Control of vibrios in ponds, hatcheries and on shrimp farms

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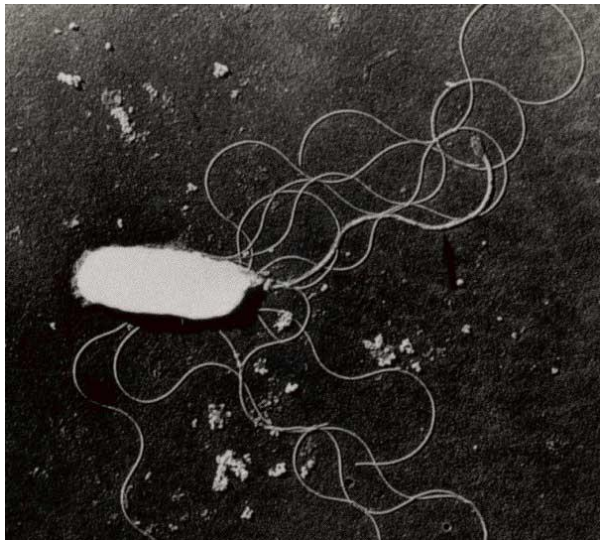
Presentation outline

- Vibrios general information
- Why control is even an issue?
- Strategies
- Conclusions

SEM of Vibrio species with multiple polar flagella



Vibrios are a part of healthy ecosystems

- Normal inhabitants of most aquatic ecosystems
- Healthy ecosystems are in balance
- This balance is dynamic changing as inputs and outputs influence the composition of nutrients and other bacteria
- Bacteria are constantly battling each other for access to nutrients
- Vibrios are essential for the degradation of chitin –the external skeleton of shrimp

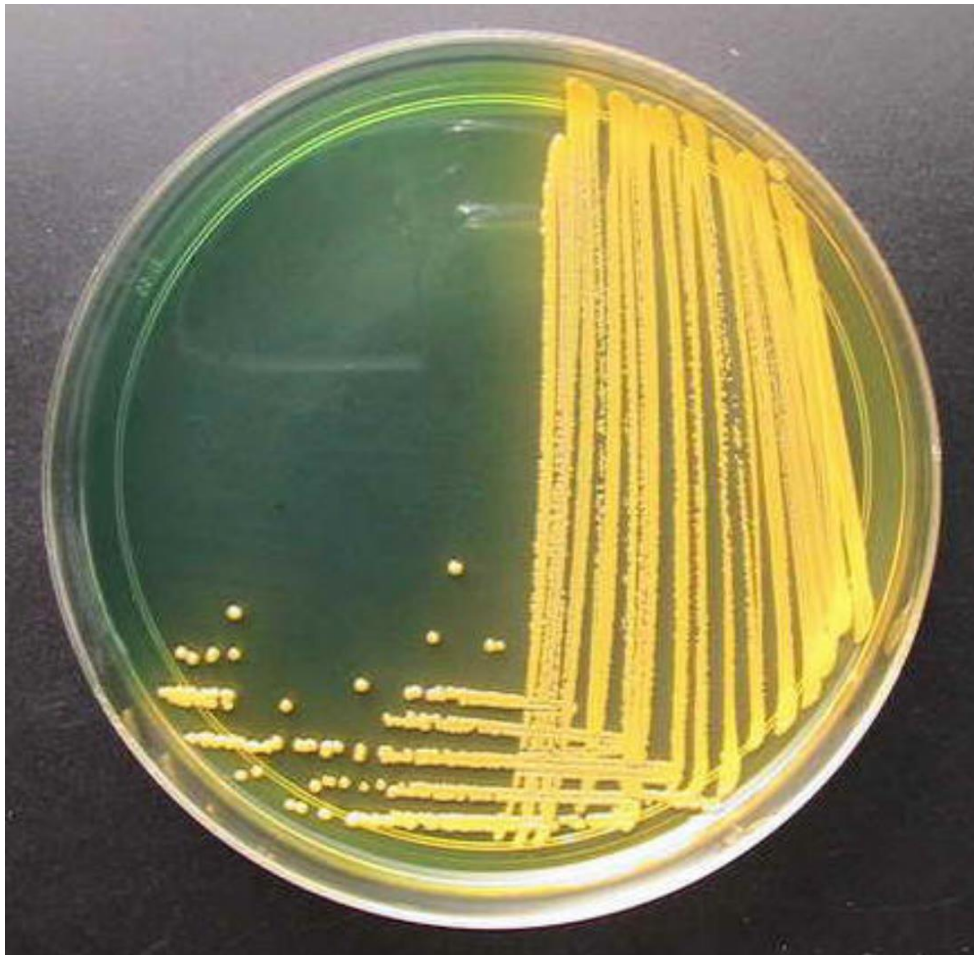
The genus vibrio general information

- More than 100 named species. 119 and counting (http://www.bacterio.net/vibrio.html)
- Diverse group of bacteria occupying marine and aquatic ecosystems.
- Very important function in recycling chitin (the most common nitrogen containing polysaccharide).
- Most are benign although various strains of certain species do cause disease in humans and animals-not just shrimp.
- Even within a species there are many strains that do not cause disease.
- In fact most strains cannot and do not cause diseases and many strains that do cause disease target specific species

Vibriosis -- infection due to vibrios

- Long history of causing disease in humans, fish and shrimp among others
- An emerging pathogen in the sense that some strains are being found with increasing frequency and new strains are being created
- Major (primary?) cause of mortality in shrimp hatcheries and farms
- Often <u>secondary to stress</u>
- Easily controlled in shrimp hatcheries and reduced on the farm (choice of technologies available; some possibly have contributed to EMS)
- No correlation between sucrose fermentation and ability to cause disease
  - (green versus yellow on selective media TCBS is a myth)

Selective media used to differentiate sucrose fermentation ability of vibrios
Thiosulphate Citrate Bile Salts Sucrose Better known as TCBS agar



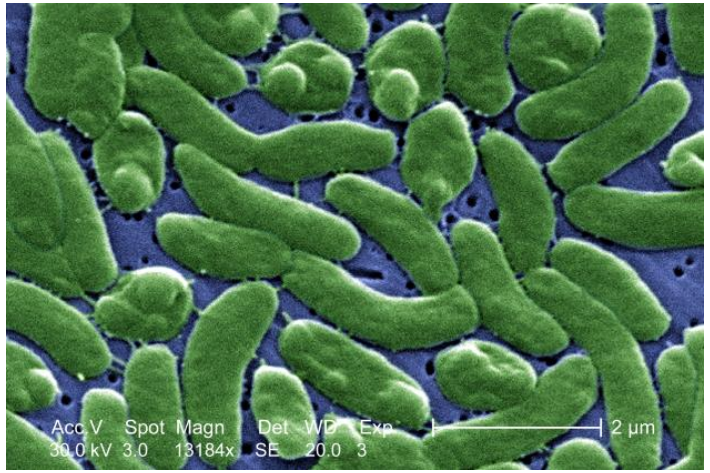
Problems with its use:

Sucrose fermentation is not related to virulence
Many bacteria besides vibrios grow in this media
Not the best selective media for vibrios alone
Useful as a general tool for determining the relative loads of vibrios in production systems

Better selective media is available

Vibrios

Vibrio vulnificus



- Some strains cause disease in humans
  - *V. cholera, V. vulnificus, V. parahaemolyticus* (not same strains that cause disease in fish and shrimp)

Children collecting cholera laden water

Cholera kills > 100,000 people per year with 3 to 5 million cases (WHO)
Prevention is simple
Filtration and or sterilization of drinking water
Separation of drinking water from sewage

An emerging pathogen causing blood poisoning and rapid death

V. parahaemolyticus strains are a major cause of seafood based food poisoning

Vibrios

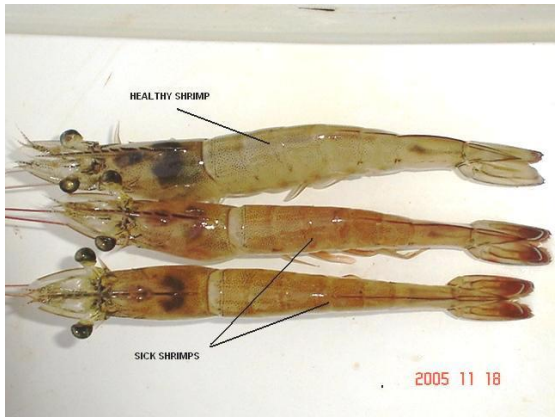
Some affect fish

V. anguillarum, V. salmonicida, V. parahaemolyticus

Characteristic lesions on body of salmonids
Major killer prior to the advent of vaccines
New strains occur all of the time
Still a problem

Vibrios

Atypical vibriosis from Belize

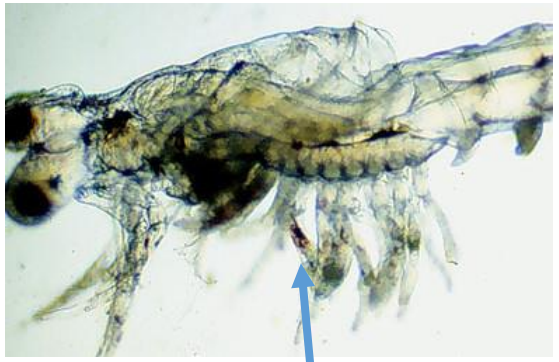


Some affect shrimp

- *V. harveyi, V. alginolyticus, V. parahaemolyticus, etc*. More than a dozen species associated with disease.

Luminescent vibrios (actually most strains are not virulent) are a problem in hatchery tanks and on farms

Characteristic lesions



Vibrio lesions are typically heavily melanized

Many vibrios cause disease direct and indirect

Partial List of vibrio species reported to cause disease in shrimp

Vibrio parahaemolyticus
Vibrio alginolyticus
Vibrio anguillarum
Vibrio damsela
Vibrio vulnificus
Vibrio penaeicida
Vibrio harveyi
Vibrio owensii
Vibrio nigropulchritudo
Vibrio campbellii
Vibrio splendidus
Vibrio fischeri
Vibrio pelagicus

Vibrio orientalis
Vibrio ordalii
Vibrio mediterrani
Vibrio logei

Most are opportunistic that cannot produce disease in healthy animals only in weakened animals

Many other bacteria species have been found to kill shrimp-the list is too long for here but includes strains of Pseudomonas, Aeromonas, Streptococcus, Shewenella, Aerococcus, etc.

At the risk of being repetitive

- Important distinction between the presence of the pathogen and disease. No absolute correlation. Potential pathogens are often present with no disease. A good example is the etiologic agent of EMS. Many shrimp carry this vibrio strain but never get ill.
- Management of disease should be done PROACTIVELY not REACTIVELY.
- Prevention is easier than treatment (Proactive)
  - Most powerful tool for preventing any problem is <u>the reduction of stress</u> on the animals at all phases of the production process
  - Most pathogens of shrimp are opportunistic. Very few are obligate.
  - What is the difference?
    - OBLIGATE Will cause disease in healthy animals merely by being present. Usually highly virulent.
    - OPPORTUNISTIC Will cause disease in stressed animals. Usually weakly virulent. May require high loads to produce disease (some strains that cause EMS are like this)

Control or elimination

Is it possible to eliminate vibrios from production systems?

No nor is this desirable

However, completely controlled nuclear broodstock production facilities can be managed to minimize the load as can indoor recirculating production systems.

Levels can be minimized and in fact kept well below detectable levels in highly controlled production systems.

Keep in mind though that when specific niches are available they will be filled and not always by the best organisms to do so

Feeding is done for the farmer not the shrimp

The manner that we feed shrimp is not consistent with the ability to deliver long term solutions in the feed, especially those that act in the gut.

Shrimp eat more or less constantly when provided with food. Feeding three times a day and not on Sundays is ????. Encourages animals to consume vectors difficult to get long term impact of additives in the feed. Short time for activity.

Metered automated feeding dividing the ration over the course of the day rather than pulsed high levels of feeding three times a day.

Automatic solar powered feeders allow shrimp to be fed throughout the day

Feeding small amounts of feed throughout the day lessens stress, lessens foraging on detritus and naturally occurring feeds that can be carrying potential pathogens and promotes a healthier and cleaner pond production system.

Tools for limiting the presence and the impact of vibrios

Control of vibrios in the environment
Control of vibrios in/on shrimp

TOOLS that can be used.

Chemicals water and feed application

Environmental manipulation not possible in typical production systems

Microbiological water and feed application

Other

Use of chemicals to control vibrio loads

Disinfectants quaternary ammonium compounds, formalin, chlorine, chloramine T, etc.

Hatchery tanks are smaller; need less product, although larval and post larval shrimp can be more sensitive to levels required for optimum efficiency

Large ponds require high levels and thus cost is more

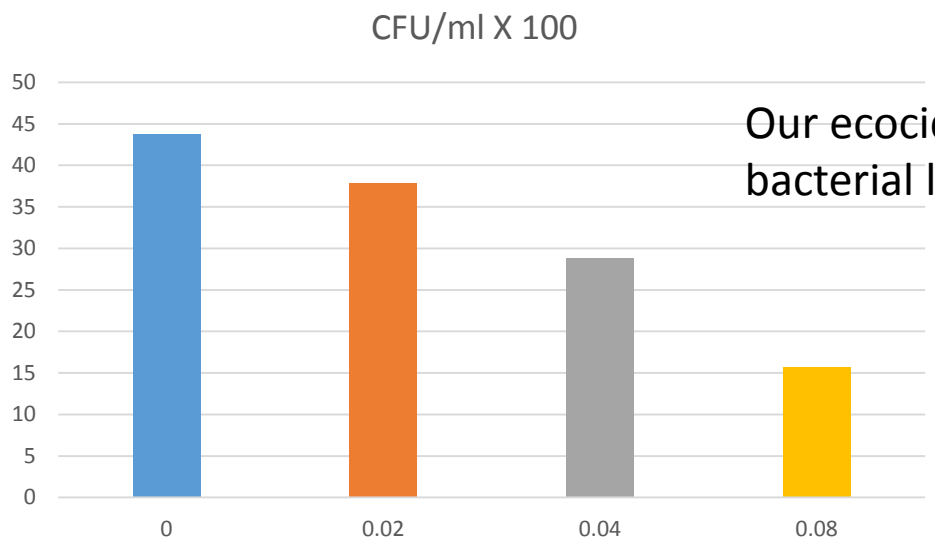
Partial control achievable although typically this is a broad spectrum approach and temporary.

Chemicals can be added to the water or used in the feed.

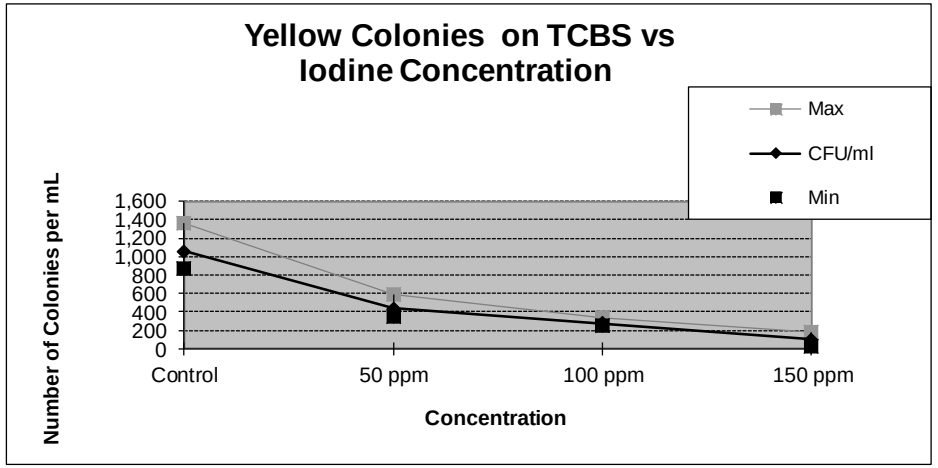
Important to control the vibrio loads that enter the production system with the animals

BIOSECURITY in the hatchery

QAC (Ecocide) added to pond reduces bacterial loads



Our ecocide lowers bacterial loads

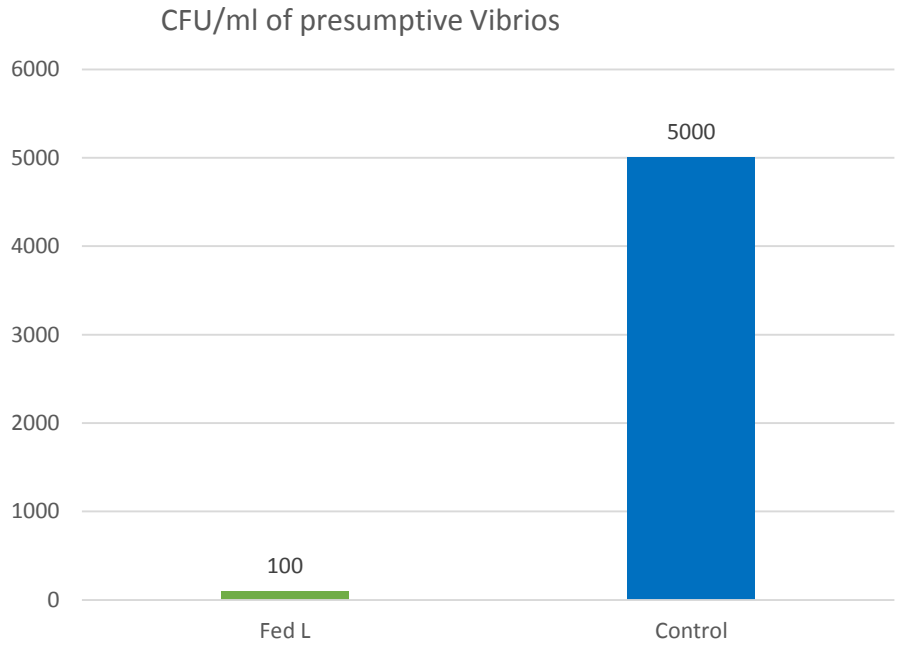


Nauplii washed with iodophor

Basic biosecurity in the hatchery is critical for vibrio control

The use of chemicals in the production environment to reduce vibrio loads

Mix of monoglycerides added to feed kills vibrios in tank



Our mixture of monoglycerides controls the levels of vibrios through the feed

Environmental Manipulation

To control bacterial loads and types in production environments.

Controlling salinity, temperature, vector control (elimination of carriers and physical carriers to prevent movement of diseased animals) all impact the types and levels of a variety of bacteria.

Not always practical because of lack of resources, site of ponds, etc.

Paradigm changes? Complete control of inputs? Technically possible in closed production systems.

<u>Makes more sense to talk about reducing susceptibility more so than altering production environments</u>

Biosecurity
Reduction of stress

Sick shrimp on pond surfaces are easy pickings for many birds who can in turn move pathogens between ponds and farms.

Competitive exclusion

Most widely used approach is through bacterial amendments popular term is probiotics.

Complete misnomer as activity is not through the gut. No need to coin the term as these are really tools for bioremediation. Started out as a sales gimmick that unfortunately stuck.

Many product types in the market. Many contain viable bacterial cells that cannot be preserved or are held in a dormant state. Most effective tools are those that contain bacterial spores.

Data on a product that we developed and sell. Not all products are the same. Purpose is to show that this is viable approach to lowering vibrio loads in shrimp hatcheries and farms. This is a tool and it has been field proven to reduce the overall loads of vibrio species including the species that causes EMS.

Can be used to control EMS.

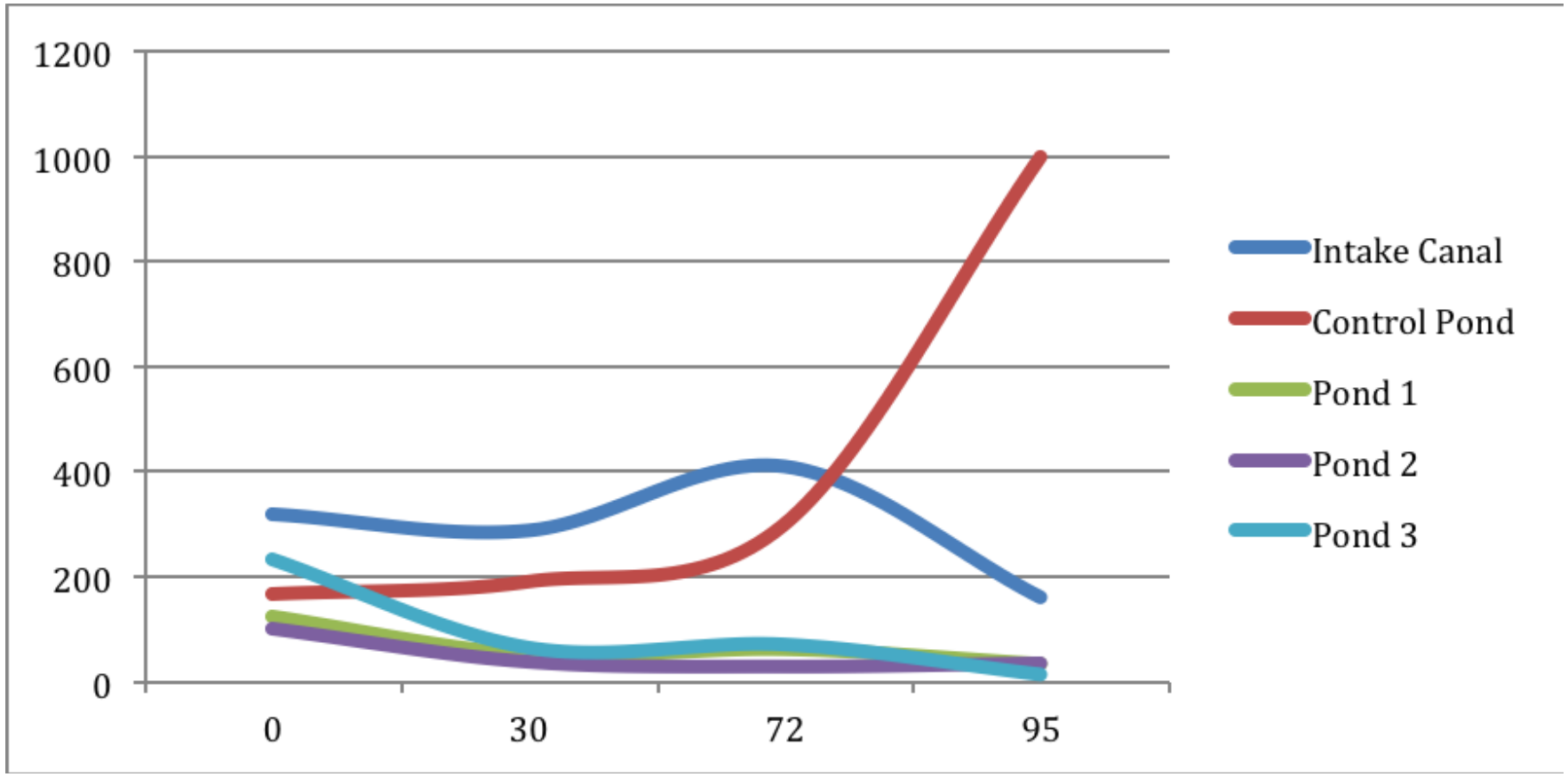
Experimental Parameters India 2013

Pond #	Animals per sq m	Area (ha)	Depth (m)	Water Exchange (%)	Cycle (days)	weight grams	MT harvest	% surv	FCRs
1	35	1	1.5	0	115	24.5	>7.7	>90	1.1
2	35	1	1.5	0	115	23.3	>7.3	>90	1.1
3	28	0.9	1.4	0	115	25.9	>6.5	>90	1.2
Control	35	1	1.5	10 to 15	122	15.1	5.3	82	1.7

Results of three ponds treated with our tableted product, PRO4000X compared with a pond that was not treated.

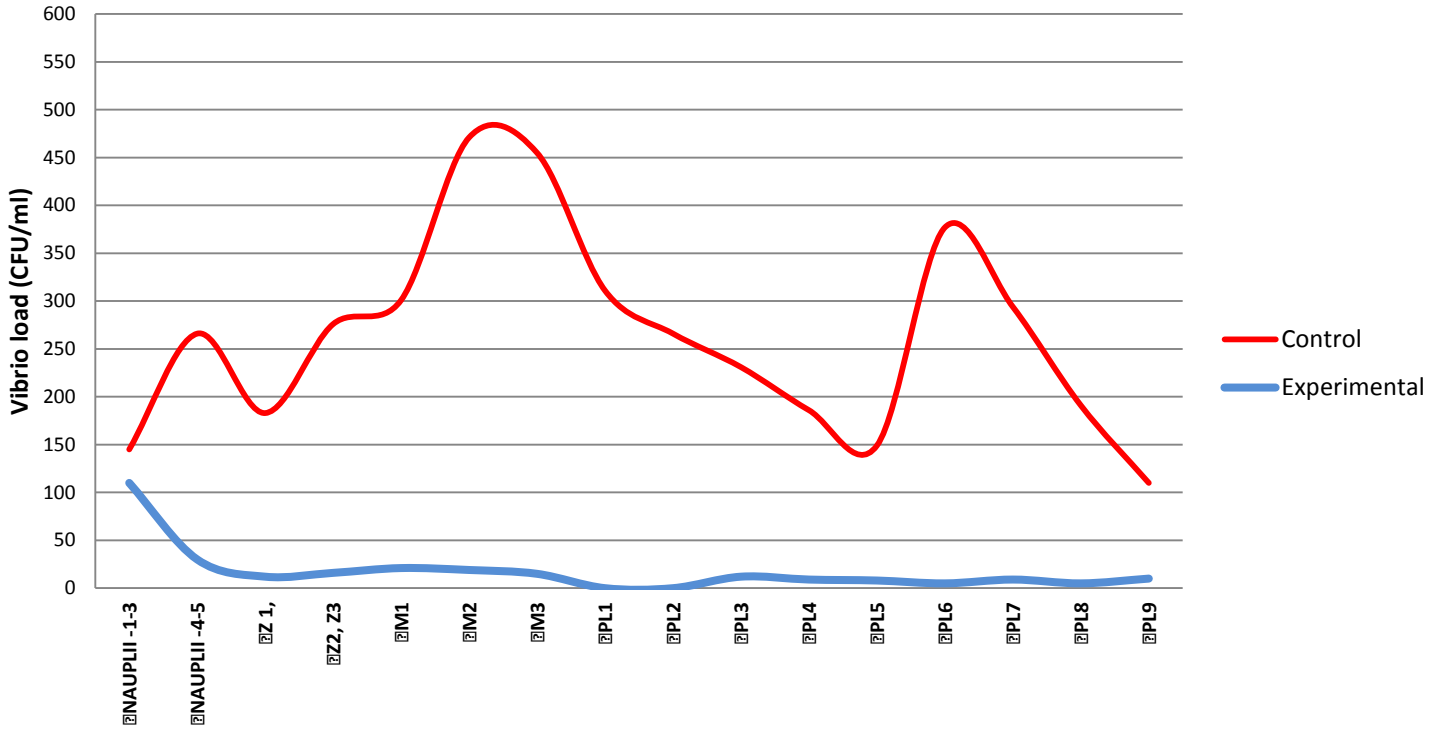
Clearly demonstrates that in this instance there was a dramatic impact on productivity. Subsequent pages show why.

Reduction of TCBS green vibrio loads over the course of the production cycle.



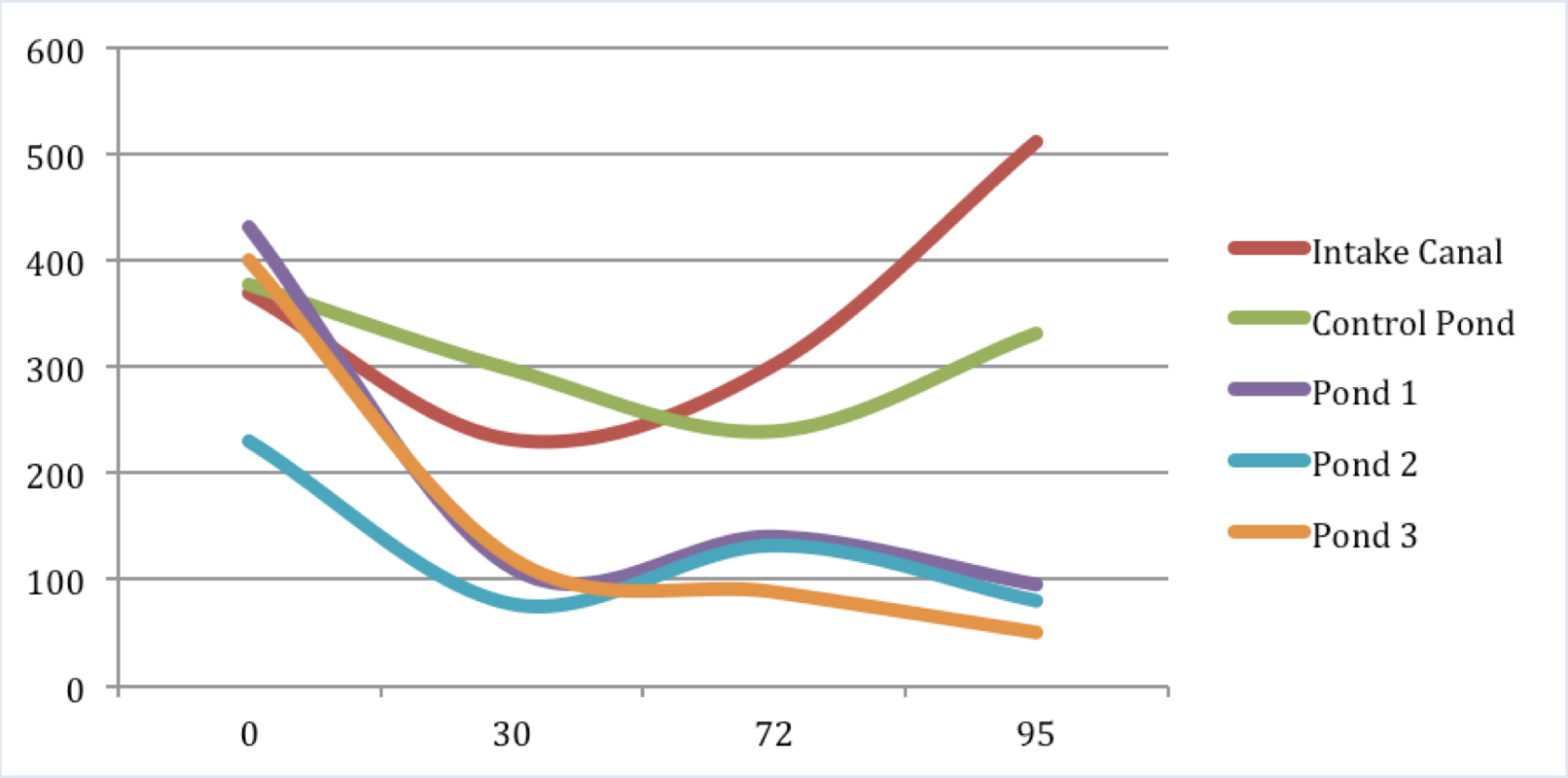
TCBS green vibrio loads were significantly reduced in ponds that used PRO4000X

PRO4000X reduces green vibrios in hatchery tanks.



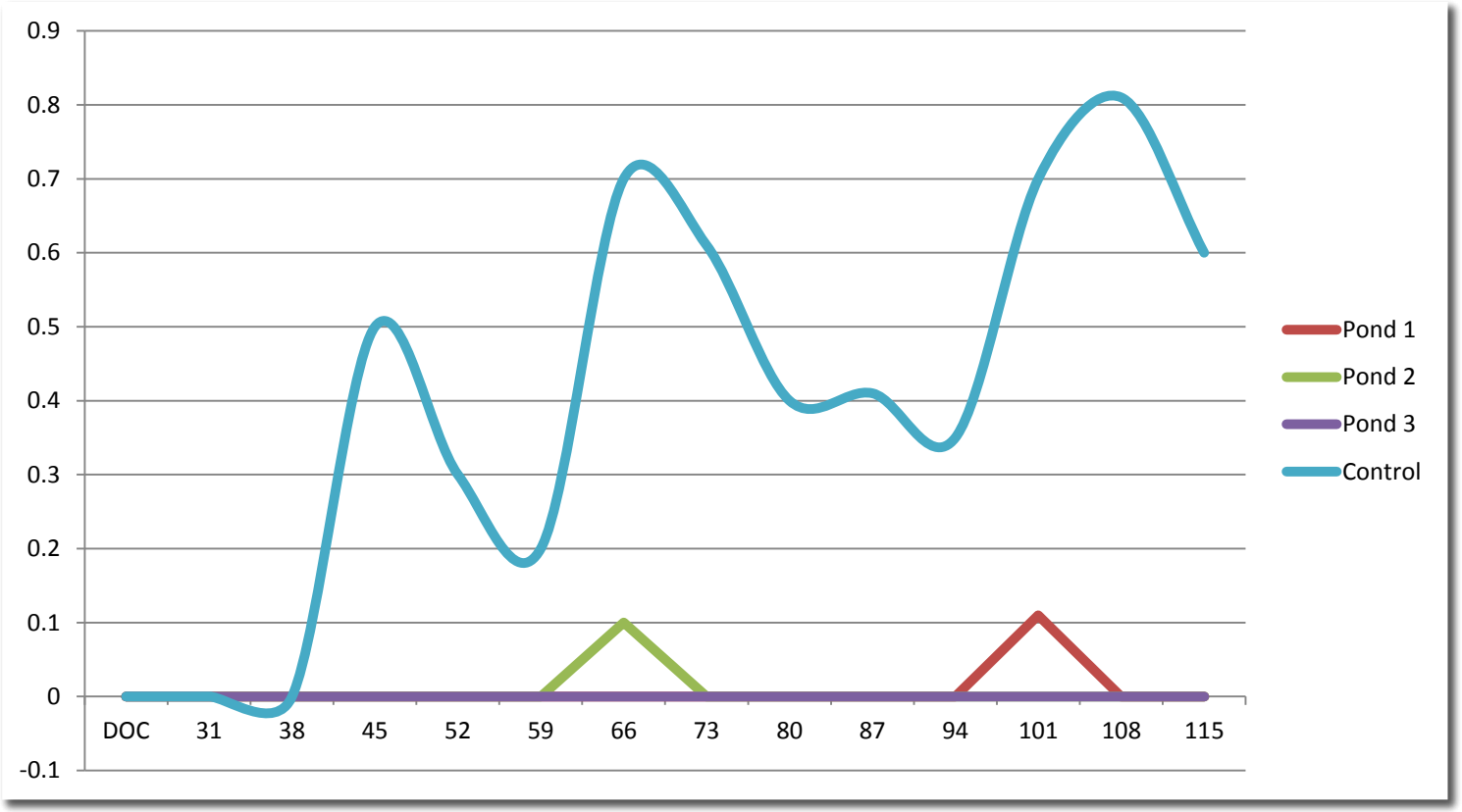
TCBS Green loads reduced to almost zero in hatchery tanks

Reduction of TCBS yellow vibrio loads over the course of the production cycle



TCBS yellow vibrio loads were significantly lower in ponds treated with PRO4000x

Weekly ammonia levels in control and experimental ponds



PRO4000X controlled ammonia levels in the experimental ponds

Other possible tools for impacting bacteria loads in production environments.

Bacteriophage bacterial viruses-highly specific

QSI **Quorum sensing inhibition**

HSP **Heat shock proteins**

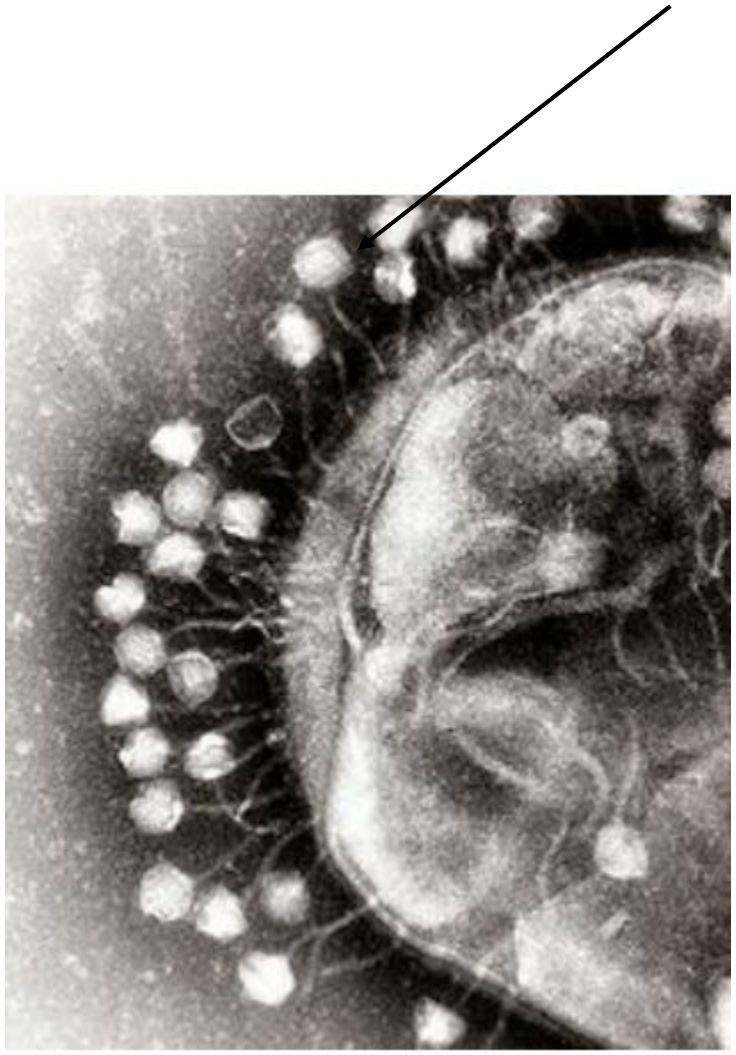
Heat shock proteins are involved in many different physiological processes. Problem with delivery.

Vaccination

No evidence of specific immunity.

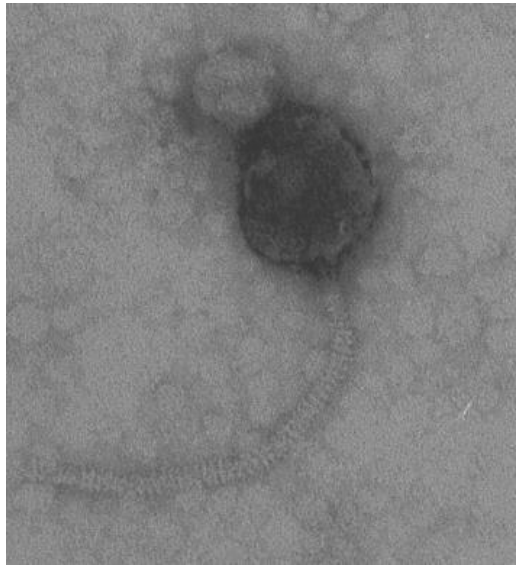
Other possible tools to use in impacting the vibrio loads

Bacteriophages viruses that attach, infect and kill bacteria.



- Specific for each bacterial strain.
- Not practical in a farm. Risky as selective killing of strains can create holes in the ecology opening the way for other bacteria.
- Will work in hatcheries. Costly though and better tools are available.

Double edged sword? Experimental and while scientifically valid not a good tool for general use. Some risks.



Quorum sensing inhibition (QSI)

- Bacteria communicate with each other through chemical signals. These signals have a profound impact on the bacteria's ability to grow, to form biofilms, to create disease, to resist environmental factors that can negatively impact growth.
- Not practical on a large scale.
- Some indications that Tilapia can mitigate EMS/APHPNS perhaps by excreting vibrio species that produce specific QSI compounds.

Co-cultivation of shrimp with Tilapia either in the ponds or in the waters that the ponds are filled with (reservoirs) can lower loads of potential pathogens

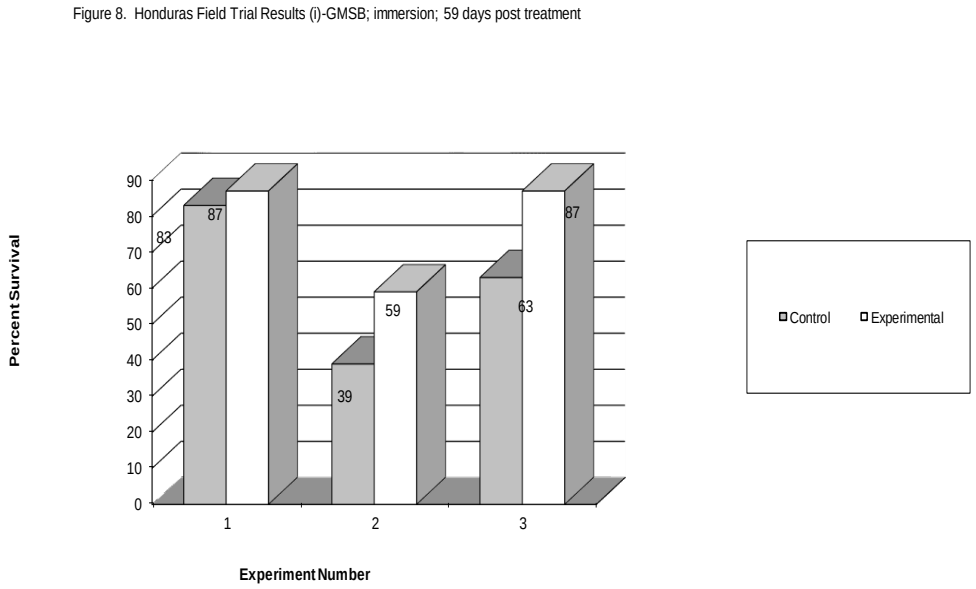
Not consistent and there are problems with mixing fish with shrimp if it is not done properly

Tools that can be used in the feed and in the water

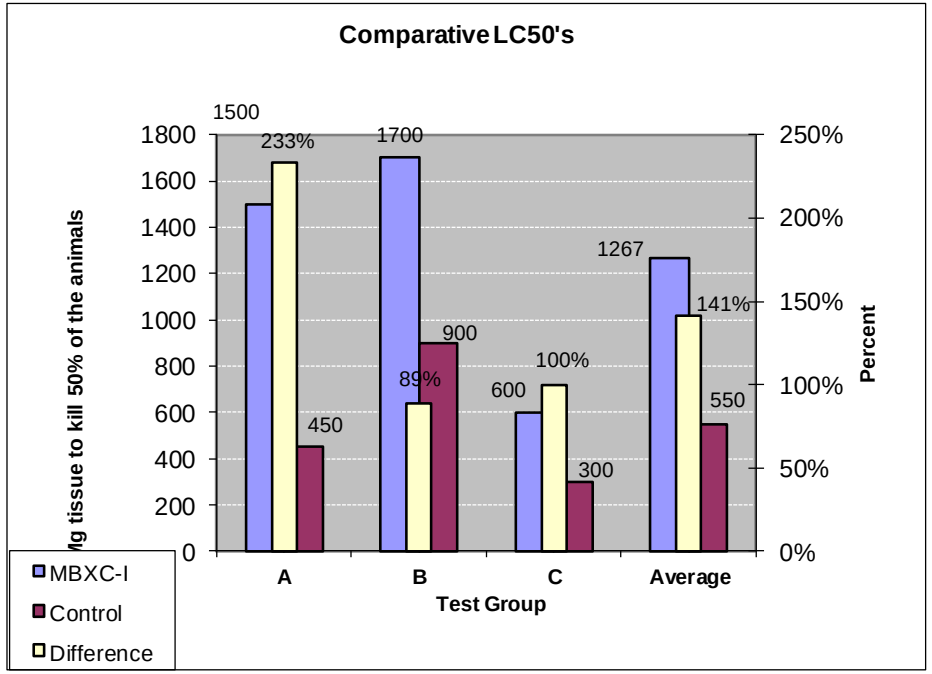
- HSP inducers
  - Compounds that induce a series of chaperone proteins called heat shock proteins
  - Evidence suggests that they may impact disease resistance
  - Could be more effective in the feed
  - Good potential that needs more research
- NSI
  - Non specific immune stimulants

Immunization --for shrimp this is the stimulation of non-specific immunity

- Little evidence of specificity
- Non specific immune response can be protective; although likely of short duration.



MBXC used to feed larval shrimp before stocking showed a strong benefit in two three side by side pond comparisons for 60 days post feeding



Animals feed MBXC and exposed to the TSV showed a statistically significant increase in their ability to tolerate the virus. It took much more virus to kill them.

Tools to impact the ability of the animals to resist disease

- Typically applied in the feed
  - Glucans, LPS, Chitin, Chitosan, sulfated polysaccharides
  - Herbal remedies
  - Neutraceuticals

Conclusions

Controlling vibrios in production systems requires a commitment of resources and a consistent use of appropriate tools.

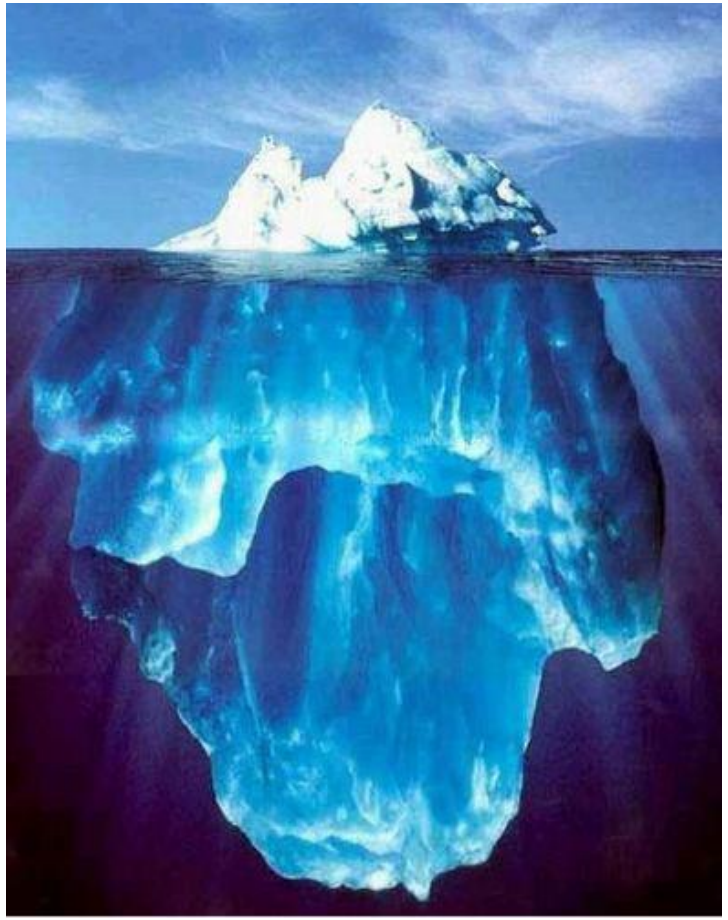
Controlling total vibrio loads in the environment is likely not normally feasible although tools exist that will allow farmers to lower the overall loads in some environments.

Controlling loads of specific vibrios can be done via the use of biosecurity measures in maturation and the hatchery.

Reducing stress and creating a more shrimp friendly environment is important.

The susceptibility of the animal to acute problems can be impacted by the selective use of the appropriate tools.

A parting thought



The top of the iceberg is the part that we see; these equate with the components of the process that we have control over and that we believe we can control.

The bottom of the iceberg are those factors we cannot control or we are unaware of the need to control them.

Successful shrimp farming depends on being able to see what is real and minimizing the impact of those things that can be controlled.

Proper production protocols also lessen the potential for serious impact from what we cannot see.