MICROBIAL MANAGEMENT AS PART OF THE OPERATIONAL STRATEGY FOR SHRIMP FARMING DISEASE CONTROL

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DISEASES, A PROBLEM OF INDUSTRIALISATION

1970 1980 1990 2000 2016

Start commercial farming

MBV

IHHNV

YHV

TSV

GAV

IMNV

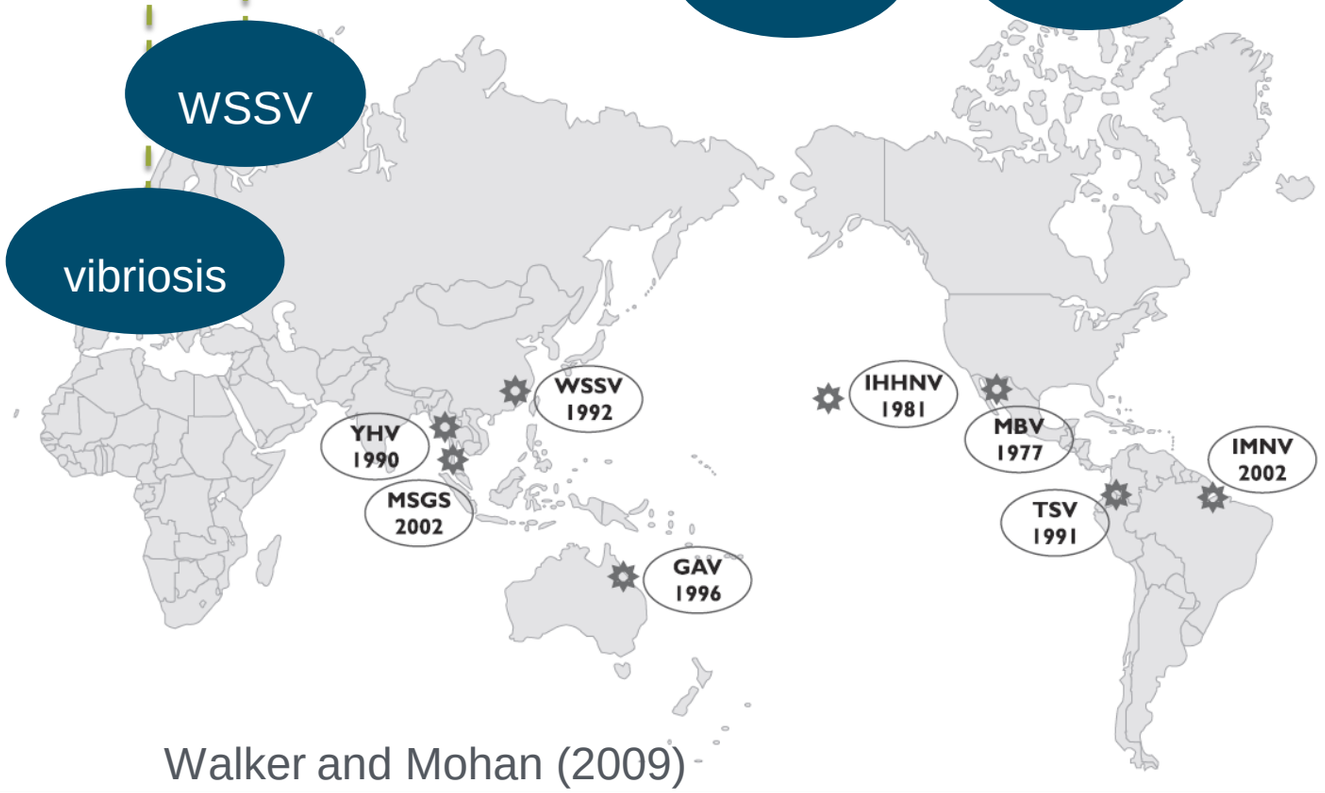
MSGS

EMS

EHP

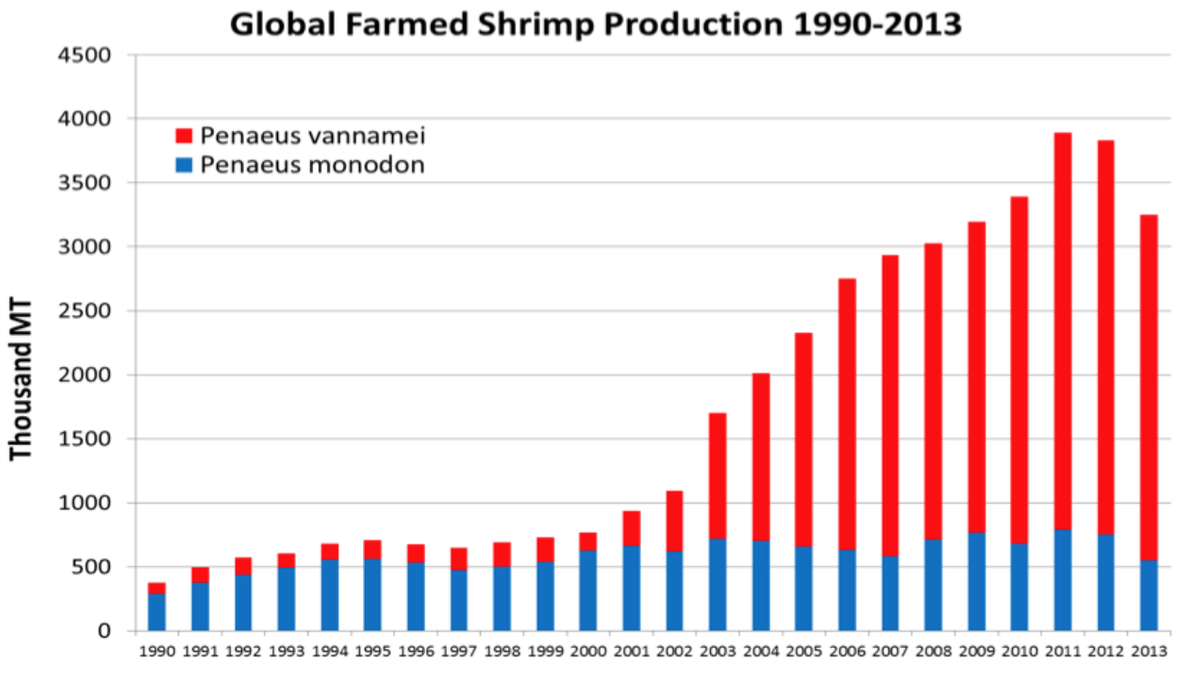
WSSV

vibriosis



Walker and Mohan (2009)

Global Production



DISEASES ARE THERE TO STAY IN AQUACULTURE

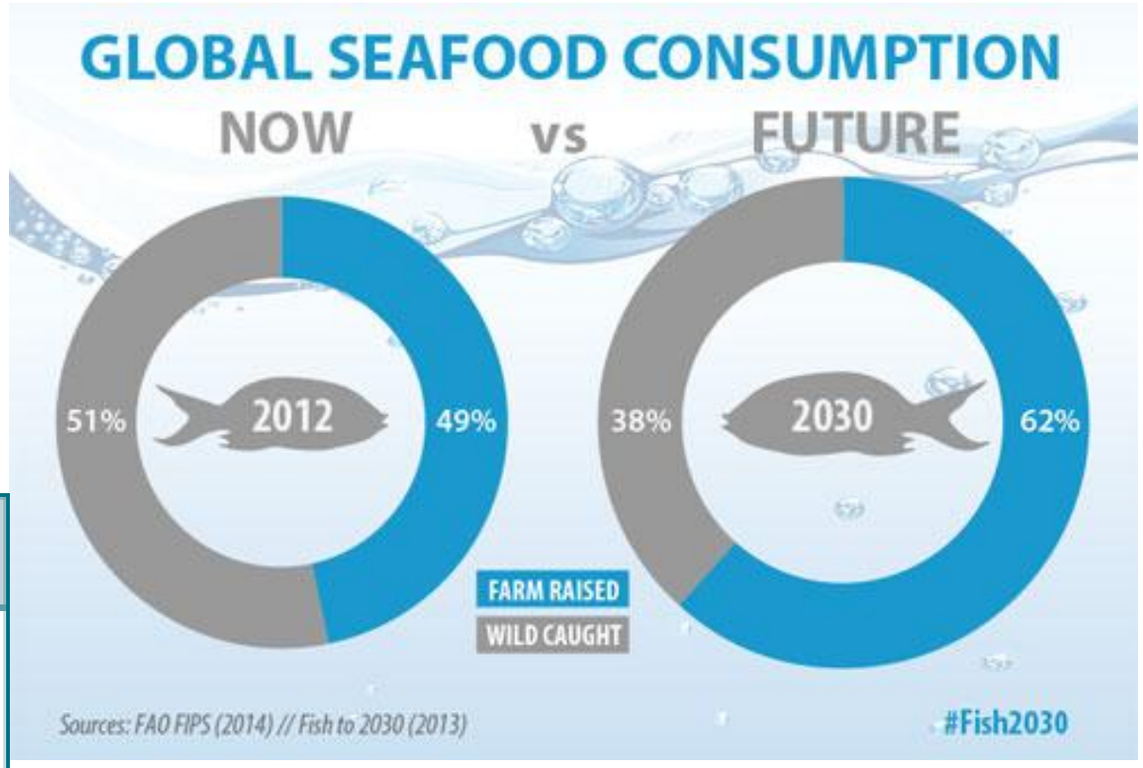
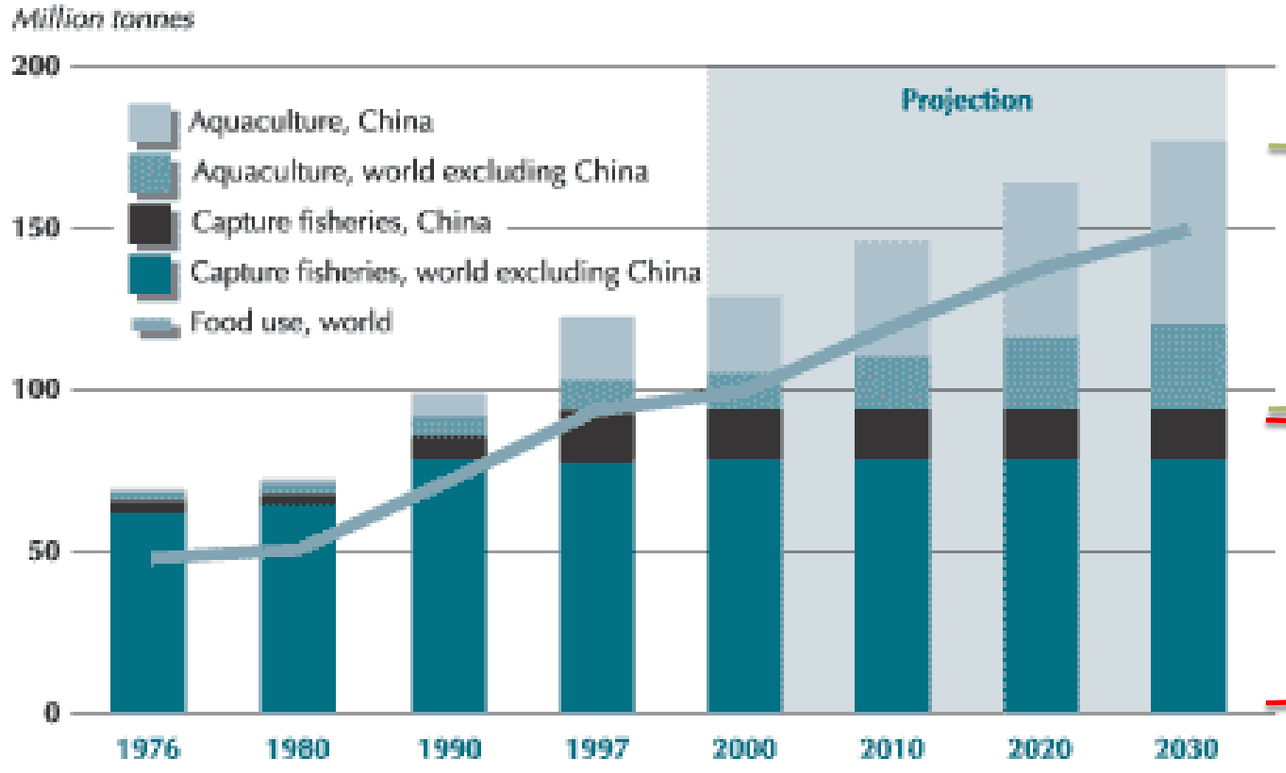


FIGURE 47
World fish production and food use consumption 1976–2030



Note: Data are from the Global 1 report; in general they are suported by the Global 2 report.

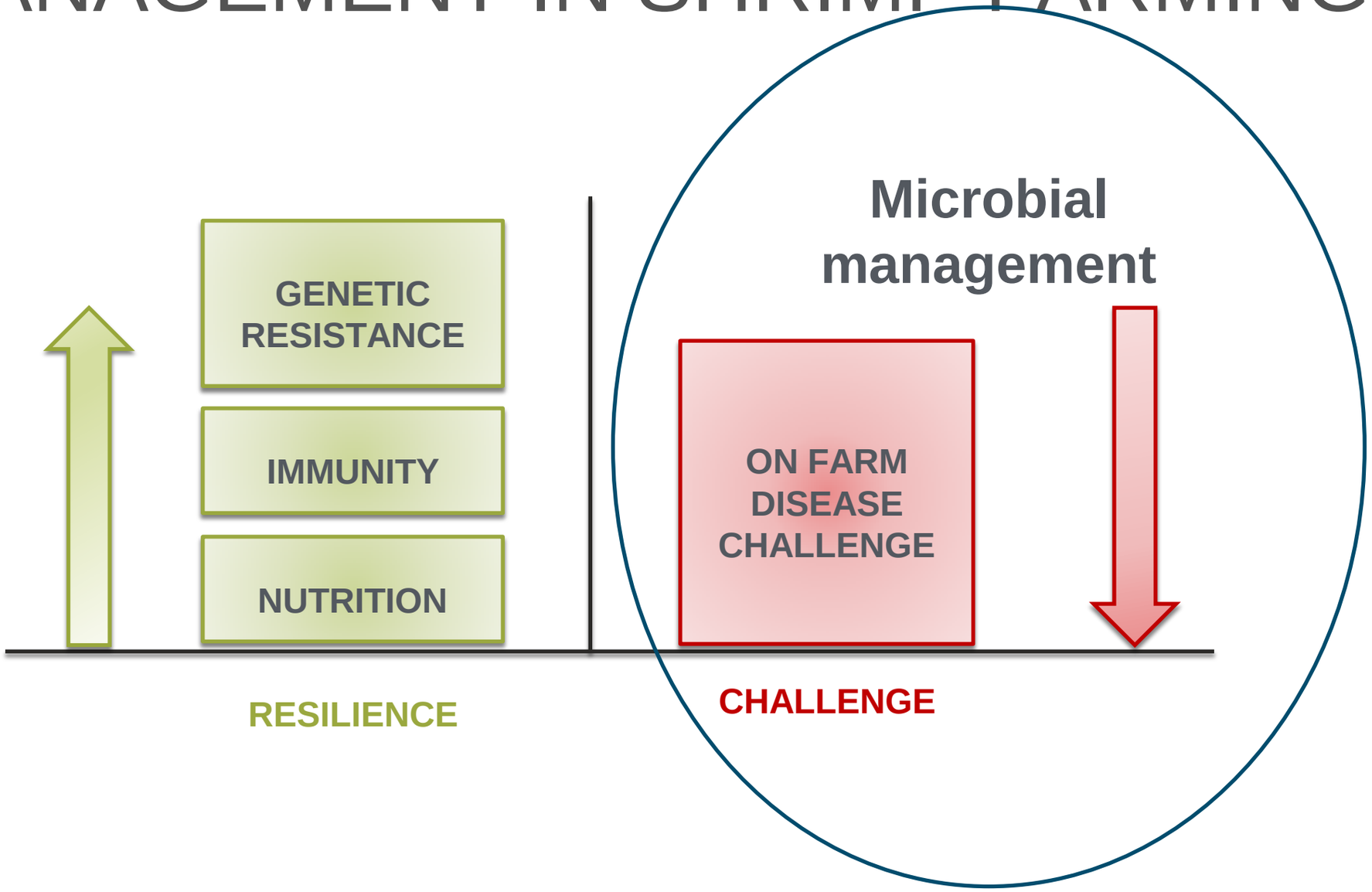
Aquaculture has to increase with another 10 million tonnes by 2030 (+ 13-14%!!)

Capture fisheries stagnate at about 90 million tonnes

INCREASING SHRIMP PRODUCTION

Towards (super-)intensive shrimp production→ health management!

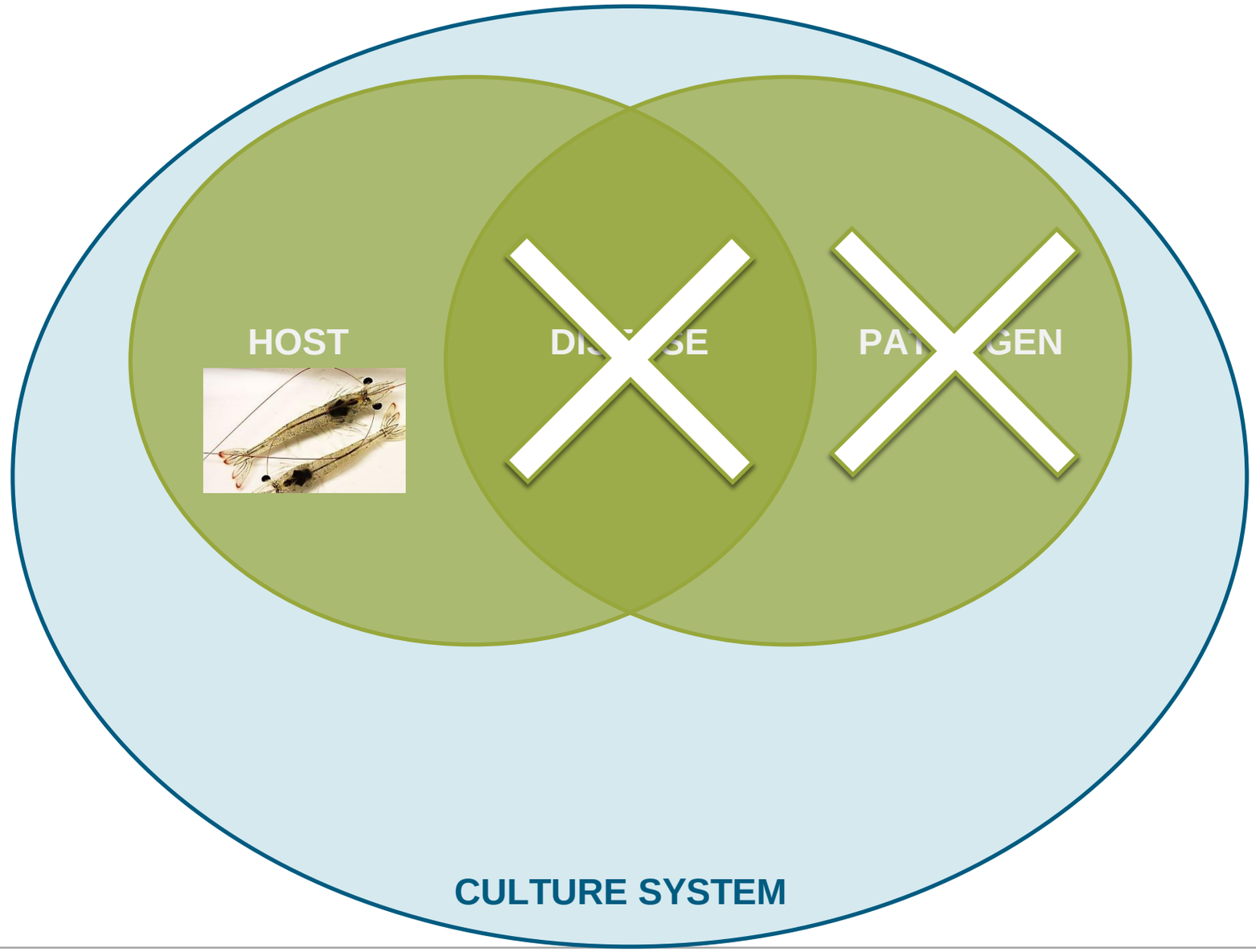
BALANCED APPROACH FOR HEALTH MANAGEMENT IN SHRIMP FARMING



- REDUCE “RED STACK” AND INCREASE “GREEN STACK”

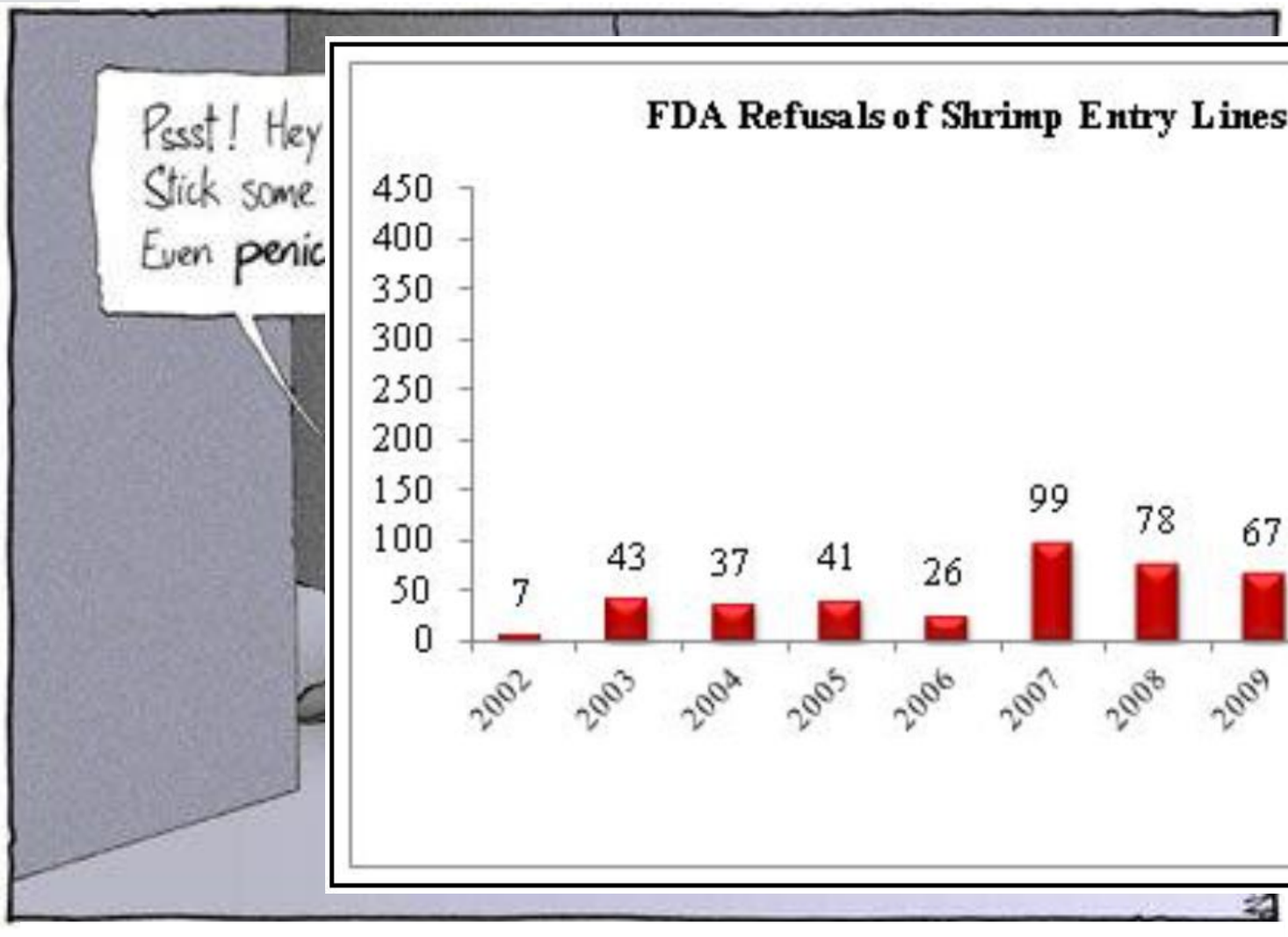
MICROBIAL MANAGAMENT IN SHRIMP FARMING

THE TRADITIONAL VISION: "BEAT THEM"



MICROBIAL MANAGEMENT IN SHRIMP FARMING

Focus on pathogens only – the “beat them” approach

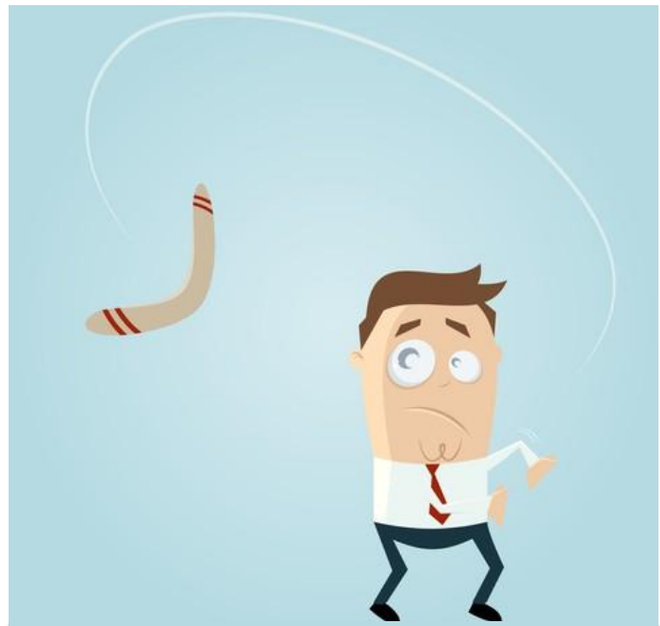


It was on a strawl through the shrimp pond that Albert was first approached by a member of the Antibiotic Resistance

MICROBIAL MANAGEMENT IN SHRIMP FARMING

Focus on pathogens only – the “beat them” approach

The evolution of bacteria on a “mega-plate” petri dish – Kishony Lab; Harvard Medical School

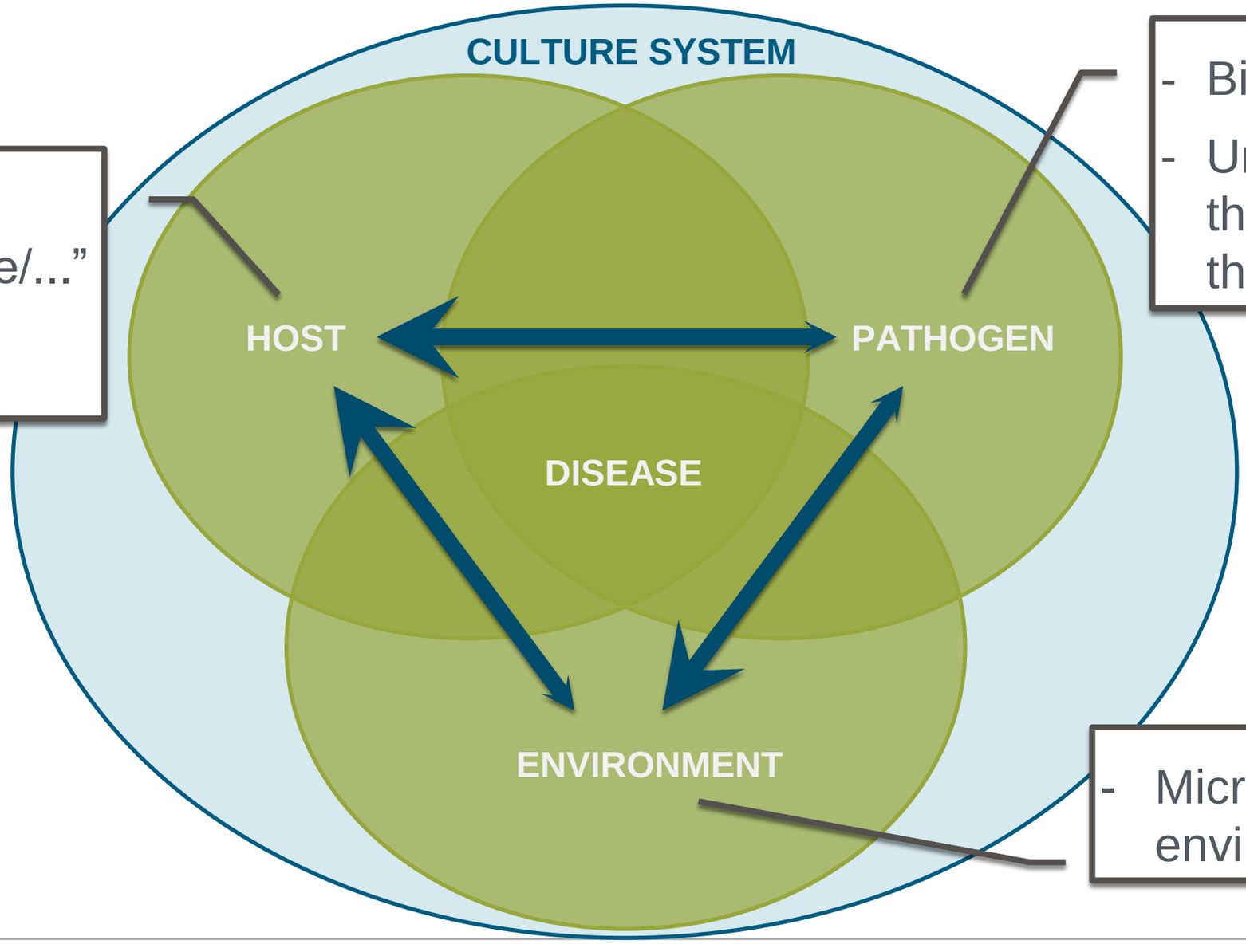


Unsustainable antibiotic use is coming back to hit you in the face!!!

MICROBIAL MANAGEMENT IN SHRIMP FARMING

THE NEW VISION: "HOLISTIC MICROBIAL MANAGEMENT"

- Host provides "housing/entrance/..." for bad and good bacteria



- Biosecurity
- Understanding the ecology of the pathogen

- Microbial environment

MICROBIAL MANAGEMENT IN SHRIMP FARMING

THE
AQUACULTURE
ROUNDTABLESERIES® 2016
A shared vision for aquaculture in Asia

TARS 2016
The Aquaculture Roundtable Series
August 17-18 2016

SHRIMP AQUACULTURE & THE NEW NORMAL

August 17-18 2016, JW Marriott Phuket Resort & Spa, Thailand

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o contributed to the success of TARS 2016! See you at TARS 2017! The organizers would like to thank the Thai Department of Fis

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Organizers

AQUA CULTURE Asia Pacific

TARS 2016 on Shrimp Aquaculture & The New Normal

The theme for the sixth Aquaculture Roundtable Series (TARS) 2016 is **Shrimp Aquaculture & the New Normal**. TARS 2016 is a follow-up to the 2014 meeting that focused on the recovery, revival and renaissance of Asia's shrimp aquaculture, and the industry's struggles to contain disease outbreaks, mainly that of the Early Mortality Syndrome (EMS) and White Spot Syndrome Virus (WSSV).

Despite some progress, in terms of management and disease preventive measures, vulnerability to disease outbreaks continues to disrupt production. Asia's production of farmed shrimp, on the whole, declined to an

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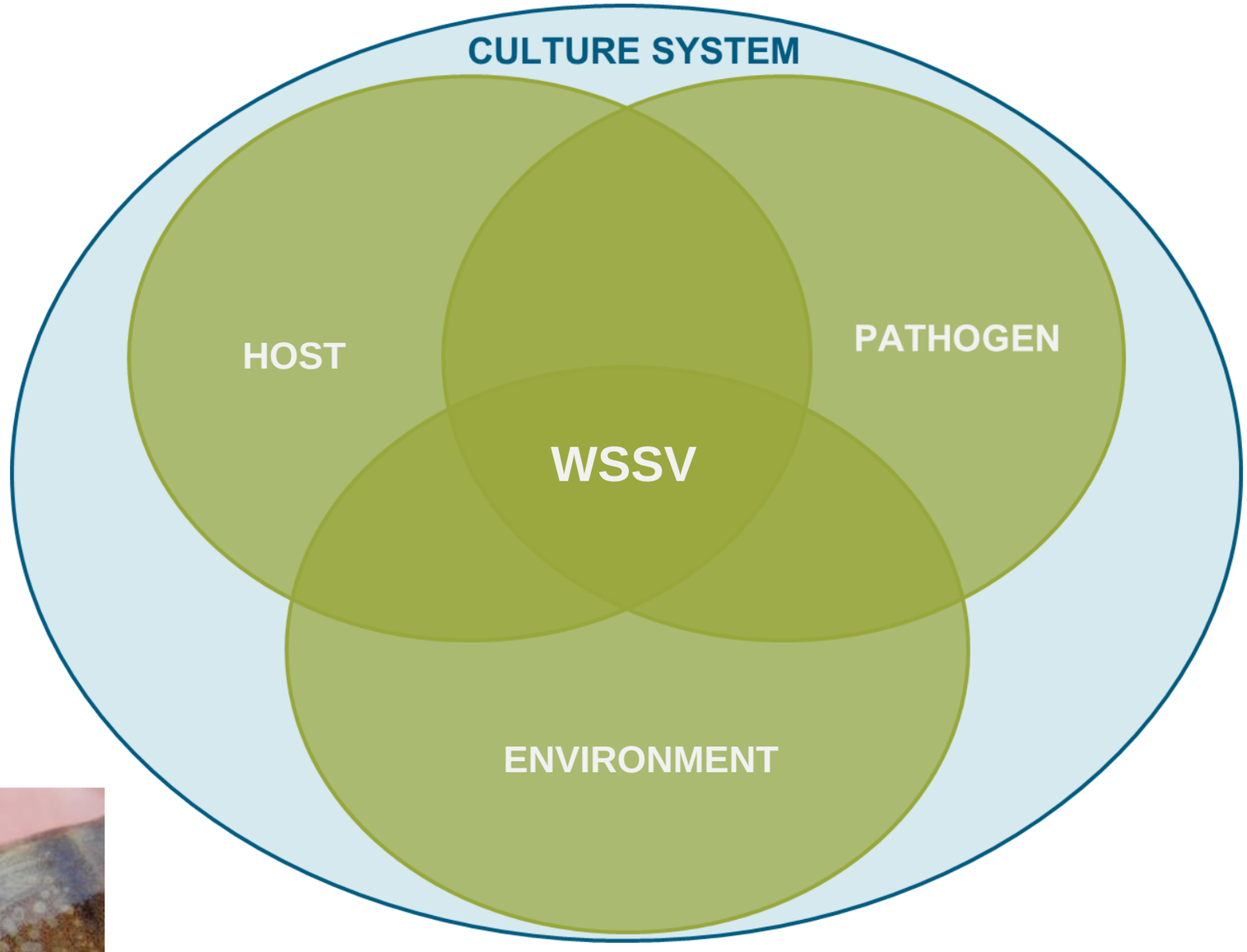
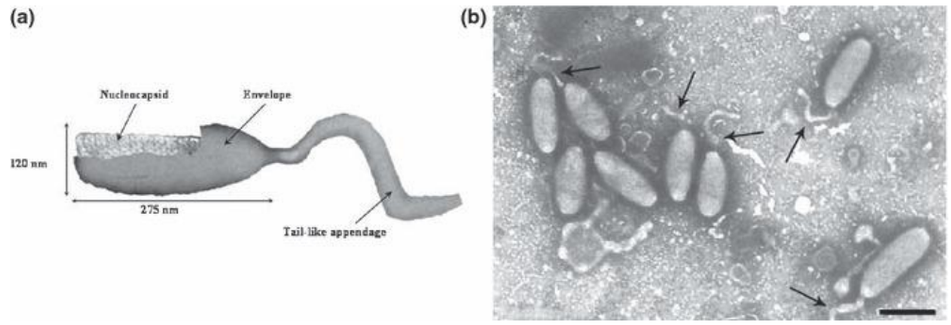
WHITE SPOT SYNDROME VIRUS



WHITE SPOT SYNDROME VIRUS



Escobedo-Bonilla et al. (2008)

- Emerged in 1992-1993, and dispersed quickly by seed, broodstock and frozen shrimp throughout Asia and the rest of the world
- Annual loss in Mekong Delta 2015: ± 8 $ mln *(Shinn and Griffiths, TARS 2016)*

Table 1 Chronology of white spot syndrome virus outbreaks in shrimp farming countries in Asia and America

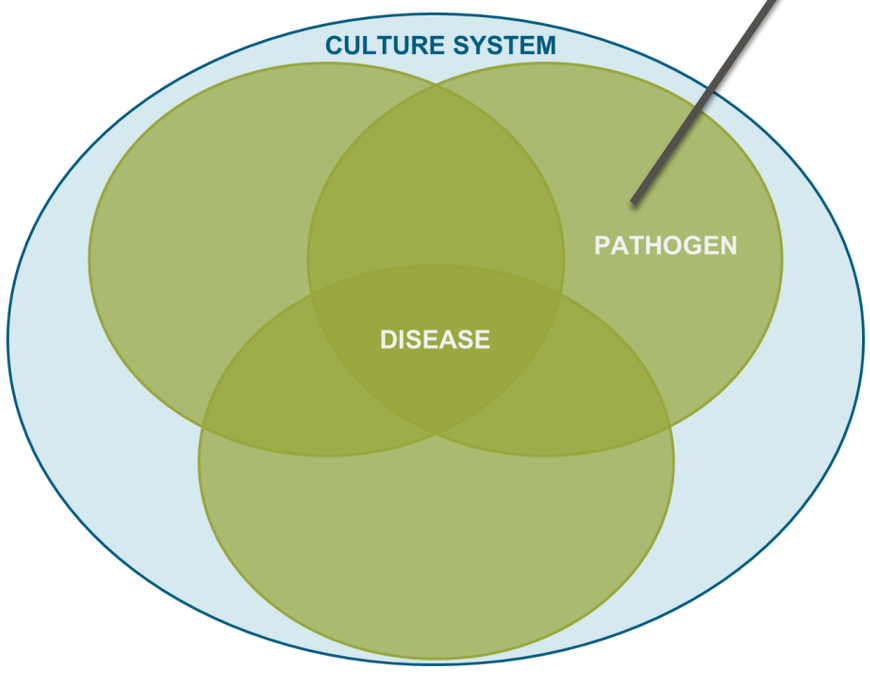
Year first reported	Country	Reference
1992	Taiwan	Chou *et al.* 1995
1993	China, Japan, Korea	Zhan *et al.* 1998; Inouye *et al.* 1994; Park *et al.* 1998
1994	Thailand, India, Bangladesh	Lo *et al.* 1996a; Karunasagar *et al.* 1997; Mazid & Banu 2002
1995	USA	Lightner 1996; Wang *et al.* 1999a
1996	Indonesia, Malaysia, Sri Lanka	Durand *et al.* 1996; Kasornchandra *et al.* 1998; Rajan *et al.* 2000
1997	Vietnam	Bondad-Reantaso *et al.* 2001
1998	Peru	Rosenberry 2001
1999	Philippines, Ecuador, Colombia, Panamá, Honduras, Nicaragua, Guatemala, Belice	Magbanua *et al.* 2000; Bondad-Reantaso *et al.* 2001; Hossain *et al.* 2001; Wu *et al.* 2001
1999–2000	México	Bondad-Reantaso *et al.* 2001
2002	France, Iran	Dieu *et al.* 2004; Marks 2005
2005	Brazil	APHIS-USDA 2005

Escobedo-Bonilla et al. (2008)

HOLISTIC MICROBIAL MANAGEMENT FOR WHITE SPOT SYNDROME VIRUS

Despite increasing knowledge and a lot of research:

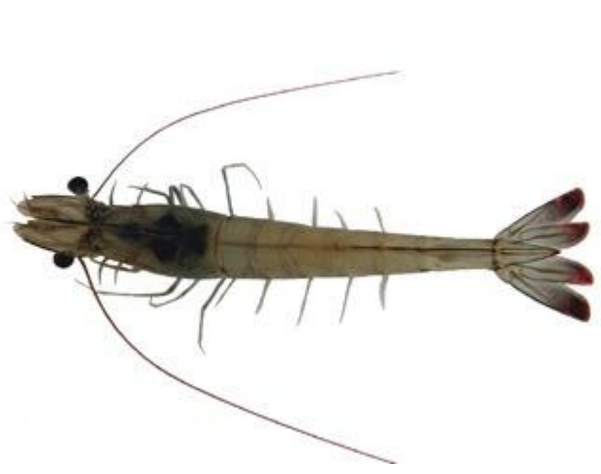
no silver bullet yet for its control in shrimp farming

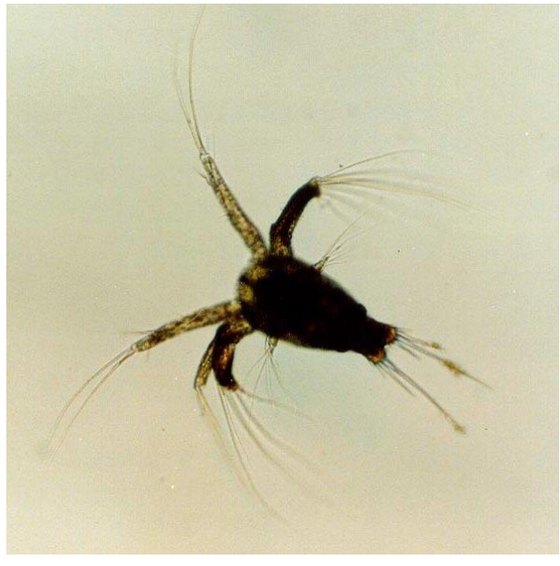


- Understanding the ecology of the pathogen

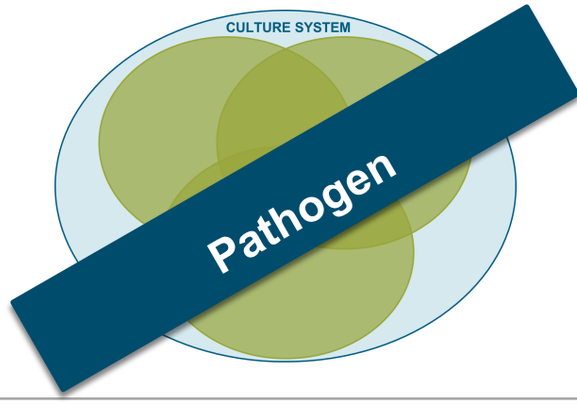
HOLISTIC MICROBIAL MANAGEMENT FOR WHITE SPOT SYNDROME VIRUS

- WSSV needs a host to replicate → how does WSSV enter into a shrimp?
  - vertical transmission: broodstock to offspring





Biosecurity: use of SPF shrimp is a must!



HOLISTIC MICROBIAL MANAGEMENT FOR WHITE SPOT SYNDROME VIRUS

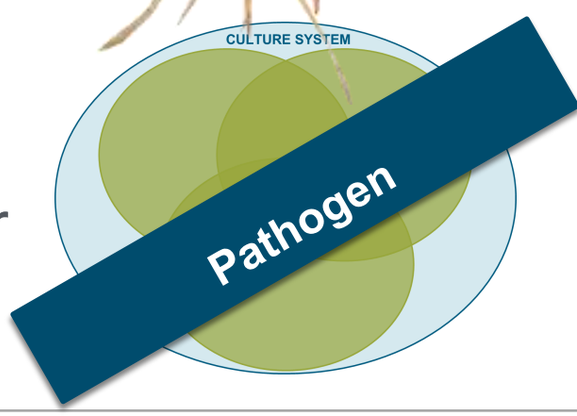
- WSSV needs a host to replicate → how does WSSV enter into a shrimp?
  - horizontal transmission: ingestion of infected tissues

Research indicates that very high doses are needed in order to cause infection: > 10^8 SID_{50}/g (im) *(Thuong et al. 2016, Veterinary Research)*

→ questions this route as trigger for disease outbreak

→ however, when virus is in shrimp becomes “virus fermentor”

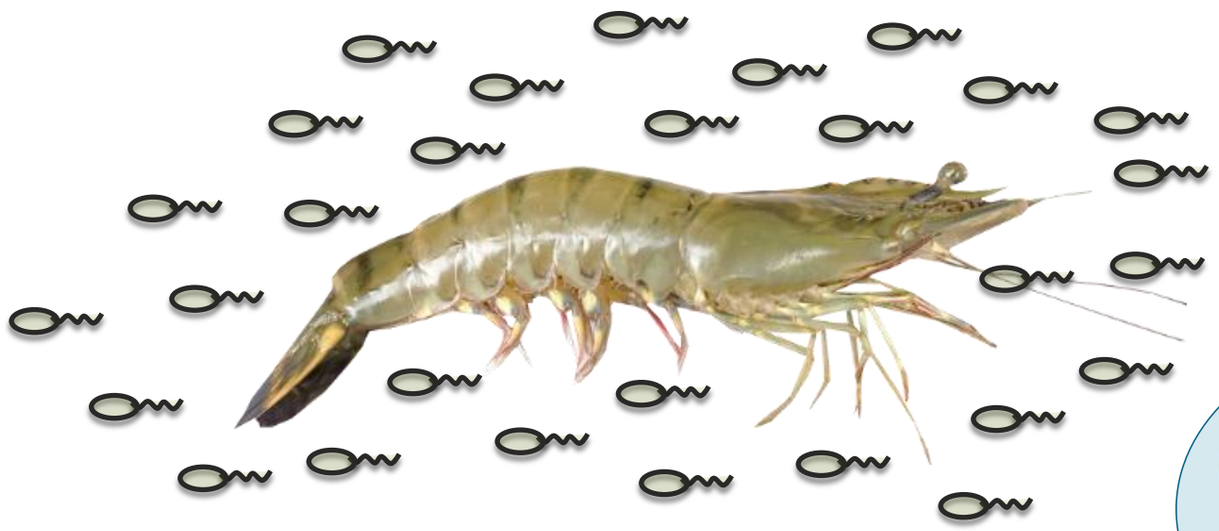
→ Biosecurity: elimination prior to stocking of intruder crustaceans that may carry WSSV

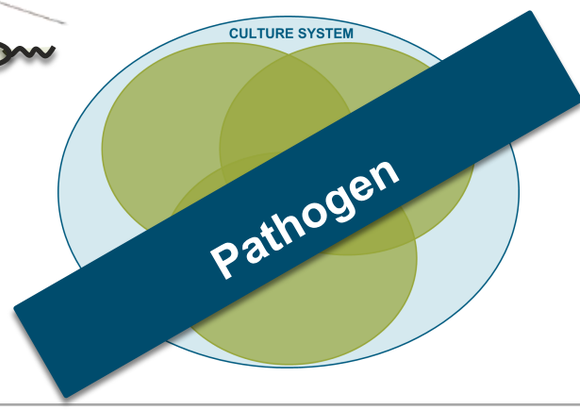


HOLISTIC MICROBIAL MANAGEMENT FOR WHITE SPOT SYNDROME VIRUS

- How are shrimp infected?
  - waterborne transmission: virus particles in the water

  Infection models indicate that high doses are needed in order to cause infection under standard conditions: > $10^{5,5}$ SID_{50}/mL (im) (≈ $10^{7.5}$ SID_{50}/g) *(Thuong et al. 2016, Journal of Fish Diseases)*





→ Biosecurity: pond preparation (sun drying and disinfection), not 100% efficient (Satheesh Kumar et al. 2013)

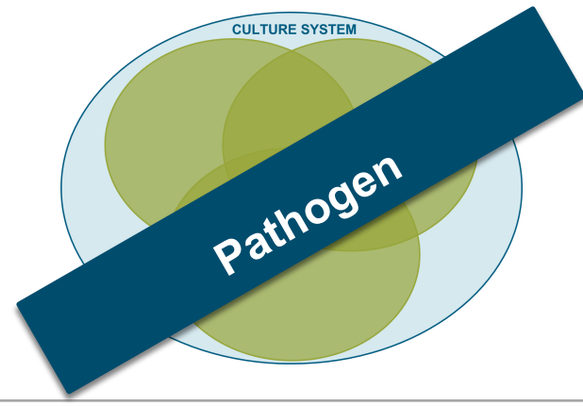
HOLISTIC MICROBIAL MANAGEMENT FOR WHITE SPOT SYNDROME VIRUS

- How are shrimp infected?
  - waterborn transmission: virus particles in the water

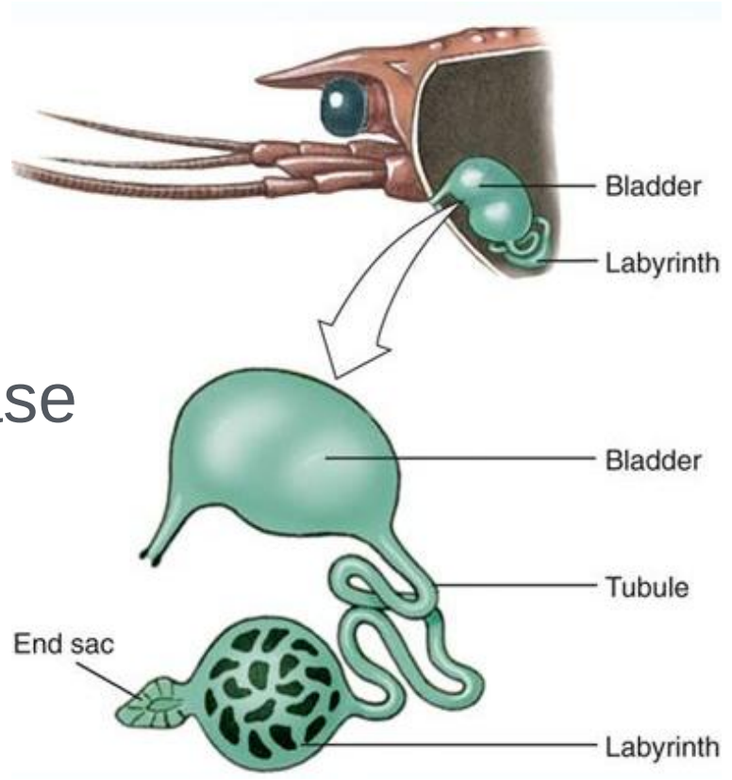
Very recent finding *(patent - Nauwynck, WO2016150931 A1):* inoculation through external pore of the antennal gland is highly effective → $10^{1.5\text{-}2.1}$ SID^{50}/g (im)

→ Could this be route that starts disease outbreak?

→ What are environmental conditions that trigger infection?



HOLISTIC MICROBIAL MANAGEMENT FOR WHITE SPOT SYNDROME VIRUS



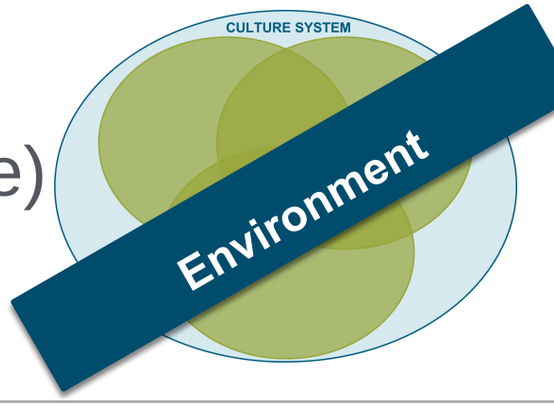
- Entrance through external pore of the antennal gland: excretory and osmoregulatory organ at base of antennae

Incidences of WSSV outbreaks have been associated with rain fall *(Peina-do-Guevara & Lopez-Meyer 2006; Thuong et al. 2016)* and cold season *(Withyachumnarnkul et al. 2003)*

→ sudden drops in salinity (WSSV uptake)

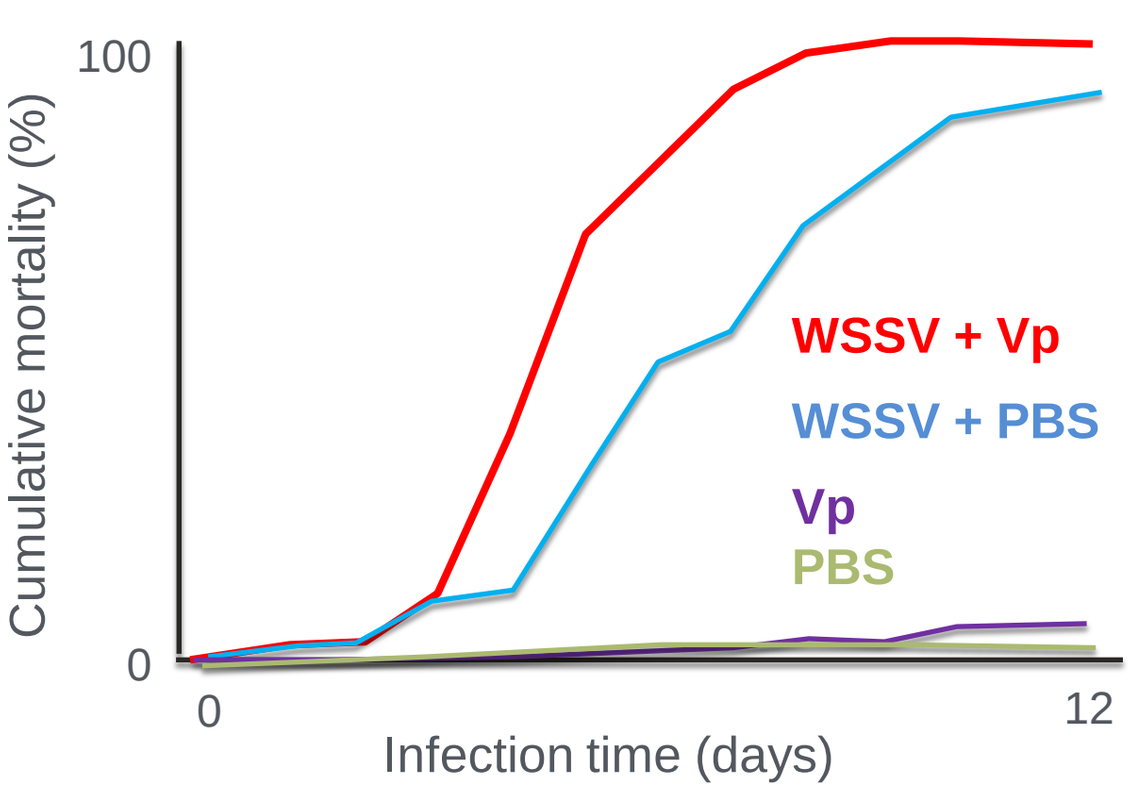
→ lower temperature in the water (immunocompetence)

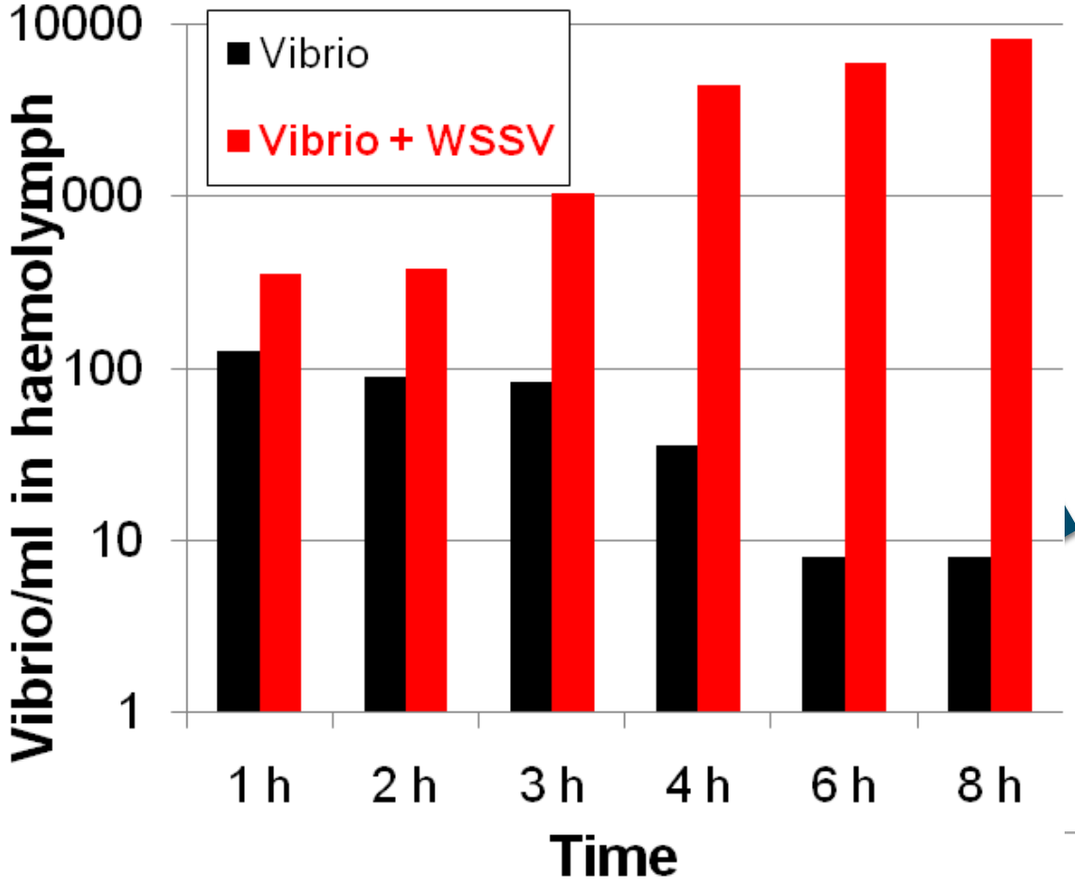
→ High presumptive vibrio count *(Tendancia and Verreth, 2010)*



HOLISTIC MICROBIAL MANAGEMENT FOR WHITE SPOT SYNDROME VIRUS

Vibrios may act as opportunistic pathogens causing secondary infection in WSSV weakened shrimp or *vice versa* *(Phuoc et al. 2008, 2009; Zhang et al. 2016)*





HOLISTIC HEALTH MANAGEMENT APPLIED TO WHITE SPOT SYNDROME VIRUS

WHITE SPOT SYNDROME VIRUS:

→ Challenge is to control

WSSV + VIBRIOSIS

- Biosecurity
- Environmental conditions? (salinity, temperature,...)
- Immunostimulation
- Vaccination (VP28)
- RNAi (VP28)
- Others?


investigación y desarrollo

Protection of Penaeid Shrimp Against WSSV Using Oral Vaccination

Jeroen Witteveldt, Just M. Vlak and Mariëlle C.W. van Hulten*


Global Aquaculture Advocate™

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ANIMAL HEALTH & WELFARE

January 7, 2016 | by C. M. Escobedo-Bonilla

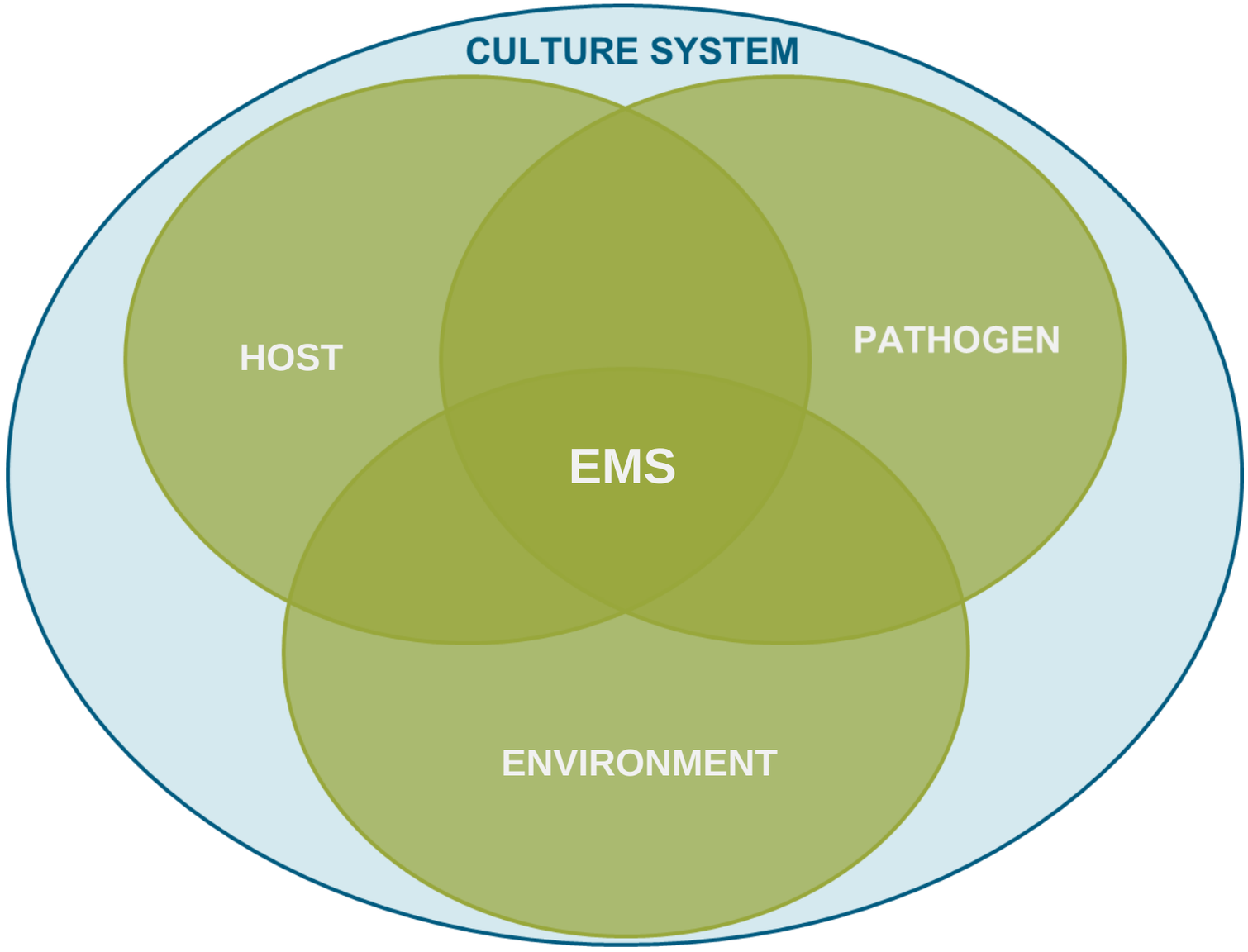
Double-stranded RNA against WSSV genes provides antiviral protection in shrimp

SHARE Print

Researchers explore solutions to major shrimp disease

The white spot syndrome virus (WSSV) has caused major economic losses to shrimp farmers worldwide since the 1990s. Various field

EARLY MORTALITY SYNDROME (AHPND)



EARLY MORTALITY SYNDROME

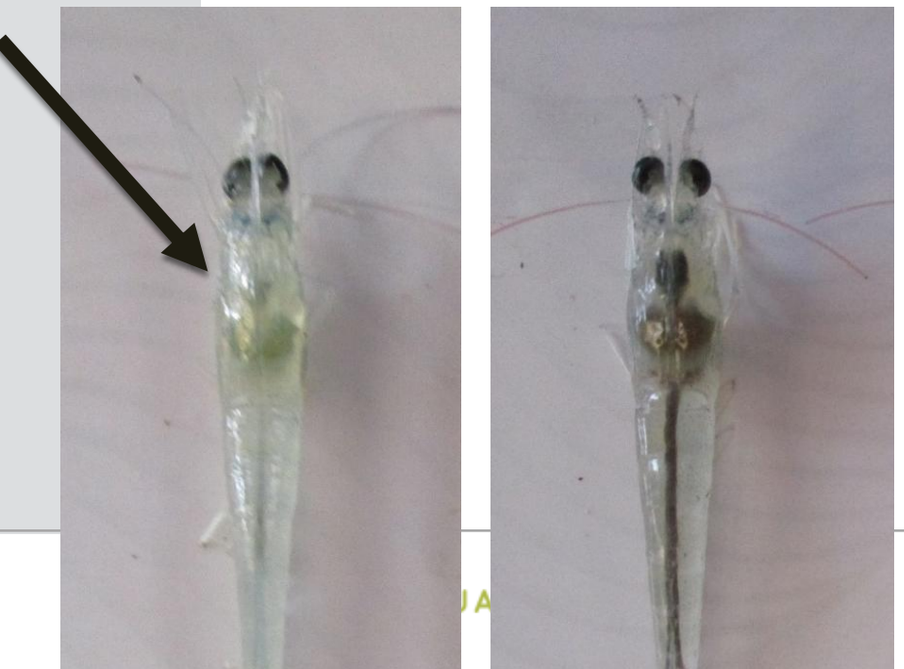
- *Vibrio parahaemolyticus*

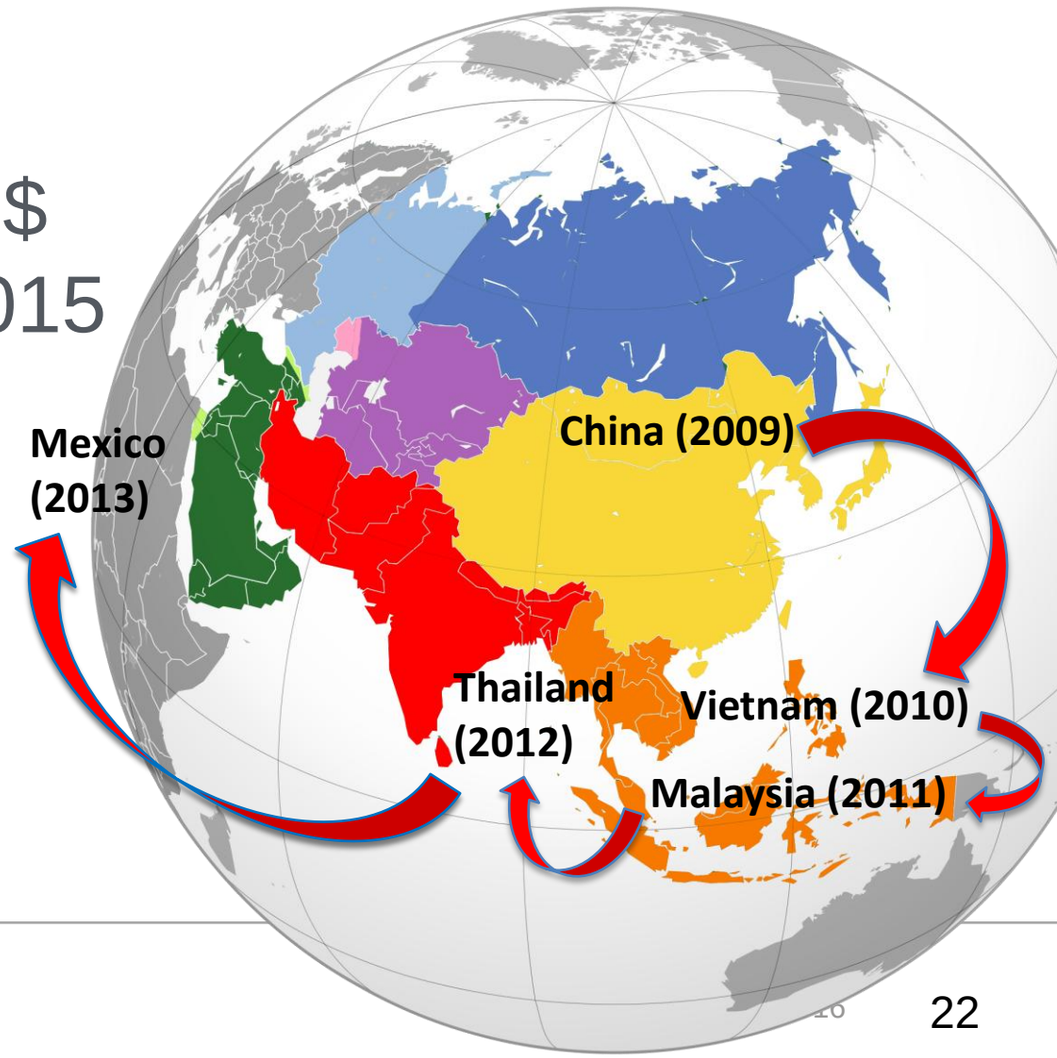
-Shrimp post-larvae

- 10 - 30 days after stocking in grow-out pond

- causes up to 100 % mortality

- 5-7 $ bn losses in Thailand; 10 $ mln losses in Mekong Delta in 2015

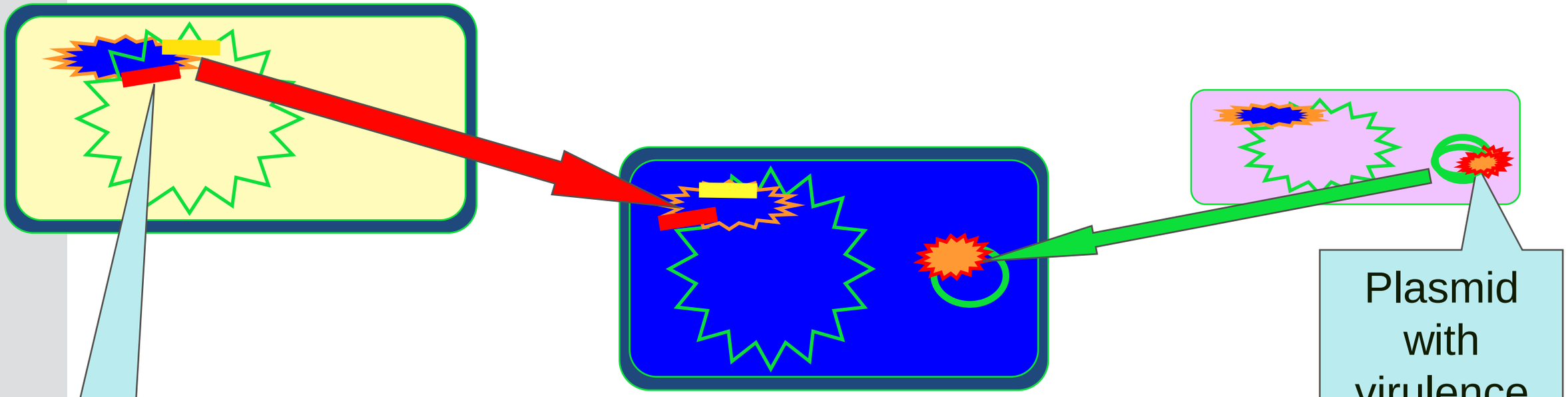




TRANSFER OF EMS TOXIN GENES TO NON V. PARAHAEMOLYTICUS STRAINS

Principal Mechanism: Horizontal Gene Transfer

Between cells of the same or different species



- Gene transfer without cell division
- Common in bacteria and viruses
- Faster when more resistant to antibiotics and at high population densities

THE UNPREDICTABILITY OF THE EMS EPIDEMIC

LOCAL SCALE

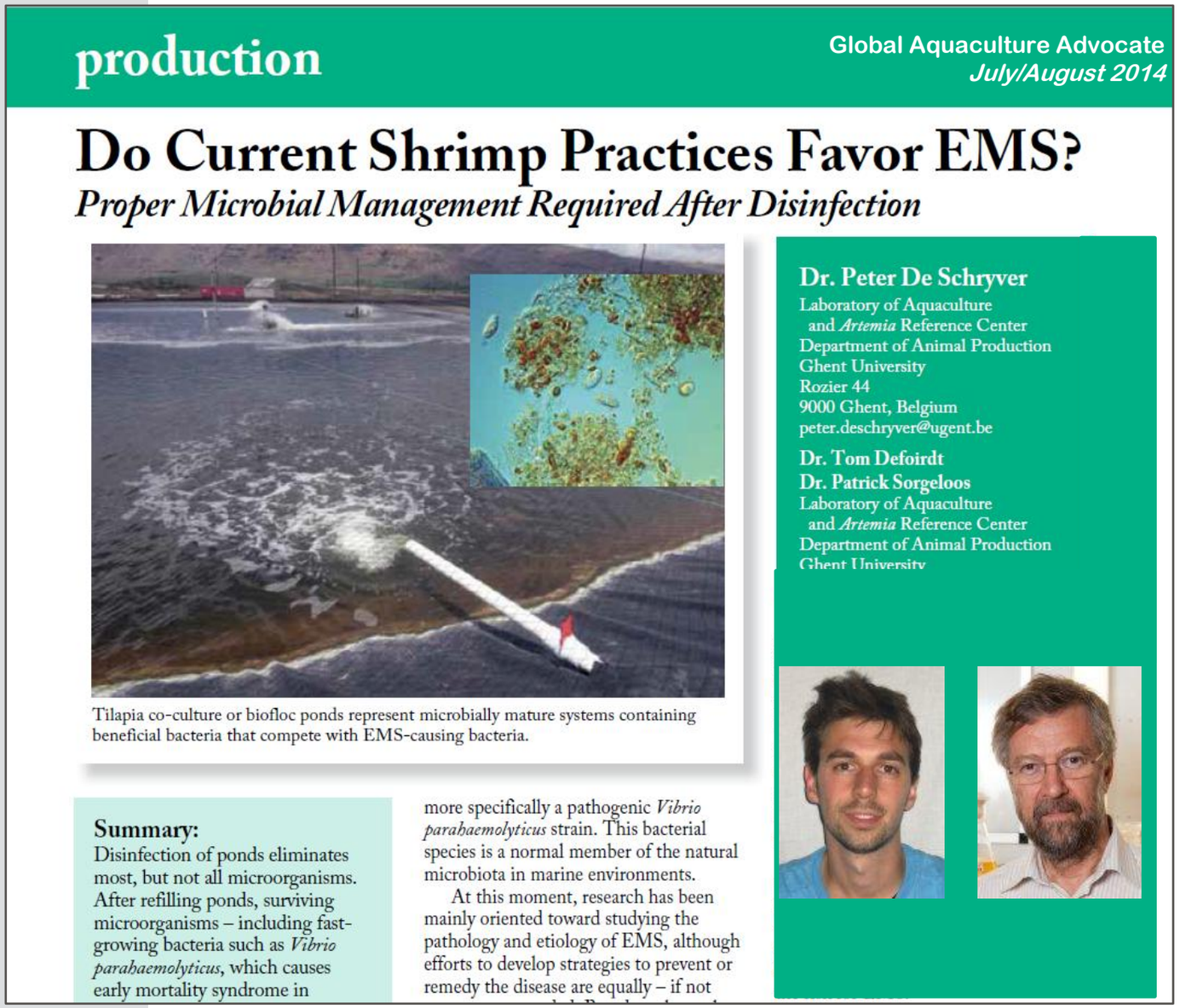
Biosecurity:

-EMS free larvae for stocking in grow out ponds

- Disinfection of water and pond sediment

HOLISTIC MICROBIAL MANAGEMENT FOR EARLY MORTALITY SYNDROME

MICROBIAL CONTROL OF VIBRIOS:



production

Global Aquaculture Advocate
July/August 2014

Do Current Shrimp Practices Favor EMS?

Proper Microbial Management Required After Disinfection

Tilapia co-culture or biofloc ponds represent microbially mature systems containing beneficial bacteria that compete with EMS-causing bacteria.

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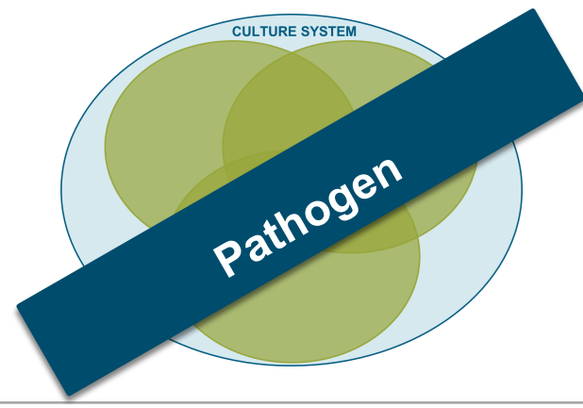
Summary:
Disinfection of ponds eliminates most, but not all microorganisms. After refilling ponds, surviving microorganisms – including fast-growing bacteria such as *Vibrio parahaemolyticus*, which causes early mortality syndrome in

more specifically a pathogenic *Vibrio parahaemolyticus* strain. This bacterial species is a normal member of the natural microbiota in marine environments.

At this moment, research has been mainly oriented toward studying the pathology and etiology of EMS, although efforts to develop strategies to prevent or remedy the disease are equally – if not

PROLIFERATION

Vibrio parahaemolyticus is a natural component of marine environments, but it is possible to set up selection to **outcompete the opportunists!**



CURRENT DISINFECTION PRACTICE

black box at the microbial level

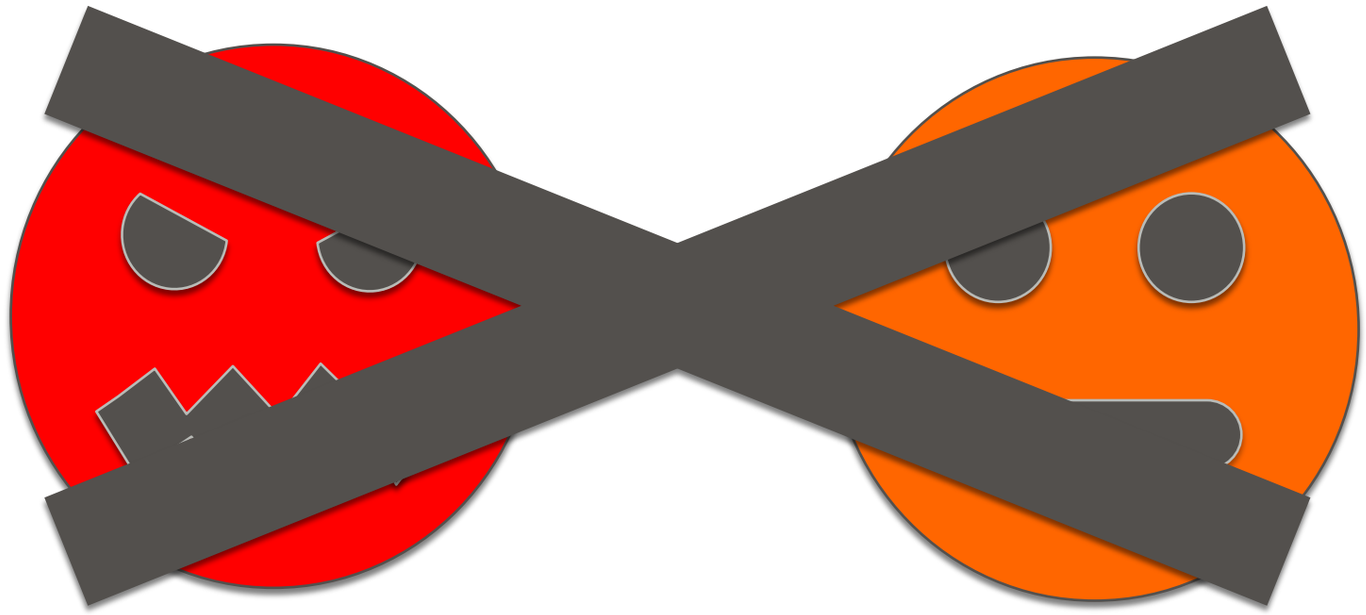
?

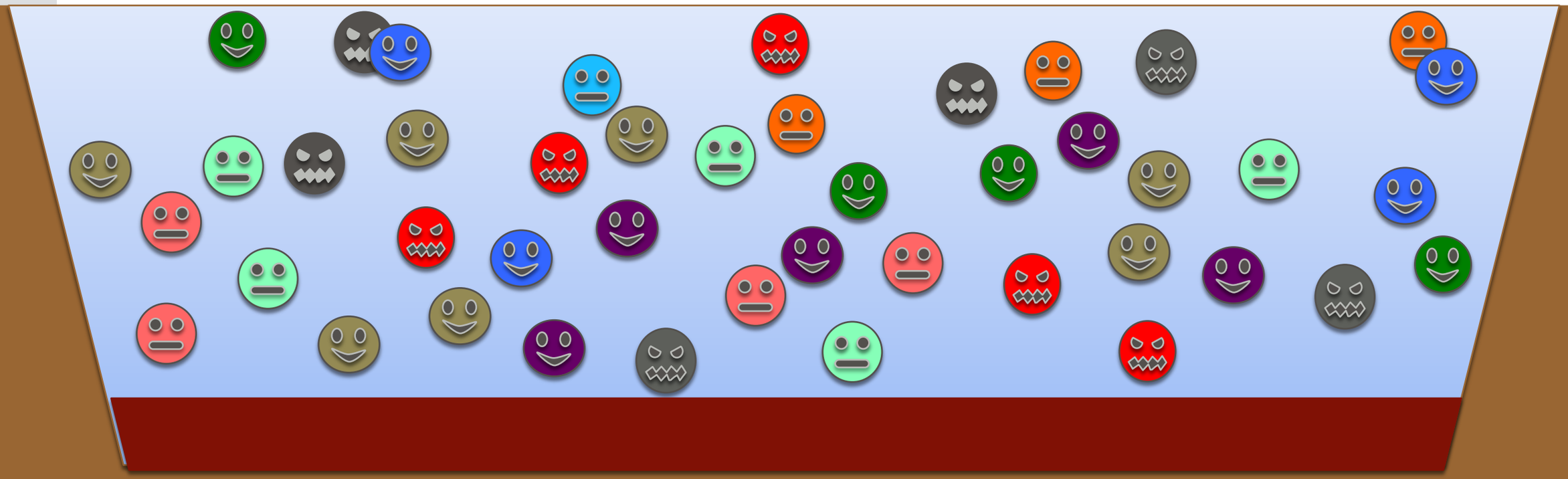
What do we want???

neutral/beneficial bacteria

harmful bacteria (obligate pathogens)

potentially harmful bacteria (opportunists)

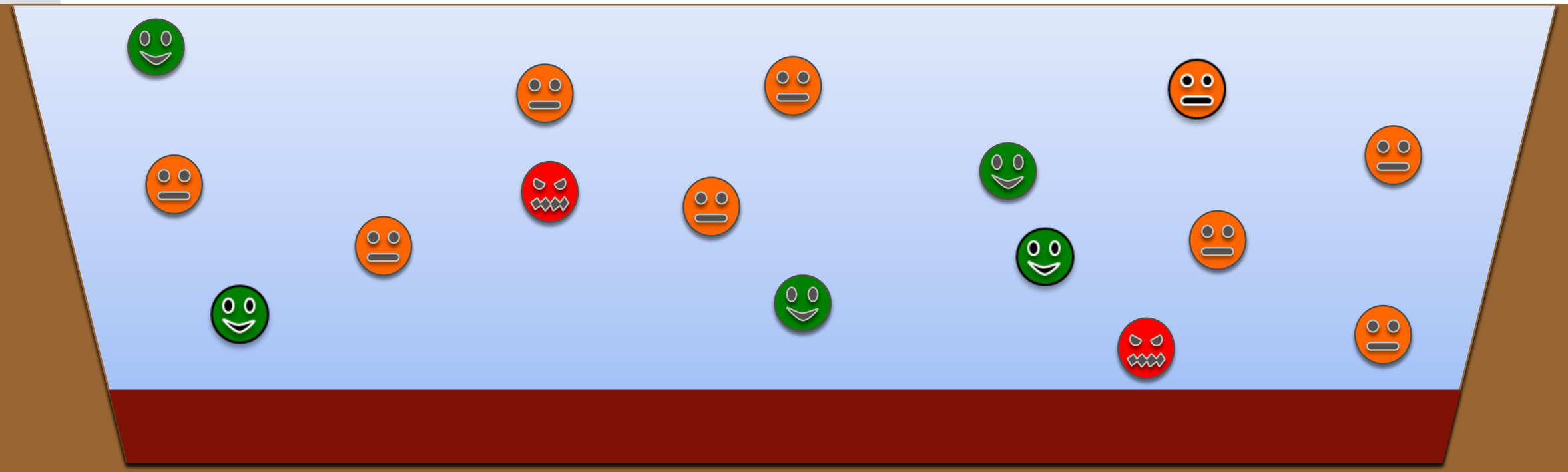




In order to eliminate bad bacteria: disinfection

- all bacteria are eliminated

- but, new bacterial colonization starts:
difference r-strategists (😐) and K-strategists (😀)!!!!



ecological characteristics of

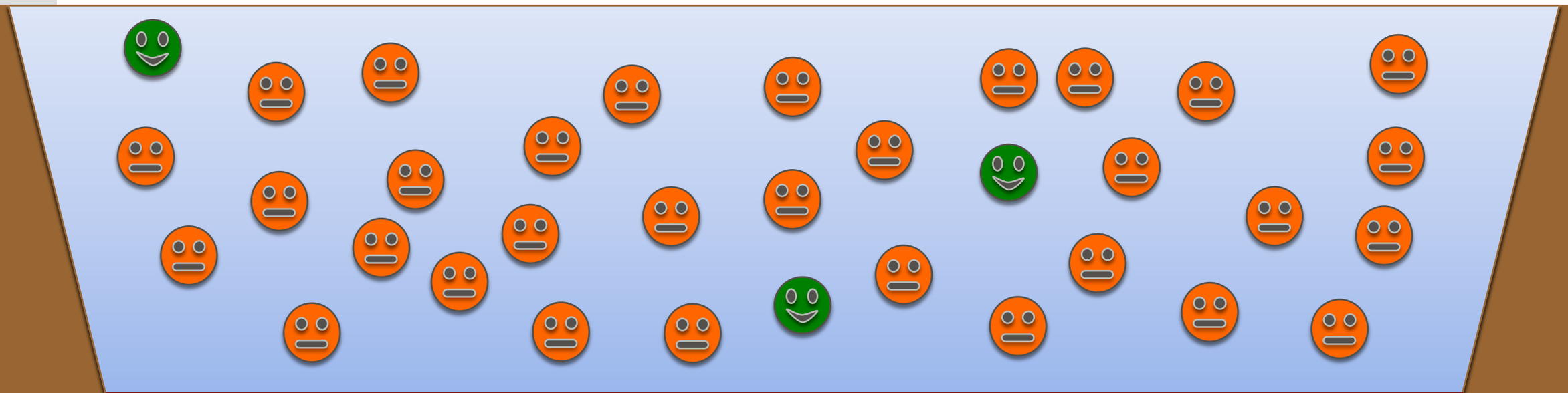
	r-strategist bacteria	**K-strategist bacteria**
Importance for shrimp	Dangerous; opportunistic pathogens	Generally harmless
Growth rate	HIGH	LOW
Effect of enrichment	RAPID GROWTH	SLOW GROWTH
Competitive ability: High substrate/indiv Low substrate/indiv	 HIGH LOW	 LOW HIGH
Dominance?	Unstable environmental conditions	Stable environmental conditions

What happens at the microbial level in grow out ponds upon refilling and restocking?

Initially: Low number of bacteria and a lot of nutrients

→ Stimulates r-strategist bacteria

Substrate per bacterium = HIGH; niches are open

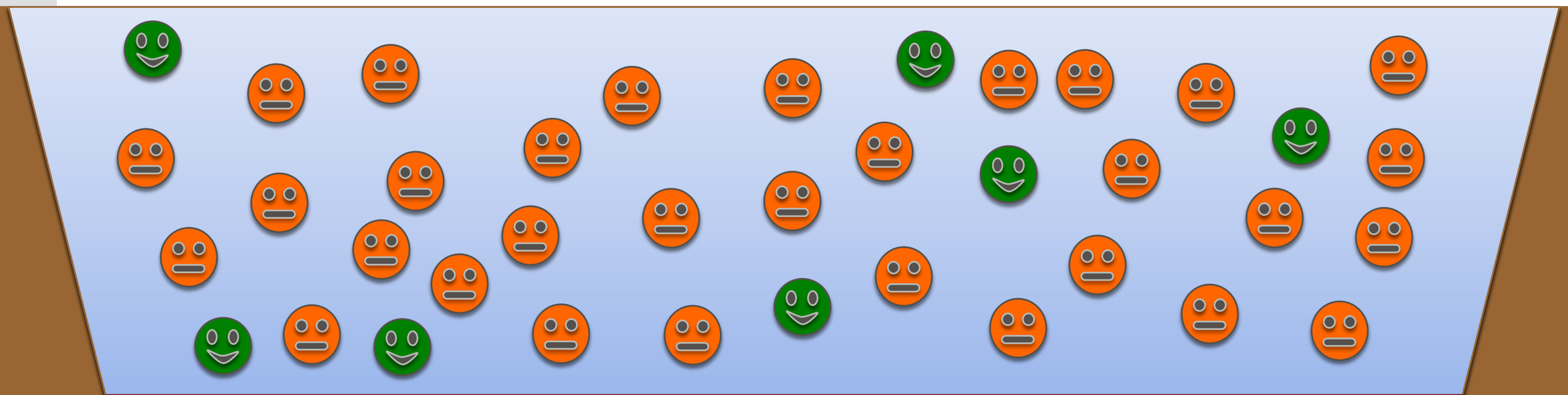


What happens at the microbial level in grow out ponds upon refilling and restocking?

In the mean time:

→ K-strategist bacteria grow slowly

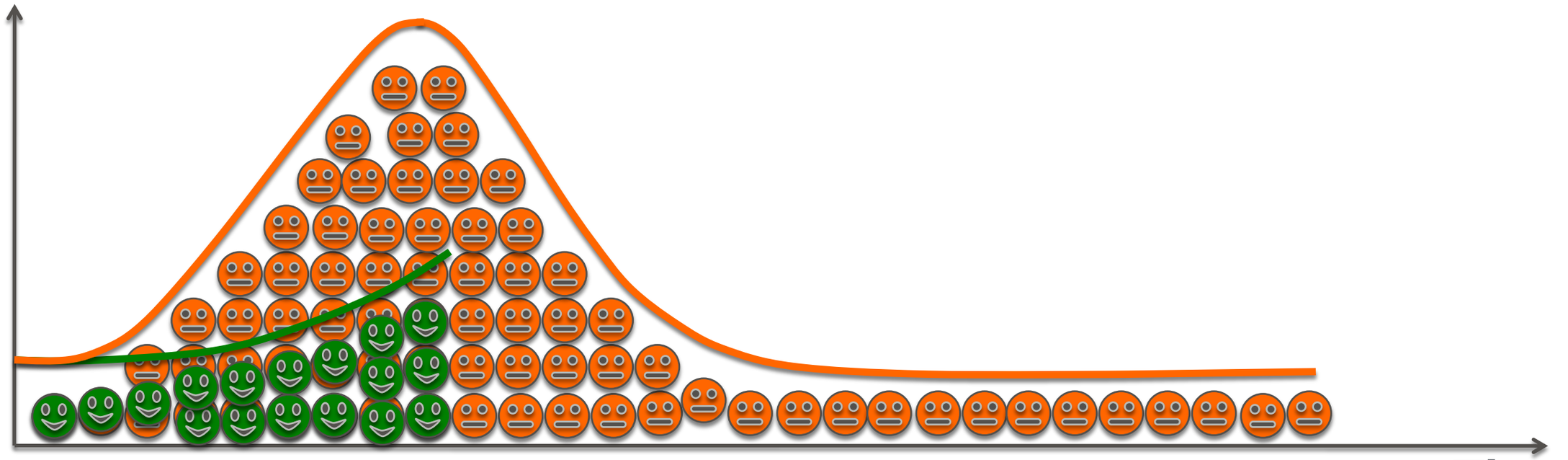
Substrate per bacterium = HIGH; niches are open



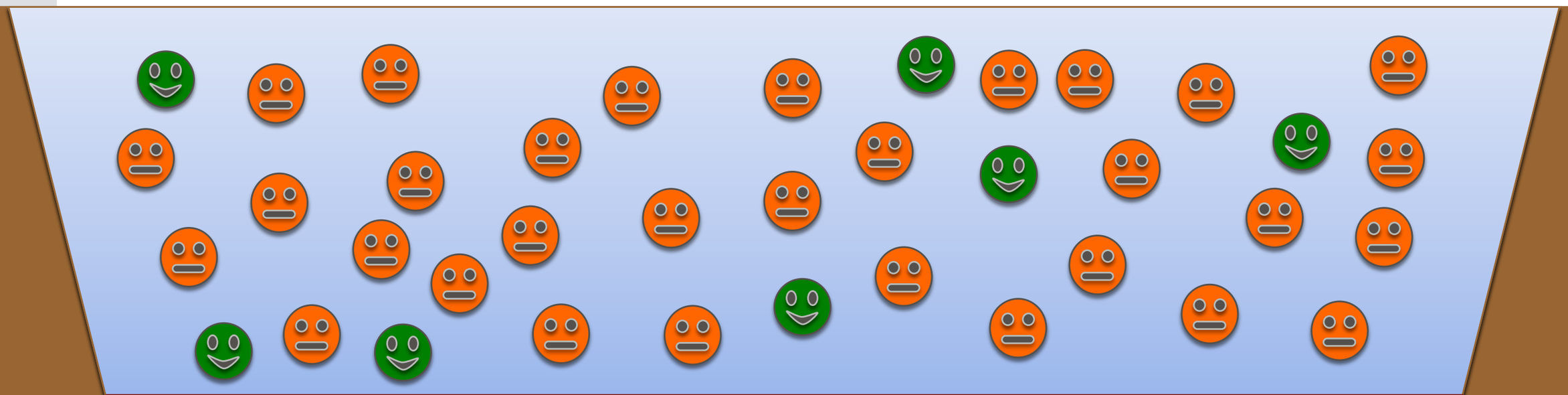
What happens at the microbial level in grow out ponds upon refilling and restocking?

Transition: High number of bacteria and less nutrients

→ Collapse of r-strategist bacteria



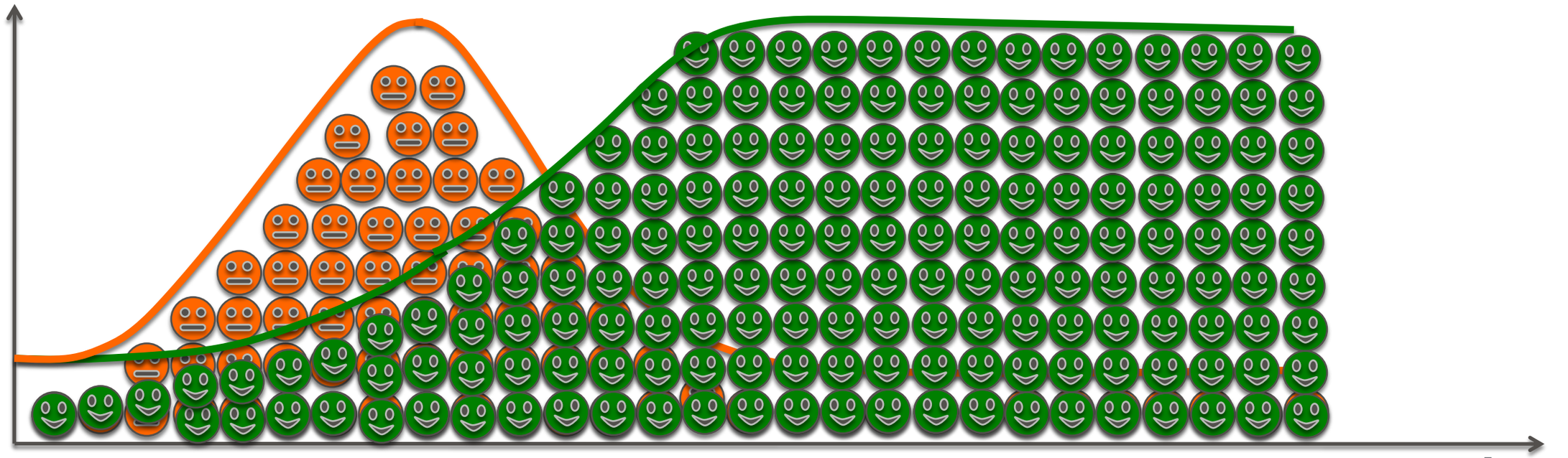
Substrate per bacterium = LOW; niches are taken



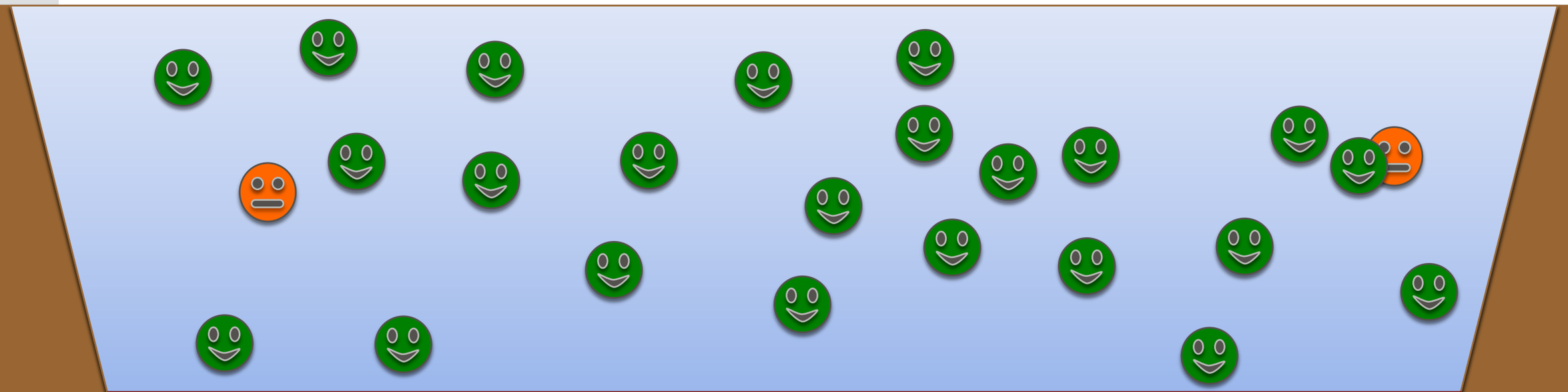
What happens at the microbial level in grow out ponds upon refilling and restocking?

In the mean time:

→ K-strategist bacteria continue to grow



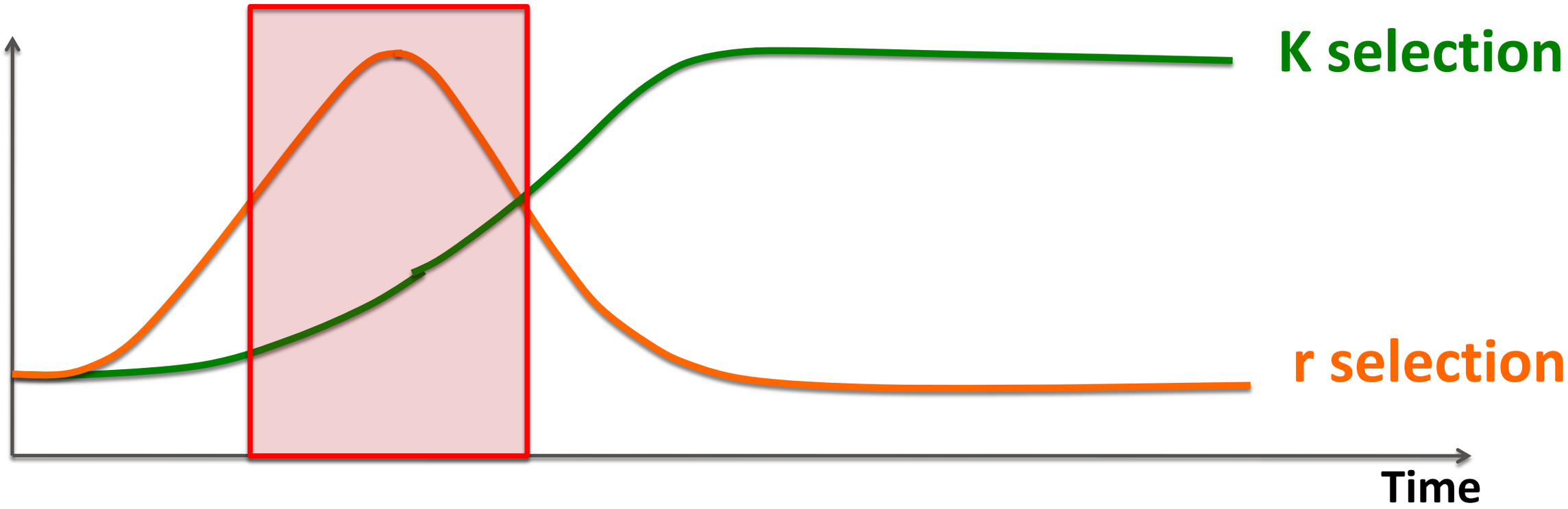
Substrate per bacterium = LOW; niches are taken

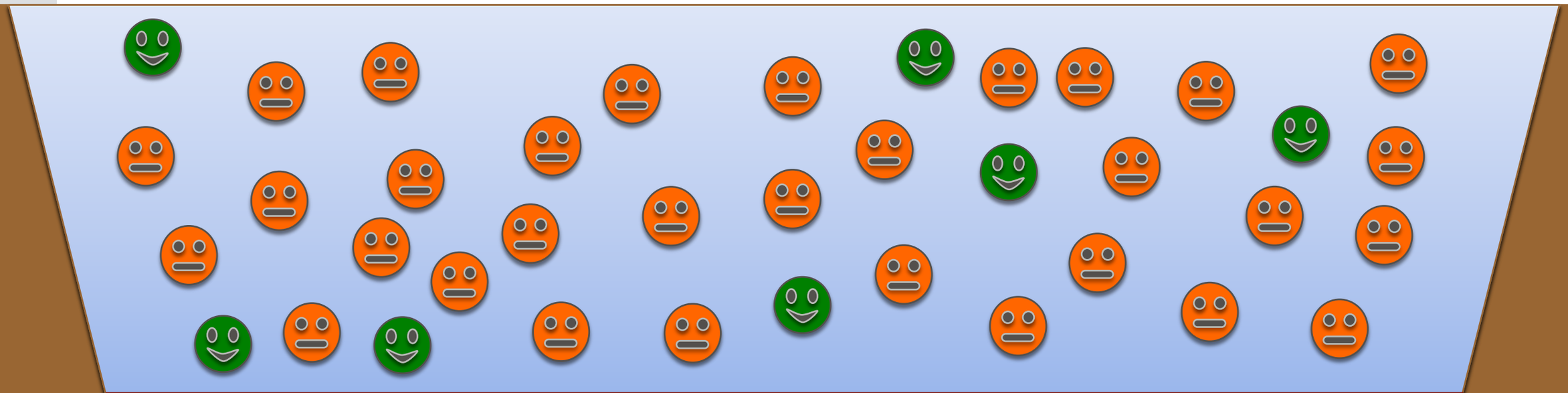


What happens at the microbial level in grow out ponds upon refilling and restocking?

introduction of animals:

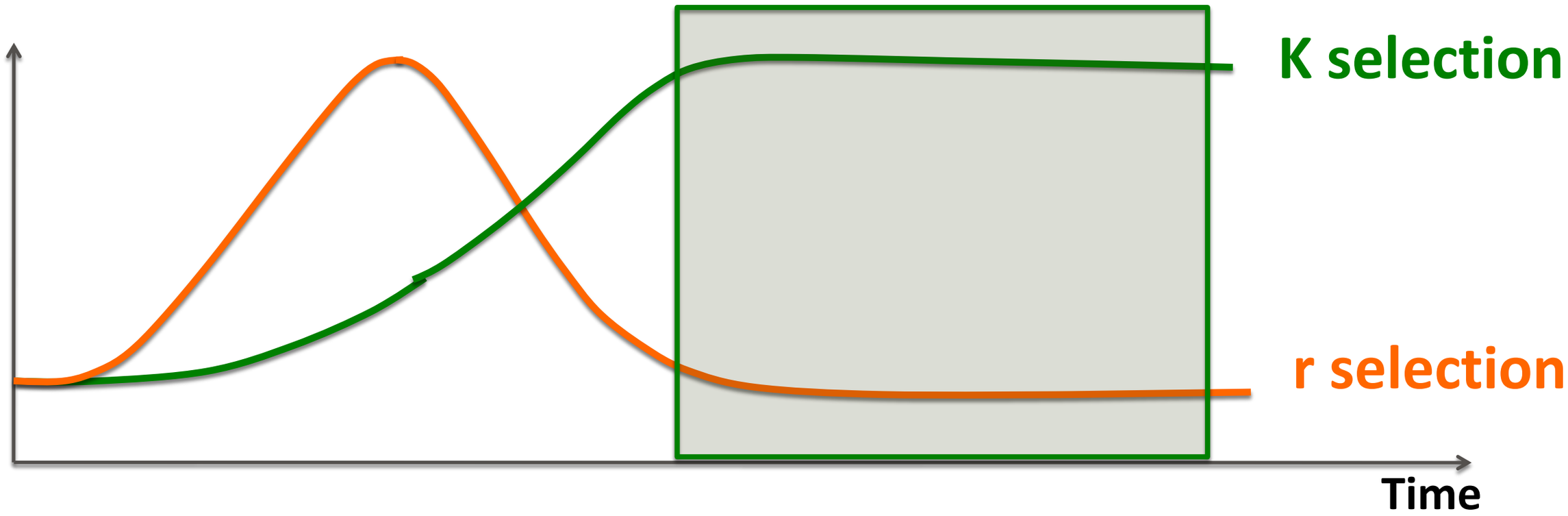
→ typically during massive microbial growth

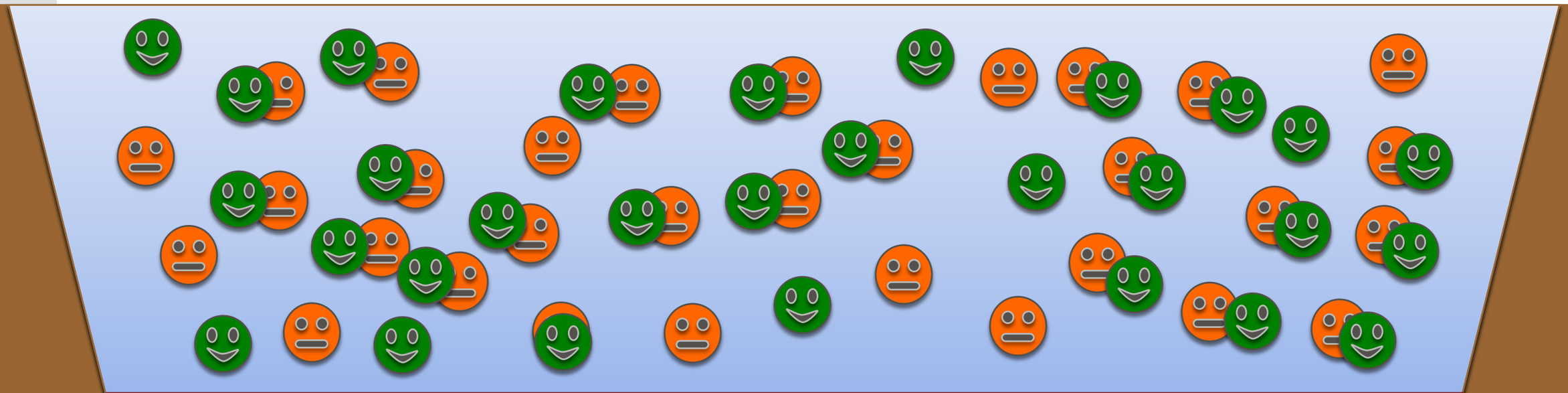




What happens at the microbial level in grow out ponds upon refilling and restocking?

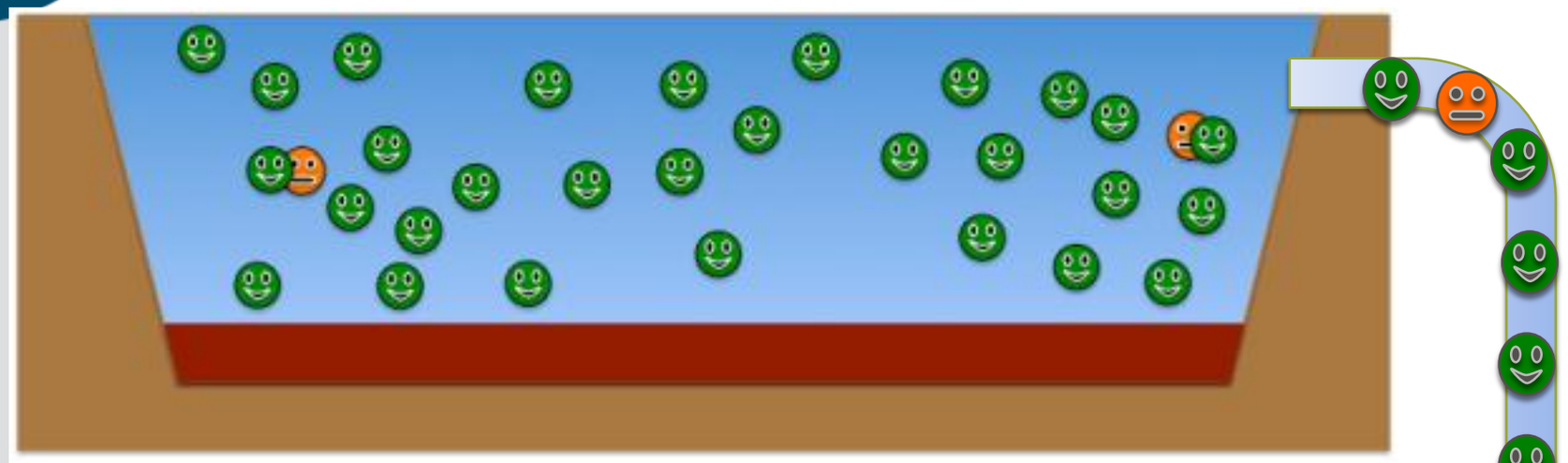
→ introduction of animals after peak of r-strategists





What happens at the microbial level in grow out ponds upon refilling and restocking?

→ use “mature” (microbially stabilized) water



pond system for preparation of microbially stabilized pond water (ensure long retention time)

Effluent from shrimp pond

Seaweed pond

Tilapia pond

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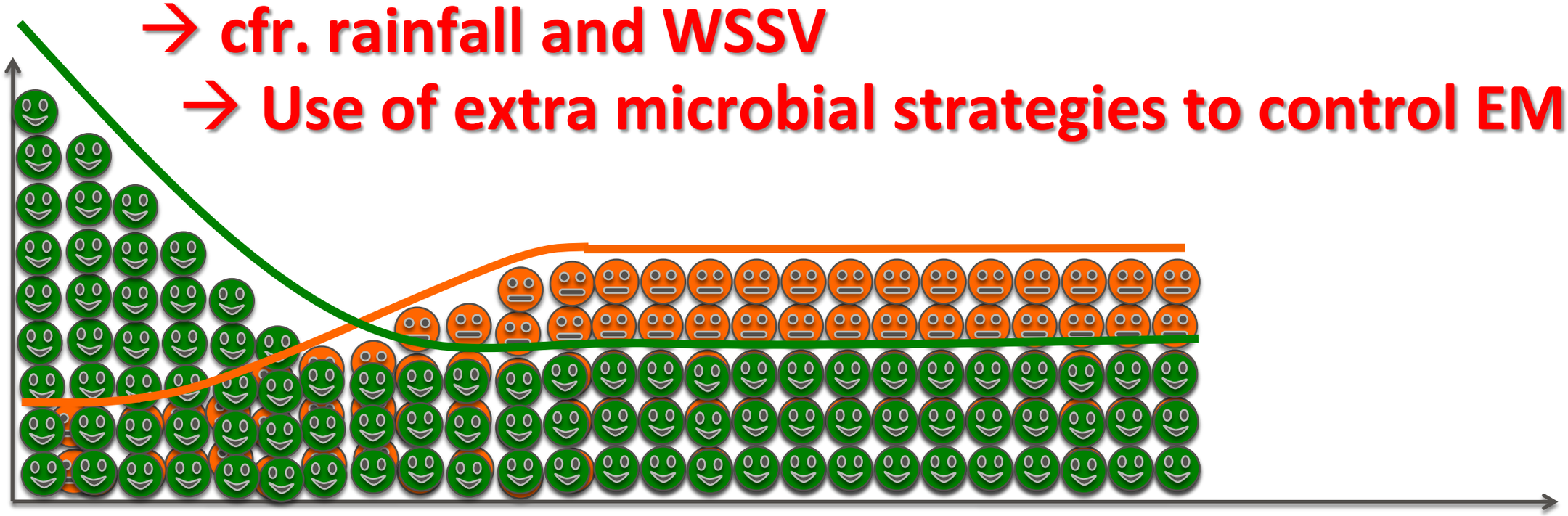
Result of sudden rainfall on microbial stability

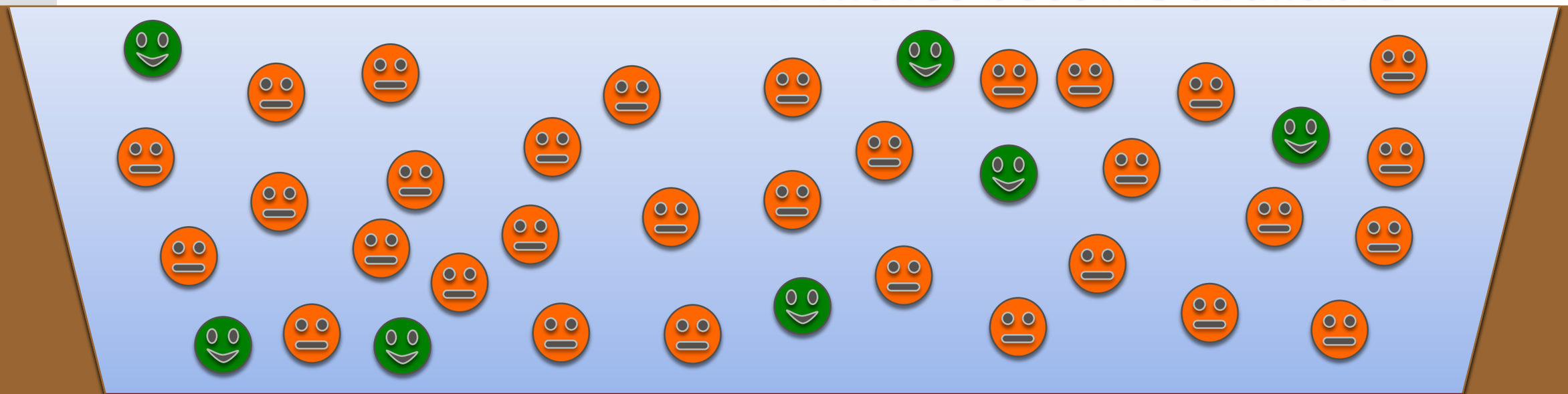
water exchange results in wash-out of bacteria
→ K-strategist bacteria cannot dominate anymore
→ cfr. rainfall and WSSV
→ Use of extra microbial strategies to control EMS



substrate/bacterium becomes HIGH

Time

→ niches become available



SUMMARY

HOLISTIC MICROBIAL MANAGEMENT IS KEY IN THE PREVENTION OF DISEASES IN SHRIMP CULTURE:

MINIMIZE RISK FOR WSSV:

- BIOSECURITY (SPECIFIC PATHOGEN)!
- INCREASED KNOWLEDGE WILL LEAD TO NEW PREVENTIVE APPROACHES

MINIMIZE RISK FOR VIBRIOSIS (EMS):

- PREVENTIVE APPROACH USING MICROBIAL MATURATION CONCEPT
- ENSURE MICROBIAL STABILITY
- ADDITIONAL BENEFIT FROM EFFECTIVE PROBIOTICS

THANKS TO A LOT TO THE COLLEAGUES AT INVE AND

THANK YOU FOR YOUR ATTENTION!!

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