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Hatchery performance of the pacific white shrimp in biofloc system using different stocking densities

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ABSTRACT

Higher rates of production per unit area, avoiding or minimizing significant environmental damages, is a challenge for aquaculture to contribute with an ascendant demand for food due to the increase in world population. The present study assessed the hatchery performance of *Litopenaeus vannamei* between the mysis 1 and postlarva 5 stages, in a zero-exchange biofloc system under 12.5:1 fixed C:N ratio with dextrose in four stocking densities: 200, 250, 300 and 350 larvae per liter (D200, D250, D300, D350 respectively). Water quality and performance parameters were compared among treatments. The mean values of the evaluated water quality parameters were appropriate for this production stage in all treatments. Fertilization with dextrose efficiently controlled ammonia levels not reaching the average concentrations considered toxic for the specie. Lower values of pH and higher values of volatile solids were finding in D350. There was no difference between groups in means of survival and dry weight of postlarva 5. Therefore, the use of biofloc systems without water exchange with dextrose as a carbon source in 12.5:1C:N ratio resulting in adequate production indexes and water quality during the mysis 1 to postlarva 5 hatchery phase of *L. vannamei*. Based in these results the density limit to a viable BFT hatchery was not reached.

1. Introduction

The intensity levels of inputs passing through aquaculture systems varies from extensive systems stocking young organisms to grow naturally, to very intensive with high stocking rates and basically commercial feed. Nowadays, aquaculture undergoes continual modification with new methods and technologies (genetic, feed formulation and water management) to achieve higher rates of production per unit area. This intensification certainly will continue in coming future (Diana et al., 2013).

Specifically, biofloc technology (BFT) allows the production intensification and avoid the water exchange of farming minimizing the flow of pathogens and discharge of nutrient-rich effluents into the environment (Samocha et al., 2007). In BFT systems without water exchange, the ammonia control (to be toxic for fish and shrimp) starts by a carbon/nitrogen balance that promotes the growth of heterotrophic bacteria, which incorporate ammonia nitrogen from the water (Moriarty, 1997; Avnimelech, 1999; Ebeling et al., 2006; Hari et al., 2006; Samocha et al., 2007). The suitable ratio for this purpose is obtained by adding organic carbon sources (such as molasses, flours, sugar and dextrose) to ponds. It requires 20 g of carbohydrate, or about 6 g of carbon, to convert 1 g of ammonia nitrogen to bacterial biomass (Avnimelech, 1999; Ebeling et al., 2006).

This approach could be applied specifically to shrimp hatchery considering pros and cons to be understood, besides the potential intensification contributions can bring to improve the knowledge at this phase taking account production and sustainability. A first systematic approach with BFT in hatchery phase, evaluated the performance of *L. vannamei* between the mysis 1 (M1) and postlarva 5 (PL5) stages in zero-exchange BFT systems with 200 larvae per liter (Lorenzo et al., 2015). Similar results in terms of water quality and performance were obtained from the standard production system with water exchange (control group) and with addiction of organic carbon sources (BFT group) to kept the total ammonia nitrogen (TAN) levels near 1 mg L^{-1}. Secondly, using the same stocking density, efficient average C/N ratio that resulted from the input of

Abbreviations: BFT, biofloc technology.

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organic matter (artemia + feed + source of carbon) to control ammonia was over 12.5:1 according to our second hatchery approach using different fertilization levels (Lorenzo et al., 2016).

Generally, the stocking density in shrimp hatchery is 150 nauplii per liter (FAO, 2003), however the BFT can increase these stocking density. Therefore, the objective of the present study was to assess the hatchery performance of *L. vannamei* using a zero-exchange BFT system, from mysis 1 to postlarva 5 stages, under four stocking densities (200, 250, 300, 350 larvae L^{-1}) looking for BFT hatchery intensification potential.

2. Materials and methods

The experiment was conducted at the Laboratório de Camarões Marinhos (LCM), Departamento de Aquicultura of the Universidade Federal de Santa Catarina, Brazil.

2.1. Biologic material

The utilized larval line was free of any pathogens that require notification of the International Organization of Epizootics (from Aquatec LTDA, Rio Grande do Norte, Canguaratema, Brazil). Before the experiment, nauplii of *L. vannamei* were raised in a 4 m^3 (stocking density of 100 larvae L^{-1}), semi-cylindrical hatchery tank in salinity of 35 ppm until they reached mysis 1. The microalgae *Chaetoceros muelleri* (5×10^4 cells mL^{-1}) was added and maintained to the culture water daily. When the larvae reached the stage of mysis 1 (average dry weight of $0{,}031 \pm 0{,}004$ mg and average length of $3{,}57 \pm 0{,}029$ mm) they were transferred to the experimental units, which were initially filled with 50% water from the hatchery tank and 50% filtered and disinfected water.

2.2. Experimental conditions

The larvae were reared under four treatments with different densities ratios: 200, 250, 300 and 350 postlarvae L^{-1} (D200, D250, D300 and D350 respectively). The organic source used was anhydrous dextrose ($C_6H_{12}O_6$, Sigma-Aldrich), and was added four times per day. The carbohydrate percentage of dextrose was assumed to be 100%.

The experimental groups were randomly distributed in a unifactorial experimental design. Semi-cylindrical plastic tanks ($92 \times 68 \times 25$ cm) with a working volume of 60 L constituted the experimental units. Four tanks were prepared for each experimental condition, resulting in 16 tanks. All tanks were equipped with linear aeration supplied by a PVC pipe (90 cm long, 20 mm diameter with 36 holes of 1 mm) to keep the solids generated during cultivation in suspension and maintain the level of dissolved oxygen in the water at the recommended concentration for *L. vannamei* larval cultivation (>5 mg L^{-1}). The water temperature was kept constant, between 29 and 30 °C using 100-W heaters connected to a thermostat.

The water supplied had the following parameters: *Chaetoceros muelleri* (5×10^4 cells mL^{-1}), oxygen = 6.0 mg L^{-1}, pH = 8,1, temperature = 31 °C, salinity = 35.0 mg L^{-1}, total ammonia = 0.6 mg L^{-1}, free ammonia = 0.06 mg L^{-1}, nitrite = 0.01 mg L^{-1}, nitrate = 2.8 mg L^{-1}, phosphate = 0.001 mg L^{-1}, total suspended solid (TSS) = 243.5 mg L^{-1}, volatile suspended solid (VSS) = 44.0 mg L^{-1}, and alkalinity = 120 mg L^{-1}.

The nauplii were reared in the matrix tank until reach the mysis 1 phase. Thereafter larvae were concentrated in a 200 L square tank with strong aeration to homogenize and count. Sample of 50 mL were counted to estimate the quantities of mysis 1 per liter and the stocking density was established by volume. Each experimental unit was stocked with 12000, 15000, 18000 and 21000 larvae in M1 stage according to density treatments D200, D250, D300 and D350 respectively. The experiment was conducted until the larvae reach the postlarva stage 5 (seven days after stoking). The water in BFT experimental units was not exchanged during the experimental period, but evaporated water was replaced with fresh water in order to maintain salinity. No suspended solids were removed from the water during the experiment.

The larval and postlarval shrimp were fed microencapsulated commercial diets (INVE) according to Lorenzo et al. (2015) based on the manufacturer's recommendation for each larval stage. The Lansy ZM diet (minimum protein 48%, minimum ether extract 13%, maximum fibrous matter 2.5%, maximum mineral matter 13%, maximum humidity 8.0%, minimum calcium 0.25%, maximum calcium 1.5%, and minimum phosphorus 1.0%) was fed from M1 to mysis 3/postlarva 1. After this period, and until harvesting, postlarvae were fed with Lansy MPL diet (minimum protein 48%, minimum ether extract 9.0%, maximum fibrous matter 2.5%, maximum mineral matter 13%, maximum humidity 9.0%, minimum calcium 1.0%, maximum calcium 2.2%, and minimum phosphorus 1.0%). The carbohydrate level for both diets was 50% for the input calculation.

The amount of feed was adjusted for each hatchery phase in grams per million larvae/postlarvae like follow: mysis 1 = 45 g/million, mysis 2 and 3 = 55 g/million, postlarva 1 = 60 g/million, postlarva 2 = 65 g/million, postlarva 3 and 4 = 70 g/million, postlarva 5 = 75 g/million.

Larvae were fed nine times a day (0800, 1000, 1200, 1400, 1600, 1800, 2100, 2300, and 0300), and *Artemia* nauplii(considered 48% protein and 2,42 μg $nauplii^{-1}$) were also provided to the larvae at a rate of six nauplii for each mysis or postlarva, five times each day (0900, 1100, 1500, 1700, and 0000).

2.3. Addition of carbohydrates

The fertilization with organic carbon for ammonia control and the consequential C:N ratio obtained was based on the feed and *Artemia* nauplii introduction daily (Table 1). The amount of nitrogen and carbon derived from the food was considered and the carbohydrate (dextrose) was administered to the experimental units to reach 12.5:1C:N ratio (Lorenzo et al., 2016).

2.4. Chemical and physical variables of the water

Dissolved oxygen, temperature (YSI 55, YSI Incorporated, Yellow Springs, OH, USA) and pH (YSI 100, YSI Incorporated, Yellow Springs, OH, USA) were measured twice a day. Salinity (YSI 30, YSI Incorporated, Yellow Springs, OH, USA), alkalinity (APHA 2005-2320 B), total suspended solids (TSS) and total ammonia were analyzed daily (APHA, 2005). Volatile suspended solids (VSS) and nitrite and nitrate were assessed every other day (APHA, 2005-2040 D and 2005-2540 E). TSS and VSS were filtered using 0.6-μm glass fiber micro-filters (GF-6, Macherey-Nagel, Düren, Germany).

Two hundred milliliters of water samples were collected from each tank. Samples were frozen until nitrate (HACH method 8039, cadmium reduction) and orthophosphate analysis. The TAN, nitrite, nitrate, and orthophosphate analyses were carried out using a spectrophotometer and analyzed according to Strickland and Parsons (1972), and following the guidelines contained in APHA (2005).

2.5. Larval quality and performance

Each day, 20 larvae from each tank were analyzed at the macro and microscopic level to assess larval quality. We observed the following parameters: swimming activity, lipid reserves and color of the hepatopancreas, intestinal contents, deformities, presence of epibionts, adhered particles, necrosis and muscular opacity (FAO, 2003; Suita et al., 2015). Performance parameters used to evaluate treatments included ultimate survival (%), final dry weight (mg,