



Reassessment of the suitable range of water pH for culture of Nile tilapia *Oreochromis niloticus* L. in eutrophic water

Vanessa Tomaz Rebouças, Francisco Roberto dos Santos Lima, Davi de Holanda Cavalcante and Marcelo Vinícius do Carmo e Sá*

Laboratório de Ciência e Tecnologia Aquícola, Departamento de Engenharia de Pesca, Centro de Ciências Agrárias, Universidade Federal do Ceará, Av. Mister Hull, s/n, 60356-000, Fortaleza, Ceará, Brazil. *Autor para correspondência. E-mail: marcelo.sa@ufc.br

ABSTRACT. The present work aimed at reassessing the suitable range of water pH for culture of Nile tilapia, *Oreochromis niloticus* L. juveniles in eutrophic water. Two hundred and forty tilapia juveniles (1.37 ± 0.04 g) were stocked in twenty 250-L polyethylene tanks (12 fish per tank) for eight weeks. In the control tanks, the pH of water was not adjusted at any time, varying freely over the entire study. In the slight acidification treatment, the culture water was acidified daily to reach a water pH between 5.5 and 6.5. In the moderate acidification treatment, there were daily applications of HCl solution to reach a water pH between 4.5 and 5.5. In the alkalization treatment, tanks received daily applications of Na_2CO_3 and NaOH to achieve a water pH between 8.5 and 9.5. Acidification of water, regardless the degree, i.e., slight or moderate, was not able to significantly affect final body weight, specific growth rate and yield of fish. It can be concluded that the acidification of water up to pH 5.5 has no negative influence on growth of Nile tilapia fingerlings in eutrophic tanks. Accordingly, the suitable range of water pH for rearing Nile tilapia should be set at 5.5 – 9.0.

Keywords: pH of water, tilapia, aquaculture.

Reavaliação da faixa adequada de pH da água para o cultivo da tilápia do Nilo, *Oreochromis niloticus* L. em águas eutróficas

RESUMO. O presente trabalho teve por objetivo reavaliar a faixa adequada de pH da água para o cultivo de juvenis da tilápia do Nilo, *Oreochromis niloticus* L. em águas eutróficas. Foram estocados 140 juvenis de $1,37 \pm 0,04$ g em 20 tanques de polietileno de 250 L por oito semanas (12 peixes por tanque). Nos tanques do grupo controle, não houve ajuste do pH da água em nenhum momento, o qual variou livremente ao longo do estudo. Nos tanques submetidos à acidificação leve, a água de cultivo foi acidificada diariamente para atingir o valor de pH entre 5,5 e 6,5. Os tanques submetidos à acidificação moderada receberam aplicações diárias de solução de HCl para manter o pH da água entre 4,5 e 5,5. Nos tanques submetidos à alcalinização, procedeu-se a aplicação diária de Na_2CO_3 e NaOH para manter o pH da água entre 8,5 e 9,5. A acidificação da água, independentemente do nível empregado, i.e., leve ou moderado, não foi capaz de afetar significativamente o peso corporal final, taxa de crescimento específico e produtividade de peixe. Concluiu-se que a acidificação da água até pH 5,5 não prejudica o crescimento de juvenis de tilápia em águas eutróficas. Por consequência, a faixa de adequação de pH da água para cultivo da tilápia do Nilo deveria ser estendida para 5,5 – 9,0.

Palavras-chave: pH da água, tilápia, aquicultura.

Introduction

The pH of water can significantly affect the physiology of aquatic animals. The degree of acidity and basicity of water can stress and disrupt the normal growth of farmed fish and shrimp. Mechanisms of ionic regulation in fish are activated by variations in water pH, seeking the homeostasis and health maintenance. Acid-base disturbances in blood and body fluids can alter important metabolic parameters in fish, such as the concentrations of glucose, glycogen and lactate

(Bolner, Copatti, Rosso, Loro, & Baldisserotto, 2014; Garcia, Gutiérrez-Espinosa, Wásquez-Torres, & Baldisserotto, 2014).

In general, the suitable range of water pH for aquaculture is 6.5 – 9.0 (Boyd, Tucker, & Somridhivej, 2016). However, some fish species prefer to live in acidic waters, such as the tambaqui, *Colossoma macropomum* (Cuvier) (Aride, Roubach, & Val, 2007), while others are acidic-tolerant, such as the Mozambique tilapia, *Oreochromis mossambicus* (Furukawa, Watanabe, Inokuchi, & Kaneko, 2011).

Therefore, it is possible to have exceptions to that general rule of suitability of water pH.

According to El-Sherif and El-Feky (2009), the optimal water pH for the culture of Nile tilapia, *Oreochromis niloticus* L., is 7 - 8. These authors, however, have reared tilapia fingerlings in oligotrophic clear waters, in tanks with 100% daily water exchange. Consequently, there were low concentrations of toxic metabolites in the water, such as ammonia and H₂S. However, Nile tilapia juveniles have grown surprisingly well on acidic organic-matter rich waters (pH < 6) in previous studies carried out in our laboratory (Nobre, Lima, & Magalhães, 2014; Rebouças, Lima, & Cavalcante, 2015; Silva, Santos Lima, Vale, & Carmo, 2013). Colt, Momoda, Chitwood, Fornshell, and Schreck (2011) have also found that *O. niloticus* could be transferred from pH 6 - 7 to as low as pH 4.2 without problems. Due to the discrepancy between these results and those reported by El-Sherif and El-Feky (2009).

The present work was carried out aiming to reassess the suitable range of water pH for culture of Nile tilapia juveniles in eutrophic waters.

Material and methods

Masculinized Nile tilapia juveniles with body weight between 1 - 2 g were obtained from a regional producer and transported to the laboratory facilities, where they were maintained for four days in one 1,000-L tank for acclimation. In this phase, the animals were fed on four times daily at 0800, 1100, 1400 and 1700 with a commercial diet for omnivorous tropical fish containing 43.4% crude protein at 10% body weight daily.

At the onset of the experiment, two hundred and forty tilapia juveniles (1.37 ± 0.04 g) were stocked in twenty 250-L polyethylene tanks (12 fish per tank) for eight weeks. Fish were fed daily with appropriate commercial diets at 1000, 1300, 1500 and 1700, on feeding rates that ranged from 8.9% (initial) to 3.9% (final) body weight. No mechanical aeration was provided to the tanks throughout the experimental period. There was also no water exchange, just replenishment to maintain the initial water level. The bottom of the tanks was filled with a 5-cm layer of gross sand to allow water-soil interactions.

The experimental design consisted of three treatments and one control group, each one with five replicates. In the control tanks, the pH of water was not adjusted at any time, varying freely over the entire study. In the slight acidification treatment, the culture water was acidified daily with a 3.6 N HCl

solution in order to reach a water pH between 5.5 and 6.5. In the moderate acidification treatment, there were daily applications of HCl solution to obtain a water pH between 4.5 and 5.5. Finally, in the alkalization treatment, tanks received daily applications of Na₂CO₃ (12 g) and 1 N NaOH (9 mL), in order to achieve a water pH between 8.5 and 9.5. At each day, the dosages of HCl, Na₂CO₃ and NaOH used were adjusted to reach the designed pH for each treatment (slight acidification, moderate acidification and alkalization). The acidic and alkaline solutions had their total volumes split in three equal doses, which were delivered at 0800, 0830 and 0900. The water pH at 0730 was used to define the amounts of the acidic or alkaline solutions used on that specific day. A second pH reading was performed daily at 1500. The reported pH of water was the mean value of those two determinations.

The water quality of the culture tanks was monitored by regular observations of the following variables: (1) temperature and specific conductance at 0800 and 1600 (conductivity meter CD-4303 - Lutron), (2) dissolved oxygen - DO (0800; Winkler method with azide modification), (3) free CO₂ (titration with Na₂CO₃ standard solution), (4) total ammonia nitrogen (TAN; indophenol method), (5) NH₃ (estimated by the Emerson's formula as presented by El-Shafai, El-Gohary, Nasr, van der Steen, and Gijzen (2004)), (6) nitrite (sulfanilamide method), (7) reactive phosphorus (molybdenum blue method), (8) total alkalinity (titration with H₂SO₄ standard solution), (9) total hardness (titration with EDTA standard solution), (10) soluble iron (colorimetric Herapath method) and (11) H₂S (titration of total sulfide - TS with standard Na₂S₂O₃ solution and estimation of H₂S according to Boyd (2000)). Water quality variables were monitored daily (1), weekly (2-6) and fortnightly (7 - 11). All water quality determinations were carried out according to APHA (2014).

The soil pH and organic carbon concentration were determined every other week following the guidelines provided by Boyd, Wood, and Thunjai (2002). In the seventh experimental week, the pH and concentrations of DO, TAN, TS and H₂S in water were observed on a diel basis. For that, water samples were taken from the culture tanks every two (pH, DO, TS and H₂S) or four (TAN and NH₃) hours. The growth performance variables analyzed were the following: survival (%), final body weight (g), specific growth rate (% day⁻¹; $SGR = [\ln(\text{final weight}) - \ln(\text{weight initial})]^{-1} \text{ days of culture} \times 100$), fish yield (g m⁻³ day⁻¹) and feed conversion ratio ($FCR = \text{feed consumed}^{-1} \text{ body weight gain}$).

Metabolic performance of fish was assessed by the respiratory rate using 2.5-L respirometers (Barbieri, Passos, & Garcia, 2005). Weekly, from the third experimental week, one fish from each treatment was allotted to one respirometer. For that, one animal was withdrawn from each tank and those individuals not used for the respirometer assays were discarded. Each respirometer was filled up with 2.0-L filtered water taken from the culture tanks. Initially, air was bubbled into the respirometer water for one hour and the resulting concentration of dissolved oxygen in water was measured. Next, one fish was placed inside the respirometer for four hours. After that, the DO concentration in the respirometer water was measured by the Winkler method with azide modification. The respiratory rate in $\mu\text{g DO g}^{-1} \text{ fish h}^{-1}$ was obtained by the following equation: respiratory rate = $[(\text{DO}_i - \text{DO}_f)^{-1} \text{ fish body weight (g)} 4\text{h}^{-1}] \times 2 \text{ L}$ (respirometer volume), where DO_i is the initial concentration of DO and DO_f is the concentration of DO in the respirometer water after 4 hours.

The results were analyzed by one-way ANOVA. When a significant difference was detected between the treatments ($p < 0.05$), the means were compared pairwise by Tukey's test, for equal-variance variables, or Games-Howell's test, for non-equal variance variables. The assumptions of normal distribution (Shapiro-Wilk's test) and homogeneity of variance (Levene's test) were checked before analysis. The SPSS v.15.0 and Windows Excel 2010 software were used for the statistical analyses.

Results and discussion

Water and soil quality

The temperature and concentration of dissolved oxygen in water were not significantly affected by water acidification (slight and moderate) and alkalization (Table 1). The average temperature of water at 0800 and 1600 were $27.6 \pm 0.40^\circ\text{C}$ ($27.0 - 28.6^\circ\text{C}$) and $30.8 \pm 1.14^\circ\text{C}$ ($28.2 - 33.3^\circ\text{C}$), respectively. In the last week, the average concentration of DO in water was $4.5 \pm 1.9 \text{ mg L}^{-1}$ ($1.8 - 7.6 \text{ mg L}^{-1}$). Therefore, it seems that the acidification and alkalization procedures have not impaired the release of O_2 by photosynthesis to the water. The acidification of water has increased the concentrations of free CO_2 in the tanks. The highest concentration of free CO_2 (23.7 mg L^{-1}) was found in the moderately acidified tanks. On the other hand, the alkalization of water has reduced the concentrations of free CO_2 in water (Table 1). It is accepted that concentrations of free CO_2 in water

above 20 mg L^{-1} may be stressful to fish (Danley, Kenney, Mazik, Kiser, & Hankins, 2005). However, it has probably not affected Nile tilapia fingerlings because they have reached the highest final body weight only in the moderately acidified tanks ($23.4 \pm 1.3 \text{ g}$; Table 2).

The specific conductance (SC) of water increased with the acidification and, mainly, alkalization of water. The ionic concentration or salinity of water is the main factor responsible for the water SC. However, once the isosmotic point for Nile tilapia is near 12 g L^{-1} (Hassan et al., 2013) and the highest TDS (total dissolved solids) of water in the present work was 870 gm L^{-1} , these minor variations in SC of water have probably not affected the tilapia physiology.

The acidification of water has decreased the total alkalinity (TA) of water in direct proportion with the level of acidification (Table 1). The average TA of water was 13.5 ± 2.0 and $5.4 \pm 1.7 \text{ mg L}^{-1} \text{ CaCO}_3 \text{ eq.}$ for the slight and moderate acidification, respectively.

A minimum TA of $20 \text{ mg L}^{-1} \text{ CaCO}_3 \text{ eq.}$ is required for an acceptable water pH buffering in aquaculture tanks (Boyd et al., 2016). In the present work, however, since the pH of water was deliberately controlled to reach certain levels, the effect of low TA on water pH was null. While the acidification of water has increased the total hardness (TH), the alkalization has reduced it (Table 1). The highest TH of $223.4 \text{ mg L}^{-1} \text{ CaCO}_3$ was found in one of the moderately acidified tanks; the lowest TH of $102.7 \text{ mg L}^{-1} \text{ CaCO}_3$ was found in one of the tanks subjected to alkalization. Boyd et al. (2016) recommended TH between $40 - 300 \text{ mg L}^{-1} \text{ CaCO}_3$ for aquaculture production. Therefore, the values of TH for both the acidified and alkaline tanks have remained within the appropriate range.

The acidification of water has increased significantly the concentrations of TAN in water. On the other hand, the alkalization of water had no significant effect on TAN (Table 1). The average TAN concentrations were $0.57 \pm 0.17 \text{ mg L}^{-1}$ ($0.47 - 0.82 \text{ mg L}^{-1}$) and $0.49 \pm 0.18 \text{ mg L}^{-1}$ ($0.28 - 0.62 \text{ mg L}^{-1}$) for the slight and moderate acidification, respectively. Boyd (2001) mentions TAN concentrations higher than $3 - 4 \text{ mg L}^{-1}$ as toxic to warm-water aquaculture organisms in waters with $\text{pH} > 8.5 - 9.0$. Therefore, the highest concentration of TAN (0.82 mg L^{-1}) verified in the present study is still far below the TAN critical levels indicated by the literature. As the water was acidified, the concentrations of NH_3 in water have almost zeroed. In the alkalized tanks, the concentrations of NH_3 were very low ($16.5 \mu\text{g L}^{-1}$).

Table 1. Water quality in Nile tilapia outdoor tanks after eight weeks of culture (mean \pm SD; n = 5).

Variable ¹	Treatments ²				ANOVA p
	Control (No action)	Acidification		Alkalinization	
		Light	Moderate		
T8 am	27.4 \pm 0.13	27.5 \pm 0.21	27.4 \pm 0.08	27.4 \pm 0.13	NS ³
T4 pm	30.2 \pm 0.71	30.1 \pm 0.89	30.2 \pm 0.52	30.3 \pm 0.57	NS
SC	965 \pm 41 c ⁴	1132 \pm 39 b	1168 \pm 41 b	1273 \pm 23 a	< 0.001
TA	137 \pm 5 b	13 \pm 2 c	5 \pm 2 d	318 \pm 17 a	< 0.001
TH	177 \pm 11 b	208 \pm 8 a	220 \pm 3 a	119 \pm 8 c	< 0.001
DO	4.12 \pm 1.64	4.80 \pm 1.98	4.64 \pm 2.57	4.55 \pm 1.81	NS
CO ₂	12.3 \pm 1.4 b	12.9 \pm 1.8 b	17.6 \pm 1.8 a	0.0 \pm 0.0 c	< 0.001
TAN	0.20 \pm 0.1 bc	0.57 \pm 0.2 a	0.49 \pm 0.2 ab	0.14 \pm 0.1 c	0.004
NH ₃	2.04 \pm 0.67 b	0.23 \pm 0.02 b	0.07 \pm 0.04 b	16.48 \pm 1.46 a	< 0.001
NO ₂ ⁻	0.06 \pm 0.02 a	0.08 \pm 0.04 a	0.07 \pm 0.03 a	0.0 \pm 0.0 b	0.002
P-react	0.07 \pm 0.03 b	0.05 \pm 0.02 b	0.24 \pm 0.10 a	0.04 \pm 0.01 b	< 0.001
Fe ²⁺	0.53 \pm 0.17 b	0.80 \pm 0.13 b	2.26 \pm 0.89 a	0.19 \pm 0.06 b	< 0.001
T sulfide	1.42 \pm 0.5 ab	1.02 \pm 0.5 ab	0.62 \pm 0.3 b	1.67 \pm 0.3 a	0.015
H ₂ S	0.15 \pm 0.07 b	0.73 \pm 0.31 a	0.62 \pm 0.28 a	0.01 \pm 0.01 b	0.001

¹T8 am and T4 pm: temperature at 8 am and 4 pm ($^{\circ}$ C), SC: specific conductance (μ S cm^{-1}), TA and TH: total alkalinity and total hardness (mg L^{-1} CaCO₃), DO: dissolved oxygen (mg L^{-1}), CO₂: free carbon dioxide (mg L^{-1}), TAN: total ammonia nitrogen (mg L^{-1}), NH₃: non-ionized ammonia ($\mu\text{g L}^{-1}$), NO₂⁻: nitrite (mg L^{-1}), P-react: reactive phosphorus (mg L^{-1}), Fe²⁺: soluble iron (mg L^{-1}), T sulfide: total sulfide (mg L^{-1}) and H₂S: unionized hydrogen sulfide (mg L^{-1}); ²Slight acidification: a 3.6 N HCl solution was routinely applied to the culture water to reach a pH between 5.5 and 6.5 (pH = 6.6 \pm 0.8). Moderate acidification: a 3.6 N HCl solution was routinely applied to the culture water to reach a pH between 4.5 and 5.5 (pH = 5.5 \pm 1.2). Alkalinization: a 1 N NaOH solution plus Na₂CO₃ were routinely applied to the culture water to reach a pH between 8.5 and 9.5 (pH = 9.2 \pm 0.4). The pH of water in the control tanks was not adjusted and averaged 8.2 \pm 0.4; ³Non-significant (ANOVA p = 0.631, 0.989 and 0.958 for T8 am, T4 pm and DO₂, respectively); ⁴For the same variable, means with distinct letters are significantly different by Tukey's (SC, TH, TAN and T sulfide) or Games-Howell's test (TA, CO₂, NH₃, NO₂⁻, P-react, Fe²⁺, H₂S).

Table 2. Growth performance of Nile tilapia juveniles after eight rearing weeks (mean \pm SD; n = 5).

Variables ¹	Treatments ²				ANOVA p
	Control (No action)	Acidification		Alkalinization	
		Light	Moderate		
Survival	94.3 \pm 7.8	97.1 \pm 6.4	97.1 \pm 6.4	91.4 \pm 7.8	NS ³
IBW	1.36 \pm 0.04	1.39 \pm 0.02	1.36 \pm 0.05	1.36 \pm 0.03	NS
FBW	20.5 \pm 2.0 ab ⁴	20.5 \pm 1.9 ab	23.4 \pm 1.3 a	19.8 \pm 21.5 b	0.023
SGR	5.3 \pm 0.2 ab	5.3 \pm 0.2 ab	5.6 \pm 0.1 a	5.2 \pm 0.2 b	0.032
FY	9.7 \pm 1.5 ab	9.9 \pm 1.1 ab	11.3 \pm 0.9 a	9.0 \pm 0.6 b	0.023
FCR	1.12 \pm 0.10	1.09 \pm 0.07	1.03 \pm 0.05	1.13 \pm 0.04	NS

¹Survival (%), IBW: initial body weight (g), FBW: final body weight (g), SGR: specific growth rate (% day⁻¹), FY: fish yield (g m^{-3} day⁻¹), FCR: feed conversion rate (feed allowance/body weight gain); ²Slight acidification: a 3.6 N HCl solution was routinely applied to the culture water to reach a pH between 5.5 and 6.5 (pH = 6.6 \pm 0.8). Moderate acidification: a 3.6 N HCl solution was routinely applied to the culture water to reach a pH between 4.5 and 5.5 (pH = 5.5 \pm 1.2). Alkalinization: a 1 N NaOH solution plus Na₂CO₃ were routinely applied to the culture water to reach a pH between 8.5 and 9.5 (pH = 9.2 \pm 0.4). The pH of water in the control tanks was not adjusted and averaged 8.2 \pm 0.4; ³Non-significant (ANOVA p = 0.547, 0.340 and 0.125 for survival, IBW and FCR, respectively); ⁴For the same variable, means with distinct letters are significantly different by Tukey's (FBW, SGR) or Games-Howell's test (FY).

The acidification of water has not significantly affected the concentrations of nitrite in water when compared to the control tanks (Table 1), which, in general, were very low (0.04 – 0.12 mg L⁻¹). Interestingly, the concentrations of nitrite have been zeroed in the alkalinized tanks. Yanbo, Wenju, Weifen, and Zirong (2006) have determined the 96-h LC₅₀ nitrite for Nile tilapia at 28.2 mg L⁻¹. Therefore, the safe concentration of nitrite for tilapia in freshwater is 0.3 mg L⁻¹ (1% 96-h LC₅₀). Accordingly, the concentrations of nitrite found herein (0.12 mg L⁻¹) have probably caused no damage to fish.

There were significant increases in the concentrations of reactive phosphorus and dissolved iron in water by the moderate acidification implemented. The slight acidification and alkalinization of water had no expressive effect on the phosphorus and Fe²⁺ concentrations (Table 1). The water acidification has led to soil acidification: the pH of soil in the control and moderately acidified tanks were 7.9 \pm 0.5 (7.3 – 8.9) and 6.4 \pm 1.1 (4.6 – 7.6), respectively. The increase in the concentrations of phosphorus and iron in water in

the moderately acidified tanks may be explained by the release of these ions from the acidic soils into the water (Falagán, Sánchez-España, & Johnson, 2014). The concentrations of organic carbon in soil were not significantly affected by the treatments (ANOVA p < 0.05). At the end, these concentrations were 0.35% \pm 0.16 (control), 0.33% \pm 0.09 (slight acidification), 0.33% \pm 0.10 (moderate acidification) and 0.30% \pm 0.08 (alkalinization).

The acidification of water, either slight or moderate, promoted an increase in H₂S concentrations of water (Table 1). The alkalinization of water had no significant effect on that variable. It is possible that the ions Fe²⁺ and S²⁻ have been released together to the water along the soil acidification (Lahav, Ritvo, Slijper, Hearne, & Cochva, 2004). While the average concentrations of H₂S in water were 0.73 \pm 0.31 mg L⁻¹ and 0.62 \pm 0.28 mg L⁻¹ for the slightly- and moderately-acidified tanks, respectively, H₂S levels as high as 0.96 mg L⁻¹ were detected in the acidified tanks. Exposure to 0.1 – 0.5 mg H₂S L⁻¹ caused severe biochemical and physiological damages in channel

catfish (Hargreaves & Tomasso, 2004). Since the tilapia growth has not been significantly affected by the high H_2S levels found in the present study, it is suggested that Nile tilapia fingerlings are H_2S -resilient organisms.

Diel monitoring

Over the diel monitoring, the pH of water remained almost constant in the moderately acidified and alkalized tanks (5.3 ± 0.1 and 9.2 ± 0.1 , respectively). On the other hand, there was an expressive increase in water pH in the slightly acidified tanks at 1400 (Figure 1). The diel pattern for the water pH in the slightly acidified tanks has resembled that verified in the control tanks. That suggests that out of the three treatments, i.e., slight acidification, moderate acidification and alkalization, the first was the one that minimally affected the phytoplankton dynamics in the tanks.

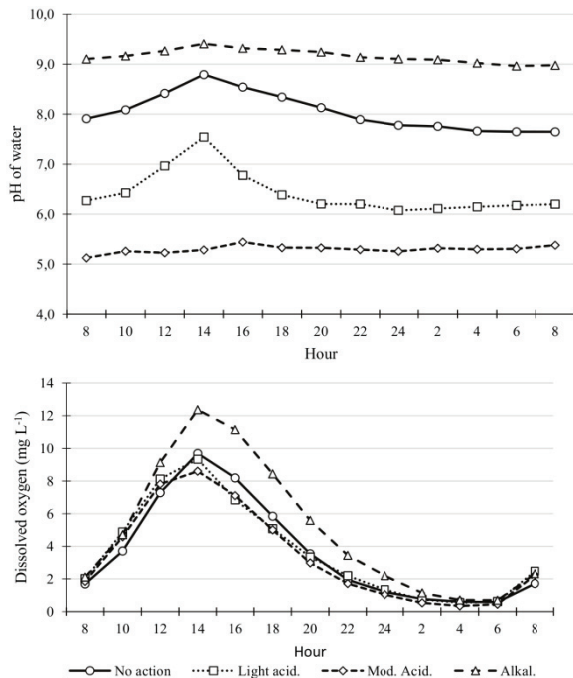


Figure 1. Diel monitoring of pH and dissolved oxygen in water of Nile tilapia tanks ($n = 5$)

DO variations in the acidified tanks (slight and moderate) in the diel cycle were very similar to those observed for the control tanks. Remarkably, the concentrations of DO in water have increased significantly more in the alkalized tanks than in the others (Figure 1). While the other tanks reached a maximum DO concentration of 8.8 ± 1.8 mg L⁻¹ at 1400, the alkalized tanks achieved 12.4 ± 2.1 mg L⁻¹ at the same time. It is difficult to explain that fact, but no advantage was found for tilapia juveniles, since the growth performance in the alkalized tanks

was similar to that observed in the control ones (Table 2).

The concentrations of TAN remained low in the control and alkalized tanks throughout the diel monitoring. On the contrary, the levels of TAN in water were higher in the acidified tanks, especially in the moderately acidified, than in the other tanks, except for the alkalized tanks. In the latter tanks, NH_3 concentrations as high as 0.14 mg L⁻¹ were observed over the diel monitoring (Figure 2).

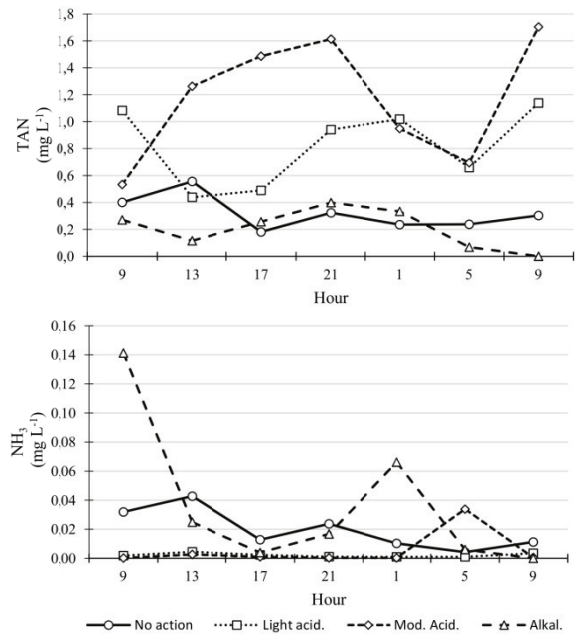


Figure 2. Diel monitoring of total ammonia nitrogen (TAN) and NH_3 in water of Nile tilapia tanks ($n = 5$).

The maximum tolerable concentration of NH_3 for fish is supposed to be 0.1 mg L⁻¹ (El-Shafai et al., 2004). Therefore, fish stocked in alkalized tanks might have suffered stress by NH_3 in some instants along the 24-h cycle.

Variations in total sulfide levels were very similar between the treatments along the diel monitoring. The total sulfide concentrations increased from 1.13 ± 0.52 mg L⁻¹ at 0800 to 4.07 ± 0.75 mg L⁻¹ at 1000 and decreased afterwards up to 0.91 ± 0.78 mg L⁻¹ at 1800. Next, there was a pronounced increase up to 5.49 ± 0.75 mg L⁻¹ at 2400 (midnight), when concentrations as high as 7.9 mg L⁻¹ were determined. Then, the total sulfide concentrations fell again up to 0.46 ± 0.40 at 0800, when no sulfide was detected in some tanks (Figure 3). For the acidified tanks (slight and moderate), the concentrations of H_2S in water have matched the concentrations of total sulfide over the diel monitoring, since the proportion of H_2S in total sulfide increases as the pH of water drops (Boyd et al.,

2016). On the other hand, lower concentrations of H_2S were registered in the control tanks ($0.42 \pm 0.42 \text{ mg L}^{-1}$) and almost no H_2S ($0.03 \pm 0.02 \text{ mg L}^{-1}$) was detected in the alkalinized ones (Figure 3).

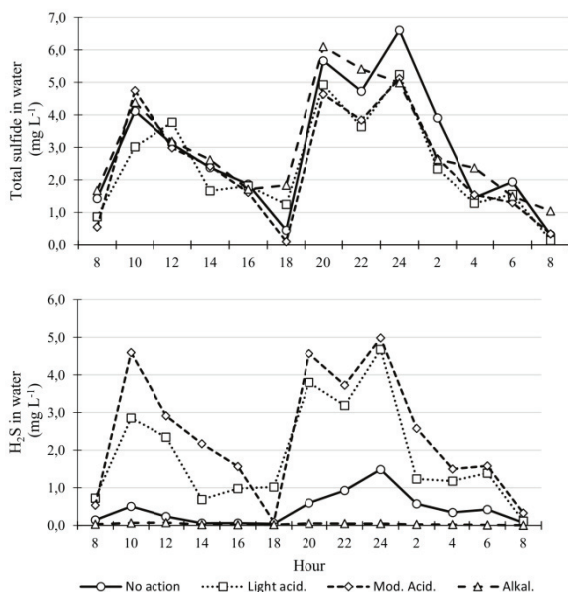


Figure 3. Diel monitoring of total sulfide and H_2S in water of Nile tilapia tanks ($n = 5$).

Respirometer performance

Except for the second experimental week, when fish in the alkalinized tanks consumed more DO than fish stocked in moderately acidified water (284.4 and $125.5 \mu\text{g DO g fish}^{-1} \text{ h}^{-1}$, respectively), the consumption rates of DO by fish allotted to the respirometers were very similar between the treatments and control group (non-action) over time (Figure 4).

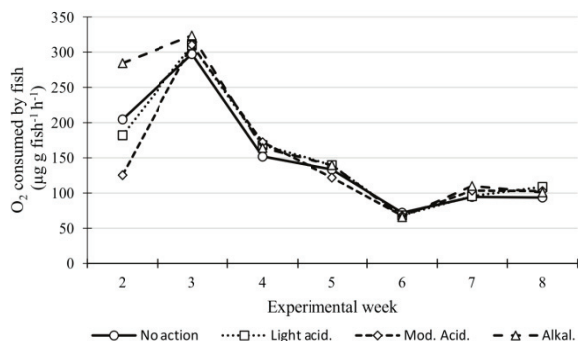


Figure 4. Consumption rate of dissolved oxygen by fish allotted to respirometers over four hours.

There was a decrease from $310.3 \pm 10.8 \mu\text{g DO g fish}^{-1} \text{ h}^{-1}$, in the 3rd week, to $101.5 \pm 6.2 \mu\text{g DO g fish}^{-1} \text{ h}^{-1}$, in the last week (8th). These results agree with Van Dijk, Van Den Thillart, and Bonga (1993),

who have shown that tilapia *O. mossambicus* reduces the metabolic rate (MR) and oxygen consumption (OC) when exposed to acidic waters. Therefore, it is suggested that (1) the initial strategy of Nile tilapia to cope with pH stress was different between the alkalinized (increased MR and OC) and acidic (decreased MR and OC) tanks and (2) over time, fish were able to adapt to the pH-manipulated waters, either acidified or alkalinized, in a very similar way between the treatments and control. The general reduction in oxygen uptake with time was probably due to the normal reduction in oxygen uptake with increasing body size (1 to 20 g).

Growth performance

Fish survival and feed conversion rate (FCR) were not significantly affected by either acidification or alkalinization of water (Table 2). On average, these results were satisfactory, $95.0\% \pm 2.7$ and 1.09 ± 0.05 , respectively. The acidification of water, regardless the degree, i.e., slight or moderate, was not able to significantly affect final body weight, specific growth rate and yield of fish.

Therefore, it can be suggested that Nile tilapia fingerlings can grow well in green waters with pH as low as 5.5. These same variables, however, were significantly lower in the alkalinized tanks compared to the moderately acidified ones (Table 2). Consequently, Nile tilapia fingerlings seem to be more tolerant to acidic than to alkalinized waters.

The results of growth performance corroborate those obtained by Colt et al. (2011), Silva et al. (2013), Nobre et al. (2014) and Rebouças et al. (2015), but disagree to El-Sherif and El-Feky (2009), who concluded that the optimum water pH for the culture of Nile tilapia, *O. niloticus*, is 7–8. As already pointed out elsewhere, the main difference between the research conducted by El-Sherif and El-Feky (2009) and the present study is the quality of rearing water. While the former authors have reared the Nile tilapia juveniles in oligotrophic waters, we used eutrophic waters. In nature, as well as in aquaculture outdoor tanks, several interactions occur between the different water quality factors, such as water pH and concentrations of NH_3 and H_2S , and fish growth (Bagarinao & Lantin-Olaguer, 1998). These interactions were probably minimal or even nonexistent in the transparent water tanks analyzed by El-Sherif and El-Feky (2009). Therefore, their conclusion that the optimum water pH for the culture of Nile tilapia, *O. niloticus*, is 7–8 might be inaccurate on a diverse culture environment, such as green waters.

Conclusion

The acidification of water up to pH 5.5 has not affected the growth performance of Nile tilapia fingerlings in eutrophic tanks. Accordingly, the suitable range of water pH for rearing Nile tilapia should be extended from 6.5 – 9.0 to 5.5 – 9.0.

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