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Effect of light limitation on the water quality, bacterial counts and performance of *Litopenaeus vannamei* postlarvae reared with biofloc at low salinity

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Abstract

The aim of this study was to evaluate the effect of light limitation on the water quality, bacterial counts and performance of *Litopenaeus vannamei* postlarvae reared with biofloc at low salinity (≈9 g L^{-1}). Two treatments were designed: T_1 = culture with natural sunlight and T_2 = culture in darkness. After 28 days, in both treatments, the final weight of shrimp was over 0.6 g with a specific growth rate over 7.4% d^{-1}, and a survival rate over 70%. In both treatments, *Vibrio* sp. concentration presented low values (culture with natural sunlight = 0.1 to 9.9 × 10^2 CFU mL^{-1}, culture in darkness = 0.4 to 11.7 × 10^2 CFU mL^{-1}) and *Bacillus* sp. had high values (culture with natural sunlight = 0.7 to 66.0 × 10^4 CFU mL^{-1}, culture in darkness = 0.7 to 65.8 × 10^4 CFU mL^{-1}). All water quality parameters remained within the ranges suitable for shrimp culture, except for alkalinity during the first stage of the study. Although in some sampling periods some significant differences were found in bacterial counts and water quality parameters, shrimp productive performance under culture with biofloc at low salinity was not affected significantly by light limitation.

Keywords: shrimp performance, biofloc, bacteria, water quality

Introduction

Different studies with penaeid confirm that the use of nurseries with the biofloc system contributes to the rapid growth of the cultured organisms (Fóes, Fróes, Krummenauer, Poersch & Wasielesky 2011; Emerenciano, Ballester, Cavalli & Wasielesky 2012; Wasielesky, Fróes, Foes, Krummenauer, Lara & Poersch 2013). Biofloc technology allows manipulating the microbial community under controlled conditions within the culture system of the raised animals (Avnimelech 1999; De Schryver, Crab, Defoirdt, Boon & Verstraete 2008; Avnimelech 2009). This system facilitates the production of aquatic animals in a sustainable and biosecure way. Azim, Little and Bron (2008) reported that one of the advantages of operating a bacterial-driven system, versus a conventional system dominated by phytoplankton, is that microbial production is limited by the availability of organic matter or substrate rather than by light, giving rise to the potential for this system in indoor conditions.

Many studies have focused on understanding the operation and potential benefits of the biofloc system (Avnimelech 1999; Azim & Little 2008; De Schryver *et al.* 2008; Brito, Chagas, da Silva, Soares, Severi & Gálvez 2016; Xu, Morris & Samocha 2016; Esparza-Leal, Amaral Xavier & Wasielesky 2016). Despite the significant progress that has been reached with the biofloc system, it is still under development. Most of the research on biofloc systems has been carried out in glasshouses in tropical or subtropical regions with an abundance of natural light (Neal, Coyle, Tidwell & Boudreau 2010).

Light is a main biofactor for aquatic animals and organisms, and several studies have investigated diverse light conditions on aquatic organisms and found significant differences in their behaviour, nutrition and growth (Jaski, Kamrani & Salarzadeh 2014). Some authors suggest that different light parameters, such as severity, spectrum and length of light period, have considerable effects on growth, survival and sexual maturity (Giri, Sahoo, Sahu, Mohanty, Mukhopadhyay & Ayyappan 2002; Wang, Dong, Huang, Wu, Tian & Ma 2003; Coyle, Bright, Wood, Neal & Tidwell 2011). Additionally, crustacean moulting frequency, food consumption, cannibalism and growth performance are directly affected by the photoperiod (Aiken, Roubichaud & Waddy 1983; Gardener & Macguire 1998; Tidwell, Coyle, Van Arnum, Bright & McCarthy 2001).

Baloi, Rafael, Schveitzer, Magnotti and Vinatea (2013) studied the performance of *Litopenaeus vannamei* juvenile raised in biofloc systems with varying levels of light exposure and demonstrated that shrimp production was higher in the treatment with light exposure although shrimp can be raised in total absence of light with acceptable performance. However, little is known about the functionality of the biofloc systems in an environment without light at nursery level in *L. vannamei* postlarvae.

Systems operating in the absence of light may require more oxygen input during daylight hours, but the risks associated with harmful algae are reduced (Baloi *et al.* 2013). Ray, Shuler, Leffler and Browdy (2009) support the idea that by eliminating the dependence on sunlight in the biofloc systems, organisms can be housed in a controlled environment of insulated buildings, leading to a reduction in energy costs during the cold months. In systems operated with light, oxygen supply can be reduced during the daylight hours, as a result of greater photosynthetic production, especially when the phytoplankton community composition is dominated by chlorophytes, which are better oxygenators of water compared to bloom-forming cyanobacteria (Ray *et al.* 2009; Schrader, Green & Perschbacher 2011; Baloi *et al.* 2013). Hence, the purpose of this study was to evaluate the effect of light limitation on the water quality, bacterial counts and performance of *L. vannamei* postlarvae reared in tanks with biofloc at low salinity.

Material and methods

Experimental design and shrimp culture system

This study was carried out in an outdoor tank facility at the *CIIDIR-Unidad Sinaloa* of the *Instituto Politécnico Nacional* in Mexico. Shrimp *L. vannamei* postlarvae used in this work were acquired from a commercial hatchery and transferred to the experimental tanks for acclimation during 36 h. The experimental design was randomized with two treatments: T_1 = culture with natural sunlight (midday $\approx$ 718lx) and T_2 = culture in darkness (the tanks were covered with black plastic). Three replicates were randomly assigned to each treatment. Each tank was stocked with 1000 orgs m^{-3} (average body weight = 0.09 $\pm$ 0.01 g). Shrimp were fed twice a day ($\approx$08:00 and 16:00 hours) with commercial feed (40% protein). Initially, the feeding rate was established according to Jory, Cabrera, Dugger, Fegan, Lee, Lawrence, Jackson, McIntosh and Castañeda (2001), and, posteriorly, feed was adjusted daily according to its consumption (7-12% of the biomass). The study lasted 28 days.

The shrimp culture system included six 0.4-m^{-3} circular tanks with 300 L of water per tank, supplied with continuous aeration with a blower of 5 hp (Sweetwater™, Aquatic Eco-System, Inc., Apopka, FL, USA). Before starting the study, all tanks were filled with diluted marine water at salinity of $\approx$9 g L^{-1} and supplied with sugar cane molasses (0.3 g L^{-1} per day) as a source of organic carbon during 7 days to promote biofloc formation. Sugar cane molasses were added to each tank to maintain the level of total ammonia at values lower than 1 mg L^{-1} according to Avnimelech (1999). In the tank system, no water

renewal was performed during the study, only replacement of the water lost due to evaporation by adding freshwater.

During the study, biometrics were performed weekly, weighing individually 50 shrimp from each experimental tank using a digital balance (precision 0.01 g; Ohaus Corporation, Parsippany, NJ, USA). The shrimp were returned to their original tanks after weighing. At end of the study, all the shrimp that survived in each experimental tank were weighed and counted to evaluate their growth [final weight, specific growth rate (SGR)], survival, feed conversion ratio (FCR), productivity and final density per treatment. The SGR (% weight increase d^{-1}) was calculated from $SGR = 100 \times [(Ln\ Wf - Ln\ Wi)]/t$, where Wf = mean weight at the end of the period, Wi = mean weight at the beginning of the period and t = time in days of the period (Ricker 1979). The FCR was calculated by dividing the feed supplied (dry weight) by the live weight gain (wet weight) (Hari, Madhusoodana, Varghese, Schrama & Verdegem 2004). Survival (%) was calculated by counting the living organisms and subtracting the stocked organisms in each tank. Survival data were transformed (arcsine of the square root) before analysis (Zar 1996).

Physicochemical parameters and bacterial analyses

During the experiment, pH (monitored with a Hanna 213 pH meter, Hanna Instruments, Woonsocket, RI, USA), temperature (°C) and dissolved oxygen (DO; mg L^{-1}) (both monitored using a YSI 55 digital oxygen meter with an integrated thermometer, Yellow Springs, OH, USA) were measured in each experimental tank twice a day (08:00 and 16:00 hours). Water salinity was monitored weekly with an Atago refractometer (Nova-Tech International, Houston, TX, USA). Total ammonia (mg L^{-1}), nitrite (mg L^{-1}), nitrate (mg L^{-1}), phosphate (mg L^{-1}), alkalinity (mg $CaCO_3$ L^{-1}) and total suspended solids (TSS, mg L^{-1}) were analysed weekly using the methods described by Strikland and Parsons (1972) and APHA (1998).

Water samples for bacterial analyses were collected weekly from each tank following standard procedures (APHA 1998; Gómez-Gil 2006). Bacteria were spread-plated on nutrient agar to obtain bacterial counts. Thiosulphate citrate bile salt agar (TCBS agar; Difco, USA) was used to count presumptive *Vibrio* sp. The plates were supplemented with 2.5% NaCl and incubated for 24 h at 30°C before counting (CFU mL^{-1}). To count presumptive *Bacillus* sp., water samples were incubated at 80°C for 10 min to favour spore-forming bacteria (bacilli) and then were spread on plates with Trypticase soya agar (TS agar, BD, Bioxon, Sparks, MD, USA) supplemented with 2% NaCl and incubated at 37°C for 24 h.

Statistical analyses

The homoscedasticity of variances and normality of data were first verified. Treatment effects on water quality parameters and bacterial counts were evaluated by one-way repeated-measures ANOVA with treatment as the main factor and the sampling date as the repeated-measures factor (Gomez & Gomez 1984). Significant differences were tested with Tukey's multiple comparison test of means. Treatment effects on production parameters were evaluated using Student's *t*-tests. The results were evaluated with a 5% significance level. The analyses were conducted using STATISTICA package v6 (StatSoft, Tulsa, OK, USA).

Results

Water quality

During the experimental period, the values of DO, temperature, pH and salinity did not differ significantly between the cultures with and without light limitation ($P > 0.05$; Table 1). Total ammonia concentrations in both treatments were low (<0.75 mg L^{-1}) and only differed significantly at 14 days of culture ($P < 0.05$) with the lowest value in the darkness treatment (<0.1 mg L^{-1};

Table 1 Physicochemical parameters (mean ± SD) in the culture of *Litopenaeus vannamei* postlarvae at low salinity with biofloc, with and without light limitation during 28 days

Trial	DO (mg L^{-1})	Temperature (°C)	pH	Salinity (g L^{-1})
With natural sunlight	7.0 ± 0.5	28.2 ± 1.0	8.1 ± 0.2	8.9 ± 0.7
In darkness	6.8 ± 0.4	29.0 ± 1.2	8.2 ± 0.2	9.1 ± 0.7

DO, dissolved oxygen.

Fig. 1). In addition, nitrite concentrations in both treatments were low (<0.75 mg L^{-1}) and only differed significantly at 7 and 14 days of culture ($P > 0.05$; Table 1) with the highest values in the darkness treatment (≈0.70 mg L^{-1}). Nitrate concentrations were not significantly different between treatments except on days 0 and 14 of culture ($P > 0.05$); in the rest of the study, the highest values were reached in the darkness treatment ($P < 0.05$; Fig. 1). Phosphate concentration in both treatments was lower than 0.4 mg L^{-1} and presented significant differences only at the 14th day of culture ($P < 0.05$; Fig. 1).

Alkalinity presented significant differences between treatments at days 7 and 14 of culture ($P < 0.05$) with the lowest values in the darkness treatment; alkalinity was maintained at less than 50 mg L^{-1} (darkness treatment; Fig. 1). Only on the 7th day of culture, the TSS concentration fluctuated between 100 and 250 mg L^{-1} and did not differ significantly between treatments except at the beginning and end of the study ($P > 0.05$). During most of the study, TSS presented lower values in the darkness treatment (Fig. 1). During the study, the total *Vibrio* sp. count presented low values in both treatments (<1.250 × 10^3 CFU mL^{-1})

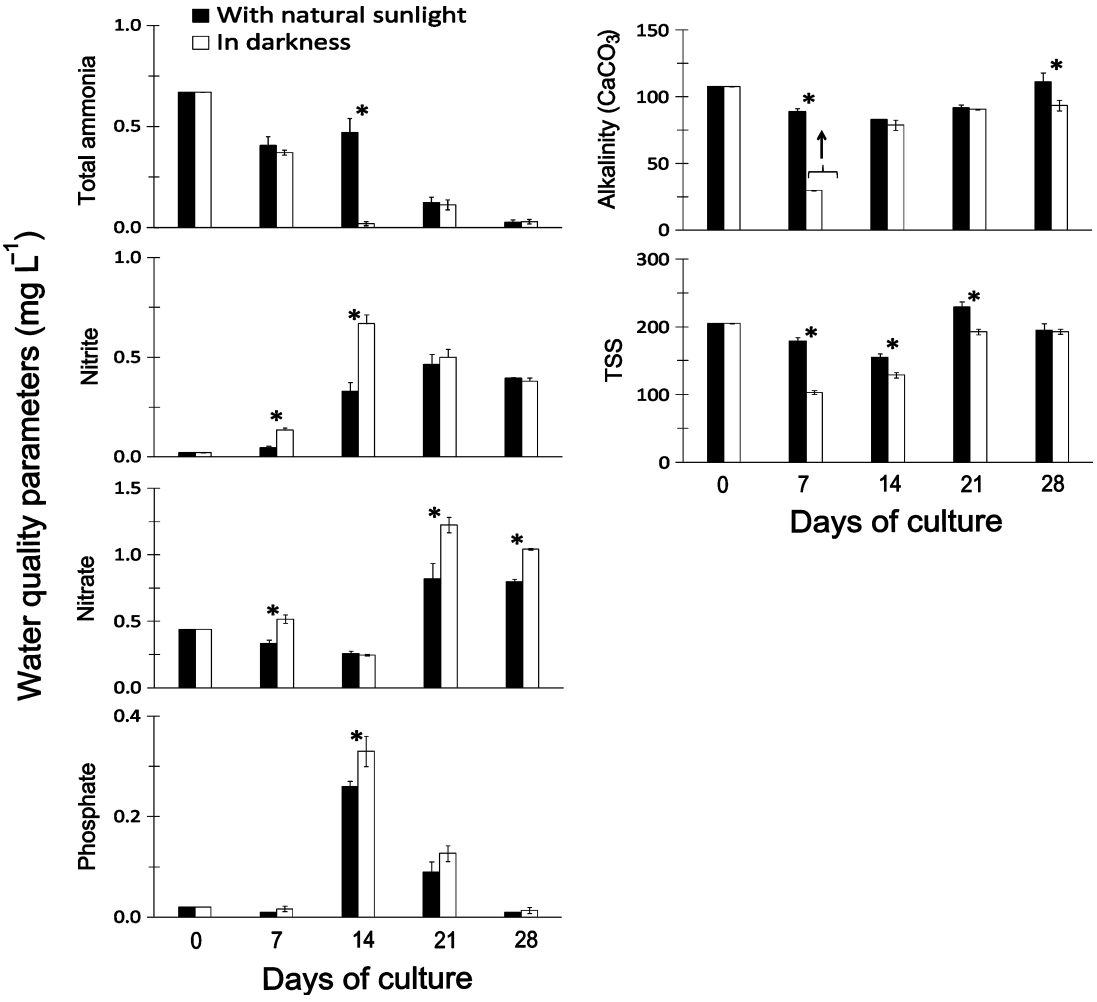

Figure 1 Temporal variations of the water quality parameters in the culture of *Litopenaeus vannamei* postlarvae at low salinity with biofloc, with and without light limitation. Each bar represents the mean (±SD) of three replicates. Arrow = to increase the alkalinity in the culture with darkness; the tanks were supplied with 0.05 g L^{-1} per day of sodium bicarbonate ($NaHCO_3$) with a 99.0% purity (Furtado *et al.* 2011, 2014) for three consecutive days after the 7th day of culture. *significant differences ($P < 0.05$).

with the lowest concentrations between the 7th and 21st days of culture (<0.250×10^3 CFU mL^{-1}). Significant differences were observed between both treatments ($P < 0.05$) with the highest values towards the end of the study in the darkness treatment (Fig. 2). Whereas the concentration of *Bacillus* sp. depicted an inverse behaviour, with the highest values between the 7th and 14th days of culture, in this case, the *Bacillus* sp. concentration did not present significant differences between both treatments except at 0 and 14 days of culture ($P > 0.05$). The concentration of *Bacillus* sp. fluctuated between 7×10^3 and 6.60×10^5 CFU mL^{-1} with the lowest values towards the study end in the darkness treatment (Fig. 2).

Shrimp performance

After 28 days, the performance of shrimp postlarvae did not differ significantly between the cultures with natural sunlight and in darkness ($P > 0.05$; Table 2 and Fig. 3). In both treatments, the final weight of shrimp was over 0.6 g with an SGR over 7.4% d^{-1}, and a survival rate over 70% (Table 2).

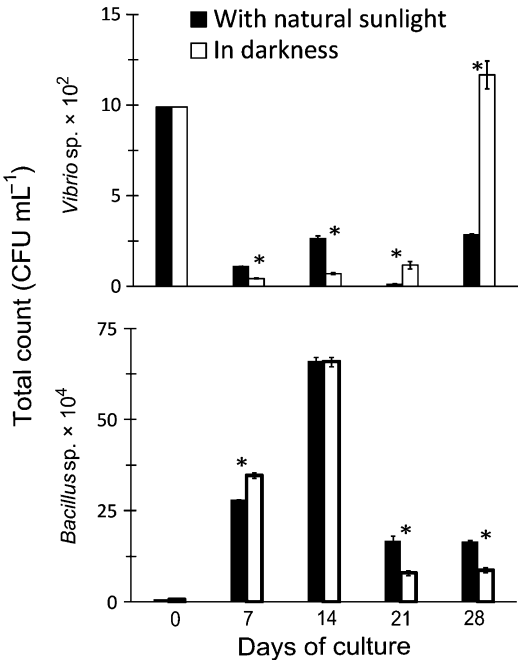

Figure 2 Effect of light limitations on presumptive *Vibrio* sp. and *Bacillus* sp. counts (CFU mL^{-1}) in the water of the culture of *Litopenaeus vannamei* postlarvae at low salinity with biofloc. Each bar represents the mean (±SD) of three replicates. *significant differences ($P < 0.05$).

Discussion

Water quality

Light limitation did not affect significantly the DO, temperature, pH and salinity, as similar values were found under natural sunlight and darkness conditions. The concentrations of the other water quality parameters (total ammonia, nitrite, nitrate, phosphate, alkalinity and TSS) presented differences between treatments only in some periods of the study, but except for alkalinity (in the first stage of the study), all parameters remained within the ranges reported as suitable for shrimp culture (Wickins 1976; Van Wyk & Scarpa 1999; Samocha, Patnaik, Speed, Ali, Burger, Almeida, Ayub, Harisanto, Horowitz & Brock 2007; Furtado, Campos, Serra, Klosterhoff, Romano & Wasielesky 2015).

At the start of the study, the alkalinity was similar in both treatments, at about 100 mg L^{-1}, but in the darkness treatment, alkalinity showed a value lower than 50 mg L^{-1} at the 7th day of culture. For this reason, all tanks of this treatment were supplied with sodium bicarbonate for three consecutive days, according to other reports (Furtado, Poersch & Wasielesky 2011; Furtado, Gaona, Poersch & Wasielesky 2014). This procedure was performed after the 7th day of culture to maintain an alkalinity above 100 mg $CaCO_3$ L^{-1} to ensure a good biofloc development (Ebeling, Timmons & Bisogni 2006; Furtado *et al.* 2014). It is important to mention that before the addition of sodium bicarbonate, the TSS concentration in the culture under darkness was lower than in the culture with natural sunlight, revealing the low biofloc performance as TSS are often used as indicators for quantitative determination of biofloc (De Schryver *et al.* 2008). However, after the addition of sodium bicarbonate, alkalinity was around 100 mg L^{-1} in both treatments; however, in the final stage of the study, the culture with natural sunlight presented a higher alkalinity than the culture in darkness. It is possible that this was related to the metabolism of bacteria that predominate in each system, as mostly heterotrophic bacteria prevailed in darkness, and Ebeling *et al.* (2006) reported that this bacterial group presents a lower yield of alkalinity

Table 2 Performance of *Litopenaeus vannamei* postlarvae reared at low salinity with biofloc, with and without light limitation during 28 days

Trial	Initial weight (g)	Final weight (g)	Survival (%)	FCR	SGR (% d^{-1})	Productivity (kg m^{-3})	Final density (orgs m^{-3})
With natural sunlight	0.09	0.72 ± 0.08	78.0 ± 16.8	0.97 ± 0.14	7.4 ± 0.4	0.17 ± 0.02	780 ± 168
In darkness	0.09	0.69 ± 0.02	73.7 ± 5.5	0.98 ± 0.13	7.3 ± 0.1	0.15 ± 0.02	737 ± 55

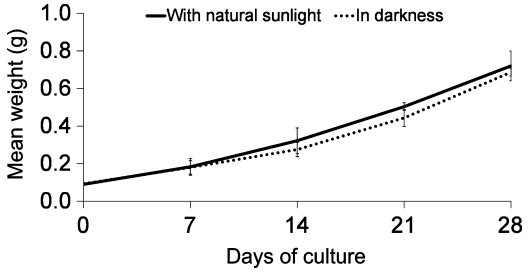

Figure 3 Weight performance (mean ± SD) of *Litopenaeus vannamei* postlarvae reared at low salinity with biofloc, with and without light limitation.

per consumed nitrogen (3.57 g alkalinity/g N) as compared with autotrophic bacteria (7.05 g alkalinity/g N).

Both treatments showed an increasing trend in nitrate concentration with the progression of the study, suggesting a greater intensity of the nitrification processes as reported by Esparza-Leal *et al.* (2016). In the culture with natural sunlight, a tendency of lower nitrate concentration prevailed than in the culture in darkness. This could be accounted for by the lower ability of heterotrophic bacteria to assimilate nitrate as concluded by other authors (Horrigan, Hagstrom, Koike & Azam 1988; Kirchman, Moss & Keil 1992; Kirchman, Ducklow, McCarthy and Garside (1994), who found that nitrate uptake by heterotrophic bacteria is usually low compared to autotrophic organism, which prevailed in the culture with natural sunlight.

Similar results were found in nitrite and phosphate concentrations. Conversely, although no significant differences were found in some sampling periods, total ammonia, alkalinity and TSS showed the highest values in the culture with natural sunlight. However, despite the observed differences in water quality variables between the two treatments, values did not exceed the limits recommended for shrimp farming. These results suggest that once a mature biofloc community is established in the culture water, water quality parameters, with exception of alkalinity, can be controlled. This control can be achieved by either heterotrophic assimilation (e.g. total ammonia assimilation into microbial biomass; culture in darkness) or autotrophic nitrification (e.g. total ammonia assimilation to nitrite and then to nitrate; culture with natural sunlight) as reported by other authors (Ebeling *et al.* 2006; Hargreaves 2006; Xu *et al.* 2016).

In shrimp culture, zero or minimal water exchange increases the amount of organic matter in the water, favouring the development of *Vibrio* sp.; in turn, high concentrations of these species are related to large amounts of organic matter in the culture (Ferreira, Bonetti & Seiffert 2011). However, despite that TSS concentration was higher than 100 mg L^{-1} in both treatments during most of the study, *Vibrio* sp. concentration had low values (culture with natural sunlight = 0.1 to 9.9×10^2 CFU mL^{-1}, culture in darkness = 0.4 to 11.7×10^2 CFU mL^{-1}) with two peaks (in both treatments at the beginning and at end in the darkness culture). This behaviour was inversely proportional to the concentrations of *Bacillus* sp. (culture with natural sunlight = 0.7 to 66.0×10^4 CFU mL^{-1}, culture in darkness = 0.7 to 65.8×10^4 CFU mL^{-1}), which is consistent with other studies that indicate that the community of *Vibrio* sp. can be displaced by *Bacillus* sp. For example, addition of *Bacillus* sp. as a probiotic to penaeid shrimp-rearing ponds has been shown to decrease luminous *Vibrio* sp. densities (Moriarty 1998). Other studies have reported that the addition of *Bacillus* spp. to the shrimp diet can reduce *Vibrio* sp. concentrations (Boonthai, Vuthiphandchai & Nimrat 2007; Nimrat, Suksawat, Maleeweach & Vuthiphandchai 2008). *Bacillus* sp. might outcompete *Vibrio* sp. for nutrients and space and exclude other harmful bacteria. They secrete either exoenzymes or metabolites that degrade the slime layers or biofilms of pathogenic *Vibrio* sp., allowing penetration of inhibitory substances, such as polymyxin, bacitracin and

gramicidin, into cells (Moriarty 1997; Rhodehamel & Harmon 1998).

Shrimp performance

In this study, the performance of shrimp postlarvae did not differ significantly between the cultures with natural sunlight and in darkness, these results were different to those reported by Baloi *et al.* (2013), who demonstrated that shrimp juvenile production is higher in the light-exposed treatment. In our work, at 28 days of culture, shrimp growth fluctuated between 0.69 (in darkness) and 0.72 g (with natural sunlight) from a starting weight of 0.09 g, with a survival range of 73.7 (in darkness) to 78.0% (with natural sunlight) at a stocking density of 1000 orgs m^{-3}. Other reports indicate that with a biofloc system in 35 days at stocking densities of 4400 to 17 600 orgs m^{-3}, *L. vannamei* postlarvae (from PL′10, mean weight ≈0.008 g) may grow between 0.23 and 0.45 g, with mean survival rates up to 87.6% (Wasielesky *et al.* 2013). In another work, *Penaeus esculentus*, reared with water exchange at a rate of 80% during 56 days and stocking densities between 5720 and 11 430 orgs m^{-3} (from PL′17; mean weight ≈0.01 g), grew from 1.46 to 1.04 g, but the mean survival rates were low (21.2% to 39.1%; Arnold, Sellars, Crocos & Coman 2006). According to these studies, our findings with both treatments were acceptable for this type of shrimp culture.

In summary, although in some sampling periods significant differences were found in the variables of water quality and bacterial counts, shrimp productive performance under culture with biofloc at low salinity (≈9 g L^{-1}) was not significantly affected by light limitation. At the nursery level, in both cultures, with natural sunlight and under darkness, growth, survival, FCR and SGR showed values that can be considered suitable for *L. vannamei* postlarvae rearing.

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