Vibriosis –the bane of shrimp farmers

by Stephen Newman

Dead muscle tissue in shrimp affected by an obligate pathogenic strain of Vibrio alginolyticus

Shrimp farmers throughout the world will be familiar with the challenges involved in preventing and dealing with diseases that could easily wipe out their stocks. Members of the Vibrio genus are foremost among the leading causes of mortality. Farmers need to understand that stress, genes and the environment play roles in susceptibility and that appropriate management strategies do not include automatically reaching for antibiotics.

Introduction

The genus *Vibrio* is a dynamic aquatic species that has, among many other traits, the ability to degrade chitin. As of this writing there are more than 125 species, the vast majority of which are benign and not associated with disease.

Given the ubiquitous nature of this genus in aquatic ecosystems, it is no surprise that they are widely believed to be the primary cause of mortality in farmed shrimp. These are highly evolved bacteria with generation times in some cases under 10 minutes and the ability to use a wide range of substrates as food and they readily exchange genetic material. There is a clear cut distinction between the cause of a disease and opportunism. A few strains are obligate pathogens in that they will cause acute disease and kill shrimp at very low levels while most strains are opportunistic and invade weakened animals.

Table 1: Some Vibrio species reported to cause disease in shrimp

Species	Colony colour on TCBS agar
Vibrio parahaemolyticus	Green
Vibrio anguillarum	Yellow
Vibrio vulnificus	Green/yellow
Vibrio harveyi	Yellow/Green
Vibrio nigropulchritudo	Green
Vibrio ordalii	Yellow
Vibrio mediterranei	Yellow
Vibrio splendidus	Yellow
Vibrio pelagius	Yellow
Vibrio alginolyticus	Yellow
Vibrio damsela	Yellow/green
Vibrio penaeicida	Green
Vibrio owensii	Yellow
Vibrio campbellii	Green
Vibrio orientalis	Faint yellow
Vibrio logei	Yellow
Vibrio fischeri	Weak yellow

A common misconception among many aquaculturists is that there is a relationship between the inability to break sucrose down and pathogenicity. This distinction, yellow versus green, is not founded in science at all. Table 1 clearly shows that this is simply not the case. Furthermore some of the worst obligate pathogens (such as *Vibrio alginolyticus*) are yellow on TCBS media.

Opportunistic and ubiquitous

The vast majority of outbreaks from *vibriosis* are secondary to stress.

Table 2: Some stressors known to weaken shrimp

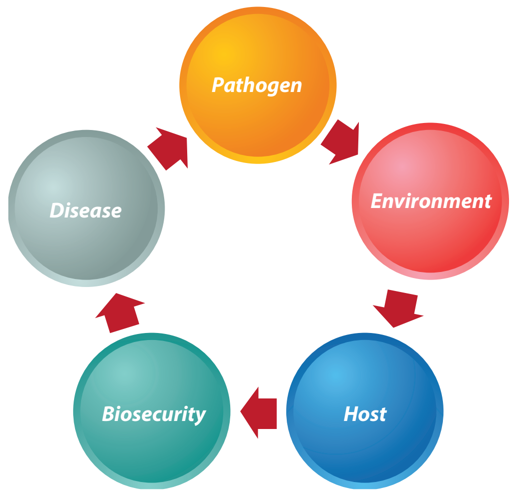
Stressor	Comments
Low dissolved oxygen (DO) levels	DO levels below 4 mg/l can cause stress but many farmers think that if the shrimp do not die, then it should be all right. Continued exposure to low levels of oxygen will weaken them, increasing their susceptibility to *Vibrio* infections
Hydrogen sulphide from anaerobic pond bottoms	This is a very toxic gas and there are impacts on animal physiology even at very low levels
Poor quality feed	Lack of proper nutrients impacts susceptibility
Accumulated organic matter on pond bottoms	*Vibrios* grow very well in this milieu. There are strong indications that this may be a critical factor in the development of EMS
Diseases such as WSSV	WSSV weakens animals, making them easy targets for opportunistic *Vibrios*. Typically, shrimp are fighting multiple pathogens
Sudden changes in salinity, temperature, etc.	Anything that stresses the shrimp can alter their ability to resist the onslaught of potential pathogens

Control of *Vibrios* is challenging at best and efforts to eliminate them are misguided. If this were even possible, the niches created would likely rapidly be filled by other bacteria that are just as able to affect stress-weakened animals as the *Vibrios*. The goal should not be to eliminate them, but to exert control over the components of the production system that can be controlled.

Figure 1 depicts a chart which is a modification of the Venn diagramme that shows how disease outbreaks are multifactorial and that stress, genes and the environment all play roles in susceptibility and outcome. It becomes obvious that the disease processes are complex and could easily be the subject of an article by itself.

Once we have some idea about what we are dealing with and we have implemented the appropriate biosecurity protocols, we can begin to mitigate the overall impact. For most pathogens the myriad of contributing factors are not well understood and all too often academic exercises, while useful, are misleading about what might be occurring in the field. The lab and the field are not the same and what works in the lab may not work in the field and vice versa.

Fig 1: Interlinked causes of disease outbreaks



Pathogen What is it? Is it obligate or opportunistic? Where is coming from in the production system (carry over from broodstock, infected PLs, etc.). How virulent is it?

Environment What factors in the environment encourage presence and its growth? Are there vectors involved in spreading the disease? What stressors are present?

Host There will be differences in susceptibility based on species and genetic variability within stocked populations

Biosecurity Carry-over of pathogens from poor biosecurity in maturation, the hatchery, the ponds, etc

Disease is the result of all of the interacting factors that allow animals to be infected and unable to defend themselves immunologically

Spread and expression of *Vibrio* genes

At this time perhaps the foremost *Vibrio* problem that is on the minds of shrimp farmers everywhere is Early Mortality Syndrome or AHPNS. Early data suggested that this is an infectious disease process by specific strains of *V. parahaemolyticus* (these form green colonies on TCBS -an indication that they cannot degrade sucrose). As our understanding of this pathological process has evolved there are some who now believe that the evidence supports this as being a toxicosis.

Specific strains of *V. parahaemolyticus* contain a plasmid (small non-chromosomal circular DNA) that encodes for the production of a bivalent protein toxin very similar to that produced by certain bacteria that kill insects. This toxin damages the target organ for the disease, the hepatopancreas, which in turn (when the damage is great enough) weakens the animals to the point where they are easily overwhelmed by bacteria, and not just by the *Vibrio* strain that produced the toxin, although they can be dominant.

Plasmids are ubiquitous and there are many examples where they are essential for the ability of a bacterial species to be virulent. They are readily and easily moved between bacterial species and there is published evidence that the plasmid can be found in other *Vibrio* species besides *V. parahaemolyticus.* Given the nature of *Vibrios* it is possible that these genes will eventually be found in other bacterial species.

While there are some who have focused on the presence of the gene (and indeed this is considered to be a critical element of confirmation of the presence of the disease process), the reality is that it is gene expression that is important. The gene can be present in any number of copies but that does not mean that the gene is being expressed. At this time there is enough variable experience in the field to lend credence to the theory that there are as yet unknown factors that may be controlling gene expression and thus loads of the toxin in production systems. It could turn out that controlling the factors that affect gene expression is going to be critical to allow mitigation of the impact.

There are a number of cases where the polymerase chain reaction (PCR) primers that are specific to the toxin genes (AP4) suggest that the gene is widespread (it is specific for the gene and says nothing about what bacterial species it might be in and whether or not any toxin is even being produced), and yet there is no pathology. It can be misleading in that some attribute this to resistance on the part of the shrimp with no evidence that the toxin is in fact present.

There are many possible things that can impact gene expression and we know that there can be strains with many copies of the plasmid (not unusual). Differential gene expression can explain why some areas are being hit so hard and others are not. This of course can also complicate proactive strategies designed to lower the overall loads of *Vibrios* in the production environment. If you have a strain that is a super toxin producer, far lower levels of it might be able to produce damage to the hepatopancreas than a strain that is a weak toxin producer. So efforts to lower the overall loads of *V. parahaemolyticus* may not be fruitful if there is a strain that produces high loads of toxins.

Gene expression can be affected by many things, such as the presence of other bacterial species that produce inhibiting or activating substances, to factors that inhibit biofilm formation, etc. At this time, much more work remains to be done to determine exactly what factors are influencing expression of the toxin. For now, it appears that no one strategy is universally successful in preventing the problem. In general, elimination of accumulated organics, the use of bioremediation, high water exchange rates and focusing on management of feed can have a positive impact.

Disease prevention strategies in general

Proactive strategies are geared towards prevention. This entails controlling the presence and movement of possible pathogens through the production process and starts with broodstock. Stress reduction is also a critical element of this. Research is ongoing to find suitable proactive management paradigms by stimulating the immune system and the use of beneficial bacteria (inaccurately coined probiotics) to potentially displace pathogens by competitive processes. The most common reactive approach, i.e. after the fact, is the use of antibiotics.

Antibiotics

The use of, and reliance upon antibiotics in global aquaculture is a common practice and for the most part, is essentially unrestricted. While they can be very valuable tools they are widely abused by farmers for many different reasons. Table 3 is a partial list of some of the main antibiotics used in shrimp culture.

Table 3. Main antibiotics used in shrimp culture

Antibiotic	Usage	Dosage (largely anecdotal)
Erythromycin phosphate	Indefinite bath against bacterial necrosis and *vibriosis* in hatchery	0.5 to 2 ppm
Tetracyclines	Indefinite bath to prevent bacterial necrosis and *vibriosis*	2 ppm
	Prophylactic treatment for broodstock	1 ppm
	Oral application in feeds for treatment of NHP	1.5 to 5 kg/MT
Furazolidone Nitrofurazone	Indefinite bath to prevent bacterial necrosis and to reduce bacterial loads in hatchery waters, rearing tanks and Artemia culture	1-2 ppm
Chloramphenicol	Indefinite bath to prevent bacterial necrosis and reduce bacteria loads	2 ppm
Oxolinic Acid	Oral for 30 days for *vibriosis*	35 mg/kg
Sarafin sarafloxacin	Oral for 5 days for *vibriosis*	
Flumequin	24 hour bath	10 ppm
Enrofloxacin	24 hour bath. Also top dressed	8-10 ml/cu^3
Ciprofloxacin	Oral for 10-14 days	

*Note: The author is **making no statement** about the suitability of any of these compounds for use in the manner and dosage described*

Properly used, antibiotics can be very useful tools. If they are applied at the right time they can stop serious bacterial disease problems quickly. However there are disadvantages to their irresponsible use some of which are highlighted in Table 4. Ultimately, wise use of antibiotics must involve processes that consider the legality of the use of particular antibiotics in the markets where the final products will be sold into.

Table 4: Advantages and disadvantages of antibiotics

Advantages	Disadvantages
Powerful tools for stopping disease	Development of resistant strains
Useful in impacting feed conversions	Environmental residues can affect microbiomes
Prophylactic use can prevent disease	Residues in animals at harvest resulting in rejection of product
	Inadequate safeguards to ensure optimum dosages
	Cannot be used late in the growth cycle because of residues in the final product

The following is a list of guidelines for the responsible use of antibiotics. The most important aspect of this is to isolate the bacteria that are the cause of the problem(s) and determine what the correct antibiotic and dosage of antibiotic is that will kill the bacteria. Use the antibiotic only as needed and per manufacturers' suggestions.

- Develop and implement management philosophies and strategies designed to minimise the potential of bacterial disease outbreaks. These include using water disinfection systems in hatcheries such as ozone or UV in combination with sand, charcoal and bag filters to minimise bacterial loads entering into hatcheries and many other steps;
- Use responsible techniques (copious washing with clean water, use of iodophors or other suitable chemicals) for disinfection of brood females, nauplii and PL's;
- In ponds, use techniques designed to maintain as high of a quality pond environment as possible. This entails controlling and minimising stressors, avoiding over-feeding and over-fertilising and the judicious use of bioremediation;
- Only use antibiotics when they are needed. They should not be used as a routine tool for preventing potential problems, although prophylactic usage in broodstock is acceptable (using proper antibiotic, correct dosage, etc.);
- Using antibiotics at lower than therapeutic levels can generate antibiotic-resistant bacteria. Bacteria constantly evolve and the development of resistance is natural and inevitable, more so when antibiotics are not used responsibly. Resistance makes it harder to treat subsequent infections which can easily be spread and can potentially pose a threat to other animals and humans;
- Use antibiotics only when you have bacterial or rickettsia infections. Do not use them to treat fungal, viral or protozoan infections unless you know that there are secondary bacterial pathogens involved in the disease process;

Proper collection and surface disinfection of nauplii is essential for controlling Vibrio loads that might enter production systems attached to nauplii

- Treat for the entire time period recommended. Do not be tempted to stop simply because animals look healthier;
- Use antibiotics that are from a reliable source;
- Wherever possible, mill the antibiotic into the feed. This provides the assurance that the antibiotics will not leach out immediately after the feed is added to the pond;
- Use an adequate withdrawal period (21 days is considered in most cases to be adequate) although this is usually dosage, species and temperature dependent; and
- Don't store feed containing antibiotics for long periods of time.

Ongoing work on immune stimulants

Immune stimulants fall into a number of different categories: nutrients that act indirectly on cell physiology; those that work in a specific manner such as vaccines; and those that are non-specific in nature such the beta 1-3 glucans, alginates and lipopolysaccharide-based materials (there are many others). Shrimp have immune systems that, though quite complex and highly evolved, are not the same as vertebrate immune systems. Shrimp do not produce any type of antibodies (specific proteins produced by white blood cells in response to specific pathogens) and there is no evidence that any component of their immune response has the degree of specificity that occurs with a vaccine. Instead, they produce proteins that are lectin-like and function in a capacity that is similar to antibodies, though largely non-specific in nature. Additionally they do not appear to have an immunological memory, at least not one that operates at the same levels that vertebrates do.

In general, the use of immune stimulants in shrimp farming is still in its early stages. While there have been some successes, these are tools and not solutions.

***Dr. Stephen Newman** is a marine microbiologist whose early career focused on the development of the first commercial fish vaccines. Since the early 1990s, he has been working closely with shrimp farmers in almost every country that farms shrimp. In 1996 he founded Aquaintech Inc., to provide a wide variety of products and consulting services to the international aquaculture community. He is an internationally recognized expert in aquaculture and has extensive experience in product development and sales and consulting with clients on a wide variety of topics ranging from business plan preparation and due diligence to auditing of operations with the goal of improving productivity. More details are available at www.aqua-in-tech.com and www.sustainablegreenaquaculture.com.*