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A holistic management approach to EMS

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Increased knowledge and novel strategies helping shrimp farmers manage the disease



An important EMS management strategy is to deny favorable substrates for the establishment and growth of populations of *V. parahaemolyticus*, such as the chitinous molts of shrimp, ponds must be removed by siphoning on a regular basis.

Early Mortality Syndrome (EMS), or acute hepatopancreatic necrosis disease (AHPND), is caused by a specific bacterium strain of the *Vibrio parahaemolyticus*. This is a well-studied bacterium species known to be widespread throughout the shrimp aquaculture industry for decades, and a significant amount of knowledge exists on *Vibrio* in aquaculture, and in other related fields, namely on *V. fisheri* and *V. harveyi*, and the human pathogens *V. cholera* and *V. parahaemolyticus*.

For more than 40 years, *Vibrionaceae* has been consistently identified as one of the dominant families in the natural intestinal flora of wild and farmed penaeid shrimp. As *Vibrio* has a natural place in the microflora of penaeid shrimp, with a large proportion of them being harmless it can by no means be considered automatically as an obligate pathogen. However, shortly after shrimp farming intensified, reports of disease and mortality in shrimp caused by *Vibrio* began to spread. *V. harveyi* was one of the first and most frequently identified culprits, later joined by *V. parahaemolyticus* and others. The disease was baptized “vibriosis” and one of its most typical disease signs was an increased luminescence. Since then, much has been written about virulence of *Vibrio* in shrimp.

Some thoughts on *Vibrio* virulence

Virulence genes are spread among *Vibrio* via horizontal gene transfer. Plasmids or temperate bacteriophages transfer genetic material from bacteria to bacteria, leading to a virulence switch and the production of toxins. This is relatively well-understood from human epidemics of *V. cholera* and *V. parahaemolyticus* and also in marine luminous *Vibrio*, such as *V. harveyi*. The types of toxins, how the

genes are transmitted among *Vibrio* and how they cause clinical signs have been extensively described for humans and shrimp. In fact, several publications have already reported on necrosis of hepatopancreas cells and midgut cells of shrimp by toxigenic *Vibrio* isolates, mainly *V. harveyi*.



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Most *Vibrio* which cause disease and mortality in shrimp have been labelled as opportunistic/secondary pathogens, which take advantage of unfavorable environmental conditions to overwhelm shrimp hosts with compromised immune systems. This is observed when culture conditions are sub-optimal and stressful. Several specific strains of *Vibrio* sp were identified as primary pathogens, capable of killing shrimp in hatcheries and grow-out ponds even under optimal conditions, and these have become a major source of concern.

It has been stated that EMS/AHPND is caused by a newly emerged, primary pathogenic *V. parahaemolyticus*, capable of adversely affecting perfectly healthy shrimp no matter how much has been the effort in the management of the farm. The fact is however, that the distinction between primary and secondary pathogens is artificial. In both cases, there is a classic interplay of bacterial virulence and infection pressure, host defense and environmental influences which ultimately decide in which direction the balance tips. In all cases published so far, high doses of *Vibrio* had to be inoculated to reproduce the disease under laboratory settings.

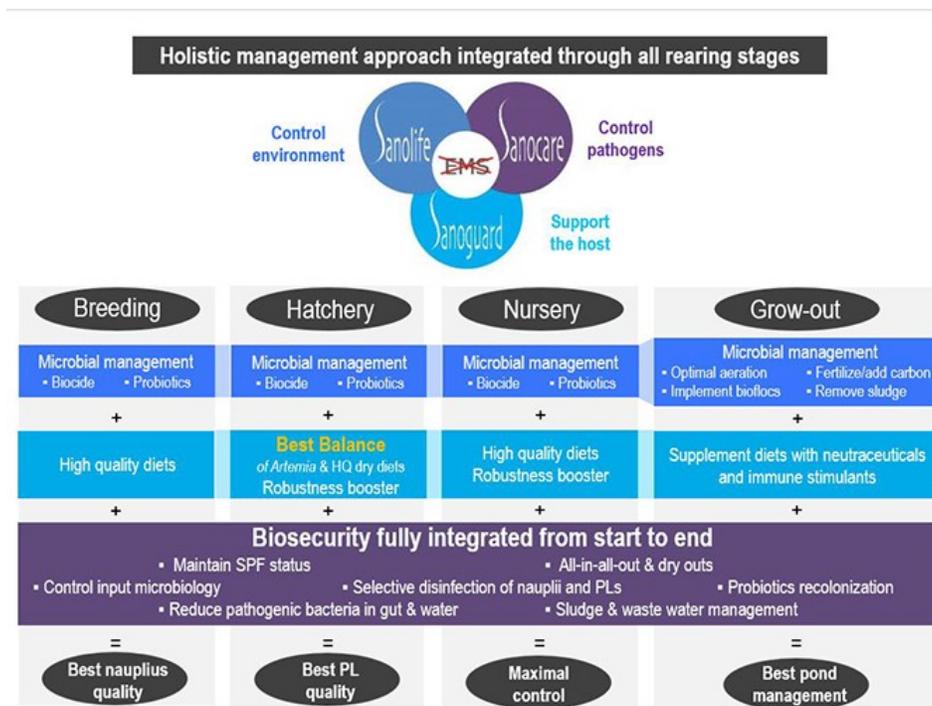


Fig 1. Holistic management approach integrated at all rearing stages.

Taking a holistic approach to EMS management

It is important to consider that EMS is caused by a bacterium, not a virus. The majority of the biosecurity measures the shrimp farming industry has developed globally during the last two decades have targeted mostly viral diseases and were significantly based on pathogen exclusion. This is much harder to achieve with such a ubiquitous pathogen as *V. parahaemolyticus*.

Based on available information and experience on *Vibrio*, we have designed a holistic management approach that can successfully minimize the damage the bacteria inflict on cultured shrimp. The approach of our company, INVE Aquaculture has always been holistic. First and foremost, basic good practices have to be established in the management of the aquaculture systems to provide an optimal and stable environment. Secondly, the host health is reinforced by optimizing nutrition and supportive supplements for the immune system. Thirdly, on the level of infectious agents, we aim to reduce the presence of viruses and virulent bacteria, while preventing opportunistic bacteria from getting a chance to overwhelm the hosts.

Breeding centers



Proper testing of shrimp postlarvae before stocking into ponds for final growout, including the use of molecular techniques like PCR, is critical to assure no infected animals go into the ponds.

A good breeding center can only be operated under strict quarantine, with a surveillance program to maintain specific pathogen free (SPF) status. A team of experts has to plan and execute a long-term program of selective breeding for desired traits, while keeping inbreeding under control. In the context of EMS, broodstock has been implicated as the cause of the problem, both that injudicious breeding which can lead to wide-spread inbreeding or that broodstock can be carriers of the EMS-causing bacterial strains. Hence, we advocate strongly for disinfection of nauplii and materials used in the hatchery and farm in order to obtain real quarantine. In this context, further improvement of formulated diets for broodstock can allow the elimination of the risk of pathogen transfer via natural feeds.

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Hatcheries and nurseries

As soon as shrimp larvae begin to feed, optimal nutrition becomes the main corner stone to ensure the best chances for survival and growth in their further life. Intuitively, most hatchery managers know that this is a complex process orchestrated by natural microbiological processes, and the administration of artificial feeds and live feeds (algae, *Artemia*). Our protocol aims to have as much control as possible over these factors by adding on the one hand probiotic bacteria, and on the other the best balance of quality-controlled *Artemia* and dry diets. Together, this results in stable and controlled conditions, which renders the use of antibiotics unnecessary. Robustness of the post-larvae grown using this hatchery protocol is maximized by supplying specific health boosters, allowing them to better cope with stressors encountered during transport and when released in new environments. This can be evaluated objectively by recording their consistently better performance in stress tests.

Recently, we have enhanced even further this stress resistance by applying a mixture of plant extracts during the hatchery cycle and in the transport water of post larvae. These specially selected plant molecules are not lethal to shrimp larvae, but boost their defense systems by inducing the production of heat-shock proteins. Additionally, they have a pronounced selective bactericidal effect, specifically against gram negative bacteria such as *V. parahaemolyticus*. As a result, post larvae with a superior quality can be delivered to grow-out ponds or nurseries.

Fig 2. The best balance between artificial diets and Artemia.

The practice of nursing post larvae to a larger size before stocking into ponds has been strongly encouraged since the onset of the EMS epizootic. Although some have stated that this avoids early mortalities in ponds, others have reported that the practice does not result in any improvement. As information is being gathered, the explanations for differences in the effectiveness of using a nursery step has been emerging. First and foremost, a good nursery requires a high investment. It has to be physically separated from the grow-out area and very strict biosecurity measures have to be in place. Due to the fear of EMS, many farmers have been making the mistake of leaving the shrimp for too long in the nursery, ignoring the carrying capacity of the system. Further, it has become clear that using cheap grow-out feeds undermines the success of a good nursery. The use of hatchery-grade diets does not only meet better the nutritional requirements of young shrimp, the difference in ingredients also has a drastic effect on the microbial flora growing in the shrimp and on the faeces. Finally, robustness of the fry stocked at high densities should be maximized through addition of health boosters.

Fig 3. Reduction of Vibrio load by use of various plant extracts during transport.

A good nursery protocol, be it in raceways or ponds, allows for better control and stabilization of growth conditions, shorter cycles in open ponds, and more crops per year. But above all, in any shrimp nursery, well-trained staff and a good understanding of water management cannot be replaced by products with exaggerated claims or with the use of antibiotics.

Fig 4. Enhanced shrimp survival during the hatchery cycle by applying various plant extracts.

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Growout

During the transference of the juvenile shrimp from the nursery to the growout ponds there is a second opportunity to selectively disinfect against *Vibrio* and to boost the stress resistance of the shrimp. We see a significant potential to optimize net profits from a shrimp crop with this practice, as even before EMS became a problem, the majority of shrimp mortality occurred during the first weeks after stocking.

Thorough pond preparation should not be underestimated and the same principles as for nursery should be applied, with attention to biosecurity and stabilizing the chemistry and microbiology of the intake water. Applying disinfectant during pond preparation reduces the risk of horizontal transfer. However, it is not sufficient and as a stand-alone treatment it might increase disease risk, as fast growing microorganism will fill up the empty niches. What we support is a matured microbial ecosystem with a wide bacterial diversity. This microbial community will prevent *V. parahaemolyticus* from becoming one of the few dominant species. Therefore, a subsequent treatment with probiotic bacteria prevents opportunistic pathogens from blooming, colonizing and invading the shrimp.

As we are dealing with an open system, algae play an important role in grow-out ponds. High quality fertilizers should be applied, in combination with a carbon source, in order to obtain the right C:N:P balance. After several weeks, depending on the stocking density, the organic load in the water will rise, and the algae phase will shift to the phase dominated by heterotrophic bacteria.

In this phase, three points are critical: *aeration, pH and sludge management*. The aerators have to be installed properly, so that they mix the whole pond (particularly important for biofloc systems), and maintain oxygen levels above 4 mg/L at all times. The pH of the water has to be buffered by adequate amounts of alkalinity (>150 mg/L) and should not be allowed to fluctuate. Excess sludge has to be

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removed by regular siphoning. In the context of sludge management, many parallels can be made between the field of waste water management and the biofloc concept in aquaculture. In order to have as much control as possible over the dynamics of the spontaneous generation of bioflocs in the ponds, addition of selected *Bacillus* sp. and substrates is one of the few tools available to farmers.

The EMS crisis has been a serious challenge for the shrimp farming industry and the supporting sectors. It is our firm belief that further integration and professional farm management are the way out of this crisis and to a sustainable future.

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The control strategies of *V. cholera* and *V. parahaemolyticus* from the point of view of human epidemics, center largely on waste water management, namely separating sewage and water sources. The cross-contamination of outlet water from shrimp farms to the inlet of the same or other farms has been a problematic issue for a long time already. With EMS/AHPND, this is more than ever a point to which farmers should pay particular attention. In order to prevent further spread and increase of the infection pressure in the environment surrounding shrimp farming areas, we advocate for a proper treatment of pond water and sludge even after the crop has died due to an EMS outbreak.

Even if the environment of the shrimp is optimally managed, ubiquitous bacteria such as *V. parahaemolyticus* and *V. harveyi* might still have an opportunity to cause disease in shrimp. A major issue is that in grow-out, the feed constitutes a big part of the operation costs. Pressure on prices results in saving on ingredients, which leads to deficiencies in vitamins, oligo elements and bio-available ingredients. This is why grow-out diets have to be supplemented with nutraceuticals, enzymes and immune stimulants, which allow the shrimp to strengthen their natural barriers such as the cuticle, and to build up more reserves in energy and innate immunity to fight off invading bacteria.

Perspectives

The EMS crisis has been a serious challenge for the shrimp farming industry and the supporting sectors. It is our firm belief that further integration and professional farm management are the way out of this crisis and to a sustainable future.

In our opinion, one single miracle cure product does not yet exist that fully attacks *V. parahaemolyticus*. Opportunistic bacteria with transferable virulence genes have always been and will always be around in shrimp farming. The use of antibiotics or disinfection protocols without immediate recolonization by

probiotics is not a sensible strategy. Our vision is that only a holistic approach, optimizing environmental and postlarvae quality, can successfully manage shrimp culture microbiology and control EMS.

This article is summarized and updated from an original version originally published in Aquaculture Asia Pacific. More information concerning EMS/AHPND, references to scientific publications and health products can be requested from the authors.

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