EMS

Early mortality syndrome or

Acute Hepatopancreatic Necrosis Syndrome

AHPNS or AHPND

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Flow of presentation

- The disease
- The pathogen
- Disease mechanism
- Disease in the lab
- Control
- Outlook

The Disease

- First named Early Mortality Syndrome (EMS)
- Initially not readily characterized as to causative agent
  - Not unusual given that shrimp farming is susceptible to many problems
  - Quite likely mixed infections cause problems in some farms (most?)
  - Problem likely present for longer than our history suggests
  - *Vibrio harveyi* ? New virus from China (covert mortality disease), microsporidian (*Enterocytozoon hepatopenaei*)

The Disease (continued)

- After confirmation that a bacterial strain causes the disease named changed to reflect the pathology (Loc at al University of AZ)
- Acute Hepatopancreatic Necrosis Disease or Syndrome (AHPNS)
- Pathology is characterized as a progressive destruction of the tubules that make up the HP
- Worst case is total destruction
- Many instances where damage is not complete and animals survive-albeit with potential growth issues

Pathology

- Characteristic pathology
  - Hepatopancreas is the target organ
  - Tubule epithelial cells slough in the early stages of the disease in the absence of apparent pathogens
  - Bacteria colonize the stomach and produce a toxin (recently characterized as being similar to insecticidal toxins produced by an insect pathogen)
  - Toxin enters the animal through the HP damaging and eventually destroying it allowing a myriad of opportunistic bacteria to then kill the starving host

The Pathogen

- To date all strains associated with disease have been characterized as being *Vibrio parahaemolyticus* strains.
- There appears to be a large amount of variability as to virulence via water borne exposure between strains.
- This is theorized as being due to the presence of a plasmid that encodes for the toxin.

What is a plasmid?

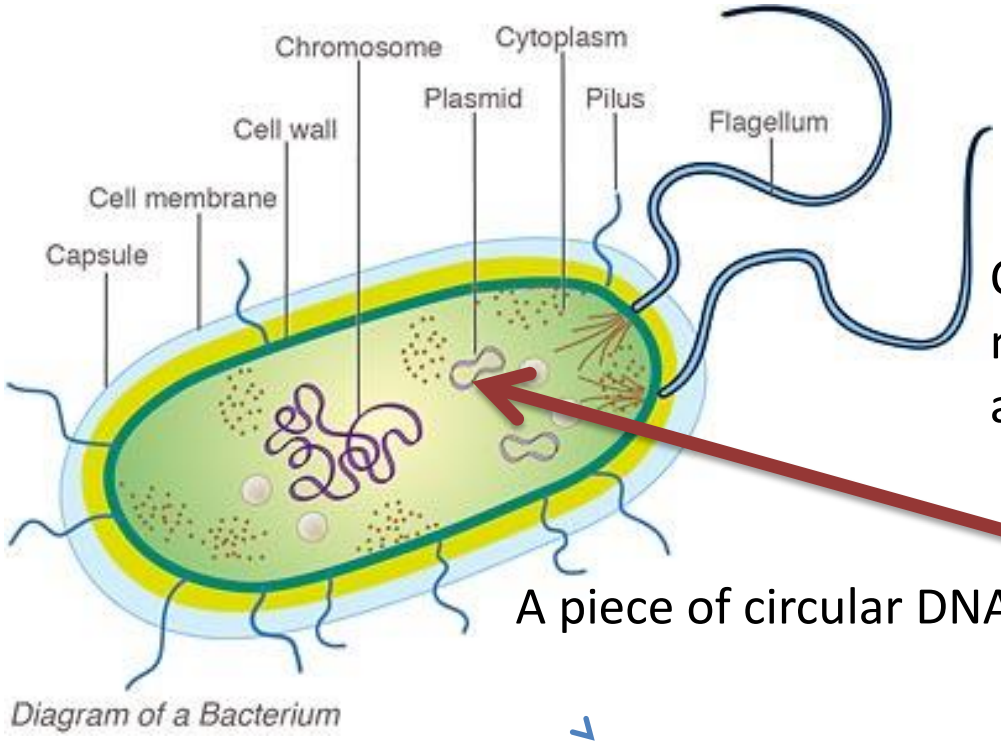


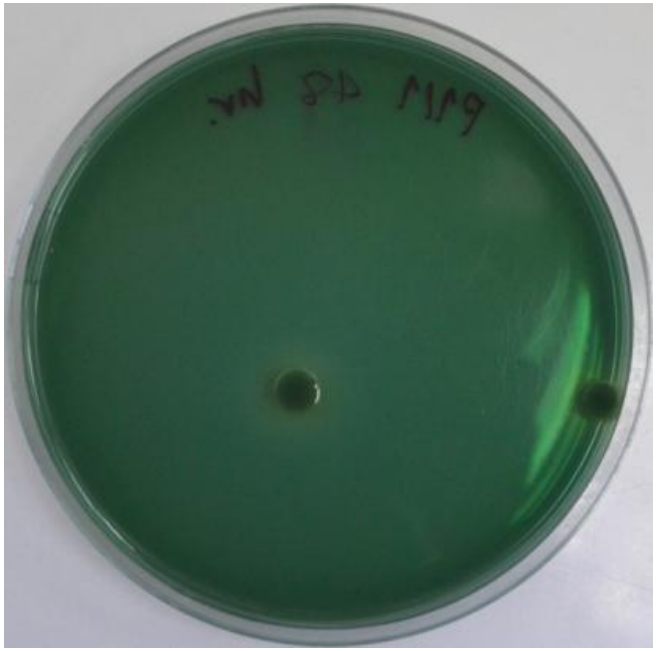
Diagram of a Bacterium

Can code for toxins, virulence mechanisms, biofilm formation, antibiotic resistance, etc.

A piece of circular DNA that is not part of the main genome.

- Easily transmitted in whole or in part between bacterial species. Plasmid DNA is promiscuous.
- Unique to individual species typically

Vibrio parahaemolyticus



Green colonies on selective media (TCBS)

Common vibrio strain, most strains are benign although some cause disease in fish, humans and crustaceans.

An emerging disease in some areas. Increased frequency and impact.

Where did the bacterial strain originate?

Vibrio parahaemolyticus is ubiquitous in the marine environment; also reported in healthy human guts and in fresh water environments where it may be viable but not culturable (VBNC)

Question should be: *Where did the genes come from that allow this usually (probably) benign bacteria to become such a threat?*

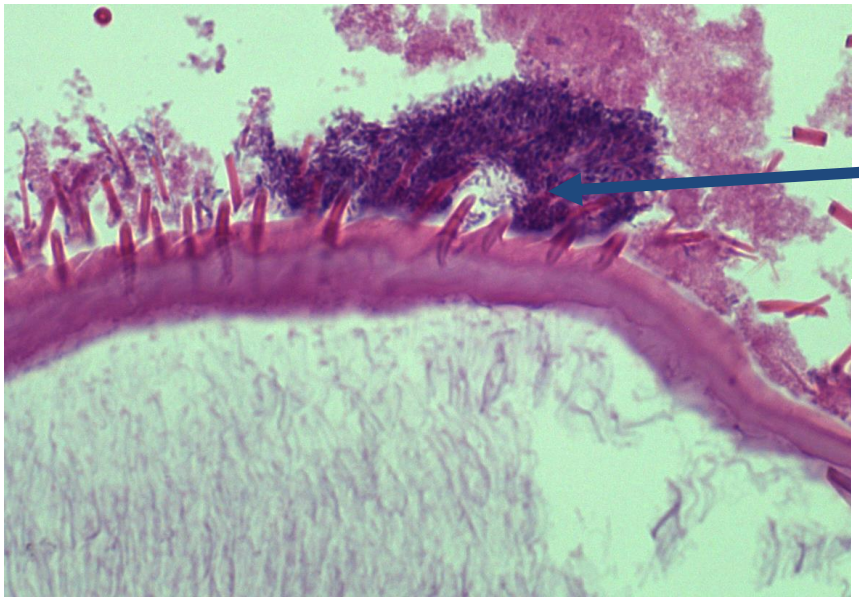
Data suggests toxin may be from an unusual bacterial species. This is a commercially available nematode/bacteria combination that is used to kill certain kinds of insects. Close contact between this and strains of VP?

Fundamental lack of use of time honored and proven biosecurity protocols combined with greed and carelessness moved the genes far and wide.

Appearance of disease

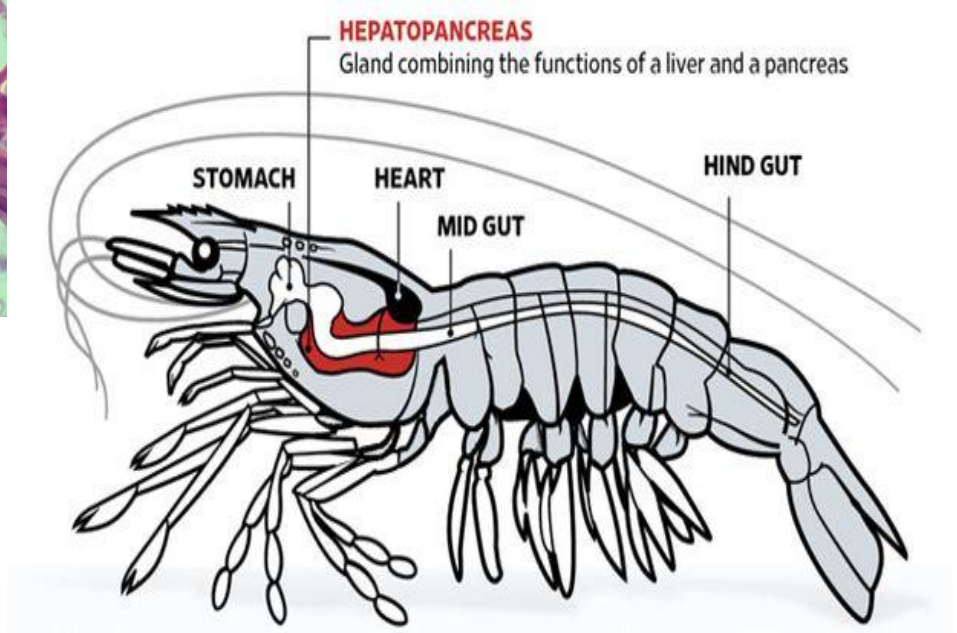
Massive numbers of dead shrimp usually within first 30 days of stocking though not always

Where is the bacteria?



Biofilm on stomach lining
Dr. James Brock Moana Technologies

bacteria growing on the chitinous surface of the gastric wall.
H&E stain, 60x with additional photo magnification

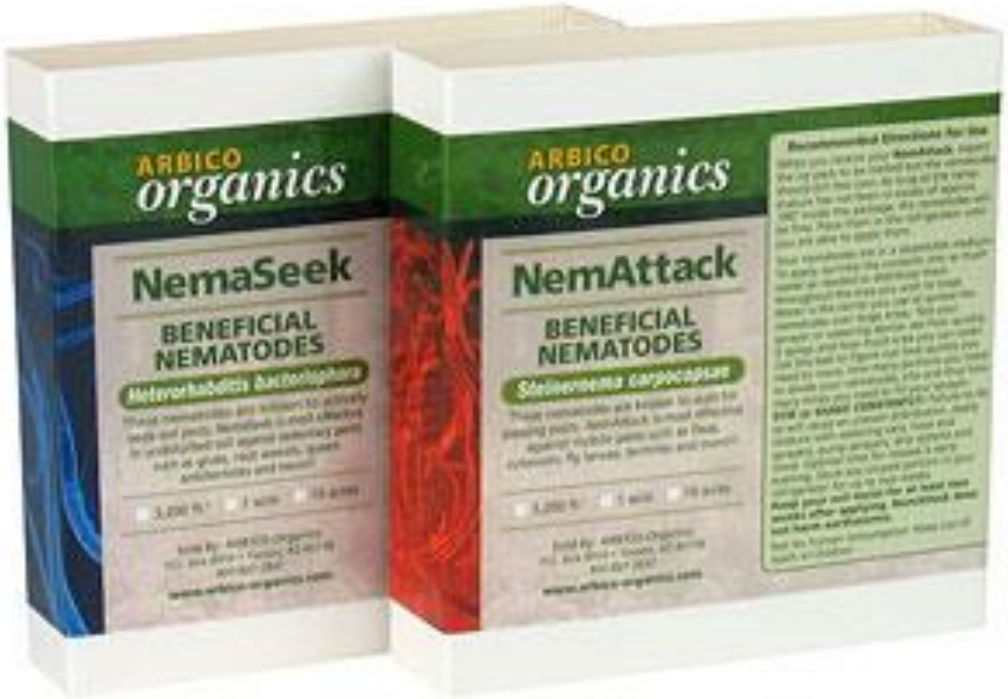


From WSJ

Mechanism of pathogenesis

- Extra-chromosomal plasmid encodes proteins homologous to *Photorhabdus* binary toxin PirAB
- These are two toxins that act together (to damage the tubules)?
- Where did this come from?
  - Photorhabdus is only found in nematodes, not free living; *Heterorhabditis bacteriophora* nematodes
  - Contact between the nematode hosts and VP?
  - Commercially available

Commercially available nematodes



Spread of the disease

Disease with characteristic pathology

First reported 2009 Southern China
2010 Vietnam
2011 Malaysia
2012 Thailand
2013 Mexico

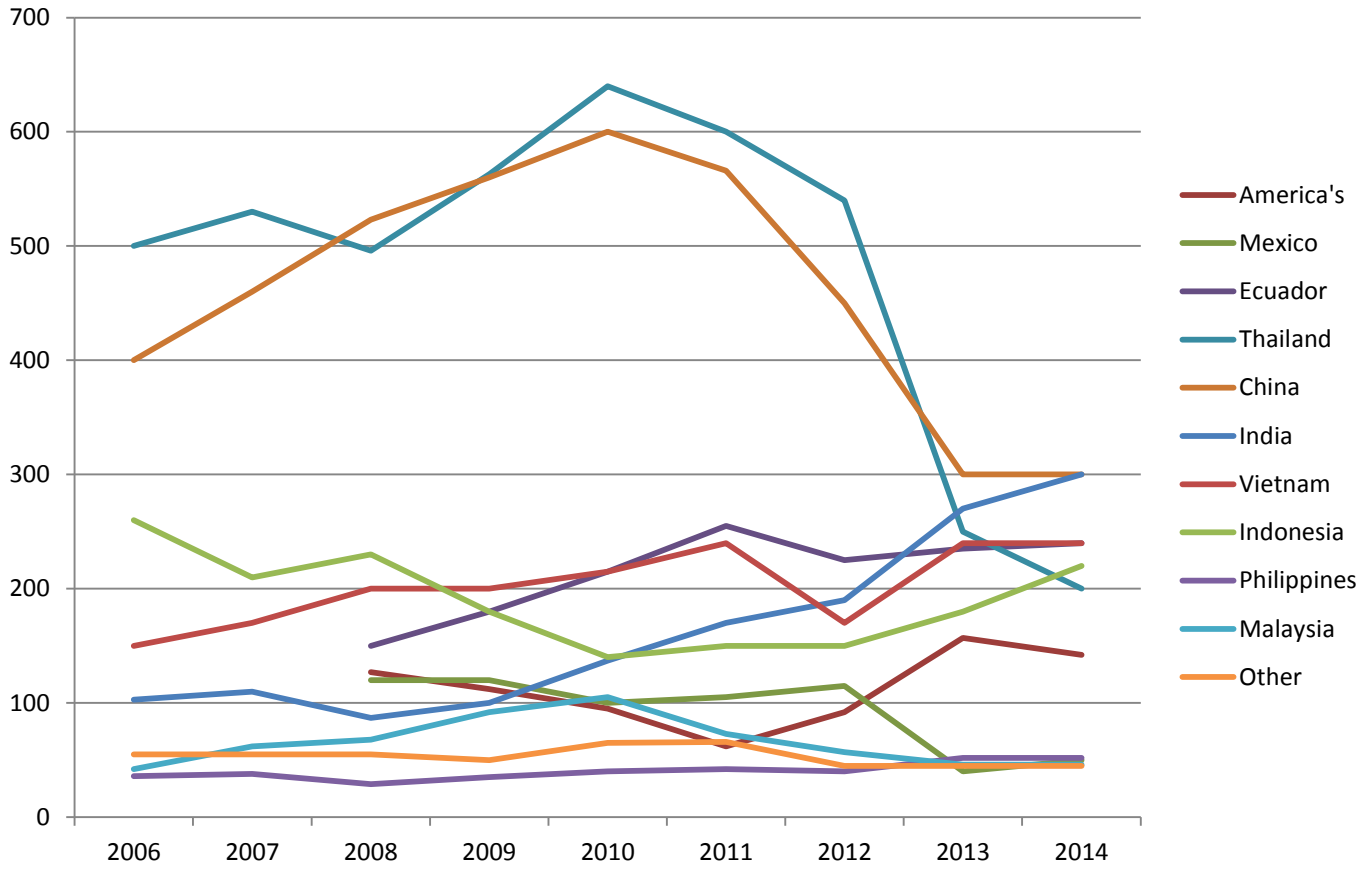
Sporadic reports India, Indonesia and the Philippines with no confirmation as of yet. Philippines has been confirmed as of late October 2014.

Highly probable that it will continue to spread.

Moved North initially

Mexico

Global Impact on Production



Exact production figures for some countries very difficult to get at

Localized impact

Mexico probably the most severely impacted percentage wise.

Thailand seriously affected. Production around 200,000 MT this year.

China as well. Some areas of local recovery.

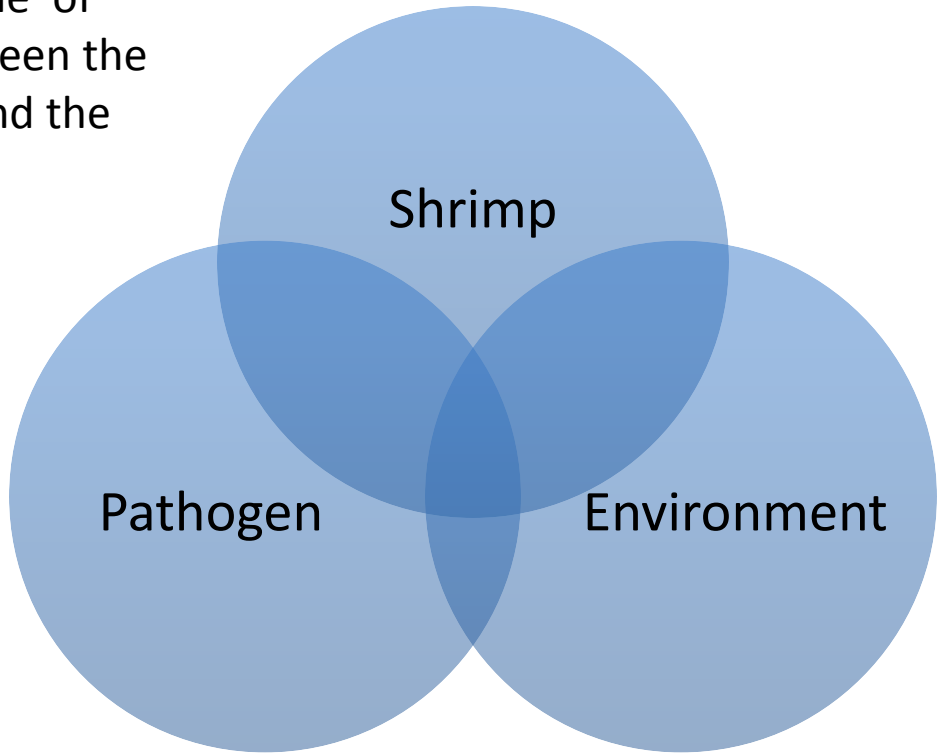
Malaysia to a lesser degree. > 60% farms are fallow

Vietnam seems to be recovering. Paradigm shifts?

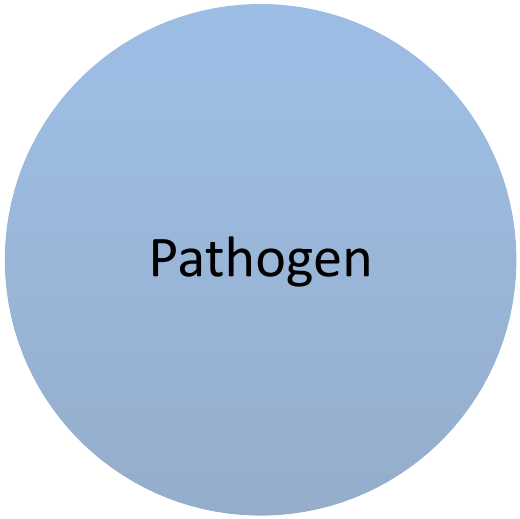
Numbers are not as important as the trends. Vietnam imports and re-exports making figures seem higher than they probably are. China exaggerates production year after year.

Disease is a complex phenomenon

Disease is the outcome of the relationship between the host, the pathogen and the environment



- Genetics? Some indications that there may be a genetic component to susceptibility although this remains to be seen. No proof-only speculation.
- Age? there are reports that very large animals appear refractory ??? PLs molt frequently possibly preventing biofilm formation.
- Overall health of the animals? Healthy and strong animals resist disease better in general.
- Stocking densities? Super high density systems less likely to have problems
- Other?

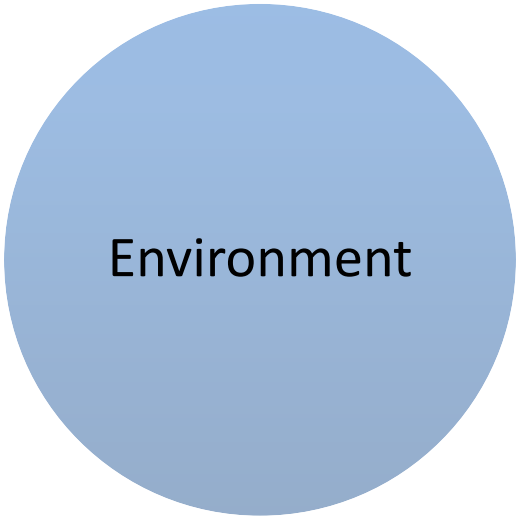


Pathogen

Presence of toxin producing genes differences could explain differences in virulence

Ability to form biofilm

Presence of amplifying vectors such as rotifers



Environment

Presence of stressors; low DO, high nitrite, high levels of un-ionized ammonia (NH3)-pH related, algal and bacterial toxins, sulfides, handling stresses, depleted ascorbic acid levels in PLs, ?

At this time not at all clear what factors might precipitate disease.

How is it spread

Not totally understood at this time. Likely multiple mechanisms once it is established. Broodstock carry it in the gut; no signs of disease. Poor biosecurity. Nauplii carry it externally and PLs may carry it as well externally.

PLs have not been reported to display the disease in hatcheries although they may be carrying it at very low levels frequent molting?

PLs may carry it into ponds and likely have played a role in disseminating the strain.

Significant role of stress in susceptibility?

Poor biosecurity practices?

Broodstock

Adult shrimp to be used for broodstock have been theorized as potential vectors. The bacteria apparently is only in the intestinal tract (or external?) and no active disease has been reported in large animals (are they refractory?)

This could explain how the disease has been moved between countries by shipping broodstock carrying the pathogen.

Threat would not be from broodstock that have never been reared outdoors-such as nuclear breeding facilities. Threat is from pond reared broodstock and from indoor animals that are not being held under proper biosecurity.

Caveat emptor—not all broodstock are the same; if animals are held outdoors in nonbiosecure environments, they should be considered potentially infected even in areas where the disease has not been reported.

Post larval shrimp

Surface contamination?

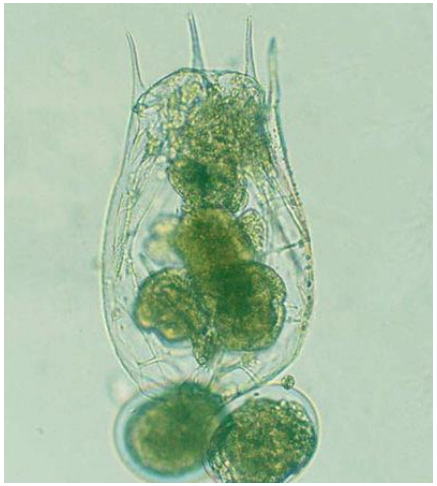
Internal?

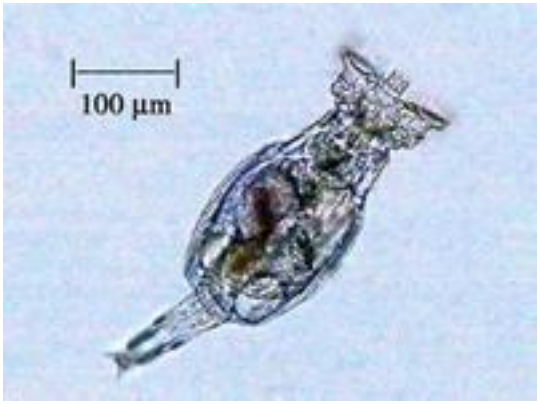
It is highly likely that this is one mechanism by which the bacteria has been introduced into ponds.

Since shrimp take a while to succumb the bacterial loads must build up- cannibalism?

Bioamplification in ponds by vectors

Rotifers (and likely any chitinous organism) have been found to harbor high levels and are capable of causing acute disease in lab challenges and likely on farms as well





Makes sense from a disease perspective. High degree of variability among SE Asian strains as to levels of lethality. Low levels not lethal. High levels are.

Polychaetes

- Chinese worms have been found to be carriers

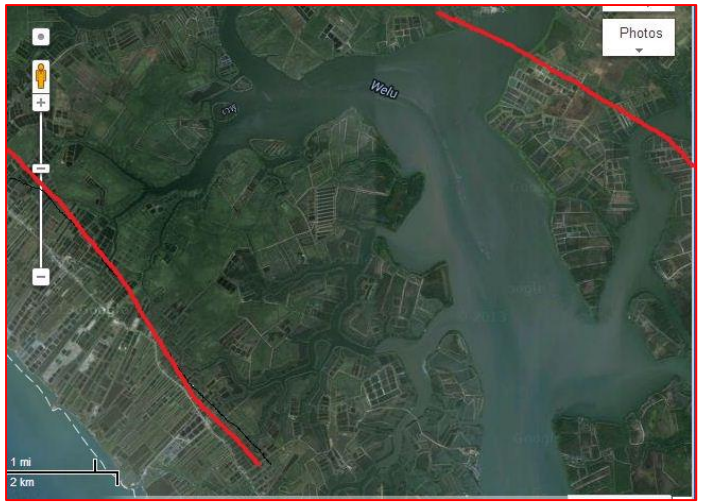
Different species of worms are routinely fed to adult shrimp to facilitate maturation and to improve the quality of the nauplii.

Wide spread use of these worms (typically tested for presence of OIE required pathogens) could have led to spread through broodstock.

Flegel et al. Detection in Thailand

Sources	Province (Positive/Total tested)			Totals (%)
	Songkhla	Trad	Rayong	
Broodstock feces	2/5	8/15	14/24	24/44 (55)
Nauplii	1/1	0/5	3/8	4/14 (29)
Polychaetes	1/2	2/3	2/3	5/7 (71)
Squid	1/1	0/3	3/5	4/9 (44)
Artemia	1/1	-	0/1	1/2 (50)
Oysters	0/1	-	0/2	0/3 (0)
Clams	1/1	1/1	-	2/2 (100)
Acetes	-	-	0/2	0/2 (0)
Blood worms	-	-	0/2	0/2 (0)

Movement through the environment



Prevention

Use of broodstock that are properly screened (using enrichment and the correct PCR)
Biosecurity in maturation facilities/hatcheries
- Copious washing and disinfection of eggs and nauplii
- Individual spawning
- Testing using enrichment before PCR test

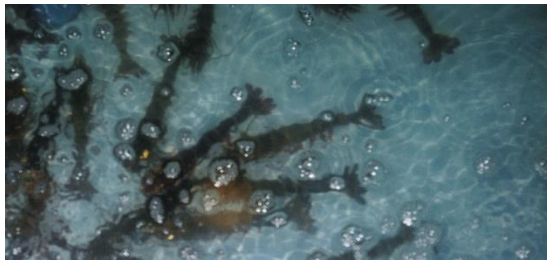
PCR using the latest probes (directed against two toxin gene containing plasmids)
Pond preparation
- Avoid the use of chlorine
- Filter the incoming water
- Use of well water where possible
- Allow ponds to mature before stocking
- Use of certain kinds of microbial bioremediation tools

Water sources
- Well water better than estuarine water
- High water exchange rates seem to favor less severe disease

Sites to break infectious process

Maturation

- Use animals that are from nuclear breeding facilities if they have not become contaminated through infected broodstock.
- Screen adults individually for the presence of the bacteria. This entails tagging the animals. This will require enrichment to increase the sensitivity of the test.
- Surface disinfect individual adults. Iodine, formalin or a QAC (Ecocide)
- Using an antibiotic pro-actively should be considered if there is a history. The proper antibiotic should be used for the proper amount of time. Ten to 14 days in the feed.
- Do not use of polychaetes from China and warm water squid.



Hatchery

Screening PLs is a hit or miss proposition

Low levels of prevalence are difficult to catch and no test can find a low level of a bacteria in a population-enrichment critical.

No illness in PLs possibly a result of constant molting keeping levels of attached bacteria too low. After stocking growth slows and the bacteria accumulates in the environment.

Keep live feeds **clean**-primarily Artemia. Outdoor culture of algae can pose risks.

Biosecurity Copious washing and disinfection of eggs and nauplii.

Farm

There is some speculation that changing pond preparation and stocking procedures will be essential to the long term control of this problem. The wide spread use of protocols intended to sterilize the production system followed by protocols that allow the bacteria to thrive must be modified.

Farm

Consider strategies that lower the load of pathogen entering the system and that encourage the development of mature ecologies in the ponds prior to stocking.

Some strategies that have "worked"

Using well water

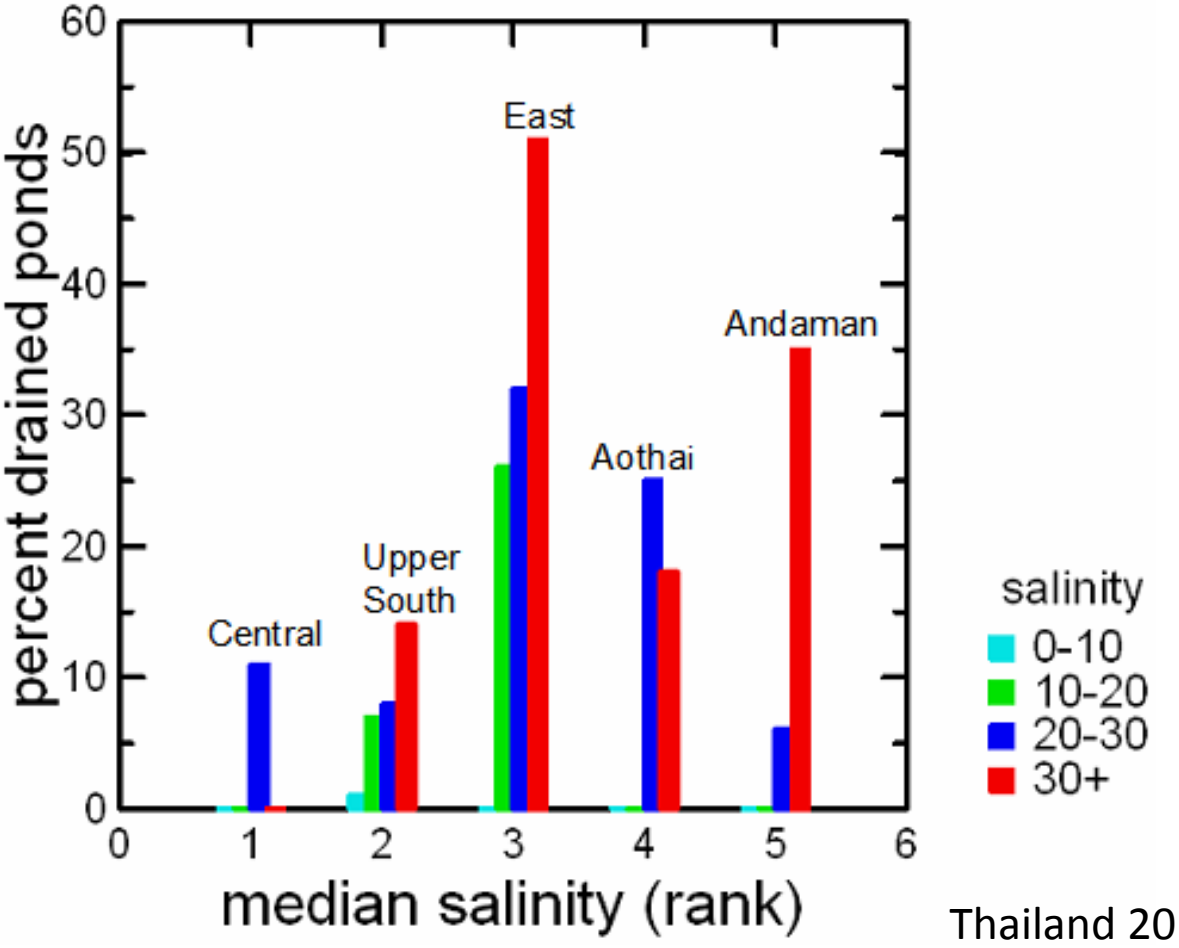
Lower salinity waters have lower prevalence (under 5 ppt)

Co culturing with Tilapia

Super high density systems only food animals can eat is what they are fed

Use of automatic feeders to spread out feed consumption

Correlation of salinity and EMS



Thailand 2013

Stock *P. monodon*

(SPF stocks now established in Gulf of Mexico)

Susceptible but apparently less so (feeding at a different trophic level?)

Stock juvenile shrimp

Some areas in China and Thailand report that stocking shrimp at 30 days or post hatchery gives better results

Other areas do not see this effect

Pathogen load?

Change the production paradigm

Paradigm shift concrete sides and lined bottoms using pumped well water close to the ocean in Vietnam eliminated the problem in some areas

Very high density production systems are typically not affected

Stress reduction

Stress plays a critical role in susceptibility in many diseases.

In lab studies fitness of animals at challenge determines the outcome NOT unexpected. Typical of other pathogens.

Important stressors to control are dissolved oxygen levels, organic matter loads (which can encourage growth of bacteria such as *V. parahaemolyticus*) ammonia and nitrite levels. ????

Blue green algae produce toxins that may also impact susceptibility

Disease in the lab

Challenge studies in the lab reproduce the pathology although there are some differences between isolates as to the levels that it takes to produce disease

Some evidence that there is a role of stressors is critical in lowering dosage of bacteria need to cause disease remains to be determined.

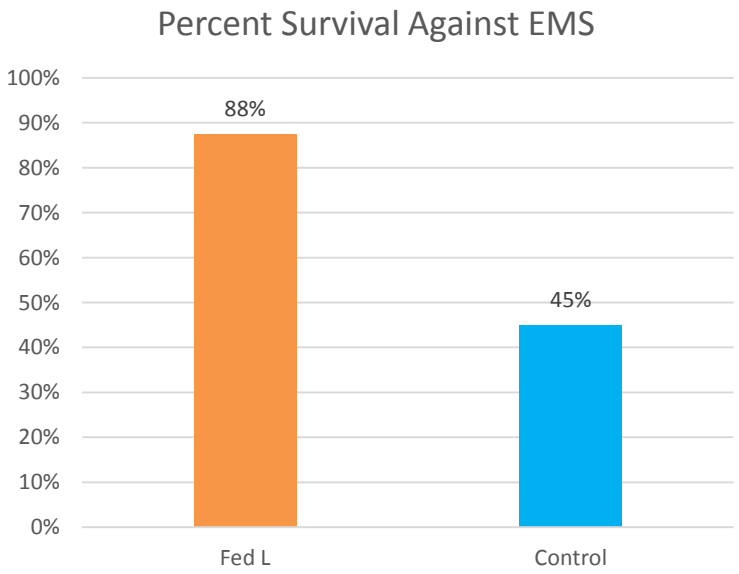
Lab studies are not always field relevant

Many claims of products that stop the disease

Lab tests rarely are performed in a manner that is consistent with what occurs naturally

Single level challenges by injection
Single variable environments
Oral application in feed

Mix of monoglycerides



Outlook-BIOSECURITY FAILURE

Highly likely that genes will spread. Conjugative plasmids are easily transferred between heterologous and homologous species. Opens door for other bacterial species to produce disease, although the role of biofilm and attachment to a target organ may limit this.

Disease outbreak is similar to that reported with a luminescent strain of vibrio in the early 1990's. A strong ecological element.

An ecological disease that is a exacerbated by less than wise pond preparation practices?

Control is possible and is being done successfully in some areas. Not hopeless.

Some tools may help as well.

Don’t Believe in a Silver Bullet Cure