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Health & Welfare

Comparing recirculating, biofloc and synbiotic systems for Pacific white shrimp in low salinity and high-density conditions

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A synbiotic system produced shrimp with higher survival, yield and lower feed conversion than the RAS and BFT systems

This study compared recirculating aquaculture, biofloc and synbiotic systems to produce Pacific white shrimp under low salinity and high-density conditions. View of the shrimp harvested from the super-intensive culture with a synbiotic system at low salinity. Photo by Otávio Augusto L. F. Pimentel.

The Pacific white shrimp (*Penaeus vannamei*) is the most widely produced invertebrate species in the global aquaculture industry and its production in inland regions using low salinity water is a reality around the world. This inland production is possible due to the species hardiness characteristics, which allows it to withstand a wide range of salinities, temperatures and stocking densities. This opens the door to *P. vannamei* culture in intensive systems such as recirculating aquaculture systems (RAS), biofloc technology (BFT) and synbiotic systems.

These systems allow for greater yield while reducing water and land use, thus being environmentally friendly alternatives to other production systems. RAS requires controlled conditions, with water treatment using various mechanical and biological filtration stages before recirculating it back into the production tanks. BFT is a microbial-based system characterized by manipulating the water's carbon:nitrogen (C:N) ratio with a simple organic carbon source, such as molasses or sugar, to control total ammonia nitrogen (TAN) by stimulating the growth of heterotrophic bacteria. These groups of bacteria assimilate ammonia into biomass, creating microbial aggregates, known as bioflocs.

The synbiotic system has been gaining ground recently, with the potential to expand fertilization management strategies for *P. vannamei* crops. This system is characterized by fertilizing water with plant brans processed by probiotic microorganisms (e.g., Bacillus, Lactobacillus and yeast). In synbiotic systems, the fertilizer improves water quality by helping control TAN, as it is a source of organic carbon and also serving as a nutritious and functional supplementary food source for shrimp. This occurs because probiotic microorganisms colonize the bran particles during processing, and when released into the water, the shrimp can consume them. Chemoautotrophic bacteria grow in both BFT and synbiotic systems, playing a key role in controlling ammonia and nitrite, which are highly toxic in low salinity conditions.

Intensive culture of *Penaeus vannamei* in a BFT system at the Federal University of Rio Grande – FURG, Brazil. Photo by Otávio Augusto L. F. Pimentel.

Given the expansion of *P. vannamei* culture to inland regions, it is important to highlight the lack of comparative studies among RAS, BFT and synbiotic systems using low-salinity water; and also, their impact on the control of nitrogenous compounds, microbial composition, and shrimp growth.

Investigations from this perspective are important to overcome the barriers posed by shrimp culture under these unique conditions. Therefore, the aim of this study was to analyze the effect of RAS, BFT and synbiotic systems on water quality, plankton composition, and *P. vannamei* growth in water with a salinity of 2 grams per liter and high stocking density.

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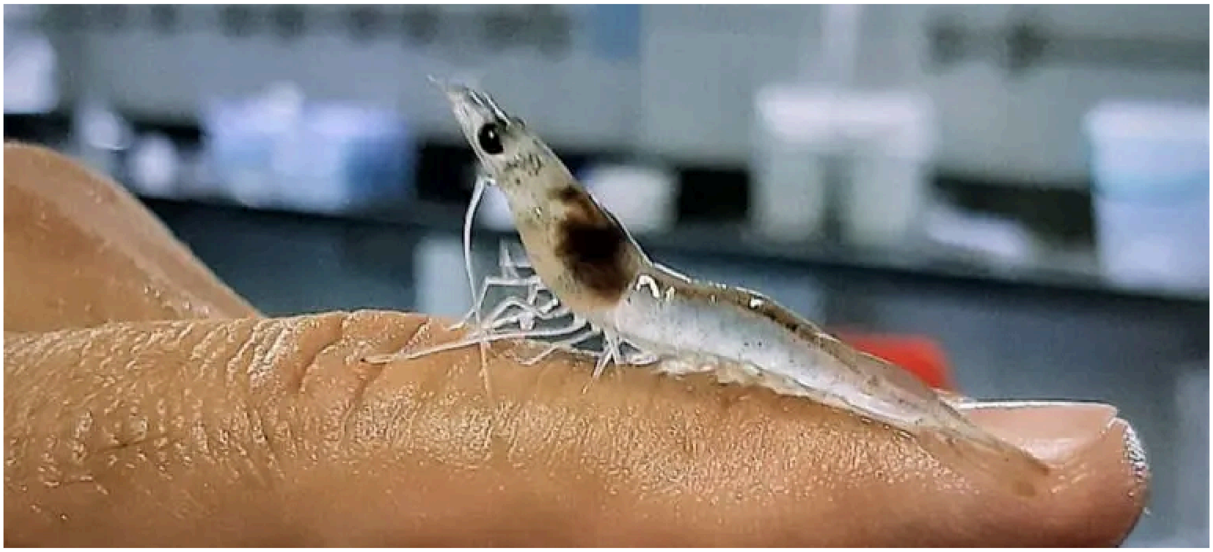

Pacific white shrimp nursery trials in seawater and low-salinity water utilizing a synbiotic system

Authors evaluate L. vannamei PL performance in a synbiotic nursery with rice and wheat bran as carbon sources in seawater and low-salinity water.

Global Seafood Alliance

Study setup

This study was conducted at the Virginia Seafood Agricultural Research and Extension Center (VSAREC), Virginia Polytechnic Institute and State University (Virginia, USA) (Fig. 1a). A *P. vannamei* culture at a density of 500 shrimp per cubic meter (initial weight: 1.27 ± 0.06 grams) was carried out for 30 days in 0.1-cubic-meter experimental units testing the following treatments, all with three repetitions: recirculating aquaculture system (RAS), biofloc technology system (BFT) and synbiotic system (Fig. 1b). The water used in the experiment was obtained by mixing dechlorinated tap fresh water with commercial artificial salt (Instant Ocean Sea Salt) to reach a salinity of 2 grams per liter.

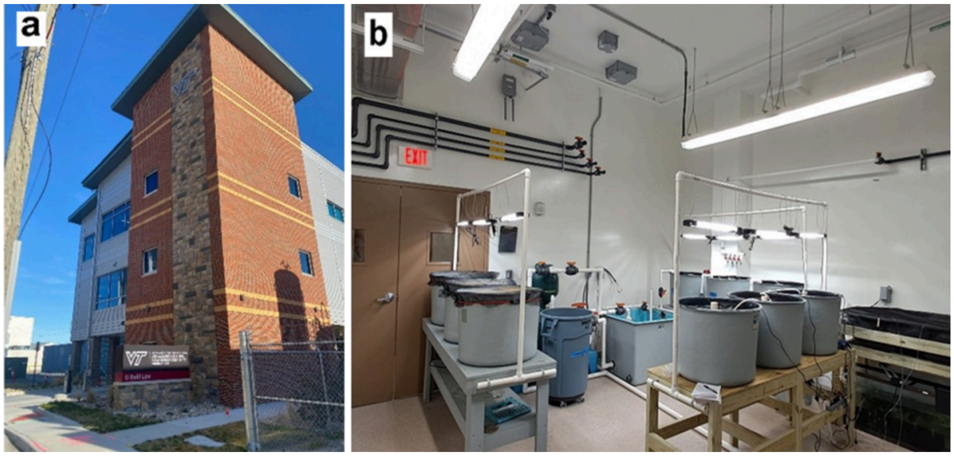

Fig. 1. External view of the VSAREC building (a) and experimental units used for super-intensive culture of *Penaeus vannamei* in low salinity RAS, BFT and synbiotic systems (b). Source: Dariano Krummenauer (a) and Otávio Augusto L. F. Pimentel (b).

The RAS system had a total volume of 0.6 cubic meters, and water was constantly recirculated at a flow rate of approximately 180 liters per hour. It was treated by mechanical filtration using a bubble bead filter and biological filtration (a sump with K1 Kaldnes Biological Media constantly aerated) before returning to the experimental units. Backwashes were conducted once a week to clean the mechanical filter. Before stocking the shrimp, the RAS system was matured with two applications of 5 mg per liter of ammonium chloride (NH_4Cl) over 10 days.

The BFT treatment was static and reused water from a previous *P. vannamei* culture at a salinity of 28 grams per liter and was gradually acclimated to a salinity of 2 grams per liter over 13 days. Dextrose was used as the carbon source and was applied at a C:N ratio of 15:1 only when the TAN was equal to or greater than 1 mg per liter. The RAS and BFT treatments received weekly applications of a commercial probiotic (Sanolife Mic, INVE Aquaculture) at a concentration of 0.4 grams per cubic meter.

The synbiotic treatment was also static and was matured with 16 daily applications of processed rice bran before the stocking the shrimp. The synbiotic fertilizer consisted of rice bran (20 grams per cubic meter), the same commercial probiotic (0.4 grams per cubic meter), dextrose (2 grams per cubic meter), sodium bicarbonate (2 grams per cubic meter), and water at a salinity of 2 grams per liter in a 10:1 ratio [volume of clean water (L): rice bran weight (kg)]. The fertilizer was processed through a 12-hour anaerobic phase followed by a 12-hour aerobic phase and then applied to the system. In addition to organic fertilizers, the synbiotic treatment also received daily applications of 1 mg per liter of NH_4Cl as an inorganic nitrogen source to aid in the establishment of nitrifying bacteria. During the trial, daily fertilizer applications were made for the first 15 days, thereafter applications three times a week. The BFT and synbiotic treatments received 15 × 15 cm pillows containing artificial substrates (K1 Kaldnes Biological Media) to aid in the nitrification process. In all treatments, the water temperature was maintained around 30 degrees-C using heaters.

During the trial, shrimp were fed twice daily with a commercial 35 percent crude protein diet (Zeigler Bros). Nitrogenous compounds, calcium and magnesium concentrations, and Mg:Ca ratio were monitored. Ten percent volume water exchanges in experimental units were performed when the TAN exceeded 1 mg per liter. At the end of the trial, the main groups of zooplanktonic microorganisms in the water were identified and quantified.

Results and discussion

At the beginning of the trial, calcium concentration varied between 90 mg per liter in the synbiotic treatment and 100 mg per liter in the RAS, and magnesium varied between 90 mg per liter in the RAS and 140 mg per liter in the BFT treatment. The Mg:Ca ratio varied from 0.9:1 in the RAS and 1.68:1 in the BFT treatment. At the end of the trial, calcium concentration was 110 mg per liter in the RAS treatment, 80 mg per liter in the BFT, and 90 mg per liter in the synbiotic treatment. Magnesium was 120 mg per liter in the RAS, 100 mg per liter in the BFT, and 130 mg per liter in the synbiotic treatment. The Ca:Mg ratio was 1.09:1 in the RAS, 1.26:1 in the BFT and 1.44 in the synbiotic treatment. Both at the beginning and at the end of the trial, the concentrations of calcium and magnesium ions were above the recommended threshold for intensive culture of *P. vannamei* in low-salinity water, which is 25 mg per liter for calcium and 89 mg per liter for magnesium. Although the Mg:Ca ratio did not follow the recommended 3:1 ratio, this was not detrimental to shrimp growth during the trial.

The mean TAN was higher in the BFT (0.83 mg per liter) and synbiotic (0.75 mg per liter) treatments than in the RAS (0.42 mg per liter). The BFT had more events where the TAN exceeded 1 mg per liter (Fig. 2a), requiring partial water changes, and suggesting that the ammonia-oxidizing bacterial community was not yet fully established.

In shrimp culture systems with low salinity water, ammonia and nitrite toxicity are high, and the system must be fully matured to avoid spike events, which can be detrimental to the system. Despite the ammonia spikes in the BFT, nitrite remained stable throughout the trial, maintaining mean concentrations below 0.50 mg per liter. The mean nitrate was higher in the BFT (53.93 mg per liter) and the synbiotic (61.27 mg per liter) treatments than in the RAS treatment (17.80 mg per liter). In the BFT and synbiotic treatments, nitrate accumulation was observed throughout the trial, particularly in the synbiotic treatment (Fig. 2b), demonstrating the high activity of nitrifying bacteria in these treatments, especially nitrite-oxidizing bacteria.

This group of bacteria is responsible for oxidizing nitrite to nitrate. The lower nitrate levels in the RAS are explained by weekly backwashes to clean the mechanical filter, which discarded much of the nitrate produced in the system. The findings of this study demonstrated that the synbiotic system was more efficient in controlling nitrogenous compounds than the BFT in super-intensive shrimp culture systems using low salinity water, as it required fewer water changes, had low nitrite concentrations (no spikes) and resulted in greater nitrate accumulation. This was only possible through the use of the synbiotic treatment maturation protocol, which included daily organic fertilization with rice bran processed by probiotic microorganisms combined with fertilization with an inorganic nitrogen source until the nitrogenous compounds stabilized.

The synbiotic system had a higher abundance of ciliates and amoebae than that found in RAS and BFT. All treatments were dominated by flagellated protozoa. The RAS treatment had the highest relative abundance of amoebae (21 percent). Ciliates represented 6.8 percent of the total zooplankton in the RAS and BFT treatments and 8 percent in the synbiotic treatment (Fig. 3).

Rotifers represented 5 percent of the total zooplankton abundance in the RAS treatment, 2.7 percent in the BFT treatment, and 2.6 percent in the synbiotic treatment (Fig. 3).

Fig. 2. Patterns of total ammonia nitrogen (TAN, a), and nitrate nitrogen (NO_3−N, b) during a super-intensive culture of *Penaeus vannamei* with RAS, BFT and synbiotic systems in low salinity water.

The presence of protozoan microorganisms indicated heterotrophic dominance, a striking feature of both BFT and synbiotic systems. Protozoan growth is mediated by the presence of bacteria, which are the primary food source for these microorganisms. This dominance is stimulated to ensure greater

Fig. 3. Relative abundance of the main zooplankton groups in the RAS (a), BFT (b), and synbiotic (c) treatments in a super-intensive culture of *Penaeus vannamei* in low salinity water.

control of phytoplankton, water quality and nutrient cycling. Overall, BFT and synbiotic had a higher abundance of microorganisms than RAS and this was expected due to the operational characteristics of the culture systems.

The constant presence of microorganisms in the culture medium is an advantage for BFT and synbiotic, and this directly influenced the production indices of *P. vannamei*. The shrimp final weight was higher in the BFT (5.14 grams) and synbiotic (4.99 grams) treatments than in RAS (4.45 grams) (Fig. 4a). Survival was higher in the synbiotic (98 percent) than in RAS (80.67 percent) and BFT (85.33 percent) (Fig. 4b).

Fig. 4. Final weight (a), survival (b), feed conversion ratio (FCR, c) and yield (d) of super-intensive culture of *Penaeus vannamei* in RAS, BFT and synbiotic with low salinity water.

The FCR was higher in RAS (2.01) and BFT (1.80) than in the synbiotic (1.38) (Fig. 4c). The yield was higher in the synbiotic (2.31 kg per cubic meter) than in RAS (1.79 kg per cubic meter) and BFT (1.93 kg per cubic meter) (Fig. 4d).

The results demonstrated the positive effect of microorganisms as a supplementary source of natural food for shrimp, improving growth, survival, immune status of the animals, reducing feed costs, and increasing yield. This effect was most striking in the synbiotic treatment. Furthermore, the treatment that employed the synbiotic system had an additional benefit: the application of processed rice bran as fertilizer, which also provided a highly nutritious and functional supplemental source of natural food, as it is rich in probiotic microorganisms.

Considering these factors is extremely important when culturing marine shrimp in low salinity water, as it is a harsh environment for the animals. Therefore, creating more comfortable conditions related to water quality and the presence of supplemental natural food are alternatives for the super-intensive culture of *P. vannamei* in inland regions with low salinity water. It is important to emphasize that these results represent a short grow-out period and that a full cycle must be performed to confirm the positive effects of the synbiotic system on the super-intensive culture of *P. vannamei*.

Conclusion

The synbiotic system can be considered a promising approach for super-intensive culture of *P. vannamei* in low-salinity water, as it provided better nitrogenous compounds control compared to the BFT and a higher abundance of zooplankton microorganisms such as ciliates and amoeba than the RAS and BFT systems. The synbiotic system also produced shrimp with higher survival, yield and a lower FCR than the RAS and BFT systems.

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