



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

Davi de Holanda Cavalcante, Francisco Roberto dos Santos Lima, Vanessa Tomaz Rebouças 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.º, 60356-000, Fortaleza, Ceará, Brazil. \*Author for correspondence. E-mail: marcelo.sa@ufc.br

**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 ( $1.56 \pm 0.07$  g; 72 fish  $m^{-3}$ ), on variables of water quality, growth performance and effluent quality after 10 weeks. The experiment was arranged in a  $2 \times 2$  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 perifiton 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 (perifiton) com o sistema baseado no ajuste da relação C: N da água (bioflocos), no cultivo intensivo de juvenis de tilápia do Nilo ( $1,56 \pm 0,07$  g; 72 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 (perifiton) 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

colonized by bacteria, fungi, protozoa, phytoplankton, zooplankton, and debris. The periphyton can be used as a food source by the farmed fish (Schweitzer 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 & Little, 2008; Schweitzer 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 ( $1.12 \pm 0.05$  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 on the 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 ( $0.90 \text{ m}^2$ ). 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 Tecnopon®), electrical 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 CO<sub>2</sub> (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 NH<sub>3</sub> 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 following: 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<sub>2</sub> 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<sub>2</sub> 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 ± SD; n = 5).

Variable	Adjustment of the C: N ratio of water	Underwater structure	
		No	Yes
pH	No	8.10 ± 0.60 A <sup>1</sup>	8.13 ± 0.61 A
	Yes	7.02 ± 0.18 B	7.03 ± 0.17 B
Electrical conductivity (µS cm <sup>-1</sup> )	No	400 ± 25 A	379 ± 23 A
	Yes	740 ± 34 B	698 ± 45 B
<i>Two-way ANOVA P</i>			
Factor		pH	Electrical conductivity
Underwater structure		ns <sup>2</sup>	ns
C: N ratio adjustment		<0.001	<0.001
Structure x C: N ratio		ns	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 ( $p < 0.05$ ); <sup>2</sup>Non-significant ( $p > 0.05$ ).

**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).

Variable	Adjustment of the C: N ratio of water	Underwater structure	
		No	Yes
Dissolved oxygen (mg L <sup>-1</sup> )	No	5.02 $\pm$ 1.40 <sup>1</sup>	5.17 $\pm$ 0.92
	Yes	6.70 $\pm$ 0.97	6.69 $\pm$ 0.99
Free CO <sub>2</sub> (mg L <sup>-1</sup> )	No	5.49 $\pm$ 0.70 A <sup>2</sup>	6.02 $\pm$ 0.68 A
	Yes	8.96 $\pm$ 0.89 B	9.49 $\pm$ 0.69 B
Total alkalinity (mg L <sup>-1</sup> eq. CaCO <sub>3</sub> )	No	148.2 $\pm$ 17.7 A	137.9 $\pm$ 12.3 A
	Yes	52.7 $\pm$ 9.6 B	38.24 $\pm$ 6.5 B
Total hardness (mg L <sup>-1</sup> eq. CaCO <sub>3</sub> )	No	80.9 $\pm$ 10.1 A	69.1 $\pm$ 10.4 A
	Yes	260.2 $\pm$ 25.9 B	236.5 $\pm$ 37.9 B
TAN <sup>3</sup> (mg L <sup>-1</sup> )	No	0.85 $\pm$ 0.19 A	0.79 $\pm$ 0.17 A
	Yes	0.23 $\pm$ 0.10 B	0.19 $\pm$ 0.09 B
Nitrite (mg L <sup>-1</sup> )	No	1.85 $\pm$ 0.20 A	1.70 $\pm$ 0.22 A
	Yes	0.70 $\pm$ 0.16 B	0.70 $\pm$ 0.17 B
Nitrate (mg L <sup>-1</sup> )	No	21.94 $\pm$ 2.34 A	18.39 $\pm$ 3.27 A
	Yes	40.20 $\pm$ 4.12 B	41.07 $\pm$ 4.05 B
Reactive phosphorus (mg L <sup>-1</sup> )	No	0.19 $\pm$ 0.09 A	0.20 $\pm$ 0.07 A
	Yes	0.59 $\pm$ 0.12 B	0.56 $\pm$ 0.15 B
Organic matter (mg L <sup>-1</sup> )	No	196.0 $\pm$ 23.0 A	189.0 $\pm$ 23.1 A
	Yes	310.1 $\pm$ 33.3 B	315.4 $\pm$ 42.2 B

Two-way ANOVA P									
Factor	DO <sub>2</sub>	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>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>3</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.

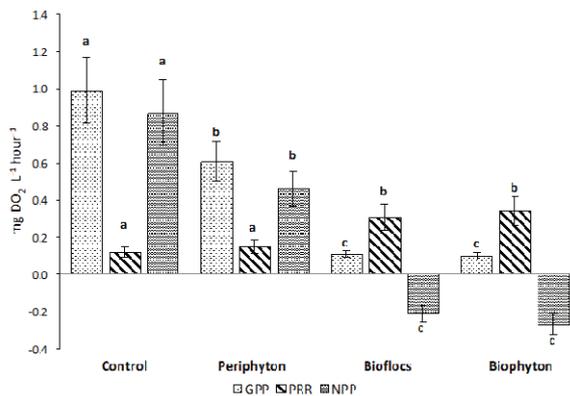
The concentrations of nitrite in water were lower ( $p < 0.05$ ) in the C: N ratio adjusted tanks. The nitrite range in the tanks was between 0.52 and 2.17 mg L<sup>-1</sup>. Liu, Hu, Dai, and Avnimelech (2014) and Luo et al. (2014) have also observed a similar response for the nitrite results. Therefore, 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<sup>-1</sup> (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, Schweitzer, 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. The 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 DO<sub>2</sub>. 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 adjusted 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, Schweitzer, & Nuñez, 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 (Schweitzer 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, their demand for DO<sub>2</sub> was also proportionately larger (Schweitzer 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 not significantly affected the concentrations of DO<sub>2</sub> 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).

**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 ( $72 \text{ fish m}^{-3}$ ). 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).

Variable	Adjustment of the C: N ratio		Underwater structure	
	of water		No	Yes
Survival (%)	No		$88.9 \pm 9.1^3$	$89.2 \pm 11.4$
	Yes		$85.5 \pm 9.3$	$84.4 \pm 9.9$
Final body weight (g)	No		$24.35 \pm 1.04 \text{ A}^4$	$26.04 \pm 2.07 \text{ A}$
	Yes		$27.79 \pm 0.82 \text{ B}$	$28.14 \pm 2.96 \text{ B}$
SGR <sup>1</sup> (% day <sup>-1</sup> )	No		$3.96 \pm 0.10 \text{ A}$	$3.98 \pm 0.11 \text{ A}$
	Yes		$4.22 \pm 0.06 \text{ B}$	$4.21 \pm 0.08 \text{ B}$
Fish yield (kg m <sup>-3</sup> cycle <sup>-1</sup> )	No		$1.56 \pm 0.13 \text{ A}$	$1.48 \pm 0.25 \text{ A}$
	Yes		$1.71 \pm 0.15 \text{ B}$	$1.70 \pm 0.09 \text{ B}$
FCR <sup>2</sup>	No		$1.33 \pm 0.06 \text{ A}$	$1.34 \pm 0.10 \text{ A}$
	Yes		$1.14 \pm 0.08 \text{ B}$	$1.09 \pm 0.07 \text{ B}$

Two-way ANOVA P					
Factor	Survival	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) =  $[(\text{Ln final body weight} - \text{Ln initial body weight})/\text{n}^\circ \text{ of days}] \times 100$ ; <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	
	of water		No	Yes
Dissolved oxygen (mg L <sup>-1</sup> )	No		$4.19 \pm 0.50 \text{ A}^2$	$4.27 \pm 0.63 \text{ A}$
	Yes		$3.40 \pm 0.45 \text{ B}$	$3.25 \pm 0.29 \text{ B}$
TAN <sup>1</sup>	No		$0.65 \pm 0.13 \text{ A}$	$0.56 \pm 0.06 \text{ A}$
	Yes		$0.33 \pm 0.08 \text{ B}$	$0.39 \pm 0.12 \text{ B}$
Organic matter (mg L <sup>-1</sup> )	No		$68.5 \pm 12.3^3$	$83.0 \pm 13.4$
	Yes		$78.4 \pm 15.5$	$75.8 \pm 14.2$
Reactive phosphorus (mg L <sup>-1</sup> )	No		$0.15 \pm 0.03 \text{ A}$	$0.14 \pm 0.06 \text{ A}$
	Yes		$0.24 \pm 0.05 \text{ B}$	$0.26 \pm 0.08 \text{ B}$

Two-way ANOVA P				
Factor	DO <sub>2</sub>	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>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.

## Referências

- American Public Health Association. (1999). *Standard methods for the examination of water and wastewater* (20th ed.). New York, NY: APHA.
- Anand, P. S. S., Kohli, M. P. S., Kumar, S., Sundaray, J. K., Roy, D. S., Venkateshwarlu, G., ... Pailan, G. H. (2014). Effect of dietary supplementation of biofloc on growth performance and digestive enzyme activities in *Penaeus monodon*. *Aquaculture*, 418-419, 108-115.
- Asaduzzaman, M., Wahab, M. A., Verdegem, M. C. J., Huque, S., Salam, M. A., & Azim, M. E. (2008). C/N ratio control and substrate addition for periphyton development jointly enhance freshwater prawn *Macrobrachium rosenbergii* production in ponds. *Aquaculture*, 280(1-4), 117-123.
- Avnimelech, Y. (1999). Carbon and nitrogen ratio as a control element in Aquaculture systems. *Aquaculture*, 176(3-4), 227-235.
- Azim, M. E., & Little, D. C. (2008). The biofloc technology (BFT) in indoor tanks: water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, 283(1-4), 29-35.
- Baloi, M., Arantes, R., Schweitzer, R., Magnotti, C. & Vinatea, L. (2013). Performance of Pacific shrimp *Liopenaeus vannamei* raised in biofloc systems with varying levels of light exposure. *Aquacultural Engineering*, 52, 39-44.
- Boyd, C. E. (2000). Effluent composition and water quality standards. *Global Aquaculture Advocate*, 3, 61-66.
- Correia, E. S., Wilkenfeld, J. S., Morris, T. C., Wei, L., Prangnell, D. I., & Samocha, T. M. (2014). Intensive nursery production of the Pacific white shrimp *Liopenaeus vannamei* using two commercial feeds with high and low protein content in a biofloc-dominated system. *Aquacultural Engineering*, 59, 48-54.

- Crab, R., Defoirdt, T., Bossier, P., & Verstraete, W. (2012). Biofloc technology in aquaculture: Beneficial effects and future challenges. *Aquaculture*, 356-357, 351-356.
- Crab, R., Avnimelech, Y., Defoirdt, T., Bossier, P., & Verstraete, W. (2007). Nitrogen removal techniques in aquaculture for a sustainable production. *Aquaculture*, 270(1-4), 1-14.
- Ekasari, J., Angela, D., Waluyo, S. H., Bachtiar, T., Surawidjaja, E. H., Bossier, P., & Schryver, P. (2014). The size of biofloc determines the nutritional composition and the nitrogen recovery by aquaculture animals. *Aquaculture*, 426-427, 105-111.
- Ekasari, J., Rivandi, D. R., Firdausi, A. P., Surawi, E. H., Zairin, M., Bossier, P., Schryver, P. (2015). Biofloc technology positively affects Nile tilapia (*Oreochromis niloticus*) larvae performance. *Aquaculture*, 441, 72-77.
- El-Shafai, S. A., El-Gohary, F. A., Nars, F. A., Steen, N. P. V., & Gijzen, H. J. (2004). Chronic ammonia toxicity to duckweed-fed tilapia (*Oreochromis niloticus*). *Aquaculture*, 232(1-4), 117-127.
- Furtado, P. S., Poersch, L. H., & Wasielesky, W. (2011). Effect of calcium hydroxide, carbonate and sodium bicarbonate on water quality and zootechnical performance of shrimp *Litopenaeus vannamei* reared in bio-flocs technology (BFT) systems. *Aquaculture*, 321(1-2), 130-135.
- Green, B. W. (2015). Performance of a temperate-zone channel catfish biofloc technology production system during winter. *Aquacultural Engineering*, 64, 60-67.
- Green, B. W., Schrader, K. K., Perschbacher, P. W. (2014). Effect of stocking biomass on solids, phytoplankton communities, common off-flavors, and production parameters in a channel catfish biofloc technology production system. *Aquaculture Research*, 45(9), 1442-1458.
- Halfhide, T., Akerstrom, A., Lekang, O. I., Gislerod, H. R., & Ergas, S. J. (2014). Production of algal biomass, chlorophyll, starch and lipids using aquaculture wastewater under axenic and non-axenic conditions. *Algal Research*, 6(B), 152-159.
- Jatobá, A., Silva, J. S., Vieira, F. N., Mourino, J. L. P., Sciffert, W. Q., & Toledo, T. M. (2014). Protein levels for *Litopenaeus vannamei* in semi-intensive and biofloc systems. *Aquaculture*, 432, 365-371.
- Krummenauer, D., Sciffert, C. A., Poersch, L. H., Foes, G. K., Lara, G. R., & Wasielesky, W. (2012). Cultivo de camarões marinhos em sistema de bioflocos: análise da reutilização da água. *Atlântica*, 34(2), 103-111.
- Kuhn, D. D., Lawrence, A. L., Boardman, G. D., Patnaik, S., Marsh, L., & Flick, G. J. (2010). Evaluation of two types of bioflocs derived from biological treatment of fish effluent as feed ingredients for Pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture*, 303(1-4), 28-33.
- Kumlu, M., Eroldogan, O. T., & Saglamtimur, B. (2001). The effects of salinity and added substrates on growth and survival of *Metapenaeus monóceros* (Decapoda: Penaeidae) post-larvae. *Aquaculture*, 196(1-2), 177-188.
- Liang, W., Luo, G., Tan, H., Ma, N., Zhang, N., & Li, L. (2014). Efficiency of biofloc technology in suspended growth reactors treating aquacultural solid under intermittent aeration. *Aquacultural Engineering*, 59, 41-47.
- Liu, L., Hu, Z., Dai, X., & Avnimelech, Y. (2014). Effects of addition of maize starch on the yield, water quality and formation of bioflocs in an integrated shrimp culture system. *Aquaculture*, 418-419, 79-86.
- 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.
- Poli, M. A., Schweitzer, R., & Nuñez, A. P. O. (2015). The use of biofloc technology in a South American catfish (*Rhamdia quelen*) hatchery: Effect of suspended solids in the performance of larvae. *Aquacultural Engineering*, 66, 17-21.
- Ray, A. J., & Lotz, J. M. (2014). Comparing a chemoautotrophic-based biofloc system and three heterotrophic-based systems receiving different carbohydrate sources. *Aquacultural Engineering*, 63, 54-61.
- Ray, A. J., Dillon, K. S., & Lotz, J. M. (2011). Water quality dynamics and shrimp (*Litopenaeus vannamei*) production in intensive, mesohaline culture systems with two levels of biofloc management. *Aquacultural Engineering*, 45(3), 127-136.
- Rostagno, H. S. (2011). *Composição de alimentos e exigências nutricionais de aves e suínos: composição de alimentos e exigências nutricionais* (3a ed.), Viçosa, MG: Universidade Federal de Viçosa.
- Schrader, K. K., Green, B. W., & Perschbacher, P. W. (2011). Development of phytoplankton communities and common off-flavors in a biofloc technology system used for the culture of channel catfish (*Ictalurus punctatus*). *Aquacultural Engineering*, 45(3), 118-126.
- Schweitzer, R., Arantes, R., Baloi, M. F., Costodio P. F. S., Arana, L. V., Sciffert, W. Q., & Andreatta, E. R. (2013). Use of artificial substrates in the culture of *Litopenaeus vannamei* (Biofloc System) at different stocking densities: Effects on microbial activity, water quality and production rates. *Aquacultural Engineering*, 54, 93-103.
- Silva, K. R., Wasielesky, W., & Abreu, P. C. (2013). Nitrogen and phosphorus dynamics in the biofloc production of the pacific white shrimp, *Litopenaeus vannamei*. *Journal of the World Aquaculture Society*, 44(1), 30-41.

Received on April 29, 2015.

Accepted on June 23, 2015.

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.