

## Condition factor and hematology of Nile tilapia from polyculture with shrimp in brackish water

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### ADDITIONAL KEYWORDS

*Oreochromis niloticus*.

Brackish water.

Nursery.

Growth.

Blood.

### SUMMARY

Polyculture systems are an alternative to promote better use of environmental resources, as well as in for prevention of diseases in aquaculture, however, its effects on the physiological status of fish are little known. This study evaluated the effect of the polyculture system in brackish water with Pacific white shrimp (*Litopenaeus vannamei*) on the hematological parameters of Nile tilapia (*Oreochromis niloticus*) on nursery and growth stages. Healthy Nile tilapia in nursery (n=50) and growth (n=50) phase were collected from polyculture ponds in Laguna, Southern Brazil, and analyzed for condition factor and hematological parameters. The condition factor was similar in both stages, with values next to 1. Significantly higher (P<0.05) values of total plasmatic protein (TPP), hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), total leukocytes and thrombocytes occurred in growth phase, compared to nursery, while the other parameters remained unaltered. These differences are probably due to the growth of fish. All the values were close to those previously reported for healthy Nile tilapia, indicating that this kind of polyculture system is appropriate and safe for the species.

### Fator de condição e hematologia de tilápias-do-nilo de policultivo com camarão em água salobra

### RESUMO

Sistemas de policultivo constituem alternativa para a melhor utilização dos recursos disponíveis no ambiente aquático, além de auxiliar na prevenção de enfermidades, porém, seu efeito sobre o estado fisiológico dos peixes é pouco conhecido. Este estudo avaliou o efeito de sistema de policultivo com camarão-branco-do-pacífico (*Litopenaeus vannamei*) em água salobra sobre os parâmetros hematológicos de tilápias-do-nilo (*Oreochromis niloticus*) em fase de berçário e engorda. Tilápias-do-nilo hípidas em fase de berçário (n=50) e de engorda (n=50) foram coletadas de viveiros de policultivo em Laguna, Sul do Brasil, e analisadas quanto ao fator de condição e parâmetros hematológicos. O fator de condição foi similar em ambas fases de cultivo, com valores próximos a 1. Houve aumento significativo (P<0,05) nos valores de proteína plasmática total (PPT), hematócrito, volume corpuscular médio (VCM), concentração de hemoglobina corpuscular média (CHCM), leucócitos totais e trombócitos na fase de engorda, em comparação com o berçário. Os demais parâmetros permaneceram inalterados. Estas diferenças são possivelmente devidas ao crescimento dos peixes. Todos os valores estiveram próximos aos previamente registrados para tilápias-do-nilo saudáveis, indicando que esta modalidade de cultivo é apropriada e segura para a espécie.

### PALAVRAS CHAVE ADICIONAIS

*Oreochromis niloticus*.

Água salobra.

Berçário.

Engorda.

Sangue.

### INFORMATION

Cronología del artículo.

Recibido/Received: 12.02.2018

Aceptado/Accepted: 09.08.2018

On-line: 07.04.2019

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### INTRODUCTION

Aquatic animals are currently the most accessible source of animal protein and essential nutrients for a large part of the world's population and aquaculture has been essential for meeting the growing demand for these products, being responsible for the production of over 50% of all fishery products consumed in the

world (FAO, 2016). Nile tilapia (*Oreochromis niloticus*) and Pacific white shrimp (*Litopenaeus vannamei*) are among the most farmed species, whose combined production corresponded to US\$ 24.9 bi in 2015, accounting for 15% of the world aquaculture income (FAO, 2017). Intensive monoculture is responsible for much of the production of these organisms. However, this modality of aquaculture imposes conditions that favor the

occurrence of diseases, such as deterioration of water quality, inadequate nutrition, stress and proximity between hosts that increases the chances of transmission of pathogens, culminating in massive losses due to diseases (Moraes & Martins, 2004).

Polyculture emerges as an alternative to this issue, proposing simultaneous farming of two or more species in the same environment. They usually present different feeding habits and preferentially occupy different spaces in the water column, optimizing the use of resources without competition for them (Arana, 2004). The presence of tilapia in shrimp ponds favors the maintenance of water quality because its feeding habit prevents the excessive proliferation of phytoplankton. The shrimp can also feed on the waste disposed of by the tilapia, which would be a problem in case of monoculture. Moreover, if there are shrimps affected by any disease, tilapia will feed on dead or dying individuals without being affected and preventing the horizontal transmission of the disease to healthy shrimp that would feed on them (Fitzsimmons & Shahkar, 2017).

Although tilapias naturally inhabit freshwater, they present tolerance to salinity, which can be explained by their probable marine origin and allows farming in brackish water (Zaniboni Filho, 2004). In 2015, the world production of Nile tilapia in brackish water was about 850,000 tonnes, corresponding to U\$ 1 billion and totalizing 21.63% of the total production volume of Nile tilapia (FAO, 2017). Nile tilapia is the favorite species for brackish waters with salinity lower than 10 psu, as it is the least saline-tolerant species among commercially relevant tilapia species (Yuan et al., 2010). Therefore, due to its zootechnical and market advantages, this species may be the best choice for polyculture with Pacific white shrimp (*Litopenaeus vannamei*) in low salinity. Other advantage is that Nile tilapia is unlikely to reproduce in salinities above 25 psu (Teichert-Coddington et al., 1997), which reduces the risk of ecological damage for the natural environment in eventual cases of escape to the estuary. The Pacific white shrimp, although occurring naturally in the marine environment can also be farmed in brackish water, as it tolerates salinities as low as 2 psu (Appelbaum et al., 2002). Finally, polyculture allows the use of large areas of brackish water for production of food items with high commercial value. It is of great interest, in particular, to take advantage of the pond structure from shrimp farms that have been deactivated due to disease outbreaks. This situation is particularly common in the local where this study was conducted, in which, after successful shrimp production periods, most farms were deactivated due to outbreaks of white spot disease (Mello & Farias, 2007).

Despite the several advantages of this modality of aquaculture, the knowledge about the effect of polyculture with shrimp in brackish water on the physiological status of Nile tilapia is still scarce. Some previous studies reported the values of red blood cell parameters for Nile tilapia juveniles in farmed in fiber-glass tanks with brackish water for 45 (Azevedo et al., 2015) and 97 days (Pereira et al., 2016). A study with Nile tilapia in polyculture with Pacific white shrimp in brackish water was performed to evaluate the effect

of probiotic supplementation on the hemogram during nursery phase for 34 days (Jatobá et al., 2011). However, there are no studies about the long-term effect of this farming environment in the hematology of Nile tilapia.

Hematology and condition factor analysis are important tools that can show how the fish organism reacts and adapts to the environment. The hematological parameters reflect the health status of fish and may vary due to changes in water quality parameters (Ranzani-Paiva et al., 2013). The condition factor is also an important health indicator that can show if the animal is in proper physiological conditions (Le Cren, 1951).

Therefore, the aim of this study was to verify the health conditions of Nile tilapia from polyculture system with Pacific white shrimp by analyzing hematological parameters and condition factor in nursery and growth phases, as well as verifying its possible variations throughout the production cycle.

## MATERIAL AND METHODS

### STUDY AREA AND FISH SAMPLING

This study was performed in an aquaculture facility called "Model Farm", in the city of Laguna, Santa Catarina state, Southern Brazil (28°22'27.4"S 48°47'24.2"W). The farm is linked to the Estuarine Complex of Laguna, more specifically to the Imaruí Lagoon, from which the water used by the farm is obtained and where it is returned.

The sampling was performed in excavated ponds, in which nursery stage tilapia (stage 1) were kept at a density of 10 juveniles.m<sup>-1</sup>, while growth (stage 2) density of 1.25 fish.m<sup>-2</sup>. The density of shrimps was 10 juveniles.m<sup>-2</sup> for both stages. The fish were collected from one nursery pond (area: 2.8 ha) and one growth pond (area: 2 ha). The water renewal was performed every night at a rate of 5-10%, through a supply channel connected to the estuary. Food management was based only on fish diet, since the prawns were not fed. Fish received commercial feed, with different protein levels and pellet sizes, depending on the fish weight: 0.5 to 5 g (powdered feed with 40% crude protein - CP), 5 to 15 g (pellet 1.7 mm with 36% CP), 5 to 50 g (pellet 2.5 mm with 36% CP), 50 to 250 g (pellet 4 mm with 32% CP) and from 250g to slaughter (pellet 6 mm with 32% PB).

Fish weighing less than 50 g were kept in the nursery tanks. The nursery stock was made in October, with final transfer in December/early January. In this way, the fish remained for approximately 60 days in the nursery ponds. Then they were transferred and kept for another 4 months in the growth ponds. The growth ponds were populated with shrimp about 30 days before the introduction of fish to ensure that the juvenile shrimp grew sufficiently that they were not predated by fish.

Regarding condition factor analysis, a total of 100 apparently healthy fish (without visible signs of disease) were analyzed, 50 of which in nursery stage (30.4 ± 7.3 g and 11.5 ± 2.3 cm) and 50 in growth stage (186.7 ± 42.9 g and 19.9 ± 1.4). After capture with dragging

net, the fish were anesthetized with eugenol (50 mg.L<sup>-1</sup>) (Vidal et al., 2008) and biometry was performed; registering the total length (Lt), standard length (Ls) and total weight (Wt). These values were used to calculate the condition factor according to Le Cren (1951).

#### HEMATOLOGICAL ANALYSIS

After biometry, blood was collected by caudal puncture from the fish still under anesthesia. About 1.0 mL of blood was withdrawn with syringes containing 10% EDTA (ethylenediaminetetraacetic acid) as anticoagulant. The hematocrit percentage was measured by the microhematocrit method (Goldenfarb et al., 1971) and the hemoglobin concentration by the cyanometahemoglobin method (Collier, 1944). The counting of red blood cells (RBC) was performed in Neubauer chamber after dilution (1:200) of the blood in saline solution (0.9%). The mean corpuscular volume (MCV) and the mean corpuscular hemoglobin concentration (MCHC) were calculated according to Wintrobe (1934) and total plasmatic protein (TPP) was measured with portable refractometer (model 301, Biobrix, São Paulo, Brazil). The total amount of thrombocytes and leukocytes and the differential counting of leukocytes were obtained by indirect method (Ishikawa et al., 2008) from blood smears stained with May-Grünwald-Giemsa according to Rosenfeld (1947) and analyzed in optic microscope.

#### WATER QUALITY PARAMETERS

The verification of physicochemical parameters from water was performed weekly, in both farming stages. Temperature and dissolved oxygen were measured with oximeter (model 55, YSI, Yellow Spring, USA), pH with pHmeter (Alfakit, Florianópolis, Brazil) and salinity with portable refractometer (model 301, Biobrix, São Paulo, Brazil). Alkalinity was determined by the method of acid titration and colorimetric kits (model AT150, Alfakit, Florianópolis, Brazil) were used to measure the concentration of total am-

monia (NH<sub>3</sub>), nitrite (NO<sub>2</sub>), nitrate (NO<sub>3</sub>), phosphorus (P), silica (SiO<sub>2</sub>), sulfide (H<sub>2</sub>S) and iron (Fe).

#### STATISTICAL ANALYSIS

The statistical analysis was performed with the software Statistica 13.0 (Statsoft Inc., Tulsa, USA). The data of condition factor, hematology and water quality were compared between the different farming stages with Student's t test. In addition, Pearson's correlation coefficients for biometric data and hematological parameters were calculated. For all tests, the assumptions were tested and a significance level of 5% was considered.

#### RESULTS

The water quality parameters (**Table I**) did not present significant difference ( $p > 0.05$ ) between the farming periods. Condition factor (Kn) also remained similar in both stages (**Table II**).

Regarding hematological parameters (**Table II**), the values of TPP, hematocrit, hemoglobin, MCV, MCHC, total leukocytes and thrombocytes were significantly higher in growth than in nursery. The amount of RBC, lymphocytes, neutrophils and monocytes did not present significant difference between the phases.

Both weight and length presented positive significant correlation with TPP, hematocrit, hemoglobin, MCV, MCHC, total leukocytes, thrombocytes, lymphocytes and neutrophils (**Table III**). The monocytes number presented positive significant correlation only with body length, while the RBC count was the only parameter without significant correlation with either weight or length.

#### DISCUSSION

In both phases, the water quality parameters were within the limits of physiological comfort from Nile tilapia (El-Sayed, 2006). The values of salinity were within the range considered as optimum for growth of Nile tilapia, according to Villegas (1990), who observed better performance between 0 and 10 psu with faster growth at 7.5 psu. The condition factor is essential to verify health status in fish, indicating if the animal is under normal physiological conditions through its growth pattern (Le Cren, 1951). In the present study, there was no difference between the farming stages, indicating that permanence in brackish water did not cause the osmoregulatory expenditure to the point of changing the condition factor of the fish. The weight gain was not as fast as in other studies with tilapia in freshwater (Brum et al., 2017). It is possible to infer that this occurred due to the energy expenditure related to osmoregulation to compensate higher salinity (Boeuf & Payan, 2001).

Both weight and length were significantly higher ( $p < 0.05$ ) in growth phase, which may have contributed to the observed hematological changes. The values of TPP, hematocrit, hemoglobin, MCV, MCHC, total leukocytes and thrombocytes were significantly higher ( $p < 0.05$ ) in growth phase. According to Ranzani-Paiva

**Table I.** Means  $\pm$  standard deviation of water quality parameters from the polyculture system, in the different stages of tilapia farming (Médias  $\pm$  desvio padrão dos parâmetros de qualidade de água no policultivo, nas diferentes fases de cultivo da tilápia).

Parameters	Nursery	Growth
Total ammonia (mg.L <sup>-1</sup> NH <sub>3</sub> )	0.12 $\pm$ 0.03	0.15 $\pm$ 0.04
Nitrite (mg.L <sup>-1</sup> NO <sub>2</sub> )	0.01 $\pm$ 0.01	0.01 $\pm$ 0.01
Nitrate (mg.L <sup>-1</sup> NO <sub>3</sub> )	0.02 $\pm$ 0.03	0.02 $\pm$ 0.02
Phosphorus (mg.L <sup>-1</sup> P)	0.57 $\pm$ 0.45	0.66 $\pm$ 0.23
Silica (mg.L <sup>-1</sup> SiO <sub>2</sub> )	0.71 $\pm$ 0.48	1.11 $\pm$ 0.42
Sulfide (mg.L <sup>-1</sup> H <sub>2</sub> S)	0.01 $\pm$ 0.01	0.01 $\pm$ 0.01
Iron (mg.L <sup>-1</sup> Fe)	0.21 $\pm$ 0.22	0.21 $\pm$ 0.13
Alkalinity (mg.L <sup>-1</sup> )	103.08 $\pm$ 16.25	99.00 $\pm$ 8.42
Salinity (ups)	6.04 $\pm$ 2.31	6.75 $\pm$ 1.56
Temperature (°C)	25.60 $\pm$ 0.70	26.07 $\pm$ 0.43
pH	8.22 $\pm$ 0.32	8.57 $\pm$ 0.32
Dissolved oxygen (mg.L <sup>-1</sup> )	6.58 $\pm$ 0.56	6.86 $\pm$ 0.34



**Table II** Means ± standard deviation of hematological parameters from Nile tilapia (*Oreochromis niloticus*) farmed in polyculture with Pacific white shrimp (*Litopenaeus vannamei*), in different farming stages. Different letters indicate significant difference between the phases (p<0.05). TPP: total plasmatic protein, MCHC: mean hemoglobin corpuscular, MCV: mean corpuscular volume. (Médias ± desvio padrão dos parâmetros hematológicos de tilápia-do-nylo (*Oreochromis niloticus*) criada em policultivo com camarão-branco-do-pacífico (*Litopenaeus vannamei*), em diferentes fases de cultivo. Letras diferentes indicam diferença significativa entre as fases (P<0,05). TPP: proteína plasmática total, MCHC: concentração de hemoglobina corpuscular média, MCV: volumen corpuscular médio).

Parameters	Nursery	Growth
Weight (g)	31.50 ± 6.92 <sup>b</sup>	202.62 ± 47.75 <sup>a</sup>
Total length (cm)	11.88 ± 1.74 <sup>b</sup>	20.21 ± 1.38 <sup>a</sup>
Condition factor (Kn)	1.00 ± 0.03	1.00 ± 0.02
TPP (g.dL <sup>-1</sup> )	5.03 ± 0.51 <sup>b</sup>	5.55 ± 0.33 <sup>a</sup>
Hematocrit (%)	23.87 ± 4.95 <sup>b</sup>	31.27 ± 3.44 <sup>a</sup>
Hemoglobin (g.dL <sup>-1</sup> )	5.92 ± 0.99 <sup>b</sup>	8.79 ± 1.24 <sup>a</sup>
RBC (10 <sup>6</sup> .µL <sup>-1</sup> )	1.50 ± 0.40	1.43 ± 0.31
MCV (fL)	168.43 ± 51.27 <sup>b</sup>	229.48 ± 62.11 <sup>a</sup>
MCHC (g.dL <sup>-1</sup> )	25.65 ± 6.03 <sup>b</sup>	28.41 ± 4.92 <sup>a</sup>
Total leukocytes (10 <sup>3</sup> .µL <sup>-1</sup> )	32.35 ± 13.26 <sup>b</sup>	59.78 ± 29.53 <sup>a</sup>
Thrombocytes (10 <sup>3</sup> .µL <sup>-1</sup> )	8.77 ± 6.39 <sup>b</sup>	14.10 ± 8.24 <sup>a</sup>
Lymphocytes (10 <sup>3</sup> .µL <sup>-1</sup> )	19.58 ± 8.23	35.09 ± 18.84
Neutrophils (10 <sup>3</sup> .µL <sup>-1</sup> )	2.55 ± 1.80	4.69 ± 3.47
Monocytes (10 <sup>3</sup> .µL <sup>-1</sup> )	1.71 ± 1.22	3.14 ± 2.18

et al. (2013), the hemogram may be influenced by exogenous factors, such as water quality, nutrition, seasonality and photoperiod, or endogenous factors such as body length and weight. In a study with farmed Nile tilapia in different weight ranges, significant influence of body size was observed on hematocrit, RBC, hemoglobin concentration and MCHC (Tran-Duy et al., 2008), corroborating the results of the present study.

In both stages, TPP was within the reference interval previously registered for healthy hybrid tilapia (*O. niloticus* x *O. mossambicus*) with mean weight 240 g (Hrubec et al., 2000). The values were slightly above the reference range for healthy Nile tilapia from recirculation system, with weight equivalent to the growth phase (Chen et al., 2003; Mauel et al., 2007). However, the values were within the range observed for *O. niloticus* grown at high at 10 psu salinity for 97 days (Pereira et al., 2016), being this condition close to that of the present study, ranging from 5 to 10 psu. Among other environmental parameters, water salinity can cause changes in hematological values in fish (Chen et al., 2003). Changes in plasma protein concentration are mainly caused by environmental changes that cause changes in plasma volume (Melo et al., 2009), TPP may vary in different salinity conditions and these adaptations are gradual. Increased salinity may initially cause dehydration as a result of the loss of water to the environment, leading to a higher concentration of the total protein levels of the exposed animals in order to maintain the osmoregulatory function of the blood

(Melo et al., 2009). Osmoregulation in fish exposed to high salinity also includes increased water ingestion and excretion of monovalent ions by active transport through chloride cells present in the gill tissue, in addition to the elimination of divalent ions through urine (Yan et al., 2012).

The percentage of hematocrit in growth phase was within the reference intervals recorded by Mauel al. (2007) and by Hrubec et al. (2000) for hybrid tilapia in the same weight range. Hematocrit values in the nursery phase were below this range, but were similar to those recorded for *O. niloticus* with similar weight in freshwater and in brackish water (Mirea et al., 2013; Azevedo et al., 2015). Regarding hemoglobin concentration, in the growth phase the values were within the reference interval registered for hybrid tilapia with equivalent weight (Hrubec et al., 2000). In nursery phase the values were lower than in growth (p <0.05), being similar to that found for *O. niloticus* in the same culture stage, at pH 6 (El-Sherif & El-Feky, 2003). However, they were lower than those recorded in other studies with *O. niloticus* at nursery stage in freshwater (Mirea et al., 2013, Hashimoto et al., 2016, Brum et al., 2017).

Corroborating the present result, significant increase in hematocrit and hemoglobin was observed in a study with Nile tilapia, due to increase in body weight. This is a necessary respiratory adaptation along the growth, because as the animal becomes larger the oxy-

**Table III.** Pearson’s correlation coefficient between biometric data and hematological parameters from Nile tilapia (*Oreochromis niloticus*) in polyculture system with Pacific white shrimp (*Litopenaeus vannamei*). TPP: total plasmatic protein, MCHC: mean corpuscular hemoglobin concentration, MCV: mean corpuscular volume (Coeficiente de correlação de Pearson entre dados biométricos e parâmetros hematológicos da tilápia do Nilo (*Oreochromis niloticus*) em sistema de policultura com camarão branco Pacífico (*Litopenaeus vannamei*). TPP: proteína plasmática total, MCHC: concentração média de hemoglobina corpuscular, MCV: Volume corpuscular médio).

Parameters	Weight (g)		Length (cm)	
	r	p	R	p
TPP (g.dL <sup>-1</sup> )	0.520	0.0001	0.539	0.0001
Hematocrit (%)	0.595	0.0001	0.630	0.0001
Hemoglobin (g.dL <sup>-1</sup> )	0.745	0.0001	0.743	0.0001
RBC (10 <sup>6</sup> .µL <sup>-1</sup> )	-0.087	0.389	-0.103	0.3079
MCV (fL)	0.404	0.0001	0.439	0.0001
MCHC (g.dL <sup>-1</sup> )	0.257	0.0097	0.225	0.0245
Total leukocytes (10 <sup>3</sup> .µL <sup>-1</sup> )	0.404	0.0001	0.432	0.0001
Thrombocytes (10 <sup>3</sup> .µL <sup>-1</sup> )	0.271	0.0069	0.299	0.0027
Lymphocytes (10 <sup>3</sup> .µL <sup>-1</sup> )	0.401	0.0001	0.427	0.0001
Neutrophils (10 <sup>3</sup> .µL <sup>-1</sup> )	0.279	0.0029	0.323	0.0011
Monocytes (10 <sup>3</sup> .µL <sup>-1</sup> )	-0.184	0.2013	0.319	0.0013

gen demand of the organism increases (TranDuy et al., 2008). In both farming stages phases, RBC values were below the reference ranges recorded for healthy tilapia (Hrubec et al., 2000), but it was similar to that observed in other studies with Nile tilapia also farmed in excavated ponds in the state of Santa Catarina, therefore, under similar environmental conditions (Azevedo et al., 2006; Jerônimo et al., 2011a,b). The RBC number in the present study was also similar to that of Nile tilapia cultivated at salinity 20 psu for 97 days (Pereira et al., 2016). The salinity elevated to 17 psu also provided reduction in RBC number in *Tilapia guineensis* (Akinrotimi et al., 2010). Higher salinities may lead to reduction in erythrocytes number due to the change in osmolarity, which causes cell fragility due to water loss and higher salt intake in cells (Emelike et al., 2008).

The MCV of the fish in the nursery phase was similar to that registered for Nile tilapia in the same weight range (Brum et al., 2017). In the growth phase, MCV values were above the reference range recorded by Hrubec et al. (2000) for hybrid tilapia, but were similar to those of tilapia *Sarotherodon melanotheron* and red hybrid tilapia in weight equivalent to the growth stage (Lea Master et al., 1990). In both farming stages, MCHC was within the reference range of Hrubec et al. (2000) for hybrid tilapia, being significantly higher in growth compared to the nursery. In both phases, MCHC was within the range reported by Hrubec et al. (2000) for hybrid tilapia, being significantly higher in growth compared to the nursery. Corroborating the present result, Bittencourt et al. (2003) analyzed Nile tilapia between 100 and 900 g and also observed significant and positive correlation of length with hematocrit and MCV, as well as between weight and MCV. The results indicate that, during this phase, the volume of RBC tends to increase as the fish grow.

In leukocyte differential count, lymphocytes predominated, followed by neutrophils and monocytes. This proportion corroborates the results of other hematological studies with Nile tilapia in different production systems (Martins et al., 2008; Jerônimo et al., 2011a; Jatobá et al., 2011; Hashimoto et al., 2016; Brum et al., 2017). Basophils and eosinophils were not observed, corroborating previous studies where these cells were also not found in blood smears of Nile tilapia (Bittencourt et al., 2003; Brum et al., 2017). In both farming stages, the total leukocyte count was within the reference interval previously reported for healthy hybrid tilapia (*Oreochromis* sp.) in growth phase (Hrubec et al., 2000; Mauel et al., 2007). The values of total leukocytes were also similar to those of Nile tilapia also farmed in Santa Catarina state, during growth phase (Jerônimo et al., 2011a). The amount of leukocytes in the blood is indicative of immunological capacity and can be influenced by environmental factors (Figueredo et al., 2014), which explains the similarity of results for fish cultured in the same region.

The amounts of lymphocytes, neutrophils and monocytes did not show significant variation between the farming stages and were within the ranges previously reported for healthy hybrid tilapia (*Oreochromis* sp.) in growth phase (Hrubec et al., 2000; Mauel et al., 2007). The values were also similar to those of Nile

tilapia farmed in excavated ponds in the state of Santa Catarina, during growth stage (Jerônimo et al., 2011a). However, in the present study, total and differential leukocyte counts presented values much higher than those of Nile tilapia grown in polyculture with shrimp at salinity of 9-12 psu (Jatobá et al., 2011). This may be due to the higher salinity in this study, that may have caused stress and reduced the amount of leukocytes, considering that the tolerance of Nile tilapia to salinity is around 10 psu (Yuan et al., 2010). In the present study the salinity was within the comfort range for the species, being always next to 6 psu.

The values of thrombocytes, total leukocytes, lymphocytes and neutrophils showed significant positive correlation with weight, whereas monocyte values showed an inverse relationship with fish growth. Lymphocytes are cells responsible for antigen recognition and specific immune response (Ranzani-Paiva et al., 2013). The higher values during growth shows that fish in the early stages of life are more dependent on the innate immune response, presenting a higher proportion of monocytes, cells with high phagocytic capacity (Ranzani-Paiva et al., 2013). Throughout the growth, the animal develops the adaptive immune response, which matches with the tendency to increase in the amount of lymphocytes.

Regarding neutrophils, in the nursery phase the values were similar to those previously reported for Nile tilapia in the same phase, but farmed in freshwater (Brum et al., 2017). Although there was no significant difference between the phases, there was positive correlation between body weight and the amount of neutrophils, cells that contribute to the innate immune response by performing phagocytosis. The same occurred with thrombocytes, cells that perform coagulation and immune response (Nagasawa et al., 2009). Jaafar et al. (2016) verified body size effect on the differential count of leukocytes in rainbow trout (*Oncorhynchus mykiss*), concluding that the size of leukocyte populations increases exponentially with body growth. For this reason, larger fish have a more effective immune response, both innate and adaptive (Jaafar et al., 2016). The increase in thrombocytes, total leukocytes and TPP in larger fish is probably due to this, and salinity did not impair the development of this response. The direct relationship between growth and amount of defense cells demonstrates that disease prevention measures should be reinforced during the earlier stages of farming in order to compensate the immunological vulnerability of fish. Despite these differences, the values remained close to those previously reported for healthy Nile tilapia.

## CONCLUSIONS

In both stages, the hematological profile of the Nile tilapia farmed in polyculture with marine shrimps was in agreement with the data previously registered for healthy tilapia. The results demonstrate that the species is able to adapt to this production system without physiological impairment, since the observed changes constitute response of adaptation to salinity. Even with these changes, the values are within the range considered as healthy for the species.

## BIBLIOGRAPHY

- Akinrotimi, OA, Uedeme-Naca, B & Agokei, EO 2010, 'Effects of acclimation on haematological parameters of *Tilapia guineensis* (Bleeker, 1862)', *The Scientific World Journal*, vol. 5, pp. 1-4. URL: <http://www.scienceworldjournal.org/article/view/8431/5950>
- Appelbaum, S, Garada, J & Mishra, JK 2002, Growth and survival of the white leg shrimp (*Litopenaeus vannamei*) reared intensively in the brackish water of the Israeli Negev desert'. *The Israeli Journal of Aquaculture*, vol. 54, no. 1, pp. 41-48. URL: <http://hdl.handle.net/10524/19041>
- Arana, LV 2004, *Fundamentos de aquicultura*. EdUFSC, Florianópolis.
- Azevedo, TMP, Martins, ML, Bozzo, FR & Moraes, FR 2006, 'Haematological and gill responses in parasitized tilapia from Valley of Tijucas River, SC, Brazil'. *Scientia Agricola*, vol. 63, no. 2, pp. 115-120. URL: <http://dx.doi.org/10.1590/S0103-90162006000200002>
- Azevedo, RV, Oliveira, KF, Flores-Lopes, F, Teixeira-Lanna, EA, Takishita, SS & Tavares-Braga, LG 2015, 'Responses of Nile tilapia to different levels of water salinity'. *Latin American Journal of Aquatic Research*, vol. 43, no. 5, pp. 828-835.
- Bittencourt, NLR, Molinari, LM, Scoaris, DO, Pedroso RB, Nakamura, CV, Ueda-Nakamura, T, Abreu-Filho, BA & Dias-Filho, BP 2003, 'Haematological and biochemical values for Nile tilapia *Oreochromis niloticus* cultured in semi-intensive system'. *Acta Scientiarum Biological Sciences*, vol. 25, no. 2, pp. 385-389.
- Boeuf, G & Payan, P 2001, How should salinity influence fish growth? *Comparative Biochemistry and Physiology*, vol. 130, pp. 411-423. URL: <http://www.sciencedirect.com/science/article/pii/S153204560100268X>
- Brum, A, Pereira, SA, Owatari, MS, Chagas, EC, Chaves, FCM, Mourinho, JLP & Martins, ML 2017, Effect of dietary essential oils of clove basil and ginger on Nile tilapia (*Oreochromis niloticus*) following challenge with *Streptococcus agalactiae*. *Aquaculture*, vol. 468, pp. 235-243. URL: <http://www.sciencedirect.com/science/article/pii/S0044848616306925>
- Chen, CY, Wooster, GA, Getchell, RG, Bowser, PR & Timmons, MB 2003, Blood chemistry of healthy, nephrocalcinosis-affected and ozone-treated tilapia in a recirculation system, with application of discriminant analysis. *Aquaculture*, vol. 213, pp. 89-102. URL: <http://www.sciencedirect.com/science/article/pii/S0044848602004994>
- Collier, HB 1944, The standardization of blood hemoglobin determinations. *Canadian Medical Association Journal*, vol. 50, pp. 550-552. URL: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1581573/>
- El-Sayed, AFM 2006, *Tilapia culture*. CABI Publishing, Alexandria.
- El-Sherif, MS & El-Feky, AMI 2009, Performance of Nile tilapia (*Oreochromis niloticus*) fingerlings. I. Effect of pH. *International Journal of Agriculture and Biology*, vol. 11, no. 3, pp. 297-300. URL: <http://www.academia.edu/download/31277142/13.pdf>
- Emelike, FO, Odeyenuma, C, Jeremiah, ZA & Obigwe, BU 2008, The use of anti-coagulated and defibrinated blood samples for the evaluation of red cell osmotic fragility. *International Journal of Natural and Applied Sciences*, vol. 4, pp. 204-208. URL: <http://dx.doi.org/10.4314/ijonas.v4i2.36271>
- Food and Agriculture Organization of United Nations 2016, *The State of World Fisheries and Aquaculture*, viewed 15 January 2018, <http://www.fao.org/3/a-i5555e.pdf>
- Food and Agriculture Organization of the United Nations 2017, *Fisheries Global Information System (FIGIS)*, viewed 15 January 2018, <http://www.fao.org/fishery/figis/en>
- Figueredo, AB, Tancredo, KR, Hashimoto, GSO, Roumbedakis, K, Marchiori, NC & Martins, ML 2014, Haematological and parasitological assessment of silver catfish *Rhamdia quelen* farmed in Southern Brazil. *Brazilian Journal of Veterinary Parasitology*, vol. 23, no. 2, pp. 157-163. URL: <http://dx.doi.org/10.1590/S1984-29612014028>
- Fitzsimmons, KM & Shahkar, E 2017, 'Tilapia-shrimp polyculture', in Perschbacher, PW & Stickney, RR (eds.) *Tilapia in intensive co-culture*. Wiley-Blackwell, Oxford.
- Goldenfarb, PB, Bowyer, FP, Hall, E & Brosius, E 1971, Reproductibility in the hematology laboratory: the microhematocrit determinations. *American Journal of Clinical Pathology*, vol. 56, pp. 35-39. URL: <https://doi.org/10.1093/ajcp/56.1.35>
- Hashimoto, GSO, Marinho-Neto, F, Ruiz, ML, Acchile, M, Chagas, EC, Chaves, FCM & Martins, ML 2016, Essential oils of *Lippia sidoides* and *Mentha piperita* against monogenean parasites and their influence on the hematology of Nile tilapia. *Aquaculture*, vol. 450, pp. 182-186. URL: <https://doi.org/10.1016/j.aquaculture.2015.07.029>
- Hrubec, TC, Cardinale, JL & Smith, SA 2000, Hematology and plasma chemistry reference intervals for cultured tilapia (*Oreochromis hybrid*). *Veterinary Clinical Pathology*, vol. 29, no. 1, pp. 7-12. URL: <https://doi.org/10.1111/j.1939-165X.2000.tb00389.x>
- Ishikawa, NM, Ranzani-Paiva, MJT & Lombardi, JV 2008, Metodologia para quantificação de leucócitos totais em peixe, *Oreochromis niloticus*. *Archives of Veterinary Science*, vol. 13, n. 1, pp. 54-63. URL: <http://revistas.ufpr.br/veterinary/article/download/11560/8050>
- Jaafar, RM, Ohtani, M, Kania, PW & Buchmann, K 2016, Correlation between leukocyte numbers and body size of rainbow trout. *Open Journal of Immunology*, vol. 6, pp. 101-110. URL: [https://curis.ku.dk/ws/files/166025477/Rzgar\\_M.\\_Jaafar\\_2016\\_Leukocytes\\_counts...OJL\\_2016091316522386.pdf](https://curis.ku.dk/ws/files/166025477/Rzgar_M._Jaafar_2016_Leukocytes_counts...OJL_2016091316522386.pdf)
- Jatobá, A, Vieira, FN, Mourinho, JLP, Silva, BC, Seiffert, WQ & Andreatta, ER 2011, Diet supplemented with probiotic for Nile tilapia in polyculture system with marine shrimp. *Fish Physiology and Biochemistry*, vol. 37, pp. 725-732.
- Jerônimo, GT, Lafitte, LV, Speck, GM & Martins, ML 2011a, Seasonal influence on the hematological parameters in cultured Nile tilapia from southern Brazil. *Brazilian Journal of Biology*, vol. 71, no. 3, pp. 719-725. URL: <http://dx.doi.org/10.1590/S1519-69842011000400017>
- Jerônimo, GT, Speck, GM, Cechinel, MM, Gonçalves, ELT & Martins, ML 2011b, Seasonal variation on the parasitic communities of Nile Tilapia cultured in three regions in Southern Brazil. *Brazilian Journal of Biology*, vol. 71, no. 2, pp. 365-373. URL: <http://dx.doi.org/10.1590/S1519-69842011000300005>
- Le Cren, ED 1951, The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). *Journal of Animal Ecology*, vol. 20, no. 2, pp. 201-219. URL: <http://www.jstor.org/stable/1540>
- Lea Master, BR, Brock, JA, Fujioka, RS & Nakamura, RM 1990, Hematologic and blood chemistry values for *Sarotherodon melanothron* and a red hybrid tilapia in freshwater and seawater. *Comparative Biochemistry and Physiology*, vol. 97A, no. 4, pp. 525-529. URL: [https://doi.org/10.1016/0300-9629\(90\)90121-8](https://doi.org/10.1016/0300-9629(90)90121-8)
- Mauel, MJ, Miller, DL & Merrill, AL 2007, Hematologic and plasma biochemical values of healthy hybrid tilapia (*Oreochromis aureus* x *Oreochromis niloticus*) maintained in a recirculating system. *Journal of Zoo and Wildlife Medicine*, vol. 38, no. 3, pp. 420-424. URL: <https://doi.org/10.1638/06-025.1>
- Mello, GL & Farias, AP 2007, Policultivo de tilápias e camarões marinhos – Os resultados das primeiras experiências em Laguna - SC. *Panorama da Aquicultura*, vol. 102, pp. 42-47.
- Melo, DC, Oliveira, DAA, Melo, MM, Junior, DV, Teixeira, EA & Guimarães, SR 2009, Proteic electrophoretic profile of chitralada tilapia nilotic (*Oreochromis niloticus*), exposed to hypoxia chronic stress. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, vol. 61, no. 5, pp. 1183-1190. URL: <http://dx.doi.org/10.1590/S0102-09352009000500022>
- Mirea, C, Cristea, V, Grecu, RI, Dediu, L & Ion, V 2013, Hematological and biochemical characterization of Nile tilapia (*Oreochromis niloticus*, Linnaeus, 1758) reared intensively in a recirculating aquaculture system in relation to water temperature. *Animal Science and Biotechnology*, vol. 46, no. 2, pp. 234-237.
- Moraes, FR & Martins, ML 2004, Favorable conditions and principal teelost diseases in intensive fish farming, in Cyrino, JEP, Urbinati, EC, Fracalossi, DM & Castagnolli, N (eds.) *Special topics in intensive tropical freshwater fish farming*. TecArt, São Paulo.



- Nagasawa, T, Somamoto, T & Nakao, M 2009, Carp thrombocyte phagocytosis requires activation factors secreted from other leukocytes. *Developmental and Comparative Immunology*, vol 52, pp. 107-111. URL: <https://doi.org/10.1016/j.dci.2015.05.002>
- Pereira, DSP, Guerra-Santos, B, Moreira, ELT, Albinati, RCB & Ayres, MCC 2016, Parâmetros hematológicos e histológicos de tilápia do Nilo em resposta ao desafio de diferentes níveis de salinidade. *Boletim do Instituto de Pesca*, vol. 42, no. 3, pp. 635-647. URL: <http://dx.doi.org/10.20950/1678-2305.2016v42n3p635>
- Ranzani-Paiva, MJT, Pádua, SB, Tavares-Dias, M & Egami, MI 2013, *Métodos para análise hematológica em peixes*. Eduem, Maringá.
- Rosenfeld, G 1947, Corante pancrômico para hematologia e citologia clínica. Nova combinação dos componentes do May-Grünwald e do Giemsa num só corante de emprego rápido. *Memórias do Instituto Butantan*, vol. 20, pp. 329-335.
- Teichert-Coddington, DR, Popma, TP & Lovshin, LL 1997, Attributes of tropical pondcultured fish, in Egna, HS & Boyd, E, *Dynamics of Pond Aquaculture*. CRC Press, Boca Raton.
- Tran-Duy, AN, Schrama, JW, Van Dam, AA & Verreth, JAJ 2008, Effects of oxygen concentration and body weight on maximum feed intake, growth and hematological parameters of Nile tilapia, *Oreochromis niloticus*. *Aquaculture*, vol. 275, pp. 152-162. URL: <https://doi.org/10.1016/j.aquaculture.2007.12.024>
- Vidal, LVO, Albinati, RCB, Albinati, ACL, Lira, AD, Almeida, TR, Santos, GB 2008, Eugenol as an anesthetic for Nile tilapia. *Pesquisa Agropecuária Brasileira*, vol. 43, no 8, pp. 1069-1074. URL: <http://dx.doi.org/10.1590/S0100-204X2008000800017>
- Vilegas, CT 1990, *Growth and survival of Oreochromis niloticus, O. mossambicus and their F1 hybrids at various salinities*, viewed 15 January 2018, <https://repository.seafdec.org.ph/handle/10862/341>
- Wintrobe, MM 1934, Variations on the size and hemoglobin content of erythrocytes in the blood various vertebrates. *Folia Haematologica*, vol. 5, pp. 32-49.
- Yan, B, Wang, ZH, Zhao, JL 2012, Mechanism of osmoregulatory adaptation in tilapia. *Molecular Biology Reports*, vol. 40, pp. 925-931. URL: <https://doi.org/10.1007/s11033-012-2133-7>
- Yuan, D, Yang, YI, Yakupitiyage, A, Fitzsimmons, K, Diana, JS 2010, Effects of addition of red tilapia (*Oreochromis spp.*) at different densities and sizes on production, water quality and nutrient recovery of intensive culture of white shrimp (*Litopenaeus vannamei*) in cement tanks. *Aquaculture*, vol. 298, pp. 226-238. URL: <https://doi.org/10.1016/j.aquaculture.2009.11.011>
- Zaniboni Filho, E 2004, Piscicultura das espécies exóticas de água doce, in Poli, CR, Poli, ATB, Andreatta, ER & Beltrame, E (Eds.). *Aquicultura: Experiências Brasileiras*. Multitarefa, Florianópolis.