INVE
AQUACULTURE

AN UPDATE ON MICROBIAL MANAGEMENT STRATEGIES DURING DIFFICULT TIMES IN SHRIMP CULTURE

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Project Leader, Innovations Department
INVE TECHNOLOGIES NV

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WHY UPDATE?

INVE

undercurrentnews
seafood business news from beneath the surface

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BLACK TIGER SUPPLY WORLD

GOAL: Disease the biggest challenge for shrimp aquaculture industry

By Louis Harkell Oct. 10, 2017 09:23 BST

Credit: ShrimpVet on Facebook

DUBLIN, Ireland -- Disease has overtaken cost of production to b
challenge facing the world's shrimp industry, according to an ind
Global Aquaculture Alliance.

Global Aquaculture Advocate

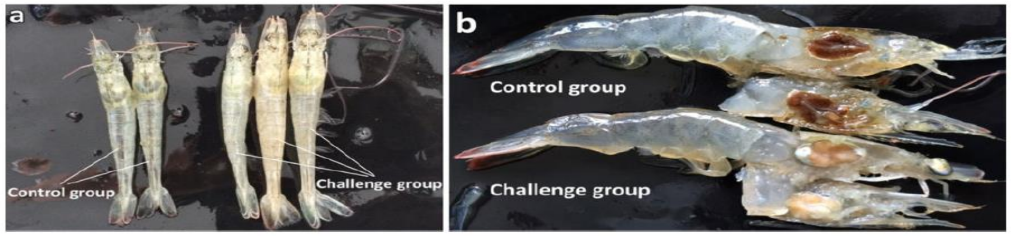
ANIMAL HEALTH & WELFARE (/ADVOCATE/CATEGORY/ANIMAL-HEALTH-WELFARE)

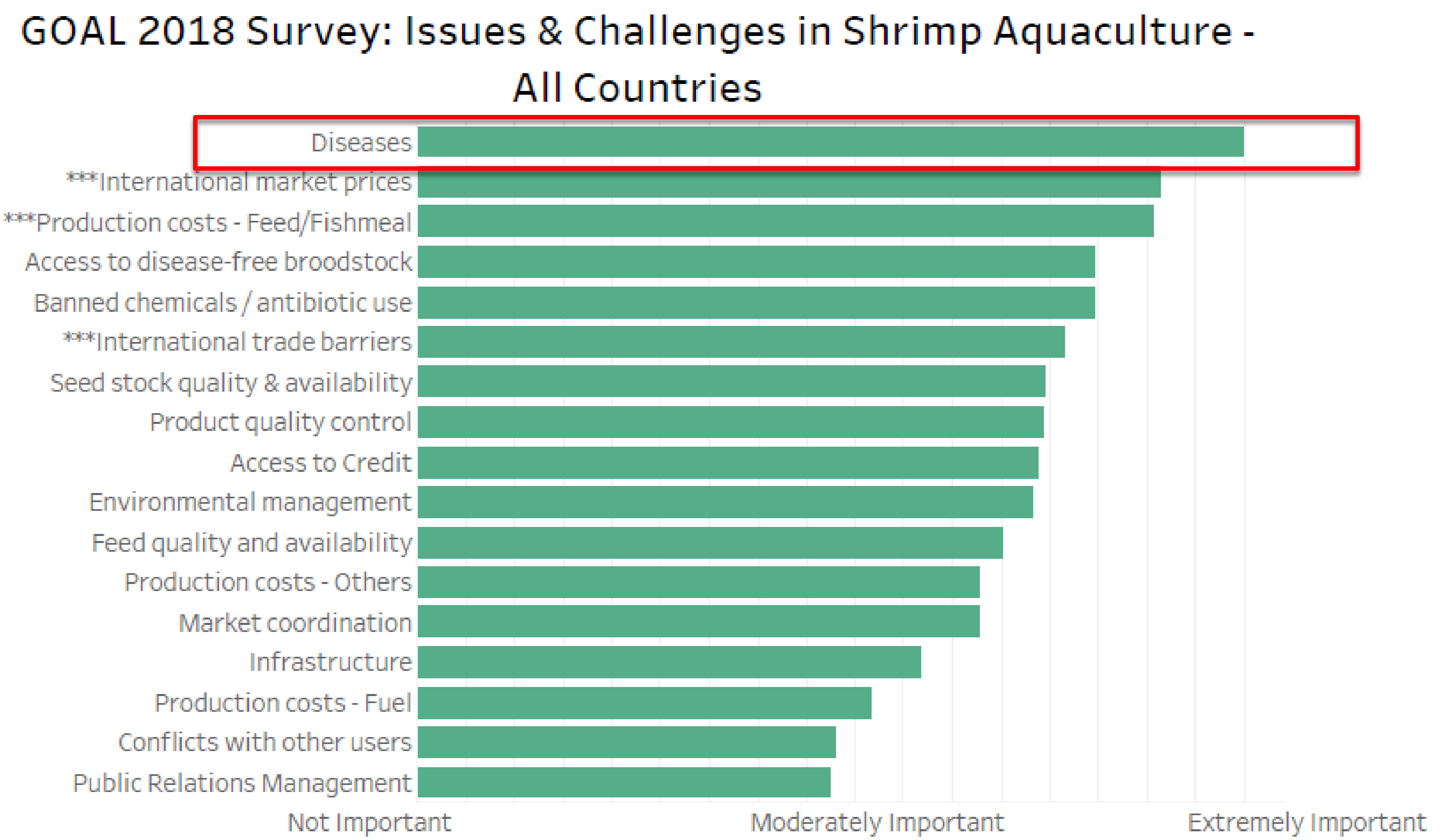
Emerging disease: Shrimp Hemocyte Iridescent Virus (SHIV)

Monday, 12 November 2018

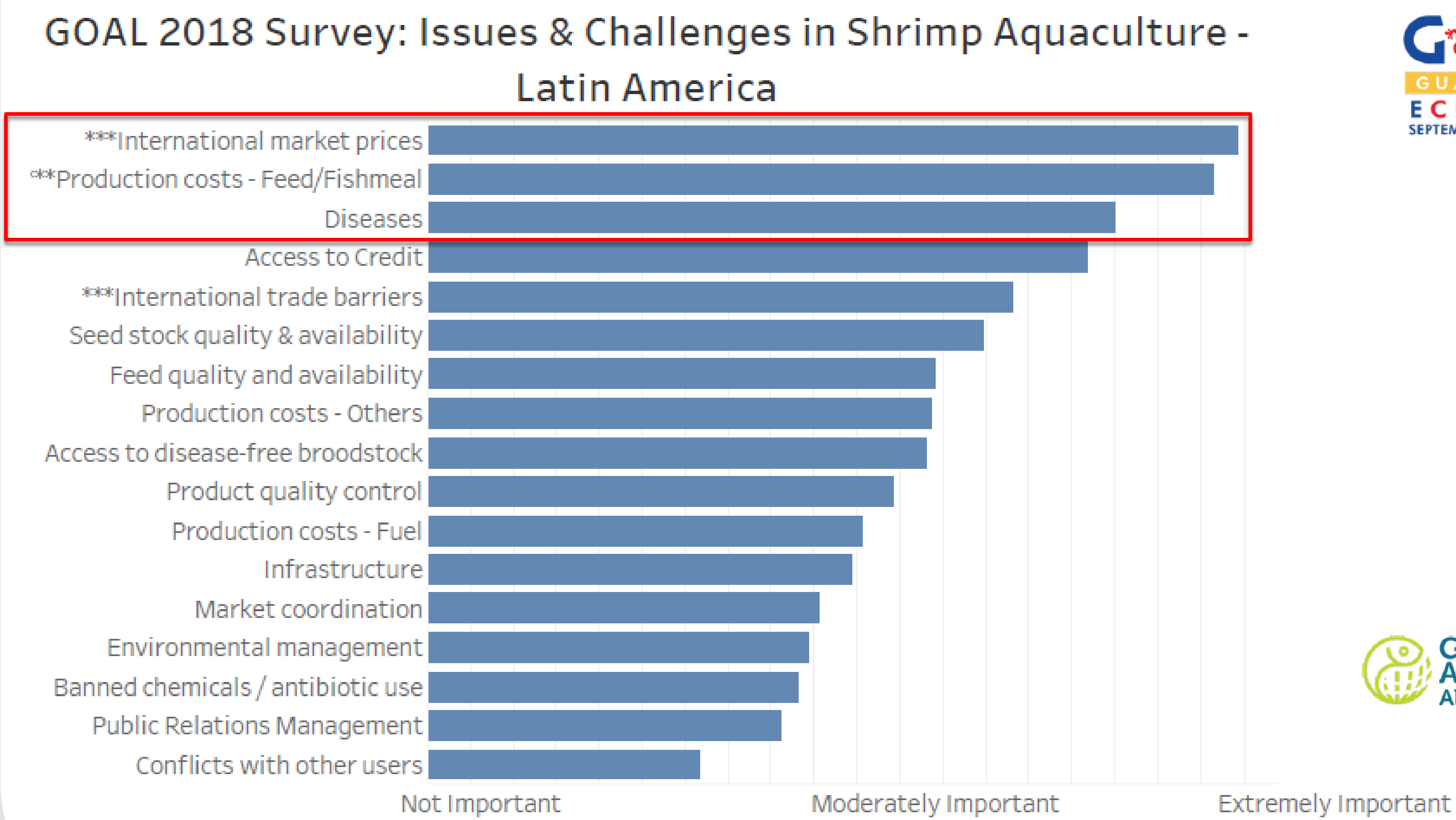
By Dr. Jie Huang

Virus causing serious mortalities in Pacific white shrimp in China





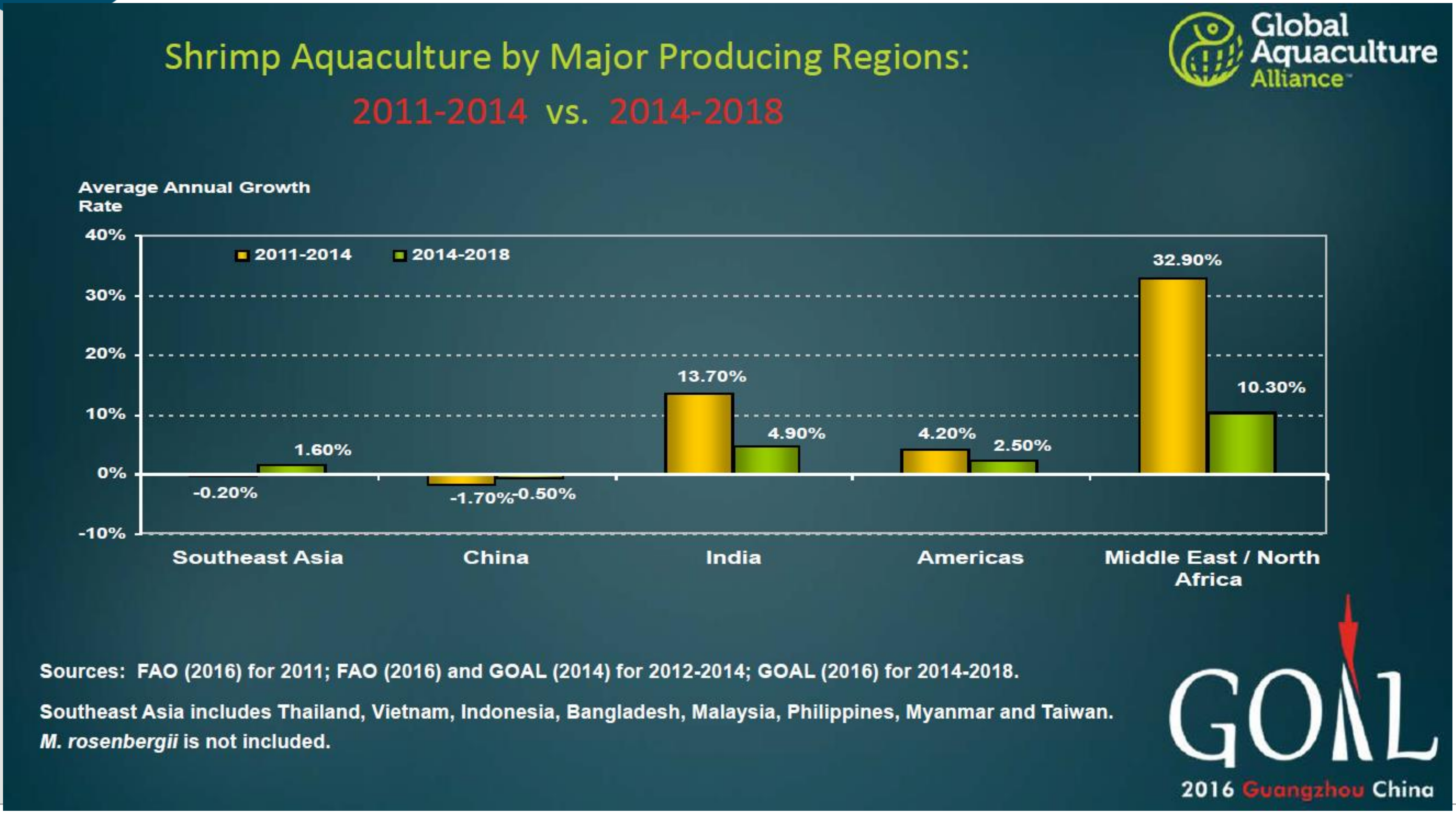
WHY UPDATE?



Presentation James Anderson - University of Florida

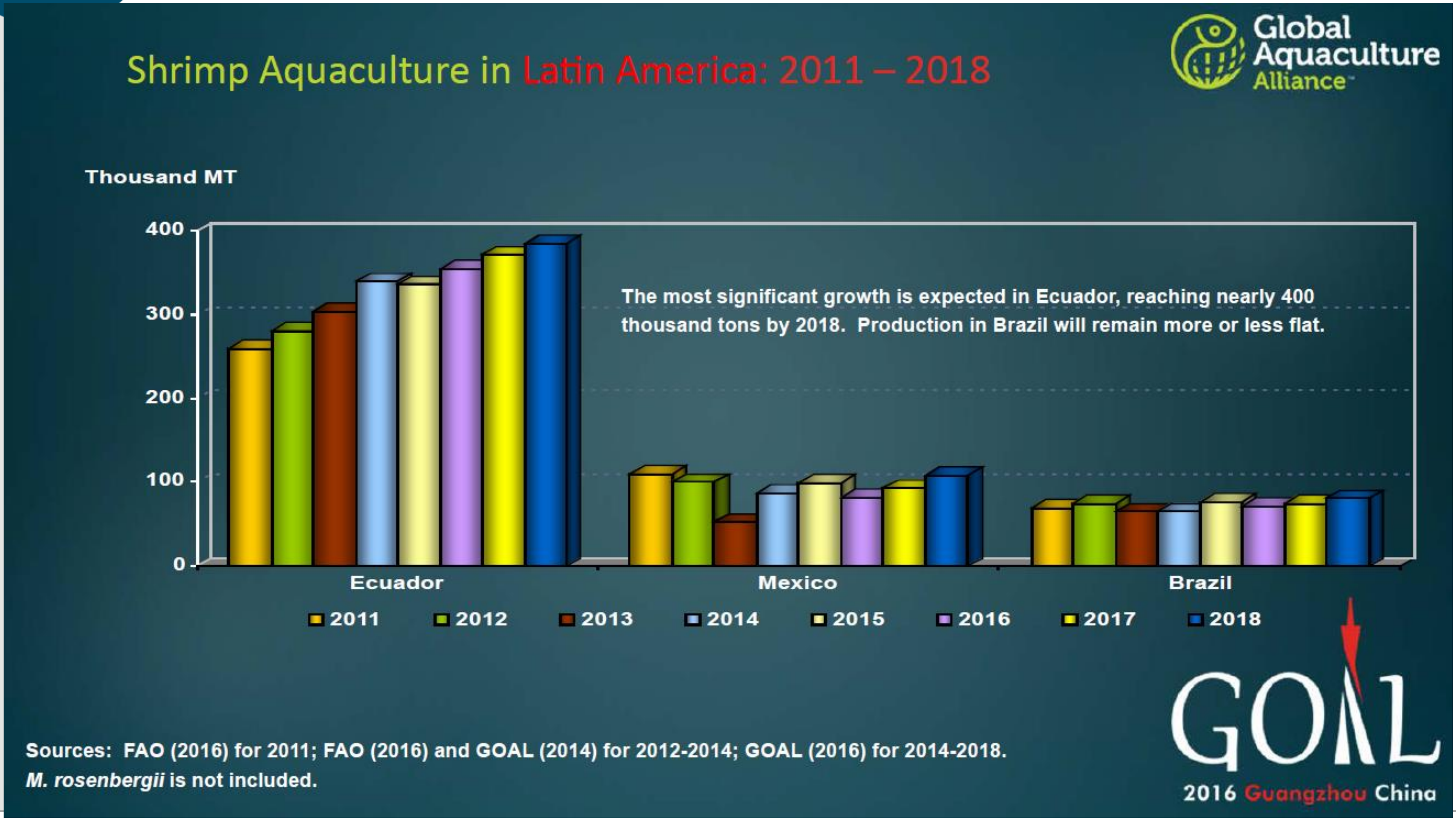
SITUATION IN AMERICAS AND BRASIL

Presentation James Anderson - University of Florida

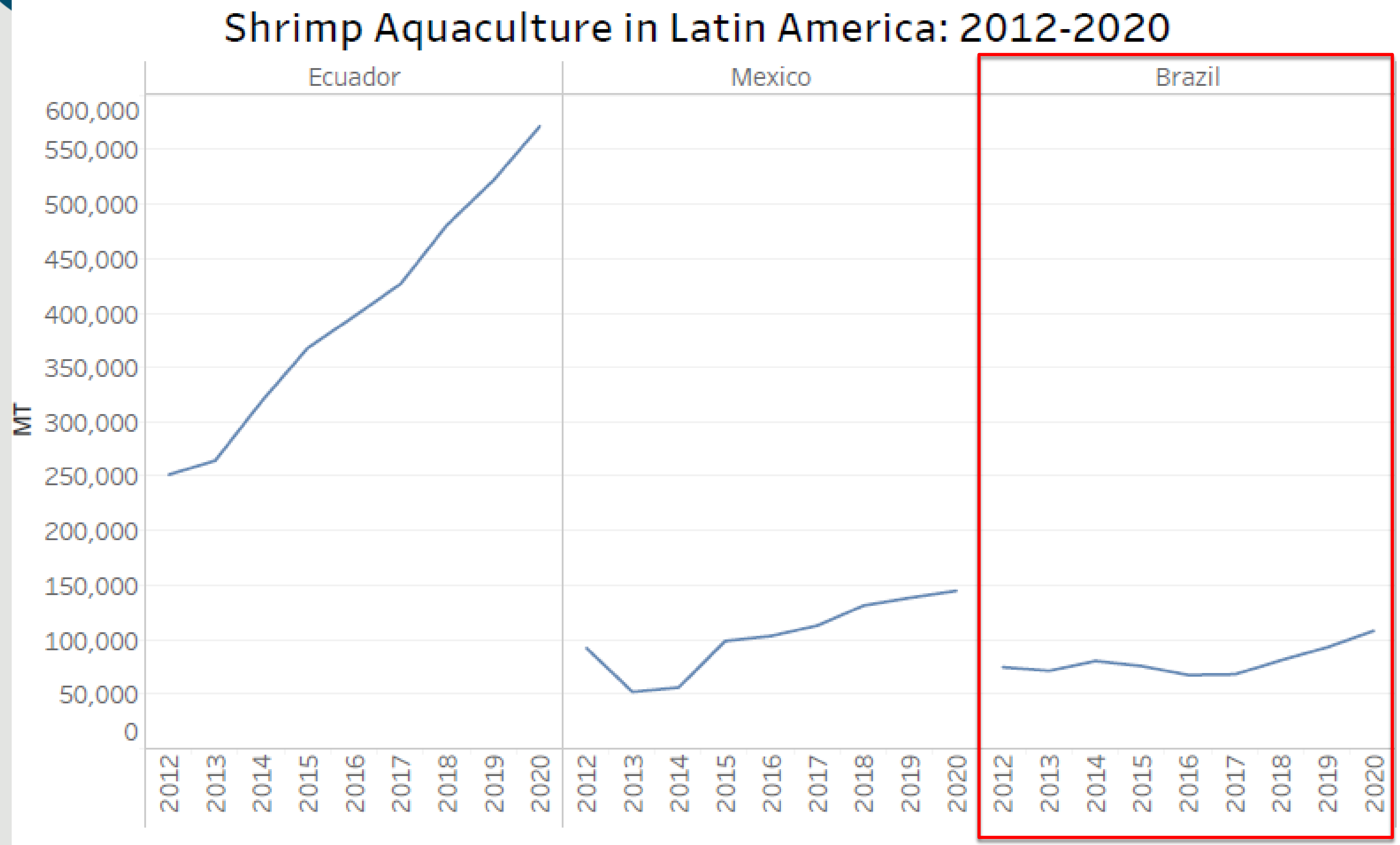


SITUATION IN AMERICAS AND BRASIL

Presentation James Anderson - University of Florida



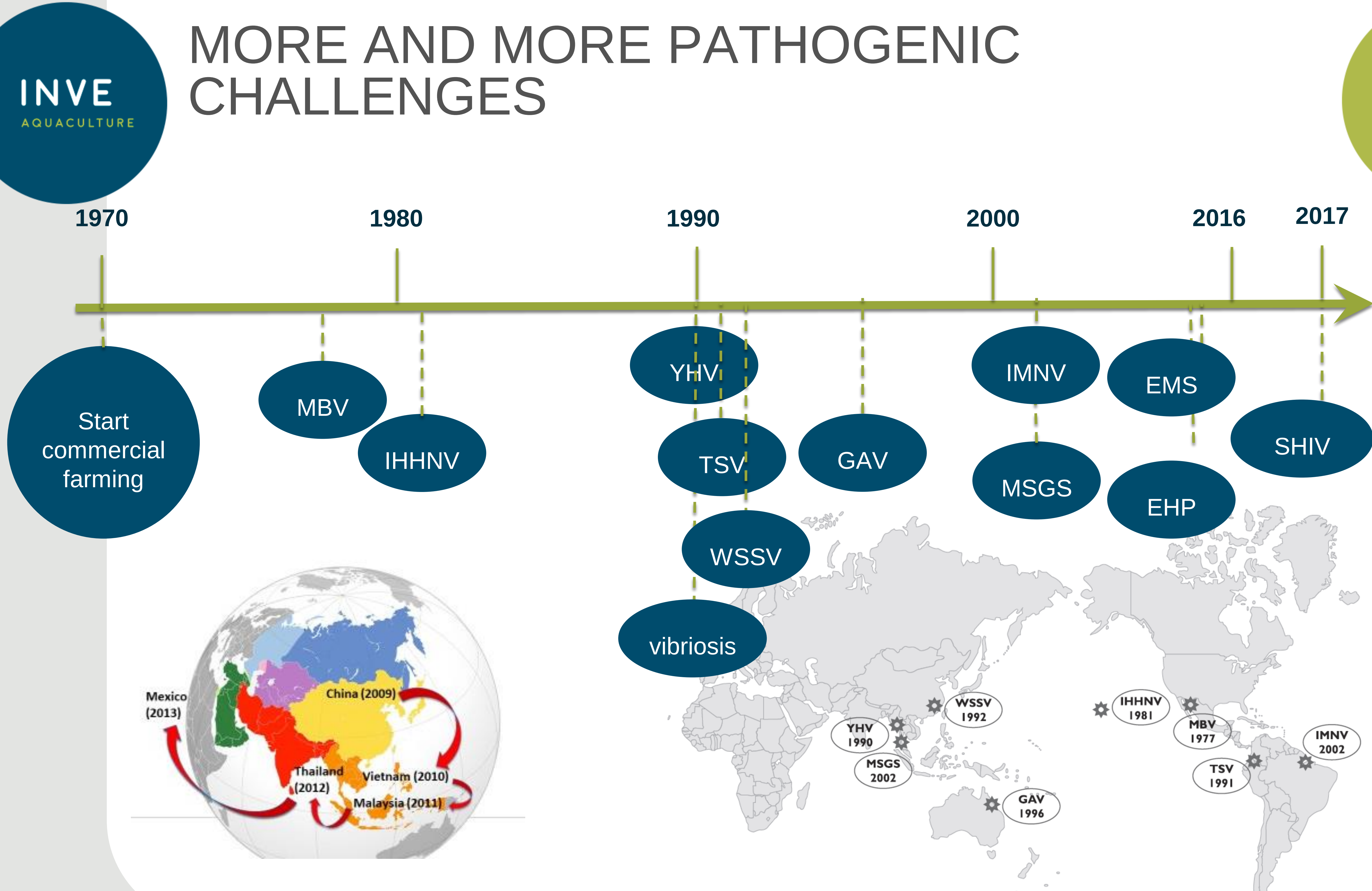
SITUATION IN AMERICAS AND BRASIL



GOAL GUAYAQUIL ECUADOR SEPTEMBER 25-27, 2018

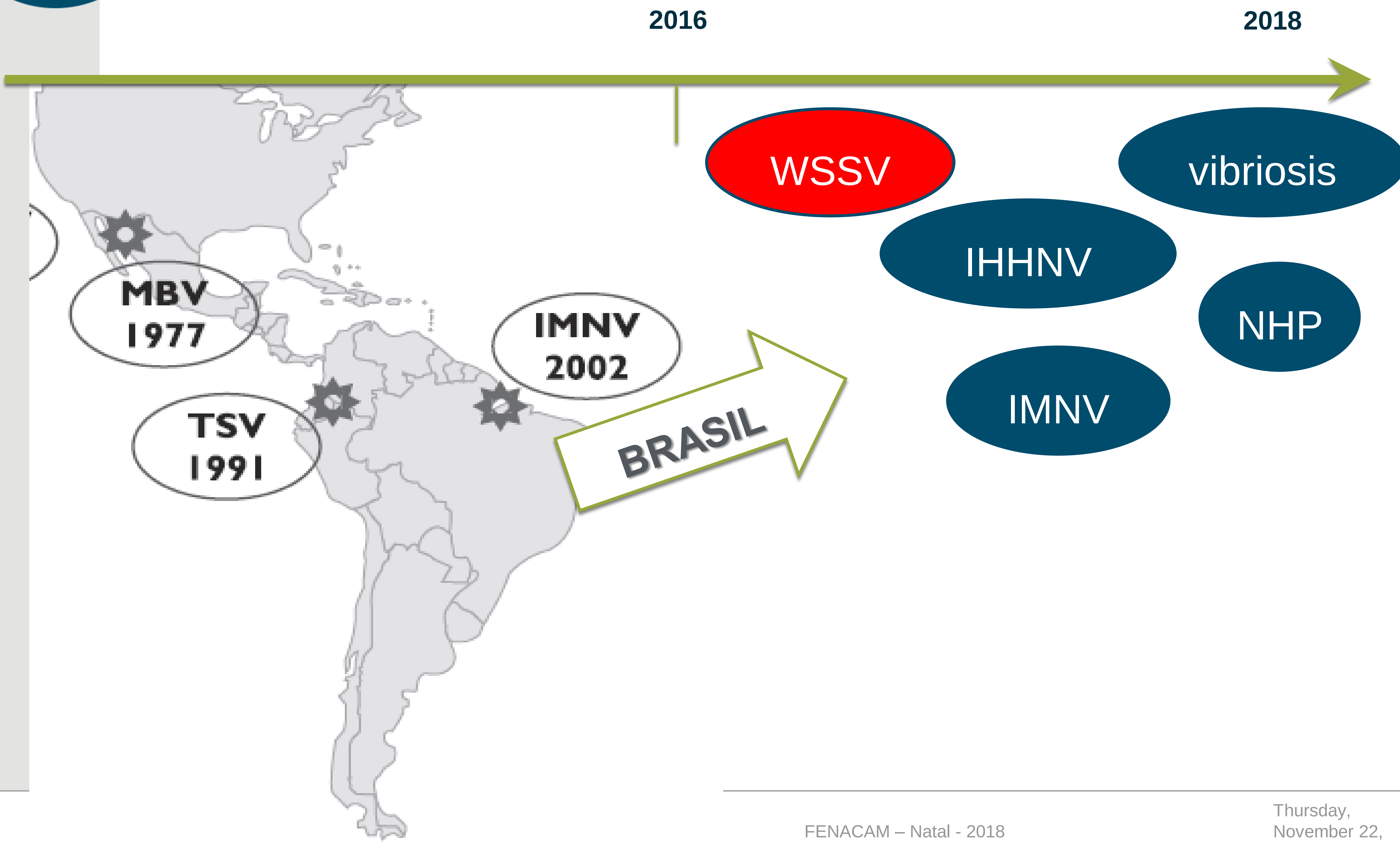
Global Aquaculture Alliance

MORE AND MORE PATHOGENIC CHALLENGES



Walker and Mohan (2009)

MORE AND MORE PATHOGENIC CHALLENGES

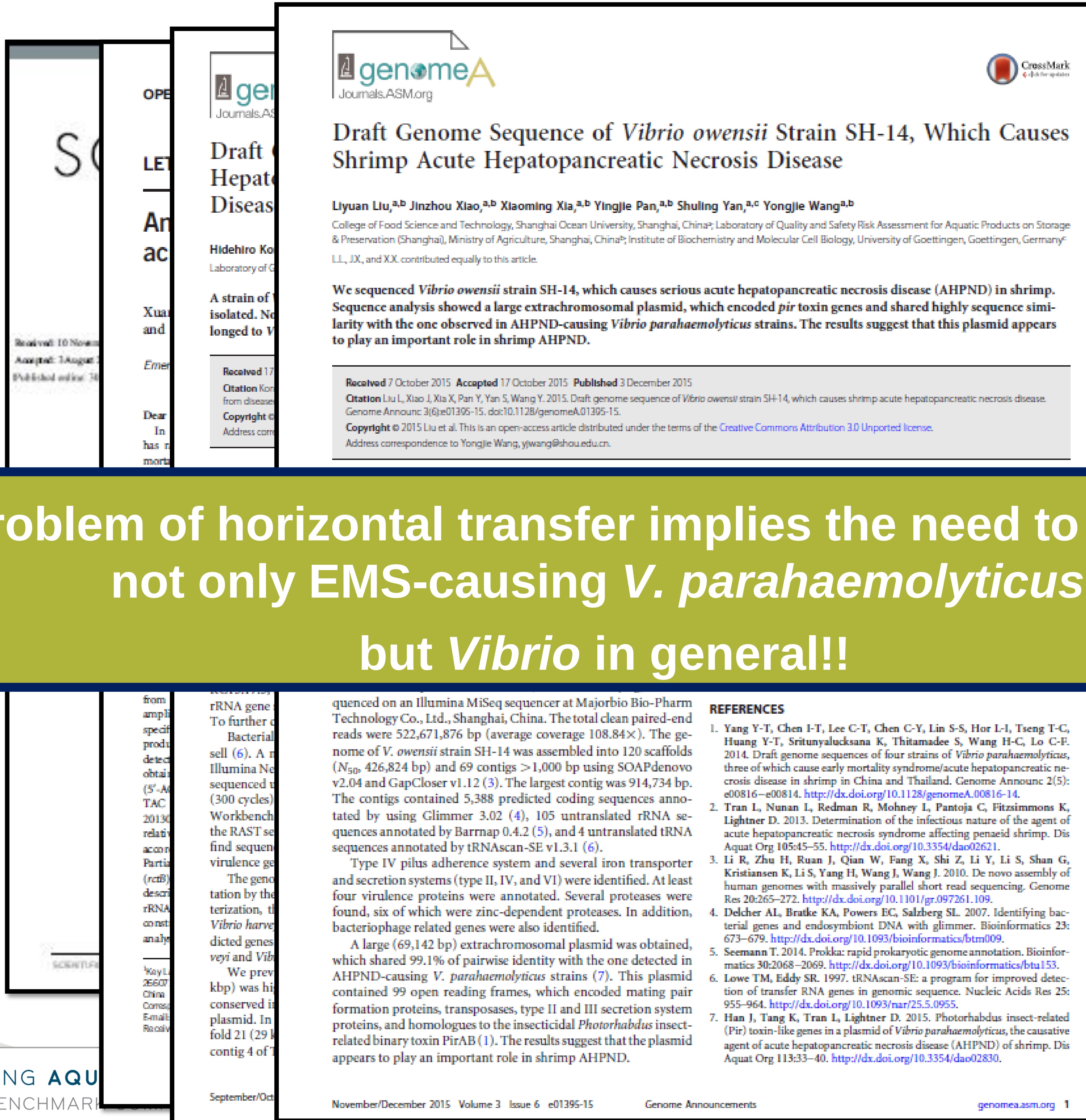


INVE AQUACULTURE

VIBRIOS

BACTERIA

Vibriosis


genomeA Journals.ASM.org

Draft Genome Sequence of *Vibrio owensii* Strain SH-14, Which Causes Shrimp Acute Hepatopancreatic Necrosis Disease

Liyuan Liu,[a,b] Jinzhou Xiao,[a,b] Xiaoming Xia,[a,b] Yingjie Pan,[a,b] Shuling Yan,[a,c] Yongjie Wang[a,b]

College of Food Science and Technology, Shanghai Ocean University, Shanghai, China[a]; Laboratory of Quality and Safety Risk Assessment for Aquatic Products on Storage & Preservation (Shanghai), Ministry of Agriculture, Shanghai, China[b]; Institute of Biochemistry and Molecular Cell Biology, University of Goettingen, Goettingen, Germany[c]

L.L., J.X., and X.X. contributed equally to this article.

We sequenced *Vibrio owensii* strain SH-14, which causes serious acute hepatopancreatic necrosis disease (AHPND) in shrimp. Sequence analysis showed a large extrachromosomal plasmid, which encoded *pir* toxin genes and shared highly sequence similarity with the one observed in AHPND-causing *Vibrio parahaemolyticus* strains. The results suggest that this plasmid appears to play an important role in shrimp AHPND.

Received 7 October 2015 **Accepted** 17 October 2015 **Published** 3 December 2015

Citation Liu L, Xiao J, Xia X, Pan Y, Yan S, Wang Y. 2015. Draft genome sequence of *Vibrio owensii* strain SH-14, which causes shrimp acute hepatopancreatic necrosis disease. Genome Announc 3(6):e01395-15. doi:10.1128/genomeA.01395-15.

Address correspondence to Yongjie Wang, yjwang@shou.edu.cn.

quenced on an Illumina MiSeq sequencer at Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China. The total clean paired-end reads were 522,671,876 bp (average coverage 108.84×). The genome of *V. owensii* strain SH-14 was assembled into 120 scaffolds (N_{50}, 426,824 bp) and 69 contigs >1,000 bp using SOAPdenovo v2.04 and GapCloser v1.12 (3). The largest contig was 914,734 bp. The contigs contained 5,388 predicted coding sequences annotated by using Glimmer 3.02 (4), 105 untranslated rRNA sequences annotated by Barrnap 0.4.2 (5), and 4 untranslated tRNA sequences annotated by tRNAscan-SE v1.3.1 (6).

Type IV pilus adherence system and several iron transporter and secretion systems (type II, IV, and VI) were identified. At least four virulence proteins were annotated. Several proteases were found, six of which were zinc-dependent proteases. In addition, bacteriophage related genes were also identified.

A large (69,142 bp) extrachromosomal plasmid was obtained, which shared 99.1% of pairwise identity with the one detected in AHPND-causing *V. parahaemolyticus* strains (7). This plasmid contained 99 open reading frames, which encoded mating pair formation proteins, transposases, type II and III secretion system proteins, and homologues to the insecticidal *Photorhabdus* insect-related binary toxin PirAB (1). The results suggest that the plasmid appears to play an important role in shrimp AHPND.

REFERENCES

1. Yang Y-T, Chen I-T, Lee C-T, Chen C-Y, Lin S-S, Hor L-I, Tseng T-C, Huang Y-T, Sritunyalucksana K, Thitamadee S, Wang H-C, Lo C-F. 2014. Draft genome sequences of four strains of *Vibrio parahaemolyticus*, three of which cause early mortality syndrome/acute hepatopancreatic necrosis disease in shrimp in China and Thailand. Genome Announc 2(5):e00816–e00814. http://dx.doi.org/10.1128/genomeA.00816-14.
2. Tran L, Nunan L, Redman R, Mohney L, Pantoja C, Fitzsimmons K, Lightner D. 2013. Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp. Dis Aquat Org 105:45–55. http://dx.doi.org/10.3354/dao02621.
3. Li R, Zhu H, Ruan J, Qian W, Fang X, Shi Z, Li Y, Li S, Shan G, Kristiansen K, Li S, Yang H, Wang J, Wang J. 2010. De novo assembly of human genomes with massively parallel short read sequencing. Genome Res 20:265–272. http://dx.doi.org/10.1101/gr.097261.109.
4. Delcher AL, Bratke KA, Powers EC, Salzberg SL. 2007. Identifying bacterial genes and endosymbiont DNA with glimmer. Bioinformatics 23:673–679. http://dx.doi.org/10.1093/bioinformatics/btm009.
5. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. http://dx.doi.org/10.1093/bioinformatics/btu153.
6. Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 25:955–964. http://dx.doi.org/10.1093/nar/25.5.0955.
7. Han J, Tang K, Tran L, Lightner D. 2015. Photorhabdus insect-related (Pir) toxin-like genes in a plasmid of *Vibrio parahaemolyticus*, the causative agent of acute hepatopancreatic necrosis disease (AHPND) of shrimp. Dis Aquat Org 113:33–40. http://dx.doi.org/10.3354/dao02830.

November/December 2015 Volume 3 Issue 6 e01395-15 Genome Announcements genomea.asm.org 1

Problem of horizontal transfer implies the need to control not only EMS-causing *V. parahaemolyticus*, but *Vibrio* in general!!

IF BACTERIA CHANGES, WE SHOULD TOO

“THE INTRODUCTION OF AHPND TO THE AMERICAS HAS ONCE AGAIN EMPHASIZED THE VULNERABILITY OF TRADITIONAL CULTURE AND THE NEED FOR A NEW PRODUCTION MODEL FOR THE 21ST CENTURY”

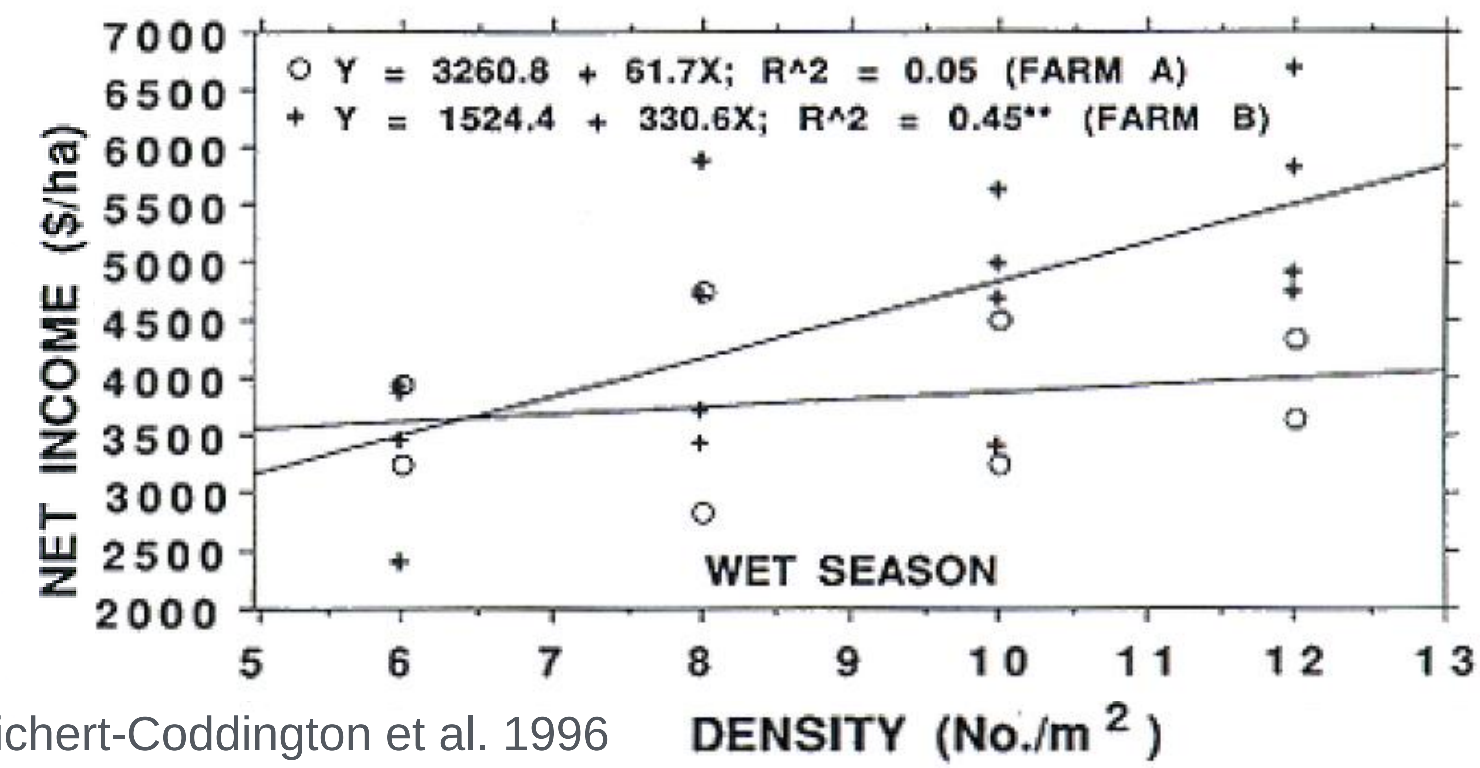
SCOTT EDWARD HORTON, MEXICO

HOW DO FARMERS DEAL WITH DISEASE IN SHRIMP CULTURE

- Lowered stocking density and less input

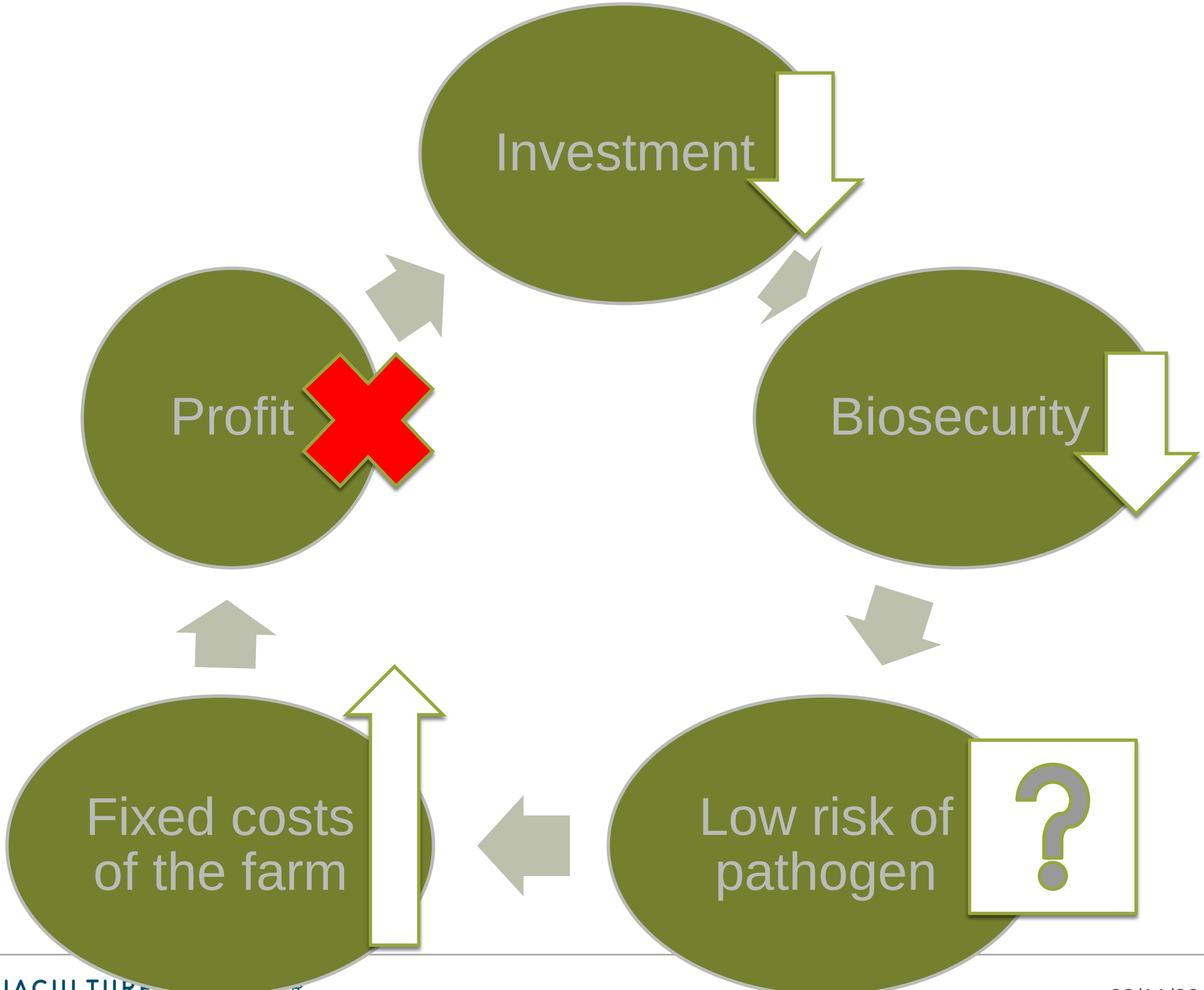
→ Minimizing costs and lowering disease risk

→ Lowers the problems caused by pathogenic microorganims but does not eliminate them; and does not result in an economically feasible shrimp culture model

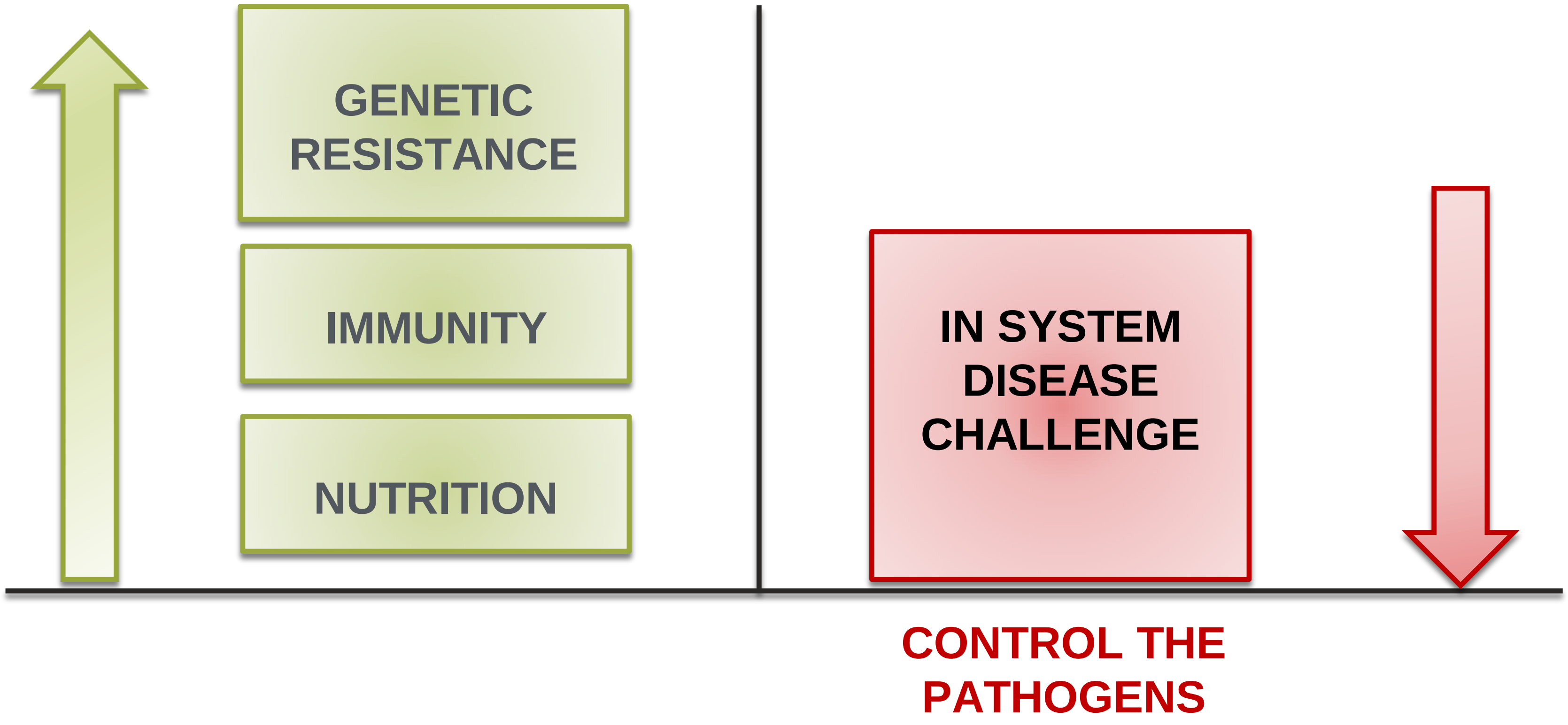


Teichert-Coddington et al. 1996

HOW DO FARMERS DEAL WITH DISEASE IN SHRIMP CULTURE -

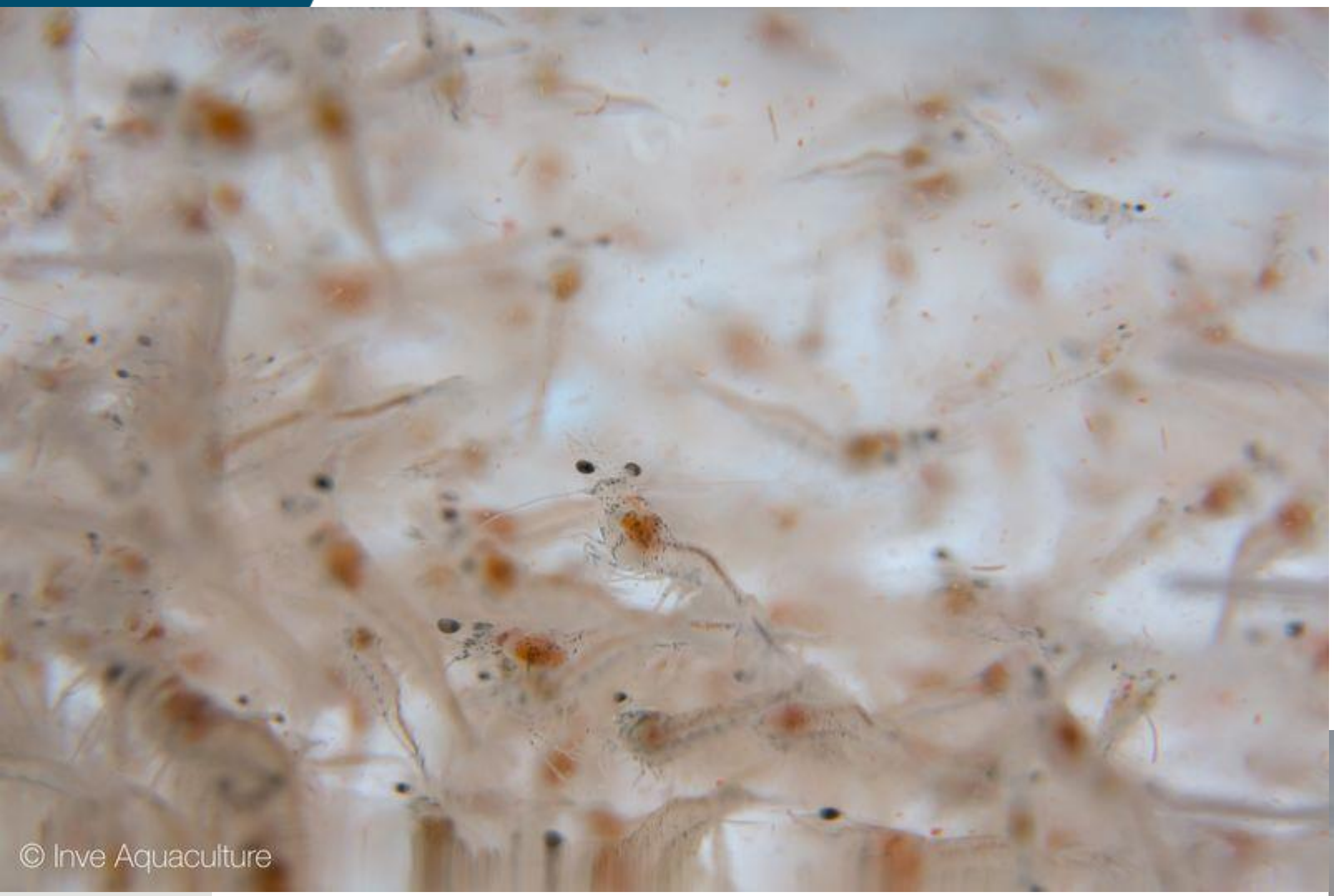


HOW TO DEAL WITH DISEASE IN SHRIMP CULTURE



BUT, this target is least compatible with an extensive and earthen pond based way of shrimp culture

HOW TO DEAL WITH DISEASE IN SHRIMP CULTURE – MAIN VECTORS OF CONTAMINATION



- ✓ PL quality

- ✓ Water

MINIMIZING DISEASE CHALLENGE

✓ Water

1) AVOID ENTRANCE OF POSSIBLE PATHOGENS

Disinfection of the culture system

Clean, remove organic matter and rinse with water. Disinfect for 15-30 minutes with Sanocare PUR 0,5%, rinse and dry.

MINIMIZING DISEASE CHALLENGE

1) AVOID ENTRANCE OF POSSIBLE PATHOGENS

Disinfection of the culture system

Clean, remove organic matter and rinse with water. Disinfect for 15-30 minutes with Sanocare PUR 0,5%, rinse and dry.

Effect of Chlorination on Bacterial Load in Brackishwater Shrimp Culture Pond

MS Khatun[1], Md. Motiur Rahman[2], M Ariful Islam[3] and Ajhar Ali[4]
[1,2,3] Bangladesh Fisheries Research Institute, Shrimp Research Station, Bagerhat, Bangladesh
[4] Bangladesh Fisheries Research Institute, Brackishwater Station, Khulna, Bangladesh

Dose of chlorination	THB count in soil(CFU/ml) x10^5		
	Before chlorination	After 2 hr.	After 2 days
No chlorination(T_1)	28±0.65	28±0.44	30±0.72
3 ppm (T_2)	23.6±0.48	14.8±0.39	21±0.63
6 ppm (T_3)	55±0.81	16±0.33	29±0.70
9 ppm (T_4)	26±0.52	19±48	24±0.42

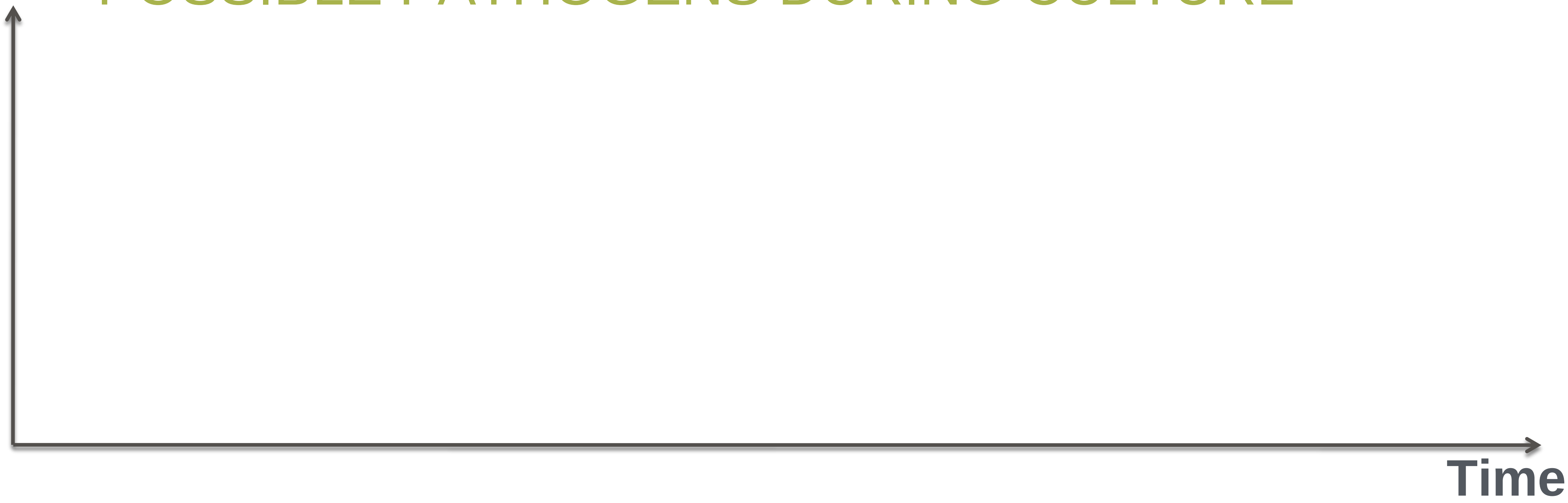
MINIMIZING DISEASE CHALLENGE

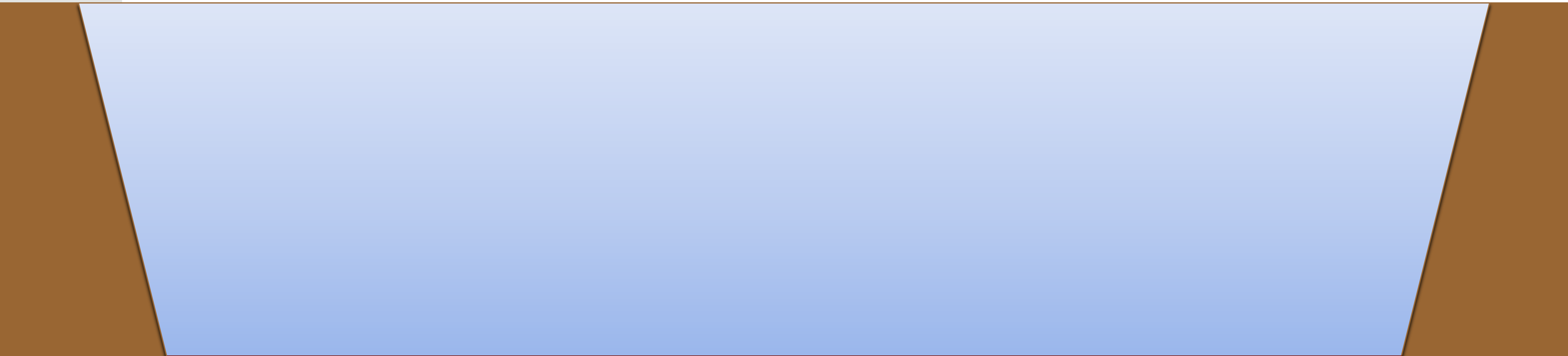
1) AVOID ENTRANCE OF POSSIBLE PATHOGENS

Additional shielding further increases level of biosecurity

MINIMIZING DISEASE CHALLENGE

2) MINIMIZE PROLIFERATION OR ACTIVITY OF POSSIBLE PATHOGENS DURING CULTURE





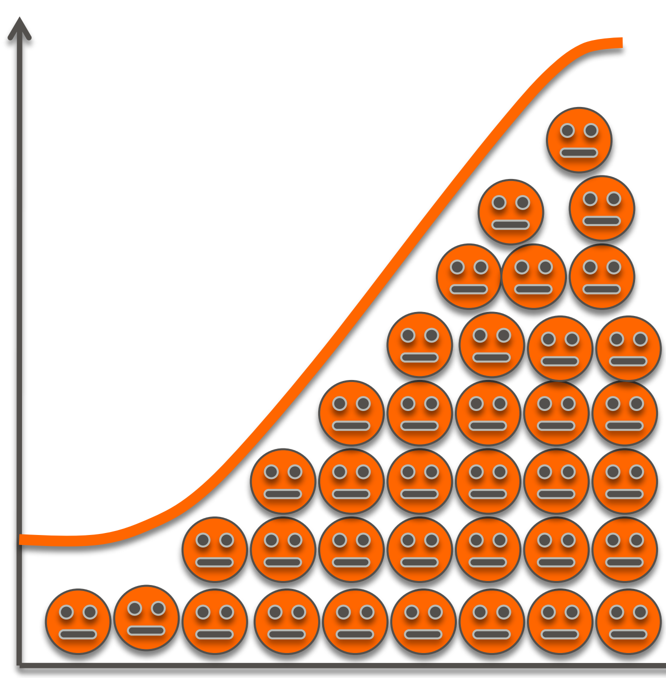
ecological characteristics of

	r-strategist bacteria	
Importance for shrimp	Dangerous; opportunistic pathogens;	
Growth rate when a lot of nutrients/bacterium	HIGH	

What happens at the microbial level after disinfection?

Initially: Low number of bacteria and a lot of nutrients

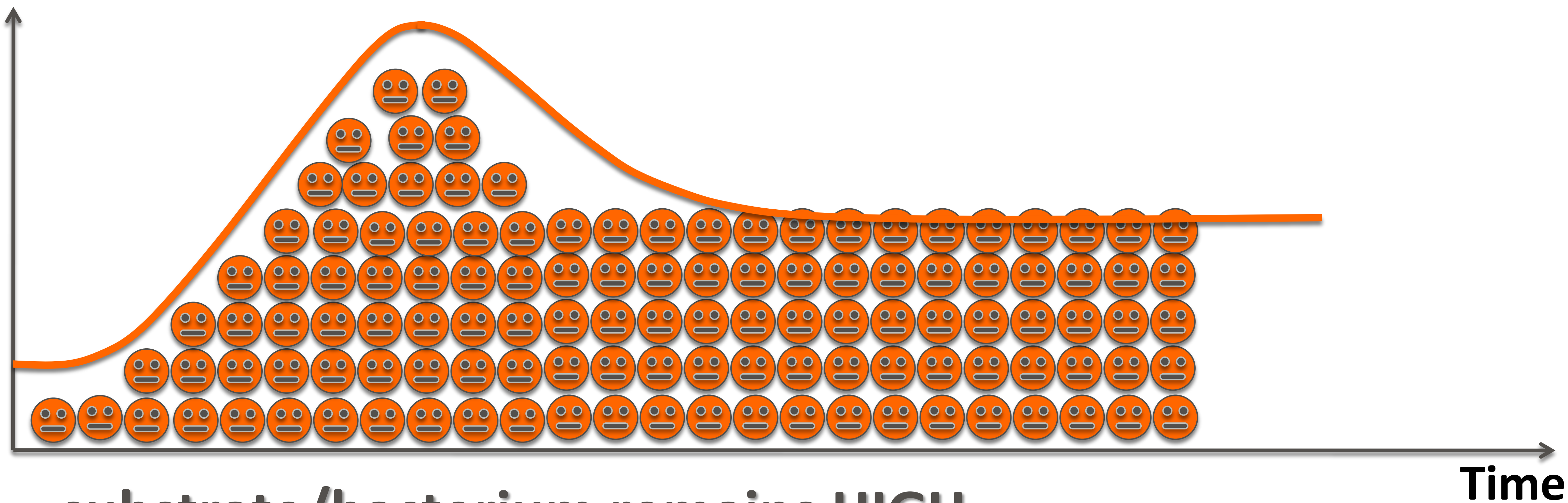
→ Peak of r-strategic bacteria



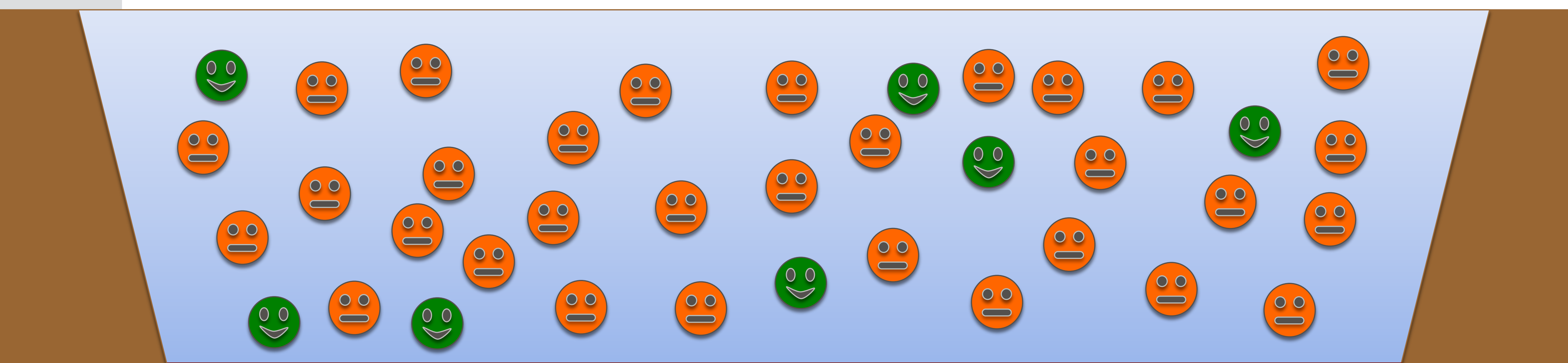
Substrate per bacterium =

What happens in the long run?

Earthen pond systems: a lot of nutrients remain in the water → r-strategist may remain in high levels

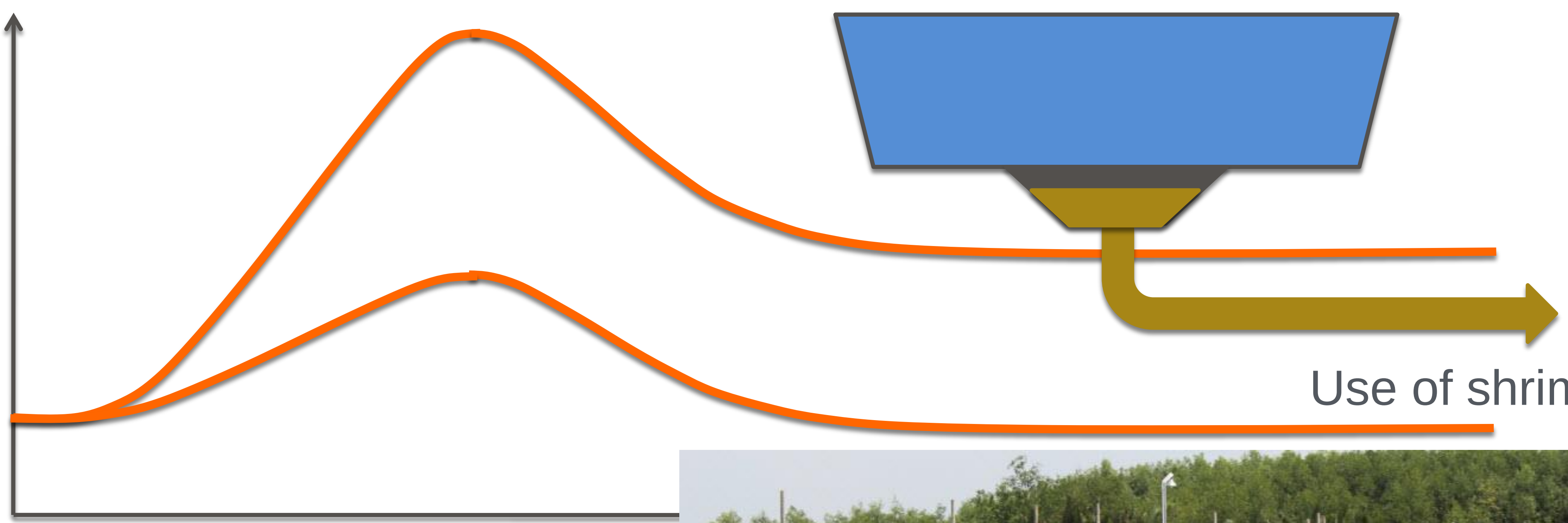


substrate/bacterium <u>remains</u> HIGH



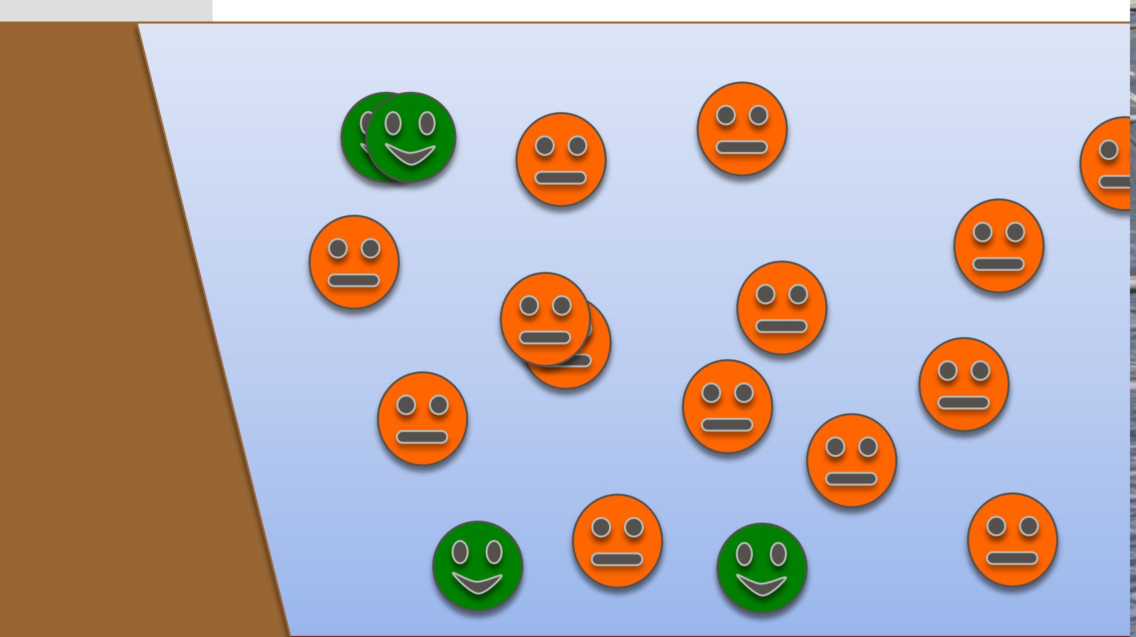
MINIMIZING DISEASE CHALLENGE

Removal of organic matter from the system: fewer nutrients for r-strategist bacteria to dominate



Use of shrimp pond toilets

substrate/bacteriu



ecological characteristics of

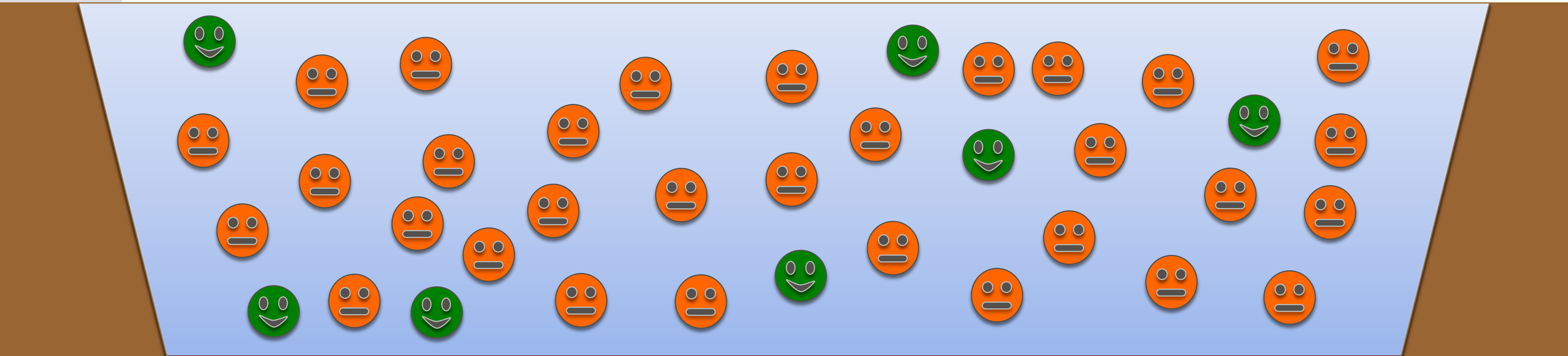
	r-strategist bacteria	K-strategist bacteria
Importance for shrimp	Dangerous; opportunistic pathogens;	Generally harmless
Growth rate when a lot of nutrients/bacterium	HIGH	LOW

What happens at the microbial level after disinfection?

In the mean time:

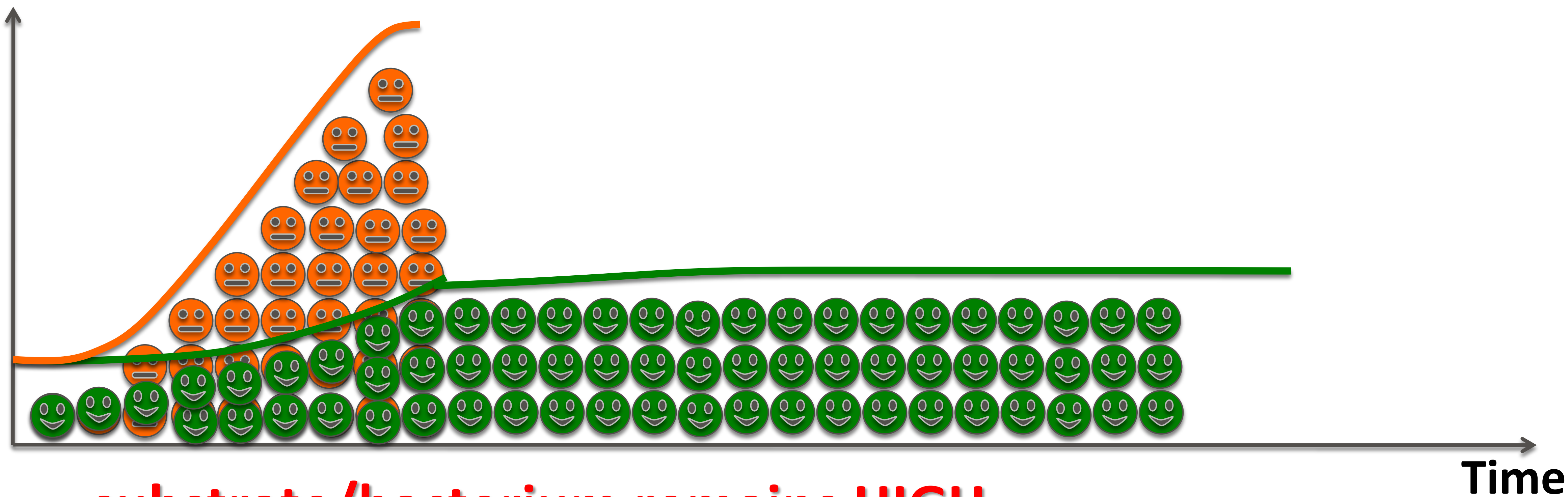
→ good bacteria grow slowly

Substrate per bacterium = HIGH; niches are open

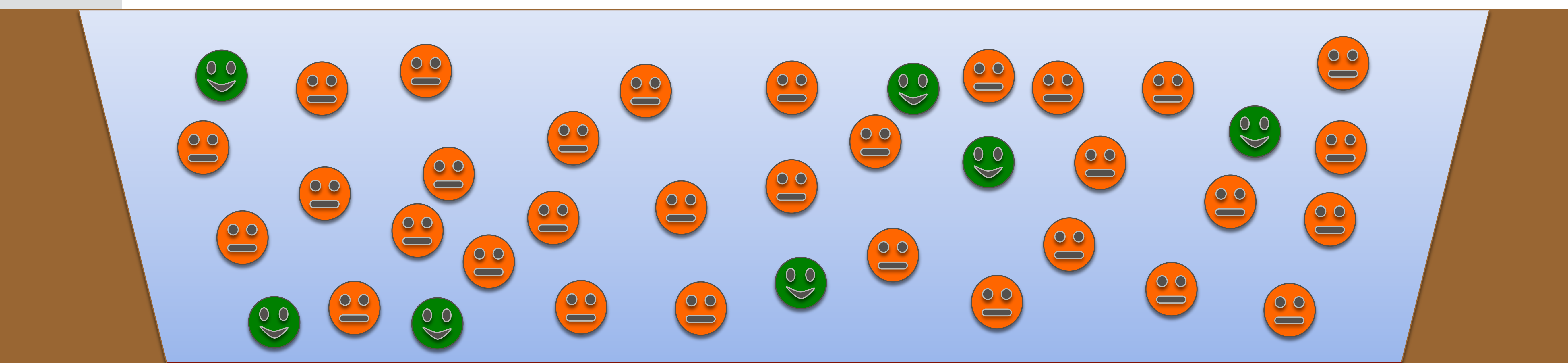


What happens in the long run?

Standard systems: water replacement to maintain water quality → partial wash-out of K-strategists

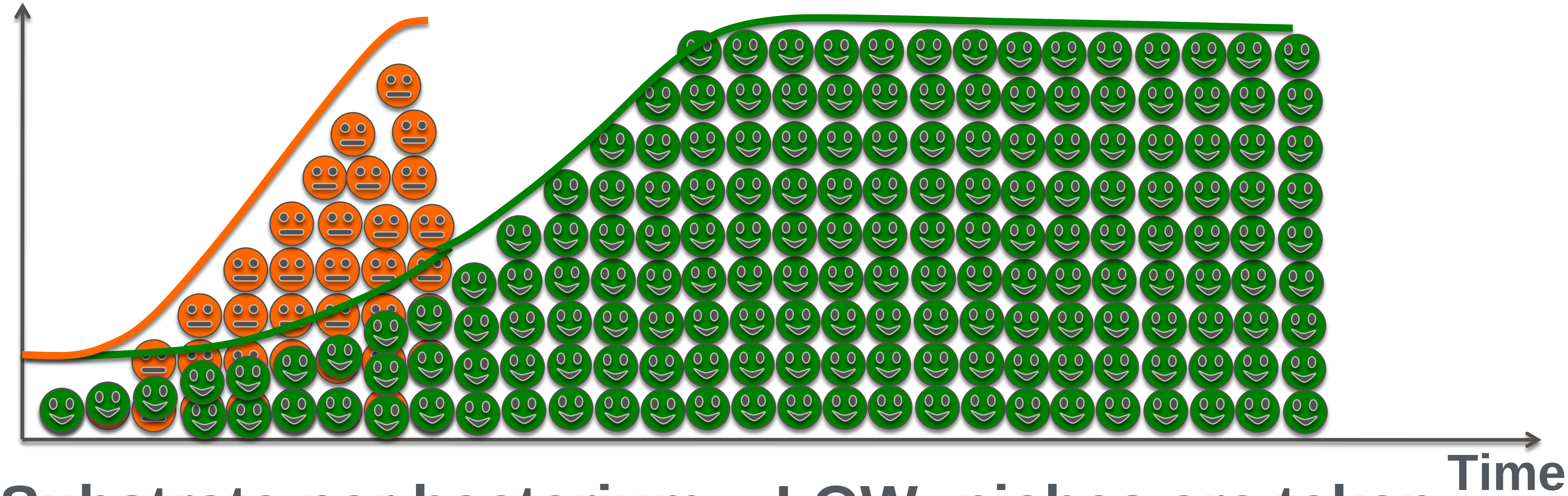


substrate/bacterium remains HIGH

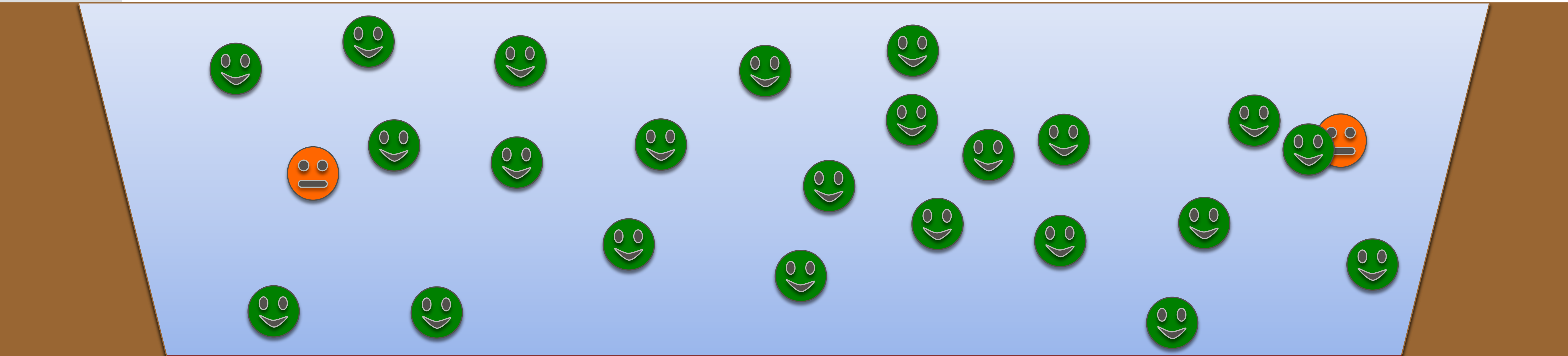


What happens in the long run?

Zero exchange systems: water quality is maintained by microbial community → K-strategist can dominate



Substrate per bacterium = LOW; niches are taken



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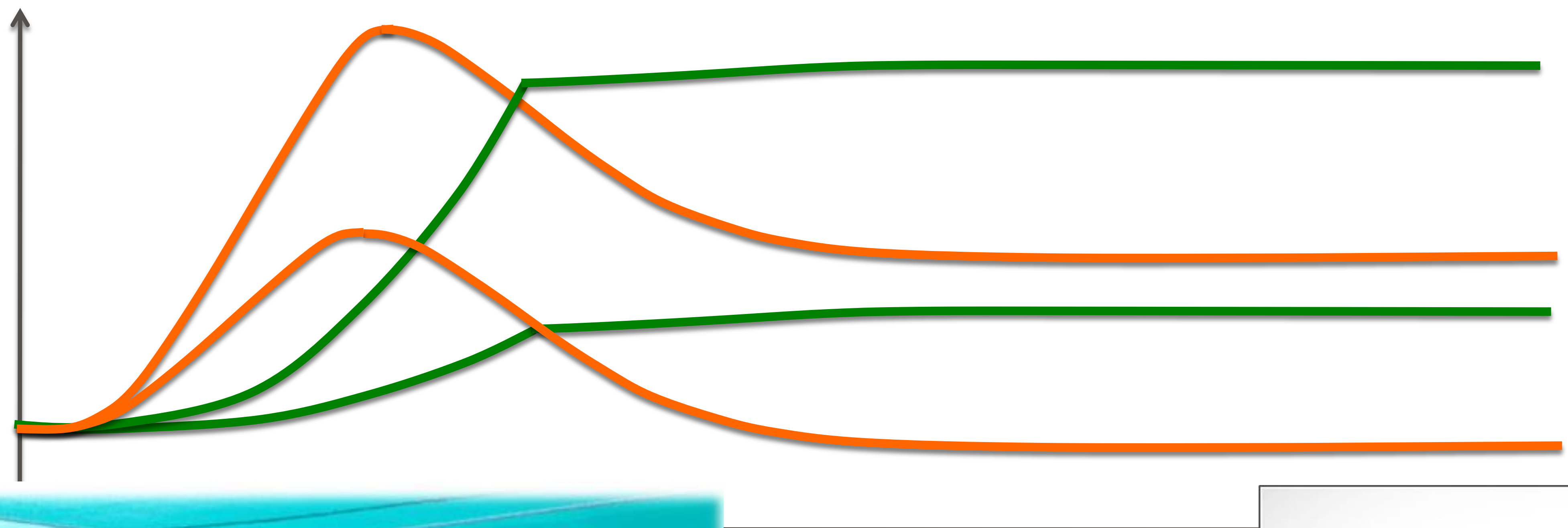
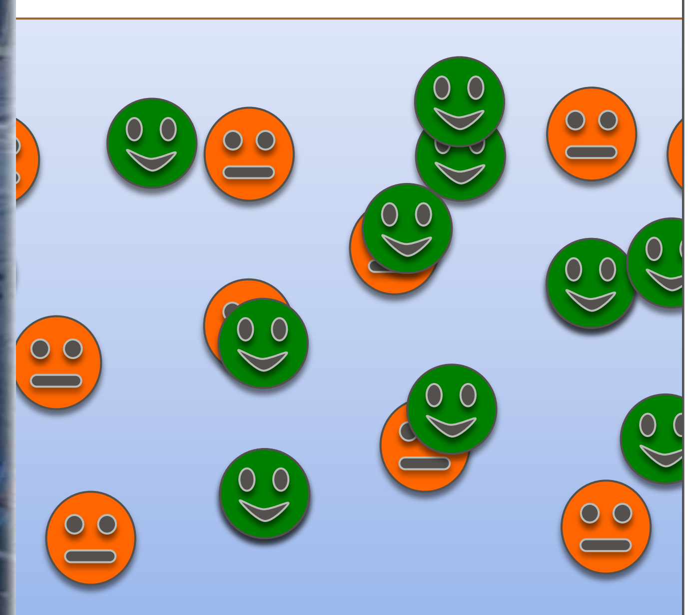
MINIMIZING DISEASE CHALLENGE

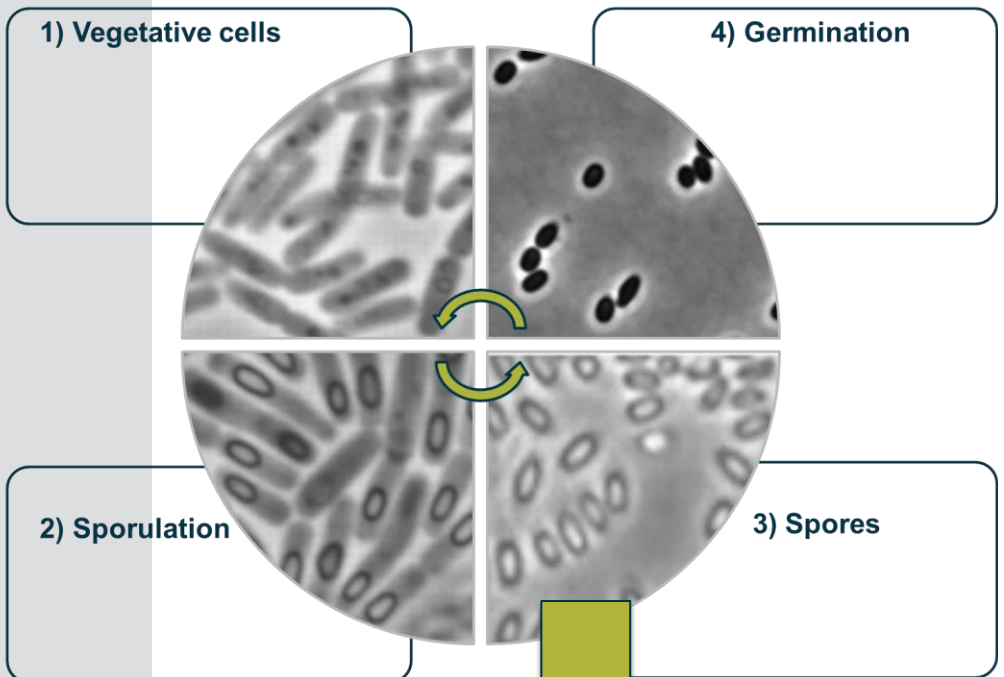


remains HIGH

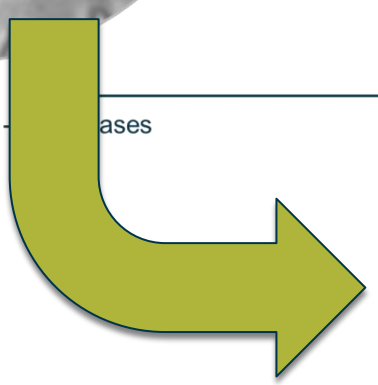


KNOWN MICROBIAL MANAGEMENT STRATEGIES BECOME MORE EFFICIENT IN CLOSED SYSTEMS

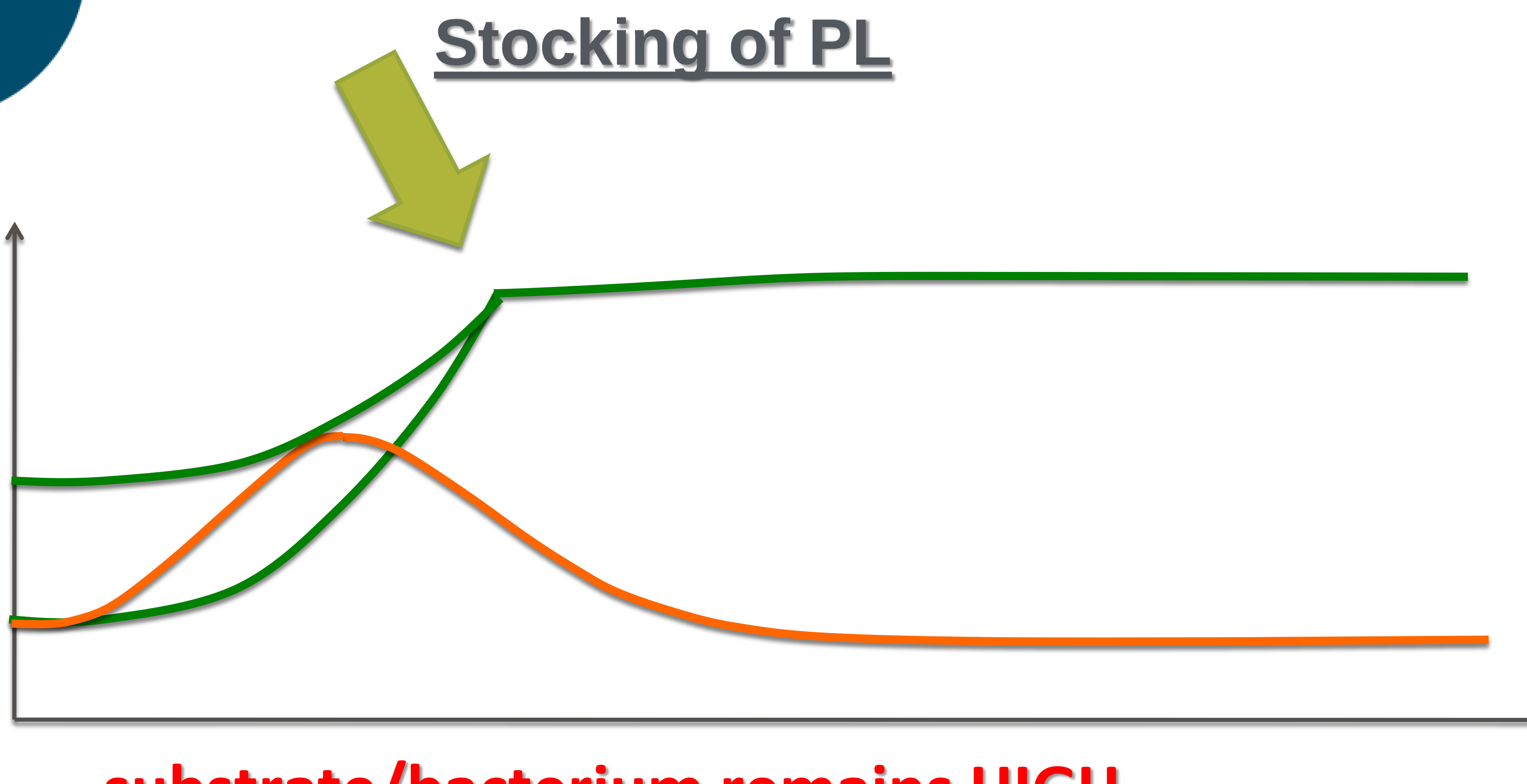
USE OF GOOD BACTERIA TO CONTROL BAD BACTERIA - PROBIOTICS



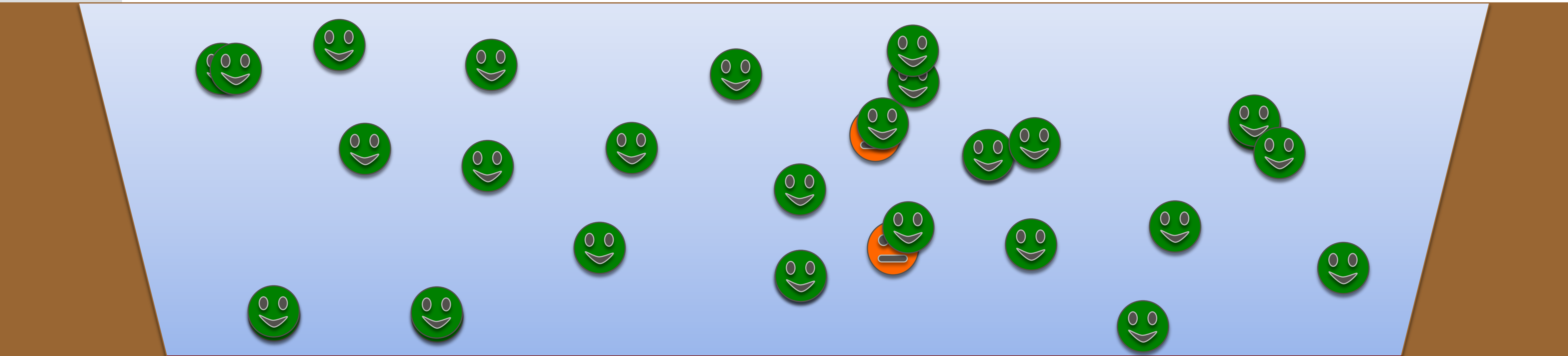
Bacillus Life Cycle - ases



MINIMIZING DISEASE CHALLENGE



substrate/bacterium remains HIGH



MICROBIAL RUSSIAN ROULETTE – THE WAY PROBIOTICS ARE PREPARED MATTER

Survival of pathogenic microorganisms in *kefir*

Sobrevivência de micro-organismos patogênicos em *kefir*

RIALA6/1452

Priscila Alves DIAS, Daiani Teixeira da SILVA, Talita Schneid TEJADA, Maria Cristina Garcia Moraes LEAL, Rita de Cássia dos Santos da CONCEIÇÃO, Cláudio Dias TIMM*

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Recebido: 10.12.2010 - Aceito para publicação: 13.03.2012

ABSTRACT

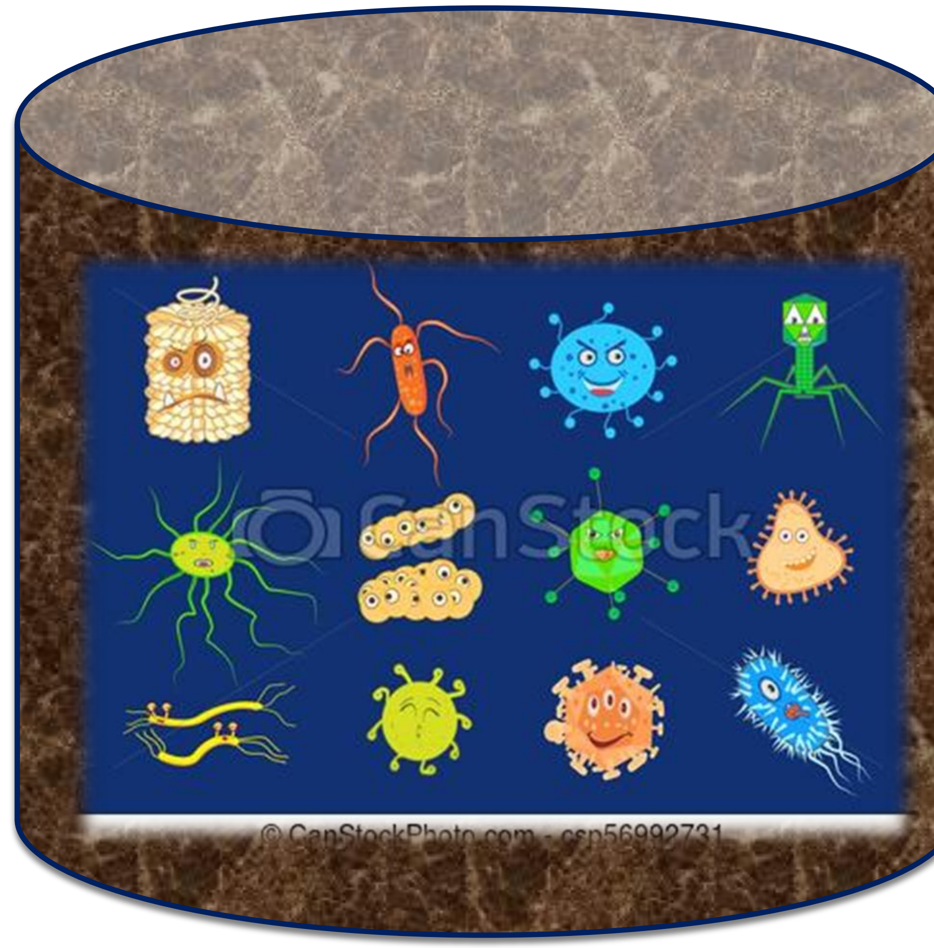
Kefir is a homemade fermented milk produced by adding kefir grains. The domestic handling and the use of raw materials from different standards and sources, and the lack of inspection by qualified professionals, all this classify kefir as a food which might represent potential risks to human health. This study aimed at evaluating the pathogens survival during the kefir fermentation process. Kefir grains were added into portions of UHT skimmed milk which were experimentally contaminated with *Escherichia coli* O157:H7, *Salmonella* Typhimurium and Enteritidis, *Staphylococcus aureus* and *Listeria monocytogenes*. Analyses of the microorganism isolation in these milk samples were carried out at 0, 6, 12, 48 and 72 hours of fermentation process. *Salmonella* Typhimurium and Enteritidis survived for a 24-hour period in fermenting kefir. *Escherichia coli* O157:H7, *Staphylococcus aureus* and *Listeria monocytogenes* were recovered in less than 72 hours after the fermentation process was initiated. Under the conditions and the microorganisms concentrations established in the present study, the analyzed pathogenic bacteria survived for a period longer than those used for homemade kefir fermentation, and this one might be a potential hazard for human consumption.

Keywords. kefir, fermented milk, inhibition, pathogens.

Salmonella Typhimurium e Enteritidis sobreviveram por 24 horas no kefir em fermentação. *E. coli* O157:H7, *S. aureus* e *L. monocytogenes* foram recuperados até 72 horas após o início da fermentação. As bactérias patogênicas estudadas, nas concentrações e condições do presente trabalho, sobreviveram por tempo superior àquele normalmente utilizado para a fermentação do kefir preparado artesanalmente, o qual representa perigo potencial para o consumo humano.

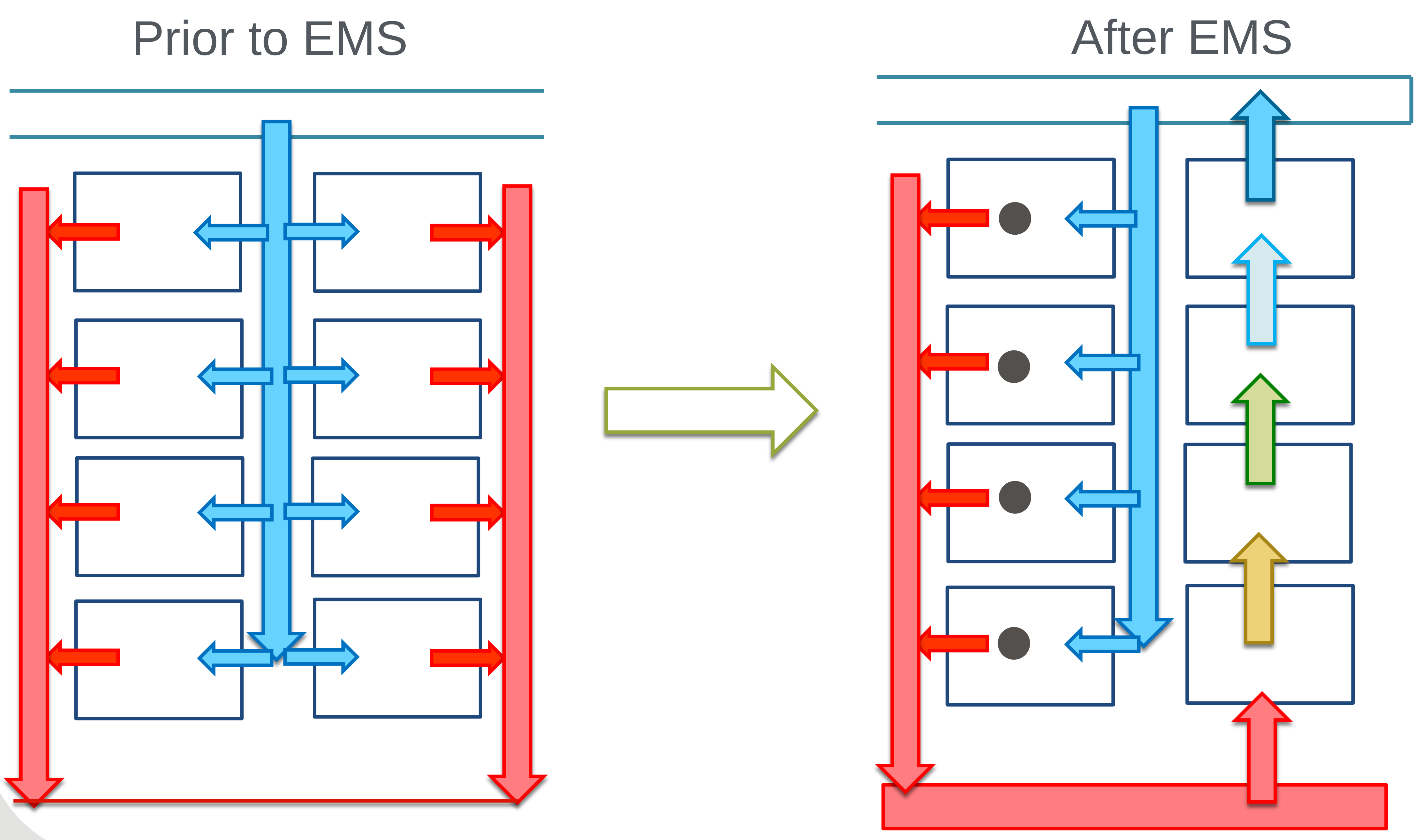
Palavras-chaves. *kefir*, leite fermentado, inibição, agentes patogênicos.

Palavras-chaves. *kefir*, leite fermentado, inibição, agentes patogênicos.



SYSTEM MANAGEMENT TO MINIMIZE DISEASE RISK

- Industrial approach in Asia



(Taw, 2017)

INVE AQUACULTURE — BENCHMARK'S ADVANCED NUTRITION DIVISION — ROLLS OUT ZERO WATER EXCHANGE PROTOCOL TO INCREASE BIOSECURITY IN SHRIMP PRODUCTION

Manuel Poulain

ONE OF THE MAIN RISK FACTORS IN SHRIMP PRODUCTION IS DISEASE DUE TO BACTERIAL CONTAMINATION. TO REACH THE HIGHEST LEVEL OF BIOSECURITY, IT IS CONSIDERED ESSENTIAL TO CONTROL THE ENTIRE PRODUCTION CYCLE WITH NO ENTRY OF POSSIBLY CONTAMINATED WATER INTO THE PONDS. THIS IS WHY INVE AQUACULTURE – ONE OF THE WORLD'S LEADING EXPERT COMPANIES – RAN EXTENSIVE TRIALS DESIGNED TO INTRODUCE HIGH DENSITY ZERO-WATER EXCHANGE PROTOCOLS.

The main principle of INVE Aquaculture's zero-water exchange protocol is based on bacterial competitive exclusion via the use of selected probiotic bacteria.

SET-UP

- The trials were realized in 500m2 fully lined ponds, 1.2m deep.
- Prior to stocking, all material and pond surfaces were disinfected with Sanocare®PUR, to ensure the complete removal of possible pathogens, including bacterial biofilms.
- After the first pond was filled, no water was added or exchanged for the entire production cycle.
- Shade cloth was installed over the ponds to minimize co[...] deviation of the physico-chemical parameters due to the [...] population

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CARE FOR GROWTH

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ABOUT INVE AQUACULTURE

For over 30 years INVE Aquaculture has been enabling growth in aquaculture. The healthy growth of fish and shrimp, the growth of our clients' local businesses and the growth of global aquaculture. Since December 2015 INVE Aquaculture has become part of Benchmark, an aquaculture biotechnology business. Together the group offers a complete package of nutrition, health and genetic solution across all the major aquaculture [...]

BENCHMARKPLC.COM

TRIAL PROTOCOL

During production, two INVE Aquaculture probiotic products were used to achieve bacterial competitive exclusion of Vibro sp.: Sanolife®PRO-W for water conditioning Sanolife®PRO-2 as feed coating to improve the shrimp's gut bacterial ecology.

During the first month of farming, INVE Aquaculture's nursery feed supplement Sano®S-PAK was used as immunostimulant to improve the shrimp's resistance against environmental stress (density).

TRIAL RESULTS

Thanks to the complete absence of water renewal and the indoor production, the impact of environmental and meteorological variables was minimal, resulting in very consistent results.

https://seafood-tip.com/shrimptails-online/march-2018/

SUSTAINABLE APPROACHES FOR SHRIMP CULTURE

INDOOR ZERO-WATER EXCHANGE SUPER-INTENSIVE PRODUCTION

Trial by Manuel Poulain – INVE Aquaculture

P. vannamei Super Intensive Farm (>200 PL/m^2)
Ø WE/day

Viet-Uc Grow-Out

VIETNAM, 2016-2017

Trial by Manuel Poulain – INVE Aquaculture

- Disinfection of lined ponds

-Shading to limit variations in phytoplankton

- Conditioned water in a separate reservoir to promote nitrification

- Solids management : settling tanks

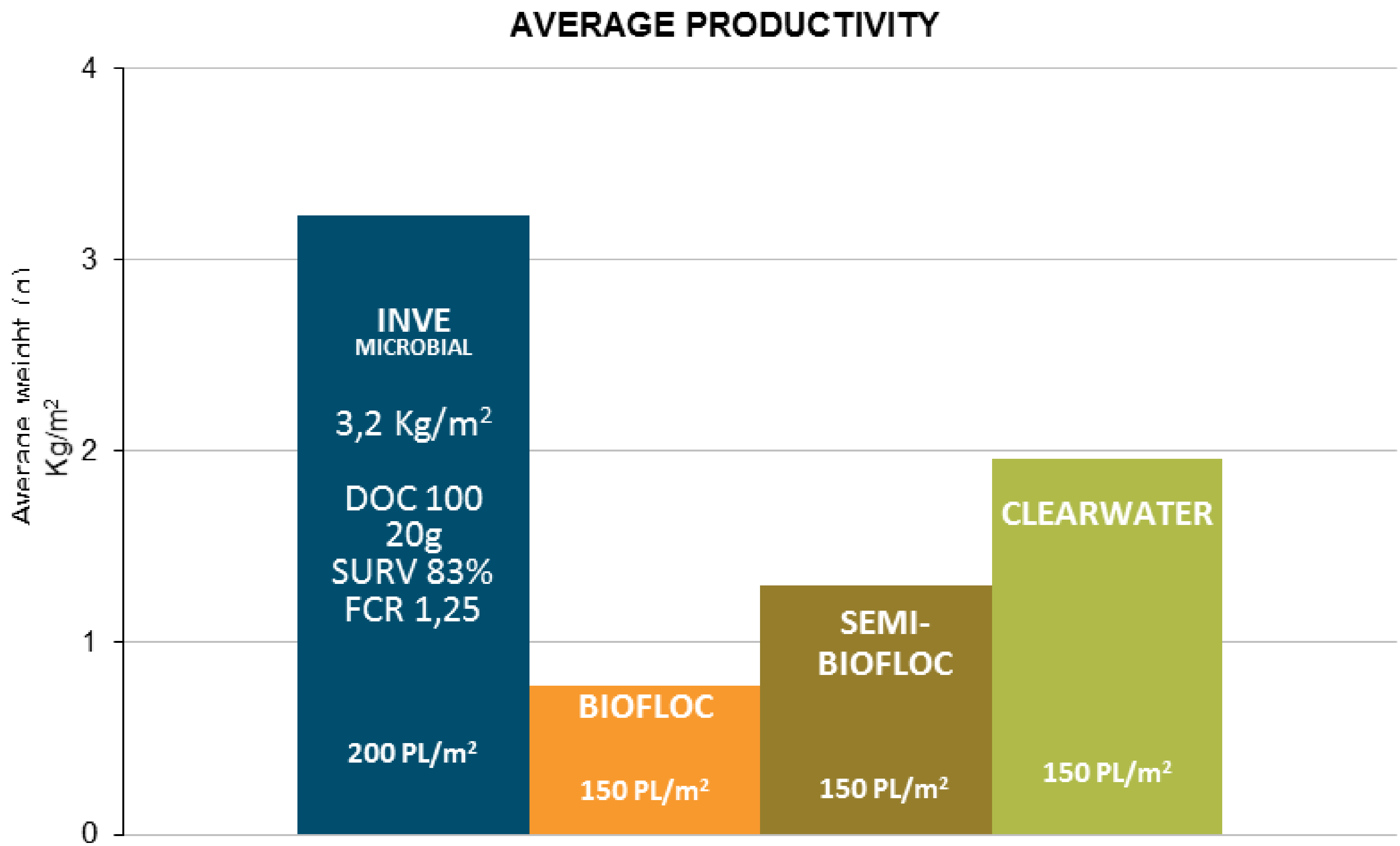
-Probiotic application: in the water and to the feed

-Close follow up in water quality parameters – no entrance of possible contaminants

INDOOR GROW-OUT, VIETNAM

150-250 PL/m² - Protocol comparison

INVE	BIOFLOC C/N	SEMI-BIOFLOC C/N	HIGH WATER ECHANGE

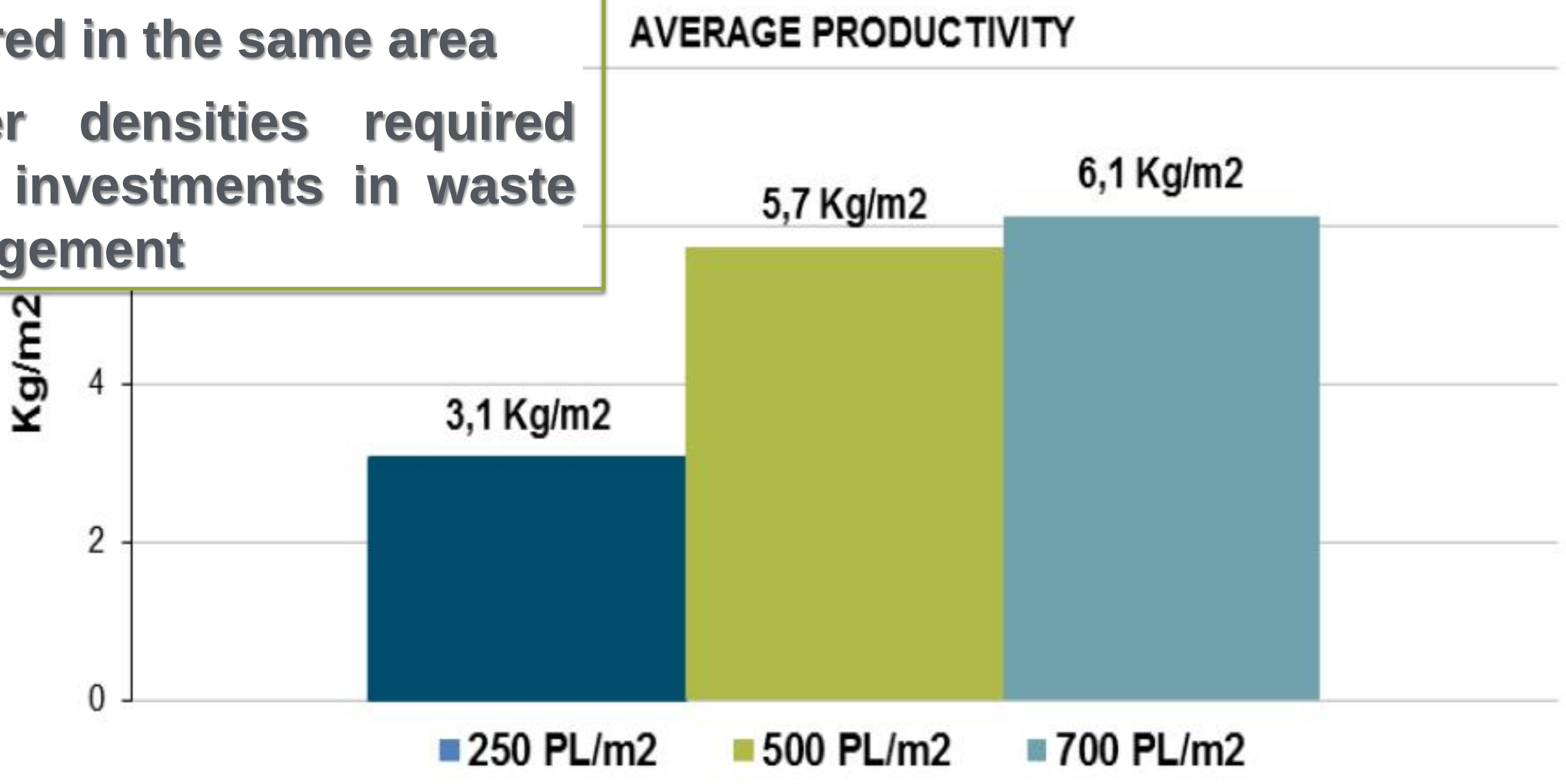


Results Q3 2016

GREENHOUSE 5, 09/2016

250+500+700 PL/M²

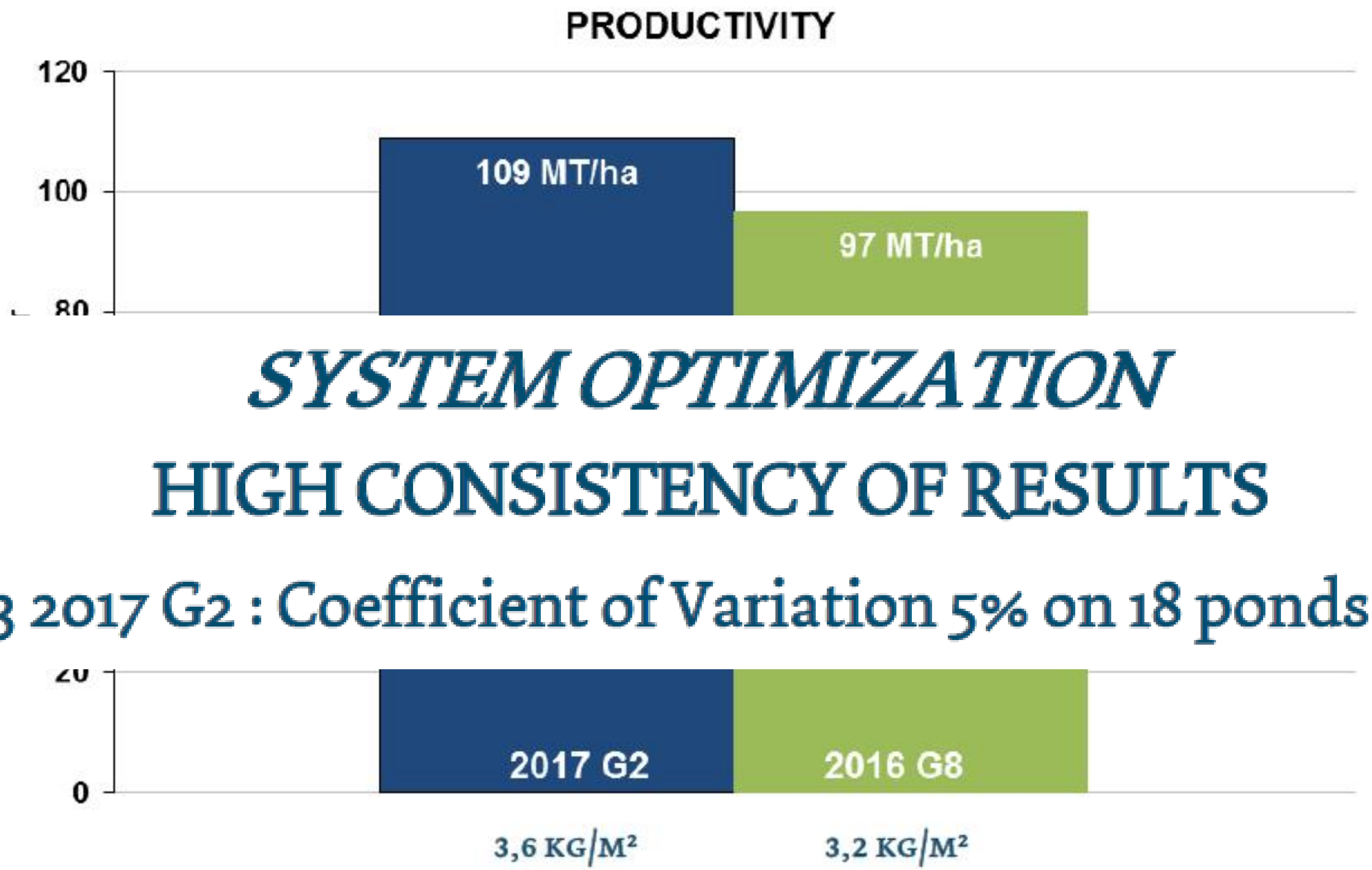
- **Optimization of the protocol: different densities cultured in the same area**
- **Higher densities required more investments in waste management**



Results Q3 2017

GREENHOUSE 2, 09/2017

200-250 PL/M²



SYSTEM OPTIMIZATION

HIGH CONSISTENCY OF RESULTS

Q3 2017 G2 : Coefficient of Variation 5% on 18 ponds !!!

- ZERO WATER EXCHANGE: 70% OF NEW DISINFECTED WATER PRIOR STOCKING

+

- 30% OF INOCULUM FROM MATURED WATER TANK – NITRIFIER COMMUNITY

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- ENTRANCE OF POSSIBLE CONTAMINANTS IN THE SYSTEM

=

- HIGHLY BIOSECURE SYSTEM

OVERVIEW

- TRADITIONAL EARTHEN POND FARMING DOES NOT PROVIDE ADEQUATE MICROBIAL CONTROL

- INTENSIVE, BUT SMALLER AND LINED PONDS OFFER HIGHER LEVEL OF CONTROL
  - BIOSECURITY
  - WASTE MANAGEMENT AND WATER QUALITY
  - MICROBIAL MANAGEMENT
  - HIGHER VALUE OF USED PRODUCTS

- INTENSIVE SYSTEMS WITH MAXIMUM LEVEL OF CONTROL ARE THE FUTURE OF SHRIMP FARMING

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