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Effect of total hardness of water on juvenile mullets (*Mugil liza*) raised in fresh water

*Efeito da dureza total em juvenis de tainha (*Mugil liza*) criados em água doce*

Fernanda Scheuer*[1], Marisa Pereira de Souza[1], Fábio Carneiro Sterzelecki[2], Filipe dos Santos Cipriano[1], Vinicius Ronzani Cerqueira[1], Mauricio Laterça Martins[3]

ABSTRACT: The aim of this study was to evaluate the effects of three fresh-water samples with different hardness values (25, 250, and 750 mg L^{-1} $CaCO_3$) and a control (sea water with salinity 15% and hardness 2500 ± 130.9 mg L^{-1} $CaCO_3$) on *Mugil liza* (mullet) juveniles by conducting a long-term test (for 50 days). Zootechnical performance [weight gain (WG), feed conversion rate, specific growth rate, and survival (%)], stress indicator parameters (glucose), and physiological parameters in gill tissues were analyzed to determine the fresh-water hardness range that can allow the optimal survival, growth, and feed conversion for the species. Fish with a mean initial weight of 22 ± 2.84 g were maintained under constant conditions of temperature, pH, alkalinity, and ammonia concentration, and they were fed four times per day with a commercial diet. When evaluating zootechnical performance, only survival (%) was not significantly different among treatments. For the other variables—weight gain, feed conversion, and specific growth rate—the best results were found in the treatment with 15% salinity (control). Blood glucose levels in animals maintained at 15% salinity (control) were significantly lower than those observed in the other treatments. Histological analyses corroborated the zootechnical data, potentially indicating stress in fish maintained at very low and very high water hardness. In conclusion, for cultivating juvenile mullets in fresh water, the most suitable hardness value was 250 mg L^{-1} $CaCO_3$, because the best final weight of fish was achieved in that condition.

KEY WORDS: Aquaculture; euryhaline species; physiological comfort; calcium carbonate.

RESUMO: O objetivo desse estudo foi avaliar o efeito de três durezas diferentes de água doce (25, 250 e 750 mg.L^{-1} $CaCO_3$) e controle (água do mar com salinidade 15 % e dureza 2500 ± 130,9 mg.L^{-1} $CaCO_3$), em juvenis de tainha *Mugil Liza* , através de um teste de longo prazo (50 dias), avaliando o desempenho zootécnico (ganho de peso (GP), conversão alimentar (CA), taxa de crescimento específico (TCE) e sobrevivência (%)), parâmetros indicadores de estresse (glicose) e avaliação de parâmetros fisiológicos nos tecidos das brânquias, a fim de determinar a faixa de dureza em água doce onde há melhor sobrevivência, crescimento e conversão alimentar dessa espécie. Os peixes com peso médio inicial de 22 ± 2,84g foram mantidos em condições constantes de temperatura, pH, alcalinidade e amônia, e alimentados quatro vezes ao dia com dieta comercial. Ao avaliar o desempenho zootécnico apenas a sobrevivência (%) não foi significativamente diferente entre os tratamentos, porém, para as demais variáveis: ganho de peso (GP), conversão alimentar (CA) e taxa de crescimento específico (TCE) os melhores resultados foram encontrados no tratamento com salinidade 15 % (controle). A glicose no sangue dos animais mantidos a salinidade 15 % (controle) foi significativamente menor em relação aos outros tratamentos. As análises histológicas corroboram os dados zootécnicos, indicando possivelmente stress nos peixes mantidos em durezas muito baixas e muito altas. Conclui-se que para cultivar juvenis de tainha em água doce, a dureza mais adequada é 250 mg.L^{-1} $CaCO_3$, pois apresentou melhor peso final dentre as durezas avaliadas.

PALAVRAS-CHAVE: Aquicultura; espécies eurialinas; conforto fisiológico; carbonato de cálcio.

[1]Laboratório de Piscicultura Marinha (LAPMAR), Departamento de Aquicultura, CCA, UFSC, Florianópolis, Brasil.
[2]Laboratório de Biossistemas Aquáticos Amazônico (ISARH), Universidade Federal Rural da Amazônia, Belém, Brasil.
[3]Laboratório de Sanidade de Organismos Aquáticos (AQUOS), Departamento de Aquicultura, CCA, UFSC, Florianópolis, Brasil.
*Corresponding author: fernandascheuer@hotmail.com
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INTRODUCTION

The mullet *Mugil liza* Valenciennes, 1836 (Teleostei: Mugilidae) is easy to farm, because it is rustic and robust, can adapt to confinement conditions, easily accepts artificial feeding, and has satisfactory resistance to temperature variations and salinity. These advantages make its breeding possible in different environmental conditions (CERQUEIRA et al., 2017; PAN et al., 2016; VIEIRA; SCALABRIN, 1991).

This fish is found from Rio de Janeiro, Brazil to parts of Argentina. It is a coastal fish (present in tropical and subtropical waters), which swims on the surface and forms schools. It is present in substantial abundance in estuarine environments, where they can reach up to 50 cm in average total length and weigh 6–8 kg (BARLETTA; DANTAS, 2016; DURAND et al., 2012; LEMOS, 2014; MENEZES et al., 2010). The species has a detritivorous feeding habit (iliophagous): they feed on benthic microorganisms, detritus, diatoms (*Skeletonema costatum*), cyanophyceans, bacteria, and decomposing plants (debris) associated with inorganic sediment (CERQUEIRA, 2004).

This euryhaline teleost fish can maintain a relatively constant osmolality and ionic composition of its internal fluids, regardless of the composition of the external environment (NORDLIE; LEFFLER, 1975). The study of osmoregulation is fundamental because most fish live in environments with ion concentrations different from those in their blood (BALDISSEROTTO, 2003). Osmoregulation demands high energy input; the ideal salinity of the species can reduce the energy spent on maintaining osmotic homeostasis and consequently promote growth (SAMPAIO & BIANCHINI 2002).

The requirements and mechanisms of ion regulatory pathways change as a function of environmental salinity, diet, activity, developmental stage, stressors, and acclimatization to salinity. Structural and metabolic reorganization is needed to fulfill the increased energy demand associated with exposure to the new environment (ANDRADE et al., 2007). Exposure to different salinities can change the cost of osmoregulation and, consequently, the amount of energy available for fish growth (ANNI et al., 2016; GRACIA-LÓPEZ; ROSAS-VÁZQUEZ; BRITO-PÉREZ, 2006).

Water hardness is of fundamental importance in this process; fish can absorb calcium and magnesium directly from water or food. The presence of free (ionic) calcium in water helps reduce the loss of other salts (e.g., sodium and potassium) from body fluids of fish (WURTS AND DURBOROW, 1992).

Hardness is also essential for biological processes, such as bone building and blood clotting (FLIK, RIJS, WENDELAAR BONGA, 1985). Because Ca^{2+} reduces gill permeability (BALDISSEROTTO, 2011), increasing water hardness may be a satisfactory strategy for improving mullet growth in fresh water. The aim of this study was to evaluate the influence of water hardness on young mullets reared in fresh water.

MATERIAL AND METHODS

Fish and Acclimatization

All animal handling procedures were approved by the Ethics Committee for the Use of Animals (CEUA/UFSC no. 3102220419). The experiment was conducted at the Marine Fish Laboratory of the Federal University of Santa Catarina, Florianópolis, Brazil. The *M. liza* juveniles used in this experiment were 8 months old and weighed 22 ± 2.84 g. The fish were obtained from reproduction in the laboratory from specimens maintained in captivity, according to methods described by PASSINI et al. (2016).

Before the experiment, the fish (n = 240) were placed in a 6000 L tank, where they were maintained at 32% salinity and 28 °C. The salinity was progressively reduced by diluting the seawater with filtered and dechlorinated water with a daily renewal of 25% water.

By dilution, a previously prepared solution was used, in which the hardness was increased to 500 mg.L^{-1} $CaCO_3$ (intermediate value between the treatments tested) and 0% salinity. The fish were maintained in a 500 L tank first with seawater (salinity 35%), and each day, 125 L prepared solution was added. After 4 days, the salinity reached 0%, and the fish were randomly distributed to the experimental units.

Experimental design

Initially, an acute test of 96 hours (h) was performed, during which seven treatments were used with three replications each, using 21 buckets of 15 L populated with 10 fish per unit. The treatments were related to water hardness and had concentrations of 20, 100, 250, 500, 750, and 1000 mg L^{-1} $CaCO_3$ and a treatment with seawater (control). The animals were abruptly transferred to the experimental units, and there was no feeding. The daily salinity (portable refractometer), dissolved oxygen, temperature (Alfakit AT-150 oximeter), pH with a portable pH (YSI EcoSense pH 10, Yellow Springs Instruments, OH, USA), alkalinity, and hardness were measured using the EDTA titration method (EATON et al., 2005). The dead fish were counted daily for survival analysis.

Based on the results of the acute test, a 50-day long-term test with pre-selected values of freshwater hardness was conducted as follows: soft water treatment (25 mg L^{-1} $CaCO_3$); hard water treatment (250 mg L^{-1} $CaCO_3$); extremely hard water treatment (750 mg L^{-1} CaCO3); and a control treatment using seawater plus soft water (directly from the treatment plant, with a hardness of 2500 mg L^{-1} $CaCO_3$), resulting in a salinity of 15%, close to the isosmotic point (13.5%) of the mullet (CUNHA et al., 2015).

Experimental Units and Conditions

Four independent water recirculation systems with a flow of 1 L min^{-1} were used, each coupled to three 100 L tanks,

totaling 12 tanks. Each system had a 100 L buffer box, which contained a water pump, heater with thermostat, UV, and biological media (bioballs), and the objective of the latter was to improve the quality of the water in the system. All ponds were covered with a screen to avoid jumping or fish loss. Each experimental unit had light, constant aeration, and a photoperiod of 12 h of light and 12 h of darkness.

The system also had four tanks filled with 80 L water stock solution; 20% of water was renewed daily for each treatment. This solution had the same water quality as that of the experimental systems, including pre-established hardness.

The initial density was 20 fish per tank (0.2 fish L^{-1} of water). After 25 days, the density was decreased by removing 10 fish from each experimental unit. In all treatments, the fish showed different behavior from the beginning (erratic swimming and decreased appetite), probably due to the high density. After thinning, fish behavior was normalized.

Feeding was performed with a commercial diet (Nutripisces) containing 45% protein offered manually to satiety four times per day (8:30 h, 11:30, 14:30, and 17:30 h). Feeding was suspended 24 h before the intermediate and final sampling. Food remains and feces were removed daily by siphoning.

Water quality parameters

Daily salinity was measured using a portable refractometer (Soma Peixes SHR10-ATC). Dissolved oxygen and temperature values were measured with an oximeter (AT-150, Alfakit, Florianópolis). pH was measured with a portable pH meter (YSI EcoSense pH 10, Yellow Springs Instruments, OH, USA). Alkalinity (APHA, 2005) and hardness values were measured using the EDTA titration method (EATON et al., 2005). Ammonia concentration was measured weekly using a calibration curve (STRICKLAND and PARSONS, 1972).

The freshwater used in the experiment was directly from the water distribution station of the municipality of Local B and had an average hardness of 33 ± 7.09 mg L^{-1} $CaCO_3$. When necessary, adjustments were made to the hardness of the treatments by using $CaCl_2.2H_2O$ and to the alkalinity by using $NaHCO_3$. The pH (below 7.0) was adjusted using a 2N NaOH solution.

Zootechnical performance

Benzocaine (50 mg L-1) was used as the anesthetic in all samples. Juveniles were weighed on a 0.01 g precision scale (Mars AD1000) and measured with a ruler. Those who had material collected (blood and gills) were previously anesthetized and euthanized with an overdose of benzocaine (200 mg.L-1). Blood was collected by caudal venipuncture using a 2 ml syringe prepared with heparin (anticoagulant). Approximately 2 ml was removed per fish, which was stored individually with no need to pool.

At the end of the experiment, biometry was performed on 100% of fish. Blood and gills were collected from five fish per experimental unit for glucose determination and the preparation of histological slides.

The following parameters were evaluated: survival (%); weight gain: WG (g) = FW - IW, FW being final weight and IW, initial weight; growth rate: GRS = [(ln FW – ln IW)/t) x100], where t is time (days); and apparent feed conversion: AFC = FO/WG, where FO is the amount of food offered (g).

Collection of biological material and analysis

Histological slides were prepared at the Sanitation of Aquatic Organisms, Federal University of Santa Catarina, Florianópolis, Brazil. For histological analysis, 5 fish per replicate per treatment were used, totaling 60 fish. The total section of the second left gill arch was standardized. Next, the samples were stored individually in cassettes and immersed in 10% buffered formalin solution until the date of histology.

The samples were subsequently dehydrated in an increasing series of ethanol solutions, diaphanized in xylene, and embedded in paraffin to obtain 3 µm-thick cross-sections, which were sectioned using a manual microtome. They were then stained with hematoxylin-eosin (H&E) according to the methodology described by Michalany (1998). For each slide, three images were captured under a microscope (LEICA DM750, Wetzlar, Germany) for further analysis using software (LEICA LAS-EZ, Wetzlar, Germany). The analysis of the gills consisted of searching for tissue alterations, such as secondary lamellar epithelial hyperplasia, interlamellar epithelial hyperplasia, secondary lamella fusion, telangiectasia, venous sinus congestion of the primary lamella, secondary lamella fusion, justalamellar edema, epithelial detachment, and sinus dilatation. For analyzing the secondary lamella and the increase in the volume of the chloride cells, the degree of severity was determined using a methodology adapted from Poleksić and Mitrović-Tutundžić (1994). I is a mild alteration, II is a moderate alteration, and III is a severe alteration. These alterations were classified based on the location of the lesions: focal, multifocal, and coalescent.

Blood was collected for glucose analysis at the beginning and end of the experimental period. Blood was obtained by caudal puncture. Immediately after collection, glucose was measured using a portable glucometer (Ultra 2 System Kit Glico Apar; Onetouch, Brazil).

Statistical analysis

First, the homoscedasticity of the variances and the normality of the data distribution were verified using the Shapiro–Wilk and Levene tests. Because the results were positive, the data were compared using one-way analysis of variance, followed by Tukey's test to compare the means. All analyses were

performed using Statistica 7.0, with a significance level of 5%. Data are presented as the mean and standard deviation.

RESULTS AND DISCUSSION

Water quality

The ammonia concentration did not exceed 0.003 mg L^{-1}. There were significant differences in the pH and temperature between the 25 mg L^{-1} $CaCO_3$ treatment and the other treatments. The dissolved oxygen in the control treatment (salinity 15%) was significantly lower than that in the other treatments. The mean values of all parameters are shown in Table 1.

Zootechnical performance

There was no significant difference in mortality between the treatments. In the first biometry, at 25 days, there was no significant difference in length and weight between the treatments.

At the end of the experiment, the control treatment (salinity 15%) showed greater gain in weight, final weight, TCE, and a lower feed conversion, than the other treatments ($p < 0.05$) (Table 2).

The results indicate that mullet juveniles tolerate a wide range of hardness in freshwater, but the freshwater environment impairs their growth. Fish raised in 15% salinity grew more than those maintained in freshwater (0% salinity). The isosmotic salinity for several teleosts is approximately 12%–15%, which may also be the salinity with the highest growth performance (ANNI et al., 2016; BOEUF; PAYAN, 2001; HERRERA et al., 2009; NORDLIE, 2009; TSUZUKI et al., 2007) because of the low cost of osmoregulation (BOEUF; PAYAN, 2001). This finding indicates that freshwater results in metabolic changes with increased osmoregulatory expenditure.

The highest feed conversion was observed in the control treatment (salinity 15%). Often, the high survival rate and enhanced growth in isosmotic environments can be explained by the low energy used for osmoregulation because the transport of ions between these media is reduced by reducing the gradient between the external and internal media. This energy saving is converted to weight gain and development (BOEUF; PAYAN, 2001). The findings of this study corroborate those of other studies, in which lower water hardness led to significantly higher feed intake and feed conversion than harder waters, for example, in largemouth bass (*Micropterus salmoides*) (ROMANO et al., 2020), kutum (*Rutilus frisii*)

Table 1. Mean values (± standard deviation) of water quality parameters in juvenile mullet in fresh water with different hardness (25, 250 and 750 mg. L^{-1} $CaCO_3$) and control (salinity 15% with hardness 2500 mg L^{-1} $CaCO_3$).

Parameters	Hardness (mg.L^{-1} $CaCO_3$)			Sal. 15 %
	25	250	750	
pH	7.57±0.27^a	7.22±0.50^b	7.35±0.31^b	7.33±0.19^b
DO (mg L^{-1})	5.4±0.73^b	5.25 ± 0.60^b	4.95±0.65^b	4.5 ± 0.57^a
T (°C)	30.0±1.23^a	29.45±1.15^b	29.7±1.12^b	29.7±0.19^b
Alkal	22±4.62^c	20±3.49^d	26±3.71^b	44.5±8.91^a
RH	33±7.09^a	246±19.32^b	738±18.40^c	2500±130.9^d

Mean ± standard deviation, dissolved oxygen (DO), Temperature (T), Alkalinity (Alkal), Actual Hardness (RH). Different letters in the lines indicate significant difference among treatments by Tukey's test ($P < 0.05$).

Table 2. Zootechnical performance of mullet juveniles cultivated for 50 days at different hardness in fresh water (25, 250 and 750 mg.L^{-1} $CaCO_3$) and control in salinity 15% (hardness 2500 mg $CaCO_3$.L^{-1}).

Parameters	Hardness (mg L^{-1} $CaCo_3$)			Sal. 15 %
	25	250	750	
FW (g)	29.16±2.62^c	34.04±3.08^b	29.89±0.61^c	40.30±2.72^a
WG (g)	7.16±2.62^b	12.03±3.08^b	7.88±0.83^b	18.3±2.72^a
FC	3.65±1.61^b	2.06±0.58^{ab}	3.34±0.29^{ab}	1.55±0.20^a
SGR (%/day)	2.88±0.08^c	3.02±0.08^b	2.91±0.01^c	3.18±0.05^a
S (%)	100 ± 0.01	93.33 ± 0.01	100 ± 0.01	100 ± 0.01

Mean values (± standard deviation) of final weight (FW), weight gain (WG), feed conversion (FA), specific growth rate (SGR) and survival (S) of *Mugil liza* juveniles. Different letters in the lines indicate significant difference among treatments by Tukey's test ($P < 0.05$).

(TAGHIZADEH et al., 2013), and rabbitfish (*Siganus guttatus*) (ZHAO et al., 2013).

The mullets achieved greater growth in the control treatment than in the other treatments. Growth rates were negatively affected by the other treatments. A possibility is that the high levels of Ca^{2+} in the treatment with 750 mg L^{-1} $CaCO_3$ and the low level of Ca^{2+} in the treatment with 25 mg L^{-1} $CaCO_3$ may have increased the energy demand for osmoregulation, due to the difference in input and output ion concentration. Calcium is notable for the regulation of ions in freshwater fish because it influences the permeability of biological membranes and prevents high ionic loss to the medium. In the treatment of 25 mg L^{-1}, there was probably a deficiency of this ion, impairing other metabolic processes. In the treatment of 750 mg L^{-1}, a possibility is that the high levels of Ca^{2+} may have increased the demand for energy for osmoregulation, owing to the difference in ion input and output, which interfered with the growth and performance of juvenile mullet.

Glucose

The mean glucose value in the initial biometrics was considered the initial glucose value. Glucose levels were the lowest in fish maintained at 15% salinity ($p < 0.05$). There was no significant difference among the other treatments at the end of the experiment, but there was a difference in the control treatment (Table 3).

Glucose plays a vital role in animal bioenergetics and is transformed into chemical energy, which can be expressed as mechanical energy (LUCAS, 1996).

Changes in glucose levels can be observed when animals are exposed to extreme salinity (ARJONA et al., 2007; HERRERA et al., 2009; ROCHA et al, 2004). The increase in glucose levels during stress is extremely important because large energy demands are necessary to activate the mechanisms presented in response to an adverse situation (WENDELAAR BONGA, 1997).

The results of this study indicate that the fish in the control (salinity 15%) were in greater physiological comfort than they were in the other treatments, which indicates a reduction in glucose reserves, which may have been used for homeostatic, metabolic, and growth (MICHELOTTI et al., 2018). By contrast, in a study evaluating the effect of different water hardness on common sea bass juveniles (*Centropomus undecimalis*), Michelotti et al. (2018) found that blood glucose did not differ significantly between treatments, which may suggest that the data from this study may be subjective because these values may have been a result of management (MICHELOTTI et al., 2018).

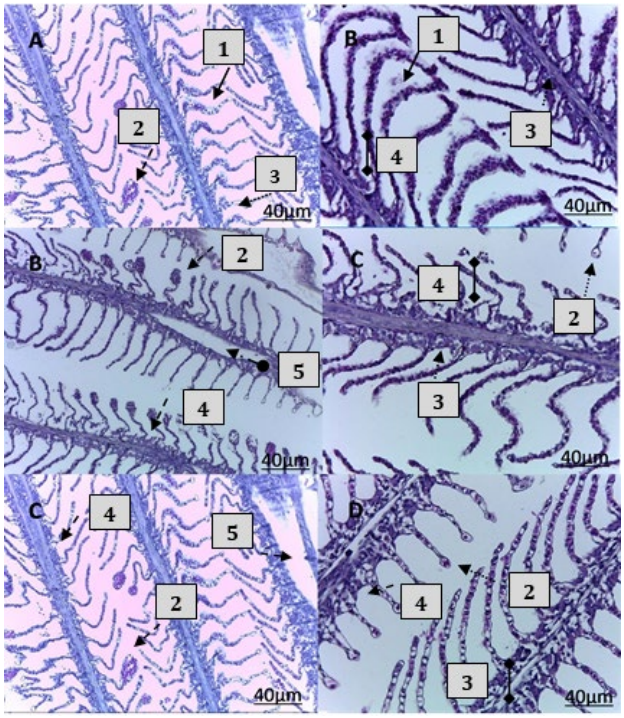
Table 3. Mean values (± standard deviation) of initial and final glucose in juvenile mullet in fresh water with different hardness (25, 250 and 750 mg L^{-1} $CaCO_3$) and control (Salinity 15% hardness 2500 mg L^{-1} $CaCO_3$).

Glucose		
Treatments	**Initial**	**Final**
Sal. 15 %	170,5 ± 41,52	82,85 ± 7,87[a]
25	170,5 ± 41,52	136,71 ± 15,67[b]
250	170,5 ± 41,52	111,85 ± 16,97[b]
750	170,5 ± 41,52	126,85 ± 32,40[b]

Mean values (± standard deviation) of blood glucose in *Mugil liza* juveniles. Different letters (a, b, c) indicate significant difference among treatments by Tukey's test ($P < 0.05$).

Histological analysis

Morphological changes were observed in all treatments, including secondary lamellar epithelial hyperplasia, interlamellar epithelial hyperplasia, secondary lamellar fusion, telangiectasia, venous sinus congestion of the primary lamella, secondary lamellar fusion, justalamellar edema, epithelial detachment, and secondary lamellar sinus dilatation. Histological analysis of the gills revealed that the animals maintained at 25 mgL^{-1} $CaCO_3$ presented the highest frequencies of intensity of severe alterations (grade III) in secondary lamellar hyperplasia, interlamellar hyperplasia, and interlamellar edema (Figure 1 - B).



A - Control (15% salinity), B - 25 mg.L^{-1} $CaCO_3$, C - 250 mg.L^{-1} $CaCO_3$ and D - 750 mg.L^{-1} $CaCO_3$. In A and B, epithelial hyperplasia of the secondary lamella was observed (arrows number 1). In A, B and D, mild and moderate telangiectasia can be observed in C (arrows number 2). In A, C and D, mild and severe interlamellar epithelial hyperplasia were observed in B (arrows number 3). In B, C and D justalamellar edema was observed (arrows number 4). In B and C, there was moderate epithelial detachment (arrows number 5). Dilation of the secondary lamella venous sinus of moderate degree was observed in B and mild degree in C and D. Hematoxylin-eosin staining. Source: Author's collection.

Figure 1. Histological alterations in the gills of juvenile mullet in fresh water with different hardness (25, 250 and 750 mg.L^{-1} $CaCO_3$) and in 15% salinity (2500 mg.L^{-1} hardness).

The animals maintained at 750 mg.L^{-1} $CaCO_3$ showed the lowest levels of alteration, remaining in most cases at zero (Figure 1 - D); the alterations with higher degree of severity were related to the treatments with lower concentrations of Ca^{2+}, and *vice versa*.

The analysis showed the appearance of moderate multifocal lesions of secondary lamellar epithelial hyperplasia, mild and multifocal telangiectasia, and mild interlamellar epithelial hyperplasia after treatment with 15% salinity (Figure 1 - A).

The data showed that the histological changes from the 25 mg.L^{-1} $CaCO_3$ treatment were more frequent and extensive than those from the other three treatments. Severe multifocal lesions of secondary lamellar epithelial hyperplasia, mild multifocal telangiectasia, severe coalescing interlamellar epithelial hyperplasia, severe justalamellar edema, moderate epithelial detachment, and moderate dilatation of the secondary lamellar sinus were also observed (Figure 1 - B).

In the 250 mg.L^{-1} $CaCO_3$ treatment group, moderate multifocal telangiectasia, mild hyperplasia of the interlamellar epithelium, mild dilatation of the venous sinus of the secondary lamella, severe justalamellar edema, moderate epithelial detachment, and mild dilatation of the secondary lamellar sinus were observed (Figure 1 - C).

In the treatment with the 750 mg.L^{-1} $CaCO_3$ treatment, there was mild hyperplasia of the interlamellar epithelium, mild justalamellar edema, and mild dilatation of the secondary lamellar sinus (Figure 1 - D).

Morphological alterations respond to environmental changes and may represent adaptive strategies for the conservation of physiological functions (LAURENT and PERRY, 1991). The gill epithelium is the main contact surface for the environment because of its extensive surface area (WONG; WONG, 2000). In this sense, analysis of histological alterations in the gills of teleosts that experience changes in salinity is of fundamental importance.

Histological analysis of the gills showed evident signs of stress, such as interlamellar epithelial hyperplasia, secondary lamellar epithelial hyperplasia, and telangiectasia, in all treatments. These alterations are examples of a defense mechanism fish use to combat different stressors and of a strategy of adaptation to environmental changes, especially in unfavorable and constant conditions (FERGUSON et al., 1990). The swelling of the lamellae, epithelial hyperplasia of the secondary lamellae, increases the diffusion distance between the environment and blood, minimizing the effect of an adverse environment (FERNANDES and MAZON, 2003).

In all treatments, an increase in chloride cell volume was not observed. An explanation for this finding is that the animals were in freshwater and that there was no excess of salts in the water, which limits the increase of cell volume.

In the control animals (salinity 15%), gill structures were close to normal, with moderate epithelial hyperplasia of the secondary lamella, an explanation for which is that the fish were in salinity close to their isosmotic point.

Incidence of moderate to severe injuries was observed in the treatment of 25 mg L^{-1} $CaCO_3$. The severe alteration of secondary lamella epithelial hyperplasia contributes to the increase in water-blood diffusion distance, which makes gas exchange difficult. The increase in the number of cells in the gill filament can reduce or prevent the passage of water between the secondary lamellae, compromising the physiological well-being of fish.

In the treatment of 250 mg L^{-1} $CaCO_3$, there was light lamellar fusion, and in the animals maintained in 25 mg L^{-1} $CaCO_3$, it was of a moderate degree. Lamellar fusion is a natural defense mechanism to protect the lamellar epithelium from direct contact with toxic agents and stressors (Heath, 1987).

The gills also showed vascular changes, such as vascular congestion in fish maintained at 250 mg L^{-1} $CaCO_3$ and mild aneurysm in all treatments. Aneurysm usually results from the collapse of the pillar cell system, which impairs vascular integrity with the release of a large amount of blood, which pushes the lamellar epithelium out (HEATH, 1987).

Hyperplasia of the gill lamellae allows a direct assessment of the relationship between fish and the aquatic environment with respect to its homeostasis. An expectation is that the lamellar area will increase when there are favorable conditions for the animal to have increased interaction with the aquatic environment. The opposite occurs when the animal is in a more hostile environment either because of the presence of chemical or biological irritants or variations in the physicochemical characteristics of the water.

In fish maintained at low hardness (25 mg L^{-1} $CaCO_3$), hyperplasia was severe. An explanation for this finding could be that because of the 0% salinity and low amount of Ca^{2+} in the water, the expansion of the lamellae area was necessary to maintain the level of O_2 circulating in the blood of the animals and to favor osmoregulation. In the other treatments, the results showed moderate hyperplasia, which can be explained by the availability of Ca^{2+} in water, which does not require the intense uptake of this ion.

CONCLUSIONS

Mullet juveniles can be cultivated in hard fresh water; however, further in-depth research on the hardness condition of 250 mg L^{-1} $CaCO_3$ is necessary. We do not recommend hardness above or below this range when the source used to generate this hardness is Ca^{2+}. However, obtaining more information on the metabolic features related to the ionic balance of mullet juveniles in fresh water is necessary. Among the tested hardness values, the histological analyses corroborated the zootechnical data, and the best result was obtained in 250 mg L^{-1} $CaCO_3$ (it led to the best final weight). The use of hard water resulted in benefits in relation to gill protection; however, zootechnical benefits were not observed.

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REFERENCES

ANDRADE, L.S. et al. Interaction of Water Alkalinity and Stocking Density on Survival and Growth of Silver Catfish, *Rhamdia quelen*, Juveniles. **Journal of the World Aquaculture Society**, v. 38, n. 3, p. 454-458, set. 2007.

ANNI, I. S. A. et al. Salinity influence on growth, osmoregulation and energy turnover in juvenile pompano *Trachinotus marginatus*, Cuvier 1832. **Aquaculture**, v. 455, p. 63-72, mar. 2016.

APHA. Standard Methods of Water and Wastewater. 21st Edition, Washington, DC.: **American Public Health Association**, p. 61, 2005.

BALDISSEROTTO, B. Osmoregulatory adaptations of freshwater teleosts. In: VAL, A. L.; KAPOOR, B. G. (Ed). Fish Adaptations. Enfield, **Science Publishers**, p. 179-201, 2003.

BALDISSEROTTO, B. Water pH and hardness affect growth of freshwater teleosts. **Brazilian Journal of Animal Science**, v. 40, p. 138-144, 2011.

BARLETTA, M.; DANTAS, D. V. Biogeography and Distribution of *Mugilidae* in the Americas. 2016.

BOEUF, G.; PAYAN, P. How should salinity influence fish growth? **Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology**, v. 130, p. 411–423, 2001.

CERQUEIRA, V. R. Cultivation of marine fish. In: POLI, C. R.; POLI, A. T.; ANDREATTA, E.; BELTRAME, E. (Ed.). Aquaculture: Brazilian Experiences. Florianópolis: **Multitarefa Editora Ltda.**, v. 1, p. 369-406, 2004.

CERQUEIRA, V. R. et al. Breeder management and breeding control of marine fish from the Brazilian coast. **Brazilian Journal of Animal Reproduction**, v. 41, p. 94-102, 2017.

CUNHA, V.L. et al. Acclimation of juvenile *Mugil liza* Valenciennes, 1836 (Mugiliformes: Mugilidae) to different environmental salinities. **Neotropical Ichthyology**, v.13, p. 591-598, 2015.

DURAND, J. D. et al. Systematic of the grey mullets (Teleostei: Mugiliformes: Mugilidae): molecular phylogenetic evidence challenges two centuries of morphology-based taxonomy. **Molecular Phylogenetics and Evolution**, v. 64, p. 79-92, 2012.

EATON, S. et al. Heat accumulation effects in femtosecond laser-written waveguides with variable repetition rate. **Optics Express**, v. 13, p. 4708-4716, 2005.

FERGUSON, H.; POPPE T.; SPEARE D. Cardiomyopathy in farmed Norwegian salmon. **Diseases of Aquatic Organisms**, v. 8, p. 225–231, 1990.

FERNANDES, M. N.; MAZON, A.F. Environmental pollution and gill morphology. In: VAL, A. L.; KAPOOR, B. G. (Ed.). Fish adaptations. USA: **Science Publishers**, v. 9, p. 203-231, 2003.

FLIK, G.; RIJS, J.H.; WENDELAAR BONGA, S.E. Evidence for high-affinity Ca^{2+} -ATPase activity and ATP-driven Ca^{2+} transport in membrane preparations of the gill epithelium of the cichlid fish *Oreochromis mossambicus*. **Journal of Experimental Biology**, v. 119, p. 335–347, 1985.

GRACIA-LÓPEZ, V.; ROSAS-VÁZQUEZ, C.; BRITO-PÉREZ, R. Effects of salinity on physiological conditions in juvenile common snook *Centropomus undecimalis*. **Comparative Biochemistry and Physiology, Part A, Molecular & Integrative Physiology**, v. 145, p. 340-345, 2006.

HEATH, A.G. Water Pollution and Fish Physiology. **C.R.C. Press**, p. 384, 1987.

HERRERA, M. et al. Osmoregulatory changes in wedge sole (*Dicologoglossa cuneata Moreau*, 1881) after acclimation to different environmental salinities. **Aquaculture Research**, v. 40, p. 762-771, 2009.

LAURENT, P.L.; PERRY, S.F. Environmental effects on fish gill morphology. **Physiological and Biochemical Zoology**, v. 64, p. 4–25, 1991.

LEMOS, V. M. et al. Migration and reproductive biology of *Mugil liza (Teleostei: Mugilidae*) in south Brazil. **Journal of Fish Biology**, v. 85, p. 671-687, 2014.

MENEZES, N. A.; OLIVEIRA, C.; NIRCHIO, M. An old taxonomic dilemma: the identity of the western South Atlantic lebranche mullet (Teleostei: Perciformes: Mugilidae). **Zootaxa**, v. 2519, p. 59-68, 2010.

NORDLIE, F.G. Environmental influences on regulation of blood plasma/serum components in teleost fishes: a review. **Reviews in Fish Biology and Fisheries**, v.19, p. 481-564, 2009.

NORDLIE, F.G.; LEFFLER, C.W. Ionic regulation and the energetics of osmoregulation in *Mugil cephalus*. **Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology**, v. 51, p. 125-131, 1975.

PAN, J. et al. Grey Mullet as Possible Indicator of Coastal Environmental Changes: the MUGIL Project. **Biology, Ecology and Culture of Grey Mullet (Mugilidae)**, p. 514–521, 2016.

PASSINI, G. et al. Induction of sex inversion in common snook (*Centropomus undecimalis*) males, using 17-ß oestradiol implants. **Aquaculture Research**, v. 47, p. 1090-1099, 2016.

POLEKSIĆ, V.; MITROVIĆ-TUTUNDŽIĆ, V. Fish gills as a monitor of sublethal and chronic effects of pollution. **Oxford: Fishing News Books**, v. 30, p. 339-352, 1994.

ROCHA, A.J.S. et al. Metabolic demand and growth of juveniles of *Centropomus parallelus* as function of salinity. **Journal of Experimental Marine Biology and Ecology**, v. 316, p. 157-165, 2004.

SAMPAIO, L.A.; BIANCHINI, A. Salinity effects on osmoregulation and growth of the euryhaline flounder *Paralichthys orbignyanus*. **Journal of Experimental Marine Biology and Ecology**, v. 269, p. 187-196, 2002.

STRICKLAND, J.D.H.; PARSONS, T.R. A Practical Hand Book of Seawater Analysis. 2nd Edition. **Ottawa: Fisheries Research Board of Canada Bulletin**, v. 157, 310 p, 1972.

TSUZUKI, M.Y. et al. Salinity tolerance of laboratory reared juveniles of the fat snook *Centropomus parallelus*. **Brazilian Journal of Oceanography**, v. 55, p. 1-5, 2007.

VIEIRA, J.P.; SCALABRIN, C. Migração reprodutiva da Tainha *Mugil platanus*, Günther, 1880 no sul do Brasil**. Atlântica**, v. 131, p. 131-141, 1991.

WENDELAAR BONGA, S.E. The stress response in fish. **Physiological Reviews**, v. 77, p. 591-625, 1997.

WONG, C.K.; WONG, M.H. Morphological and biochemical changes in the gills of Tilapia (*Oreochromis mossambicus*) to ambient cadmium exposure. **Aquatic Toxicology**, v.48, p. 517-527, 2000.