Clearing WSSV and all other pathogens

- **Understanding biosecurity at farm level**

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TCRS Shrimp SUMMIT
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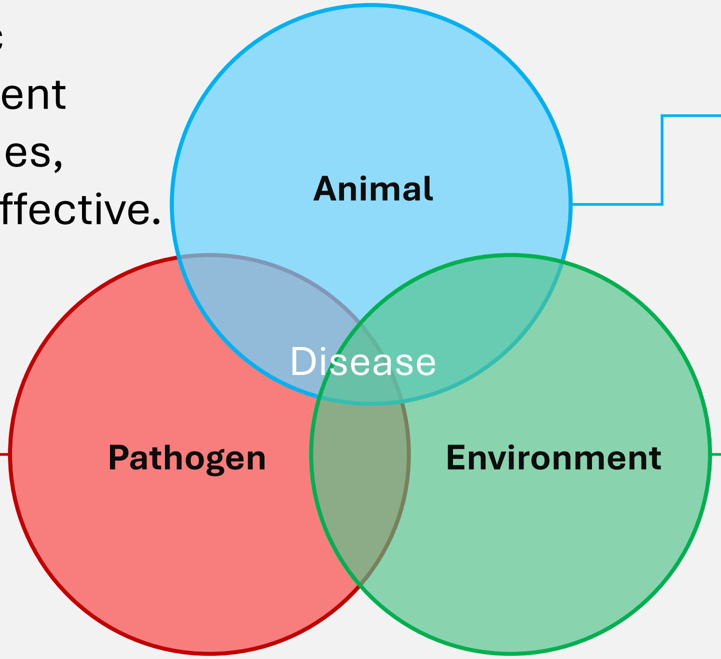
What is biosecurity in aquaculture?

PMP/AB (FAO): Biosecurity is the **cost-effective** management of risks presented by **pathogenic agents** through a strategic approach at **farm, national and international levels** with shared **public and private responsibilities.**

Layers
International
National
Farm Level

Economic Frame
- Cost efficiency
- Profit protection

The objective is to reduce the economic impact of diseases and protect investment and profit. It is about combining strategies, focusing on main risks and being cost-effective.



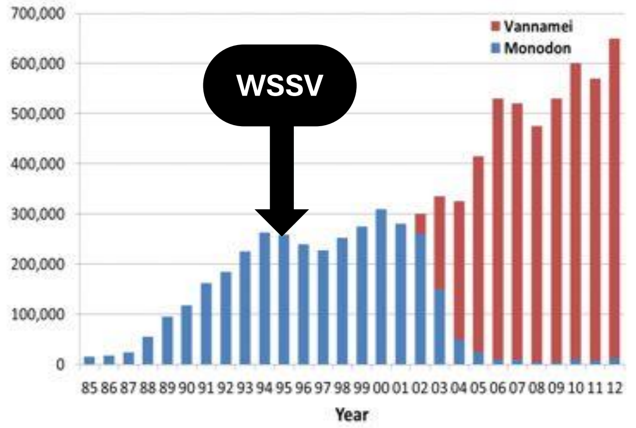
- Health status: SPF, “clean seed”
- Genetics: tolerance, resistant, susceptible
- Epigenetics
- Vaccines
- Nutritional status
- Growth
- Etcetera

- Translocations
- Transmission
- Diagnostics
- Pathogenesis
- Surveillance program
- Microbial management
- Cleaning and disinfections
- Exclusion of pathogen carriers
- Dry out and fallowing
- Etcetera

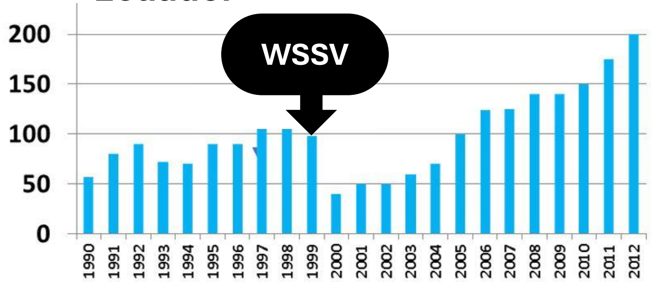
Culture conditions
- Husbandry: welfare
- Infrastructure: tanks, ponds, shrimp toilets, aerators
- Feed and feed management
- Water and bottom quality
- Densities
- Hatchery hygiene
- Etcetera

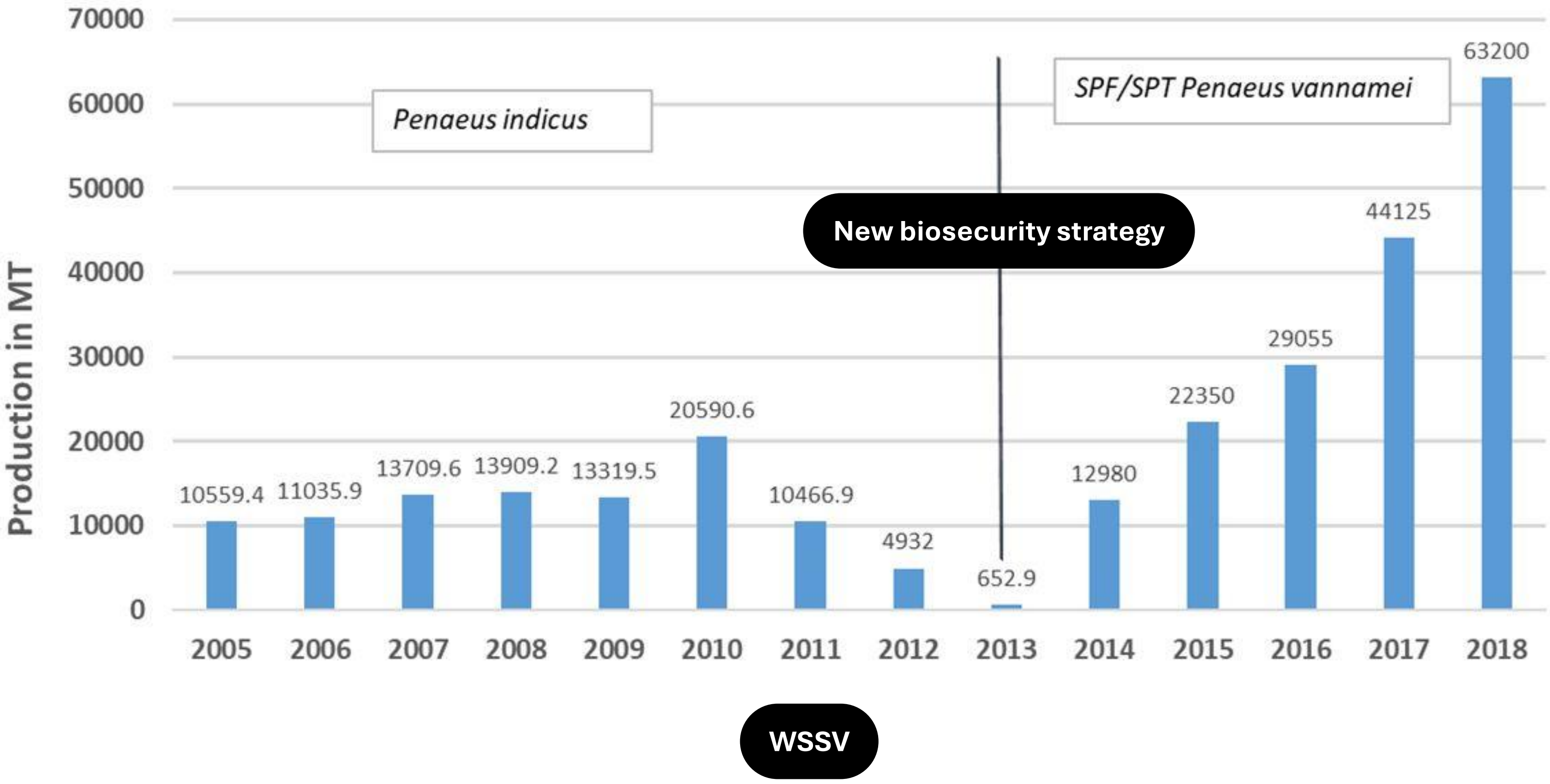
WSSV and shrimp production

Thailand



Ecuador

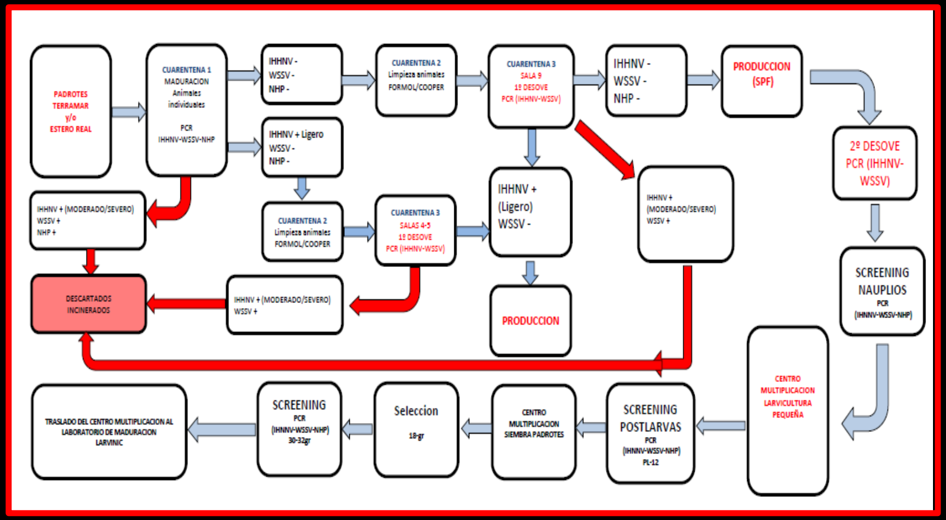




Cleared: WSSV, BP, IHHNV, HPV, TSV

“Reverse” SPF development

- Concept based on the Accommodation hypothesis (Prof Flegel)
- 10 years of naturally developed WSSV tolerance (Also to IHHNV, TSV, BP, NHP)
- Prior to the introduction of EMS and EHP (already present in Asia)
- Selection from APE ponds at 23-30gr from a wide geographic area
- Individual reception and cold challenge: nested PCR
- Testing for endemic (individual) and exotic pathogens (pools)
- Histology of broodstock after spawning
- 2 years of continuous negative results
- External certification
- New product: SPF-SPT/SPR



Pillars of farm Biosecurity

Animal quality

1. SPF/SPT breeding program

Diagnostic tools

1. Surveillance for primary pathogens
2. Animal health monitoring
3. Clinical sign diagnosis (histopathology)

Data analysis

1. Syndromic surveillance

Breeding program components

Health status

- SPF: animals, facilities and procedures
- Government inspection and sampling every 4 months to achieve annual certification

Husbandry

- Optimization of culture conditions and performance (target minimum survival over 85% in each step) = “welfare”

Genetic selection

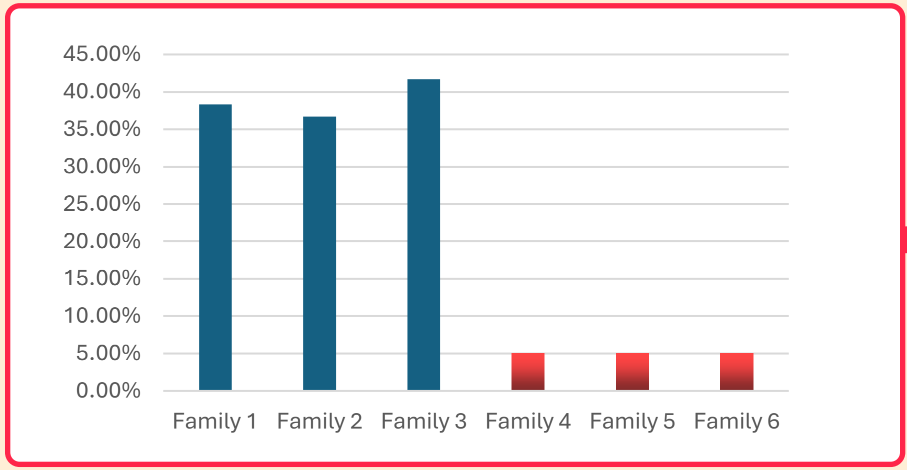
- Up to 2021, we tried to maintain maximum genetic diversity because of lack of tools for selection
- Construction of Challenge Facility
- BLUP based selection: started 2021
- Two criteria: growth and WSSV tolerance: survival and viral replication (next trait: growth at high salinity)
- Molecular markers: in development

Epigenetics (heritable): mainly Broodstock and larval stages

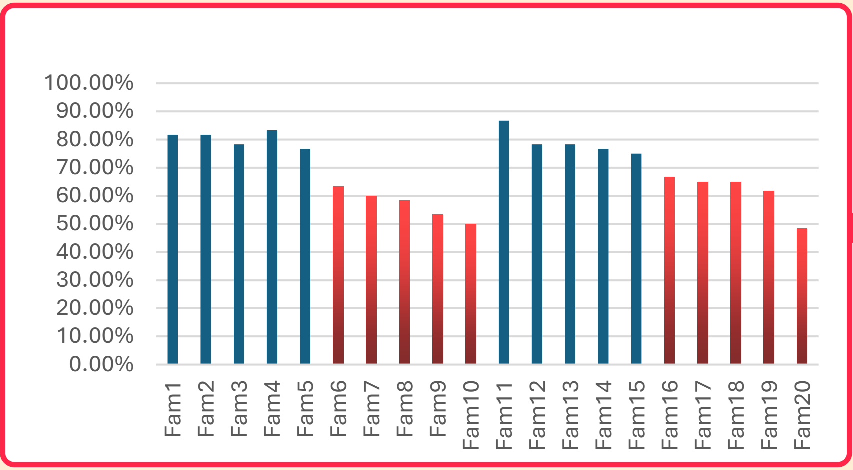
- Heat Shock Proteins (HSP) triggering: Robustness
- Specific pathogen tolerance

Improving growth without losing tolerance

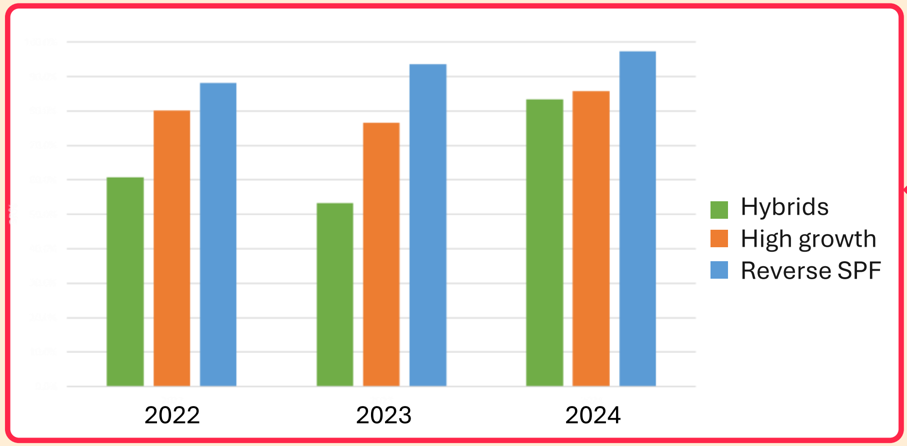
Reverse SPF vs fast growth SPF



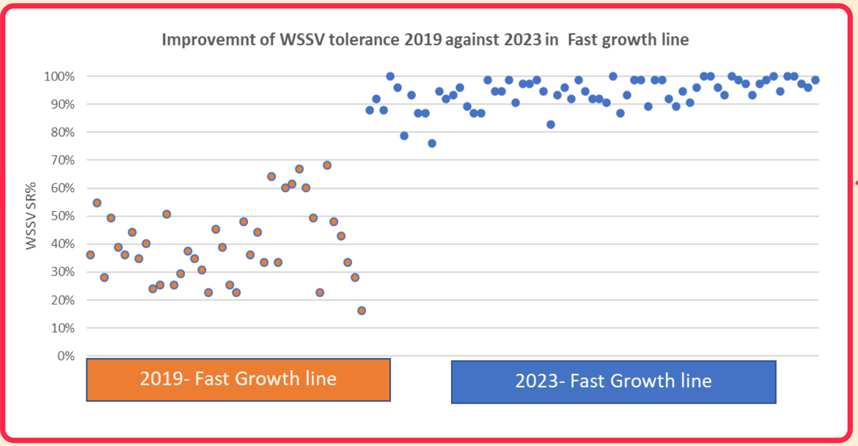
Hybrids vs Reverse SPF



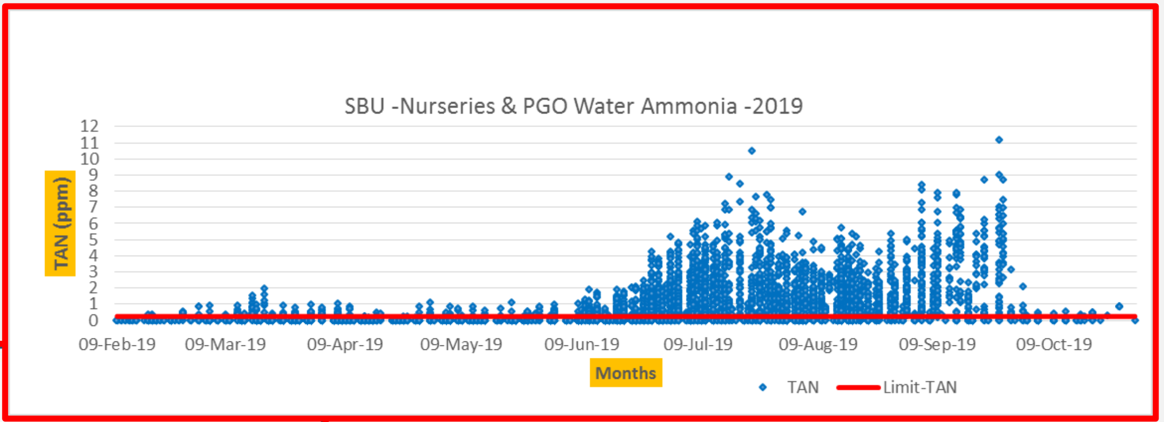
Three breeding programs

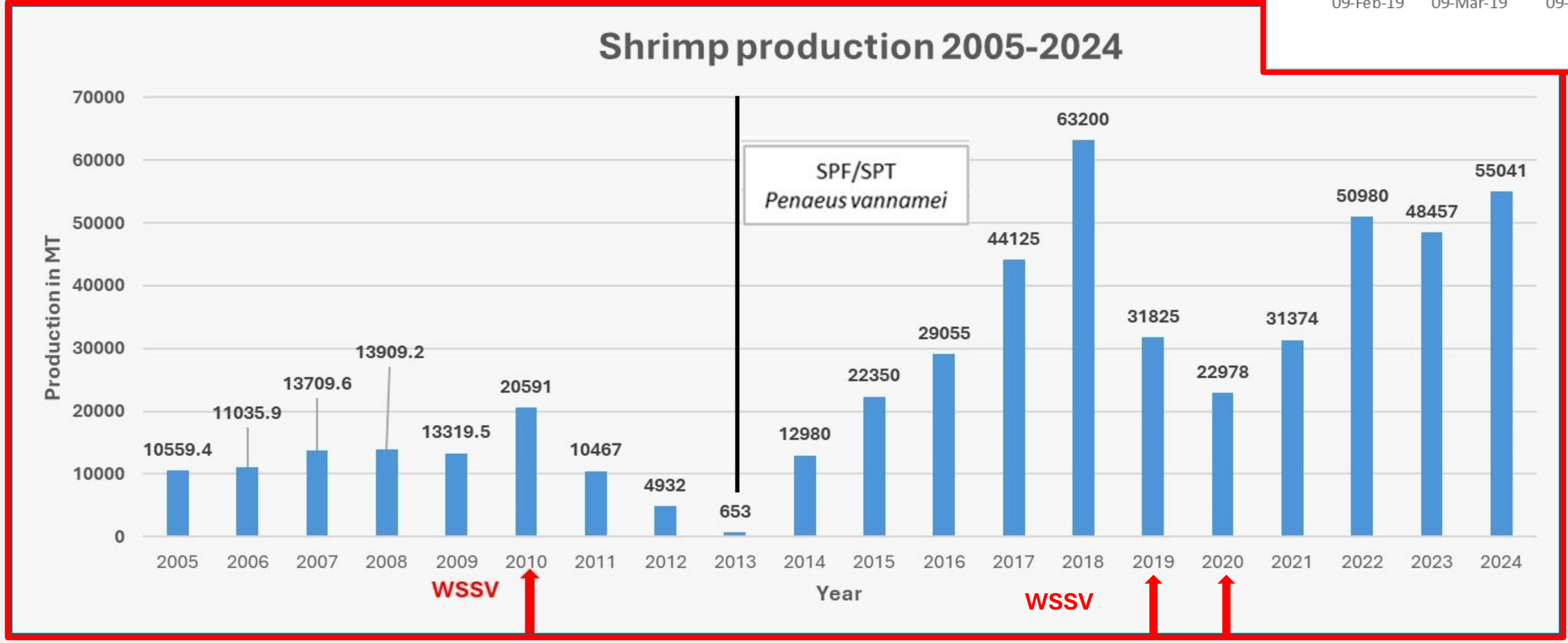


Hybrids 2019 vs hybrids 2023



Genetics does not solve poor management





Ammonia levels in nurseries up to 12ppm (intensive culture)
Leading to immune depression

No impact in GO ponds

Animals do not get infected even if WSSV is in the "environment"

No biosecurity

Abuse of the system

Improving WSSV tolerance (Biotec)

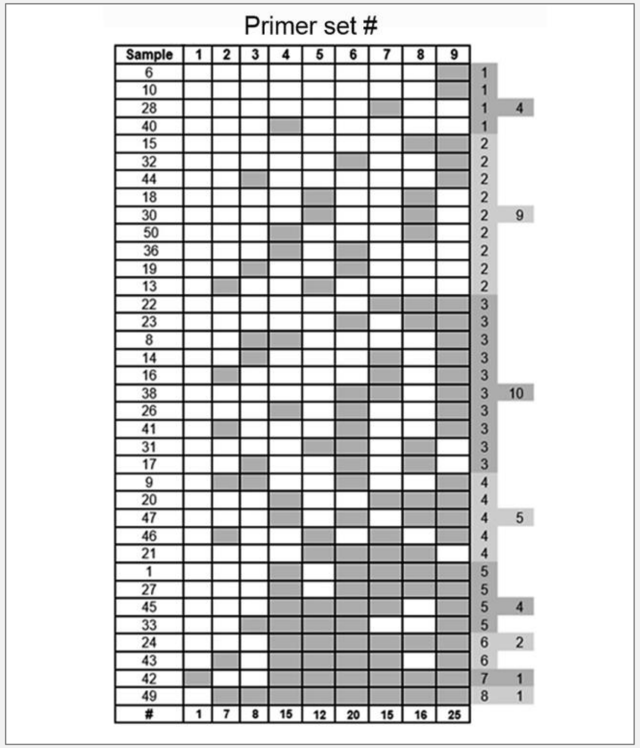
Objective: Identification of protective EVE's of WSSV that produce cvcDNA against WSSV.

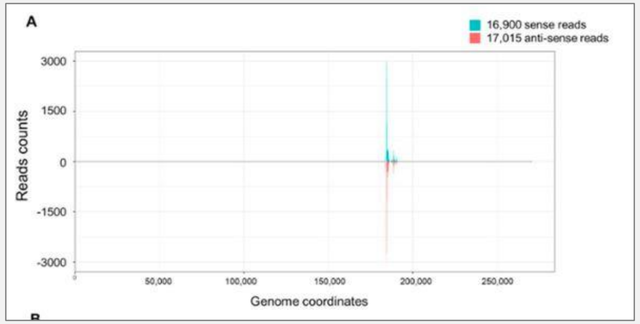
Applications: The identification of protective cvcDNA opens the possibility of developing molecular markers for WSSV tolerance and vaccines.

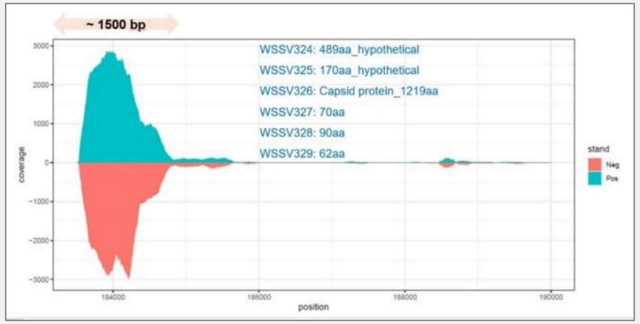
Many WSSV EVEs have been detected in Naqua SPF/SPT stocks. Surprisingly most come from a small region of the genome (1500bp). Considering the whole length of the genoma (>300,000 bp)

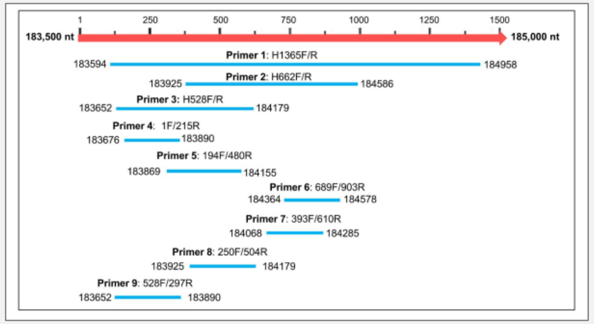
EVEs from the same region have been found in WSSV tolerant *P. monodon*

Next step: Understanding the heritability and expression



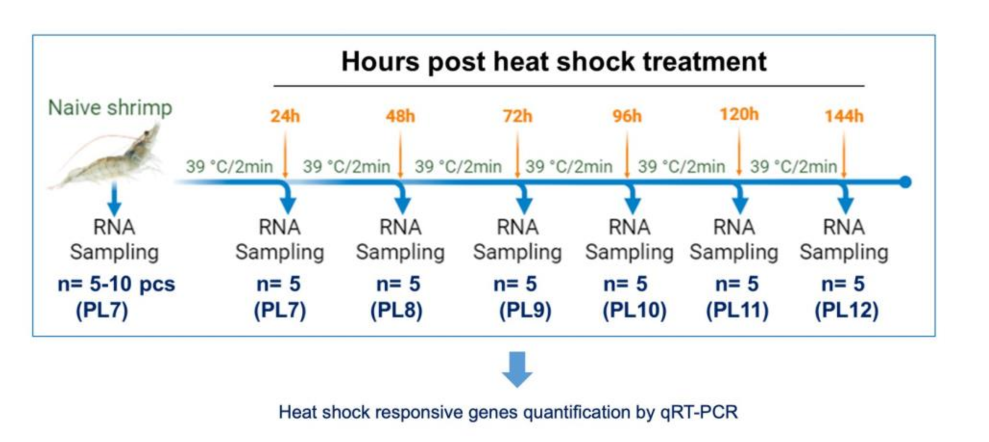


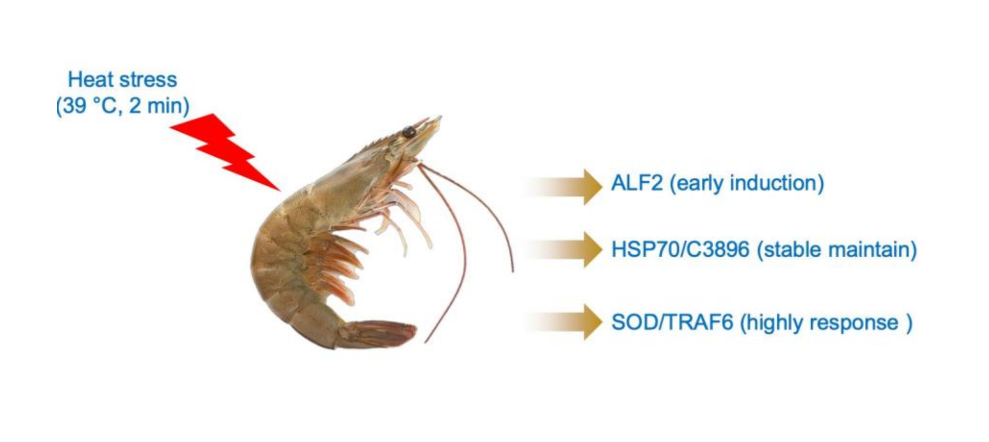




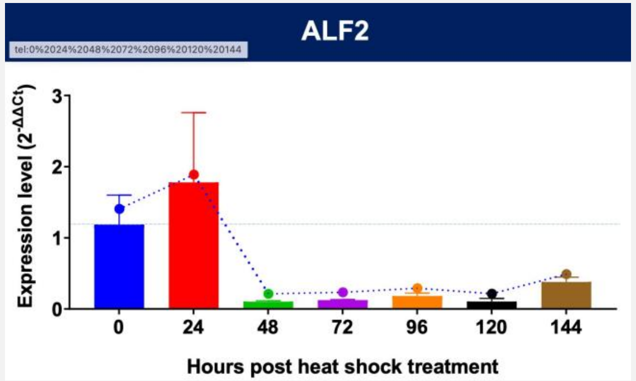
Heat shock treatment validation (Centex)

Prof Bossier, Ghent University

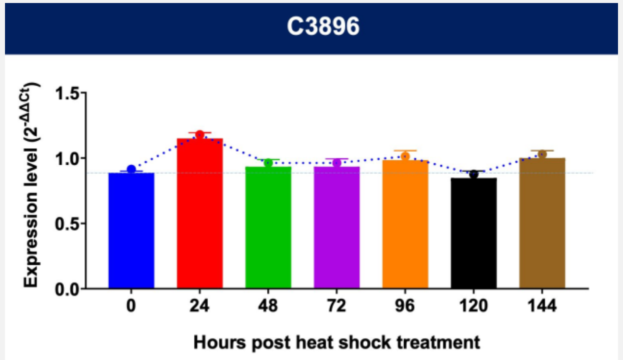


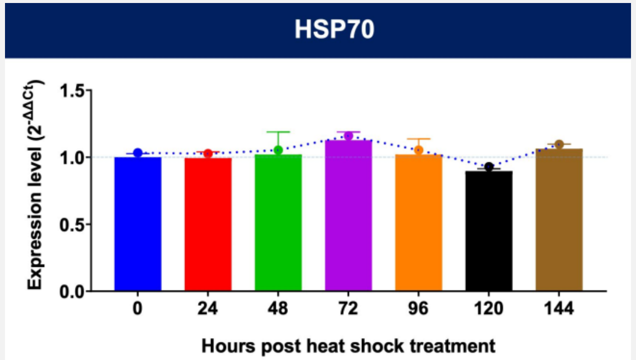


Early induction

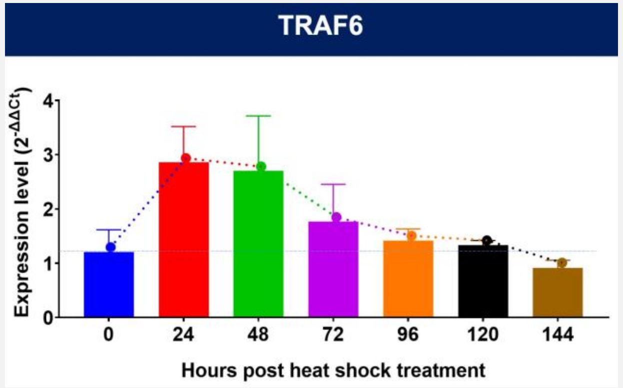


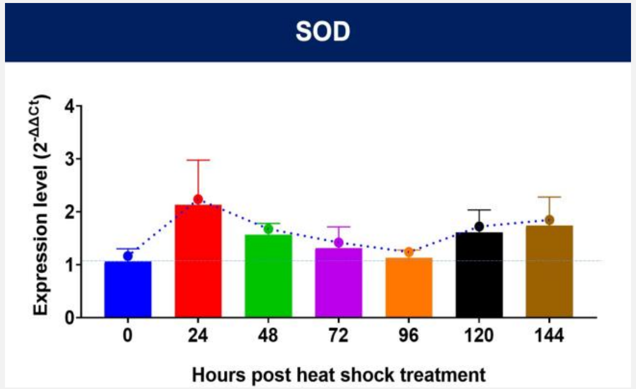
Maintained response



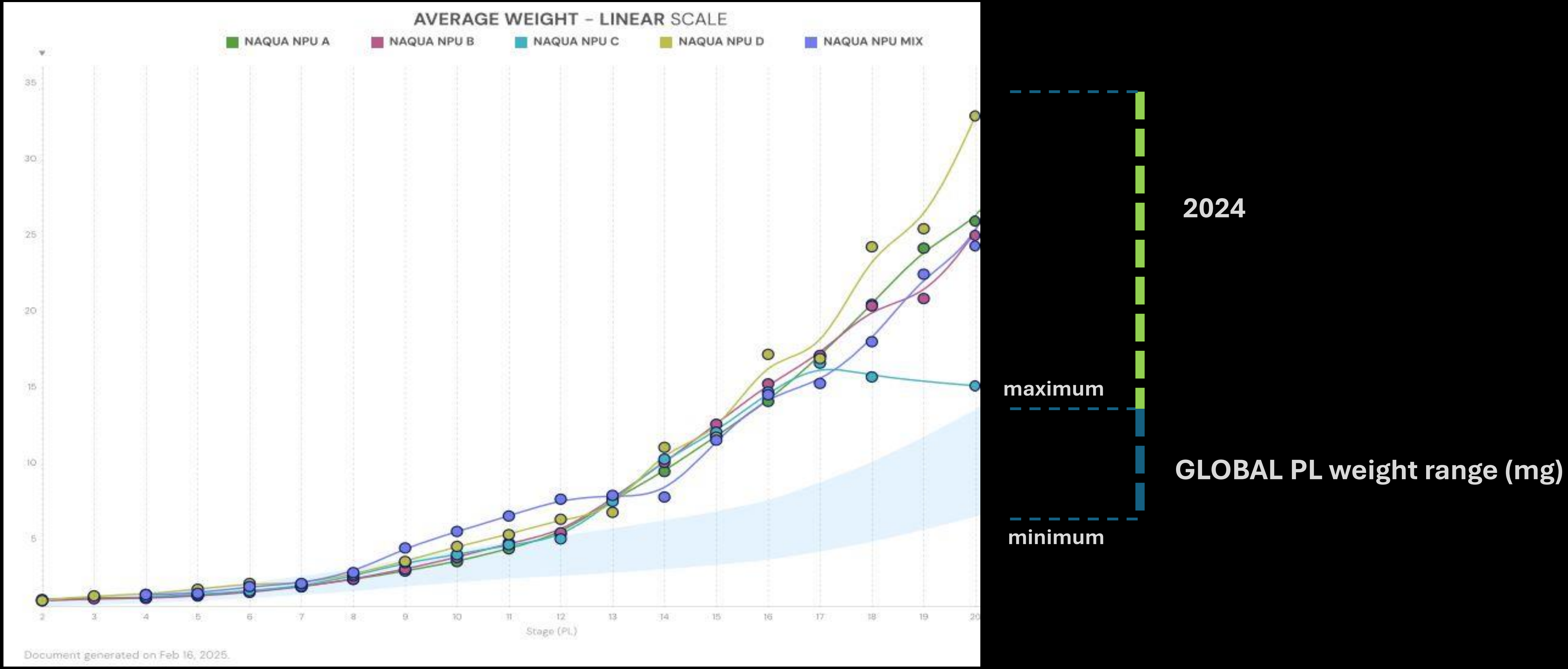


High response





Genetic potential 2024 postlarvae (LarvlA)



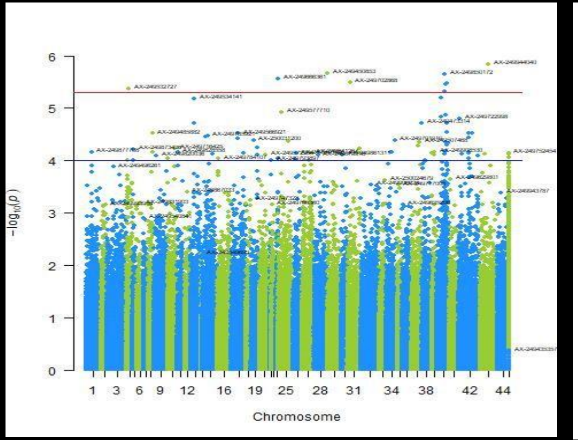
Selection inside a NBC has advantages and disadvantages

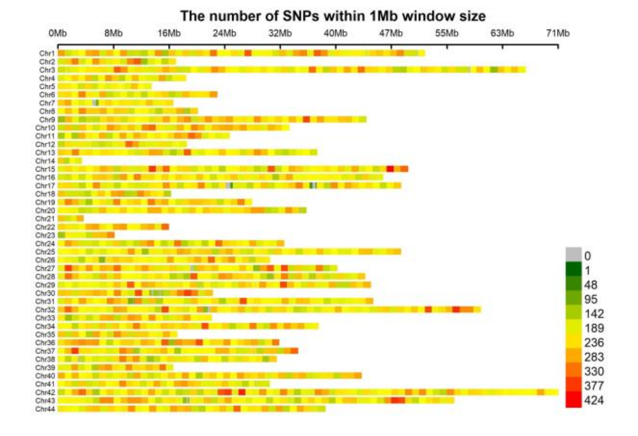
From pedigree breeding to molecular breeding and epigenetics modulation (Prof Xiang)

- Specific conditions for KSA
- Domestication: 2009-2011
  - SPF
  - WSSV tolerance
- Phenotypic selection: 2020
- Epigenetics modulation: 2020-…
- Marker assisted selection: 2026
- Genomic selection: 2028
- Fully sequenced *P. vannamei* genome, Prof Xiang, et al. IOCAS, China
- Identified: WSSV genetic tolerance, immune and molting related genes, high salinity tolerance

Most traits have polygenic architecture:

- Chip with 600Ksnps (cost vs prediction accuracy)
- Validation for KSA conditions
- Design of lower density chips (0.5K-10K)





Super females

Identification of molecular markers for maturation productivity

Higher mortality between the low production females

High production females keep the quality despite repeated spawning

Commercial spawning frequency	
Spawners frequency	Total no.of samples
1	26
2	37
3	32
4	44
5	49
6	31
7	27
8	33
9	17
10	12
11	13
12	4
13	8
14	1
15	2
Total	336

Tank #	Spawner	Tagged	Total # of eggs	Hatching %
1	2nd	481white	220500	66%
2	2nd	580gray	324000	86%
3	3rd	540gray	308000	79%
4	3rd	378white	253000	60%
5	4th	306lightpurple	396000	89%
6	5th	249white	302500	72%
7	5th	575gray	383000	87%
8	5th	233yellow	361500	83%
9	6th	508white	279500	71%
10	6th	182green	274000	74%
11	7th	445white	222500	51%
12	7th	412gray	316500	76%
13	8th	487orange	394000	86%
14	8th	273darkpurple	321000	63%
15	14th	235white	262500	60%
16	10th	509white	171000	65%
17	10th	179red	317000	79%
18	15th	374red	336500	67%
19	12th	433white	461500	85%
20	11th	427mint	385500	81%
21	13th	327white	416000	84%

Spawner	1st	2nd	3rd	4th	5th	6th	8th
# of Mortality	16	10	9	4	2	4	1
%	35%	22%	20%	9%	4%	9%	2%

Diagnostic tools – choosing the appropriate technique
A lab result is not a diagnosis

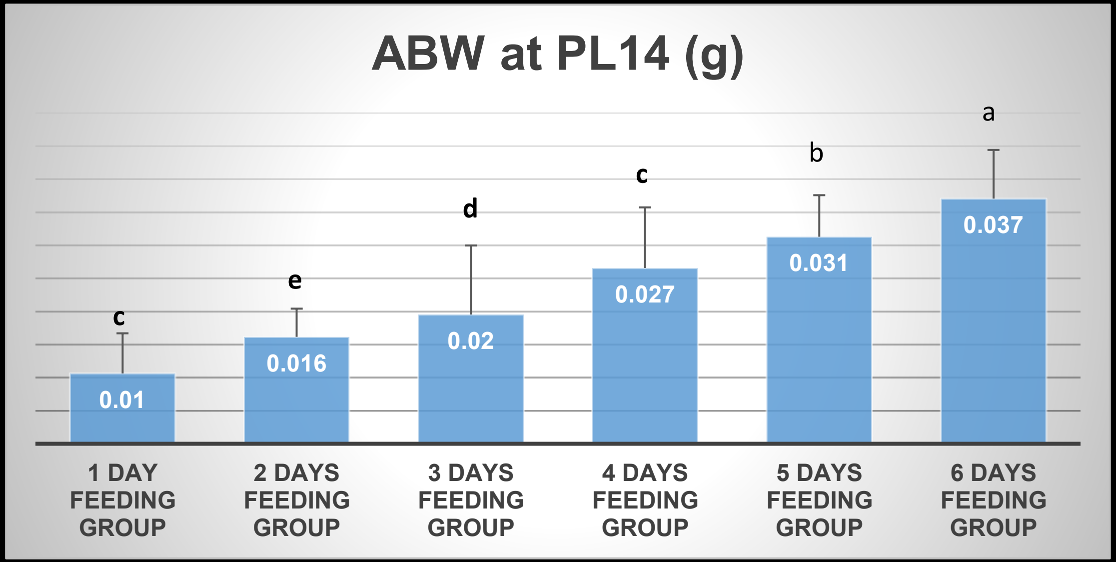
Techniques	Advantages	Disadvantages
Histopathology	**Most informative**, to be used for any mortality event or presence of clinical signs (**Gold standard**)	Slow, expensive, scarce knowledge available, takes years of training, only few animals can be analyzed, suitable samples are key
PCR	Quick, sensitive, easy to perform, validates biosecurity measures in place	Limited information: -Presence or absence -Active or inactivated False positives (EVEs),
Wet mounts	Quick, cheap, allows determine prevalence	
Microbiology	Validation of disifection procedures	

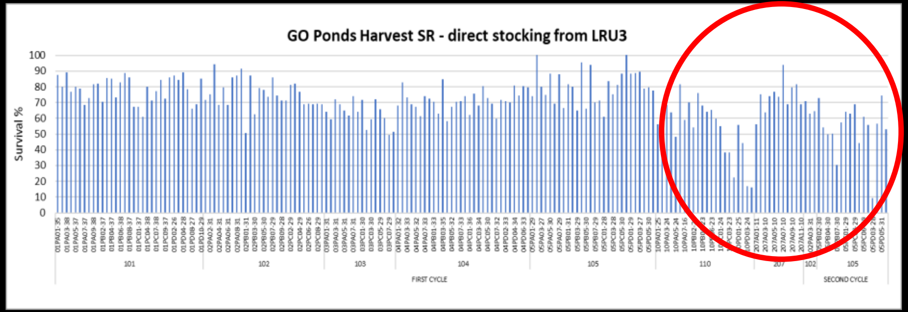
- The "distance" between the farm and the diagnostic lab: Poor communication in both directions leads to wrong diagnosis: anamnesis. The time to deliver results is often too late to implement mitigation measures
- Most diseases are multifactorial and often, there is an anthropogenic component
- The lack of aquaculture experience/understanding of the diagnostic technician
- Histopathology, remains the gold standard, absolutely necessary for unknown and emerging diseases. The expertise is being lost worldwide!
- Quality assurance of a diagnostic technique is essential

Lessons from syndromic surveillance

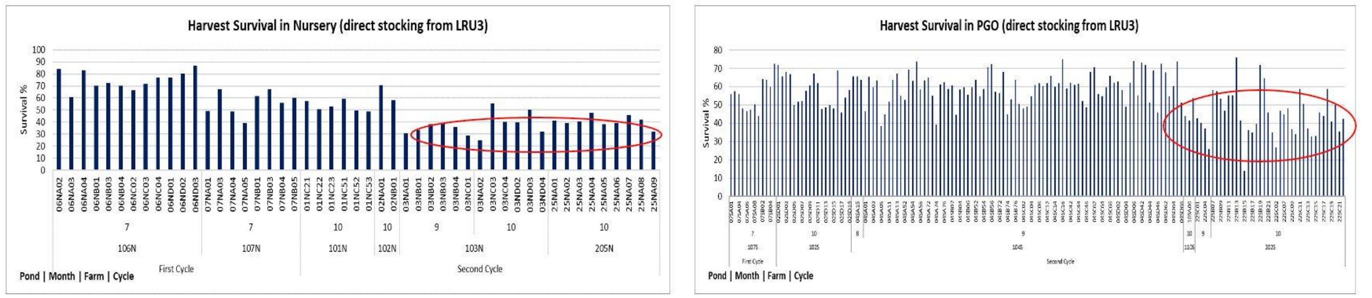
The money saved in the hatchery is money lost in GO

- Poor quality PLs, give poor performance in GO
  - High quality live feeds:
  - Thalassiosira (TCBS free)
- Artemia (instar II, TCBS green vibrio free)
- Feed accumulation alert
- Wet mount assessment
- Manage organics and nitrogen cycle to control bacterial issues





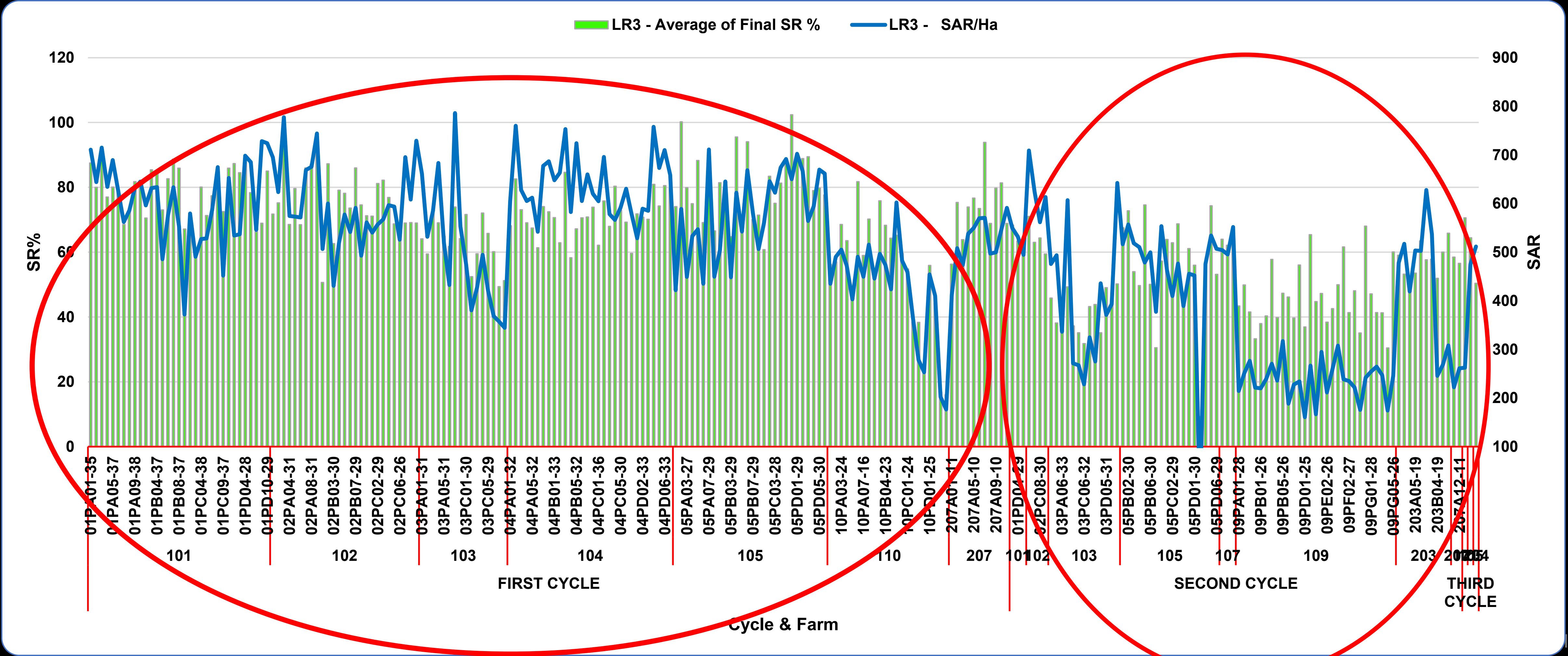
Second crop direct stocking-low survival in nursery, PGO and GO ponds



Is lower survival in an earlier stage a strategy for the selection of stronger animals?

No

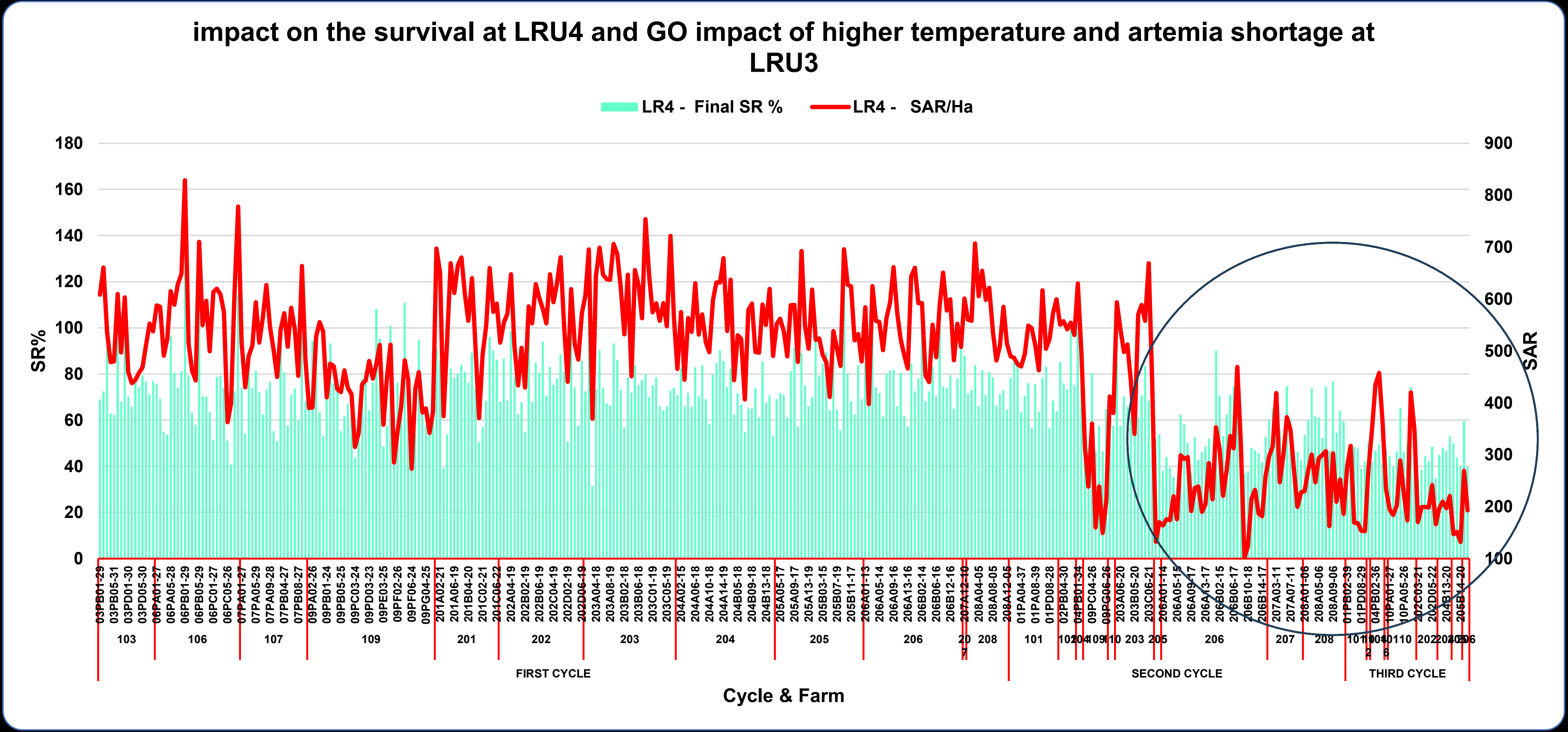
Profitability in GO is directly related to PL survival



Will the nursery "filter out" the poor quality PLs and keep the good ones?

No

Low survival in hatchery, low profitability in GO despite of the “filter” in nursery



Profitability comparison between farms and source.
Same genetics, same infrastructure, same “management”

The effect of ***caring***

Biosecurity needs to be cost-efficient

SPF/SPT Broodstock production

- Includes water filtration, disinfection (UV & Ozone)
- Fresh feeds etc…

Diagnostic cost includes

- Routine animal health monitoring
- Surveillance for primary pathogens
- Emergency response

Diagnostic techniques used

- Wet mounts
- PCR (animals & fresh feeds)
- Histology

Year	Cost diagnostics /kg of shrimp (USD)	Cost of SPF+SPT /kg of shrimp (USD)	Total cost of biosecurity/kg of shrimp (USD)
2016	0.011	0.126	0.137
2017	0.004	0.064	0.068
2018	0.004	0.041	0.045
2019	0.015	0.068	0.083
2020	0.014	0.101	0.114
2021	0.007	0.065	0.071
2022	0.005	0.054	0.059
2023	0.007	0.055	0.061
2024	0.007	0.044	0.051

WSSV

- ✓ Production management and animal health goes hand in hand. Biosecurity needs to be integrated to achieve commercial success.
- ✓ Biosecurity is tailor made and requires a multidisciplinary approach.
- ✓ Health is a measure of productivity.
- ✓ Biosecurity is a shared responsibility.
- ✓ Health is not a competitive advantage.
- ✓ Data based decisions—Science based strategies.

No WSSV nor other primary pathogens.
No antibiotics used.

Terima kasih
Thank you