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Testing an intensive shrimp hatchery system using biofloc technology

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Study in Brazil gauges adequate production indexes and water quality results for Pacific white shrimp



Generic PLs.

The hatchery phase is a critical and complex first step in the commercial farming of Pacific white shrimp, and constant attention is required to produce shrimp postlarvae of the best quality possible.

The use of biofloc technology (BFT) is increasing in the aquaculture of many commercially-important aquatic species. BFT is used to intensify production and to minimize or prevent the exchange of culture water, with a consequent reduction in potential pathogens and the discharge of nutrient-rich effluents into the environment.

The objective of this study was to assess the hatchery performance of Pacific white shrimp (*Litopenaeus vannamei*) between the larval stages of M1 and PL5 (seven days after experimental units stocking) using a BFT system with the addition of organic carbon (molasses or dextrose) and without water exchange.

Shrimp hatcheries and BFT technology

The hatchery stage is a critical stage in the production of Pacific white shrimp, and where constant attention is required. The hatchery stage extends from the nauplii phase to the postlarva 5 (PL5) phase. At this stage, shrimp are extremely susceptible to physical, chemical and biological stressors, such as vibriosis outbreaks.

Hatchery shrimp production is traditionally performed in a predominantly autotrophic medium, with high rates of daily water exchange. At this stage, microalgae rich in polyunsaturated fatty acids are added every day, after water exchange. These microalgae not only contribute to the nutrition of the larval shrimp, but also enable the control of ammonia nitrogen levels in the tanks.

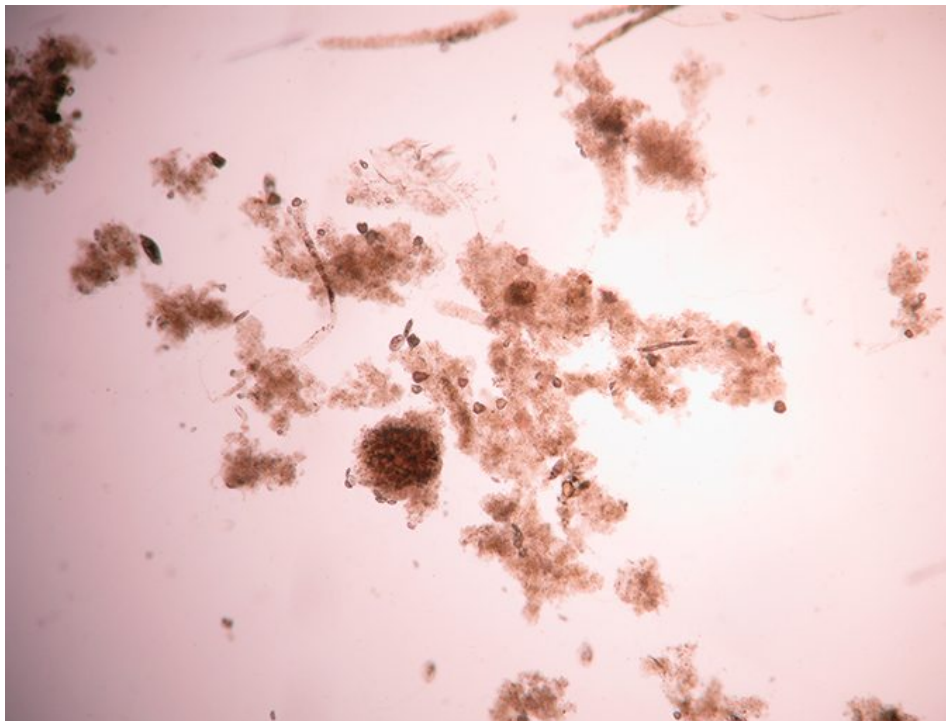
Undesirable impacts associated with such production systems – such as the discharge of large volumes of water with high levels of ammonia nitrogen and phosphorus (microalgae, feces, and uneaten feed) – may be unwanted in many coastal ecosystems and could lead to other risks. In this context, the sometimes considerable economic costs of the energy required to capture, heat, and distribute large volumes of water must be considered too.

Reducing water exchange requires control of the ammonia from protein catabolism, as it is toxic to shrimp. Ionized and non-ionized ammonia are present in the water of tanks in variable proportions that are influenced by factors such as pH, temperature and salinity. The non-ionized form of ammonia is more toxic to shrimp than the ionized form, and causes a variety of physiological damage due to its affinity for the non-polar compounds of the plasma membrane.

In BFT systems without water exchange, the ammonia control starts on the establishment of a carbon-nitrogen balance that facilitates the growth of heterotrophic bacteria, which incorporate ammonia nitrogen from the medium. This relationship is established by adding organic carbon sources (molasses, flours, sugar, and dextrose) to aquaculture media. It requires 20 g of carbohydrate, or about 6 g of carbon, to convert 1 g of ammonia nitrogen to bacterial biomass.

In BFT culture systems, chemoautotrophic and heterotrophic bacteria participate in the formation of bioflocs, which also include an aggregate of algae, fungi, protozoa, rotifers, and nematodes. Therefore, in addition to providing ammonia control, bioflocs may represent a food source in the shrimp production tanks.

The use of BFT systems in the pre-nursery and growout stages of marine shrimp aquaculture have been extensively studied. However, systematic studies with BFT without water exchange during the hatchery phase as an alternative to the standard production systems of penaeid shrimp larvae is just beginning.



In BFT culture systems, bioflocs can be a food source for the cultured animals.

Study setup

The experiment was conducted at the Laboratório de Camarões Marinhos (LCM), Departamento de Aquicultura, Universidade Federal de Santa Catarina, Brazil. Before the experiment, nauplii of *L. vannamei* were raised in a 20 m³ (stocking density of 100 larvae/L), semi-cylindrical hatchery tank at a salinity of 35 ppm until they reached mysis1.

The microalgae *Chaetoceros muelleri* (at 5×10^4 cells/mL) was added to the culture water daily. When the larvae reached the stage of M1 they were transferred to the experimental 60 L units (200 M1/L), which were initially filled with the same water from the hatchery donor tank. Larvae were fed nine times a day (0800, 1000, 1200, 1400, 1600, 1800, 2100, 2300, and 0300) and were provided quantities according to the feed manufacturer's recommendation for each larval stage. *Artemia* nauplii 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).

Three groups of larvae were set up: a control group was reared in a conventional autotrophic system with daily water exchange and the addition of microalgae, and two experimental groups were reared in a heterotrophic system without water exchange.

To keep total ammonia nitrogen (TAN) below the established maximum of 1 mg/L, the water of the control units was exchanged at rates that ranged from 50 percent per day at the start of the experiment to 200 percent in the final stage of cultivation. After water exchange, *Chaetoceros muelleri* was counted and added to maintain a concentration of 5×10^4 cells/mL.

Sources of organic carbon were added (divided in four times per day) to the biofloc tanks to maintain ammonia levels of <1 mg/L. Anhydrous dextrose (100 percent carbohydrate) was added for one group, and sugar cane molasses (55 percent carbohydrate, 3 percent crude protein) was added to the second group.

The fertilization with organic carbon was done in two ways:

- 1) The amount of carbohydrate required to neutralize the ammonium excreted was estimated assuming that shrimp assimilate 25 percent of the food nitrogen and 75 percent of this nitrogen is transformed into ammonia dissolved in water. Sources of carbon were added daily to each tank at a ratio of 20 g carbohydrate for each gram of TAN expected.
- 2) When TAN levels exceeded 1 mg/L, additional carbohydrate (molasses or dextrose) was added to the system at a ratio of 20 g carbohydrate for each excess gram of TAN.

View of the experimental setup used in the study.

Results

Daily physical and chemical analysis of water was performed, as well as microbiological water analysis at the end of the experiment. All water quality parameters remained within the appropriate range for the hatchery stage of *L. vannamei*. These parameters were similar in the conventional production system with high rates of daily exchange (control), and the BFT production systems with both sources of organic carbon (dextrose and molasses).

The mean values of TAN and non-ionized ammonia remained below toxic levels (total ammonia <1.3 mg/L and free ammonia <0.05 mg/L). The molasses treatment results in the lowest values of ammonia, as shown in Fig. 1, which also presents the profile variation of TAN and free ammonia by treatment during the experiment.

Total ammonia increased somewhat in the dextrose group on the last two days of the experiment (Fig. 1A). However, the average levels of toxic ammonia in the BFT groups did not significantly differ from those of the control group on any day of the experiment (Fig. 1B).

The measurements of nitrite (< 0.02 mg/L) suggest that nitrification was not established during the course of our experiment and that the activity of the heterotrophic bacterial community successfully controlled ammonia in the BFT groups (Figure 1B).

The number of heterotrophic bacteria in the water was greater in the molasses and dextrose groups than in the control group. However, there was no difference in vibriionaceae count between groups.

Larval quality, zootechnical performance, low salinity stress survival (19 mg/L) and water usage were also evaluated (Table 1). We did not observe any difference in larval quality parameters between treatments. All larvae were active (high swimming activity), had lipid reserves, normal hepatopancreas coloration and full intestines. We found no deformities, epibionts, adhered particles necrosis or

Fig. 1: Daily mean total (A) and free ammonia (B) in Pacific white shrimp (*Litopenaeus vannamei*) hatcheries between the mysis 1 and postlarva 5 phases. White bars represent controls maintained using a conventional water exchange technique; gray bars represent biofloc systems supplemented with dextrose; and black bars represent biofloc systems supplemented with sugar cane molasses. Water was not exchanged in either of the biofloc systems. Different letters on the same day indicate significant differences, as indicated by Tukey's test of mean separation ($p < 0.05$).

muscular opacity.

Final survival, body length, dry weight and survival to a salinity stress test did not differ between groups, showing homogeneity to the productive parameters and larval resistance (Table 1).

BFT treatments required approximately 12 percent of the water used by the control group. Such a reduction in the amount of water required for the hatchery phase of shrimp aquaculture would proportionally reduce the costs associated with the capture, disinfection and neutralization, heating and pumping of water for hatcheries.

Parameter	Control	Dextrose	Molasses	p
Survival (%)	90.58 ± 5.40a*	90.23 ± 10.51a	85.13 ± 11.15a	0.7058
Stress survival (%)	97.45 ± 2.01a	95.39 ± 3.25a	93.67 ± 6.11a	0.4731
Final length (mm)	6.14 ± 0.21a	6.11 ± 0.19a	6.20 ± 0.23a	0.5093
Final weight (mg)	0.155 ± 0.02a	0.197 ± 0.06a	0.178 ± 0.01a	0.3206
Water consumption (L per thousand postlarvae 5)	56.22 ± 3.31a	6.49 ± 0.79b	6.89 ± 0.95b	<0.0001

Perspectives

The use of biofloc systems without water exchange that are supplemented with molasses or dextrose as a carbon source results in adequate production indexes and water quality during the hatchery phase of *L. vannamei*. Because water is not exchanged in these biofloc systems, it requires approximately 12 percent of the water used in the conventional autotrophic system, and is an appropriately hygienic, economic and ecological approach that can be further developed.

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