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Evaluation of biofloc technology in pink shrimp *Farfantepenaeus duorarum* culture: growth performance, water quality, microorganisms profile and proximate analysis of biofloc

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Abstract In a 210d experiment, the potential of biofloc technology (BFT) was evaluated for *Farfantepenaeus duorarum*. Water quality parameters, microorganisms profile and proximate analysis of biofloc were also assessed. BFT did not improve the growth performance in *F. duorarum* when compared to conventional clear-water water exchange system (final weight and survival of 13.3 g and 63.2 %; and 13.9 g and 81.4 %, respectively). Microorganism assessment suggested a higher presence of filamentous cyanobacteria followed by protozoa, nematodes and copepods. Proximate analysis of biofloc showed crude protein and crude lipid means levels of 25 and 0.6 %, respectively, and these values varied during the experiment. *F. duorarum* seemed to be susceptible to high stocking density and high levels of suspended solids (>15 mL L^{-1}).

Keywords Solids · Floc · Nutrition · Water exchange · *F. duorarum*

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Introduction

Indigenous shrimp species could be more suitable for aquaculture as they are more disease resistant in local environment, as well as may demonstrate higher growth rates under specific environmental conditions with better acceptance in local markets (Peixoto et al. 2011).

The pink shrimp *Farfantepenaeus duorarum* is distributed in the western Atlantic Ocean from Maryland (EUA), through Gulf of Mexico until Ascensión Bay in Quintana Roo, Mexico (Pérez-Farfante 1969). Once abundant in natural waters, pink shrimp fishery in southern Gulf of Mexico has collapsed (Arreguin-Sanchez et al. 2008). During the early 1970s, the total shrimp production was approximately 27,000 t per year of which 90 % comprised of *F. duorarum.* The production dropped to below 3000 t per year recently (Arreguin-Sanchez et al. 2008). The possible reasons explaining this collapse include overfishing (Gracia 1995, 1997), as well as pollution in nursery areas (Arreguín-Sánchez et al. 1997). Consequently, the need to farm *F. duorarum* increased due to the fact that this species has shown signs of depletion.

Recently, government regulations have initiated the development of aquaculture in southern Gulf of Mexico with indigenous aquatic species. Considering the valuable local market, previous studies have been carried out investigating the *F. duorarum* farm (Cripe 1994; Samocha et al. 2008; Gullian et al. 2010). However, high costs associated with wild broodstock capture to guarantee the availability of quality PLs have been the most important constraint for aquaculture development for this species. To overcome this problem, preliminary research efforts have been done in order to domesticate such species (Lopez-Tellez et al. 2000; Gullian et al. 2010); however, poor results in terms of growth were observed. Alternative means for sustainable broodstock farming need to be developed.

Biofloc technology (BFT) was initially developed in early 1970s at Ifremer-COP, French Polynesia (for review, see Emerenciano et al. 2011a). Nowadays, BFT limited water exchange system has become a popular grow-out technology in shrimp farming (Avnimelech 2012), however, little is known about BFT benefits to the indigenous species *F. duorarum*. Furthermore, with the global spread of viruses, biosecurity appears as a priority to avoid vertical infections. BFT limited water exchange culture method can help improve biosecurity (Taw 2010), besides minimize the effluent discharge, protecting the surrounding areas. In addition, BFT provides in situ nutrients such as "native protein" (Emerenciano et al. 2011a), lipid (Wasielesky et al. 2006), aminoacids (Ju et al. 2008) and fatty acids (Izquierdo et al. 2006; Ekasari et al. 2010) in a form of diverse microbiota. Microorganisms, that is, grazers and bacteria (Ballester et al. 2010) play a key role in recycling nutrients and maintaining water quality (McIntosh et al. 2000; Ray et al. 2010a, b). Control of bacterial community over autotrophic microorganisms is achieved using a high carbon to nitrogen ratio (C:N) (Avnimelech et al. 1994; Asaduzzaman et al. 2008), in which nitrogenous by-products are taken up by heterotrophic bacteria (Avnimelech 1999). Moreover, microbial particles as a rich food source are available 24 h per day, reducing artificial feed inputs and costs (Avnimelech 2007; Samocha et al. 2007).

The objective of the present study was to evaluate the potential of biofloc technology (BFT) on enhancement of *F. duorarum* zootechnical and water quality parameters when compared to conventional water exchange system during grow-out phase.

Materials and methods

Experimental design and culture conditions

This experiment was performed at UMDI, Universidad Nacional Autónoma de México (UNAM), located at Sisal Beach (21°09′5/N and 90°02′5/W), Yucatán, Mexico. *F. duorarum* postlarvae (G1) were obtained from routine larviculture carried out at UMDI using Galveston technique (Smith et al. 1992). Juveniles (initial weight ~7.4 g) were stocked in six 20,000 L round outdoor lined tanks covered by shade cloth (~80 % light reduction) with stocking density of 38 shrimp m^{-2} (760 shrimp per tank). Shrimp were maintained in two conditions (treatments): water exchange in a rate of 50 % daily using three tanks (CW) and biofloc limited water exchange not exceeding 0.5 % daily using the three remaining tanks (FLOC). After 7 months (210 days), survival (%), final weight (g), weight gain per week (g), productivity (kg m^{-2}) and final biomass (kg) were measured.

In FLOC conditions, C:N ratio of 20:1 (for review, see Avnimelech 1999; Emerenciano et al. 2007) was maintained using sugarcane molasses and wheat bran as a carbon source, added daily after feed addition in a ratio of 90:10, respectively. External carbon source was applied to ensure optimal heterotrophic bacterial growth (Avnimelech 2007) until floc volume (FV; measured in Imhoff cones, Avnimelech 2012) achieved the concentration of 5 mL L^{-1}. Once FV achieved this concentration, carbon source addition was stopped. If FV dropped (<5 mL L^{-1}) or total ammonium nitrogen (TAN) reached more than 3 mg L^{-1}, the carbon addition was restarted. Water was vigorously aerated using a hose circle (3 inches, 0.8 m diameter) finely perforated positioned in the center of the tanks. Water exchange (<0.5 %) was carried out with sludge removal by a central drain to prevent accumulation. Dechlorinated freshwater and marine water (~35 ppt) were added to compensate sludge removal and evaporation losses. Shrimp were fed five times per day (00:00, 04:00, 09:00, 14:00 and 20:00 h) in both treatments using commercial feed with 35 % crude protein content (Malta Clayton, Inc., Culiacán, Sinaloa, Mexico) at 2–4 % of biomass (Jory 2001) and according to consumption.

Water quality parameters such as temperature, salinity, pH and dissolved oxygen (DO) (Hach HQ40d, Hach Company, Loveland, Colorado, USA) were monitored five times per day (00:00, 04:00, 09:00, 14:00 and 20:00 h). FV was monitored daily, whereas TAN, nitrite ($N\text{-}NO_2$) and nitrate ($N\text{-}NO_3$) were monitored three times per week (Hach test kits cat. 20686-00, Hach Company, Loveland, Colorado, USA).

Assessment of microorganisms

Microorganisms profile was assessed from month 2–6. Well-mixed water samples were collected from CW and FLOC tanks and preserved with a buffered formalin solution (4 %) (Thompson et al. 2002) for further analysis. Microorganisms were identified and quantified using microscopy (Barnes 1963; Wimpenny 1966), and the abundance of zooplankton and large microorganisms such as filamentous cyanobacteria, protozoa, nematodes and copepods was determined using a Sedgwick-Rafter counting chamber (Azim and Little 2008; Muangkeow et al. 2011) with a 1-mL sample previously concentrated. Counts were made in at least 10 fields chosen at random with three replicates per sample.

Proximate analysis of biofloc

Proximate analysis was performed in biofloc biomass each month (except month 1). Samples (tank water previously filtered in a 100-µm mesh) were dried in an oven at 55 °C (constant weight), stored at −20 °C and processed following protocols described by AOAC (2000). The total carbohydrate was estimated by difference, and gross energy content was calculated according to Tacon (1990).

Statistical analysis

Student's *t* test was applied to find differences between treatments in water quality and grow-out parameters when data were homogeneous and normality distributed (Zar 1984). Percentage data were arcsine transformed, but only original values were presented. Differences were considered significantly at $P < 0.05$ (Sokal and Rohlf 1995).

Results

Water quality parameters

Descriptive statistics of water quality parameters are shown in Table 1. Temperature, DO, salinity, pH, TAN, N-NO_2 and N-NO_3 were similar between treatments and presented no significant differences ($P > 0.05$). FV measured in FLOC tanks presented mean values of 9.0 mL L^{-1} with maximum and minimum of 17.3 and 5.3 mL L^{-1}, respectively (Fig. 1). Fig. 2 is presented the daily variations of temperature, DO and pH, and no significant differences ($P > 0.05$) were observed.

Growth performance

Growth performance of shrimp is given in Table 2. Final weight and weight gain $week^{-1}$ presented no significant differences between treatments. Final biomass, productivity and survival were significantly higher in CW (8.4 kg $tank^{-1}$, 0.42 kg m^{-2} and 81 %) when compared to FLOC treatment (6.3 kg $tank^{-1}$, 0.32 kg m^{-2} and 63 %). Fig. 3 is presented the evolution of weight and weight gain per $week^{-1}$ throughout the experiment and showed similar values ($P > 0.05$). The highest weight gain was performed by FLOC treatment in month 1 with a growth rate of 0.5 g $week^{-1}$.

Table 1 Means, SD, maximum and minimum values of water quality parameters performed during *F. duorarum* grow-out (38 shrimp m^{-2}) in biofloc (FLOC) and clear-water (CW) treatments

Parameters	CW (control)				FLOC			
	Means	SD	*Max.*	*Min.*	Means	SD	*Max.*	*Min.*
Temperature (°C)	26.2	2.1	28.9	22.8	25.8	2.2	28.3	22.6
Dissolved oxygen (mg L^{-1})	6.1	0.3	7.6	5.6	6.2	0.3	6.6	5.6
Salinity (ppt)	38.4	0.4	39.0	37.7	39.3	1.4	41.0	37.4
pH	8.4	0.2	8.6	8.1	8.3	0.3	8.8	7.9
TAN (mg L^{-1})	0.3	0.1	0.6	0.2	0.4	0.1	0.6	0.2
N-NO_2 (mg L^{-1})	0.3	0.1	0.5	0.2	0.2	0.2	0.6	0.1
N-NO_3 (mg L^{-1})	7.3	1.9	10	5.6	12.7	4.8	20.0	7.0
Floc volume (mL L^{-1})	–	–	–	–	9.0	6.4	17.3	5.3

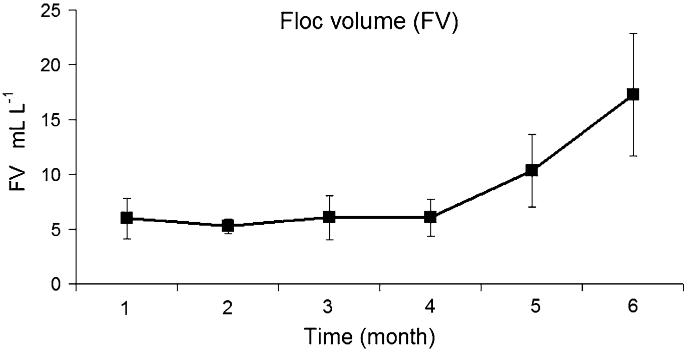

Fig. 1 Means (±SD) of floc volume (FV) during *F. duorarum* grow-out

Assessment of microorganisms and proximate analysis of biofloc

Microorganism profile is showed in Fig. 4. Data suggested a high presence of filamentous cyanobacteria (FC) when compared to the other groups, mainly in the initial phase, and then decreased over time until month 4. Protozoa (second most representative group) followed the same FC trend with high levels in the initial period and then decreased, but reached to a peak when FC achieved low levels. Nematodes and copepods reached high levels both in month 2, and then decreased over time, increased again in month 5 and 6, respectively.

Proximate analysis of biofloc is given in Table 3. Mean value of crude protein was 24.7 % (ranging from 18.2 to 29.3 %); lipid content and crude fiber were 0.6 and 3.6 % (ranging from 0.4 to 0.7 % and 1.7 to 3.5 %, respectively). Moreover, carbohydrates, ash and gross energy values were 26.3, 47.3 % and 10.2 kJ g^{-1} DW. Variation of crude protein and crude lipid throughout the experiment presented in Fig. 5 showed a decrease in both levels in month 3, achieving 18 and 0.4 %, respectively. Peak in terms of protein and lipid were achieved in month 7 and 5, with 29 and 0.74 %, respectively.

Discussion

Growth performance and water quality

In the present study, growth parameters were not improved using BFT system. CW treatment demonstrated better survival, productivity and final biomass ($P < 0.05$). Final weight and weight gain week^{-1} presented no significant differences, and shrimp growth was very slow in both systems. Emerenciano et al. (2007) also did not report differences in nursery phase of *F. paulensis* using BFT when compared to clear-water system. On the other hand, Krummenauer et al. (2011) in a 120d of *Litopenaeus vannamei* BFT culture reported superior survival and productivity when compared to our study, with 92, 81 and 75 % and 2.1, 4.1 and 3.0 kg m^{-2}, for a stocking density of 150, 300 and 450 shrimp m^{-2}, respectively.

Usual values expected for weight gain ranges ~1 g week^{-1} in tropical penaeid species. For example, *L. vannamei* juveniles fed with 35 % CP commercial feed at 300 shrimp m^{-2}, growth rates were 1.25 and 0.85 g week^{-1} in BFT and clear-water, respectively, although no

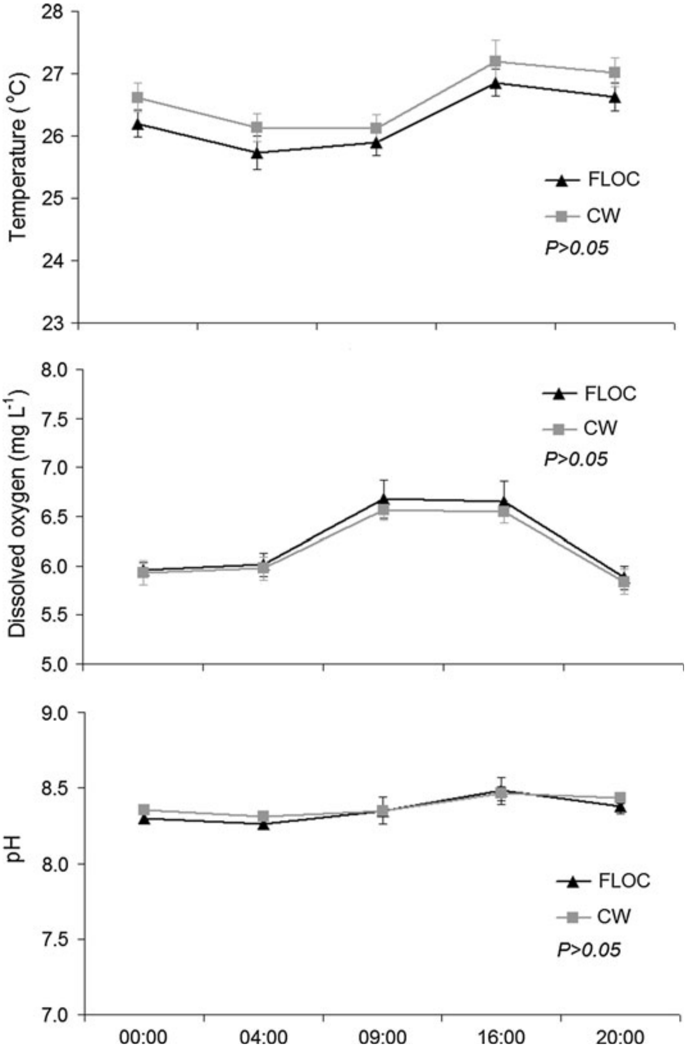

Fig. 2 Daily variation (mean ± SE) of temperature, DO and pH during *F. duorarum* grow-out in biofloc (FLOC) and clear-water (CW) treatments

Table 2 Means (±SD) of *F. duorarum* growth performance in biofloc (FLOC) and clear-water (CW) treatments during grow-out (38 shrimp m^{-2}). Within rows, superscript letters indicate significant differences by Student's t-test

Parameter	CW	FLOC	*P value*
Initial weight (g)	7.6 (±1.2)	7.3 (±1.3)	ns ($P = 0.097$)
Final weight (g)	13.9 (±2.8)	13.3 (±2.7)	ns ($P = 0.136$)
Weight gain (g $week^{-1}$)	0.25 (±0.13)	0.26 (±0.24)	ns ($P = 0.407$)
Final biomass (kg $tank^{-1}$)	8.4^{A} (±0.9)	6.3^{B} (±0.3)	$P = 0.021$
Productivity (kg m^{-2})	0.42^{A} (±0.04)	0.32^{B} (±0.02)	$P = 0.020$
Survival (%)	81.4^{A} (±9.2)	63.2^{B} (±1.2)	$P = 0.045$

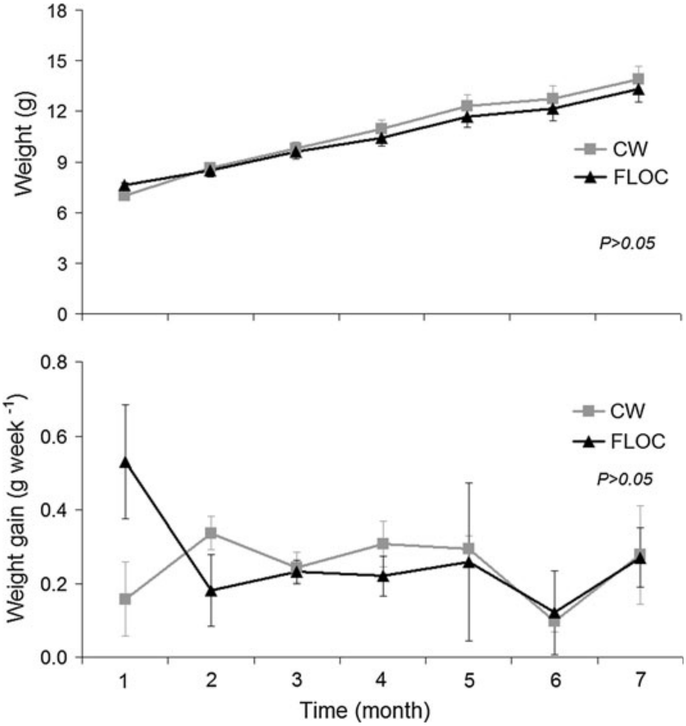

Fig. 3 Evolution of weight and weight gain per week (±SE) of *F. duorarum* in biofloc (FLOC) and clear-water (CW) treatments, during grow-out (38 shrimp m^{-2})

differences were observed in survival (Wasielesky et al. 2006). Lopez-Tellez et al. (2000) reported a growth rate of ~0.5 g $week^{-1}$ at 10 shrimp m^{-2} in 57d of *F. duorarum* continuous water exchange culture. In the present study, growth rate in both treatments was ~0.2 g $week^{-1}$, fourfold lower than expected for most penaeid species (Fig. 3). On the other hand, the best growth rate was performed by FLOC treatment in month 1 with ~0.5 g $week^{-1}$, similar to Lopez-Tellez et al. (2000) that used low stocking density.

BFT has been demonstrated benefits for many aquaculture species in terms of growth and production such as in *L. vannamei* (Burford et al. 2003, 2004; Wasielesky et al. 2006), *L. setiferus* (Emerenciano et al. 2009), *P. monodon* (Arnold et al. 2009), *Litopenaeus stylirostris* (Emerenciano et al. 2011a), *F. brasiliensis* (Emerenciano et al. 2012), *F. paulensis* (Ballester et al. 2010; Emerenciano et al. 2011b), *Macrobrachium rosenbergii* (Crab et al. 2010) and tilapia (Avnimelech 2007, Azim and Little 2008; Crab et al. 2009). The better adaptation of species to BFT seems to be related to the tolerance of high stocking densities, solids and N compounds, capacity to ingest–digest detritus and microbial particles, as well as presence of an adequate morphological apparatus to capture the microbial aggregates.

Water quality parameters did not differ between treatments and levels were within acceptable ranges for most penaeid species (Wickins 1976; Van Wyk and Scarpa 1999). Although no significant differences were found, FLOC seems to be more efficient to control daily temperature fluctuations when compared to CW (Fig. 2). This finding

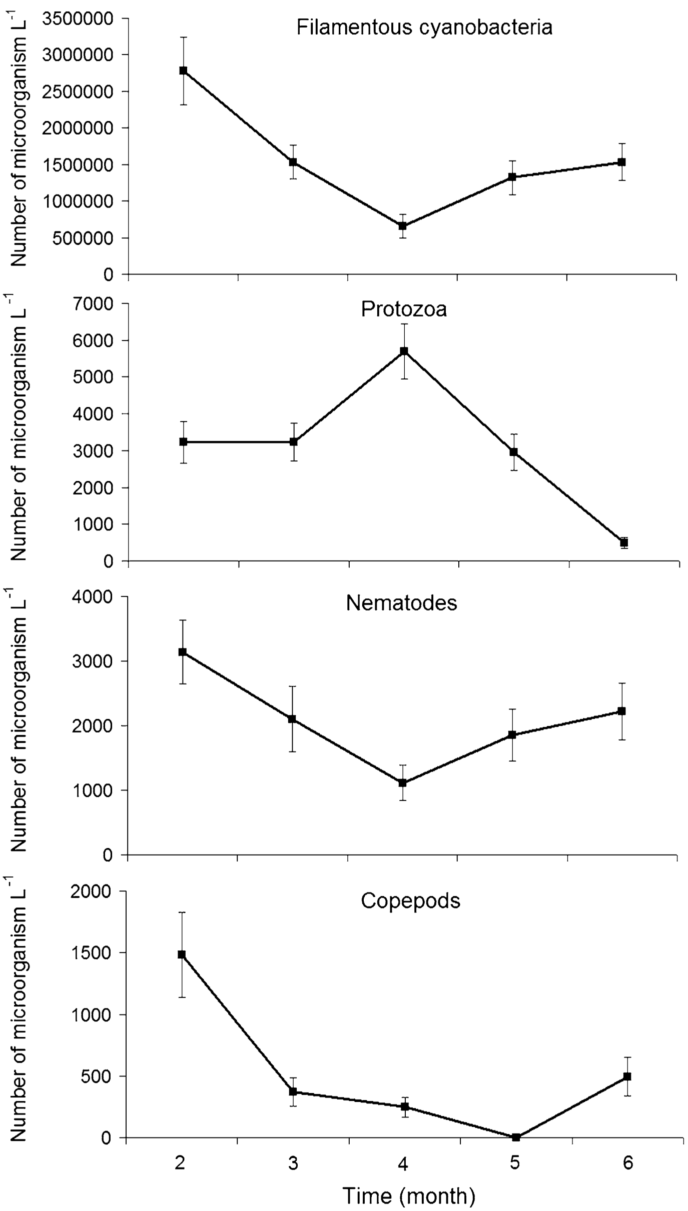

Fig. 4 Microorganisms assessment (mean ± SE) in biofloc (FLOC) tanks during *F. duorarum* grow-out

corroborates with Crab et al. (2009) and also with Emerenciano et al. (2011a) that proposed BFT as a system to control water quality fluctuations typical presented year-round in ponds.

Control of solids in BFT is closely related to optimum levels of DO and inorganic N compounds (Vinatea et al. 2010). High levels of solids were observed at the end of the experiment (17 mL L^{-1}) in FLOC treatment, partially due to the high temperatures

Table 3 Proximate analysis of biofloc performed during *F. duorarum* grow-out

Month	Crude protein (%)	Crude lipid (%)	Crude fiber (%)	Carbohydrates (%)	Ash (%)	Gross energy (kJ g^{-1} DW)
2	25.3	0.71	2.1	27.6	44.4	10.8
3	18.2	0.40	3.0	26.7	51.8	8.9
4	27.1	0.66	1.7	26.7	43.7	11.1
5	25.0	0.74	3.5	22.8	48.0	10.0
6	23.4	0.73	1.5	23.8	50.5	9.8
7	29.3	0.59	2.5	29.9	45.3	10.4
Means (± SD)	24.7 ± 3.8	0.6 ± 0.1	2.4 ± 0.8	26.3 ± 2.6	47.3 ± 3.4	10.2 ± 0.8

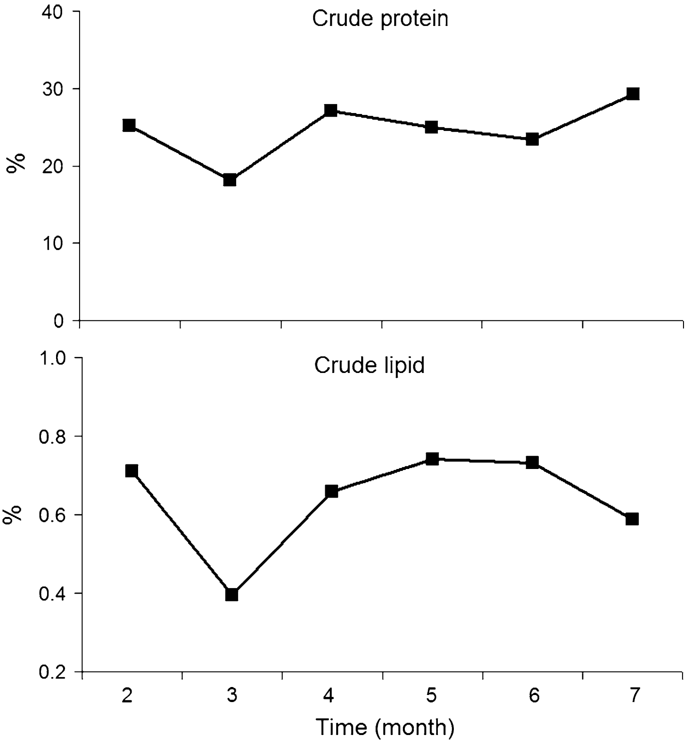

Fig. 5 Crude protein and crude lipid variation (%) measured in biofloc (FLOC) tanks during *F. duorarum* grow-out

(~28 °C). As a result, gills of shrimp were clogged and mortalities detected, suggesting negative effects in survival. Taw (2010) recommended FV below 15 mL L^{-1} for *L. vannamei* reared in outdoor lined ponds. In addition, Ray et al. (2010b) demonstrated benefits on shrimp yields by controlling solids with a simple settling low-tech solids removal device. Our FV values certainly affected *F. duorarum* performance.

Some species are more susceptible to high stocking density than others as a result of their natural behavior, that is, burrowing in close-telicum species. At high density occur negative behavioral interactions leading to cannibalism that could drop survival rates (Arnold et al. 2005). In wild, adults of *F. duorarum* are often observed in deep waters (Gracia 1995), suggesting that this species could be more sensitive to N compounds when compared to other penaeid species. Moreover, *F. duorarum* posses a strong burrowing behavior, as observed in other close-telicum species (Penn 1981). These findings suggest that a "sum of factors" such as an adequate temperature, stocking density and control of solids levels ($\sim$5–10 mL L^{-1}) could enhance *F. duorarum* performance in BFT. Thus, three major points raised with poor performance of *F. duorarum*: (1) species not domesticated (G1); after several generations (i.e., >G9), weight gain could be enhanced by a process of selection (Goyard et al. 2002); (2) susceptibility to high stocking density suggesting, that is, the use of artificial substrates (sand, AquaMats^{TM}, etc.) to enhance shrimp performance (Moss and Moss 2004; Ballester et al. 2007; Asaduzzaman et al. 2008; Arnold et al. 2009); and finally (3) poor tolerance to N compounds and suspended solids when compared to other penaeid species (Wickins 1976).

Microorganisms profile and proximate analysis of biofloc

Filamentous cyanobacteria (FC) compete by substrate with other microorganisms such as phytoplankton, in which antagonistic substances are excreted and inhibitory effects can occur (Yusoff et al. 2002; Hargreaves 2006). In addition, FC take competitive advantage in water with high P and N concentrations (Yusoff et al. 2002; Burford et al. 2003) and high turbidity (Case et al. 2008). Organic carbon addition also contributes to cyanobacteria growth (Esteves 1998), as well as protozoa (Thompson et al. 1999). On the other hand, small bacteria (i.e., cocoids) seem to take advantage on nutrients assimilation due to their small size and higher surface:volume ratio (Suita 2009). In the present study, high concentration of FC in the initial period could be related to a more availability of organic carbon and, while biofloc did not achieve high concentrations, due to the light penetration (Kirchman 2008).

A decrease in FC concentration over time was followed by nematodes and copepods (Fig. 4), suggesting a predation or substrate competition with small bacteria and other microorganisms (Kirchman 2008). In addition, high concentration of protozoa was related to the low concentration of FC and nematodes in month 4, also suggesting an inverse relationship between these groups. Protozoa act as predators of phytoplankton, fungi and bacteria, or as a food source for metazoans (Nagano and Decamp 2004). Bacteria and protozoa play a key role in the energy budge of microbial community, suggesting an alternative flow of nutrients into higher trophic levels well described as "microbial loop" (Pomeroy and Wiebe 1988). Predominance in certain group of protozoa (i.e., ciliates *vs.* flagellates) could be caused, that is, by salinity variation (Pedrós-Alió et al. 2000; Decamp et al. 2003; Maicá et al. 2012). Both cyanobacteria and protozoa (Bombeo-Tuburan et al. 1993; Decamp et al. 2001; Gamboa-Delgado et al. 2003), as well as nematodes and copepods (Soares et al. 2004; Rajkumar and Kumaraguru-Vasagam 2006; Ballester et al. 2007), are well known as natural food items for farmed organisms.

Some factors could modify the nutritional quality of biofloc such as salinity (Ekasari et al. 2010; Maicá et al. 2012), carbon source (Crab et al. 2010; Ekasari et al. 2010), changes in microbial community (Ray et al. 2010a), as well as light source and intensity (Coyle et al. 2011). In the present study, proximate analysis showed crude protein and crude lipid reached 24.7 and 0.6 %, respectively. These values varied throughout the experiment with low levels observed in month 3 (Fig. 5), which was corroborated with relative low concentration of copepods, nematodes and FC. Microorganism dynamics certainly affect the nutritional quality of biofloc, and consequently, further shrimp growth (Ray et al. 2010a). More research is needed in this field.

Conclusion

Although biofloc particles presented an added value to be used as shrimp food, in our experimental conditions, BFT system did not improve growth performance of *F. duorarum* when compared to conventional clear-water water exchange system. Furthermore, *F. duorarum* seems to be susceptible to high stocking density and high levels of suspended solids (>15 mL L^{-1}). Use of advanced generations (i.e., >G9), artificial substrates and rigorous control of suspended solids should be evaluated aiming to enhance *F. duorarum* performance during grow-out phase.

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References

AOAC (2000) Official methods of analysis, 16th edn. AOAC International, Washington

Arnold SJ, Sellars MJ, Crocos PJ, Coman GJ (2005) Response of juvenile brown tiger shrimp (*Penaeus esculentus*) to intensive culture conditions in a flow through tank system with three-dimensional artificial substrate. Aquaculture 246:231–238

Arnold SJ, Coman FE, Jackson CJ, Groves SA (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

Arreguin-Sanchez F, Zetina-Rejón M, Ramírez-Rodríguez M (2008) Exploring ecosystem-based harvesting strategies to recover the collapsed pink shrimp (*Farfantepenaeus duorarum*) fishery in the southern Gulf of Mexico. Ecol Model 214:83–94

Arreguín-Sánchez F, Schultz-Ruíz LE, Gracia A, Sánchez JA, Alarcón T (1997) Las pesquerías de camarón de altamar, explotación, dinámica y evaluación. In: Flores-Hernández D, Sánchez-Gil P, Seijo JC, Arreguín-Sánchez F (eds) Análisis y diagnóstico de los recursos pesqueros críticos del Golfo de México, EPOMEX Serie Científico 7. Universidad. Autónoma de Campeche, Campeche, pp 145–172

Asaduzzaman M, Wahab MA, Verdegem MCJ, Huque S, Salam MA, Azim ME (2008) C/N ratio control and substrate addition for periphyton development jointly enhance freshwater prawn *Macrobrachium rosenbergii* production in ponds. Aquaculture 280:117–123

Avnimelech Y (1999) Carbon and 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 bioflocs technology ponds. Aquaculture 264:140–147

Avnimelech Y (2012) Biofloc technology, a practical guide book (2nd ed.). The world. Aquaculture Society, Baton Rouge, Louisiana, EUA

Avnimelech Y, Kochva M, Diab S (1994) Development of controlled intensive aquaculture systems with limited water exchange and adjusted carbon to nitrogen ratio. Bamdigeh 46:119–131

Azim ME, Little DC (2008) The biofloc technology (BFT) in indoor tanks: water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). Aquaculture 283:29–35

Ballester ELC, Wasielesky W Jr, Cavalli RO, Abreu PC (2007) Nursery of the pink shrimp *Farfantepenaeus paulensis* in cages with artificial substrates: biofilm composition and shrimp performance. Aquaculture 265:355–362

Ballester ELC, Abreu PC, Cavalli RO, 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. Aquac Nut 16:163–172

Barnes RD (1963) Invertebrate zoology. W. B. Saunders, London

Bombeo-Tuburan I, Guanzon NG Jr, Schroeder GL (1993) Production of *Penaeus monodon* (Fabricius) using four natural food types in an extensive system. Aquaculture 112:57–65

Burford MA, Thompson PJ, Bauman RH, Pearson DC (2003) Nutrient and microbial dynamics in high-intensive, zero-exchange shrimp ponds in Belize. Aquaculture 219:393–411

Burford MA, Thompson PJ, McIntosh RP, Bauman RH, Pearson DC (2004) The contribution of flocculated material to shrimp (*Litopenaeus vannamei*) nutrition in a high-intensity, zero-exchange system. Aquaculture 232:525–537

Case M, Leca EE, Leitao SN, Sant'Anna EE, Schwamborn R, de Moraes AT Jr (2008) Plankton community as an indicator of water quality in tropical shrimp culture ponds. Mar Pollut Bull 56:1343–1352

Coyle SD, Bright LA, Wood DR, Neal RS, Tidwell JH (2011) Performance of Pacific White Shrimp, *Litopenaeus vannamei*, reared in zero-exchange tank systems exposed to different light sources and intensities. J World Aquac Soc 42:687–695

Crab R, Kochva M, Verstraete W, Avnimelech Y (2009) Bio-flocs technology application in over-wintering of tilapia. Aquac Eng 40:105–112

Crab R, Chielens B, Wille M, Bossier P, Verstraete W (2010) The effect of different carbon sources on the nutritional value of bioflocs, a feed for *Macrobrachium rosenbergii* postlarvae. Aquac Res 41:559–567

Cripe G (1994) Induction of maturation and spawning of pink shrimp, *Penaeus duorarum,* by changing water temperature, and survival and growth of young. Aquaculture 128:255–260

Decamp O, Moss S, Nagano N (2001) Live protozoa: suitable live food for larval fish and shrimp? Glob Advocate 4:28–29

Decamp O, Cody J, Conquest L, Delanoy G, Tacon AGJ (2003) Effect of salinity on natural community and production of Litopenaeus vannamei (Boone) within experimental zero-water exchange culture systems. Aquac Res 34:345–355

Ekasari J, Crab R, Verstraete W (2010) Primary nutritional content of bio-flocs cultured with different organic carbon sources and salinity. HAYATI J Biosci 17:125–130

Emerenciano MGC, Wasielesky W, Soares RB, Ballester EC, Cavalli RO, Izeppi EM (2007) Crescimento e sobrevivêcia do camarão-rosa Farfantepenaeus paulensis na fase de berçário em meio heterotrófico. Acta Sci Biol Sci 29:1–7

Emerenciano M, Vinatea L, Gálvez AO, Shuler A, Stokes A, Venero J, Haveman J, Richardson J, Thomas B, Leffler J (2009) Effect of two different diets fish meal based and "organic" plant based diets in *Litopenaeus setiferus* earlier postlarvae culture under bio-floc, green-water and clear-water conditions. CD of abstracts World Aquaculture Society Meeting 2009, Veracruz, Mexico

Emerenciano M, Cuzon G, Goguenheim J, Gaxiola G, Aquacop (2011a) Floc contribution on spawning performance of blue shrimp *Litopenaeus stylirostris. Aquaculture Research* (published online first doi: 10.1111/j.1365-2109.2011.03012.x)

Emerenciano M, Ballester ELC, Cavalli RO, Wasielesky W (2011b) Effect of biofloc technology (BFT) on the early postlarval stage of pink shrimp *Farfantepenaeus paulensis*: growth performance, floc composition and salinity stress tolerance. Aquac Int 19:891–901

Emerenciano M, Ballester ELC, Cavalli RO, Wasielesky W (2012) Biofloc technology application as a food source in a limited water exchange nursery system for pink shrimp *Farfantepenaeus brasiliensis* (Latreille, 1817). Aquac Res 43:447–457

Esteves FA (1998) Fundamentos de limnologia, 2nd edn. Interciência, Rio de Janeiro

Gamboa-Delgado J, Molina-Poveda C, Cahu C (2003) Digestive enzyme activity and food ingesta in juvenile shrimp *Litopenaeus vannamei* (Boone, 1931) as a function of body weight. Aquac Res 34:1403–1411

Goyard E, Patrois J, Peignon J, Vanaa V, Dufour R, Viallon J, Bedier E (2002) Selection for better growth of *Penaeus stylirostris* in Tahiti and New Caledonia. Aquaculture 204:461–468

Gracia A (1995) Impact of artisanal fishery on production of the pink shrimp Penaeus *Farfantepenaeus duorarum* Burkenroad, 1939. Ciencias Marinas 21:343–359

Gracia A (1997) Pesquería artesanal de camarón. In: Flores-Hernández D, Sánchez-Gil P, Seijo JC, Arreguín-Sánchez F (eds) Análisis y diagnóstico de los recursos pesqueros críticos del Golfo de México, EPOMEX, Serie Científico 7. Universidad. Autónoma de Campeche, Campeche, pp 173–184

Gullian M, Aramburu C, Sanders B, Lope R (2010) Viability of culturing pink shrimp *Farfantepenaeus duorarum* in low-salinity groundwater from the Yucatán Peninsula (SE, México). Aquaculture 302:202–207

Hargreaves JÁ (2006) Photosynthetic suspended-growth systems in aquaculture. Aquac Eng 34:344–363

Izquierdo M, Forster I, Divakaran S, Conquest L, Decamp O, Tacon A (2006) Effect of green and clear water and lipid source on survival, growth and biochemical composition of Pacific white shrimp *Litopenaeus vannamei*. Aquac Nut 12:192–202

Jory DE (2001) Feed management practices for a healthy pond environment. In: The new wave, proceedings of the special session on sustainable shrimp culture (ed. by Browdy CL, Jory DE), Aquaculture 2001. The World Aquaculture Society, Baton Rouge, USA, pp 118–143

Ju ZY, Forster I, Conquest L, Dominy W, Kuo WC, Horgen FD (2008) Determination of microbial community structures of shrimp floc cultures by biomarkers and analysis of floc amino acid profiles. Aquac Res 39:118–133

Kirchman DL (2008) Microbial ecology of the oceans, 2nd edn. Wiley-Liss, NY

Krummenauer D, Peixoto S, Cavalli RO, Poersch LH, Wasielesky W (2011) Superintensive culture of white shrimp, *Litopenaeus vannamei*, in a biofloc technology system in southern brazil at different stocking densities. J World Aquac Soc 42:726–733

López-Téllez N, Hernández-Rodríguez J, Ramírez-Ligonio H, Seca-Escalante J (2000) Crecimiento del camarón rosado *Farfantepenaeus duorarum* en estanques rústicos. Ciencia Pesquera 14:29–32

Maicá PF, Borba MR, Wasielesky W (2012) Effect of low salinity on microbial floc composition and performance of *Litopenaeus vannamei* (Boone) juveniles reared in a zero-water-exchange super-intensive system. Aquac Res 43:361–370

McIntosh D, Samocha TM, Jones ER, Lawrence AL, McKee DA, Horowitz S, Horowitz A (2000) The effect of a bacterial supplement on the high-density culturing of *Litopenaeus vannamei* with low-protein diet in outdoor tank system and no water exchange. Aquac Eng 21:215–227

Moss KRK, Moss SM (2004) Effects of artificial substrate and stocking density on the nursery production of pacific white shrimp *Litopenaeus vannamei*. J World Aquac Soc 35:537–542

Muangkeow B, Ikejima K, Powtongsook S, Gallardo W (2011) Growth and nutrient conversion of white shrimp *Litopenaeus vannamei* (Boone) and Nile tilapia *Oreochromis niloticus* L. in an integrated closed recirculating system. Aquac Res 42:1246–1260

Nagano N, Decamp O (2004) Ingestion of a ciliated protozoa by first-feeding larval stage of pacific white shrimp *Litopenaeus vannamei* (Boone). Aquac Res 35:516–518

Pedrós-Alió C, Calderon-Paz JI, MacLean MH, Medina G, Marrasé C, Gasol JM, Guixa-Boixereu N (2000) Themicrobial food web along salinity gradients. Microb Ecol 32:143–155

Peixoto S, Wasielesky W, Cavalli RO (2011) Broodstock maturation and reproduction of the indigenous pink shrimp *Farfantepenaeus paulensis* in Brazil: an updated review on research and development. Aquaculture 315:9–15

Penn JW (1981) The behavior and catchability of some commercially exploited penaeids and relationship to stock and recruitment. In: Penaeid shrimp—their biology and Management (ed. by Gulland and Rothschild), pp 173–186, Fishing News Books Ltd., Farnham

Pérez-Farfante I (1969) Western Atlantic shrimps of the genus *Penaeus*. Fish Bull 67:461–591

Pomeroy LR, Wiebe WJ (1988) Energetics of microbial food webs. Hydrobiologia 159:7–18

Rajkumar M, Kumaraguru-Vasagam KP (2006) Suitability of the copepod, *Acartia clausi as* a live feed for seabass larvae (*Lates calcarifer* Bloch): compared to traditional live-food organisms with special emphasis on the nutritional value. Aquaculture 261:649–658

Ray AJ, Seaborn G, Leffler JW, Wilde SB, Lawson A, Browdy CL (2010a) Characterization of microbial communities in minimal-exchange, intensive aquaculture systems and the effects of suspended solids management. Aquaculture 310:130–138

Ray AJ, Lewis BL, Browdy CL, Leffler JW (2010b) 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

Samocha TM, Patnaik S, Speed M, Ali AM, Burger JM, Almeida RV, Ayub Z, Harisanto M, Horowitz A, Brock DL (2007) Use of molasses as carbon source in limited discharge nursery and grow-out systems for *Litopenaeus vannamei*. Aquac Eng 36:184–191

Samocha TM, Gandy RL, Morris TC, Patnaik S, Kim JS, Davis AD, Richardson JR, Browdy CL (2008). Development of viral pathogen free broodstock populations of the Atlantic pink shrimp *Farfantepenaeus duorarum* and the Atlantic white shrimp *Litopenaeus setiferus*. Abstract of Aquaculture America 2008, pp 399, World Aquaculture Society Meeting, Florida, USA

Smith LL, Beidenbach JM, Lawrence AL (1992). Penaeid larviculture: Galveston method. In: *Marine Shrimp Culture: Principles and Practices* (ed. by Fast A.W. & Lester J.L.), pp. 171–191, Elsevier Science Publishers, Amsterdam, the Netherlands

Soares R, Peixoto S, Bemvenuti C, Wasielesky W, D'Incao F, Murcia N, Suita S (2004) Composition and abundance of invertebrate benthic fauna in *Farfantepenaeus paulensis* culture pens (Patos Lagoon estuary, Southern Brazil). Aquaculture 239:199–215

Sokal R, Rohlf J (1995) Biometry, the principles and practice of statistics in biological research. WH Freeman, New York

Suita SM (2009) O uso da dextrose como fonte de carbono no desenvolvimento de bio-flocos e desempenho do camarão-branco (*Litopenaeus vannamei*) cultivado em sistema sem renovação de água. Dissertação de mestrado, Universidade Federal do Rio Grande, 44p

Tacon AGJ (1990) Standard methods for the nutrition and feeding of farmed fish and shrimp. Argent Laboratories Press, Washington

Taw N (2010) Biofloc technology expanding at white shrimp farms. *Global Advocate* may/june, 24–26 (available in http://www.gaalliance.org/mag/May_June2010.pdf)

Thompson FL, Abreu PC, Cavalli RO (1999) The use of microorganisms for water quality and nourishment in intensive shrimp culture. Aquaculture 203:263–278

Thompson FL, Abreu PC, Wasielesky W (2002) Importance of biofilm for water quality and nourishment in intensive shrimp culture. Aquaculture 203:263–278

Van Wyk P, Scarpa J (1999) Water quality requirements and management. In: Van Wyck P (ed) Farming marine shrimp in recirculating freshwater systems. Florida Department of Agriculture and Consumer Services, Tallahasee, pp 128–138

Vinatea L, Gálvez AO, Browdy CL, Stokes A, Venero J, Haveman J, Lewis BL, Lawson A, Shuler A, Leffler JW (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. Aquac Eng 42:17–24

Wasielesky W Jr, Atwood H, Stokes A, Browdy CL (2006) Effect of natural production in a zero exchange suspended microbial floc based super-intensive culture system for white shrimp *Litopenaeus vannamei*. Aquaculture 258:396–403

Wickins JF (1976) The tolerance of warm-water prawns to recirculated water. Aquaculture 9:19–37

Wimpenny RS (1966) The plankton of the sea. Faber and Fabe, London

Yusoff FM, Zubaidah MS, Matias HB, Kwan TS (2002) Phytoplankton succession in intensive marine shrimp culture ponds treated with a commercial bacterial product. Aquac Res 33:269–278

Zar JH (1984) Biostatistical analysis, 2nd edn. Prentice Hall, Englewood Cliffs