

**Use of PRO4000X to control vibriosis in shrimp farming**

Members of the vibrio genus are ubiquitous members of the natural marine microbial ecology. They occupy a wide variety of niches with the vast majority of strains not being pathogenic. Many of the strains that do cause acute disease are opportunistic, taking advantage of weakened and stressed hosts. To date more than 90 distinct species have been identified and characterized. There are hundreds, if not thousands of variants of these distinct species. Most are not pathogens.

Vibrios play a critical role in the recycling of chitin in many marine environments. They attach to chitin and are found associated with copepods, bivalves (as a result of filtration of the water), corals and a myriad of other fish and shrimp species. They are a part of a healthy well-balanced microbial flora in fish and shrimp. They are an element of biofilms in most marine and estuarine systems and are often found as normal inhabitants of the digestive tracts of both terrestrial and marine animals.

Among those that are pathogenic at least a dozen strains have been associated with clinical disease in human beings. Typically most people think of cholera due to species of *Vibrio cholerae* when they hear the word vibrio. This is however only one of many species that are of economic importance. Perhaps the second most important member of the genus is *Vibrio parahaemolyticus*. As with most bacterial species it is genetically diverse and there are many different strains with different properties. Only a small percentage of these strains are capable of producing disease. Strains have been implicated in human acute gastro enteritis (food poisoning) and play an ever-increasing role in seafood-based food poisoning. Strains have also been reported to cause serious and occasionally fatal infections from wounds. Immune suppressed individuals are at the greatest risk.

Vibrios have long been described as potential pathogens of fish and shrimp and likely have had a substantial impact over the years on productivity. Most strains are thought to be opportunistic in that their mere presence is not usually sufficient to cause disease. However when combined with stressors disease can occur often with serious consequences.

*V. parahaemolyticus* is often associated with biofilms although it occurs in aquatic environments independent of them and is often found in a variety of shellfish species where it lives commensally. While they can be found in colder waters, they prefer warmer aquatic environments. They are typically found in marine and estuarine environments, although there are strains adapted to low sodium environments such as fresh-water. Hundreds of strains have been identified and a relatively small percentage carry toxin genes are potentially problematic.

In shrimp reports of disease outbreaks due to *V parahaemolyticus* date back to the 1970's. They have been implicated in serious outbreaks in hatcheries (zoea syndrome) and on farms (Seagull syndrome). Reports continue of outbreaks from *Vibrio harveyi*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus* and many others. While the specter of panzootic viral diseases such as White Spot Syndrome has been of major concern in recent years, the role of vibrios in killing affected animals is not to be discounted.

AquaInTech Inc. recognizes that the use of antibiotics while a valuable husbandry tool to control these diseases has been the target of abuse and that this serves the industry and humanity very poorly. While most antimicrobial resistance is likely a result of human abuse of antibiotics we are all better off minimizing the use of the antibiotics.

Controlling vibrio loads in the environment, as one can see from their huge roles in the aquatic ecosystem is a daunting task. As a intricate part of stable ecosystems they belong there and attempts to eradicate them as a whole are likely to cause other, even worse, problems as the niches that they exploit open up for others to take advantage of.

Using our proprietary blend of bacteria, we have been able to show that we can control the loads of what are considered traditionally to be potentially harmful vibrios in the hatchery. Figures 1 and 2 show the results of trials in which tanks



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were treated with PRO4000X tablets. By PL5, the level of bacteria (green on TCBS) presumptive *Vibrio harveyi* and *V. parahaemolyticus* contrasted with competitor treated control tanks was a tiny fraction of the total vibrios. Both hatchery tank water and the bodies of PLs in tanks treated with PRO4000X showed a 99.99% or greater reduction in green on TCBS vibrio counts. PRO4000X substantially reduced loads of potential pathogenic vibrios in the hatchery. A single tablet per 10 MT of water used daily is inexpensive, safe and a rapid tool for controlling vibrio loads in hatchery tanks.

Figure 1. 13000 fold reduction in potentially pathogenic vibrios in water

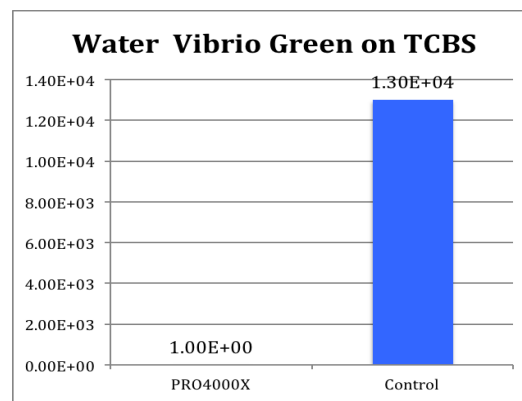
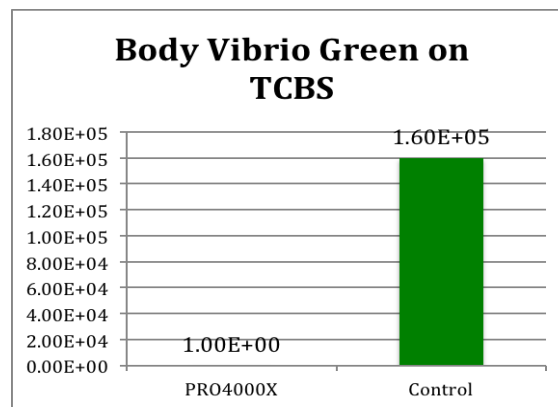


Figure 2. 160000 fold reduction in potentially pathogenic vibrios attached to the animals.



In another series of trials, this affect was shown to be reproducible. Figures 3, 4, and 5 show this. Figure 3 demonstrated that hatchery tanks containing the tablets showed almost a 100% reduction in the loads of green vibrio colonies (presumptive potential pathogens). Substantial reductions were also seen in yellow vibrios and potential *V. cholera* loads (figures 4 and 5).



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Figure 3. Large reduction in green colonies in tanks treated with PRO4000X.

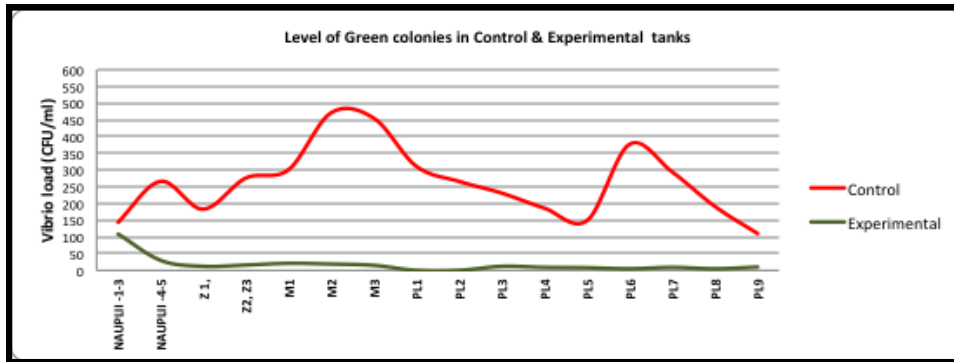


Figure 4. Large reduction in yellow colonies in tanks treated with PRO4000X.

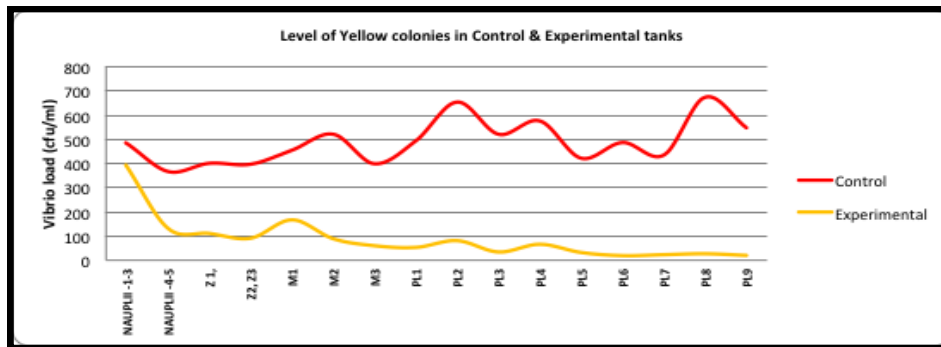
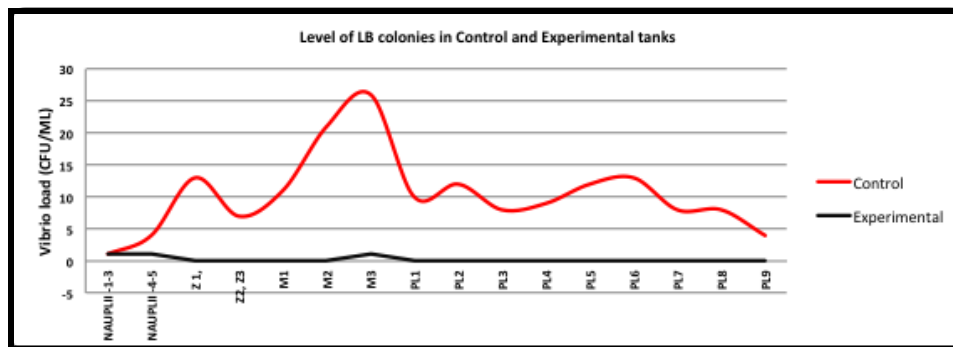


Figure 5. Large reduction in LB positive colonies (presumptive Vibrio cholerae )



It is evident that the bacteria and the respective very high loads (>64 billion CFU/tablet) in the tablets are able to effectively compete against vibrios in hatchery tanks. We expect to see the same result on farms. For more information contact us at:

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