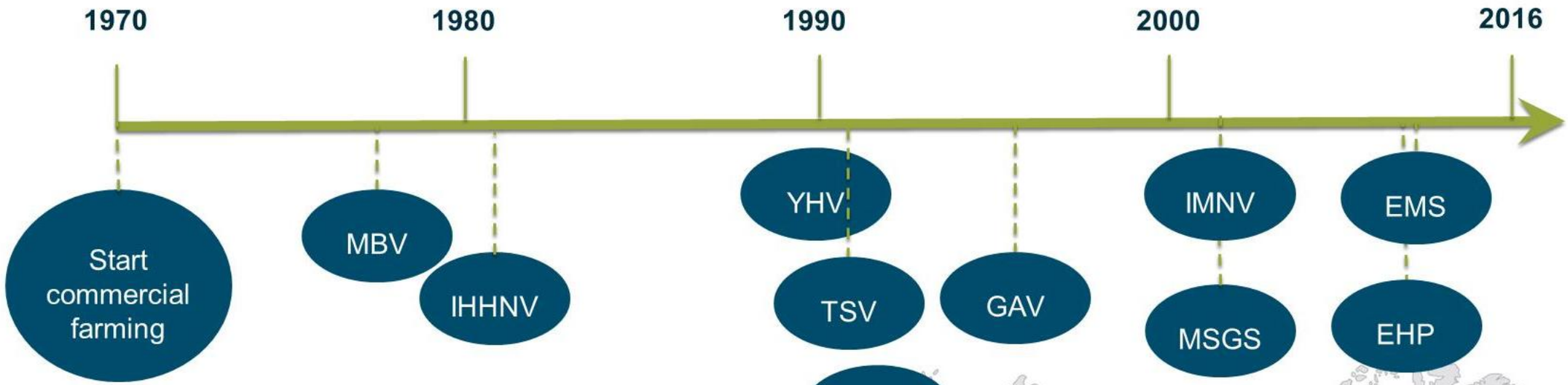
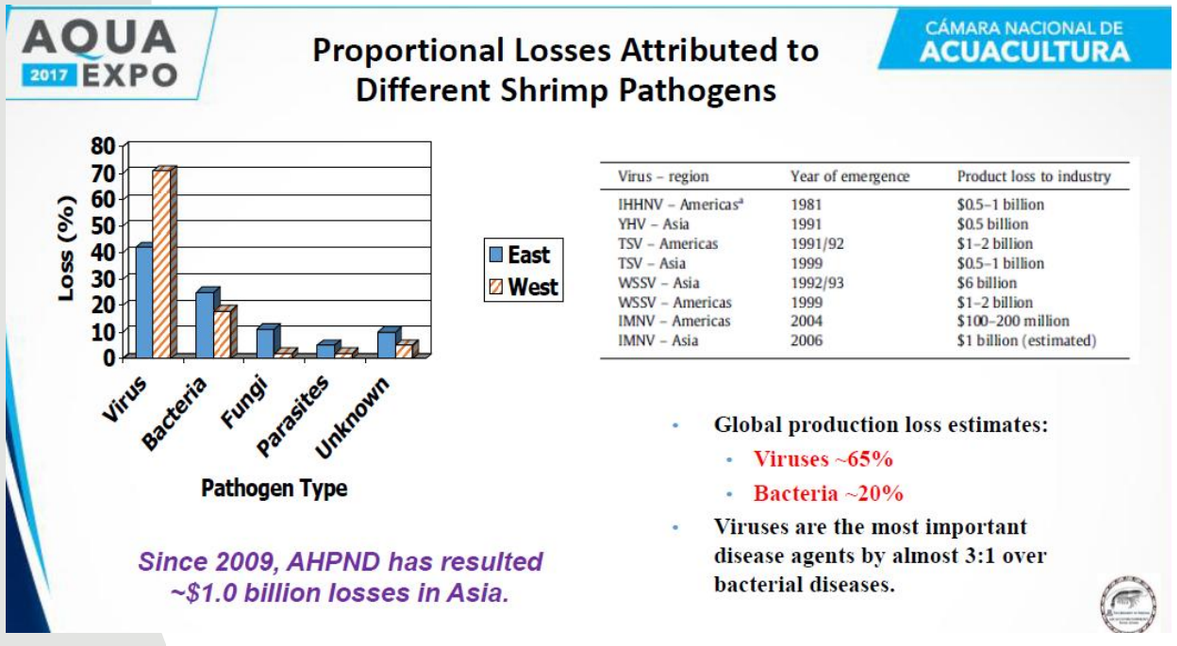
DISEASE CONTROL THROUGH MANAGEMENT OF THE WHOLE PRODUCTION CYCLE

Olivier Decamp, PhD

Farm & Feedmill Product Manager

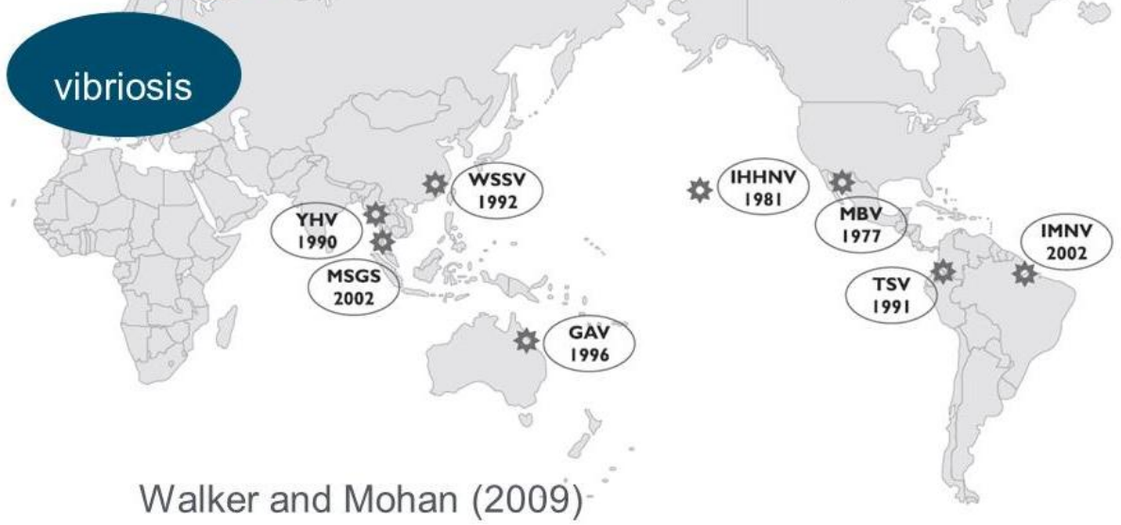
DISEASE CONTROL IS A FOCUS POINT





Virus – region	Year of emergence	Product loss to industry
IHHNV – Americas[a]	1981	$0.5–1 billion
YHV – Asia	1991	$0.5 billion
TSV – Americas	1991/92	$1–2 billion
TSV – Asia	1999	$0.5–1 billion
WSSV – Asia	1992/93	$6 billion
WSSV – Americas	1999	$1–2 billion
IMNV – Americas	2004	$100–200 million
IMNV – Asia	2006	$1 billion (estimated)

- **Global production loss estimates:**
  - **Viruses ~65%**
  - **Bacteria ~20%**
- **Viruses are the most important disease agents by almost 3:1 over bacterial diseases.**



Walker and Mohan (2009)

Dhar, 2017

HOW TO HANDLE DISEASES?

- Know your enemy: example of EMS/AHPND
- Understand your enemy

NACA

DISEASE ADVISORY

Asia Regional Aquatic Animal Health Programme

Early Mortality Syndrome (EMS)/Acute Hepatopancreatic Necrosis Syndrome (AHPNS): An emerging threat in the Asian shrimp industry

Eduardo M. Leaño and C.V. Mohan
NACA, Bangkok, Thailand

Vol. 105: 45–55, 2013
doi: 10.3354/dao02621

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published July 9

Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp

Loc Tran[1,2], Linda Nunan[1], Rita M. Redman[1], Leone L. Mohney[1], Carlos R. Pantoja[1], Kevin Fitzsimmons[2], Donald V. Lightner[1,*]

[1]Aquaculture Pathology Laboratory, School of Animal and Comparative Biomedical Sciences, Department of Veterinary Science and Microbiology, and [2]Department of Soil, Water and Environmental Science, University of Arizona, Tucson, Arizona 85721, USA

frontiers in Microbiology

ORIGINAL RESEARCH
published: 05 October 2017
doi: 10.3389/fmicb.2017.01959

$pirAB^{vp}$-Bearing *Vibrio parahaemolyticus* and *Vibrio campbellii* Pathogens Isolated from the Same AHPND-Affected Pond Possess Highly Similar Pathogenic Plasmids

OPEN ACCESS

Edited by:
Dongsheng Zhou,
Beijing Institute of Microbiology and Epidemiology, China

Xuan Dong[1†], Dexi Bi[2†], Hailiang Wang[1], Peizhuo Zou[1,2], Guosi Xie[1], Xiaoyuan Wan[1], Qian Yang[1], Yanping Zhu[1], Mengmeng Chen[1,2], Chengchang Guo[1], Zhen Liu[4], Wenchao Wang[1] and Jie Huang[1*]

AMERICAN SOCIETY FOR MICROBIOLOGY | genomeAnnouncements™

Complete Genome Sequence of Acute Hepatopancreatic Necrosis Disease-Causing *Vibrio campbellii* LA16-V1, Isolated from *Penaeus vannamei* Cultured in a Latin American Country

Ye Som Ahn,[a,b] Patharapol Piamsomboon,[c] Kathy F. J. Tang,[d] Jee Eun Han,[e] Ji Hyung Kim[a]

Infectious Disease Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Republic of Korea; Department of Biochemistry, Chungnam National University, Daejeon, Republic of Korea; Faculty of Veterinary Science, Prince of Songkla University, Songkhla, Thailand[c]; ShrimpVet Laboratory, Ho Chi Minh City, Vietnam[d]; CJ CheilJedang Feed and Livestock Research Institute, Suwon, Republic of Korea[e]

www.nature.com/scientificreports

SCIENTIFIC REPORTS

OPEN

: 5 May 2017
: 1 August 2017
Online: 24 August 2017

Microbiome Dynamics in a Shrimp Grow-out Pond with Possible Outbreak of Acute Hepatopancreatic Necrosis Disease

Wei-Yu Chen[1], Tze Hann Ng[2], Jen-Horng Wu[3], Jiun-Wen Chen[1] & Han-Ching Wang[2,4]

HOW TO HANDLE DISEASES?

- Know your enemy: example of EMS/AHPND
- Understand your enemy

Species	Signals	Quorum sensing-regulated virulence factors
Aeromonas hydrophila	BHL, HHL	biofilm formation, exoprotease production
Edwardsiella tarda	probably HHL and HeHL	"virulent strain-specific protein"
Vibrio alginolyticus	AI-2	extracellular protease production, haemolytic activity and siderophore production, lethality to *Pagrus major*
Vibrio anguillarum	ODHL, AI-2 and at least 2 other AHLs	unknown
Vibrio campbellii	HAI-1, AI-2 and CAI-1	lethality to brine shrimp
Vibrio harveyi	HAI-1, AI-2 and CAI-1	siderophore production, production of type III secretion system components, extracellular toxin production, metalloprotease production, lethality to brine shrimp and rotifers
Vibrio mimicus	AI-2	protease
Vibrio parahaemolyticus	HAI-1, AI-2 and CAI-1	production of type III secretion system components, lethality to brine shrimp
Vibrio scophthalmi	AI-2, OHdDHL	unknown
Vibrio vulnificus	AI-2, BHL	protease and haemolysin production,
Yersinia ruckeri	OOHL, OHL	unknown

HOW TO HANDLE DISEASES?

- Know your enemy: example of EMS/AHPND
- Understand your enemy

PCR results from AP2 detection with enrichment specimens

Sources	Province (Positive/Total tested)			Totals (%)
	Songkhla	Trad	Rayong	
Broodstock feces	2/5	8/15	14/24	24/44 (55)
Nauplii	1/1	0/5	3/8	4/14 (29)
Polychaetes	1/2	2/3	2/3	5/7 (71)
Squid	1/1	0/3	3/5	4/9 (44)
Artemia	1/1	-	0/1	1/2 (50)
Oysters	0/1	-	0/2	0/3 (0)
Clams	1/1	1/1	-	2/2 (100)
Acetes	-	-	0/2	0/2 (0)
Blood worms	-	-	0/2	0/2 (0)

- : no specimens

Serious biosecurity risk!

Tim Flegel (2014)

Laboratory analysis of samples collected in 2014

Types of sample	AHPND Plasmids		AHPND Toxin gene		WSSV	
	Total sample	% positive	Total sample	% positive	Total sample	% positive
Broodstock	73	0	73	0	112	0
Broodstock feces	27	19	27	0	ND	ND
Nauplii	53	0	53	2	ND	ND
Post larvae	2174	11	1363	5	1779	5
Juvenile	1490	28	1261	18	1246	4
Farm water	3116	29	2166	22	22	5
Hatchery water	542	19	23	0	0	0
Farm sediment	1614	36	1054	17	172	19
Others	33	21	15	0	4	25

ND= not detect.

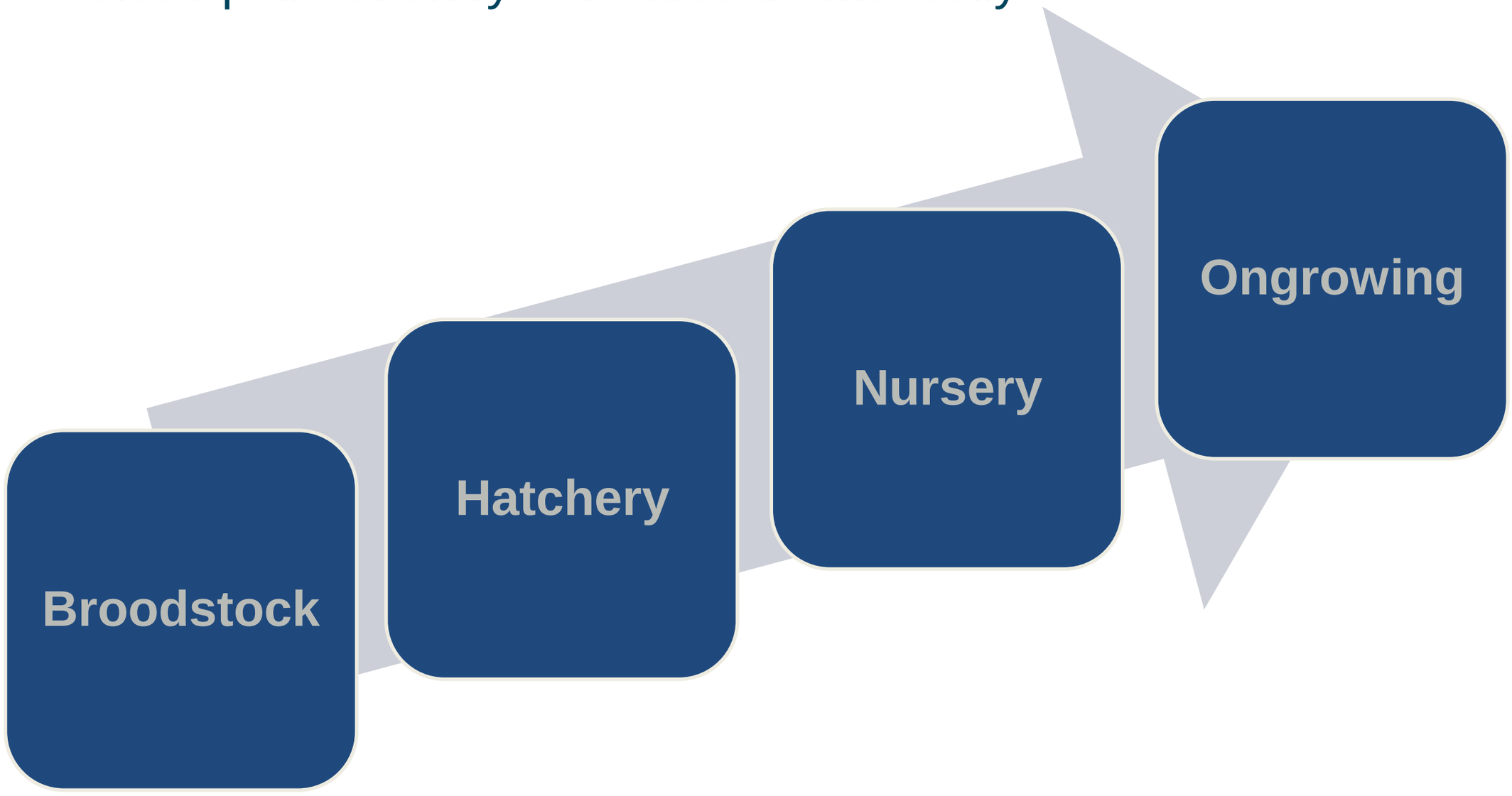
Putth Songsangjinda (2016)

HOW TO HANDLE DISEASES?

- Know your enemy
- Understand your enemy
- Genes associated with toxin production jumping from 1 species to another
- Mechanisms of communication between Vibrio and other bacteria may interfere with virulence mechanisms
- Distribution/location in hatchery/farm

MANAGEMENT DURING CYCLE

- Identify points of entry (SWOT)
- Close point of entry or reduce dramatically



HOW TO HANDLE DISEASES?

- Establish prophylactic protocols to prevent/reduce disease
- Establish protocols to cure or reduce disease
- Establish protocol to control spread of disease
- Understand lack of efficacy and risks associated with past protocols

Aquaculture Reports 2 (2015) 17–21

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journal homepage: www.elsevier.com/locate/aqrep

Short communication

Plasmid mediated tetracycline resistance of *Vibrio parahaemolyticus* associated with acute hepatopancreatic necrosis disease (AHPND) in shrimps

Jee Eun Han*, Leone L. Mohney, Kathy F.J. Tang, Carlos R. Pantoja, Donald V. Lightner

School of Animal and Comparative Biomedical Sciences, University of Arizona, Tucson, AZ, USA

MANAGEMENT DURING CYCLE

Broodstock:

- SPF animals (for existing pathogens including WSSV, AHPND and EHP)
- Disinfection of water and facilities, including low pH treatment (vs EHP) and efficient biocides (vs biofilm of Vibrio)
- Use of confirmed pathogen-free feed/food. Focus on certified/registered diet
- Water treatment to control accumulation of organic waste (vs Vibrio, including AHPND, and EHP)

FAO Second International Technical Seminar/Workshop on Acute hepatopancreatic necrosis disease (AHPND) There is a way forward!

FAO Technical Cooperation Programme: TCP/INT/3501 and TCP/INT/3502

Risk factors

The most important risk factors for the international spread of AHPND are:

- mouvement of live shrimp from a geographic region where AHPND is prevalent to an unaffected region for aquaculture (AHPND is thought to have been transmitted to Mexico from Asia by this route).
- the importation of live animals (e.g. polychaetes, clams) as feeds for shrimp broodstock (polychaetes imported from P.R. China may have been the major route for introduction of AHPND to Thailand).

MANAGEMENT DURING CYCLE

Hatchery – stocking of nauplii:

- To reduce Vibrio outbreak, disinfect water and recolonize prior to stocking
- To reduce EHP (possibly transferred from spawning tank), transfer with minimum amount of water from the spawning tank, as nauplii mouths are closed

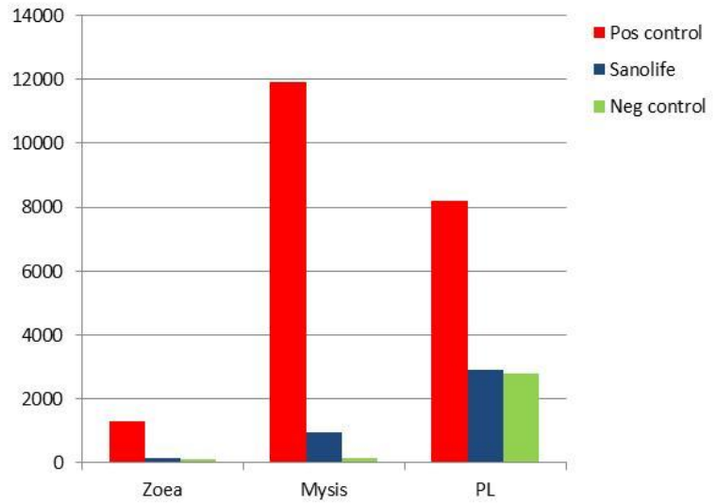
MANAGEMENT DURING CYCLE

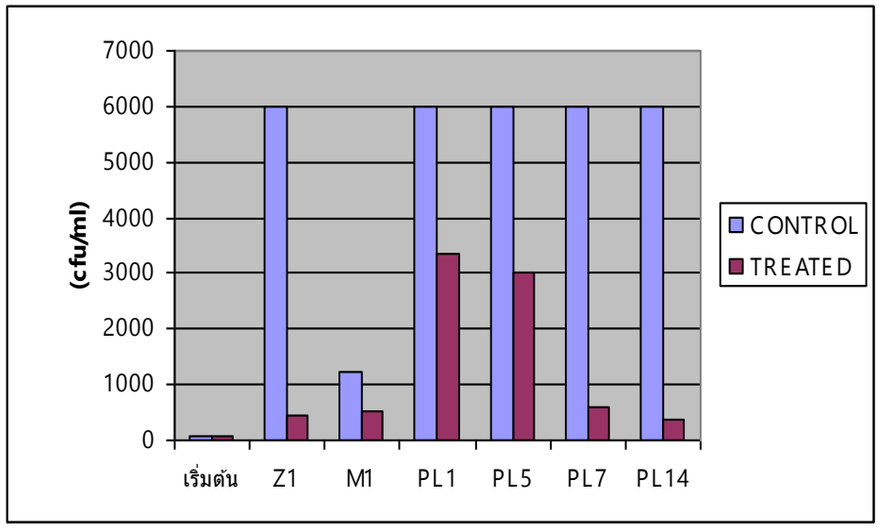
Hatchery – during larval rearing:

- Use quality feed to improve growth and reduce the accumulation of organic waste
- Control the entry of bacterial pathogens via algae and artemia
- Use microbial products to degrade organic waste
- Use microbial product to compete with bacterial pathogens

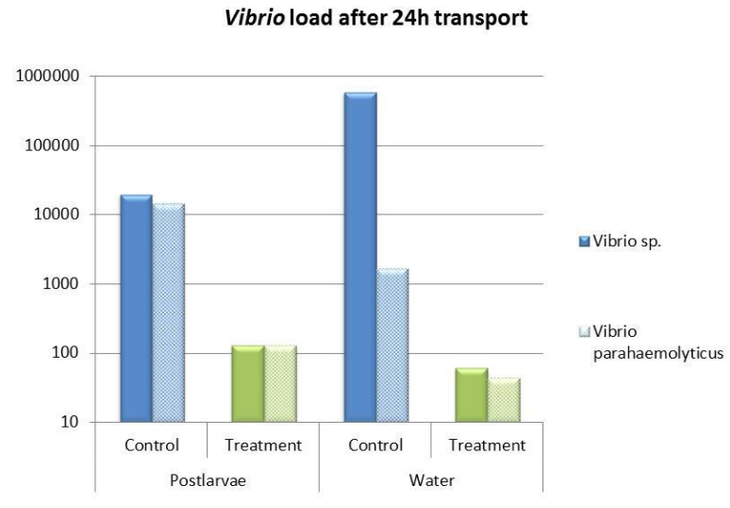
MANAGEMENT DURING CYCLE

Control of vibrio during culture, using probiotic treatment





Control of Vibrio at harvest, using disinfectant or plant-based treatment

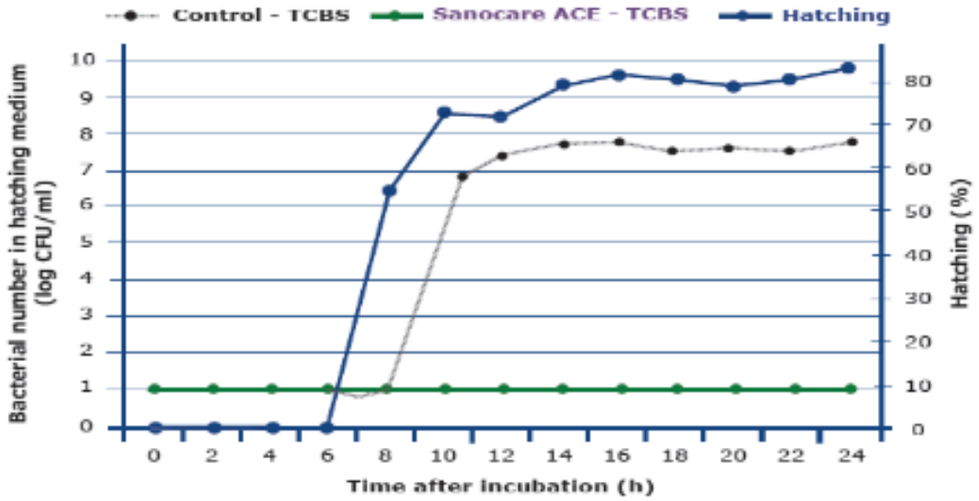


Application	Treatment
Nauplii disinfection	3 dips (max 45 seconds) in 40ppm Sanocare PUR bath, before stocking in larval tank
PL4 transfer	Dip PL4 for max 1 minute in 10-15ppm Sanocare PUR bath
PL10 harvest	Dip PL4 for max 1 minute in 10-15ppm Sanocare PUR bath

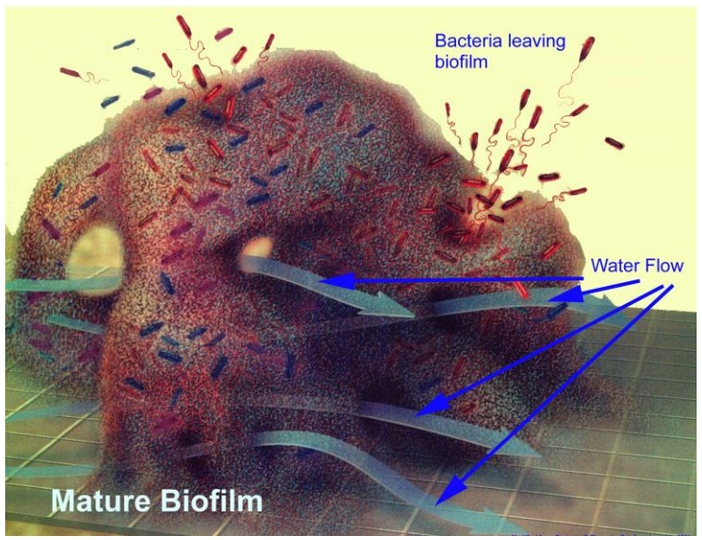
BIOSECURITY AT HATCHERY

Control of Vibrio in harvested algae (30 min biocide treatment)

Control of Vibrio in Artemia (treatment during hatching)



Control of Vibrio in biofilm



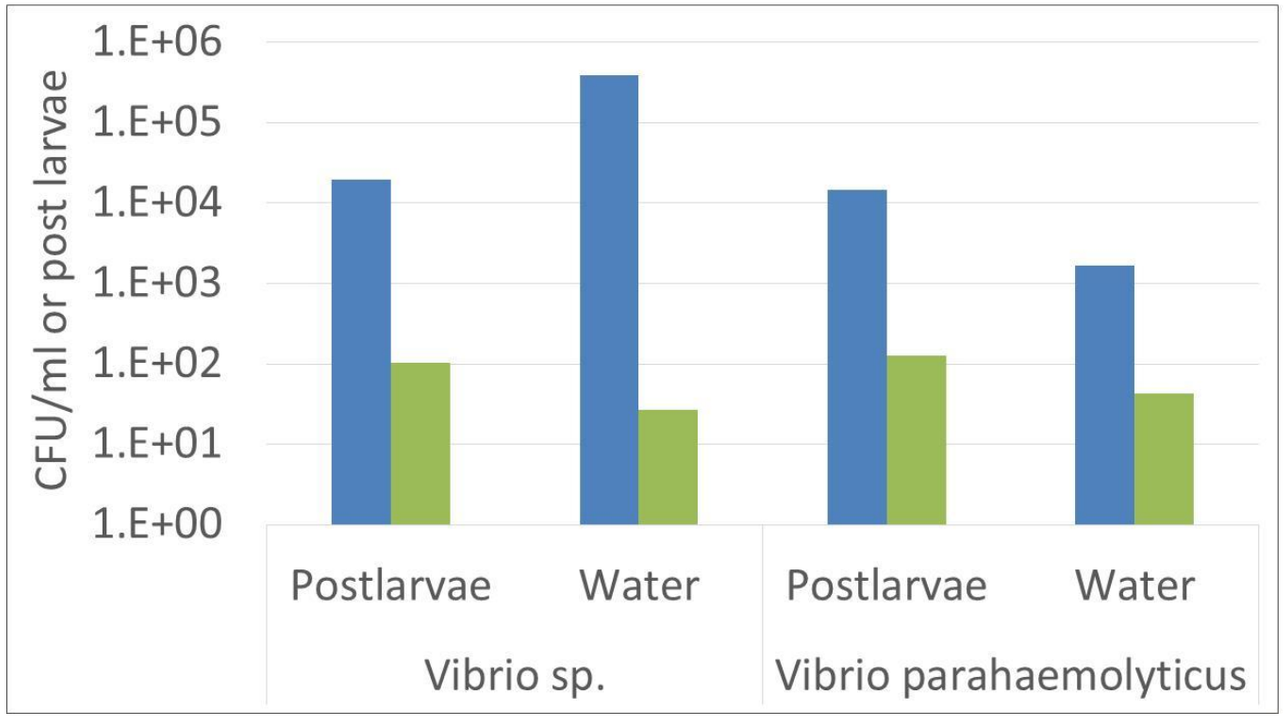
MANAGEMENT DURING CYCLE

Hatchery – preparation for transfer to nursery:

- Use health booster to strengthen PLs before transfer
- “Colonize” the gut of PLs with good bacteria prior to transfer
- Reduce the Vibrio load before transfer

Sanocare FIT applied in the culture water during 24h PL10 transport.

➢ Drastic reduction of *Vibrio* in transport water and shrimp.



MANAGEMENT DURING CYCLE

Hatchery – preparation for transfer to nursery:

- Use health booster to strengthen PLs before transfer
- “Colonize” the gut of PLs with good bacteria prior to transfer
- Reduce the Vibrio load before transfer

More robust shrimp by stimulating protein chaperones

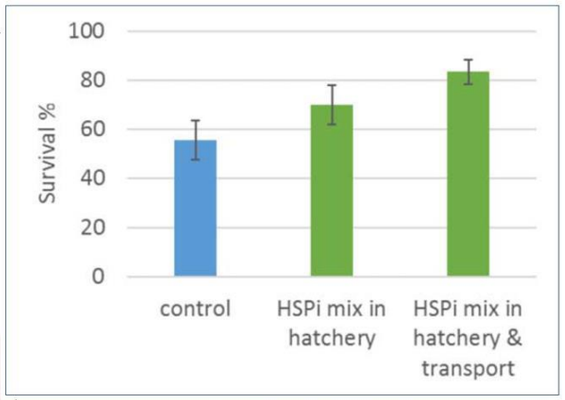
By Eva Bequé[1], Geert Rombaut,[1]Patrick Lavens[1],Eddy Naessens[1] Mathieu Wille[2], Peter Bossier[2]

1. INVE Technologies, Belgium. 2. Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Belgium

Loss of production due to bacterial and viral infections is a recurring problem in shrimp aquaculture. The fact that shrimp mortality is often perceived as uncontrollable, is mainly due to a lack of a holistic approach and tools in developing control strategies which assists in preventing diseases. Only focusing on the pathogens by biosecurity management does not suffice, monitoring and controlling stress is critical as well. As stressed animals are more susceptible to diseases, both the environment and the host’s defense have to be measurably improved.

Attaining robust animals throughout the entire production cycle is an essential part in the holistic approach to combat diseases for all aquaculture organisms.

reduce mortality during stress incidents in nursery facilities or in ponds.

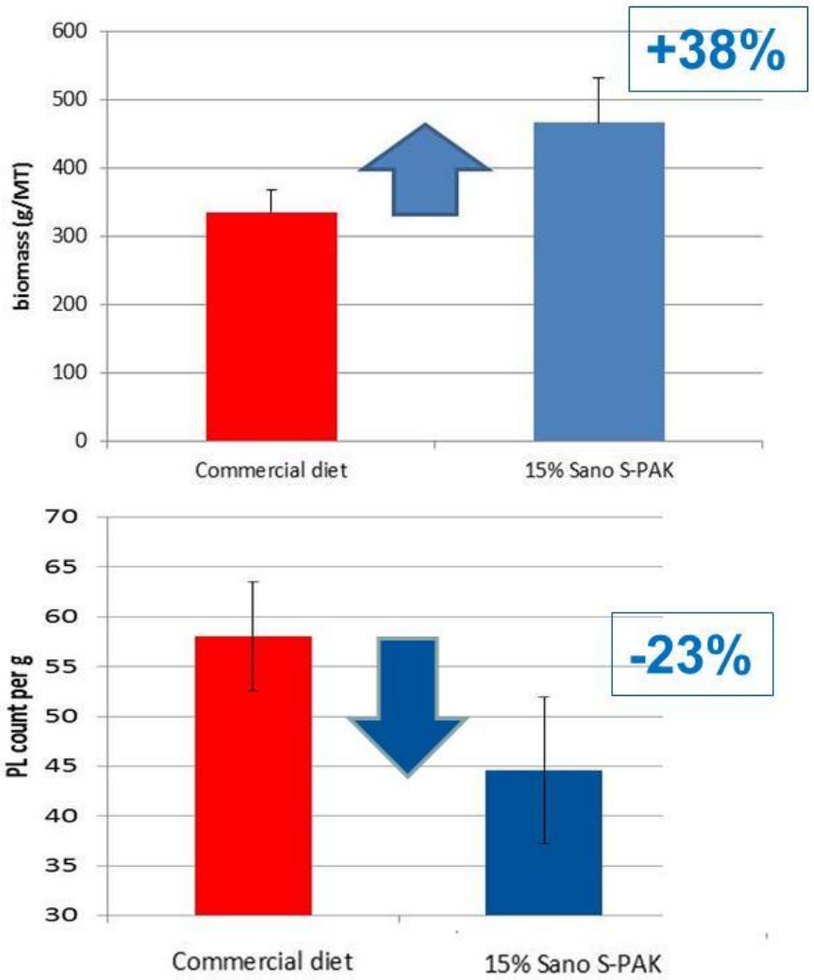


PL quality ➔ effect on performance in grow-out

MANAGEMENT DURING CYCLE

Nursery

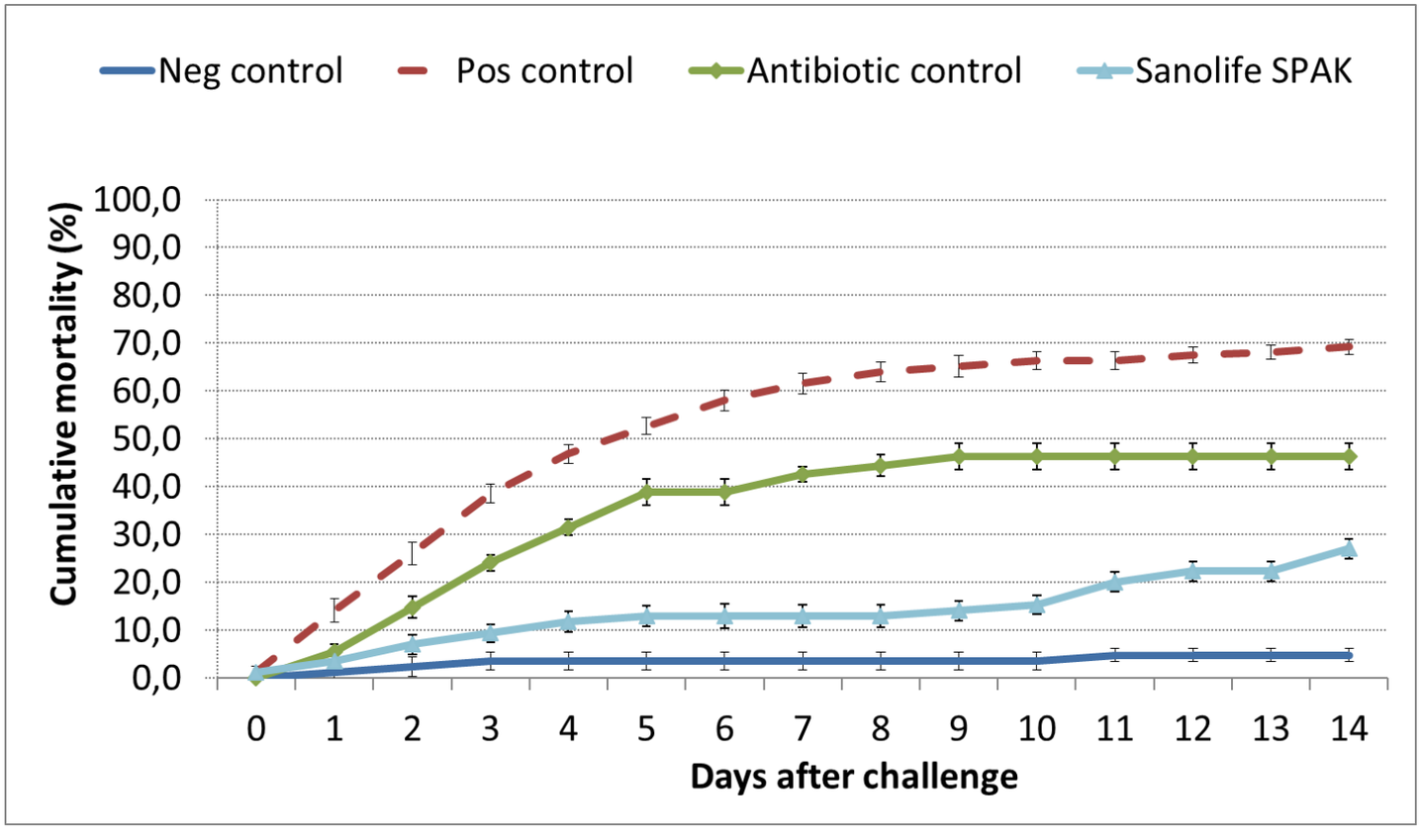
- Use quality diet to improve growth and reduce waste
- Apply microbial product to degrade wastes and control Vibrio
- Use health booster to strengthen PLs before transfer



MANAGEMENT DURING CYCLE

Nursery

- Use quality diet to improve growth and reduce waste
- Apply microbial product to degrade wastes and control Vibrio
- Use health booster to strengthen PLs before transfer and bacterial challenge



Disease challenge test in collaboration with Can Tho University (Vietnam)

MANAGEMENT DURING CYCLE

Nursery

- Use quality diet to improve growth and reduce waste
- Apply microbial product to degrade wastes and control Vibrio
- Use health booster to strengthen PLs before transfer and bacterial challenge

The second phase of test rearing took place in the raceways of the Fitmar hatchery.

Bacillus Probiotics Improve Hatchery, Nursery Production In EMS-Hit Mexico

Summary:
In early 2014, a trial to evaluate the effects of a mixture of *Bacillus* strains on early mortality syndrome bacteria during the larviculture and nursery phases for shrimp was carried out at a commercial hatchery in Mexico. The application of the *Bacillus* mixture during the initial growth of nauplii through $P.L._{1}$, led to a clear improvement in survival. At the end of a second phase reflecting growth to $P.L._{19}$, continued probiotic treatment increased overall postlarvae production as well as animal size.

J. Jaime Munoz M.
INVE Aquaculture México, S.A. de C.V.
Avenida Camarón Sábalo # 51
Local 6, Interior, Plaza Riviera Zona
Dorada Mazatlán
Sinaloa 82110, México
j.munoz@inveaquaculture.com

F. Marino Pinzon M.
Rodolfo Rivera F.
Proveedora de Larvas, S.A. de C.V.
Mazatlán, Sinaloa, México

Olivier Decamp, Ph.D.
INVE Aquaculture
Tambon Nong Lum,
Amphoe Wachirabarumi, Phicit, Thailand

The ongoing outbreak of early mortality syndrome/acute hepatopancreatic necrosis (EMS/AHPN) has had dramatic impacts on shrimp producers in affected countries in Asia and Mexico. Following reports of the disease being associated with broodstock management – for example, through the use of contaminated polychaetes –

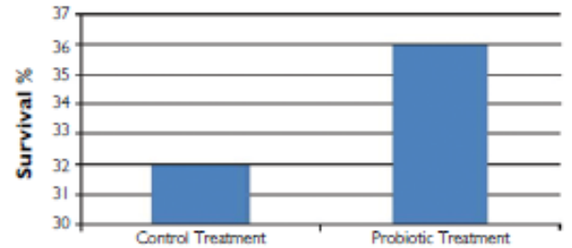


Figure 1. Survival of shrimp postlarvae at the end of the first phase of rearing.

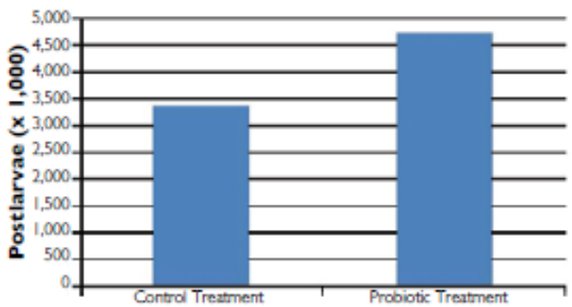


Figure 2. Number of postlarvae harvested at the end of the second phase of rearing.

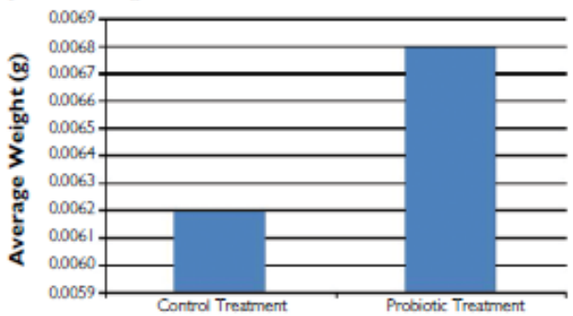


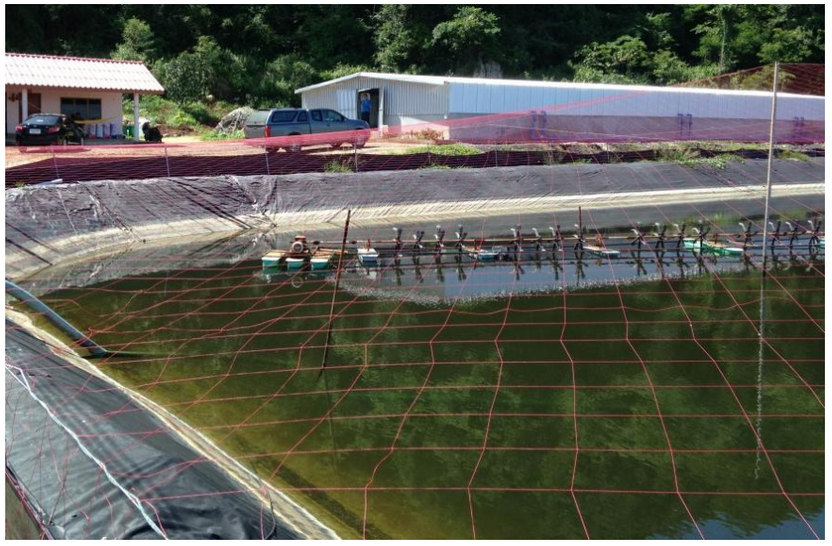
Figure 3. Average wet weight of postlarvae harvested from the control and treatment raceways.

Munoz et al. 2014. Global Aquaculture Advocate. Sept-Oct 2014 issue. 50-52

MANAGEMENT DURING CYCLE

Ongrowing

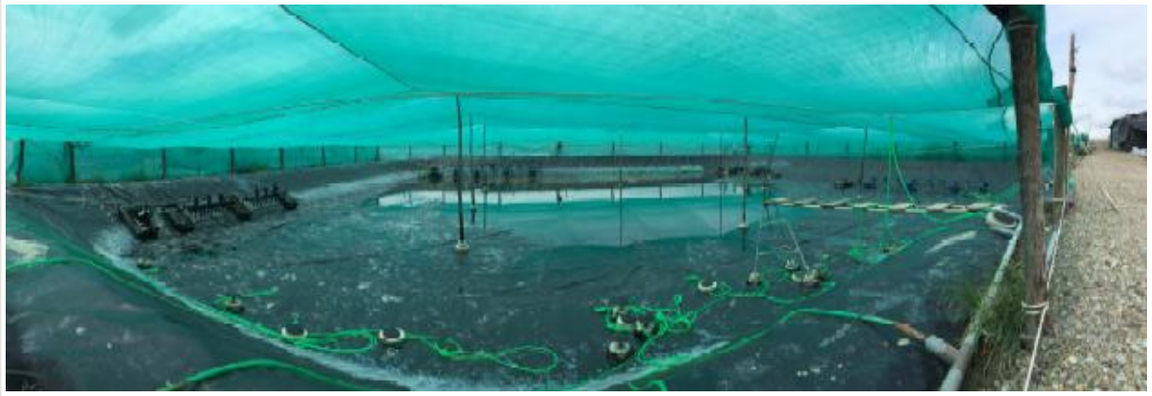
- Adapt farm design/management to conditions



MANAGEMENT DURING CYCLE

Ongrowing

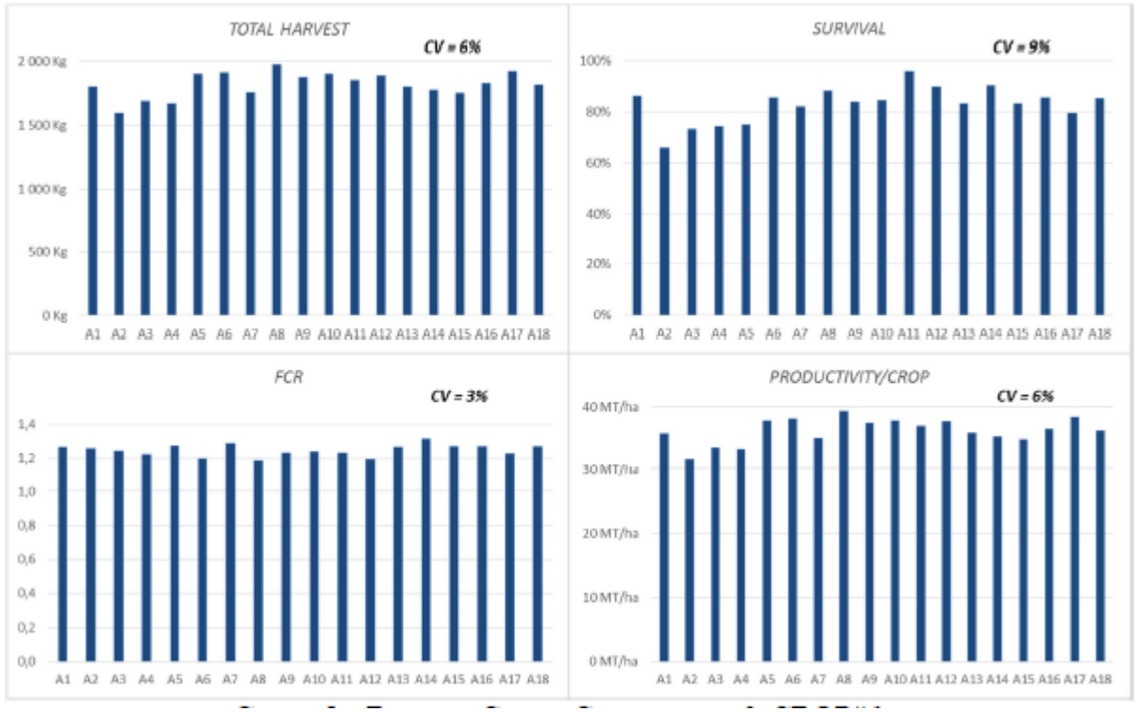
- Adapt nursery and ongrowing protocols



MANAGEMENT DURING CYCLE

Ongrowing

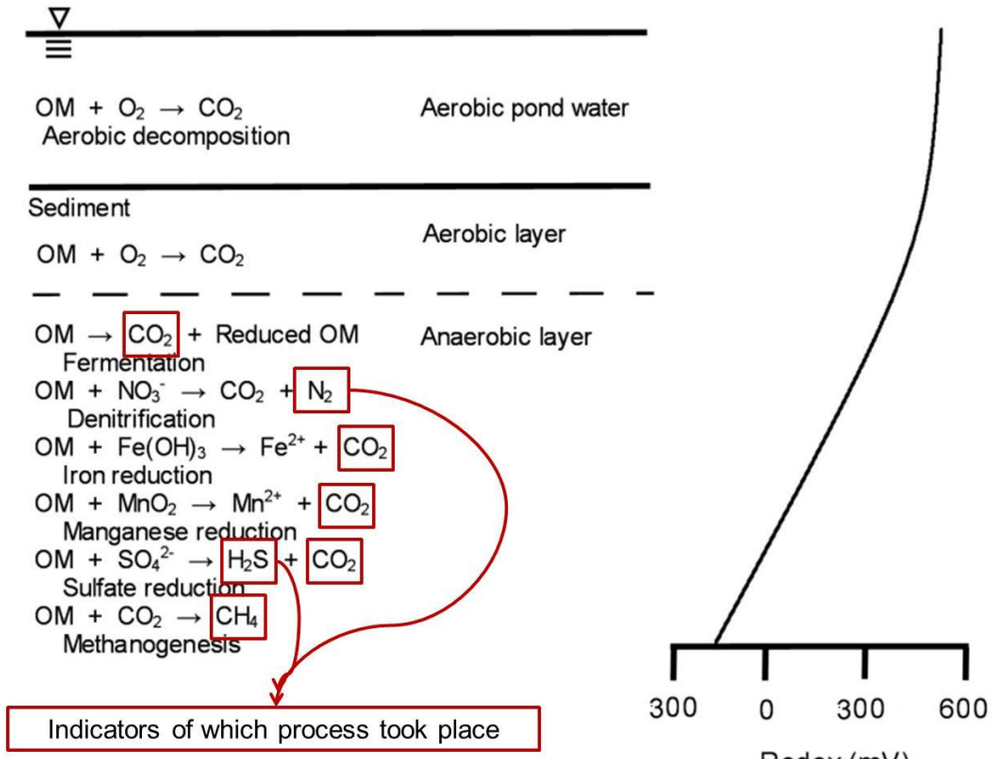
- Establish most cost efficient and predictable protocols



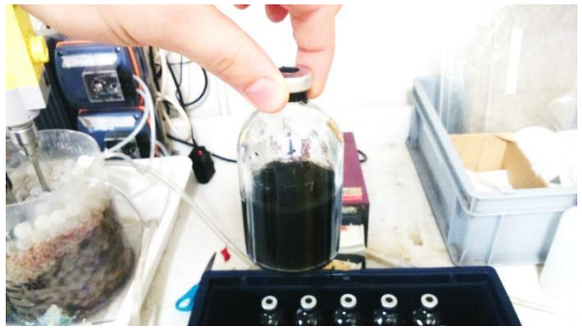
(Poulain, 2017)

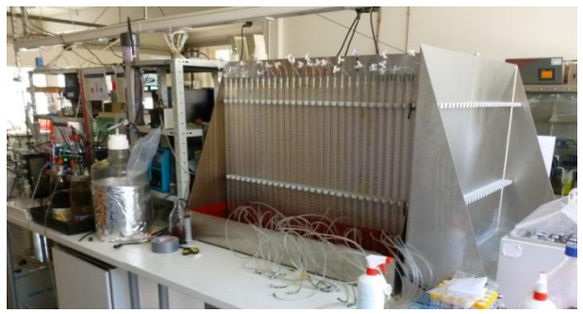
MANAGEMENT DURING CYCLE

Ongrowing
- Control organic waste, using microbial products alone or in combination with oxidizing agents



Biochemical Methane Production model at LabMET

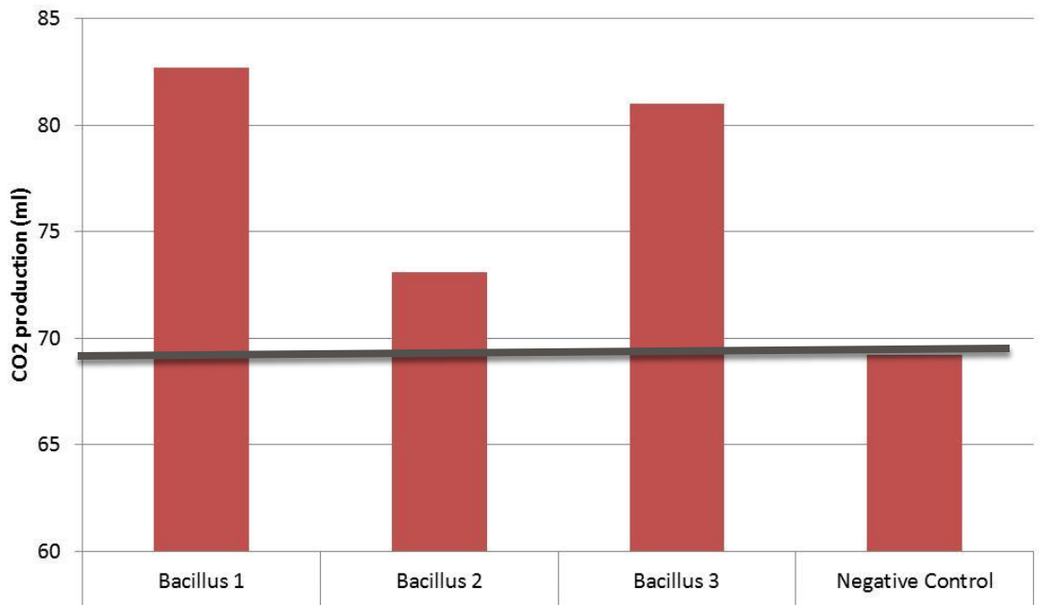


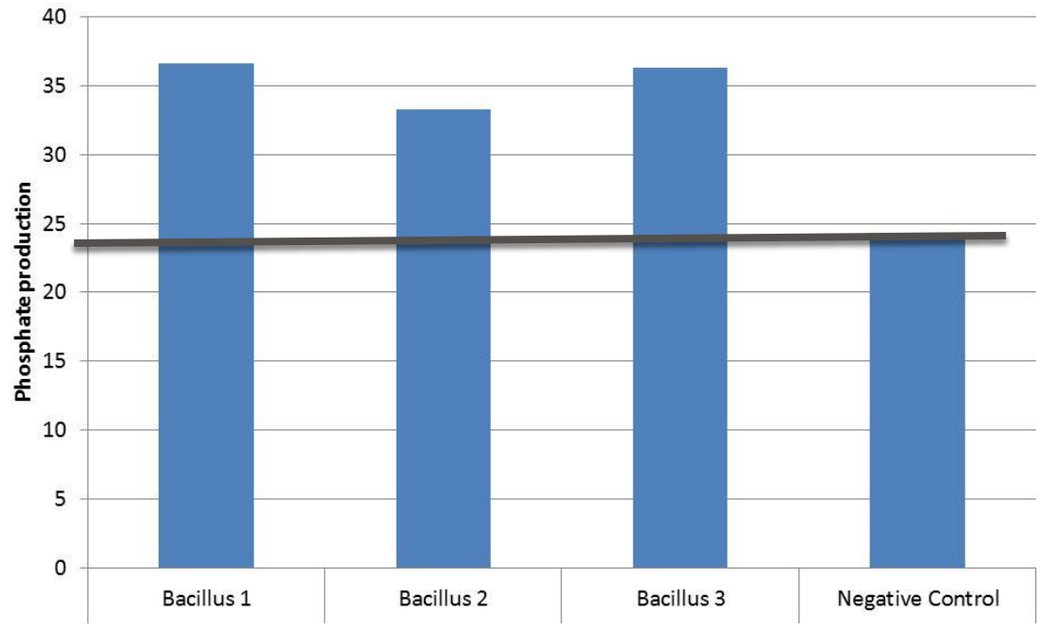


(INVE Technologies and University of Ghent)

MANAGEMENT DURING CYCLE

Ongrowing
- Control organic waste, using microbial products alone or in combination with oxidizing agents





(INVE Technologies and University of Ghent)

MANAGEMENT DURING CYCLE

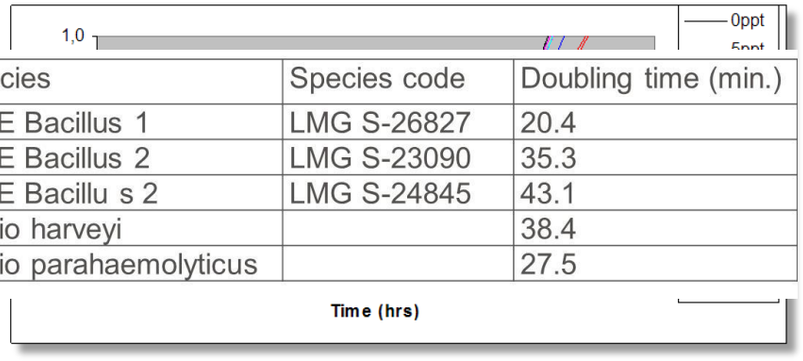
Ongrowing
- Control organic waste, using microbial products alone or in combination with oxidizing agents

MANAGEMENT DURING CYCLE

Ongrowing

- Control Vibrio load in the sediment/water, using probiotics that are and remain active in the farm conditions

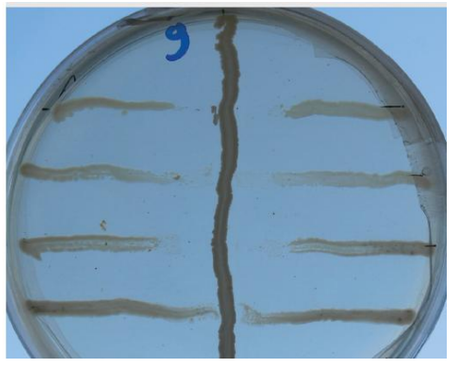
Active/alive in pond



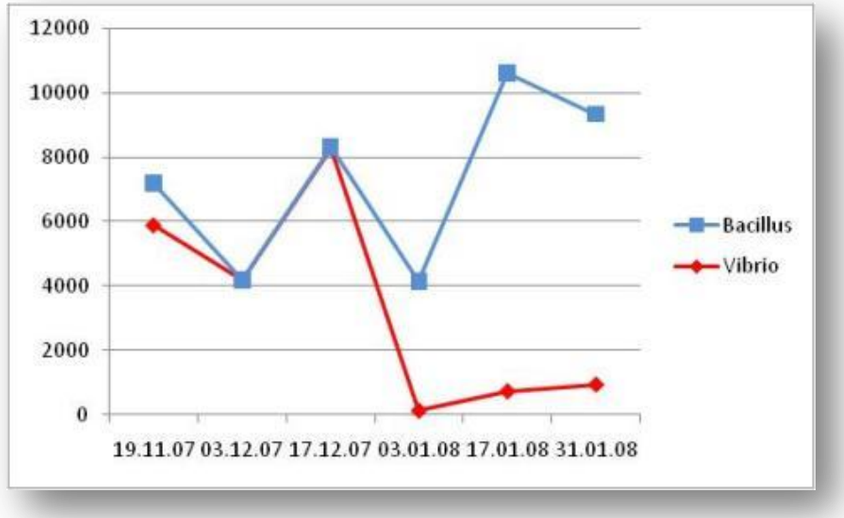
Species	Species code	Doubling time (min.)
INVE Bacillus 1	LMG S-26827	20.4
INVE Bacillus 2	LMG S-23090	35.3
INVE Bacillu s 2	LMG S-24845	43.1
Vibrio harveyi		38.4
Vibrio parahaemolyticus		27.5

degrade wastes

kills Vibrio



➔ Less Vibrio in the sediment



Santos et al. 2009.

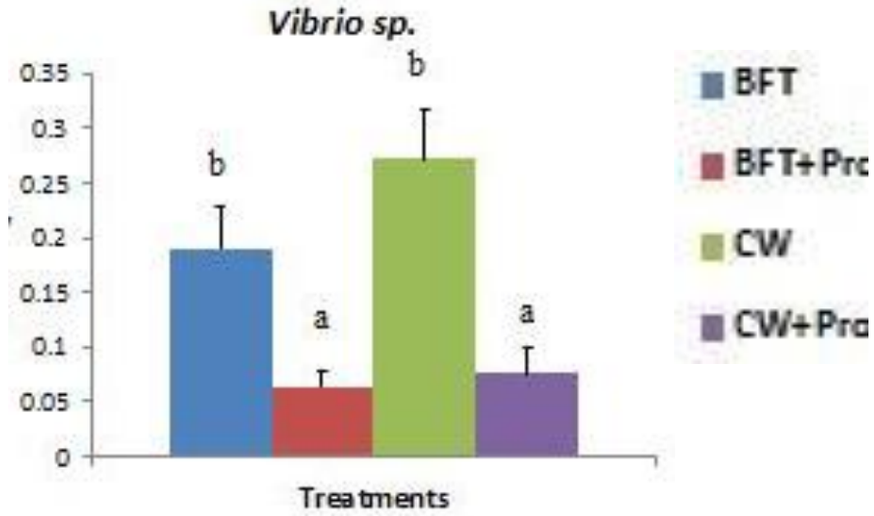
MANAGEMENT DURING CYCLE

Ongrowing

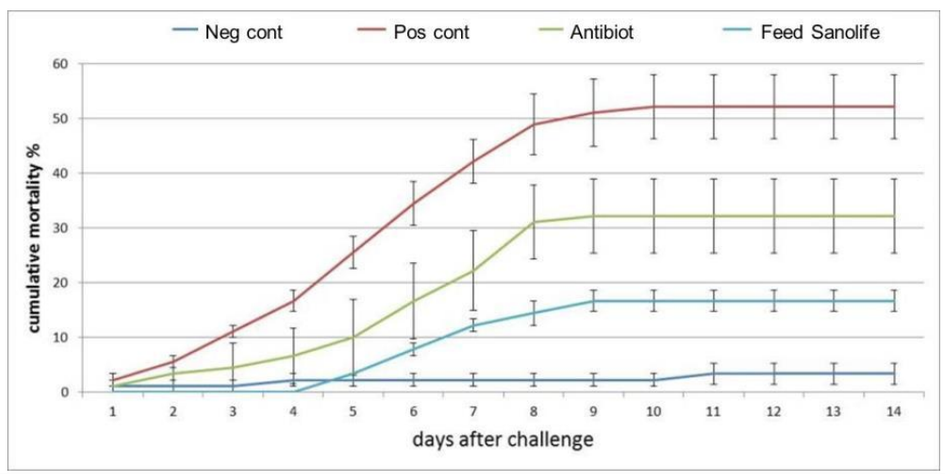
- Control Vibrio load in the shrimp gut

Bacillus in feed

displace Vibrio from shrimp gut



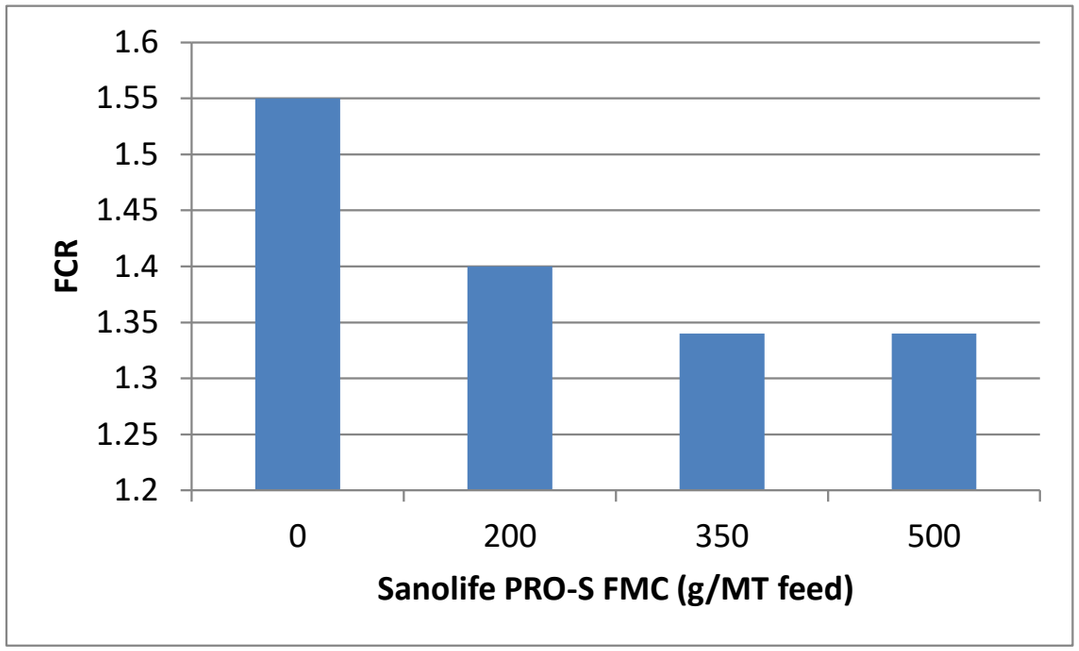
➔Improved survival with AHPND

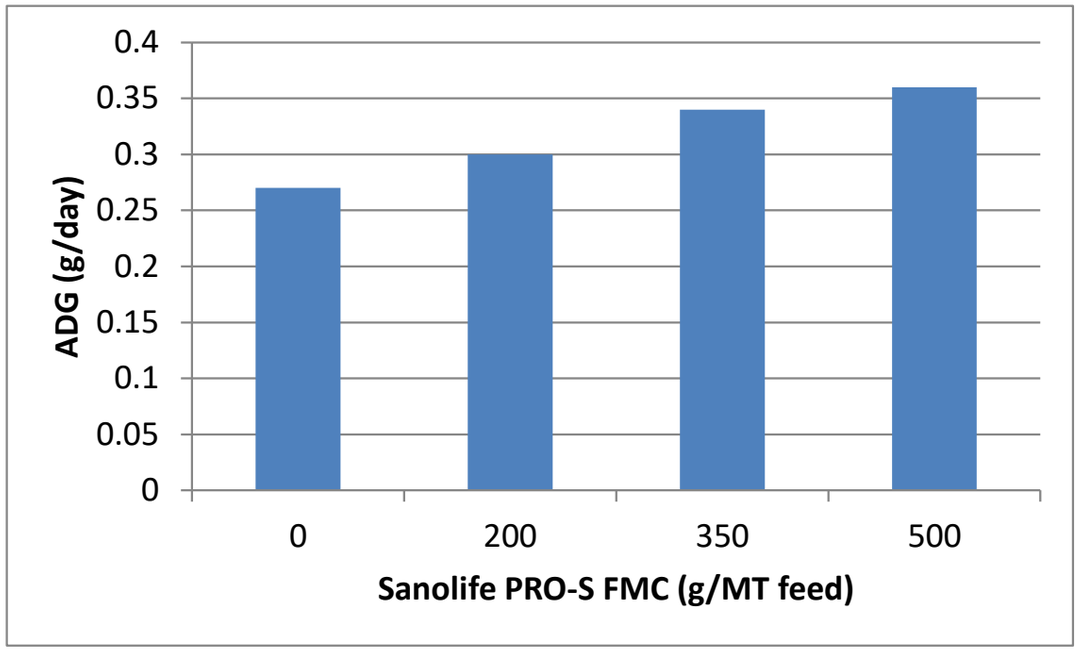


MANAGEMENT DURING CYCLE

Ongrowing
- Reduced Vibrio and improved utilization of nutrients

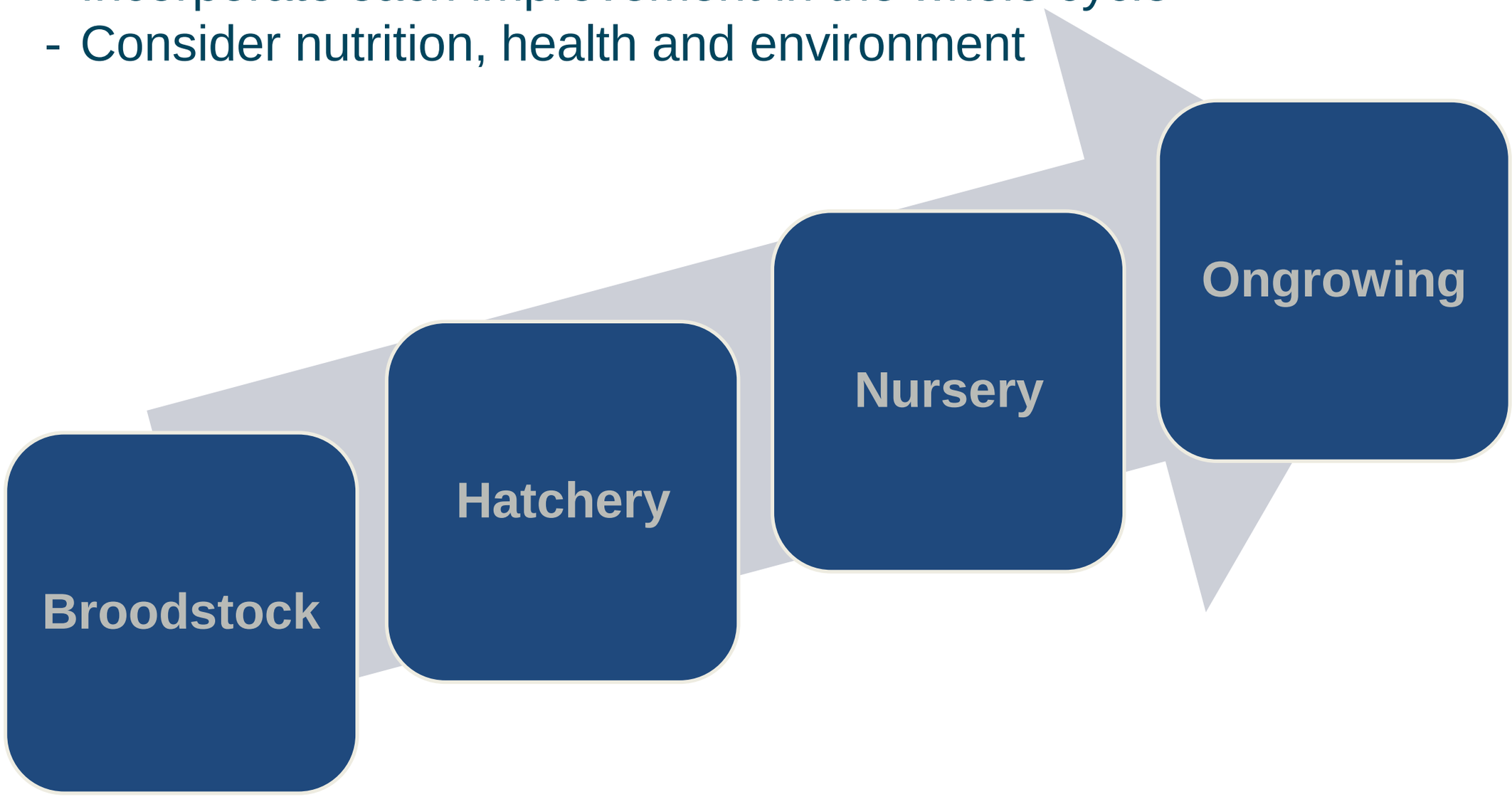
➔ Better growth and FCR





MANAGEMENT DURING CYCLE

- Prevent pathogen transfer from one phase to the other
- Incorporate each improvement in the whole cycle
- Consider nutrition, health and environment



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