Biosecurity along the shrimp value chain

Andy Shinn
INVE Thailand

a.shinn@inveaquaculture.com

1

How did I learn about diagnostics?

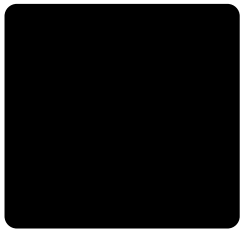
Me – aged 5 (no picture of the stick!)

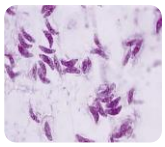
2

How did I learn about diagnostics?

Me – aged 5 (no picture of the stick!)

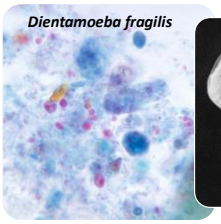
Headlice





Toxoplasma (high risk grp; 23-33% in UK inf.; 50% by age 60)

Enterobius pinworm



Giardia

Athlete's foot

Leeches

So, what did this teach me?

I learnt how infection affects me

Food & water safety

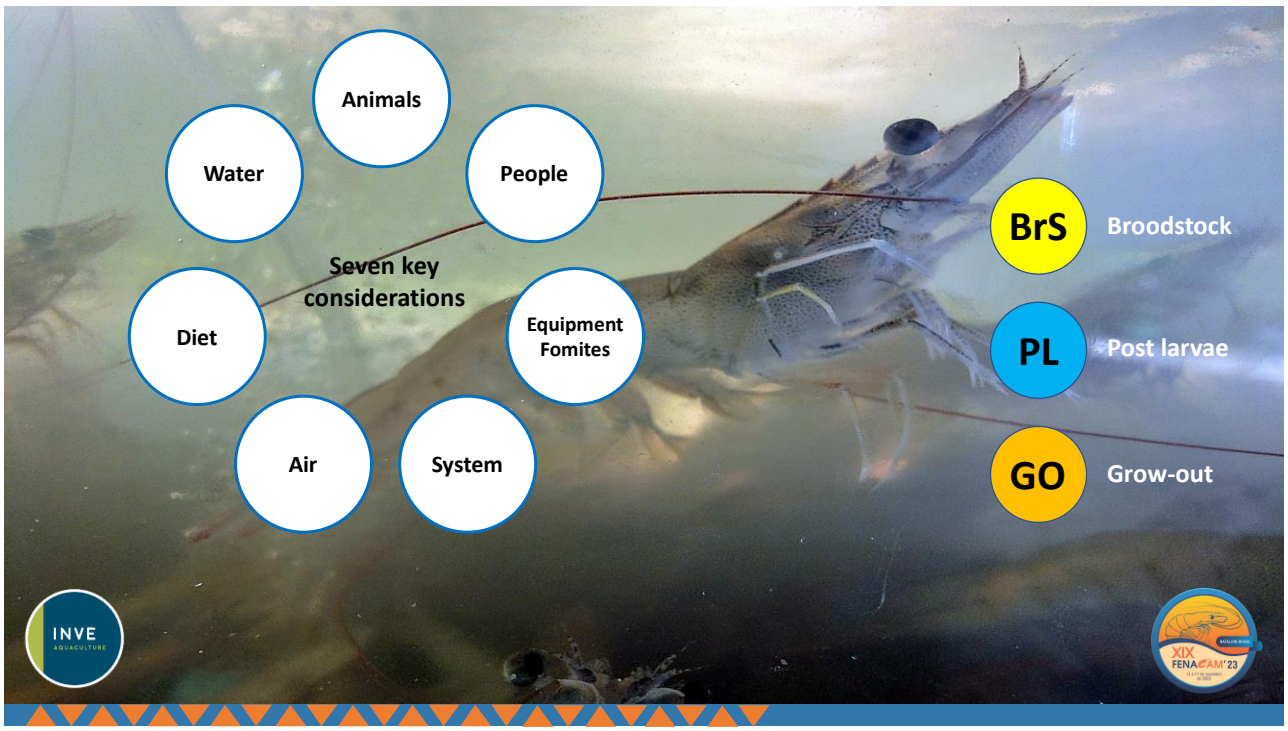
Chemotherapy & sanitary measures

Biosecurity

Images from the internet


"Teddy" the tick
(here played by an actor)
https://edition.cnn.com/2023/07/01/health/tick-season-diseases/index.html

5


Animals
Water
People
Seven key considerations
Diet
Equipment Fomites
Air
System
BrS
Broodstock
PL
Post larvae
GO
Grow-out

6

BrS

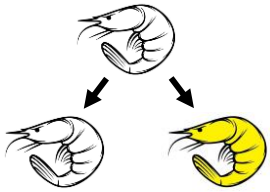
Genetic selection and breeding for resistance (important to understand the mechanism of resistance)

Specific Pathogen Free

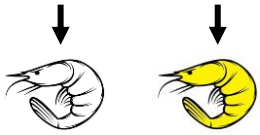
Neg for specific pathogen for 2 yrs
(not free of all pathogens)
Surveillance must be in place
Reared in biosecure facilities
Water treatment in place
Fed biosecure feeds
Needs transparency and auditing

If stocked into a non-biosecure system, however, stock can get infected, develop disease, have mortality

Specific Pathogen Resistant

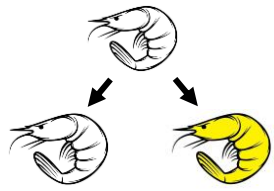


Resistant to infection by pathogen
A - qualitative term as might get infected or might not

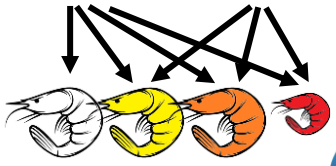


Animals limit infection so that mortalities do not result

Specific Pathogen Tolerant



Tolerant to infection by pathogen
A - qualitative term as might get infected or might not. Those that get infected may develop disease at levels lower than "normal" stocks



BrS

Use of live/fresh feeds in maturation

Polychaetes

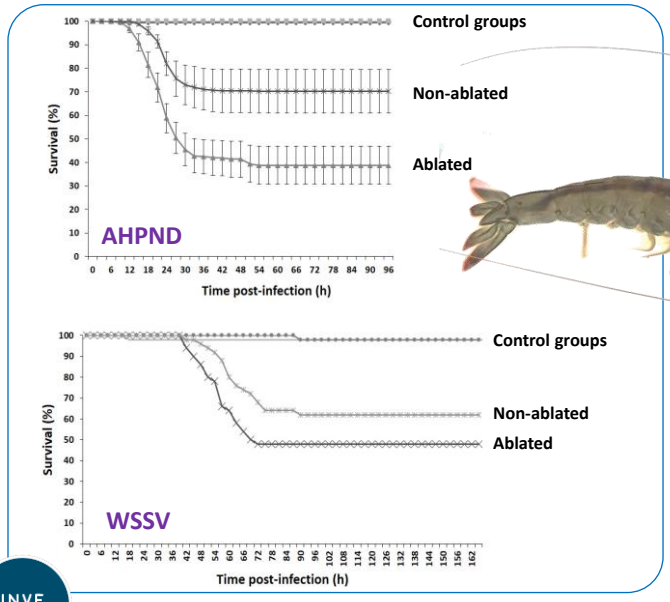
Biosecurity of polychaetes - minimising vertical transmission of disease

Commercial maturation trial

100FF: 100% mixed fresh food (with polychaetes)
40SP: 40% semi-moist pellet plus 60% mixed fresh food (with polychaetes)
60SP: 60% semi-moist pellet plus 40% mixed fresh food (no polychaetes)

Exp1	100FF	40SP	60SP
Spawns per female	3.6	3.3	3.6*
Fecundity	291,346	277,647	306,636
Egg hatching (%)	63	67	75*
Zoea (%)	93	94	96*

* Statistically significant difference $p<0.05$



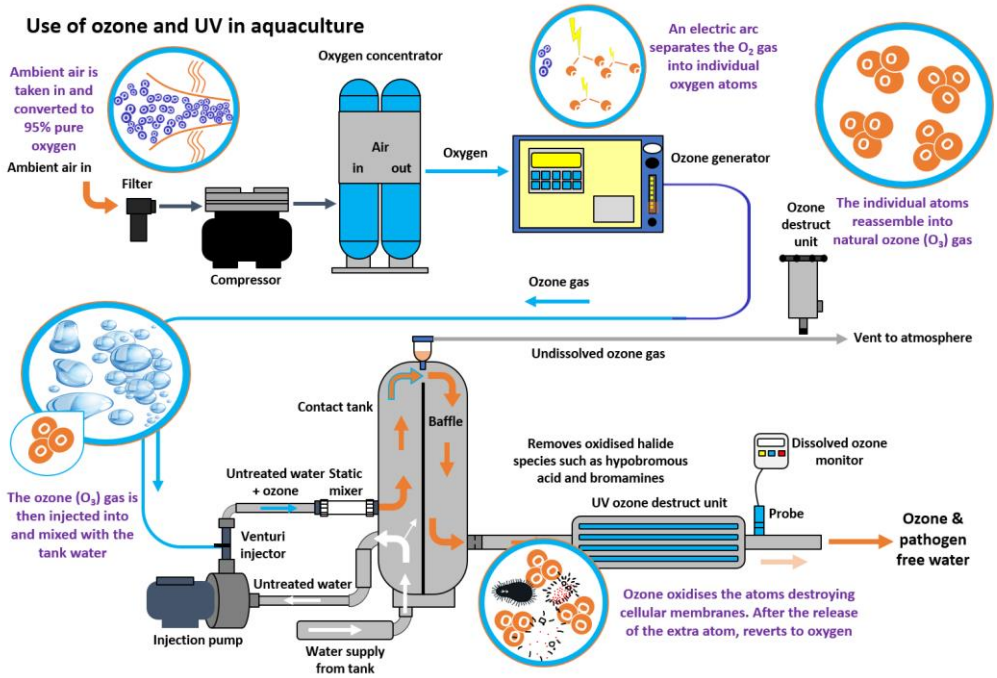
Increased robustness of offspring from non-ablated broodstock

BrS

AHPND: PL17
70.4% (NAF) survival vs 38.8% (AF)
***p* = <1.3E-36**

WSSV: Av. 1.4 g
62% (NAF) survival vs 48% (AF)
***p* = NS**

Zacarias *et al.* (2020)
Aquaculture, 532, 736033.



Disinfectant effect of electrolyzed saltwater

Bacteriocidal and virucidal effects of hypochlorite produced by electrolysis (3.5 m^3/h, 0.1 A) are greater than using the chemical reagent. After electrolysis, the chlorine needs to be removed with charcoal due to its toxicity.

The bacteriocidal effect of electrolysis is almost the same as that of UV irradiation (1× 10^5 µW.sec/cm^2) or ozonation (TROs 1 mg/L, 1 min). Electrolization can be used to treat larger volumes of wastewater compared to ultraviolet irradiation or ozonation.

$NaCl + H_20 + Energy$

$\downarrow$

$NaOCl + H_2$

Kill doses of chlorine, UV, ozone for key pathogens need establishing

11

Ultrafiltration can retain particles down to 0.03 microns, i.e., bacterial filtration

12

Microbial management at the hatchery level

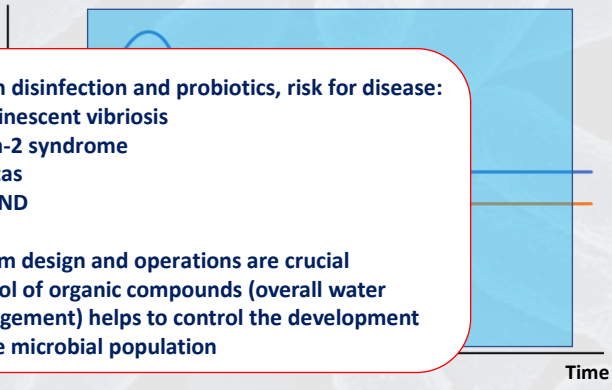
Balance of r-strategists versus K-strategists determines the risk for bacterial interference, but this is unpredictable

Even with disinfection and probiotics, risk for disease:

- Luminescent vibriosis
- Zoea-2 syndrome
- Bolitas
- AHPND
- …

→ System design and operations are crucial

→ Control of organic compounds (overall water management) helps to control the development of the microbial population



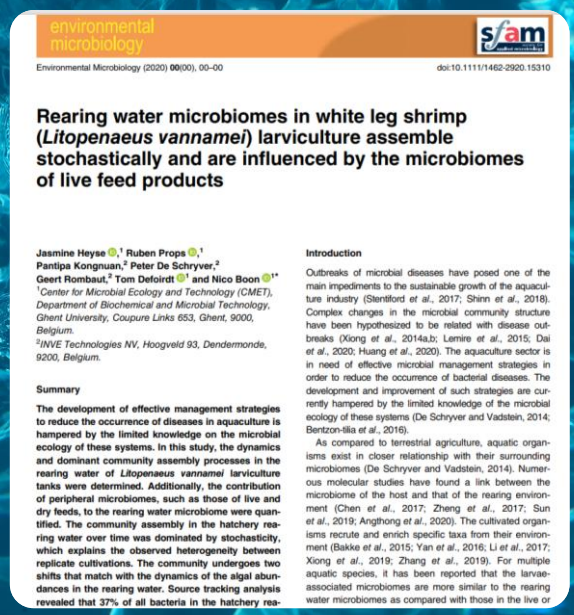
Organisms living in unstable and unpredictable environments - they undergo rapid reproduction to stabilise themselves. Can be dangerous opportunistic pathogens.

Organisms living in stable environments. Low / slower growth and are generally harmless.

13

Biosecurity is not only reducing risk of pathogen entry but – even more importantly – creating a stable microbiome ecosystem that reduces the potential development of pathogen populations

The phytoplankton community is an important driver of the rearing water microbiome



environmental microbiology

Environmental Microbiology (2020) 00(00), 00–00 doi:10.1111/1462-2920.15310

Rearing water microbiomes in white leg shrimp (*Litopenaeus vannamei*) larviculture assemble stochastically and are influenced by the microbiomes of live feed products

Jasmine Heyse,[1] Ruben Props,[1] Pantipa Kongnuan,[2] Peter De Schryver,[2] Geert Rombaut,[2] Tom Defoirdt[1] and Nico Boon[1]*
[1]*Center for Microbial Ecology and Technology (CMET), Department of Biochemical and Microbial Technology, Ghent University, Coupure Links 653, Ghent, 9000, Belgium.*
[2]*INVE Technologies NV, Hoogveld 93, Dendermonde, 9200, Belgium.*

Summary

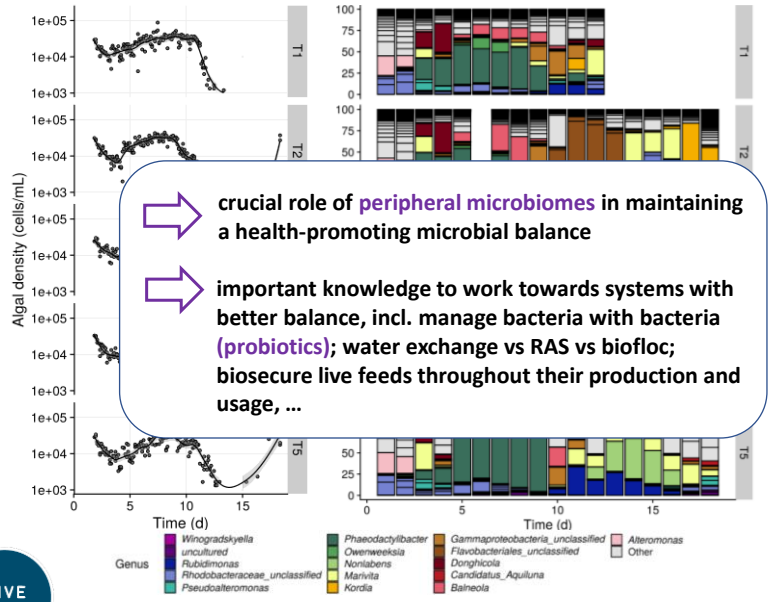
The development of effective management strategies to reduce the occurrence of diseases in aquaculture is hampered by the limited knowledge on the microbial ecology of these systems. In this study, the dynamics and dominant community assembly processes in the rearing water of *Litopenaeus vannamei* larviculture tanks were determined. Additionally, the contribution of peripheral microbiomes, such as those of live and dry feeds, to the rearing water microbiome were quantified. The community assembly in the hatchery rearing water over time was dominated by stochasticity, which explains the observed heterogeneity between replicate cultivations. The community undergoes two shifts that match with the dynamics of the algal abundances in the rearing water. Source tracking analysis revealed that 37% of all bacteria in the hatchery rea-

Introduction

Outbreaks of microbial diseases have posed one of the main impediments to the sustainable growth of the aquaculture industry (Stentiford *et al.*, 2017; Shinn *et al.*, 2018). Complex changes in the microbial community structure have been hypothesized to be related with disease outbreaks (Xiong *et al.*, 2014a,b; Lemire *et al.*, 2015; Dai *et al.*, 2020; Huang *et al.*, 2020). The aquaculture sector is in need of effective microbial management strategies in order to reduce the occurrence of bacterial diseases. The development and improvement of such strategies are currently hampered by the limited knowledge of the microbial ecology of these systems (De Schryver and Vadstein, 2014; Bentzon-tilia *et al.*, 2016).

As compared to terrestrial agriculture, aquatic organisms exist in closer relationship with their surrounding microbiomes (De Schryver and Vadstein, 2014). Numerous molecular studies have found a link between the microbiome of the host and that of the rearing environment (Chen *et al.*, 2017; Zheng *et al.*, 2017; Sun *et al.*, 2019; Angthong *et al.*, 2020). The cultivated organisms recrute and enrich specific taxa from their environment (Bakke *et al.*, 2015; Yan *et al.*, 2016; Li *et al.*, 2017; Xiong *et al.*, 2019; Zhang *et al.*, 2019). For multiple aquatic species, it has been reported that the larvae-associated microbiomes are more similar to the rearing water microbiomes as compared with those in the live or

14



crucial role of peripheral microbiomes in maintaining a health-promoting microbial balance

important knowledge to work towards systems with better balance, incl. manage bacteria with bacteria (probiotics); water exchange vs RAS vs biofloc; biosecure live feeds throughout their production and usage, …

absolute abundance of 39 taxa is significantly correlated to the algal densities

"Source tracking analysis revealed that 37% of all bacteria in the hatchery rearing water were introduced either by the live or dry feeds, or during water exchanges. The contribution of the microbiome from the algae was the largest, followed by that of the *Artemia*, the exchange water and the dry feeds."

15

16

Live feeds
Artemia Sep-Art & tools

Biosecure *Artemia*
A complete separation of the cyst from nauplii

By removing shells, we also get a higher yield, and the approach is more environmentally friendly

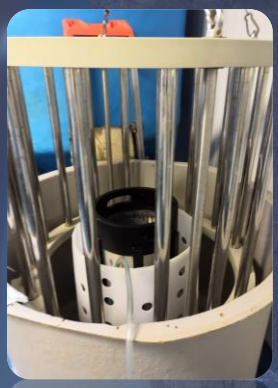
Empty shells are a perfect substrate for *Vibrio*

17

SEP-Art by INVE

Innovation in nauplii separation use of magnetic trapping of cysts

SEP-*Art*
HandyMag
NEW TOOL



SEP-*Art*
CysTM 2.0
NEW TOOL

SEP-*Art*
AutoMag
NEW TOOL

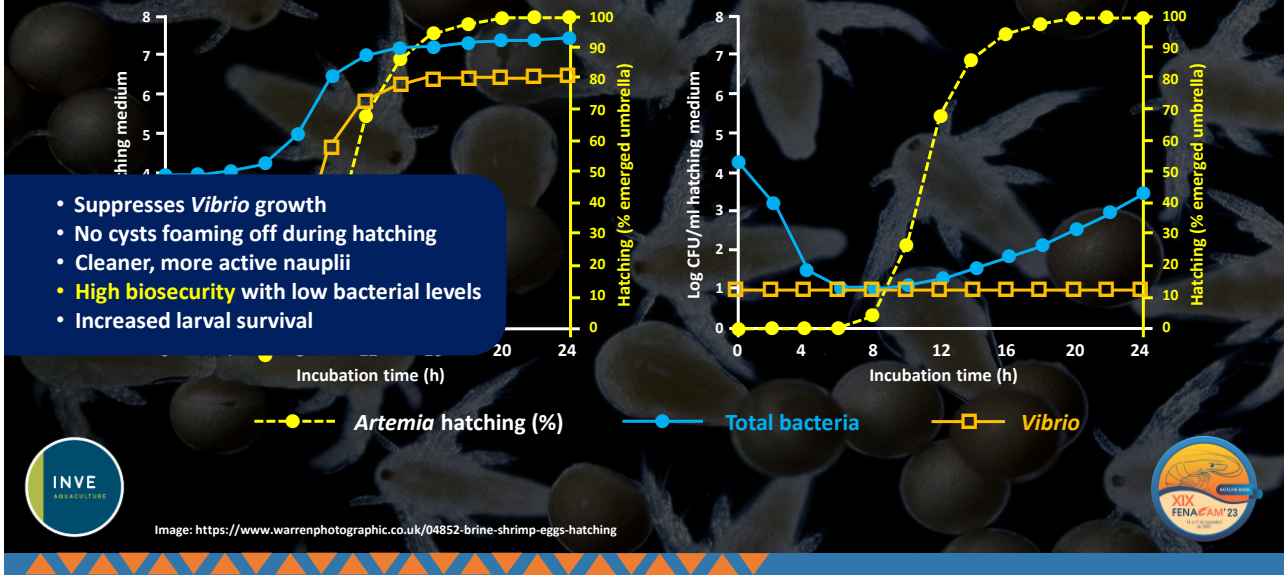
Have questions?

SCAN ME

18

Improved water quality and suppression of *Vibrio* during hatching

Sanocare® ACE PRO



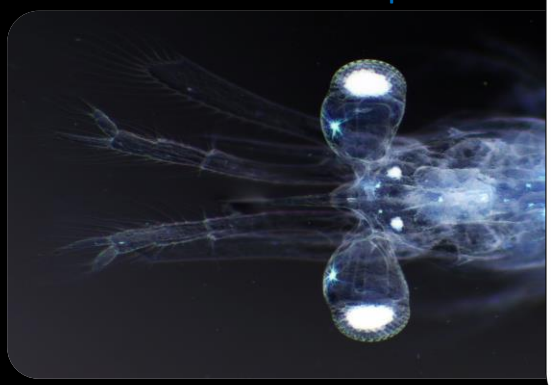
- Suppresses *Vibrio* growth
- No cysts foaming off during hatching
- Cleaner, more active nauplii
- High biosecurity with low bacterial levels
- Increased larval survival

Image: https://www.warrenphotographic.co.uk/04852-brine-shrimp-eggs-hatching

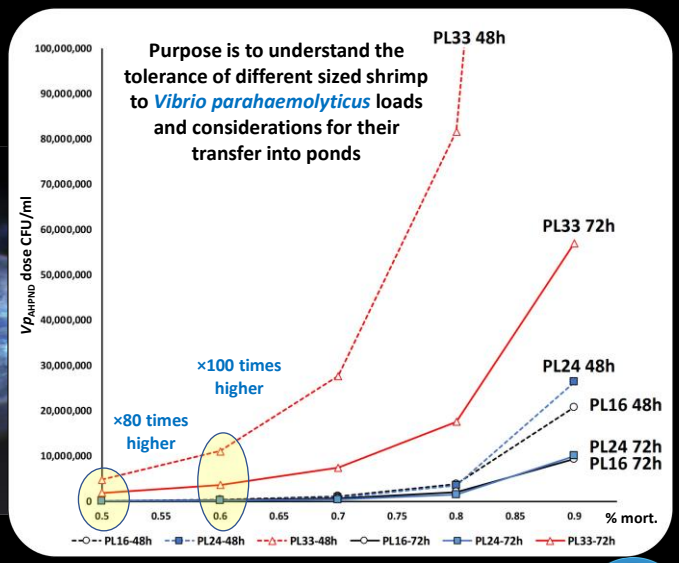
19

PL transfer: timing = crucial

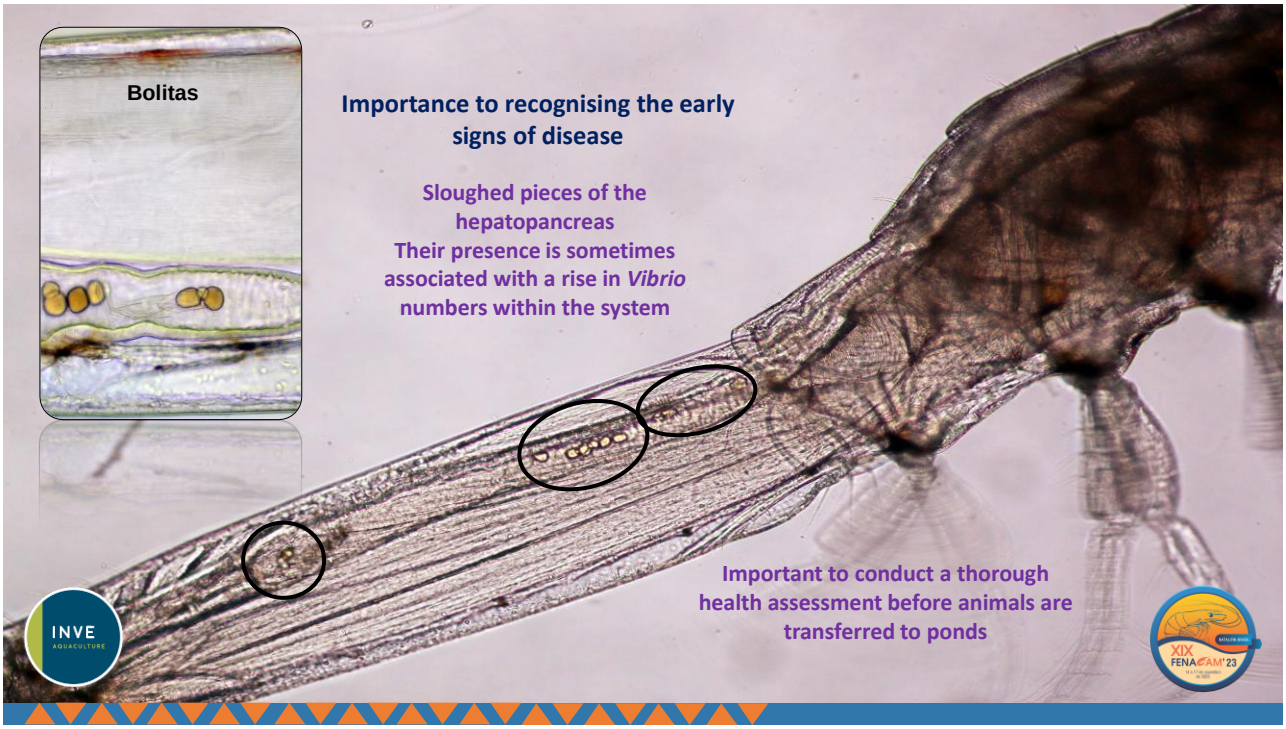
Understanding lethal *Vibrio* doses for different sized shrimp



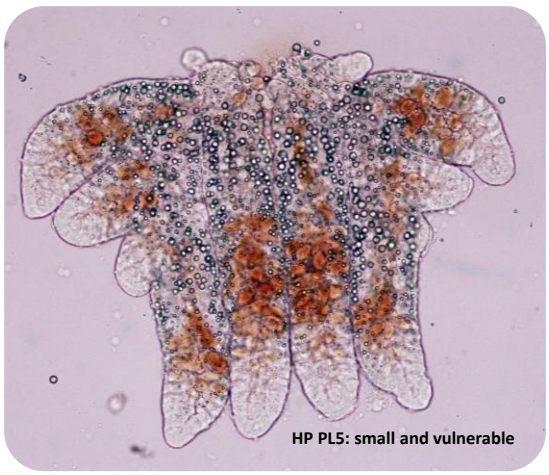
Shinn *et al.* (in prep) study



20

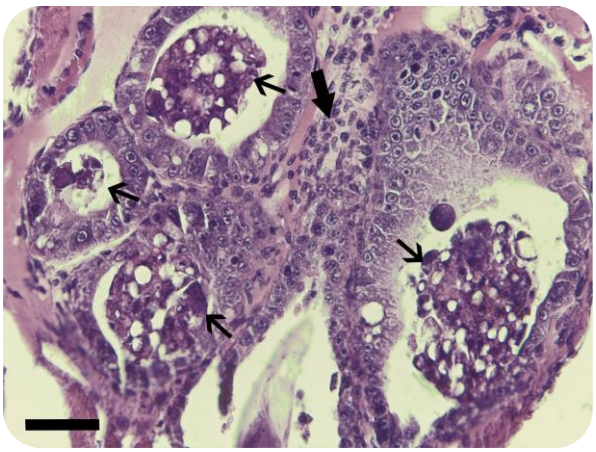


21



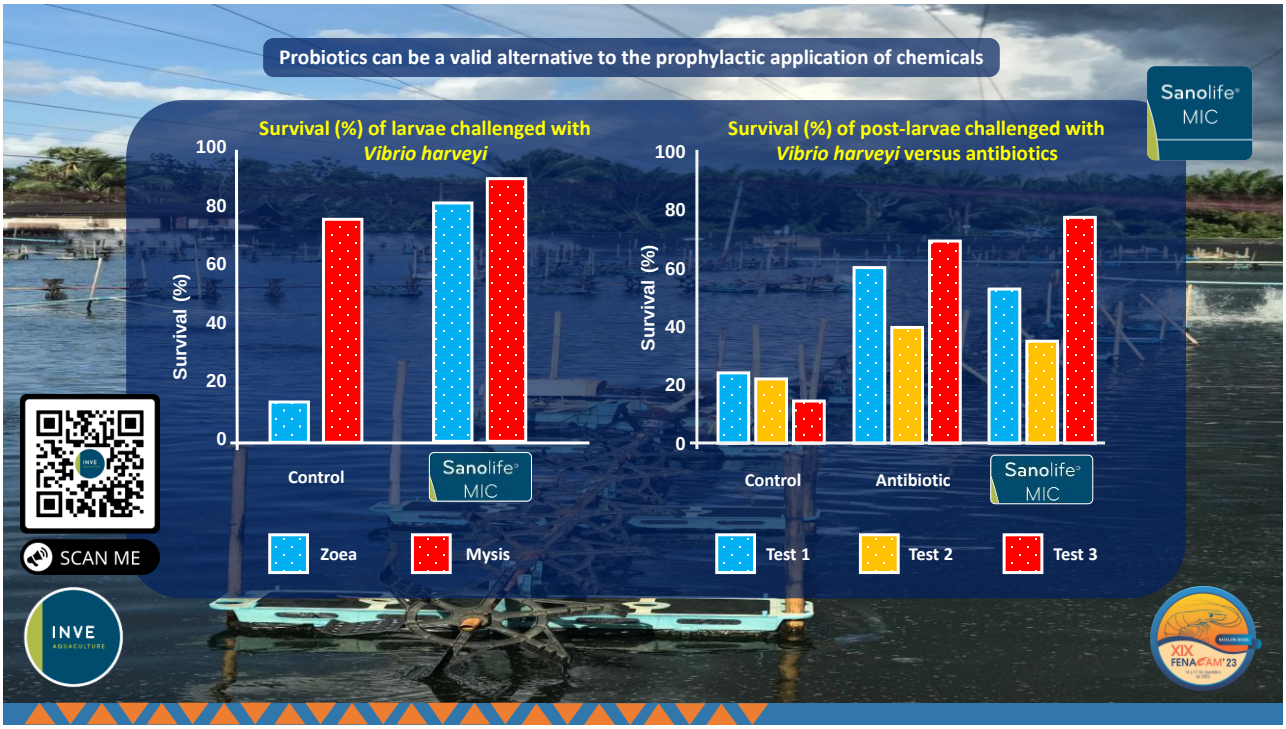
Occasionally, mortalities occurred within 2 hours from appearing normal until experiencing 100% mortality

Hepatopancreas with severe epithelial sloughing within the tubules

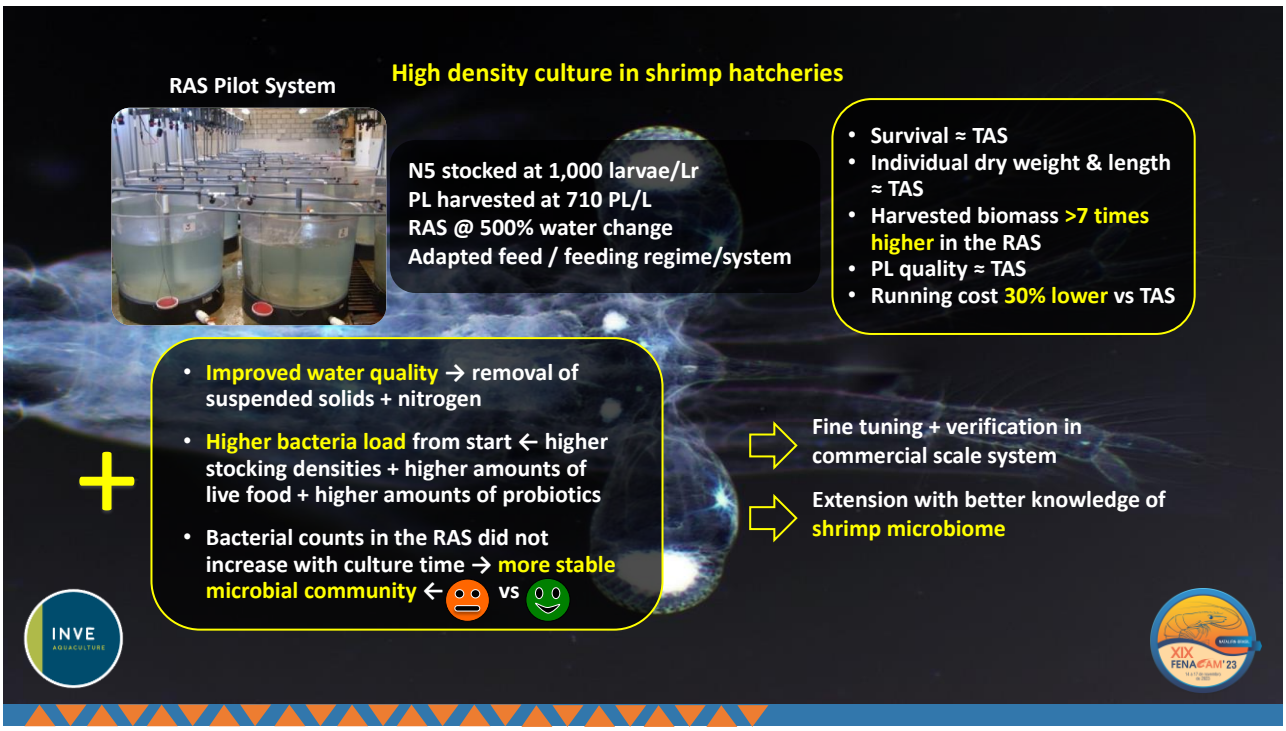


Intriago *et al.* (2023) *Aquaculture International*

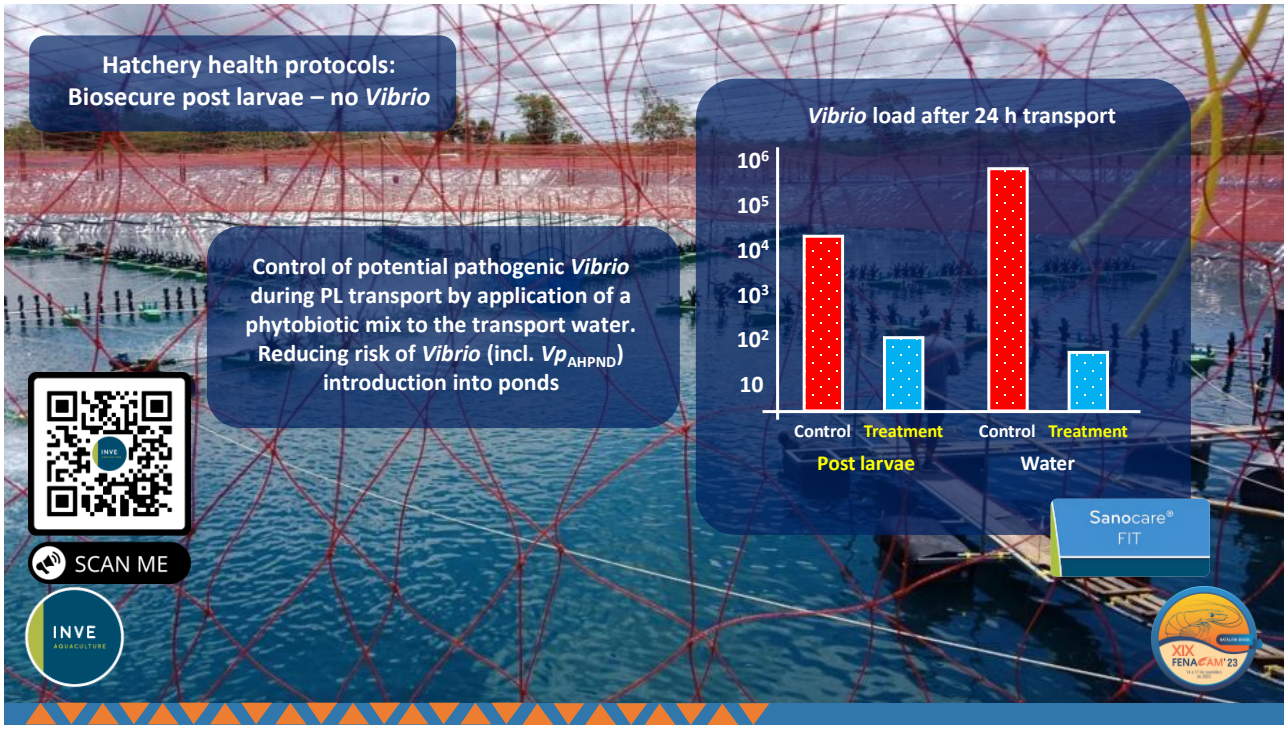
22


Probiotics can be a valid alternative to the prophylactic application of chemicals
Sanolife MIC
Survival (%) of larvae challenged with Vibrio harveyi
Survival (%)
100
80
60
40
20
0
Control
Sanolife MIC
Zoea
Mysis
Survival (%) of post-larvae challenged with Vibrio harveyi versus antibiotics
Survival (%)
100
80
60
40
20
0
Control
Antibiotic
Sanolife MIC
Test 1
Test 2
Test 3
SCAN ME
INVE AQUACULTURE
XIX FENACAM'23

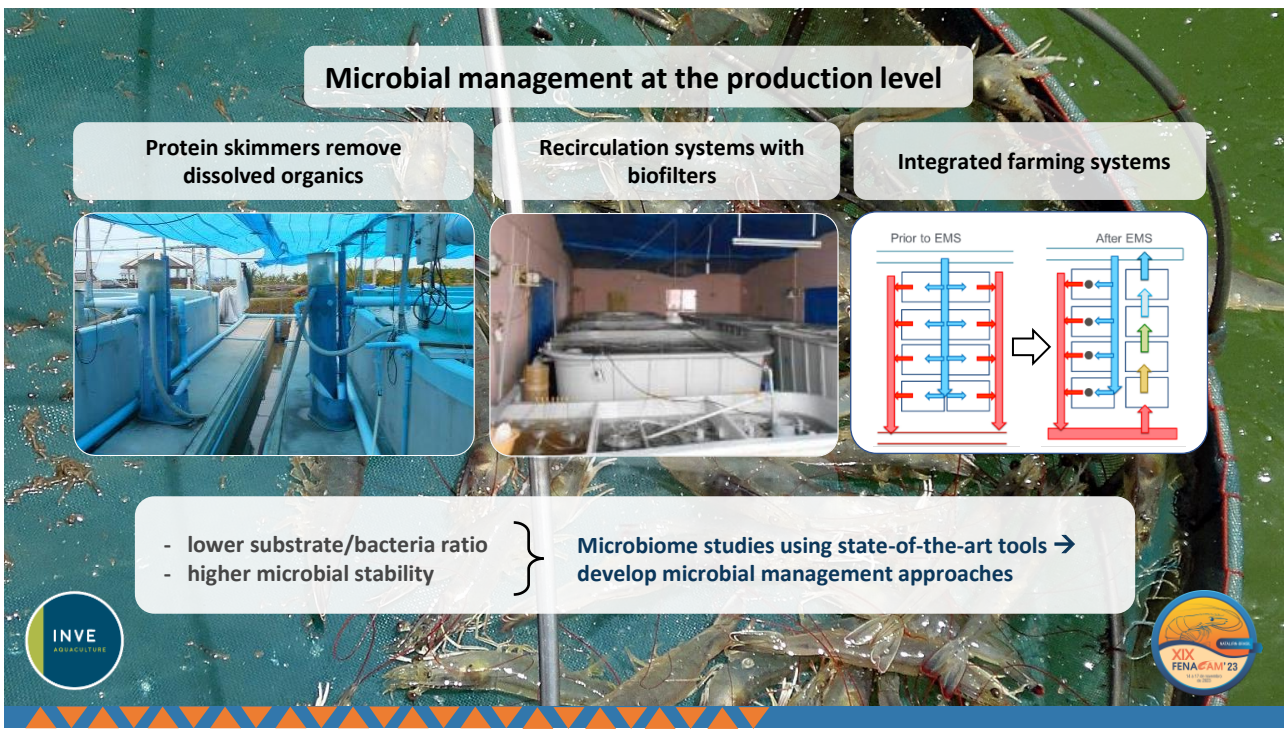
23


RAS Pilot System
High density culture in shrimp hatcheries
N5 stocked at 1,000 larvae/Lr
PL harvested at 710 PL/L
RAS @ 500% water change
Adapted feed / feeding regime/system
Survival ≈ TAS
Individual dry weight & length ≈ TAS
Harvested biomass >7 times higher in the RAS
PL quality ≈ TAS
Running cost 30% lower vs TAS
Improved water quality → removal of suspended solids + nitrogen
Higher bacteria load from start ← higher stocking densities + higher amounts of live food + higher amounts of probiotics
Bacterial counts in the RAS did not increase with culture time → more stable microbial community ← vs
Fine tuning + verification in commercial scale system
Extension with better knowledge of shrimp microbiome
INVE AQUACULTURE
XIX FENACAM'23

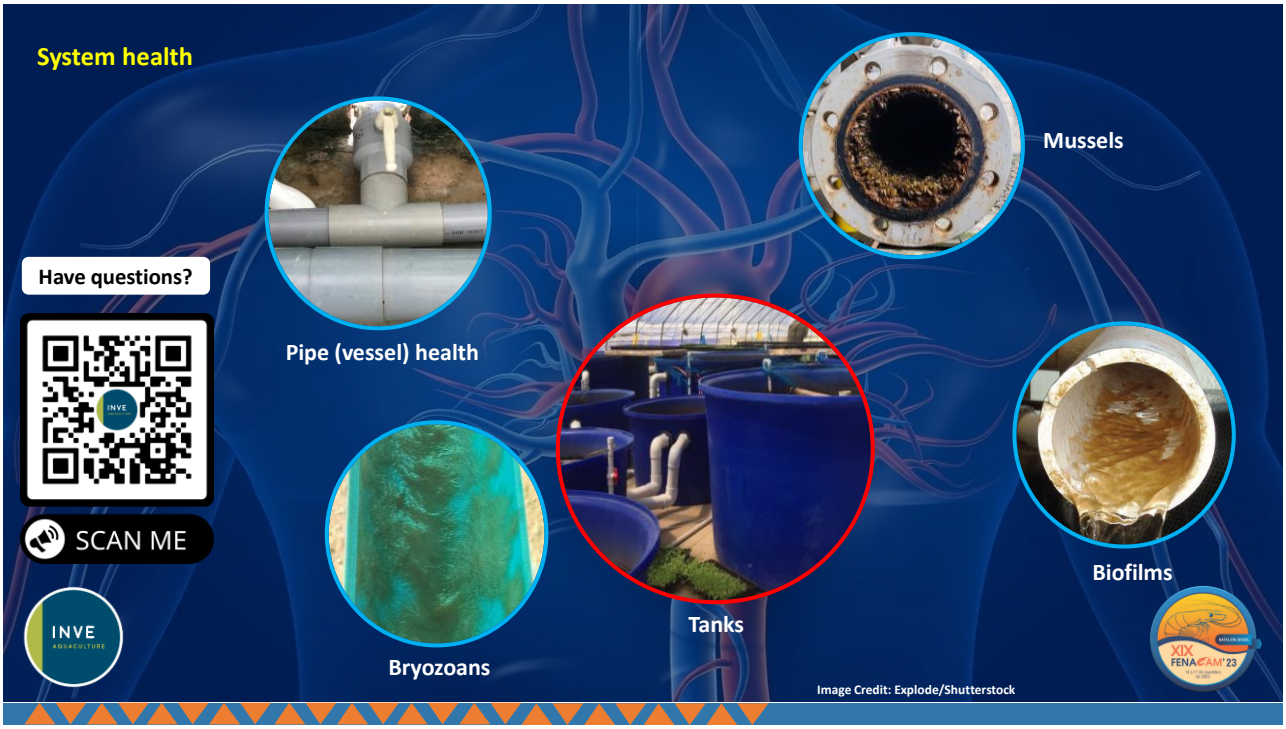
24


Hatchery health protocols:
Biosecure post larvae – no *Vibrio*
Control of potential pathogenic *Vibrio* during PL transport by application of a phytobiotic mix to the transport water. Reducing risk of *Vibrio* (incl. Vp_{AHPND}) introduction into ponds
Vibrio load after 24 h transport
10^6
10^5
10^4
10^3
10^2
10
Control Treatment
Post larvae
Control Treatment
Water
Sanocare® FIT
SCAN ME
INVE AQUACULTURE
XIX FENACAM'23

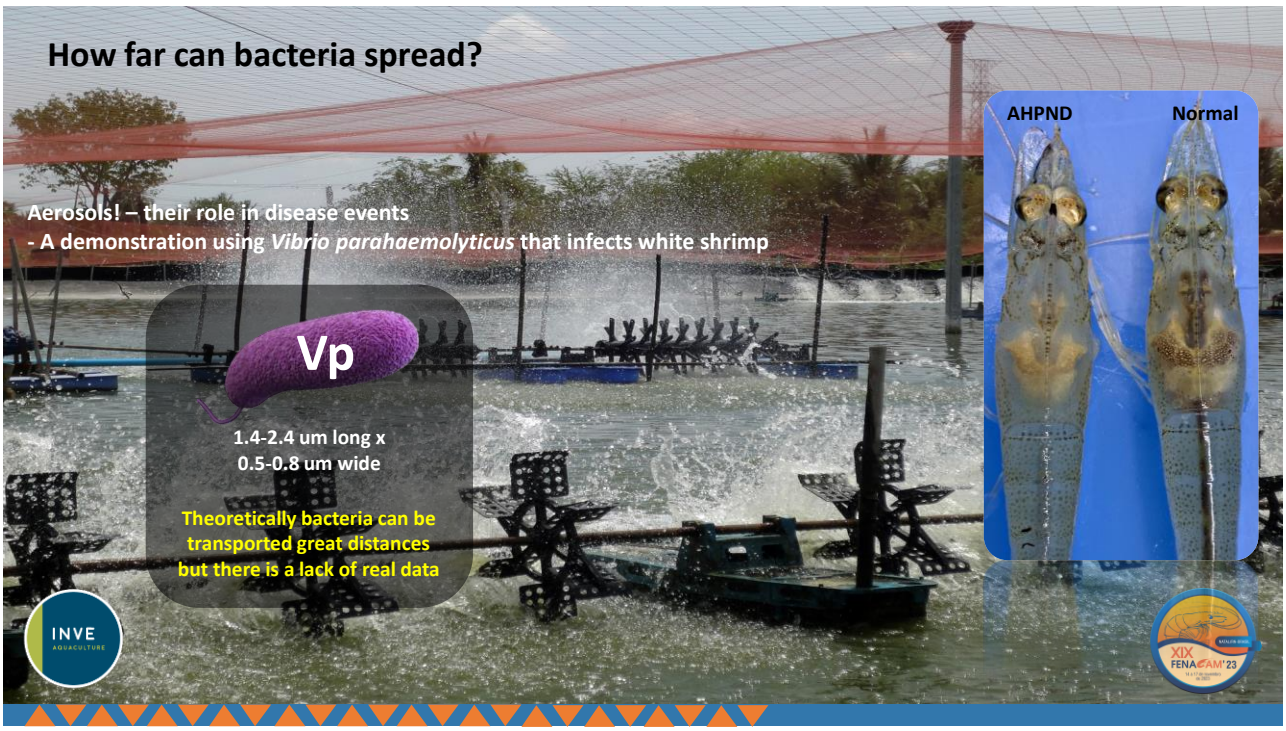
25


Microbial management at the production level
Protein skimmers remove dissolved organics
Recirculation systems with biofilters
Integrated farming systems
Prior to EMS
After EMS
- lower substrate/bacteria ratio
- higher microbial stability
Microbiome studies using state-of-the-art tools → develop microbial management approaches
INVE AQUACULTURE
XIX FENACAM'23

26


System health
Pipe (vessel) health
Mussels
Have questions?
SCAN ME
Biofilms
Tanks
Bryozoans
Image Credit: Explode/Shutterstock

27


How far can bacteria spread?
AHPND
Normal
Aerosols! – their role in disease events
- A demonstration using *Vibrio parahaemolyticus* that infects white shrimp
Vp
1.4-2.4 um long x
0.5-0.8 um wide
Theoretically bacteria can be transported great distances but there is a lack of real data

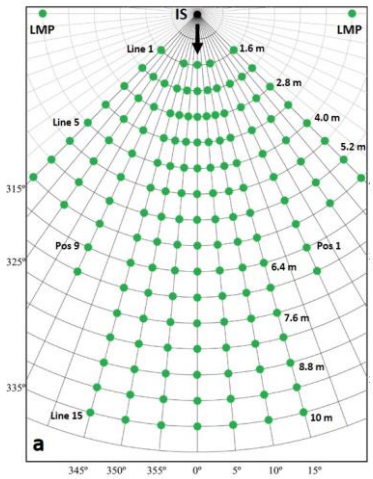
28

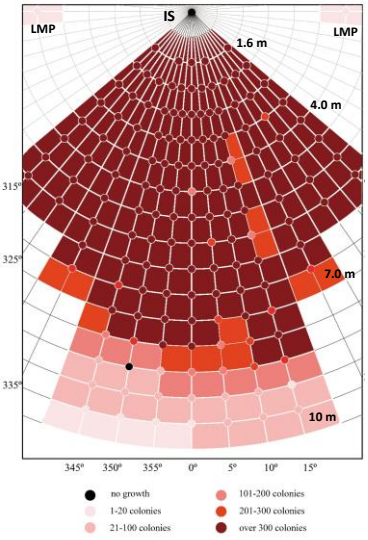
Trial 1: 10 m spread

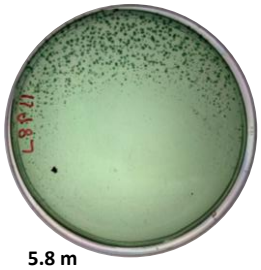
Vp_{AHPND} inoculum 1.46 × 10^9 cfu mL^{-1}

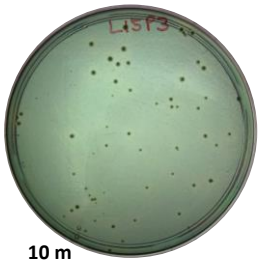
TCBS plate

33.99 ± 0.41°C
humidity 63.29 ± 1.38%
6 hours







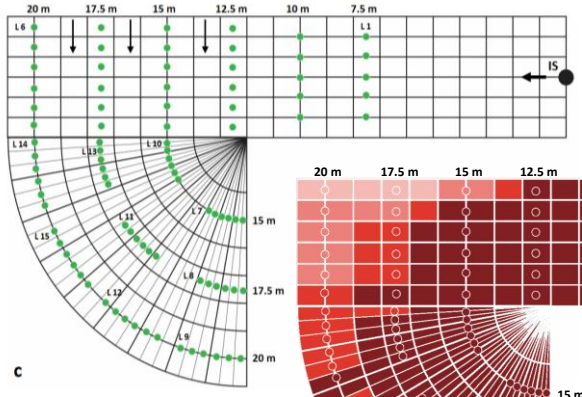


9.81 km h^{-1} wind 43 cm high
Bacteria spread at least 10 m

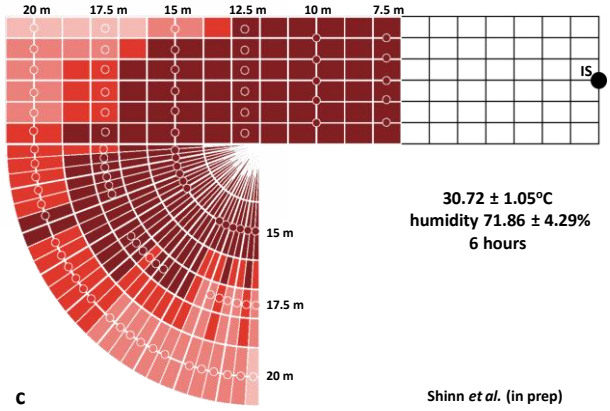
Shinn *et al.* (in prep)

29

Trial 3: 20 m spread + cross winds



5 m s^{-1} wind 220 cm high
Bacteria spread at least 20 m + round walls



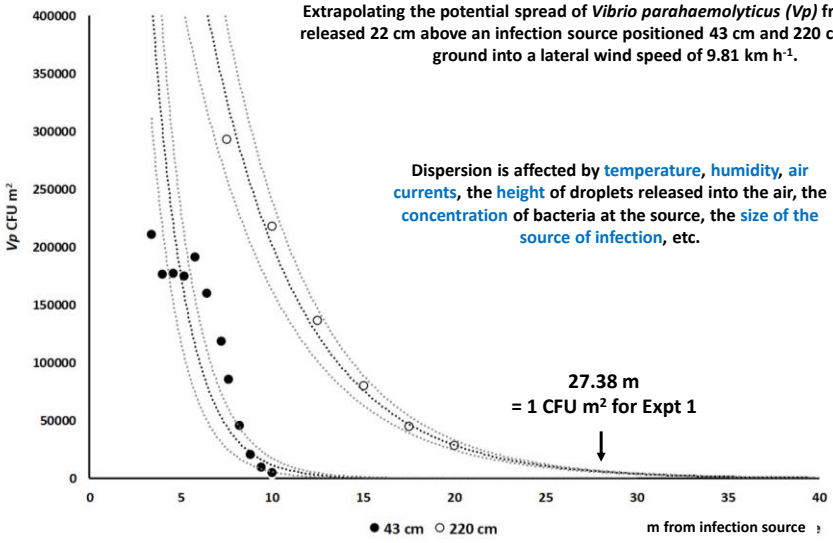
30.72 ± 1.05°C
humidity 71.86 ± 4.29%
6 hours

Shinn *et al.* (in prep)

30

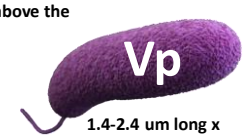
So what?

Extrapolating the potential spread of *Vibrio parahaemolyticus (Vp)* from aerosols released 22 cm above an infection source positioned 43 cm and 220 cm above the ground into a lateral wind speed of 9.81 km h^{-1}.



27.38 m
= 1 CFU m^2 for Expt 1

72.92 m
= 1 CFU m^2 for Expt 3



1.4-2.4 um long x
0.5-0.8 um wide

Dispersion is affected by temperature, humidity, air currents, the height of droplets released into the air, the concentration of bacteria at the source, the size of the source of infection, etc.

Shinn *et al.* (in prep)

31

"Teddy" the tick

For a few days, we were really close ….. I mean really close
Then he had to go
During that time together we crossed vast distances together
I calculated, I travelled approximately >200 km across several provinces
In the same time, infected batches of shrimp could have crossed Continents

A good lesson in biosecurity and the need for comprehensive health screening of batches of shrimp

https://edition.cnn.com/2023/07/01/health/tick-season-diseases/index.html

32

Any questions?

SCAN ME

A Benchmark Company

Many thanks for the invitation to speak at this conference here today and to all of you for listening.

Thank you very much

Andy Shinn, INVE Aquaculture
a.shinn@inveaquaculture.com