Aquacult Int (2011) 19:891–901
DOI 10.1007/s10499-010-9408-6

# Effect of biofloc technology (BFT) on the early postlarval stage of pink shrimp *Farfantepenaeus paulensis*: growth performance, floc composition and salinity stress tolerance

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Received: 19 April 2010 / Accepted: 15 December 2010 / Published online: 15 February 2011


**Abstract** Biofloc rearing media provides a potential food source for shrimp reared in limited or zero water exchange systems. This culture system is environmentally friendly as it is based on limited water use and minimal effluent is released into the surrounding environment. In this study, we evaluated the survival, growth performance and salinity stress tolerance of pink shrimp *Farfantepenaeus paulensis* postlarvae reared from $PL_{10}$ to $PL_{25}$ in a biofloc technology limited water exchange system. PL (mean ± SD weight and length of 14 ± 10 mg and 8.10 ± 0.7 mm, respectively) were reared in nine 40-L plastic tanks with a stocking density of 10PL/L. Three culture treatments were applied (1) culture in the presence of bioflocs and commercial feed supply (FLOC + CF); (2) culture in the presence of biofloc without feed supply (FLOC) and (3) culture in clear water with feed supply (control). Final biomass and survival were significantly higher in FLOC + CF treatment than the control ($P < 0.05$), but did not differ from FLOC. PL reared in the FLOC + CF treatment achieved a significantly higher final weight, weight gain and length in comparison with the other two treatments ($P < 0.05$). No significant difference ($P > 0.05$) between treatments was found for salinity tolerance over 24 and 48 h durations. The proximate analysis of floc shown high levels of crude protein (30.4%), but low levels

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of crude lipids (0.5%). The continuous availability of bioflocs had a significant effect on growth and survival of *F. paulensis* postlarvae cultured in BFT nursery systems.

**Keywords** Biofloc · *Farfantepenaeus paulensis* · Limited water exchange · Nursery · Sustainability

## Introduction

According to the Food and Agriculture Organization of the United Nations (FAO 2003, 2008), shrimp is the most important and profitable commodity among all seafood trades. It is expected that shrimp farming will maintain high growth rates in the next decade. The use of high water exchange rates is a common strategy to maintain suitable water quality in intensive shrimp production systems (Wang 1990; Hopkins et al. 1993; Moss et al. 1999). However, incoming water is the main pathway for pathogen introduction in most shrimp culture systems (Lotz and Lightner 1999). Considerable impact of disease outbreaks on commercial shrimp farming operations during the past two decades greatly affected the operational management of shrimp farms worldwide (Wasielesky et al. 2006). Thus, crop losses forced shrimp farmers to look for more biosecure culture practices, such as minimal or zero water exchange, to minimize the risk associated with exposure to pathogens (Browdy et al. 2001). Limited water exchange technologies will also enable the shrimp farming industry to avoid excessive damage to natural habitats (Naylor et al. 2000; Ray et al. 2010).

Biofloc technology (BFT) systems were developed to minimize effluent discharge, protect the surrounding water resources and improve farm biosecurity (Weirich et al. 2002; Burford et al. 2003; Avnimelech 2007). The microorganisms play major roles with respect to natural productivity, nutrient cycling, water quality and the nutrition of the cultured animals (Moriarty 1997; McIntosh et al. 2000). Control of the predominantly heterotrophic bacterial community over autotrophic microorganisms is achieved by the use of high carbon to nitrogen ratios (C:N) (Avnimelech et al. 1994). The uptake of ammonia by bacteria improves water quality and increases microbial biomass production (Avnimelech 1999; Moss et al. 1999). These processes serve as fuel for operating the ''floc system'' (Burford et al. 2004; Cohen et al. 2005). Furthermore, a nutrient-rich feed source is available 24 h per day and could reduce artificial feed inputs and costs (Browdy et al. 2001; Avnimelech 2007; Samocha et al. 2007).

Shrimp farm biosecurity programs based on minimal or zero water exchange is an emerging technology mainly developed for grow-out ponds, but could be easily applied as a management tool in the nursery phase. This phase is defined as the intermediate step between hatchery-reared, early postlarvae (PL) and the grow-out phase (Mishra et al. 2008). Previous studies on nursery systems, mainly with the Pacific white shrimp *Litopenaeus vannamei*, have reported several benefits such as optimization of farm land and increased shrimp survival and growth performance in the grow-out phase (Apud et al. 1983; Sandifer et al. 1991; Samocha et al. 2000, 2007). Nursery systems can be characterized by high stocking densities, high water exchange rates and the use of high quality artificial feeds (Speck et al. 1993; Mishra et al. 2008).

Despite PL quality, the higher survival rates at the end of PL larviculture were often associated with higher grow-out performance (Aquacop et al. 1991), or at least during PL pond stocking (Fegan 1992). Different criteria are used to evaluate PL quality. Survival of a salinity stress test is a widely used low-cost criterion to predict the quality of PL reared under diverse conditions (i.e., diets, environmental condition, culture schedule, etc.) (Rees et al. 1994; Racotta et al. 2003, 2004).

Little is known about the rearing of other penaeid species in BFT systems. Usually, endogenous species show greater adaptation to local conditions and may be used in restocking programs or cultured in their natural environment (Wasielesky et al. 2004). Furthermore, they often have particular market niches (such as live sport fishing bait) and high social and local importance. The pink shrimp *Farfantepenaeus paulensis* is a relatively cold-tolerant species that has been considered for shrimp farming in southern Brazil (Wasielesky 2000; Wasielesky et al. 2003; Peixoto et al. 2003; Cavalli and Wasielesky 2003). Thus, the aim of the present work is to evaluate the growth, survival and salinity stress tolerance of *F. paulensis* PL reared during the nursery phase in a BFT limited water exchange culture system.

## Materials and methods

This study was conducted at the Marine Aquaculture Station (EMA), Federal University of Rio Grande (FURG), located at Cassino Beach (32°12/S and 51°50/W), Rio Grande, RS, Brazil. An outdoor 7,000-L fiberglass round tank was used for biofloc production under limited water exchange conditions (*macrocosm tank*). The tank was inoculated with the diatom *Thalassiosira weissflogii* at $5 \times 10^4$ cells/ml and stocked with *F. paulensis* juveniles ($3.54 \pm 0.88$ g) at a density of 40 shrimp/m$^2$. These animals were fed twice a day (40% crude protein Cargill Purina$^{TM}$) at 3% of the estimated shrimp biomass. The tank was covered with shade cloth to reduce the light. Sugar cane molasses and wheat bran were added as the carbon source. A C:N ratio of approximately 20:1 was maintained during the study to optimize heterotrophic bacterial growth (Avnimelech 1999; Asaduzzaman et al. 2008).

The experiment was initiated when bioflocs (measured as total suspended solids TSS) was higher than 100 mg L$^{-1}$. The floc-rich water was pumped from the macrocosm tank to the experimental units by a submerged pump and returned by gravity. Limited water exchange was carried out during the experimental period in the macrocosm tank. Accumulated sludge was removed by a central drain and did not exceed 0.5% water exchange daily. Seawater (35 ppt) was previously filtered in a 125-μm sand filter. Dechlorinated freshwater was added to compensate losses from sludge removal and evaporation.

*F. paulensis* PL (mean ± SD weight and length of $14 \pm 10$ mg and $8.10 \pm 0.7$ mm, respectively) were reared at a density of 10PL/L over 15 days (from $PL_{10}$ to $PL_{25}$) in a completely randomized experimental design with three treatments and three replicates each: (1) bioflocs plus a commercial feed (FLOC + CF), (2) bioflocs with no feed supply (FLOC) and (3) bioflocs cultured in clear water with commercial feed supply (control). Experimental units consisted of nine 40-L rectangular plastic bins each one containing five air stones to ensure oxygenation and to keep the bioflocs in suspension. The water recirculation rate in the experimental units was approximately 300% per day. In clear water treatment, seawater was previously filtered in a sand filter (125 μm). An 800-L tank was used as a reservoir and water exchange and recirculation was the same as described for the floc treatments. The shrimp were fed with a 40% commercial crude protein feed (Cargill Purina$^{TM}$), which was administered twice daily (09:00 and 18:00) at 100% of shrimp biomass.

After 15 days, all experimental tanks were harvested and shrimp were individually counted and weighed to the nearest 0.01 g. Survival, weight, weight gain, total length and biomass were estimated. Every 5 days, microbial floc samples were collected with a 100-μm mesh. Proximate analysis of organic fertilizers, feed and a pooled sample of the bioflocs were performed. All samples were dried at 102°C until constant weight and then

maintained at 4°C. Samples were then ground and the contents of protein, lipids, fiber and ash were determined following AOAC (2000). Carbohydrate levels were estimated by difference (Tacon 1990).

Temperature (mercury thermometer, precision ±0.5°C), salinity (Atago™ optical refractometer, ±1 ppt), pH and dissolved oxygen (YSI model 556) were measured daily between 08:00 and 10:00 h. Total ammonia nitrogen (TAN), nitrite ($NO_2$–N) and reactive phosphorous RP ($PO_4^{-3}$) were measured three times a week (UNESCO 1983), while TSS was determined every 3 days (Strickland and Parsons 1972).

At the end of the experiment, a salinity stress test was carried out. This test consisted of transferring PL from each treatment to five replicated units (20 PL per unit) containing salinity levels of 0, 1, 3, 5, and 10 ppt (Tsuzuki et al. 2000). To prepare the different salinities, 35 ppt filtered seawater and dechlorinated tap water (<0.1 ppt) were used. Experimental conditions were 28.0 ± 0.5°C and pH 7.8 ± 0.1. Twenty-five 2-L plastic bottles were used as experimental units. Gentle aeration was provided by one air stone per bottle. After 24 and 48 h of exposure, shrimp not responding to mechanical stimuli were considered dead. Based on the mortality rates of shrimp exposed to different salinities, the medium lethal concentrations for 50% of the population (24- and 48-h SL50) were estimated using the Trimmed–Spearman–Karber method statistical package (Hamilton et al. 1977). Water quality and shrimp performance results were analyzed by one-way ANOVA. If significant differences were found, Tukey's multi-comparison test was applied at a 5% significance level.

## Results

Water quality data are summarized in Table 1. Temperature, dissolved oxygen, and nitrite presented no significant differences between treatments. However, salinity was significantly higher in the treatments with flocs, while pH values were lower in these treatments. Concentrations of total ammonia (TAN) and reactive phosphorous were significantly higher in the treatments containing flocs. Fluctuations of TAN, $NO_2$–N and RP and of pH and TSS in the macrocosm tank are shown in Figs. 1 and 2, respectively. Levels of pH ranged from 6.05 to 6.70. Mean (±SD) TSS was 290 (±143) mg $L^{-1}$. Although TSS values varied widely (181–449 mg $L^{-1}$), it is clear that suspended particulate matter (bioflocs) were constantly available throughout the study period (Fig. 2).

**Table 1** Mean (±SD) water quality variables during the experimental period

| | FLOC + CF | FLOC | Control | *P* |
|---|---|---|---|---|
| Temperature (°C) | 23.8$^a$ (±1.8) | 23.7$^a$ (±1.8) | 23.9$^a$ (±2.0) | 0.915 |
| Salinity (g $L^{-1}$) | 33.9$^a$ (±1.3) | 34.0$^a$ (±1.3) | 33.1$^b$ (±1.6) | 0.005 |
| Dissolved oxygen (mg $L^{-1}$) | 7.85$^a$ (±0.77) | 7.92$^a$ (±0.75) | 8.03$^a$ (±0.69) | 0.478 |
| pH | 7.33$^a$ (±0.17) | 7.33$^a$ (±0.16) | 8.31$^b$ (±0.16) | 0.000 |
| TAN (mg $L^{-1}$) | 1.80$^a$ (±0.67) | 2.16$^a$ (±0.69) | 0.26$^b$ (±0.17) | 0.000 |
| $NO_2$–N (mg $L^{-1}$) | 0.52$^a$ (±0.87) | 0.52$^a$ (±0.92) | 0.02$^a$ (±0.02) | 0.072 |
| $PO_4^{-3}$ (mg $L^{-1}$) | 1.88$^a$ (±0.30) | 1.70$^a$ (±0.51) | 0.15$^b$ (±0.07) | 0.000 |

Within rows, different superscript letters indicate significant differences ($P < 0.05$). Treatments: (FLOC + CF) presence of bioflocs and a commercial feed supply; (FLOC) presence of bioflocs with no feed supply; and (control) cultured in clear water with feed supply

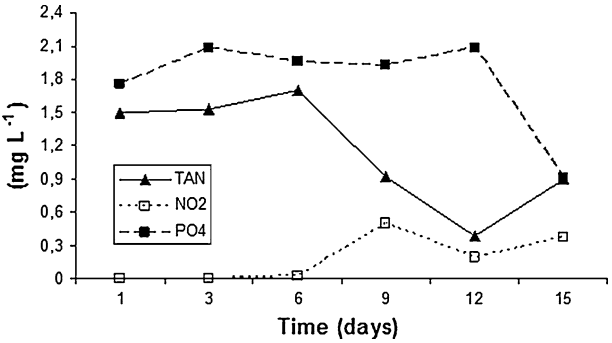


**Fig. 1** Fluctuations of total ammonia nitrogen (TAN), nitrite nitrogen ($NO_2$–N) and reactive phosphorous ($PO_4^{-3}$) on the macrocosm tank

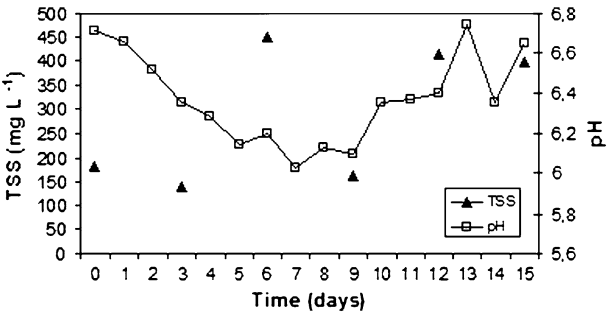


**Fig. 2** Fluctuations of pH and total suspended solids (TSS, mg $L^{-1}$) on the macrocosm tank

**Table 2** Mean (±SE) growth performance parameters during the experimental period

| | FLOC + CF | FLOC | Control | *P* |
|---|---|---|---|---|
| Final length (mm) | 11.96$^{a}$ (±0.16) | 10.83$^{b}$ (±0.21) | 9.78$^{c}$ (±0.12) | 0.000 |
| Final weight (mg) | 6.80$^{a}$ (±0.39) | 4.94$^{b}$ (±0.33) | 3.57$^{c}$ (±0.18) | 0.000 |
| Weight gain (mg) | 5.40$^{a}$ (±0.39) | 3.54$^{b}$ (±0.33) | 2.17$^{c}$ (±0.18) | 0.000 |
| Survival (%) | 47.75$^{a}$ (±3.53) | 25.75$^{ab}$ (±4.13) | 17.58$^{b}$ (±1.62) | 0.018 |
| Final biomass (mg) | 1,266.7$^{a}$ (±29.8) | 491.4$^{ab}$ (±11.8) | 248.1$^{b}$ (±20.9) | 0.019 |

Within rows, different superscript letters indicate significant differences ($P < 0.05$). Treatments: (FLOC + CF) presence of bioflocs and a commercial feed supply; (FLOC) presence of bioflocs with no feed supply; and (control) cultured in clear water with feed supply

Final weight, length and weight gain were significantly higher in PL from the FLOC + CF than those from FLOC, which, in turn, were significantly higher in comparison with control (Table 2). Final biomass and survival were significantly higher in the FLOC + CF treatment than the control ($P < 0.05$), but did not differ from FLOC. No differences were detected between treatments in terms of medium lethal salinity levels after 24 and 48 h (Table 3).

Proximate analysis of organic fertilizers (wheat bran and molasses), feed and biofloc are shown in Table 4. The commercial feed and microbial flocs presented comparatively higher levels of protein (39.7 and 30.4%, respectively). As expected, high levels of

**Table 3** Mean lethal salinity (SL50) and 95% confidence intervals after 24 and 48 h of *Farfantepenaeus paulensis* postlarvae

| | FLOC + CF | FLOC | Control |
|---|---|---|---|
| SL50 24 h | 3.32 (3.0–3.62) | 3.41 (3.07–3.62) | 3.85 (2.71–4.95) |
| SL50 48 h | 3.26 (2.97–3.55) | 3.16 (2.35–3.96) | 2.93 (2.39–3.47) |

Treatments: (FLOC + CF) presence of bioflocs and a commercial feed supply; (FLOC) presence of bioflocs with no feed supply; and (control) cultured in clear water with feed supply

**Table 4** Proximate analysis (% dry weight basis) of the commercial feed, organic fertilizers (wheat bran and molasses) and biofloc

| Composition (%) | Feed | Wheat bran | Molasses | Biofloc |
|---|---|---|---|---|
| Crude protein | 39.7 | 18.9 | 5.6 | 30.4 |
| Carbohydrate | 35.6 | 61.1 | 81.6 | 29.1 |
| Crude lipid | 13.1 | 2.7 | 0.16 | 0.5 |
| Crude fiber | 2.6 | 11.3 | 0.2 | 0.8 |
| Ash | 9.0 | 6.0 | 12.4 | 39.2 |

carbohydrate were found in wheat bran and molasses (61.1 and 81.64%, respectively) and intermediate levels in the feed and floc (35.6 and 30.4%, respectively). Lipid levels in the flocs were low (0.5%). Wheat bran had the highest crude fiber content with 11.3%.

## Discussion

Experimental tanks receiving flocs had significantly higher salinity and lower pH levels than clear water tanks. Salinity was probably higher due to the evaporation in the macrocosm tank, while lower pH values were possibly a result of high respiration rates by the large quantities of microorganisms (heterotrophic community), which may have increased $CO_2$ concentrations. A similar trend was observed by Tacon et al. (2002) and Wasielesky et al. (2006). In addition, Chen et al. (2006), Ebeling et al. (2006) and Rijn et al. (2006) reported a decrease in pH during the chemolithotrophic nitrification process as a result of $CaCO_3$ consumption and the release of $CO_2$ and $H^+$ into the culture medium.

It is well known that ammonia and nitrite are highly toxic to shrimp, though their toxicity is species specific and depends on water characteristics and duration of exposure (Tomasso 1994; Hargreaves 1998; Barajas et al. 2006; Mishra et al. 2008). Although nitrite levels were not different between treatments, ammonia levels were higher in the FLOC + CF and FLOC treatments. This was probably due to the high input of nutrients in the macrocosm tank that supplied floc-rich water and the subsequent accumulation of particulate organic matter in the tanks from these treatments. Nevertheless, the levels of ammonia observed in this study were below than the lethal levels reported for *F. paulensis* (Wasielesky et al. 1994). However, growth could be affected accords to the same author. Overall, water quality parameters in this study were considered to be within suitable ranges for the culture of *F. paulensis* (Poersch and Marchiori 1992; Santos and Marchiori 1992; Wasielesky et al. 1994; and Tsuzuki et al. 2000).

Similar to observed for ammonia, the significantly higher levels of reactive phosphorous (RP) in the FLOC + CF and FLOC treatments may also be a result of the input of nutrients

from the macrocosm tank and the accumulation of particulate organic matter. The buildup of RP in floc-based treatments may also occurs because of the lower assimilation of this nutrient by the bacterial community in comparison with systems where phytoplankton is predominant. Under traditional pond-based aquaculture systems, most RP is normally retained in the sediment. However, in floc-based systems where lined tanks with no sediment are commonplace, RP tends to accumulate and is eventually dissolved in the water column. Hargreaves (2006) showed that algal assimilation could temporally reduce RP levels, but these could increase again following algal crashes. In limited water exchange systems, a higher accumulation of RP occurs in lined ponds (with no sediment) compared to earthen ponds in which RP binds to the sediment (Burford et al. 2003). The water renewal could be the only effective way to prevent the accumulation of RP (Hopkins et al. 1993).

Since the early 1960s (Baylor and Sutcliffe 1963) and more recently (Moriarty 1997), particulate organic matter as well as other organisms in the microbial food web have been proposed as potential food sources for aquatic animals. Microorganisms present in floc-based systems are thought to have an important role in the nutrition of cultured animals. In this study, microbial flocs were found to have relatively high crude protein levels (30.4% CP), which is comparable to 31.2 and 31.1% CP found by Tacon et al. (2002) and Wasielesky et al. (2006), respectively. Ash and crude lipid contents in the present study averaged 39.2 and 0.5%, respectively, and were also close to the values reported by Wasielesky et al. (2006), which were 44.8 and 0.47%. According to Avnimelech (1999, 2007), microbial flocs provide an excellent source of nutrients to shrimp that is available 24 h per day. However, improvement of floc nutritional value (i.e., using different carbon sources or a mixture of phytoplankton and bacteria) needs further investigations (Kuhn et al. 2009). Crab et al. (2010) reported that glucose or a combination of glycerol and *Bacillus* as a carbon sources led to microbial floc higher protein content and also higher n-6 fatty acids.

Concentrations of TSS in this study ranged between 181 and 449 mg $L^{-1}$. For tilapia in BFT system, Azim and Little (2008) reported TSS values between 560 and 597 mg $L^{-1}$, while Avnimelech (2007) detected a greater variation (460–643 mg $L^{-1}$). The authors suggested the need of further investigation to determine the optimal range of TSS on fish. For penaeids, Samocha et al. (2007) recommended TSS values below 500 mg $L^{-1}$, which is above than the average reached in the present study. Differences in TSS values between studies may be due to cultured growth, consumption and stocking density, tank and aeration design, microbial community and ecological succession of the microorganisms in each situation. Moreover, studies on the ecology of microorganisms have demonstrated that organic particles (like bioflocs) may suffer changes in size and structure due to several biological and physicochemical factors (Eisma 1986; Alldredge and Gotschalk 1988; Biddanda and Pomeroy 1988; Riebesell 1991; Cowen 1992; Kepkay 1994).

Several studies have demonstrated positive effects of microorganism communities on *F. paulensis* culture (Thompson et al. 1999, 2002; Abreu et al. 2007; Ballester et al. 2007). The average survival found in the present study was rather low in comparison with previous studies with PL of the same species (Speck et al. 1993; Thompson et al. 2002; Jensen et al. 2006). Nevertheless, floc treatments resulted in higher survival probably due to the provision of essential nutrients, such as aminoacids, vitamins, and minerals present in the flocs (Decamp et al. 2002). Microbial flocs could provide nutritional supplement to *F. paulensis* PL as has been demonstrated for *L. vannamei* (McIntosh et al. 2000; Browdy et al. 2001; Moss 2002; Tacon et al. 2002; McAbee et al. 2003; Samocha et al. 2004; Otoshi et al. 2006). Burford et al. (2004) estimated that more than 29% of the daily food

intake of *L. vannamei* consisted of microbial flocs. Additionally, several studies with BFT culture systems have shown that levels of protein in the feeds may be significantly reduced (Avnimelech 2007; Azim and Little 2008; Ballester et al. 2009). In the present study, the presence of biofloc resulted in increases of 50% in weight and almost 80% in final biomass when compared to the control.

Tackaert et al. (1989) proposed a salinity stress test to define PL quality standards. However, there is no reference in the literature describing the effect of biofloc-based systems on the stress tolerance of shrimp. We found no differences between treatments in salinity stress test. In general, higher survival and growth rates are closely related to PL that are more resistant to stress tests (Castille et al. 1993; Rees et al. 1994; Gallardo et al. 1995; Kontara et al. 1997; Paibulkichakul et al. 1998). Therefore, we conclude that the survival and growth benefits of BFT were not expressed in the tolerance to salinity changes.

## Conclusion

The presence of bioflocs increased survival and growth rates of shrimp. This was observed even when postlarvae were not fed with a commercial feed. Thus, BFT systems play an important role as a nutritional source for *F. paulensis* early postlarval stages. The BFT systems can be a key to environmentally friendly aquaculture via minimal water exchange and a decrease in commercial feed requirements.

**Acknowledgments** The authors would like to thank Roberta Soares, Paulo Abreu, Luis Vinatea, Gerard Cuzon, Jeannot Gogueinhein and all EMA-FURG research team for their contribution toward this study. We also like to acknowledge Stuart Arnold and Andrew Ray for critical reading of the manuscript, the Brazilian Council for Scientific and Technological Development (CNPq) for financial support and CAPES-Brazil for the M.Sc. grant to the first author. W. Wasielesky and R. O. Cavalli are research fellows of CNPq.

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