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ORIGINAL

Protozoan and metazoan parasites of Nile tilapia *Oreochromis niloticus* cultured in Brazil

Parásitos protozoarios y metazoarios de la tilapia del Nilo *Oreochromis niloticus* criadas en Brasil

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ABSTRACT

Objective. This study describes the parasitic fauna and relative condition factor (Kn) in Nile tilapia *Oreochromis niloticus* L. (Cichlidae) from fish farms in the State of Amapá. **Material and methods.** 123 fish from four fish farms in the state of Amapá, Brazil were necropsied for parasitological and Kn analysis. **Results.** 64.2% of the examined fish, had the gills infected with *Cichlidogyrus tilapiae* Paperna, 1960 (Monogenoidea: Dactylogyridae); *Ichthyophthirius multifiliis* Fouquet, 1876 (Protozoa: Ciliophora), *Trichodina* Ehrenberg, 1830 and *Paratrichodina africana* Kazubski & El-Tantawy, 1986 (Protozoa: Trichodinidae). The highest prevalence found corresponded to Monogenoidea *C. tilapiae* while the lowest corresponded to Trichodinidae. However, *I. multifiliis* was the parasite that presented the greatest intensity and abundance. The differences found in the infection rates of the different fish farms due to causes further discussed. The parasitism did not influence the relative condition factor (Kn) of fish. This was the first record of *P. africana* in Brazil and occurred in the Eastern Amazon. **Conclusions.** In Brazil, *Lamproglena* sp. is an emerging parasite in the Southern and Southeastern regions, but this crustacean was not found in the Nile tilapia in the State of Amapá. The parasitic infections in Nile tilapia farmed in Brazil are caused by protozoan, monogenoidea, crustacea and digenea species, and the regional differences on their prevalence and intensity rates are discussed in this study.

Key words: Freshwater fishes, parasites, prevalence, *Oreochromis niloticus* (*Source: CAB*).

RESUMEM

Objetivo. Describir la parasitofauna y el factor de condición relativa (Kn) de la tilapia del Nilo *Oreochromis niloticus* L. (Cichlidae) en granjas piscícolas del estado de Amapá. **Materiales y métodos.** 123 peces, de cuatro granjas piscícolas del Estado del Amapá, Brasil, fueron necropciados para realizarles un análisis parasitólogico y el análisis Kn. **Resultados.** De los peces examinados, 64.2% estaban con las branquias infectadas por *Cichlidogyrus tilapiae Paperna*, 1960 (Monogenoidea: Dactylogyridae), *Ichthyophthirius multifiliis* Fouquet, 1876 (Protozoa: Ciliophora), *Trichodina* Ehrenberg, 1830 y *Paratrichodina africana* Kazubski & El-Tantawy, 1986 (Protozoa: Trichodinidae). La mayor prevalencia fue de Monogenoidea *C. tilapiae*, mientras que la menor fue de los parásitos Trichodinidae. Sin embargo, *I. multifiliis* fue el parásito que mostró la mayor intensidad y abundancia. Las diferencias encontradas estuvieron en las tasas de infección parasitaria de diferentes granjas piscícolas debido a las causas aquí discutidas. El parasitismo no influenció el factor de condición relativa (Kn). Este fue el primer registro de *P. africana* para el Brasil, en la Amazonía Oriental. **Conclusiones.** En el Brasil, *Lamproglena* sp es un parásito emergente en las regiones del Sur y Suroeste, pero este crustáceo no fue encontrado en la tilapia del Nilo del Estado de Amapá. En la tilapia del Nilo criada en el Brasil, las infecciones parasitarías son causadas por especies de protozoarios, monogenoideas, crustáceos y digenéticos; las diferencias regionales en las tasa de prevalencia e intensidad son discutidas aquí.

Palabras clave: Parásitos, peces de agua dulce, prevalencia, *Oreochromis niloticus* (*Fuente:CAB*).

INTRODUCTION

The freshwater aquaculture in Brazil has been growing, driven especially by fish farming, which represents the bulk of the domestic production. The Nile tilapia *Oreochromis niloticus* is the species making the greatest contribution to the growth of this production, representing 39% of all fish from freshwater fish farming (1). Culture of this fish occurs mainly in the Northeastern, Southern, Midwestern and Southeastern areas, but the largest production is found in the Northeast (1). This production at high stocking densities is done mainly in tanks, besides ponds.

In northern Brazil, the production of Nile tilapia is small, since it is cultured only in the States of Rondônia, Acre, Pará and Amapá. In the state of Amapá, the Nile tilapia was introduced in the early 90's by the former Aquiap (Aquaculturers of Amapá Association). The choice for the cultivation of this non-native fish in the state of Amapá was due to its rapid reproduction rate which allows the quick replenishment of the tanks of the pirarucu *Arapaima gigas*. Thus, the production of the Nile tilapia grew from 2004 to 2007, going from 10 to 30 tons (1).

The fish live in balance with the parasites, but this balance can be broken, mainly by environmental disturbances, among which the changes in the water quality have a relevant role (2,3), as well as inadequate management and high stocking densities of fish (3,4). Therefore, in systems of intensive culture, problems of infections caused by protozoan and metazoan are quite frequent. Protozoan parasites are common in farmed fish and can cause economic losses in fish farms. Metazoan are parasites that can cause gill infections, damage to eyes and internal organs, starvation, inflammation of the swim bladder, and inhibited oxygen exchange across gill lamella. They provide portals of entry for bacteria in fish. Therefore, these parasites can be limiting factors for the development of fish farm as they cause low growth of fish and diseases, reducing profitability and increasing the costs of production due to treatments. Thus, epidemiological studies in fish farms are important for adapting the management techniques and providing sanitary guidelines.

In Brazil, the parasitic fauna in Nile tilapia has been studied primarily in fish farms in the states of São Paulo, Santa Catarina and Paraná, but the information are scattered in the literature. In addition, there is no information about the parasitic fauna in this cichlid cultured in the North, so some important issues remain open. Thus, the objectives of this study were to determine the prevalence rate and mean intensity of protozoan and metazoan parasites, as well as the condition factor in *O. niloticus* cultured in the State of Amapá (Northern region).

MATERIAL AND METHODS

Study site. Specimens of Nile tilapia were collected from August 2009 to March 2010 in four fish farms in the city of Macapá, state of Amapá (Brazil) for parasitological analysis.

Conservation fishes. In ponds of different sizes, the fish were maintained with an artificial diet and ignored stocking density, since they were not sexually reversed.

Parasitological analysis. All fish were collected with net, weighed (g) and measured (cm). Then, they were necropsied for parasitological analysis. Each specimen had its mouth, opercula, gills and gastrointestinal tract examined. The methodology used for collection, fixation and quantification of parasites followed previous recommendations (5,6). Identification of parasites was done in accordance to suggestions from the literature (7-9). The ecological terms were according to Bush et al (10) and Rhode et al (11).

Data analysis. With the weight and total length data, the relative condition factor (Kn) of the parasitized and non-parasitized fish was determined. The differences between parasitized and non-parasitized fish were compared through the test t ($p<0.05$). Spearman's rank (rs) correlation coefficient was used to determine possible correlations between the total length and weight of the hosts and the number of parasites. At each fish collection, the potential for hydrogen (pH), the temperature and dissolved oxygen concentration (DO) of the nurseries were measured with digital (YSI) equipments, respectively.

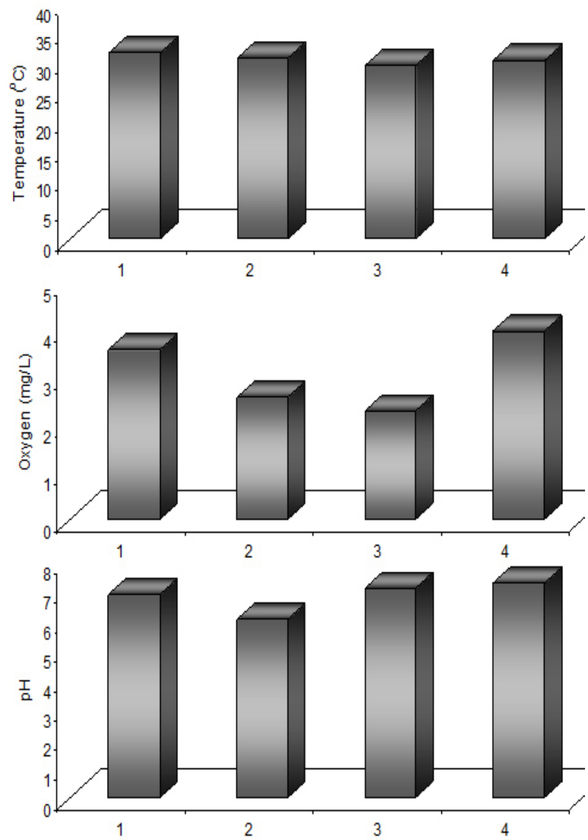

Figure 1. Physicochemical parameters of water quality in ponds from four fish farms in the State of Amapá.

RESULTS

The temperature and the pH of ponds water were similar; however, the dissolved oxygen levels were lower in the fish farm 2 and 3 (Figure 1).

A total of 123 Nile tilapia were examined in four fish farms in Macapá (State of Amapá) and weigh and total length mean ± standard deviation are described on Table 1. In the four fish farms, 64.2% of fish were parasitized by one or more parasites (Table 1), such as: *Ichthyophthirius multifiliis* Fouquet, 1876 (Protozoa), *Paratrichodina africana* Kazubski & El-Tantawy, 1986 (Protozoa: Trichodinidae), *Trichodina* Ehrenberg, 1830 (Protozoa: Trichodinidae) and *Cichlidogyrus tilapiae* Paperna, 1960 (Monogenoidea: Dactylogyridae). The highest prevalence of parasitic infection was observed in the fish farm 2 and the lowest prevalence in the fish farm 4. In the other fish farms (1 and 3) there was not a significant difference in the prevalence, which was of 73.6% and 76.0% respectively (Table 1).

Infections by *I. multifiliis* were observed in Nile tilapia cultured in three of the four fish farms investigated. However, the lowest rates of parasitism occurred in the fish farm 1 and the highest in the fish farm 3. The rates of infection by Trichodinidae were similar in the three fish

Table 1. Mean values ± standard deviation of weigh and total length of Nile tilapia collected in four fish farms from the state of Amapá. EF: examined fish; PF: parasitized fish, P: Prevalence.

Fish farms	Geographic coordinates	Weight (g)	Length (cm)	EF	PF	P (%)
1	0°02'31.4"S, 051°07'34.4"W	44.0 ± 31.7	12.6 ± 2.7	38	28	73.6
2	0°00'58.1"S, 051°06'31.8"W	51.1 ± 44.9	12.9 ± 3.7	32	29	90.6
3	0°00'36.8"S, 051°06'13.7"W	135.8 ± 36.7	19.1± 2.0	25	19	76.0
4	0°00'04.5"N, 051°05'52.1"W	55.2 ± 68.8	12.6 ± 4.2	28	3	10.7
Total	-	-	-	**123**	**79**	**64.2**

Table 2. Parasitological indices of *Ichthyophthirius multifiliis* and Trichodinidae on the gills of Nile tilapia from four fish farms from the state of Amapá.

Parasites	Ichthyophthirius multifiliis				Trichodinidae			
Fish farms	1	2	3	4	1	2	3	4
EF	38	32	25	28	38	32	25	28
PF	13	17	19	0	3	1	1	0
P (%)	34.2	53.1	76.0	0	7.9	3.1	4.0	0
MI	700.8	7183.9	75.198,4	0	1957.3	6800	9894	0
MA	239.7	3816.5	57.150,8	0	1545	206.1	395.7	0
Range	120-2550	4.100-14.800	13.108-282.785	0	735-3180	-	-	0
TNP	9110	122.127	1.428.770	0	5872	-	-	0

EF: examined fish; PF:parasitized fish; P:Prevalence; MI:Mean intensity of infection; MA:Mean abundance; TNP:Total number of parasites.

farms in which there was parasitism (Table 2) and two species were identified. *P. Africana* was found in the fish farm 1 and *Trichodina* sp. was found in the fish farms 2 and 3.

In tilapia from the fish farms 3 and 4, infection rates for *C. tilapiae* were lower than the ones from the fish farms 1 and 2 (Table 3).

Table 3. Parasitological indices of *Cichlidogyrus tilapiae* on the gills of Nile tilapia from four fish farms in the state of Amapá.

Fish farms	1	2	3	4
EF	38	32	25	28
PF	28	22	2	3
P (%)	73.7	68.7	8.0	10.7
MI	12.3	7.6	3.5	11.0
MA	9.0	5.2	0.28	1.2
Range	2-51	3-17	1-6	4-23
TNP	343	168	7	33

EF:examined fish; PF:parasitized fish; P:Prevalence; MI:Mean intensity of infection; MA:Mean abundance; TNP:Total number of parasites.

The Protozoan *I. multifiliis* was the parasite with the greatest abundance and relative dominance (Table 4) and it showed a positive correlation with the weigh and length of *O. niloticus* (Figure 2).

Table 4. Total parasitological indices in Nile tilapia in the state of Amapá.

Parasites	*C. tilapiae*	*I. multifiliis*	Trichodinidae
EF	123	123	123
PF	55	49	5
P (%)	44.7	39.8	4.1
MI	10.0	31.836,9	4513.2
MA	4.5	12.683	183.5
TNP	551	1.560,007	22.566
MRD	0.0003	0.9854	0.01425

EF: examined fish; PF: parasitized fish; P: Prevalence; MI: Mean intensity of infection; MA: Mean abundance; TNP: Total number of parasites; MRD: Mean relative dominance.

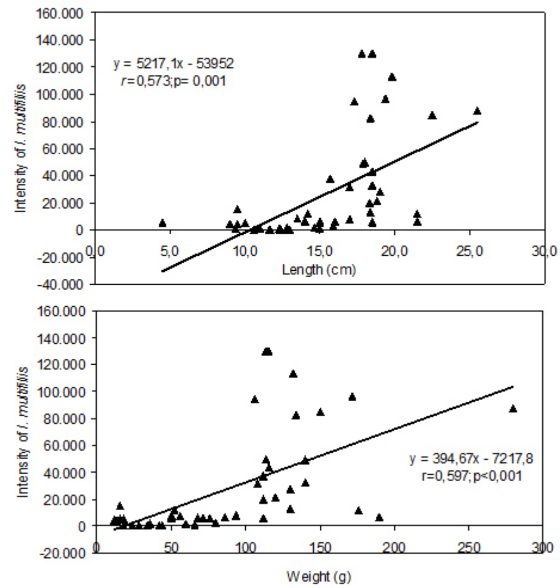

Figure 2. Intensity ratio of *Ichthyophthirius multifiliis* with the total length and weigh in Nile tilapia (N=48) cultured in the state of Amapá.

On the other hand, Trichodinidae *P. africana* and *Trichodina* sp. were the less prevalent parasites and showed a higher relative dominance when compared to *C. tilapiae* (Table 4).

Kn of parasitized (0.999±0.012) and non-parasitized (1.00±0.03) *O. niloticus* showed no significant difference (p=0.676).

DISCUSSION

In Brazil, Trichodinidae are the most common protozoan affecting cultivated Nile tilapia, especially in the South where their culture has been intensified (Table 5). However, few species are described in Brazil, since in general, they are described as *Trichodina* sp (3,5,12-23). These parasites are important agents causing diseases in Nile tilapia and most Trichodinidae species do not show host specificity (24). In Nile tilapia grown in Bangladesh, Trichodinidae were the most frequent parasites, with a prevalence ranging from 24.2–90.2%, depending on the fish farm and the time of the year. In addition, the high prevalence proved to be correlated with the high stocking density of fish and with the physicochemical parameters of the water (2). Therefore, these results indicate the aggregate pattern of distribution of the Trichodinidae, causing these high prevalence rates when fish are kept in high stocking densities during culture.

Table 5. Parasites of Nile tilapia cultured in different Brazilian states.

Group/Species	Culture/State	Prevalence (%)	Mean Intensity	References
PROTOZOA				
I. multifilis	Feefishing (SP)	4.0	76.0	5
I. multifilis	Net cage (SP)	3.2	-	14
I. multifilis	Fish farm and Feefishing (PR)	21.3-25.0	-	15
I. multifilis	Feefishing (PR)	1.8-2.5	-	12
I. multifilis	Fish farm (PR)	26.0	-	16
Trichodina sp.	Feefishing (PR)	15.8-18.3	-	12
Trichodina sp.	Fish farm (PR)	43.0	-	16
Trichodina sp.	Fish farm (PR)	62.5-72.5	-	15
Trichodina sp.	Fish farm (PR)	17.0-72.0	-	17
Trichodina sp.	Net cage (PR)	13.3-50.0	-	18
Trichodinidae	Net cage (PR)	13.9-17.4	-	19
Trichodina sp.	Net cage (SP)	24.0-38.1	-	14
Trichodina sp.	Fish farm (SP)	8.0	243	5
Trichodina sp.	Feefishing (SC)	1.6	-	20
Trichodina magna	Fish farm (SC)	24.7	-	22
Trichodina compacta	Fish farm (SC)	24.7	-	23
T. compacta and *T. magna*	Fish farm (SC)	10.0-51.0	55.1-621.9	21
T. compacta and *T. magna*	Fish farm (SC)	81.0	-	13
T. compacta and *T. magna*	Fish farm and Feefishing (SC)	0.6-1.7	-	3
MONOGENOIDEA				
Cichlidogyrus sp.	Fish farm (RJ)	12.8	1.1	25
Dactylogyrus sp.	Net cage (SP)	52.8-83.3	65.6-112.8	26
Cichlidogyrus sclerosus and *Cichlidogyrus* sp.	Fish farm (SP)	6.7-43.8	3.6-7.3	4
Dactylogyridae	Fish farm (PR)	3.3-10.0	-	18
Dactylogyridae	Feefishing (PR)	25.8-53.3	-	12
Dactylogyrus sp.	Fish farm (PR)	49.0	0.8	16
Gyrodactylogyridae	Feefishing (PR)	0.8	-	12
Cichlidogyrus sp. and *C. sclerosus*	Feefishing (SC)	13.3	4.2	20
Cichlidogyrus sp., *C. sclerosus* and *Gyrodactylus* sp.	Fish farm (SC)	28.0-83	1.3-34.5	21
Cichlidogyrus sp., *C. sclerosus* and *Gyrodactylus*	Fish farm (SC)	76.0	-	13
Cichlidogyrus sp. and *C. sclerosus*	Feefishing (SC)	13.2-16.5	0.8-2.6	3
DIGENEA				
Clinostomum complamatum	Fish farm (RS)	100	-	27
Diplostomum sp.	Net cage (SP)	4.8	-	14
CRUSTACEA				
Ergasilidae	Fish farm (SP)	18.0	3.4	6
Ergasilus sp.	Fish farm (RJ)	18.2	2.0	25
Lamproglena sp.	Fish farm (RJ)	60.0	3.4	25
Lamproglena sp.	Fish farm (SP)	67.4	5.2	4
Lamproglena sp.	Feefishing (SC)	3.3	1.5	20
Lamproglena sp.	Fish farm (SC)	3.0-22.0	0.3-0.8	21
Lamproglena sp.	Fish farm (SC)	9.0	-	13
Lamproglena sp.	Fish farm (SC)	0.5-1.7	0.1-0.2	3
Argulus spinulosus	Fish farm (SC)	33.0	1.7	21
Dolops carvalhoi	Net cage (SP)	1.6	-	14

Several species of Trichodinidae are distributed worldwide due to the transcontinental introduction of fish (24). Martins & Ghiraldelli (22) mention that the *Trichodina*, *Trichodinella* and *Paratrichodina* have been described parasitizing tilapia. *Trichodina* compacta is common in the skin and gills of several families of freshwater fish from Africa, Taiwan and Philippines, but it has a clear preference for Cichlid species (24). *Paratrichodina africana* occurs in 100% of tilapia from Lake Vitoria in Kenya and in 64.7% of tilapia from the Nile Delta in Egypt (8,9). In tilapia *O. niloticus* from three fish farms in the state of Amapá, rates of infection by *Trichodina* sp. and *P. Africana* were similar. However, the prevalence was lower than that reported for the same host grown in other regions of Brazil, while the intensity was higher (Table 5). Trichodinidae reproduction is favored by the excess of organic matter in the culture ponds (3,21,22) and by the high temperatures (2,3) such as the ones that occur in the region of this study.

Ichthyophthirius multifiliis is one of the biggest responsible for significant economic losses in fish farms worldwide (28). In Nile tilapia reared in several localities in Brazil, it is the second protozoan causing infections (Table 5), which proves its great adaption also in tropical areas. In the gills of *O. niloticus* cultivated in the State of Amapá, the intensity of *I. multifiliis* was positively correlated with weigh and length, which indicates an increase of parasitism according to the growth of fish. An increase in the number of parasites is due to cumulative process since the gills increase their surface area in proportion to an increase in fish growth (25). There is a proportional increase in habitat for reproduction of this protozoan.

The ichthyophthiriasis often manifests itself after handling operations during cold seasons and in other stressful situations (5). High rates of infections by *I. multifiliis* were found in tilapia from three fish farms in the state of Amapá, in the eastern Amazon, where temperatures are higher and more constant than in other Brazilian regions. However differences in the abundance and prevalence for the same host in different regions may be due to the balance between the host immune system and the performance of the parasite.

Monogenoidea is the main metazoan parasite infecting cultured tilapia in Brazil, mainly *Cichlidogyrus* Paperna, 1960 (Table 5). However there are few records of mortality caused by severe infections in cichlid fish. These parasites have been responsible for 80.0% of the infections in Nile tilapia grown in the state of Santa Catarina; 40.0% in the ones grown in the state of São Paulo and 16.0% in the ones grown in the state of Paraná (29). Parasitism by *C. tilapiae* was high only in tilapia from two of the investigated fish farms. However the indices were similar to the ones described for this same host grown in the southern Brazil (Table 5). Banu & Khan (2) have demonstrated that in tilapia grown in Bangladesh, Monogenoidea was the second most frequent parasite throughout the year and that their prevalence was correlated with the physicochemical parameters of the water. In a polluted environment, there is a decrease in the abundance of *Cichlidogyrus sclerosus*, as well as in the immunological resistance of tilapia, thereby increasing the persistence of this infection. Therefore, in tropical environments, this parasite and its host are useful bio indicators of the impact of environmental quality (30). Nevertheless, the severity of the disease also depends on the pathogenicity of the Monogenoidea species (3) and the nutritional conditions of the host.

In Nile tilapia from the states of São Paulo, Rio de Janeiro and Santa Catarina (Brazil), the emerging parasite *Lamproglena* sp. (Table 5) has been found since 2000. In the southeast, this crustacean parasite has arisen with the intensification in tilapia culture in cages. However, *Lamproglena* sp. has not been reported in the Brazilian Amazon, including the state of Amapá. Besides, other parasites have been known to infect this cichlid species in Brazil (Table 5). In Brazil, the diversity of digenean and other crustacean parasitizing Nile tilapia is low (Table 5), but it has also acquired parasites common in native fish, such as o *Clinostomum* sp., *Diplostomum* sp., *Ergasilus* sp., *Argulus spinulosus* and *Dolops carvalhoi*.

In conclusion, this study highlights that the diversity of parasites reported for *O. niloticus* grown in Brazil was higher than the one found in the state of Amapá, probably due to differences in size and age of fish, water quality, management and culture system for each fish farm. In Brazil, the parasitic fauna in tilapia is composed by protozoans, monogenoideans, crustaceans and digeneans. However, Trichodinidae are the most frequent protozoan in fish in the south and southeast, while *I. multifiliis* was more abundant in the state of Amapá, in the north. This was the first report of *P. africana* in *O. niloticus* in the eastern Amazon, what broadens its distribution and confirms the presence of this protozoan in Brazil.

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