Technical sessions :

Emergency response to emerging disease: AHPND in shrimp

Kallaya Sritunyalucksana-Dangtip

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National Center for Genetic Engineering and Biotechnology (BIOTEC),

National Science and Technology Development Agency (NSTDA),

Thailand

Diseases of Crustaceans — **Acute Hepatopancreatic Necrosis Syndrome (AHPNS)**

Juvenile *Penaeus vannamei* from Vietnam: left with AHPNS; right appears normal.
Source: D Lightner

- ✓ The precise case definition is a major breakthrough for further study.
- ✓ It is important because research progress depends on analysis of identical cases.

Disease signs at pond level

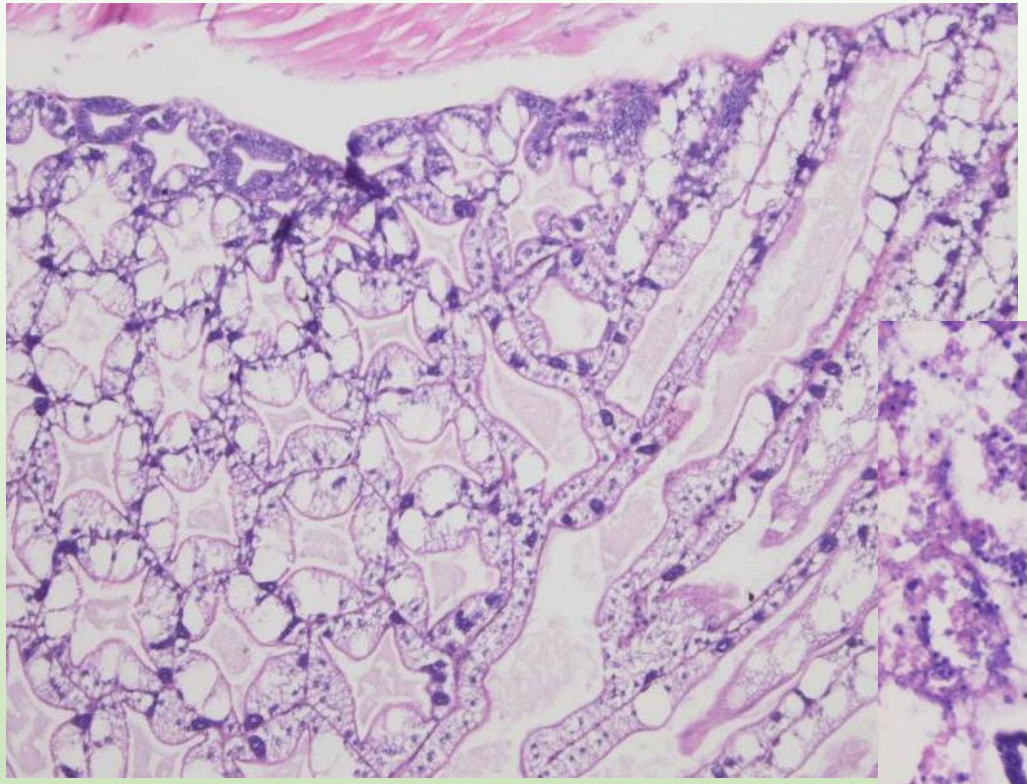
- Often pale to white hepatopancreas (HP) due to pigment loss in the connective tissue capsule.
- Significant atrophy (shrinkage) of HP.
- Often soft shells and guts with discontinuous contents or no content.
- Black spots or streaks sometimes visible within the HP.
- HP does not squash easily between thumb & finger.
- Onset of clinical signs and mortality starting as early as 10 days post stocking.
- Moribund shrimp sink to bottom.

Pale atrophied hepatopancreas of juvenile *Penaeus vannamei*, indicative of AHPNS.
Source: D Lightner

Pale atrophied hepatopancreas of juvenile *Penaeus monodon* from Vietnam with AHPNS.
Source: D Lightner

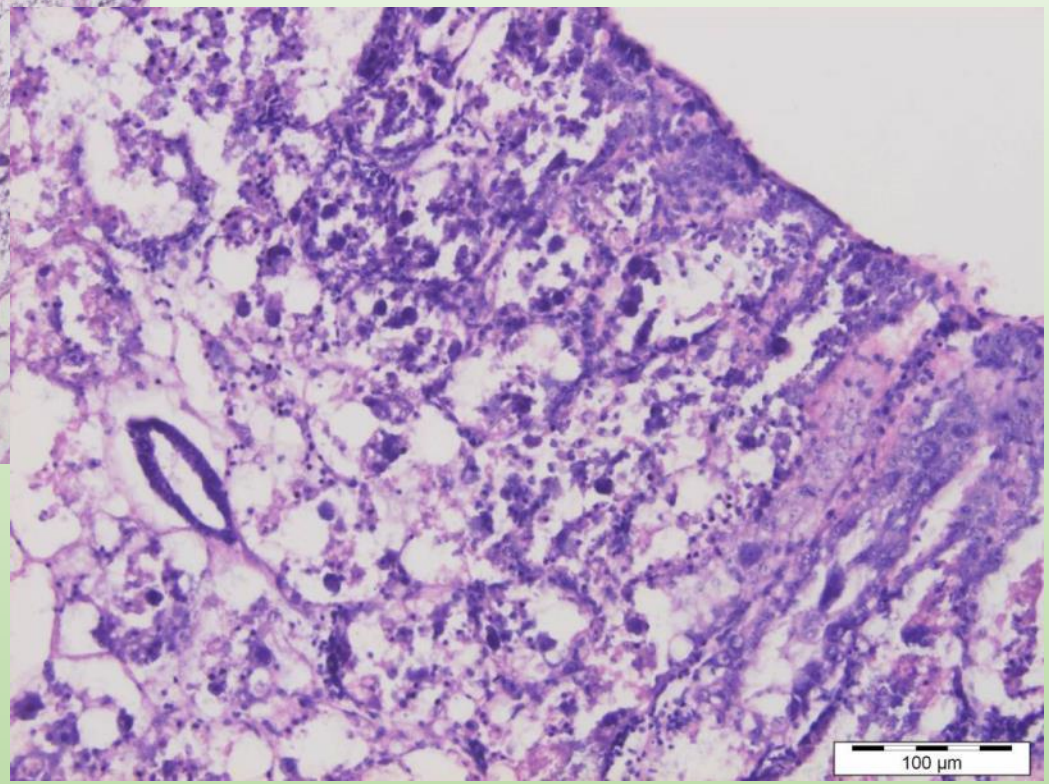
Medial sloughing of HP cells

The key diagnostic feature needed for confirmation



Normal hepatopancreas

AHPND hepatopancreas



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

2014

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

A specific strain of *Vibrio parahaemolyticus* (VP_{AHPND})

is a cau

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Variation in *Vibrio parahaemolyticus* isolates from a single Thai shrimp farm experiencing an outbreak of acute hepatopancreatic necrosis disease (AHPND)

Jyoti Joshi [a,b], Jiraporn Srisala [a,c], Viet Hong Truong [a,b], I-Tung Chen [d], Bunlung Nuangsaeng [e], Orasa Suthienkul [f], Chu Fang Lo [d], Timothy W. Flegel [a], Kallaya Sritunyalucksana [a,b,c], Siripong Thitamadee [a,b,*]

Aquaculture 428–429 (2014) 297–302

Contents lists available at ScienceDirect

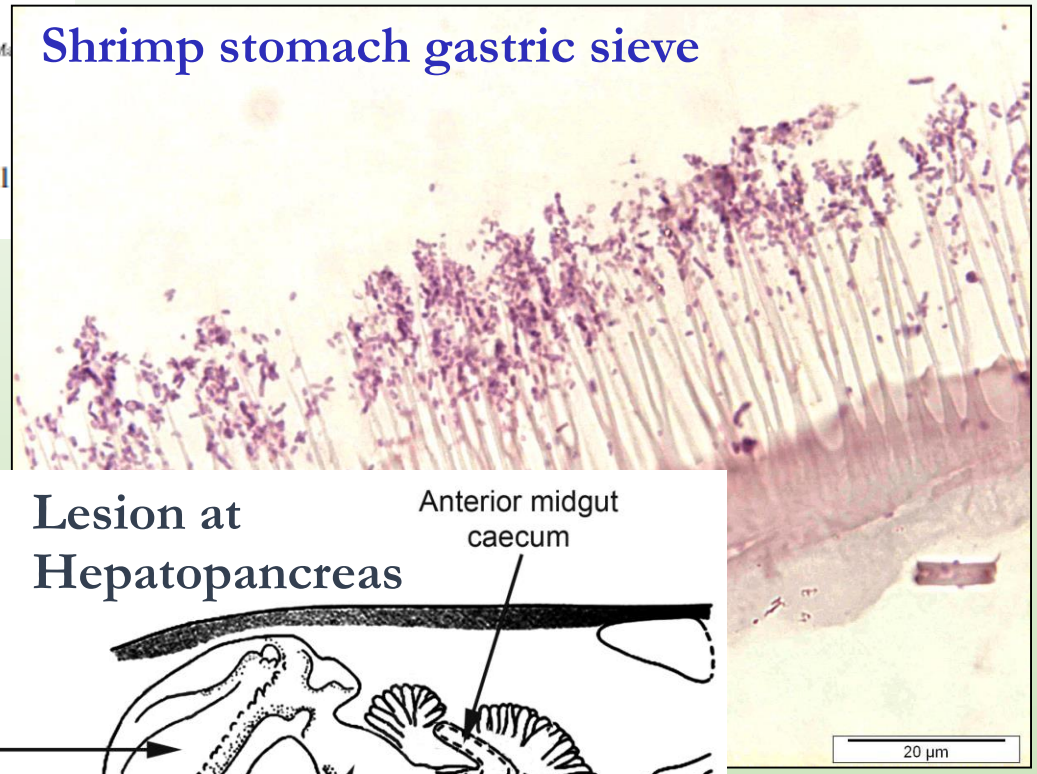
Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Bacteria colonize the stomach

Variation in *Vibrio parahaemolyticus* isolates from a single Thai shrimp farm experiencing an outbreak of acute hepatopancreatic necrosis disease (AHPND)

Jyoti Joshi [a,b], Jiraporn Srisala [a,c], Viet Hong Truong [a,b], I-Tung Chen [d], Bunlung Nuangsaeng [e], Orasa Suthienkul
Chu Fang Lo [d], Timothy W. Flegel [a], Kallaya Sritunyalucksana [a,b,c], Siripong Thitamadee [a,b,*]

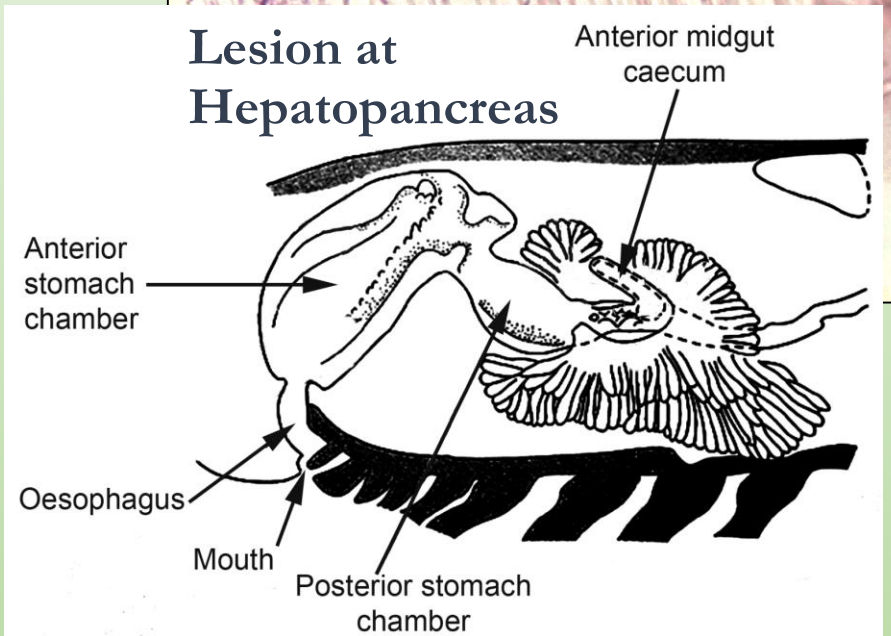


PLOS | ONE

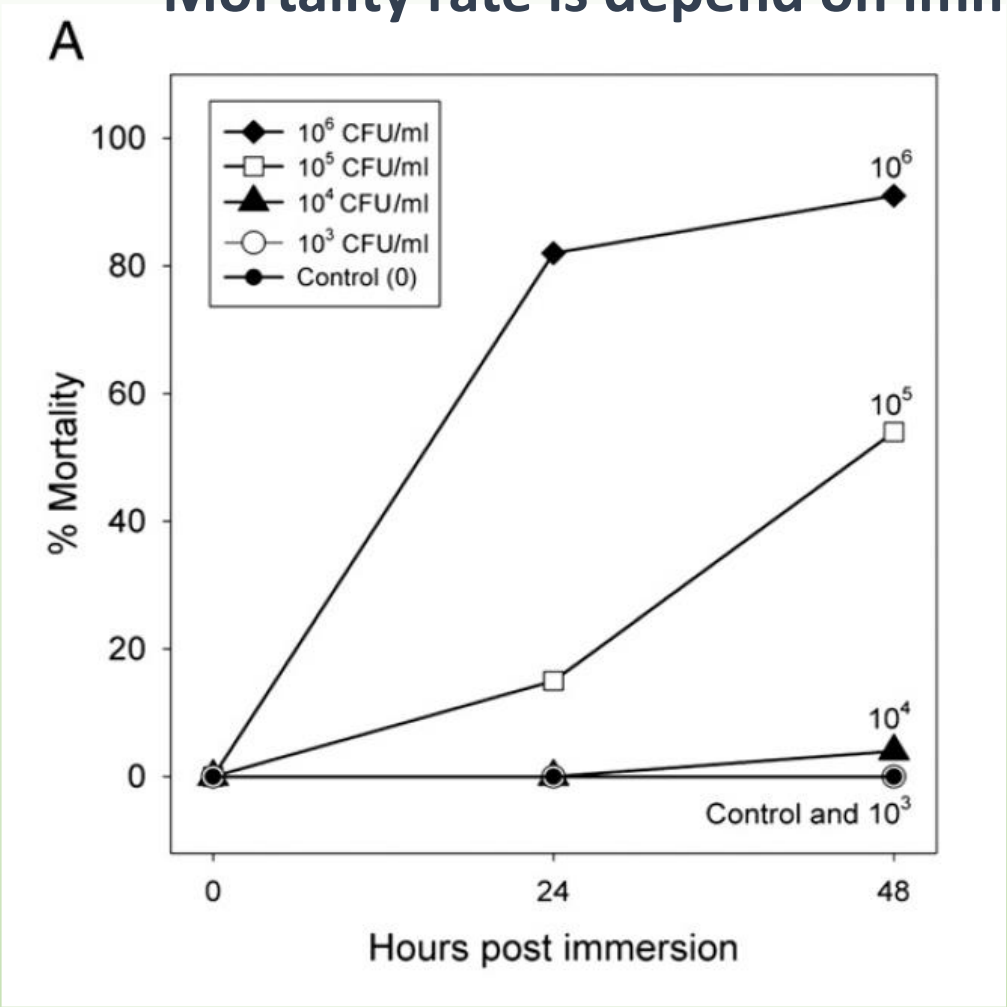
RESEARCH ARTICLE

Characterization and PCR Detection Of Binary, Pir-Like Toxins from *Vibrio parahaemolyticus* Isolates that Cause Acute Hepatopancreatic Necrosis Disease (AHPND) in Shrimp

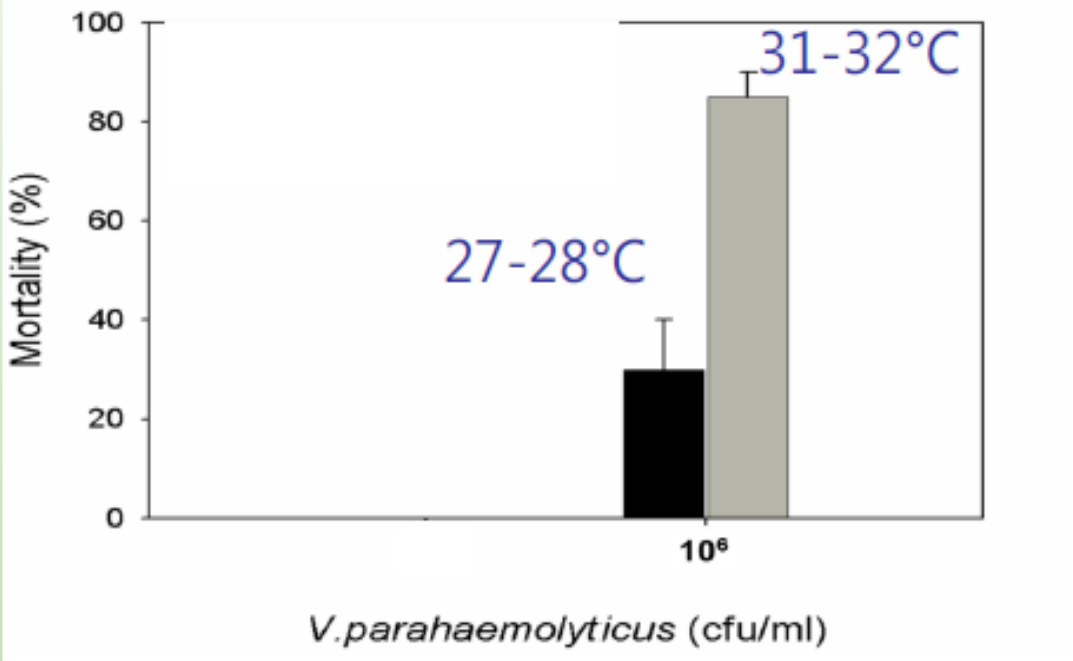
Ratchanok Sirikharin[1,2,3], Suparat Taengchaiyaphum[1], Piyachat Sanguanrut[1,2], Thanh Duong Chi[1,2], Rapeepat Mavichak[4], Porranee Proespraiwong[4], Bunlung Nuangsaeng[5], Siripong Thitamadee[2,3], Timothy W. Flegel[2], Kallaya Sritunyalucksana[1,2]*



Mortality rate is depend on immersion dose



High mortality rate at high temperature



Development of detection methods

Aquaculture Reports 2 (2015) 158–162

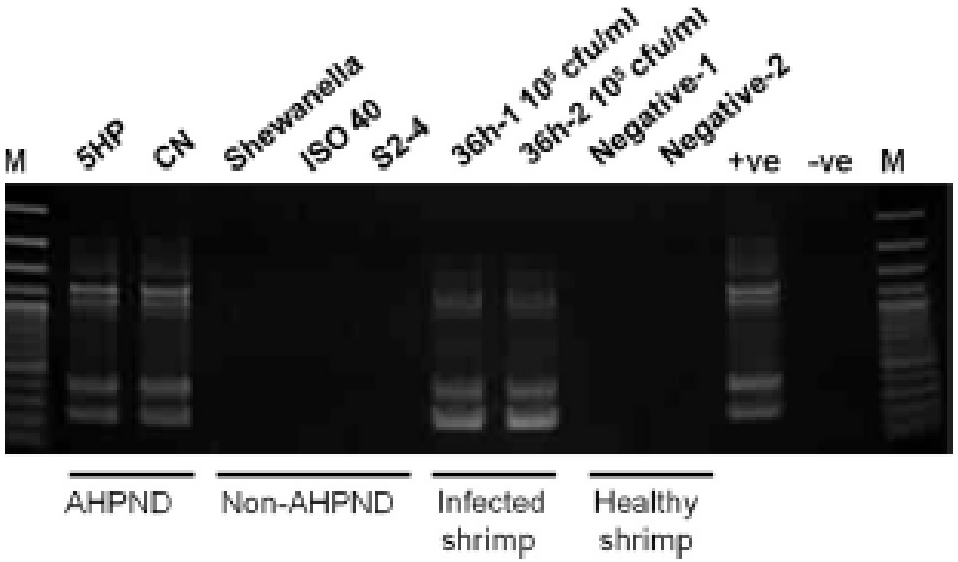
Contents lists available at ScienceDirect

Aquaculture Reports

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

AP4 method for two-tube nested PCR detection of AHPND isolates of *Vibrio parahaemolyticus*

Sirintip Dangtip[a,1], Ratchanok Sirikharin[b,c,1], Piyachat Sanguanrut[c], Siripong Thitamadee[d], Kallaya Sritunyalucksana[b,c], Suparat Taengchaiyaphum[b,*],



Aquaculture 474 (2017) 75–81

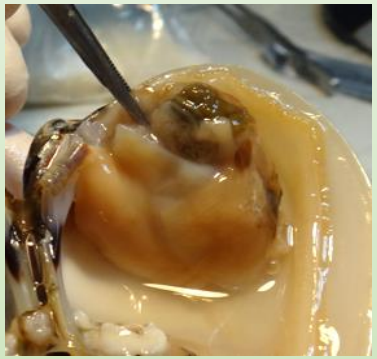
Contents lists available at ScienceDirect

Aquaculture

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

Development of monoclonal antibodies specific to ToxA and ToxB of *Vibrio parahaemolyticus* that cause acute hepatopancreatic necrosis disease (AHPND)

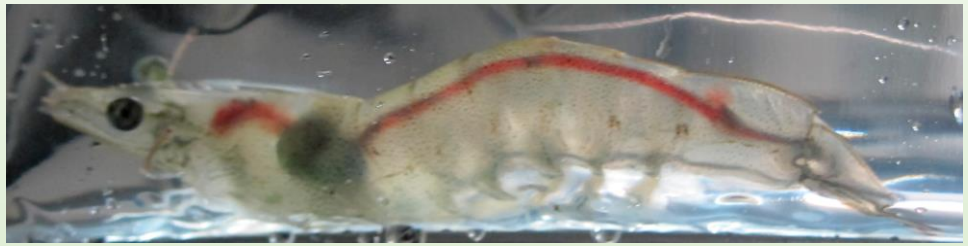
Pradit Wangman[a,b], Parin Chaivisuthangkura[a,b], Kallaya Sritunyalucksana[c], Suparat Taengchaiyaphum[c], Saengchan Senapin[d,e], Chalinan Pengsuk[f], Paisarn Sithigorngul[a,b], Siwaporn Longyant[a,b,*]

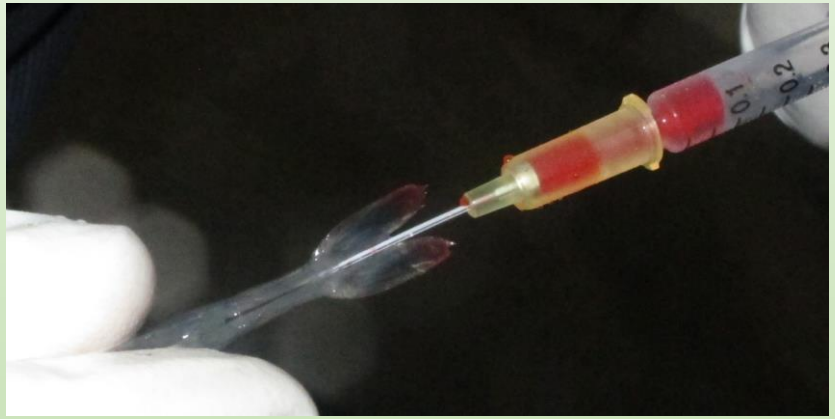


Industrial applications

Initial : 10 µg each

Recent : 50 µg each





Selection of Toxin-tolerant stock

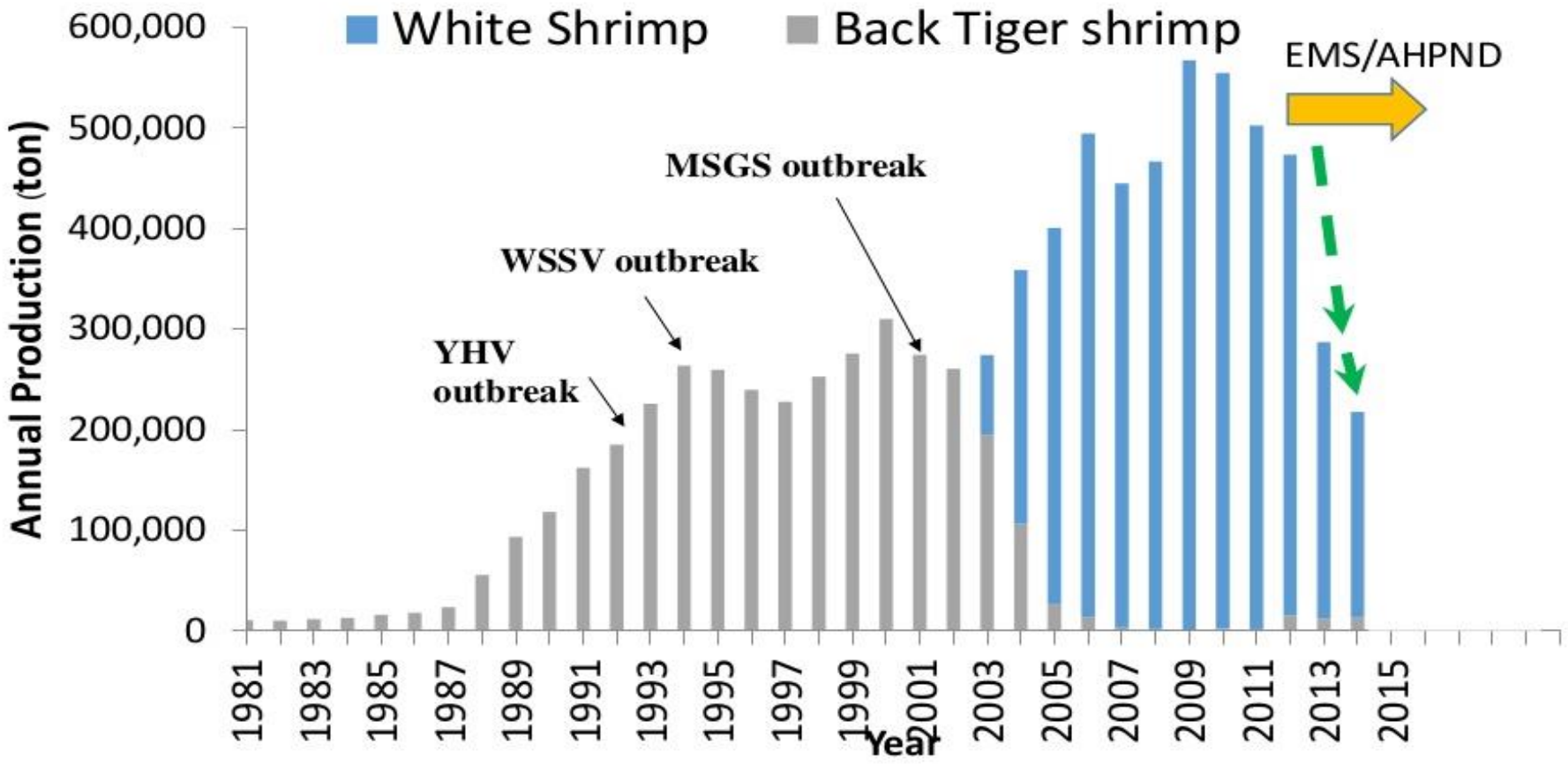
with recombinant toxins

Remove excess organic waste
to limit bacterial growth

Acclimating shrimp postlarvae before pond stocking: Nursery pond

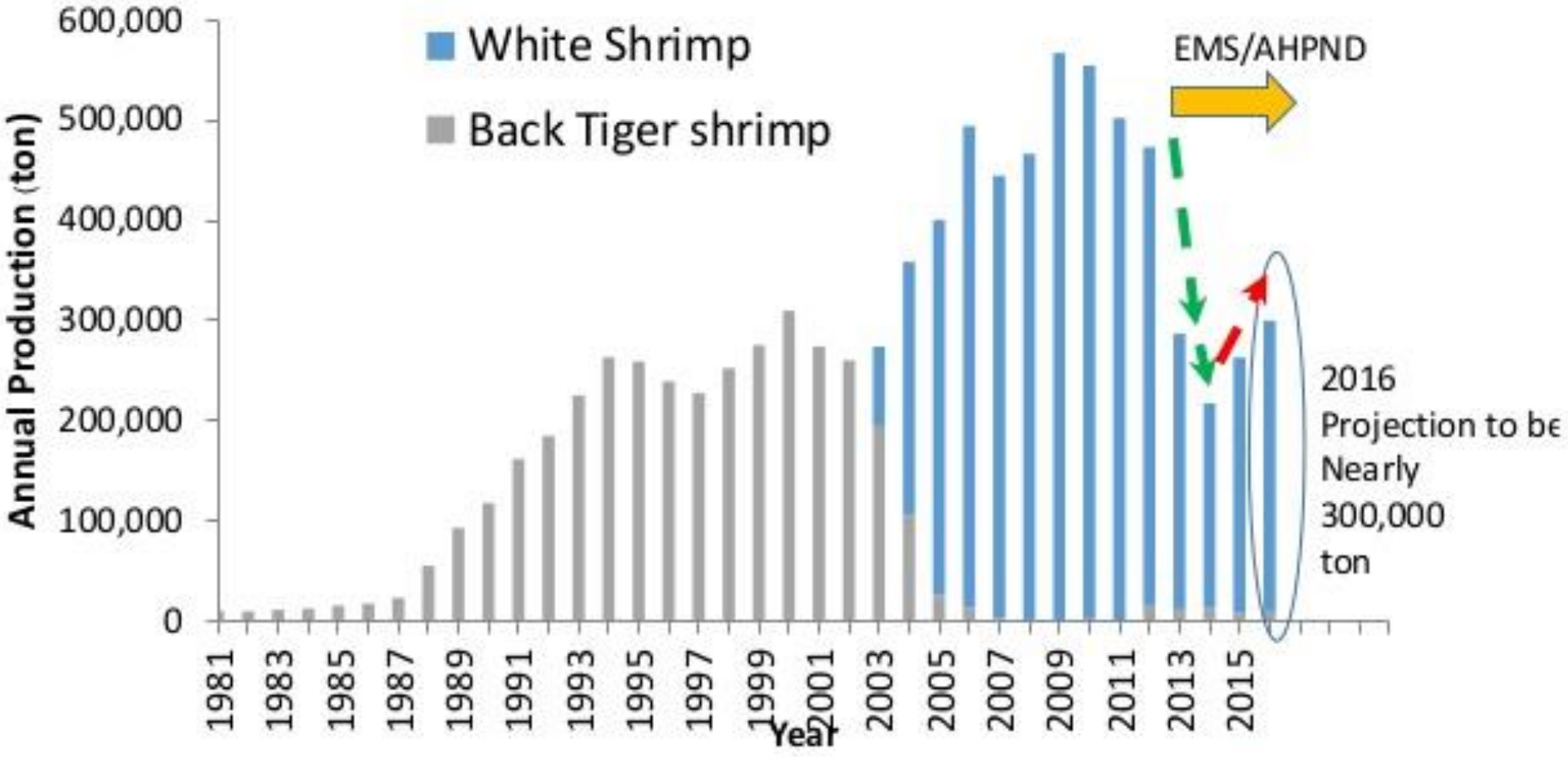
Modern, permanent acclimation station at shrimp farm (left); the new nursery systems (center, right) can also be used as acclimation stations at the beginning of the growout cycle, when the PL arrive at the farm. Photo by Darryl Jory.

Thailand annual shrimp production 1981-2014



Food and Agriculture Organization of the United Nations

Recovery of Thailand annual shrimp production 2015-2016



Food and Agriculture Organization of the United Nations

Aquaculture 493 (2018) 26–36

Contents lists available at ScienceDirect

Aquaculture

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

A cohort study of 196 Thai shrimp ponds reveals a complex etiology for early mortality syndrome (EMS)

Piyachat Sanguanrut[a,b], Natthinee Munkongwongsiri[a,b], Janejit Kongkumnerd[c], Jumroensri Thawonsuwan[d], Siripong Thitamadee[b,e], Visanu Boonyawiwat[f], Varin Tanasomwang[g], Timothy W. Flegel[b,h], Kallaya Sritunyalucksana[a,*]

AHPND is part of EMS.

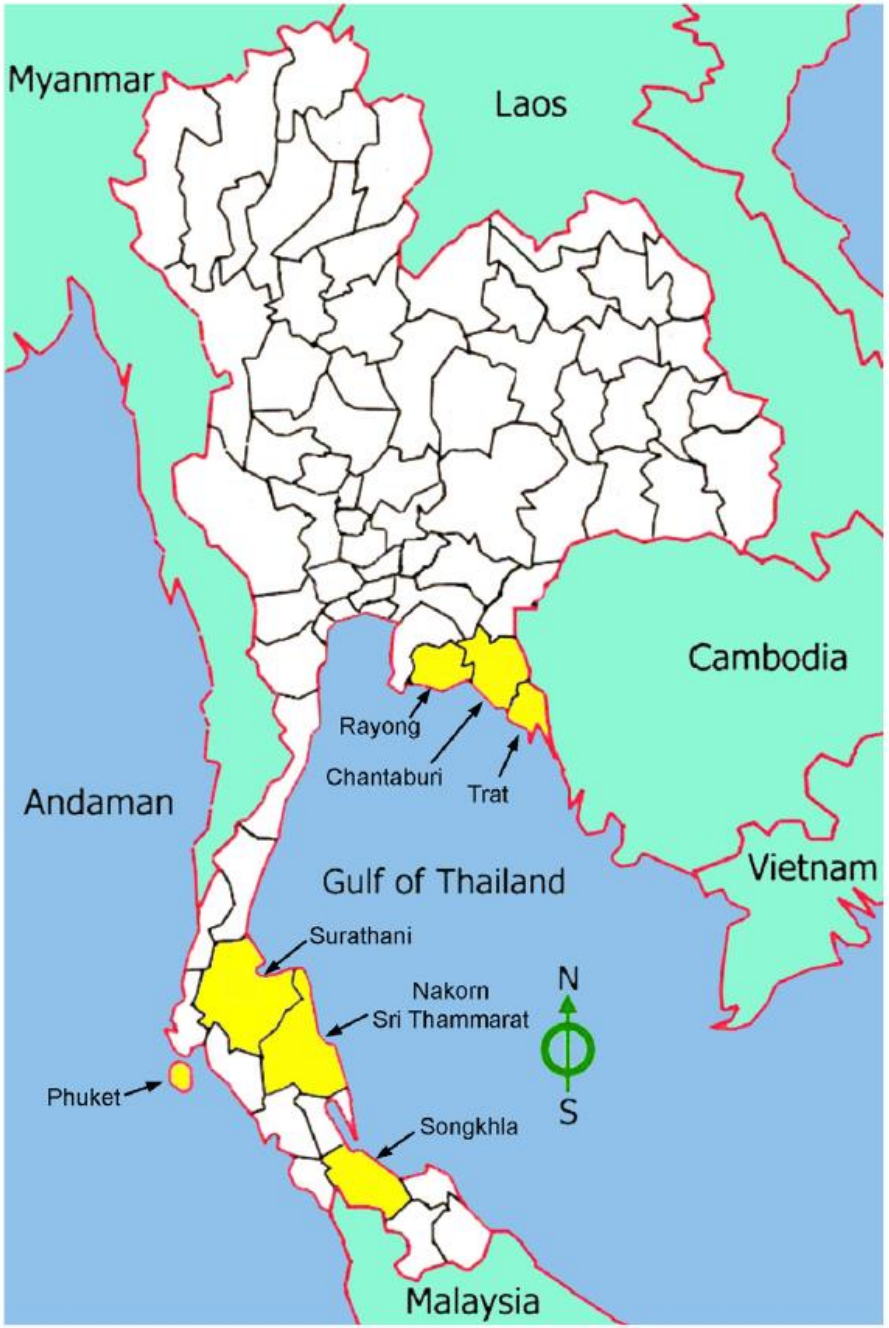


Fig. 1. Map of Thailand showing the provinces of major shrimp cultivation where the sampling sites for this study were located.

Table 1
Breakdown of the number of ponds sampled per farm in this study of 200 sample ponds from 133 farms (an average of 1.5 ponds per farm).

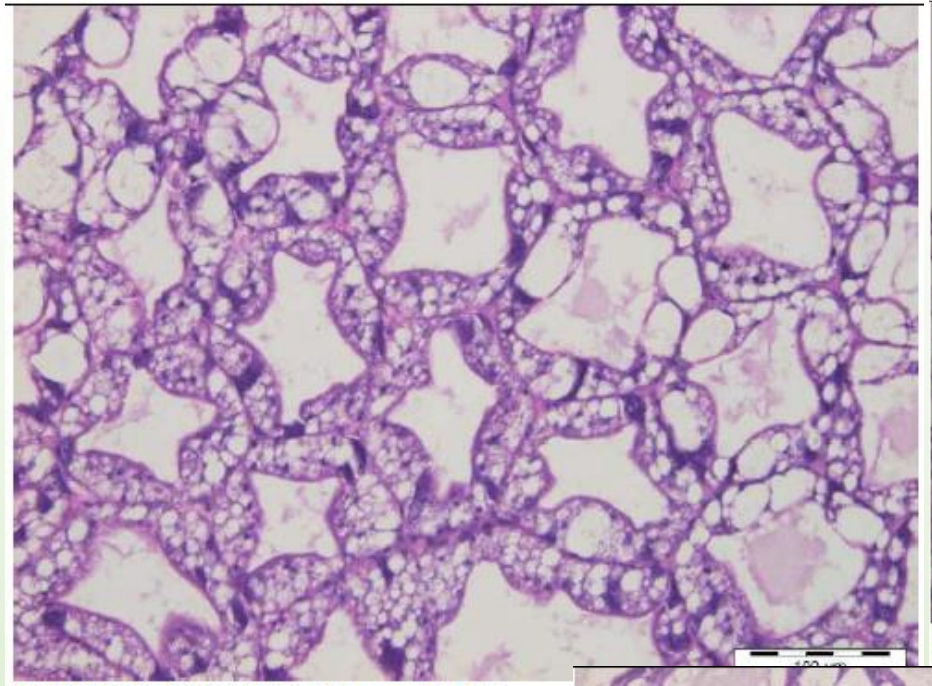
#Ponds sampled	#Farms	% of Farms	#Ponds	% of Ponds
1	101	75.9	101	50.5
2	15	11.3	30	15.0
3	7	5.3	21	10.5
4	6	4.5	24	12.0
5	1	0.8	5	2.5
6	2	1.5	12	6
7	1	0.8	7	3.5
Totals	133	100	200	100

Table 2

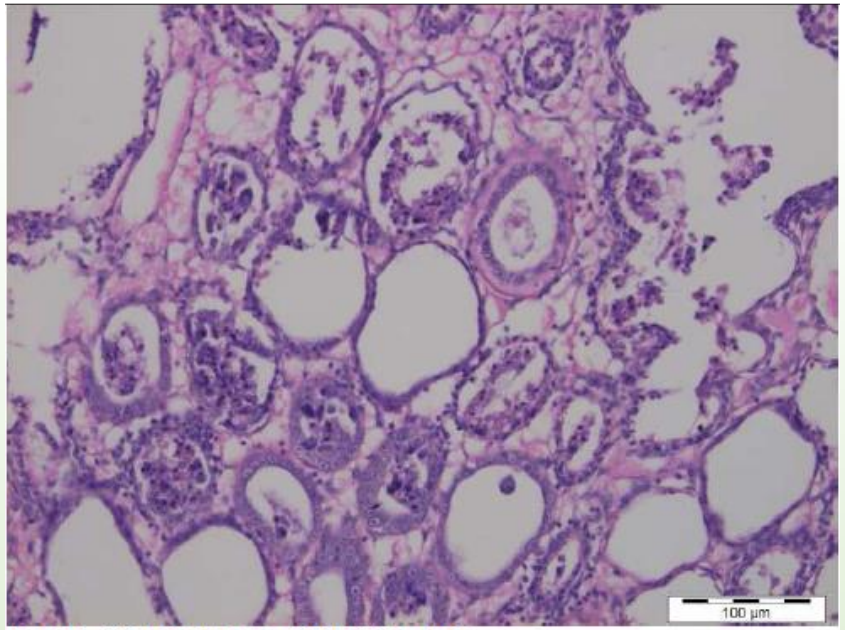
Summary of histological and PCR test results for 196 shrimp ponds included in this study.

AHPND by histology and/or PCR	No AHPND but bacterial lesions	Collapsed HP tubule epithelia	Normal HP histology
03, 06 **07**, **11**, 12, **16**, **17**, 19, 20, **32**, 40, 47, 50, 52, **54**, **74**, 84, **91**, 93, **110**, **112**, 116, **119**, **122**, **126**, 131, 136, 144, **150**, 160, 161, 162, **166**, **167**, 168, 169, 172, 181, **191**, 199, 200	04, 05, 21, 24, 44, 45, 51, 58, 69, 75, 79, 86, 87, 88, 96, 99, 101, **117**, **130**, 133, 139, 143, 170, 174, 186, **192**, 196, 197, 198	02, 15, 25, 26, 29, **30**, 37, 38, 46, 48, 49, 53, 64, 65, 76, 80, 81, 82, 83, 90, 95, 97, 104, 109, 113, 114, 115, 123, 125, 127, 128, 134, 135, 158, 159, 163, 165, 171, 173, 175, 178, 179, 182, 184, 185, 187, 188, 189, 193	01, 08, 09, 10, 13, 14, 18, 22, 23, 27, 28, 31, 33, 34, 35, 36, **39**, 41, 42, 43, 55, 56, 57, 59, 60, 61, 62, 63, 66, 67, 68, 70, 71, 72, 73, 77, 85, 89, 92, 94, **98**, **100**, 102, 103, 105, 106, 107, 108, **111**, 118, 120, 121, 124, 129, 132, 137, 138, 140, 141, 142, 145, 148, 149, 152, 153, 154, 155, **156**, **157**, 164, 176, 177, 180, 183, **190**, **194**, 195
18	3	1	10
41/196 (20.9%)	29/196 (14.8%)	49/196 (25.0%)	77/196 (39.3%)

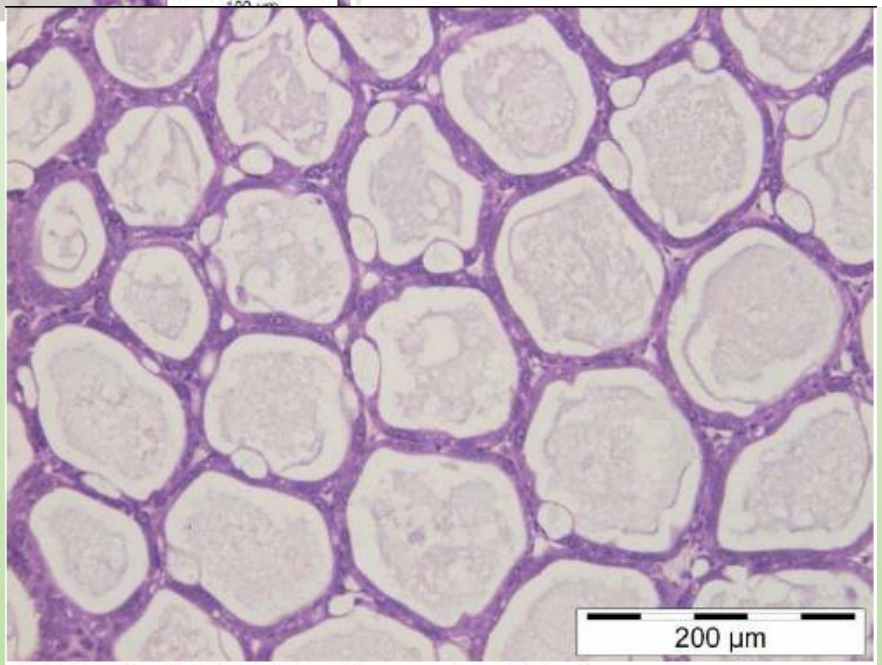
Pond number in bold, underlined typeface indicate ponds designed as EMS by the study definition.



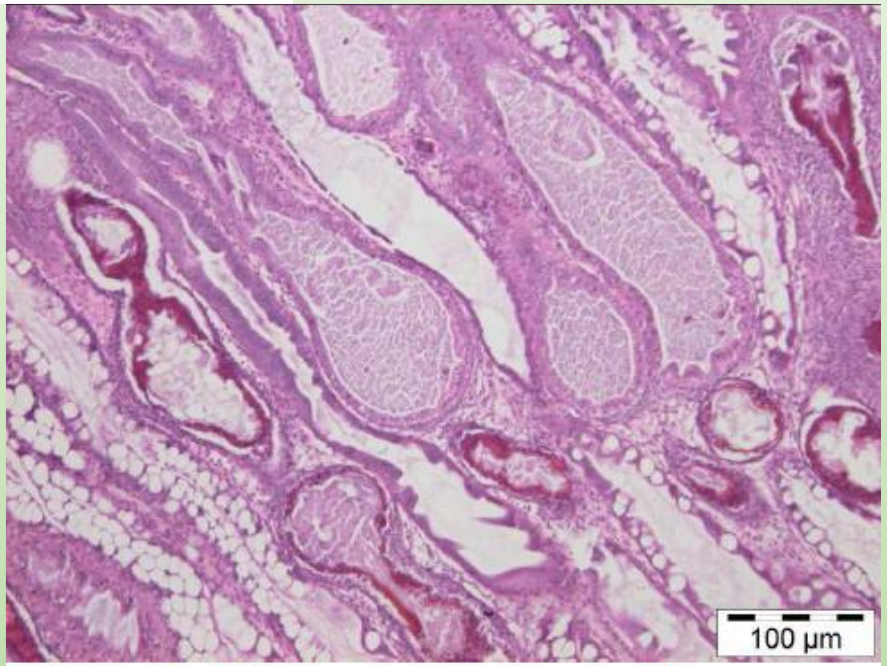
DOF 13-09-30-3 Normal HP



DOF 13-09-50-7 AHPND- M2



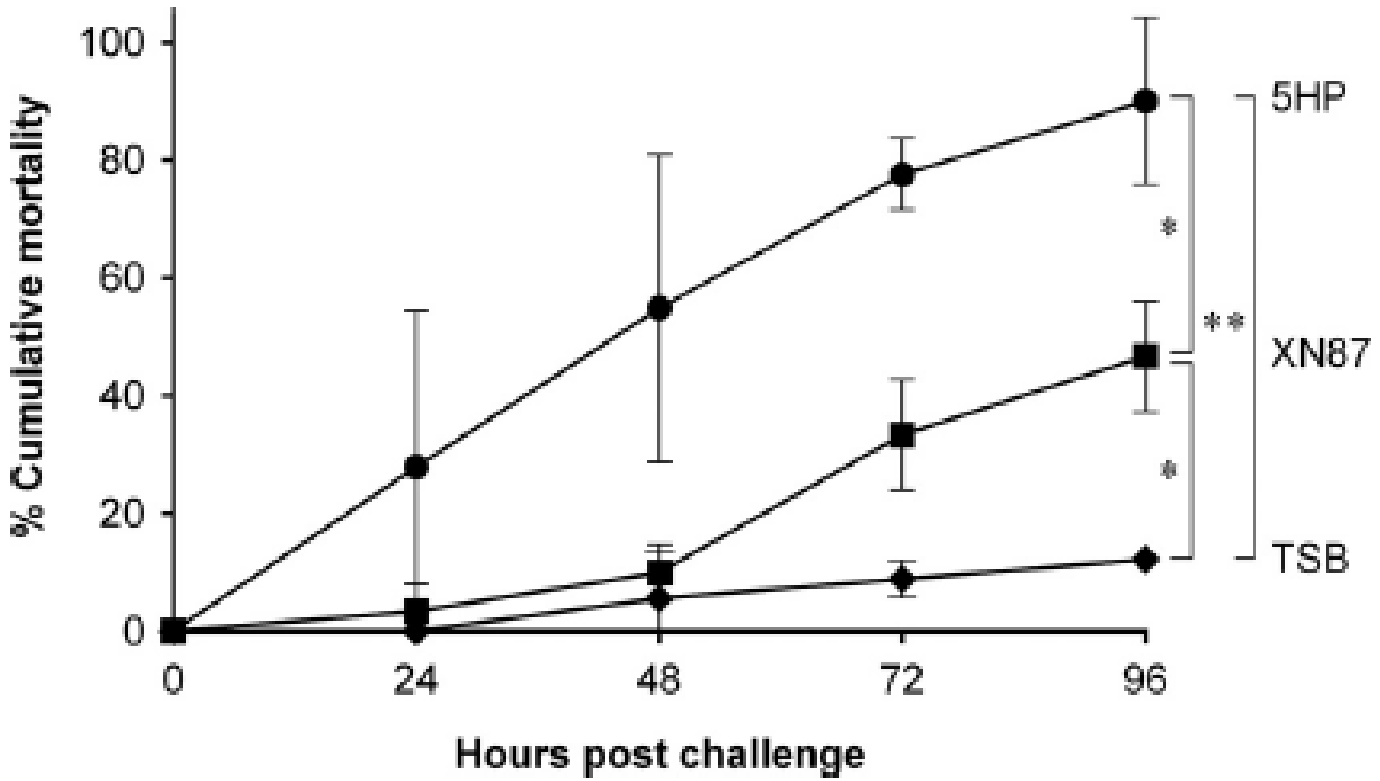
DOF 13-10-95-10 collapsed epithelia

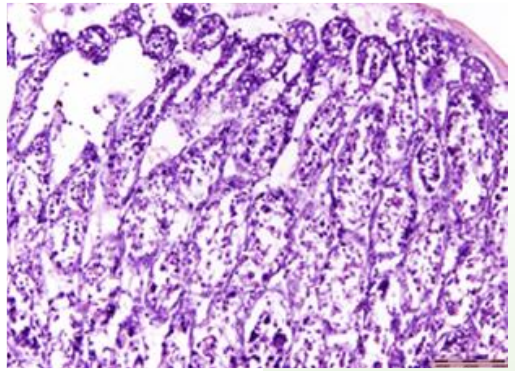


DOF 13-10-117-2 bacterial lesions in HP

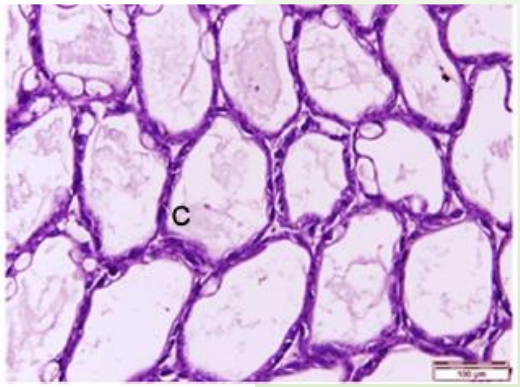
A Natural *Vibrio parahaemolyticus* $\Delta pirA^{Vp}$ $pirB^{Vp+}$ Mutant Kills Shrimp but Produces neither Pir^{Vp} Toxins nor Acute Hepatopancreatic Necrosis Disease Lesions

Kornsunee Phiwsaiya,[a,b] Walaiporn Charoensapsri,[a,b] Suwimon Taengphu,[b] Ha T. Dong,[c] Pakkakul Sangsuriya,[a,d] Giang T. T. Nguyen,[e] Hung Q. Pham,[e] Piti Amparyup,[a,d] Kallaya Sritunyalucksana,[a,f] Suparat Taengchaiyaphum,[a,f] Parin Chaivisuthangkura,[g] Siwaporn Longyant,[g] Paisarn Sithigorngul,[g] Saengchan Senapin[a,b]





5HP



XN87

FIG 6 Cumulative percent mortality resulting from shrimp challenged with *V. parahaemolyticus* 5HP and XN87 isolates. The control group was mock challenged with TSB medium. Each group started with 9 to 15 *P. vannamei* shrimp, and experiments were conducted in duplicate. Statistical significance was calculated by one-way analysis of variance, and *P* values of <0.05 (*) and <0.01 (**) were considered significant.

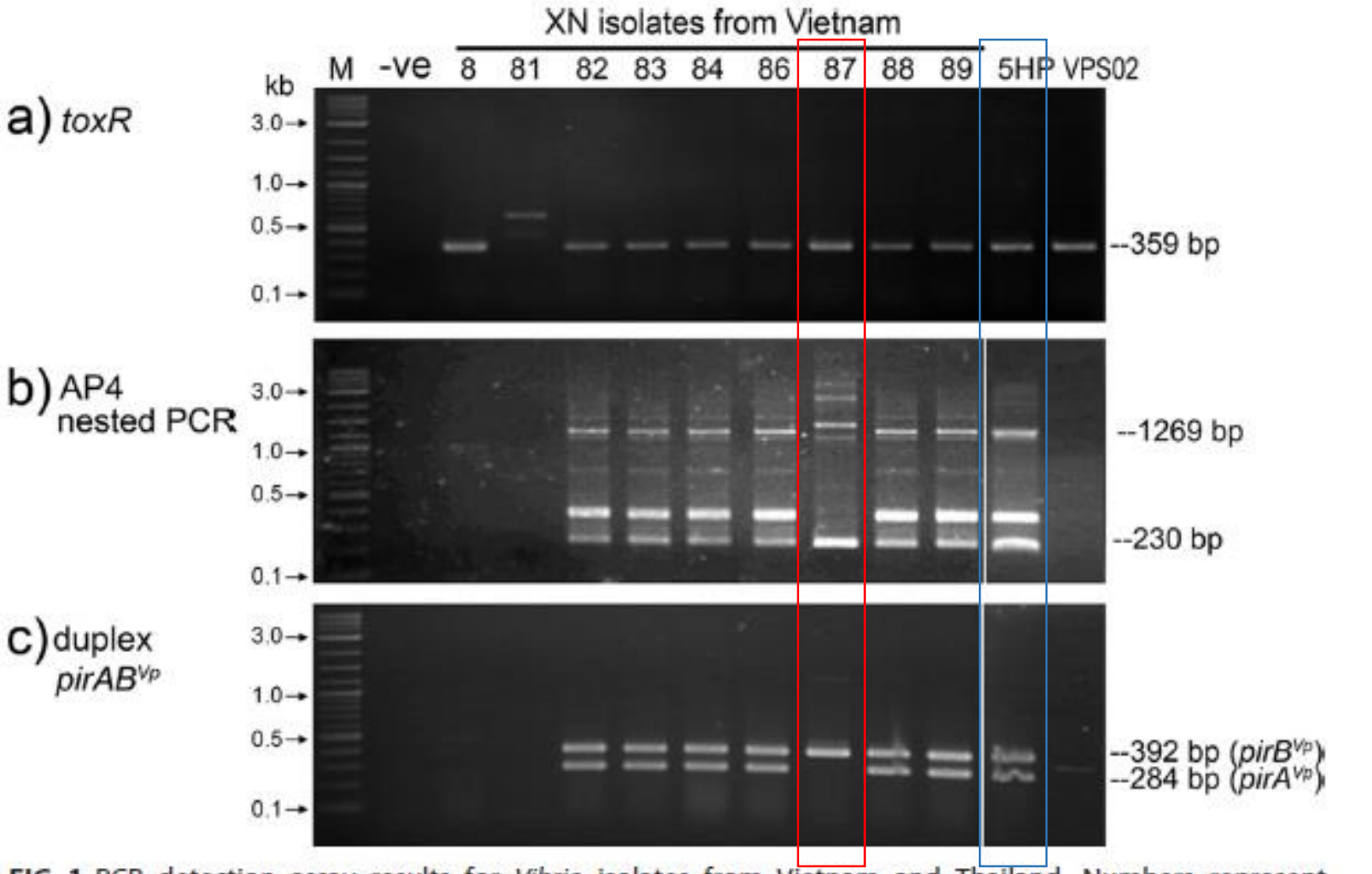
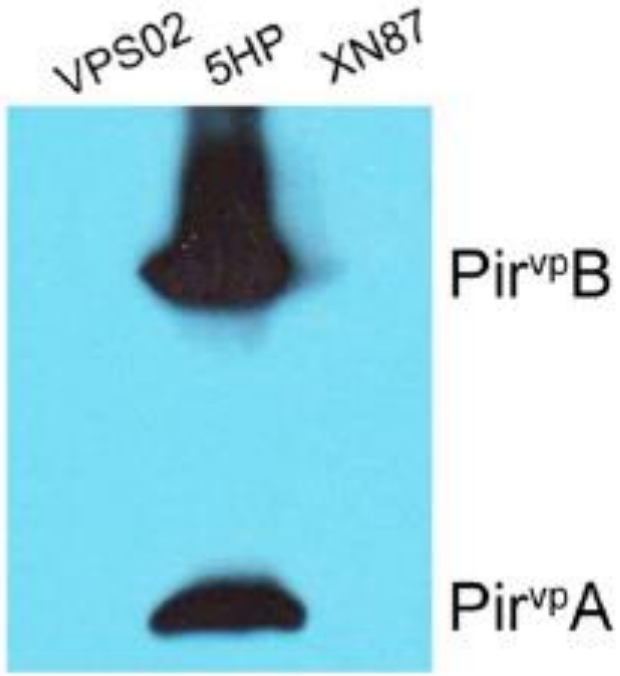


FIG 1 PCR detection assay results for *Vibrio* isolates from Vietnam and Thailand. Numbers represent

Western blot



Indirect ELISA

Sample	OD_{450}	$Pir^{vp}B$ toxin concentration (ng) per µg total protein
5HP	0.311 ± 0.048	4.815 ± 1.025
XN87	0.113 ± 0.002	Below detection limit
VPS02	0.110 ± 0.007	Below detection limit

Expression and quantification of the Pir^{Vp} toxins.

Other vibrio species reported to produce Pir^{VP} toxins

Vibrio owensii

Vibrio harveyi

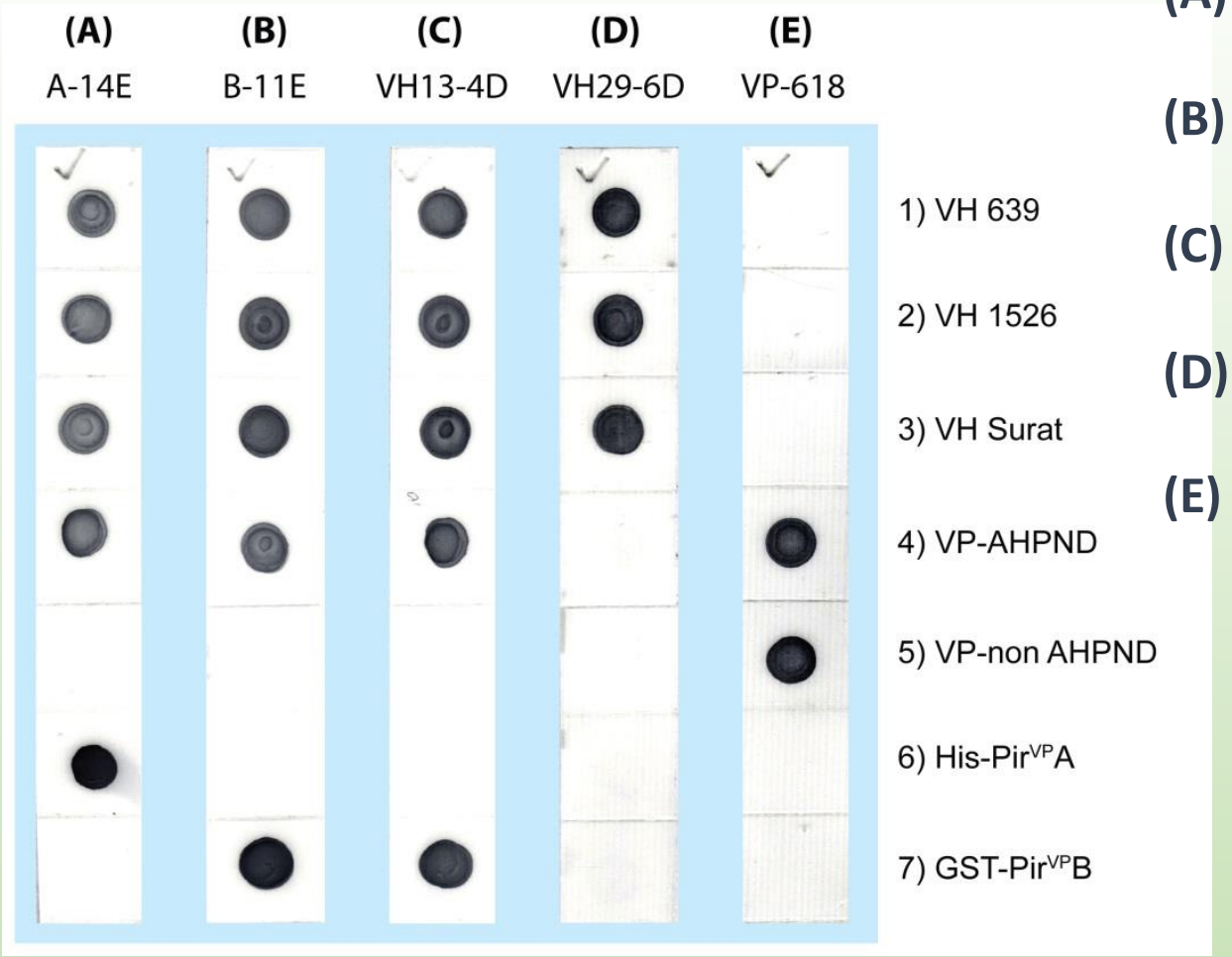
Vibrio campbellii

ACCEPTED MANUSCRIPT

PirA & B toxins discovered in archived shrimp pathogenic *Vibrio campbellii* isolated long before EMS/AHPND outbreaks

Pradit Wangman [a,b], Siwaporn Longyant [a,b], Suparat Taengchaiyaphum [c], Saengchan Senapin [d,e], Paisarn Sithigorngul [a,b], Parin Chaivisuthangkura [a,b,*]

***Vibrio campbellii* already carried *PirA* and *PirB* toxin genes since 2002.**



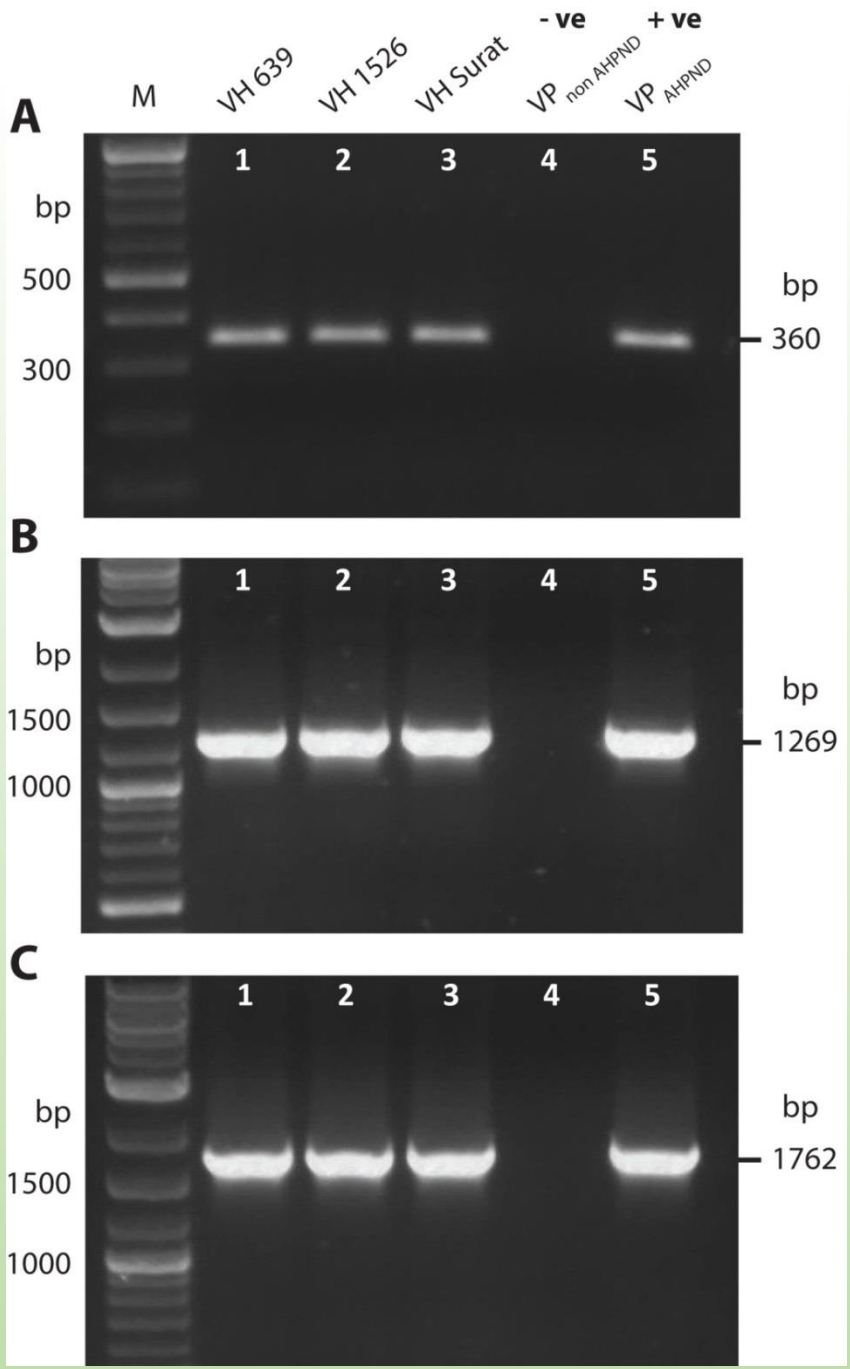
(A) A-14E (recognize PirA),

(B) B-11E (recognize PirB),

(C) VH13-4D recognize a ~ 49 kDa protein,

(D) VH29-6D bound to LPS of VH 639, VH 1526, VH Surat

(E) VP-618 specific to *V. parahaemolyticus*

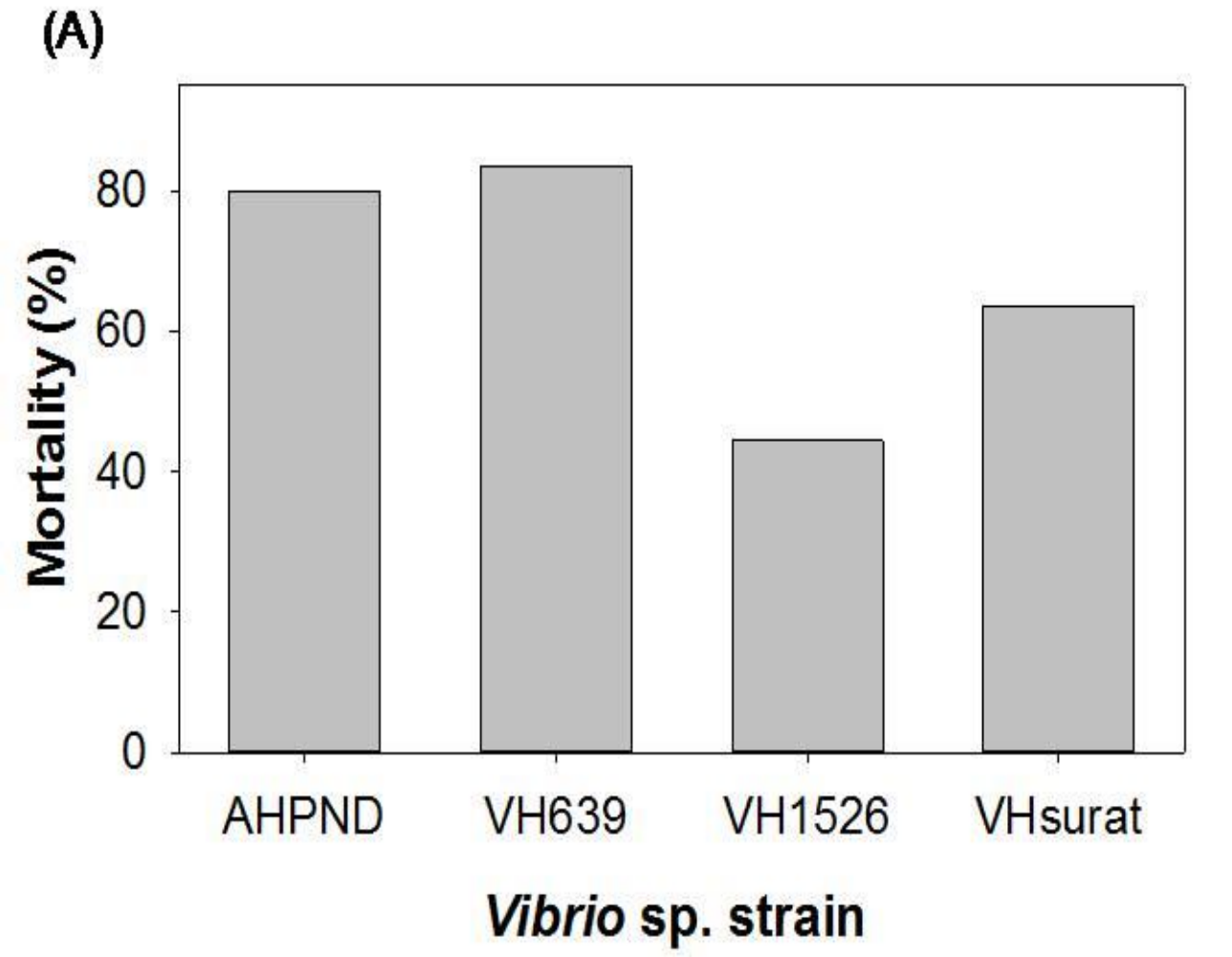


PCR identification.

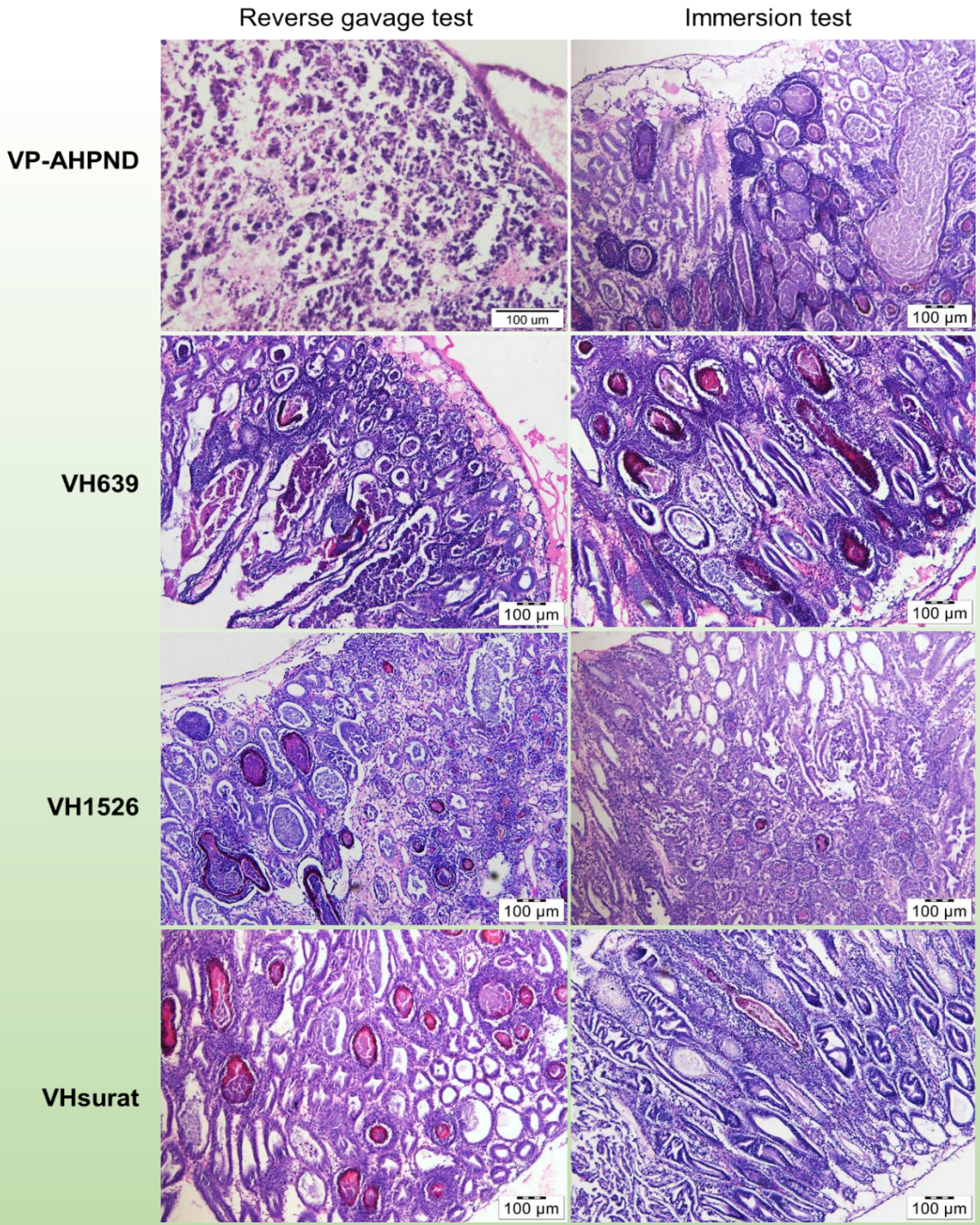
(A) TUMSAT-Vp3 primers (360 bp),

(B) first step of AP4 primers (1269 bp)

(C) Full length PirA/B primers (1762 bp)



The cumulative mortality in shrimp at 48 h post administered by 80% ammonium sulfate protein fraction obtained from three *vibrio* isolates. The VP-AHPND was performed as a positive control.



Histological examination of moribund shrimp post challenging test

- Cell sloughing
- Necrosis
- Encapsulation
- Nodule formation

Received: 21 March 2018 | Revised: 26 June 2018 | Accepted: 27 June 2018

DOI: 10.1111/jfd.12874

Check for updates

ORIGINAL ARTICLE

WILEY Journal of Fish Diseases

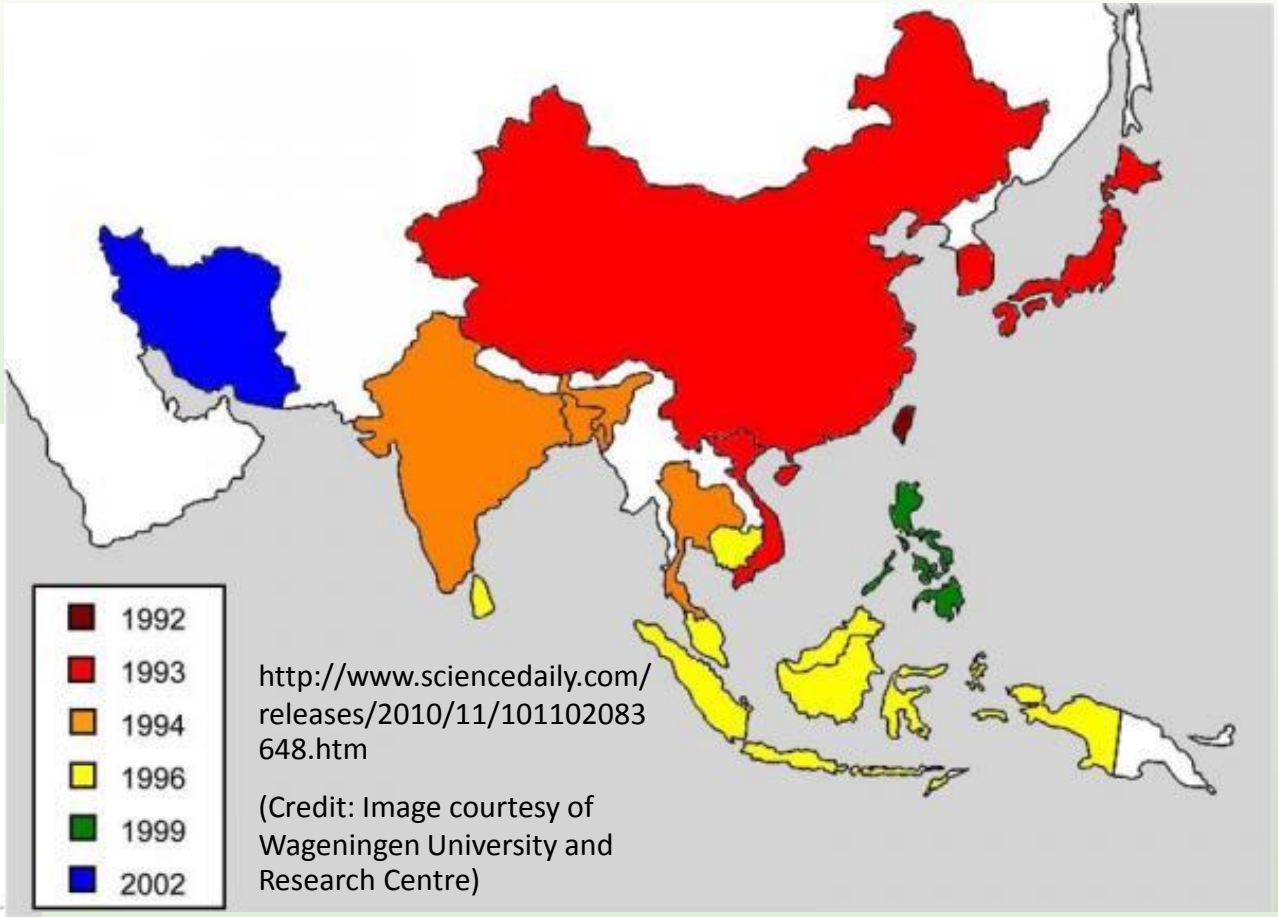
*pir*A- and *pirB*-like gene identification in *Micrococcus luteus* strains in Mexico

Ma. de Jesús Durán-Avelar | Alejandro Vázquez-Reyes | Ana Lourdes González-Mercado | José Francisco Zambrano-Zaragoza | Miriam Fabiola Ayón-Pérez | Juan Manuel Agraz-Cibrián | Jorge Gutiérrez-Franco | Norberto Vibanco-Pérez

Summary for the technical part

- Different VP_{AHPND} isolates have different virulence.
- Not only VP that can cause AHPND.
- The causes of EMS, not EMS/AHPND is under investigation.

Surveillance program for shrimp pathogens

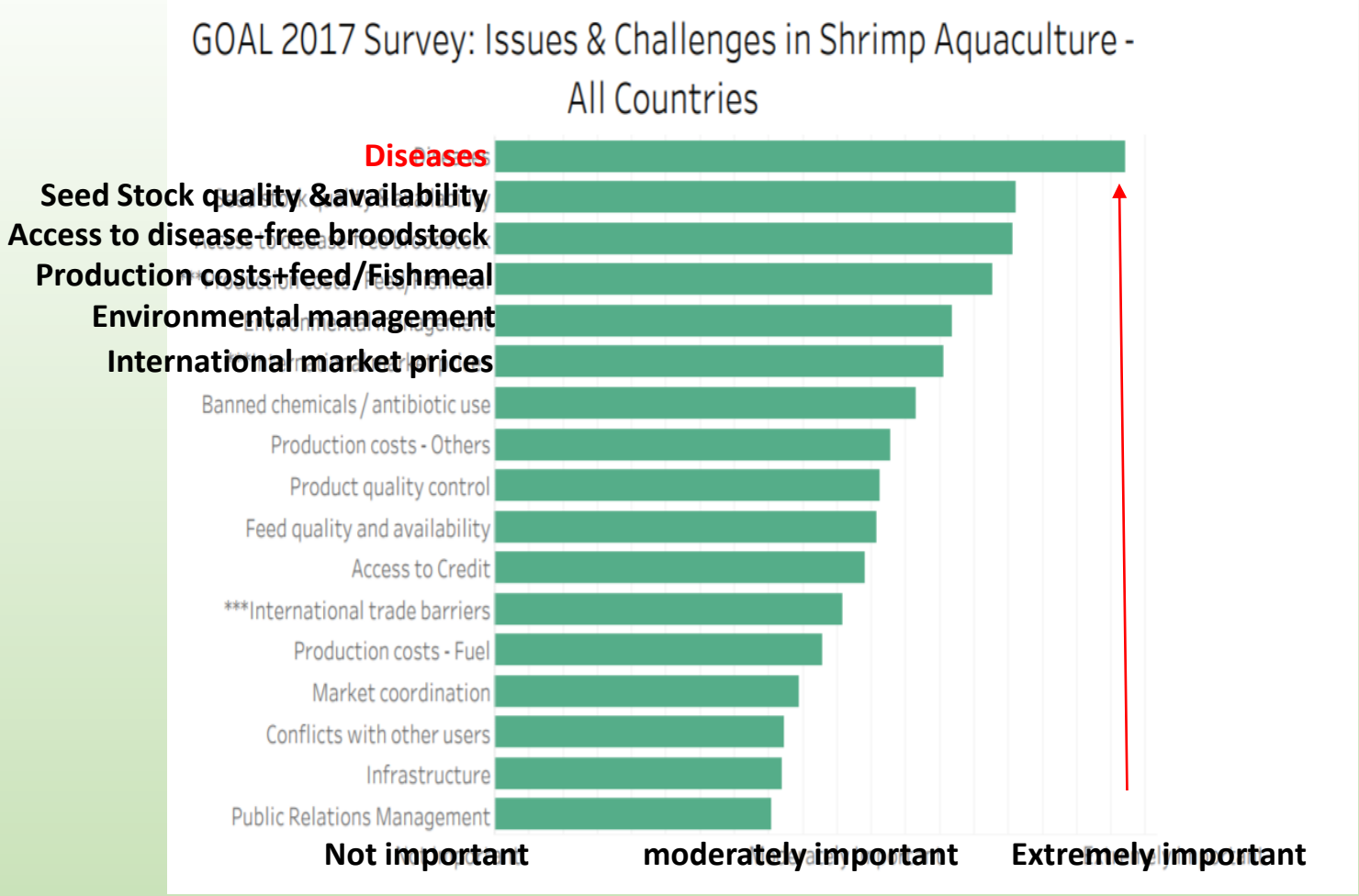


http://www.sciencedaily.com/releases/2010/11/101102083648.htm

(Credit: Image courtesy of Wageningen University and Research Centre)

Spread of the WSSV in Southern Asia. The virus spread over the entire region from its source in Taiwan within several years.

Global Aquaculture Alliance™



INSH

BIOTEC a member of NSTDA

Cefas

Newton Fund Institutional Links

An International Network for Shrimp Health (INSH) to bridge the gap between cutting-edge research, policy and the farmer

INSH

INSH

A network in shrimp health designed to provide a platform to mitigate/avoid future epidemics by learning from current issues and aligning more rapidly on future.

Goal of INSH

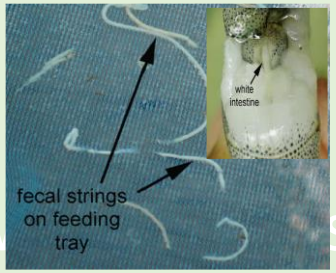
To create an inter-country, evidence-based community that jointly supports sustainable expansion of Thai, regional and global aquaculture

- To assist in diagnostics, detection, surveillance and control of shrimp diseases to the industry and Thai competent authority
- Establish a strong collaborative research network on shrimp diseases between shrimp diseases laboratories at national and international level

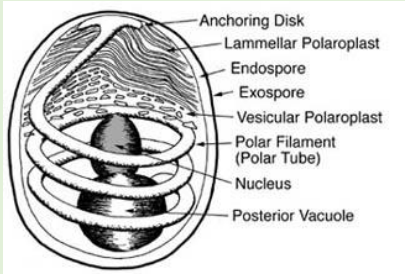
Strategies: Working on collaborative projects on urgent shrimp diseases and related issue

Research projects of INSH

White Feces Syndrome



Microsporidian EHP



Interference of viral inserts to PCR detection

Farmer-friendly detection device

Industry ⟷ INSH ⟷ Government

INSH

Centre for Environment Fisheries & Aquaculture Science

Cefas

BIOTEC a member of NSTDA

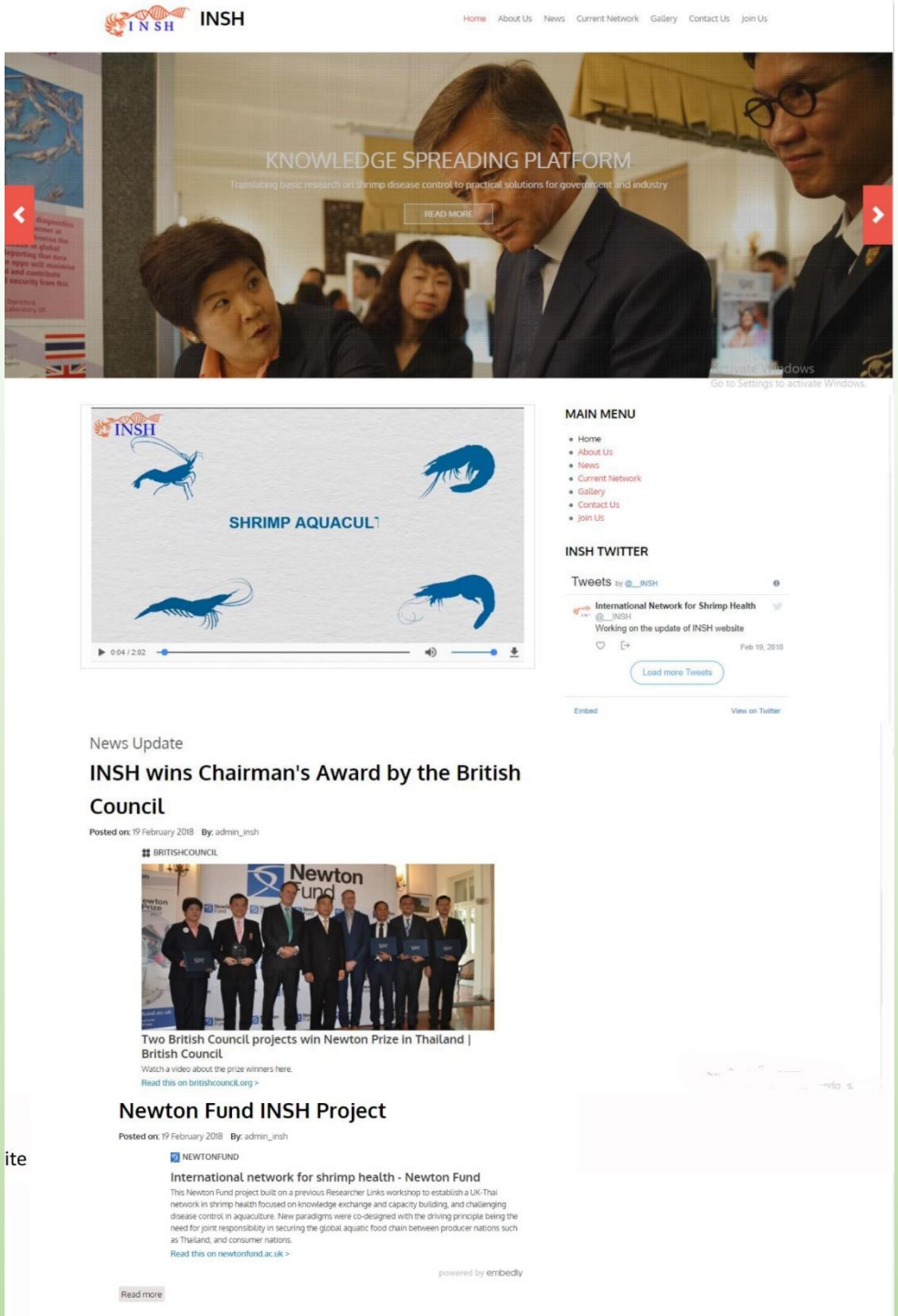
MAHIDOL UNIVERSITY Center for Shrimp BIOTEC-NSTDA

BRITISH COUNCIL

Newton Fund

UK-Thai Summit on Healthy Aquaculture to 2050

- ✓Industry-Operated Network of Aquaculture Council
- ✓Multinational Disease Surveillance Program: Emerging Disease management
- ✓A sustainable, high density and insurable shrimp farm for the future
- ✓Mechanism to maintain and improve INSH



http://www.insh.info

Research Team

Thank You!

for your attention