Loc Tran

FOUNDER & DIRECTOR
SHRIMPVET LABORATORY

Loc is founder and director of ShrimpVet Laboratory in Vietnam. He leads a team of more than 70 researchers in shrimp health management, focusing on genetics, breeding and seed production, diagnostics and disease management, and farming technologies. He has a Ph.D. in aquaculture pathology from the University of Arizona, where he studied under renowned aquaculture pathologist Don Lightner.

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Global Aquaculture Alliance

Science in Shrimp Farming and Recent Innovations in Hatchery and Production Systems in Vietnam

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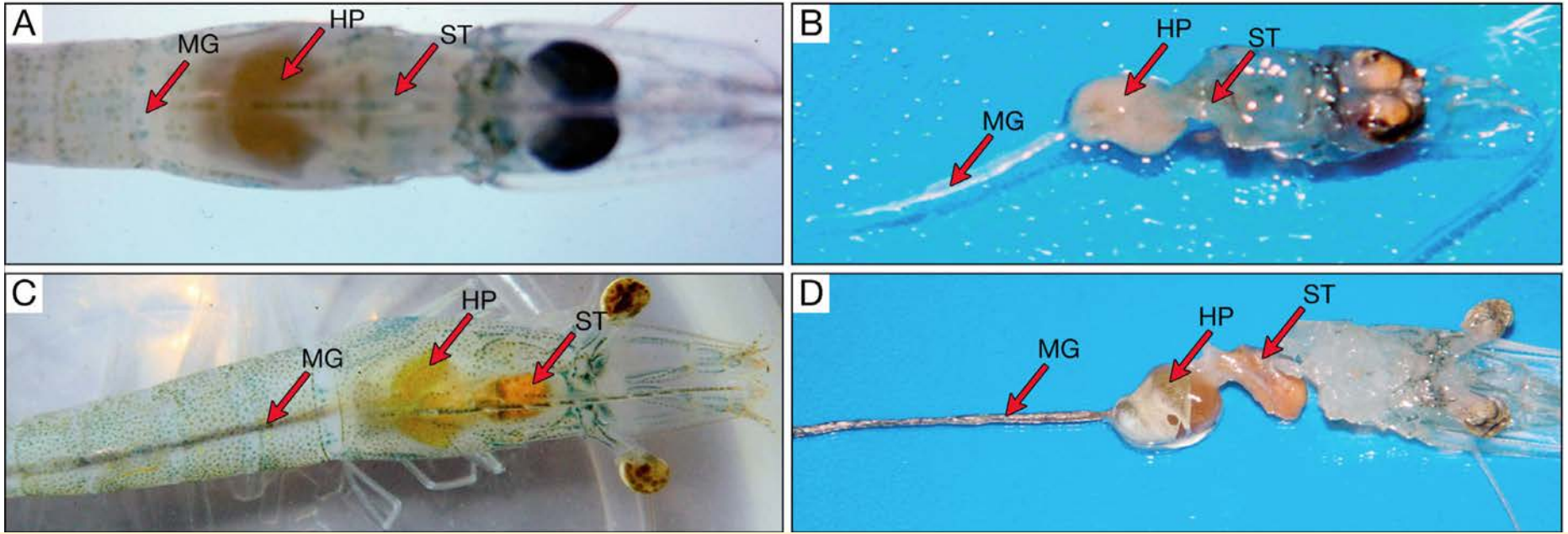
Some Recent Updates

- Asian shrimp production in 2017-2018:
  - Increasing; prices plunged (second quarter).
- Major challenges:
  - EMS/AHPND, WSSV, EHP, White Feces Disease, SHIV?
  - Antibiotics residue.

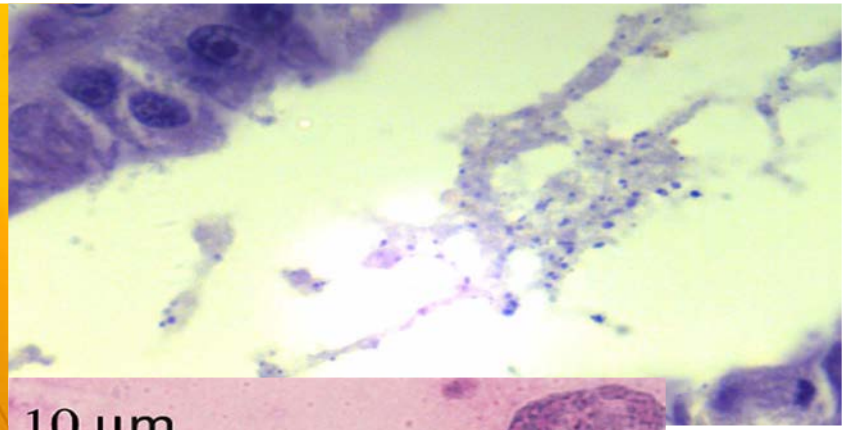
Back to the Old School EMS/AHPND

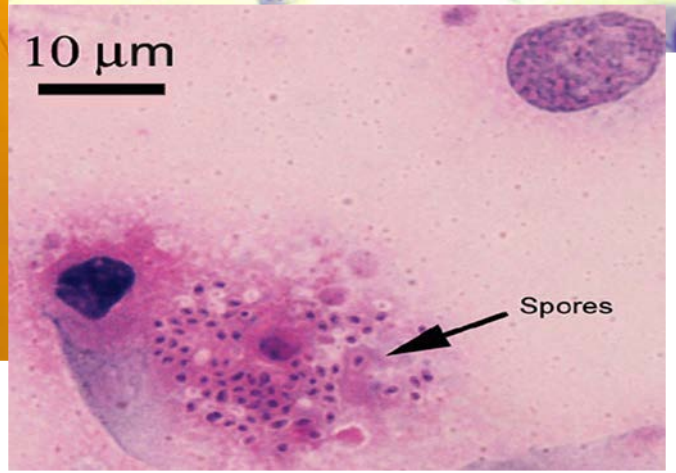


Gross signs of EMS/AHPND affected *Penaeus vannamei*.

- ✓A and B display a pale, atrophied hepatopancreas (HP), and an empty stomach (ST) and midgut (MG).
- ✓C and D show a normal size HP with dark orange color, and a full stomach and midgut.

Enterocytozoon hepatopenaei





SPF SHRIMP
(Control)

WFD-INFECTED SHRIMP
(LABORATORY CHALLENGED)

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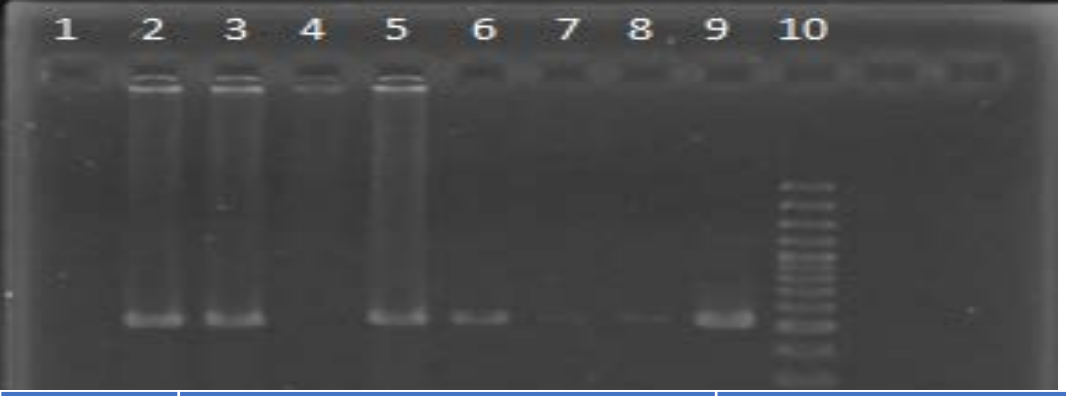

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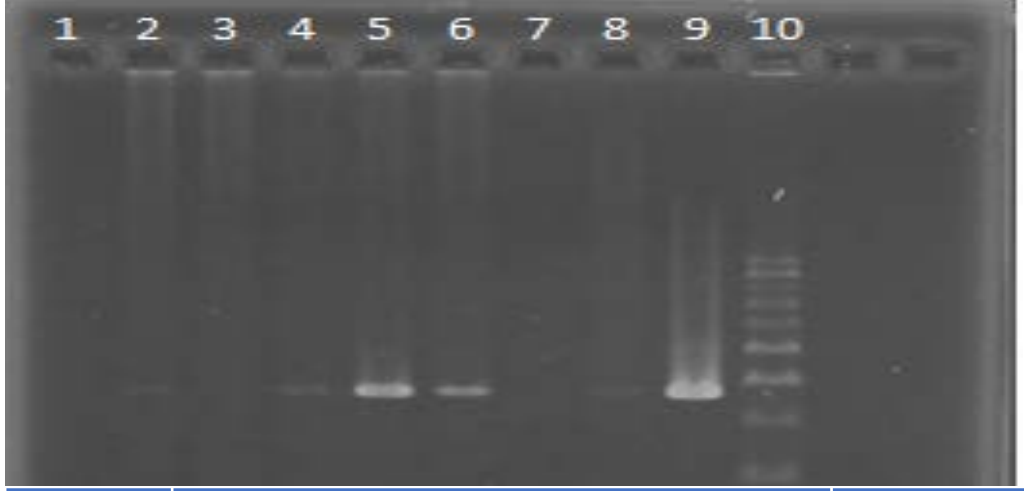
WELL	SAMPLE NAME	*Enterocytozoon hepatopenaei*	MOTE
1	NEGATIVE CONTROL	-	
2	GROW OUT SHRIMP	+	
3	GROW OUT SHRIMP	+	
4	PL_5 (Shrimp)	-	
5	PL_9 (Shrimp)	+	
6	BROOD STOCK FECES	+	
7	BROOD STOCK FECES	+	
8	BROOD STOCK FECES	+	
9	POSITIVE CONTROL	+	*DNA* *Enterocytozoon hepatopenaei*
10	LADDER		100bp



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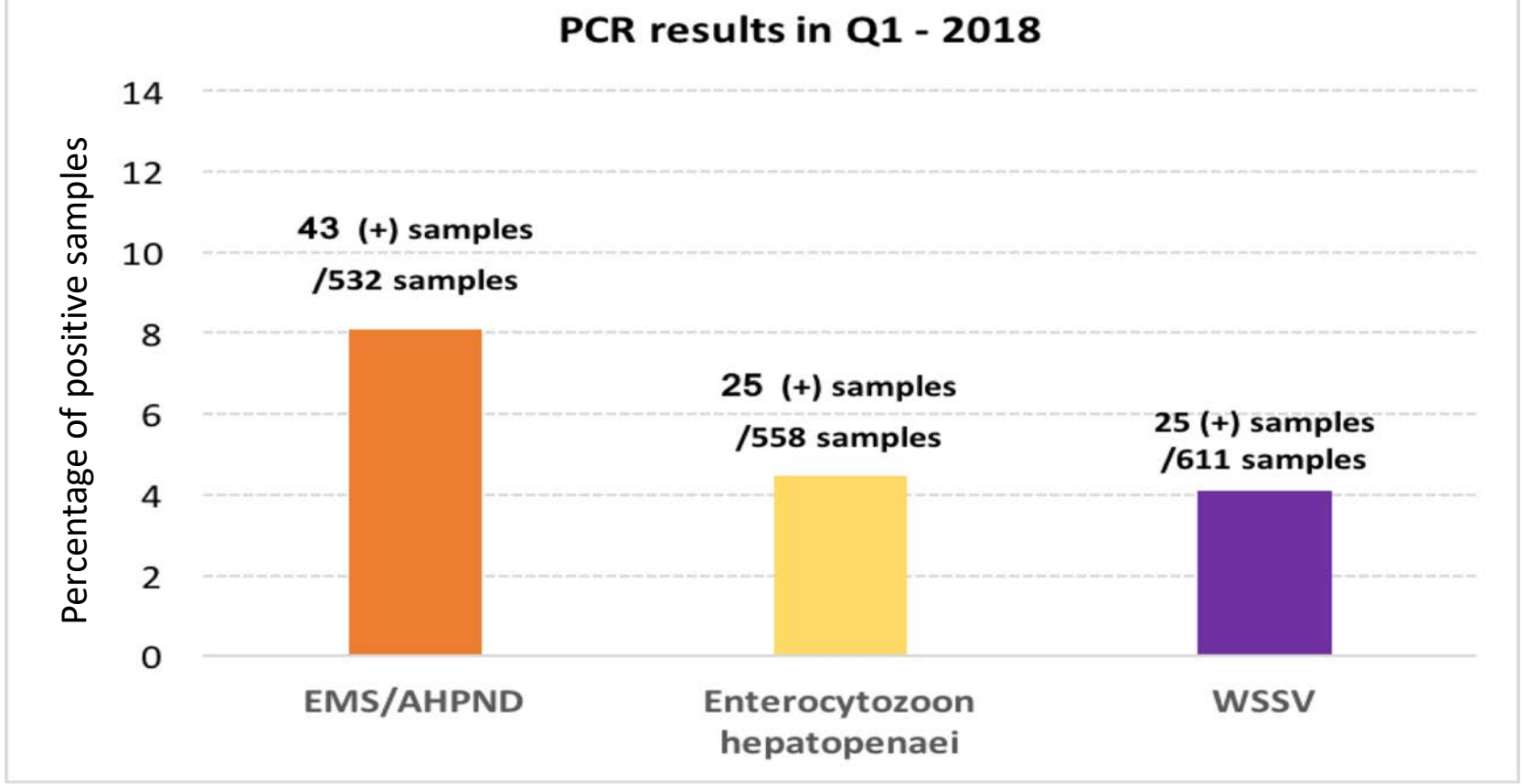
WELL	SAMPLE	*Enterocytozoon hepatopenaei*	NOTE
1	NEGATIVE CONTROL	-	
2	BLOOD WORM 1	+	
3	BLOOD WORM 2	-	
4	OYSTER	+	
5	SQUID	+	
6	BROOD STOCK MATURATION	+	
7	BROOD STOCK AT QUARANTINE	-	
8	GROW OUT SHRIMP	+	
9	POSITIVE CONTROL	+	*DNA* *Enterocytozoon hepatopenaei*
10	LADDER		150bp

Horizontal Transmission

- Negative control (SPF shrimp) after 45 DOC)
- EHP-challenged shrimp after 45 DOC)

Warning?


PCR results in Q1 - 2018
Percentage of positive samples
14
12
10
8
6
4
2
0
43 (+) samples
/532 samples
25 (+) samples
/558 samples
25 (+) samples
/611 samples
EMS/AHPND
Enterocytozoon hepatopenaei
WSSV

Good PLs (*Penaeus vannamei*)?

1. Have to be clean of OIE-listed diseases (usually not an issue).
2. Have to be clean of EMS/AHPND and EHP (**most important**).
3. Antibiotics-free hatchery protocol.
4. Size variation (CV < 12%).
5. ADG: minimum at 0.3 g/day to reach breakeven point.

What Can We Do About It?

Transmission from hatcheries:

1. Better biosecurity.
2. Check all inputs and outputs (broodstock, fresh feed, nauplii, water, and PLs).
3. Diseases of concern: EMS/AHPND, EHP, WSSV.

How to Control EMS/AHPND and EHP in Hatcheries?

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⇒ PCR screening (enriched fresh samples): broodstock feces, live feeds, nauplii, PLs).

⇒ Freezing live feeds before feeding - alternatives for live feeds are desperately needed.

⇒ Impossible to keep the hatchery sterile - need to use probiotics to out compete Vibrios.

⇒ Biosecurity and microbiota balance in larval rearing are the two keys to keep PLs clean.

How to Achieve a Healthy "Microbiota Balance"

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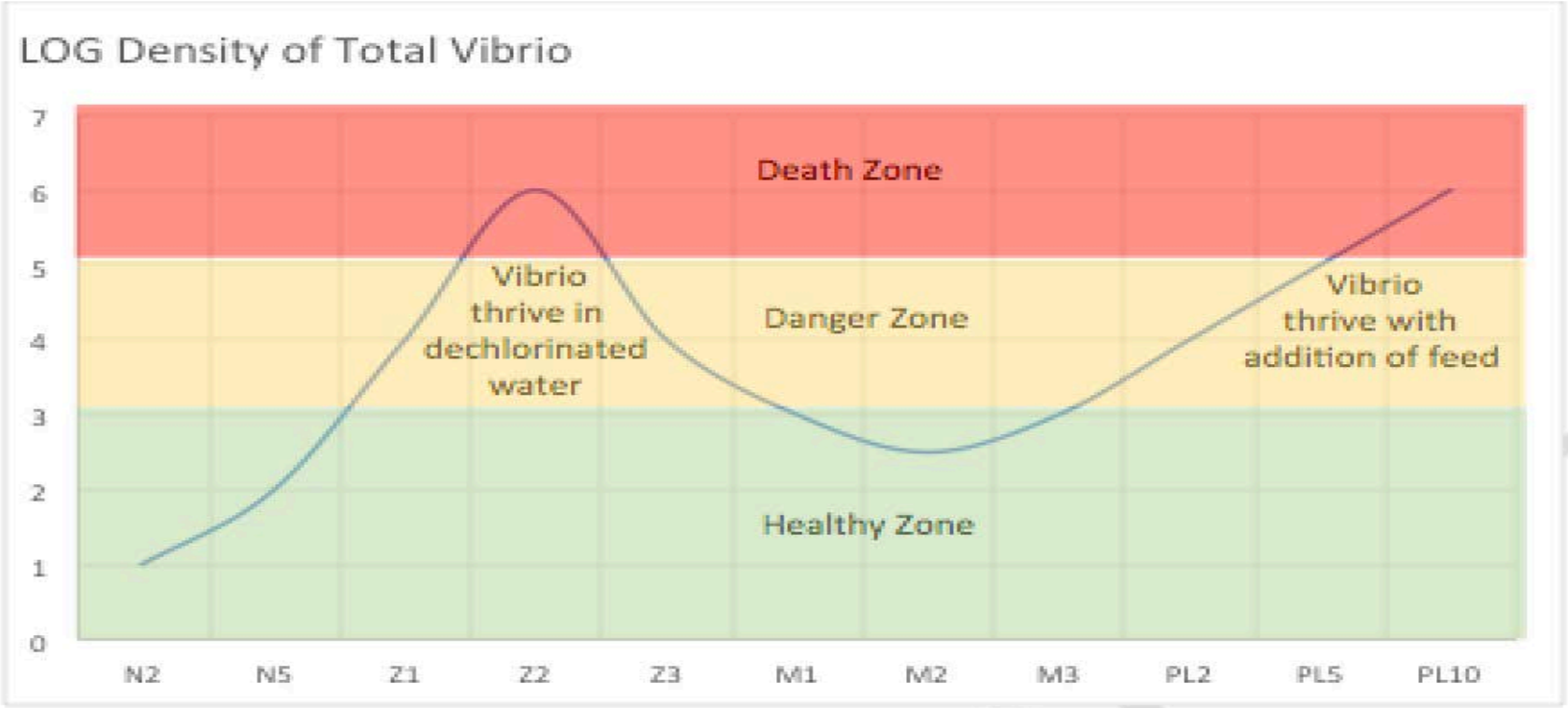
- 

Figure 3. Typical pattern of Total Vibrio bacteria growth in larval tanks within shrimp hatcheries.

Some Facts About Bioremediation / Probiotics vs. Vibrio

How to Fix This?

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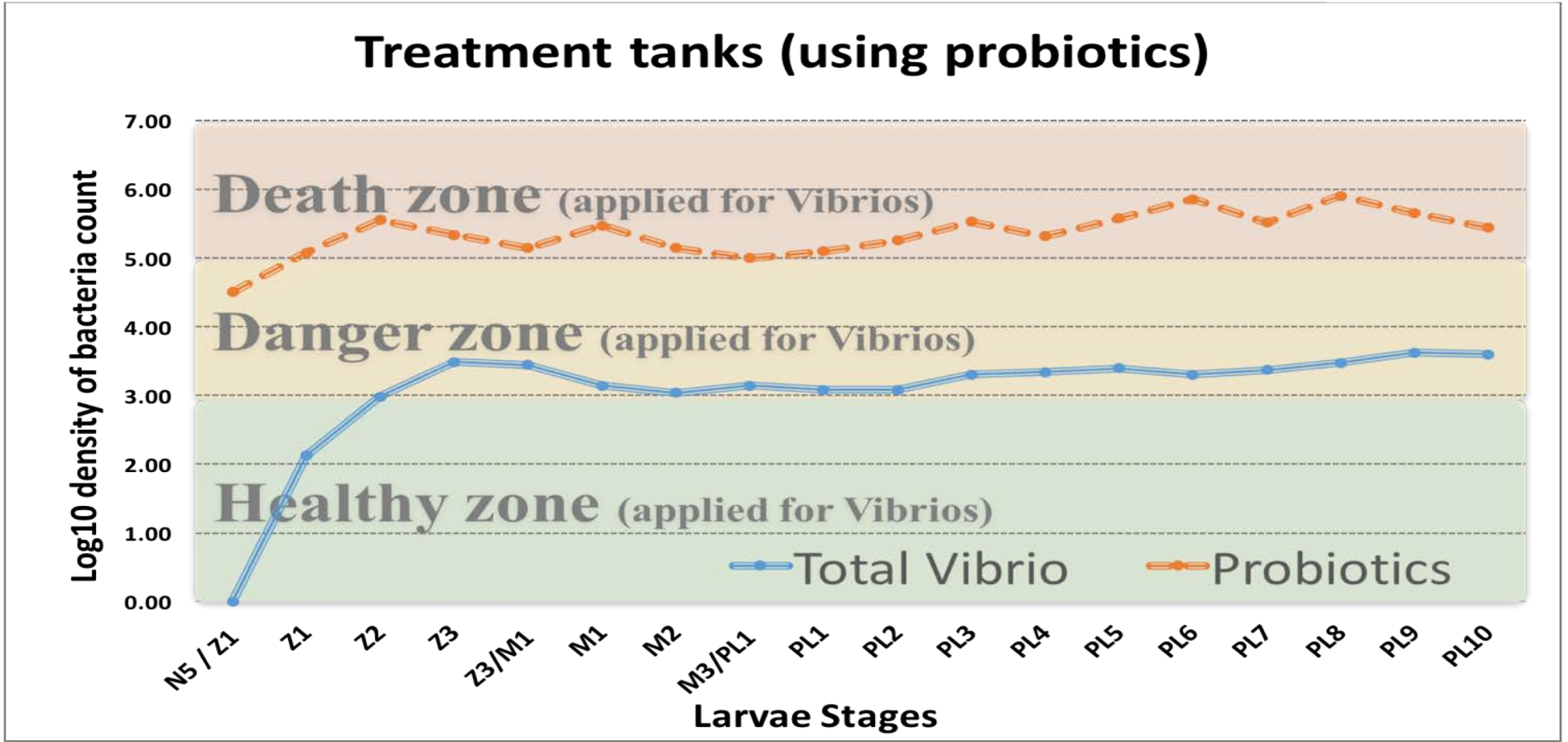

How to Manage EMS/AHPND and WFD at the Farm Level

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1. Clean PLs (checked for EMS/AHPND, EHP, WSSV).
2. Clean pond – Clean water – Clean PLs.
3. Go back to the concept of “Microbiota Balance.”
4. Waste management: siphoning, probiotics, RAS, bioflocs, etc.
5. Probiotics consistently in feed and in water.
6. Nursery.
7. Functional diets.

Nursery

- 1000 – 5000 PLs/m^3.
- 20-30 days.
- Harvest at 0.8-1.2 grams.
- Reduce risks (shorten the culture period), reduce cost.
- Faster rotation of the shrimp ponds.

Facts and Solutions

- The Bad: obligate pathogens - The Good: probiotics - The Ugly: opportunistic.
- The Bad/Ugly proliferate a lot faster than the Good.
- The Bad USUALLY WINs in 1 vs. 1 combat vs. the Good.
- Probiotics/Bioremediation is **never** the remedy for bacterial diseases, but it is a powerful **tool!**
- How to provide some advantages to the Good?
- => Give the Good a jump start (10^{4-5} CFU/mL inoculation), daily application (at 10^{4-5} CFU/mL) continuously. This has to be done BEFORE STOCKING.
- => No providing the Bad/Ugly with what they need (nutrient, inoculation, etc.).
- => Rule of Thumb: always have the Good at a proportion of at least **100/1** to the Ugly/Bad. If not, correct it.


See anything wrong?

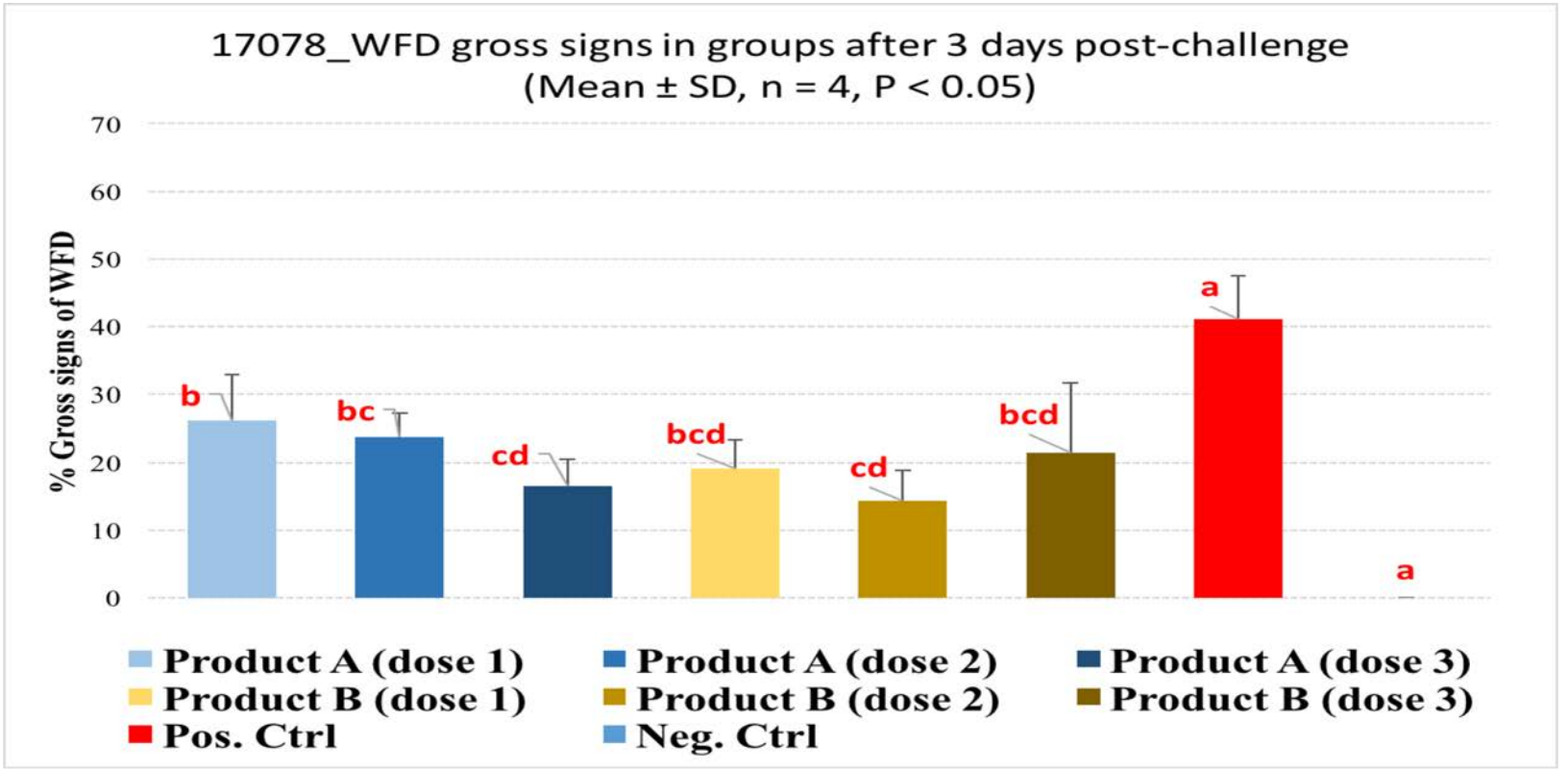
Fermentation of Feed Ingredients?

Feeding shrimp with fermented corn/soybean?

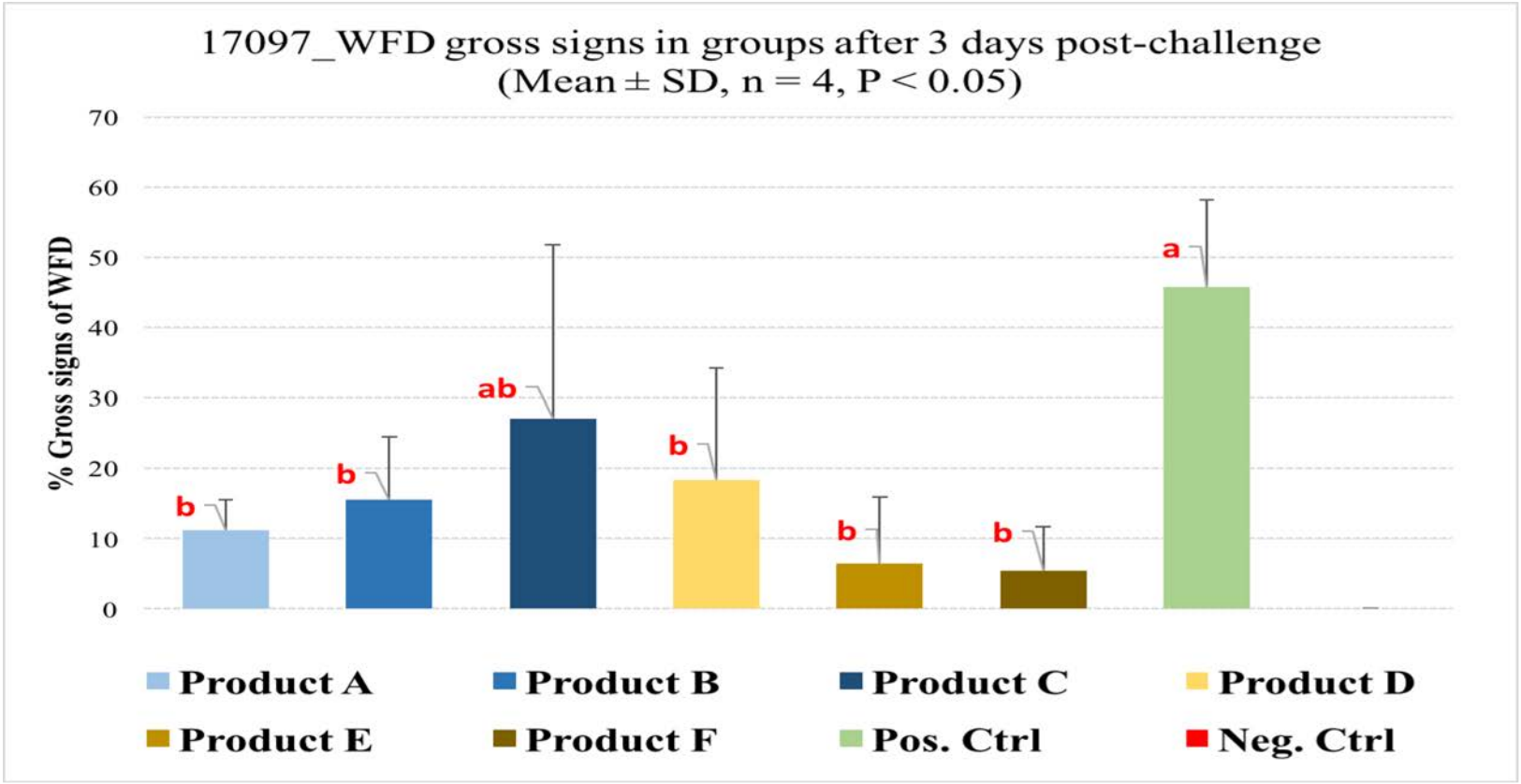
- Disrupt nutrients needed for Vibrio.
- “Flush out” Vibrios in the shrimp gut.
- Provide an abundance of probiotics community.

Functional Diets?

How to Translate Lab Trial to Pond Trial



Functional Diets?



A Pond Trial with a Functional Diet

- Location: Dong Nai, Vietnam.
- Duration: Dec-2017 – March-2018.
- DOC: 100 -- 70 days of grow out, plus 30 days of nursery.

Treatment	Control (T1)		Treatment (T2)	
Pond designation	Pond#1 (T1-1)	Pond#3 (T1-2)	Pond#2 (T2-1)	Pond#4 (T2-2)
Area (m^2)	2350	2380	2420	2180
Feed	Control		Functional diet	
Administration method	-		Incorporated in feed ingredients	
No. shrimp stocked	117,500	119,000	121,000	109,000
Density (pcs/m^2)	50	50	50	50

Disease Diagnostics Using Routine Histology and PCR Methods

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Week			1		2		3		4	
Ctrl.	T1-1	HIS	WFD (G1-3)	WFD (G1)	-		WFD (G1)		WFD (G1-3)	
		PCR	-		-		-		EMS	
Ctrl.	T1-2	HIS	WFD (G1-3)	WFD (G1-3)	-		WFD (G1)		EMS (G3)	WFD (G1-4)
		PCR	-		-		-		-	
Treatment	T2-1	HIS	WFD (G1-3)	WFD (G1)	WFD (G1)		WFD (G1-2)		WFD (G1-2)	
		PCR	-		-		-		-	
Treatment	T2-2	HIS	WFD (G1-3)	WFD (G1-2)	EMS (G1-3)	WFD (G1)	EMS (G1)	WFD (G1-2)	EMS (G1-2)	WFD (G1)
		PCR	-		-		-		EMS	

Disease Diagnostics Using Routine Histology and PCR Methods

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Week			5		6		7			8		
Ctrl.	T1-1	HIS	EMS (G2)	WFD (G1)	EMS (G1-4)	WFD (G2)	EMS (G1-3)	WFD (G3)	EHP (G1)	EMS (G1-3)	WFD (G1-4)	EHP (G1)
		PCR	EHP		-		EHP			-		
Ctrl.	T1-2	HIS	WFD (G1-2)		WFD (G2-3)		EMS (G1-2)	WFD (G1-2)		EMS (G1-3)	WFD (G1-2)	
		PCR	-		-		EHP			EMS	EHP	
Treatment	T2-1	HIS	-		WFD (G1-3)		WFD (G1)			WFD (G2-3)		
		PCR	-		-		-			-		
Treatment	T2-2	HIS	EMS (G1)	WFD (G1-2)	EMS (G1)	WFD (G2-3)	EMS (G2)	WFD (G2)		EMS (G1)	WFD (G1-3)	
		PCR	-		EMS		-			-		

Growth Performance, FCR and Survival in Ponds

Parameters	Ctrl. T1-1 (Pond 1)	Ctrl. T1-2 (Pond 3)	T 2-1 (Pond 2)	T2-1 (Pond 4)
Initial weight (g)	1.14 ± 0.03a	1.14 ± 0.03a	1.14 ± 0.03a	1.14 ± 0.03a
Final weight (g)	17.30 ± 2.95	18.63 ± 3.21	23.77 ± 2.85	19.32 ± 3.85
Weight gain (g)	16.10[c]	16.72[c]	22.12[a]	18.86[b]
ADG (g/day)	0.22	0.24	0.31	0.25
Survival rate	75.30%	86.85%	96.44%	78.03%
FCR	1.60	1.38	1.20	1.40

Take Home Messages

⇒ Biosecurity is a **MUST.**

⇒ Keeping a healthy microbiota balance is a **MUST.**

⇒ Probiotics/bioremediation is a **TOOL** - Probiotics need a **jump start.**

⇒ **Functional diets** have big potential.

⇒ **Antibiotics-free** hatchery and growout protocol are both achievable.

⇒ Make shrimp farming more science-based, more predictable, simple, and more **cost effective**.

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