Aquaculture Research

Aquaculture Research, 2015, 1–10 doi: 10.1111/are.12949

Effect of different total suspended solids levels on a *Litopenaeus vannamei* (Boone, 1931) BFT culture system during biofloc formation

Carlos Augusto Prata Gaona[1], Marcos Souza de Almeida[1], Veronica Viau[2], Luis Henrique Poersch[1] & Wilson Wasielesky Jr[1]

[1]Marine Station of Aquaculture, Institute of Oceanography, Federal University of Rio Grande, Rio Grande (RS), Brazil
[2]Biology of Reproduction and Growth in Crustaceans, Department of Biodiversity and Experimental Biology, FCEyN, University of Buenos Aires, Ciudad Universitaria, Buenos Aires, Argentina

Correspondence: C A P Gaona, Marine Station of Aquaculture, Institute of Oceanography, Federal University of Rio Grande, C.P. 474, Rio Grande (RS), CEP 96201-900, Brazil. E-mail: carlosgaona@ig.com.br

Abstract

In a Biofloc Technology System (BFT), there is constant biofloc formation and suspended solids accumulation, leading to effects on water quality parameters that may affect the growth performance of cultured shrimp. This study aimed to analyse during biofloc formation the effect of different total suspended solids (TSS) levels on water quality and the growth performance of *Litopenaeus vannamei* shrimp in a BFT system. A 42-day trial was conducted with treatments of three ranges of TSS: 100–300 mg L^{-1} as low (TL), 300–600 as medium (TM) and 600–1000 as high (TH). The initial concentrations of 100 (TL), 300 (TM) and 600 mg L^{-1} (TH) were achieved by fertilization before starting the experiment. *Litopenaeus vannamei* juveniles with an average weight of 4.54 ± 1.19 g were stocked at a density of 372 shrimp m^{-3}. Physical and chemical water parameters and shrimp growth performance were analysed. After 6 weeks, TSS mean concentrations were 306.37, 532.43 and 745.2 mg L^{-1} for, respectively, TL, TM and TH treatments. Significant differences ($P < 0.05$) were observed in TSS, settleable solids, pH, alkalinity and nitrite, especially between the TL and TH treatments. Similarly, differences ($P < 0.05$) were observed in the growth performance parameters, specifically final weight, survival, feed conversion and productivity. The water quality parameters at lower range of total suspended solids concentration (TL) treatment resulted in a better performance of *L. vannamei* in the BFT system. The maintenance at range of 100–300 mg L^{-1} TSS is thus important to the success of shrimp culture.

Keywords: suspended solids, biofloc, *Litopenaeus vannamei*, clarifier

Introduction

A super-intensive marine shrimp culture system (BFT) works by stimulating natural productivity, consisting in the formation of bioflocs, which are microbial aggregates (Samocha, Patnaik, Speed, Ali, Burger, Almeida, Ayub, Harisanto, Horowitz & Brook 2007; Avnimelech 2009). The suspended bioflocs constitute the suspended solids, which remain distributed throughout the water column according to the dynamics caused by aeration. These aggregate particles distinguish intensive culture systems from natural environments by the large amount of particulate organic carbon distributed among different taxa of microorganisms (Moss & Pruder 1995; Burford, Thompson, McIntosh, Bauman & Pearson 2004; Ray, Seaborn, Leffler, Wilde, Lawson & Browdy 2010).

In a BFT system, where stocking densities can reach 450 shrimp m^{-3} with higher feeding rates (Krummenauer, Cavalli, Poersch & Wasielesky 2011), the aeration system that diffuses air through the water column promotes vertical motion, distributing particulate matter throughout the tank via intensive mixing and keeping the biofloc suspended (Hargreaves 2006; De Schryver, Crab, Defoirdt, Boon & Verstraete 2008). This mixing becomes more important in impermeable structures where there is minimal or no water culture renewal, providing access to the solids and organic matter accumulated from offered food and the natural productivity in the suspended aggregates (Ray, Lewis, Browdy & Leffler 2010; Gaona, Poersch, Krummenauer, Foes & Wasielesky 2011; Schveitzer, Arantes, Costódio, Santo, Vinatea, Seiffert & Andreatta 2013). The natural biota maintained in suspension acts in the recycling of nitrogen compounds (ammonia and nitrite) and supplements the feeding of penaeid shrimp (Wasielesky, Atwood, Stokes & Browdy 2006; Arnold, Coman, Jackson & Groves 2009; Ballester, Abreu, Cavalli, Emerenciano, Abreu & Wasielesky 2010; Megahed 2010).

Simultaneously, there is an increase in nutrient concentrations, which can result in rapid eutrophication in closed systems (Thakur & Lin 2003). Silva, Wasielesky and Abreu (2013) analysed the nitrogen and phosphorus dynamics in BFT systems and found that 39% of the nitrogen input into the system is in dissolved form and 7.7% of that fraction is in inorganic form. Of the phosphorus that remained in the system, 34.1% was in dissolved form, with 50.7% of that portion being inorganic. However, the bacterial community in the water culture assimilates and converts inorganic nitrogen species, improving water quality (Avnimelech 2009; De Schryver & Verstraete 2009; Luo, Avnimelech, Pan & Tan 2013).

The capacity of an intensive biofiltration system is dependent on a continuous oxygen supply (Hargreaves 2006). Nitrifying bacteria, which maintain water quality by reducing nitrite concentrations, depend on an environment with high dissolved oxygen (Ebeling, Timmons & Bisogni 2006; Avnimelech 2009). Organic carbon is the energetic substrate for many microorganisms, and its consumption contributes to the use of dissolved oxygen, posing a risk of inadequate oxygen supply for the animals reared in the system if facilities are inadequate (Avnimelech 2009; Mook, Chakrabarti, Aroua, Khan, Ali, Islam & Abu Hassan 2012). In addition, the interactions between the biofloc and water quality may interfere with the stability of the system, potentially causing changes in pH, alkalinity and carbon dioxide concentration (Ebeling *et al.* 2006; Furtado, Poersch & Wasielesky 2011; Furtado, Gaona, Poersch & Wasielesky 2014).

As the density of suspended solids increases, a culture's success will depend on a balance between waste production and the capacity of the environment of the cultured species to assimilate nutrients. One of the strategies for formation of biofloc is to stimulate heterotrophic bacteria growth and metabolism by adding organic carbon (C) sources, balancing this substrate with the total ammonia nitrogen (N) through the C:N ratio (Avnimelech 1999; Ebeling *et al.* 2006). Nitrifying bacteria require an inorganic carbon substrate for nitrite assimilation to reduce the concentrations of this compound (Ebeling *et al.* 2006). Due to concerns about the establishment of ammonium-oxidizing and nitrite-oxidizing bacteria in cultures, some studies have focused on the analysis of suspended solids concentrations and zootechnical performance using biofloc inoculum from previous crops (Gaona *et al.* 2011; Ray, Dillon & Lotz 2011; Schveitzer *et al.* 2013).

Apart from the nutritional benefits of bioflocs and their ability to maintain water quality, monitoring of biofloc formation must be conducted through a specific analysis of the progression of the concentration of suspended solids and water quality parameters. However, none study started with different concentration of total suspended solids on biofloc system without inoculum. In this sense, the objective of this study was to analyse during biofloc formation, the effect of different suspended solids levels on water quality and the growth performance of the penaeid shrimp *Litopenaeus vannamei* in a BFT system.

Materials and methods

Location and period of the study

The study was conducted at the Marine Station of Aquaculture (EMA), Institute of Oceanography, Federal University of Rio Grande, located at Cassino Beach in Rio Grande, RS, Southern Brazil (32°11′S; 52°10′W). A 42-day trial was conducted from December 2010 to January 2011.

Experimental design

The experimental design was completely randomized, consisting of three treatments with three replicates each. Nine tanks (1.0 m^3) with a useful capacity of 0.86 m^3 were installed in a greenhouse for shrimp growth via a BFT system. The aeration system consisted of an Aero-Tube™ (a hose with micropores distributed evenly across its length) to optimize oxygen transfer and aeration efficiency. Forty-centimetre lengths of this hose were cut and installed in pairs in each experimental unit, coupled to a PVC pipe (20 mm diameter) and supplied by mechanical aeration from a 2 hp blower.

Three treatments were defined by ranges of TSS: 100–300 mg L^{-1} as low (TL), 300–600 as medium (TM) and 600–1000 as high (TH). The water used in the experiment was obtained by pumping directly from Cassino Beach, which has a salinity of 33 g L^{-1}. Before the beginning of the experiment, the water was treated with 10 mg L^{-1} of chlorine and neutralized with ascorbic acid 24 h later and then fertilized with sugar cane molasses in a 20:1 ratio (C:N) to achieve the initial suspended solids concentrations: 100, 200 and 500 mg L^{-1}. A first adjustment was made to the total suspended solids concentrations before the start of the experiment. Further adjustments to the suspended solids levels were made (third and fifth week) by suspended solids removal (6 h each removing) in all treatments, based on sedimentation methods (clarification) used in previous studies (Ray *et al.* 2010, 2011; Gaona *et al.* 2011). Clarifiers were assembled into a conical-cylindrical glass fibre chamber with a diameter of 0.48 m, height of 0.50 m and useful volume of 48 L, representing 5.5% of the total volume of the experimental unit. A PVC pipe (100 mm diameter) was placed inside this chamber to reduce the turbulence of the water pumped from the culture tank by a submerged pump with a flow of 1500 L h^{-1}.

Biological material and feeding

The juveniles of *L. vannamei* used in this study were first kept for 60 days in the nursery and grow-out BFT system inside the greenhouse at the Marine Station of Aquaculture (EMA). Each experimental unit was stocked at a density of 372 shrimp m^{-3} (265 individuals m^{-2}) with an average weight per shrimp of 4.54 ± 1.19 g. Shrimp were fed twice a day (at 9:00 and 16:00 hours) with commercial feed (Potimar Active 38; Centro Oeste Rações SA, Campinas, Brazil) containing 38% crude protein and 8% lipid. The food was offered on feeding trays (Wasielesky, Atwood, Stokes *et al.* 2006) at an initial rate of 10% of shrimp biomass and was adjusted according to consumption observed in a period of 24 h. This value was adjusted posteriorly according to the consumption observed in the trays within each interval between feedings.

Physical and chemical water parameters

Monitoring of dissolved oxygen, pH, temperature and salinity was performed daily. Samples were collected daily for total ammonia nitrogen (TAN) and nitrite (NO_2^-–N) analysis and once a week for nitrate (NO_3^-–N), orthophosphate (PO_4–P) and alkalinity. Total suspended solids (TSS) and settleable solids (SS) were monitored twice a week. Dissolved oxygen, pH, temperature and salinity were measured using multiparameter YSI® mod. 556 (YSI Incorporated, Yellow Springs, OH, USA). The concentrations of ammonia (TAN) and nitrite (NO_2^-–N) were measured according to UNESCO (1983) and Bendschneider and Robinson (1952) respectively. The method used for nitrate (NO_3^-–N) and orthophosphate (PO_4–P) was Aminot and Chaussepied (1983), and alkalinity was measured according to APHA (1998). The TSS was determined through gravimetry by filtering aliquots of 20 mL of water through GF 50-A glass fibre filters, according to Strickland and Parsons (1972) and AOAC (2000). Settleable solids was analysed using Imhoff cone and the volume of floc on the bottom of the cone was measured after 15 minutes of sedimentation (Avnimelech 2009).

Growth performance

Shrimp growth was monitored every 15 days, and used to adjust the amount of supplied feed according to Jory, Cabreras, Durwood, Fegan, Lee, Lawrence, Jackson, Mcintosh and Castañeda (2001). For that purpose, 30 shrimps were collected randomly from each experimental unit, individually weighed and returned to their respective tank. After weighing, the mean individual weight (g) was calculated.

At the end of the experiment the following variables were calculated:

$$\text{Survival (\%)} = (\text{final shrimp number}/\text{initial shrimp number}) \times 100.$$

$$\text{Feed conversion ratio (FCR)} = \text{offered feed (g)}/(\text{final biomass (g)} - \text{initial biomass (g)});$$

$$\text{Productivity } (\text{kg m}^{-3}) = (\text{final biomass} - \text{initial biomass})/\text{volume } (\text{m}^3)$$

Statistical analysis

The water quality parameters and growth performances in the different treatments were submitted to a one-way analysis of variance (ANOVA), taking into account the assumptions (Levene and Kolmogorov–Smirnov tests) necessary for its implementation. Tukey's test was applied when significant differences were detected ($P < 0.05$). The survival data were transformed (arcsine $x^{0.5}$) before analysis (Zar 1996).

Results

Water quality

Temperature and dissolved oxygen were maintained above 26°C and 5 mg L^{-1}, respectively, throughout the experiment and did not exhibit significant differences ($P > 0.05$) between treatments. Salinity increased at the end of the study; however, the mean salinity values were similar ($P > 0.05$) between groups.

Means and standard deviations of the parameters monitored throughout the experiment are presented in Table 1.

The concentrations of total suspended solids (TSS) outside of setting ranges of the treatments were recorded during the culture. Significant differences ($P < 0.05$) in total suspended solids concentrations were found between the TL and TH treatments throughout the study, whereas the TM group exhibited intermediate values (Fig. 1a). However, after 6 weeks of the experiment, the TSS concentrations did not present a significant difference ($P > 0.05$) between the TM and TL treatments (Fig. 1a).

From de second week onward, the settleable solids (SS) presented significant differences ($P < 0.05$) among all treatments (Fig. 1b).

The pH was significantly higher ($P < 0.05$) in TL compared to TH tanks from the third week onward (Fig. 1c).

The variations in alkalinity observed in the study (Fig. 1d) were marked by significant differences ($P < 0.05$) between treatments in the last 2 weeks, when the concentrations were significantly higher ($P < 0.05$) in TL with respect to TH.

Ammonia and nitrate concentrations were not differences ($P > 0.05$) between treatments during the experiment. However, the nitrite concentration increased throughout the experiment for the TM and TH treatments while it remained low for the TL group (Fig. 1e). Concentrations were significantly lower ($P < 0.05$) in the low TSS treatment (TL) relative to the other two groups during the final 2 weeks.

The highest orthophosphate concentration was recorded for the TH treatment during the last week, being significantly different ($P < 0.05$) from the TL group.

Growth performance

Shrimp kept at low (TL) and medium (TM) TSS concentrations exhibited similar ($P > 0.05$) growth

Table 1 Physical and chemical parameters data for *L. vannamei* in a BFT system with three suspended solids levels (low = TL, medium = TM, and high = TH), with mean values ± standard deviation

	Treatment		
	TL	TM	TH
Temperature (°C)	27.86 ± 1.37	28.07 ± 1.42	28.17 ± 1.45
Dissolved oxygen (mg L^{-1})	5.85 ± 0.64	5.76 ± 0.69	5.68 ± 0.71
pH	7.93 ± 0.23	7.86 ± 0.25	7.74 ± 0.31
Alkalinity (mg $CaCO_3$ L^{-1})	159.29 ± 19.96^{a}	136.43 ± 20.88^{ab}	116.71 ± 43.75^{bc}
Salinity (g L^{-1})	37.23 ± 3.13	37.69 ± 2.92	38.49 ± 2.95
TSS (mg L^{-1})	306.37 ± 119.71^{a}	532.43 ± 321.00^{ab}	745.20 ± 283.85^{bc}
SS (mg L^{-1})	11.48 ± 3.79^{a}	57.00 ± 41.22^{ab}	210.83 ± 161.47^{c}
Ammonia (mg L^{-1})	3.42 ± 3.01	3.33 ± 3.03	2.54 ± 3.25
Nitrite (mg L^{-1})	0.73 ± 1.08	3.66 ± 8.00	10.28 ± 14.25
Nitrate (mg L^{-1})	0.38 ± 0.63	0.46 ± 0.72	1.73 ± 2.11
Orthophosphate (mg L^{-1})	0.51 ± 0.27^{a}	1.39 ± 0.84^{ab}	4.82 ± 1.99^{bc}

Different letters in the same row indicate significant difference ($P < 0.05$) between treatments.

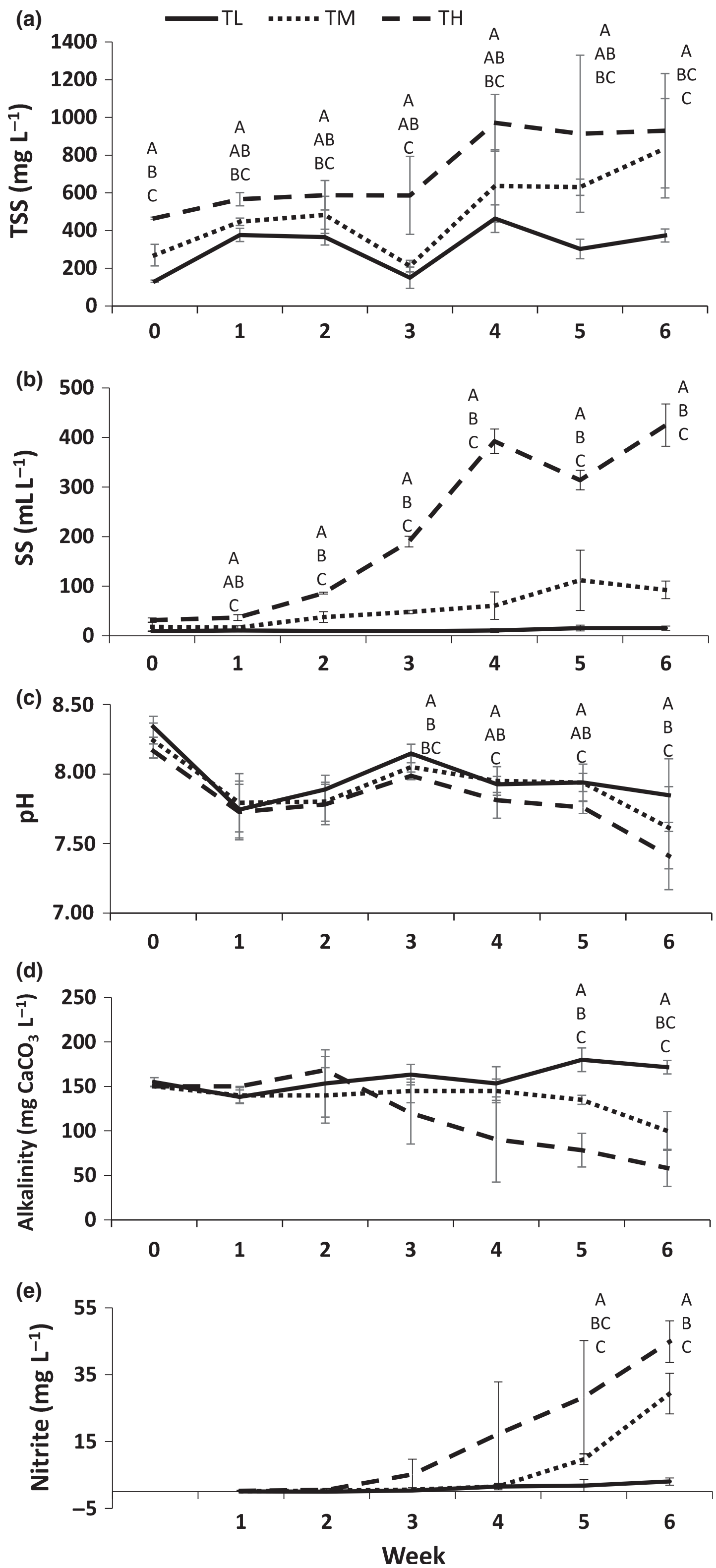

Figure 1 Variations in total suspended solids – TSS (a), settleable solids – SS (b), pH (c), alkalinity (d) and nitrite (e) throughout the experiment. Significant differences ($P < 0.05$) are indicated by the letters A, B and C, for Low, Medium and High treatments, respectively. Different letters indicate significant differences ($P < 0.05$) between treatments for each sample period.

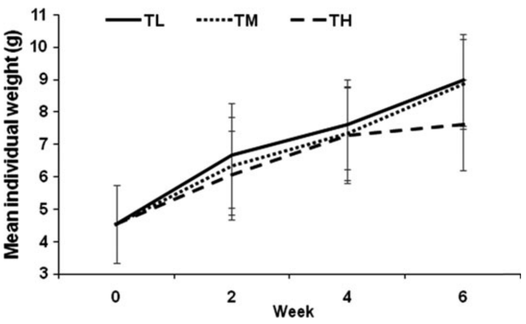

Figure 2 Shrimp growth during the 42-day trial in the presence of low (TL), medium (TM) and high (TH) suspended solids levels.

during the experiment, reaching the highest ($P < 0.05$) weights relative to the TH treatment after 42 days (Fig. 2).

The growth performance results in terms of final mean weight, survival, feed conversion ratio (FCR) and productivity presented significant differences between treatments, as indicated in Table 2.

Significant differences ($P < 0.05$) were observed in the last week, distinguishing the final weight of TH treatment shrimp from the other two treatments. The survival for the TL and TM treatments were similar ($P > 0.05$), both being significantly higher ($P < 0.05$) than that for the TH treatment. The FCR and productivity exhibited significance differences ($P < 0.05$) among all treatments at the end of the trial, with the best feed conversion ratio (FCR) and productivity obtained for the TL group in relation to the other groups (Table 2).

Table 2 Growth performance data for *L. vannamei* in a BFT system with three suspended solids levels (low = TL, medium = TM and high = TH), with mean values ± standard deviation

	Treatment		
	TL	TM	TH
Mean initial weight (g)	4.54 ± 1.19	4.54 ± 1.19	4.54 ± 1.19
Mean final weight (g)	8.98 ± 1.42[a]	8.85 ± 1.39[a]	7.60 ± 1.41[b]
Survival (%)	94.79 ± 1.41[a]	84.17 ± 7.73[a]	20.73 ± 8.58[b]
FCR	1.08 ± 0.04[a]	1.21 ± 0.08[b]	5.28 ± 1.92[c]
Productivity (kg m^{-3})	3.40 ± 0.08[a]	2.98 ± 0.21[b]	0.65 ± 0.37[c]

FCR, feed conversion ratio.
Different letters in the same row indicate significant differences ($P < 0.05$) between treatments.

Discussion

This study observed the effects of TSS of the three ranges along the experiment into BFT water quality and its consequences on *L. vannamei* growth performance. The changes on water quality occurred together with the evolution of every range of TSS concentrations.

Parameters such as temperature and oxygen availability directly affect the growth performance of *L. vannamei* by regulating metabolic functions, determining the growth of the animals. In this study, temperature and oxygen availability were maintained at levels favourable to the growth of the species (Van Wyk & Scarpa 1999; Zhang, Zhang, Li & Huang 2006). Salinity varied over the course of the experiment within the tolerance range of the species (Van Wyk & Scarpa 1999). A previous study of the combined effect of temperature and salinity on the culture of the shrimp *L. vannamei* observed the great osmoregulatory ability of this species, which quickly adapted to variations in salinity between 10 and 40 g L^{-1} (Re, Díaz, Ponce-Rivas, Giffard, Muñoz-Marquez & Sigala-Andrade 2012).

It is possible to estimate the dry matter present in settleable solids, estimating the proportion of TSS as dry weight (i.e. 1.4% TSS) present in biofloc, but this may not be the rule (Avnimelech 2007). According Schveitzer *et al.* (2013) the degree of correlation between TSS and SS may be affected by independent variations of each parameter. These same authors observed that even in stable levels of TSS, changes occurred in the SS measures. In this study, the SS had similar behaviour to TSS with increasing throughout the experiment, as noted by Furtado *et al.* (2011). According to Avnimelech (2009) typical floc volumes for shrimp culture are between 2 and 40 mL L^{-1}, whereas in the present study the values of TL treatment were within these ranges.

Litopenaeus vannamei has the capacity to feed on the natural biota and also contributes to the generation of a significant amount of biofloc in a BFT system (Burford *et al.* 2004). This was observed by Ferreira (2008) studying microbial floc formation in two cultures of marine shrimp species, who reported higher TSS concentrations in an *L. vannamei* culture than a *Farfantepenaeus paulensis* culture. The most notable variations in the average concentrations of total suspended solids were observed throughout the study in the TH and TL treatments, which, respectively, varied below and above 500 mg L^{-1}, a value recommended by Samocha *et al.* (2007). Suspended solids are involved in the organic matter decomposition processes and reflect changes in water quality (Vinatea, Galvez, Browdy, Stokes, Venero, Haveman, Lewis, Lawson, Shuler & Leffler 2010). Changes in pH and alkalinity during the study correlated inversely with TSS levels; a similar pattern was noted by Furtado *et al.* (2011) for TSS levels above 850 mg L^{-1}. The respiration in the water column by microorganisms present in the biofloc results in the excretion of carbon dioxide, which drives a reduction in pH (Wasielesky, Atwood, Stokes *et al.* 2006; Vinatea *et al.* 2010; Ray, Seaborn *et al.* 2010). Simultaneously, the dissociation of carbonate and bicarbonate ions reduces the alkalinity of the water culture (Ebeling *et al.* 2006). The alkalinity and pH remained above 100 mg $CaCO^3$ L^{-1} and 7, respectively, and within the recommended values for *L. vannamei* in a BFT system (Ebeling *et al.* 2006; Wasielesky, Atwood, Kegl, Bruce, Stokes & Browdy 2006).

Nitrogen cycling by heterotrophic and nitrifying bacteria resulted, respectively, in similar reductions in ammonia concentrations and increases in nitrite with increasing organic matter at the higher TSS levels. Due to the addition of molasses, heterotrophic bacteria had a substrate for obtaining carbon and subsequently metabolizing ammonia (Avnimelech 1999; Samocha *et al.* 2007), reducing the ammonia concentrations to levels tolerable to *L. vannamei* (Lin & Chen 2001). Luo *et al.* (2013) observed that the effect of the organic substrate in the inhibition of nitrifying bacteria was not toxic, but stimulated rapid heterotrophic growth and competition for dissolved oxygen, space, total ammonia and micronutrients. Inhibition of nitrifying bacteria may have led to slowed growth, and the reduction in alkalinity in the TH treatment may have limited the availability of inorganic carbon (Ebeling *et al.* 2006). In the present study, there were increased concentrations of nitrite in treatments TM and TH, in which the oxidation necessary to decrease and stack nitrate did not occur. Nitrite concentrations in the TL treatment also increased, but it was a very gentle rise. It was clear that the length of the experiment was most likely not sufficient for the establishment of characteristic nitrification in BFT systems. According to Silva *et al.* (2013), the route of nitrification occurs with successive conversions of ammonia to nitrite and nitrate prevalence at the end of the crop cycle.

Phosphorus concentrations remained at concentrations that accompanying TSS levels, remaining accumulated in the water culture. Barak, Cytryn, Gelfand, Krom and Van Rijn (2003) reported that the source of phosphorus in the culture is mainly due to unconsumed feed and nutrients excreted by cultured organisms. In cultures with biofloc technology, *L. vannamei* can incorporate up to 35% of the total phosphorus that enters the system, whereas the majority remains in the dissolved and particulate forms, with accumulation expected in a BFT system (Silva *et al.* 2013).

The shrimp's performance was determined by the water quality during the experiment. The lower total suspended solids concentration (at range of 100–300 mg L^{-1}) used in the study provided the best conditions for the shrimp, most likely due to the low levels of nitrites maintained throughout the 42-day trial. Lin and Chen (2003) verified a nitrite (NO_2^-–N) safe level of 25.7 mg L^{-1} for *L. vannamei* juveniles at a salinity of 35 g L^{-1} indicating the high tolerance of the species, as the concentration indicated by these authors was only achieved in the sixth week for the TM treatment in higher salinity. The TH treatment exceeded this concentration from the fifth week, exposing the animals for longer toxicity of nitrite. At low salinity to 48 h LC50 (median lethal concentration) studies, Schuler, Boardman, Kuhn and Flick (2010) in salinity of 10 g L^{-1} observed 154 mg L^{-1} NO_2^-–N, while Lin and Chen (2003) at 15 g L^{-1} recorded 143 mg L^{-1} NO_2^-–N. However, the nitrite concentration was determinant for the growth rate of the animals, and the two variables may have an inverse relationship (Vinatea *et al.* 2010). These interactions were reflected in the better growth and survival in treatment TL, which was observed to have the lowest nitrite concentration. In an *L. vannamei* culture with a TSS concentration of 465 mg L^{-1}, Ray, Lewis *et al.* (2010) recorded a maximum nitrite concentration of 5.4 mg L^{-1}, obtaining a final mean weight of 11.6 g and mean survival of 71% at a stocking density of 460 shrimp m^{-3} after 12 weeks. Gaona *et al.* (2011) observed, after 16 weeks of culture, that shrimp stocked at a density of 250 individuals m^{-2} reached 10.76 g with a survival of 81%, maintaining the TSS level at 500 mg L^{-1}. Schveitzer *et al.* (2013) observed 83% survival in *L. vannamei* reared under suspended solids conditions kept within the range of 400–600 mg L^{-1}. Concomitantly, there was a better conversion of feed consumed to shrimp growth in the present study. The best FCR was observed in the treatment with low TSS, with a ratio of 1.08. In accordance with this result, Samocha *et al.* (2007) obtained the best FCR in a culture with a total suspended solids concentration near 100 mg L^{-1} but with a lower stocking density than in the current study. Ray *et al.* (2011), testing two TSS levels (197 and 313 mg L^{-1}), obtained a FCR of 2.5 in the treatment with lower TSS. However, some studies have observed variations in productivity between 2.15 and 4.10 kg m^{-3} at different stocking densities (Gaona *et al.* 2011; Krummenauer *et al.* 2011; Ray *et al.* 2011; Baloi, Arantes, Schveitzer, Magnotti & Vinatea 2013; Schveitzer *et al.* 2013). In the present study, an intermediate value of productivity (3.40 kg m^{-3}) was observed at the lowest concentration of TSS with range of 100–300 mg L^{-1}. To keep TSS in such range becomes a strategy to await the establishment of nitrite oxidizers which occurs around 4–6 weeks (Avnimelech 2009) and to start a cycle without inoculum-containing nitrifiers.

Conclusion

Total suspended solids concentrations about 100–300 mg L^{-1} during biofloc formation are important for maintaining water quality, particularly when the nitrification process is not well established. In addition, negative impacts on water quality parameters can be reduced by using low TSS concentrations, starting the culture at approximately 100 mg L^{-1}, reducing variations over culture. Obviously, a culture environment with better physical and chemical conditions favours a better growth performance of cultured organisms. As a recommendation, preventive actions to maintain a TSS concentration by means of clarification are indicated for water quality improvement in a BFT system.

Acknowledgments

The authors are grateful for the financial support provided by the National Council for Scientific and Technological Development (CNPq), Ministry of Fishery and Aquaculture (MPA) and Coordination for the Improvement of Higher Level Personnel (CAPES). Special thanks to Centro Oeste Rações S.A. (GUABI) for donating the experimental diets. W. Wasielesky, and L.H. Poersch are research fellows of CNPq.

References

Aminot A. & Chaussepied M. (1983) *Manuel des analyses chimiques en milieu marin*. CNEXO, Brest 395p.

AOAC (Association of Official Analitycal Chemists) (2000) *Official Methods of Analysis of AOAC* (16th edn). Patricia Cunniff (editora), Washington, DC.

APHA (American Public Health Association) (1998) *Standard Methods for the Examination of Water and Wastewater* (20th edn), Washington, DC 1193 pp.

Arnold S.J., Coman F.E., Jackson C.J. & Groves S.A. (2009) High-intensity, zero water-exchange production of juvenile tiger shrimp, *Penaeus monodon*: an evaluation of artificial substrates and stocking density. *Aquaculture* **293**, 42–48.

Avnimelech Y. (1999) Carbon/Nitrogen ratio as a control element in aquaculture systems. *Aquaculture* **176**, 227–235.

Avnimelech Y. (2007) Feeding with microbial flocs by tilapia in minimal discharge bio-flocs technology ponds. *Aquaculture* **264**, 140–147.

Avnimelech Y. (2009) *Biofloc Technology - A Pratical Guide Book*. The World Aquaculture Society, Baton Rouge, Louisiana, United States.

Ballester E.L.C., Abreu P.C., Cavalli R.O., Emerenciano M., Abreu L. & Wasielesky W. (2010) Effect of practical diets with different protein levels on the performance of *Farfantepenaeus paulensis* juveniles nursed in a zero exchange suspended microbial flocs intensive system. *Aquaculture Nutrition* **16**, 163–172.

Baloi M., Arantes R., Schveitzer R., Magnotti C. & Vinatea L. (2013) Performance of Pacific white shrimp *Litopenaeus vannamei* raised in biofloc systems with varying levels of light exposure. *Aquacultural Engineering* **52**, 39–44.

Barak Y., Cytryn E., Gelfand I., Krom M. & Van Rijn J. (2003) Phosphorus removal in a prototype, recirculating aquaculture system. *Aquaculture* **220**, 313–326.

Bendschneider K. & Robinson R.J. (1952) A new spectrophotometric method for the determination of nitrite in sea water. *Journal of Marine Research* **11**, 87–96.

Burford M.A., Thompson P.J., McIntosh R.P., Bauman R.H. & Pearson D.C. (2004) The contribution of flocculated material to shrimp (*Litopenaeus vannamei*) nutrition in a high-intensity zero-exchange system. *Aquaculture* **232**, 525–537.

De Schryver P. & Verstraete W. (2009) Nitrogen removal from aquaculture pond water by heterotrophic nitrogen assimilation in lab-scale sequencing batch reactors. *Bioresource Technology* **100**, 1162–1167.

De Schryver P.D., Crab R., Defoirdt T., Boon N. & Verstraete W. (2008) The basics of bio-flocs technology:

The added value for aquaculture. *Aquaculture* **277**, 125–137.

Ebeling J.M., Timmons M.B. & Bisogni J.J. (2006) Engineering analysis of the stoichiometry of hotoautotrophic, autotrophic, and heterotrophic control of ammonia-nitrogen in aquaculture production systems. *Aquaculture* **257**, 346–358.

Ferreira L.M.M.H.M. (2008) *Formação de flocos microbianos em cultivo do camarão-rosa Farfantepenaeus paulensis e do camarão-branco Litopenaeus vannamei*. MSc Thesis, Federal University of Rio Grande, Brazil.

Furtado P.S., Poersch L.H. & Wasielesky W.J. (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**, 130–135.

Furtado P.S., Gaona C.A.P., Poersch L.H. & Wasielesky W.J. (2014) Application of different doses of calcium hydroxide in the farming shrimp *Litopenaeus vannamei* with the biofloc technology (BFT). *Aquaculture International* **22**, 1009–1023.

Gaona C.A.P., Poersch L.H., Krummenauer D., Foes G.K. & Wasielesky W.J. (2011) The effect of solids removal on water quality, growth and survival of *Litopenaeus vannamei* in a Biofloc Technology Culture System. *International Journal of Recirculating Aquaculture* **12**, 54–73.

Hargreaves J. (2006) Photosynthetic suspended-growth systems in aquaculture. *Aquacultural Engineering* **34**, 344–363.

Jory D.E., Cabreras R.T., Durwood M.D., Fegan D., Lee G.P., Lawrence A.L., Jackson J.C., Mcintosh P.R. & Castañeda A.J. (2001) *A Global Review of Shrimp Feed Management: Status and Perspectives*. Aquaculture the World Aquaculture Society, Baton Rouge, LA USA.

Krummenauer D., Cavalli R.O., Poersch L.H. & Wasielesky W. (2011) Superintensive Culture of White Shrimp, *Litopenaeus vannamei*, in a Biofloc Technology System in Southern Brazil at Different Stocking Densities. *Journal of the World Aquaculture Society* **42**, 726–733.

Lin Y.C. & Chen J.C. (2001) Acute toxicity of ammonia on *Litopenaeus vannamei* (Boone) juveniles at different salinity levels. *Journal of Experimental Marine Biology and Ecology* **259**, 109–119.

Lin Y.C. & Chen J.C. (2003) Acute toxicity of nitrite on *Litopenaeus vannamei* (Boone) juveniles at different salinity levels. *Aquaculture* **224**, 193–201.

Luo G., Avnimelech Y., Pan Y. & Tan H. (2013) Inorganic nitrogen dynamics in sequencing batch reactors using biofloc technology to treat aquaculture sludge. *Aquacultural Engineering* **52**, 73–79.

Megahed M.E. (2010) The effect of microbial biofloc on water quality, survival and growth of the green tiger shrimp (*Penaeus semisulcatus*) fed with different crude protein levels. *Journal of the Arabian Aquaculture Society* **5**, 119–142.

Mook W.T., Chakrabarti M.H., Aroua M.K., Khan G.M.A., Ali B.S., Islam M.S. & Abu Hassan M.A. (2012) Removal of total ammonia nitrogen (TAN), nitrate and total organic carbon (TOC) from aquaculture wastewater using electrochemical technology: a review. *Desalination* **285**, 1–13.

Moss S.M. & Pruder G.D. (1995) Characterization of organic particles associated with rapid growth in juvenile white shrimp, *Penaeus vannamei* Boone, reared under intensive culture conditions. *Journal of Experimental Marine Biology and Ecology* **187**, 175–191.

Ray A.J., Seaborn G., Leffler J.W., Wilde S.B., Lawson A. & Browdy C.L. (2010) Characterization of microbial communities in minimal-exchange, intensive aquaculture systems and the effects of suspended solids management. *Aquaculture* **310**, 130–138.

Ray A.J., Lewis B.L., Browdy C.L. & Leffler J.W. (2010) Suspended solids removal to improve shrimp (*Litopenaeus vannamei*) production and an evaluation of a plant-based feed in minimal-exchange, superintensive culture systems. *Aquaculture* **299**, 89–98.

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**, 127–136.

Re A.D., Díaz F., Ponce-Rivas E., Giffard I., Muñoz-Marquez M.E. & Sigala-Andrade H.M. (2012) Combined effect of temperature and salinity on the Thermotolerance and osmotic pressure of juvenile white shrimp *Litopenaeus vannamei* (Boone). *Journal of Thermal Biology* **37**, 413–418.

Samocha T.M., Patnaik S., Speed M., Ali A.M., Burger J.M., Almeida R.V., Ayub Z., Harisanto M., Horowitz A. & Brook D.L. (2007) Use of molasses as carbon source in limited discharge nursery and grow-out systems for *Litopenaeus vanammei*. *Aquacultural Engineering* **36**, 184–191.

Schuler D.J., Boardman G.D., Kuhn D.D. & Flick G.J. (2010) Acute toxicity of ammonia and nitrite to Pacific white shrimp, *Litopenaeus vannamei*, at low salinities. *Journal of the World Aquaculture Society* **41**, 438–446.

Schveitzer R., Arantes R., Costódio P.F.S., Santo C.M.E., Vinatea L.A., Seiffert W.Q. & Andreatta E.R. (2013) Effect of different biofloc levels on microbial activity, water quality and performance of Litopenaeus vannamei in a tank system operated with no water exchange. *Aquacultural Engineering* **56**, 59–70.

Silva K.R., Wasielesky W.J. & 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**, 30–41.

Strickland J.D.H. & Parsons T.R. (1972) *A Practical Handbook of Seawater Analysis* (2nd edn), Bulletin 167. Fisheries Research Board of Canada, Ottawa.

Thakur D.P. & Lin C.K. (2003) Water quality and nutrient budget in closed shrimp (*Penaeus monodon*) culture systems. *Aquacultural Engineering* **27**, 159–176.

UNESCO. (1983) *Chemical Methods for Use in Marine Environmental Monitoring*. Manual and Guides 12, Intergovernamental Oceanographic Commissiony, Paris, France.

Van Wyk P. & Scarpa J. (1999) Water Quality and Management. In: *Farming Marine Shrimp in Recirculating Freshwater Systems* (ed. by P. Van Wyk, M. Davis-Hodgkins, R. Laramore, K.L. Main, & J. Scarpa), pp. 128–138. Florida Department of Agriculture and Consumer Services, Tallahassee.

Vinatea L., Galvez A.O., Browdy C.L., Stokes A., Venero J., Haveman J., Lewis B.L., Lawson A., Shuler A. & Leffler J.W. (2010) Photosynthesis, water respiration and growth performance of *Litopenaeus vannamei* in a super-intensive raceway culture with zero water exchange: interaction of water quality variables. *Aquacultural Engineering* **42**, 17–24.

Wasielesky W.J., Atwood H.I., Stokes A. & Browdy C.L. (2006) Effect of natural production in brown water super-intensive culture system for white shrimp *Litopenaeus vannamei*. *Aquaculture* **258**, 396–403.

Wasielesky W.J., Atwood H., Kegl R., Bruce J., Stokes A. & Browdy C. (2006) Efeito do pH na sobrevivência e crescimento do camarão branco *Litopenaeus vannamei* em cultivos superintensivos. Aquaciência 2006. Anais do congresso.

Zar J.H. (1996) *Biostatistical Analysis* (3rd edn). Prentice Hall, New Jersey 662 pp.

Zhang P., Zhang X., Li J. & Huang G. (2006) The effects of body weight, temperature, salinity, pH, light intensity and feeding condition on lethal DO levels of white shrimp, *Litopenaeus vannamei* (Boone, 1931). *Aquaculture* **256**, 579–587.

