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Research Article

Hematological analysis and relative condition factor in naturally parasitized Nile tilapia

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ABSTRACT. This study aimed to evaluate the hematological profile and relative condition factor of naturally parasitized Nile tilapia from 12 fish farms in southern Brazil. A total of 240 fish were collected from four regions of the state of Santa Catarina. They were anesthetized, and the blood was collected by puncturing the caudal vein. Subsequently, the fish were necropsied for parasitological analysis. The results were compared with the Kruskal-Wallis test, and the correlations were verified with the Spearman test ($P < 0.05$). The parasitological analysis revealed high infestations by *Ichthyophthirius multifiliis* on the fish gills of the south and west regions. The presence of *Trichodina magna* and *Trichodina compacta* was observed in low intensity on the body surface of fish from all facilities. *Trichodina* spp. was found in high intensity parasitizing the fish gills of northern, Itajaí valley and western regions, nonetheless, in those regions, the presence of Monogenea, *Cichlidogyrus sclerosus*, and *Cichlidogyrus halli*, on fish gills was observed. The hematological profile presented altered parameters, featuring monocytosis and thrombocytopenia, possibly associated with high parasitic intensities. The observed correlations suggest that neutrophils and monocytes are directly involved in an organic defense against parasites. Monocytes are mainly targeted in the defense against *I. multifiliis*.

Keywords: *Oreochromis niloticus*; Monogenea; *Trichodina* sp.; *Ichthyophthirius multifiliis*; monocytosis; thrombocytopenia

INTRODUCTION

Aquaculture currently accounts for half of all fish for human consumption in the world, reaching a record in 2014, with *per capita* consumption of 20 kg/inhabitant/year (FAO, 2016). The preference for fish comes from several factors such as nutritional quality (rich in proteins and polyunsaturated fatty acids), sensory (pleasant and mild flavor), economic and convenience, presenting the potential for the market (MPA, 2015). In this context, Nile tilapia (*Oreochromis niloticus*) has characteristics favorable for culture and consumption (Khaw *et al.*, 2012) and is widely marketed worldwide (Fitzsimmons *et al.*, 2011). Nevertheless, it is the second most widely cultivated fish species in the world and the first in Brazil (Vicente *et al.*, 2014).

With the intensification of culture system, a favorable environment for epizootic outbreaks is created, due to several factors that contribute to the onset of diseases (Tavares-Dias *et al.*, 2009). Parasitic diseases are a limiting factor in fish production. Also, lesions caused by parasites serve as gateways for secondary infections (Takemoto *et al.*, 2013; Valladão *et al.*, 2014). Some studies suggest that parasites are also vectors of other pathogens, which can lead to acute mortality of fish (Xu *et al.*, 2012; Pilloux *et al.*, 2015). Ectoparasites are common in tilapia culture and have been reported by several authors (Ghiraldelli *et al.*, 2006a; Jerônimo *et al.*, 2011; Tavares-Dias *et al.*, 2013; Nunes *et al.*, 2016) and according to Moraes & Martins (2004) are directly related to water quality and animal management.

Hematological analysis is a diagnostic tool that has been used in fish studies and shows normal and pathological equilibrium conditions (Azevedo *et al.*, 2006). Blood parameters can be used as indicators to

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monitor the degree of fish health, effectively identifying responses to breeding challenges, such as the stress that the environment and pathogens cause to fish (Tavares-Dias *et al.*, 2009; Ranzani-Paiva *et al.*, 2013). Tavares-Dias *et al.* (2002) evaluating Nile tilapia from a fishery located in southeastern Brazil have observed that the parasitism by *Ichthyophthirius multifiliis* Fouquet, 1876 (Protozoa: Ciliophora) and *Saprolegnia* sp. caused anemia and an increase in the percentage of neutrophils and monocytes in fish. On the other hand, Ranzani-Paiva *et al.* (2005) and Ghiraldelli *et al.* (2006b) in parasitological studies with Nile tilapia originating respectively from cultures and dam, did not observe significant changes in hematological variables, a fact attributed to low parasite intensity. The authors concluded that the fish were in good health.

Another commonly used parameter to determine fish welfare is the relative condition factor (Kn), measured by the relationship between the observed weight and the expected weight for a given length (Guidelli *et al.*, 2009). It is expected that, under normal conditions, Kn is equal to 1, but it is known that it can be influenced by numerous factors such as nutrition, contamination and parasites (Yamada *et al.*, 2008). Analyzing the relative condition factor (Kn), Ranzani-Paiva *et al.* (2000) and Tavares-Dias *et al.* (2002) have reported that the parasites did not significantly affect the health condition of the hosts. In contrast, Singhal *et al.* (1990) found that high infestation by *Argulus indicus* Weber, 1892 (Crustacea: Branchyura) and infection by *Saprolegnia* sp. significantly suppressed the growth of *Cyprinus carpio*. Tavares-Dias *et al.* (2000) reported that high parasite infection reduced welfare in *O. niloticus*, *Leporinus macrocephalus* and *Piaractus mesopotamicus*, which may retard fish growth and cause damage to the producer in different culture systems.

Although Nile tilapia is one of the most cultured and studied fish in the world, there are few parasitological studies associated with hematological characteristics and relative condition factor in this species. Thus, this study aimed to evaluate the health status through the hematological profile and the relative condition factor in Nile tilapia naturally parasitized in fish farms from southern Brazil.

MATERIALS AND METHODS

Ethics statement

The procedures adopted for this study were approved by the Committee on Ethics in the Use of Animals of the Federal University of Santa Catarina-CEUA NºPP00928.

Study area

Adult tilapia from 12 fish farms were collected, covering four regions of the State of Santa Catarina: north, south, Itajaí valley and west, with three fish farms per region. In each, 20 specimens were studied, totaling 240 fish.

During the year 2015, punctual collections were carried out in each of the 12 fish farms, located in the cities of Braço do Norte (28°16'30"S, 49°09'57"W) in the south of the state, Joinville (26°18'14"S, 48°50' 45"W) in the northern region, Gaspar (26°55'51"S, 48°57'32"W) in the Itajaí Valley, and three other fish farms west of Santa Catarina, in the cities of Caxambú do Sul (27°09'39"S, 52°52'44"W), Pinhalzinho (26°0'52"S, 52°59'31"W) and Barra Bonita (26°39'14"S, 53°26'24"W).

Physical and chemical parameters of water

During the sampling, the following water quality parameters were evaluated: transparency with Secchi disk, temperature, pH and dissolved oxygen with the aid of multiparameter (model HI 9828 - Hanna instruments), and ammonia with commercial Hanna® kit.

Hematological analysis

After fish capture with the aid of net, they were anesthetized with Eugenol Vetec® (75 mg L^{-1}) during one minute of exposition and the blood (1 mL) was collected by puncturing the caudal vessel, using a syringe with a 20×0.55 mm (24 G x 3/4") needle containing a drop of 10% EDTA. Blood smears were performed in duplicates, later stained with May-Grunwald/Giemsa/Wright, for total thrombocyte and leukocyte counts and differential leukocyte count. The counts of these cells were calculated by the indirect method, from the blood extensions (Ishikawa *et al.*, 2008). An aliquot of the blood was used to determine the percentage of hematocrit (Ranzani-Paiva *et al.*, 2013). The total erythrocyte count was performed in a Neubauer chamber, after dilution (1:200) in Dacie solution (Blaxhall & Daisley, 1973).

Parasitological analysis

After blood sampling, the animals were euthanized by a rapid cerebral concussion, followed by a detailed macroscopic examination and biometry. The body surface mucus was scraped to make two slides each in duplicates that were later stained with silver nitrate and Giemsa to identify the protozoans. The remainder of the mucus was conditioned in flasks and fixed in 5% formalin for further parasite counting. The eyes, gills and gastrointestinal tract were collected separately and fixed, according to Jerônimo *et al.* (2011).

For protozoan quantification, the body surface and gill contents were homogenized and subsequently three 1 mL aliquots were taken for counting in the Sedgewick-Rafter chamber to estimate the volume of the fixed, as proposed by Martins *et al.* (2011). Monogeneans were quantified under a stereomicroscope in a labeled Petri dish and later mounted on slides with Hoyer's medium for identification.

Prevalence rate, mean intensity and mean parasite abundance were calculated according to Bush *et al.* (1997) for each parasite species. The identification of *Trichodina* sp. was performed according to Ghiraldelli *et al.* (2006c) and Martins & Ghiraldelli (2008), and Monogenea according to Paperna & Thurston (1969) and Pariselle & Euzet (1995).

Relative condition factor (Kn)

Kn values were calculated according to the method described by Le Cren (1951). Thus, with the logarithms of the total length (Lt) and total individual weight (Wt) values, the curve of the Wt/Lt relationship was adjusted, and the values of the regression coefficients a and b were estimated. The values of these coefficients were used to estimate the theoretically expected values of body weight (We), using the equation $We = a\ Lt^b$. Then Kn was calculated, corresponding to the ratio between the observed weight and the theoretically expected weight for a given length (Kn = Wt/We).

Statistical analysis

Statistical analyses were performed using Statistica 10.0® software. Since normality and homoscedasticity were not reached, the non-parametric Kruskal-Wallis test was used to compare the means. The possible correlations were verified with Spearman's correlation coefficient. The level of significance was $P \leq 0.05$.

RESULTS

The fish farms in this study were generally characterized by monocultures of tilapia, polyculture and catch and fee fishing systems (Table 1), and no mortalities were found.

The water quality values, measured only on the collection day, showed variations among the fish farms (Table 2). The mean weight of the animals ranged from 250.2 to 868.7 g, while the mean maximum and minimum total lengths were 22.2 and 35.6 cm, respectively.

The condition factor presented an average of one (Kn = 1) in all fish farms in the present study, except for one in which the mean was 0.99 (Table 3).

The parasitological analysis revealed the presence of *Trichodina magna* Van as & Basson, 1989 and *Trichodina compacta* Van as & Basson, 1989 on the fish body surface of all fish farms, with a maximum prevalence of 95% and a minimum of 35%, with a relatively low average intensity. Already the parasitism by *Trichodina* sp. Ehrenberg, 1830 on the gills, was reported in fish from all fish farms in the north, Itajaí valley and west, with prevalences of 40 to 100% and the high mean intensity of infestation (Table 4).

Ichthyophthirius multifiliis was observed in tilapia gills of all fish farms in the south and west regions, and only one of the northern regions of the state. The prevalence of *I. multifiliis* ranged from 5 to 100%, and the mean intensity followed the variation, being more intense in fish farms with higher prevalences (Table 5).

Only two species of Monogenea were observed on the gills, *Ciclidogyrus sclerosus* Paperna & Thurston, 1969 and *Ciclidogyrus halli* Price & Kirk, 1967, well as *Trichodina* sp. were present in all fish farms in the north, valley and west region, and absent in fish farms in the south. The lowest prevalence observed was 15%, where the lowest values for intensity and average infection abundance were also observed (Table 6). Prevalence of 100% was observed in fishery 3 of the Itajaí valley. However, the highest mean infestation intensity by *Ciclidogyrus* sp. was in the northern fish farm with 100.42 ± 65.32 parasites per fish.

The hematological parameters (Table 7) revealed variations in the red blood cells that presented minimum and maximum values of 1.4×10^6 and 2.4×10^6 μL^{-1}, respectively, as well as the hematocrit, which ranged from 25.1 to 36.5%, remaining within the reference values for the species (Azevedo *et al.*, 2006; Ghiraldelli *et al.*, 2006b).

Thrombocytes presented low values in all fish farms, characterizing thrombocytopenia. The total white blood cells count showed variations among the fish farms, with the most abundant leucocytes being lymphocytes, followed by monocytes and neutrophils. The monocytes presented marked values in all fish farms, which were consistent with a monocytosis. However, the lymphocytes and neutrophils, despite a wide variation, were within the reference values for the species (Azevedo *et al.*, 2006; Ghiraldelli *et al.*, 2006b).

When all the parasites were compared with each hemogram parameter, no correlation was observed between the hematological variables and the parasitism. However, monocytes were positively correlated with protozoan *I. multifiliis* (ρ = 0.19) and neutrophils showed positive correlations with *I. multifiliis* (ρ = 0.25), *Trichodina* sp. on the gills (ρ = 0.18) and Monogenea (ρ = 0.15). The relative condition factor (Kn)

Table 1. Management characteristics in the different regions and fish farms in the state of Santa Catarina. *One year ago, was consortium with pigs. **In the winter the animals were not receiving feed, which coincided with the date of collection.

South			
Characteristics	Fish farming 1	Fish farming 2	Fish farming 3
System	Fish-pay	Monoculture*	Monoculture
Density (fish m^{-3})	2	-	4-5
Feeding	2 times daily**	3 times daily**	5 times daily**
Complementary Aeration	No	Yes	Yes
Water Quality Monitoring	No	Monthly	No
Prophylaxis	No	No	No
Treatment of tanks	Disinfection between crops	Disinfection between crops	No
North			
Characteristics	Fish farming 1	Fish farming 2	Fish farming 3
System	Polyculture	Polyculture	Tank tank
Density (fish m^{-3})	4	2	-
Feeding	2 times daily	2 times daily	1 time daily
Complementary Aeration	Yes	Yes	Yes
Water Quality Monitoring	Monthly	1 time per week	No
Prophylaxis	Salt	Salt	Salt
Treatment of tanks	Fertilization	Disinfection and fertilization (uses manure)	No
Itajaí Valley			
Characteristics	Fish farming 1	Fish farming 2	Fish farming 3
System	Polyculture	Fish-pay	Polyculture
Density (fish m^{-3})	3	8-10	5
Feeding	3 times daily	1 time daily	5 times daily
Complementary aeration	2 times daily	2 times daily	2 times daily
Water Quality Monitoring	2 times per week	Não	Monthly
Prophylaxis	Salt	Salt	Salt
Treatment of tanks	Disinfection and fertilization between crops	Disinfection between crops	Disinfection between crops and fertilization sometimes
West			
Characteristics	Fish farming 1	Fish farming 2	Fish farming 3
System	Polyculture	Fish-pay	Polyculture
Density (fish m^{-3})	3.6	4	2.5
Feeding	-	2 times daily	8 times daily - automatic
Complementary aeration	Yes	No	Yes
Water Quality Monitoring	Monthly	No	Monthly
Prophylaxis	No	Salt e and syrup with pine	No
Treatment of tanks	Drying, disinfection, and fertilization between crops	Drying, disinfection, and fertilization between crops	Drying, disinfection, and fertilization between crops

when related to hematological indices and parasitism presented only negative correlation (ρ = -15) with *Trichodina* sp. in the mucus.

DISCUSSION

Nile tilapia is a rustic fish capable of supporting low-quality water environments (Zaniboni-Filho, 2004). However, dissolved oxygen was relatively low in fish farms 1 and 2 in the south region and fish farming 2 in the west region. Likewise, the water temperature in most fish farms was below the ideal limit for their cultivation (Kubitza, 2000). In general, changes in water quality, high stocking density, inadequate management or unbalanced nutrition are factors capable of producing stress in the fish, predisposing them to various infestations and parasitic infections (Zanolo & Yamamura, 2006).

The parasites reported in this study are common to tilapia culture, especially Monogenea and *Trichodina* sp. (Azevedo *et al.*, 2006; Ghiraldelli *et al.*, 2006a; Jerônimo *et al.*, 2011; Zago *et al.*, 2014; Nunes *et al.*, 2016) and the protozoans have been shown to be the most prevalent group in Santa Catarina. Ranzani-Paiva *et al.* (2005), in a study with Nile tilapia from a reservoir in São Paulo, observed among other parasites, *Trichodina*

Table 2. Physical and chemical parameters of water quality in the nurseries of the different fish farms studied. P: fish farming, DO: dissolved oxygen.

Parameters	South			North		
	P1	P2	P3	P1	P2	P3
DO (mg L^{-1})	1.8	1.4	8.3	7.3	6.6	6.1
Transparency (cm)	21	20	19	11	13	41
pH	6.1	7.0	7.0	6.6	6.8	7.0
Ammonia (mg L^{-1})	1	0.5	0.2	0.1	0	0.1
Temperature (°C)	20.7	23.6	22.0	22.8	23.7	20.9
Parameters	Itajaí Valley			West		
	P1	P2	P3	P1	P2	P3
DO (mg L^{-1})	2.2	7.8	3.3	7.6	0.6	3.1
Transparency (cm)	14	6	20	21	19	20
pH	6.4	7.0	6.0	6.7	5.9	5.8
Ammonia (mg L^{-1})	1.5	0.1	1.0	1.6	0.2	0.6
Temperature (°C)	28.7	22.4	22.9	29.2	25.3	30.9

Table 3. Biometric indices (mean ± standard deviation) and relative condition factor (Kn) of Nile tilapia. Different letters indicate a significant difference in the columns by the Kruskal-Wallis test ($P < 0.05$). P: fish farming.

Fish farming	Weight (g)	Length (cm)	Kn
P1 south	362.3 ± 124.3^{abc}	25.4 ± 3.1	1.0^{a}
P2 south	347.0 ± 76.1^{abc}	24.2 ± 1.6	1.0^{b}
P3 south	250.2 ± 52.0^{a}	23.3 ± 1.6	0.9^{c}
P1 north	407.5 ± 86.9^{bcd}	26.4 ± 1.8	1.0^{a}
P2 north	488.7 ± 170.7^{cd}	28.3 ± 3.3	1.0^{a}
P3 north	422.8 ± 166.3^{abcd}	28.4 ± 3.7	1.0^{a}
P1 valley	261.5 ± 50.6^{ab}	22.2 ± 1.6	1.0^{ab}
P2 valley	868.7 ± 123.7^{e}	35.6 ± 1.5	1.0^{a}
P3 valley	372.9 ± 71.9^{abc}	26.7 ± 1.6	1.0^{ab}
P1 west	815.3 ± 113.8^{e}	33.6 ± 1.8	1.0^{a}
P2 west	588.1 ± 116.6^{de}	31.2 ± 2.7	1.0^{ab}
P3 west	511.0 ± 7.7^{cde}	29.4 ± 1.5	1.0^{ab}

sp., *Ichthyophthirius multifiliis* and Monogenea on gills and *Trichodina* sp. on the fish skin, corroborating the parasitic fauna found in the present study. On the other hand, previous studies did not report the presence of *I. multifiliis* in fish from Santa Catarina while other ectoparasites such as *Piscinoodinium pillulare* (Schäperclaus 1954) Lom 1981 were found to be recurrent (Azevedo *et al.*, 2006; Ghiraldelli *et al.*, 2006a; Jerônimo *et al.*, 2011; Nunes *et al.*, 2016). The above suggests a possible variation of the parasitological fauna of *Oreochromis niloticus* among the fish farms and along with culture cycles in the same region.

The highest values of prevalence and the average intensity of *Trichodina magna* and *Trichodina compacta* on the body surface of fish from fish farms 3 in the southern region may be associated with the fact that drying and treatment of tanks between harvests were not performed. These management practices contribute to an environment conducive to the proliferation of these protozoa, since they are indicators of water quality, and are directly related to the concentration of organic matter in the environment (Jerônimo *et al.*, 2011). In addition to poor water quality, its proliferation is also associated with the total number of bacteria and the ecological aspects of the host (Martins *et al.*, 2015). Similar fact occurs in fish farming 2 of the northern region, which is the only one of the fish farms in this study that uses manure for fertilization of the tanks, contributing to a higher average intensity of *Trichodina* sp. in the gills of the fish at that site.

Ichthyophthirius multifiliis was the parasite that affected the fish of the west region with higher intensity. It is known to be a widespread infestation in times of lower temperatures in the southern and south-

Table 4. Parasitological indices of *Trichodina* sp. in Nile tilapia. Different letters indicate a significant difference in the lines by the Kruskal-Wallis test ($P < 0.05$). P%: prevalence, MI: mean intensity and MA: mean abundance (mean ± standard deviation), SII: site of infestation/infection, G: gills, M: mucus of body surface, P: fish farming.

Fish farming	SII	P (%)	MI	MA
P1 south	G	0	0^{a}	0
	M	65	12.3 ± 15.2^{abc}	8.0 ± 13.5
P2 south	G	0	0^{a}	0
	M	70	8.1 ± 6.9^{abc}	5.7 ± 6.8
P3 south	G	0	0^{a}	0
	M	95	97.2 ± 144.7^{d}	92.3 ± 142.5
P1 north	G	75	486.6 ± 533.4^{bcd}	365.0 ± 505.1
	M	65	4.3 ± 3.6^{abc}	2.8 ± 3.2
P2 north	G	40	11250.0 ± 29540.3^{ab}	4500.0 ± 18800.8
	M	60	2.7 ± 3.4^{abc}	1.6 ± 2.9
P3 north	G	90	983.3 ± 1001.3^{bcd}	885.0 ± 994.3
	M	35	2.5 ± 4.1^{a}	0.9 ± 2.6
P1 valley	G	70	1004.7 ± 3033.8^{ab}	703.3 ± 2553.5
	M	40	2.2 ± 3.1^{ab}	0.9 ± 2.2
P2 valley	G	80	283.3 ± 189.3^{bcd}	226.6 ± 204.4
	M	80	6.8 ± 5.6^{abcd}	5.5 ± 5.7
P3 valley	G	75	275.5 ± 260.4^{abc}	206.6 ± 254.8
	M	45	6.4 ± 8.8^{abc}	2.9 ± 6.3
P1 west	G	100	4956.4 ± 11594.9^{b}	4956.4 ± 11594.9
	M	55	4.6 ± 5.9^{abc}	2.5 ± 4.8
P2 west	G	100	4136.3 ± 8661.8^{cd}	4136,3 ± 8661.8
	M	90	15.1 ± 25.2^{cd}	13.6 ± 24.3
P3 west	G	100	566.3 ± 375.6^{bcd}	566.3 ± 375.6
	M	45	4.3 ± 6.5^{abc}	1.9 ± 4.7

Table 5. Parasitological indices of *Ichthyophthirius multifiliis* in gills of Nile tilapia. Different letters indicate a significant difference in the lines by the Kruskal-Wallis test ($P < 0.05$). P: fish farming, P%: prevalence, MI: mean intensity and MA: mean abundance (mean ± standard deviation).

Fish farming	P (%)	MI	MA
P1 south	45	377.7 ± 210.8^{ab}	170 ± 236.4
P2 south	50	330.0 ± 231.1^{ab}	165 ± 232.3
P3 south	30	116.6 ± 40.8^{a}	35 ± 58.7
P1 north	0	0^{b}	0
P2 north	5	100 ± 0^{b}	5 ± 22.3
P3 north	0	0^{b}	0
P1 valley	0	0^{b}	0
P2 valley	0	0^{b}	0
P3 valley	0	0^{b}	0
P1 west	95	1564.5 ± 988.9^{a}	1486.3 ± 1024.1
P2 west	95	571.6 ± 554.4^{a}	543.0 ± 554.5
P3 west	100	2419.6 ± 1515.1^{a}	2419.6 ± 1515.1

eastern regions of Brazil as well as in stressful situations (Martins & Romero, 1996; Tavares-Dias *et al.*, 2001). The temperature is a factor that influences the duration of the cycle of this parasite. In colder climates it can last several months, thus explaining the fact that, in apparently unaffected populations, massive infestations may develop suddenly as a consequence of the increase in water temperature, which demonstrates the need for constant surveillance in fish during the summer months (Eiras, 1994). The indexes reported in

Table 6. Parasitological indices of Monogenea in gills of Nile tilapia. Different letters indicate a significant difference in the lines by the Kruskal-Wallis test ($P < 0.05$). P: fish farming, P%: prevalence, MI: mean intensity, MA: mean abundance (mean ± standard deviation).

Fish farming	P(%)	IM	AM
P1 south	0	0^{ab}	0
P2 south	0	0^{ab}	0
P3 south	0	0^{ab}	0
P1 north	15	1 ± 0^{ab}	0.1 ± 0.3
P2 north	25	2 ± 1.7^{ab}	0.5 ± 1.1
P3 north	95	100.4 ± 65.3^{e}	95.4 ± 67.4
P1 valley	70	3.3 ± 3.5^{bcd}	2.3 ± 3.3
P2 valley	60	5.2 ± 6.7^{bcd}	3.1 ± 5.8
P3 valley	100	9.1 ± 9.1^{de}	9.1 ± 9.1
P1 west	15	1.3 ± 1.1^{b}	0.2 ± 0.7
P2 west	40	3.2 ± 4.8^{bc}	1.3 ± 3.3
P3 west	80	13.9 ± 22.3^{cde}	13.1 ± 20.6

Table 7. Hematological characteristics (mean ± standard deviation) of Nile tilapia cultivated in fish farms in the state of Santa Catarina. Different letters indicate a significant difference between the columns by the Kruskal-Wallis test ($P < 0.05$). RBC: red blood cells, WBC: white blood cells, P: fish farming.

Parameters	South			North		
	P1	P2	P3	P1	P2	P3
Hematocrit (%)	34.3 ± 4.9^{ab}	32.7 ± 3.6^{abc}	27.2 ± 3.3^{e}	34.1 ± 3.1^{a}	35.2 ± 4.2^{a}	32.3 ± 3.3^{abcd}
RBC ($\times 10^6$ µL^{-1})	2.3 ± 5.2^{ab}	2.3 ± 2.6^{a}	2.4 ± 3.91^{a}	2.0 ± 5.6^{ab}	1.8 ± 6.0^{bc}	1.4 ± 2.8^{c}
WBC ($\times 10^3$ µL^{-1})	63.8 ± 2.5	112.0 ± 2.9	88.6 ± 2.9	70.9 ± 3.1	53.0 ± 2.3	37.3 ± 1.5
Lymphocytes ($\times 10^3$ µL^{-1})	99.4 ± 2.4^{a}	103.0 ± 2.4^{a}	108.0 ± 1.6^{a}	98.5 ± 2.8^{a}	88.4 ± 3.1^{abc}	63.6 ± 1.9^{c}
Neutrophils ($\times 10^3$ µL^{-1})	4.4 ± 3.0^{ab}	1.4 ± 1.6^{bcd}	1.2 ± 1.5^{cd}	1.6 ± 2.5^{cd}	0.1 ± 0.2^{d}	1.7 ± 2.2^{bcd}
Monocytes ($\times 10^3$ µL^{-1})	9.9 ± 4.2^{a}	17.8 ± 13.2^{a}	12.2 ± 6.4^{ab}	1.2 ± 1.2^{c}	2.5 ± 4.5^{bc}	1.8 ± 2.3^{bc}
Thrombocytes ($\times 10^3$ µL^{-1})	4.3 ± 3.9^{bc}	6.6 ± 5.4^{abcd}	2.0 ± 3.7^{d}	13.2 ± 8.8^{ab}	9.6 ± 8.1^{abcd}	2.4 ± 4.1^{cd}
Parameters	Itajaí Valley			West		
	P1	P2	P3	P1	P2	P3
Hematocrit (%)	28.1 ± 4.3^{cde}	30.3 ± 3.4^{abcde}	25.1 ± 7.1^{de}	36.5 ± 2.5^{ab}	32.7 ± 2.9^{abc}	29.1 ± 3.9^{bcde}
RBC ($\times 10^6$ µL^{-1})	2.2 ± 4.9^{ab}	2.0 ± 3.6^{ab}	1.9 ± 4.5^{abc}	2.3 ± 5.1^{ab}	2.1 ± 3.5^{ab}	2.1 ± 3.1^{ab}
WBC ($\times 10^3$ µL^{-1})	104.0 ± 3.0	78.6 ± 3.4	47.4 ± 2.2	69.9 ± 1.9	60.4 ± 1.8	83.0 ± 1.9
Lymphocytes ($\times 10^3$ µL^{-1})	99.8 ± 2.6^{a}	94.1 ± 1.9^{a}	83.8 ± 2.0^{abc}	104.0 ± 2.4^{a}	87.6 ± 1.7^{abc}	85.4 ± 1.1^{abc}
Neutrophils ($\times 10^3$ µL^{-1})	1.3 ± 1.7^{cd}	3.0 ± 3.3^{abc}	6.9 ± 4.8^{a}	6.3 ± 6.1^{abc}	3.6 ± 3.5^{abc}	4.1 ± 3.6^{abc}
Monocytes ($\times 10^3$ µL^{-1})	15.7 ± 8.1^{a}	10.4 ± 6.5^{a}	7.8 ± 4.5^{ab}	2.8 ± 3.4^{bc}	12.3 ± 5.4^{a}	14.9 ± 6.1^{a}
Thrombocytes ($\times 10^3$ µL^{-1})	13.8 ± 10.2^{ab}	11.6 ± 11.4^{abc}	13.2 ± 8.6^{ab}	22.7 ± 9.9^{a}	8.9 ± 6.6^{abcd}	12.5 ± 9.7^{ab}

this study are higher than observed by Zago *et al.* (2014) that reported the maximum prevalence of 38% and meant intensity of 100 parasites per fish in Nile tilapia parasitized by *I. multifiliis*. According to Lemos *et al.* (2007), this shows that, in Brazil, the same host may present a different pattern of this infestation, depending on the region in which it is being cultivated.

Monogenea helminths had higher average infestation intensity in fish culture 3 in the north region, which was characterized by a storage tank, in which several species of fish were allocated, that stocking density was unknown. The fact of not controlling the amount of fish in a nursery contributes to the dissemination of this parasite, which has a direct life cycle and has its proliferation and dissemination facilitated in high storage densities and poor water quality (Moraes & Martins, 2004). The above mentioned may justify the results of the present study, mainly associated with poor sanitary management and mechanisms of parasite permanence in fish (Buchmann & Lindenstrom, 2002).

In a study with *Mugil platanus* from an estuarine-lagoon region in the state of São Paulo (Brazil), Ranzani-Paiva & Silva-Souza (2004) observed that parasitism of the gills by Monogenea affects the weight

of fish, especially when co-infected with *Trichodina* sp. and copepods, demonstrating the negative correlation of parasitism with the condition factor (Kn). Contrary to what was observed in this study, in which Kn showed an only negative correlation (ρ = -15) with *Trichodina* sp. in mucus and when associated with all parasites, there was no correlation. However, all fish farms analyzed in this study showed a Kn equal to or very close to 1, which indicates indirectly that the observed parasitism did not affect the growth and well-being of the fish, a fact that was also reported by Ranzani-Paiva *et al.* (2000) and Tavares-Dias *et al.* (2002).

In the present study, the number of monocytes exceeded the number of neutrophils, contrary to that observed in other studies (Hrubec *et al.*, 2000; Tavares-Dias & Moraes, 2003; Ghiraldelli *et al.*, 2006b). However, the same situation was reported by Azevedo *et al.* (2006) in *O. niloticus* in fish-pay. On the other hand, the higher lymphocyte rates in relation to monocytes and neutrophils suggest that the fish were not submitted to stressors before blood withdrawal, since several studies have indicated lymphopenia and neutrophilia in fish under stress (Martins *et al.*, 2002, 2004, 2006).

In *O. niloticus* highly infested by *I. multifiliis* and *Saprolegnia* sp, the neutrophil and monocyte percentages were significantly higher in the parasitized group, but the number of thrombocytes was equivalent between the two groups (Tavares-Dias *et al.*, 2002). On the other hand, Tavares-Dias *et al.* (1999) reported the occurrence of thrombocytopenia and monocytosis in *P. mesopotamicus* parasitized with *Argulus* sp., corroborating the results of the present study, in which the same changes in the hematological profile of Nile tilapias parasitized with *Trichodina* sp., Monogenea and *I. multifiliis* were observed.

The values of thrombocytes in the present study were lower than those reported in several studies with tilapias (Hrubec *et al.*, 2000; Tavares-Dias *et al.*, 2002; Tavares-Dias & Moraes, 2003; Azevedo *et al.*, 2006; Ghiraldelli *et al.*, 2006a). Thrombocytes are responsible for blood coagulation and play an essential role in phagocytosis, especially of cellular debris (Ranzani-Paiva *et al.*, 2013). According to Nagasawa *et al.* (2015), in their study with carp, thrombocytes require activation factors secreted by other activated leukocytes to perform phagocytosis. Possibly thrombocytes have been activated and recruited from their reserve compartments to contribute to the mechanisms of organic defense (Tavares-Dias *et al.*, 1999), justifying the thrombocytopenia observed in the present study. On the other hand, monocytes, which are the principal fish phagocytes (Tavares-Dias & Moraes, 2004), in cases of infectious processes, migrate from the blood vessels to the inflammatory focus (Martins *et al.*, 2009 and Santos *et al.*, 2009), increased production in the bloodstream in order to supply the requirements for organic defenses, unlike thrombocytes that possibly were only sequestered for the focus of inflammation.

The correlations observed in the present study suggest that neutrophils and monocytes are involved in the organic defense of fish against the parasites found. Studies with *P. mesopotamicus* related the increase in the number of circulating monocytes against parasite infection (Tavares-Dias *et al.*, 1999, 2008). However, monocytosis observed in this study refers in particular to *I. multifiliis* parasitism, given the positive correlation observed between the two.

Tavares-Dias *et al.* (1999), when studying the hematology and relative condition factor (Kn) of parasitized *L. macrocephalus* and *P. mesopotamicus*, observed that despite the high level of Monogenea infestation, *Trichodina* sp., *Lernaea cyprinacea* Linnaeus, 1758 (Crustacea: Copepoda), *P. pillulare* and *I. multifiliis*, there were no changes in the studied parameters, possibly having a balance between parasites and hosts. In contrast to the report mentioned above, in the present study, the high infestations did not influence the relative condition factor, but the alterations observed in the blood count suggest a direct relationship with the parasitic infestation.

Even without apparent losses in production, such as inadequate growth and/or mortality, the parasites of this study had a direct influence on fish health, as could be observed through changes in the blood count. The hematological profile of the tilapias showed the sequestration and use of cellular components of organic defense, possibly as a function of parasitism. Thus, parasites can divert the immune response of the fish, compromising them against any future adverse events.

CONCLUSIONS

The tilapia organic defense system was effective against the observed parasitism, so as not to affect the physiological state of animals and growth. Thus, the monocytosis and thrombocytopenia conditions were possibly due to the high parasitic intensities caused by *Trichodina* spp., *I. multifiliis*, and Monogenea. The correlations observed in the present study suggest that neutrophils and monocytes are directly involved in the organic defense of fish against parasites, and monocytes are primarily related to *I. multifiliis* infection.

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