

¿Biorremediación o Probióticos?





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Today's talk

- Introduction
- Definitions
- Different product types
- Representative data demonstrating effectiveness/highlights
- What is real and what is not? Challenges.
- Conclusions

Introduction (1)

The widespread use and abuse of antibiotics has resulted in their use being banned outright in many countries (not necessarily the country of origin)

- Important to find possible substitutes
- “Probiotics” are being proposed as one possible tool in terrestrial agriculture and aquaculture
- Unfortunately while probiotics for shrimp are an interesting idea, the science lags far behind the speculation that is the result of poorly designed experiments. Science is not at all clear as to whether probiotics really exist based on agreed upon definitions.
- After today's talk you will agree with me that the actual mechanisms are bioremediation and bioaugmentation.

Introduction (2)

Many different products commercially available. Literally thousands of them.

- Many organisms are used with varying degrees of effectiveness. The best are those using Bacillus spores. Why?
- The mechanism of action appears to be through the degradation of organic matter and metabolites like $\text{NH}_3/4^+$ and competition for nutrients.
- Although it is certain that the shrimp consume vegetative cells of the Bacillus and other bacteria and that these are not harmful, this does not mean that they colonize the guts or that there would be a health benefit if they did. **The microbiome in the gut is in a constant state of flux.**

Definitions

- Microbiome
- Probiotic
- Bioremediation
- Bioaugmentation

Microbiome (1)

Definition is evolving (play on words intended)

Human Genome Project

The microbiome is defined as the collective genomes of the microbes (composed of bacteria, bacteriophage, fungi, protozoa and viruses) that live inside and on the human body.

Widely used definition

A community of microorganisms (such as bacteria, fungi, and viruses) that inhabit a particular environment and especially the collection of microorganisms living in or on the human body.



Ignore human bias in the definition and the term can be used regarding any environment, any animal, plant, etc. Microbiomes are everywhere.

Microbiome (2)

A very exciting area of research

Role of the microbiome in animal health, stress resistance, growth, etc. is the subject a great deal of research and we are in the early stages of understanding how the microbiome impacts the host.

A **FACT** is that the microbiome is constantly evolving in response to changes in the conditions that engender its formation. It will not be static but will be constantly changing.

Caveat emptor is Latin for "let the buyer beware".

Shrimp farming is largely a non-science based agricultural practice. Many who claim to be using and or selling tools of science are not. Unfortunately, many of the snake oil salesmen that permeate the supply chain in aquaculture are already making outrageous claims about how their products impact the microbiome and the astonishing impacts one sees on production.

Expect more of this. There are no silver/magic bullets.

Probiotic

Definition is that agreed upon by FAO/UN



1. **Living** microorganisms-typically bacteria, although can be yeast (fungi) and possibly others (bacterial viruses).
2. Administered **orally**, i.e. by ingestion (not through passive up take).
3. **Colonize** the gut (they must do this to be considered a probiotic). Impacting the microbiome.
4. Have a **beneficial impact** on the consuming **animals' health**. Vague at best. Are we talking about disease resistance, tolerance ? Typically meant to increase disease resistance.

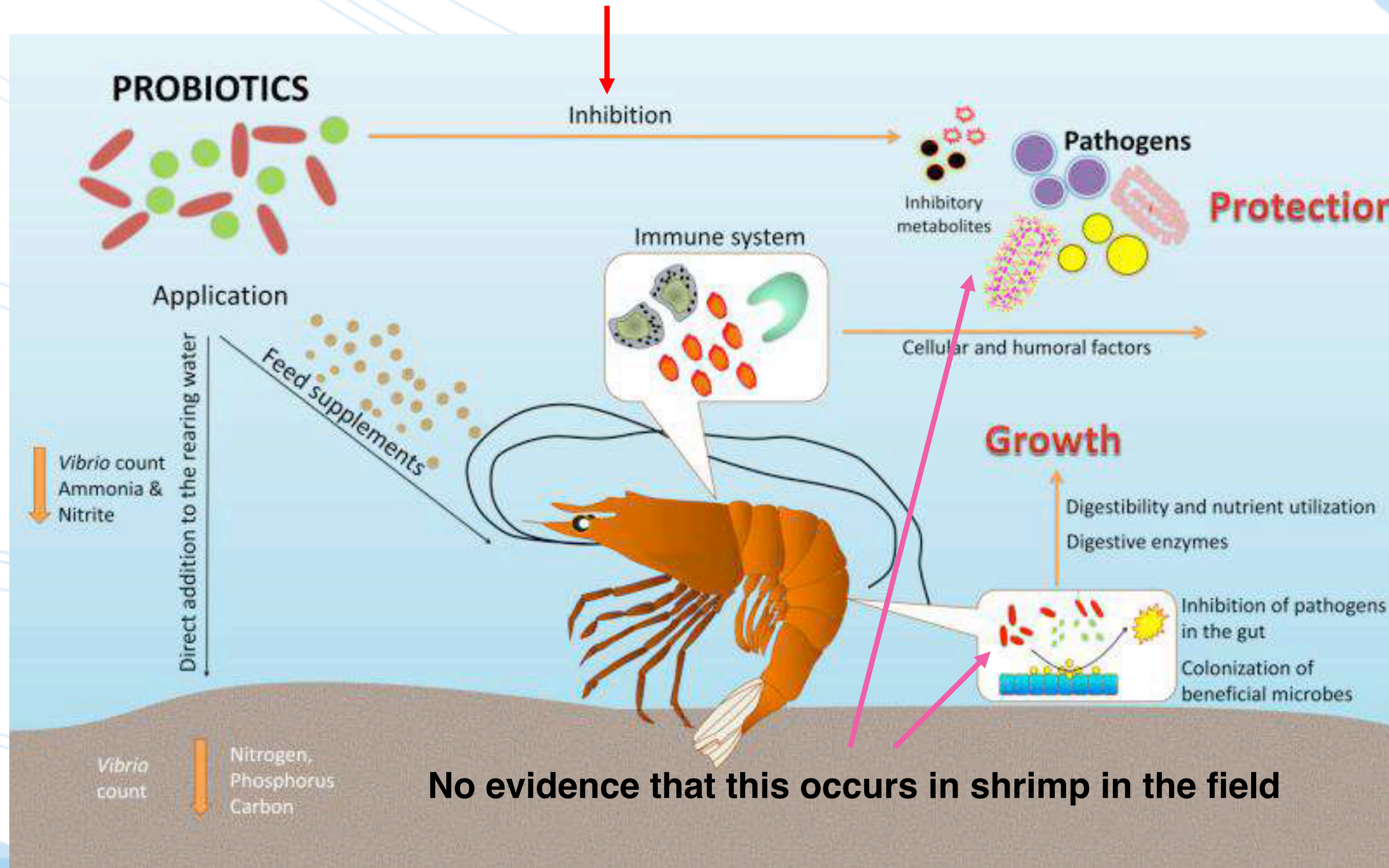
These claims could force registration with the FDA as claims are made about impacting a disease process.

Reality is that these things do not exist. For shrimp it may not even be possible. Very difficult to prove for human beings.

Reason being the plasticity of the microbiome. Correlation does not mean causation. Lab results do not translate to field results.

We know that what colonizes the intestinal tract of shrimp is what is in the environment.

No evidence that this occurs in shrimp in the field –more than likely indirect competitive inhibition (bacteriocins?)



Under most field conditions of use the mode of action through bioremediation and bioaugmentation

Bioremediation

The treatment of pollutants or waste (as in an oil spill, contaminated groundwater or an industrial process) via microorganisms (such as bacteria) that break down the undesirable substances.

This is what **ALL** products that are being marketed as probiotics for aquaculture do. The pollutants are accumulated organic matter from feces, uneaten feed, decaying plant matter (algae), detritus, ammonia, chitin, etc.

Bioaugmentation

Biological augmentation is the addition of micro-organisms, typically bacteria, to speed up the rate of degradation of what is deemed to be a contaminant. These organisms could (in theory) become a component of a specific microbiome although they are typically temporary (require repeated application).

This is what **ALL** products that are being marketed as probiotics for aquaculture do. The contaminants are accumulated organic matter from feces, uneaten feed, detritus, ammonia, etc.

In an ideal world

We would be able to add bacteria for bioremediation and/or bioaugmentation that would become a permanent part of the microbiome.

Single application would bring about real change.

This (probably) WILL NEVER HAPPEN.

Why? Aquatic environments are dynamic and constantly changing. The microbiome is dynamic and is constantly evolving in response to a changing environment.

Shrimp farming, like all agricultural practices, whether terrestrial or aquatic, is NOT NATURAL. (monoculture, high density production environments, artificial feeds, etc.)

Best that can be hoped for is to find strains of bacteria that colonize the environment for a short period of time and require repeated application to maintain numbers (for most production environments).

Approaches to the use of these tools

Liquid

Powder

- With and without added nutrients

Tablets/disks

Bricks

Pondside growout

Advantages
versus risks

Many different products available

For many reasons, Bacillus species are the safest and smartest choice

Can be formulated in a manner where they can not be contaminated or adulterated by unethical companies/salesmen

Form stable spores with indefinite storage under right conditions

Produce a wide array of powerful enzymes at very high levels.

This allows them to degrade a wide range of food sources.

Widely used in bioremediation and bioaugmentation.

Some bacterial species claimed to be in commercial products:

Nitrosomonas, Nitrobacter, Lactobacillus, Rhodobacterium, Pediococcus, Vibrio, Pseudomonas, Aeromonas, Bacillus (spores) species, etc.

Caveat emptor

More species is not better. Small numbers do most of the work. Do not be misled by the strain game.

Higher count is not better. Not about how much you add as much as it is about what nutrients are present that may limit growth.



Liquid products > 98% water



Rhodobacterium (made in China)

Stabilized via pH manipulation

May or may not contain viable vegetative cells.

Very limited shelf lives in general. Contain high levels of dead bacteria.

Not all bacterial strains are suitable for these applications.

Relatively expensive because they are mainly water.

First product used widely in aquaculture was liquid based.

Powdered products (focusing on Bacillus based products)

Can be spray dried, freeze dried, drum dried, air dried, etc.

Can be in salt, on wheat, rice or soybean hulls, mixed with nutrients (sugars), or blended with any number of other materials.

May be sold in biodegradable bags (best suited for nonaerated environments).

Often sold as requiring activation.

Must be kept away from moisture as this will stimulate germination.

Composition

Expert opinion is that products that contain *Bacillus* spores cannot (and should not) be mixed with products that contain viable cells.

Viable cells may be spray dried, freeze dried, etc. Die in the absence of water and nutrients.

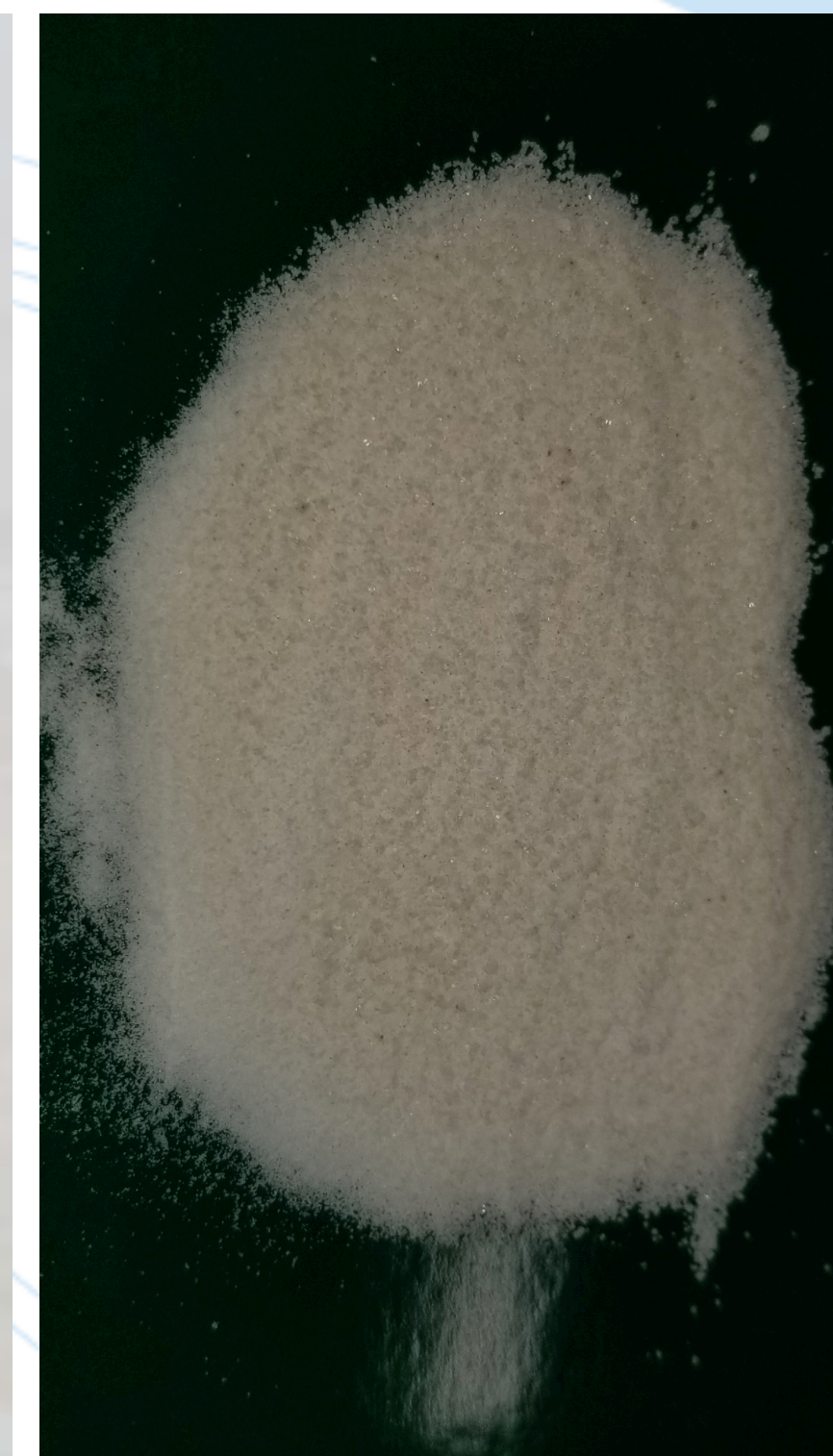
This would require products to be held under refrigerated conditions at best.

Not necessary with spore-based products.

Great deal of interest in *Lactobacillus* strains as possible probiotics. Most are from human tested strains (yogurt)

Again no evidence from the field that these act as probiotics or even can function this way in shrimp (outside of the lab).

POWDERED PRODUCTS COME IN DIFFERENT FORMS



Field Results using a powdered Bacillus spore product

Five Bacillus spores on a wheat mid substrate.

Where: South Florida, USA

Details: Four (4) 1/2-acre lined ponds stocked at 50 to 55 animals per square meter

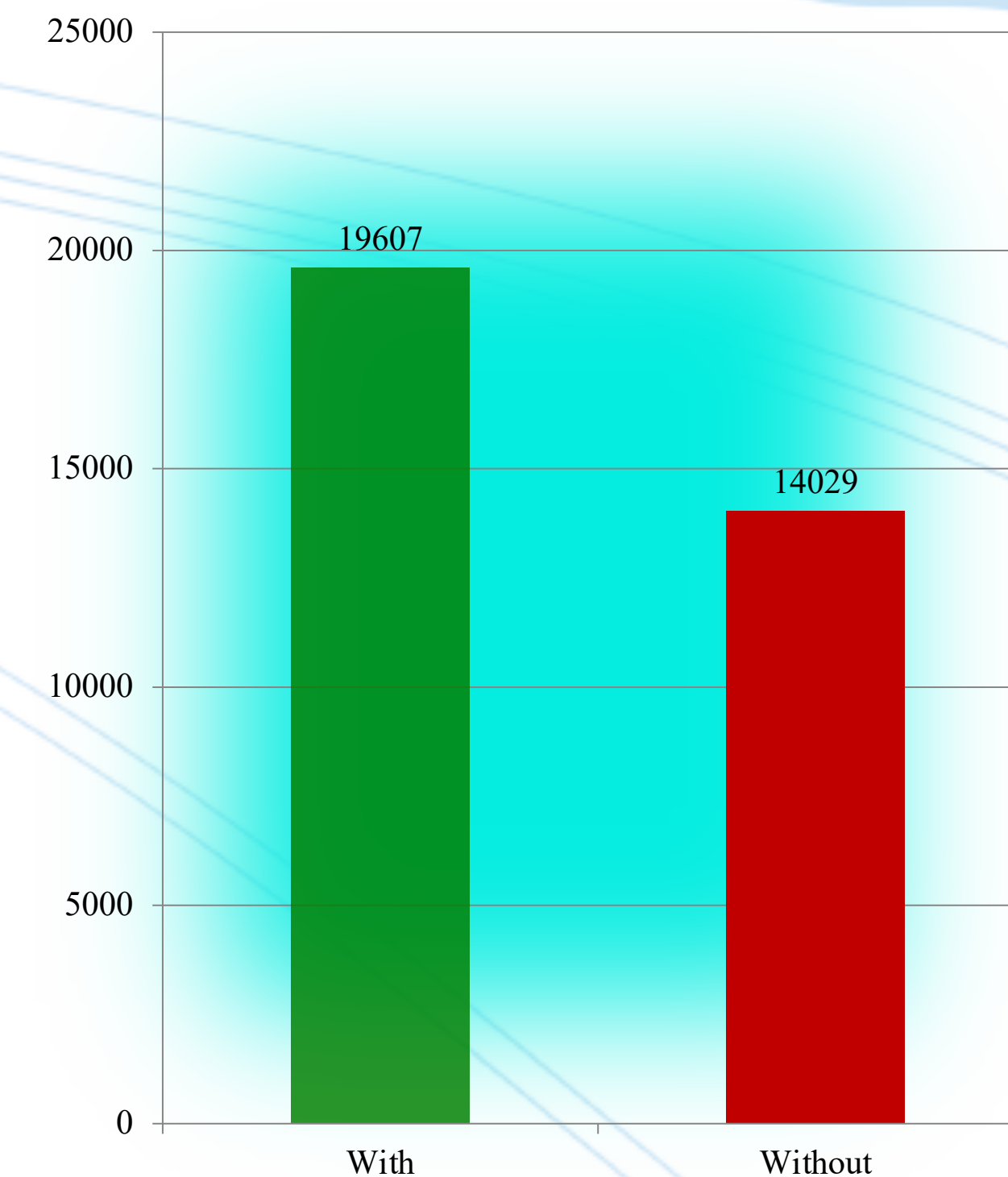
Seven-month cycle

21 kgs of material per pond for entire cycle

Results: 40% yield increase at harvest from treated ponds

Controlled sludge build up; control ponds needed to be dredged before next cycle

Reduced stress and less disease in pond treated with bacteria



Tablets/disks/pellets



Easy and convenient way to use product.



Prevents unethical distributors from diluting products.



Work better than powdered products in many instances.



Targeted delivery.

Untreated pond New Caledonia

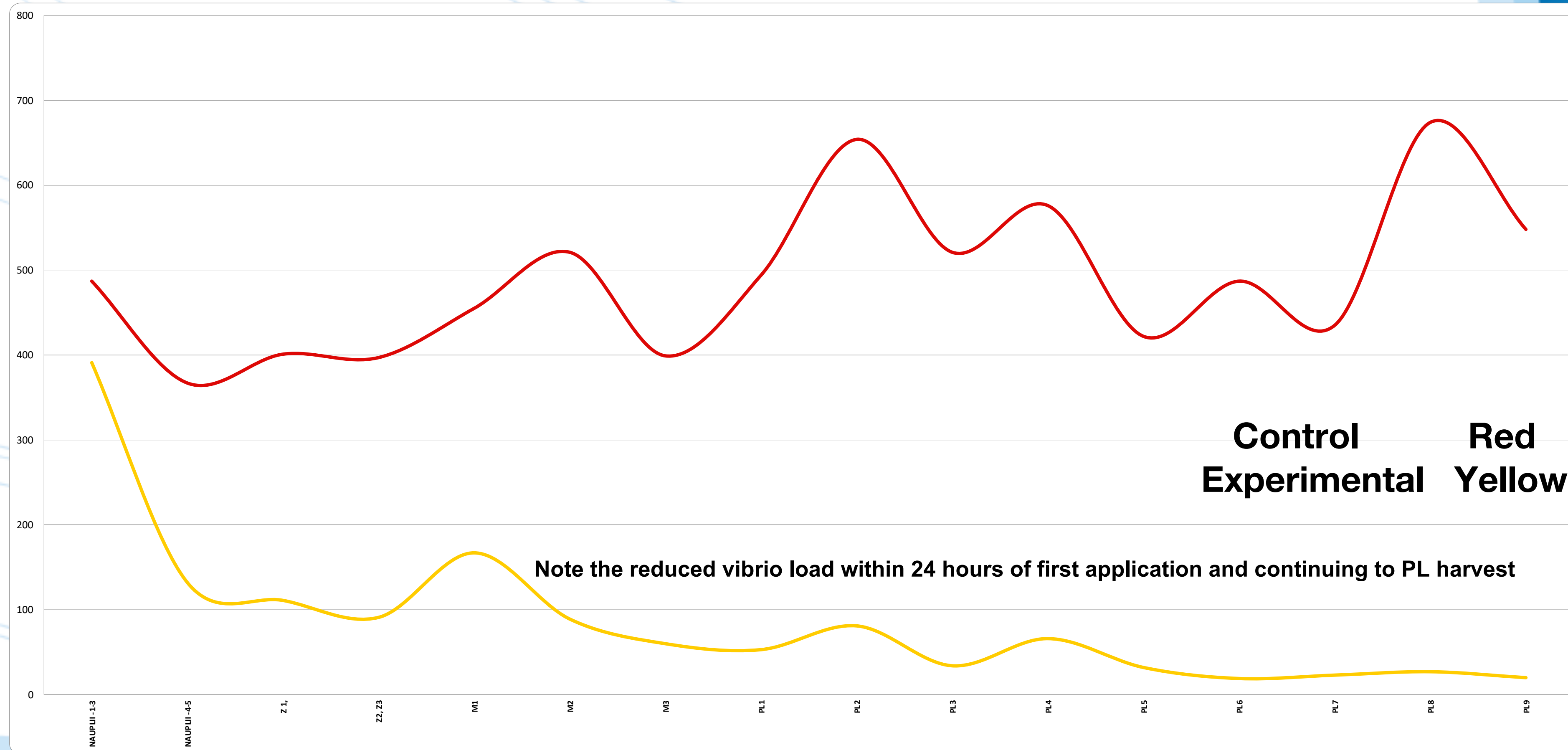


Treated pond New Caledonia



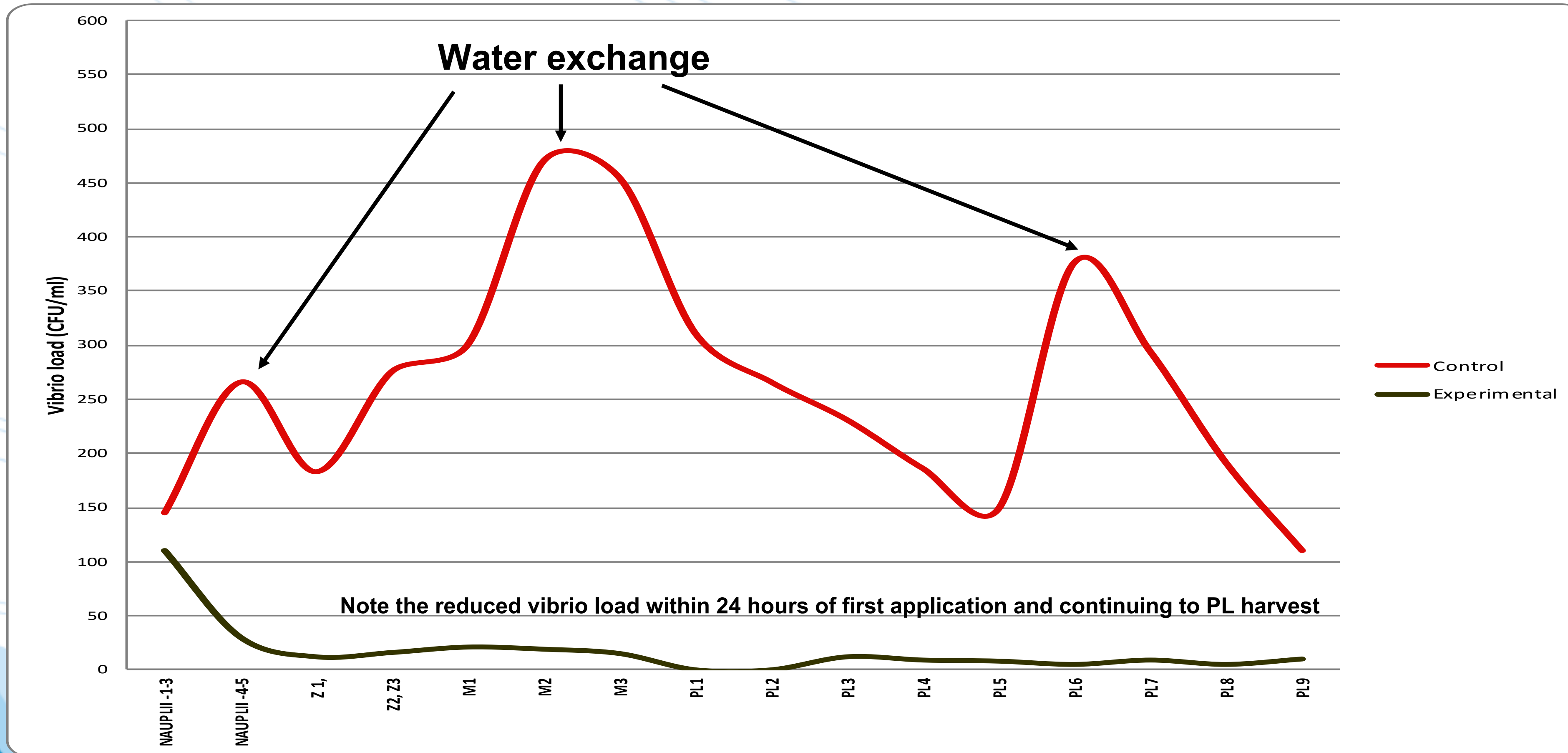
Impact on TCBS yellow colonies-hatchery

Vibrio load (cfu/ml)



Daily application of tablets at 1 ppm. Control tanks were flushed. Experimental tanks were closed.

Impact on TCBS green colonies-hatchery



Daily application of tablets at 1 ppm. Control tanks were flushed. Experimental tanks were closed.

Pond side growout (activation)

Not recommended without proper quality control. A full-time microbiologist on staff who is monitoring growth rates, contaminants present, etc.



Consensus among those who know:

A very dangerous practice that could unleash disastrous results. Not worth the risk. No real benefits.

Bacteria exchange genetic material readily and easily.

This includes genes that allow them to produce potent toxins, antibiotic resistance, etc.

Virulence is not determined by gram reaction or the ability to grow on specific types of bacteriological media.

Can easily result in localized acute die offs.

No evidence that it is economically justified. An added cost. The idea behind adding them to the pond is to allow the addition of high levels of metabolically active vegetative cells. Too many factors impacting outcomes.

Might be useful in highly controlled environment if done properly.

Major source of vibriosis in PL tanks is from contaminated Artemia and algae. Airborne.

Generalities regarding observations on efficacy in the field*



Farm	
Lower vibrio loads	Reduced cyanophytes
Lower heterotrophic bacteria loads	Lower vibrio loads
Cleaner water	Cleaner pond bottoms
Cleaner animals	Visibly reduced organic loads
Higher survivals	Less water exchange needed
Less water exchange	Less disease

*These observations are from client observations. Many products likely have similar impacts, but many also do nothing (perception is reality). I cannot comment on others unsupported claims. Animal health impacts are likely the result of a cleaner environment and lower pathogen loads not via a probiotic effect.

WHAT ARE SOME OF THE CHALLENGES USING THESE PRODUCTS?

Aquatic production environments are highly variable.

Companies selling products that contain dead organisms or false label claims as to content and cherry picked data (not representative of scope of activity of the product)

Variable nature of production systems

Myriad production paradigms equates with many different possible approaches to how best to use these tools.

Cost benefit critical

High priced products are often able to achieve optimum cost benefits

How well tools work depends on how they are used

Frequency

Dosage





CONACUA '19
Congreso de Acuicultura de Camarón



Primary challenges to optimal use and maximum cost benefit from the use of these tools

Aquaculture is agriculture in aquatic ecosystems.

The use of science is largely ignored by the vast majority of farmers and those who provide them with seed, feed and the other tools that can help make production sustainable.

Some examples:

Failure to understand that animals from true nucleus breeding centers are safe.

Screening of broodstock for pathogens is not adequate for ensuring that the specific pathogens being screened for are not present using conventional approach.

Constant perpetuation of myths. Almost universal disregard for scientific method.

Thinking that a magic bullet exists to solve problems that are inherent in the production paradigm.

What is my problem with the use of the term probiotics?

Misleading. Farmers with no understanding or interest in using the scientific method think about yogurt.

Term does not apply to environment. Never did and never will. Bioremediation and bioaugmentation are the correct terms.

Accepted definition would not apply to the ingestion of spores, dead bacteria, etc.

Contentious term at best. Evidence in human beings is not supported by much of the data.

Marketing hype. Just another example of pseudo-science foisted on an industry that has enough problems without spending money on things that claim to do things that they cannot possibly do.

Some of the many challenges (summary)

Promotion by agents and distributors and unethical companies as solutions to problems that cannot be fixed by the use of these tools.

- Effectiveness often impacted by product price mark-ups that make the product too expensive for the end users to use properly.
- Variability of the environments that they are used in.
- Variability of the conditions under which they are being applied.

Many companies sell whatever the customer wants with no regard as to scientific basis of activity. Claims abound about things that cannot possibly be real or true.

Some of the many challenges (summary)

Encouraging companies to grow large amounts of bacteria to add to ponds and tanks is **IRRESPONSIBLE**

Genetic drift.

Ready exchange of genetic material can result in newly toxigenic strains, antibiotic resistance, super pathogens.

Contamination by faster growing bacteria (vibrio generation time is 8-15 minutes).

No evidence that is necessary.

Conclusions

Bacillus spore based microbial products are valuable tools with field proven benefits.

- They provide a reproducible impact.
- Safe and effective consistently.
- Not true probiotics (although vegetative cells are commonly ingested).
- Can they replace antibiotic usage?
Yes and No. They cannot replace the illegal and inappropriate use of antibiotics; 99% of usage.
- **Perception is reality in absence of science-based analytics.**

About the author

BSC University of Maryland 1973 Conservation and Resource Development
Ph.D. University of Miami 1979 Role of a plasmid in virulence of *Vibrio anguillarum* in salmonids

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(Aquaculture/Information/Technology)**

Founded in May of 1996 to market and sell first commercial shrimp vaccine. Focused largely now on tools for bioremediation. Provide range of consulting services for entire value chain from genetics programs (Super Shrimp and Seaquest) to processing plants (ACC/GAA) to maturation, hatcheries and farms in every major shrimp producing country. Work closely with clients in Mexico, USA, Ecuador, Vietnam, Indonesia, and elsewhere. Consulting work geared largely towards reduction of prevalence of disease and proactive management strategies. Written many articles geared towards helping farmers be better at what they do.

**www.aqua-in-tech.com
www.bioremediationaquaculture.com**





Gracias

por su atención

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