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EMS update August 2015

A hot topic of discussion among shrimp farmers everywhere has been the disease known as EMS, or Early Mortality Syndrome. It has now spread into most shrimp farming countries although the impacts remain variable in some areas. Not solely an early mortality problem it can kill shrimp at almost any life stage (large shrimp seem to be refractory although this may be related to the amount of toxin needed to cause sufficient damage to a larger animals hepatopancreas (HP) rather than a straight issue of susceptibility).

It has been determined that the etiologic agent is a strain of a common marine bacterium, *Vibrio parahaemolyticus* (reports that other strains may also be able to produce the syndrome though not common are not entirely unexpected). This strain contains small circular pieces of DNA (these are ubiquitous among bacteria) called plasmids that in this strain have genes on them that code for a pair of proteins (Han, et al. 2015. Photorhabdus insect-related (Pir) toxin—like genes in a plasmid of *Vibrio parahaemolyticus*, the causative agent of acute hepatopancreatic necrosis disease (AHPND) of shrimp, DAO 113:33-40. 2015).

These proteins are toxins. They evidently bind to the HP tissue damaging the structural integrity of this vital organ, likely in a dose dependent manner. This can explain the gradations in pathology noted in the field. This damage can weaken the animal to the point that where it cannot defend itself against the onslaught of invading bacteria that accompany the damage. This vibrio strain is among the bacteria that can be found in sick and dying shrimp although this is not at all unexpected since chitinous surfaces are one of the bacteria's niches. This is not the usual type of bacterial disease process where a specific strain attaches and gains entry into the shrimp killing them from within. The toxin is the critical component of this. Without the toxin the strain apparently does not cause pathology.

It is important to understand some of the observations from the field in the context of what types of approaches might be useful in mitigating the impact of this vibrio and its toxin genes.

1. This is a disease process that appears to hinge solely on the presence of a toxin that damages the HP. No toxin-no disease.
2. The toxins except that they are similar to those of produced by another group of bacteria that kills insects (PirA and PirB toxins). (Han, et al. 2015.)



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3. During an acute outbreak it has been repeatedly observed that when one stops feeding the shrimp the mortality stops.
4. This bacteria readily attaches to chitinous surfaces.
5. It can be present with no evidence of toxin production. One can isolate the bacteria from the chitinous mouth structures.
6. Vibrios play a wide range of diverse roles in aquatic ecosystems. Many vibrios have doubling times of ten minutes or less. At this rate, in a very short time there can be very high levels of a vibrio present under the right conditions.
7. There are reports of some areas showing signs of recovery. There are likely different reasons for each area. Shrimp farming production paradigms are highly variable and each of the affected paradigms has different issues that potentially affect the disease process.
8. Early reports demonstrated that the bacteria could pass into the production environment via contaminated (not infected) post larval shrimp because of inadequate biosecurity in the broodstock maturation and hatchery facilities. Field observations suggest that it can move via other routes through the environment into ponds. The relative contribution of each method to the disease process remains to be clarified.
9. Using well water limits the disease impact. The vibrio does not grow in fresh water so for farms using fresh water this would explain the lack of problems. For those who are pumping brackish seawater from wells there seems to be a lower incidence as well possibly related to negligible loads of the bacteria entering into the production system via this route contrasted with the use of surface water.

There are increasing reports of successful cultivation in areas where this was not possible a cycle ago. Mexico is reporting a resurgence in production this first cycle of 2015. While the macroeconomic environment has impacted the number of companies stocking (not just in Mexico) a number of farms that were seriously affected last year are not showing any evidence of the problem. Most farms in the Northern part of Mexico dry out for the cold winter months. Most likely a proper dry out including intake canals will lower the overall environmental load of this vibrio. Combined with proper biosecurity to keep the bacteria out and off of PLs it is not unreasonable to expect to see a decrease in the overall incidence of the disease process. However as organics accumulate (feed waste, chitin, feces, etc.) in production environments it would not be an unreasonable theory that one should expect to see an increase in the problem again in those areas where the animals are still susceptible and conditions favor the presence and growth of the specific strain of vibrio.



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Some farms in Thailand are reporting that by completely changing the production paradigms they have been able to eliminate the impact of this vibrio. Closing the ponds, exchanging water with water from adjacent ponds containing Tilapia and the use of techniques to remove the organic matter as it accumulates and stocking clean PLs have resulted in some startling recoveries in areas that could not grow any shrimp at all last year.

For many though recovery still remains elusive. Based on what we have observed and been told we suggest that farmers consider the following:

Do not use PLs from hatcheries that use broodstock held in outdoor ponds unless testing definitively demonstrates a very low to no load of the etiologic agent. This testing must be done by a competent well trained microbiologist who understands the need to enrich and how to ensure that the testing is realistic and meaningful. It is too easy to miss this organism using conventional approaches. PCR must target the toxin gene.

In areas where farms can be dried out, do so. Dry out all inlet and outlet canals. Remove any accumulated organic matter in the ponds and your system. If you can work with your neighbors try to coordinate an area wide dry out and if intake canals can be dried, do so. The use of lime (to elevate the pH) and the sun combined with tilling (8 to 10 inches minimum) should eliminate most of the bacteria. Not drying out poses a serious and ongoing continuing risk and while there may be variable impacts one should expect the problem to get worse as organic materials accumulate and the vibrio produces large amounts of toxin.

Divide larger ponds into smaller ponds. Large ponds are affected as readily as smaller ponds are. Stocking density is not a factor. This is not an infectious disease process. It is easier to control stress and inputs in smaller ponds. Ponds with dirt bottoms will be harder to keep free of organics. As organics accumulate remove them. Smaller ponds can be engineered with sumps and aeration systems that push the accumulated organics into an area where they can be pumped out ASAP. The use of bioremediation can be focused on these areas. These can be very heavily contaminated materials so it must be handled responsibly. It must be kept away from the shrimp ponds and hauled off or destroyed in some other manner. The less time these are in the pond the less milieu there is for growth of the toxin producing strains and thus less toxin production.



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Tilapia appear to have an impact on the vibrio in some manner. At this time the mechanism is speculative but it may center around competitive vibrio species being excreted by the Tilapia or the presence of other bacteria that inhibit the growth and/or toxin production. The most effective use does not seem to be growing the Tilapia in the same ponds as the shrimp are in. Successes have been observed when water from Tilapia ponds is used as makeup and exchange water for shrimp ponds.

Given the successes being seen in some areas it is likely that we may be approaching the time where we know enough to be able to lessen the overall impact of EMS. It remains to be seen to what extent farmers will be able to modify their production paradigms to lessen stress and limit the niches that favor the growth of this bacterial strain and its ability to produce toxins. Those farmers who do not change will continue to be impacted by the disease until such a time as there are shrimp that have become tolerant of the toxin (if this turns out to be a part of why we are seeing recovery).

Much work is ongoing on this pathogen and as with research of this nature, our knowledge will increase with time and experience. It is critical to bear in mind that from all appearances so far, this disease process appears to be a toxicosis rather than strictly an invasive process. Controlling the bacterial loads and gaining a better understanding of what factors influence growth and production of the toxin(s) are going to be critical for shrimp farmers to learn how to live with AHPNS.



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