The Importance of <u>Shrimp Health</u> and <u>Breeding</u> in Evolving the Modern Shrimp Industry





Re-Learning What Health Is and Is Not

Has Poor shrimp health been normalized?





- Not Healthy is not "only being dead"
- Lack of Healthy is not always inbreeding
- Lack of Healthy is not always feed issues
- Lack of Healthy is not always management issues
- Consider Environment, Stresses, and Greed as reasons for declining shrimp health



Myth: Low survival in a Hatchery results in your best post larvae (Darwinian Theory)

Bragging rights in Hatcheries "Low Hatchery survival= Strong PI's Cheap cost and sales price" 66The strong survive.99

Fellow Hatchery man circ 1994

Early Shrimp Culture technology in the Americas was based on Large Extensive and Semi-Intensive Farms with minimum controls and no biosecurity

Ecuadorian 20 Ha ponds

Central American 10 Ha Ponds



No aeration, high water exchange rates, few feed rate controls And highly variable profitability

McIntosh goes to Belize 1996; Advise from leading Ecuadorean Farmers – DON'T DO IT!!!!



Healthy Harvest of SPF from Belize

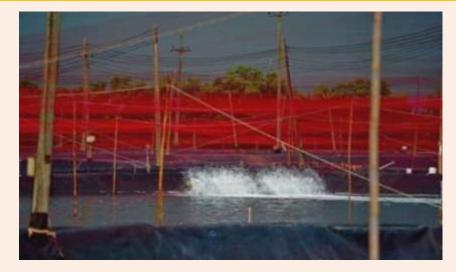


- SPF is weak; has no disease resistance
- SPF grows fast and DIES fast
- Shrimp must have exposure to a wide array of pond pathogens and undergo pond selection pressures to succeed

Needed Biosecurity and good culture conditions for "SPF" to Succeed

Exclusion:

virus/microsporidea

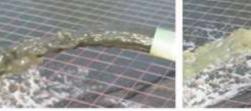


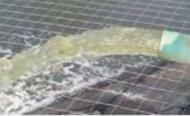


Limitation:

Bacteria/toxin







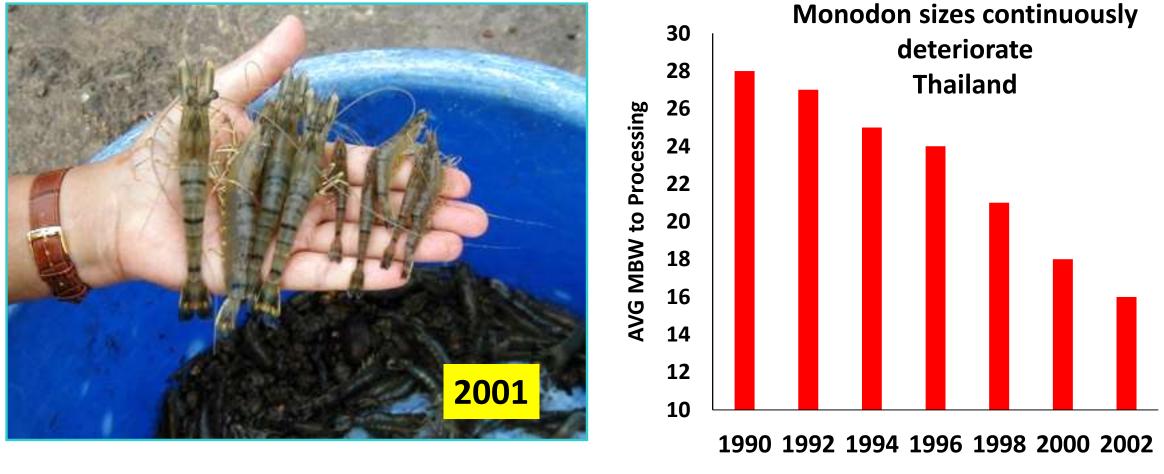
Prior to 2000: All post larvae were from wild pls and broodstock Why did it need to change?



In 1998-1999: America Died of WSSV (Vannamei)

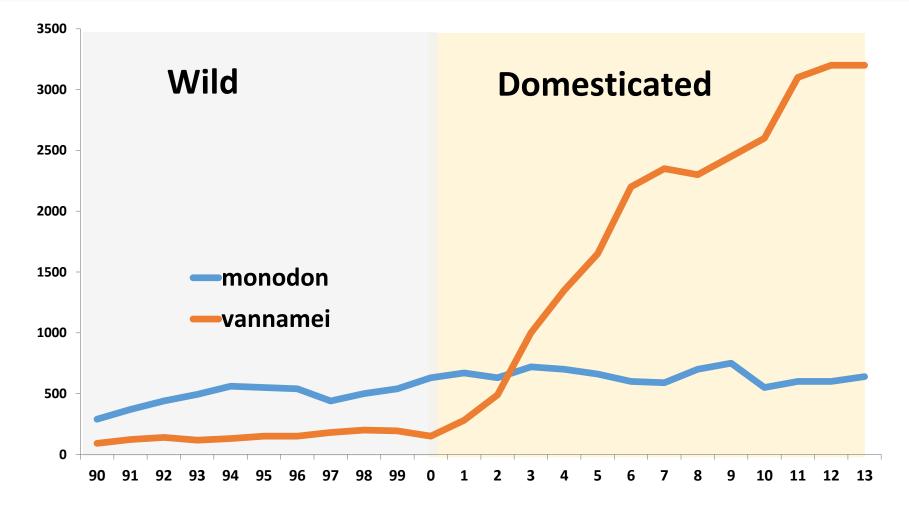


In 1999-2000 Asia lost growth and survival (Monodon)



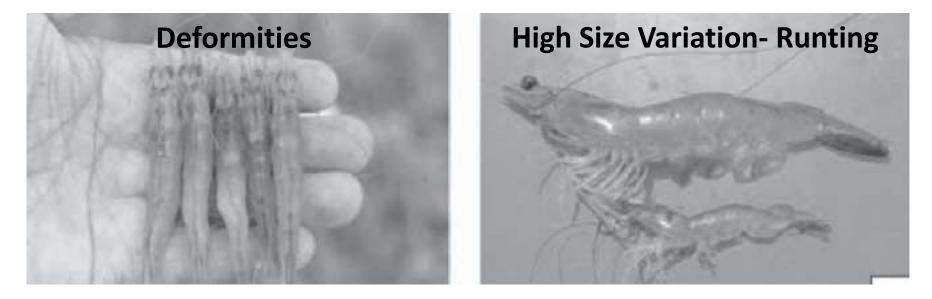
Year

Adoption of Domestication results in surge in shrimp industry growth 2003-2010



2000: The year where domestication became a dominant theme in Shrimp

SPF Concept Developed(1990-1991) in Hawaii Response to introduction of IHHNV



IHHNV introduced and infects post larvae Hawaiian pond production declines

Myth I: Must have an island for SPF

SPF Spreads; and mostly Fail Second Myth: SPF shrimp are Weak



<mark>Taiwan</mark>



Belieze

Texas, USA

Ecuador

Thailand

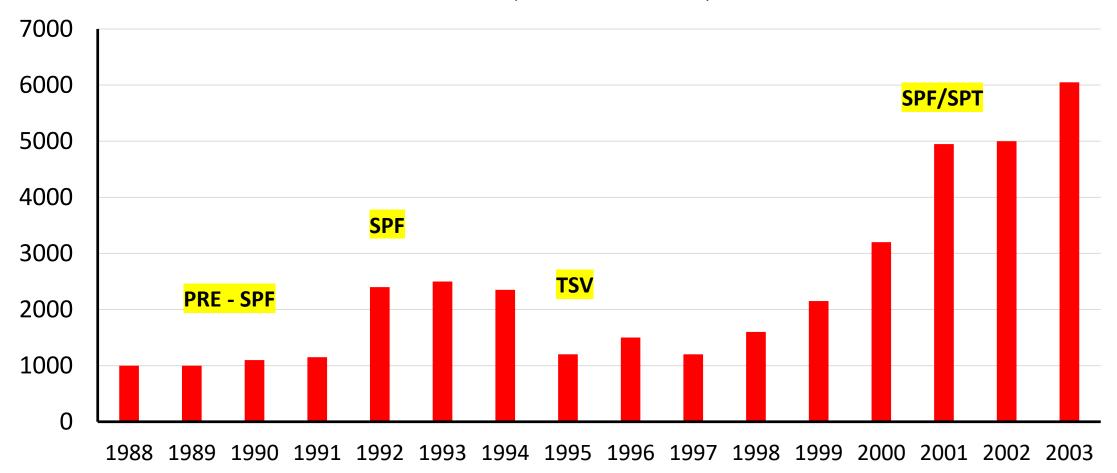
GoogleTH

Texas: Shrimp Farmers succeed, then failed Ecuador: Disaster- TSV wipes out first 500 million pls Belize: BAL successful; NOVA not? Taiwan : Successful (initial) China: Successful (initial) Thailand : Disaster

Hawaii

History of Texas Shrimp Production Demonstrates the strengths and weakness of SPF shrimp model

Texas Shrimp Farm Production History



Healthy Shrimp are not "just" Shrimp without disease BUT

Shrimp with a fully developed immune system to prevent disease

Understanding SPF/SPR/SPT Definition of SPF/SPR/SPT

- Specific Pathogen Free Shrimp refers to the Health Status of a stock and not a genetic characteristic. To be SPF a shrimp should be free of all known shrimp virus (not only OIE listed pathogens).
- Specific Pathogen Resistant Shrimp refers to a Genetic characteristicbeing resistant to infection of a specific pathogen; a shrimp may be SPF and SPR.
- Specific Pathogen Tolerant Shrimp refers to a Genetic Characteristic where the shrimp can get infected but does not express the disease

Important Lesson:

The description SPF refers to health Status only; The shrimp is free of specified pathogens

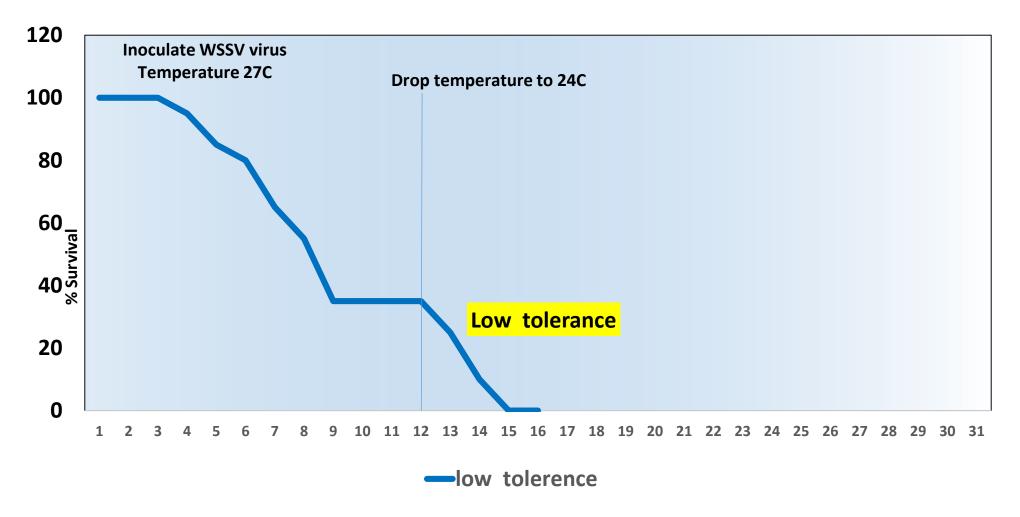
Tolerance to Disease (SPT) is Genetic and must be developed and selected

Both SPF and Tolerance being independent; can be in the same shrimp or not

There can be SPF only—original Hawaiian SPF There can be SPF combined with SPT: There can be SPT only—APE is an example

Tolerance is not Resistance Either SPF or APE: but not Both

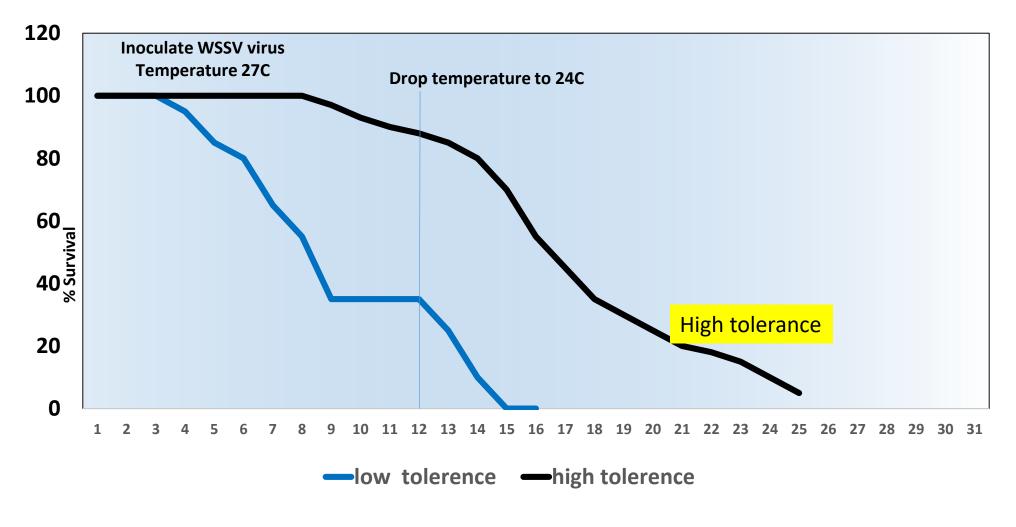
Comparison of WSSV Tolerance and Resistance



Day of Culture

Tolerance is not Resistance Either SPF or APE; but not Both

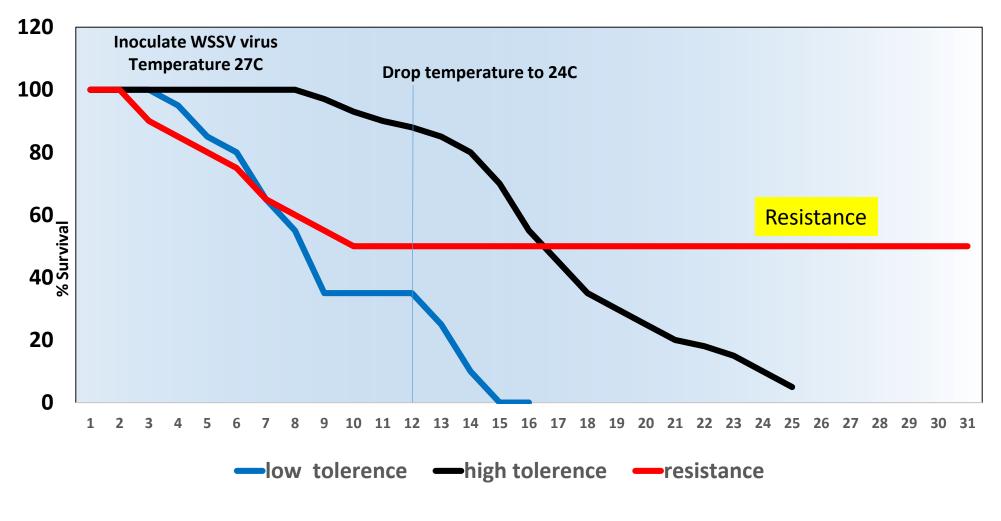
Comparison of WSSV Tolerance and Resistance



Day of Culture

Tolerance is not Resistance Either SPF or APE: but not Both

Comparison of WSSV Tolerance and Resistance



Day of Culture

Domesticated Pathogen free shrimp stocks are essential for efficient genetic selections

Vannamei



CV < 15%



<mark>35 grams in 105 days</mark>

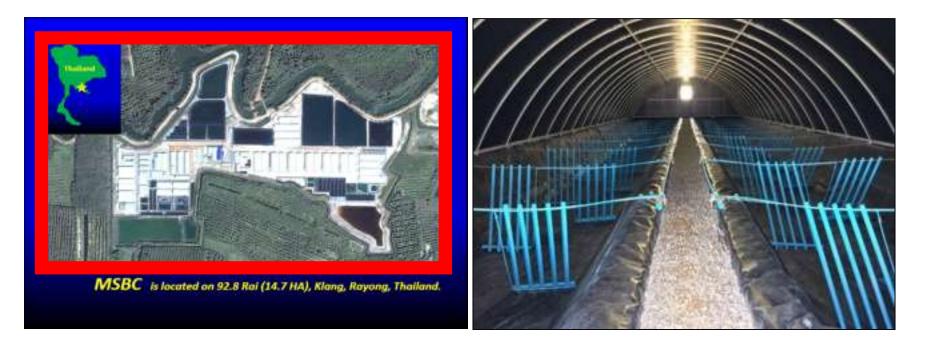




CV <12%

42 grams in 100 days

SPF requires Nucleus Breeding Concepts certified disease free compartments



- **1.** Strict Quarantine for Founders before entry
- 2. Nucleus Breeding Compartment; regularpathogen surveillance
- 3. List of pathogens being surveilled
- 4. Strictest of biosecurity;

Two contrasting methods of initiating SPF populations: Hawaiian and Reverse SPF

Screening from wild sea caught shrimp: quarantine (original Hawaiian Protocol)



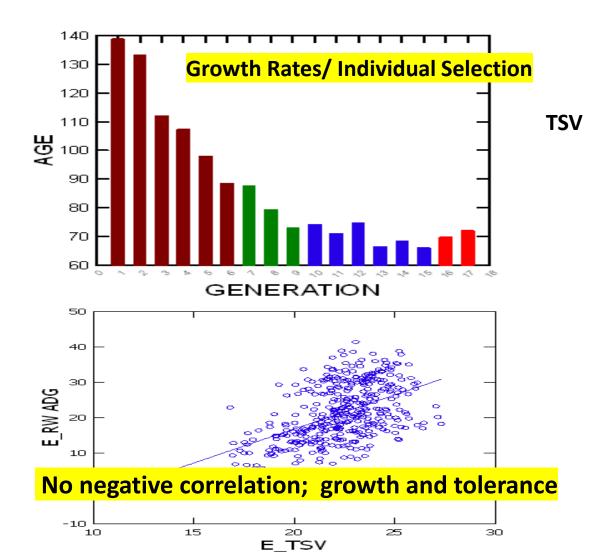
Screening from shrimp exposed to pathogens Many generations: quarantine (referred to as reverse SPF)

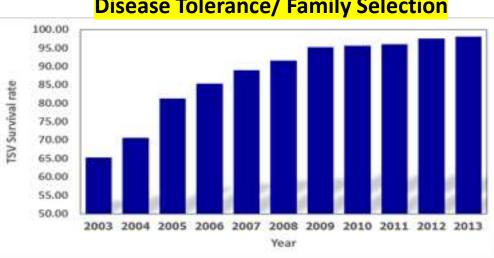


Must select spf from tolerant shrimp

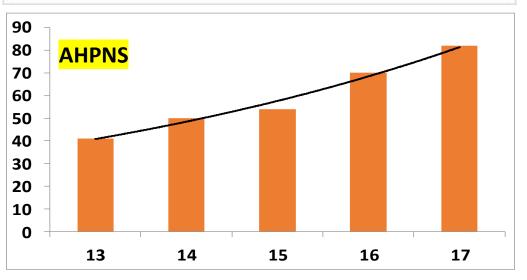
SPF is a good breeding platform: Growth and Tolerance (SPF/SPT)

(all data from CPF vannamei Program/Hawaiian Concept)



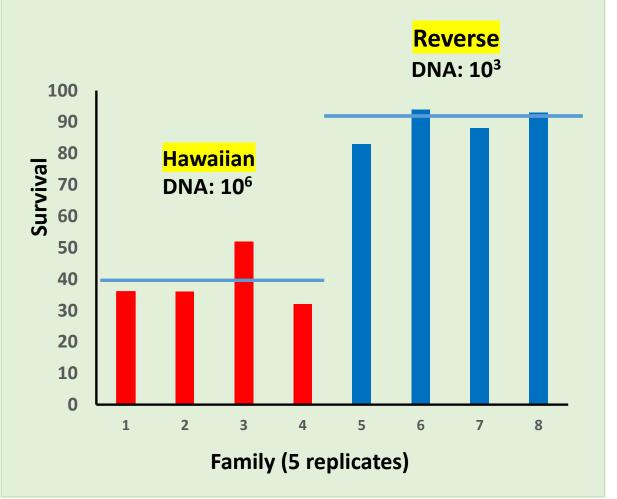


Disease Tolerance/ Family Selection

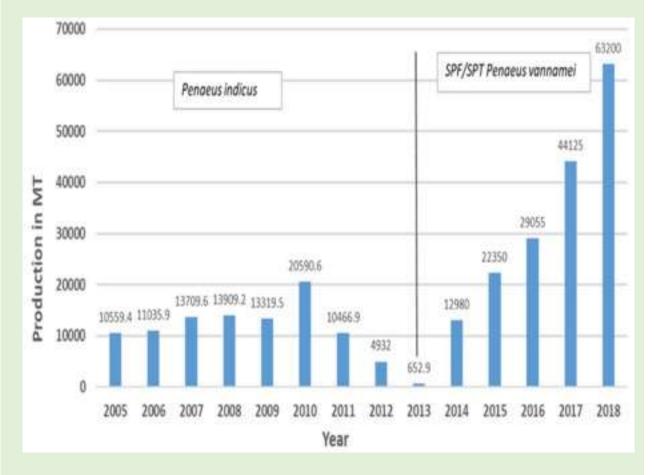


Reverse SPF proves to be an efficient way to create disease free/healthy + disease tolerant shrimp (SPF/SPT)

Thailand : WSSV Lab Challenge



Saudi Arabia: Real World Proof



Specific Pathogen Resistant (SPR): Does it exist?





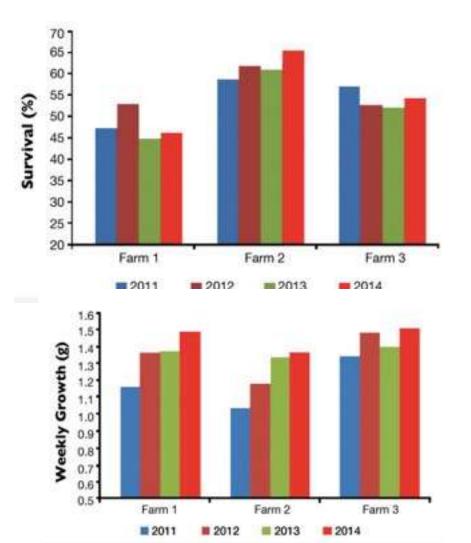
True resistance has been found as a homozygous trait in P. Monodon off Madagascar: but only individuals- entire families

SPT/APE: Effective concept for Low Density (not secure for international Trade)

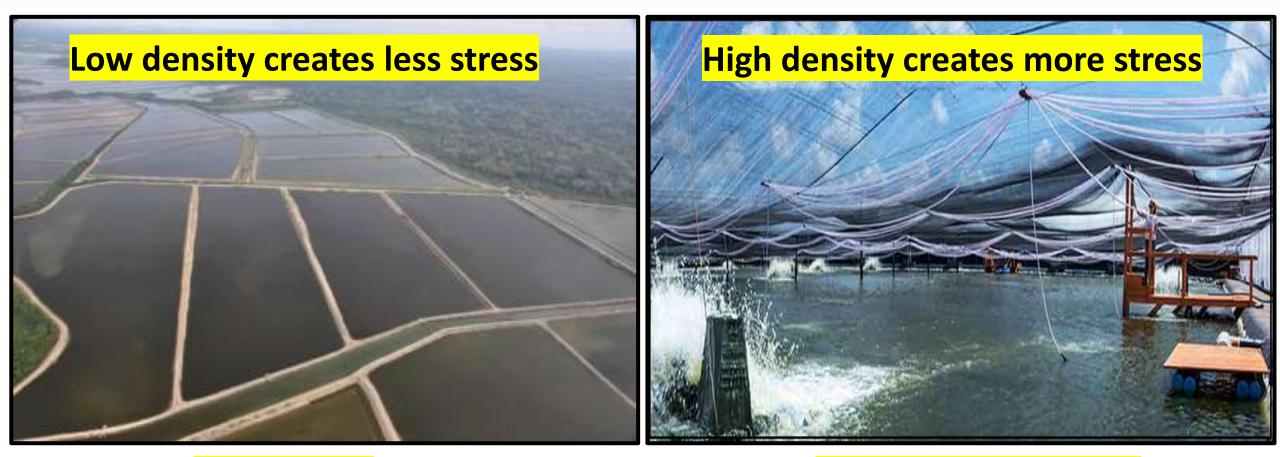
After the 2000 Industry collapse from WSSV Industry recovered using APE domestication



Birds on WSSV dying shrimp in Ecuadorian Pond (small percentage) **Selection from the Pond environment**



Disease (Health) is a function of stress, genetics and the pathogen

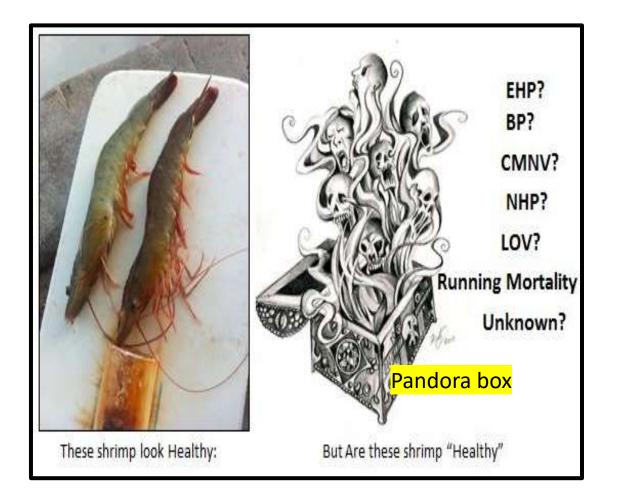


No disease

IMNV; EHP Disease

Camanor Farms; Brazil using same non SPF post larvae

Why Does it Matter: SPF/SPT/SPR



- Only "genuine" SPF guarantees freedom of pathogens
- Shrimp marketed today as SPR; are not SPR— they are SPT and SPT carries pathogens if not also Genuine "SPF"
- The myth persists that SPF is weak and SPR is strong- this is a dangerous and wrong idea

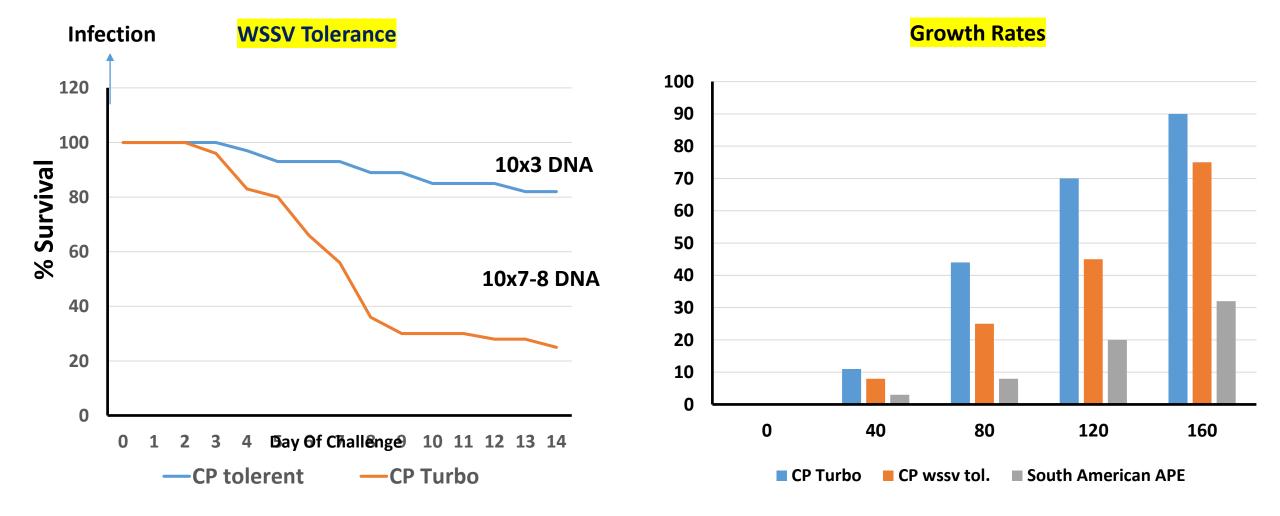
Is it True?

"APE" survives better against EMS - SPF dies much faster?

Parameter	APE I	APE II	APE III	SPF	SPF +
AHPNS CHALLENGE	21	60	55	62	78
MBW (DOC 35)	3.6	2.5	2.2	4.8	4.8
MBW (DOC 80)	17.5	15.5	13.5	29.6	29.6

+ =immune primed

Combining APE tolerance with SPF Health and Growth



SPF applies to Monodon as Well!!!



Before: 2001







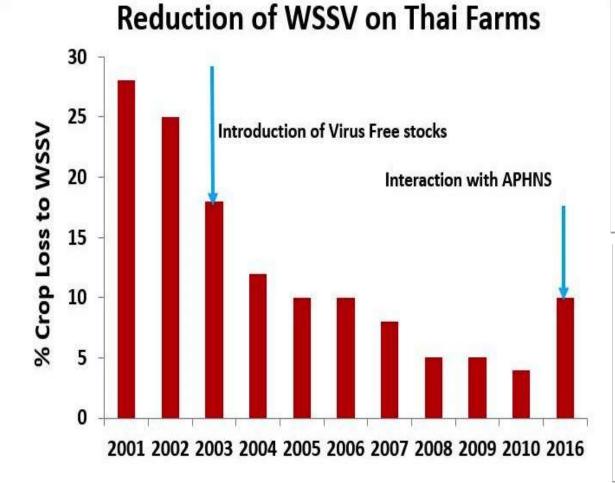
CPF thinking and Processes



CPF executives searching for Island to locate original SPF nucleus in Thailand (2002)

- Dead Shrimp don't grow
- Growth is important for lower costs (FCR, Capital,)
- Size Matters
- Never Never Ever sell or give a shrimp to any farmer that contains a pathogen-
- Broodstock and post larvae are major responsibilities

Pathogen Free Animal STOCKS A MUST FOR Heathy shrimp and is the Basis of Biosecurity











Nucleus Breeding in disease free compartments is the Only WAY

Pond broodstock are not cheap WSSV IHHNV TSV Healthy appearance but INFECTED INBRED

Pond Reared Appears to be CHEAP

Nucleus SPF Broodstock are not Expensive



But for sustainable and "intensified " systems only pathogen free will succeed

Thailand adopts Nucleus Breeding for SPF 2003



- **1.** Strict Quarantine for Founders before entry
- 2. Nucleus Breeding Compartment; constant pathogen surveillance
- 3. List of pathogens being surveilled
- 4. Strictest level of biosecurity

Marine Shrimp Broodstock Program requires Multiple Facilities



Nucleus Breeding Should have constant Surveillance of all known/possible pathogens Not Just OIE pathogens



CHAROEN POKPHAND FOODS PUBLIC CO., LTD

135/1 M.8, Nongkhanan, Mueang District, Phetchaburi 76000, Thailand Establishment standard : Sor-Aor. 3 No: TH 7623160002

CERTIFICATE OF ANALYSIS

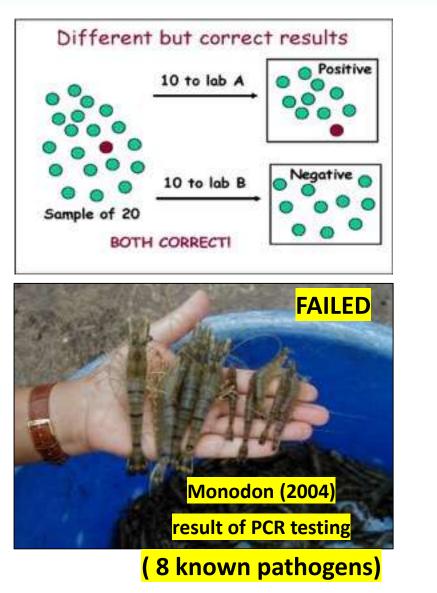
Year	No. of Sample												. li	All Disease			
	IHHNV	IMNV	TSV	wssv	YHV	EHP	AHPND	ннрв	DIV1	HPV	CMNV	BP	MBV	MrNV	No. of Sample	% Negative	% Positive
2013	604	604	604	604	604	6,113	60								9,193	100%	0%
2014	873	873	873	873	873	5,928	840	(((l i	11,133	100%	0%
2015	654	654	654	654	654	3,955	900								8,125	100%	0%
2016	512	472	472	472	472	3,174	736								6,310	100%	0%
2017	457	432	433	434	435	4,607	1,326								8,124	100%	0%
2018	467	442	442	467	467	4,287	1,262	680	114	680					9,308	100%	0%
2019	2,613	1,156	1,156	2,984	2,613	5,439	3,780	2,306	688	2,642	19				25,396	100%	0%
2020	3,363	2,801	2,801	3,363	3,363	6,525	5,189	5,020	886	6,801	30	20			40,162	100%	0%
2021	1,510	1,510	1,510	1,510	1,510	4,119	2,469	2,591	268	3,934	40	50	40	40	21,101	100%	0%
Total	11,053	8,944	8,945	11,361	10,991	44,147	16,562	10,597	1,956	14,057	89	70	40	40	138,852		

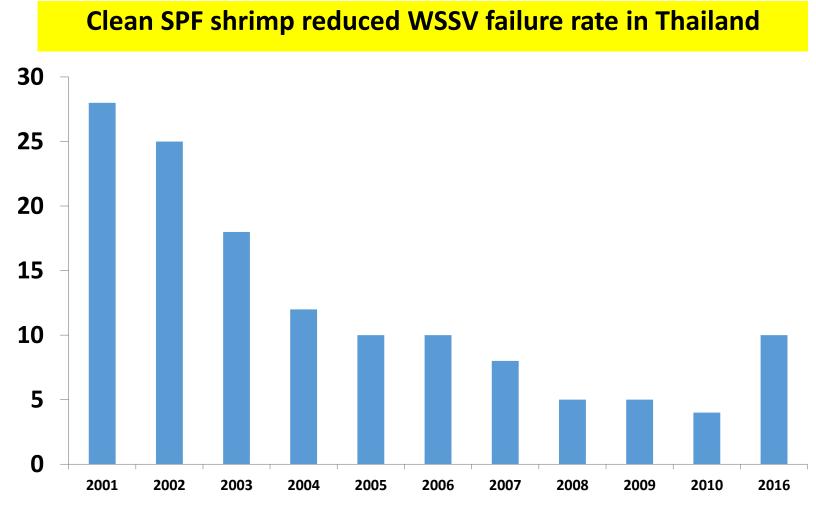
This is certify that the processed white shrimp broodstock quality analysis which listed above has been analysed by CPF Central Laboratory



SPF is a process and not a PCR test result

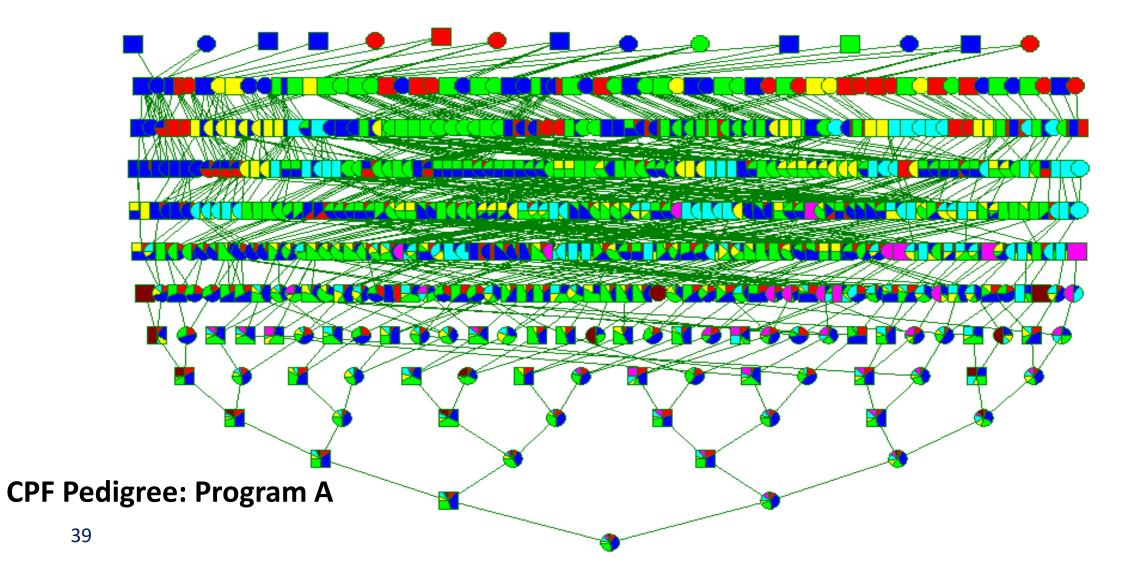
PCR only + short term quarantine does not replace SPF shrimp





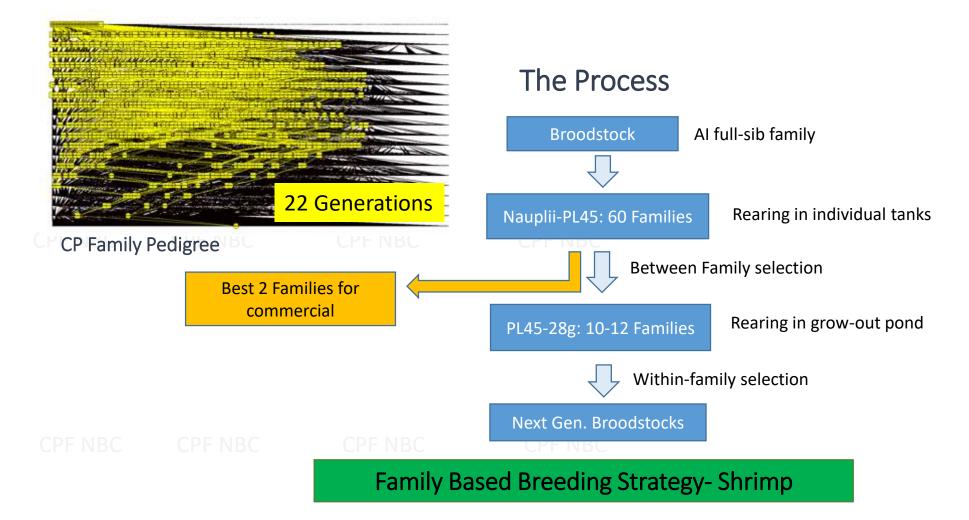
Before PCR tested wild broodstock and post larvae

Pedigrees define a breeding program



CPF Genetics breeding program

CPF NBC CPF was the first company in Asia to develop and operate SPF genetic centers and broodstock multiplication centers; starting operations in 2003. Since operations began CPF broodstock have been maintained disease free.



How to breed for multiple characteristics: Use of Indexing

Indexing weight depends on the needs of Farmers

Years	Growth	TSV	APHNS	WSSV	Robustness	Reproduction
2004-2007	++	++++				+
2008-2012	++++	++			++	+
2013-2017	++		++++		+++	+
2018- present	+++		++	++++	++++	+

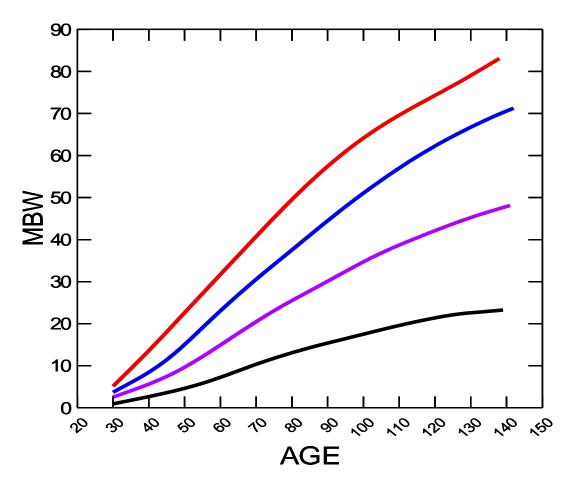
Turbo Pl was Born: 2008



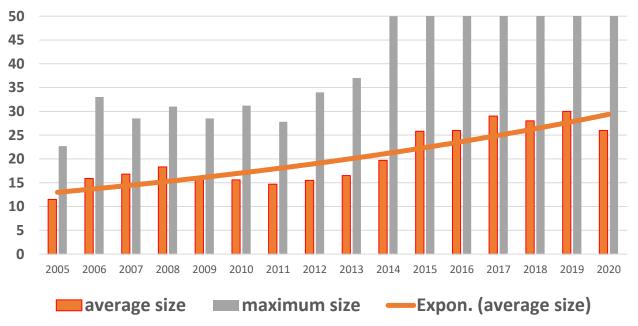
"DEAD SHRIMP NEVER GROW FAST"

Growth Improvement and Farm Performance

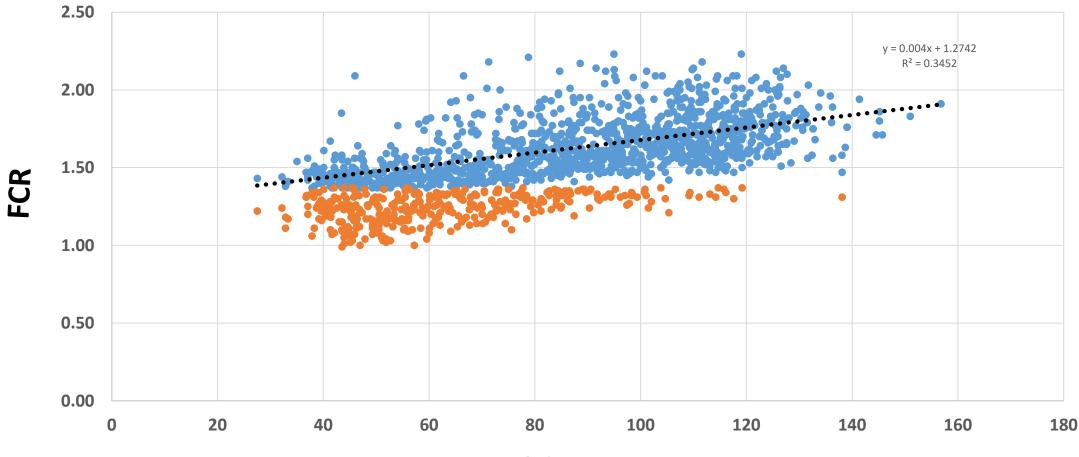




Thailand Farm Performance

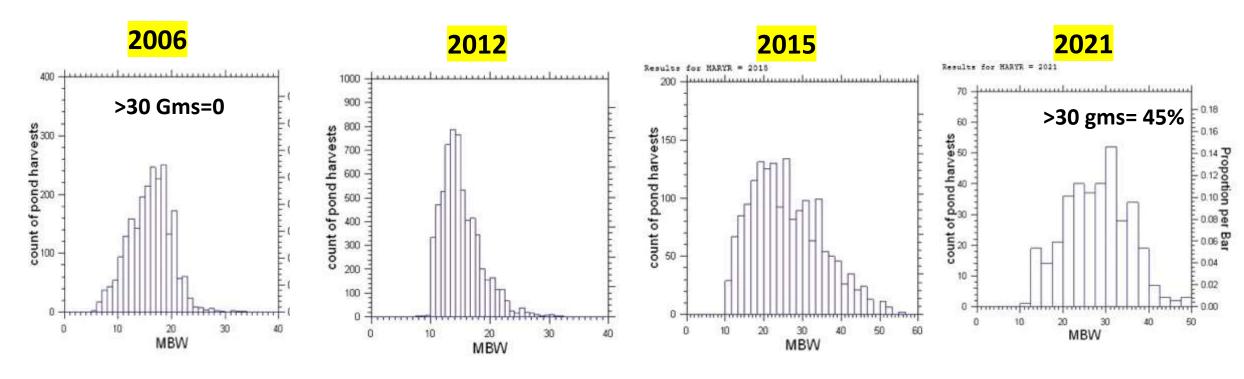


Growth Rate Improves FCR (assuming survival stays constant)



DOC

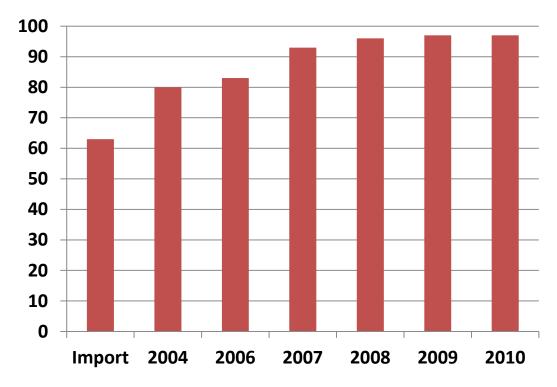
Harvest Sizes have increased over the time of the program providing higher farmer values



30 gms= 0 percent

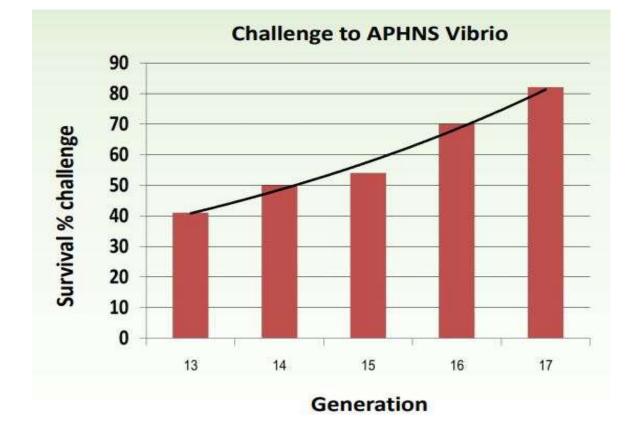
>30 gms= 40%percent

Disease Tolerance: Laboratory challenge

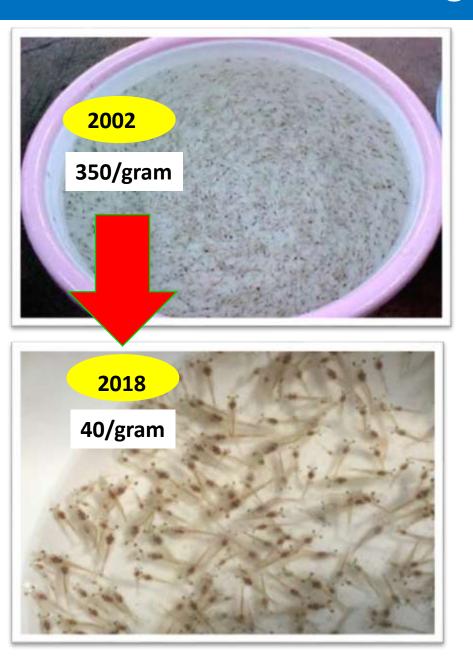


TSV >99%

EMS> 80%

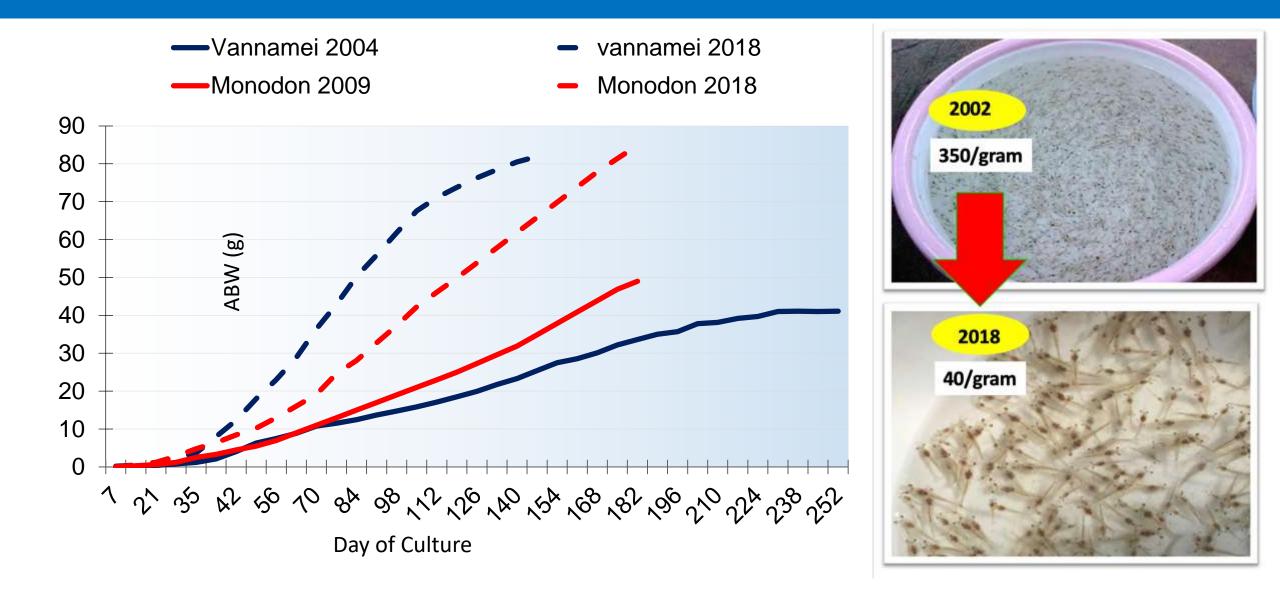


Changes in Turbo over the Years



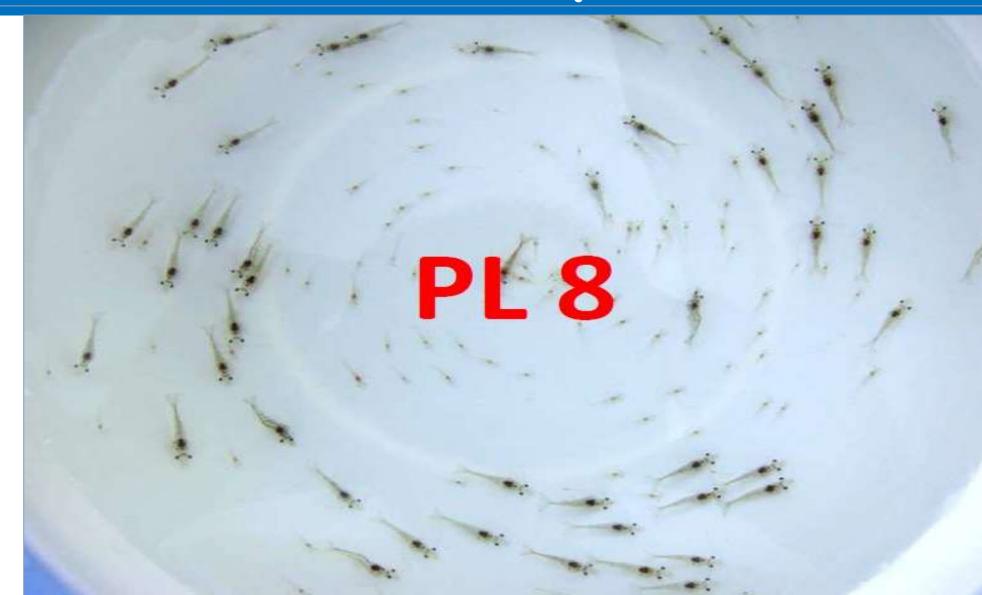


Maximize Growth and Survival depends on the "E" in GxE.



GxE in Hatchery

Same larvae : grown in different conditions



CPF "Turbo" requires Pond Biosecurity, good pond Conditions

-5-

New Farm in Central America

윾

50.00

Farmers do not buy Broodstock; Farmers buy Post Larvae



Modern hatchery is modularized: **Consistent operations and production**





Disinfection of Water and Air become essential with todays environment

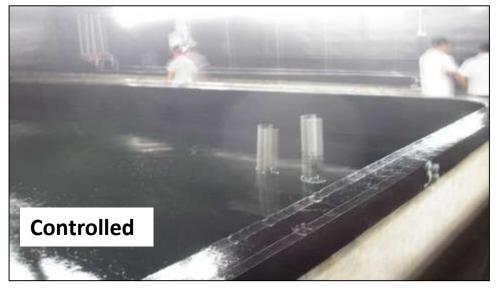


Today with the challenge of fungal and parasite spores; new cost effective technologies are required

Maturation Operations require pathogen free feeds

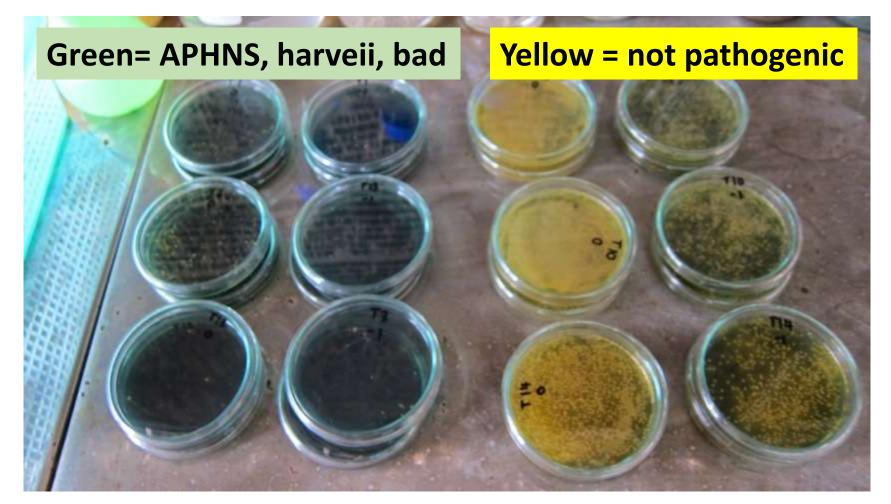






EFFICIENCY= 30-45 MILLION NAUPLII/1000 FEMALES/DAY

TCBS Monitoring is Good QC

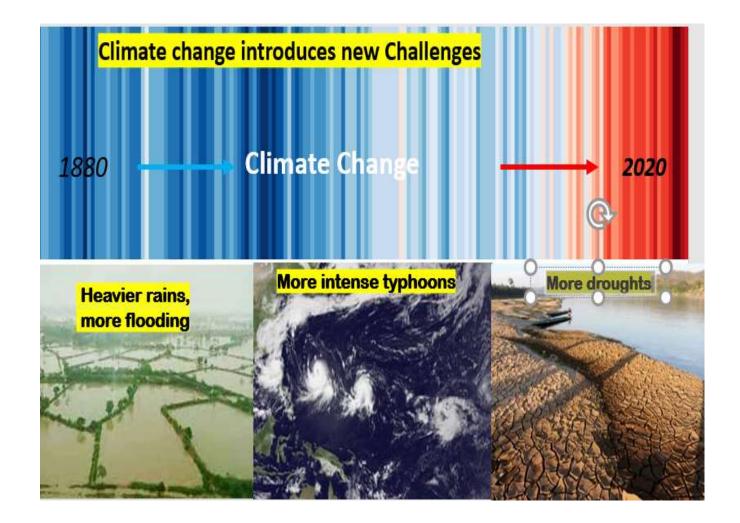


<10³/gram pl

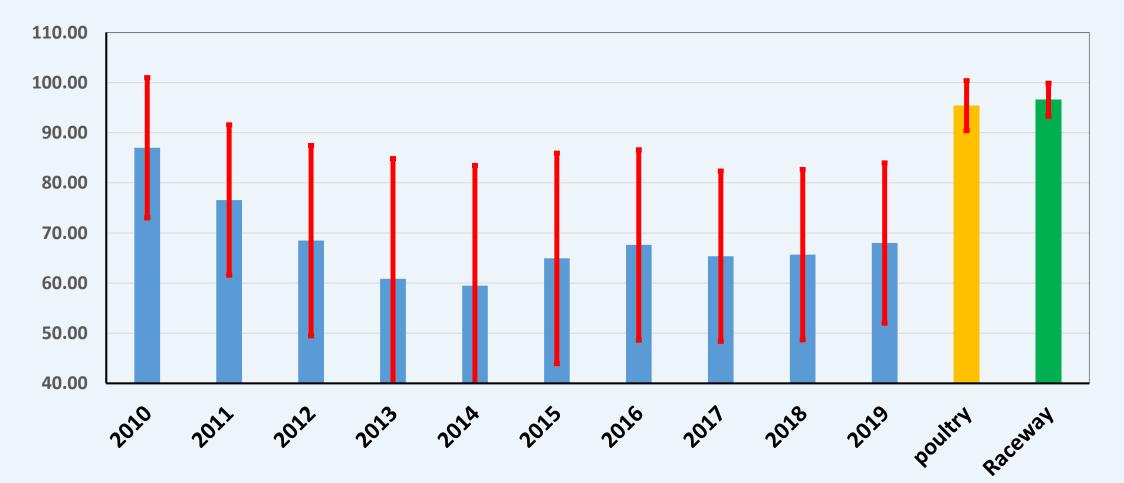
< 10⁴/gram/pl

The FUTURE

- Environmental and Climatic Change
- Increasing Prevalence of DISEASE
- Necessity for Efficiency
- Balance of Markets and Consumption
- New Technologies



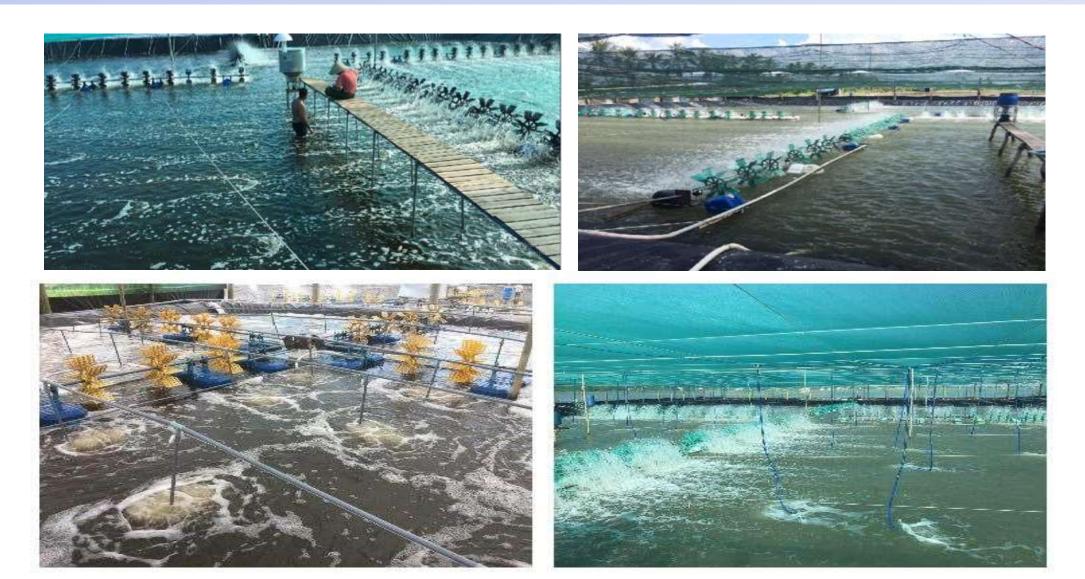
Survivals can be Improved: Improving Immune and Culture systems



Increasing Environmental Stresses



Trend to smaller, more intensive ponds for additional control (Vietnam, Thailand, China)

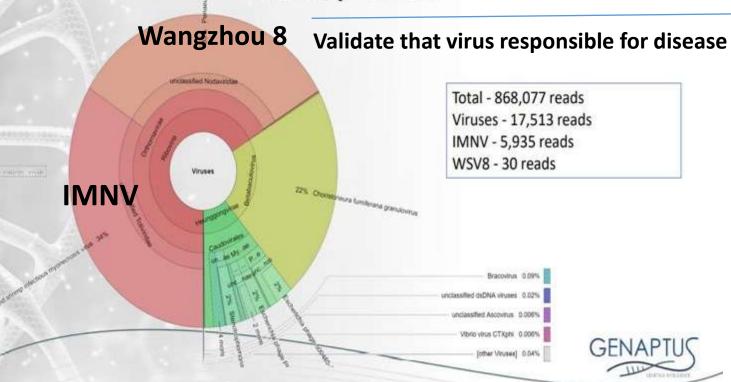


Metagenomics is changing the way we

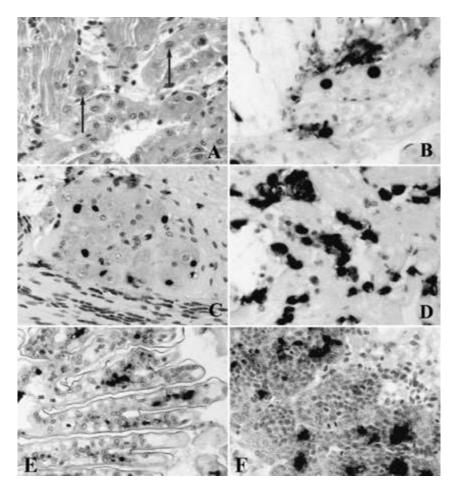
identify new emerging pathogens and define microbiology of "healthy environments"

Metagenomic Taxonomy Blast

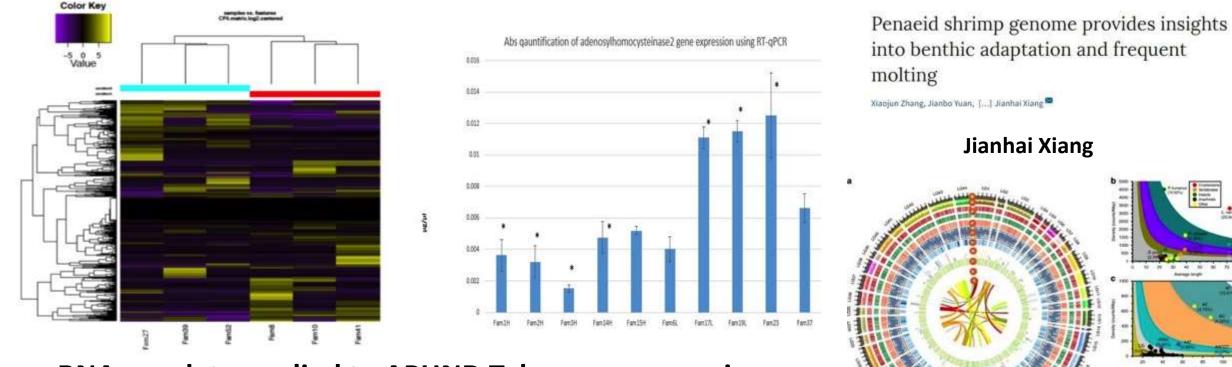
Evaluation of a pool of 15 symptomatic animals by RNAseq - Viruses



In-Situ Hybridization



Genomics will increase the speed and accuracy with which we apply genetics to low heritability traits (disease stress tolerance, and robustness)

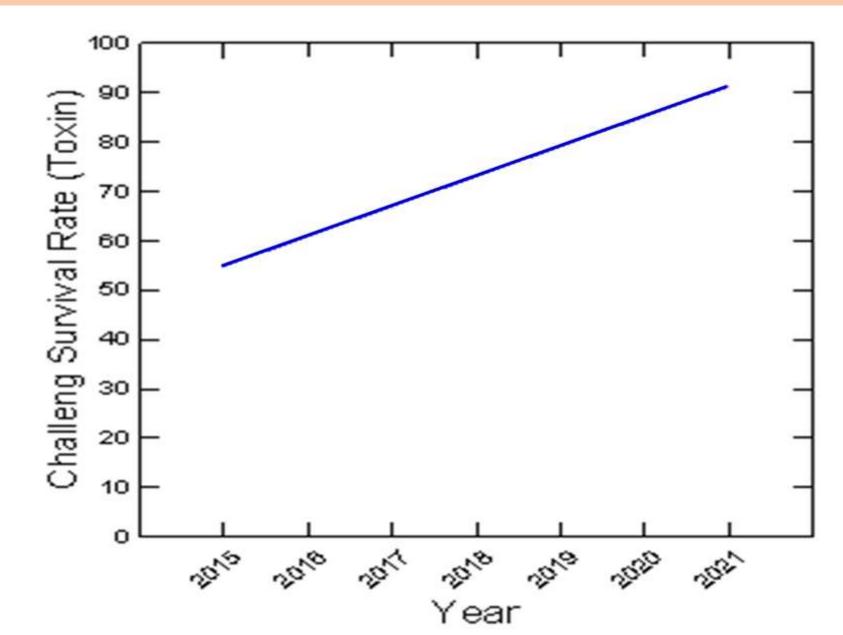


RNA seq data applied to APHND Tolerance expression

Dr. Anchalee Tassanakajon

Genetic Epigenetic selection can increase survival (robustness) of shrimp under stress

Increased tolerance to APHNS toxins over generation of selective breeding in the presence of NO₂ stress

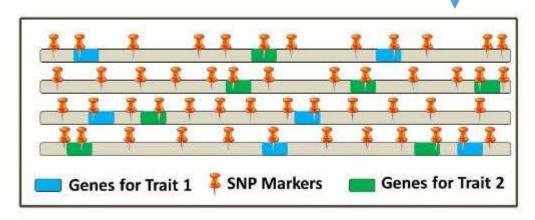


2022: Introducing CP KONG: more robust, wssv tolerence

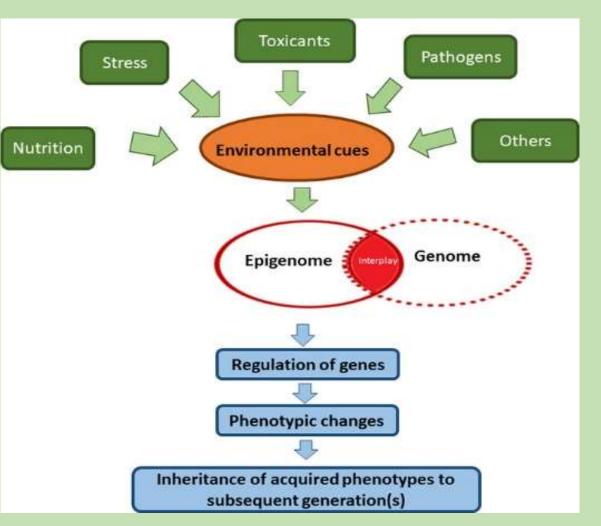
Increase robustness through selective Genetics/ family and individuals



Classic challenge provides Inputs to develop multi trait SNP chips



Increase tolerance through manipulation of the genome (epigenetics)



C.P. KONG WSSV tolerance with Greater Robustness

• Growth Rate: 15 gms 0.17 (85) 30 gms 0.29 (105)

Requirements :

- Less Biosecurity and Pond controls
- Oxygen> 5.0
- Best when stocked <40/m2



Reminder: This is Healthy

Healthy Post Larvae



Healthy Shrimp









Holistic Approach for Success

