host-parasite-environment relationship in two fish farms located in Mato Grosso do Sul, Central Brazil, South America. A total of 120 hybrids from two different farms, 60 in each season (30 in the hot and 30 in cold season) were examined during a year. Water quality was weekly measured to evaluate the interaction among environmental conditions and parasitism. Histopathology was used to observe the effects of the parasites and environment on the fish gills. The ciliate protozoan *Ichthyophthirius multifiliis* and the monogeneans (*Ameloblastella* sp., *Amphocleithrium paraguayensis*, *Vancleaveus ciccinus*, *V. fungulus* and *V. janacauensis*) were the most prevalent parasites detected in both seasons in both farms, with prevalence above 80%. It was stated that parasites did not cause important damage in the health status of the hybrid surubim. These results might be related to general good management practices and environmental quality implemented by the fish farmers. The presence of uncommon monogenean parasites to this hybrid compared to their parents causing an environmental and ecological concern is here discussed.

Keywords: Fish farming, catfish, helminthes, protozoans, water quality.

Resumo

Este estudo avaliou a fauna de parasitos do surubim híbrido cultivado (*Pseudoplatystoma reticulatum* x *P. corruscans*) e a relação hospedeiro-parasito-ambiente em duas pisciculturas localizadas no Estado do Mato Grosso do Sul, região Centro-Oeste, Brasil. Um total de 120 híbridos de duas fazendas, 60 em cada estação (30 na estação quente e 30 na fria), foram examinados durante um ano. A qualidade da água foi medida semanalmente para avaliar a interação entre as condições ambientais e o parasitismo. Histopatologia foi usada para observar os efeitos dos parasitos e do ambiente nas brânquias dos peixes. O protozoário ciliado *Ichthyophthirius multifiliis* e Monogenea (*Ameloblastella* sp., *Amphocleithrium paraguayensis*, *Vancleaveus ciccinus*, *V. fungulus* e *V. janacauensis*) foram os parasitos mais prevalentes detectados em ambas estações nas duas fazendas, com prevalências acima de 80%. Observou-se que os parasitos não causaram danos ao estado de saúde do surubim híbrido. Esses resultados estão relacionados às boas práticas de manejo e qualidade ambiental implementada pelos produtores. É discutida a presença incomum de Monogenea para esse híbrido, comparado com seus progenitores, podendo causar preocupação ambiental e ecológica.

Palavras-chave: Piscicultura, bagre, helmintos, protozoários, qualidade de água.

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Introduction

The production of native catfish is an expanding activity in Brazil, where production is concentrated in the central region of the country. Due to the spread of hybridization in aquaculture, one of the most common farmed fish are the carnivores belonging to the genus *Pseudoplatystoma* (CARVALHO-COSTA et al., 2011).

The genus *Pseudoplatystoma* comprises eight species (BUITRAGO-SUÁREZ & BURR, 2007), which includes the spotted surubim (*Pseudoplatystoma corruscans* Spix & Agassiz, 1829) and the "cachara" (*Pseudoplatystoma reticulatum* Eigenmann & Eigenmann, 1889). They are migratory fish, with great ecological importance and high commercial value in South America (BIGNOTTO et al., 2009). In nature, *P. corruscans* and *P. reticulatum* coexist in the Paraná watershed and their genetic proximity infers the ability to generate artificial hybrid catfish (BIGNOTTO et al., 2009). Currently, hybrid surubim catfish (*Pseudoplatystoma* sp.) stands in intensive production due to the great potential for industrial exploitation, rapid growth, efficient feed conversion, organoleptic quality and standard export (CREPALDI et al., 2006).

However, few studies have been carried out on parasitological assessment in hybrid surubim in Brazil. Naldoni et al. (2009) described the myxozoan *Henneguya pseudoplatystoma* from the gills of the hybrid surubim farmed in the of Mato Grosso do Sul. Jerônimo et al. (2013) evaluated the distribution of ectoparasites in the gill arches of this host under farming conditions. In the early stages of farming, Pádua et al. (2012) reported *Ichthyophthirius multifiliis, Epistylis* sp., *Trichodina* sp., *Henneguya* sp. and Monogenea as the main parasites affecting larval fish. Even in fish hatcheries, Pádua et al. (2013) showed higher parasitism by *Epistylis* sp., characterizing an emerging disease in Brazil. On the other hand, Ventura et al. (2013) have related *Epistylis* sp., *Trichodina* sp. and digenean metacercariae in post-larvae and fingerlings of the hybrid surubim.

In order to consolidate the production of this fish more studies are needed to understand the host/parasite/environment relationship to improve the knowledge of diseases responsible for economic losses (MARTINS et al., 2015). This study aimed to contribute to the knowledge of the parasite fauna in the hybrid catfish (*P. reticulatum* female x *P. corruscans* male) and to establish the host/parasite/environment relationship during the cycle of two production systems.

Materials and Methods

Sampling, fish farms and water quality

One hundred and twenty hybrid surubim were collected between June 2010 and May 2011, 60 fish in each fish farm during warm and cold seasons, 30 fish per season. Both fish farms are located in Grande Dourados region (Farm A 22° 19' 42,7" S; 54° 43' 55,2" W and Farm B 21° 52' 48,1" S; 54° 20' 39,2" W), Mato Grosso do Sul, Brazil. Fish farms are designed as A and B and their characterization is reported in Table 1.

Water quality was weekly measured: dissolved oxygen, temperature, pH and electrical conductivity were measured with multiparameter HANNA® (Hanna instruments, Inc, USA), transparency with the Secchi disk and alkalinity by titration method. Samples of farm output water were collected for orthophosphate, total ammonia, nitrite and nitrate determination by colorimetric kit (Alfakit®, Brazil).

Fish capture and parasitological survey

The fish were caught by trawl, transferred to shipping boxes with aeration and transported to the Aquaculture Laboratory of *Embrapa Western Region Agriculture* to posterior euthanasia by deep in clove oil, 75 mg L^{-1} (Ethic Committee 29979/2009-05/CEUA/UFSC 23080.0). Biometry and necropsy for parasitological analysis was according to Jerônimo et al. (2011). Helminthes

Table 1. Characteristics of fish farms used in this study.

	Fish farm A	Fish farm B
Size	20 ha	170 ha
Total water area	5 ha	130 ha
Size of pond	0.48 ha	1.2 ha
Fish farm purpose	Sportive fee fishing	Intensive production
Water supply	Water captured from Engano Stream	Individual entrance, water captured in São Domingos River
Fish origin	Commercial hatcheries	Own hatcheries
Density	1 fish.m^{-2}	4 fish.m^{-2}
Feeding	Once daily	Twice daily
Feed/Offer	36% CP, 1.5% body weight	32% CP, 1.5 - 2% body weight
Aeration	Without aeration	If necessary
Water renewal	Yes	Yes, basically by replacing
Drying of ponds	No	Yes
Fish stage production	Juvenile/Adult	Juvenile
Consumer market	Fee fishing	Domestic market and export

CP = Crude Protein.

infection levels were determined under a stereomicroscope, and the protozoans in a Sedwick Rafter chamber.

Parasites identification followed the methodology proposed by Eiras et al. (2006) and Thatcher (2006) and the parasitological descriptors were determined according to Bush et al. (1997).

Histological analysis

Gill fragments were collected and fixed in 10% phosphate-buffered formalin, dehydrated in ascending series of alcohol, embedded in historesin (hydroxyethyl metacrylate – Leica historesin embedding kit – Leica Instruments, Germany), sectioned at 2 µm thickness, stained with toluidine blue and observed under light microscopy.

Statistical analysis

Statistical analysis were performed using the Software IBM® SPSS® Statistics. Comparison of prevalence between the fish farms and between the seasons, was made by Chi-square test. The nonparametric Mann-Whitney *U* test was also used to evaluate the biometric data and the intensity of infestation. The level of significance was set at $p<0.05$. For water quality a Principal Component Analysis (PCA) of each fish farming was plotted.

Results

The mean weight, length and range of surubim collected from two fish farms in two sampling periods are reported in Table 2. Fish from farm A were significantly higher ($p = 0.001$) than that observed in fish farm B. The biggest fish were found in fish farm A in cold season ($p = 0.01$).

Analyzing the water quality of fish farm A by Principal Components Analysis (PCA) 62% of variance was explained by the two main factors (Figure 1). The results showed a factor strongly related to season and other related to water quality, highlighting a higher correlation between cold season and high levels of transparency, orthophosphate and dissolved oxygen, whereas the hot season was strongly correlated with higher levels of nitrite, nitrate and temperature. On the other hand, for fish farm B (Figure 2) PCA these factors accounted for 68% of variance, with a relationship between cold season and electrical conductivity.

In this study, were identified the ciliate protozoan *I. multifiliis* Fouquet, 1876, *Trichodina* sp. and *Epistylis* sp., the myxosporeans *Henneguya pseudoplatystoma* Naldoni, Arana, Maia, Ceccarelli, Tavares, Borges, Pozo & Adriano, 2009 and *Myxobolus* sp., the monogeneans *Ameloblastella* sp. Kritsky, Mendoza-Franco & Scholz, 2000; *A. paraguayensis* Price & Romero, 1969; *Vancleaveus ciccinus* Kritsky, Thatcher & Boeger, 1986; *V. fungulus* Kritsky, Thatcher & Boeger, 1986; and *V. janacauensis* Kritsky, Mendoza-Franco & Scholz, 2000. The cestodes were identified as *Choanoscolex abscissus* Riggenbach, 1896 and *Nomimoscolex sudobin* Woodland, 1935 and *Spatulifer rugosa* Woodland, 1935. The crustaceans *Lernaea cyprinacea* Linnaeus, 1758 and *Dolops carvalhoi* Lemos de Castro, 1949 were also identified in this study.

Ichthyophthirius multifiliis and monogeneans were the most prevalent parasites (Table 3) in both fish farms during the two seasons. *Ichthyophthirius multifiliis* showed an increase in the mean intensity of infection ($p = 0.008$) in cold season in fish farm A. Monogeneans showed a greater mean intensity in hot season in both fish farms ($p = 0.008$, $p = 0.001$) (Table 4). The myxozoan *H. pseudoplatystoma* presented the highest prevalence ($p = 0.001$) in fish farm A in hot season. In contrast, *Myxobolus* sp. did not show significant difference between the seasons and fish farms.

Among the crustacean parasites, copepodids of *L. cyprinacea* showed increased prevalence ($p = 0.001$) in fish farm B in cold season, as well as *D. carvalhoi* ($p = 0.004$). Prevalence of cestodes was higher ($p = 0.006$) in fish farm B, but did not show seasonal variation. These worms also presented the highest mean intensity of infections ($p = 0.002$) in cold season in fish farm B (Table 4).

Histological analysis revealed that all gills presented hyperplasia at the base of the secondary lamellae (Figure 3a), which may be attributed to the ammonia levels detected in both fish farms in both seasons. Monogenean parasites also caused hyperplasia and fusion of secondary lamellae (Figure 3b).

Discussion

This study reports an important a survey of parasite species affecting farmed hybrid surubim in South America. Dactylogyrids are monogeneans presenting high host specificity (LAMBERT & EL GHARBI, 1995; WHITTINGTON et al., 2000). The dactylogyrids *A. paraguayensis* and *V. fungulus* were originally described infecting native *P. corruscans* (SURIANO & INCORVAIA, 1995; TAKEMOTO et al., 2009), and this is the first report of these parasites in the hybrid surubim. However, *V. ciccinus* is originally found in *Phractocephalus hemioliopterus* (Pimelodidae), while *V. janacauensis* can be found parasitizing

Table 2. Means, standard deviation and range of hybrid surubim weight and length collected in cold and hot season in fish farms A and B, and significance of differences ($p < 0.05$) in host size between seasons and farms.

Parameters	Fish farm A		*p*	Fish farm B		*p*	Total		*p*
	Cold season (n = 30)	Hot season (n = 30)		Cold season (n = 30)	Hot season (n = 30)		Fish farm A (n = 60)	Fish farm B (n = 60)	
Weight (g)	1,222±426 (252-2250)	747±248 (300-1,170)	0.01	613±121 (338-910)	554±152 (328-816)	NS	984±421 (252-2,250)	584±140 (328-910)	0.001
Length (cm)	50.5±5.0 (36-62)	46.4±4.3 (36-57)	0.001	42.3±3.0 (36-50)	42.0±3.0 (36-47)	NS	48.5±5.0 (36-62)	42.2±3.0 (36-50)	0.001

NS = no significant.

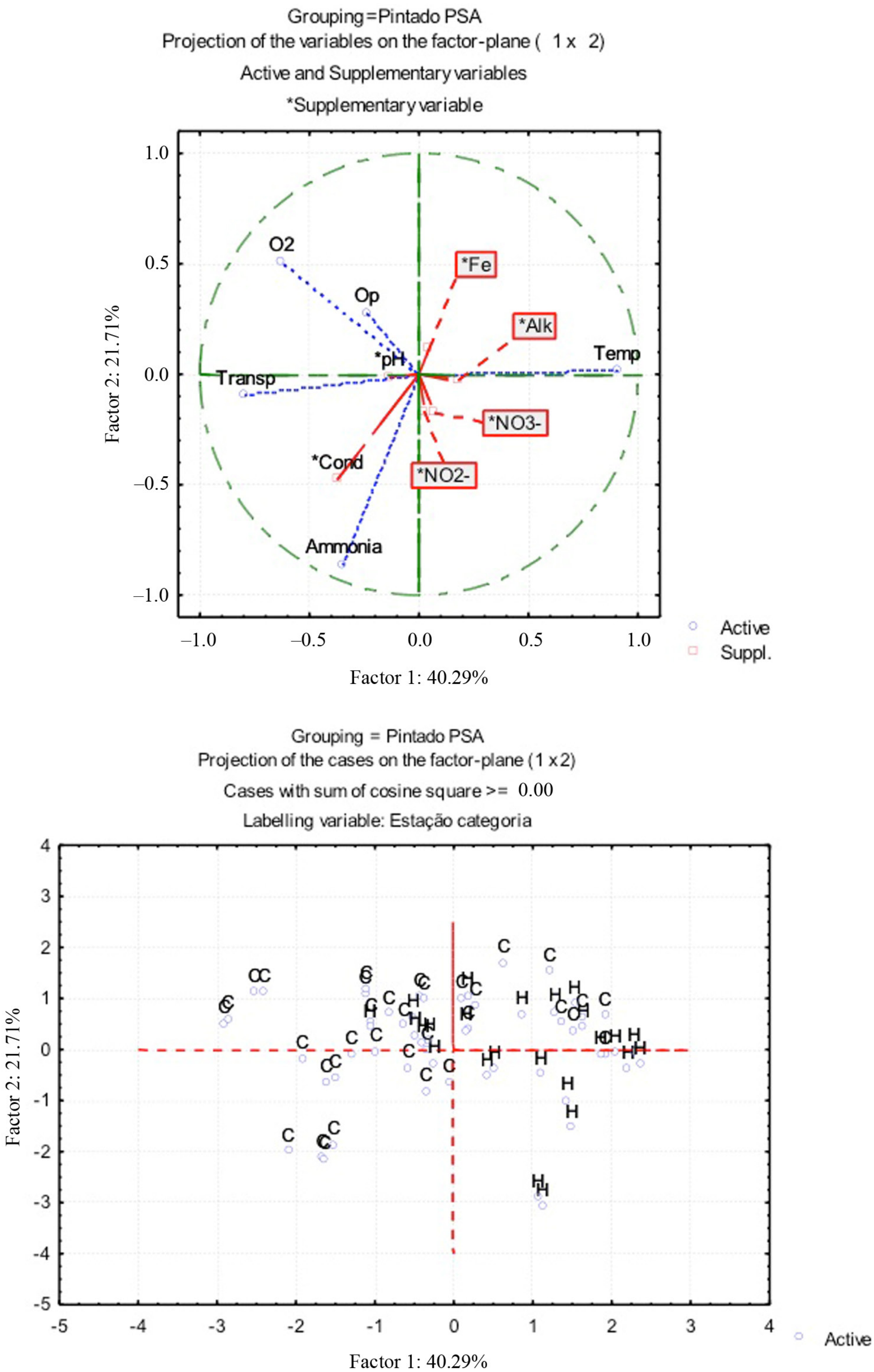

Figure 1. Principal components analysis (PCA) of water quality parameters from fish farm A (sportive fee fishing) of hybrid surubim. O_2: dissolved oxygen; Op: orthophosphate; Transp: transparency; Cond: electrical conductivity; Temp: temperature; Alk: alkalinity; Fe: iron; C: cold season; H: hot season.

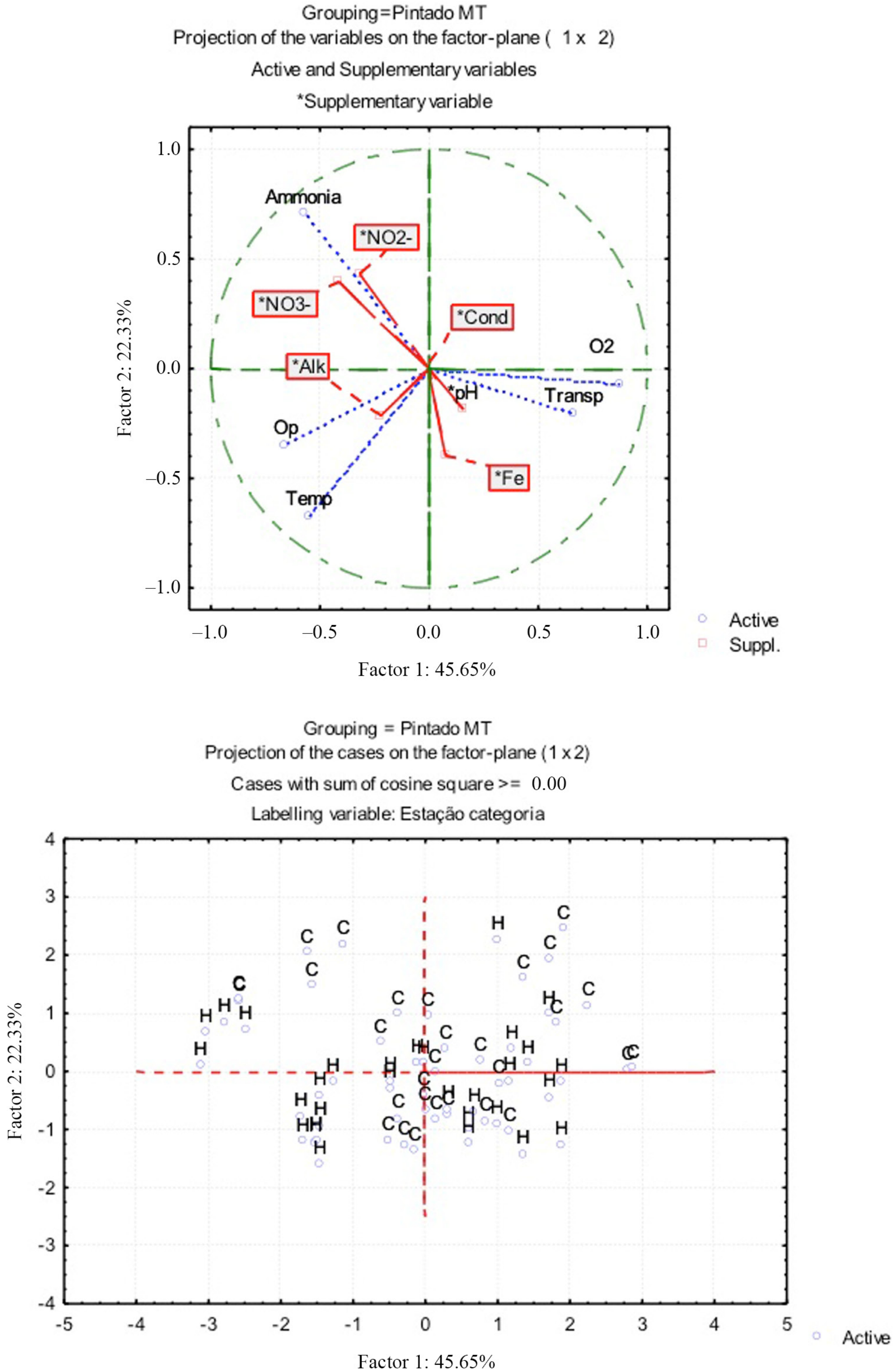

Figure 2. Principal components analysis (PCA) of water quality parameters from fish farm B (intensive production) of hybrid surubim. O_2: dissolved oxygen; Op: orthophosphate; Transp: transparency; Cond: electrical conductivity; Temp: temperature; Alk: alkalinity; Fe: iron; C: cold season; H: hot season.

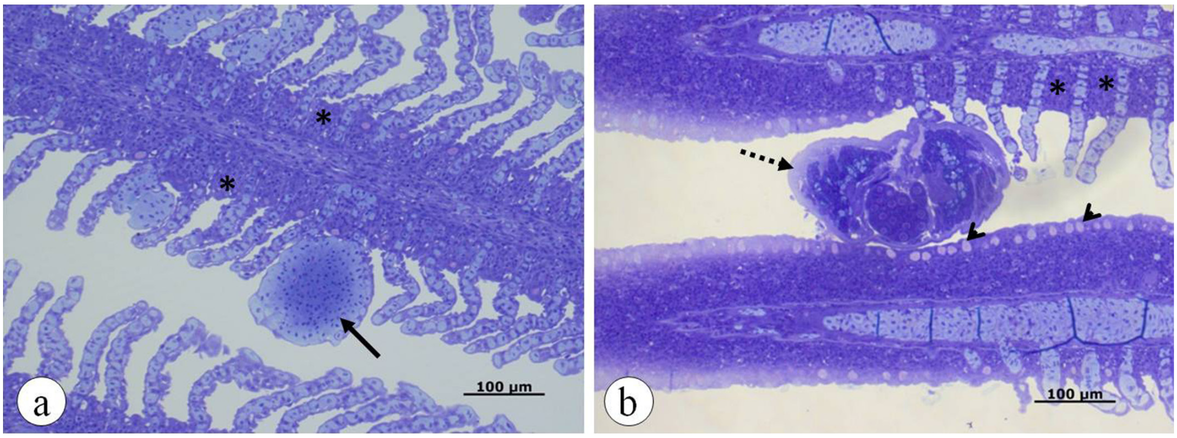

Figure 3. Histological sections of the gills of hybrid surubim. Telangiectasia (a - arrow) hyperplasia at the base of the secondary lamellae (a, b - asterisk), presence of monogenean between the gill filaments (b - dotted arrow) with hyperplasia and fusion of secondary lamellae, and hyperplasia of mucosal cells adjacent to the parasite (b fabric - arrowheads). Staining: Toluidine Blue.

Table 3. Prevalence (%) of hybrid surubim parasites. In cold and warm season in fish farm A and B and significant differences (p < 0.05) detected.

Parasites	Fish farm A			Fish farm B			Total			Site of infection
	Cold Season (n = 30)	Warm Season (n = 30)	*p*	Cold Season (n = 30)	Warm Season (n = 30)	*p*	Fish farm A (n = 60)	Fish farm B (n = 60)	*p*	
CILIOPHORA										
I. multifiliis	100	96.7	NS	100	93.3	NS	98.3	96.7	NS	Gills and skin
Trichodina sp.	0	40	0.001	0	6.7	NS	20	3.3	0.010	Gills
Epistylis sp.	*	*	*	50	30	NS	0	40	0.001	Skin
MYXOSPOREA										
Henneguya sp.	20	80	0.001	73.3	53.3	NS	50	60	NS	Gills, Liver and Kidney
Myxobolus sp.	40	20	NS	23.3	16.7	NS	30	20	NS	Liver and Kidney
MONOGENEA	100	83.3	NS	100	100	NS	91.7	100	NS	Gills
CESTODA	0	26.7	NS	50	23.3	NS	13.3	36.7	0.006	Intestine
ACANTOCEPHALA	0	3.3	NS	0	0	NS	1.7	0	NS	Intestine
CRUSTACEA										
L. cyprinacea	3.3	3.3	NS	53.3	3.3	0.001	3.3	28.3	0.001	Gills
D. carvalhoi	36.7	3.3	0.004	10	0	NS	20	5	0.027	Skin

*No observed. (NS) No significant; (*p < 0.05*) indicate significant difference between seasons for each parasite in each facility.

Table 4. Mean intensity (average ± standard deviation, minimum and maximum values in parenthesis) of parasitism in hybrid surubim from different fish farms in Central Brazil.

Parasites	Fish farm A			Fish farm B			Total		
	Cold season (n = 30)	Warm season (n = 30)	*p*	Cold season (n = 30)	Warm season (n = 30)	*p*	Fish farm A (n = 60)	Fish farm B (n = 60)	*p*
PROTOZOA									
I. multifiliis	13,351±13,971 (147-56,140)	1,919±1,402 (157-5,890)	0.004	3,258±2,909 (480-13,800)	3,265±2,600 (143-11,267)	NS	7,732±11,479 (147-56,140)	3,261±2,737 (143-13,800)	0.002
Trichodina sp.	*	344.9±221.4 (107-960)	NS	*	155±0.0 (155-155)	NS	344.9±221.4 (107-960)	155±0,0 (155-155)	NS
MONOGENEA	88±84.4 (7-378)	44.3±48.5 (2-160)	0.008	117.4±78.2 (17-383)	252.1±1.88 (65-934)	0.001	68.6±73.5 (2-378)	184,8±158,0 (17-934)	0.001
CRUSTACEA									
L. cyprinacea	3±0 (3-3)	1±0 (1-1)	NS	5.5±3.7 (1-12)	2±0 (2-2)	NS	2.0±1.4 (1-3)	5.3±3.7 (1-12)	NS
CESTODA	*	3.4±3.5 (1-10)	NS	14.2±9.4 (3-31)	3.0±2.5 (1-7)	0.002	3.4±3.5 (1-10)	10.6±9.5 (1-31)	0.024

* No observed. (NS) No significant; (*p < 0.05*) indicate significant difference between seasons for each parasite in each facility

Pterodoras granulosus (Doradidae) (SURIANO & INCORVAIA, 1995), both South American catfishes of the order Siluriformes. Dupont & Crivelli (1988) observed that cyprinid hybrids may harbor parasites from both parental species and even some from non-related species.

The occurrence of *V. ciccinus* and *V. janacauensis* in the hybrid surubim was uncommon and may be related to interspecific hybridization, which makes them the target of ecological concern by its ability to harbor unusual parasites. Šimková et al. (2013) argued that the role of fish hybridization on parasite specificity is not yet clear, there may be changes in parasitic fauna due to host co-adaptation, though hybrids may harbor parasites from both parental species. We note that this may pose an important ecological issue, because with higher numbers of cultured fish, natural stocks may be greatly affected by parasites from cultured stocks, a situation seen before in salmonids (MCVICAR, 1997; TORRISSEN et al., 2013).

Although in this study the mean intensity of monogeneans varied from 44.3 to 252.1 parasites per host, the histopathological analyzes did not show severe tissue injury associated with the parasitism. Proliferative changes in the gill epithelium are among the main disorders observed during the infestation by monogenean (MANSELL et al., 2005; DEL RIO-ZARAGOZA et al., 2010; JERÔNIMO et al., 2014) and can progress to degeneration and necrosis of respiratory tissue, especially in mixed infections with other parasites (YARDIMCI & PEKMEZCI, 2012). The severity of injuries caused by dactylogyrids are directly related to the parasite intensity (DEL RIO-ZARAGOZA et al., 2010). Sub-lethal lesions in fish were observed in varied degrees of infestations. However, higher infestation intensities result in more pronounced lesions (DEL RIO-ZARAGOZA et al., 2010). Nevertheless, in this study histopathological lesions were not limited to parasite infestation site, thus may also be due to poor water quality. These histopathological lesions are observed in fish submitted to culture conditions, including those exposed to high ammonia levels (PAUL & BANERJEE, 1997).

The proliferation of *I. multifiliis* is strongly related to periods of lower temperatures in tropical regions (MARTINS et al., 2015) coinciding with the highest water transparency, higher levels of dissolved oxygen and orthophosphate. Crustaceans were also observed in periods of low temperature. In contrast, in periods of higher temperature, an increase in the aquatic productivity occurs reflecting in nitrogen and conductivity alterations as observed on the monogenean parasitism in fish from farm B. In fact, an increase in the number of monogenean might be associated with environmental quality deterioration (MODU et al., 2012; ZARGAR et al., 2012) favoring its reproduction.

Although proteocephalid cestodes are commonly found parasitizing native *Pseudoplatystoma* spp. (SANTOS et al., 2003; CAMPOS et al., 2009), no reports were until now observed in the hybrid surubim. Its occurrence in farmed hybrid surubim was lower than that observed in cachara (*P. reticulatum*) from the natural environment (CAMPOS et al., 2008).

The undissociate ammonia molecule NH_3, is highly toxic for fish and even low levels can cause gill hyperplasia (see ROBERTS, 2012) as observed in the present study. It is strongly advisable that fish farmers increase water renewal in order to improve fish health status of this economically important fish culture.

We also highlight that the role of fish hybridization in parasite specificity is a promising field of study and understanding about it, may contribute to the development of sanitary control measures and prevent possible ecological impacts.

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