



Nile tilapia fingerling cultivated in a low-salinity biofloc system at different stocking densities

Priscilla C. M. Lima, Jéssika L. Abreu, Allyne E. M. Silva, William Severi, Alfredo O. Galvez, and Luis O. Brito
Universidade Federal Rural de Pernambuco (UFRPE), Dept. Pesca e Aquicultura (DEPAq), Rua Dom Manuel de Medeiros, Dois Irmãos, 52171-900, Recife, PE, Brazil.

Abstract

A 42-day trial was conducted to evaluate the effects of a low-salinity biofloc system with different stocking densities on water quality and zootechnical performance of Nile tilapia fingerlings (10 g/L). Four treatments were tested at different densities: 500 fish/m³, 750 fish/m³, 1,000 fish/m³ and 1,250 fish/m³, all in triplicate. Fingerlings of *Oreochromis niloticus* (initial mean weight of 1.17 ± 0.05 g) were stocked in twelve experimental black-plastic tanks (40 L) with no water exchange during the experimental period. Molasses was added daily to the system at 30% of the amount of feed, and fish were given four daily rations of a formulated feed composed of 36% crude protein and 9% lipids. Water quality variables (dissolved oxygen, pH, salinity, TAN, NO₂, NO₃ and PO₄³⁻) did not demonstrate significant differences between the treatments. However, significant influences ($\alpha \leq 0.05$) of the stocking densities were observed for total suspended solids, settleable solids, final weight, yield, and protein efficiency ratio. The results showed survival over 96%, final weight values between 12 and 18 g, yield between 9.49 and 15.27 kg/m³, water consumption of 52 to 101 L/kg fish, and total time of settling chambers between 238 and 305 h/kg fish. These results indicate a negative effect of stocking density on final weight, survival, alkalinity, NO₂, PO₄³⁻ and water consumption, and a positive effect on yield in Nile tilapia fingerling culture (1-20 g) in a low-salinity biofloc system with densities up to 1000 fish/m³.

Additional keywords: aquaculture; growth; water quality; BFT; fish.

Abbreviations used: BFT-500 (500 fish/m³); BFT-750 (750 fish/m³); BFT-1000 (1,000 fish/m³); BFT-1250 (1,250 fish/m³); FCR (feed conversion ratio); PER (protein efficiency ratio); SGR (specific growth rate); SS (settleable solids); TAN (total ammonia nitrogen); TSS (total suspended solids).

Authors' contributions: All authors contributed equally to this work (conception; acquisition, analysis, data interpretation; drafting of the manuscript; critical review of the manuscript and statistical analysis).

Citation: Lima, P. C. M.; Abreu, J. L.; Silva, A. E. M.; Severi, W.; Galvez, A. O.; Brito, L. O. (2018). Nile tilapia fingerling cultivated in a low-salinity biofloc system at different stocking densities. Spanish Journal of Agricultural Research, Volume 16, Issue 4, e0612. <https://doi.org/10.5424/sjar/2018164-13222>

Received: 24 Mar 2018. **Accepted:** 11 Dec 2018.

Copyright © 2018 INIA. This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International (CC-by 4.0) License.

Funding: Brazil's National Council for Scientific and Technological Development, CNPq (grant to AOG, PQ 311058/2015-9); Coordination for the Improvement of Higher Education Personnel, CAPES; Funding Authority for Studies and Projects FINEP/RECARCINA.

Competing interests: The authors have declared that no competing interests exist.

Correspondence should be addressed to Luis O. Brito: engpescalo@hotmail.com

Introduction

Oreochromis niloticus (Nile tilapia) is the most cultivated fish species in Brazil, representing 47.1% (239.09 × 10³ metric tons) of total Brazilian fish production. This is linked to its resistance and adaptability to different growing environments, and the species' rapid growth rate (Costa & Fróes, 2012; NG & Romano, 2013).

Despite these interesting zootechnical attributes found in cultivation systems using net cages and ponds with water exchange, more study of the zootechnical performance of Nile tilapia raised in intensive systems

with minimal or no water exchange is necessary, and of the use of resources in systems with low flow and high levels of dissolved salts not suitable for human consumption, mainly due to the scarcity of freshwater in various parts of Brazil and the world.

Nile tilapia culture in biofloc systems is a very promising alternative, since it uses minimal or zero water exchange, high stocking densities and can reach high yields, due to the C:N ratio in the water, strong aeration and alkalinity control using microorganisms to remove and recycle nutrients (De Schryver *et al.*, 2008; Crab *et al.*, 2012; Emerenciano *et al.*, 2013; Avnimelech, 2012).

Biofloc systems have been used with great success for shrimp (Brito *et al.*, 2014, 2016; Marinho *et al.*, 2017; Ray & Lotz, 2017) and tilapia cultivated in freshwater (Ekasari *et al.*, 2015; Long *et al.*, 2015; Pérez-Fuentes *et al.*, 2016; Alves *et al.*, 2017; Miranda-Baeza *et al.*, 2017; Zapata-Lovera *et al.*, 2017). Tilapia can adapt to a biofloc system because of their resistance to high solids levels and filter-feeding ability, thus allowing the absorption of the suspended flocs (Avnimelech, 2011), although more information on tilapia performance in brackish water is needed.

Tilapia are excellent for cultivation not only in freshwater, but also in brackish water, due to their tolerance for salinity between 0 and 12 g/L (Likongwe *et al.*, 1996; El-Sayed, 2006). Other studies have shown salinity tolerance of 22.5 g/L (Kamal & Mair, 2005) and up to 35 g/L (Mapenzi & Mmochi, 2016). In addition, freshwater fish cultivated in brackish water may save energy, which could be made available for growth (Takata & Luz, 2015), and increased salinity may also reduce the toxicity of nitrogen compounds (ammonia-NH₃ and nitrite-NO₂) (Colt, 2006), which increases at higher stocking densities.

Nevertheless, higher stocking densities of tilapia cultivated in traditional freshwater systems have been found to cause the following negative effects: decreased growth (Abou *et al.*, 2007), decreased survival (Ferdous *et al.*, 2014), increased FCR (feed conversion ratio) (Ridha, 2006), decreased crude protein content of the fish carcass (Osofero *et al.*, 2009); decreased cost-benefit ratio (Moniruzzaman *et al.*, 2015), decreased levels of hematological parameters: red blood cells, white blood cells, hemoglobin, hematocrit and platelets (Kpundeh *et al.*, 2013), and decreased plasma cortisol concentrations, which may indicate chronic stress (Barcellos *et al.*, 1999), despite the positive effect on increased yield (Suresh & Lin, 1992).

The effects of stocking densities (25, 50, 100 fish/m³) of *Oreochromis* sp. in culture systems with and without bioflocs have been reported by Widanarni *et al.* (2012), with higher zootechnical performance obtained in the biofloc system. In this sense, this study evaluated the effects of a low-salinity biofloc system with different stocking densities on water quality and zootechnical performance of Nile tilapia fingerlings.

Material and methods

Experimental conditions

A 42-day indoor trial was conducted at the Sustainable Mariculture Laboratory (LAMARSU) of the Department of Fisheries and Aquaculture (DEPAq) of the Federal Rural University at Pernambuco (UFRPE),

Recife, Brazil (08°01'00.16"S, 034°56'57.74"W). The experimental design was completely randomized with four stocking densities: BFT-500 (500 fish/m³); BFT-750 (750 fish/m³); BFT-1000 (1,000 fish/m³) and BFT-1250 (1,250 fish/m³), all in triplicate. All procedures were previously approved by the Ethics Committee on Animal Use of UFRPE under license number 129/2016.

To prepare the biofloc system, a fiberglass matrix tank was filled with 1.4 m³ of water with a salinity of 10 g/L, previously disinfected with 13 mg/L of active chlorine. After 72 hours of aeration, the water was fertilized with urea and triple superphosphate at concentrations of 3 and 0.3 mg/L, respectively. Organic fertilization was also conducted by adding 196 g of sugarcane molasses and 37 g of pulverized feed (36% crude protein) to produce bioflocs (15 mL/L of settleable solids). The carbohydrate:nitrogen ratio was maintained at 12:1 and was calculated according to De Schryver *et al.* (2008).

The experimental units (50 L volume, 0.20 m² bottom surface area) were filled with the homogenized bioflocs from the matrix tank up to ~50% of their volume, and the remaining volume was filled with previously treated salt water (10 g/L). All experimental units were maintained under constant aeration by three air stones per tank. No water exchange was conducted during the experimental period, except for the addition of dechlorinated freshwater to compensate for evaporation losses. Light intensity was kept at 2000 lux using a fluorescent lamp with a 12 h light/12 h dark photoperiod. Molasses was added once a day at an amount of 30% of the feed offered. Hydrated lime (Ca(OH)₂ - 81% neutralization power) was added twice a week at 20% (by weight) of the total weekly feed.

Water quality

Dissolved oxygen, temperature, salinity and pH (YSI model 556, Yellow Springs, OH, USA) were monitored twice a day (at 08:00 am and 04:00 pm). Total ammonia nitrogen (TAN) (Hansen & Koroleff, 2007), nitrite (NO₂) (Golterman *et al.*, 1978), nitrate (NO₃) (Mackereth *et al.*, 1978), total suspended solids (TSS) (APHA *et al.*, 2005), orthophosphate (PO₄³⁻) (APHA *et al.*, 2005) and alkalinity (mg/L CaCO₃) (Felföldy *et al.*, 1987) were monitored once a week. Settleable solids (SS) were monitored three times per week with an Imhoff Cone (Avimelech, 2012), and when their volume in the experimental tanks reached 30 mL/L, a settler was used to maintain SS values below this limit.

Fish stocking, feeding and monitoring

Sex reversed male fingerlings of Nile tilapia (*O. niloticus*) (1.02 ± 0.02 g body weight) were obtained

from a commercial hatchery (Piscicultura Vale da Mina, Paulista, Pernambuco, Brazil) and maintained in a fiberglass tank, with a useful volume of 360 L ($0.4 \times 1.5 \times 0.6$ m), at a density of 1,000 fish/m³ and under a natural photoperiod. Throughout the acclimatization period (5 days), salinity was increased at a rate of 2 g/L day until reaching a salinity of 10 g/L by replacing freshwater from experimental units with seawater (35 g/L). All animals (1.17 ± 0.05 g) were maintained at the desired salinity for seven days prior to the experiment, and then relocated into experimental units of rectangular polypropylene tanks (50 L) at densities of 500, 750, 1,000 and 1,250 fish/m³.

Tilapia fingerlings were fed a commercial feed composed of 36% crude protein, 4% crude fat, 5% crude fiber and 12% moisture, at a feeding level of 8% fish biomass/day in the first week (powder feed), with a gradual reduction in the amount of feed until reaching a rate of 5% biomass/day (2.6 mm feed). Daily feed rations were split into four equal quantities and fed at 08:00 am, 11:00 am, 02:00 pm and 05:00 pm in all experimental units.

Fish weight (BEL Engineering M503, 0.001 g) and length (ichthyometer) were monitored weekly (30% of population) in each experimental unit to determine biomass and survival. All fish were counted weekly in each experimental unit to determine available survival. At the end of the experiment, biomass gain, final mean weight (W), final length, survival, FCR, specific growth rate (SGR), protein efficiency ratio (PER), yield, water consumption (WC) and sedimentation time (ST) were calculated, based on the following equations:

Biomass gain (g) = (Final weight (g) * Final population) – (Initial weight (g) * Initial population);

SGR (%/day) = $100 \times [(\text{Ln Final weight (g)} - \text{Ln Initial weight (g)}) / \text{Time (days)}]$;

FCR = Feed supplied (g)/Biomass gain (g);

Survival (%) = (Final population/Initial population) \times 100;

Yield (kg/m³) = Final biomass (kg)/Volume (m³);

PER = Biomass gain (g)/Total protein intake (g);

WC (L/kg) = Total water consumed (L)/ Final biomass (kg);

ST (h/kg) = Total time of settling chambers (h)/ Final biomass (kg).

Statistical analysis

Statistical analyses of the data were performed using Statistica software version 10.0 (StatSoft). Data were checked for the homogeneity of variances with the Cochran test ($\alpha \leq 0.05$) and normality using the Shapiro-Wilk test ($\alpha \leq 0.05$). One-way variance analysis (ANOVA) was conducted to evaluate the zootechnical

performance variables and repeated ANOVAs were used to compare water quality data, followed by the Tukey test to compare means ($\alpha \leq 0.05$). Nitrogen compounds (TAN, NO₂ and NO₃) were evaluated with the Kruskal-Wallis non-parametric test followed by Dunn's multiple comparison test ($\alpha \leq 0.05$). The Pearson correlation coefficient test (r) ($\alpha \leq 0.05$) was used to verify the relationship between water quality variables and zootechnical performance variables of the fingerlings.

Results

The water quality variables of temperature (28.8 to 29.5°C), dissolved oxygen (4.3 to 6.5 mg/L), pH (6.2 to 8.8) and salinity (10.4 to 11.5 g/L), were not significantly affected ($\alpha > 0.05$) by stocking density (Table 1).

TSS increased during culture time with increasing stocking densities, reaching average values close to 600 mg/L (573.49 mg/L in BFT-1000; 677.19 mg/L in BFT-1250) (Table 1). SS also increased during culture time and were significantly affected ($\alpha < 0.05$) by stocking density (Table 1).

Significant differences in alkalinity were found in BFT-1250 and BFT-1000 as compared to BFT-750 and BFT-500 (Table 1). A reduction in alkalinity occurred in the second week, although the addition of inorganic carbon (hydrated lime-Ca(OH)₂) twice a week at 20% (by weight) of the total weekly feed was sufficient to maintain alkalinity levels above 100 mg/L.

The principal dissolved inorganic nitrogen compounds were NO₃, which ranged from 0.85 to 0.90 mg/L, followed by NO₂ with concentrations ranging from 0.48 to 0.49 mg/L, and TAN ranging from 0.30 to 0.44 mg/L. No significant differences ($\alpha < 0.05$) were observed in the nitrogen compounds with stocking density increases (Table 1). PO₄⁻³ concentrations were significantly higher in BFT-1250 (2.82 mg/L) and BFT-1000 (2.85 mg/L) as compared to BFT-500 (2.60 mg/L) (Table 1).

In relation to water consumption, BFT-1250 and BFT-1000 treatments had the highest consumption (101.54-94.77 L/kg) and BFT-500 the lowest (52.48 L/kg). The use of settling chambers was necessary in all experimental units, but its sedimentation time in BFT-1250 was significantly higher (305 h/kg) than in the other treatments (238-271 h/kg) (Fig. 1).

Fish survival rates were all above 96% during the 42-day experimental period and significant differences ($\alpha \leq 0.05$) were found in BFT-500, BFT-750 and BFT-1000 compared to BFT-1250. Fish growth (final weight and final length) were more accelerated at the lowest

Table 1. Water quality variables of Nile tilapia fingerling (*Oreochromis niloticus*) culture in a low-salinity biofloc system at different stocking densities during a 42-day experimental period.

Water quality variables ⁽¹⁾	Treatments ⁽²⁾			
	BFT-500	BFT-750	BFT-1000	BFT-1250
Temperature (°C)	28.84 ± 0.73	29.22 ± 0.59	28.84 ± 0.46	28.47 ± 0.52
DO (mg/L)	5.13 ± 0.21	5.10 ± 0.24	4.98 ± 0.19	4.95 ± 0.23
pH	7.64 ± 0.26	7.62 ± 0.25	7.57 ± 0.32	7.76 ± 0.23
Salinity (g/L)	10.53 ± 0.47	10.80 ± 0.68	10.97 ± 0.45	11.01 ± 0.53
TSS (mg/L)	457.74 ± 40.68 ^b	476.24 ± 46.22 ^b	573.49 ± 38.23 ^a	677.19 ± 50.63 ^a
SS (mL/L)	13.51 ± 1.62 ^c	19.10 ± 2.54 ^b	22.29 ± 7.61 ^{ab}	26.00 ± 2.63 ^a
Alkalinity (mg/L)	159.05 ± 9.29 ^a	151.10 ± 7.24 ^a	112.23 ± 6.80 ^b	119.09 ± 9.12 ^b
TAN (mg/L)	0.44 ± 0.07	0.35 ± 0.07	0.39 ± 0.09	0.30 ± 0.08
NO ₂ (mg/L)	0.49 ± 0.02	0.49 ± 0.03	0.48 ± 0.01	0.48 ± 0.03
NO ₃ (mg/L)	0.85 ± 0.01	0.85 ± 0.01	0.86 ± 0.01	0.90 ± 0.04
PO ₄ ⁻³ (mg/L)	2.60 ± 0.13 ^b	2.78 ± 0.06 ^{ab}	2.85 ± 0.08 ^a	2.82 ± 0.07 ^a

⁽¹⁾DO= dissolved oxygen; TSS= total suspended solids; SS= settleable solids; TAN= total ammonia nitrogen. ⁽²⁾The data correspond to the mean of six weeks ± standard deviation. Different letters on the same row indicate a significant difference ($\alpha < 0.05$).

stocking density (BFT-500) as compared to the highest density (BFT-1250), although resulted in a lower yield. No significant differences ($\alpha > 0.05$) were observed for SGR and FCR, but significant differences ($\alpha \leq 0.05$) were observed for PER between BFT-1250 and the other stocking densities (Table 2).

Weight gain and length had positive correlations with alkalinity and TSS, and negative correlations with density, NO₃ and PO₄⁻³. For FCR, the inverse was found, negative correlations with alkalinity and TSS, and positive correlations with PO₄⁻³. As for yield, a positive correlation with density and TSS was recorded, and a negative correlation with survival and density. Water consumption had a negative correlation with density, TSS and PO₄⁻³ (Table 3).

Discussion

The water quality variables (dissolved oxygen, temperature, salinity and pH) in the culture water were within the range recommended for Nile tilapia culture (El-Sayed, 2006).

TSS and SS increased in higher stocking densities, probably due to increasing amounts of feed, molasses and feces. Peaks of TSS (1,005 mg/L) were found in BFT-1250 on the fourth week of culture, but its mean value was close to 500-600 mg/L for the stocking densities evaluated. These amounts are similar to those observed by Long *et al.* (2015) who investigated the effects of biofloc technology on growth, digestive

activity, hematology, and immune response at a C:N ratio of 15, and Zapata-Lovera *et al.* (2017) who evaluated C:N ratios of 10, 15 and 20. According to Avnimelech (2012) TSS can reach 1,000 mg/L in tilapia culture with bioflocs.

SS also presented the same rise as stocking density increased, however, the values were within the range (5 to 50 mL/L) recommended by Avnimelech (2011), possibly due to the settler used to maintain SS values under this limit. High concentrations of solids are not favorable for fish growth, since they contribute to a greater consumption of oxygen, and may cause accumulation of organic matter and obstruction in fish gills (Hargreaves, 2006; Avnimelech, 2011).

Although the treatments with higher stocking densities presented lower alkalinity values, these were higher than 100 mg/L, as recommended by Hargreaves (2013), so as not to interfere with nitrification processes by nitrifying bacteria and absorption of ammonia by heterotrophic bacteria. The alkalinity values were maintained at ideal values by corrections made with calcium hydroxide.

The concentrations of TAN and NO₂ were low and related to the use of water from a biofloc matrix tank (50% of the tank volume), so that only 30% molasses was used in relation to the quantity of feed supplied. Low TAN and NO₂ concentrations were also observed in other studies that used previously prepared biofloc water (Pérez-Fuentes *et al.*, 2016; Zapata-Lovera *et al.*, 2017). The use of such water prior to fish stocking led to an increase in heterotrophic and nitrifying bacteria,

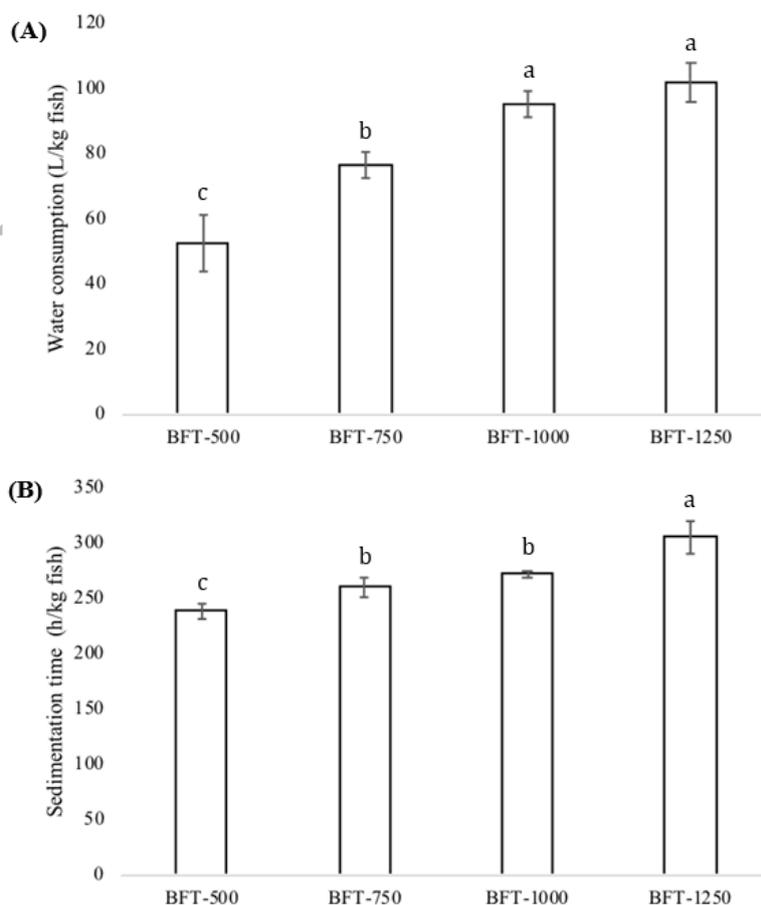


Figure 1. Water consumption (A) and sedimentation time (B) of tilapia farming in a low salinity biofloc system with different densities (500, 750, 1000 and 1250 fish/m³) in 42 days.

Table 2. Performance of zootechnical variables of Nile tilapia fingerling (*Oreochromis niloticus*) cultivated in a low-salinity biofloc system at different stocking densities during a 42-day experimental period.

Zootechnical variables ⁽¹⁾	Treatments ⁽²⁾			
	BFT-500	BFT-750	BFT-1000	BFT-1250
Survival (%)	100 ± 0.00 ^a	100 ± 0.00 ^a	99.33 ± 0.67 ^a	96.82 ± 1.42 ^b
Final weight (g)	18.99 ± 1.94 ^a	17.47 ± 0.48 ^a	15.36 ± 0.13 ^b	12.40 ± 0.14 ^c
Final length (cm)	9.80 ± 0.10 ^a	9.85 ± 0.08 ^a	9.31 ± 0.18 ^b	8.84 ± 0.04 ^c
Yield (kg/m ³)	9.49 ± 0.97 ^c	13.28 ± 0.52 ^b	15.27 ± 1.20 ^a	15.12 ± 0.25 ^a
SGR (%/day)	5.72 ± 0.42 ^a	5.54 ± 0.16 ^a	5.22 ± 0.29 ^a	4.73 ± 0.64 ^a
FCR	1.24 ± 0.11 ^a	1.30 ± 0.06 ^a	1.40 ± 0.12 ^a	1.32 ± 0.29 ^a
PER	1.93 ± 0.17 ^a	1.81 ± 0.08 ^a	1.71 ± 0.16 ^a	1.45 ± 0.02 ^b
WC (L/kg fish)	52.48 ± 8.66 ^c	76.32 ± 3.80 ^b	94.77 ± 4.03 ^a	101.54 ± 5.91 ^a
ST (h/kg fish)	238.33 ± 6.67 ^c	260.00 ± 8.66 ^b	271.67 ± 3.33 ^b	305.00 ± 15.27 ^a

⁽¹⁾SGR= specific growth rate; FCR= feed conversion ratio; PER= protein efficiency ratio; WC= water consumption; ST= sedimentation time. ⁽²⁾The data correspond to the mean of three replicates ± standard deviation. Different letters on the same row indicate a significant difference ($\alpha < 0.05$).

Table 3. Pearson coefficient (r) of the correlations between water quality variables and the zootechnical performance variables of Nile tilapia fingerlings (*Oreochromis niloticus*) cultivated in a low-salinity biofloc system at different stocking densities. In parenthesis, *p* values.

Zootechnical variables	Water quality variables										
	Density	Alkalinity	TSS	SS	TAN	NO ₂	NO ₃	PO ₄ ⁻³	Temperature	DO	pH
Survival	-0.4057* (0.001)	0.028 (0.835)	-0.197 (0.131)	0.026 (0.842)	0.175 (0.181)	0.102 (0.440)	-0.191 (0.143)	-0.012 (0.928)	0.047 (0.723)	0.201 (0.123)	-0.021 (0.873)
Weight	-0.263* (0.043)	0.492* (0.000)	0.343* (0.007)	0.007 (0.956)	-0.220 (0.092)	0.214 (0.100)	-0.343* (0.007)	-0.404* (0.001)	-0.089 (0.500)	-0.181 (0.167)	0.178 (0.175)
Length	-0.2078 (0.111)	0.497* (0.00)	0.353* (0.006)	0.038 (0.771)	-0.218 (0.095)	0.206 (0.115)	-0.342* (0.008)	-0.370* (0.004)	-0.054 (0.684)	-0.200 (0.125)	0.159 (0.226)
Yield	0.470* (0.000)	0.156 (0.233)	0.379 (0.003)	-0.071 (0.588)	-0.197 (0.131)	0.037 (0.782)	-0.180 (0.168)	-0.151 (0.249)	-0.044 (0.740)	-0.154 (0.239)	0.110 (0.405)
SGR	0.028 (0.833)*	0.060 (0.650)	-0.187 (0.153)	0.049 (0.713)	-0.135 (0.304)	0.015 (0.910)	0.000 (0.998)	-0.091 (0.489)	0.022 (0.867)	-0.002 (0.990)	-0.037 (0.779)
FCR	0.020 (0.879)	-0.328* (0.010)	-0.349* (0.006)	-0.022 (0.866)	0.063 (0.631)	-0.041 (0.756)	0.179 (0.171)	0.377* (0.003)	-0.142 (0.278)	0.055 (0.677)	-0.029 (0.825)
PER	-0.193 (0.140)	0.331* (0.010)	0.277* (0.032)	0.066 (0.616)	-0.200 (0.126)	0.128 (0.331)	-0.283 (0.028)	-0.391 (0.002)	-0.048 (0.717)	-0.225 (0.084)	0.137 (0.298)
WC	-0.662* (0.000)	0.274 (0.034)	-0.256* (0.048)	0.105 (0.423)	0.185 (0.156)	0.130 (0.321)	-0.142 (0.278)	-0.264* (0.041)	-0.107 (0.415)	-0.094 (0.477)	0.145 (0.270)
ST	-0.016 (0.905)	0.074 (0.113)	0.175 (0.182)	0.040 (0.763)	-0.002 (0.988)	0.100 (0.448)	-0.168 (0.089)	-0.096 (0.099)	-0.092 (0.484)	-0.206 (0.114)	0.193 (0.140)

* indicates a significant difference ($\alpha < 0.05$). For abbreviations of water quality and zootechnical variables see Tables 1 and 2, respectively.

which transform ammonia into microbial biomass and NO₃, respectively. A higher concentration of NO₃ than other nitrogen compounds indicates that the controlled addition of molasses does not inhibit the development of nitrifying bacteria. The stocking densities tested did not seem to influence these nitrogen compounds, contrasting with results obtained in a system without addition of an organic carbon source (Luz *et al.*, 2012).

In biofloc systems there is an increase of PO₄⁻³ concentrations in the water (Luo *et al.*, 2014; Day *et al.*, 2016). According to Thakur & Lin (2003), a large portion (38.8-66.7%) of the phosphorus that enters pond systems is deposited in the sediment, which does not exist in biofloc systems. This accumulation was influenced by the increased stocking density, with increasing input of phosphorus by feed addition.

Effects of increased stocking density on WC and ST were observed, where the treatments with higher densities of 1,000 and 1,250 fish/m³ (respectively 94.77 and 101.54 L/kg) presented significantly higher values (Fig. 1). These higher values are related to the higher concentration of TSS and SS, leading to the need for a longer total time of settling chambers, and consequently greater water replenishment. Despite the higher use of water at the higher densities tested,

the amount is still much lower than in traditional systems with water renewal. Zapata-Lovera *et al.* (2017) evaluated water consumption in tilapia culture in a water exchange (1,800 L/kg) and a biofloc system (90-190 L/kg) and found that a biofloc system can be used to produce tilapia, especially in places with limited water availability.

The lowest survival (96.82%), final weight (12.4g) and final length (8.84 cm) was found in BFT-1250, indicating the negative effect of high density. Water-flow systems, such as raceways (Silva *et al.*, 2002) and cages (Maeda *et al.*, 2010), also have reductions in final weight at increased stocking densities. These results suggest greater competition for feed and space due to cannibalism and stress, influencing the performance of the fingerlings.

SGR and FCR were not influenced by the stocking density, and our results were similar to those observed in other studies with tilapia in freshwater biofloc systems (Zapata-Lovera *et al.*, 2017), thus indicating that the effect of a salinity of 10 g/L in a biofloc system does not seem to influence the productive performance of tilapia at the densities studied. However, PER was influenced by the increased stocking density, since the lowest values were found in the BFT-1250 treatment, due to the lower performance of the organisms.

In relation to salinity, some studies have found a decrease in the hematological parameters and histopathological alterations at increased water salinity levels (Azevedo *et al.*, 2015), however, in biofloc systems salinity does not seem to be the main stress factor for fish. According to Lima (2017), tilapia grown in a biofloc system with salinity of 10 g/L and high levels of settleable solids (55 mL/L) show signs of stress (reddish body extremities) and hemorrhaging, leading to reduced growth and decreased survival. However, the higher solids levels did not appear to have negatively influenced yield, since the treatments with higher stocking density presented a yield of 15 kg m⁻³. Biomass produced in biofloc systems can result in a yield of 10-40 kg/m³ (Avnimelech, 2007), similar to the results observed in this study (9.4-15 kg/m³), therefore indicating good results even at a salinity of 10 g/L.

To summarize, the results of this study confirm the zootechnical potential of Nile tilapia fingerling cultivated in a low-salinity biofloc system, with a positive effect of stocking density increase on yield, but a negative effect on zootechnical performance (survival, final weight and final length), water quality (TSS, SS and PO₄⁻³), WC and ST. It is possible to increase the stocking density of Nile tilapia fingerlings up to 1000 fish/m³ in a low-salinity biofloc system, since this has no effect on the zootechnical potential. However, studies are needed to determine the economic viability and compensatory growth for Nile tilapia fingerling culture in the growth stage.

References

- Abou Y, Fiogbe ED, Micha JC, 2007. Effects of stocking density on growth, yield and profitability of farming Nile tilapia, *Oreochromis niloticus* L., fed Azolla diet, in earthen ponds. *Aquacult Res* 38: 595-604. <https://doi.org/10.1111/j.1365-2109.2007.01700.x>
- Alves GFO, Fernandes AFA, de Alvarenga ÉR, Turra EM, de Sousa AB, Teixeira EA, 2017. Effect of the transfer at different moments of juvenile Nile tilapia (*Oreochromis niloticus*) to the biofloc system in formation. *Aquaculture* 479: 564-570. <https://doi.org/10.1016/j.aquaculture.2017.06.029>
- APHA, AWWA, WEF, 2005. Standard methods for the examinations of the water and wastewater. 12th ed. APHA, Washington, DC, 560 pp.
- Avnimelech Y, 2007. Feeding with microbial flocs by tilapia in minimal discharge bioflocs technology ponds. *Aquaculture* 264 (1): 140-147. <https://doi.org/10.1016/j.aquaculture.2006.11.025>
- Avnimelech Y, 2011. Tilapia production using biofloc technology (BFT). *Proc 9th Int Symp on Tilapia in Aquaculture*; Liping L & Fitzsimmons K (eds.). pp: 362-366. AquaFish Collaborative Research Support Program, Shanghai.
- Avnimelech Y, 2012. *Biofloc technology-A practical guide book*. 2nd ed. The World Aquaculture Society, Baton Rouge, USA. 272 pp.
- Azevedo RV, de Oliveira KF, Flores-Lopes F, Teixeira-Lanna EA, Takishita SS, Tavares-Braga LG, 2015. Responses of Nile tilapia to different levels of water salinity/Respuestas de la tilapia del Nilo a diferentes niveles de salinidad del agua. *Lat Am J Aquat Res* 43 (5): 828-835.
- Barcellos LJG, Nicolaiewsky S, De Souza SMG, Lulhier F, 1999. The effects of stocking density and social interaction on acute stress response in Nile tilapia *Oreochromis niloticus* (L.) fingerlings. *Aquacult Res* 30 (11-12): 887-892. <https://doi.org/10.1046/j.1365-2109.1999.00419.x>
- Brito LO, Arana LAV, Soares RB, Severi W, Miranda RH, da Silva SMBC, Coimbra MRM, Gálvez AO, 2014. Water quality, phytoplankton composition and growth of *Litopenaeus vannamei* (Boone) in an integrated biofloc system with *Gracilaria birdiae* (Greville) and *Gracilaria domingensis* (Kützing). *Aquacult Int* 22 (5): 1649-1664. <https://doi.org/10.1007/s10499-014-9771-9>
- Brito LO, dos Santos IGS, de Abreu JL, de Araújo MT, Severi W, Gálvez AO, 2016. Effect of the addition of diatoms (*Navicula* spp.) and rotifers (*Brachionus plicatilis*) on water quality and growth of the *Litopenaeus vannamei* postlarva e reared in a biofloc system. *Aquacult Res* 47 (12): 3990-3997. <https://doi.org/10.1111/are.12849>
- Colt J, 2006. Water quality requirements for reuse systems. *Aquacult Eng* 34 (3): 143-156. <https://doi.org/10.1016/j.aquaeng.2005.08.011>
- Costa A, Fróes R, 2012. *Produção de tilápias*. Secretaria de Agricultura e Pecuária. Niterói, Brazil. Technical Manual 31.
- Crab R, Defoirdt T, Bossier P, Verstraete W, 2012. Biofloc technology in aquaculture: beneficial effects and future challenges. *Aquaculture* 356-357: 351-356. <https://doi.org/10.1016/j.aquaculture.2012.04.046>
- Day SB, Salie K, Stander HB, 2016. A growth comparison among three commercial tilapia species in a biofloc system. *Aquacult Int* 24 (5): 1309-1322. <https://doi.org/10.1007/s10499-016-9986-z>
- De Schryver P, Crab R, Defoirdt T, Boon N, Verstraete W, 2008. The basics of bio-flocs technology: the added value for Aquaculture. *Aquaculture* 277 (3-4): 125-137. <https://doi.org/10.1016/j.aquaculture.2008.02.019>
- Ekasari J, Rivandi DR, Firdausi AP, Surawidjaja EH, Junior ZM, Bossier P, de Schryver P, 2015. Biofloc technology positively affects Nile tilapia (*Oreochromis niloticus*) larvae performance. *Aquaculture* 441: 72-77. <https://doi.org/10.1016/j.aquaculture.2015.02.019>

- El-Sayed Abdel-Fattah M, 2006. Tilapia culture. Oxfordshire: CABI Publ, Wallingford, UK. 277 pp. <https://doi.org/10.1079/9780851990149.0000>
- Emerenciano M, Gaxiola G, Cuzon G, 2013. Biofloc technology (BFT): A review for aquaculture application and animal food industry. In: Biomass now-cultivation and utilization; Matovic MD (ed.). pp: 301-328. InTech, Manhattan. <https://doi.org/10.5772/53902>
- Felföldy L, Szabo E, Toth L, 1987. A biológiai vizminőség. Vizügyi Hidrobiológia Vizdok, Budapest, Hungary. 258 pp.
- Ferdous Z, Masum Md A, Ali Md M, 2014. Influence of stocking density on growth performance and survival of monosex tilapia (*Oreochromis niloticus*) fry. Int J Res Fish Aquacult 4 (2): 99-103.
- Golterman HL, Clymo RS, Ohnstad MAM, 1978. Methods for physical and chemical analysis of freshwaters. Blackwell Sci Publ, London. 213 pp.
- Hansen HP, Koroleff F, 2007. Determination of nutrients. In: Methods of seawater analysis; Grasshoff K, Kremling K, Ehrhardt M (eds.). pp: 159-226. Blackwell Sci, Oxford.
- Hargreaves JA, 2006. Photosynthetic suspended-growth systems in aquaculture. Aquacult Eng 34 (3): 344-363. <https://doi.org/10.1016/j.aquaeng.2005.08.009>
- Hargreaves, J.A., 2013. Biofloc production systems for aquaculture. Southern Regional Aquaculture Center, SRAC Publication No. 4503. Stoneville, USA. 12 pp.
- Kamal AH Md M, Mair GC, 2005. Salinity tolerance in superior genotypes of tilapia, *Oreochromis niloticus*, *Oreochromis mossambicus* and their hybrids. Aquaculture 247 (1-4): 189-201. <https://doi.org/10.1016/j.aquaculture.2005.02.008>
- Kpundeh MD, Xu P, Yang H, Qiang J, He J, 2013. Stocking densities and chronic zero culture water exchange stress' effects on biological performances, hematological and serum biochemical indices of gift tilapia juveniles (*Oreochromis niloticus*). J Aquac Res Dev 4 (5): 1-6. <https://doi.org/10.4172/2155-9546.1000189>
- Likongwe JS, Stecko TD, Stauffer Jr. JR, Carline RF, 1996. Combined effects of water temperature and salinity on growth and feed utilization of juvenile Nile tilapia *Oreochromis niloticus* (Linnaeus). Aquaculture 146: 37-46. [https://doi.org/10.1016/S0044-8486\(96\)01360-9](https://doi.org/10.1016/S0044-8486(96)01360-9)
- Lima PCM, 2017. Efeito da adição de *Chlorella vulgaris* e melação na qualidade da água e crescimento de alevinos de tilápia do nilo (*Oreochromis niloticus*) em sistema de bioflocos com baixa salinidade. Master's thesis. Universidade Federal Rural de Pernambuco, Recife, Brazil. 61 pp.
- Long L, Yang J, Li Y, Guan C, Wu F, 2015. Effect of biofloc technology on growth, digestive enzyme activity, hematology, and immune response of genetically improved farmed tilapia (*Oreochromis niloticus*). Aquaculture 448: 135-141. <https://doi.org/10.1016/j.aquaculture.2015.05.017>
- Luo G, Gao Q, Wang C, Liu W, Sun D, Li L, Tan H, 2014. Growth, digestive activity, welfare, and partial cost-effectiveness of genetically improved farmed tilapia (*Oreochromis niloticus*) cultured in a recirculating aquaculture system and an indoor biofloc system. Aquaculture 422-423: 1-7. <https://doi.org/10.1016/j.aquaculture.2013.11.023>
- Luz RK, Silva WS, Filho RM, Santos AEH, Rodrigues LA, Takata R, de Alvarenga ÉR, Turra EM, 2012. Stocking density in the larviculture of Nile tilapia in saline water. R Bras Zootec 41 (12): 2385-2389. <https://doi.org/10.1590/S1516-35982012001200001>
- Mackereth FJH, Heron J, Talling JF, 1978. Water analysis: some revised methods for limnologists. Blackwell Sci Publ, Oxford, England. 120 pp.
- Maeda H, Silva PC, de Castro Oliveira RP, da Silva AM, Pádua DMC, Machado NP, da Silva RH, 2010. Densidade de estocagem na alevinagem de tilápia-do-nilo em tanque-rede. Ciênc Anim Bras 11 (3): 471-476.
- Mapenzi LL, Mmochi AJ, 2016. Role of salinity on growth performance of *Oreochromis niloticus* ♀ and *Oreochromis urolepis* urolepis ♂ hybrids. J Aquacult Res Dev 7 (6): 431.
- Marinho YF, Brito LO, Campos CVFS, Severi W, Andrade HA, Gálvez AO, 2017. Effect of the addition of *Chaetoceros calcitrans*, *Navicula* sp. and *Phaeodactylum tricorutum* (diatoms) on phytoplankton composition and growth of *Litopenaeus vannamei* (Boone) postlarvae reared in a biofloc system. Aquacult Res 48 (8): 4155-4164. <https://doi.org/10.1111/are.13235>
- Miranda-Baeza A, Mariscal-López MA, López-Eliás JA, Rivas-Vega1 M, Emerenciano M, Sánchez-Romero A, Esquer-Méndez JL, 2017. Effect of inoculation of the cyanobacteria *Oscillatoria* sp. on tilapia biofloc culture. Aquacult Res 48 (9): 4725-4734. <https://doi.org/10.1111/are.13294>
- Moniruzzaman M, Uddin KB, Basak S, Mahmud Y, Zaher M, Bai SC, 2015. Effects of stocking density on growth, body composition, yield and economic returns of monosex tilapia (*Oreochromis niloticus* L.) under cage culture system in Kaptai Lake of Bangladesh. J Aquacult Res Dev 6 (8): 1-7. <https://doi.org/10.4172/2155-9546.1000357>
- NG WK, Romano N, 2013. A review of the nutrition and feeding management of farmed tilapia throughout the culture cycle. Rev Aquacult 5 (4): 220-254. <https://doi.org/10.1111/raq.12014>
- Osofero SA, Otubusin SO, Daramola JA, 2009. Effect of stocking density on tilapia (*Oreochromis niloticus* Linnaeus 1757) growth and survival in bamboo - net cages trial. Afr J Biotechnol 8 (7): 1322-1325.
- Pérez-Fuentes JA, Hernández-Vergara MP, Pérez-Rostro CI, Fogel I, 2016. C:N ratios affect nitrogen removal and production of Nile tilapia *Oreochromis niloticus* raised in a biofloc system under high density cultivation.

- Aquaculture 452: 247-251. <https://doi.org/10.1016/j.aquaculture.2015.11.010>
- Ray AJ, Lotz JM, 2017. Comparing salinities of 10, 20, and 30‰ in intensive, commercial-scale biofloc shrimp (*Litopenaeus vannamei*) production systems. Aquaculture 476: 29-36. <https://doi.org/10.1016/j.aquaculture.2017.03.047>
- Ridha MT, 2006. Comparative study of growth performance of three strains of Nile tilapia, *Oreochromis niloticus*, L. at two stocking densities. Aquacult Res 37 (2): 172-179. <https://doi.org/10.1111/j.1365-2109.2005.01415.x>
- Silva PC, Kronka SN, Tavares LHS, Souza VL, 2002. Desempenho produtivo da tilápia do Nilo (*Oreochromis niloticus* L.) em diferentes densidades de trocas de água em raceway. Acta Scientiarum Anim Sci 24 (4): 935-941.
- Suresh AV, Lin CK, 1992. Effect of stocking density on water quality and production of red tilapia in a recirculated water system. Aquacult Eng 11 (1): 1-22. [https://doi.org/10.1016/0144-8609\(92\)90017-R](https://doi.org/10.1016/0144-8609(92)90017-R)
- Takata R, Luz RK, 2015. Água salinizada na produção de peixes de água doce. In: Aquicultura no Brasil: novas perspectivas; Tavares-Dias M, Mariano WS (eds.). pp: 523-543. Editora Pedro & João, São Carlos.
- Thakur DP, Lin CK, 2003. Water quality and nutrient budget in closed shrimp (*Penaeus monodon*) culture systems. Aquacult Eng 27 (3): 159-176. [https://doi.org/10.1016/S0144-8609\(02\)00055-9](https://doi.org/10.1016/S0144-8609(02)00055-9)
- Widanarni W, Ekasari J, Maryam S, 2012. Evaluation of biofloc technology application on water quality and production performance of red tilapia *Oreochromis* sp. cultured at different stocking densities. Hayati J Biosci 19 (2): 73-80. <https://doi.org/10.4308/hjb.19.2.73>
- Zapata-Lovera KP, Brito LO, Lima PCM, Vinatea-Arana LA, Galvez AO, Cárdenas JMV, 2017. Cultivo de alevines de tilapia en sistema biofloc bajo diferentes relaciones carbono/nitrógeno. Boletim do Instituto de Pesca 43 (3): 399-407. <https://doi.org/10.20950/1678-2305.2017v43n3p399>