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Health & Welfare

Challenging Pacific white postlarvae with AHPND

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Penaeus vannamei in biofloc water had higher survival rates

Penaeus vannamei postlarvae challenged with VPAHPND in biofloc had higher survival rates than animals similarly challenged but in clear water. Photo by Darryl Jory.

The Acute Hepatopancreatic Necrosis Disease (AHPND) that affects Pacific white shrimp (*Penaeus vannamei*) and black tiger shrimp (*P. monodon*) has had devastating effects on global production of farmed shrimp and caused significant economic losses. Since its onset, concerted research efforts have been made to better understand the causative agent of AHPND: the bacterium *Vibrio parahaemolyticus* with a plasmid containing the toxin genes; its mode of action and host pathology; field studies and conditions where mortalities occurred; and investigations on practices that mitigate the infection, slow its spread or affect its treatment.

Regarding biosecurity, management of the culture environment, management practices and the application of appropriate probiotics have been central themes. The beneficial effects of biofloc culture (flocculated aggregations of protein-rich organic material consisting of bacterial biomass, microalgae, fecal material, protozoa and others) on the growth rate, robustness and immune parameters of the shrimp are well documented. The use of biofloc is favored in closed intensive culture systems, since microorganisms can recycle the organic material to convert it into microalgae and bacterial biomass that forms additional flocculated material.

This biofloc stimulates the growth of heterotrophic and autotrophic microorganisms to process organic waste, converting waste carbon and nitrogen (for example, ammonia) into new bacterial biomass. Therefore, the need for water exchange in the growth phase of the commercial production of marine shrimp, therefore, is reduced.

A reduction in the abundance of *Vibrio* in biofloc systems has been reported, and the application of selected probiotics on the nutritional profile of biofloc in the culture of a variety of shrimp species, including Pacific white shrimp, has been studied. These changes in the microbial community and in the shrimp condition improve its growth and survival rates. And the use of disinfectants without proper pond preparation before stocking of shrimp postlarvae can lead to impoverished microbial communities, creating conditions that may facilitate the proliferation and dominance of certain bacterial species such as *V. parahaemolyticus*.

This article – adapted and summarized from *Aquacultura* (Ecuador) Issue 128, April 2019 – reports on a study in Thailand to investigate the possible protective effects under shrimp culture conditions of biofloc and shrimp before and during infection with a pathogenic strain of *V. parahaemolyticus* ($_{VP}$AHPND).

Study setup

A single cohort of *P. vannamei* postlarvae (PL12 from an anonymous, specific pathogen-free facility for confidentiality) was obtained and quarantined at the Fish Vet Group Asia Research Aquarium Limited (FVGAL) in Chonburi. The animals were disinfected, and samples stress-tested and evaluated for the presence of various pathogens, including the microsporidium *Enterocytozoon hepatopenaei* (EHP); AHPND; Infectious Hypodermic and Haematopoietic Necrosis Virus (IHHNV); Infectious Myonecrosis Virus (IMNV); Taura Syndrome Virus (TSV); White Spot Syndrome Virus (WSSV); and Yellow Head Virus (YHV), following World Organization for Animal Health (OIE) polymerase chain reaction (PCR) methodologies.

Once it was determined that the PL were of these diseases, the animals were stocked into aerated, 400-liter tanks containing a biofloc culture, and maintained at 15 ppt salinity and 28 to 29 degrees-C. For detailed information on the setup and management of the various tests carried out, including biofloc and statistical analyses, please refer to the original publication.

Results and discussion

For the first challenge test, after the experimental challenge with $_{VP}$AHPND, water samples were randomly collected from the test vessels and used to verify the dose of added bacteria. Shrimp were subsequently evaluated every three hours and any mortality was recorded. The trial ended 96 hours after infection, once the mortality trend stabilized, and Fig. 1 shows the cumulative mortality curves determined.

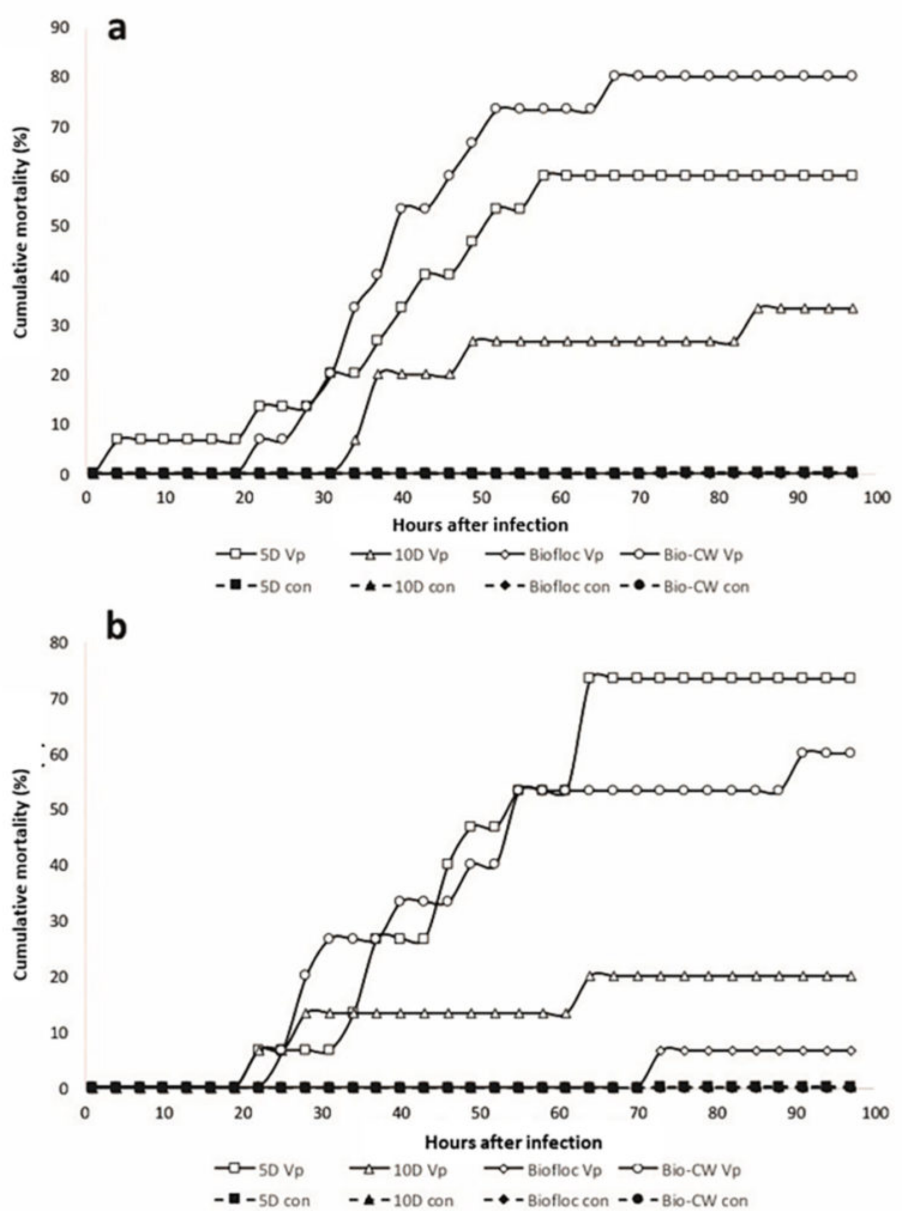

Fig. 1: Cumulative mortality curves for *Penaeus vannamei* cultured in biofloc and then challenged with two different doses of *Vibrio parahaemolyticus* (Vp isolate FVG0001) and compared with the corresponding controls. Fig. 1a = 1.2 mL of VPAHPND culture (dose 1). Fig. 1b = 1.8 mL of VPAHPND (dose 2) with an initial inoculum concentration of 1.28 × 108CFU/mL.

Two doses of $_{\text{VP}}$AHPND were used to test the shrimp (2.11 to 2.63 × 10^5CFU/mL and 2.84 to 4.54 × 10^5 CFU/mL; n = 60 shrimp per dose) against a control group (n = 60 shrimp). The highest mortalities were observed in the groups of shrimp that had been transferred more recently from biofloc, that is, those that were immediately transferred from biofloc to water and challenged, and those that had been reared in biofloc but kept for five days in clear water and then challenged.

Mortality rates were lower in shrimp that were transferred from biofloc and then kept in clear water for 10 days before being challenged in clear water, but the lowest mortalities occurred in the two groups of shrimp that were reared and tested in biofloc. All shrimp were handled identically, since they were transferred from their culture environment to their relevant containers approximately 4 hours before the challenge tests. The lowest mortality rate in the shrimp reared and tested in biofloc was significantly lower than for the other test groups.

The second challenge test used $_{VP}$AHPND doses of 1.33 to 1.44 × 10^{6}CFU/mL and produced results similar to those of the first challenge (Fig. 2). The assay, however, included filtered biofloc as a test condition. Again, the highest level of mortality, 73.3 percent, was observed in shrimp transferred from biofloc to clear water before pathogen exposure, which differed significantly from those reared and challenged in biofloc, which had the lowest level of mortality (13.3 percent). A high level of mortality (53.3 percent) was also observed in shrimp cultured in water for seven days before the challenge. Lower levels of mortality were observed in the other groups tested with biofloc, that is, 26.7 percent for shrimp reared in clear water for seven days but then tested in biofloc, and only 20 percent mortality in animals shrimp reared in biofloc but tested in filtered (< 2µm) biofloc.

Fig. 2: Cumulative mortality curves of *Penaeus vannamei* in the second challenge study with *Vibrio parahaemolyticus*.

Research has demonstrated that biofloc has a positive impact not just on the survival and growth of, but also the activity of digestive enzymes in *P. vannamei*, and other studies found that the activity levels of digestive enzymes were significantly higher in the stomachs of shrimp grown with biofloc vs. in shrimp reared in water without biofloc.

These findings show that *P. vannamei* shrimp, when challenged in biofloc conditions and regardless of their previous culture conditions, have lower mortality levels than shrimp challenged in clear waters.

The results suggest that the transfer to clear waters exerts a stress on the shrimp, having an immediate impact on its feeding activity and its feed consumption, with possible consequent impacts on the enzymatic activity within the hepatopancreas and the immunological status of the shrimp. The change of the culture environment will also affect the gut bacterial community, changing the resistance of the shrimp to the pathogenic bacteria, for example, affecting their virulence by suppressing quorum sensing (the ability of bacteria to detect and respond to cell population density by gene regulation).

The lower mortality rates correlated with the time the animals spent in clear waters suggest that an adaptation to the new environment is required to: 1) restore a stable gut microflora; and 2) change from the option of feeding continuously with biofloc supplemented with a commercial pelleted diet, to a diet where the diet is not continuously available. At the end of our trial, the shrimp kept in biofloc had dark guts, which indicated that they had continued feeding throughout the trial, while the shrimp kept in clear water had very little in their intestines despite being fed throughout the tests.

In our study, shrimp that were reared in biofloc but were then transferred to clear water for the $_{\mathrm{VP}}$AHPND test had the highest mortality rates. This suggests that a pre-culture period in biofloc and the gut bacterial community of shrimp do not offer protection when transferred and then challenged in clear water under the experimental conditions we used.

However, the biofloc benefits seem to be immediate. There was only a 26.7 percent mortality in shrimp that were reared in clear water for seven days, but were then immediately transferred to biofloc for the $_{\mathrm{VP}}$AHPND test, while there was a 73.3 percent mortality in shrimp reared in biofloc but then transferred to clear water for the $_{\mathrm{VP}}$AHPND test (Fig. 2). Under the conditions used in our study, it appears that the biofloc has an impact on the virulence of $_{\mathrm{VP}}$AHPND, possibly by interrupting the detection of quorum sensing.

Perspectives

In conclusion, *P. vannamei* challenged with $_{\mathrm{VP}}$AHPND in biofloc had the highest survival rate – 86.7 percent survival for shrimp tested in biofloc vs. 43.4 percent for shrimp in the clear water test. The benefits seem immediate, but the protection is immediately lost once the shrimp are transferred to clear water to be tested. Our findings suggest that careful management of the microbial community within aquaculture ponds can help control bacterial infections.

References available from original publication.

Editor's note: there are nine co-authors in this study, but we only list the corresponding author. Please consult the original publication for names and affiliations of all co-authors.

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