Managing Vibrio and EHP Infections in Shrimp Farming

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Overview

Concept of critical control points (CCPs)

Stress defined

Vibrio description

Properties (toxins) essential for virulence

EHP description

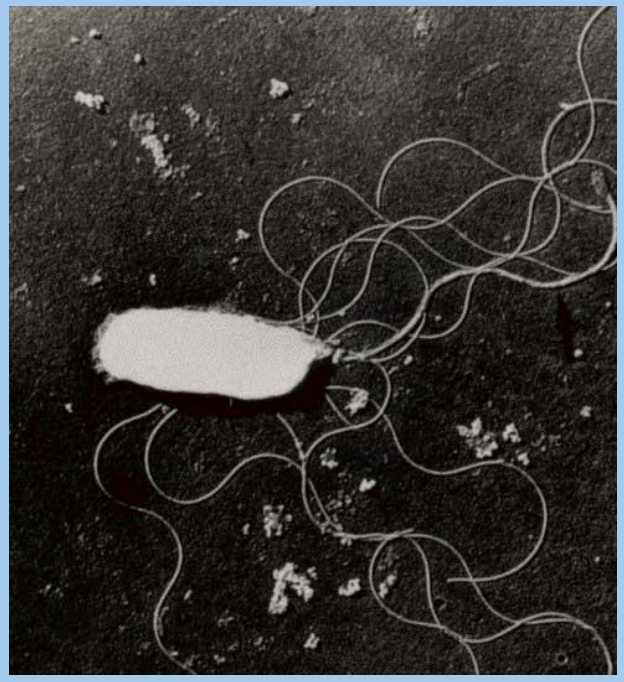
Factors that impact disease processes in shrimp

Mitigation and prevention

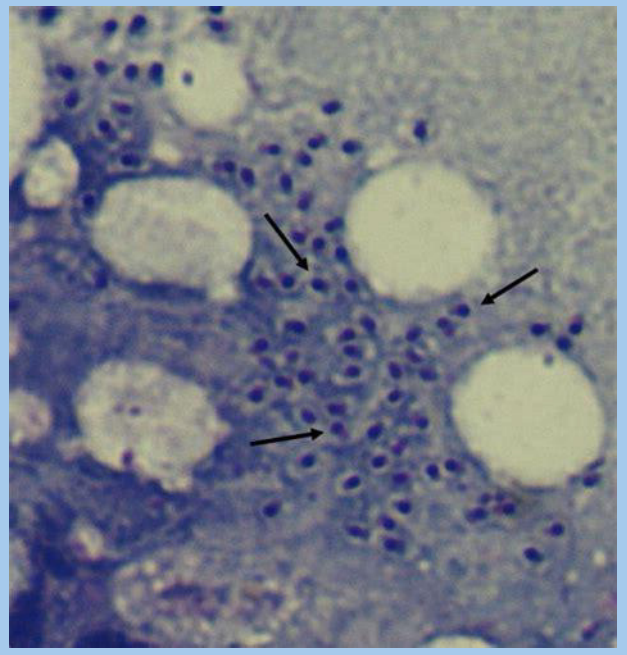
Proactive versus reactive (prevention versus treatment)

- Conclusions

Vibrio with flagella

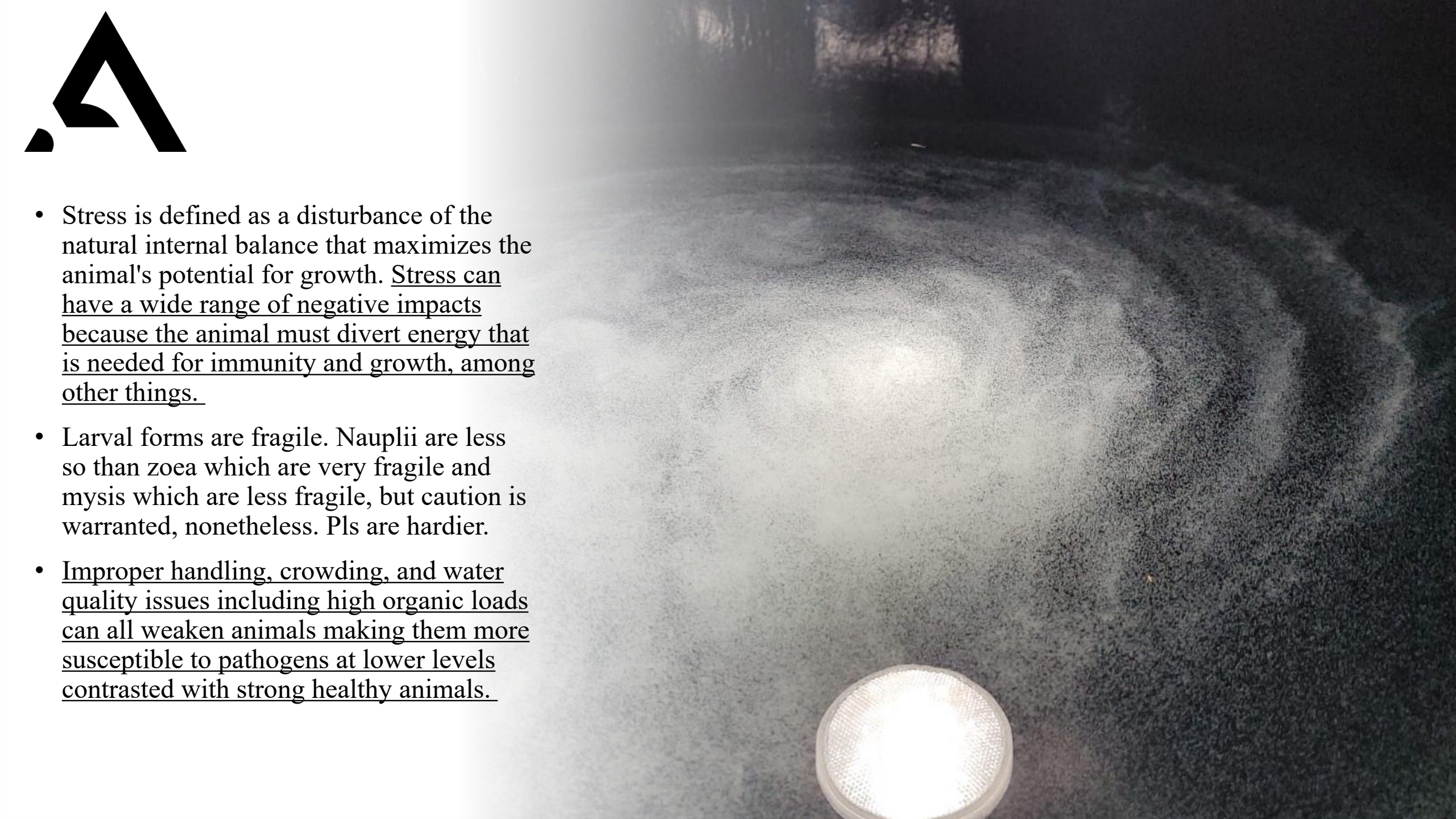


EHP spores

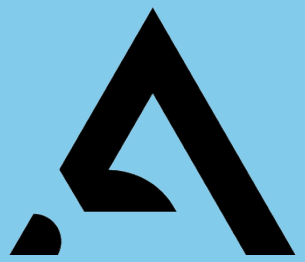


Critical Control Points (CCPs)

Area	Critical Control Point
Maturation:	Eggs and nauplii surface contaminated Elimination of carriers that are qPCR positive for possible horizontally transmitted pathogens. Live feeds sterilization and testing. Individual animal testing.
Hatchery:	PLs contaminated. Discard with EHP and EMS use probiotics at high levels throughout the cycle.
PL transport:	PLs stressed. Use high levels of Vitamin C.
Stocking:	PLs stressed. Inadequate acclimation time.
Pond preparation:	Elimination of potential pathogens and carriers. Probiotics and liming between cycles for EHP spores. Crab and bird barriers. Lined ponds.
Pond management:	Stress management, feed management, probiotics usage based on impacts not cookbook formulas
Proactive animal health management:	Identifying potential problems before they kill animals
Stress mitigation:	Stress management (aeration, diffusers, automatic feeders, reduced densities, probiotics)
Harvest:	



- Stress is defined as a disturbance of the natural internal balance that maximizes the animal's potential for growth. Stress can have a wide range of negative impacts because the animal must divert energy that is needed for immunity and growth, among other things.
- Larval forms are fragile. Nauplii are less so than zoea which are very fragile and mysis which are less fragile, but caution is warranted, nonetheless. Pls are hardier.
- Improper handling, crowding, and water quality issues including high organic loads can all weaken animals making them more susceptible to pathogens at lower levels contrasted with strong healthy animals.



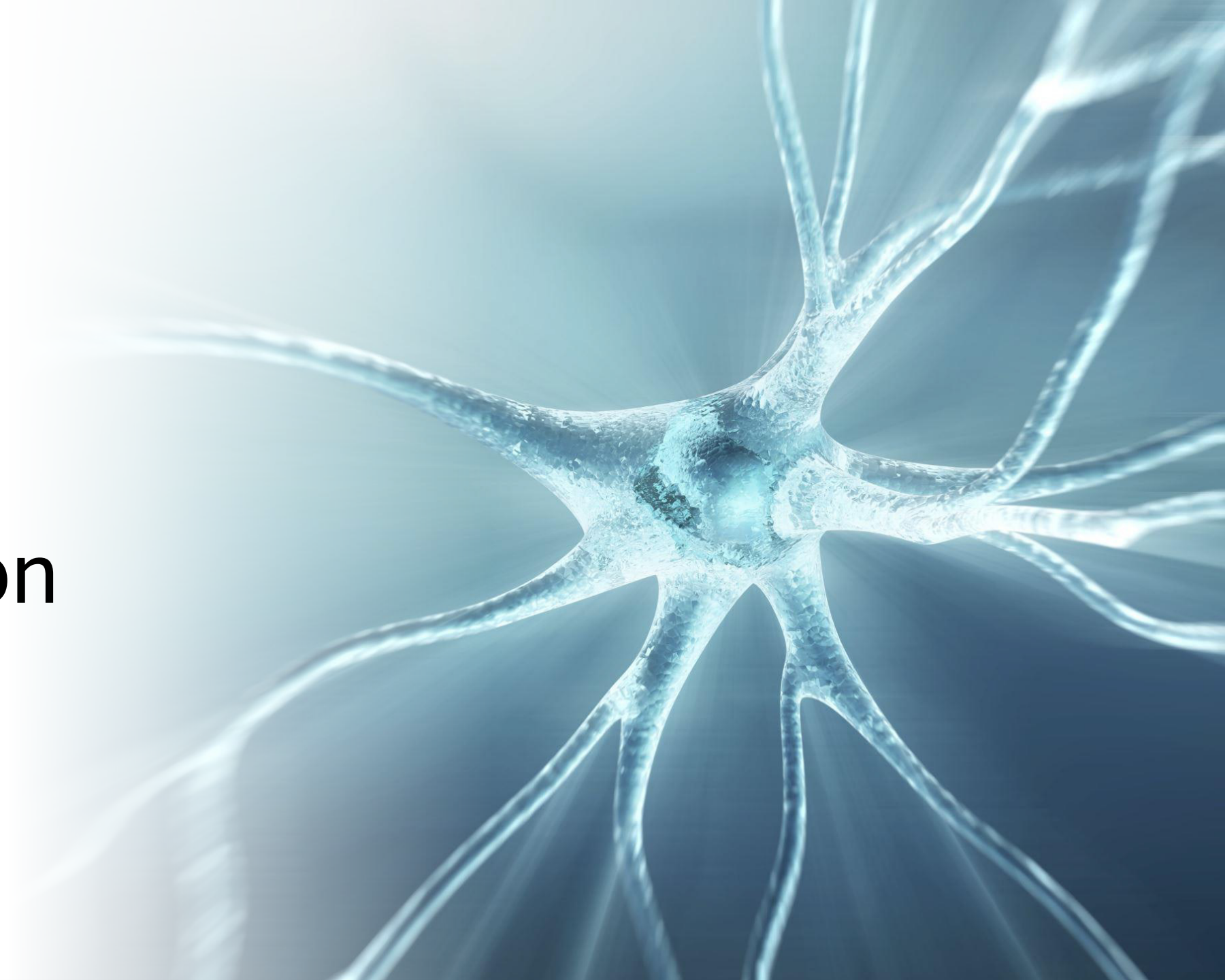
Stress contributes to susceptibility. This can be a little or a lot.

Environment (stress)

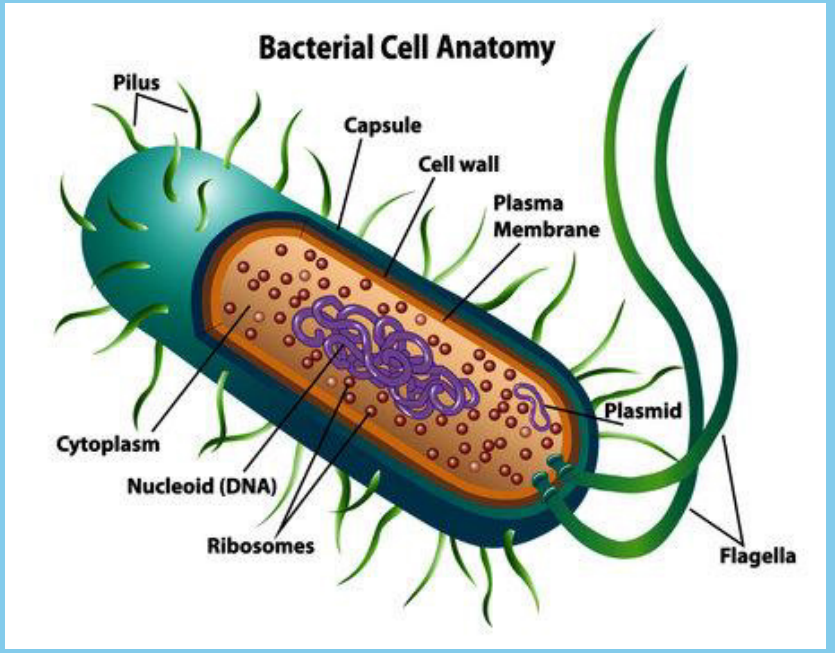
- Temperature-Too low or too high
- Salinity-Sudden changes in salinity.
- Nutrient loads-Excessive feed – sludge habitat for vibrio growth and EHP spores deposition
- DO levels-Too low
- Presence of H_2S/CH_4


PREVENTING DISEASE
IS BETTER THAN TREATING IT.
Focus on biosecurity to protect your shrimp farm.

Pathogen description



Vibrio description



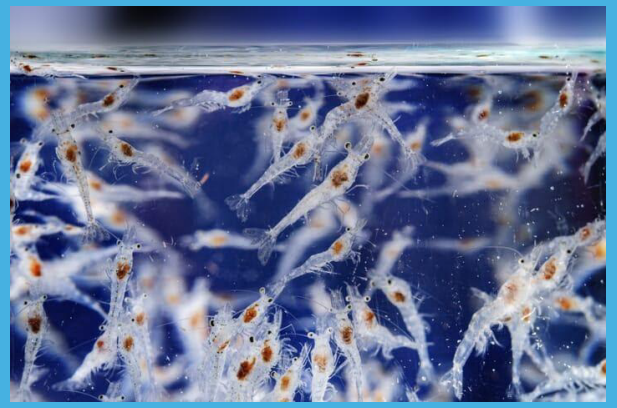
- Copiotrophs
  - Found in nutrient rich environments -organic matter that accumulates from shrimp and fish farming.
  - **High sediment loads = high levels of vibrios.**
  - Rapid growth.
  - Prefer simple substrates like amino acids, sugars and organic acids as nutrients
  - Enzymatically versatile; adapt readily to changing nutrients.
  - More than 178 vibrio species identified to date.
  - Some strains cause acute disease in fish and shrimp. Most are opportunistic. Very few are obligate.

A given species may have many strains some of which are obligate (produce disease in healthy animals), others opportunistic (require coinfection, stress or something that weakens host) and others benign.

Vibriosis can be caused by multiple species

Only a few are obligate in the sense that their presence in low numbers usually leads to acute disease. Invariably toxin related.

- All life stages can be affected.
  - Zoeae hatchery.
  - Hatchery post larval shrimp
  - Nursery
  - Grow out
  - Broodstock



Creating an environment where they cannot grow to the levels required to cause disease and minimizing stress helps the animal fight it off at low levels.

Species impacting 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*

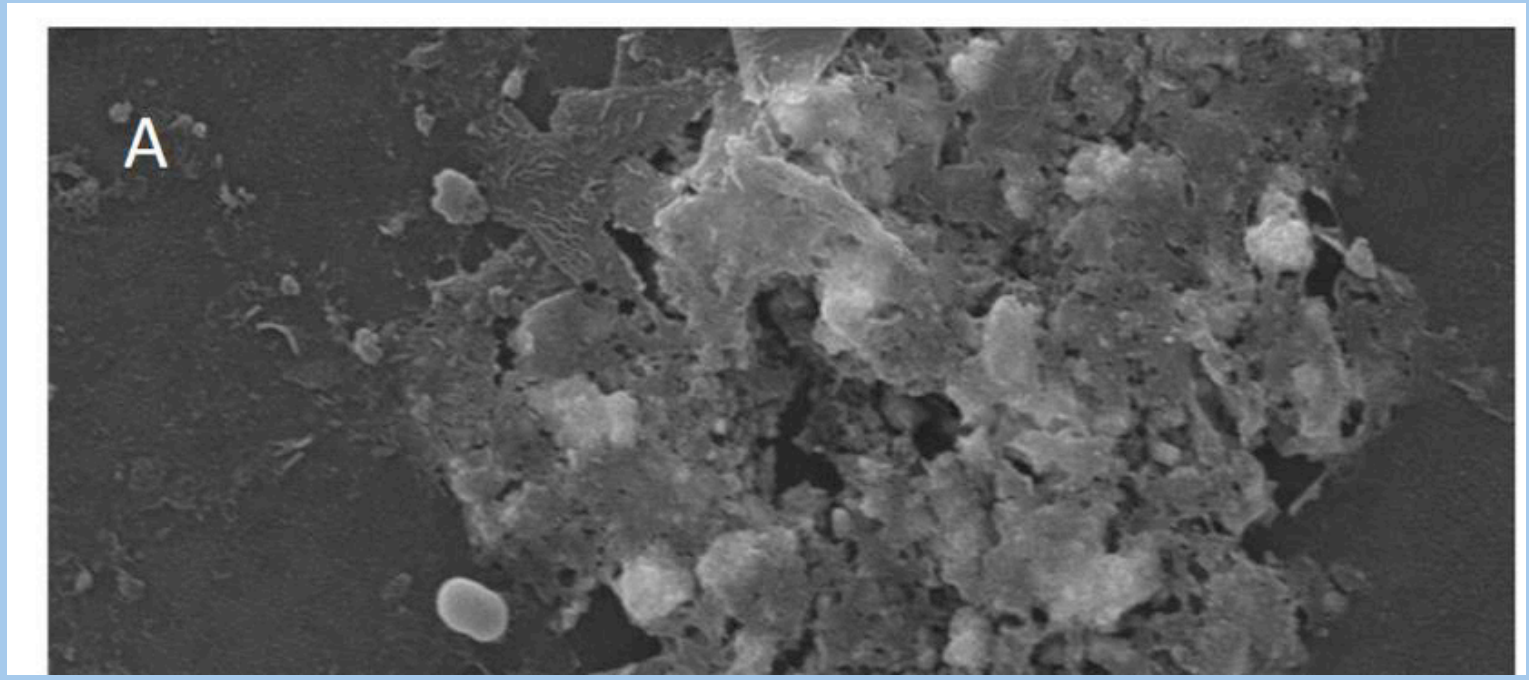
Vibrio pelagicus
Vibrio orientalis
Vibrio ordalii
Vibrio mediterrani
Vibrio logei

The characteristic lesions are black because of an immune reaction that results in melanin production to kill bacteria, viruses, etc.

Vibrio habitats

Vibrios are important in the recycling of chitin via chitinase.
Form biofilms (essential for virulence) due to quorum sensing
Some species form symbiotic relationships bioluminescent (glow in the dark)
Found on algae, in and on crustaceans and fish
Critical role of toxins in disease

Biofilm appearance



Toxins

Note that controlling organic matter loads is essential if one wants to reduce vibrio loads.

Toxin / factor	Typical species	What it does in shrimp	Fast diagnostics	First moves
PirAB^Vp (AHPND)	*Vp*, *V. campbellii*, *V. owensii* (pVA1/pVH)	Pore-forming; HP epithelial necrosis → acute mortality	**PCR pirA/pirB** (AP3 or similar); emerging **PirAB LFIAs**	Isolate tanks/ponds, **Bacillus boost**, C:N steering, sanitize lines/biofilms, cull worst tanks early (PubMed Central)
Hemolysins (TDH/TRH/VHH)	*Vp* (TDH/TRH), *V. harveyi* (VHH), *V. alginolyticus*	Cytolysis, barrier damage; classic larval/grow-out vibriosis	Hemolysin gene screens (**vhh/hly**, **tdh/trh**); phenotypic hemolysis	**Lower organics, biofloc/C:N** to out-compete vibrios; **probiotics**; manage salinity swings (SpringerLink)
Proteases (metallo/serine)	*V. harveyi* clade, *V. alginolyticus*	Tissue degradation, spread & nutrient access	Virulence gene panels; enzyme assays (lab)	Reduce stress & injuries; **probiotics**; floc solids control; siphon sludge (SpringerLink)
T3SS effectors (e.g., VopZ)	*V. parahaemolyticus*	Hijack cytoskeleton/signaling; aid colonization	Species ID + T3SS markers (lab)	Stocking density discipline; limit shocks; **pair with Bacillus and good C:N management** (PubMed Central)

CFU correlation with disease vibrios

Pond compartment	Normal range (healthy pond)	Outbreak / disease	Notes
Water column	10^2–10^4 CFU/mL	>10^5–10^6 CFU/mL	Sudden blooms often precede AHPND, luminous vibriosis, or septicemic outbreaks.
Sediment (sludge)	10^2–10^5 CFU/g (dry weight)	>10^6–10^7 CFU/g	**Sediment acts as reservoir**; disturbed pond bottoms can reseed water column.
Shrimp hepatopancreas & gut	10^3–10^5 CFU/g tissue	>10^6–10^8 CFU/g	Pathogenic vibrios often dominate hepatopancreas during AHPND or SHPN.
Larval tank water (hatcheries)	10^3–10^5 CFU/mL	>10^6 CFU/mL	Hatcheries are especially sensitive—luminescent vibrios may dominate quickly.

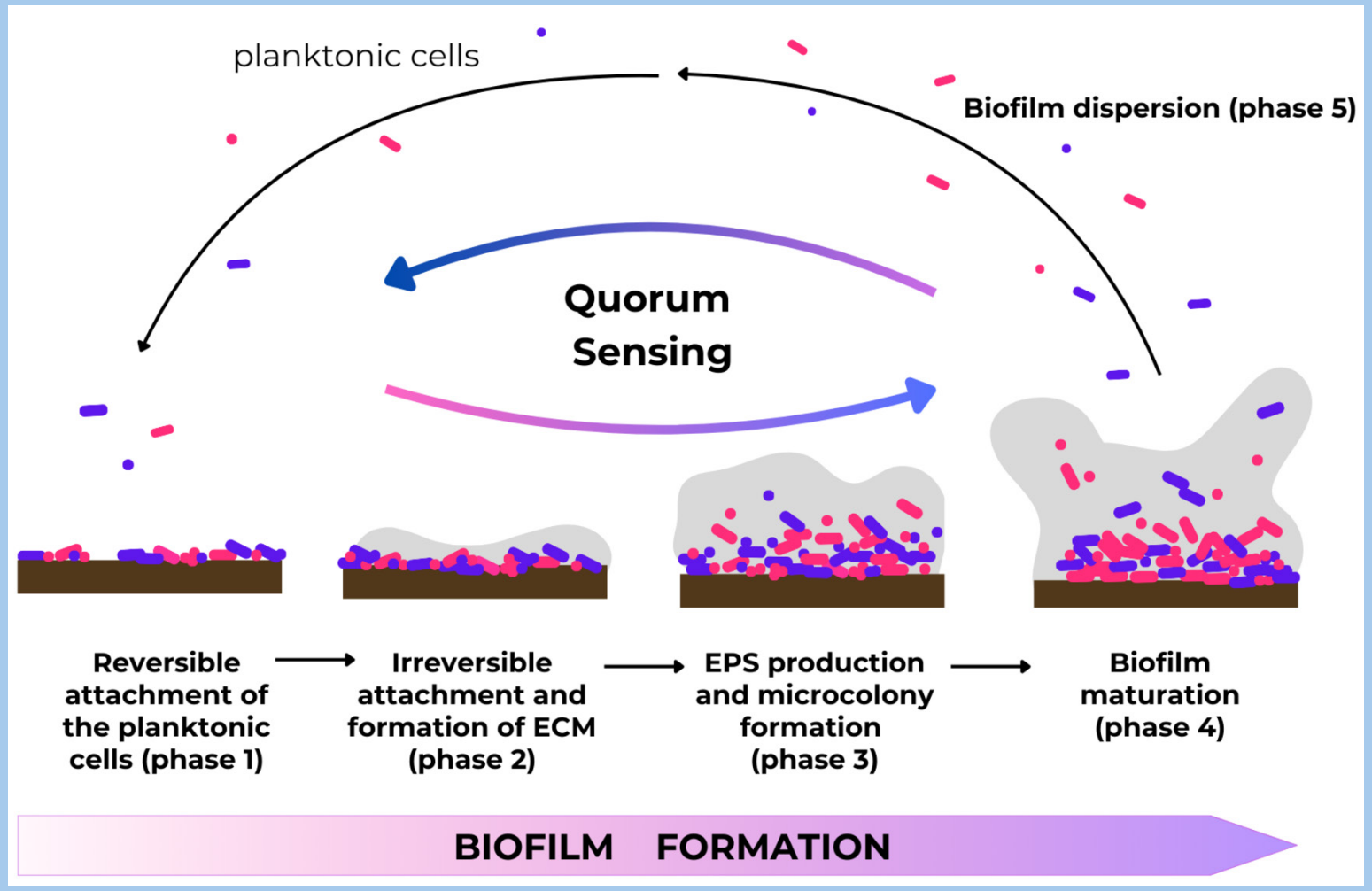
Quorum sensing

Communication between cells via the use of specific types of molecules (autoinducers) that bind to receptors and result in gene translation

Allows bacteria to work together as a cohesive group.

Play critical roles in microbial ecology and how disease is produced.

<u>Critical first stage in biofilm formation.</u>

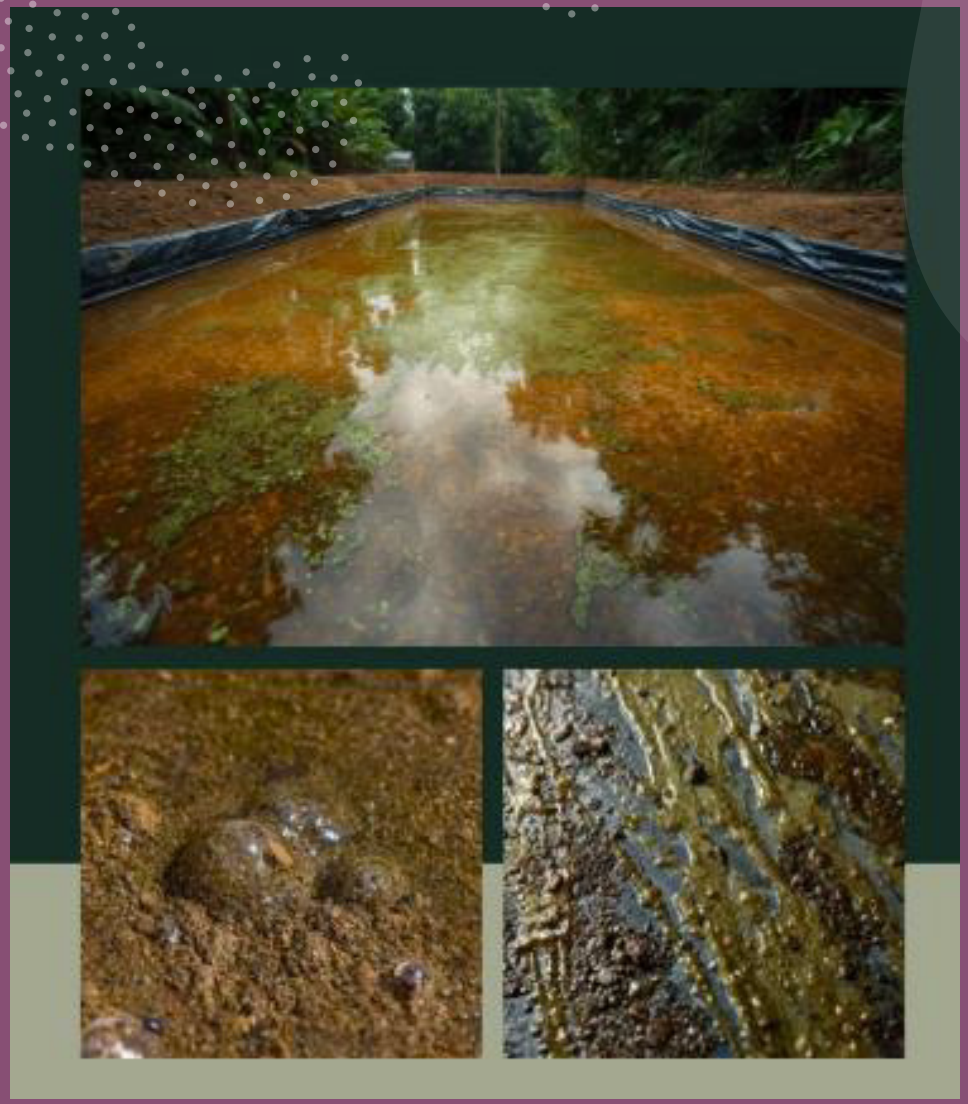


This is a critical control point (CCP). Blocking quorum sensing can prevent biofilm formation and reduce disease. This is being evaluated.

ECM-extracellular matrix
EPS-extracellular polymer

Biofilm

- Critical for pathogenesis
- Affects toxin production and protects the bacteria from competitive inhibition.
- Many bacteria do this, and the biofilm can often be an assemblage of different species/strains living together in “harmony”.
- The biofilm can be eliminated although typically it is not there by itself, and elimination of all biofilms can result in increased risks.

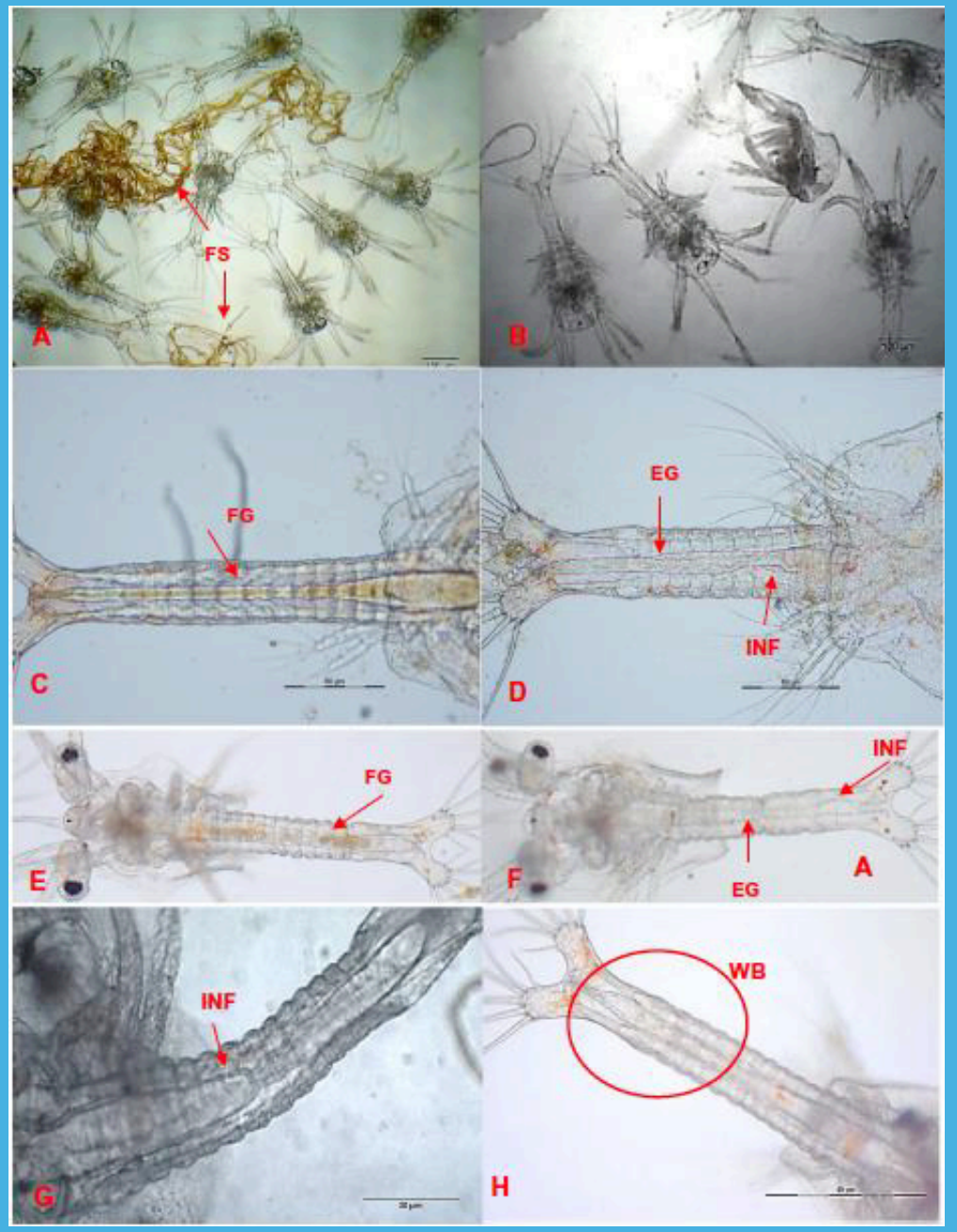


Vibriosis-infection with vibrios

Zoea syndrome

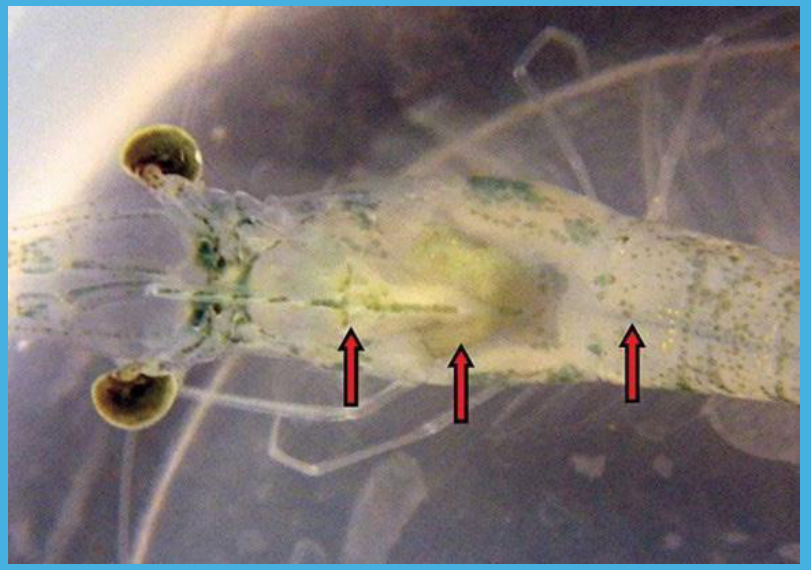
Zoea-2 syndrome of *P. vannamei* in shrimp hatcheries

- Light microscopic observations of normal and Zoea-2 syndrome affected larvae.
  - A — Normal zoea with full gut and fecal strands;
  - B — affected zoea with empty gut and absence of fecal strand;
  - C & E — normal zoea with full gut with no abnormalities, D, F, G —infected zoea showing empty gut with inflammation like disruptions in intestinal epithelium; H — infected zoea showing sloughed of epithelial cells as white balls or white sphere like structures (circle). FS — fecal stands; FG — full gut; EG — empty gut; INF — Inflammation, WB — White ball or sphere like structures.



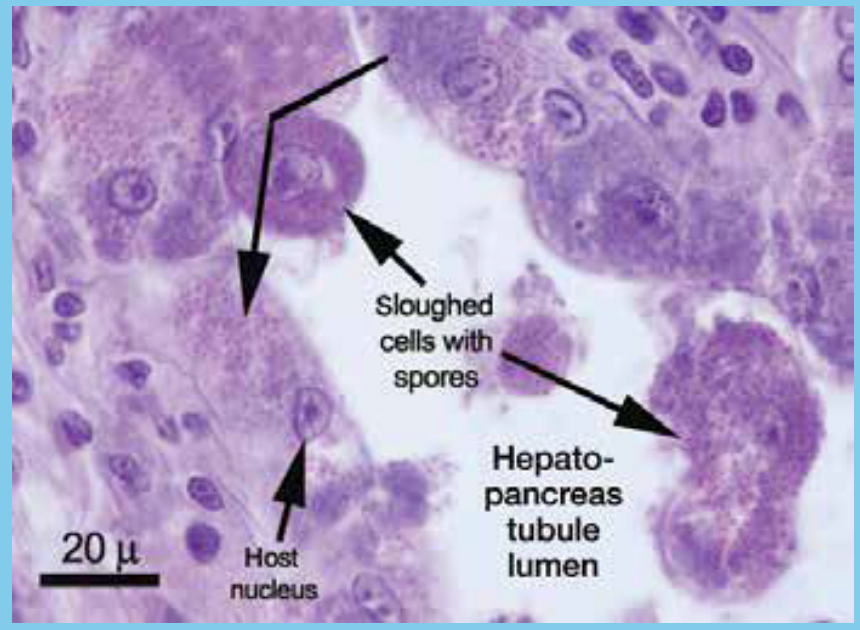
Melanin deposition and reddish coloration are pathognomonic

Early mortality syndrome EMS and AHPNS/D (acute hepatopancreatic necrosis syndrome or disease)



EHP

A microsporidian closely related to fungi. First reported in Thailand 2004 in *P. monodon*; spread globally. Can occur from 3 to > 30 ppt salinity.

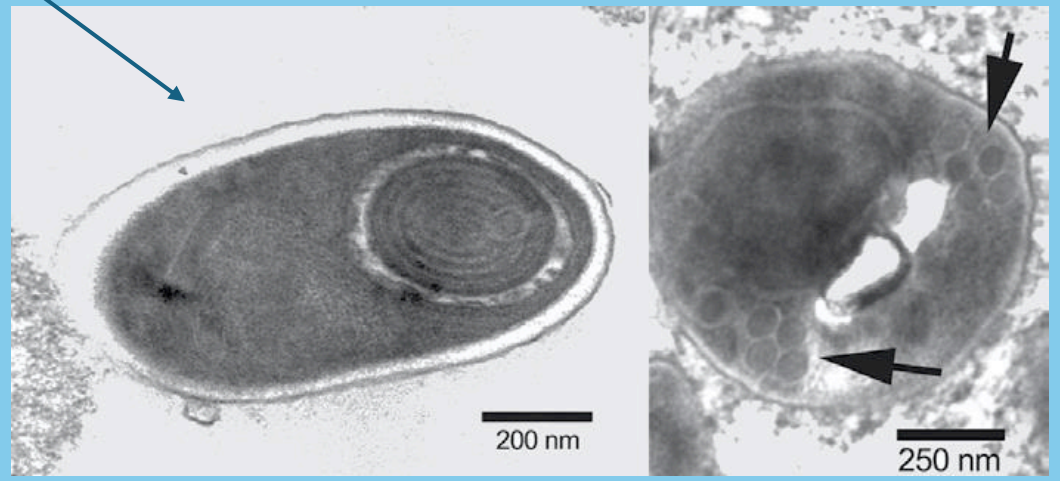


Forms spores-these are the infectious component

Vectors: Found in the water, sediment, marine crabs, feed (natural), insects (dragonfly), live fresh feeds; polychaetes, mollusks, artemia, squid, etc.

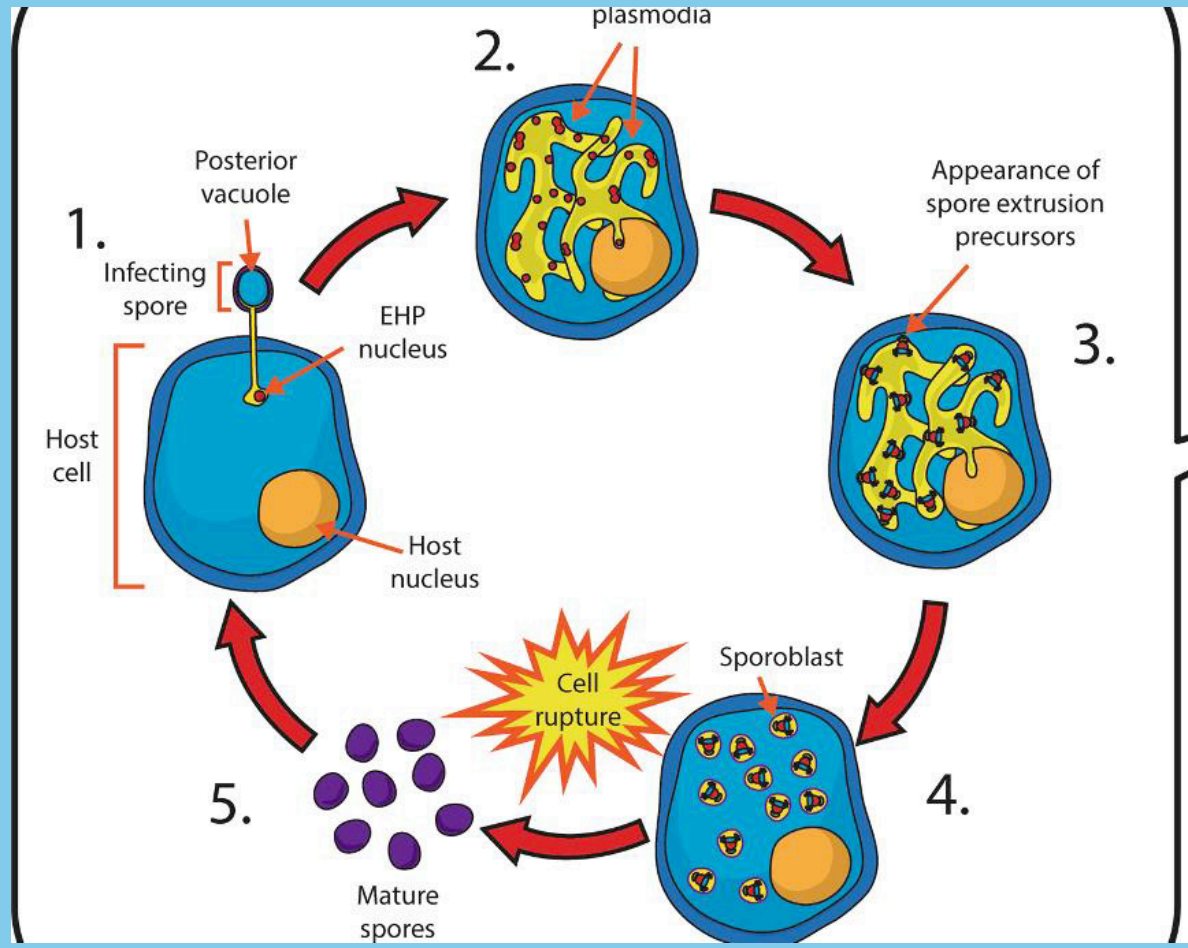
Primary source is infected animals shedding spores from feces and gut mucus and cannibalism of dead and dying animals.

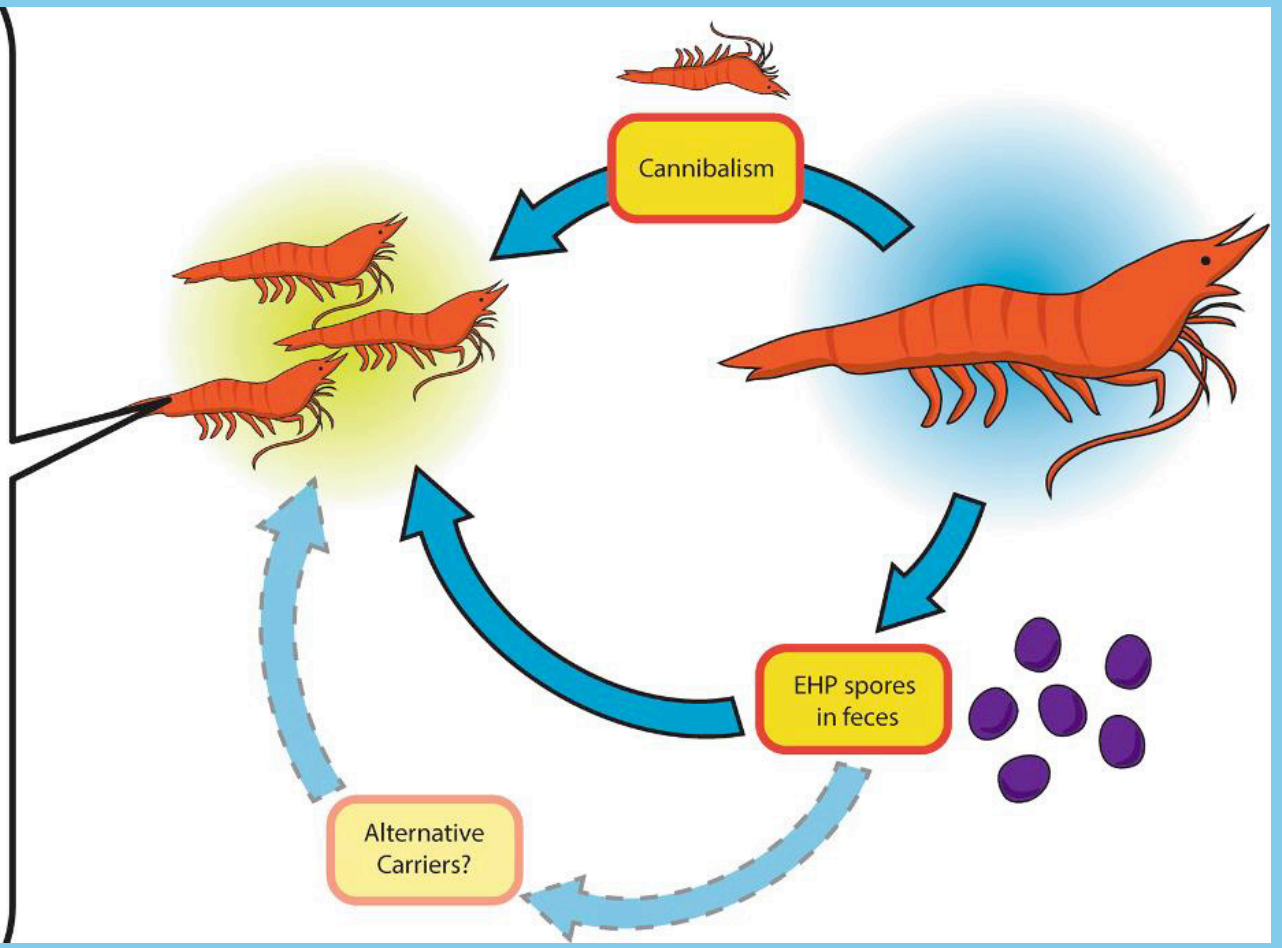
Spore containing sediments not adequately treated during and between cycles ensuring constant problems. Liming and removal of substrates when bioremediation (probiotic) cannot reduce the levels sufficiently.



Typical spores: photos courtesy Tim Flegal

EHP *Ecytonucleospora (formerly Enterocytozoon) hepatopenaei* Life cycle



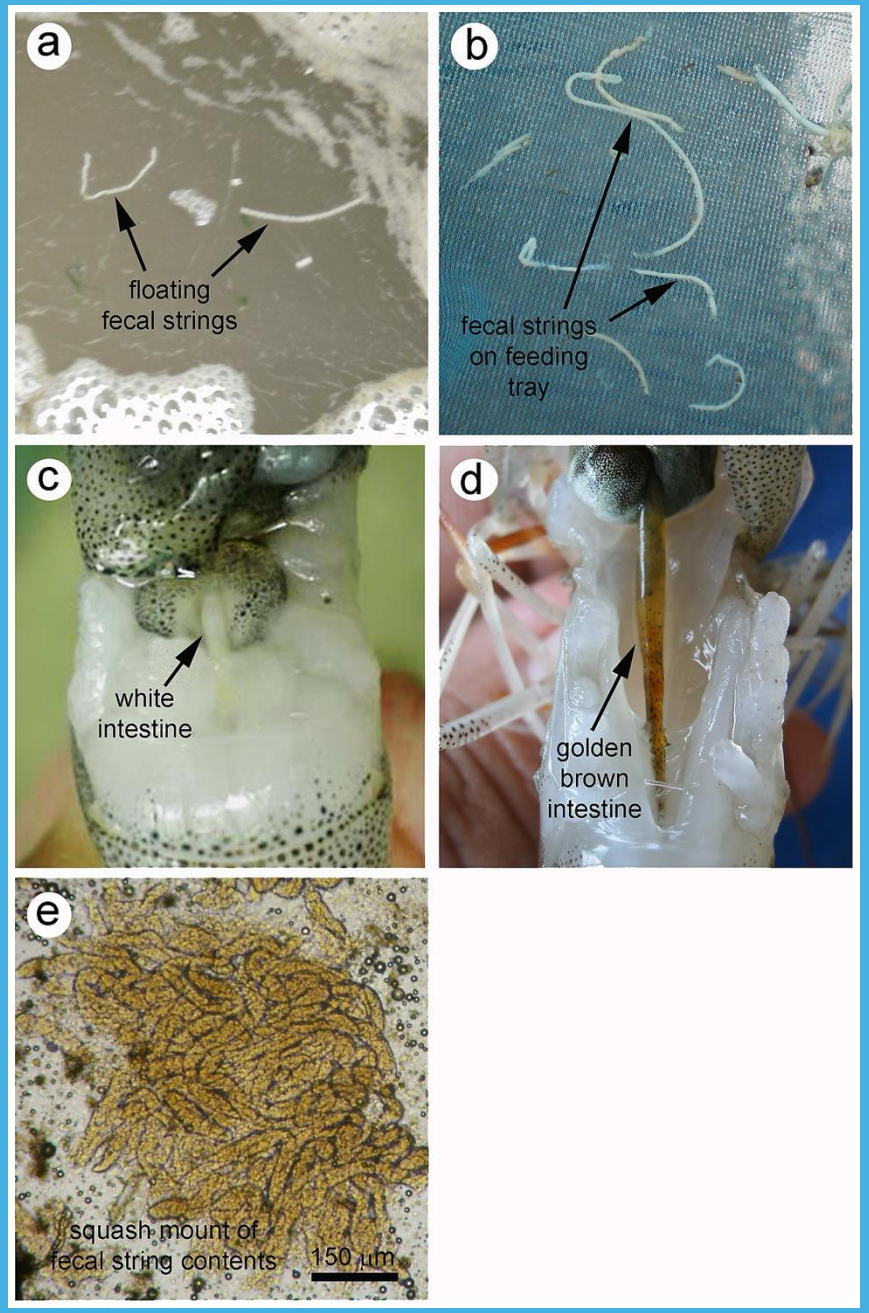


The shrimp microsporidian Enterocytozoon hepatopenaei (EHP): Biology, pathology, diagnostics and control. Chaijarasphong, T. et al. , J. Invertebrate Pathology 186 (2021). https://doi.org/10.1016/j.jip.2020.107458

EHP results in extreme size variation

- Damage to digestive tissues (HP) results in slow growth, size disparity and increased susceptibility to vibrios (AHPNS).
- Empty guts and runting in heavily infected populations.
- Not typically fatal by itself

White Feces Syndrome



Data strongly supports the idea that this is due to a coinfection of EHP and vibrio species including the etiologic agent of EMS/AHPNS (and other toxin containing bacteria).

Disease prevention and management strategies

Proactive approaches

Prevention (proactive) does not mean your animals cannot get ill.

- Ideally, it reduces the loads that are present from sources that can be controlled.
- This does not necessarily impact their susceptibility.
- Stress weakens animals.
- Vectors and other sources of the pathogens pose risks.
- Potent pathogens can cause disease at very low levels (LD50 is small)
- CCPs. Starts with broodstock.
- Biosecurity at all phases including ensuring proper treatment of wastewater discharge

“An ounce of prevention is worth a pound of cure”

Use crab fences and bird netting to reduce presence of birds/crabs and other vectors.

Use of reservoirs that are source of incoming water and are properly treated to ensure that that they are not sources of infectious material.

Ensure proper use of disinfectants on equipment and on personnel (boots, lab coats, etc.)

An example of no biosecurity ensuring that PLs are stressed and that pathogens are readily moved between animals.

Consider polyculture with tilapia or other fish to reduce accumulated wastes (among other potential benefits)

- Use SPF broodstock that are from NBCs. Do not rely on population testing. Test individual adults with and without stress.
- Critical to keep it out of hatcheries. Even low levels (AHPNS) can result in mortality post stocking in ponds.
- Test PLs using qPCR and plating on TCBS or CV media repeatedly during cycle with stress.

Change the production paradigm and deeper ponds, liners, sumps (toilets), increased aeration (density dependent -70 to 100 hp/ha) and reservoir designs.

Work with neighbors to ensure that their ponds post harvest (drying and removal of remaining sediments) are properly treated.
Bioremediation during production and if needed physical removal of sludge.
Sludge collection in dedicated treatment ponds. (5 to 7% of pond surface area)

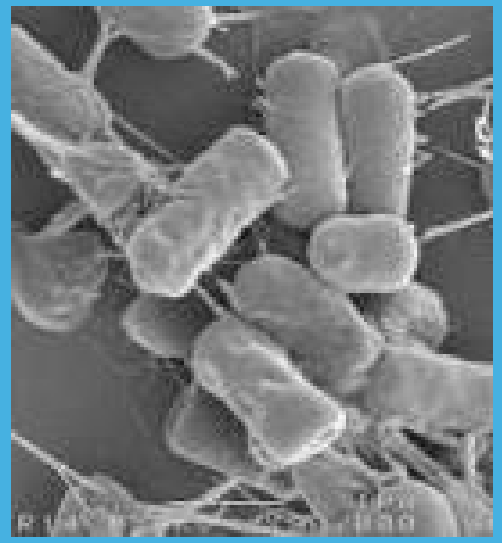
Automatic feeders

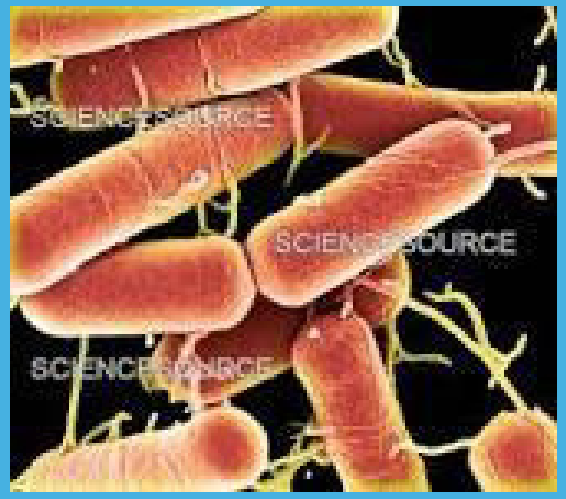
- Monitor shrimp health by taking samples at least 2X a week. Focus on apparently ill animals. Look for cramping do wet mounts of the HPs. Use TCBS/CV to culture. QPCR?
- Avoid over feeding. Use of (on-demand) automatic feeders.

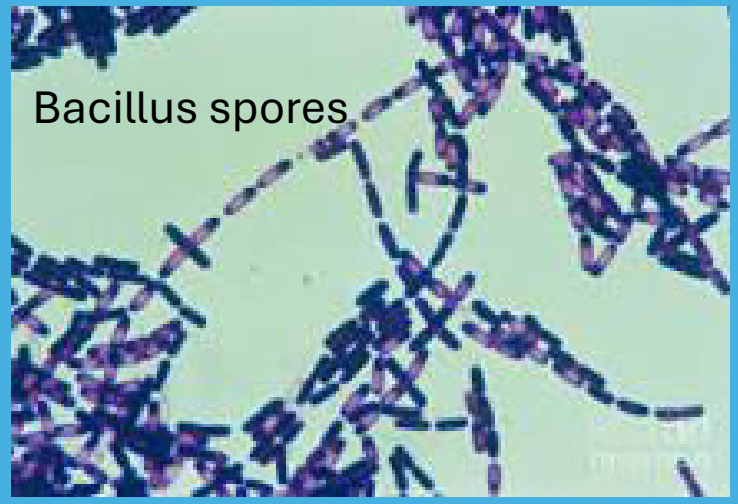
Feed with tableted probiotics

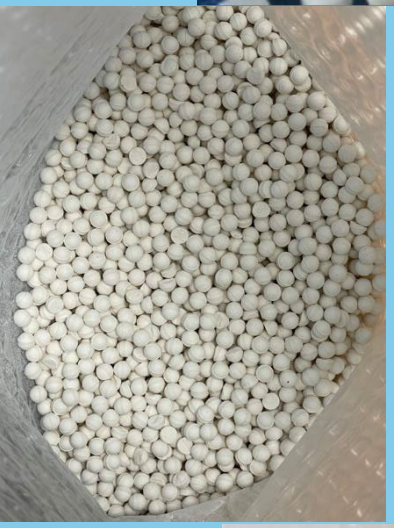
Reduction of accumulated organic matter

- Probiotics such as specifically selected strains of Bacillus species can mitigate the impacts by reducing organic matter that accumulates, robbing vibrios of nutrients and EHP spores of substrate from which to infect foraging shrimp.
- High water exchange rates.





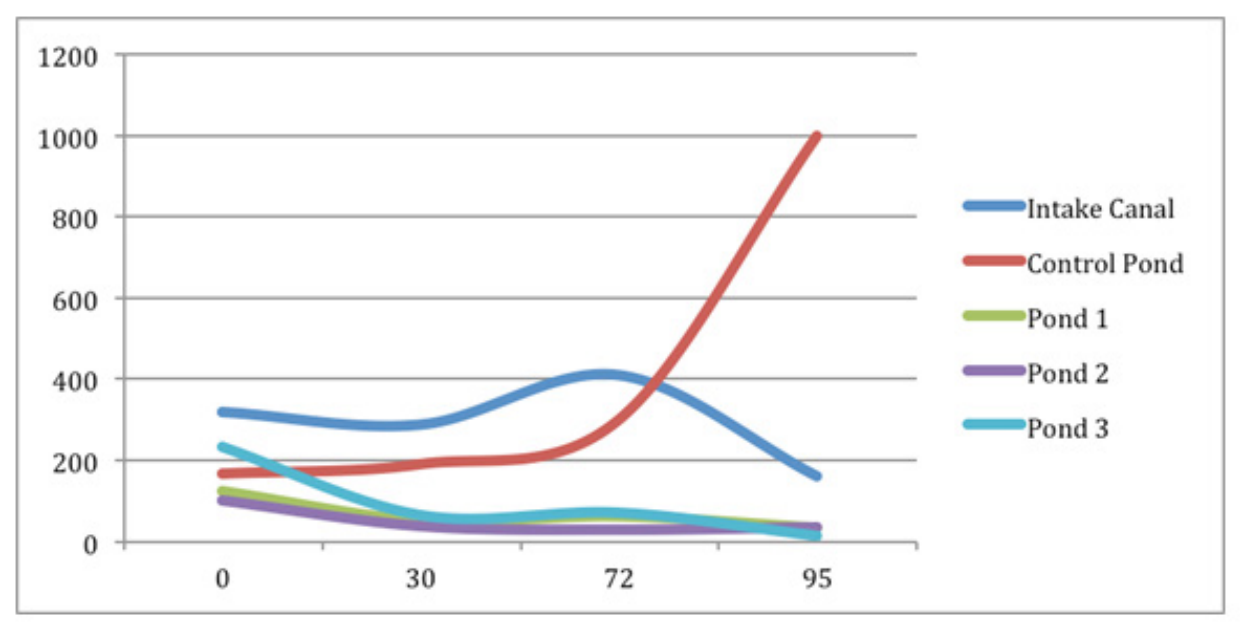


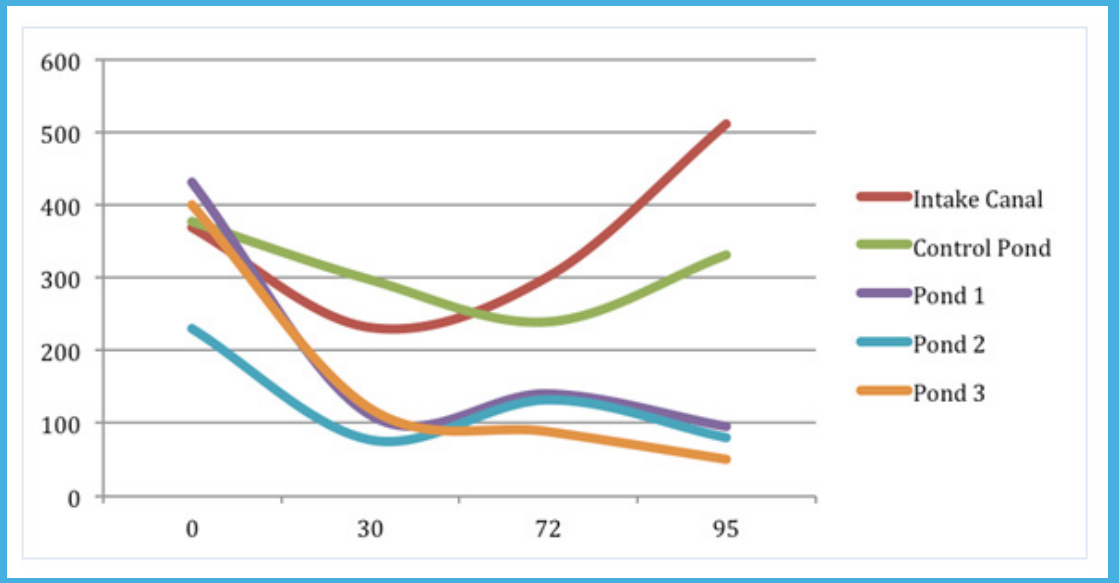


Proper use of probiotics

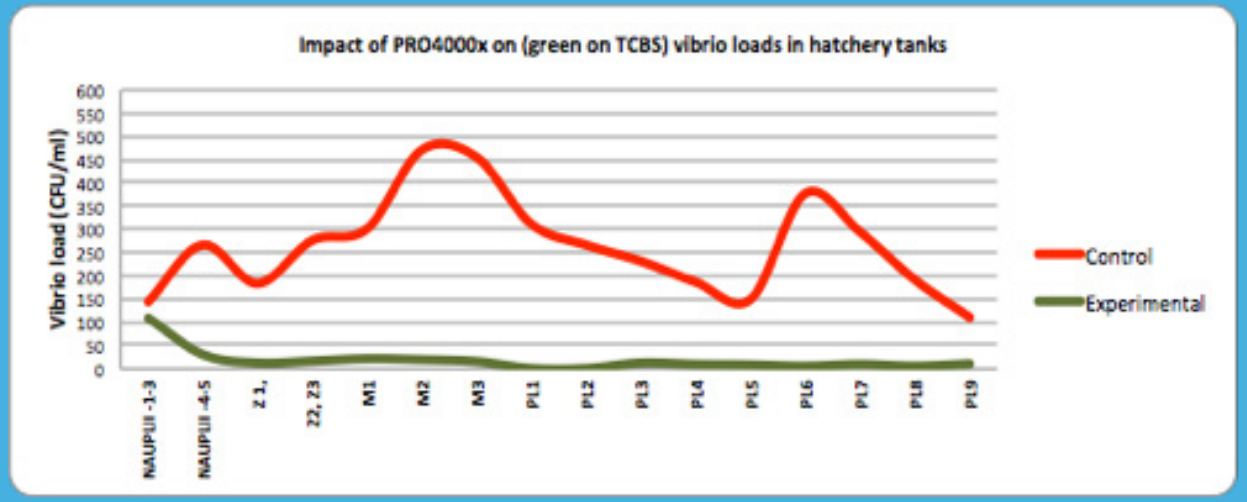
- Don’t use multiple products at the same time. They can inhibit each other, and many do. More is not better.
- Focus on Bacillus based products because of ease of use (spores), enzymatic versatility of strains and resistance to contamination.
- Goal is to reduce organic loads and by doing so reduce nutrients for pathogenic vibrios and substrate for EHP spores.
- Where, when, how much and what strains are in them determine efficacy.
- Let the buyer beware.

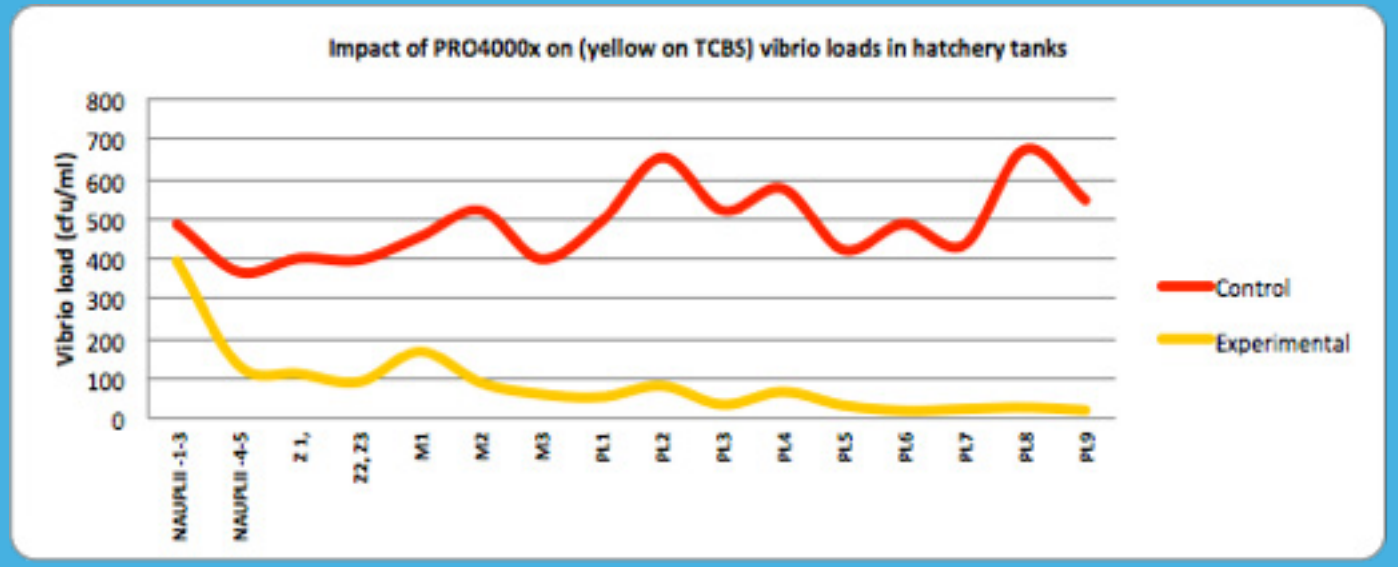
India Pro4000X treated ponds





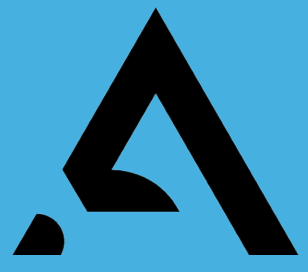
India Pro4000X treated hatchery





Before and after application of probiotic to pond bottoms -Ecuador

Data strongly supports controlling overall vibrio levels by reducing nutrients for them and ensuring that they must compete for growth limiting nutrients.



Reactive–treatment of problems once they occur

- Antibiotic usage is not acceptable because antibiotics have been widely abused.
- Residues and resistance from improper usage.
- Only work against bacteria.
- Requires isolation of culprit and confirmation of susceptibility.
- Subject to regulatory oversight-complex registration processes and strict enforcement of residues

Prevention by exclusion

- **Exclusion.** Keep pathogens out of broodstock. Mass spawning can make it challenging to prevent this unless you are working in a clean environment with SPF for EHP animals. Minimize the loads in every way possible.
- The spores are defecated and infect other animals. The use of nucleus breeding facilities, thorough regular screening using qPCR (EHP can not be cultured on agar) and keeping tanks/ponds free of organic material through Bacillus based probiotics and frequent testing are all helpful with achieving the goal of keeping them out of your Pls.
- **Note that the primary concern with the broodstock being infected is that this poses a serious biosecurity risk. There does not appear direct vertical transmission--infected broodstock do not infect nauplii through ovarian tissues.**
- **The spores cannot infect the larval stages although they can carry them.** Early PLs are less affected as the spores target hepatic tissue. As the tissues grow this increases tissue for the spores to infect.

Other tools for reactive approaches

Phage-bacterial viruses-Not typically able to kill all the bacteria and when they kill strains containing toxins these are released. Bacteria become resistant requiring constant isolation of new phage strains. Limited impact on internal loads of target pathogens.

Other tools for reactive approaches

Many compounds, drugs, chemicals, plant extracts, algal extracts, etc. have been tested for their ability to inhibit bacteria (vibrios) and EHP.

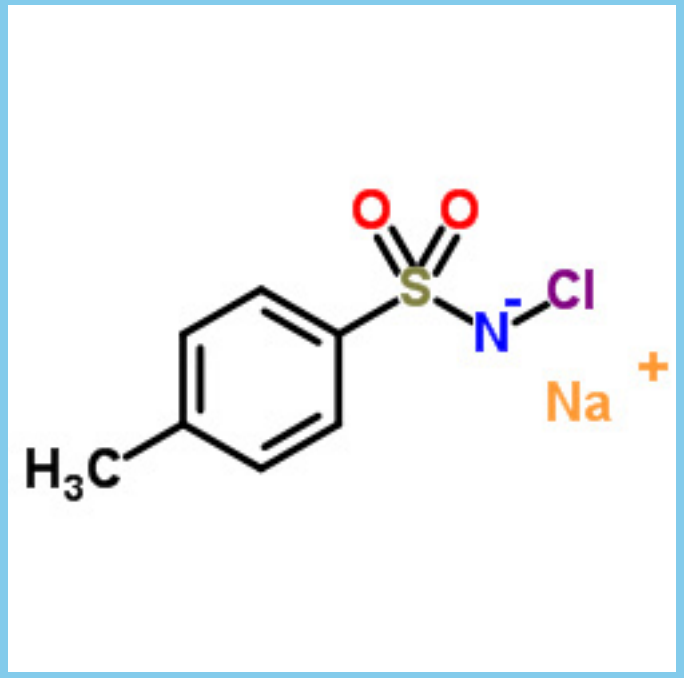
Some lab studies see positive results.

Cannot be readily converted to the field, however.

Fucoidan-sulfated polysaccharide derived from algae. Some reports suggesting that heparin, a sulfated polysaccharide used as an anti-coagulant in humans binds to EHP spores preventing them from infecting cells.

Other tools for reactive approaches

- Some disinfectants such as Tosylchloramide sodium can be used in hatcheries to stop acute outbreaks. Kill pathogens on contact. Do not impact pathogens in infected animals.
- Chlorine based with low toxicity.



Conclusions

- Best overall approach is to ensure that CCPs are properly addressed to ensure that the overall loads of potential pathogens are reduced or eliminated where possible.
- Ensure that all broodstock are SPF for all toxin causing strains of Vibrio parahaemolyticus and other species and EHP spores.
- Vibriosis (AHPNS) and EHP can be managed via proactive strategies.
- Biosecurity and reducing/mitigating stress are essential.
- Using probiotics to reduce organic loads will lower both vibrio and EHP spore loads. Not all probiotics work the same. Many products in the marketplace with ranges of efficacy.

Thank you.