



# Dynamics of the water quality parameters in the super-intensive culture of *Litopenaeus vannamei* in BFT system on artificial brackish water

Anna Carolina Ferreira Spelta, et al. [full author details at the end of the article]

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## Abstract

The study aimed to characterize the dynamics involved in the water quality parameters in a biofloc system (BFT) during the super-intensive cultivation of *Litopenaeus vannamei* using two levels of artificial brackish water. The test was designed with two salinity levels, T<sub>16</sub> (16‰) and T<sub>8</sub> (8‰), with 4 replicates, and 250 shrimps/m<sup>2</sup> by 60 days, water analysis and animal performance were studied in each treatment. No significant differences were observed between the two salinities for biochemical oxygen demand (BOD<sub>5</sub>), total chemical oxygen demand (COD<sub>t</sub>), and fixed suspended solids (FSS). Using COD<sub>t</sub>/BOD<sub>5</sub>, it was possible to identify the water biodegradability and manage the concentration of organic and inorganic matter in the medium. Filtered chemical oxygen demand (COD<sub>f</sub>) was used to monitor the dissolved organic matter, which was higher in T<sub>16</sub>. Carbohydrate (molasses) did not control total ammonia reaching in T<sub>8</sub> = 1.16±0.64 mg/L. This organic matter addition reduced the growth of chemoautotrophic nitrifying bacteria and interfered in the nitrogen dynamics. Regarding total solids and suspended solids, there was a significant difference between treatments, except for FSS. Dissolved oxygen (DO) and oxygen saturation (sO<sub>2</sub>) were significantly different between the treatments. The maintenance of a more neutral pH and greater alkalinity were observed, with significant differences between the treatments throughout the whole cultivation. Regarding the shrimp growth performance, the high salinity presented more weight gain, specific growth rate, feed conversion and final biomass, and lower mortality than lower salinity. These results showed that shrimps presented a higher performance in salinity 16‰.

**Keywords** Pacific white shrimp, · Biofloc, · Dissolved organic matter, · Low salinity

## Introduction

The intensification of aquaculture, which invariably leads to generation of considerable waste formation, aims to increase food production to meet the increased demand for a growing global population (Muhler et al. 2013).

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According to Bossier and Ekasari (2017), nutrient waste can be recycled by natural biogeochemical processes involving microorganisms, that may be consumed by cultured animals. This is the principle of the Biofloc Technology (BFT), in which the nutrients and organic matter may be recycled, mainly by heterotrophic bacterial growth through the addition of extra carbon to the aquaculture system (Avnimelech 2015; Emerenciano et al. 2017; Abakari et al. 2020).

The BFT system is considered sustainable because the water renewal is zero or minimal, reducing costs of pumping water, minimizing the introduction of pathogens from external water (biosecurity), and avoiding environmental contamination with the disposal of large volumes of wastewater (Avnimelech 1999; Burford et al. 2003).

From the relationship between oxygen demand analysis (BOD<sub>5</sub>) and filtered chemical oxygen demand (COD<sub>f</sub>), it is possible to identify the biodegradability of culture water and the availability of organic matter to the heterotrophic bacterial community (von Sperling 1996); however in the literature, little is known about organic concentration added to the BFT system.

Thus, observing the behavior of organic parameters over time can contribute to the management of the BFT system and the consequent zootechnical performance of the animals (Kumar et al. 2018; Liu et al. 2018). With the increase in organic load, total solids in the system tend to increase, changing the biofloc composition, the water quality (dissolved oxygen, carbon dioxide, pH, alkalinity, etc.), which can affect animal health (Crab et al. 2007).

The control of total suspended solids (TSS) could be effective to control organic and inorganic matter, suspended microbial biomass, and food available to bacteria. According to Souza et al. (2019), smaller particles in the BFT system tend to negatively interfere with the nitrification process. The use of clarifiers is effective to maintain the TSS in the optimum level for shrimp culture (Gaona et al. 2016a, 2016b, 2017).

The salinity of the water is another point to be addressed because, despite having euryhaline characteristics, the Pacific white shrimp *Litopenaeus vannamei* may have lesser osmoregulatory expenditure when in brackish water (Decamp et al. 2003). Moreover, information about *L. vannamei* tolerance to low salinity is needed, once this is demanding to reduce expenses with salts on shrimp farming in inland areas far from the coast. However, low salinity stress can cause problems related to growth and mortalities of *L. vannamei*, as described in the literature (Zhao et al. 2015; Esparza-Leal et al. 2016; Fregoso-López et al. 2017).

The objective of this study was to characterize and evaluate the water quality parameters during the intensive cultivation of *L. vannamei* in brackish water (artificially produced), using the BFT system without water renewal. We also studied shrimp performance after 60 days under indoor conditions.

## Materials and methods

The experiment was conducted for 60 days at the Mariculture and water quality Laboratory and the physical-chemical analyses were carried out at the Environmental Sanitation Laboratory and at the Mariculture and water quality Laboratory of the Federal University of Minas Gerais.

## Experimental design

Post-larvae of *L. vannamei* were obtained from Aquatec® Ltd (RN-Brazil). The post-larvae were kept in nursery units in a BFT system until they reached a mean weight of 1.0 g. After that they were transferred to grow-out units where they were maintained at a density of 250 shrimp/m<sup>2</sup> until the beginning of the study. During this period, they were fed using commercial feed with 38% crude protein (Potimar, Guabi, Brazil) composed of moisture (10%), crude protein (38%), etheric extract (7.5%), fibrous matter (5%), mineral matter (13%), calcium (3%), phosphorus (1.45%), vitamin a (13,000 IU), vitamin d3 (2,500 IU), vitamin e (200 IU), and vitamin c (500 mg).

The design was completely randomized with two treatments (salinity 16‰ = T<sub>16</sub> and 8‰ = T<sub>8</sub>) and four replicates. Additionally, each treatment had a matrix tank (external to maintain water volume for the treatments), developed as described by Wasielesky et al. (2006, 2013), Emerenciano et al. (2011, 2012), and Lara et al. (2017). The polyethylene tanks used for shrimp production had 100 L (0.23 m<sup>2</sup>), and for matrix purposes had 500 L (0.71 m<sup>2</sup>). The volume used was 80 L, maintained by the matrix tank. There was no water renewal, only the replacement of evaporated water. Each tank had diffused aeration supplied by a blower to keep dissolved oxygen close to water saturation. The photoperiod was natural.

The flow of water between the tanks was continuous, i.e., water recirculated through the system without the obstruction of flux, through a pump with a flow rate of 21 L/min. The density of shrimps (4.05 g ± 0.62 g) in each tank with biofloc was 250 animals/m<sup>2</sup>, totalizing 60 shrimps per experimental unity. The feed was given twice daily (8:00 a.m. and 4:00 p.m.) with a commercial feed 38% CP (Potimar, Guabi) at 3% of total biomass during the experimental period. Biometrics was performed weekly on 20 randomly sampled animals to determine growth and monitoring of feed use and adjustments.

To achieve the salinity in each treatment (8‰ and 16‰), we used mature biofloc from previous shrimp cultivation with 8‰ salinity. In T<sub>16</sub> treatment, salinity was adjusted to 16‰ in 24 h. The brackish water was artificially produced according to ionic concentration described by Grasshoff et al. (1983) with brief modifications. Corrections were made to keep pH values above 7.8 and alkalinity above 150 mg/L of CaCO<sub>3</sub>, by adding hydrated lime and dolomitic lime (Rural MF, Brazil). When ammonia concentrations were greater than 0.5 mg/L, a source of carbon, molasses (Rural MF, Brazil) was added in sufficient quantity to maintain the C:N ratio 6:1, as previously reported (Avnimelech 1999; Ebeling et al. 2006).

Clarification was performed in treatments where settleable solids (SetS) concentrations were higher than 15 mL/L by the use of polystyrene Imhoff graduated settling cone (1000 mL), based on the methodology described by Gaona et al. (2011) and was built in PVC with a volume of 53 L. The operating principle considered gravitational action for solid sedimentation in the clarifier bottom and lighter water returned to the system. A submerged pump (19 W, BOYU) was used to capture the medium to be clarified and was maintained for 3 to 5 h.

## Water quality monitoring

To characterize water quality of the shrimp cultivation, the following physicochemical variables were analyzed: dissolved oxygen (DO) (mg/L) and oxygen saturation (sO<sub>2</sub>) (%) were monitored daily in the morning using a digital oximeter (Hanna, HI9146, Czech Republic); pH and temperature (T) with multiparameter apparatus (Hanna, HI98129, Czech Rep.); salinity using refractometer (Atago, ATC-S, Mill 2440, Japan); total ammonia (TA-N) and nitrite

(NO<sub>2</sub><sup>-</sup>-N) determined by spectrophotometric method (UNESCO 1983); and settleable solids (SetS) with Imhoff cone (Avnimelech 2007).

The total chemical oxygen demand (CODt), filtered oxygen demand (CODf), total solid (TS), total fixed solid (TFS), total volatile solid (TVS), total suspended solid (TSS), volatile suspended solids (VSS), and fixed suspended solids (VSS) were analyzed twice weekly, while the 5-day biochemical oxygen demand (BOD<sub>5</sub>) was analyzed once a week using the APHA methods (APHA 1999).

To evaluate the organic contribution of molasses, BOD<sub>5</sub> and CODt of 20 g of this fertilizer were analyzed and the values obtained were 8432 mg/L and 3,8974 mg/L, respectively.

## Performance parameters

Every week a random sample of 20 shrimps from each experimental unit was individually dried in paper towel, weighed on a precision balance, and then returned to their home tanks. At the end of the experiment, all shrimps were quantified and total biomass estimated. The performance of *L. vannamei* juveniles subjected to different treatments of salinity was evaluated according to the following parameters: survival rate (%), weekly weight gain, specific growth rate, feed conversion factor, and final biomass.

- Survival rate (%) = Number of surviving animals / Initial number of animals x 100
- Weekly weight gain (g/week) = Weight gain / week of cultivation
- Specific growth rate (SGR) (%/day) =  $100 \times (\ln \text{ final weight (g)} - \ln \text{ initial weight (g)}) / \text{time in days}$
- Feed Conversion Factor (FCF) = Feed consumption / final biomass
- Final biomass (g) = Final number of animals x final weight (g)

## Statistical analysis

The data were tested by Lilliefors and Cochran tests to verify normal distribution and homoscedasticity of variances respectively. Analysis of variance (ANOVA) and Tukey's test were performed to compare the means of qualitative factors using the software INFOSTAT (2008). The type I error rate of 5% probability was assumed.

## Results and discussion

### Water quality monitoring

Table 1 shows the averages for BOD<sub>5</sub>, CODt, and CODf in the two treatments. It was observed that there was no significant difference between the treatments for BOD<sub>5</sub> and CODt, possibly due to the amount of molasses added during the experimental period. From BOD<sub>5</sub> and CODt values, it was possible to show that CODt/BOD<sub>5</sub> ratio was higher than 3.5 in both treatments, showing high concentrations of inert or non-biodegradable material in the systems (von Sperling 1996). Therefore, little organic material available for bacteria was present in the medium. According to von Sperling (1996), this inert organic

**Table 1** Water quality parameters during the cultivation of *Litopenaeus vannamei* in salinity 8 and 16‰

Variables	Treatments		CV (%)
	T8 (8‰)	T16 (16‰)	
CODt (mg L <sup>-1</sup> )	1266.55 ± 607.23 <sup>a</sup>	1338.28 ± 641.74 <sup>a</sup>	13.98
CODf (mg L <sup>-1</sup> )	741.63 ± 330.19 <sup>a</sup>	1278.13 ± 599.09 <sup>b</sup>	14.75
BOD <sub>5</sub> (mg L <sup>-1</sup> )	38.59 ± 25.42 <sup>a</sup>	42.75 ± 34.66 <sup>a</sup>	38.21
NO <sub>3</sub> <sup>-</sup> (mg L <sup>-1</sup> )	6.56 ± 0.95 (5.55 – 8.12) <sup>a</sup>	6.74 ± 1.06 (5.53 – 7.73) <sup>b</sup>	3.40
NO <sub>2</sub> <sup>-</sup> (mg L <sup>-1</sup> )	0.49 ± 0.26 (0.20 – 0.94) <sup>a</sup>	0.44 ± 0.15 (0.31 – 0.69) <sup>b</sup>	28.51
TA-N (mg L <sup>-1</sup> )	1.16 ± 0.64 (0.31 – 1.94)	0.80 ± 0.42 (0.25 – 1.50) <sup>b</sup>	59.84
PO <sub>4</sub> <sup>3-</sup> -P (mg L <sup>-1</sup> )	4.02 ± 0.98 (3.02 – 5.44) <sup>a</sup>	3.86 ± 1.08 (2.63 – 4.91) <sup>b</sup>	8.76
TS (mg L <sup>-1</sup> )	8871.85 ± 1126.62 <sup>a</sup>	17450 ± 1240.12 <sup>b</sup>	11.67
TVS (mg L <sup>-1</sup> )	2009.95 ± 414.58 <sup>a</sup>	2357.26 ± 368.34 <sup>b</sup>	3.83
TFS (mg L <sup>-1</sup> )	6861.89 ± 882.53 <sup>a</sup>	15092.73 ± 963.34 <sup>b</sup>	13.90
TSS (mg L <sup>-1</sup> )	914.95 ± 255.00 <sup>a</sup>	920.87 ± 304.32 <sup>b</sup>	1.58
VSS (mg L <sup>-1</sup> )	656.34 ± 193.72 <sup>a</sup>	515.69 ± 178.10 <sup>b</sup>	6.95
FSS (mg L <sup>-1</sup> )	258.61 ± 95.34 <sup>a</sup>	405.18 ± 156.23 <sup>a</sup>	16.54
DO (mg L <sup>-1</sup> )	5.81 ± 0.20 <sup>a</sup>	5.49 ± 0.38 <sup>b</sup>	11.21
sO <sub>2</sub> (%) <sup>*</sup>	85.08 ± 2.93 <sup>a</sup>	80.10 ± 5.85 <sup>b</sup>	11.56
T (°C)	27.76 ± 0.57 <sup>a</sup>	27.53 ± 0.86 <sup>b</sup>	1.41
pH	7.76 ± 0.24 <sup>a</sup>	7.67 ± 0.17 <sup>b</sup>	2.14
CaCO <sub>3</sub> (mg L <sup>-1</sup> )	88.54 ± 29.80 <sup>a</sup>	104.83 ± 39.68 <sup>b</sup>	29.50
CO <sub>2</sub> (mg L <sup>-1</sup> )	1.74 ± 0.88 <sup>a</sup>	2.48 ± 1.40 <sup>b</sup>	58.54
SetS	51.27 ± 21.34 <sup>a</sup>	46.78 ± 22.21 <sup>b</sup>	5.41

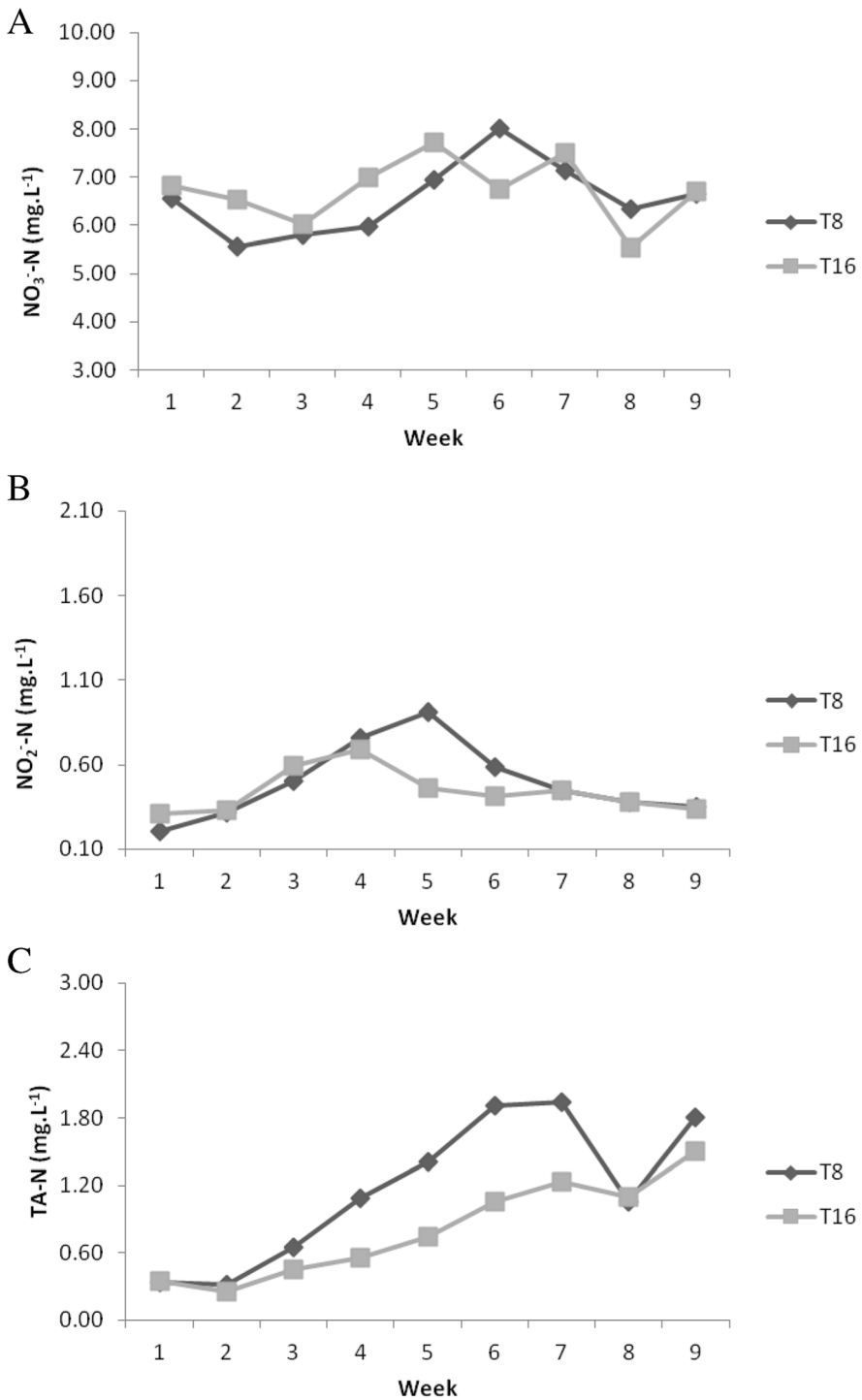
Different superscript letters within a row indicate statistically significant differences ( $P < 0.05$ ) between treatments. Data are presented as mean ± SD

material is formed through the decay of bacterial biomass by endogenous metabolism, death, predation, and others.

Ray et al. (2011) also worked with mesohaline salinity (16‰) aimed inland shrimp cultivation, and comment on the importance of salinity in shrimp growth. According to Medeiros et al. (2005), salinity can cause adverse effects on the microbial flora, causing plasmolysis and loss of cell activity, which may explain the high concentration of inert material in both treatments.

Besides, it was found that the amount of organic material dissolved (CODf) was different between the two treatments. In T<sub>16</sub>, the mean was 1,278.13 ± 599.09 mg/L, which represented 95.5% of soluble material and in T<sub>8</sub>, the average was 741.63 ± 330.19 mg/L, which represented 58.55% of soluble material. This difference was possibly due to the amount of feed provided during the experiment that varied according to the biometry, in T<sub>16</sub> due to the gain of animals' weight, the amount of feed was increased. When observing Fig. 1, it was noticed that the concentration of CODf in T<sub>16</sub> increased in greater proportions than in T<sub>8</sub>.

The higher soluble matter in T<sub>16</sub> cannot be attributed to the addition of molasses, since in T<sub>8</sub>, the total molasses added to control the ammonia concentration was higher than in T<sub>16</sub>. Thus, in addition to not being biodegradable and consequently not being available for bacterial consumption, it accumulates in the system. Despite a large amount of dissolved organic matter, it was not biodegradable and probably was originated from the decomposition of the bacterial community (von Sperling 1996). It justified the high CODf concentration throughout the experiment.



**Fig. 1** Concentrations of nitrate (A), nitrite (B), and ammonia total (C) during the cultivation of *Litopenaeus vannamei* in salinity 8 and 16‰

Although molasses was not directly related to COD<sub>f</sub> difference between the treatments, its participation in the increase of organic matter soluble was clear, since in 20 g of molasses diluted in 100 mL of water, about 73% was soluble fraction of organic material (COD<sub>f</sub>). The BOD<sub>5</sub> and COD<sub>t</sub> values obtained for molasses showed lower concentrations of biodegradable material, which may also have an influence on inert organic matter in the water. Although Arantes et al. (2017) associated high values of biodegradable material, via BOD<sub>5</sub>, with the amount of feed and the continuous intake of carbohydrate added in the system throughout the culture, the two carbon fertilizations (inputs) not only presented biodegradable organic matter, but also contributed to the increase in inert material.

Zhao et al. (2014) verified low values of COD<sub>t</sub> in carp biofloc systems, they showed that addition of corn starch can increase the C:N ratio, maintaining low concentrations of COD<sub>t</sub> in the control tank, with C:N = 7:1. The COD<sub>t</sub> mean was  $11.53 \pm 0.74$  mg/L, with no significant difference among treatments with higher C:N ratios. In this way, molasses may not be the best input for the maintenance of the C:N ratio, since it considerably increases COD<sub>t</sub> concentrations in the cultivation system.

In addition to inert organic material, the presence of inorganic material in the water was also observed through solids analysis. In relation to total solids, there was a significant difference between treatments, both in relation to organic solids (TVS) and inorganic solids (TFS) (Table 1). Among the two treatments, most of these solids corresponded to the inorganic fraction (TFS), being 86% in T<sub>16</sub> and 77% in T<sub>8</sub>, which demonstrated high concentrations of inert material in the tanks. As salinity increases, the amount of inorganic salt in the water was increased, as demonstrated by the higher TFS value in the 16‰. Inputs used for pH correction, maintenance of the C:N ratio, and feeding of the animals may also contribute to this inorganic fraction.

From the values of total and suspended solids, it was possible to infer that there was a fraction of dissolved solids higher than the particulate fraction. However, most dissolved solids were inorganic and therefore not used by the bacterial community present in the medium. Only 17% of the dissolved solids were biodegradable and consumed immediately by the organisms as carbon source since there was no need for hydrolysis.

Concerning the suspended solids, there was a significant difference between the treatments for TSS and VSS (Table 2). By relating these parameters, it was possible to determine that most of the particulate matter had an organic origin, being 71% in T<sub>8</sub> and 56% in T<sub>16</sub>. In Table 2 it is possible to observe that the increase of microbial biomass occurred rapidly. Also, in T<sub>8</sub>, microbial growth was favored, due to the higher values of VSS, demonstrating that in low salinities, growth of heterotrophic and chemoautotrophic bacteria was facilitated. A different response was observed by Maicá et al. (2012), when evaluating the effect of low salinities on biofloc composition and performance of *L. vannamei* in the BFT system. The authors described that with increasing salinity, there was an increase of suspended solids, and in the salinity of 25‰, the mean concentration of TSS was  $256.0 \pm 12.71$  mg/L. Thus, we cannot attribute in isolation that salinity was responsible for the amount of TSS.

Values close to those found in the present study were also reported by Ray et al. (2010) working with diets based on fish oil and vegetable meal in BFT (without clarification), with a mean TSS concentration of  $820 \pm 135$  mg/L and  $745 \pm 165$  mg/L; and VSS of  $509 \pm 99$  and  $485 \pm 114$  mg/L, respectively. The use of a clarifier is advisable because it can reduce biofloc, BOD<sub>5</sub> concentration, decrease animal stress, or alter the microorganism community, favoring the shrimp production (Ray et al. 2010, 2011). According to Schweitzer et al. (2013b), TSS

**Table 2** Water quality parameters during the cultivation of *Litopenaeus vannamei* in salinity 8 and 16‰

Variables	Salinity		Weeks of experiment								
	8	16	1	2	3	4	5	6	7	8	9
<b>PO<sub>4</sub>-P (mg L<sup>-1</sup>)</b>	8	4.36 ABa	3.02 Ba	4.09 ABa	3.54 ABa	3.73 ABa	3.53 ABa	4.44 ABa	4.06 ABa	5.44 Aa	
	16	3.27 ABa	3.47 ABa	2.63 Bb	4.67 Aa	4.91 Aa	4.70 Ab	3.06 ABb	3.28 ABa	4.74 Aa	
<b>TSS (mg L<sup>-1</sup>)</b>	8	1032.91 ABa	941.66 ABa	576.66 Ca	791.66 ABCa	1011.66 ABa	1098.33 Aa	1276.66 Aa	853.33 ABCa	651.66 BCa	
	16	821.25 ABa	746.66 Ba	1075.00 ABb	910.00 ABa	666.66 Bb	980.00 ABa	1285.0Aa	820.0 ABa	983.33 ABb	
<b>VSS (mg L<sup>-1</sup>)</b>	8	675.41 ABCa	600.00 ABCDa	425.00 Da	553.33 BCDA	715.00 ABCa	828.33 ABa	961.66 Aa	635.00 ABCDa	513.33 CDa	
	16	369.58 BCb	353.33 Cb	586.66 ABCa	505.00 ABCa	341.66 Cb	566.66 ABb	793.33 Aa	508.33 ABCa	616.66 Aa	
<b>DO (mg L<sup>-1</sup>)</b>	8	5.68 BCa	5.81 ABCa	5.94 ABa	5.82 ABCa	5.80 ABCa	5.71 BCa	5.48 Ca	6.00 ABa	6.08 Aa	
	16	5.49 Aa	5.89 Aa	5.82 Aa	5.21 CDb	5.69 ABa	5.33 Cb	4.88 Db	5.34 BCb	5.39 BCb	
<b>sO<sub>2</sub> (mg L<sup>-1</sup>)</b>	8	83.89 BCa	86.30 ABa	86.76 ABa	85.43 ABa	85.75 ABa	82.38 BCa	79.79 Ca	89.03 Aa	86.35 ABa	
	16	85.76 Aa	86.77 Aa	84.25 Aa	76.23 Bb	83.35 Aa	77.66 Bb	70.68 Cb	78.30 Bb	77.93 Bb	
<b>T (°C)</b>	8	27.66 CDa	27.51 Da	26.81 Fa	28.33 Ba	27.77 Ca	27.35 Ea	28.65 Ea	28.42 Ba	27.34 Ea	
	16	26.81 Db	27.00 Cb	26.30 Eb	28.23 Bb	27.11 Cb	26.83 Db	28.67 Aa	28.72 Ab	28.12 Bb	
<b>pH</b>	8	7.20 Ea	7.61 Da	7.76 Ca	7.77 Ca	7.67 Da	8.01 Aa	7.85 Ba	8.01 Aa	7.97 Aa	
	16	7.26 Eb	7.65 Cb	7.69 BCb	7.81 Ab	7.72 Bb	7.80 Ab	7.57 Db	7.82 Ab	7.71 BCb	
<b>TA-N (mg L<sup>-1</sup>)</b>	8	0.338 Da	0.313 Da	0.646 Da	1.084 Ca	1.406 BCa	1.905 Aa	1.941 Aa	1.058 Ca	1.800 ABa	
	16	0.352 DEa	0.258 Ea	0.454 DEa	0.552 DEb	0.741 CDb	1.057 BCb	1.235 ABb	1.091 BCa	1.502 Ab	

Means followed by lowercase letters in the column indicate significant differences by Tukey test ( $p < 0.05$ ) for salinity and different upper-case letters in the line differ by Tukey's test ( $p < 0.05$ ) for the interaction of salinity and week



between 400 and 600 mg/L prevents damage in *L. vannamei* during its superintensive cultivation.

The treatments were clarified by measuring SetS above 15 mL/L. However, the mean concentration of SetS exceeded this assumption, with mean values of  $51.27 \pm 21.34$  mL/L for T<sub>8</sub> and  $46.78 \pm 22.21$  mL/L for T<sub>16</sub>. The clarification time was not sufficient to efficiently remove the particulate biofloc volume. Ray and Lotz (2017) presented mean values of  $7 \pm 1$  mL/L in salinity 10‰ and  $9 \pm 1$  mL/L in salinity 30‰ for SetS since they maintained the clarifier (settling chambers) with a continuous flow rate of 15 L/min, except once a week in which the water flow was interrupted for 1 hour to allow the complete sedimentation of the biofloc particles.

In the Fig. 1, it was possible to verify that nitrification was not the main route of ammonia removal throughout the shrimp cultivation, since the by-product of the nitrification reactions remained constant throughout the time, with few variations. In addition to slower growth of nitrifying bacteria, competition for space and oxygen occurs with heterotrophic bacteria, as mentioned by Hargreaves (2006).

Therefore, adding organic matter to the system eventually inhibited the growth of chemoautotrophic bacteria. In addition, salinity interferes with nitrogen dynamics in the system, since there was a significant difference between treatments for ammonia, nitrite, and nitrate, with higher means in the lower salinity, except for nitrate, whose average was higher in T<sub>16</sub>. Decamp et al. (2003) working with *L. vannamei* in BFT did not observe significant differences of these parameters in different salinities and associated the accumulation of nitrogen to the absence of water renewal and nitrification processes that occur in both salinities.

Regarding phosphate, there is an increase in its concentration over time (Table 2) since feed is the main source of phosphorus loading in aquaculture. Only about 13% of the phosphorus present in the food is usually incorporated into shrimp biomass, the rest could compromise water quality (Briggs and Fvng-Smith 1994), but phosphate is considered to be slightly toxic to aquatic organisms and the concentrations found in the present study can be considered harmless according Kim et al. (2013).

During the experiment, the method of carbon addition as a form of ammonia control was not efficient, since the tendency of ammonia concentration was to increase in both treatments, as observed in Fig. 1 and in Table 2, with a significant difference between the treatments from the fourth week. This corroborates the previous finding regarding the amount of inert waste in the system, superior to active biomass (VSS) responsible for the biological degradation of organic matter. That is, the assimilation of ammonia in the biomass of heterotrophic bacteria was also low, perhaps due to the C:N ratio or to the removal of active biomass by the clarifier.

Ammonia toxicity is mainly dependent on pH levels, with ammonia (NH<sub>3</sub>) concentrations increasing in alkaline medium (Colt 2006). In this way, control of pH is extremely important. With the increase of salinity, the maintenance of a more neutral pH was observed (Table 2), with significant differences between treatments throughout whole shrimp cultivation. The highest pH may have contributed to animal mortality, mainly in T<sub>8</sub>, since *L. vannamei* are less tolerant to ammonia at lower salinities (Li et al. 2007). The alkalinity of T<sub>16</sub> was higher than T<sub>8</sub>, showing that in higher salinities, alkalinity tends to be higher. This difference may be related to cellular plasmolysis at higher salinities, decreasing the number of active bacteria that consume alkalinity. However, Ray and Lotz (2017) did not find a significant difference in alkalinity between salinity of 10‰, 20‰, and 30‰

in *L. vannamei* production using BFT. The alkalinity values in the present study did not negatively affect the shrimp, because, in both treatments, they were higher than 75 mg/L, as described by Furtado et al. (2015).

It was possible to observe that DO and sO<sub>2</sub> were significantly different between treatments, with the increase of salinity. The oxygen solubility in the water decreases, as observed by Maicá et al. (2012), who verified DO reduction in higher salinities in BFT. However, it is noteworthy that in both treatments, DO concentration was considered ideal for shrimps, that is, above 3 mg/L (Boyd 2015). According to McGraw et al. (2001), DO levels close to saturation result in maximum productivity and levels below 50% saturation should be avoided. In addition, oxygen is required in biological stabilization of the organic matter, so in the T<sub>16</sub>, whose BOD<sub>5</sub> was higher, more oxygen was demanded for biological degradation of organic matter.

### Zootechnical performance

The means and standard deviations of shrimp performance are presented in Tables 3 and 4. It was possible to verify that the weight gain was higher in T<sub>16</sub> with averages of 5.99 ± 0.54 g and 2.91 ± 0.93 g in T<sub>8</sub>, as well as specific growth rate and final biomass which were also higher in T<sub>16</sub>. Higher mortality was observed in T<sub>8</sub>, showing that salinity affected shrimp performance. It is noteworthy that total ammonia peaks also contributed to mortality in both treatments.

As observed in Fig. 2, lower zootechnical performances at lower salinities in BFT systems have also been noted by Decamp et al. (2003), which attributed a lower growth and survival rate in salinity 9‰ due to the relationship between alkalinity greater than 150 mg/L of CaCO<sub>3</sub> and high concentrations of chlorides (> 300 mg/L) for shrimp success at low salinities.

Maicá et al. (2012) also observed a positive influence of increased salinity on shrimp survival, weight gain and final biomass. Smaller growths at lower salinities may be related to the use of proteins not only for feeding, but also for the maintenance of osmotic equilibrium (Rosas et al. 2001).

Jatobá et al. (2014) in a comparative study between zootechnical indexes in conventional intensive system and BFT, with the same density of 250 animals/m<sup>2</sup>, observed that when feeding rations with 36.7% CP, the best performance was obtained in BFT system, with a weight gain of 9.18 ± 1.14 g and a weekly growth rate of 1.84 ± 0.23 g/week. This confirms the efficiency of BFT cultivation with high densities and that the reduction of the protein

**Table 3** Mean and standard deviations of the zootechnical performance of *Litopenaeus vannamei* in BFT system at 8 and 16‰

Variables	Treatments	
	T <sub>8</sub> (8‰)	T <sub>16</sub> (16‰)
Weight gain (g)	2.91±0.93 <sup>b</sup>	5.99±0.54 <sup>a</sup>
Specific growth rate (%/day)	0.82±0.27 <sup>b</sup>	1.64±0.13 <sup>a</sup>
Mortality (%)	24±4.12 <sup>b</sup>	14.7±2.52 <sup>a</sup>
Final biomass (g)	147.93±47.2 <sup>b</sup>	338.46±37.12 <sup>a</sup>
Feed conversion	1.7±0.93 <sup>a</sup>	1.7±0.23 <sup>a</sup>

Means followed by distinct letters on the same line are significantly different by the Tukey test ( $p \leq 0.05$ )

**Table 4** Weight of *Litopenaeus vannamei* during nine weeks of experiment

Variable	Salinity (%)	Weeks of shrimp cultivation									
		0	1	2	3	4	5	6	7	8	9
Weight (g)	8	4.555 Da	4.998 Da	6.033 Ca	6.572 BCa	6.812 AB-Ca	7.116 AB-a	7.514 AB-a	6.961 AB-Ca	7.578 Aa	7.475 AB-a
	16	3.553 Ib	4.608 Ha	5.654 Ga	6.193 FGa	6.872 EFa	7.261 DEa	7.850 CD-a	8.402 BCb	9.126 AB-b	9.556 Ab

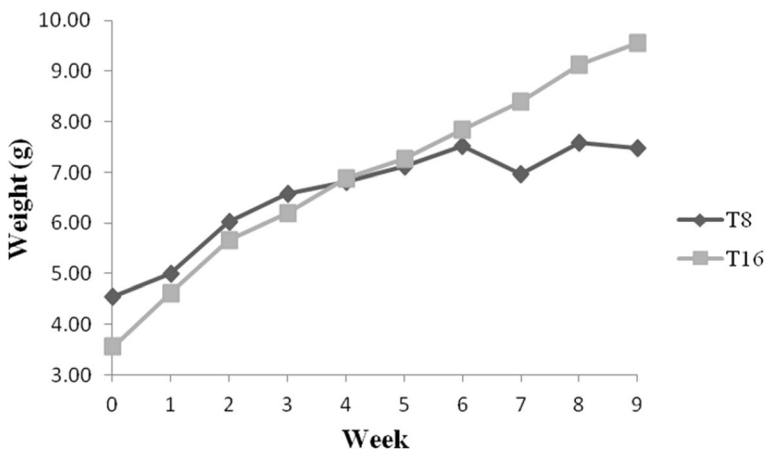
Means followed by lowercase letters in the column indicate significant differences by Tukey test ( $p < 0.05$ ) for salinity and different upper-case letters in the line differ by Tukey’s test ( $p < 0.05$ ) for the interaction of salinity and week

percentage in the feed to 30.3% did not significantly alter the weight gain and weekly growth rate, demonstrating the economic viability allied to the productivity.

The feed conversion rates did not show a significant difference between treatments, since in both there was formation of bacterial communities, which help to reduce feed intake and feed conversion of shrimps when compared to animals raised in clear water (Wasielesky et al. 2006). However, feed conversion rates were relatively high when compared to other works in BFT system. Maicá et al. (2012) verified rates of 0.81 to 0.87 at low salinities with density of 300 shrimp/m<sup>2</sup>; Krummenauer et al. (2011) with density of 300 shrimp/m<sup>2</sup> observed a rate of  $1.29 \pm 0.05$  in BFT system.

Also, biofloc can improve weight gain of shrimps by providing nutrients that would be found only in the feed. Ekasari et al. (2014) evaluated the nutritional composition of biofloc and observed that particles greater than 100 µm and less than 48 µm present high nutritional value for shrimp, with high concentrations of lipids and proteins in larger biofloc and essential amino acids in smaller biofloc.

Souza et al. (2019) described that particle size of the biofloc did not interfere in the nitrification process and when the TSS > 500 mg/L negatively affect the productivity of *L. vannamei*. Several authors observed that artificial substrates could increase the nitrification



**Fig. 2** Weight variations during the cultivation of *Litopenaeus vannamei* in salinity 8 and 16‰

(Santos et al. 2019) and area for the shrimps (Schveitzer et al. 2013a) in BFT systems. However, more studies are needed relating the biofloc and the nitrification process, taking into account the concern with water quality.

## Conclusion

Assessing the water quality of the BFT system allows management of the non-biodegradable material that accumulates in the system. The higher salinity (16‰) was better for the development of *L. vannamei*. Besides, use of molasses as a form of ammonia control was not so efficient and contributed to the increase of organic matter in the environment. It was observed that water quality changes during shrimp cultivation in biofloc can interfere in shrimp performance.

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## Declarations

**Ethics approval** The study with invertebrate species does not require an ethical approval.

**Conflict of interest** The authors declare no competing interests.

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## Affiliations

Anna Carolina Ferreira Spelta<sup>1</sup> · João Paulo Silva Lorenzini<sup>1</sup> ·  
Luanna do Carmo Neves<sup>1</sup> · Luciano dos Santos Rodrigues<sup>2</sup> ·  
Ângela Maria Quintão Lana<sup>1</sup> · Cintia Labussière Nakayama<sup>1</sup> ·  
Rebeca Valentim Marques<sup>1</sup> · Marco Yuri Rodrigues Napoli<sup>2</sup> ·  
Gustavo Moreira Alves<sup>1</sup> · Kleber Campos Miranda Miranda-Filho<sup>1</sup>

✉ Kleber Campos Miranda Miranda-Filho  
kmiranda2010@ufmg.br; kleber08@gmail.com

<sup>1</sup> Escola de Veterinária, Departamento de Zootecnia, Laboratório de Aquacultura, Universidade Federal de Minas Gerais-UFMG, Av. Antônio Carlos, 6627, Belo Horizonte, MG, 31270-901, Brazil

<sup>2</sup> Escola de Veterinária, Departamento de Medicina Veterinária Preventiva, Laboratório de Saneamento Ambiental, Universidade Federal de Minas Gerais-UFMG, Av. Antônio Carlos, 6627, Belo Horizonte, MG, CEP 31270-901, Brazil