

Research Article

Responses of Nile tilapia to different levels of water salinity

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ABSTRACT. A 45 day experiment was carried out to evaluate the effect of water salinity on the performance, haematological parameters and histological characteristics of the gills of the Nile tilapia, *Oreochromis niloticus*. The water salinity levels evaluated were: 0, 7, 14 and 21 g L⁻¹. Nile tilapia specimens (1.62 ± 0.01 g), distributed into 20 fibreglass tanks (100 L) at a density of 15 fish per tank. There were no significant differences of the water salinity levels on daily feed intake; however, there were differences ($P < 0.05$) on the daily weight gain, feeding conversion rate and survival. The best results were observed for the water salinity levels of 0 and 7 g L⁻¹. There were no differences ($P > 0.05$) between these levels. Regarding the haematological parameters, it was observed that the percentage of the haematocrits and the erythrocyte count were influenced ($P < 0.05$) by the water salinity level, which was not observed for the leukocyte count. The observed histopathological alterations were chloride cell hypertrophy, epithelial lifting, structure alteration, telangiectasia, primary lamellae cells aggregation, fusion and occurrence of aneurisms of different sizes in some secondary lamellae. Regarding the frequency of gills infection intensity, there were slight changes between the salinities of 0, 7 and 14 g L⁻¹ and moderate changes at 21 g L⁻¹. It is concluded that Nile tilapia can be reared in water salinities of up to 7 g L⁻¹ without damage to the parameters evaluated in this work.

Keywords: *Oreochromis niloticus*, haematology, histopathological alterations, aquaculture.

Respuestas de la tilapia del Nilo a diferentes niveles de salinidad del agua

RESUMEN. Se realizó un experimento de 45 días para evaluar el efecto de la salinidad sobre el crecimiento, parámetros hematológicos y características histológicas de las branquias de la tilapia del Nilo, *Oreochromis niloticus*. Los niveles de salinidad fueron: 0, 7, 14 y 21 g L⁻¹. Los especímenes de tilapia del Nilo (1,62 ± 0,01 g) se distribuyeron en 20 estanques de fibra de vidrio (100 L) a una densidad de 15 peces por estanque. No hubo diferencias significativas en los niveles de salinidad sobre el consumo diario de alimento, pero hubo diferencias ($P < 0,05$) en la ganancia diaria de peso, conversión alimenticia y supervivencia. Los mejores resultados se observaron en los niveles de salinidad de 0 y 7 g L⁻¹, sin diferencias ($P > 0,05$) entre ellos. En cuanto a los parámetros hematológicos, se observó que el porcentaje de hematocrito y número de eritrocitos fueron influenciados ($P < 0,05$) por el nivel de salinidad, que no se observó para el recuento de leucocitos. Las alteraciones histopatológicas observadas fueron: hipertrofia de las células de cloruro, elevación epitelial, cambio en la estructura, telangiectasia, agregación de las células primarias de las laminillas, fusión y aparición de algunos aneurismas de diferentes tamaños en laminillas secundarias. En cuanto a la frecuencia de la intensidad en la infección de las branquias, hubo ligeros cambios entre las salinidades de 0,7 y 14 g L⁻¹ y cambios moderados a 21 g L⁻¹. Se concluye que la tilapia del Nilo se puede cultivar en salinidades de hasta 7 g L⁻¹ sin daño a los parámetros evaluados en este trabajo.

Palabras clave: *Oreochromis niloticus*, hematología, cambios histopatológicos, acuicultura.

INTRODUCTION

In regions where fresh water is scarce, fish farming in brackish or salt water can provide a source of extra income (Dimaggio *et al.*, 2009; Marengoni *et al.*, 2010; Jesus *et al.*, 2011). Tilapias can be reared in this type of environment provided gradual acclimatization procedures are adopted (Likongwe *et al.*, 1996; Dominguez *et al.*, 2004). The Nile tilapia (*Oreochromis niloticus*) is widely cultivated in fresh water; however, it tolerates certain levels of salinity, being considered as euryhaline. Despite its good adaptation capacity to salinity, it is less tolerant than other species of tilapia such as *O. aureus* and *O. mossambicus* (Kamal & Mair, 2005).

The way that each species of fish responds to different salinity levels allows evaluation of the best place for its culture. Thus, several studies have evaluated the influence of this parameter on the performance of euryhaline fish (Likongwe *et al.*, 1996; Boeuf & Payan, 2001; Tsuzuki *et al.*, 2006; Luz *et al.*, 2008; Riche & Williams, 2010). Besides the performance it is necessary to evaluate the effect of the salinity on fish health and organs (Árnason *et al.*, 2013). The digestive tube and the gills that remain in direct contact with water and under physical and chemical environment changes may suffer morphologic alterations (Reis *et al.*, 2009; Yuan *et al.*, 2010). The gills are vital structures for the fish health, being involved in the processes of osmoregulation and nitrogen composites excretion, as well as being the main site for gaseous exchanges. Some functional changes such as the gill epithelium chloride cells and $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity were observed during the adaptation of tilapia to saline water (Güner *et al.*, 2005). Thus, any damage to the filaments and branchial lamellae that can interfere with their function, will compromise the survival of these animals (Winkaler *et al.*, 2001; Reis *et al.*, 2009).

Histopathological studies have been developed to evaluate the effects of contaminants on fish health in the environment and to help establish a causal relationship between exposure to toxic substances and various biological responses (Schwaiger *et al.*, 1997). There is an increased incidence of diseases and pathological conditions in fish, as well as a variety of aetiologies. This increase is an indicator of environmental stress and provides a definitive biological endpoint of the history of exposure to a pollutant (Schwaiger *et al.*, 1997).

The aim of this study was to evaluate the performance, haematologic parameters and histological characteristic of Nile tilapia gills submitted to different levels of water salinity.

MATERIALS AND METHODS

The experiment was carried out at Ilhéus city, Bahia, Brazil (14°47'20"S, 39°02'58"W), during 45 days, with 300 juvenile (1.62 ± 0.01 g) Nile tilapia of Thai ancestry, sexually reversed, bred in fresh water, in a completely randomised design with four treatments (salinity levels) and five repetitions.

The fish were distributed into 20 cylindrical fibreglass tanks, with a useful volume of 100 L, at a density of 15 fishes per tank. The tanks were placed in a closed recirculation system in four groups, used with biological filters through four water pumps, one for each salinity level. Each tank received individual aeration by means of porous stones fed by a 1 hp air blower.

During the fish acclimatization period, the salinity was raised by $2 \text{ g L}^{-1} \text{ day}^{-1}$ with seawater (35 g L^{-1}) replacing the fresh water from the experimental tanks. The water salinity levels evaluated were 0, 7, 14 and 21 g L^{-1} .

The fish were fed an extruded commercial feed with 360 g kg^{-1} crude protein, 70 g kg^{-1} ether extract, 130 g kg^{-1} moisture, 120 g kg^{-1} mineral matter, 20 g kg^{-1} calcium, 10 g kg^{-1} phosphorus and 250 mg vitamin C (guarantee levels). The feed was ground in a knife mill with 1.0 mm sieve and supplied *ad libitum* four times a day (07:00 am, 10:00 am, 01:00 pm and 04:00 pm).

Water-quality parameters were measured daily. Dissolved oxygen, pH and temperature were measured through multi-parameter devices (YSI model 55-12FT, YSI Corporation, Owings Mills, MA, USA); salinity was measured with a refractometer (Atago S/Mill-E, Atago Co. Ltd., Tokio, Japan).

At the beginning of the experiment and after 45 days, all fish were weighed. The individual daily consumption of feed was obtained by the ratio between the total consumption of feed of each fish and the experimental period. The daily weight gain was calculated by difference between the final and initial weights of each fish relative to the experimental period. The feed conversion rate was calculated by the ratio between the feed consumed and the weight gain at the end of the experiment. The survival was calculated through the ratio between dead and live individuals.

At the end of the experiment, after biometrics measurements, four specimens were randomly selected from each repetition for cardiac puncture blood collection (after anaesthesia with benzocaine 0.1 g L^{-1}) with a syringe containing 10% EDTA (Ethylenediamine tetraacetic acid). The erythrocyte and leukocyte counts were determined by dilution and counting in a haemocytometer. The percentage of

haematocrits was carried out according to the microhematocrit method.

One specimen (tilapia) of each treatment was sacrificed (benzocaine) for the histopathological examination of the gills. The material was fixed in 10% formalin for a week and then preserved in 70% alcohol. The gills of the most external gill arches were removed, always on the right-hand side of the fish. The gills were decalcified with EDTA for a period ranging from three days to a week. The specimens were prepared for histological analysis using an ethanol routine dehydration technique, then impregnated and embedded in paraffin. Sections of 5-7 μm were made with a microtome. The sections were stained with haematoxylin and eosin (H&E) for visualisation of the affected tissues and organs.

For a better understanding of the results, the histopathological alterations were classified as scores of 0 to 3, where 0 = no alteration, 1 = slight alteration, 2 = moderate alteration, and 3 = severe alteration. The definitions slight, moderate and severe were modified from Poleksic & Mitrovic-Tutundzic (1994) and are characterised as follows: slight alteration (1) involves changes that do not damage gill tissues so that the restructuration and recovery of normal gill function can occur with improvement of the environmental conditions. These changes are limited to small parts of the gills or some filaments; for example, slight alteration of the epithelium of the primary lamella. Moderate alteration (2) involves more severe changes that lead to effects in tissues associated with the functioning of the organ. These lesions are reparable, but if wide areas of the gills are affected or maintained in situations of chronic pollution, they can lead to severe alterations, on practically the whole surface of the gills, for example, the epithelial lifting of secondary lamella. In cases of severe alteration (3), the recovery of the gill structure is not possible, even with improvement in water quality or no further exposure to a toxic stimulus, for example, aneurysms. This scale was used to determine the mean values of alteration intensities for each treatment.

The presence of histopathological alterations for gills was determined semi-quantitatively by the degree of tissue alteration (Histopathological Alterations Index - HAI), based on the severity of the injuries. In the determination of the HAI (modified from Poleksic & Mitrovic-Tutundzic, 1994), the alterations in each organ were classified in progressive stages of tissue damage (Table 1).

The HAI value was calculated for each animal using the following formula:

$$\text{HAI} = (1X SI) + (10X SII) + (100X SIII),$$

where I, II and III correspond to the number of stages of alterations 1, 2 and 3; and S represents the sum of the number of alterations for each particular stage.

HAI values between 0 and 10 indicate normal functioning of the organ; values 11 to 20 indicate slight damage to the organ; 21 to 50 indicate moderate alterations in the organ; values 50 to 100 indicate severe lesions; and values >100 indicate irreparable lesions of the organ (Poleksic & Mitrovic-Tutundzic, 1994). Some cases were selected and photographed with the use of a photomicroscope.

Data were analysed by ANOVA followed by Tukey's test ($P < 0.05$). To set the best water salinity level was carried out the Regression analysis ($P < 0.05$). The non-parametric Mann-Whitney test for independent samples with $P < 0.05$ was used for comparison of anomaly intensity means between five specimens of each treatment. The differences between treatments were tested using non-parametric ANOVA. The degree of significance was 95%. Statistical analyses were carried out using the software R.

RESULTS

The addition of seawater to the fresh water did not change the parameters of water quality, dissolved oxygen, temperature and pH, monitored during the experimental period, which had been similar ($P > 0.05$) between the treatments and remained inside the acceptable range for fish breeding (Table 2).

There was no effect ($P > 0.05$) of salinity levels on daily feed intake, obtaining an average 0.54 g day⁻¹. The salinity levels affected the other variables of fish performance (Table 3).

Water salinity levels had significant effect on the daily weight gain ($P = 0.0267$), feed conversion rate ($P = 0.0072$) and survival ($P = 0.0005$) of Nile tilapia. A significant reduction of these parameters was observed at 14 and 21 g L⁻¹ of water salinity by Tukey's test analysis. Regression analysis showed a quadratic behavior with better values for, respectively, daily weight gain, feed conversion rate and survival of 5.62, 2.08 and 4.19 g L⁻¹ of water salinity (Figs. 1, 3).

Regarding the haematological parameters evaluated, it was observed that the haematocrit ($P = 0.0001$) and the erythrocyte count ($P = 0.0040$) were influenced by the water salinity level with higher values in 0 and 7 g L⁻¹; however, the leukocyte count did not change ($P = 0.5376$) regardless of the water salinity level (Table 4).

Regression analysis showed a quadratic behavior with better values for, respectively, haematocrit and erythrocyte count of 0.92 and 2.39 g L⁻¹ of water salinity (Figs. 4, 5).

Table 1. List of histopathologic alterations observed in the gills of *Oreochromis niloticus*. I, II and III – Severity stages of alterations.

Stage	Histopathologic alterations in the gills
I	Hypertrophy and hyperplasia of gill epithelium Sanguineous congestion Dilation of marginal vascular channels Lifting of respiratory epithelium Fusion and disorganisation of secondary gill lamellae Shortening of secondary lamellae Leukocyte infiltration of gill epithelium Aggregation of cells of the primary lamella
II	Haemorrhage and rupture of lamellar epithelium Hypertrophy and hyperplasia of mucous cells Empty mucous cells or their disappearance Hypertrophy and hyperplasia of chloride cells
III	Lamellar aneurism Necrosis and cell degeneration Lamellar telangiectasis

Table 2. Mean values and standard deviation of the physical and chemical parameters of the water of the experimental tanks, in accordance with the treatment.

Parameter	Salinity (g L ⁻¹)			
	0	7	14	21
Dissolved oxygen (mg L ⁻¹)	4.75 ± 0.63	4.58 ± 0.13	4.51 ± 0.48	4.32 ± 0.45
Temperature (°C)	28.19 ± 1.25	28.33 ± 1.10	28.27 ± 0.96	28.31 ± 0.99
pH	7.06 ± 0.71	7.16 ± 0.11	7.14 ± 0.13	7.14 ± 0.14
Salinity (g L ⁻¹)	0.02 ± 0.01	7.06 ± 0.71	13.75 ± 1.17	20.73 ± 1.59

Table 3. Performance and survival of Nile tilapia cultured in different salinity levels. *Significant, NS: not significant. Means followed by different letters in the lines differ by Tukey's test ($P < 0.05$).

Variable	Salinity (g L ⁻¹)				F
	0	7	14	21	
Initial weight (g)	1.60 ± 0.21 ^a	1.63 ± 0.19 ^a	1.61 ± 0.15 ^a	1.62 ± 0.22 ^a	2.54 ^{NS}
Daily feed intake (g day ⁻¹)	0.52 ± 0.01 ^a	0.57 ± 0.07 ^a	0.52 ± 0.07 ^a	0.53 ± 0.06 ^a	2.79 ^{NS}
Daily weight gain (g day ⁻¹)	0.46 ± 0.01 ^a	0.52 ± 0.01 ^a	0.41 ± 0.02 ^b	0.42 ± 0.02 ^b	3.64 [*]
Feed conversion (g g ⁻¹)	1.15 ± 0.10 ^a	1.10 ± 0.02 ^a	1.28 ± 0.05 ^b	1.29 ± 0.05 ^b	8.24 [*]
Survival (%)	95.00 ± 5.53 ^a	97.33 ± 3.65 ^a	83.99 ± 3.64 ^b	80.83 ± 9.25 ^b	15.87 [*]

Regarding the histological analysis, Nile tilapia gills have the same pattern as in other teleosts. The filament is covered by a stratified epithelium in the interlamellar region in which there are epithelial cells, melanocytes, lymphocytes, macrophages, granulocytes and eosinophils, as well as chloride cells. The structure of the gill arch and gill filaments shows mucosal cells. The secondary lamella is covered by a squamous epithelium that generally shows a thickness of one or two cell layers. Below the epithelium there are lamellar blood spaces that are bordered by pillar cells, which have a contractile function. In the outermost region of the

secondary lamella, there is a blood vessel that has an internal covering of endothelium (Fig. 6).

The histopathological alterations observed in *Oreochromis niloticus* were chloride cell hypertrophy, epithelial lifting, structure alteration, telangiectasia, aggregation of cells of primary lamellae, fusion and occurrence of various sizes aneurysms in some secondary lamellae.

Slight alterations were observed in the first treatment, such as epithelial lifting and fusion of secondary lamellae and severe alterations such as telan-

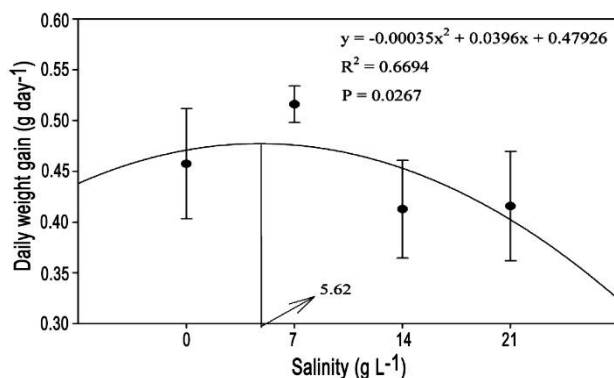


Figure 1. Effect of water salinity levels on the daily weight gain of Nile tilapia.

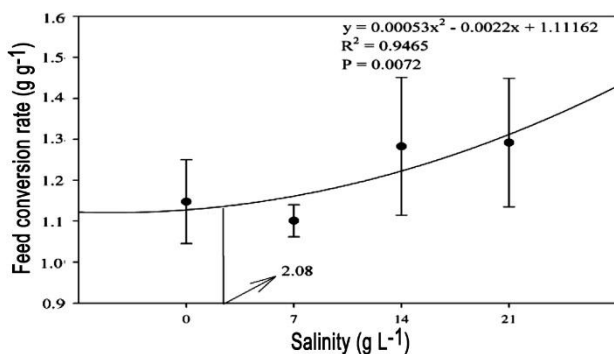


Figure 2. Effect of water salinity levels on feed conversion rate of Nile tilapia.

giectasia in a few secondary lamellae. In salinity of 7 g L⁻¹, slight alterations were observed such as epithelial lifting, fusion of secondary lamellae, aggregation of cells in the primary lamella and severe alterations such as telangiectasia and aneurysms in a few secondary lamellae. In salinity of 14 g L⁻¹ slight alterations were observed such as bifurcation of secondary lamellae, fusion of secondary lamellae, epithelial lifting and severe alterations such as telangiectasia and aneurysms with frequency higher than in previous treatments. In salinity of 21 g L⁻¹ a greater number of slight alterations was observed, which were epithelial lifting, fusion of secondary lamellae, change in the structure of secondary lamellae, chloride cell hypertrophy, and aggregation of cells of the primary lamella. Telangiectasia and aneurysms were observed among the severe alterations.

Only slight alterations (1) were found in salinities of 0, 7 and 14 g L⁻¹ when we analysed the gills' frequency of infection intensity; in salinity of 21 g L⁻¹ we observed a high frequency of individuals with moderate alterations (2) (Fig. 7).

The results of the Histopathological Alteration Index (HAI) showed that in all treatments there were averages above 100 (102.0, 136.7, 137.7 and 172.3,

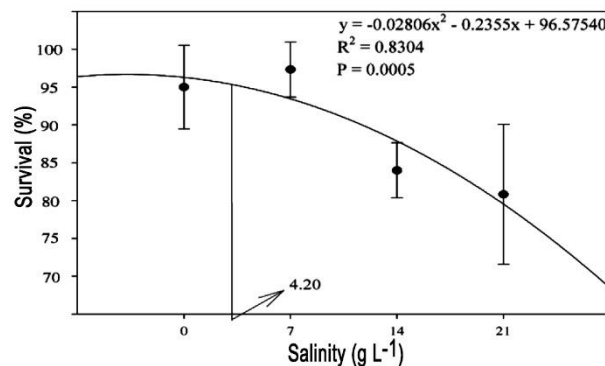


Figure 3. Effect of water salinity levels on the survival of Nile tilapia.

respectively), indicating that individuals present irreparable injury in the body and that even if water conditions improve, the structure of the body cannot be recovered (Table 5). The high average values is due to the presence of aneurysms, which are regarded as a serious injury, in almost all the individuals.

DISCUSSION

The fish survival and growth can be influenced by the water quality (Likongwe *et al.*, 1996). The relationship between salinity and fish growth seems to be complex, because studies with different species have presented varied results. Similar results were reported by Likongwe *et al.* (1996), who evaluated different salinity levels (0, 8, 12 and 16 g L⁻¹) and water temperature (24, 28 and 32°C) on the performance of juvenile Nile tilapia (4.6 to 4.8 g), observing a better performance in the highest temperatures evaluated, and a decrease from 8 g L⁻¹ of water salinity. They also observed that the combination between the salinity of 8 g L⁻¹ and water temperature of 32°C resulted in better feed conversion rate.

The best performance of the fish cultivated in the lower water-salinity levels in this experiment can be related to the energy cost for the ionic regulation, which is lower when the fish are kept in an isotonic environment, where ionic gradients between the blood and the water are minimum, and this energy economy can be directed towards growth (Boeuf & Payan, 2001).

Besides osmoregulation, the effect of water salinity on the performance of fish can be explained by its action upon digestive enzymes, where the exposure to different salinities modifies the water ingestion, altering the salinity of the intestinal content and affecting the activity of digestive enzymes (Moutou *et al.*, 2004). This process can explain the worsening in the alimentary conversion and consequent worsening of the weight gain of tilapias in the highest salinities in this experiment.

Table 4. Hematological variables to Nile tilapia cultivated in different salinity levels. *Significant, NS: not significant. Means followed by different letters in the lines differ by Tukey's test ($P < 0.05$).

Variable	Salinity (g L ⁻¹)				F
	0	7	14	21	
Haematocrit (%)	25.4 ± 1.82 ^a	27.6 ± 1.72 ^a	18.4 ± 1.34 ^b	16.8 ± 3.11 ^b	16.95*
Erythrocyte (x10 ⁶ μL ⁻¹)	1.58 ± 0.20 ^a	1.86 ± 0.29 ^a	1.08 ± 0.15 ^b	1.08 ± 0.15 ^b	7.77*
Leukocyte (x10 ³ μL ⁻¹)	23.01 ± 8.63 ^a	23.05 ± 10.21 ^a	23.31 ± 7.04 ^a	19.75 ± 3.11 ^a	0.42 ^{NS}

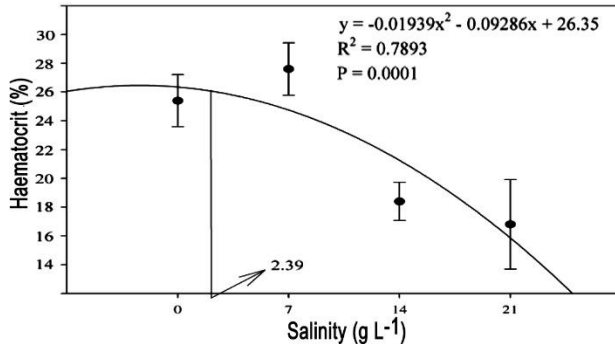


Figure 4. Effect of water salinity levels on the haematocrit of Nile tilapia.

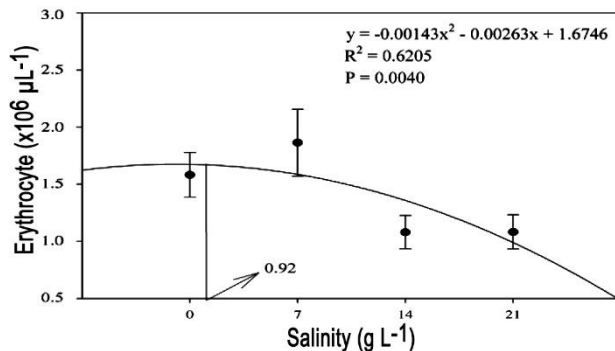


Figure 5. Water salinity levels effect of upon erythrocyte count of Nile tilapia.

In this study, although the leukocyte values were not influenced ($P > 0.05$) by the different water salinity levels, there was a decrease of these values with increased salinity. This small difference between the compared values may be due to the high standard deviation.

The haematocrit and erythrocyte count found in this study for the water salinity levels of 0 and 7 g L⁻¹ were similar to the reference erythrogram values for Nile tilapia observed by Barros *et al.* (2009) and Teixeira *et al.* (2012). However, for the salinity levels of 14 and 21 g L⁻¹, the values were lower than those found by these authors. The study of the haematological analyses allows understanding of the influence of the nutritional and environmental conditions on fish health (Ranzani-

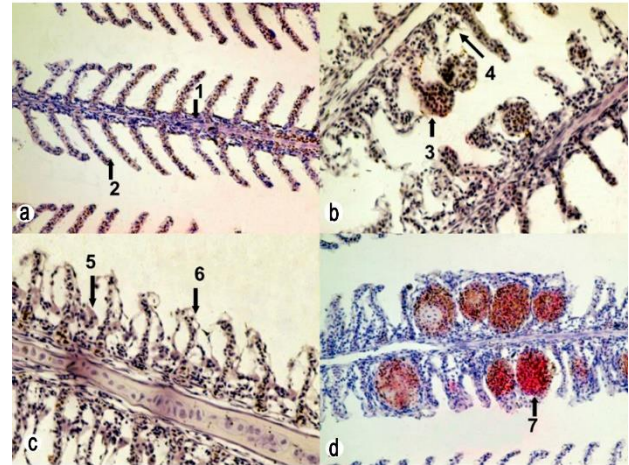


Figure 6. Histological sections of gills of *Oreochromis niloticus* specimens: a) Normal gill (1: primary lamella, 2: secondary lamella, H&E, 400x), b-d) Histopathological alterations. b and d) H&E 400x; c) H&E, 1000x; 3: telangiectasis; 4: aggregation of cells of the primary lamella; 5: hypertrophy of chloride cells; 6: intensive epithelial lifting; 7: lamellar aneurysms.

Paiva & Silva-Souza, 2004). In this study, for the water salinity levels of 0 and 7 g L⁻¹, the blood parameters for haematocrit percentage and erythrocyte and leukocyte counts were adequate and indicated healthy fish as described by Barros *et al.* (2009). In contrast, the amounts obtained for the water salinity levels of 14 and 21 g L⁻¹ were inadequate, suggesting that the fish may have suffered stress caused by the higher levels of water salinity.

The high variation between the haematological parameters has also been shown by Ranzani-Paiva & Silva-Souza (2004), who attributed this variation to internal and external factors.

According to Aird (2000), the anaemia may be characterised when two or more blood parameters are below the normal average for the species. In this case, the haematocrit and erythrocyte values support this hypothesis for the water salinity levels of 14 and 21 g L⁻¹. The increased water salinity probably damaged the erythropoiesis of juvenile tilapia, which could not main-

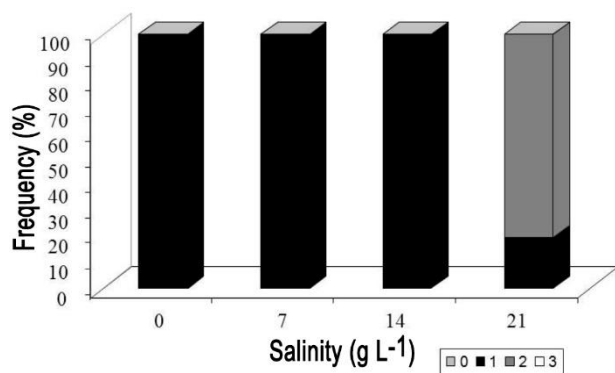


Figure 7. Frequency of intensity of anomalies in gills, per treatment, during the study period. 0: without alterations, 1: slight alteration, 2: moderate alteration, and 3: severe alteration.

tain the minimum necessary production of erythrocytes for health maintenance (Barros *et al.*, 2009). Likewise, the number of leukocytes, which are part of the organism's defence system, may have been reduced (although not presenting a significant difference) as a result of the stress caused by the highest salinity levels evaluated, damaging the organism's defence functions, which can explain the highest mortality rates with the highest water salinity levels.

Only a few micrometres separate the blood from the water in the gills (Yada *et al.*, 2012), which not only facilitates the exchange of gases, but also allows the gill tissue to be exposed to variations in the environment. Consequently, the presence of toxic substances in the environment causes alterations in the vital functions carried out by the gills and alterations in the morphologic structure of these organs (Poleksic & Mitrovic-Tutundzic, 1994). Thus, the histopathological analysis of fish gills has been used as a tool that is extremely important in the evaluation of the quality of aquatic ecosystems.

Physical and chemical changes in this ecosystem are rapidly reflected as quantifiable physiologic measurements in the fish. In general, reactions of the fish gills due to an irritant include inflammation, hyperplasia, lamellar fusion, excessive production of mucus, epithelial lifting, flattening of the secondary lamella and formation of aneurysms. Inflammation, hyperplasia, secretion of mucus and aneurysms were also observed in *O. niloticus*, demonstrating that the gills of these individuals had been affected by the action of various stressors (Schwaiger *et al.*, 1997). Because of the epithelial lifting, there is an increase in the distance between the water and blood, impairing oxygen uptake. However, in these conditions, the fish increase their rate of respiration by compensating for the low entrance of oxygen (Emmanouil *et al.*, 2008).

Table 5. Results of histopathological alteration index (HAI) in each treatment. N: Number of individuals, M: means, SD: standard deviation.

Salinity (g L ⁻¹)	N	Means of HAI	SD
0	5	102.0	54.4
7	5	136.7	45.3
14	5	137.7	44.5
21	5	172.3	0.9

According to Winkaler *et al.* (2001), these types of histopathological lesions indicate that the fish respond to the effects of toxic agents present in the water.

In this study, the high frequency of occurrence of telangiectasia and aneurysms may be associated with salinity. Stentford *et al.* (2003) found an increased frequency of aneurysms in specimens captured in contaminated areas. These authors observed that these lesions, which cause disturbances in blood flow in the fish gills, can be associated with the presence of irritants in the water. Therefore, the presence of these alterations can be useful biomarkers for certain substances. Histopathological alterations such as lamellar aneurysm and epithelial lifting were also observed by Winkaler *et al.* (2001) in different species.

The results of this study demonstrate that Nile tilapia can be raised in environments with moderate levels of water salinity. Up to 7 g L⁻¹ were not observed negative effects on growth, survival and haematological parameters by Tukey's test, although by Regression analysis were observed the best results for daily weigh gain, feed conversion rate and survival, respectively, with 5.62, 2.08 and 4.20 g L⁻¹ of water salinity; and worsening trend in the haematological parameters from 0.92 g L⁻¹ (erythrocyte count) and 2.39 g L⁻¹ (haematocrit). Regarding the histological changes in the gills slight alterations were found in salinities of 0, 7 and 14 g L⁻¹ and moderate alterations were observed in salinity of 21 g L⁻¹, showing the deleterious effects of higher salinities over the gills of Nile tilapia.

REFERENCES

- Aird, B. 2000. Clinical and hematological manifestations of anemia. In: B.F. Feldman, J.G. Zinkl & N.C. Jain (eds.). Schalm's veterinary hematology. Lippincott Williams & Wilkins, Philadelphia, 140-142 pp.
- Árnason, T., B. Magnadóttir, B. Björnsson, A. Steinarsson & B.T. Björnsson. 2013. Effects of salinity and temperature on growth, plasma ions, cortisol and immune parameters of juvenile Atlantic cod (*Gadus morhua*). *Aquaculture*, 380-383(4): 70-79.
- Barros, M.M., M.J.T. Ranzani-Paiva, L.E. Pezzato, D.R. Falcon & I.G. Guimarães. 2009. Haematological

- response and growth performance of Nile tilapia (*Oreochromis niloticus*) fed diets containing folic acid. *Aquacult. Res.*, 40(8): 895-903.
- Boeuf, G. & P. Payan. 2001. How should salinity influence fish growth? *Comp. Biochem. Physiol. C, Toxic. Pharmacol.*, 130(4): 411-423.
- DiMaggio, M.A., C.L. Ohs & B.D. Petty. 2009. Salinity tolerance of the Seminole killifish, *Fundulus seminolis*, a candidate species for marine baitfish aquaculture. *Aquaculture*, 293(1-2): 74-80.
- Dominguez, M., A. Takemura, M. Tsuchiya & S. Nakamura. 2004. Impact of different environmental factors on the circulating immunoglobulin levels in the Nile tilapia, *Oreochromis niloticus*. *Aquaculture*, 241(1-4): 491-500.
- Emmanouil, C., R.M. Green, F.R. Willey & J.K. Chipman. 2008. Oxidative damage in gill of *Mytilus edulis* from Merseyside, UK, and reversibility after depuration. *Environ. Pollut.*, 151(3): 663-668.
- Jesus, L.S.F., R.V. Azevedo, J.S.O. Carvalho & L.G.T. Braga. 2011. Farellos da vagem da algaroba e da folha da mandioca em rações para juvenis de tilápia do Nilo mantidos em água salobra. *Rev. Bras. Saúde Prod. Anim.*, 12(4): 1116-1125.
- Güner, Y., O. Özden, H. Çagırgan, M. Altunok & V. Kizak. 2005. Effects of salinity on the osmoregulatory functions of the gills in Nile tilapia (*Oreochromis niloticus*). *Turk. J. Vet. Anim. Sci.*, 29: 1259-1266.
- Kamal, A.H.M.M. & G.C. Mair. 2005. Salinity tolerance in superior genotypes of tilapia, *Oreochromis niloticus*, *Oreochromis mossambicus* and their hybrids. *Aquaculture*, 247(1-4): 189-201.
- Likongwe, J.S., T.D. Stecko, J.R. Stauffer-Jr. & R.F. Carline. 1996. Combined effects of water temperature and salinity on growth and feed utilization of juvenile Nile tilapia, *Oreochromis niloticus* (Linnaeus). *Aquaculture*, 146(1-2): 37-46.
- Luz, R.K., R.M. Martínez-Álvarez, N. De-Pedro & M.J. Delgado. 2008. Growth, food intake regulation and metabolic adaptations in goldfish (*Carassius auratus*) exposed to different salinities. *Aquaculture*, 276(1-4): 171-178.
- Marengoni, N.G., D.M. Albuquerque, F.L.S. Mota, O.P. Passos-Neto, A.A. Silva-Neto, A.I.M. Silva & M. Ogawa. 2010. Performance and sexual proportion in red tilapia under inclusion of probiotic in mesohaline water. *Arch. Zootec.*, 59(227): 403-414.
- Moutou, K.A., P. Panagiotaki & Z. Mamuris. 2004. Effects of salinity on digestive protease activity in the euryhaline sparid *Sparus aurata* L.: a preliminary study. *Aquacult. Res.*, 35(9): 912-914.
- Poleksic, V. & V. Mitrovic-Tutundzic. 1994. Fish gills as a monitor of sublethal and chronic effects of pollution. In: R. Müller & R. Lloyd (eds.). *Sublethal and chronic effects of pollutants on freshwater fish*. Cambridge University, Cambridge, pp. 339-352.
- Ranzani-Paiva, M.J. & A.T. Silva-Souza. 2004. Hematologia de peixes brasileiros. In: M.J. Ranzani-Paiva, R.M. Takemoto & M.A.P. Lizama (eds.). *Sanidade de organismos aquáticos*. Editora Varela, São Paulo, pp. 89-120.
- Reis, A.B., D.M.G. Santana, J.F. Azevedo, L.S. Merlini & E.J.A. Araújo. 2009. The influence of the aquatic environment in tanks sequentially interconnected with PVC pipes on the gill epithelium and lamellas of tilapia (*Oreochromis niloticus*). *Pesq. Vet. Bras.*, 29(4): 303-311.
- Riche, M. & T.N. Williams. 2010. Apparent digestible protein, energy and amino acid availability of three plant proteins in Florida pompano, *Trachinotus carolinus* L. in seawater and low-salinity water. *Aquacult. Nutr.*, 16(3): 223-230.
- Schwaiger, J., R. Wanke, S. Adam, M. Pawert, W. Honnen & R. Triebkorn, R. 1997. The use of histopathological indicators to evaluate contaminant-related stress in fish. *J. Aquat. Ecosyst. Stress Recovery*, 6(1): 75-86.
- Stentiford, G.D., M. Longshaw, B.P. Lyons, G. Jones, M. Green & S.W. Feist. 2003. Histopathological biomarkers in estuarine fish species for the assessment of biological effects of contaminants. *Mar. Environ. Res.*, 55(2): 137-159.
- Teixeira, C.P., M.M. Barros, L.E. Pezzato, A.C. Fernandes, J.F.A. Koch & C.R. Padovani. 2012. Growth performance of Nile tilapia, *Oreochromis niloticus*, fed diets containing levels of pyridoxine and haematological response under heat stress. *Aquacult. Res.*, 43(8): 1081-1088.
- Tsuzuki, M.Y., J.K. Sugai, J.C. Maciel, C.J. Francisco & V.R. Cerqueira. 2006. Survival, growth and digestive enzyme activity of juveniles of the fat snook (*Centropomus parallelus*) reared at different salinities. *Aquaculture*, 271(1-4): 319-325.
- Winkaler, E.U., A.G. Silva, H.C. Galindo & C.B.R. Martinez. 2001. Histological and physiological biomarkers to assess fish health in Londrina streams, state of Paraná. *Acta Sci.*, 23(2): 507-514.
- Yada, T., S.D. McCormick & S. Hyodo. 2012. Effects of environmental salinity, biopsy, and GH and IGF-I administration on the expression of immune and osmoregulatory genes in the gills of Atlantic salmon (*Salmo salar*). *Aquaculture*, 362-363(1): 177-183.
- Yuan, X., H. Yang, L. Wang, Y. Zhou & H.R. Gabr. 2010. Effects of salinity on energy budget in pond-cultured sea cucumber *Apostichopus japonicus* (Selenka) (Echinodermata: Holothuroidea). *Aquaculture*, 306(2): 348-351.