INVE AQUACULTURE

shapingaquaculturetogether

Disease in Shrimp Culture: How to Contain a Global Threat

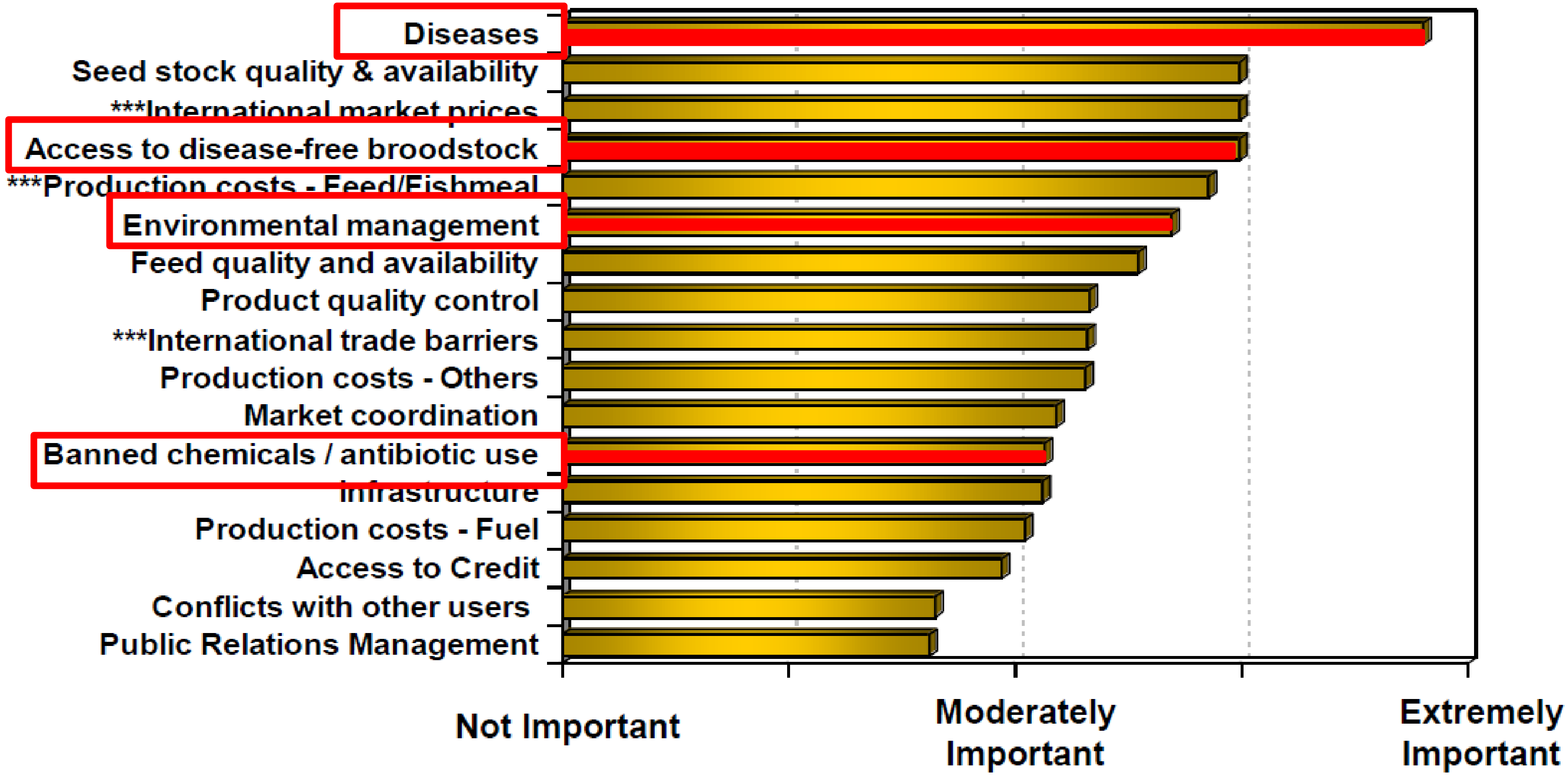
Olivier Decamp and colleagues

INVE

GOAL 2014 Survey

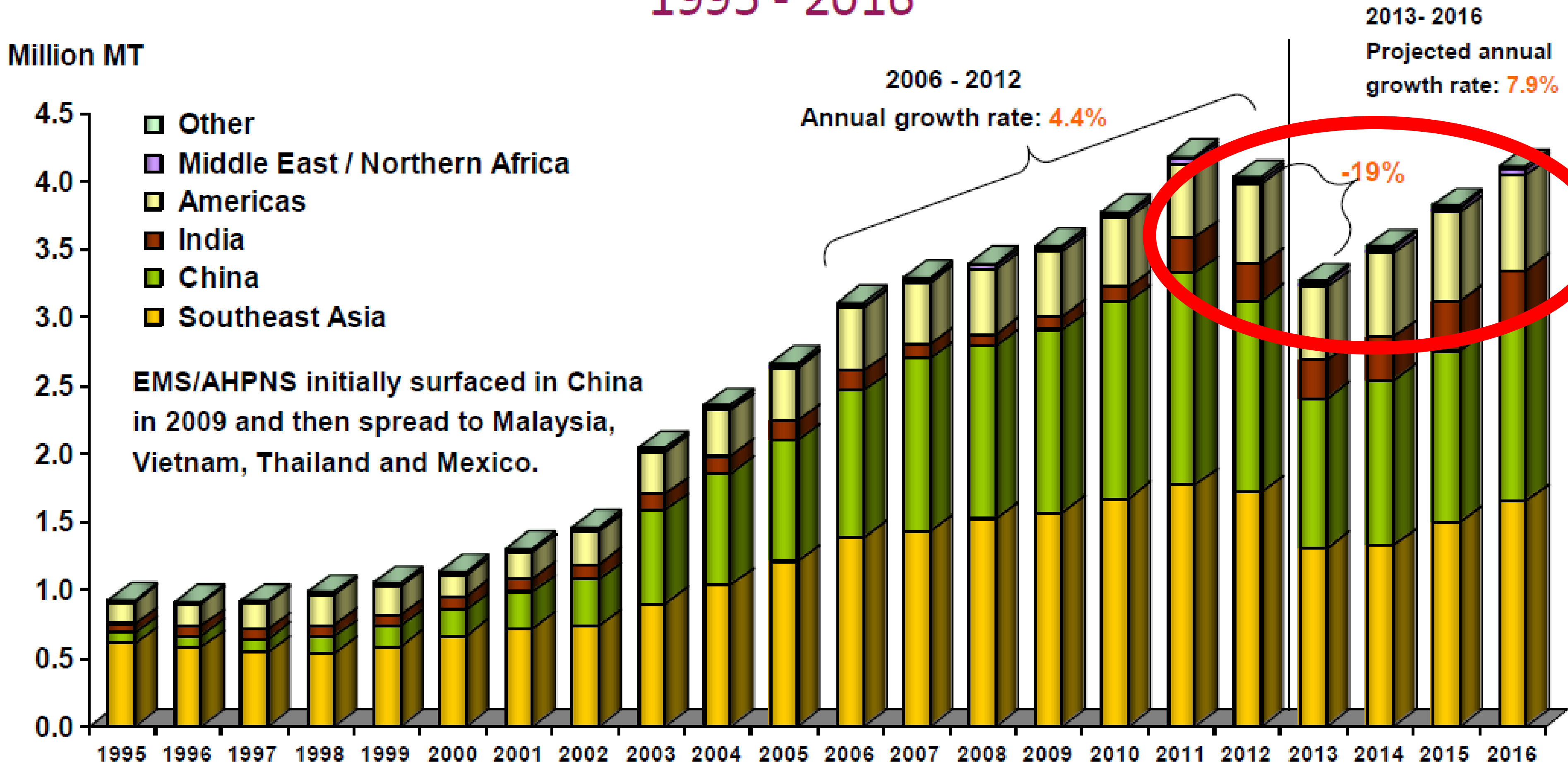
Issues & Challenges in Shrimp Aquaculture

All Countries



Asterisk indicates a Top 3 issue in GOAL 2007 Survey

GOAL 2014 Survey
Shrimp Aquaculture Production by World Region: 1995 - 2016



Sources: FAO (2014) for 1995-2012; GOAL (2014) for 2013-2016.

Southeast Asia includes Thailand, Vietnam, Indonesia, Bangladesh, Malaysia, Philippines, Myanmar and Taiwan.

M. rosenbergii is not included.

Current situation

AHPND, EHP, WSSV, Running Mortality Syndrome, Covert Mortality Disease, etc

Improvement in many countries regarding AHPND

Additional issues:

- Pathogens such as microsporidians (EHP) and WSSV
- Undefined syndrome like Running Mortality Syndrome in India
- Use of antibiotics to control pathogens
- Switch to small size shrimp
- Fluctuation in shrimp price
- Production/country incorrect due to translocation
- Loss of European tariff preference and anti-dumping duties

Running Mortality Syndromes (RMS)

Prolonged chronic mortalities during the crop

- Starts 1 to 2 months after stocking
- Mortality rate increasing year by year
- Mortalities increasing with temperature
- Severity varies depending on the source water
- Nothing seems to work when the mortality commences

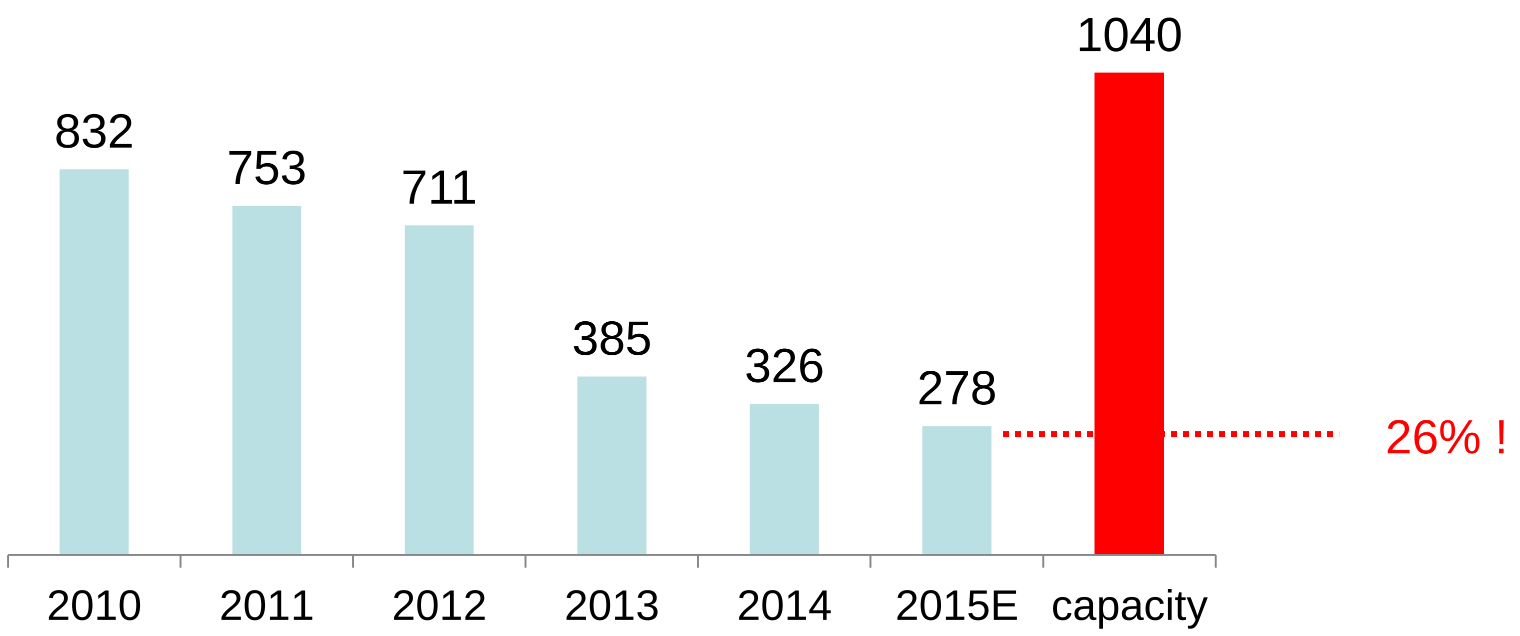
Multifactorial? covert mortality disease by CMNV, white muscle syndrome, EHP/white faeces syndrome

Impact of RMS in Indian shrimp culture

- Major harvest done in 50 to 70 count.
- Lower price for higher counts.
- Reduced income/loss for farmers
- Cycle period reduced to 60 – 70 days
- Frequent stocking (3-4 crops/year) increasing the demand for PLs!

Impact of disease (and other factors)

- Thai shrimp feed market ('000 MT)



(Diener, TARS 2015)

How to cope with diseases?

Preventive measures

- Biosecurity measures (incl monitoring)
- SPF animals
- Quality feed
- Water/soil management
- Health booster

Curative measures

- Contingency plans
- Treatment with biocides or antibiotics

How to cope with diseases?

- Potential vertical routes of infection: contaminated broodstock, contaminated larvae and postlarvae

➔ Focus on the hatchery!

- Potential horizontal routes of infection: water, cannibalism, biofilm, plankton, mollusks, birds, etc.

➔ Focus on the farm management

BACK TO BASIC

Biosecurity issues with broodstock

"Since typical water treatment in hatcheries removes bacterial and viral pathogens, the most likely transmission route for VPAHPND was to broodstock via live feeds"

"… shortages in the supply of imported SPF broodstock led some entrepreneurs to employ post larvae (PL) of imported SPF stocks to produce 2nd generation broodstock in open shrimp ponds where they became contaminated…"
(Thitamadee et al. in press)

→ Reduce use of live feed
→ Screening of broodstock

Bio-security at hatchery

IN THEORY

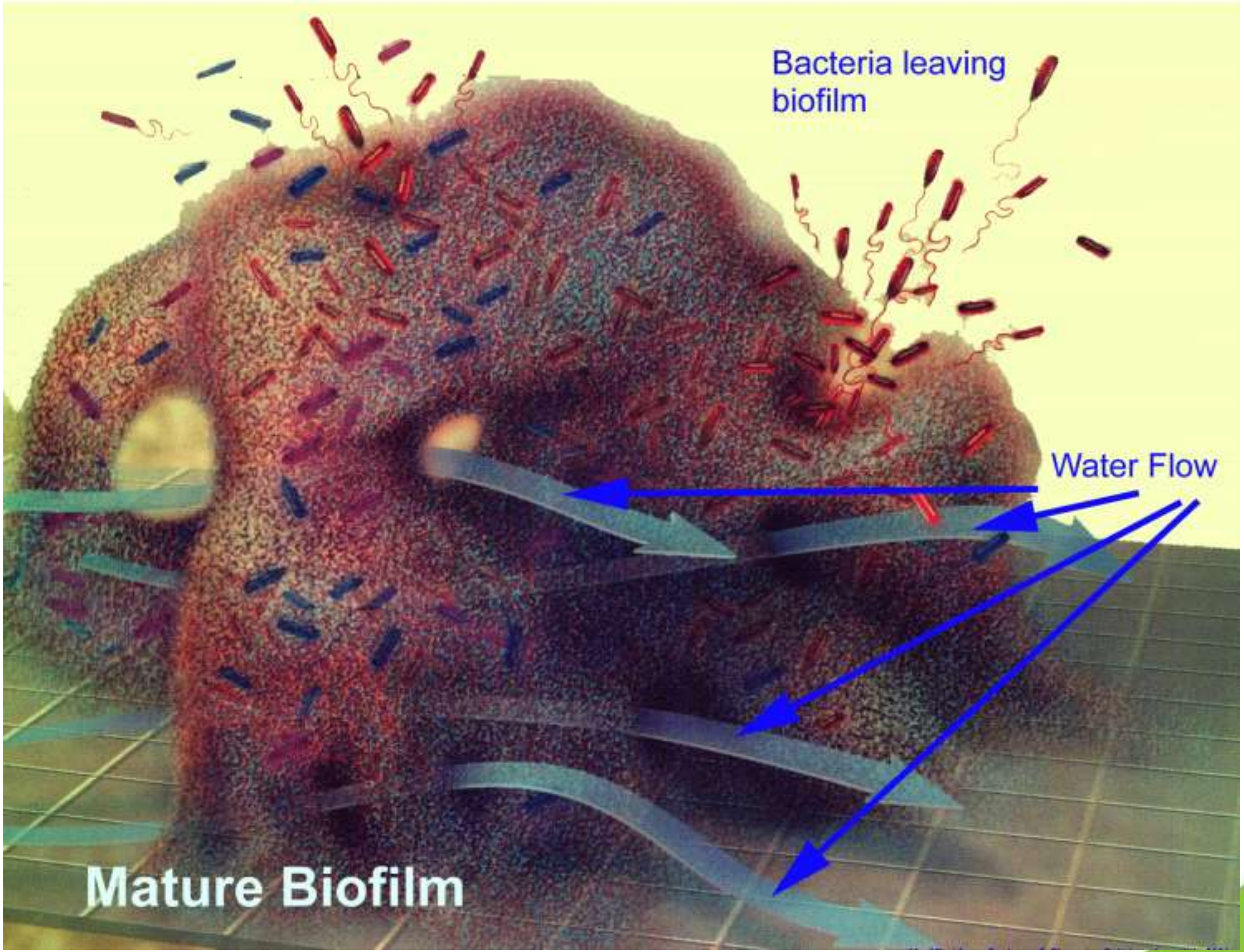
Bio-security is defined as *a set of practices that will reduce the probability of introducing a pathogen and its subsequent spread from one place to another*.

IN PRACTICE

Bio-security needs to be an integral part of the operations. Bio-security theory is translated into specific actions by virtue of the development of a **Bio-security Plan** and the daily implementation of the steps specified in the protocols of the plan. While the principals applied in Bio-security activities can be considered as global; the application of those principals is influenced by animal species, specified pathogen(s) and site specific characteristics.

- ❑ Parent animals
  - ❑ Nauplii
  - ❑ Postlarvae
- ❑ Rearing Facilities
- ❑ Water
- ❑ Feed

Bacteria released by biofilm



Biosecurity – bacterial biofilm

Antibiotics do not kill Vibrio in biofilm

Type of substrate	Control	Tetracycline	Chloramphenicol
Plastic	5.34×10^7	5.59×10^7	3.08×10^6
Cement slab	1.23×10^7	1.17×10^7	1.14×10^7
Steel coupon	2.44×10^6	7.18×10^6	1.08×10^7

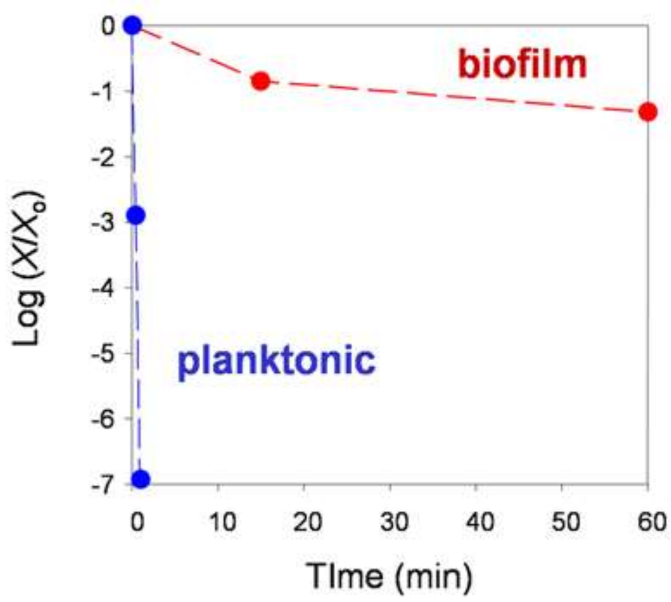
V. harveyi in water is sensitive to chloramphenicol and tetracycline. But V. harvery in biofilm will survive

➔ These Vibrio from the biofilm could be a source of infection for the larvae.

Karunasagar et al.1996. Aquaculture 140:241-245

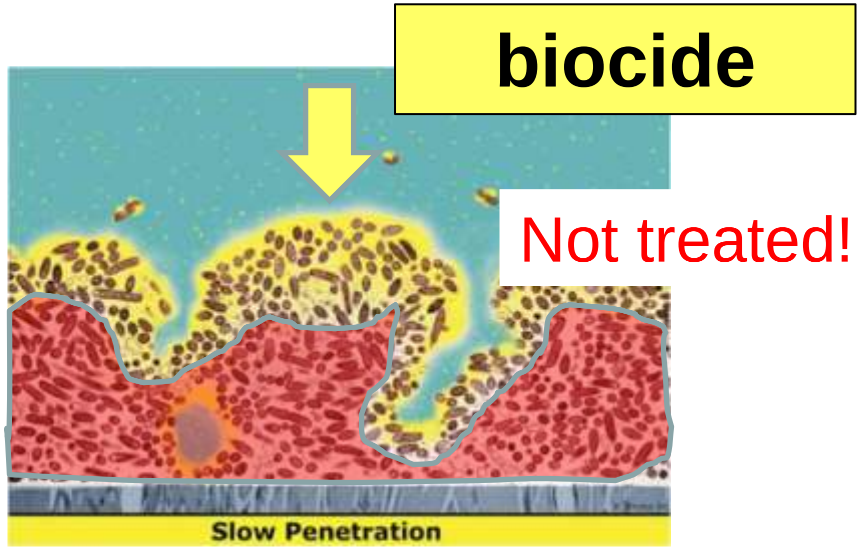
Biosecurity
How to cope with Vibrio?

Reduction in viable bacteria in planktonic vs biofilm



Omar et al. 2008. Annual Conference of the Canadian Society of Microbiologists

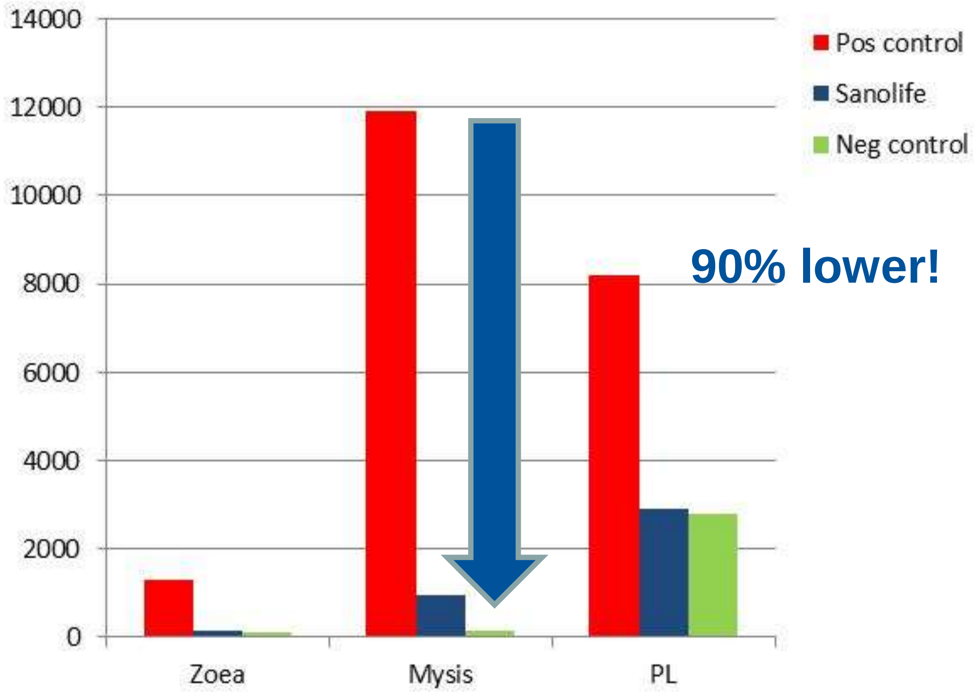
Polymeric substances in the matrix of a biofilm retard the diffusion of chemicals



Costerton et al. 1999. Science 284:1318-1322

Control Vibrio during larviculture

Vibrio's entry through algae, artemia, water, aerosol, staff, etc.



(Vogeley et al. 2011)

➔ Microbial management during culture

Issues with PLs

"Inferior postlarvae have played a part in almost 80% of EMS occurrences in Malaysia"

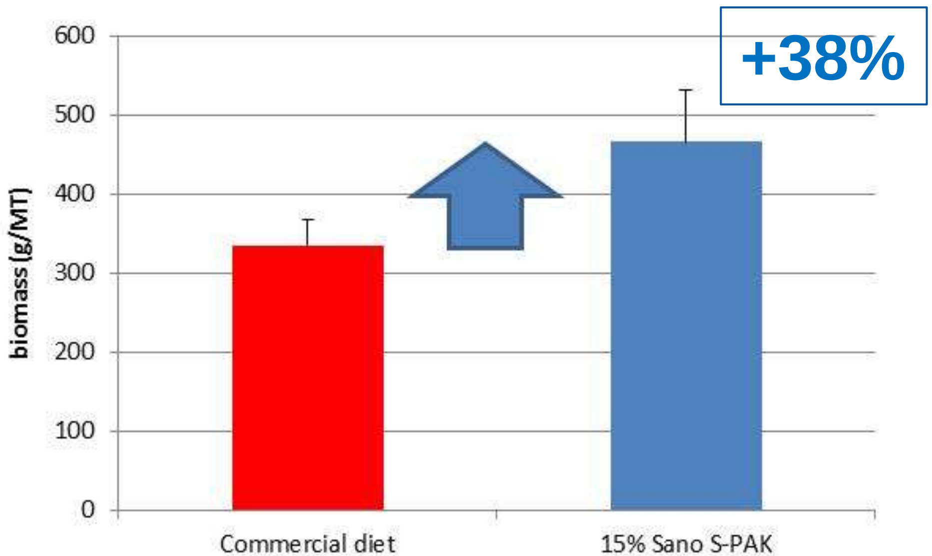
"Once the postlarvae are transferred to grow-out ponds, various changes in pond parameters such as low minerals, low dissolved oxygen, variations in temperature, pH, salinity and unpredictable weather could result in the already stressed postlarvae quickly succumbing to infections."

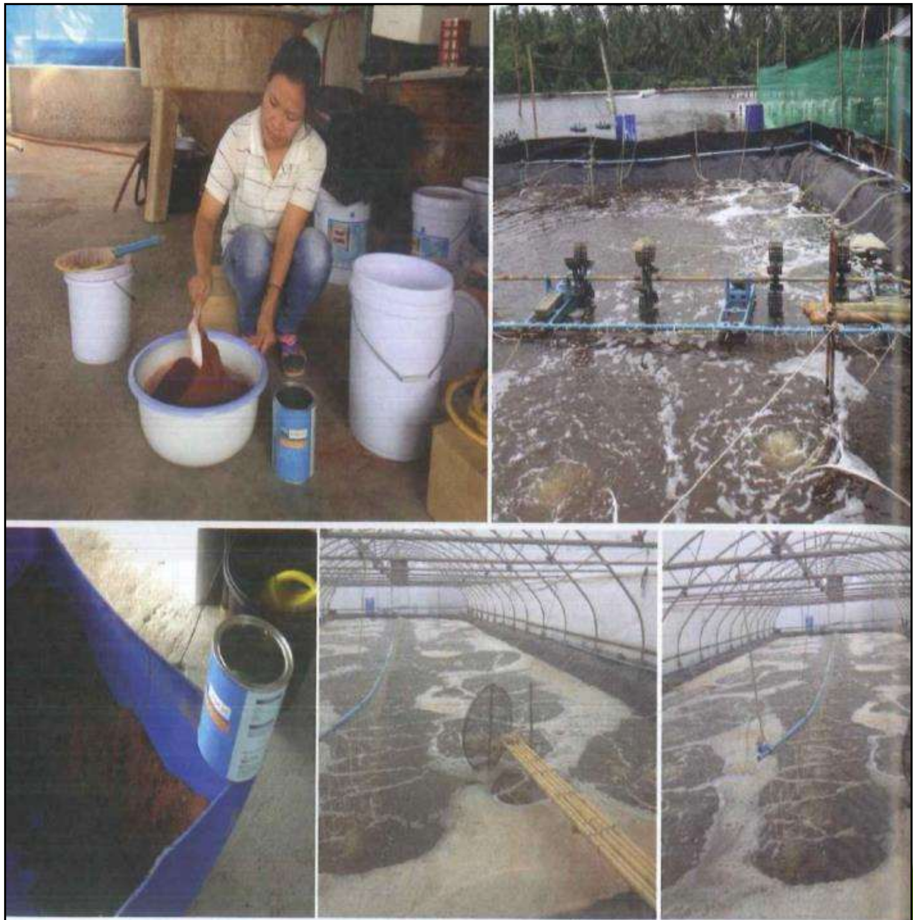
Karunanithi Muthusamy, 2013

- ➔ Produce stronger PLs
- ➔ Disinfect PLs before transfer

Use of nursery (Mexico)

Use of nursery in Asia





Sanoguard S-PAK ประกอบไปด้วยวิตามินหลายชนิด กรดไขมัน เม็ดสีและนิวคลีโอไทด์ที่มีความเข้มข้นสูงเพื่อช่วยในการปรับสมดุลภายในร่างกายการเจริญเติบโตรวมทั้งกระบวนการต่างๆ ที่มีความสำคัญในช่วงที่กุ้งเกิดความเครียด มีสารที่ช่วยในการกระตุ้นระบบภูมิคุ้มกัน ทำให้ระบบภูมิคุ้มกันของกุ้งสามารถทำงานได้อย่างมีประสิทธิภาพ ยับยั้งการเจริญเติบโตของเชื้อ *Vibrio* และเชื้อไวรัสชนิดต่างๆ ที่มีอยู่ภายในตัวกุ้งได้ดี ซึ่งในจุดนี้เองที่ทำให้กุ้งมีอัตราการรอดตายที่สูงขึ้น

Control Vibrio during nursery

Nursery rearing of the pink shrimp *Farfantepenaeus brasiliensis* in a zero exchange aerobic heterotrophic culture system. Three replicate tanks randomly assigned to the 3 probiotic treatments vs control.

Treatament	Survival	Final Weight	Specific Growth rate
Inve	91.65 ± 11.02^a	1.42 ± 0.40^a	0.036 ± 0.007^a
Other probiotic	81.92 ± 2.40^a	1.39 ± 0.8^a	0.035 ± 0.009^a
Other probiotic	81.90 ± 13.4^a	1.34 ± 0.36^a	0.034 ± 0.004^a
Control	88.86 ± 6.36^a	1.22 ± 0.38^b	0.030 ± 0.003^b

*Different superscript letters indicate significant difference

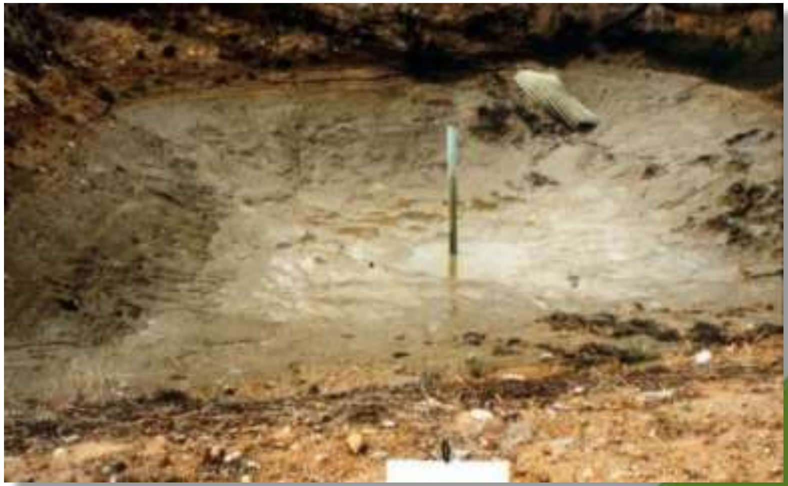
Benefit of probiotic treatment:
- Concentration of presumptive *Vibrio* spp. significant lower ($p<0.05$)
- Higher levels of total protein and granular hemocyte

Souza et al. 2011. THE USE OF PROBIOTICS DURING THE NURSERY REARING OF THE PINK SHRIMP Farfantepenaeus brasiliensis IN A ZERO EXCHANGE SYSTEM. World Aquaculture 2011 - Meeting Abstract. https://www.was.org/WasMeetings/meetings/ShowAbstract.aspx?Id=24044

What to do? At the farm…

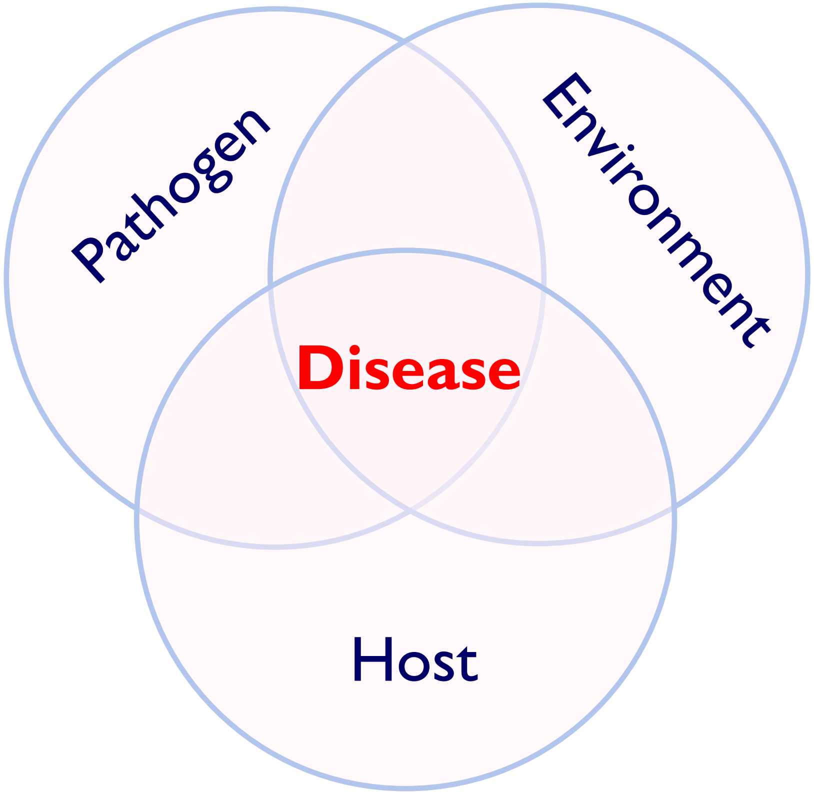
Improved management:

- consider the carrying capacity of the pond
- manage the nitrogen/phosphate ratio to control algae
- control anaerobic zone in the pond sediment



Control of disease with microbial management

Reduced presence of pathogen through inhibition or competition



Improved rearing conditions with less stress on animals, and lower risk of bacterial bloom

Stronger animal trough nutrition and immunostimulation

Danger of ammonia – disease

Shrimp, when exposed to ammonia prior to immersion challenge with *Vibrio, suffered* more frequent and earlier pathological changes

Alday-Sanz, V., Roque, A., and Turnbull, J.F. 2002. Clearing mechanisms of *Vibrio vulnificus biotype I in the black tiger* shrimp *Penaeus monodon. Dis Aquat Organ* ***48: 91–99.***

Liu CH, Chen JC. Effect of ammonia on the immune response of white shrimp Litopenaeus vannamei and its susceptibility to Vibrio alginolyticus. Fish Shellfish Immunol 2004;16:321–34.

5 mg l−1 ammonia-N reduced the immunocompetence of *P. japonicus*.

Modulatory effects of ammonia-N on the immune system of Penaeus japonicus to virulence of white spot syndrome virus. Aquaculture 241:61-75. 2004

Ammonia: management strategies

Convert toxic ammonia into nitrite and then nitrate
But nitrification requires a substrate for growth of nitrifying bacteria, high dissolved oxygen and maintained alkalinity

Convert ammonia into bacterial cell
But frequent application of molasses can lead to waste accumulation. Microbial growth require oxygen.

Remove sludge from pond bottom (siphon)
But this requires water exchange. Toxic waste can be re-suspended in water. Higher disease risk

Microbial management and disease

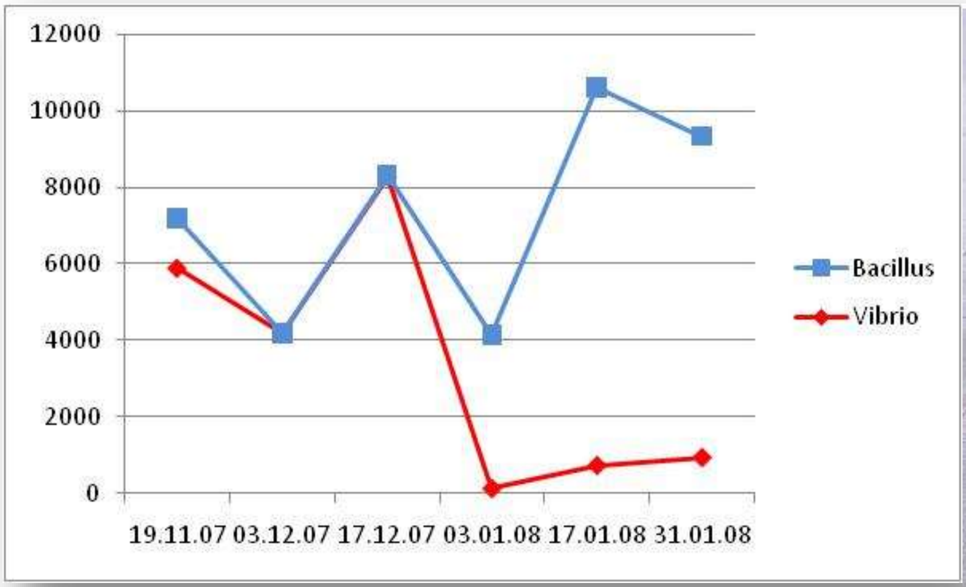
Control of waste material on pond bottom

Nearly all vibrios can metabolize chitin (Hunt et al., 2008, *Science* 320, 1081–1085; Grimes et al., 2009, *Microb.Ecol.* 58, 447–460)

Vibrio can survive long-term under resource-limited conditions; recover from starvation and grow rapidly in response to substrate pulse; and actively seek out nutrient patches (Thompson and Polz, 2006, *The Biology of Vibrios*, 190–203)

Pond bottom management

Bacillus probiotics and lower Vibrio abundance in sediment



Microbial dynamics in shrimp ponds

By Marcos H. S. Santos, Tadeu de Silva, Jose Torres, Roseli Pimentel Silva, David Moriarty and Olivier Decamp

Understanding the microbial ecological process in ponds and in the shrimp intestinal tract is vital to maintain and optimize productivity without prophylactic use of hazardous substances. The maintenance of good water quality and the control of disease are closely linked to managing the communities of microbes and phytoplankton.

Abudance of **Bacillus** and **Vibrio** in soil (cfu/g)

Santos et al. 2009. AQUAculture Asia Pacific July pp 25-26

Benefits of selected Bacillus strains

Bacteria release extracellular enzymes to break down particles in order to make them more readily available.

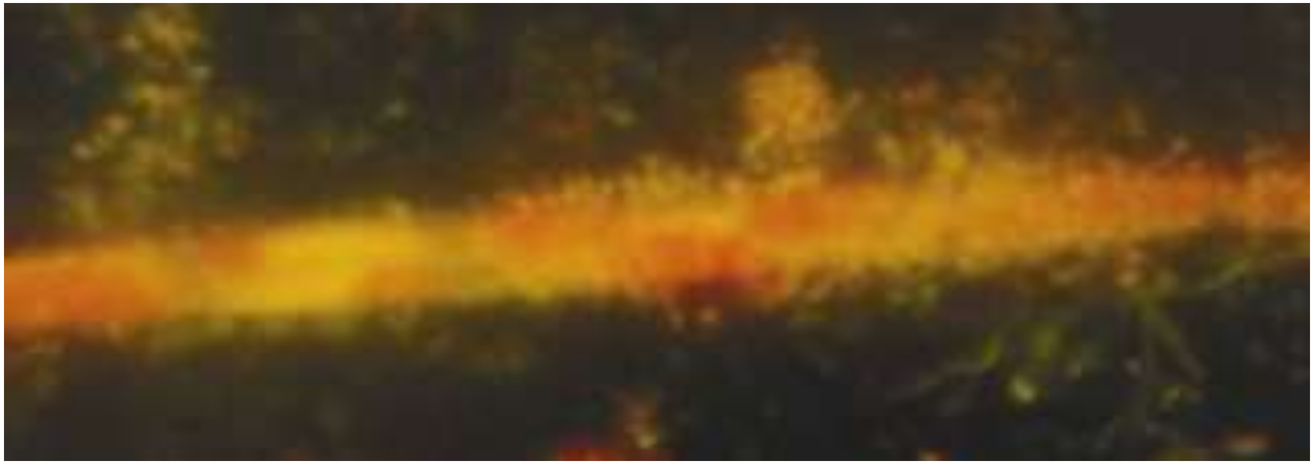
Enzymes produced by Sanolife Bacillus strains
- protease (uneaten feed, faeces, dead shrimp, etc)
- amylase (uneaten feed)
- cellulose (algal material, uneaten feed)
- xylanase (algal material, uneaten feed)
- mannanase (algal material)

Pond bottom management

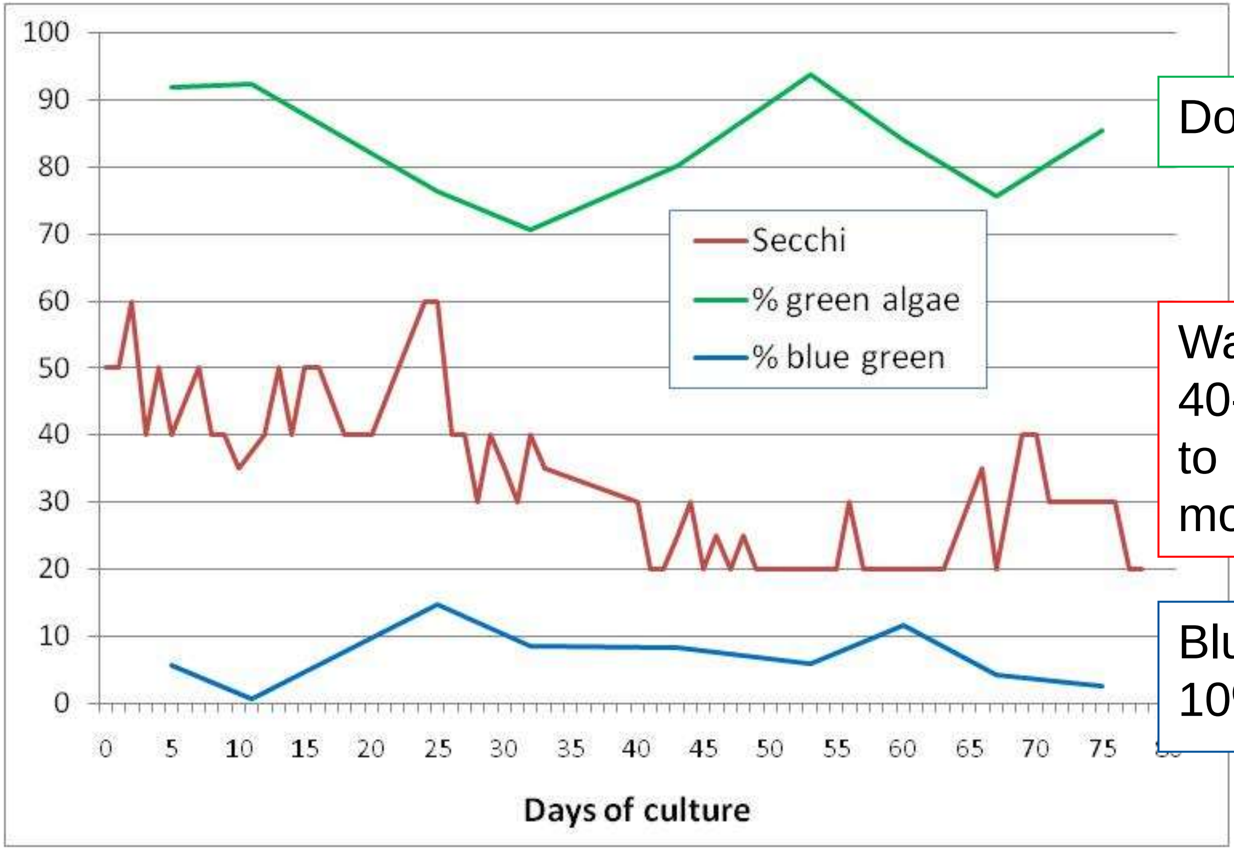
Microbial management and disease

Control of phytoplankton

Cyanobacteria as a long-term reservoir of *Vibrio cholerae* (Islam et al., 2004. Can. J. Microbiol. 50: 127–131)



Control of phytoplankton through the use of water probiotic, control of C:N:P ratio in semi-biofloc



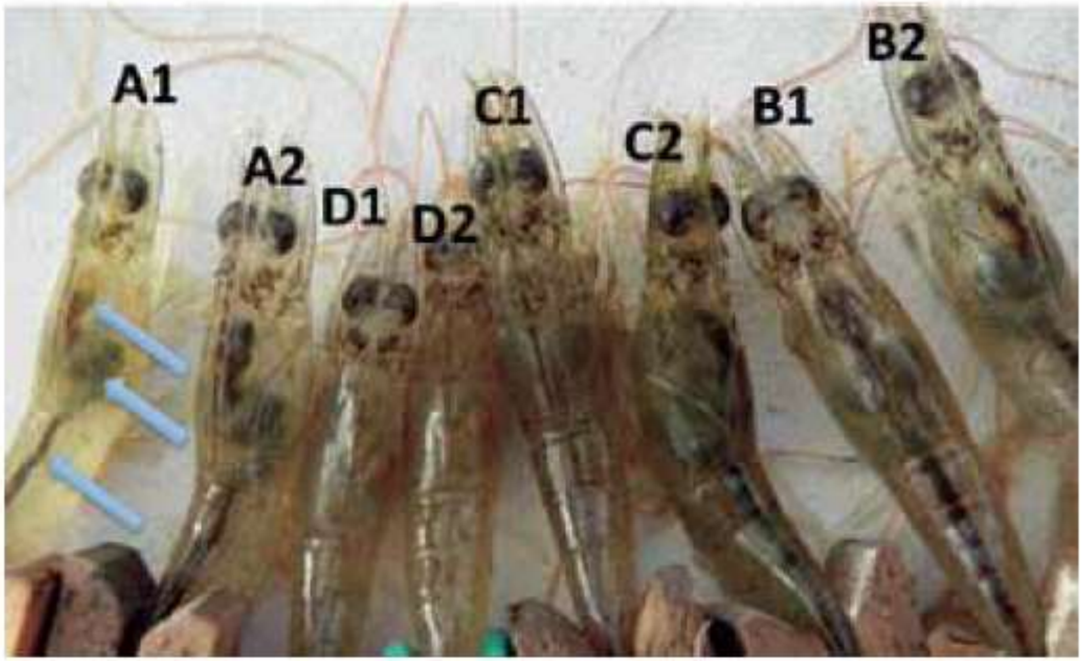
Dominated by green algae

Water transparency from 40-60 cm in the first weeks to 20-30cm in the last 2 months of the crop

Blue-green algae below 10% most of the time.

(Huda et al. 2013. AQUA Culture Asia Pacific Magazine March-April, pp 8-12 production

Tilapia Could Enhance Water Conditions, Help Control EMS In Shrimp Ponds



Ten days after exposure to pathogenic *Vibrio parahaemolyticus*, shrimp A1, A2, C2, B1 and B2 show normal stomachs, hepatopancreases and midguts (arrows from top to bottom). The remaining shrimp show signs of AHPN infection: empty stomachs, pale hepatopancreases and empty midguts.

Loc H. Tran, Ph.D.
School of Animal and Comparative Biomedical Sciences
Department of Soil, Water and Environmental Sciences
University of Arizona
1401 East University Boulevard
Tucson, Arizona 85721 USA
thuuloc@email.arizona.edu

Kevin M. Fitzsimmons, Ph.D
Department of Soil, Water and Environmental Sciences
University of Arizona

Donald V. Lightner, Ph.D
School of Animal and Comparative Biomedical Sciences
University of Arizona

AHPN Challenge Study

A laboratory study was conducted at

Microbial management via feed

- Probiotic coated on feed at farm

- Probiotic coated on feed at feedmill

- Alternative method of delivery of gut probiotic

Key issues to understand probiotics

- Product description (composition, concentration)
- Product claims (validated by studies)
- Product registration
- Product QC (consistency)
- Product safety (covered by registration and subsequent issues)

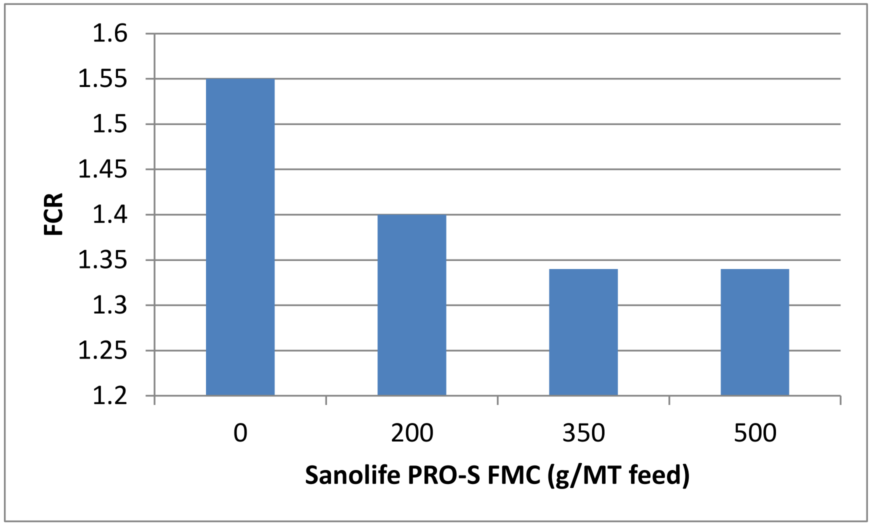
Microbial management and disease

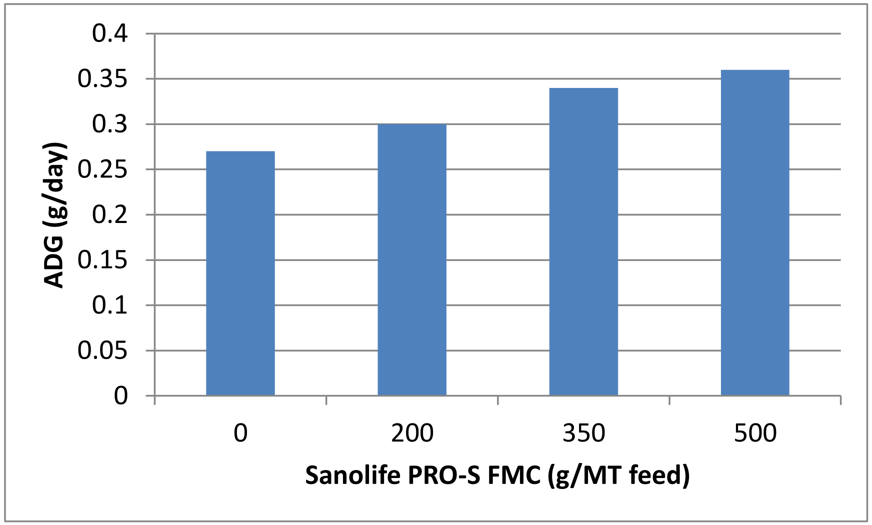
Improved nutrition – production of enzymes

- Higher growth when protease-producing *Bacillus* added to shrimp diet (Liu et al, 2009. J Appl Microbiol. 107:1031-41)
- Increased activity of protease and amylase of the shrimp midgut gland and the intestine in *Bacillus*-treated feed (Geovanny & Shen, 2008. J Ocean Univ China 7:215-218)

Benefit of feed probiotics

Improved nutrition – Improved FCR and growth



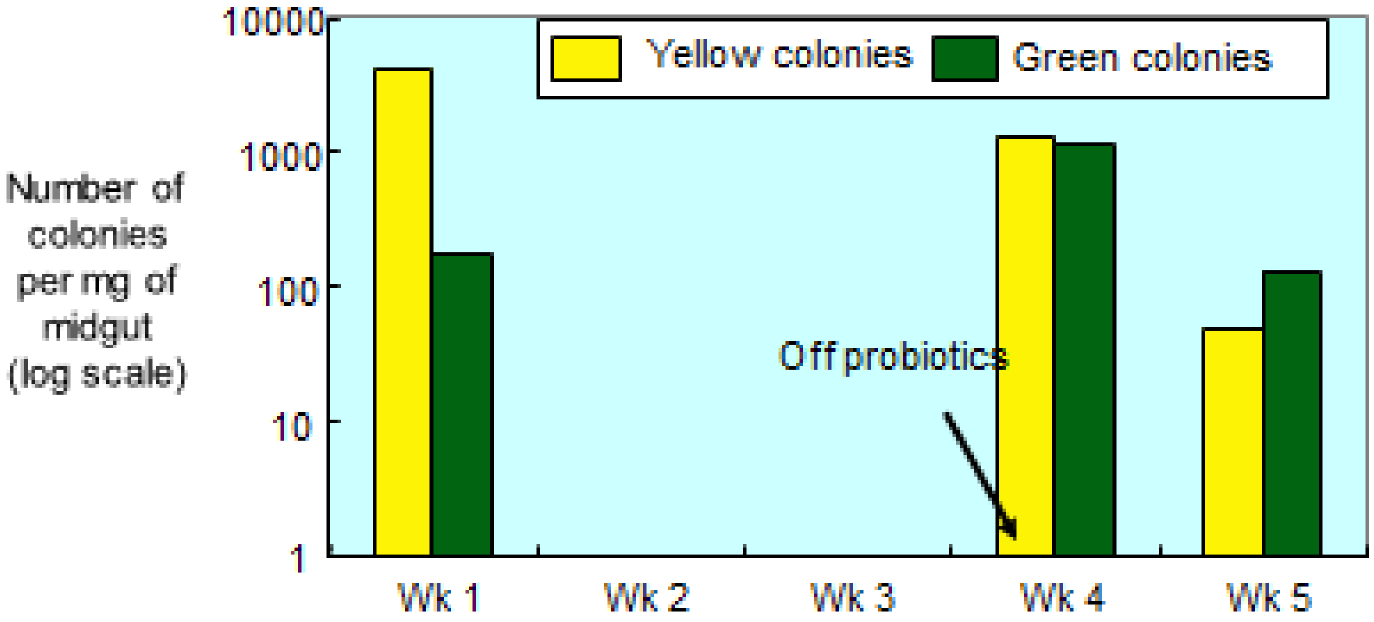


INVE Aquaculture. 56 DOC. Test run at the Fisheries Faculty of Kasetsart University, Thailand

Microbial management and disease

Reduced abundance of (potential) pathogens in gut

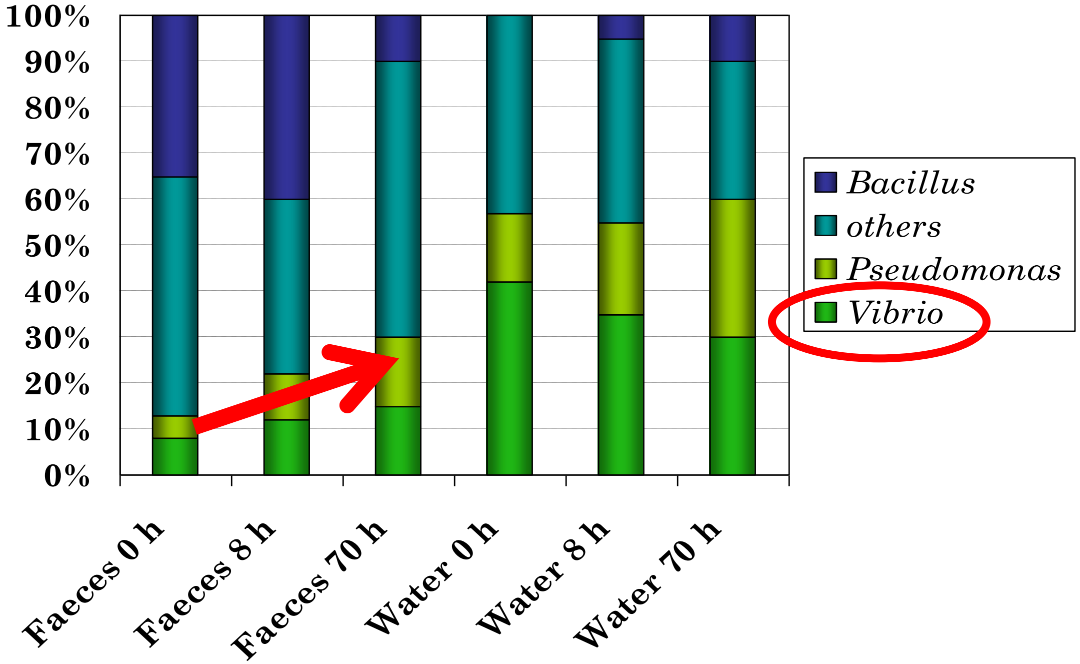
"Colonization" (=occupy) of the environment



Moriarty & Barnes. University of Queensland, Australia.

Microbial management and disease

Reduced abundance of (potential) pathogens in faeces



Hansen & Bech. 1996. J. Plankton Res. 18: 257-273

- Faeces contain undigested feed in addition to waste
- *Bacillus* in the feed pass into the faeces and secrete enzyme, increasing its digestibility.
- *Bacillus* in the feed/ faeces compete with vibrios and inhibit pathogens

Link between water probiotic and gut microflora

Evaluation of presence and efficiency of probiotic bacteria in the gut of *Litopenaeus vannamei* reared in BFT system

Bárbara Hostins *, Gabriele Lara, Dionéia E. Cesar, Paulo C. Abreu, Wilson Wasielesky Jr.

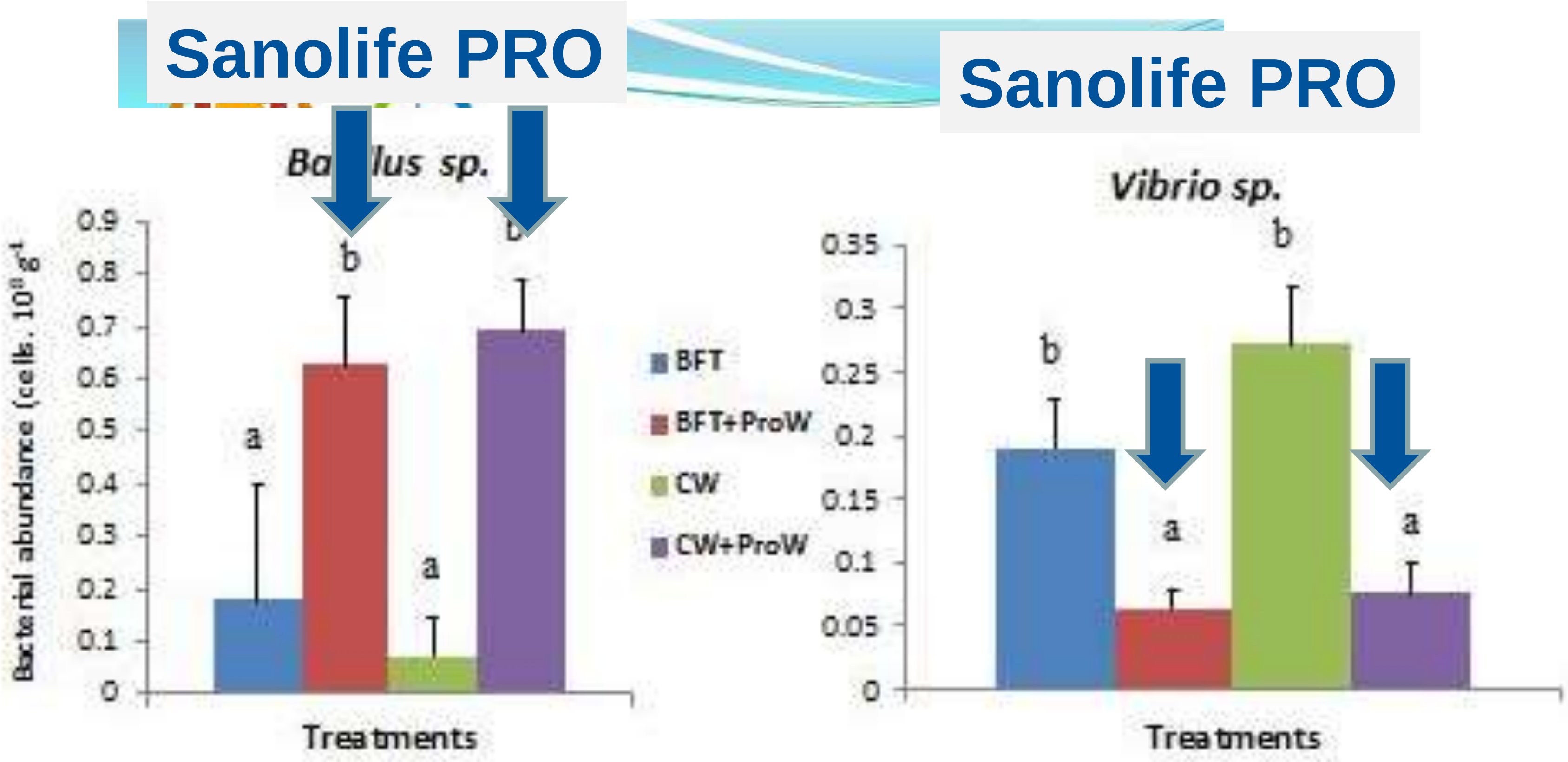
Aquaculture America, New Orleans 2015

Material and methods

- ✓ 50 L experimental tanks
- ✓ 150 shrimps/m^2
- ✓ Initial weight 0,64 ± 0,02g
- ✓ Commercial Probiotic
- ✓ *Bacillus subtilis* and *Bacillus licheniformis* (5 x 10^6 cfu/mL)

Reduced Vibrio with Sanolife!

High Bacillus and low Vibrio inside shrimp gut



GLOBAL SHRIMP FARMING SITUATION

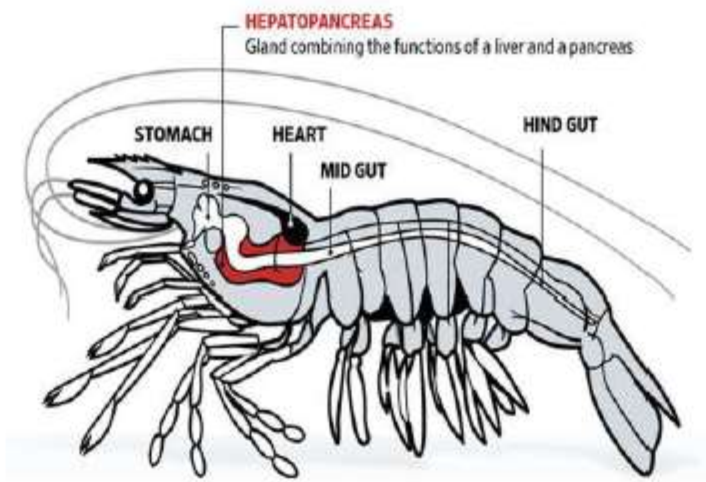
George Chamberlain
President

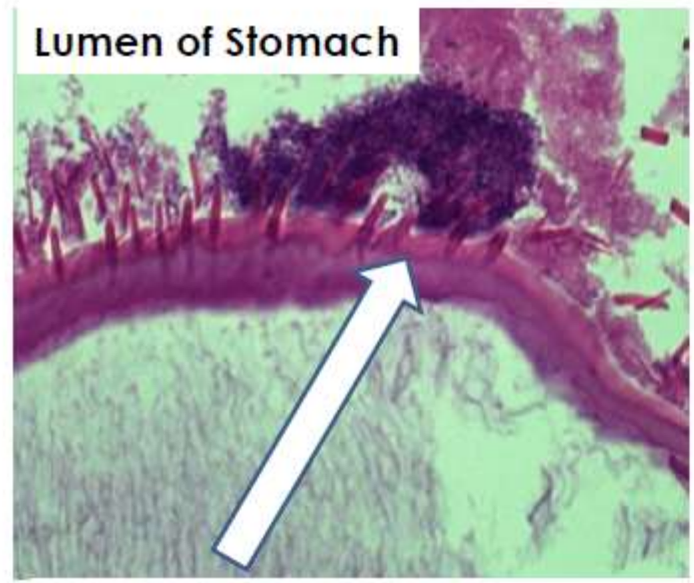
global aquaculture
the alliance

iAqua
IMPROVING AQUACULTURE

INVE AQUACULTURE

EMS COLONIZES THE STOMACH AND PRODUCES A TOXIN THAT DAMAGES THE HEPATOPANCREAS

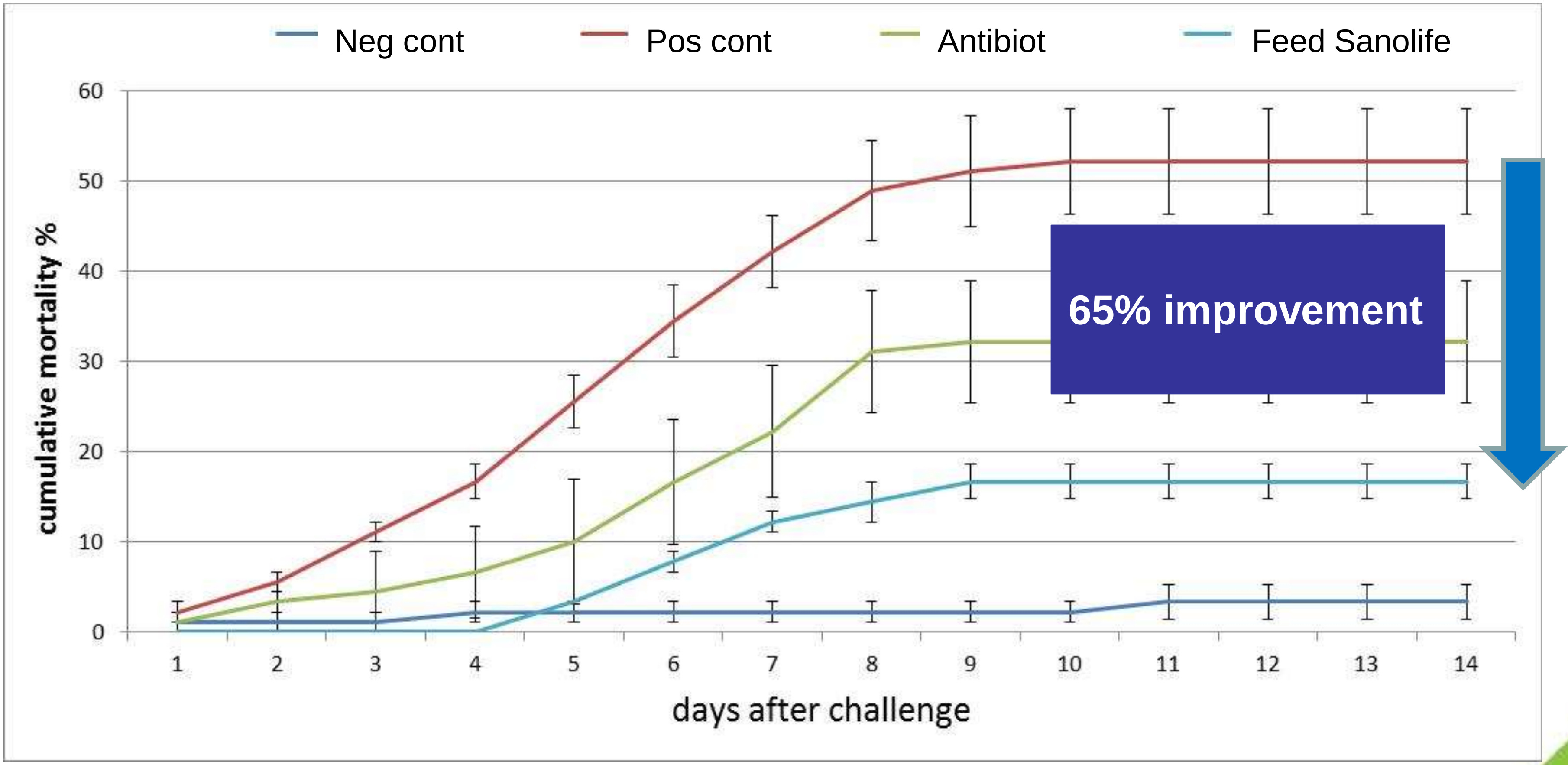




Microcolony of bacteria growing on epicuticle of gastric wall

INVE

Benefit in AHPND challenge test



How could feed Bacillus help against AHPND or white faeces?

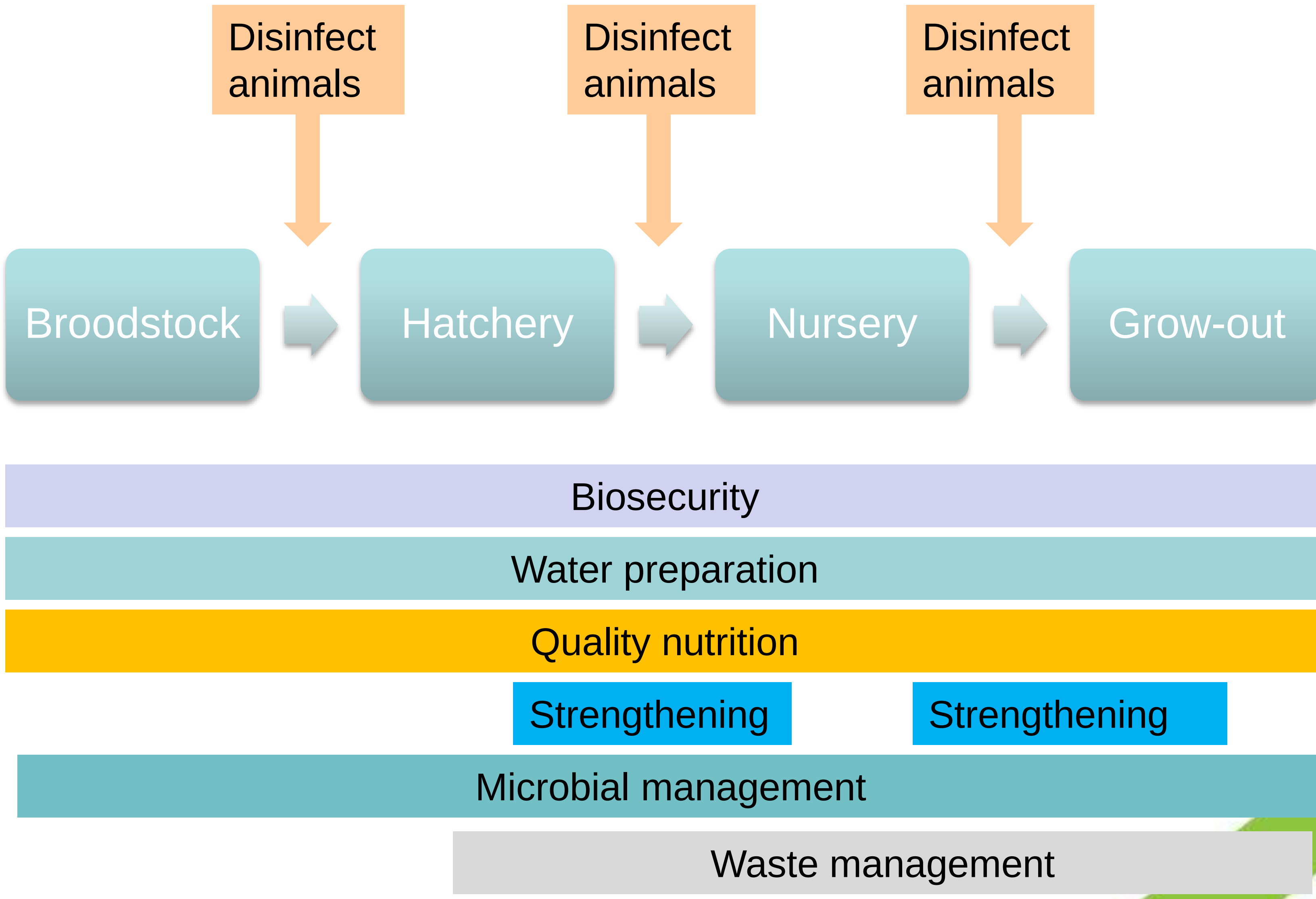
- Stimulate immune system ➔ better self defense by shrimp

- Kill directly pathogens

- Displace pathogens by occupying gut

- Improve nutrient uptake (stronger shrimp)

Conclusion – need for holistic approach



Conclusion – need for holistic approach

- Improved screening of PLs
- Use of health boosters (feed/additive/probiotic)
- Changes to farm set-up (development of nurseries)
- Changes to pond set-up (central drain)
- Changes to water treatment and management

Thank you