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# Association between periphyton and bioflocs systems in intensive culture of juvenile Nile tilapia

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**ABSTRACT.** The present work aimed at determining the effects of the association between the periphyton-based system with the bioflocs-based system in the intensive culture of juvenile Nile tilapia  $(1.56 \pm 0.07~\rm g; 72~\rm fish~m^{-3})$ , on variables of water quality, growth performance and effluent quality after 10 weeks. The experiment was arranged in a 2 x 2 factorial randomized block design with four treatments and five repetitions each. The factors tested were the following: 'underwater structure' (absence and presence) and 'adjustment of the C: N ratio of water' (no and yes). The final fish body weight, specific growth rate and yield were higher (p < 0.05) in the C: N-adjusted tanks. The presence of submerged structures in the tanks had no significant influence on those same variables. It was concluded that the periphyton-based system is not indicated for intensive farming of Nile tilapia, in which there is a high allowance of artificial feed to fish.

Keywords: aquaculture, Oreochromis, limnoculture, limnology.

# Integração dos sistemas perifíton e bioflocos no cultivo intensivo de juvenis de tilápia do Nilo

**RESUMO.** O presente trabalho teve por objetivo determinar os efeitos da integração do sistema baseado em substrato (perifíton) com o sistema baseado no ajuste da relação C: N da água (bioflocos), no cultivo intensivo de juvenis de tilápia do Nilo  $(1,56 \pm 0,07 \, \text{g}; 72 \, \text{peixes m}^{-3})$ , sobre variáveis de qualidade de água, desempenho zootécnico e qualidade de efluentes, após 10 semanas. O delineamento experimental utilizado foi em blocos ao acaso, em arranjo fatorial  $2 \times 2$ , com quatro tratamentos e cinco repetições cada. Os fatores em teste foram os seguintes 'estruturas submersas' (ausência e presença) e 'ajuste da relação C: N da água' (não e sim). Os resultados de peso corporal final, taxa de crescimento específico e produtividade de peixe foram maiores (p < 0,05) nos tanques nos quais se fez o ajuste da relação C: N da água, quando comparado aos tanques sem esse ajuste. A presença das estruturas submersas nos tanques não afetou de modo significativo essas mesmas variáveis. Concluiu-se que o sistema de cultivo de peixe baseado em substrato (perifíton) não é indicado para cultivos intensivos de juvenis de tilápia do Nilo, nos quais há grande entrada de alimento artificial nos tanques de cultivo.

Palavras-chave: aquicultura, Oreochromis, limnocultura, limnologia.

# Introduction

Usually, intensive systems of fish farming are based on the supply of large amounts of artificial food (balanced rations) to the farmed animals. However, those systems have high costs and may cause environmental problems, since most of the feed given to the animals is not consumed and accumulate in the environment as fish feces (Avnimelech, 1999). Such organic debris, which are rich in nitrogen and phosphorus, can cause eutrophication of the natural ecosystems when they are directly discharged into the receiving water bodies (Green, Schrader & Perschbacher, 2014). An

alternative to the intensive use of artificial feed in fish farming is the increased supply of natural food to the farmed animals (Azim & Little, 2008). Among the solutions currently proposed, it is highlighted the use of artificial substrates and the bioflocs technology (BFT).

Diverse underwater substrates can be used in fish and shrimp tanks to promote the development of periphyton, and obtain a better water quality and improved feed efficiency (Asaduzzaman et al., 2008; Azim & Little, 2008). The periphyton is formed by a complex community of aquatic organisms that adhere to the underwater substrates, which are

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colonized by bacteria, fungi, protozoa, phytoplankton, zooplankton, and debris. The periphyton can be used as a food source by the farmed fish (Schveitzer et al., 2013). Some studies, however, have not found clear benefits derived from the submerged substrates on fish growth and water quality (Kumlu, Eroldogan, & Saglamtimur, 2001).

The bioflocs technology (BFT) in aquaculture aims at removing toxic compounds from the water, such as ammonia and nitrite, immobilizing them into the bacterial biomass (bioflocs) by adjusting the C: N ratio of water. The bioflocs can control the water quality and be used as a natural food source by the reared animals (Avnimelech, 1999; Crab, Avnimelech, Defoirdt, Bossier, & Verstraete, 2007). However, the knowledge currently available on the BFT system is still undeveloped. The contribution of bioflocs on fish production has not been properly quantified yet (Crab, Defoirdt, Bossier, & Verstraete, 2012.), nor it is clear the relative importance of the heterotrophic bacteria as a food source (Azim & Little, 2008; Anand et al., 2014).

An alternative to the single use of those systems, not fully evaluated yet, is the association between periphyton and bioflocs into a same system. Some authors have suggested that periphyton and bioflocs can act synergistically, improving the water quality and nutrition of farmed animals (Azim & Litlle, 2008; Schveitzer et al., 2013). This study aimed at determining, in an intensive culture of juvenile Nile tilapia, the effects of associating periphyton-based system with the BFT system on variables of water quality, growth performance and effluent quality.

## Material and methods

The study was conducted at the Laboratório de Ciência e Tecnologia Aquícola (LCTA, Departamento de Engenharia de Pesca, Centro de Ciências Agrárias, Universidade Federal do Ceará, Fortaleza, Ceará). One-thousand sexually reversed Nile tilapia juveniles (1.12 ± 0.05 g) were obtained from one local fish farmer. Fish were maintained for five days in one 1,000-L tank provided with constant aeration. Fish were fed four times a day (08, 11, 14 and 17h) with a powdered diet containing 45% crude protein at 10% total biomass daily.

At the beginning of the experiment, fish with  $1.56 \pm 0.07$  g were transferred to twenty polyethylene 250-L tanks at a density of 18 fish per tank. The experiment was carried out for ten weeks from April 14<sup>th</sup> 2014 to June 23<sup>rd</sup> 2014.

The fish were fed on a commercial diet four times a day at 8, 11, 14 and 17 h. The feeding rates were adjusted every 14 days according to the fish

weighings, as follows: 1<sup>st</sup> - 2<sup>nd</sup> weeks (10.5%); 3<sup>rd</sup> - 4<sup>th</sup> weeks (5.8%); 5<sup>th</sup> - 6<sup>th</sup> weeks (4.2%); 7<sup>th</sup> - 8<sup>th</sup> weeks (3.9%); 9<sup>th</sup> - 10<sup>th</sup> weeks (2.6% of the stocked biomass). Initially, the fish were fed on a powdered commercial 45%-crude protein (CP) diet for omnivorous fish. After four weeks, fish received a 0.8-1.2 mm pelleted diet with 39% CP. In the last two weeks, it was allowed a 2-3 mm diet with 35% CP. In the fish weighings, all fish from a same tank were pooled and weighed quickly in one plastic beaker with a small amount of water.

The experiment was arranged in a randomized block design with four treatments and five repetitions each. Each one of the five experimental blocks had one replicate of each treatment, which were positioned sequentially along the tank bench. This was required to control the influence of unmonitored environmental factors experimental variables, such as winds and insolation. The treatments under evaluation were the following: periphyton (absence and presence) and bioflocs (absence and presence). In that way, the treatments consisted of: (1) control no underwater substrate and no adjustment of C: N ratio of water; (2) periphyton use of underwater substrate but no adjustment of the C: N ratio of water; (3) bioflocs no underwater substrate but adjustment of the C: N ratio of water; (4) biophyton association of periphyton and bioflocs in the same tank.

Two flat polyethylene boards were arranged vertically into each tank for the periphyton development, providing a total substrate area equal to 135% of the tank's bottom area (0.90 m<sup>-2</sup>). The growth of bioflocs was stimulated by adjusting the C: N ratio of water to 15: 1 with daily applications of dry molasses. The amounts of molasses applied to the bioflocs and biophyton tanks were calculated following the guidelines presented by Avnimelech (1999), which are based on the percentage of crude protein and on the feeding rates applied. Besides, it was considered that the dry molasses contained 40% carbon. The crude protein content of the diets was determined by chemical analysis.

In the biofloc and biophyton tanks, there was 24-h aeration of water throughout the experiment, provided by one 2.5-CV radial compressor (air blower). In the control and periphyton tanks, nocturnal aeration (18-6 h) of water was carried out from the 3<sup>rd</sup> week up to the end. In all tanks, no water exchange was performed over the entire experiment, just maintenance of the initial level. The replacement of dead fish for a new one was allowed only in the first experimental week. Forty-eight hours after the final fish weighing, samples of the tank effluents were obtained for chemical

analyses (dissolved oxygen, total ammonia nitrogen, organic matter and reactive phosphorus).

Daily, the water pH (pH meter mPA210 - MS electrical Tecnopon®), conductivity (conductivimeter CD-850) and temperature were determined at 8 and 15h. Weekly, the following variables were measured: dissolved oxygen (8h; DO<sub>2</sub>; Winkler method with azide modification); free CO2 (titration with standard solution of sodium carbonate); total ammonia nitrogen (TAN; indophenol method) and organic matter (KMnO<sub>4</sub> consumed method). Fortnightly, the determinations were the following: nitrite (sulfanilamide method), nitrate (reducing Cd column method), reactive phosphorus (molybdenum blue method), total alkalinity (titration with H<sub>2</sub>SO<sub>4</sub> standard solution), total hardness (titration with EDTA standard solution), primary phytoplankton productivity (light and dark bottle method) and total dissolved sulfide (titration with Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> standard solution). The concentrations of NH3 were calculated by applying the TAN, pH and water temperature results to the Emerson's Formula (El-Shafai, El-Gohary, Nars, Steen, & Gijzen, 2004). The concentrations of H<sub>2</sub>S were calculated from the total dissolved sulfide results according to Boyd (2000). These water quality determinations were performed according to the American Public Health Association's methods (APHA, 1999).

The variables of growth performance monitored over the study were the followeing: fish's final body weight (g), survival (%), food conversion ratio (FCR = feed consumed/body weight gain), specific growth rate (SGR = [Ln (final weight) - Ln (weight initial)]/days of culture) x 100), fish yield and protein efficiency ratio (PER = weight gain/protein consumed).

The results were presented as mean ± standard deviation. Assumptions of normal distribution (Shapiro-Wilk's test) and homogeneity of variance (Levene's test) were checked before analysis. The results of growth performance and water quality were submitted to the two-way ANOVA (periphyton x bioflocs) and the significantly different means were compared by the Tukey's test. The SPSS v.15.0 software and the Windows Excel

2010 were used in these analyses. The level of significance was set at p < 0.05.

#### Results and discussion

#### **Water Quality**

The presence of underwater structures has not affected the water pH (p > 0.05). On the other hand, significantly lower pH values were observed in the C: N ratio-adjusted tanks (Table 1). The electrical conductivity (EC) of the water was significantly affected by the adjustment of the C: N ratio of water, in which the C: N-adjusted tanks presented the higher values of EC (Table 1). Dry molasses after decomposition releases CO<sub>2</sub> to the water, reducing the water alkalinity and pH (Ray, Dillon, & Lotz, 2011).

The concentrations of free  $CO_2$  were higher (p < 0.05) in the C: N-adjusted tanks (Biofloc and Biophyton). The presence of the underwater structures has not affected the concentrations of free  $CO_2$  in water (Table 2). The total alkalinity of water was significantly higher in the non-adjusted C: N ratio tanks. Therefore, it is important to determine systematically the total alkalinity of water in BFT tanks because those tanks are prone to acidification (Furtado, Poersch, & Wasielesky, 2011).

There were no significant differences between the treatments for dissolved oxygen (DO<sub>2</sub>). Therefore, the artificial aeration provided to the tanks that received daily applications of molasses was sufficient to meet the increased demand for DO<sub>2</sub> in those tanks. The total hardness of water was higher in the C: N-adjusted tanks. Dry molasses contains calcium in its composition, which explain the hardness increase in those tanks (Rostagno, 2011). The underwater structures have not significantly affected the hardness of water (Table 2).

The tanks with adjusted C: N ratio had significantly lower concentrations of TAN. The underwater structures have not affected the concentrations of TAN in the tanks (p > 0.05, Table 2).

**Table 1.** Water pH and electrical conductivity in Nile tilapia juvenile tanks. Tanks were provided or not with underwater structures for periphyton colonization and had or not the C: N ratio adjusted to 15: 1 (mean  $\pm$  SD; n = 5).

Variable	Adjustment of the C: N ratio	Underwater structure		
variable	of water	No	Yes	
	No	$8.10 \pm 0.60 \mathrm{A}^1$	8.13 ± 0.61 A	
pН	Yes	$7.02 \pm 0.18 \mathrm{B}$	$7.03 \pm 0.17 \mathrm{B}$	
Electrical conductivity	No	$400 \pm 25 \text{ A}$	$379 \pm 23 \text{ A}$	
(μS cm <sup>-1</sup> )	Yes	$740 \pm 34 \mathrm{B}$	$698 \pm 45 \mathrm{B}$	
	Two-way ANG	OVA P		
Factor	рŀ	I	Electrical conductivity	
Underwater structure	ns	ns <sup>2</sup>		
C: N ratio adjustment	< 0.001		< 0.001	
Structure x C: N ratio	ns	5	ns	

<sup>1</sup> For a same variable, means followed by different capital letters in the same column are significantly different by Tukey's test (p < 0.05); 2Non-significant (p > 0.05).

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**Table 2.** Water quality of Nile tilapia tanks provided or not with underwater structures for development of periphyton and with or without the C: N ratio of water adjusted to 15: 1 (mean  $\pm$  SD; n = 5).

X7 : 11		Adjustment of the C: N ratio		ratio	Underwater structure					
Variable		of water			No			Yes		
Dissolved oxygen		No			$5.02 \pm 1.40^{\circ}$		$5.17 \pm 0.92$			
(mg L <sup>-1</sup> )		Yes			$6.70 \pm 0.97$			$6.69 \pm 0.99$		
Free CO <sub>2</sub>		No			$5.49 \pm 0.70 \mathrm{A}^2$			$6.02 \pm 0.68 \mathrm{A}$		
(mg L <sup>-1</sup> )		Yes			$8.96 \pm 0.89 \mathrm{B}$			$9.49 \pm 0.69 \mathrm{B}$		
Total alkalinity		No			$148.2 \pm 17.7 \mathrm{A}$			$137.9 \pm 12.3 \mathrm{A}$		
(mg L <sup>-1</sup> eq. CaCO <sub>3</sub> )		Yes $52.7 \pm 9.6 \mathrm{B}$			9.6 B	$38.24 \pm 6.5 \mathrm{B}$				
Total hardness			No $80.9 \pm 10.1 \text{A}$ 69.1			$69.1 \pm 10.4$	· A			
(mg L <sup>-1</sup> eq. CaCO <sub>3</sub> )			Yes $260.2 \pm 25.9 \mathrm{B}$			$236.5 \pm 37.9 \mathrm{B}$				
TAN <sup>3</sup>			No $0.85 \pm 0.19 \text{A}$			$0.79 \pm 0.17 \mathrm{A}$				
(mg L-1)			Yes			$0.23 \pm 0.10 \mathrm{B}$		$0.19 \pm 0.09 \mathrm{B}$		
Nitrite		No			$1.85 \pm 0.20 \mathrm{A}$			$1.70 \pm 0.22 \mathrm{A}$		
(mg L <sup>-1</sup> )			Yes			$0.70 \pm 0.16 \mathrm{B}$		$0.70 \pm 0.17 \text{ B}$		
Nitrate			No			$21.94 \pm 2.34 \mathrm{A}$		$18.39 \pm 3.27 \mathrm{A}$		
(mg L <sup>-1</sup> )			Yes $40.20 \pm 4.12$			4.12 B	$41.07 \pm 4.05 \mathrm{B}$			
Reactive phosphorus			No $0.19 \pm 0.09 \text{A}$ $0.20 \pm 0.00 \text{A}$			$0.20 \pm 0.07$	Α			
(mg L <sup>-1</sup> )			Yes	Yes $0.59 \pm 0.12 \mathrm{B}$ $0.56 \pm 0.12 \mathrm{B}$			$0.56 \pm 0.15$	В		
Organic matter			No			$196.0 \pm 23.0 \mathrm{A}$			$189.0 \pm 23.1 \mathrm{A}$	
(mg L <sup>-1</sup> )			Yes $310.1 \pm 33.3 \text{ B}$				$315.4 \pm 42.2 \mathrm{B}$			
				Two-way AN	OVA P					
Factor	$DO_2$	CO <sub>2</sub>	Alkal.	Hardn.	TAN	Nitri.	Nitra.	Phos.	O.M.	
Structure	ns <sup>4</sup>	ns	ns	ns	ns	ns	ns	ns	ns	
C: N ratio	ns	< 0.001	< 0.001	< 0.001	0.001	< 0.001	< 0.001	< 0.001	< 0.001	
Struc. x C:N	ns	ns	ns	ns	ns	ns	ns	ns	ns	

<sup>&</sup>lt;sup>1</sup>Absence of letters indicates non-significant differences between the mean values (p > 0.05); <sup>2</sup> For the same variable, means followed by different capital letters in the same column are significantly different by Tukey's test (p < 0.05); <sup>2</sup>Total ammonia nitrogen; <sup>4</sup> Non-significant (p > 0.05).

The range of TAN variation was between 0.09 and 1.12 mg L<sup>-1</sup>. These results indicate that bioflocs have a greater capacity to remove ammonia from water than periphyton, as also observed by Ekasari et al. (2014) and Green (2015).

Besides, the association of periphyton with bioflocs into a single system does not significantly improve the water quality when compared to the standard BFT system. Therefore, it is not advisable the employment of underwater structures in intensive fish farming tanks because that do not bring clear benefits to the farmer.

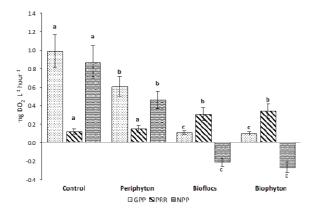
The concentrations of nitrite in water were lower (p < 0.05) in the C: N ratio adjusted tanks. The nitrite range in the tanks was between 0.52 and 2.17 mg L<sup>-1</sup>. Liu, Hu, Dai, and Avnimelech (2014) and Luo et al. (2014) have also observed a similar response for the nitrite results. Therefore, the bioflocs are capable to remove, beside ammonia, nitrite from the water, which is also a toxic compound for fish and shrimp (Krummenauer et al., 2012). There was no significant difference between the tanks with or without the submerged structures for nitrite (Table 2). The bioflocs and biophyton tanks presented higher concentrations of nitrate than the other tanks (control and periphyton; Table 2). Krummenauer et al. (2012) argued that, unlike ammonia and nitrite, nitrate is a virtually non-toxic compound for aquatic animals. Therefore, the increase of nitrate in the C: N ratio adjusted tanks is not a reason for concern among the BFT farmers. Nitrate will be toxic to fish only at very high concentrations, above 1000 mg L -1 (Kuhn et al., 2010). The installment of underwater structures

in the tanks has not significantly affected the concentrations of nitrate in water.

The concentrations of reactive phosphorus in the water were significantly higher in the C: N ratio adjusted tanks. This suggests an increase in the organic matter's mineralization rate due to the higher heterotrophic bacterial biomass present in the BFT tanks (Baloi, Arantes, Schveitzer, Magnotti, & Vinatea, 2013; Silva, Wasielesky, & Abreu, 2013). That supposition is supported by the results of EC that were higher in the C: N ratio adjusted tanks. underwater structures for periphyton colonization have not affected the concentrations of reactive phosphorus in water (p > 0.05, Table 2). Similarly, the concentrations of organic matter in the water were significantly affected only by the adjustment of the C: N ratio of water. There were more organic matter in C: N ratio adjusted tanks (p < 0.05). The concentrations of organic matter in the water increase exponentially with the bioflocs blooms, which create a greater demand for DO2. This explains why BFT systems have a high demand for DO<sub>2</sub> (Crab et al., 2007).

The gross primary productivity (GPP) was significantly higher in the control tanks (0.99  $\pm$  0.15 mg DO<sub>2</sub> L<sup>-1</sup> hour<sup>-1</sup>). The second highest GPP was found in the periphyton tanks (0.61  $\pm$  0.05 mg DO<sub>2</sub> L<sup>-1</sup> hour<sup>-1</sup>; Figure 1). The differences between the GPP results for the bioflocs tanks (0.11  $\pm$  0.05 mg DO<sub>2</sub> L<sup>-1</sup> hour <sup>-1</sup>) and the biophyton tanks (0.10  $\pm$  0.03 mg DO<sub>2</sub> L<sup>-1</sup> hour<sup>-1</sup>; Figure 1) were not significant. The plankton respiration rate (PRR) was higher (p < 0.05) in the bioflocs and biophyton

tanks (0.31  $\pm$  0.08 and 0.34  $\pm$  0.02 mg DO<sub>2</sub> L<sup>-1</sup> hour<sup>-1</sup> respectively) when compared to the control and periphyton tanks (0.12  $\pm$  0.04 and 0.15  $\pm$  0.02 mg DO<sub>2</sub> L<sup>-1</sup> hour<sup>-1</sup>, respectively). The net primary productivity (NPP) was higher in the control tanks (NPP = 0.87  $\pm$  0.15 mg DO<sub>2</sub> L<sup>-1</sup> hour<sup>-1</sup>). The second highest NPP was observed in the periphyton tanks (0.46  $\pm$  0.07 mg DO<sub>2</sub> L<sup>-1</sup> hour<sup>-1</sup>). There were no significant differences between the bioflocs and biophyton tanks for NPP (-0.21  $\pm$  0.04 and -0.27  $\pm$  0.03 mg DO<sub>2</sub> L<sup>-1</sup> hour<sup>-1</sup>, respectively).



**Figure 1.** Gross primary productivity (GPP), plankton respiration rate (PRR) and net primary productivity (NPP) of Nile tilapia tanks. The rearing tanks were provided or not with submerged structures for the development of periphyton and had or not the C: N ratio adjusted to 15: 1. For a same variable, columns with different letters indicate significantly different means (p < 0.05).

These results demonstrate that phytoplankton, bioflocs and periphyton compete between themselves for the abiotic resources available in the tanks. The growth of one of those groups probably leads to the impairment of the others. Besides, the high water turbidity in the bioflocs and biophyton tanks limits the phytoplankton and periphyton productivity due to the scarcity of underwater light (Schrader, Green, & Perschbacher, 2011; Green et al., 2014; Halfhide, Akerstrom, Lekang, Gislerod, & Ergas, 2014).

#### **Growth performance**

Fish survival was not significantly affected by the two factors evaluated in this study, with final results higher than 84% (Table 3). The final body weight, specific growth rate and fish yield were higher (p < 0.05) in the C: N ratio adjusted tanks. The superior fish performance observed in the C: N ratio adusted tanks may be explained by their better water quality (less TAN and nitrite) and greater availability of natural food (bioflocs) to fish (Correia et al., 2014; Ekasari et al., 2015; Poli, Schveitzer, & Nuñer, 2015).

The presence of the underwater structures had no significant influence on the variables of growth performance (p > 0.05, Table 3). It is hypothesized that these structures might have improved the fish growth performance whether some restriction in the feed allowances had been carried out (Schveitzer et al., 2013; Jatobá et al., 2014). It is also suggested that the feeding allowances performed in the present work with artificial diets fully met the nutritional requirements of fish. Therefore, the substrate-based systems seem to be advantageous to fish culture only when there is some level of restriction in the use of artificial diets.

Fish reared in the bioflocs and biophyton tanks exhibited better FCR results than those stocked in the control and periphyton tanks (p < 0.05). The underwater structures have not significantly affected the FCR results (Table 3).

# **Effluent quality**

The concentrations of dissolved oxygen in the C: N adjusted tanks' effluents were lower than in the other tanks. As the concentrations of organic matter were much higher in the bioflocs and biophyton tanks, demand DO2 their for was proportionately larger (Schveitzer et al., 2013). Therefore, the tank effluents from BFT systems should not be discharged directly into the environment but reused in further production cycles after a proper treatment (Correia et al., 2014; Ray & Lotz, 2014.). The installment of the underwater structures has significantly not affected the concentrations of DO2 in the tank effluents (Table 4).

The concentrations of TAN in the C: N ratio adjusted tanks were lower than in the other tanks. On the other hand, the use of underwater structures has not affected the concentrations of TAN in the effluents (p > 0.05; Table 4). The concentrations of organic matter in the effluents were not affected by any of the factors evaluated in this study (p > 0.05; Table 4). There were more reactive phosphorus in the effluents of the C: N ratio adjusted tanks. These results indicate that, except for the lower concentrations of TAN, the effluents of BFT tanks are able to cause a greater eutrophication impact than the effluents derived from conventional aquaculture tanks (Crab et al., 2012; Silva et al., 2013; Liang et al., 2014). Therefore, that is one more reason for not discharging the effluents of BFT tanks directly in the nature but reusing them in recirculation aquaculture systems. There were no significant effects of the installment of submerged structures on the concentrations of reactive phosphorus of the tank effluents (p > 0.05. Table 4).

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**Table 3.** Growth performance of Nile tilapia, *Oreochromis niloticus*, with initial body weight of  $1.56 \pm 0.07$  g, kept for 10 weeks in 250 L tanks, at high stocking density (72 fish m<sup>-3</sup>). The tanks were provided or not with submerged structures for the development of periphyton and had or not the C: N ratio of water adjusted to 15: 1 for bioflocs growth (mean  $\pm$  SD; n = 5).

	_					
	Adjustment of the C: N ra	itio	Underwater structure			
Variable	of water		No	Yes		
Survival	No		88.9 ± 9.1 <sup>3</sup>	89.2 ± 11.4		
(%)	Yes		$85.5 \pm 9.3$	$84.4 \pm 9.9$		
Final body weight	No		$24.35 \pm 1.04 \text{A}^4$	$26.04 \pm 2.07 \mathrm{A}$		
(g)	Yes		$27.79 \pm 0.82 \mathrm{B}$	$28.14 \pm 2.96 \mathrm{B}$		
SGR <sup>1</sup>	No		$3.96 \pm 0.10 \mathrm{A}$	$3.98 \pm 0.11 \mathrm{A}$		
(% day <sup>-1</sup> )	Yes		$4.22 \pm 0.06 \mathrm{B}$	4.21	$4.21 \pm 0.08 \mathrm{B}$	
Fish yield	No		$1.56 \pm 0.13 \text{ A}$	$1.48 \pm 0.25 \mathrm{A}$		
(kg m <sup>-3</sup> cycle <sup>-1</sup> )	Yes		$1.71 \pm 0.15 \mathrm{B}$	$1.70 \pm 0.09 \mathrm{B}$		
FCR <sup>2</sup>	No		$1.33 \pm 0.06 \mathrm{A}$	$1.34 \pm 0.10 \mathrm{A}$		
rck	Yes		$1.14 \pm 0.08 \mathrm{B}$	$1.09 \pm 0.07 \mathrm{B}$		
		Two-way ANO	VA P			
Factor	Survival I	Body weight	SGR	Yield	FCR	
Underwater structure	ns <sup>5</sup>	ns	ns	ns	ns	
Adjus. the C: N ratio	ns	< 0.05	< 0.05	< 0.05	< 0.05	
Structure x C: N ratio	ns	ns	ns	ns	ns	

<sup>1</sup>Specific growth rate (SGR) = [(Ln final body weight - Ln initial body weight)/n° of days] x 100; <sup>2</sup> feed conversion ratio (FCR) = feed allowance (g)/fish weight gain (g); <sup>3</sup>Absence of letters indicates non-significant differences between the mean values (p > 0.05); <sup>4</sup> For the same variable, means followed by different capital letters in the same column are significantly different by Tukey's test (p < 0.05); <sup>5</sup> Non-significant (p > 0.05).

**Table 4.** Effluent quality of Nile tilapia tanks. The rearing tanks were provided or not with underwater structures for the development of periphyton and had or not the C: N ratio adjusted to 15: 1 (mean  $\pm$  SD; n = 5).

Variable	Adjustment the C: N ratio	Underwater structure				
variable	of water	No		Yes		
Dissolved oxygen	No	$4.19 \pm 0.50 \mathrm{A}^2$		$4.27 \pm 0.63 \mathrm{A}$		
(mg L <sup>-1</sup> )	Yes	$3.40 \pm 0.45 \mathrm{B}$		$3.25 \pm 0.29 \mathrm{B}$		
TAN <sup>1</sup>	No	$0.65 \pm 0.13 \mathrm{A}$		$0.56 \pm 0.06 \mathrm{A}$		
	Yes	$0.33 \pm 0.08 \mathrm{B}$		$0.39 \pm 0.12 \mathrm{B}$		
Organic matter	No	$68.5 \pm 12.3^{3}$		$83.0 \pm 13.4$		
(mg L <sup>-1</sup> )	Yes	$78.4 \pm 15.5$		$75.8 \pm 14.2$		
Reactive phosphorus	No	$0.15 \pm 0.03 \mathrm{A}$ $0.14 \pm 0.06 \mathrm{A}$		$0.14 \pm 0.06 \mathrm{A}$		
(mg L <sup>-1</sup> )	Yes	$0.24 \pm 0.05 \mathrm{B}$		$0.26 \pm 0.08 \mathrm{B}$		
	Т	wo-way ANOVA P				
Factor	$DO_2$	TAN	Organic matter	Phosphorus		
Underwater structure	ns <sup>4</sup>	ns	ns	ns		
Adjus. the C: N ratio	< 0.05	< 0.001	ns	< 0.05		
Structure x C: N ratio	ns	ns	ns	ns		

<sup>&</sup>lt;sup>1</sup> Total ammonia nitrogen; <sup>2</sup> For the same variable, means followed by different capital letters in the same column are significantly different by Tukey's test (p < 0.05); <sup>3</sup> Absence of letters indicates non-significant differences between the mean values (p > 0.05); <sup>4</sup> Non-significant (p > 0.05).

#### Conclusion

There is no indication for the use of underwater structures in intensive tilapia farming, either alone or associated with bioflocs, due to the lack of limnological and growth performance benefits. The cleaning capacity of periphyton to remove pollutants from the rearing water is lower when compared to the bioflocs ability.

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