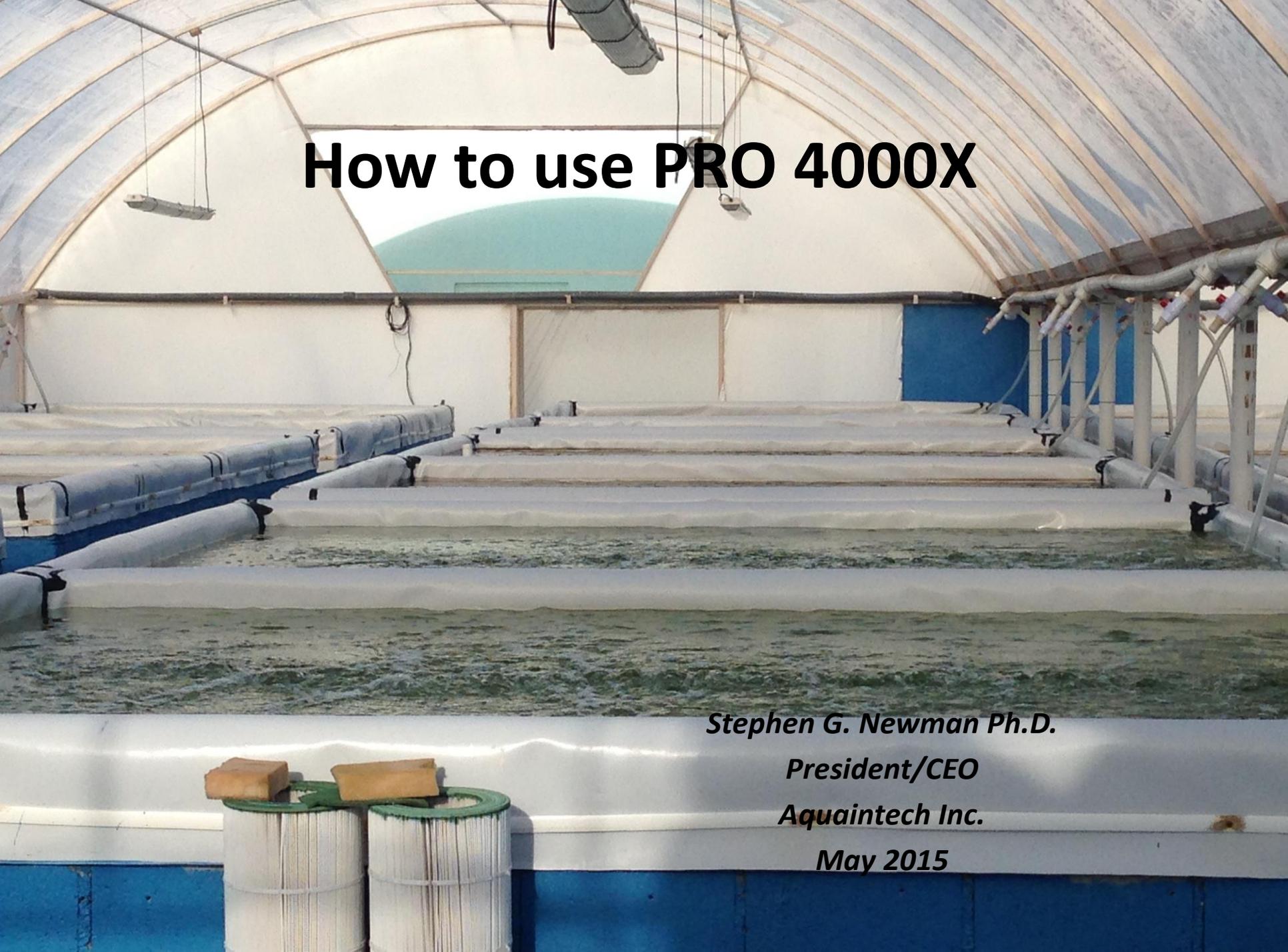


How to use PRO 4000X



Stephen G. Newman Ph.D.

President/CEO

Aquaintech Inc.

May 2015

There are no magic bullets
(only a lot of people trying to sell them).
Our products are tools not solutions.



Bacillus based products

Why bacillus?

- Long term shelf stability due to spore formation. If product is kept dry, shelf life is many years.
- Broad range of enzymatic and metabolic activities. Enzyme factories. Very high production levels.
- Naturally occurring strains and safe to use.
- Proprietary strains with proven track record for improving water quality and increasing productivity.
- Compete against other bacteria such as vibrios and blue green algae.
- Many products claim to contain strains that are not present or cannot be preserved in a shelf stable dried form. Bacillus spores can.

PRO 4000X Appearance



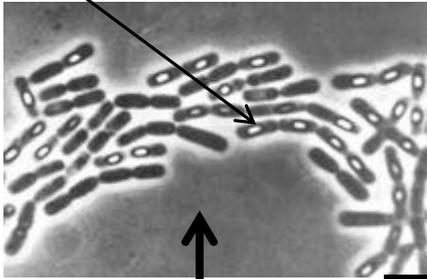
Properties

- Approximately 16 grams apiece
- 2 primary strains of Bacillus selected for ability to degrade organic matter and ammonia
- > 64 billion spores per tablet.
- Work from the pond bottom up. Tablets sink to the bottom of the pond and germinate where the shrimp and organic matter accumulate. The bacteria then move through the water column.
- No activation required (although some may still choose to do so).
- Targeted delivery to problem areas.
- No fuss, easy to use. Inexpensive.

How does this product work? See next slide.

Remember that these are tools. Like any tool they have to be used correctly for optimum impact.

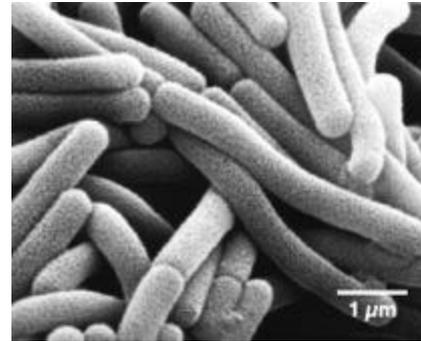
Spore forming Bacillus



Targeted Delivery to problem areas. Spores germinate.



TABLET
16 gram
>64 billion
CFU



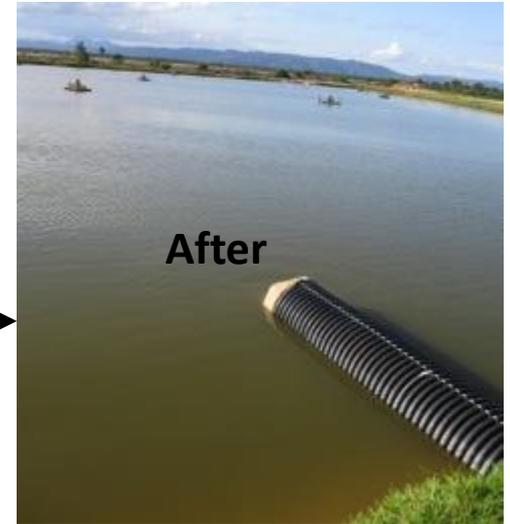
Degradation of organic matter resulting in improvement of water quality.

Used properly can compete against other bacteria (vibriosis) and blue green algae.

Before



After



Look at the color difference!

Farm usage



Benefits of using PRO4000X on the farm

Benefits reported by different clients*

Less accumulated organic matter

Better growth

Higher survivals

No need to use antibiotics

Lower vibrio loads

Lower ammonia levels

Less water exchange required

Healthier ponds with less blue green algae

Lower hydrogen sulfide levels



* Benefits vary. When used correctly all clients experience a reduction in organic matter. Secondary benefits depend on your environment and culture conditions. Support letters available on line.

- Each pond on a farm is unique like a fingerprint. Each farm is thus unique as well.
- There are many site specific properties that relate to location, the type of production paradigm (species, density, etc.), the water quality, the stocking densities, the pond age and history, pond type (lined versus dirt, etc.)
- There is no one correct way to use the product in all circumstances.
- Users should adapt their usage to the type of system.
- Flexibility in application protocols will result in the best long term benefits.



Indoor enclosed biofloc based systems can benefit by *Bacillus* colonizing the biofloc.



Smaller lined ponds with high levels of aeration and using well water for makeup and exchange can benefit by using tablets in drainage areas.



Larger lined ponds can benefit as well by targeted delivery.



The principle of titration

- Clients are urged to start from our guidelines (next slide) and modify their usage pattern to optimize the products functionality.
- This can involve starting out high levels and backing off to those levels that work the best. This is a common approach to use of the product.
- The bacteria in the product do what we claim they do. This is a scientifically validated fact.
- How well they work in a production environment is controlled by the user. Tablet application levels and frequency of application should ultimately be determined by the user.
- If products are over priced (as they are with many products), this discourages using the product in a manner consistent with optimum benefits.

Guidelines for usage in different density production systems*

Day	Tablets per ha	
	High (> 75)	Low (< 75)
Density/m ³		
0	10	0
7	15	5
14	20	7
21	20	10
28	25	12
35	30	13
42	35	15
49	40	17
56	50	20
63	50	25
70	60	30

Day	Tablets per ha	
	High (> 75)	Low (< 75)
Density/m ³		
77	60	27
84	60	30
91	75	40
98	90	40
105	90	45
112	90	45
119	90	45
Tablet #	925	431
kgs	15	7

* Application rates should be fine tuned by the end user based on their production environments and their observations

Things to consider

- These are guidelines only. Farmers are expected to modify them to suite their applications and needs.
- In many farm environments the addition of molasses can help. Usually 10-20 liters per ha added several hours (6 to 12) after the tablet application.
- The bacteria in the product can be used in pipes. If the pipes are clean to start these bacteria can colonize the pipes keeping them clean and free of other problematic bacteria species.

Field observations

- It is very difficult to get high quality consistent and scientifically valid data from the field.
- This is because of the inherent variability in the production environment.
- Invariably when clients report that there are no benefits (rarely) it is because they are not using the product correctly for their system.

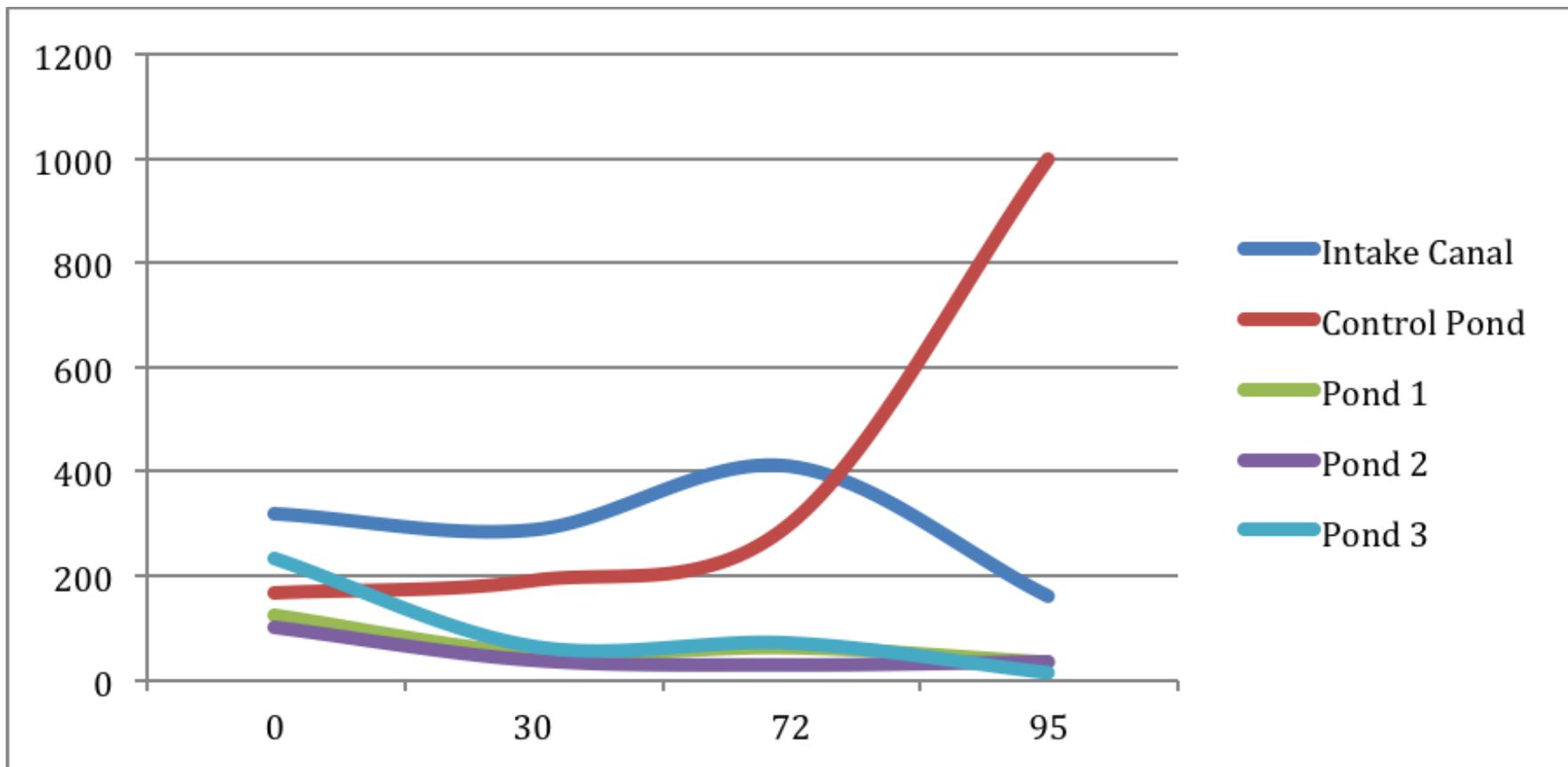
Experimental Parameters India 2013 PRO4000X

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

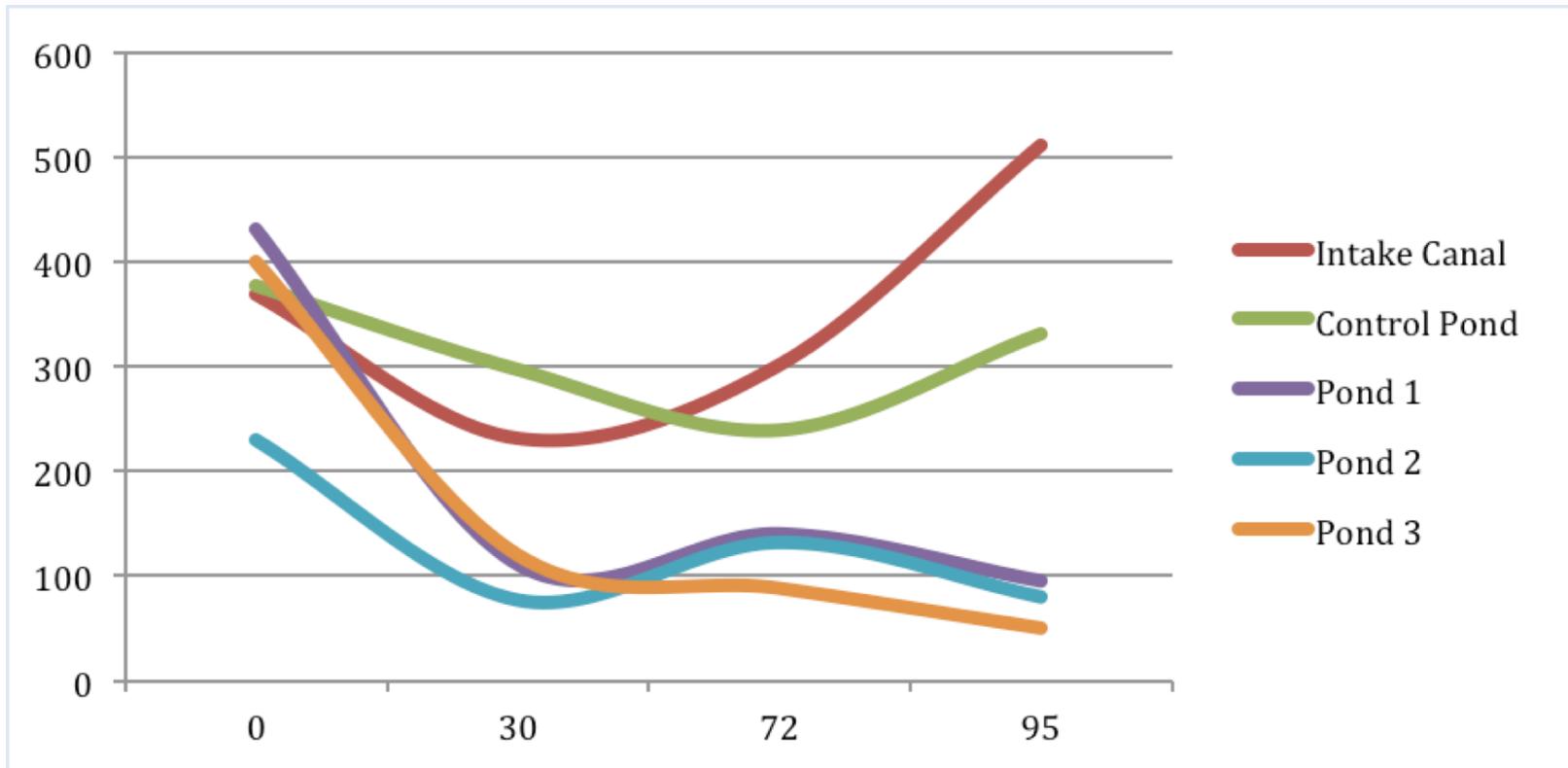
Results of three ponds treated with our tableted product, PRO4000X, compared with a pond that was not treated (control was treated with local products).

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.

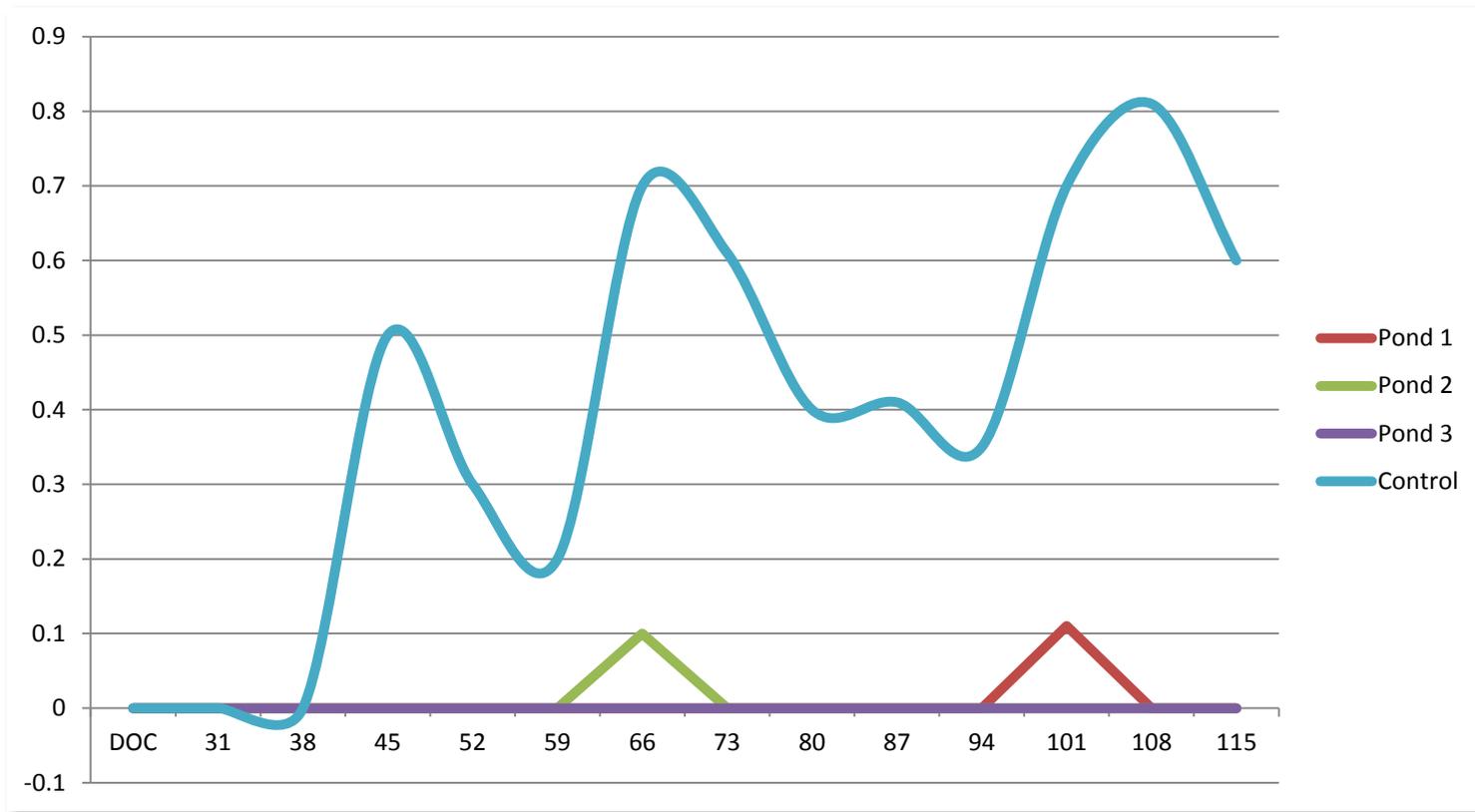


Reduction of TCBS yellow vibrio loads over the course of the production cycle



Note: TCBS is useful for differentiating sugar utilization by vibrios. It has nothing to do with pathogenicity. Not all greens are pathogens just as not all yellows are harmless.

Weekly ammonia levels in control and experimental ponds



PRO4000X controlled ammonia levels in the experimental ponds. In fact the experimental ponds could be closed (no exchange) and the control pond could not, further creating biosecurity risks.

Comments from Indian trial

- Depending upon the water quality slight modifications were made to application rates. For example at 77 DOC we applied 40 tablets, in the meantime if we noticed increased ammonia levels then repeated half of the dosage (20 tablets).
- We noted that in the control ponds the growth rate was comparable to the experimental ponds up to the 38 days of culture. After that the growth rate was reduced because of pond conditions. *The Pro4000X helped in reducing the stress factors in pond environment including ammonia, nitrite, and Vibrio loads. A stable plankton bloom was noticed in the three experimental ponds up to the harvest.*
- We were using creek water, a contaminated source with the risk of WSSV. While in pond preparation we treated the water with chlorine and following all biosecurity measures.
- We recommended the use of Pro4000X because there was no need for water exchange and we could avoid the risk. The farmer has one reservoir pond which was treated with chlorine and used for his control pond for minimum water exchange.

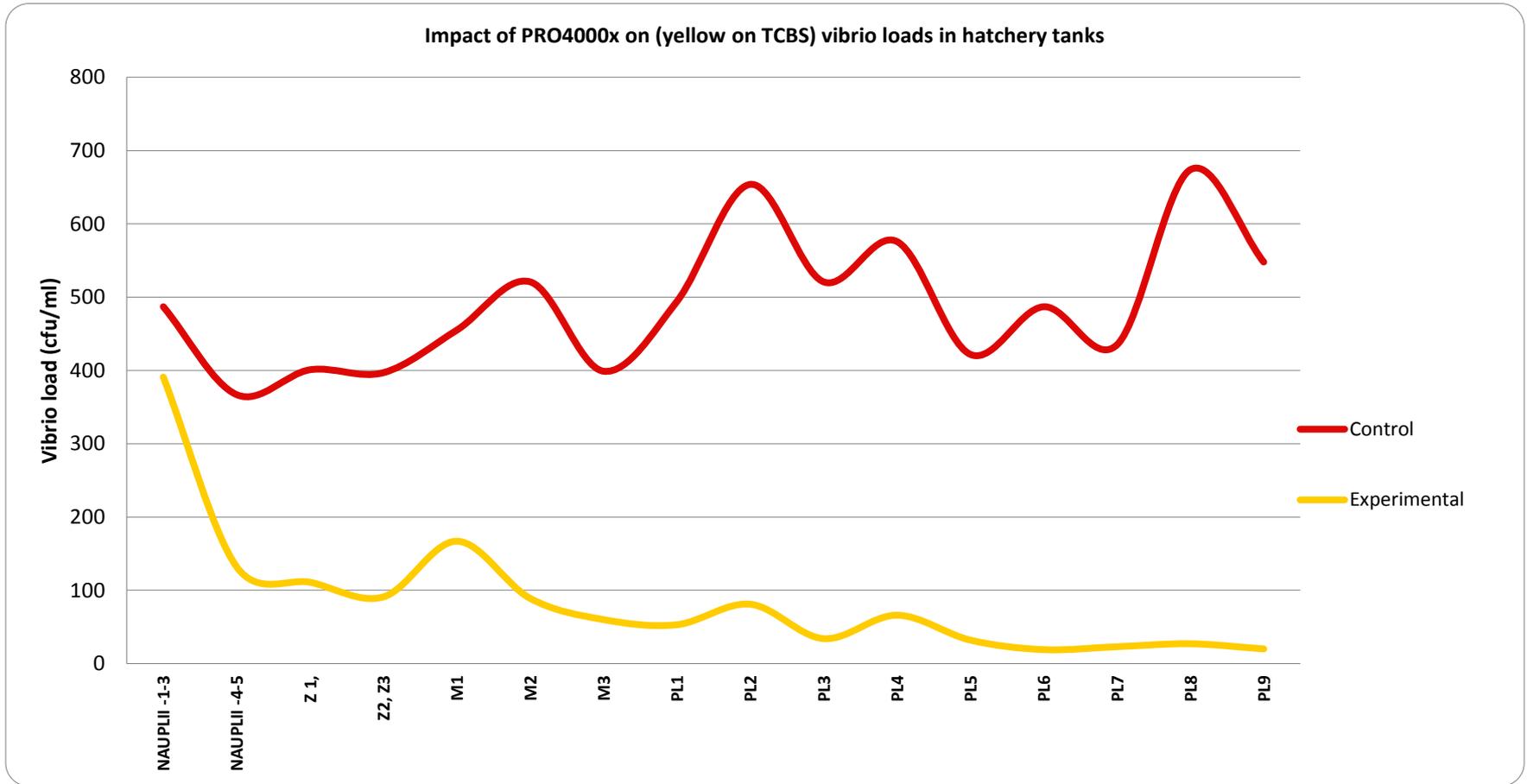
The hatchery



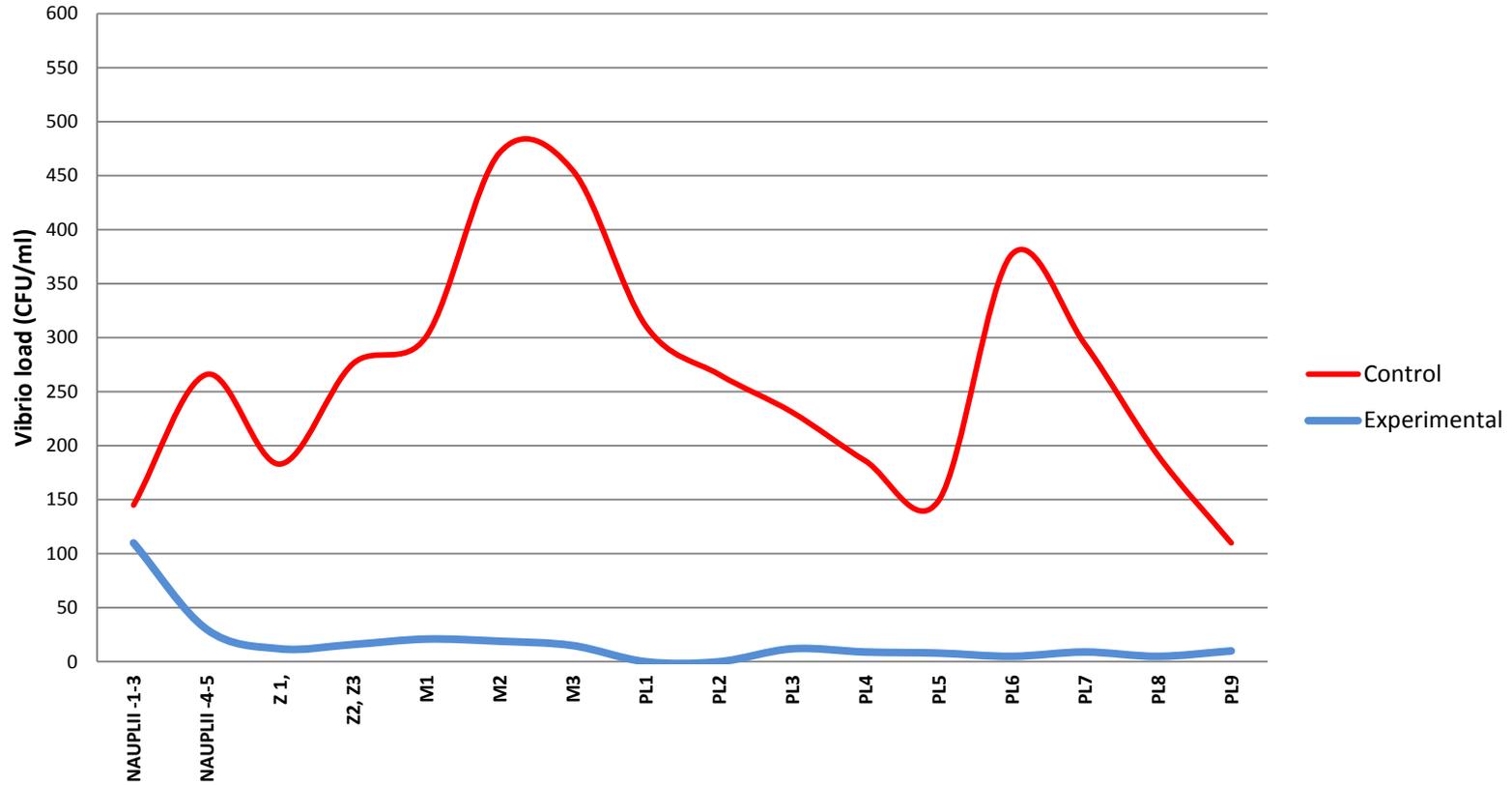
Guidelines for hatchery usage

- One to two tablets daily from stocking of nauplii to day before harvesting PLs for 5 to 10 MT of water.
- Nutrient levels in tanks will affect bacterial growth.
- Higher nutrient levels as cycle progresses.
- Can be used in Artemia culture (one tablet added before cysts)
- Can be used in algal tanks (lessens vibrio loads in out door production tanks)
- Can be used in maturation one tablet 10 MT of tank water per day.
- Will not harm most biological filters.

Reduced TCBS yellow vibrios



Reduced TCBS green vibrios



TCBS green loads reduced to almost zero in hatchery tanks.

Comments from users in hatchery

Observations

Excellent control of yellow, green and LB colonies were noted after application.

Growth rate is progressive when compared to control tanks. Increase in size 3 mm. PLs grew faster in tanks with PRO4000X added.

Animals fed more aggressively in PRO4000X tanks. No conversion problems in experimental tanks.

Presence of dead animal were controlled in experimental tanks.

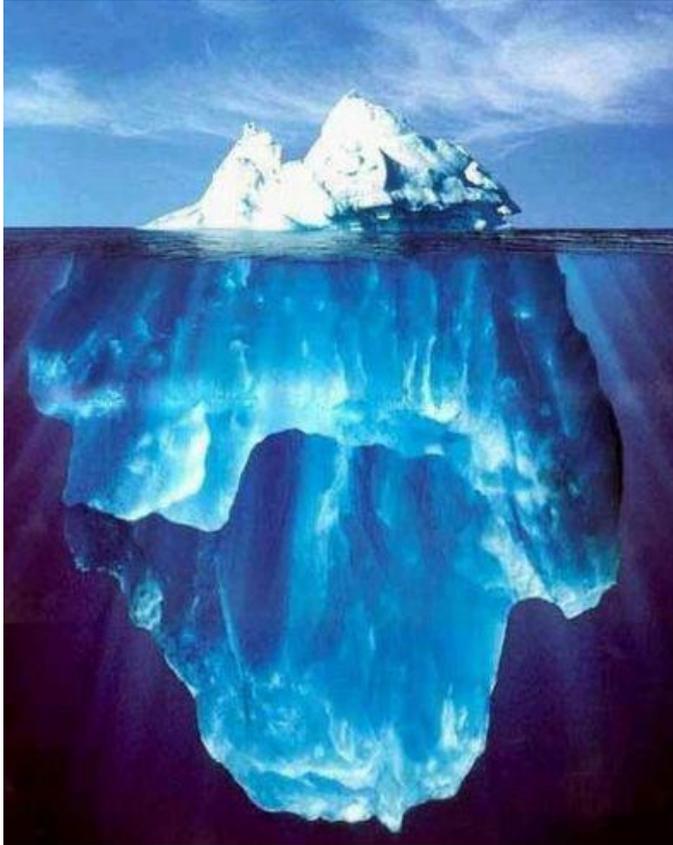
Fouling organism were controlled in experimental tanks.

Observed lower levels of waste accumulated on the tank bottom area.

Very low levels of ammonia, nitrite were observed and levels were controlled.

No other bacterial amendments were needed in tanks using PRO 4000X. Control tanks were treated with other popular products.

Final thoughts.....



What is taking place in production environments is in many ways akin to an iceberg.

What you see is only a part of what is actually happening.

Those things that contribute to the problems we see are not always obvious or easily understood. In some instances they are never figured out and in some cases no matter what we do they override our efforts.

Taking reasonable cost effective measures to limit the potential impact of any disease problem is smart.

PROACTIVE disease management.

Pro4000X tablets, by lowering available nutrient loads, can be an important tool for proactive disease management.