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Full Length Research Paper

Cultivation of black tiger shrimp *Penaeus monodon* (Fabricius 1798) at the nursery stage in biofloc technology (BFT) system

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A 120-day experiment was conducted to evaluate the performance of *Penaeus monodon* postlarvae (PL) reared during the nursery phase in a BFT limited water exchange system. The PL25 (0.19 ± 0.01g) were divided into two treatments: clear water (CW) and Biofloc system (BFT) at a stock density of 200 PL m^{-2}. Three replications were applied in each treatment. The PLs were fed three times per day applying a 38% commercial crude protein diet. Significant differences ($P < 0.05$) were found between treatments in all water quality parameters except in terms of water temperature. The BFT treatment showed higher water quality parameters with the exception of dissolved oxygen. The mean final weight, growth rate and biomass in the BFT treatment was higher than the CW treatment ($P < 0.05$); however, survival did not differ between treatments ($P < 0.05$). FCR was lower ($P < 0.05$) in the BFT treatment than the CW treatment. Results indicate that a BFT system improved the growth performance of *P. monodon* and maintained the water quality within a range which is considered optimal for the species.

Keywords: BFT, *Penaeus monodon*, postlarvae, water quality, growth performance.

INTRODUCTION

The Black tiger shrimp *Penaeus monodon* is the most valuable aquaculture species in Mozambique. Native to the Indo-West Pacific Ocean, from the eastern coast of Africa, South-East Asia and Northern Australia, *P. monodon* is one of the largest penaeid shrimp in the world reaching 260 mm in body length or 250 g in weight (Motoh 1981).

For years, the shrimp trade has been the most important internationally traded fishery commodity in terms of value and the most valuable fishery export for many tropical developing countries (Bondad-Reantaso et al. 2012). Therefore, in the last decade, aquaculture has gained the main force behind the increased shrimp trade in the world.

The rapid expansion of the shrimp farming industry has however generated environmental problems such as eutrophication of receiving streams with potential introduction of pathogens and the development of antibiotic resistance of pathogens in natural waters. In that context, the industry has been criticized by a number of organizations as being environmentally irresponsible and fast becoming an issue of great concern (Cowey and Cho, 1991).

Biofloc technology (BFT) systems is based on zero or minimal water exchange created in order to maximize bio-

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security while minimizing external environmental effects in high animal stocking densities (Emerenciano et al. 2013). The BFT is a new concept in Mozambique, which can represent an alternative to overcoming the problems of biosecurity, such as viral diseases that particularly shrimp aquaculture faces at present. Growing shrimp through BFT was proposed as a tool to minimize the introduction of viral pathogens through incoming water. Additionally, observations have been made on positive effects of BFT in reducing viral disease outbreaks (Avnimelech, 2012).

The great benefit of the BFT is the capability to rinse animals at high stocking densities generating a large amount of nutrients in the water from feedings with limited exchanging of water. As a consequence, those nutrients accumulate in the systems, contributing to the proliferation of a community of microscopic organisms. This microbial community functions to remove excess nutrients, mineralize wastes, improve protein utilization and reduce opportunities for the dominance of pathogenic strains (Avnimelech 1999, 2012; De Schryver et al. 2008). As a result, BFT results in high levels of biosecurity; lower feed conversion ratios; the possibility of intensive animal culture with potential for indoor operation; reduced costs for water treatment; and the potential for the inland production of marine species (Burford et al. 2004; Wasielesky et al. 2006; Emerenciano et al. 2013).

Hence, the BFT is an emerging technology mainly developed for grow-out ponds, which could be easily applied as a management tool in the nursery phase (Emerenciano et al. 2011). The nursery phase is defined as the intermediate step between the early post larval (PL) stage and the grow-out phase (Mishra et al. 2008). This phase is usually characterized by high water renewal rates, high stocking densities, and the use of high quality artificial diets (Speck et al., 1993 *apud* Mishra et al. 2008). Studies of different species of penaeid shrimp have reported several benefits from the incorporation of a nursery phase in the shrimp production cycle (Arnold et al. 2009, Fóes et al. 2011, Emerenciano et al. 2012, Viau et al. 2013).This provides improvements of feed conversion and survival, eliminating the need to overstock in anticipation of high mortality, more robust, healthy and uniform shrimp juveniles at harvest, tolerance of shrimp to environmental fluctuations as well as improves feeding efficiencies and enhanced growth performance (Mishra et al. 2008; Emerenciano et al. 2011; Viau et al. 2013; Wasielesky et al. 2013). Nurseries have also been used as a biosecurity measure to mitigate losses caused by diseases (Fóes et al. 2011). Therefore, the aim of this study was to evaluate the performance of *Penaeus monodon* postlarvae reared during the nursery phase in a BFT limited water exchange system.

MATERIALS AND METHODS

Study area

The study was carried out at AQUAPESCA Farm, located in Inhassunge, Zambézia province, Mozambique (18^{o}4´57.353´´S and 36^{o}49´30.098´´W).

Twenty-fiveday-old post-larvae Penaeus monodon (PL25 0.19 ± 0.01 g) used in this study were produced in the breeding laboratory of AQUAPESCA.

Biofloc production

Before starting the trial, a 20000 L (20 tons) concrete tank was used for biofloc production under limited water exchange conditions (matrix tank). The tank was inoculated with diatom (*Thalassiosira weissflogii* at 5*10^4 cells. mL^{-1}) and stocked with 15.000 PL15 (500 PL.m^{-2}).

Experimental design

The PL25 were distributed in a completely randomized experimental design with two treatments: clear water (CW) and Biofloc system (BFT), applying three replications per treatment. The shrimp were stocked at a density of 200 PL m^{-2} and reared for 120 days. The experimental units consisted of six 400 L rectangular polyethylene tanks (bottom surface area of 1.2 m^2). A central aeration (aero-tubeTM) was provided to maintain the solids in suspension and to ensure that dissolved oxygen remained at a saturation level. Each BFT experimental unit was filled with matrix tank water. The BFT treatment was operated with zero water exchange; dechlorinated freshwater was only added to compensate losses from sludge removal and evaporation. In the CW treatment, seawater was previously filtered in a sand filter (8 µm). The CW treatment was designed to simulate an intensive cultivation with regular renewal of cultivation of water at a daily average rate of 10%.

Culture conditions

Each experimental unit was stocked with 240 shrimps at a density of 200 PLm^{-2}.The shrimp were fed three times a day (07:00, 13:00 and 17:00 h) with commercial feed (38 % crude protein, Livestock Feed Ltd, Mauritius). The daily feeding rate was 9% body weight at the start of experiment, and declined gradually to 3% body weight at the end of trial. Sugarcane molasses was added as carbon source in C:N ratio of 20:1 to optimize heterotrophic bacteria growth (Avnimelech 1999).

Water quality parameters analyses

Dissolved oxygen, water temperature (model 85, YSI Inc., Yellow Springs, OH, USA) and pH (pHTestr10, OAKTON Instruments, IL USA)were measured twice a day (07:00 and 17:00 h). Salinity (model 85, YSI Inc., Yellow Springs, OH, USA)was measured daily. Total ammonia nitrogen, TAN (Salicylate Method-10031), nitrite (Diazotization Method-8507), nitrate(Cadmium Reduction Method-8039)total phosphorus (Ascorbic Acid Method-10210) and total suspended solids (Photometric Method-8006) were measured weekly using DR 2800 Spectrophotometer – HACH. To maintain the pH levels at values higher than 7, and alkaline levels above 120 mgL^{-1} calcium carbonate was added in both treatments.

Productive shrimp performance

During the study, biometric assessments were performed every two weeks. 50 shrimps were individually weighed from each experimental tank using digital scales(precision 0.001 g, Ohaus-Scout Pro ®) and returned to their tanks after weighing. At the end of the trial, all the shrimp that survived in each experimental tank were weighed and counted to evaluate their performance (final weight, average weight gain, survival, feed conversion ratio, final biomass and productivity).

Statistical analyses

After the homoscedasticity and normality of the data were verified, shrimp biological performance data were analyzed with one-way analysis of variance (ANOVA). Significant differences between treatments were evaluated using the Tukey test. Percentage data were transformed (arcsine of the square root) before their analysis. The differences were considered significant at 95%, and the results are presented as the mean ± standard deviation (SD).

RESULTS

Water quality

Water quality parameters are presented in Table 1. There were significant differences ($P < 0.05$) between treatments in all water quality parameters except for water temperature ($P>0.05$).Apart from dissolved oxygen, all water quality parameters were significantly higher in the BFT treatment.

Productive shrimp performance

The shrimp growth and yield parameters in both groups are presented in Table 2. After the 120-day experiment, the mean final weight, growth rate and biomass of shrimp in the BFT treatment was higher than in the CW treatment ($P <0.05$);however, survival did not differ between treatments ($P < 0.05$).The FCR value of the bioflocs treatment group was lower than that ofthe relative control group($P < 0.05$).

DISCUSSION

Despite differences in water quality parameters between treatments, all water quality parameters observed during the trial were within the acceptable limits for the survival and growth of *P. monodon* shrimp (Thakur and Lin 2003; Arnold et al. 2009; Shailender et al. 2012). Salinity was slightly higher in the BFT treatment compared with the CW treatment. This possibly occurred due to evaporation in BFT limited water exchange systems.

The mean values of TAN remained below toxic levels throughout the experimental period (Chen et al. 1990). The higher concentrations of ammonia, nitrite and nitrate observed in the BFT treatment compared with the CW treatment were likely due to the high input of nutrients. This also indicates that the microbial community has been successfully established and carried out intense processes of nitrification from chemoautotrophic bacteria as well as the conversion of ammonia into microbial protein (Ebeling and Timmons 2006). Similar to observations for nitrogenous compounds, the significantly higher levels of phosphorous in the BFT treatment may also be a result of the input of nutrients and the accumulation of particular organic matter. The buildup of phosphorous in the BFT treatment may also occur due to the lower assimilation of this nutrient by the bacterial community in comparison with systems where phytoplankton is predominant (Emerenciano et al. 2011). It is well known that in traditional pond-based aquaculture systems, phosphorous is normally retained in the sediment, However, in BFT systems where lined tanks with no sediment are commonplace, phosphorous tends to accumulate and is eventually dissolved in the water column.

The TSS values recorded in this study are lower than those observed by several studies with zero or limited water-exchange. Schveitzer et al. (2013) reported that TSS between 400 and 600 mgL^{-1} are more suitable to super intensive culture of *Litopenaeus vannamei*. The suitable concentration of TSS of *P. monodon* is unknown. In BFT systems, reduced water exchange, high organic matter input, and high growth rates of heterotrophic bacteria contribute to an increase in TSS (Van Wyk 2004). The consequences of high TSS concentrations include poor water quality, reduced growth rates, lower feed efficiency, changes in the biofloc composition and negative effects on health of the cultivated organisms (Van Wyk 2004; Hargreaves 2006; Vinatea et al. 2010). During visual observation of the shrimp, the presence of animals with brownish gills was noted, deemed to be a product of solid filtration in the water; but no mortality was however related to the TSS.

Compared to conventional technologies used in aquaculture, BFT provides a more economical alternative regarding the use of water. In our study, the water exchange was done regularly in the CW treatment and reached an average daily percentage of 10.3%, versus 0.3% in the BFT treatment (addition of water to compensate for the losses by evaporation). The water consumption in the BFT treatment was equivalent to 15 liters per kg of shrimp produced, which is significantly

Table 1. Water quality variables of *P. monodon* over the 120-days period.

Parameter		BFT	CW
Temperature (°C)	am	26.5 ± 1.2	26.4 ± 1.2
	pm	27.6 ± 1.2	27.5 ± 1.1
Oxygen (mgL^{-1})	am	6.06 ± 0.42^b	6.36 ± 0.28^a
	pm	6.08 ± 0.46^b	6.32 ± 0.29^a
pH	am	7.48 ± 0.35^a	7.26 ± 0.26^b
	pm	7.49 ± 0.28^a	7.32 ± 0.20^b
Salinity (gL^{-1})		31.40 ± 1.37^a	30.19 ± 2.65^b
TAN (mgL^{-1})		1.17 ± 0.60^a	0.58 ± 0.30^b
NO_2^-N (mgL^{-1})		7.38 ± 4.36^a	1.19 ± 2.50^b
NO_3-N (mgL^{-1})		21.50 ± 11.34^a	9.89 ± 8.80^b
PO_4^{-3} (mgL^{-1})		31.30 ± 12.63^a	13.68 ± 8.45^b
TSS (mgL^{-1})		192.90 ± 101.71^a	40.13 ± 19.88^b

Within rows, different superscript letters indicate significant differences ($P < 0.05$)
BFT: Biofloc treatment; CW: Clear Water treatment; TSS: Total Suspended Solids.

Table 2. Growth performance parameters of *P. monodon* over the 120-day period.

Parameter	BFT	CW
Mean final weight (g)	16.14 ± 0.30^a	11.1 ± 0.31^b
Growth rate (g $week^{-1}$)	0.56 ± 0.24^a	0.41 ± 0.15^b
Biomass (Kgm^{-2})	2.79 ± 0.10^a	1.35 ± 0.36^b
FCR	1.51 ± 0.01^b	1.81 ± 0.06^a
Survival (%)	86.00 ± 2.20	60.40 ± 15.20

Initial weight: 0.19 ± 0.01g
Within rows, different superscript letters indicate significant differences ($P < 0.05$)
BFT: Biofloc treatment; CW: Clear Water treatment; FCR: feed conversion rate.

lower than in the case of shrimp produced in the CW treatment which required 120 liters per kg.

In our study, all growth performances were better in BFT treatments when compared with the CW treatment (*P < 0.05*) which shows that *P. monodon*in nursery phase can well utilize the additional protein derived from the BFT system.The BFT is considered as a suitable approach for sustainable and efficient aquaculture production of high shrimp biomass. The utilization of microbial protein depends on the ability of the target animal to harvest the bacteria and its ability to digest and utilize the microbial protein (Avnimelech 1999). Results from extensive pond and tank trials indicate that the juvenile *P. monodon* utilize heterotrophic bacteria (a major component of microbial floc) as a protein source (Hari et al. 2004, 2006).Burford et al. (2004)and Wasielesky et al. (2006)demonstrated that the microbial floc from the heterotrophic culture system is a significant nutrient source for juvenile *Litopenaeus vannamei*.

The FCR was lower in the BFT treatment (1.51 ± 0.01) compared to the CW (1.81 ± 0.06). Wasielesky et al.(2006) had already indicated that natural productivity in BFT system can significantly improve FCR. In the present study, the growth rate was 0.56 g and 0.41g in 120 days, producing a biomass of 2.79 and 1.35 kgm^{-2} in BFT and CW treatments respectively.A relatively high growth rate (0.85 g in 30 days) has been achieved at a stocking density of 400 m^{-3}producing a biomass of 0.23 kg m^{-3}(Yusufzai and Singh 2005). Arnold et al. (2006)obtained agrowth rate of 0.77 g in 56 days at stocking densities up to 2000 $m^{-3}$and biomass of 1.27 kgm^{-3}. Despite the high biomass obtained in our study, our growth rates are lower when compared with the rates obtained by the researchers cited above.

During the larval and nursery phases, survival is considered the most important parameter for culturing success.Speck et al. (1993) compared stocking densities of *Farfantepenaeus paulensis* postlarvae (150, 300, and 600 shrimp.m^{-2}) in an indoor nursery and obtained survival rates of 85%, 84%, and 16%, respectively. Emerenciano et al. (2007) compared an *F. paulensis* nursery in a BFT culture system with and without a feed supply with an *F. paulensis* nursery in a conventional system with water exchange (100% per day) and they were no significant differences found in the survival rates under these conditions (93.7%, 93.2%, and 82.2%, respectively). The survival of 86% obtained in BFT treatment is within the range obtained by other authors(Arnold et al. 2006; Baloi et al. 2013; Schveitzer et al. 2013; Esparza-leal et al. 2015)applying the BFT system, demonstrating the stability of this system in *P.monodon* shrimp farming during the nursey phase.

The study demonstrates that the use of a BFT culture system may enable the culture of this species in nursey phase.

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REFERENCES

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.

Arnold SJ, Sellars MJ, Crocos PJ, Coman GJ (2006). Intensive production of juvenile tiger shrimp *Penaeus monodon*: An evaluation of stocking density and artificial substrates. Aquaculture 261:890–896.

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

Avnimelech Y (2012). Biofloc Technology - a practical guide book, 2nd Ed. The World Aquaculture Society Baton Rouge, Louisiana, USA, pp 258.

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. Aquac Eng 52:39–44.

Bondad-Reantaso MG, Subasinghe RP, Josupeit H Cai J, Zhou X (2012). The role of crustacean fisheries and aquaculture in global food security: past, present and future. J Inverteb. Pathol. 110: 158–165.

Burford MA, ThompsonPJ, 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.

Chen JC, Liu PC, Lei SC (1990). Toxicities of ammonia and nitrite to *Penaeus monodon* adolescents. Aquaculture 89:127–137.

Cowey CB, Cho CY (1991). Nutritional strategies and aquaculture waste. in Cho CY, Cowey CB, editors. Proceedings of the 1st International Symposium on Nutritional Strategies in Management of Aquaculture Wastes using biological approaches. University of Guelph, Ontario, Canada.

De Schryver P, 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 JM, Timmons MB, Bisogni JJ. (2006). Understanding photoautotrophic, autotrophic, and heterotrophic bacterial based systems using basic water quality parameters. In: Proceedings of the 6th International Conference on Recirculation Aquaculture. Roanoke, VA. 2006. p. 270-279.

Emerenciano M, Ballester ELC, Cavalli RO, Wasielesky W (2011). 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). Aquacult. Res. 43:447–457

Emerenciano M, Gaxiola G, Cuzon G (2013). Biofloc technology (BFT): a review for aquaculture application and animal food industry. In: Matovic MD (ed.) Biomass Now - Cultivation and Utilization, InTech, Queen's University, Belfast, Canada. pp.301–328.

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

Esparza-leal HM, Pereira A, Wasielesky W (2015).Performance of *Litopenaeus vannamei* postlarvae reared in indoor nursery tanks at high stocking density in clear-water versus biofloc system. Aquac Eng 68:28–34.

Fóes GK, Fróes C, Krummenauer D, Poersch L, Wasielesky W (2011). Nursery of Pink Shrimp *Farfantepenaeus paulensis* in Biofloc Technology Culture System: Survival and Growth at Different Stocking Densities. J Shellfish Res 30(2):367–373.

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

Hari B, Kurup BM, Varghese JT, Schrama JW, Verdegem MCJ (2004). Effects of carbohydrate addition on production in extensive shrimp culture systems. Aquaculture 241:179–194.

Hari, B Kurup BM, Varghese JT, Schrama JW, Verdegem MCJ (2006). The effect of carbohydrate addition on water quality and the nitrogen budget in extensive shrimp culture systems. Aquaculture 252:248–263.

Mishra JK, Samocha TM, Patnaik S, Speed M, Gandy RL, Ali AM (2008). Performance of an intensive nursery system for the Pacific white shrimp, *Litopenaeus vannamei,* under limited discharge condition. Aquac Eng 38:2–15.

Motoh H (1981). Studies on the fisheries biology of the giant tiger prawn, *Penaeus monodon* in the Philippines. (Technical Report No. 7). Tigbauan, Iloilo, Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center.

Schveitzer R, Arantes R, Costódio PFS, Espírito Santo CM, Vinatea L, Seiffert WQ,Andreatta ER (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. Aquac Eng 56:59–70.

Shailender M, Suresh-Babu CH, Srikanth B, Kishor B, Silambarasan D, Jayagopal P (2012). Sustainable Culture method of Giant Black Tiger Shrimp , *Penaeus Monodon* (Fabricius) in Andhra Pradesh, India. IOSR J Agric Vet Sci 1:12–16.

Speck RC, Cavalli RO, Marchiori MA (1993). Efeito de diferentes densidades de estocagem sobre o crescimento e a sobrevivência de pós-larvas de *Penaeus paulensis* (Pérez-Farfante, 1967) em sistema de berçário. In: Encontro Rio-Grandense de técnicos em aquicultura, 4, 1993, Porto Alegre, RS. Anais, Porto Alegre: UFRGS, 1993. pp. 31–39.

Thakur DP, Lin CK (2003). Water quality and nutrient budget in closed shrimp (*Penaeus monodon*) culture systems. Aquac Eng 27:159–176.

Van Wyk P (2004). Production of *L. vannamei* in recirculating aquaculture systems: management and design considerations. In: Rakestraw TT, Douglas LS, Marsh L, Granata L, Correa A, Flick GJ (Eds.). In: Proceedings of the 6th International Conference on Recirculation Aquaculture. Roanoke, VA, pp.38–47

Viau VE, Souza DM, Rodríguez EM, Wasielesky W, Abreu PC, Ballester EL (2013).Biofilm feeding by postlarvae of the pink shrimp *Farfantepenaeus brasiliensis* (Decapoda, Penaidae). Aquac Res 44:783–794.

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, 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.

Wasielesky W, Froes C, Fóes G, Krummenauer D, Lara G, Poersch L (2013). Nursery of *Litopenaeus vannamei* Reared in a Biofloc System: The Effect of Stocking Densities and Compensatory Growth. J Shellfish Res 32:799–806.

Yusufzai SI, Singh H (2005). Rearing of *Penaeus monodon* (Fabricius) postlarvae in floating cages at different stocking densities. Aquac Res 36:405–408.