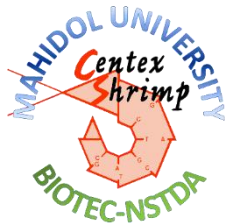


Identification and characterization of the causative agents of fish diseases

Dr. Saengchan Senapin

(saengchan@biotec.or.th)



Thai aquaculture production



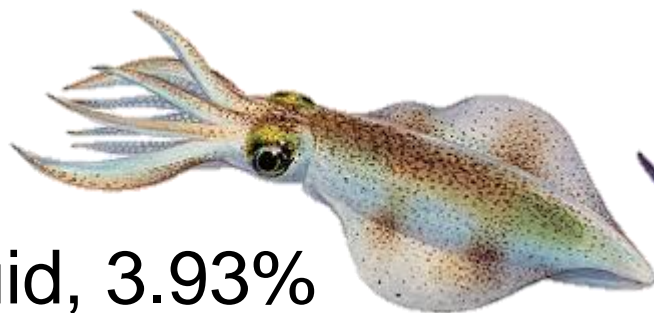
Fish, 71.22%



Shrimp, 13.17%



Mollusks, 7.85%



Squid, 3.93%



Crab, 1.11%



Production 2.56 M ton
Value 145,414 M Baht
(DOF 2014)

Fish aquaculture in Thailand

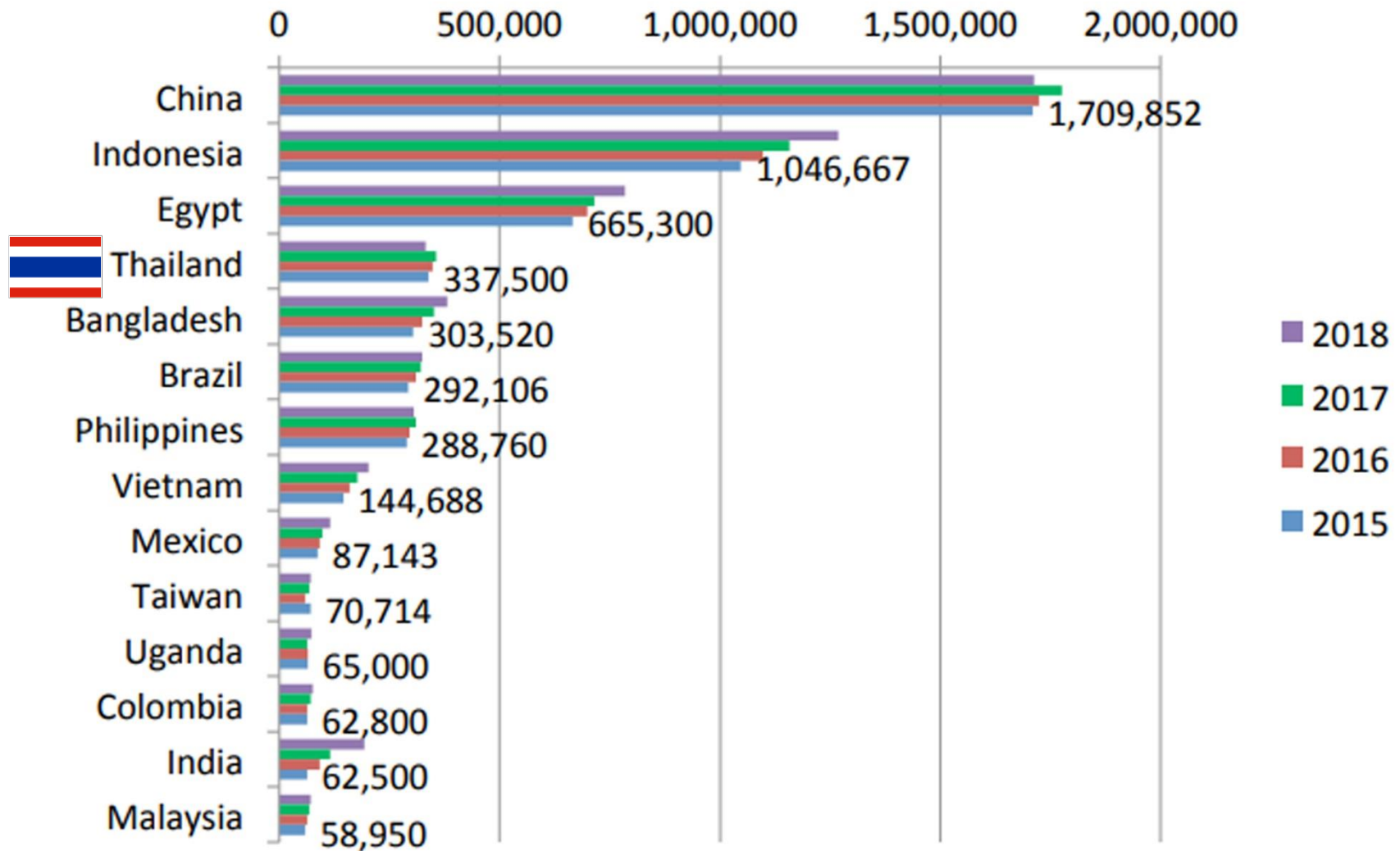
Freshwater species	% Production
Nile tilapia	45.8
Catfish	29.8
Silver barb	11.2
Snakeskin gourami	5.7
Striped catfish	4.6
Snakehead murrel	1.8
Giant gourami	1.0
Mrigal carp	0.2

Total production ~ 400,000 tonnes

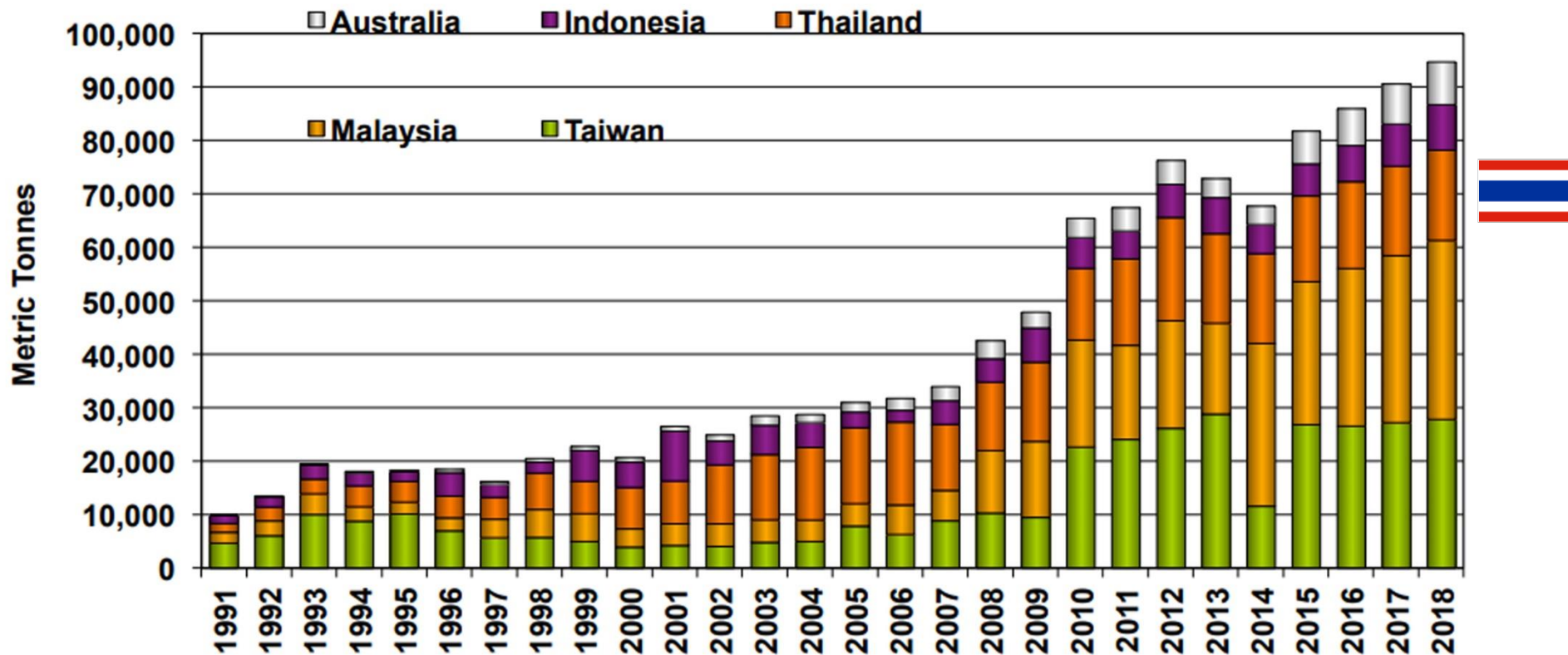
Brackish water species	% Production
Asian seabass	80.7
Grouper	19.3

Total production ~ 20,000 tonnes

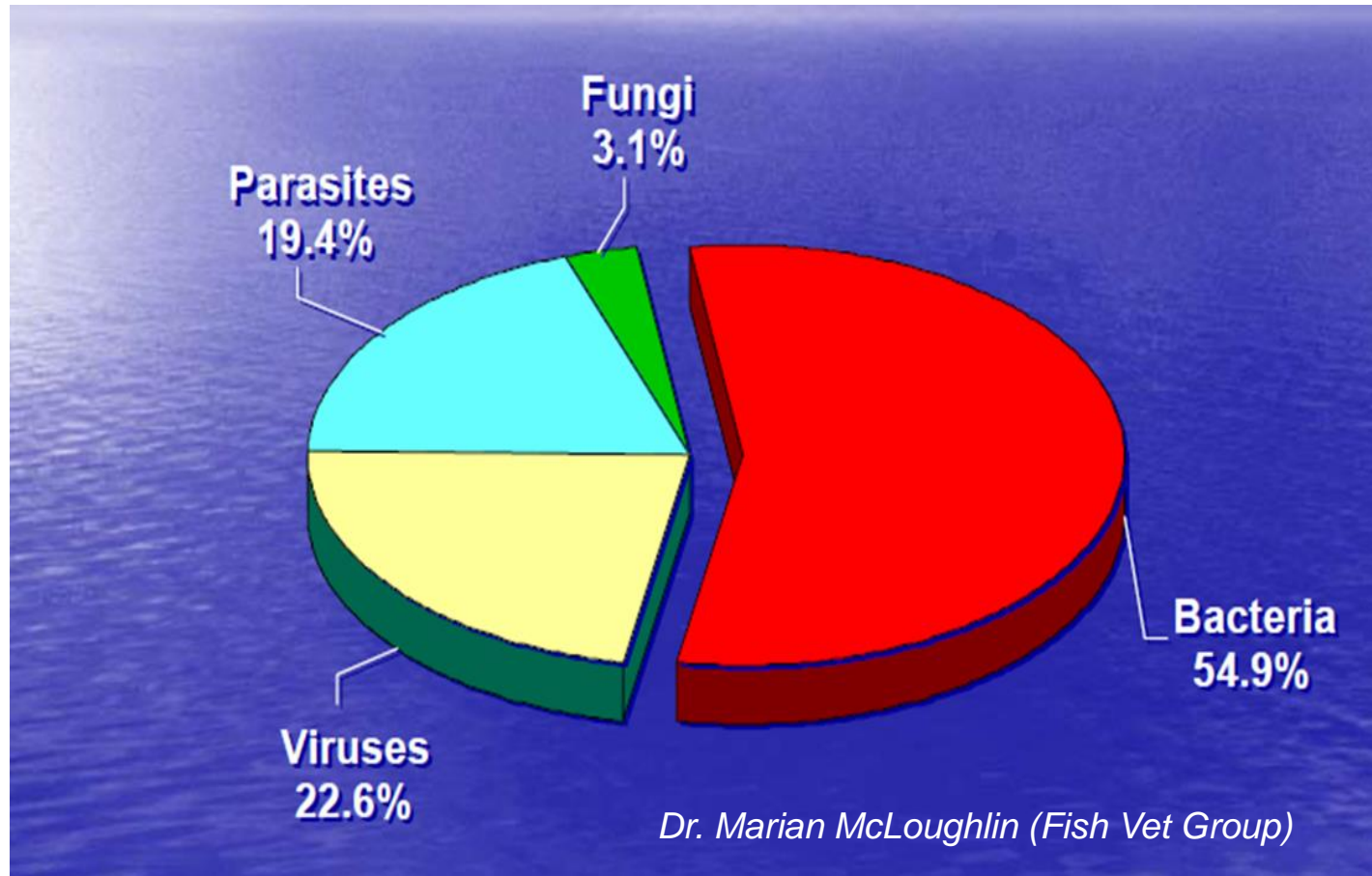
Tilapia production by major producing countries



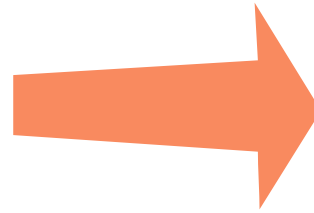
Asian seabass production by major producing countries



Disease is considered as one of the major problems to aquaculture production globally



From Research to Solutions



Solutions to disease problems



Isolation, Identification & Proof of causation

Fish pathogen detection kits & service



Research and Development of Fish Vaccines, Immunostimulants



Bio-resource & Culture collection



Comprehensive analysis of Molecular pathogenesis (Omics analysis)



Lab training & Reference Lab



Research on fish diseases



Nile tilapia (*Oreochromis niloticus*)

Hybrid red tilapia (*O. mossambicus* x *O. niloticus*)

- *Flavobacterium columnare*
- *Francisella noatunensis*
- *Streptococcus agalactiae*
- *Aeromonas veronii*
- *Hahella chejuensis*
- Tilapia lake virus (TiLV)
- Iridovirus



Barramundi / Asian Seabass
(*Lates calcarifer*)

- *Vibrio harveyi*
- Scale drop disease virus (SDDV)



Striped catfish
(*Pangasianodon hypophthalmus*)

- *Flavobacterium columnare*
- *Edwardsiella ictaluri*



Siamese fighting fish (*Betta splendens*)

- Skin nodule syndrome
- Big belly syndrome

Research on fish diseases



Nile tilapia (*Oreochromis niloticus*)
Hybrid red tilapia (*O. mossambicus* x *O. niloticus*)

- *Hahella chejuensis*
- Tilapia lake virus (TiLV)



Barramundi / Asian Seabass
(*Lates calcarifer*)

- *Vibrio harveyi*
- Scale drop disease virus (SDDV)

Novel disease in tilapia hatcheries

Red eggs

- Since 2000
- Eggs turn to red and fail to hatch
- Cumulative loss of 10% and up to 50% in cold season
- The cause is unknown, but appears to be infectious



Egg collection



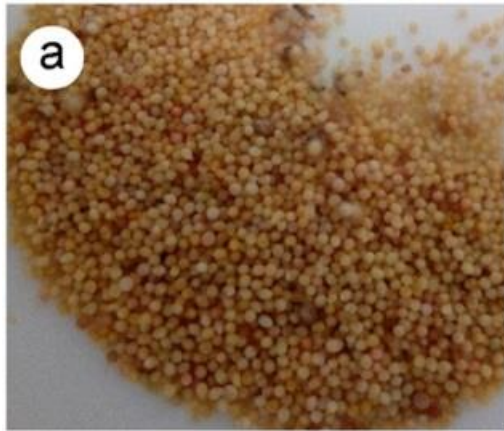
Artificial incubation



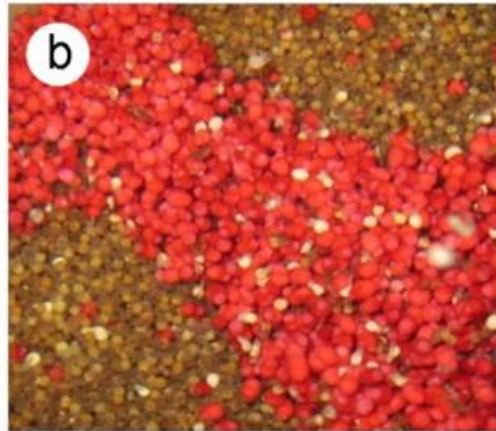
Red eggs

Novel disease in tilapia hatcheries

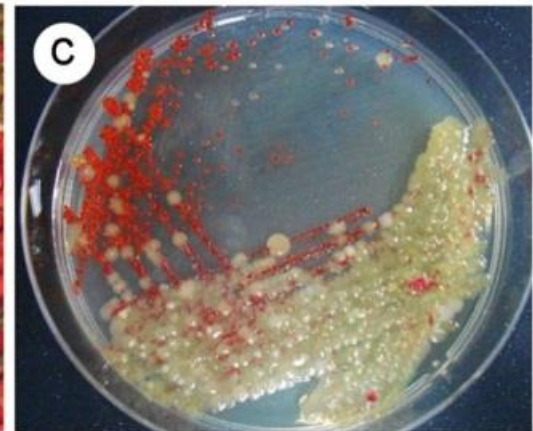
Infectious disease of red eggs caused by *Hahella chejuensis*



Normal eggs



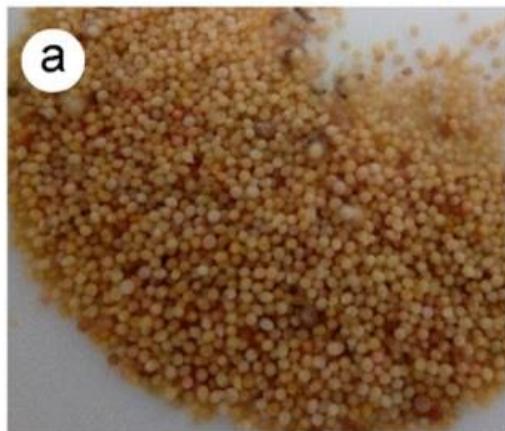
Red egg disease (10-50% loss)
(We named it "Hahellosis")



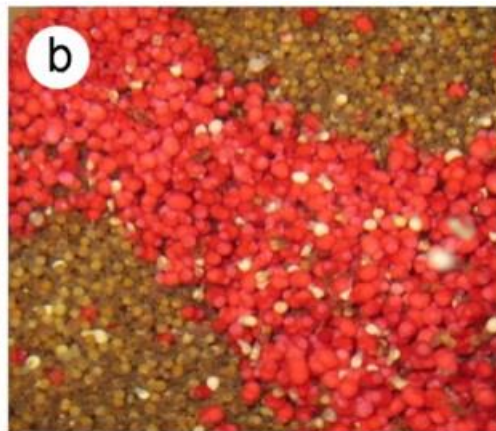
Red bacteria on TSA
(*Hahella chejuensis*)

Novel disease in tilapia hatcheries

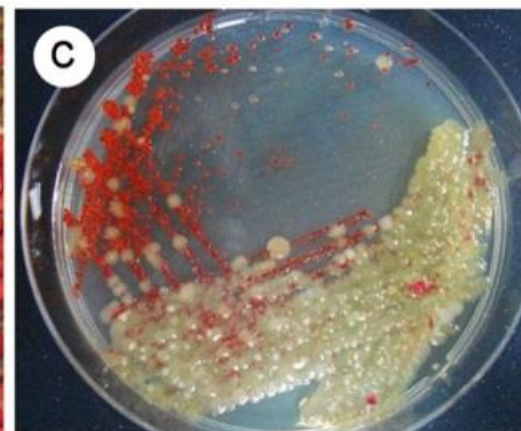
Infectious disease of red eggs caused by *Hahella chejuensis*



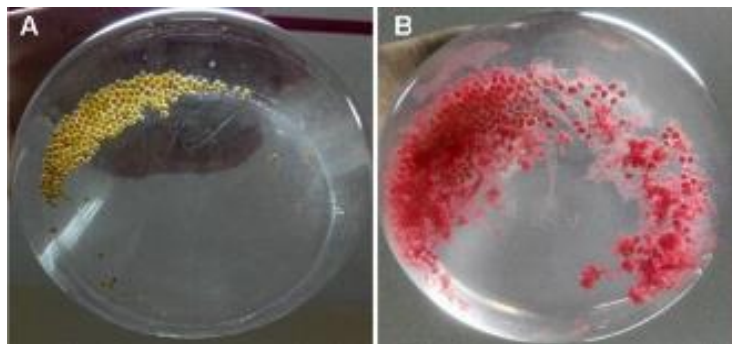
Normal eggs



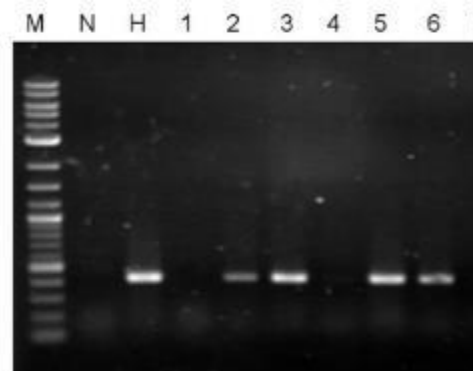
Red egg disease (10-50% loss)
(We named it "Hahellosis")



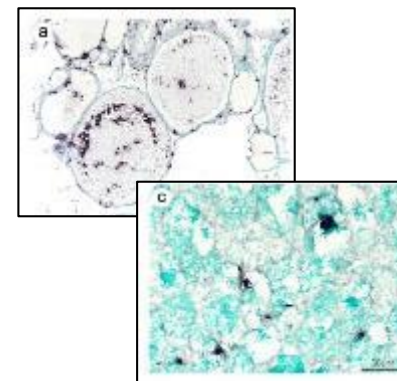
Red bacteria on TSA
(*Hahella chejuensis*)



Bioassay



PCR detection



In situ hybridization

Red egg disease “Hahellosis”

Knowing the enemy leading to the solutions

- *Hahella chejuensis* is a marine bacterium
- It is a halophilic bacteria
- Red egg problem occurs at temperature below 24 °C



- 💡 Reduce salinity from 7 ppt to 4 ppt
- 💡 Increase temperature (30 °C) by wrapping the hatcheries with plastic and using transparent roof
- 💡 Expose sand from the filter system to sunlight



Problem reduced to < 1%



Red egg disease “Hahellosis”

Economic impact

รายการ	จำนวน
(1) จำนวนไข่ปลาที่ผลิต การผลิตของฟาร์มที่ประสบปัญหา 2 ฟาร์ม (เฉพาะฟาร์มที่ส่งตัวอย่างในงานวิจัย) จำนวนไข่ปลา 1,000,000,000 ไข่/ฟาร์ม/ปี X 2 ฟาร์ม	2,000,000,000 ไข่
(2) จำนวนไข่ปลาในช่วงเวลาที่มีปัญหาไข่ปลานิลไม่ฟักตัวและเปลี่ยนเป็นสีแดง (ช่วงอากาศเย็น) 3 เดือน 2,000,000,000 ไข่ / 12 เดือน X 3 เดือน	500,000,000 ไข่
(3) จำนวนการลดการสูญเสียไข่ปลาที่ติดเชื้อไม่ฟักตัว (ก่อนมีโครงการสูญเสีย 30-50% หลังมีโครงการสูญเสียน้อยกว่า 1% =500,000,000 X30%)	150,000,000 ไข่
(4) มูลค่าการลดจำนวนไข่ปลาที่ติดเชื้อไม่ฟักตัว (จำนวนการลดการสูญเสียไข่ปลาที่ติดเชื้อไม่ฟักตัว X มูลค่าลูกปลา 0.125 บาท/ตัว*)	<u>18,750,000 บาท</u>



550,000 USD per year

Tilapia lake virus (TiLV)

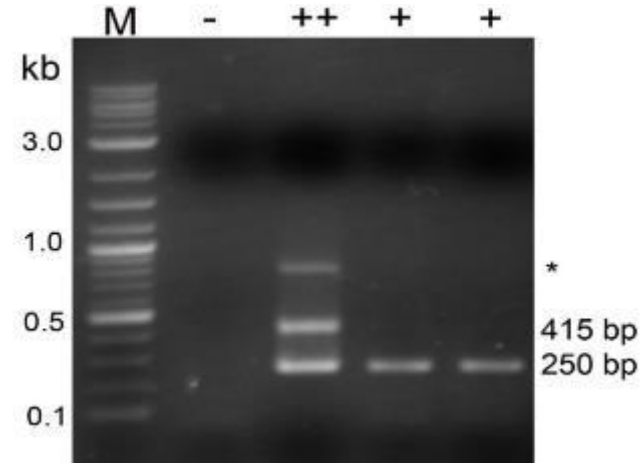
Novel Orthomyxo-like virus



- Syncytial Hepatitis of Tilapia (SHT) in Ecuador (Ferguson et al. 2014)
- TiLV in Israel (Eynigor et al. 2014)
- Complete genome of TiLV (Bacharach et al. 2016)
- TiLV reported in Colombia, Egypt, Thailand, Taiwan, Malaysia, India, Philippines, Uganda, Tanzania (Kembou Tsofack et al. 2017; Fathi et al. 2017; Nicholson et al. 2017; Surachetpong et al. 2017; Dong et al. 2017; OIE 2017; Amal et al. 2017; Behera et al. 2017, Mugimba et al. 2018)

Tilapia lake virus (TiLV)

Occurrence in Thailand and alternative RT-PCR protocol



- Primers from Eyngor et al. 2014
- Semi-Nested PCR (Dong et al 2017)

Free positive control plasmid for non-commercial use
in response to 36 requests from 20 countries

Tilapia lake virus (TiLV)

Publications & Training



Emergence of tilapia lake virus in Thailand and an alternative semi-nested RT-PCR for detection

H.T. Dong^{a,b,*}, S. Siriroob^b, W. Meemetta^b, W. Santimanawong^b, W. Gangnonngiw^{b,c}, N. Pirarat^d, P. Khunrae^a, T. Rattanarojpong^a, R. Vanichviriyakit^{b,c}, S. Senapin^{b,d,e,*}



Short communication

Evidence of TiLV infection in tilapia hatcheries from 2012 to 2017 reveals probable global spread of the disease

H.T. Dong^{a,b,*}, G.A. Ataguba^c, P. Khunrae^a, T. Rattanarojpong^a, S. Senapin^{b,d,e,*}

Aquaculture 487 (2018) 51–55



Short communication

Inapparent infection cases of tilapia lake virus (TiLV) in farmed tilapia

Saengchan Senapin^{a,b}, K.U. Shyam^d, Watcharachai Meemetta^d, Triwit Rattanarojpong^c, Ha Thanh Dong^{a,*}



Training on TiLV diagnosis (PCR and histology)

Research on fish diseases



Nile tilapia (*Oreochromis niloticus*)
Hybrid red tilapia (*O. mossambicus* x *O. niloticus*)

- *Hahella chejuensis*
- Tilapia lake virus (TiLV)



Barramundi / Asian Seabass
(*Lates calcarifer*)

- *Vibrio harveyi*
- Scale drop disease virus (SDDV)

Scale drop and muscle necrosis (SDMN) disease

Caused by *Vibrio harveyi*



Up to 40%
cumulative
mortality

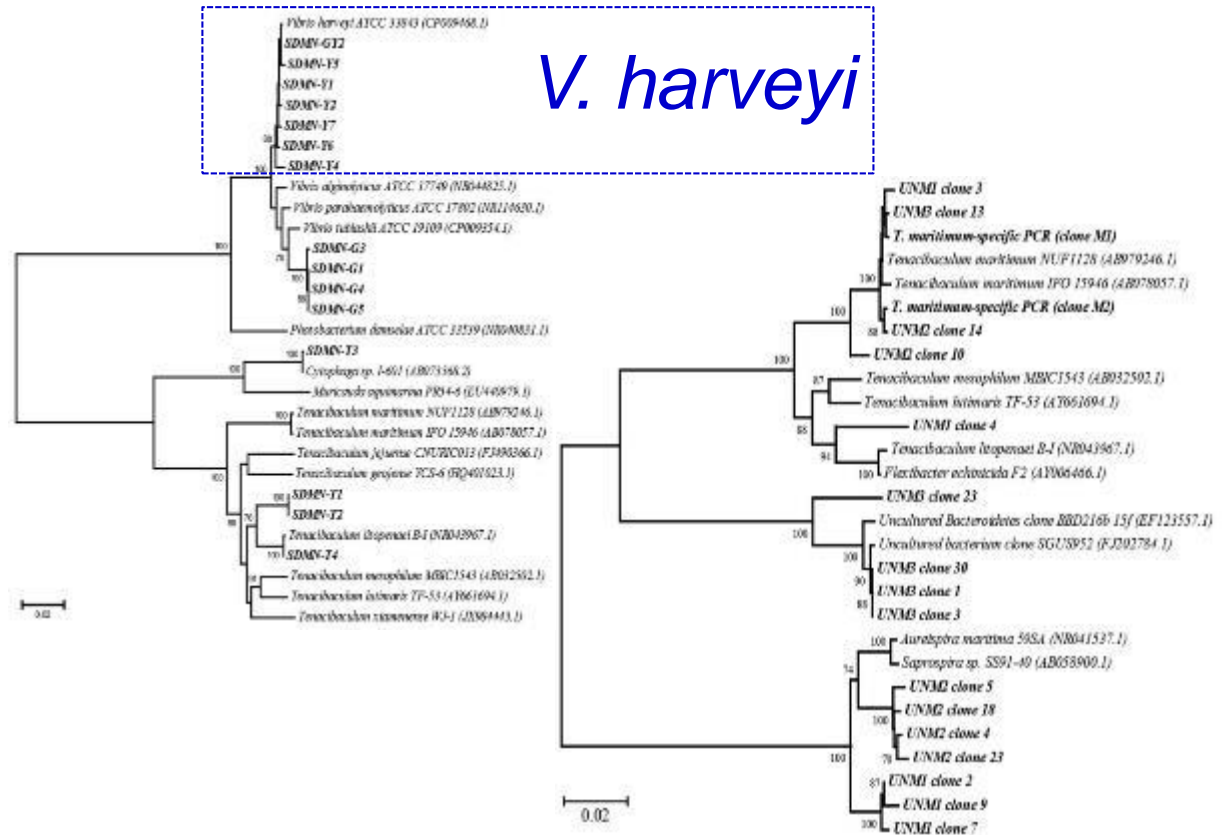
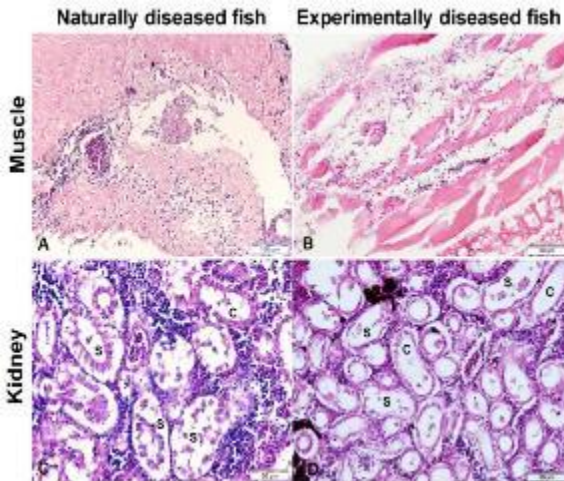
Scale drop and muscle necrosis (SDMN) disease

Caused by *Vibrio harveyi*



Scale drop and muscle necrosis (SDMN) disease

Caused by *Vibrio harveyi*



Scale drop and muscle necrosis (SDMN) disease

Caused by *Vibrio harveyi*

Aquaculture 473 (2017) 89–96



Contents lists available at ScienceDirect

Aquaculture

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



Recovery of *Vibrio harveyi* from scale drop and muscle necrosis disease in farmed barramundi, *Lates calcarifer* in Vietnam



H.T. Dong^{a*}, S. Taengphu^b, P. Sangsuriya^{cd}, W. Charoensapsri^{bcd}, K. Phiwsaiya^{bcd}, T. Sornwatana^e, P. Khunrae^a, T. Rattanaojpong^a, S. Senapin^{bd,*}

Journal of Applied Microbiology



Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Genome characterization of piscine 'Scale drop and Muscle Necrosis syndrome'-associated strain of *Vibrio harveyi* focusing on bacterial virulence determinants

P. Kayansamruaj^{1,2}, H.T. Dong³, I. Hirano⁴, H. Kondo⁴, S. Senapin^{5,6} and C. Rodkhum^{1,7}

¹ Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

² Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand

³ Aquaculture Vaccine Platform, Department of Microbiology, Faculty of Science, King Mongkut's University of Technology Thonburi, Bangkok, Thailand

⁴ Laboratory of Genome Science, Tokyo University of Marine Science and Technology, Tokyo, Japan

⁵ Center of Excellence for Shrimp Molecular Biology and Biotechnology (Cemex Shrimp), Faculty of Science, Mahidol University, Bangkok, Thailand

⁶ National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency, Pathumthani, Thailand

⁷ Fish Infectious Diseases (FID) – Special Task Force for Activating Research (STAR), Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

Highlights

- A pathogenic *V. harveyi* strain was identified as the main causative agent.
- Coinfections of culturable and unculturable bacteria were uncovered from diseased fish.
- The role of unculturable bacteria needs further investigation.

Highlights

- Genome of *V. harveyi* Y6 was incorporated by a bacteriophage.
- 17 potential virulence genes were present exclusively in the strain Y6.

Scale drop disease

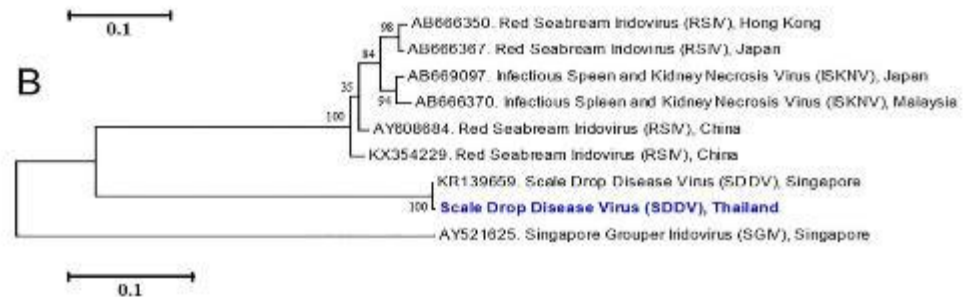
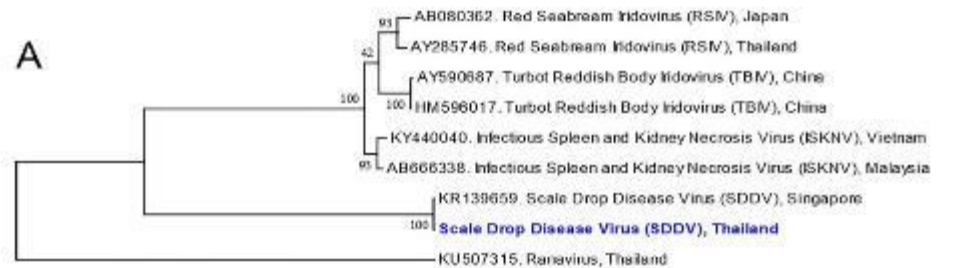
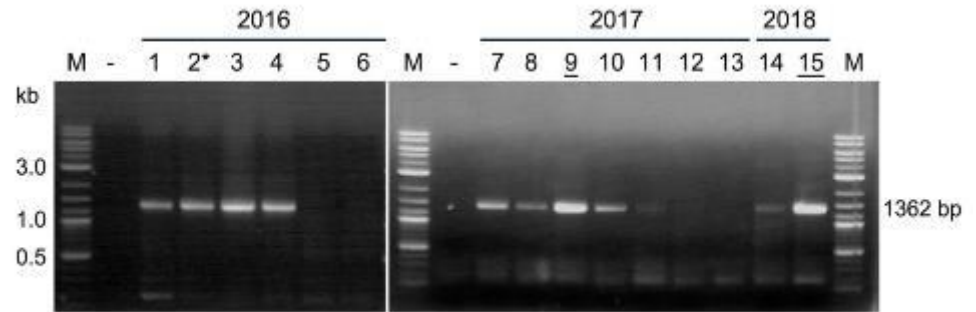
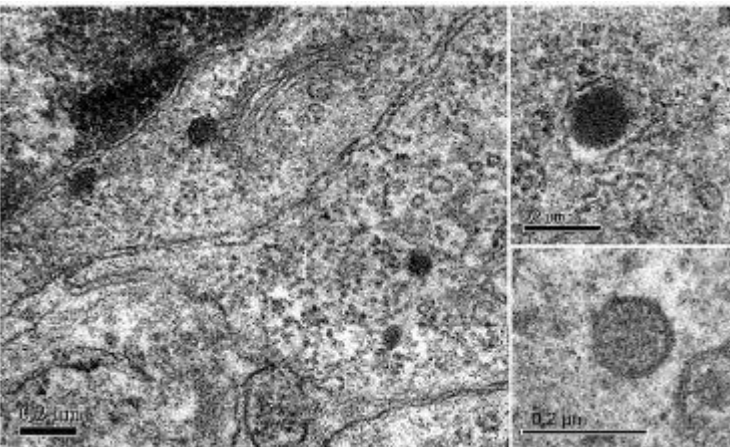
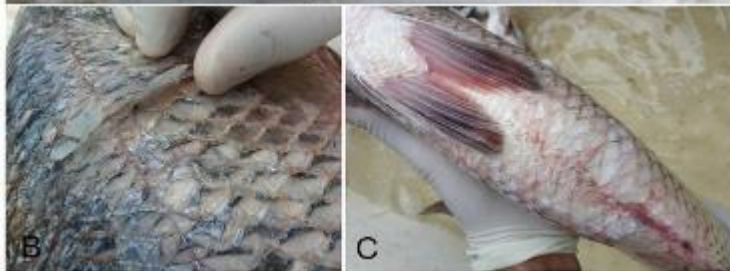
Caused by Scale drop disease virus (SDDV)



40-50% cumulative mortality

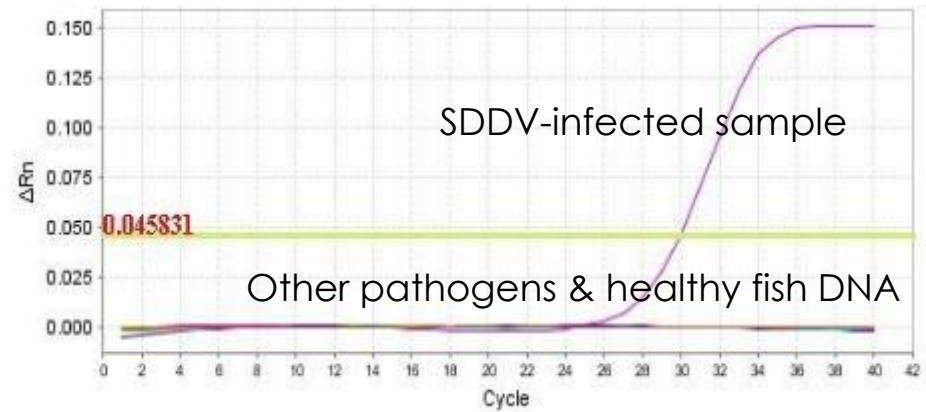
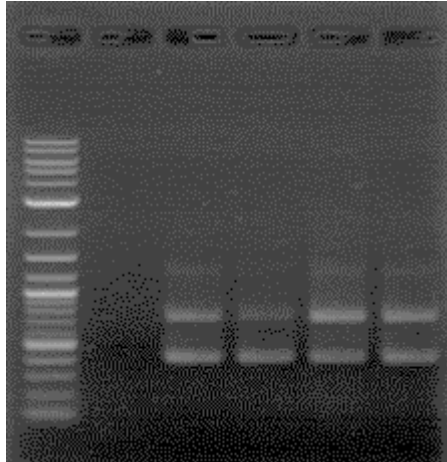
Scale drop disease

Caused by Scale drop disease virus (SDDV)

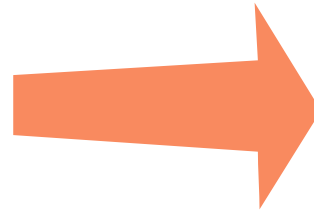


Scale drop disease virus (SDDV)

Occurrence in Thailand and PCR detection protocols



From Research to Solutions



Solutions to disease problems



Isolation, Identification & Proof of causation

Fish pathogen detection kits & service



Research and Development of Fish Vaccines, Immunostimulants



Bio-resource & Culture collection



Comprehensive analysis of Molecular pathogenesis (Omics analysis)



Lab training & Reference Lab



Research Team

Centex Shrimp/BIOTEC
Mahidol University



Chulalongkorn University

Kasetsart University
Burapha University

Asian Institute of Technology

Private companies & Non-profit organizations

Dr. Saengchan Senapin
Dr. Vanvimon Saksmerprome
Mr. Warachin Gangnonngiw
Ms. Kornsunee Phiwsaiya
Ms. Sarocha Jitrakorn
Prof. Tim W. Flegel
Prof. Boonsirm Withyachumnarnkul
Assist. Prof. Rapeepun Vanichviriyakit
Assoc. Prof. Bhinyo Panijpan
Assoc. Prof. Pintip Ruenwongsa
Dr. Pakkakul Sangsuriya

KMUTT

Assist. Prof. Triwit Rattanarojpong
Assist. Prof. Pongsak Khunrae
Dr. Ha Thanh Dong

Assist. Prof. Dr. Channarong Rodkhum
Assoc. Prof. Dr. Nopadon Pirarat
Mr. Vuong Viet Nguyen

Dr. Pattanapon Kayansamruaj
Dr. Molruedee Sonthi
Dr. Krishna R Salin

Acknowledgments

- ❖ Information and media from the internets
- ❖ Granting agencies

