




Seasonal occurrence and variation of ectoparasites in Nile tilapia (*Oreochromis niloticus*) in the Federal District, Brazil

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ABSTRACT. The present study assesses the diversity and seasonal variation of parasites in *Oreochromis niloticus* (Nile tilapia) cultivated in excavated tanks in the Federal District, Brazil. A total of 120 specimens of *O. niloticus* were collected in 12 monthly collections. Water quality parameters were checked at all tanks. The animals, sacrificed by immersion into anesthetic solution, had the gills scraped, in which the mucus was analyzed under an optical microscope. Parasitological examination showed *Trichodina* sp., *Epistylis* sp., *Ichthyophthirius multifiliis*, and Monogenea helminths in the fish gills. We found statistically significant differences between Monogenea parasites collected in the fall and winter and between *I. multifiliis* protozoans collected in fall-winter and winter-summer periods. Except for *Epistylis* sp., all parasites showed abundance peaks in *O. niloticus* specimens collected during the winter, which may characterize the seasonality of these parasites in the Federal District region. Monogenea helminths were the most prevalent among the parasites found, with the highest prevalence during the fall. The mean abundance of parasites was similar between the fall and winter. However, the abundance of monogenetic trematodes was higher between the winter and summer. *I. multifiliis* showed significant variation between fall-winter and winter-summer periods.

Keywords: parasite infestation; helminths; protozoans; fish farming; Brazil.

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Introduction

The growth of fish farming in Brazil has been driven mainly by the international market demand and the increase in fish consumption nationally, which in 2016 was 23% higher than in 2010 (Pavanelli, Machado, & Takemoto, 1997). However, to maintain the market demand, Brazilian fish farming requires significant technical advances concerning the prophylactic control of production lots. One of the major threats to production in neotropical environments is the considerable number of pathogens in the region that can cause extensive economic losses, especially parasites (Thatcher & Brites-Neto, 1994; Dias, Neves, Marinho, Pinheiro, & Tavares-Dias, 2015).

The susceptibility to parasitism is mainly due to stress caused by inadequate management and abrupt changes in temperature and water quality, which cause an increase in plasma cortisol concentration (Strange, Schreck, & Golden, 1977). The collapse of the immune resistance in animals can cause outbreaks of parasite infestations, leading to the death of the whole lot. According to Ueda, Karling, Takemoto, and Pavanelli (2013), in the United States, parasite diseases were responsible for the loss of around US\$ 23 million in 1989. In Brazil, the economic loss caused by parasite diseases was never measured accurately (Zimmermann, Moreira, Vargas, & Ribeiro, 2001); however, parasites have a substantial role, being the leading cause of mortality and economic losses in Brazilian fish farming.

The normal development of fish, guaranteed by strict sanitary control, is crucial for the commercial acceptability of the products, where the inspection of all fish organs and structures is essential. Among the most susceptible organs, fish gills and body structure are vital targets since once parasitized, the gas exchange and the animal development might be compromised (Morgan & Towell, 1973).

Although fish farming is currently a highly profitable activity, both for domestic and international markets, there are few studies reporting the parasite fauna of freshwater fish farming. Bhuiyan and Musa

(2008) studied the intensity of infestation in carp fingerlings (*Cyprinus carpio*) by ectoparasites in Bangladesh. Thien, Dalsgaard, Thanh Nhan, Olsen, and Murrell (2009) characterized parasites in larvae and juveniles of 15 freshwater fish species in Vietnam, including a species native of Brazil, the tambaqui (*Colossoma macropomum*).

In Brazil, Vargas, Povh, Ribeiro, and Moreira (2000) characterized ectoparasites in tilapia breeders in the municipality of Maringá, Paraná, where 10% of the parasites found were monogeneans and only 2% were specimens of *Trichodina*. They also analyzed the breeders' fry, where 87% of the parasites belonged to the genus *Trichodina* and 31% to the class Monogenea. The occurrence during the spring (100%) was higher than in the summer (75%). Araújo et al. (2009) evaluated parasite infections in the fish pirarucu (*Arapaima gigas*) in the Amazon and recorded a mortality rate of 36.76% caused by monogeneans and *Trichodina* sp. Tavares-Dias, Martins, and Moraes (2001) described that all parasites found during their study of quantification and seasonality were detected in fish gills, showing a preference for many parasite class, such as Monogenea and Myxozoa, by this organ.

Therefore, it is necessary to diagnose the epidemiological situation of parasites in fish farms to develop adequate and efficient sanitary control techniques, avoiding losses in any production stage. In this study, we evaluate the parasite fauna in freshwater fish gills in properties located in the Federal District, Brazil, especially in Nile tilapia (*Oreochromis niloticus*), which has the highest production rate in the region.

Material and methods

Farming Conditions

The experiment was conducted in 12 properties located in five regions of Brasília and the Federal District (Alexandre Gusmão, Brazlândia, Ceilândia, Planaltina, Gama, Paranoá, São Sebastião, Sobradinho, Vargem Bonita, Tabatinga – Federal District and Ocidental City – Goiás State, Brazil)-Brazil. Collections were performed monthly for a year, from April 2016 to April 2017, to analyze the variation among seasons. Thirty fish were collected per season, totaling 120 fish. The analyzed fish specimens were obtained using the semi-intensive production method in excavated tanks. The fish had no special preparation for the experiment and were randomly collected in the tanks using fishing nets and then submitted to parasitological exams.

During each capture, the water temperature was measured with a bulb thermometer, and pH was measured using an electronic portable pH meter (Corning). The monthly mean precipitation was recorded based on INMET (National Institute of Meteorology of Brazil) data.

Sample collection and parasitological examination

All the procedures were conducted at the Aquaculture Laboratory and Laboratory of Parasitology and Parasitic Diseases (FAV), both from the *Universidade de Brasília* (UnB). The fish were taken to the laboratory and sacrificed through anesthetic immersion in 70 mg L⁻¹ clove oil and examined externally for possible morphological and structural changes caused by parasites. The collections were approved by the Animal Use Ethics Committee number 67/2017 of UnB.

Parasite collection from fish gills was performed by scraping the mucus off the gills with a scalpel, preserving in 10% formalin solution. The gill mucus was subsequently analyzed under an optical microscope (TIM 2008T) with 100x magnification to identify the parasites found (Tavares-Dias, 2009). The parasites were classified at the lowest possible taxonomic level, according to Thatcher (2006).

Parasite quantification was performed using a McMaster's chamber to assess the parasite infestation. After the mucus homogenization in formalin solution, 0.3 mL of this solution was placed in a counting chamber, and parasites were quantified using an optical microscope (TIM 2008T). The slides were stained with hematoxylin and eosin.

Statistical analysis

All data obtained were classified into collection season and prevalence rate. The prevalence coefficient (PC), mean intensity (MI), and mean abundance (MA) parameters for each parasite were calculated according to Bush, Lafferty, Lotz, and Shostak (1997).

Once there was no evidence to reject the hypothesis of normality of the data, according to the Shapiro-Wilk test ($\alpha = 0.05$), the seasonality effect on parasite occurrence data was analyzed using a PROC ANOVA

(one-way Analysis of Variance) procedure in SAS (Statistical Analysis System) version 9.4. Mean values were compared by Tukey's test using 5% of significance.

Results

Water analysis

Mean water temperature and pH, as well as the mean precipitation of each season, measured during collection days in all properties analyzed, are shown in Figure 1. The pH value was higher during the winter, which can be attributed to the high stocking density in excavated tanks during the colder months. However, there was no significant difference ($p > 0.05$) between the studied parameters.

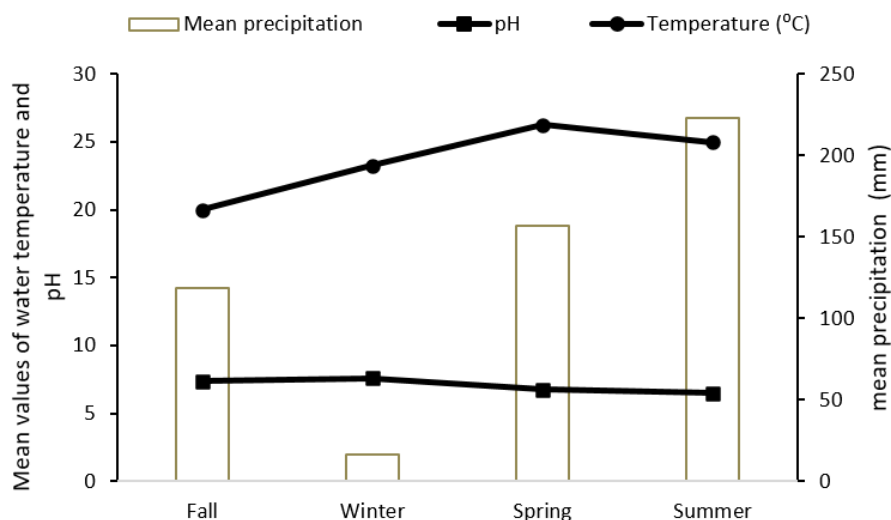


Figure 1. Mean values of water temperature (°C) and pH and mean precipitation rate (mm), evaluated in every farm visited during the experiment between April 2016 and April 2017 in the Federal District, Brazil.

Prevalence, intensity, and abundance of parasites

Parasitological examination of the specimens revealed the presence of *Trichodina* sp., *Ichthyophthirius multifiliis*, *Epistylis* sp., and Monogenea trematodes. Example of an ectoparasite detected in the gills mucus of Nile tilapia is shown in Figure 2.

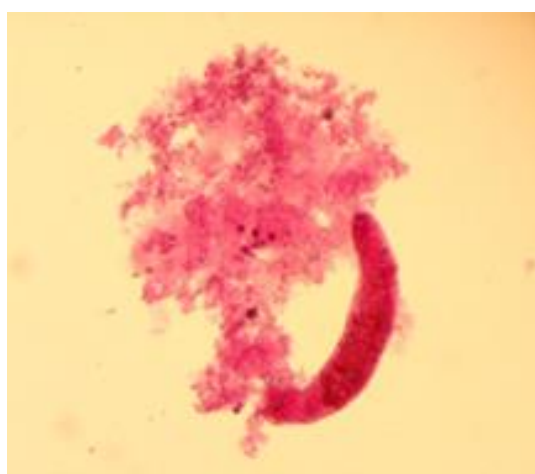


Figure 2. Monogenea helminths detected in the gills mucus of Nile tilapia using 100x magnification.

The prevalence of parasites in Nile tilapia is shown in Table 1. The parasitological analysis showed that of the 120 fish analyzed, 104 (86.6%) were parasitized. The prevalence of Monogenea, monogenetic trematode, was consistently high in all seasons. Besides, Trichodinidae occurred in all seasons. *Epistylis* sp. had low prevalence values in the fish collected in this study. Infections caused by two parasites occurred in 14.8% of the specimens, while concomitant infections by three parasites occurred in 1.4% of the fish specimens.

Table 1. Prevalence (%) of parasites in Nile tilapia (*Oreochromis niloticus*) in the Federal District region.

Parasites	Fall	Winter	Spring	Summer
<i>Trichodina</i> sp.	0	45	7.5	5
Monogenea	59.8	45	60	38.33
<i>Ichthyophthirius multifiliis</i>	0	30	0	3.33
<i>Epistylis</i> sp.	0	0	5	5

The mean intensity and abundance of parasites in Nile tilapia are shown in Table 2. The mean intensity and abundance values of Monogenea parasites varied significantly among seasons ($p < 0.05$), with winter months showing higher values than fall and summer months; however, they did not significantly differ between winter and spring. Although we found helminth infestation during all seasons, the highest prevalence occurred during the spring.

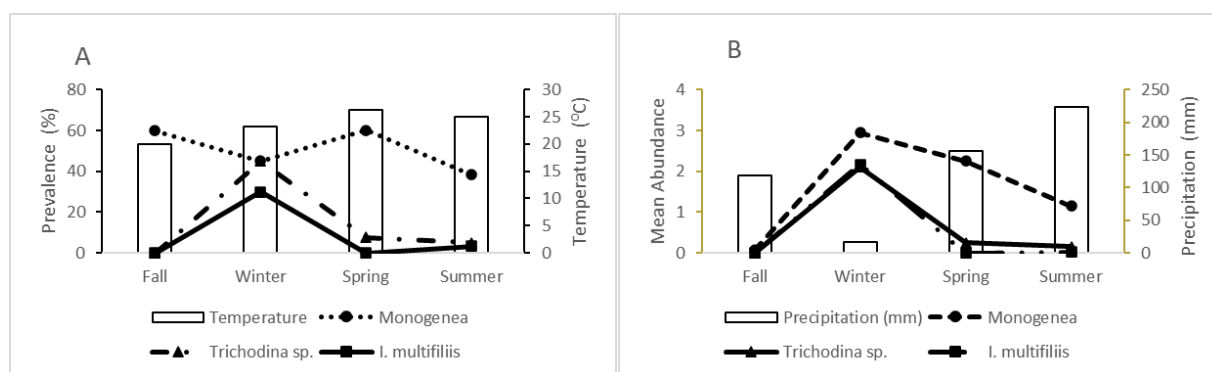
Table 2. Mean of parasite intensity and abundance in Nile tilapia (*Oreochromis niloticus*) in the Federal District region.

Parasites	Mean intensity				Mean abundance			
	Fall	Winter	Spring	Summer	Fall	Winter	Spring	Summer
<i>Trichodina</i> sp.	*	3.3	1.66	1.5	*	2.1	0.25	0.15
Monogenea	1.26 ^b	6.56 ^a	3.59 ^{ab}	2.70 ^b	0.66 ^b	2.95 ^a	2.25 ^{ab}	1.15 ^b
<i>Ichthyophthirius multifiliis</i>	*	4.75	*	0.5	*	2.17	*	0.02

(-) Indicates that the parasite was not quantified because of its colonial form; (*) Indicates that the parasite was quantified, but its presence was not detected; Mean values with different letters (^{a-b}) in the same row are significantly different ($p < 0.05$).

Epistylis sp. occurred during spring and summer with no significant variation in prevalence between the two seasons. The total number of specimens during the fall declined in the winter and spring, with a prevalence drop of 10% and 5%, respectively (Figure 3). Although the parasitism by *I. multifiliis* occurred in the winter and summer, there was no significant difference ($p > 0.05$) between the seasons. We found the highest prevalence of *I. multifiliis* during the winter, which was 88.9% higher than in the summer.

Mean intensity, abundance, and prevalence values did not vary significantly among seasons for the protozoa *Trichodina* sp. This parasite occurred during the winter, spring, and summer, with prevalence peaking in the winter, followed by a decline during the spring and summer.

**Figure 3.** (A) Water temperature and prevalence and (B) precipitation and mean abundance of *Trichodina* sp., *Ichthyophthirius multifiliis*, and Monogenea in fish examined between April 2016 and March 2017.

Discussion

The analysis of the water physical-chemical parameters did not show a statistically significant difference among seasons. However, water quality, temperature, and pH directly correlated with parasite occurrence in fish (Tavares-Dias et al., 2001). Besides, infestations by *I. multifiliis*, *Trichodina* sp., and Monogenea are directly correlated to poor water quality.

The prevalence of Monogenea helminths recorded in the present study was high, with the highest percentage (60%) in the spring and the total number of parasites 254% higher than the other parasites. The high incidence of this parasite may be due the low temperature variation and the high susceptibility to infections by this parasite in fish raised in excavated nurseries. Tavares-Dias et al. (2001) and Martins et al. (2002) showed the monogenean flatworms are among the parasites that affect fish more often. These parasites

have a predilection for the gills and are vectors for infection by other pathogens, such as bacteria and viruses (Cone, 1995). Infections by those parasites indicate poor water quality and sanitation (Pavanelli et al., 1997). The results here obtained corroborated Ghiraldelli et al. (2006), who studied gill ectoparasites in *O. niloticus* in the state of Santa Catarina, Brazil. They found a mean prevalence of 28%, 58%, and 83% of Monogenea helminths in the municipalities of Ituporanga, Blumenau, and Joinville, respectively. In another study, Tavares-Dias et al. (2001) observed a 71% prevalence of Monogenea helminths in *Piaractus mesopotamicus* and a 12.8% prevalence in *O. niloticus*.

The prevalence of *Trichodina* protozoa was 45% during the winter. The low parasitism by this protozoan may be due to the high water quality in all tanks, which hinders disease outbreaks related to environments rich in organic matter. The low parasitism caused by *Trichodina* species does not cause significant losses in fish farms. However, when the infestation is considerable, lesions may occur in the gills and cause hypersecretion of mucus, leading to death (De Pádua, Ishikawa, Kasai, Jerônimo, & Carrijo-Mauad, 2012; Hossain, Hossain, Rahman, Akter, & Khanom, 2008). Tavares-Dias et al. (2001) analyzed the parasite fauna in fish species in the state of São Paulo, Brazil, and observed higher mean intensity values of *Trichodina* sp. However, they found similar results of prevalence in *P. mesopotamicus*. The prevalence values of *Trichodina* sp. in *O. niloticus* obtained in this study are higher than the results obtained by Azevedo, Martins, Bozzo, and De Moraes (2006) and Tavares-Dias et al. (2001), while our values of intensity and abundance were lower. The highest prevalence rate observed can be explained by the different management to which these animals are subjected, such as high stocking density and constant entry of animals from other farms. This hypothesis is supported by the fact that a large amount of organic matter favors the proliferation of this parasite (Ghiraldelli et al., 2006).

For *Ichthyophthirius multifiliis*, a ciliate parasite, the mean prevalence and abundance values observed were low, reaching their peak in the winter, with a prevalence of 30%. This parasite is probably the protozoan with the highest potential to cause damage to fish. Although the low values may result from an environment that is not conducive to this parasite development, the mean temperature of 26°C is within the ideal range for a rapid life cycle of this animal. Ventura et al. (2013) observed a low prevalence of this protozoan in post-larva of jundiara (*Pseudoplatystoma* sp.) under a mean water temperature of 26°C. These results corroborate Araújo et al. (2009), which studied pirarucu (*Arapaima gigas*) fry from environments with water temperatures above 28°C and did not find *I. multifiliis*. Correlating the outbreak of *I. multifiliis* with water temperature is essential to aquaculture. The rapid reproductive capacity of this animal combined with the lesions caused by intense infestations that, when located in the gills, cause hypersecretion of mucus and compromise the gas exchange, causing extensive economic losses for fish farming (Buchmann & Bresciani, 1997).

The prevalence of the protozoan *Epistylis* sp. was 5% during the spring and summer. The low prevalence of this parasite indicates that water quality and the amount of organic matter present in the environment did not favor infestation. This parasite lodges itself in the host and feeds on suspended particles in the water, causing no direct damage to the fish's health when in low parasitic load. However, in environments with high organic matter concentration, excessive growth of *Epistylis* sp. population may favor the appearance of ulcers, which can serve as an entry point for bacterial infections (De Pádua et al., 2012) and obstruct the flow of water through the gills, causing death by asphyxiation. That shows how the prevalence of this protozoan can vary among fish in the same production system. The prevalence values of *Epistylis* sp. obtained by Ghiraldelli et al. (2006), of 4.1 and 7.9% for Blumenau and Joinville, Santa Catarina State, Brazil, respectively, corroborate the values obtained in this study.

Monogenea helminths were present in all seasons; however, they showed temporal differences regarding their mean prevalence and abundance. Reduced feeding activity in the winter, combined with low water temperatures, could explain the higher values during June, July, and August (Jansen & Bakke, 1995; Tavares-Dias et al., 2001) since the reduction of fish metabolism impairs their immunity and, consequently, increases the susceptibility to diseases.

Infections by *Trichodina* sp. occurred in the winter, spring, and summer and did not differ significantly between these seasons. The steep increase of this protozoan in the winter is supported by the literature, which shows that the infestations caused by *Trichodina* sp. usually occur in the winter (Meyer, 1970) due to low temperatures. The study of Tavares-Dias et al. (2001) reinforces this hypothesis since they also observed an increase of this parasite prevalence in the winter, decreasing again during the spring.

The ciliated protozoa *I. multifiliis* was recorded only in the winter and summer, showing the seasonal influence on this parasite occurrence. These results corroborate the literature, which suggests that the

window of optimal temperature for the parasite to complete its cycle varies from 20 to 25°C (Meyer, 1970), supporting the peak prevalence of *I. multifiliis* during the winter. Fall had the lowest temperature recorded; however, *O. niloticus* specimens did not show infection by *I. multifiliis*. According to Thatcher and Brites-Neto (1994), this parasite blooming occurs in tanks with poor water quality after sudden temperature fluctuations or inadequate handling due to stress, indicating that the prophylaxis was used efficiently by fish farmers in the Federal District region.

Conclusion

Parasitological examination of the specimens showed the presence of *Trichodina* sp., *Ichthyophthirius multifiliis*, *Epistylis* sp., and Monogenea trematodes. Monogenea helminths were the most prevalent among the parasites found, with the highest prevalence in the fall. The mean abundance of parasites was similar between fall and winter, when the mean pH value was higher. However, the abundance of monogenetic trematodes was higher during the winter and summer. *Ichthyophthirius multifiliis* showed significant variation between fall-winter and winter-summer.

The results reinforce the influence of temperature on this parasite occurrence. *Trichodina* sp. and *I. multifiliis* reached their parasitic peaks in the winter. Infections by *Trichodina* sp. occurred in the winter, spring, and summer and did not differ significantly between these seasons. *Epistylis* sp. occurred with a low prevalence in the spring and summer.

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