



Vibrios 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 and taking advantage of weakened and stressed hosts. To date more than 110 distinct species have been identified and characterized. There are many thousands of variants of these distinct species.

Vibrios play a critical role in the recycling of chitin in many marine environments. They are a part of a healthy well-balanced microbiome in fish and shrimp. 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 an element of biofilms in most marine and estuarine systems and are often found as normal inhabitants in 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. Another important member of the genus are strains of *Vibrio parahaemolyticus*. 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.

Vibrios have long been described as pathogens of fish and shrimp and 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 does 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 strains 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 including fresh-water. Hundreds of strains have been identified and a relatively small percentage that carry toxin genes are potentially problematic. Early Mortality Syndrome (EMS or AHPNS) is a result of exposure to a toxin produced by specific strains and has had a dramatic impact on global productivity.

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 causing acute mortality is widely underappreciated.

AquaInTech Inc. recognizes that the use of antibiotics, while a valuable husbandry tool to control some 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 (and abuse) 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 could 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 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

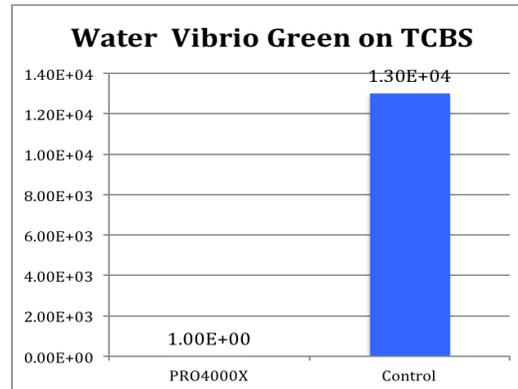
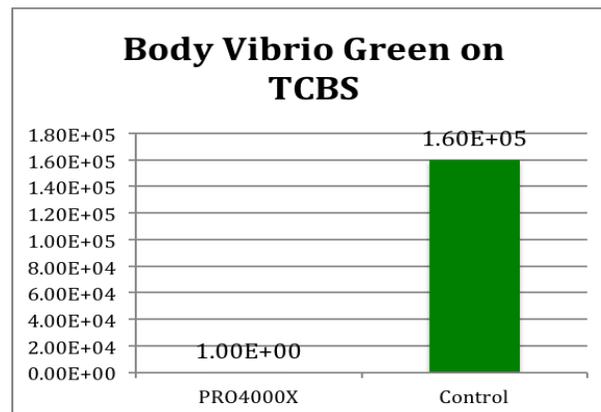


Figure 2. 160000 fold reduction in potentially pathogenic vibrios attached to the animals.



In another series of trials, this effect was shown to be reproducible. Figures 3, 4, and 5 show this. Figure 3 demonstrated that hatchery tanks treated with 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).



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

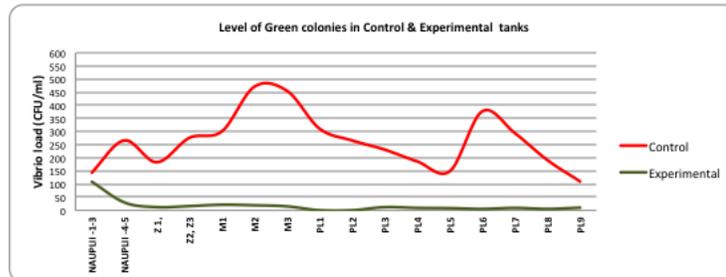


Figure 4. Large reduction in yellow colonies in tanks treated with PRO4000X.

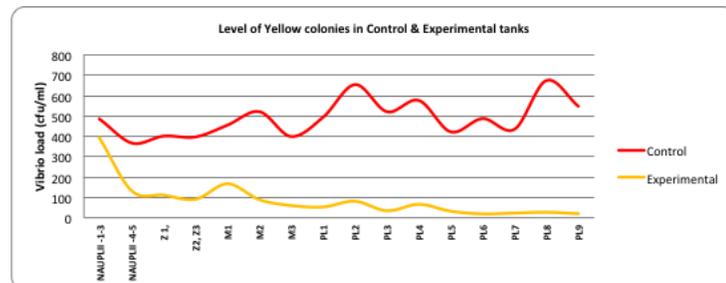
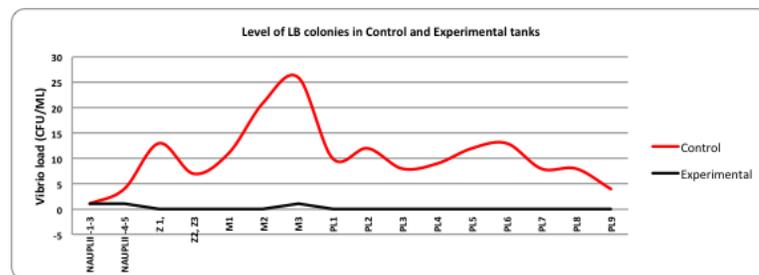


Figure 5. Large reduction in LB positive colonies (presumptive *Vibrio cholerae*)



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. WE have testdata that verifies this as well.

**Cautionary statement:** These are tools. For optimum benefit they need to be used correctly. Despite being told to the contrary there is actually no reason to believe that every tank and every pond must be treated in the same way. Every effort must be made as well to control undesirable inputs from elsewhere in order to gain the best possible impact from the use of PRO4000X.