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

Sukosol Hotel
Bangkok, Thailand
23-25 June 2016

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BACKGROUND

Outbreaks of acute hepatopancreatic necrosis disease (AHPND), commonly known as "early mortality syndrome" (EMS), caught the entire shrimp industry, the academe and the government sectors by surprise, and it took a long while to unravel its mystery because the disease broke through all biosecurity measures. While the industry has been dealing with vibriosis in all phases of culture for decades, nobody thought that a *Vibrio* would become an industry game-changer. The disease calls for a combination of basic, new and innovative strategies in biosecurity and control, and since the pathogen is ubiquitous in the environment, an exclusion strategy may not be possible. Sharing the responsibility among the government, academe and producer sectors has become essential.

The “Second International Technical Seminar/Workshop on AHPND: There is a way forward” (Bangkok AHPND June 2016) is a follow-up one year after the “First International Technical Seminar/Workshop: EMS/AHPND – Government, scientist and farmer responses” held in Panama City from 22-24 June 2015 (Panama EMS/AHPND June 2015, see **Annex 1 for summary highlights**). The Bangkok AHPND 2016 is also the 3rd international event organized by FAO on EMS/AHPND; the first one was held in Hanoi, Viet Nam from 25-27 June 2013 (Hanoi EMS/AHPND June 2013, see http://www.fao.org/docrep/018/i3422e/i3422e00.htm).

PURPOSE

The purpose of the Bangkok AHPND June 2016 is to update knowledge and exchange experiences in dealing with AHPND, to validate current concepts and models under different systems and environmental conditions and to put into action the responsibilities of the different sectors (i.e. government, producer and academe) as a way forward to deal with AHPND.

PROCESS

The event will last for three days (23-25 June 2016) with three technical sessions looking at EMS/AHPND through the lens of the industry, the academe and the government sectors and a fourth session on the way forward.

- Technical Session 1: Industry Update on AHPND
- Technical Session 2: Academic Update on AHPND
- Technical Session 3: National and International Framework Update

See **Annex 2** for some details of a tentative programme.

PARTICIPANTS

Official delegates from at least 15 FAO member countries participating in two inter-regional projects under the FAO Technical Cooperation Programme (TCP/INT/3501 and TCP/INT/3502); other FAO member countries participating on a self-funding mechanism; experts, scientists and producers; and representatives from regional and international organizations and other interested stakeholders. See **Annex 3** for tentative list of participants.

Venue:

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Annex 1
Highlights of the First International Technical Seminar/Workshop: "EMS/AHPND: Government, Scientist and Farmer Responses" (June 2015, Panama City, Panama) under the FAO project TCP/INT/3502[1]

[1] This highlight was based on the 21 technical presentations of government, academe and producers. The Panama EMS/AHPND June 2015 event was participated by more than 100 stakeholders from 21 countries representing the government, academe and producer sectors

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Annex 1

Highlights of the International Technical Seminar/Workshop: "EMS/AHPND: Government, Scientist and Farmer Responses" (22–24 June 2015, Panama City, Panama) under the FAO project TCP/INT/3502[2]

AHPND is currently the most important non-viral disease threat for cultured shrimp. It is characterized by mass mortality during the first 35 days of culture where affected shrimp show massive sloughing of hepatopancreatic epithelial cells followed by death. This emerging disease is unlike most diseases affecting farmed penaeid shrimp, in that it is caused by the ingestion of toxins (PirA and PirB) generated by a specific plasmid carried by certain strains of *Vibrio parahaemolyticus*, a bacterium that is ubiquitous in marine and brackishwater environments. The pathogen can thus be present both in cultured shrimp and in the water, sediments and associated organisms of the culture ponds.

The genus *Vibrio* comprises about 30 species of bacteria that generally require sodium chloride supplementation of the medium for growth. *Vibrio parahaemolyticus* occurs naturally in coastal and estuarine environments, in both tropical and temperate parts of the world, and has been isolated from water, sediment, molluscs, crustaceans, finfish and other animals. Environmental conditions such as temperature, salinity, zooplankton, dissolved oxygen and tidal flushing may affect the survival, establishment and growth of this organism. *V. parahaemolyticus* genome has several clusters of genes that have been acquired by horizontal gene transfer. Some of them (called tdh and trh gene clusters) are associated with pathogenicity to humans. AHPND-causing strains lack the gene clusters involved in pathogenicity to humans.

AHPND is a reportable disease in the Network of Aquaculture Centres in Asia-Pacific (NACA) Quarterly Aquatic Animal Disease (QAAD) reporting system: (http://www.enaca.org/modules/library/publication.php?tag_id=279&label_type=1&title=quarterly-aquatic-animal-disease-report). A request for the inclusion of AHPND in the List of Notifiable Diseases of the World Organisation for Animal Health's (OIE) was submitted in 2014, but the OIE Aquatic Animal Health Standards Commission (AAHSC) did not endorse the listing during its February 2014 meeting since AHPND did not meet all the criteria for disease listing. However, in March 2015, the AAHSC endorsed the listing of AHPND and a final decision to this effect was made during the OIE General Session held in May 2015. Reporting of AHPND to OIE commenced in January 2016.

Current state of knowledge about AHPND

The causative agent was discovered in 2013 as unique isolates of *Vibrio parahaemolyticus* (VP_{AHPND}) that carry a plasmid (pAP1) of approximately 69 kbp. This plasmid contains two genes that produce toxins (one 12.7 kDa and one 50.1 kDa) that are capable of acting together to cause AHPND. The Pir A/B toxin genes that code for the two toxin proteins that induce AHPND in shrimp have been reported to be similar to PirA/B toxin genes known from *Photorhabdus* spp. (Gram-negative, luminescent, rod-shaped bacteria that are members of the Family Enterobacteriaceae). In nature, *Photorhabdus* spp., that live in obligate, symbiotic relationship with the entomopathogenic nematode *Heterorhabditis* spp. and a closely-related genera *Heterorhabditis* spp., are parasites of insect larvae. *Heterorhabditis*/*Photorhabdus* have a wide geographic distribution and, since the 1980s, have been researched extensively for application in insect control. Fortunately, the VP_{AHPND} isolates characterized so far pose no threat to human health.

Current host and geographic distribution

AHPND first appeared in the People's Republic of China (around 2009 and was called Covert Mortality Disease), and has since been recorded from Viet Nam (2011), Malaysia (2011), Thailand (2012), Mexico (2013 from the scientific literature) and the Philippines (2015). It is suspected to be present in, but unreported from other countries in both Asia and Latin America and the Caribbean (LAC).

The disease infects mainly whiteleg shrimp (*Penaeus vannamei*), but has also been reported from giant tiger prawn (*P. mondon*) and fleshy prawn (*P. chinensis*).

[2] This highlight was based on the 21 technical presentations of government, academe and producers. The Panama EMS/AHPND June 2015 event was participated by more than 100 stakeholders from 21 countries representing the government, academe and producer sectors

Current status of detection methods to diagnose AHPND

The presumptive gross signs of AHPND in penaeid shrimp include an empty stomach and midgut, a pale and shrunken hepatopancreas, and mortality within approximately 35 days after stocking of postlarvae (PL). However, similar gross signs may occur with other diseases, thus, confirmation requires histological examination of the hepatopancreas to reveal the unique feature of the acute stage of AHPND, i.e. massive sloughing of cells of the tubule epithelium in the absence of any clear evidence of a causative agent.

To aid in the identification of reservoirs and potential transmission routes, two interim polymerase chain reaction (PCR) detection methods based on primers designated as AP1 and AP2 were introduced at the NACA website in December 2013 and later updated. AP2 turned out to be the better primer with about 3 percent false-positive results. Despite this weakness, the method was used successfully to reveal a high prevalence of VP_{AHPND} in live broodstock feeds (e.g. polychaetes and bivalves), in pond-reared and hatchery broodstock, and in PL used to stock shrimp farms. Testing in Thailand also provided evidence that specific pathogen free (SPF) stocks that had tested free of VP_{AHPND} became positive after use for PL production in some local shrimp hatcheries, providing clear evidence of biosecurity failures.

To overcome the problem of false-positive PCR test results, an improved PCR detection method (AP3) was developed based on discovery of the two AHPND toxins and on use of the gene sequence of the smaller 12.7 kDa toxin. The AP3 method, which was released at the NACA Website in June 2014, gave no false-positive or false-negative results with 104 bacterial isolates tested. Since the AP1 to AP3 methods for VP_{AHPND} detection were one-step PCR detection methods and could not be successfully modified into nested-PCR methods, samples with low pathogen loads had to be subjected to an enrichment step by culture in broth medium for 4 hr before separation of bacterial cells to prepare DNA template for the PCR assays.

To overcome problems with samples that could not be subjected to the enrichment step (e.g. samples preserved in alcohol or archived DNA samples), a nested-PCR method (AP4) was developed and announced at the NACA Website on 20 February 2015. It targeted the whole sequence of the 12.7 kDa toxin gene and 70 percent of the large toxin gene, and it gave 100 percent positive and negative predictive values for the same 104 isolates used to validate the AP3 method. However, it had 100 times higher detection sensitivity (down to 100 fg template DNA).

By cooperation between Centex Shrimp and the Sakarindrwirote University in Bangkok, antibodies have been produced against heterologously expressed AHPND toxins and used for detection by enzyme-linked immunosorbent assay (ELISA). This will allow for quantification of the toxins in feeds and the environment and for more convenient laboratory testing for therapeutic measures and resistant shrimp stocks.

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).

Other potential but as yet unconfirmed routes of disease transfer are by:

- crabs, crayfish and other crustaceans
- predatory birds and mammals
- attachment of flocs to zooplankton that are carried long distances by ocean currents
- attachment on crustaceans and in ships' ballast waters
- via untreated wastes from infected shrimp in processing plants
- via use of infected shrimp

Environmental factors that are believed to promote infection by VP_{AHPND} in shrimp ponds include:

- high concentration of nutrients in pond water by addition of fertilizers, molasses, etc.
- high water temperature, salinity >5 ppt and pH >7
- low water turnover coupled with low planktonic biodiversity

- presence of soluble nutrients (feed), unconsumed pelleted feed, shrimp carcasses, leading to accumulation of organic-rich sediment

Most cases of VP_{AHPND} have shown co-infection with other shrimp pathogens, for example, Monodon baculovirus (MBV), White spot disease (WSD), Hepatopancreatic virus (HPV), *Enterocytozoon hepatopenaei* (EHP) and unidentified gregarine-like entities.

Disease management

Several innovations in shrimp management have been targetted at reducing the number of VP_{AHPND} in the shrimp and its environment by promoting bacterial diversity and control high numbers of pathogenic VP_{AHPND}. This have been achieved by disinfection of water, use of reservoirs to microbially mature water, use of probiotics, clean feeds and screening of broodstock and PL.

Other effective management measures are primarily at the farm-level. These include:

- ensuring good farm biosecurity and best management practices (BMPs)
    - beginning with PL derived from broodstock verified to be free of AHPND (i.e. PL derived from SPF or high health (HH) broodstock)
    - avoid overfeeding as uneaten pellets are substrate for AHPND bacteria to grow
    - remove sediment as often as possible as it also serves as substrate
    - ensuring that all facilities and equipment are properly desinfected before stocking of PL (e.g. implementing cyclical dry-out and clean-up routines after every production cycle, involving careful cleaning and disinfection of all facilities, including the insides of air lines, pipes, water pumps and air pumps)
    - ensuring that live and treated feeds are free of infection (e.g. by sterilization of frozen material via gamma irradiation or pasteurization)
    - modifications to farm and pond designs to allow better biosecurity (e.g. use of smaller-sized ponds with plastic liners that can be fully drained, dried and disinfected between culture cycles)
    - using an increased number of reservoirs and water filtration to eliminate fish and other disease carriers
    - using water of a salinity of 5 ppt for growing shrimp
    - using water drawn from a deep well for growing shrimp
    - avoiding heavy chlorination pre-treatment of water
    - avoiding traditional fertilization schedules with commonly used products, especially if these strategies have been used previously and were found to not reduce AHPND losses
    - avoiding stocking ponds during the high-temperature season
    - applying “designer” pre- or probiotic preparations (if available)
    - applying “designer” phages that specifically target the VP_{AHPND} (if available)
- Management of culture systems to delay infections where AHPND is present in the culture environment by, e.g.:
    - stocking larger-size PL
    - co-culture of shrimp with finfish (e.g. tilapia) or use water from tilapia pond
    - use of appropriately designed grow-out systems which mitigate the environmental conditions that support high densities of VP_{AHPND} (i.e. central drainage)
    - stocking at appropriate density according to farm capacity
    - monitoring of shrimp health and removal of infected animals
    - if diseased shrimp are found, conducting laboratory analyses to aid decision making

The international spread of AHPND can be prevented or at least, reduced, by moving only live penaeid shrimp broodstock or PL that have been tested detected free from AHPND by use of the AP4 test. Another important measure is the use of fresh feeds that are free from infection. This could involve the use of treatment methods to ensure any bacteria present in the feeds is destroyed (e.g. by heating) or by the development of specific pathogen free (SPF) lines of polychaetes and clams for use in shrimp culture.

Actions of international and regional organizations

- **Network of Aquaculture Centres in Asia-Pacific:** NACA has listed AHPND as reportable by NACA member countries to its Quarterly Aquatic Animal Disease Reporting System. NACA has also prepared a AHPND Disease Card (updated June 2014) (http://www.enaca.org/publications/health/disease-cards/ahpnd-disease-card-2014.pdf) and routinely provides new information on AHPND on its Website (www.enaca.org).

- **Organismo Internacional Regional de Sanidad Agropecuaria (OIRSA, the Regional International Organization for Plant Protection and Animal Health):** OIRSA began actions related to APHND in 2013 with an official notice through its Website, complemented by virtual lectures to all member countries, with the aim of preventing the entry of this emerging disease into the region. A proposed "Regional Epidemiological Surveillance Program for EMS (RESPE)" was then put forward, in order to have a tool that includes suggestions for the development and establishment of AHPND surveillance.

- **World Organisation for Animal Health (OIE)**: AHPND has been on the OIE Aquatic Animals Health Standards Commission agenda since its emergence in 2010. In December 2013, the OIE developed an OIE Technical Fact Sheet on AHPND that was available on the OIE website. The information provided in this Fact Sheet reflected the epidemiological observations and research information available at that time on aetiology, epidemiology, diagnosis and prevention and control measures. In May 2015, after several years of discussion, OIE Member Countries agreed that AHPND met the OIE criteria for listing an OIE listed disease (as per Chapter 1.2. of the *Aquatic Code*) and adopted its listing as an OIE listed aquatic animal disease (in Chapter 1.3. of the *Aquatic Code*). Consequently, as of 1 January 2016 OIE Member Countries' must report to the OIE the presence or absence of this disease in their country. The objective of listing a disease is to support Member Countries efforts to prevent transboundary spread of important diseases of aquatic animals through transparent and consistent reporting. New chapters on AHPND to be included in the *Aquatic Code* and *Manual* are currently under development and have been circulated to Member Countries' for comments. Consequently, the factsheet has been removed from the OIE website.

- **Food and Agriculture Organization of the United Nations (FAO):** The FAO initiated work on understanding "early mortality syndrome" via the project TCP/VIE/3304 "Emergency assistance to control the spread of an unknown disease affecting shrimps in Viet Nam." The project produced a better understanding of the cause of the disease and identified a number of risk management measures and key areas for future research (Hanoi EMS/AHPND June 2013, see (http://www.fao.org/docrep/018/i3422e/i3422e00.htm). More recently, FAO is currently funding an inter-regional TCP project TCP/INT/3502 "Reducing and Managing the Risk of Acute Hepatopancreatic Necrosis Disease (AHPND) of Cultured Shrimp" aimed at providing a platform to improve the understanding of the disease through the lens of governments, scientists and producers and collectively generate practical management and control measures. The project's activities in 2015 with the holding of two back-to-back major interregional meetings in Panama City, were: (i) the International Technical Seminar/Workshop "EMS/AHPND: Government, Scientist and Farmer Responses", 22–24 June 2015 (Panama EMS/AHPND June 2015) and (ii) the First Inter-regional Workshop on EMS/AHPND Risk Management and Risk Reduction Strategies at National and Regional Levels, 25–27 June 2015.

Expert thoughts

"AHPND will serve as another game changer like WSD, necessitating a major change in the future direction of shrimp aquaculture towards relatively closed culture systems. In my opinion, the ultimate cause of the AHPND disaster was due to erosion of biosecurity due to complacency as a result of the overwhelming success of using domesticated and genetically improved SPF stocks of Penaeus vannamei for aquaculture in Asia". (Dr Tim Flegel, Bangkok)

"VP_{AHPND} and V. parahaemolyticus which do not have the capacity to induce AHPN have been reportedly isolated from shrimp sampled from the same pond in an AHPND outbreak. These results, as well as other observations indicate that VP_{AHPND} makes up only one of a mosaic of genetic strains of V. parahaemolyticus associated with shrimp and the pond environment where VP_{AHPND} occurs and in AHPND outbreak". (Dr Jim Brock, Hawaii)

"The introduction of AHPND to the Americas has once again emphasized the vulnerability of traditional culture systems and the need for a new production model for the 21st Century". (Mr Scott Edward Horton, Mexico)

"Excessive use of antibiotics to control AHPND has been reported in some countries. Alternate technologies such as those based on the use of bacteriophage therapy need to be developed for management of AHPND". (Dr Iddya Karunasagar, FAO)

"Understanding gives us reason to believe and not just hope". (Mr Robins McIntosh, Bangkok)

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Annex 2
Annotated Programme

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Annotated Programme

<table>
<tr><th>Date</th><th>Activities</th></tr>
<tr><td>22 June 2016, Wednesday</td><td>• Arrival of participants
• Registration open from 15:00-19:00</td></tr>
<tr><td>23 June 2016, Thursday</td><td>Day 1</td></tr>
<tr><td>07:30-08:30</td><td>• Registration</td></tr>
<tr><td colspan="2">Opening Session</td></tr>
<tr><td>08:45-10:30</td><td>• FAO Regional Office of Asia and the Pacific (Mr Vili A. Fuavao, Deputy Regional Representative for Asia and the Pacific)
• Thailand Department of Fisheries (Mr Adisorn Promthep, Director-General or representative)
• Self-introduction of participants
• Introduction to the Technical Seminar/Workshop and Highlights of the Panama Workshop (June 2015)</td></tr>
<tr><td>10:30-11:00</td><td>Coffee break</td></tr>
<tr><td colspan="2">Technical Session 1: Industry Update on AHPND</td></tr>
<tr><td>11:00-11:25</td><td>Presentation 1.1 Basic pond management to reduce current disease risks (Dr Pornlerd Chanratchakool)</td></tr>
<tr><td colspan="2">One major risk of AHPND and EHP outbreak is coming from infected/contaminated post-larvae (PL). Health checks and testing of PL for known pathogens before stocking is critical. Water treatment for ponds or closed recirculation systems should be adopted to reduce pathogen entry during the production cycle. To be profitable, farms need to increase production; therefore, overall farm management needs to be carefully adjusted.</td></tr>
<tr><td>11:25-11:50</td><td>Presentation 1.2 New paradigm for controlling AHPND in intensive culture systems: what works, what doesn’t and why
(Mr David Kawahigashi)</td></tr>
<tr><td colspan="2">The traditional management practices of “competitive exclusion” through the use of beneficial bacteria/biofloc, zero or low water exchange, and probiotic use continues to fail in culture systems where EMS Vibrio bacteria are ubiquitous. However, a new paradigm in shrimp culture, originating out of Thailand, has proven to be effective in controlling APHND outbreaks. This presentation will discuss this “new revolution” of intensive shrimp farming, where farmers in Asia and the Americas are changing to smaller grow-out ponds, center drains or “shrimp toilets”, high water exchange or recirculation capacity, polyculture, and nursery phases, and the genetic implications.</td></tr>
<tr><td>11:50-12:15</td><td>Presentation 1.3 Living with AHPND: an Asian experience
(Mr Mati Nitibhon)</td></tr>
<tr><td colspan="2">Since 2015, Thailand has shown promising increases in shrimp production. The recovery is largely the results of improvements by several business sectors such as the broodstock suppliers, PL producers and especially the shrimp growers. The new culture concepts can be grouped into three major systems, i.e.: (1) the clean/clear water system, (2) the brown water system and (3) the green water system.</td></tr>
<tr><td>12:15-13:45</td><td>Lunch break</td></tr>
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13:45-14:10	**Presentation 1.4 The industry response to AHPND in Mexico: a case study (Mr Dan Fegan)**
This presentation will look at the development of AHPND and the responses of the Mexican industry to the disease since 2013. Data from Mexican producers will be used to assess the effectiveness of some of the measures adopted, and some observations made on how the shrimp industry could use these experiences to improve the overall approach to health management in commercial shrimp operations.	
14:10-14:35	**Presentation 1.5 Infection trials with both AHPND and EHP: a hatchery protocol that can exclude AHPND (Dr Loc Tran)**
Laboratory studies on the efficacies of probiotics, bacteriophages, immunostimulants, herbal extracts, quorum quenching, acidifiers and toxin absorbents using a standardized challenge model revealed significant improvement in terms of survival rates when some oral probiotics, herbal extracts, bacteriophages and toxin absorbents were mixed in feed. .	
14:35-15:00	**Presentation 1.6 AHPND: Interaction, control and pond management (Dr Pikul Jiravanichpaisal)**
This presentation deals with the effects of *Vibrio* spp. (with emphasis on *V. parahaemolyticus*) in the digestive system on pathogenesis and immunity of shrimp and suggests a holistic approach to control.	
15:00-15:25	**Presentation 1.7 Transforming farm managers into the "family physicians" of their own ponds (Dr Grace Chang)**
Equipping farm managers with on-site molecular and microbiological diagnostic tools as well as AHPND-treating protocols is key to control and prevent AHPND outbreaks effectively.	
15:25-15:55	**Coffee break**
15:55-16:20	**Presentation 1.8 Surveillance and animal health monitoring, early detection of diseases (Mr Shuaib T. Muhammad)**
This presentation will focus on the design of a surveillance program for primary pathogens and a shrimp health monitoring system for early detection of diseases.	
16:20-17:45	• **Industry Panel Discussions**

24 June 2016, Friday	Day 2
Technical Session 2: Academic Update on AHPND	
08:15-08:40	**Presentation 2.1 Update June 2016 on AHPND and EHP research in Thailand (Dr Tim Flegel)**
The two new diseases of most current concern in Asia are acute hepatopancreatic necrosis disease (AHPND) caused by *Vibrio* species that produce PirVp toxins and hepatopancreatic microsporidiosis (HPM) caused by *Enterocytozoon hepatopenaei*. Progress in research on the pathogens and control measures is discussed.	
08:40-09:05	**Presentation 2.2 Polychaetes as a potential risk for shrimp pathogen transmission (Dr Desrina)**
Studies on the role of polychaetes in WSSV transmission revealed that these worms can harbor the virus without clinical signs of disease, and that most probably, polychaetes became infected inside the pond. The infection in polychaetes is related to the WSSV infection in shrimp. This information can be used as reflection to elucidate the role of polychaetes in APHND and EHP transmission.	
09:05-09:30	**Presentation 2.3 Heritability, cross-breeding and inbreeding effects on resistance of *Penaeus vannamei* to AHPND and WSSV in Mexico (Dr Hector Castillo)**
Experiments designed to estimate genetic parameters, crossbreeding and inbreeding effects on resistance of *Penaeus vannamei* to AHPND and WSSV in México, reveal that it is possible to develop lines of shrimp with greater genetic resistance to these diseases using both selection and crossbreeding.	
09:30-10:00	**Coffee break**
10:00-10:25	**Presentation 2.4 Genomic analysis of the strains of AHPND-Vp (global) and genomic target (Dr Bruno Gomez-Gil)**
AHPND is caused by more than one *V. parahaemolyticus* strain or even by another *Vibrio* species; these strains are probably local strains that have been "infected" by a plasmid that acquired a transposon. The plasmids have mutations in many genes that suggest a fast evolutionary process.	
10:25-10:50	**Presentation 2.5 Ecology, virulence factors and global spread of pathogenic *Vibrio parahaemolyticus* and related *Vibrio* spp. (Dr Iddya Karunasagar)**
This presentation outlines genetic elements described in AHPND-causing *V. parahaemolyticus* and *V. harveyi*. Ecological factors influencing survival and persistence in the environment are discussed. Evidence for global dissemination of *V. parahaemolyticus* through oceanic currents and El Nino is presented.	
10:50-11:15	**Presentation 2.6 Research progress and biosecurity control strategies against EHP and AHPND for shrimp farming in the People's Republic of China (Dr Huang Jie)**
This presentation introduces the research progress on the relationship between EHP infection and shrimp growth by qPCR, AHPND caused by new non-*V. parahaemolyticus* strains of *Vibrio* sp. and biosecurity control strategies for shrimp diseases.	
11:15-11:40	**Presentation 2.7 Epidemiology of AHPND: experiences in Viet Nam and Thailand (Dr Visanu Boonyawiwat)**
Results of cross-sectional epidemiological studies conducted in Viet Nam (2112-2013) and Thailand (2012-2015) are presented. Pond- and farm-level risk factors are discussed in this presentation.	
11:40-12:05	**Presentation 2.8 Characterization of non-*Vibrio* bacteria as potential associates of AHPND bacteria in *Penaeus vannamei* (Dr Kallaya Sritunyalucksana)**
Bacteria of the genera *Delftia* and *Shewanella* were investigated as either direct causes of shrimp mortality or as acting synergistically or in an additive manner to increase the virulence of AHPND bacteria. Laboratory challenges with natural isolates from diseased shrimp revealed high shrimp mortality accompanied by unique histopathology. Synergistic effects with AHPND bacteria were observed.	

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<tr><td>12:05-12:30</td><td>Presentation 2.9 Asian shrimp production and the economic cost of disease (Dr Andy Shinn)</td></tr>
<tr><td colspan="2">This presentation will focus on the major pathogens impacting Asian shrimp production and their effects on the host, the economic losses, and the significance of biosecurity and farm strategies for their control and management.</td></tr>
<tr><td>12:30-14:00</td><td>Lunch break</td></tr>
<tr><td>14:00-14:25</td><td>Presentation 2.10 Latest research on AHPND and measures to combat it (Dr Ikuo Hirono)</td></tr>
<tr><td colspan="2">This presentation will answer these three questions: (1) Is it only Vibrio parahaemolyticus causing AHPND?; (2) What is the virulence factor of it?; and (3) How to prevent it?</td></tr>
<tr><td>14:25-14:50</td><td>Presentation 2.11 Specific pathogen-free (SPF), specific pathogen-resistant (SPR) and specific pathogen-tolerant (SPT) as part of a biosecurity strategy (Dr Victoria Alday-Sanz)</td></tr>
<tr><td colspan="2">The use of SPF stocks has achieved levels of productivity never seen before which proves that heath status of the stocked PLs is key for the development of the shrimp industry. The type of pond biosecurity implemented will determine the use of SPT/SPR or susceptible animals to disease.</td></tr>
<tr><td>14:50-16:00</td><td>Academic Panel Discussions</td></tr>
<tr><td>16:00-16:30</td><td>Coffee break</td></tr>
<tr><td colspan="2">Technical Session 3: National and International Framework Update</td></tr>
<tr><td>16:30-16:55</td><td>Presentation 3.1 Global production and trade in shrimp and risks of pathogen transfer (Dr Rohana Subasinghe)</td></tr>
<tr><td colspan="2">This presentation will focus on the trends in global aquaculture production of shrimp over the past two decades and examines the consequences of disease outbreaks in loss production, trade and revenue.</td></tr>
<tr><td>16:55-17:20</td><td>Presentation 3.2 Aquaculture biosecurity challenges in the light of the Ballast Water Management Convention (Dr Guillaume Drillet)</td></tr>
<tr><td colspan="2">Ballast water exchange is evaluated to be responsible for about 1/3 of bio-invasions. To decrease the rate of new invasions, the Ballast Water Convention from the International Maritime Organization was developed and contains a biological Standard (Regulation D-2) as an endpoint. This standard, however, mainly focuses on large organisms that are not representing most of the biosecurity risks that aquaculture is facing. There are modeling capacities which can be used that can help define mitigation measures to be offered to the aquaculture industry.</td></tr>
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25 June 2016, Saturday	Day 3
Technical Session 3: National and International Framework Update (continued)	
08:15-08:40	**Presentation 3.3 Dealing with AHPND: Viet Nam experience (Dr Dang Thi Lua)**
The presentation will inform about the AHPND situation in Viet Nam, including the first outbreak, its spread throughout the country, the government's actions and the support from international organizations. It also describes how the shrimp industry in Viet Nam recovered from the disease. Lesions learnt as well as the way forward to deal with AHPND are also included in this presentation.	
08:40-09:05	**Presentation 3.4 Dealing with AHPND: Thailand update from 2015 (Dr Putth Songsangjinda)**
The presentation will focus on the reduction of production of farmed shrimp after the outbreak of AHPND in Thailand and its recovery. The prevalence of AHPND outbreak and monthly shrimp productivity were compared Launching of good aquaculture practices for disease-free Pacific white shrimp (*Litopenaeus vannamei*) in hatcheries as a mandatory standard is also discussed.	
09:05-09:30	**Presentation 3.5 AHPND biosecurity measures: Kingdom of Saudi Arabia experience (Dr Saad) (waiting confirmation)**
09:30-09:55	**Presentation 3.6 Avoiding AHPND: Iran experience (Dr Ghahari and Dr Reza)**
This presentation will focus on the history of Iran shrimp culture and its progress in this decade. Faced with an outbreak of WSSV in 2003, shrimp culture in Iran has tried to overcome disease problems. In this way, we learned how to implement an effective surveillance system and act promptly inspite of strengthened biosecurity in shrimp establishments. Our experiences with WSSV will help us avoid AHPND.	
09:55-10:25	**Coffee**
10:25-10:50	**Presentation 3.7 Dealing with shrimp diseases in Brasil (Public Policies for Marine Shrimp Health) (Dr Rodrigo Roubach and Dr Eduardo Cunha) to be presented by Dr Thales Passos de Andrade**
The presentation will focus on the major public policies implemented in Brazil for shrimp disease as well as the major constraints in the area.	
10:50-11:15	**Presentation 3.8 The World Organisation for Animal Health (OIE): relevant activities regarding AHPND (Prof. Mohammed Shariff)**
The presentation will provide information about relevant activities of the OIE regarding the prevention and control of AHPND.	
11:15-11:40	**Presentation 3.9 Status of AHPND in the NACA/FAO Quarterly Aquatic Animal Disease Reporting System (Dr Eduardo Leano)**
11:40-11:05	**Presentation 3.10 Progress on AHPND actions in OIRSA countries: 2015 onwards (Dr Vielka Morales)**
The presentation is about the strategies and supporting documents developed and implemented by OIRSA and its 9 member countries in Central America, for the prevention, control and eradication of endemic and emerging farmed penaeid shrimp diseases with emphasis on AHPND.	
11:05-12:30	**Presentation 3.11 Updates on AHPND in Southeast Asia: outcomes on a recent regional technical consultation on AHPND (Dr Rolando Pakingking)**
The presentation will cover outputs of the 'ASEAN Regional Technical Consultation on EMS/AHPND and other Transboundary Diseases for Aquatic Animal Health Management' organized by SEAFDEC/AQD in February 2016 particularly the current status of AHPND in farmed shrimps in ASEAN Member States and Japan (prevalence of disease, diagnostic methods employed, approaches used in managing the disease), scientific research done and research gaps; and policy recommendations and priority areas for R&D collaboration to address the gaps.	

12:30-14:00	**Lunch**
14:00-14:25	**Presentation 3.12 AHPND Contingency Plan under the framework of the Andean Community of Nations (CAN)** **(Dr Margy Aliethe Villanueva Soto on behalf of CAN)**
Activities carried out by member countries of the Andean Community of Nations (CAN) in the establishment of an Andean regional legal framework for the prevention, surveillance, control and eradication of diseases in aquatic animals and the Andean Contingency Plan for AHPND of farmed shrimp will be presented.	
14:25-15:30	**National and International Framework Panel Discussions**
15:30-16:00	**Coffee**
Closing Session	
16:00-17:00	• Presentation of Seminar Highlights and Discussions on the Way Forward
17:00-17:30	• Thailand Department of Fisheries Closing Remarks
	• FAO Closing Remarks

Annex 3

List of Participants

(as of 17 June 2016)

List of Participants (as of 17 June 2016)

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Annex 4
Extended Abstracts of Presentations
Technical Session 1:
Industry Update on AHPND

NOTES

Basic pond management to reduce current disease risks

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Shrimp farmers are aware that health problems in shrimp ponds are caused by a multitude of factors such as postlarval quality, pond water and soil management, feed management and pathogens. Disease prevention requires an understanding of the dynamic interactions between these factors. Failure to manage one factor, especially water and soil quality can have negative impacts on the others and lead to severe problems in the pond culture system. Recently, many studies on AHPND and EHP have confirmed that these diseases can be caused by the introduction of infected postlarvae (PL) or contaminated water, as well as by pathogens already present inside the facility. To avoid these diseases, care should be taken to eliminate or reduce pathogen contamination during the culture period.

As the introduction of contaminated/infected PL has proven to be a major risk factor for AHPND and EHP outbreaks, conducting a general health check, the testing of PL for known pathogens and quarantining nursery tanks and ponds is becoming more common in Asia. Most farmers request that the hatchery provide a PL test report before buying. The general health check includes PL > 10, fulll gill development, and healthy and normal looking hepatopancreatic tubules. Some hatcheries also provide a *Vibrio* spp. count test report, which should show <1 000 CFU/g of total *Vibrio* and <100 CFU/g of green colony *Vibrio*. In addition, some farmers are also testing for other pathogens such as white spot syndrome virus, yellow head virus, and AHPND strains, as well as EHP. A salinity stress test made by placing PLin freshwater for 30 minutes and then returning them to 30 ppt for another 30 minutes, is commonly used, with an acceptable survival rate being above 95 percent.

The major cause of stress to shrimp is usually due to toxic conditions in the pond. During the early stage of the production cycle, dead algae, uneaten feed and dead phytoplankton deteriorate rapidly, causing a build-up of ammonia, nitrites or hydrogen sulphide, creating toxic conditions for the shrimp at around 20-30 days post stocking. A similar condition occurs at around 50-60 days of the culture period, when organic wastes increase. During these risky periods, shrimp become vulnerable to disease, especially stocks where pathogen numbers are increasing inside the pond.

To prevent such infectious diseases, the organic wastes remaining in the pond soil from previous harvests should be removed during pond and water preparation. During the cycle, there is a large amount of dead phytoplankton, algae and other microorganisms. This can have several consequences for the pond environment. Therefore, it is vital to minimize these adverse effects. This can be done by scalping the top soil using a metal chain or by other means (which is sometimes inpractical), to keep the top soil in an aerobic condition and to try to eliminate the dead plankton by enhancing the decomposition process. Recent studies have identified novel bacterial strains that have shown strong a confirmation of field activity to minimize the organic wastes or prevent or control toxic conditions. Practical and beneficial applications are being realized from diverse types of microbe, including the more common heterotrophs (*Bacillus*), as well as less common types such as chemolithotrophs (nitrifiers) and anaerobic H_2S oxidizers (*Paracoccus*). Heterotrophic *Bacillus* strains grow rapidly and are highly competitive for nutrients in the pond environment. The effective *Bacillus* strains can grow efficiently under a wide range of salinity and temperature conditions, producing a spectrum of complex enzymes which can reduce the organic wastes in the pond. The result is a much better balance between the heterotrophs and the phytoplankton, as well as a more balanced microbial population, minimizing the possibility of a single pathogen becoming dominant in the pond.

A major cause of phytoplankton over bloom is the accumulation of organic matter in the pond. In intensive white shrimp farms, over feeding during high temperature periods is very common, due to faster feeding and the higher metabolic rate of the shrimp. Organic wastes left in the pond can cause an over bloom of plankton, which finally crashes. Feeding management must be very tightly controlled during periods when the temperature is higher than 33 °C. Uneaten feed needs to be removed via a central drainage pipe or by siphoning.

Pond soil and water preparation to remove organic matter, as well as water treatment is essential to reduce the potential risk of disease, especially AHPND and EHP. Currently, culture systems are moving toward closed, semiclosed and recirculation systems to avoid pathogen contamination during the production cycle. In these systems, areas for sedimentation, biological treatment and water storage may require up to 60 percent of the total farm area. Since the production area is reduced, farmers compensate by increasing stocking densities (2-3 times) in deeper ponds (with liners) and with heavy aeration set up to maintain dissolved oxygen levels as high as 6 ppm at all times. To reduce or prevent pathogen growth and avoid the build-up of toxic conditions, the daily removal of sludge from the culture pond is widely recommended, However, this system requires a higher investment and high PL quality. On the other hand, many farmers are still using the old culture module, but are trying to split the culture cycle into two to three stages or to do partial harvesting once the biomass becomes close to the pond's maximum carrying capacity. Many farmers now stock PL in the nursery pond or tank for 10-45 days before transferring them to the grow-out pond. They try to keep the shrimp in the grow-out pond for a maximum of 60 days, then decide whether to transfer them to new pond, split the pond or make a partial harvest before the pond water and soil deteriorate or become unmanageable.

For the nursery system, PL 8-10 from the hatchery can be tested for the specific AHPND strains and for EHP, total *Vibrio* and total bacterial counts before being introduced to a nursery tank (3-200 tonnes), cage/pen inside the culture pond, tank or small pond, which can be indoors or outdoors.

The main objective is to make sure that the PL or juveniles are more robust and have less contamination by pathogens. Water treatment in the nursery tank is required before stocking to reduce the possibility of contamination. PL can be treated with specific treatments (disinfectants, antimicrobials, etc.) or given special feed during this phase. The PL will be in this phase for at least a few days or until up to 45 days, depending on the farm's requirement. The stocking density during this phase also varies (10-30 PL/L) based on the nursery period and design and the stocking size (0.2 - 3 g). The most critical points are water quality and feed management, since the stocking density is much higher than with direct stocking. Farm technicians' experience has become essential, since often they have neverworked with larval culture before.

For indoor tanks, maintaining the PL at high temperature (>32 °C for 7 days) has been recommended to reduce the risk from white spot disease and this seems to work in some countries during low-temperature crop.

One possible risk that could impact the results is the spread of pathogens into the entire population if there is contamination in the stock. So, PL must be tested against specific pathogens that could be of risk.

The criteria that the farmer uses to determine whether to transfer stock or to conduct a partial harvest is the maximum shrimp biomass in each pond. This number is variable and depends upon the farm facility and management. In general, each farm must have accurate information regarding the pond maximum biomass that can be maintained without causing water quality and shrimp health problems. At this point, if the shrimp have not yet reached a marketable size, a partial harvest can be performed. This method can help to reduce the risk of poor water and soil quality and resulting disease problems. For example, in Thailand, with common practice and standard farm operation, a rough estimation of maximum biomass is around 1.3-1.5 kg of shrimp per m^2 .

In conclusion, for profitable and sustainable shrimp farming, the major factors that need to be managed very well are: a stocking plan to avoid weather fluctuations, good pond preparation, selection of clean PL, maintaining optimal water and soil parameters through good feed management and avoiding the use of unapproved drugs and chemicals.

New paradigm for controlling AHPND in intensive culture systems: what works, what doesn't, and why

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Shrimp farmers throughout Asia have been trying to overcome the devastating affects of early mortality syndrome (EMS) or APHNS since 2010 with little success. Traditional management practices such as low-exchange biofloc systems, enhanced biosecurity and competitive exclusion in traditional outdoor ponds continue to have high failure rates.

However, over the past couple of years, a new paradigm shift in conventional pond management has emerged in Thailand and is spreading to shrimp culture areas worldwide. This new wisdom in pond management is likened to how shrimp hatcheries are managed, where larval tank environments are kept clean and all parameters monitored throughout the cycle.

This presentation will discuss the reasons why traditional management practices no longer work in culture systems where EMS *Vibrio* bacteria are ubiquitous. Successful *Vibrio* control strategies now involve limiting inputs and eliminating organic accumulation on the pond bottom. The essential criteria and techniques that these "new age" farmers are now employing, such as modified pond bottoms or "shrimp toilets", water exchange and recycle capacity, polyculture, feeding strategies, nursery phases, and optimizing genetic potential are all addressed in this presentation.

Living with AHPND: an Asian experience

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After the outbreak of EMS in the Southeast Asian countries, all stakeholders in the shrimp business have been suffering from this disease. Shrimp growers are a majority among these stakeholders; many have tried several available anti-EMS products; however, most of them have not achieved a successful outcome in their crop as they had expected. The so called “quick & easy” method could not solve the EMS problem.

Thailand is one of the countries that suffered from this disease. Thai shrimp production was reduced from over 600 000 tonnes to less than 250 000 tonnes. It is only recently that many published papers have educated the sector about the real cause of EMS and the nature of the disease. With helpful assistance from the Thai Government and the academic institutes, shrimp growers have learned about the diseases and adapted their farming practices to cope with the problem.

Since 2015, Thailand has shown a promising recovery in shrimp production. Feed sales and PL sales have increased as a result, and both of them still show a significant upward trend in 2016. Thailand's recovery is largely also due to several business sectors such as the broodstock suppliers, the hatchery and PL producers and the grow-out farms.

Broodstock suppliers continue to research and enhance their animals; however, they are still not tolerant enough. Farmers still need to stock PLs in a clean environment and manage the water quality and pond bottom accordingly. After the outbreak, many of the hatcheries are consistently monitoring EMS in their PLs by PCR technique. Quality control of PLs by microscopic examination for any sign of tubular constriction and melanosis in the hepatopancreas is necessary before their release.

The greatest improvement has come from the shrimp-growing sector. Many farmers have rearranged their farm layout and have reconstructed the pond bottoms. HDPE plastic lining is widely used to cover slopes and bottoms of the ponds. Some farmers have invested in industrial water treatment in order to treat the in-coming water. In the farm, an on-site nursery is often used in order to condition the PLs before stocking.

Success stories at the farm level using different techniques against EMS have been widely published in local papers and social media. Some have even presented their papers in the international venues. The approach can be generally grouped into three major systems: 1) the clean/clear water system, 2) the brown water system and 3) the green water system.

The clean/clear water system is generally referred to as the system that concentrates on controlling organic load in the pond. Sometimes it is also called “an aquarium technique”. No waste is left in the pond, and feeding is strictly controlled, the feed commonly being distributed by an automatic feeder. Feed is not be left in the water for too long before being consumed by the shrimp. Uneaten feed, fecal matter and molt castings are mechanically removed via a sludge pump.

In the clear water system, high water transparency is appreciated by farmers. A transparency of 50-60 cm is a target in the first month and not less than 40 cm in the following month. High water exchange rates (sometimes up to 500 percent per crop) are used to achieve this goal. With low organic matter in the pond and water column, bacterial growth (including that of EMS toxin-producing *Vibrio*), can be minimized at a level that is not harmful to overall production.

The brown water system is an opposite approach to the clear water system, but many famers have demonstrated a benefit against EMS similar to that of the clear water system. The brown water system is sometimes called the bio-floc system. With prebiotics and inoculation of friendly bacteria, farmers promote a friendly heterotrophic bacterial population to overcome the growth of EMS-*Vibrio*. However, this approach is not easily replicated. Farmers with large ponds often fail to establish a healthy floc. However, high-yield shrimp production can be achieved in relatively smaller-sized ponds (less than0.8 ha).

The last group is called “the green water system”, which includes the use of freshwater, tilapia water and the bore (well) water technique. This approach originated from the tilapia farms. Many tilapia farms in Thailand also stock a small number of PLs of *Penaeus vannamei* in the fish ponds in order to gain extra income, with no EMS outbreaks reported in these farms. In Viet Nam, the benefit of co-culture has also been reported. Some scientific papers have also reported the benefit of tilapia water against vibriosis. For low-salinity (5 ppt or less) areas or where underground water is used, the benefit of freshwater is that it clearly limits the virulence of EMS V*ibrio*.

In conclusion, the core of living with EMS can be summarized into three parts, starting with healthy PL, stocking into ponds that have stable conditions, and the swift management of wastes in the pond bottom by any means. All of these actions are needed to achieve the goal of low *Vibrio* count in the pond. With relentless improvement in all sectors, especially for Thai farmers, better overall production of shrimp, a higher survival percentage and better growth are being achieved.

The industry response to AHPND in Mexico: a case study

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Acute hepatopancreatic necrosis disease ("early mortality syndrome" or EMS) was first detected in Mexico in the 2013 shrimp production cycle. That year, losses amounted to around 40 percent of the crop (from approximately 100 000 tonnes of production in 2012 to around 60 000 tonnes in 2013).

At the same time, Asia was also experiencing serious losses due to EMS that had been ongoing since 2010-11. Globally, several groups were involved in research to determine the causes and risk factors for the mortalities, and producers were adopting various measures in an attempt to limit losses. Throughout 2013, much of this information was shared with growers in Mexico through the efforts of individuals and businesses with connections to both regions. This gave producers in Mexico the opportunity to keep up to date with the latest thoughts on the disease in Asia and to try out some of the measures that had been suggested from Asia.

Following the 2013 crop cycle, a two-day workshop was held in Ciudad Obregon sponsored by Cargill Animal Nutrition.This brought together 42 key players from industry, government and academia together with experts from the United States of America and Asia and included PL suppliers, producers, research centers, national aquaculture health committees, aquaculture authorities and feed companies. The objective of the meeting was to review the then current state of knowledge of EMS, identify information gaps and plan activities for the 2014 crop cycle. Following a day of updates on the global and Mexican EMS situation, participants shared experiences and worked on a series of action plans for the 2014 season which were shared with groups that did not attend the workshop.

The review of the 2013 crop cycle raised some interesting questions, although rigorous epidemiological data were lacking, partly through the numerous potential factors and the relatively small database of farms/ponds available. There was some indication that different genetic stocks varied in their susceptibility to EMS, that different stocking strategies (direct stocking vs 2-phase growout) could have an impact, and that the use of some feed additives to reduce the colonization of the hepatopancreas and toxin production of *Vibrio parahaemolyticus* could reduce losses.

Research, much of which either conducted by or funded by the industry, was carried out before and during the 2014 crop cycle. Information gained from these trials and further review of the available information led to the adoption of a number of measures intended to reduce the risk factors that were thought to be associated with EMS outbreaks.

The application of these measures during 2014 was followed in a number of farms. This provided further insights into possible ways to mitigate EMS, as well as further testing the consistency of measures thought to be successful in 2013. Production in 2014 increased to over 70 000 tonnes due, at least in part, to some of the strategies and measures adopted following the 2013 season. The improvement continued in the 2015 crop season, as shrimp production further increased to over 90 000 tonnes.

Several key factors have likely played a role in the rapid recovery of the Mexican industry, not least the fact that each crop season is distinct, with a weather-controlled "fallow period" from November to March. This may have provided a buffer to reduce the exposure of individual farms to the pathogen. The level of collaboration and willingness to share information between the state and private sectors to address the problem also improved the ability to investigate quickly disease outbreaks and take remedial measures. The availability of detailed records for each farm and district is an invaluable resource for epidemiological investigations of disease that can give significant insights into potential causes and contributing factors. Finally, the high level of technical capability in the industry and the willingness to invest significant time and resources to address EMS is likely to have been one of the most important factors in Mexico's rapid recovery.

Infection trials with both AHPND and EHP: a hatchery protocol that can exclude AHPND

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Since early 2014, the Shrimp Vet Laboratory in Viet Nam has been involved in research related to the diseases' nature, and measures for prevention and control.

With regard to EMS/AHPND, the laboratory has studied the efficacies of probiotics, bacteriophages, immunostimulants, herbal extracts, quorum quenching, acidifiers and toxin absorbents using a standardized challenge model. In summary, some oral probiotics, herbal extracts, bacteriophages, and toxin absorbents mixed in feed showed significant improvement in terms of survival rates. With regard to field practices in managing EMS/AHPND, so far the only key to success is a holistic approach, which includes these key factors: disease-free and robust postlarvae (PL), corrrect pond preparation practices, conditioning of pond water (encouraging a balanced pond ecology with diverse populations of microorganism) before stocking, stocking with PL that have been reared in nurseries, better feed management, waste management (with central drainage), gut probiotics, and stable pond-water quality.The transmission of EHP, antiparasitic drugs, and chemicals against EHP were also studied at the lab. The pathogen has been detected from various sources of live feed, including bloodworms, bivalves and gastropods harvested from nearby hatchery areas by local fishermen. These seem to be carriers for this parasite. Transmission at hatcheries occurs after broodstock have been fed with contaminated live feed, with various stages of larvae and PL found positive to EHP.

A challenge model for EHP was developed at the Shrimp Vet Lab. in which live infected shrimp were used as inoculae (co-habitation). This challenge model seems to be effective in testing for efficacies of chemicals and drugs against the pathogen.

Based on the nature of the pathogens studied in the laboratory, some advances in hatchery practices were proposed in order to reduce the risk of disease transmission from hatcheries. Farming protocols that reduce the risk of these diseases were also developed and applied in the field.

AHPND: Interaction, control and pond management

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In addition to digestion of food and absorption of nutrients, the shrimp intestine establishes a strong defense against aggressions from the external environment. This important defensive task is composed of three essential parts: the mucosal barriers, the immune system and probably, the microflora. As the intestine is the entry point for many pathogens, the digestive or gastrointestinal (GI) tract and its defense mechanism are central to the health of the animal. Several diseases of shrimp, for example AHPND/EMS, vibriosis, gregarines and white faeces syndrome and very recently, *Enterocytozoon hepatopenaei* or EHP, originate or show clinical signs in the GI tract. Recently, it has been concluded that the causative agent of AHPND/EMS is *Vibrio parahaemolyticus*. To understand pathogenesis of shrimp under natural conditions, the interactions between shrimp and a pathogenic bacterium in the GI tract were investigated. Shrimp were infected via ingestion with pathogenic *Vibrio harveyi* (Vh) or *V. parahaemolyticus* (Vp) contained in *Artemia* as a feed, and by using scanning electron microscopy (SEM) and histopathological studies, we were able to show that shrimp had numerous bacteria attached randomly across the surface of the stomach, both as single and in large biofilm-like clusters at 6 h post ingestion. The persistence of highly virulent Vh or Vp resulted in the development of severe infections in the hepatopancreas and the upper part of the midgut, but not in the posterior midgut and hindgut or even in the circulatory system. In contrast to these observations, no such adherences or linkages were found when experiments were conducted with non-pathogenic *Vibrio* spp.

To understand the immune response in the stomach of shrimp to colonization of AHPND pathogen, transcriptome analysis was carried out to identify differentially expressed transcripts (DETs) in the stomach during *V. parahaemolyticus* strain 3HP (Vp3HP) infection. In total, 42 998 contigs were found. Of these, 1 585 contigs representing 1 513 unigenes were differentially expressed where 1 122 and 391 unigenes were up- and down-regulated, respectively. A total of 141 unigenes were recognized as immune-related genes. Expression profiles of 20 of 22 genes inferred from RNA sequencing were in accordance with those from qRT-PCR. Additionally, a novel isoform of an anti-lipopolysaccharide factor, PmALF7 was also discovered. Its full-length cDNA was 575 bp in length containing an ORF of 369 bp resulting in a polypeptide of 122 amino acids. Phylogenetic analysis indicated that it was allocated in the same group of a recently characterized PmALF6. PmALF7 was more abundantly expressed in the stomach and lymphoid organs than in other tissues ($P < 0.05$). Its expression level in the stomach was significantly increased for 26.8 and 52.8 fold at 6 and 12 hpi ($P < 0.05$) in shrimp fed with *Artemia*+Vp3HP. The results suggest that it is likely related to the immune response in the stomach against 3HP infection in *Penaeus monodon*.

Although shrimp have a very efficient immune system against bacterial pathogens which are ubiquitously found in natural waters, when shrimp are cultured under intensive conditions this often results in a decreased growth performance, reduced immune response and increased disease susceptibility. To control diseases caused by pathogenic bacteria, farmers have extensively used antibiotics and chemicals in the past. However, the non-sustainable strategy of antibiotic application is no longer recommended practice due to the appearance of bacterial resistance and the negative environmental impact, as well as threats to human welfare. Then the health and enhancement of the shrimp immune system are a prime concern and priority. Using probiotics, phage therapy or immune-stimulants is a more environmentally friendly approach, and these alternative approaches for the prevention and control of diseases in aquaculture are in high demand. The beneficial effects associated with the use of probiotics and Phage therapy to prevent and control AHPND-Vp in shrimp culture will be discussed in detail.

The ecosystem in shrimp-culture ponds comprises three key components, which are the environment, the host and the microbiota. The latter consists of both pathogenic and beneficial microorganisms. In nature, Vp has a wide range of niche specializations, from free-living forms to those attached to biotic and abiotic surfaces, from symbionts to pathogens, and it has the ability to form biofilms. The short generation time of Vp facilitates colonization where large numbers of cells can dominate the host in a short period of time, giving the bacterium an advantage that allows it to outcompete other bacteria.

When shrimp are growing, metabolic wastes and biological pollutants such as pathogens are accumulated in the pond, and this increases the risk of disease outbreak. Recently, many shrimp farmers have attempted to prevent pathogens (especially Vp) from invading and becoming persistent in the shrimp pond environment. They regularly remove the causing agent Vp, which accumulates within organic wastes on the pond bottom throughout the culture period. This approach has been successful in reducing the pathogen numbers and in maintaining a better environment for shrimp and as a result, farmers have been able to obtain high shrimp production without using antibiotics.

To allow us to understand the complex interplay between immunity, microbiota and feed in the shrimp gut, which can be considered as a small ecosystem, it is necessary to obtain more information on how they beneficially contribute to shrimp health Therefore, it is necessary to perform two types of research: basic research on one hand and applied research on the other hand. Basic research is needed to investigate the importance of the intestinal flora and the host immune system (particularly intestinal immunity) to understand the mechanisms of this amazing ecosystem present in the intestine. Applied research is required to select and elucidate the mode of action of probiotics, immune-stimulants or feed compositions for their practical use and efficiency.

While the impacts of AHPND are in decline, another disease which causes the condition called hepatopancreatic microsporidiosis (HPM) in *Penaeus vannamei* and *P. monodon* is caused by the microsporidian parasite *Enterocytozoon hepatopenaei* (EHP), which is increasingly being reported in association with white faeces syndrome, slow growth, morbidity and or mortality is having severe economic impacts on Asian shrimp production. Whether EHP infection is causing economic losses in penaeid shrimp hatchery and grow-out systems has become a hotly debated topic among both farmers and researchers.

Transforming farm managers into the “family physicians” of their own ponds

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Our family physicians take care of our daily healthy issues. They know us well, understand our health conditions, and provide the right medical treatment to us in a timely manner. For companion animals, poultry, or large consumable animals, qualified veterinarians are capable to identify the potential or ongoing health problems via diagnosis, and they then immediately provide advice or treatment based on the competence and experience. However, who should play the family physician’s role in shrimp culture? There are three basic criteria of this candidate– understanding the “patients” a.k.a the culture ponds very well, available all the time, and competent to apply the right and timely “first aid” to the “patients”.

In Northern China, an emerging concept of shrimp culture is getting popular. A natural biosecurity system by disinfecting the potential threats, such as the AHPND pathogenic strains and EHP, by the frozen sea water at winter time is applied. Combining the high price of the live shrimp, free geothermal heat, indoor greenhouse, and stocking pathogen free PLs, this model becomes practical and profitable, and provides consecutive successes since 2014. To avoid the potential threat from AHPND, pond managers are trained to familiarize the on-site molecular and microbiological diagnostics, and interpret the data by a unique guideline. When there is any potential risk, a prevention or even treatment protocol will start immediately.

Besides, the viral-free and EHP-free, AHPND-free PLs is the key of this system. To avoid any potential risk from live feed, such as artemia, a proper *Vibrio*-controlling protocol is applied in this system. A combination of treatment protocols is the backup for emergency needs. Some protocols focus on removal of the *Vibrio*, some are for toxin neutralization. Pond managers are trained to understand those protocols, and apply them as the first aid when the AHPND pathogenic strain is found. Those protocols have been tested in different countries under artificial infection or real infected ponds. The data suggested that the AHPND outbreak could be controlled by those treatment protocols, when the disease was at the early stage.

After equipping the pond managers with on-site diagnostic tools, including the hand-held PCR devices and ready-to-use microbiological culture agar plates, and the AHPND treatment protocols, they would be able to play the role of as family physicians of their own ponds. This strategy can help them to confirm and take care of the AHPND infection at the point of need, reduce the chance of disease spreading, and relieve the work load of the central laboratories.

Surveillance and animal health monitoring, early detection of diseases

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Disease in aquaculture systems are the outcome of three major components, namely, health of the animal, culture environment conditions and presence of pathogen in the system. The early detection of signs of diseases or poor health is crucial to take measures to minimize the economic impact of diseases. Routine animal health monitoring allows to generate this information for immediate decision-taking such as feeding rate correction, water exchange or even emergency harvest. A well-established surveillance program is the key to achieve the above mentioned results. The surveillance program is designed to focus mainly on primary endemic pathogens in key stages of production as well as in wild. It also includes exotic pathogens which are emerging or present a higher risk as these could be find the way through into the culture system via various means including water currents, importation of shrimp and fish from infected countries or ballast water.

This presentation describes about the current surveillance program at National Aquaculture Group, NAQUA, having around 4500 ha of culture surface. Since the main damage to shrimp industry of Saudi Arabia was caused by WSSV disease in recent years, the major part of the program is adopted for an early detection of WSSV and then the priority goes to TSV as the disease was present in the south of Saudi Arabia few years back. The frequency of sampling for endemic pathogens varies based on the economic impact of the disease, the type of rearing conditions (indoor or outdoor) and also depends on weather conditions as low temperature that does pose a risk factor. The frequency of exotic pathogen surveillance is based on their distribution, economic impact and the risk of introduction. Wild crustacean samples from the premises of farm (intakes, feeder canals etc.) is incorporated as part of surveillance and these samples are tested for endemic pathogens: WSSV and TSV.

This surveillance program also covers the existing *Penaeus vannamei* SPF program for all the OIE listed pathogens (WSSV, AHPND, IMNV, IHHNV, YHV and NHP) and other economically important pathogens (EHP, CMNV, HPV, MBV and BP).

This presentation also describes the shrimp health monitoring system and strategies. The objective of this program is to assess the health status of the animals in production systems and detect any possible buildup of diseases. The program describes the importance of obtaining the right sample, the different techniques used for analysis and interpretation of outcomes taking to account environmental factors. It also includes monitoring of critical points of culture systems like microbiological quality of algae and artemia (absence of green colonies in TCBS), quality of the post larvae for hatcheries and bird activity for the nursery and grow out ponds. It also elaborates the criteria of morphological changes that indicate deviation from optimum health, covering from macroscopic to microscopic changes, the possible causes that may lead to these changes and mitigation measures to decrease the risk of disease outbreak. It is emphasized that health is directly related to productivity and therefore, close monitoring and optimization of animal health is a key tool for profitable farming.

NOTES

Annex 4
Extended Abstracts of Presentations
Technical Session 2:
Academic Update on AHPND

NOTES

Update June 2016 on AHPND and EHP research in Thailand

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It has been reported that newly emerging acute hepatopancreatic necrosis disease (AHPND) of shrimp is caused by unique isolates of *Vibrio parahaemolyticus* (VPAHPND) (Tran *et al*., 2013), and that AHPND is one of several diseases that might be included under the heading "early mortality syndrome" (EMS) as it is used by shrimp farmers. Since the FAO meeting on EMS/AHPND in Panama in June 2015, the countries reporting the occurrence of AHPND outbreaks have not changed, although there are rumors that outbreaks have occurred but not been reported from India and countries in Central America in addition to Mexico (Enríquez-Espinoza *et al*., 2016; Nunan *et al*., 2014; Soto-Rodriguez *et al*., 2015). There is also one published report of a *Vibrio harveyi* (VH) isolate that causes AHPND and carries the pAP plasmid containing the PirAvp and PirBvp binary toxin genes (Kondo *et al*., 2015), possibly as a result of horizontal transfer from a VPAHPND isolate. This suggests that additional isolates of VH and perhaps isolates of other *Vibrio* species or even species of other bacterial genera may eventually be found to carry this plasmid and cause AHPND.

Recent work has shown variation in the virulence of VPAHPND isolates for reasons as yet unknown (Joshi *et al.*, 2014; Lai *et al*., 2015). It has been suggested that such variation may be related to variation in pAP copy number (e.g., 7 to 121 copies per cell) (Han *et al*., 2015) or to other virulence factors that may or may not be carried by pAP (Sirikharin *et al.*, 2015). These other factors may act to potentiate the Pirvp binary toxins or they may kill shrimp directly without causing the pathognomonic histopathology characteristic of AHPND [i.e., massive sloughing of hepatopancreatic (HP) tubule epithelial cells]. Support for this proposal can be found in a newly characterized VP isolate from Vietnam (unpublished). It contains a mutated PirAvp/PirBvp gene region on its pAP plasmid and does not produce either of the Pirvp toxins, but still causes 50% shrimp mortality without characteristic AHPND histopathology. Instead, the moribund shrimp show collapsed HP tubule epithelia. Similarly, an uncharacterized VP isolate from Thailand that gives a positive PCR test result for the PirAvp gene (AP3 method) also causes only 50% shrimp mortality accompanied by only collapsed HP tubule epithelia (Joshi *et al*., 2014). At the same time, dilution of VPAHPND isolate 5HP results in reduced shrimp mortality and the moribund shrimp also show collapsed HP tubule epithelia instead of AHPND histopathology (Joshi *et al.*, 2014). Similar results are obtained when mixed, heterologously expressed PirAvp and PirBvp toxins are diluted in shrimp challenge tests (Sirikharin *et al.*, 2015). More important, the concentration of these combined, heterologously-expressed PirAvp and PirBvp toxins needed to cause AHPND (20 µg/g shrimp) was 20 times higher than that needed from of the crude ammonium sulfate protein precipitate fraction (1 µg/g shrimp) obtained from the culture broth of the VPAHPND isolate (5HP) used as the positive control (Sirikharin *et al.*, 2015).

Obviously, it will be important to identify and characterize the other virulence factors in isolates that cause AHPND. In addition, the description of pathology from AHPND bacteria should be expanded to include the possibility of reduced morality accompanied by collapsed HP tubule epithelia rather than massive sloughing of HP tubule epithelial cells. These examples add to the data indicating that using the term "early mortality syndrome" (EMS) interchangeably with AHPND should be discouraged. We already know that a good proportion of early mortality in shrimp ponds is not the result of AHPND. For example, it may be caused by viruses such as WSSV and YHV, by environmental factors (e.g., low dissolved oxygen) or by bacterial pathogens other than those carrying an intact pAP plasmid. Thus, farmer reports of early mortality syndrome (EMS) cannot be equated with AHPND and reports of AHPND must be considered suspect if they are not accompanied by confirmatory laboratory tests.

In addition to variation in virulence of AHPND bacteria, it has been reported that some bacterial genera not previously associated with shrimp occur in a higher proportion in EMS ponds than in normal ponds (FAO, 2013). Using this information as a starting point, a new species of bacterium from the Order Berkholderiales has been isolated from AHPND ponds in Thailand (unpublished). This bacterium (tentatively called Delftia) is lethal to shrimp with virulence equal to that of highly virulent VPAHPND isolate 5HP (i.e., 100% mortality in 5 days at a concentration of 105 cfu/ml in bath culture for both bacteria). However, the Delftia isolate does not cause AHPND histopathology but instead a unique histopathology of its own. However, when the two isolates are mixed together in a bath challenge at a total of 2 x 103 cfu/ml (50 times lower concentration than either alone), 100% mortality occurred within 3 instead of 5 days, again without AHPND histopathology, revealing synergistic virulence by mixed bath challenge. Also being studied is an isolate of Shewanella obtained from an AHPND pond in Thailand (unpublished). Although less virulent for shrimp than the Delftia isolate above, it also acts in a synergistic manner to increase the virulence of 5HP. As above, mortality from such interactions could contribute to early mortality in shrimp but would not be called examples of AHPND according to current case definitions. Both of these isolates lack pAP and give negative PCR test results for the PirAvp and PirBvp toxin genes. Thus, we suspect that these bacteria have their own virulence factors that somehow potentiate the virulence of AHPND bacteria. These virulence factors also need to be identified.

The other pathogen of major concern in Asia at this time is hepatopancreatic microsporidiosis (HPM) caused by *Enterocytozoon hepatopenaei* (EHP). From reports from Thailand (Thitamadee *et al*., 2016), China (Liu *et al*., 2016), Vietnam (Ha *et al.*, 2010) and India (Rajendran *et al*., 2016), it is clear that this pathogen is endemic in the region and has been transmitted to imported, exotic stocks of *P. vannamei* that are derived from specific pathogen free stocks that do not carry the pathogen. It is now highly prevalent in cultivated P. monodon and *P. vannamei* in the region. It has been discovered in Thailand (Thitamadee *et al*., 2016) and China (Liu *et al.*, 2016) that HPM is associated with retarded shrimp growth that may not become clearly visible until the 2nd or 3rd month of cultivation. It has also been found that there is no apparent impact on growth if the copy number by quantitative PCR is not above 1000 per µg DNA, after which degree of retardation is directly proportional to the increase in copy number (Liu *et al*., 2016) and may result in progressive, slow mortality at numbers above 108 per µg DNA (unpublished).

There is a danger that living shrimp stocks exported from Asia or translocated within Asia for aquaculture may carry EHP, so it should be added to the list of pathogens to me monitored by quarantine authorities. PCR detection methods based on the ssu rRNA gene of EHP are available (Tang *et al.*, 2015; Tangprasittipap *et al*., 2013; Tourtip *et al*., 2009; Liu *et al*., 2016) and are suitable for testing post larvae and hepatopancreatic tissue of cultivated shrimp specimens. However, we now know that closely related microsporidians give cross reactions with these detection methods and that they are therefore not suitable for use in testing shrimp feeds or feed ingredients, in surveys for potential carrier species or in other environmental samples, since they may give false positive results. Instead, a recently developed nested-PCR method based on the spore wall protein gene of EHP is recommended instead (unpublished). It has been tested with DNA from microsporidian species closely related to EHP and does not cross-react as the ssu rRNA method does.

So far, there is no recommended or approved chemotherapy for HPM and the main focus for control is on supply of PCR negative post-larvae cultivated in ponds where appropriate biosecurity measures have been taken to exclude EHP. However, EHP is horizontally transmissible by feeding of infected hepatopancreatic tissue or by cohabitation of infected shrimp with naïve shrimp in the laboratory. The latter method is particularly suitable for testing of proposed chemicals and reagents designed to prevent transmission.

The spores have been purified from infected shrimp and the DNA has been purified for sequencing. It is hoped that sequence information will provide insights suitable for use in developing potential targets for HPM prevention and therapy. It was the source of information used to design the PCR method targeting the spore wall protein gene.

Polychaetes as potential risk for the shrimp pathogen transmission

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Polychaetes comprise the dominant meiofauna in shrimp ponds. Polychaetes have long given several advantages to the shrimp farming industry as nutritious natural food for shrimp and by providing ecosystem services through bioturbation, and removal of organic waste during feeding. However, currently there is a growing concern about the role of polychaetes in the shrimp disease transmission. Results of our study showed that *Dendronereis* spp. (Nereidae) are reservoir host of WSSV in shrimp pond (Desrina *et al.,* 2013, Haryadi *et al.,* 2015). Recently, the DNA of two newly emerging pathogens of shrimp, *Enterocytozoon hepatopenaei* (EHP, etiological agent of hepatopancreatic microsporidiasis, HPM) and a strain *Vibrio parahaemolyticus* (associated with acute hepatopancreatic necrosis disease, AHPND) was detected in polychaetes using PCR, indicating that polychaete may act as host/carrier/vector that play a role in the spreading of these pathogens at pond and global level (Thitamadee *et al*., 2016).

There are several reasons why polycaetes are potential vector/carrier/host of shrimp pathogens. Firstly, polychaetes live in burrow in the pond bottom and are relatively immobile, thus, providing opportunity for polychaete-shrimp pathogen encounter for an extended time. In the case where polychaetes are not the natural host, continuous exposure may trigger the adaptation between the pathogen and polychaetes. Secondly, being a burrower enable polychaetes to avoid the chemicals used to control pathogen and pest in ponds, thus causing them to sustain the pathogen as long as they live. Thirdly, polychaetes are detritofeeder and may acquire the pathogen through feeding. Pathogens such as WSSV may sink in to the sediment and retain the infectivity for an extended period (Kumar *et al*., 2014) and enter polychaete through feeding (Vijayan *et al*., 2005). Fourthly, polychaetes are the natural prey of shrimp that transfer pathogen to healthy shrimp upon feeding (Vijayan *et al*., 2005; Haryadi *et al*., 2015).

The benefit of polychaetes as nutritious natural food has resulted in polychaete movement at the global level, leading to the potential route of transmission of APHND and EHP. In retrospect, HPM and APHND agents are ubiquitous in the pond environment and may naturally gain entry into the polychaetes. We isolated *Vibrio* sucrose fermenter and nonsucrose fermenter from the body fluid of healthy *Dendronereis spp*. obtained from pond, showing that polychaetes can harbor the bacteria outside the gut. Likewise, EHP spores were released to the environment in the fecaesof infected shrimp (Rajendran *et al*., 2016) and may be ingested by polychaetes, even though this animal is not the target host. Our group just started astudy to determine status of polychaetes in HPM outbreak in shrimp ponds. Findings from our previous study on WSSV in *Dendronereis spp*. Showed that: (1) WSSV occurrence in polychaete *Dendronereis* spp. were widely distributed and this polychate can harbor the virus without outward signs of disease; (2) polychaetes grt the infection inside the pond, showing the influence of the pond environment as the source of infection; (3) WSSV occurrence in *Dendronereis* spp. Is related to the WSSV infection in shrimp. Likewise, similar situation may be applicable to APHND and EHP. Current infromation about occurrence of APHND and HPM disease agents in polychaetes need further clarification, e.g. type and source of the polychaete(s), and most important of all is transmission study to show that the disease indeed is transmitted from polychaete to healthy shrimp.

In conclusion, the niche and feeding habit of polychaetes may prompt the animal to acquire the shrimp pathogen and transmit the pathogen to shrimp upon feeding. However, it may be started with the unnatural abundance of the pathogen in the environment that caused the accumulation of pathogen in the polychaete or the pathogen may have undergone genetic changes and adapted to the polychaetes. Study on epidemiology of the shrimp pathogen in the polychaetes and pond management factors that influenced this condition is badly needed. Since HPM and APHND causative agents are normal inhabitants in the pond, control measures may include sound shrimp health management (BMPs), crop rotation to cut the pathogen cycle and lower stocking density.

Heritability, cross-breeding and inbreeding effects on resistance of *Penaeus vannamei* to AHPND and WSSV in Mexico

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Data from challenges to acute hepatopancreatic necrosis disease (AHPND) and white spot syndrome virus (WSSV) conducted in Maricultura del Pacífico, Mazatlán, Sinaloa, Mexico in 2015 were analyzed. The objective was to estimate heritability, crossbreeding, and inbreeding effects for survival time in the test. Purebred shrimp and crosses between Ecuadorian shrimp with a history of WSSV resistance, and a Mexican line with high growth ability were analyzed. Additionally, animals with an approximate inbreeding level of 25% were produced from sibling mating. Genetic groups were purebred Ecuadorians (ECU), purebred Mexican (MEX), MEX × ECU (F_1), and reciprocal backcrosses 75% MEX - 25% ECU (75MEX), ECU 75% - MEX 25% (75ECU). Inbred animals were obtained from $F_1 \times F_1$ and Ecuadorean families. The AHPND trial involved 5,171 animals from 182 sib families (119 sires), with an average initial age of 75.7 days and an average initial body weight of 2.63 g. The test was performed in six tanks using inoculation by immersion with a Mexican *Vibrio parahaemolyticus* pathogenic strain (M0904) at 3.2×10^5 CFU / mL. The test duration was 74 hours. The WSSV trial involved 6 231 animals from 181 sib families (118 sires), with an average initial age of 89.7 days and an average initial body weight of 4.32 g. The test was performed in six tanks *per os* infestation by using a dose at 10^7 copies of infective viral / g DNA. The test duration was 144 hours. A multi-trait linear mixed model with random additive genetic direct and maternal effects/full-sib common environmental effects, and fixed effects of tank, initial age, crossbreeding (direct, maternal and heterosis) and inbreeding effects was used to analyze survival time in hours for AHPND (STA) and WSSV (STW). Results are displayed in Table 1. Averages (SD) for STA and STW were 22.2 (23.7) and 41.5 (36.2) hours, respectively. Direct and maternal heritability estimates for STA were 0.16 ± 0.04 and 0.04 ± 0.03, respectively. Direct heritability for STW was 0.08 ± 0.03. The direct-maternal genetic correlation for STA was -0.80 ± 0.13. Genetic correlation between STA and STW was close to zero and not significant. Direct, maternal and heterosis crossbreeding effects for STA were not significant ($P > 0.80$). Inbreeding effects for STA were not significant ($P > 0.95$). Direct crossbreeding effects (ECU-MEX) for STW (12.0 ± 3.9 hours) were significant ($P < 0.01$). Heterosis effects were negative (-5.6 ± 3.9 hours) ($P < 0.09$). Inbreeding effects for STW were not significant ($P > 0.94$). It is concluded that for resistance to AHPND, there are individual additive genetic effects that can be used in selection programs, albeit complicated by the presence of correlated maternal genetic effects. There was no evidence of differences between the studied lines or crosses. For resistance to WSSV, there were differences in favor of animals from the Ecuadorian line compared to the Mexican line. The heterosis effect was negative, so the performance of the F_1 is closer to the Mexican line, which suggests a recessive genetic mechanism. No effects of inbreeding were found in genetic resistance to either of the diseases studied. Genetic effects for AHPND and WSSV resistance were independent. It is possible to develop lines of shrimp with greater genetic resistance to these diseases using both selection and crossbreeding.

Table 1. Genetic parameter estimates, crossbreeding and inbreeding effects (hours) for survival time to AHPND and WSSV in a mixed population of Pacific white shrimp.

	Estimate	SE	P-value
Genetic parameters			
Direct heritability			
Survival time to AHPND (STA)	0.16	0.04	
Survival time to WSSV (STW)	0.08	0.03	
Maternal heritability (STA)	0.04	0.03	
Direct-maternal genetic correlation (STA)	-0.80	0.13	
Direct genetic correlation STA-STW	-0.05	0.12	
Crossbreeding effects			
Direct Effect (Ecuador-Mexico)			
Survival time to AHPND (STA)	-0.31	2.93	0.910
Survival time to WSSV (STW)	12.04	3.90	0.003
Heterosis effects			
Survival time to AHPND (STA)	-0.05	2.59	0.983
Survival time to WSSV (STW)	-5.63	3.29	0.092
Maternal effects (Ecuador-Mexico)			
Survival time to AHPND (STA)	0.61	2.51	0.801
Survival time to WSSV (STW)	-1.78	3.22	0.577
Inbreeding effects			
Survival time to AHPND (STA)	-0.01	0.13	0.958
Survival time to WSSV (STW)	-0.01	0.19	0.948

Genomic analysis of the strains of AHPND-Vp (global) and genomic target

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Whole genome sequencing has been done to AHPND *V. parahaemolyticus* strains from China (Yang *et al*., 2014), Thailand (Kondo *et al*., 2014; Yang *et al*., 2014), Vietnam (Tang and Lightner, 2014), and Mexico (Gomez-Gil *et al.,* 2014), and also to a non-*V. parahaemolyticus* strain (Kondo *et al*., 2015). Additionally, five strains of *V. parahaemolyticus* were isolated from shrimps at an early stage of the disease in northwestern Mexico. The genomes of these strains were sequenced in an Ion Torrent PGM platform.

Bioinformatic genomic comparisons were done with the sequenced Mexican isolates and related *V. parahaemolyticus* strains publicly available in GenBank for a total of 22 genomes. Genome of the highly pathogenic Mexican isolate M0904 was used a reference genome for further genomics comparisons.

Phylogenetic relationships

The multilocus sequence analysis (MLSA) concatenated tree (11,961 bp) of seven housekeeping genes clearly showed that most of the isolates are independent strains although some could not be differentiated at this level and thus considered MLSA clones because they have similarly values equal or above 99.9 %.

.Three groups could be differentiated, one of strains only from Mexico, another only from Thailand and the mayor group that includes from diverse origins. Strain KC13.17.5 was identified a *Vibrio campbellii* and not as *Vibrio harveyi* as reported by Kondo *et al*., 2015.

Genomic comparison

Apart from the common two chromosomes found in all vibrios, extra chromosomal elements found in the strain M0904 were, 1) a 73.3 Kbp IncP type plasmid (pVp-AP, 45 % GC), 2) a 73.0 Kbp IncP plasmid (pVp-AP2, 45.6 % GC), and 3) a 37.0 Kbp 45.4 % GC circular tailed phage. Several inserted sequences were also detected in the chromosomes of M0904.

All major pathogenicity mechanisms were detected in strain M0904, classified according to the Virulence Factor of Pathogenic Bacteria database (VFPB). In chromosome I, up to 55 mechanisms were located, and in chromosome II 45 mechanisms were observed.

Plasmids

In all pathogenic strains, two conjugative plasmids were found of similar size and genetic composition. Both carry a type 4 secretion system (T4SS) expanding almost half of their genomes. These plasmids have one or two transposons inserted, one of which harbors the PirAB operon responsible for producing the delta endotoxin.

Proteome comparisons

Vibrio parahaemolyticus pathogenic strains show diverse proteomic similarity values ranging from as low as 49.4 % to 98.8%. Strains isolated from Asia have more similar proteomes (74.5 - 98.9 %) than Mexican strains (53.6 – 86.5 %). The core genome of all *V. parahaemolyticus* strains is composed of 3,284 genes, 24.3 % of the total pangenome. The core genome of only the pathogenic strains has 3,558 genes.

Conclusions

AHPND is caused by more than one *V. parahaemolyticus* strain or even by another *Vibrio* species; these strains are probable local strains that have been "infected" by a plasmid that acquired a transposon. The plasmids have mutations in many genes that suggest a fast evolutionary process.

Ecology, virulence factors and global spread of pathogenic *Vibrio parahaemolyticus* and related *Vibrio spp.*

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Genetic elements responsible for causing acute hepatopancreatic necrosis disease (AHPND) i.e PirA and PirB toxin genes have been found mostly in *Vibrio parahaemolyticus* but also in some strains of *V. harveyi* and *V. owensii*. Since these are plasmid borne genetic elements, it is possible that they are found in members of *V. harveyi* clade. Strains of *V. parahaemolyticus* carrying this plasmid, but being negative for PirAB toxin genes have also been described. The plasmid has been reported to carry few transposase genes and several insertion sequences. These could lead to excision or relocalisation of genes in the plasmid. Genetically diverse strains of *V. parahaemolyticus* have been reported to carry this virulence plasmid. Mortalities of shrimp in Australia, which did not meet the case definition of AHPND yielded a *V. harveyi*-like strain carrying PirAB toxin genes, but possibly lacking the plasmid, since they were negative by PCR with primers AP1 and AP2. This suggests the possibility of chromosomal integration of PirAB toxin genes. However, so far, none of the AHPND producing *V. parahemolyticus* studied carried tdh and trh genes associated with virulence to humans.

V. parahaemolyticus and *V. harveyi* are natural inhabitants of coastal and estuarine environments in both tropical as well as temperate parts of the world. The organisms has been isolated from water, sediment, molluscs, crustaceans and finfish and in animals, the organism may be associated with their gut. The environmental conditions such as temperature, salinity, zooplankton, dissolved oxygen, tidal flushing and others may effect the survival, establishment and growth of these organisms. In temperate region, often, *V. parahaemolyticus* is not detectable in water during winter, but could be isolated from sediment. In tropical environments, the organism is found throughout the year in areas where other environmental factors like salinity are favorable. The probability of detection of *V. parahaemolyticus* decreases in areas with salinity higher than 30 ppt and temperature below 15°C. *V. parahaemolyticus* and *V. harveyi* can grow at salt concentrations ranging from 0.5-10% with optimum being between 1-3%.

V. parahaemolyticus can adhere to and degrade chitin and adsorption of the organism to chitin or plankton occurs with higher efficiency under conditions of low estuarine salinity. Adherence to chitin could explain the relation between abundance of zooplankton and presence of this organism. About 80% of *V. parahaemolyticus* biomass in seawater was associated with zooplankton. Though cnidarians account for only a tiny proportion of zooplankton, they account for about 50% of abundance of zooplankton and copepods seem to have a smaller role. Adherence to chitin may influence survival of this organism at low temperatures. In the presence of chitin, *V. parahaemolyticus* even multiplied at 10°C. Shrimp aquaculture facilities, where chitin is available following molting, may thus favour *V. parahaemolyticus*. Biofilm formation could be one of the mechanisms of persistence of these organisms in hatchery environments. Chitin regulated pili are involved in biofilm formation in *V. parahaemolyticus*.

Aquatic birds may be carriers of *V. parahaemolyticus*. Studies in Japan and other countries show the presence of this organism in feces of aquatic birds, though the concentrations could be low. In temperate waters, sudden emergence of *V. parahaemolyticus* in certain regions may be related to water temperature. Some insights into the global spread of *V. parahaemolyticus* has been provided by studies performed on the strains that caused outbreaks of infection in Chile in 2004. One of the often cited explanations has been the discharge of ballast water from areas with high *V. parahaemolyticus* endemicity. Rapid spread of strains that are genetically similar to the O3:K6 strain that caused a number of illnesses in India in 1996 provided an impetus to the study of global spread. The pandemic strain belonging to O3:K6 serovar arrived in Peru in 1997 and infections were reported in northern part of the country, but spread southward along more than 1500 KM of the coast until it reached the Chilean city of Antofagasta. Molecular studies of the strains suggest that the 1997 El Nino episode provided the corridor for the displacement of the Asian pandemic *V. parahaemolyticus* strains to America. The El Nino phenomenon is characterized by the arrival of equatorial warm waters in a sequence of waves. In 1997, El Nino affected South American coast for about 6 months. It has been suggested that recurrent invasion of tropical masses of water might have resulted in repetitive sources of *V. parahaemolyticus* populations that would have established there. Further, an outbreak of *V. parahaemolyticus* infection in a cruise vessel in Spain yielded strains of a sequence type never found in Europe before, but involved in outbreaks in Pacific Northwest of United States. This further supports transoceanic spread of *V. parahaemolyticus*.

Research progress and biosecurity control strategies against EHP and AHPND for shrimp farming in the People's Republic of China

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Infection with *Enterocytozoon hepatopenaei* (EHP) and acute hepatopancreatic necrosis disease (AHPND) cause significant losses to Chinese shrimp farming industry in recent years. SYBR Green I and Taqman probe based real-time PCR methods were established for quantitative detection of EHP. The analytic sensitivity limitation of SYBR Green I qPCR-EHP was 8.3 × 101 copies/μL, and that of Taqman qPCR-EHP was 6.1× 101 copies/μL, which was about 4 times and 7.3 times more sensitive than the nested PCR, respectively. In comparison with Taqman qPCR-EHP, the nest PCR had a diagnostic sensitivity (DSe) at 82.8% and diagnostic specificity (DSp) at 100%, while the SYBR Green I qPCR-EHP had a DSe at 96.6% and DSp at 100% in detection with 62 samples. High prevalence of EHP in sampled slow growth populations was detected. Different levels of negative correlation between EHP loading in hepatopancreas and body length were observed in three infected populations with large different EHP loading level (100.8—106.4, 101.1—106.9, and 100.3—104.7 copies/ng HpDNA), but no significant correlation showed in other three populations with minor different EHP loading level (100.7—103.2, 100.9—103.4, and 102.3—103.8 copies/ng HpDNA). It indicates 103 copies/ng HpDNA of EHP loading in hepatopancreas may represent a high risk level. Rohrer's ponderal index (Rpi=W/L3), which represents the immediate biological feature of the aquatic animal body related to specific species, life stage, nutritional condition, and health status, were introduced for the analysis of the relationship between body weight and biological length. The EHP positive populations had significant lower Rpi at (5.19±0.26)×10-3 g/cm3, higher deviation ratio of actual weight to the estimated weight at (13.0±2.6)%, and higher coefficient of variation (C.V.) in both of body length at (20.1±7.8)% and body weight (57.5±24.1)%, in comparison with the EHP negative population of which the values are at (7.96±0.51)×10-3 g/cm3, 4.84%, 8.39%, and 28.0%, respectively. The analysis indicated that EHP infection may cause more than 2 times percentage of size variation, 2—3 times percentage of unexpected weight fluctuation, and about 30% of weight losses for same size individuals. The qPCR method and experimental infection provide technological approaches for selection of control medicines. Preliminary chemical therapy showed promising decreasing of EHP loading in hepatopancreas of infected shrimp both by immersion and feeding administration in experimental tanks and pond operation.

AHPND is still a major problem for shrimp farm during the early stage of stocking. Positive of PirVP were detected in few isolates of *V. harveyi*. Probiotics with inhibitory activity to isolates of VPAHPND were selected. More and more farmers and hatcheries adopt early detection by diagnostic kits or TCBS plates to monitoring the pathogens or possible *V. parahaemolyticus* in PLs and broodstocks. The prevalence of PirVP detection seems to be decreased in the samples of last year. Biosecurity control strategies recommended for breeding programs, hatcheries and shrimp farms become more practical due to availability of detection kits for different pathogens and relevant control techniques.

Epidemiology of AHPND: experiences in Viet Nam and Thailand

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During 2012-3013, cross-sectional study was conducted on 2508 ponds of 1254 farms in the Mekong Delta area of Viet Nam. This study was conducted under the FAO project TCP/VIE/3304. More than half of the interviewed farms (i.e. 78.5%) experienced AHPND. The data were obtained from cultured *Penaeus monodon* and *P. vannamei* as at 87.09% and 12.91%, respectively. Prevalence of AHPND occurred in *P. monodon* farms types such as intensive, semi-intensive and improve extensive were 60.9%, 71.1% and 75.9%, respectively. Prevalence of AHPND in intensive and semi-intensive *P. vannamei* farms were 57.6% and 62.5%, respectively. The farm-level risk factors identified include large culture area farm, farm have separated water inlet and outlet for each pond, farm using dry sediment method to clean pond bottom during pond preparation and farm site near other nearby farms using the same water source affected by AHPND. In the opposite way, farm using water supplied from seawater, treat water before using by holding water in reservoir pond could reduce the occurrence ofAHPND. The pond factors that cause increase risk of AHPNDoccurring include pond with large culture area and pond with water level depth of 1.2 meter or below, abnormal change of climate events that induced AHPND outbreak. Ponds supplied post-larvae (PL) from some provinces were more likely got AHPND than the other province. The protective manners include ponds using probiotic feed shrimp during culture period and ponds with aeration that could reduce the risk of AHPND occurrence.

Several epidemiological studies aimed to investigate the risk factors related to outbreak of EMS/AHPND at farm and pond levels were conducted in coastal shrimp culture areas in Thailand during 2012 – 2015. The project was supported financially by the Agriculture Research Development Agency (a public organization) and with excellent cooperation of the the Coastal Aquaculture Research and Development Center of Department of Fisheries, Thailand. The specific hatcheries providing PL for grow-out shrimp culture showed the strongest correlation with AHPND outbreaks from almost all of the study areas. Therefore, it is suggested that infected PL was the major source of infection of AHPNDoutbreaks and their association might be due to the introduction of the pathogen into the shrimp production system especially hatchery and nursery stages via contaminated water and live feed of broodstock and their offspring. We also found stocking older PL into the pond and increasing in stocking density associated with increasing the prevalence of AHPND. We found that farms, sited in areas affected with AHPND outbreak or sharing the same water supply source with an outbreak farm, were more likely to get the problem. So, the disease had the possibility of being spread via the contaminatedwater. Good pond preparation and better water management, i.e., longer duration of keeping water in reservoir pond and using water from water source of healthy shrimp pond exhibited lower the risk of AHPND outbreak. The manner of controlling the organic matter load in culture water by reducing the feeding rate during the first month after stocking and dissolving organic load by increasing time of adding water during first month of culture showed preventive effect for AHPND outbreak. Good management practice, i.e., post-larva quality assessment, good pond preparation before stocking, natural feed enrichment in pond before stocking, nursery PL in hapa and supplemented with natural feed during early part of stocking, promoting the growthof biocontrol bacterial to support optimum water quality and control the number of *Vibrio parahemolyticus* in water were valuable measures to prevent the problem. In addition, enhancing shrimp gut health with probiotics and other natural extraction products might reduce the possibility of AHPND outbreak in culture shrimp.

The epidemiological study could not identify other relevant information regarding the causative agent. However, the study gave the valuable information on how the disease was transmitted, what are the important sources of infection and which management practice can be done or to be avoided in the real situation.

Characterization of non-*Vibrio* bacteria as potential associates of AHPND bacteria in *Penaeus vannamei*

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Our collaborative project with the Thai Department of Fisheries on assessment of epidemiological risk related to known pathogens on shrimp "early mortality syndrome" has been performed during the year 2014-2015. Ten specimens each were collected from a randomly, pre-selected group of 200 ponds from several shrimp culture regions in Thailand with a previous history of problems with EMS. A cutoff day of 35 days of culture was chosen. Ponds experiencing problems in ≤35 days post-stocking were defined as EMS ponds while ponds without problems by 35 days were considered as normal ponds. From the total number of 200 ponds approximately 2 000 microscope slides (10 specimens per pond) were prepared and studied for histopathology while pooled shrimp samples (10) were used for molecular diagnostic methods. Of the 200 ponds, only 29 (14.5%) fell into the EMS category. For these EMS ponds, a higher proportion than in normal ponds were positive for white spot syndrome virus (WSSV) by PCR or for acute hepatopancreatic necrosis disease (AHPND) caused by specific isolates of *Vibrio parahaemolyticus* (VP_{AHPND}), by histological analysis. In addition, many shrimps in both the EMS and "normal" shrimp groups showed pathology of the hepatopancreas including hepatopancreatic infections caused by unidentified bacteria. The concern is a new emerging bacteria in the genus of *Delftia* which was isolated from shrimp with reported EMS. The synergistic effect of *Delftia* and VP_{AHPND} on shrimp mortality has been found. The culture medium and the PCR detection method for shrimp *Delftia* have been developed and used to study the risk of the new shrimp *Delftia* in shrimp. During our previous isolation of VP_{AHPND} in the AHPND shrimp, bacteria of genera *Shewanella* has been co-isolated. Preliminary results suggest that *Shewanella* has also synergistic effect with VP_{AHPND} to cause shrimp mortality. Whole genome sequence of shrimp *Shewanella* has been elucidated for better understanding of its virulence mechanism. Our on-going project is to evaluate the prevalence of *Delftia* and *Shewanella* in shrimp pond.

Asian shrimp production and the economic costs of disease

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Asia's combined crustacean aquaculture, which consists of at least 17 nominal species (21 classes of product as classified by FAO) is expected to exceed 5.08 million tons in 2016 compared to a global production of 5.91 million tons based on a 5.57% year-on-year growth. Yet despite Asia's 5.46% p.a. growth, production over the last three decades has been erratic and marred by episodes of disease that have resulted in major economic loss. Asia produces >2.66 million tons of whiteleg shrimp, *Ppenaeus vannamei*, worth ca. US$ 13.1 billion and employs >2 million people including casual or seasonal labour. Growth of the industry over the last few years has also been erratic (7.93± 6.35% yr-on-yr over 2008-2013; range 1.46-15.86%). The latest comprehensive figures, which are for 2013, indicate an annual growth of only 1.46% which if maintained would suggest that production for Asia in 2016 is expected to be ca. 2.77 million tons. Shrimp disease has resulted in huge national income losses (despite compensatory price rises in response to supply shortage), amounting to billions of dollars annually and at least US$ 20 billion globally over the last decade. White spot syndrome virus (WSSV) remains one of the most serious pathogens – infections are rapid and typically result in an 80-100% loss of stock. Infections in 3,907 ha of shrimp ponds in the Mekong Delta in 2015, for example, resulted in losses estimated at US$ 8.02 million.

Since its emergence in China in 2009, acute hepatopancreatic necrosis disease (AHPND) caused by the bacterium *Vibrio parahaemolyticus* with a toxin gene bearing plasmid, has had devastating economic impacts for Asian shrimp. In Thailand, shrimp productivity fell from a peak of 10.6 tons ha^{-1} in 2010 to only 4.13 tons ha^{-1} in 2013 following the AHPND outbreak with an estimated 22% drop in land use dedicated for *L. vannamei* culture. National losses, over the period 2012 to 2015, are estimated to be more than US$ 5.01 billion, with the value added by processing reduced by between US$ 1.7 to US$ 2 billion annually and with an estimated 100,000 jobs lost as a result of infections. A recent survey of 150 Thai ponds by the Thai DoF found infection prevalence at 24%. Elsewhere, AHPND in the Vietnamese Mekong Delta affected 4,627 ha of shrimp resulting in losses of >US$ 10.77 million in 2015 (*i.e.* infections in 2,318 ha of *P. monodon* ponds with resultant losses estimated at US$ 1.8 M and also in 2,309 ha of *L. vannamei* ponds with consequential estimated losses of US$ 8.9 M). Moreover, spreading infections of the fungal microsporidian *Enterocytozoon hepatopenaei* (EHP), the causative agent of hepatopancreatic microsporidiosis (HPM) have caused severe growth retardation, morbidity and mortality in shrimp is also causing serious concern within the industry. Infections in *P. vannamei* and *P. monodon* are reported from China, India, Malaysia, Thailand and Vietnam where the parasite infects not only the tubule epithelial cells of the hepatopancreas as previously reported but are also found within intestinal cells. EHP spores are small (ca. 1 × 0.67 µm), persistent and can be transmitted horizontally between shrimp, particularly in earthen rearing ponds - a Thai survey of 150 pre-stocked ponds found prevalence at 49%. With the consequential slowed growth (*i.e.* growth arrests at ca. 12 g) and enforced early harvest means that production costs are not covered (*i.e.* ca. US$ 4,000 ha^{-1}) and losses can be ca. US$ 32,000 ha^{-1} per culture cycle on normal production economics (*i.e.* 9 tons ha^{-1} as opposed to a targeted 12 tons ha^{-1}). If these losses are applied, then losses to the Thai economy could be in the order of US$ 76.4 M, however, this requires substantiating through a structured survey and a comparative evaluation of industry production data between infected and uninfected sites. The management and containment of EHP lies in strict biosecurity practices and disease testing. The faeces from broodstock and/or larger shrimp can be screened for EHP spores either by molecular and/or by histochemical means, while batches of smaller sized shrimp should be regularly screened by PCR at key steps in commercial production, e.g. on leaving the hatchery/nursery and before entering on-growing sites. The use of quality feeds is important as contaminated live feeds have been shown to be a potential source of infection. The environmental accumulation of spores can be managed through the removal of pond sludge/sediment management and its subsequent disinfection to prevent the return of spores to culture systems.

The presentation will focus on the major pathogens impacting Asian shrimp production and their effects on the host, the economic losses, the significance of biosecurity and farm strategies for their control and management.

Latest research on AHPND and measures to combat it

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1: Is only Vp causing it?. We found a non-*Vibrio parahaemolyticus* bacteria which carrying the plasmid containing AHPND toxin genes. We characterized genome of it. We identified that this bacteria is V. harveyi. This finding suggested that the virulent plasmid might be spreading to non-*Vibrio parahaemolyticus* bacteria.

2: What is the virulence factor of it? The copy number of the virulent plasmid is different in different strains. Some Vp have more than 30 copies but some have 1 or 2 copies of it. However, there is no relationship between the copy number of plasmid and virulence of Vp. The high virulent strains secreted toxin proteins. The toxin genes are existed with transposase which is a common transposon in Vibrio species. During culture of AHPND Vp, the toxin genes will be removed fully or partly from plasmid.

3: How to prevent it? We are conducting several experiments for development of prevention methods against AHPND. We are trying to find receptors of AHPND toxins for selection breeding of AHPND resistant family. We tried to develop a kind of vaccines against AHPND. However, unfortunately, our developed FKC vaccine did not work for small shrimp (under 1g) although it worked very well for large shrimp (over 5g). These studied suggested that immune system of small shrimp is not well developed. We are also studying chicken egg yolk antibody IgY and nanobubbles. I will give a talk about these studies in the seminar.

We are developing a new technology which can detect max 12 pathogens in one time by PCR for shrimp diseases. The name of this technology is PRINTED ARRAY-STRIP (PAS). I will also show you about PAS.

Specific pathogen-free (SPF), specific pathogen-resistant (SPR) and specific pathogen-tolerant (SPT) as part of a biosecurity strategy

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The concepts represented by the acronyms SPF, SPR and SPT have been used in a rather confused manner over the last few years, often with commercial interest. Specific Pathogen Free (SPF) refers to the sanitary status of a stock. Meaning that the stocks are free of certain pathogens (to be defined by the provider and the supplier). These are not necessary free from all pathogens, which would be referred as Pathogen Free (PE). SPF animals are not simply PCR negative. SPF status is obtained through history and culture conditions and does not refer to the tolerance/resistance or susceptibility to diseases, which are genetic traits. SPF stocks must come from a facility that has proven records of pathogen free status for a period of at least 2 years, complies with certain specific biosecurity measures and has a suitable surveillance program in place to prove freedom from pathogens. SPF stocks are free of certain pathogens regardless of its tolerance/resistance/susceptibility to any pathogen. Specific Pathogen Resistant (SPR) and Specific Pathogen Tolerant (SPT) refer to their genetic characteristics that allow them to be resistant to infection to a particular pathogen or tolerant to the development of the disease caused by a particular pathogen (not to all pathogens). These are genetic characteristics regardless of their sanitary status, whether the stocks are infected or not. Therefore, stocks can be both SPF and SPR/SPT, in other words they can be free of pathogens and be resistant to infection from a particular pathogen or tolerant to specific diseases.

The use of SPF stocks has achieved levels of productivity never seen before which proves that the health status of the stocked PLs is key for the development of the shrimp industry. SPR/SPT may, in some cases, have the draw back of lower productivity performance, when compared to SPF stocks which may have been involved in a long standing genetic selection program. While resistance, tolerance, susceptibility or higher productivity is a choice based on the biosecurity risk of the farm that will use them, the sanitary status should not be a choice. Why would anyone want to stock (invest) in infected animals?

NOTES

Annex 4

Extended Abstracts of Presentations Technical Session 3: National and International Framework Update

NOTES

Aquaculture biosecurity challenges in the light of the Ballast Water Management Convention

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The Ballast Water Management Convention (BWMC) has been prepared by the International Maritime Organization (IMO) in 2004 to ensure safer and more effective ballast water management options to continue the prevention, the minimization and ultimately the transfer of harmful aquatic organisms and pathogens that are one source of biosecurity issues for the aquaculture industry. Since the BWMC preparation, multiple guidelines and resolutions have been taken in order to support the preparedness of parties and the fast implementation of the regulations. With the recent ratification from Indonesia, Belgium, Finland and others, the BWMC is about to enter into force.

The BWMC and its future enforcements are a great step towards the protection of the environments and the economy associated to sensitive areas worldwide. Nevertheless, there are pitfalls and limitations on how this tools developed by the IMO and its member states can assure a complete protections. The convention nevertheless is likely to decrease the risk of transfer of microbes and viruses that could affect the aquaculture industry but the Risk Zero does not exist. An overview of the regulations, the ballast water management systems testing guidelines, and the port state control guidelines will be presented.

In a second part, tools that are already used to evaluate biosecurity through the studies of connectivity between aquatic areas will be introduced. A lot of these tools based on modelling have permitted the development of zones to exchange untreated ballast waters to decrease the risk of invasions. A step by step model development approach will be introduced together with the comparison of hydrodynamic, ecological and agent-based models. When used with parsimony, the same modelling approaches can be used for aquaculture; the tools exist, are powerful, but our industry (aquaculture) doesn’t use them to the extent which would raise confidence in investors to deal and mitigate biosecurity risks.

Dealing with AHPND: Viet Nam experience

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In Vie Nnam, aquaculture plays an important role for the national economy. Shrimp industry, in particular, is one of very dynamic and important sector of aquaculture because shrimp is not only a valuable source of aquatic food protein, but also a seafood species with the highest trade value. Viet Nam seafood sector export brought home USD 7.9 billion in 2014 and the share of shrimp export was about 49% (USD 3.9 billion). Black tiger shrimp (*Penaues monodon*) and white-leg shrimp (*P. vannamei*) are two main shrimp species cultured in Viet Nam. Among them, white-leg shrimp is an increasingly important species but tiger shrimp still remains as a main species for culture. The total volume of shrimps produced in 2010 was 469,700 tonnes with the share of tiger shrimp was 71% while this volume increased to 660 000 tonnes in 2014 with the share of white-leg shrimp was 61% (NSO and D-Fish).

Besides many advantages, the shrimp industry in Viet Nam has been faced with many issues, including disease problems. The main disease in recent years which most seriously affected shrimp production is AHPND. AHPND was officially reported in April 2011 from Soc Trang province, the highest shrimp-producing province in Viet Nam. There was about 70% of shrimp cultured areas affected with AHPND in this province in 2011. Based on epidemiological observation and other relevant field data, it was confirmed that the disease outbreak occurred with high mortalities among black tiger shrimp and white-leg shrimp. The disease was then reported in all most provinces that culture shrimp in Viet Nam. No statistic data is available on the affected areas reported during 2010-2011 at the national level. But, in 2012, AHPND occurred in 192 communes belonging to 54 districts, 16 provinces with the affected shrimp areas of about 28,005 ha. In 2015, the disease occurred in 292 communes belonging to 76 districts, 22 provinces with the affected areas of about 9,284 ha. Although the affected communes increased in 2015, the affected areas decreased more than 3 times in 2015 compared to it was in 2012.

A number of actions taken by the Government of Viet Nam in responding to “AHPND disaster” included the following: 1) established the National Task Force responsible for prevention and control of shrimp diseases; 2) performed an intensive epidemiological survey; 3) carried out screening for shrimp pathogens in order to narrow suspicious agents causing AHPND; 4) carried out diagnostic investigations done by national and regional laboratories; 5) distributed water treatment materials/disinfectants to provinces affected by AHPND outbreaks; 6) granted fund for emergency research done by national institutions and universities; and 7) solicited technical assistance from regional and international organizations. Subsequently, experts from OIE, FAO visited affected shrimp farms and Viet Nam further received the support from FAO through the TCP/VIE/3304 (E) project “*Emergency assistance to control the spread of an unknown disease affecting shrimps*” (see details at FAO Fisheries and Aquaculture Report No. 1053, Rome 2013).

The National Task Force (NTF) is established by the Minister of the Ministry of Agriculture and Rural Development, Viet Nam by Decision No. 1254/QD-BNN-TCTS. The NTF consisted of 11 persons who are representatives for the Directors of the Directorate of Fisheries (D-Fish), the Department of Animal Health (DAH), the Research Institutes for Aquaculture (RIA1, RIA2, RIA3) and the Directors of Departments of Agriculture and Rural Development at Soc Trang, Bac Lieu and Ca Mau provinces. The functions of the NTF include responsibility for determination of the cause of shrimp diseases, finding the solutions and policies for prevention and control of shrimp disease at the national scale. The NTF developed specific emergency research projects done by different institutions, such as epidemiological surveys on AHPND, screening pathogens from affected shrimp, identification of the cause of AHPND and solutions for disease control. At first, weekly and then monthly meetings also organized by the NTF in order to update on AHPND situation, the research results on AHPND and to discuss about the follow-up works.

Based on current knowledge of AHPND and the outcomes from research projects, the following solutions have been done by the Government of Viet Nam and relevant stakeholders in order to be recovery from “AHPND disaster”: 1) through improvement of technology (applying VietGAP, GlobalGAP, BAP and selective breeding program); 2) applying disease surveillance program. The surveillance results are monthly reported to DAH and then the Directors of DAH presents these results to the Minister of MARD during monthly meeting. At farm level, farmers also apply several solutions, such as, screening PLs for disease before stocking or using SPF-stocks; applying suitable stocking density; daily monitoring some environmental parameters (temperature, pH, salinity); monitoring/control total *Vibrio* by probiotics; maintaining feeding especially reducing the amount of food during the first month stocking and feeding shrimp by several times per day with small amount of foods; applying bioflocs and re-cycling water technology; and applying polyculture system and/or rotational farming with shrimp-tilapia, shrimp-marine fish.

Dealing with AHPND: Thailand update from 2015

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Shrimp production

The first finding of EMS/AHPND was during late August, 2011 in the pond located in the eastern province of Thailand. The outbreak of EMS/AHPND was reported at the end of 2012 causing a significant reduction of shrimp production to around 217,437 tons by the end of 2014. The recovery of shrimp production was found in 2015 to about 230,338 tons showing increasing production YoYof about 5.9% and from Jan-May, 2016, the production was about 84,387 tons showing increasing production about 9.3% (Table 1). In addition, the Department of Fisheries had also developed a method to estimate a shrimp production without report of national MD database from the number of PL stocked, average shrimp productivity and prevalent of disease in shrimp farming areas. The major portion of this amount of shrimp production is assumed as a production that small-scale farmers have sold for local consumption which was estimated at about 32,745 ton/yr in 2015 and about 11,999 ton in 2016 (from Jan-May).

Table 1. Production of farmed shrimp in Thailand from 2011-2016 (Jan-May) analyzed from the National shrimp movement document (MD) database indicating a slightly recovery in 2015- early of 2016.

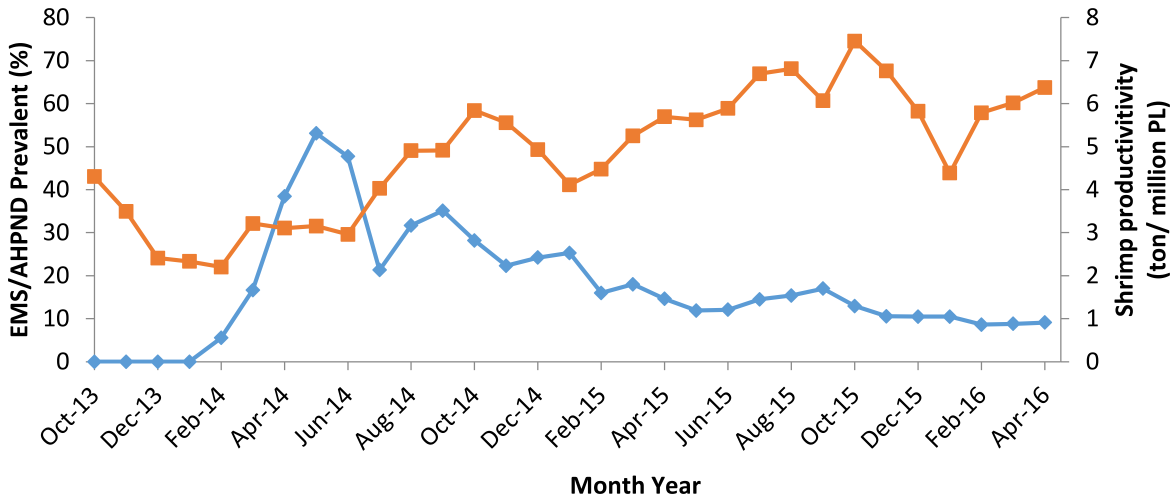
Year	Production (ton/yr)			% production change YoY
	P. monodon	*L. vannamei*	Total	
2011	1,469	500,719	502,188	-
2012	15,219	458,012	473,231	-5.8
2013	12,124	274,755	286,879	-39.4
2014	13,053	204,385	217,438	-24.2
2015*	7,828	222,510	230,338	5.9
2016*	3,543	80,844	84,387	9.3

*Note: in 2015 the shrimp production of without report in national MD database was estimated about 32,745 ton/yr. In 2016 (from Jan- May) this production was estimated about 11,999 ton.

The current EMS/APHND situation

The Department of Fisheries (DoF) has provided a laboratory service for farmer using PCR detection in 23 centers of Coastal Fisheries Research and Development Division in the entire country in order to control the outbreak of EMS/APHND. About 31,700 samples were analyzed annually and the % of prevalence was calculated every month. The results revealed that EMS/AHPND prevalence increased to the peak in April, 2014 about 18.0 % (range 5.6-53.1) after the outbreak of EMS/AHPND was severely increased from the beginning of year 2014 (Fig 1). The productivity of shrimp during that period was in the average of about 3.02 ton/million PL (range 2.2 - 4.3). In the same period, the DoF started to carry out a screening program in hatchery and pond to eliminate infected shrimp PL out of the production areas. The % EMS/APHND prevalence was about 27.1%, 14.9% and 9.3% for the second half year of 2014, in 2015 and, the first four months of 2016, respectively – indicating a a gradual decrease in the outbreak of EMS/AHPND. This situation showed in a similar trend with shrimp productivity and the recovery of shrimp production (Fig 1.). However, there was a seasonal effect that can influence the severity of EMS/AHPND outbreak. In addition, due to prolonged outbreak of the disease, the *Vibrio parahaemolyticus* EMS/AHPND strain which is the causative bacteria, has now widely contaminated into water and soil of the shrimp pond as well as the natural water body in the shrimp farming areas.

Fig 1. Percentage prevalent of the EMS/AHPND outbreak and monthly average of shrimp productivity in Thailand investigated from October, 2013 – April 2016.



Mandatory standard for disease free white shrimp hatchery

Disease outbreak such as EMS/AHPND and the other shrimp viral diseases have provedto obstruct the sustainable development of the shrimp industry and caused economic lossed in producing country, marketing and food security issues. After the outbreak of EMS/AHPND, the Ministry of Agricultural and Cooperative, Thailand launched a Good Aquaculture Practices (GAP) f as a mandatory standard from the beginning until the end of 2016. The standard (TAS 7415-2008) aims to establish GAP for hatcheries of disease-free Pacific white shrimp in order to prevent disease transmission of target diseases from broodstock to the nauplii. The target diseases are white spot syndrome virus (WSSV), yellowhead virus (YHV), Taura syndrome virus (TSV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), infectious myonecrosis virus (IMNV) and acute hepatopancreatic necrosis disease (AHPND). The requirements of good practice are:

- availability and implementing the Standard Operating Procedures of hatchery to control target diseases.
- participation in the targeted surveillance program of the DoF
- availability of surveillance report for freedom of the target diseases for at least six consecutive months,
- operators in nauplii hatchery shall obtain knowledge, training and/or experience in Pacific white shrimp hatchery operations.
- biosecurity measures in place for controlling personnel and visitors that may be the carrier of target diseases into the hatcheryselection of target disease-free Pacific white shrimp broodstock and their source shall be traceable.
- availability of effective quarantine system for Pacific white shrimp broodstock, biosecurity operating manual for broodstock, nauplius production, feed and feeding, water, tools, equipment, hatching system, vehicle disinfection.
- pest and pet control within the production areas of the hatchery.
- inspection report and record indicating the absence of the target diseases, and for traceability purpose.

This standard will be implemented with all relevant stakeholders and is very important to obtain good quality and disease-free nauplii from the well-practiced hatchery and could be a significant tool for sustainability of shrimp industry in Thailand.

Avoiding AHPND: Iran experience

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Shrimp culture in Iran began in the 1990s. Thanks to government funding and support, the new industry developed very quickly and many farms were built. Most were located in the southern part of Iran near the Persian Gulf and the Oman Sea and later offshore in the Caspian Sea. During this period the main genus of shrimp was *Peneaus indicus*. The first outbreak of WSSV occurred in Iran in 2001. Since then, it has been reported frequently and up until now has been the main problem for shrimp culture.

In Iran, the Veterinary Organization (IVO) under the supervision of the Ministry of Agriculture, is responsible for animal diseases. According to vast experience in animal disease management the Department for Aquatic Health was established in 1993. The first challenge for this department was the WSSV outbreaks in shrimp farms. These days there are many challenges in aquaculture health management especially regarding shrimp culture.

To protect aquatic animals against exotic and emerging diseases, we first need to know more about the natural behavior of disease. In regard to AHPND/EMS, we collaborate with other scientists on research to better understand the pathogen. Educating farmers to report any unusual mortality is more important for us although some of them try to hide any problems in their farm but there is some kind of self-justification between them. There is an updated active surveillance system in collaboration with laboratories equipped with verified tests (molecular tracing and other traditional isolation of *Vibrio parahemolyticus* on agar media). The Central Veterinary Laboratory of IVO in Tehran and the shrimp diagnostic center in Bushehr are the main laboratories in this field. This program was updated according to our new findings in March 2016. If WSSV breaks out at hatcheries, farmers go forward to re-building their establishment for biosecurity measures despite upgrading their knowledge on shrimp health via training courses that are almost supported by the private sector. At shrimp farms there is some complicated problem that needs to take in force to launch biosecurity measures but now most of them accept roles and obey sanitary measures. The IVO has a strict risk analysis for import of any kind of animal including shrimp brood stock. Some hatcheries, however, import fresh feed illegally, or artemia flake from unknown sources that can be a threat to our job. We are aware of them and use force if necessary. Avoiding AHPND/EMS needs close cooperation between private and government sectors on a scientific basis and implementing effective acts and regulations.

Dealing with shrimp diseases in Brasil (Public Policies for Marine Shrimp Health)

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Brazil has favorable conditions to increase its aquaculture production but it still faces animal health and technical and structural limitations. Brazil shrimp industry has faced some serious health constraints over the last years. Transboundary diseases caused by White Spot Syndrome Virus, Infectious Myonecrosis Virus and Infectious Hypodermal and Hematopoietic Necrosis Virus can be considered the most important viral diseases of penaeid shrimp in terms of economic losses during the last decade. The industry and the government have made huge investments in technical assistance, husbandry practices including closed systems, probiotic and biofloc technology as well as specific-pathogen-free and specific resistance strains. On the other hand, the Federal Government has awaken to the need for setting a legal framework for the sector regarding health issues as part of a strategy for increasing shrimp production and competitiveness. Public policies have been strengthened mostly considering human, physical and financial resources; technical authority and capability; interaction with interest parties and access to market.

Given the socio-economic importance of aquatic animal production related activities and their increased investments, Brazil´s Federal Government has established a partnership with research groups on veterinary epidemiology aiming at implementing epidemiology into a national program for aquatic animal diseases. The strengthening of the Brazilian fisheries and aquaculture production market is related to high quality and sanitary standards.

It has been considered essential to implement official diagnostics and the necessity for better knowledge on shrimp disease epidemiology, mostly emerging and reemerging diseases. It has also been taken into account the need for the improvement of biosecurity measures, the development of a sensitive risk-based surveillance system with efficient early detection alerts implemented, the development of geographical information systems and the modelling of disease spread into farmed shrimp and the environment among other actions.

In 2010, Brazil began to carry out import risk analysis of aquatic animal products, following the OIE methodology, including risk assessment of shrimp import for human consumption. Other noteworthy measures were the onset of quarantine procedures; the national program for aquatic animals and the establishment of the national network of laboratories for fisheries and aquaculture (RENAQUA), essential for the implementation of the health programs and import control. These policies are being implemented with the support of regional agricultural agencies in states and municipalities. A national program for the certification of post-larvae of marine shrimp is currently under discussion and it will probably lead the shrimp industry to another level of production and international competitiveness.

Therefore, the application of epidemiological concepts and tools become fundamental to the development of a sustainable production process, which might guide the achievement of world-class healthy products. The range of activities planned involves the proposition of a national active surveillance system; the design of prevalence studies for crustaceans; the assessment of import risk analysis; economical assessment of official health programs; specialization and movement of products of risk. The successful implementation of such programs is expected to address the specific needs of a decision-making process based on objective and scientific criteria, which is essential to any country wishing to protect their internal supply chain against the entry of existing diseases found in exporting countries such as Acute hepatopancreatic necrosis disease (AHPND). It is also relevant to structure effective control programs and surveillance systems based on quantified disease impacts, as well as to spread the use of veterinary epidemiology to aquatic animal health professionals.

Amongst the major hurdles, the low awareness of the productive sector regarding the need to invest in surveillance and disease control tops the list. Moreover, environmental permits still limit the access of shrimp farmers to increased availability of credit. It should also be stressed that the states' veterinary services have limited knowledge on aquatic animal health and have most of their operational capability dedicated to terrestrial animals. Other major constraints on implementing public health policies in shrimp productions are: professional competencies of veterinary or aquatic animal health professionals and other technical personnel, and their continuing education; disease prevention, control and eradication; epidemiological surveillance; veterinary medicines and biologicals for shrimp; crustacean movement control within the country and traceability of products of aquatic animal origin; zoning and compartmentalization for access to markets. In conclusion, Brazil is putting up serious measures to improve aquatic health but there is plenty to be done in order to implement an effective system of epidemiological surveillance of aquatic animals in the country.

The World Organisation for Animal Health (OIE): relevant activities regarding AHPND

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The World Organisation for Animal Health (OIE) is an intergovernmental organisation with 180 Member Countries with a mandate to "improve animal health, veterinary public health, animal welfare, and consolidate the animal's role worldwide".

The OIE *Aquatic Animal Health Code* (*Aquatic Code*) and *Manual of Diagnostic Tests* (*Aquatic Manual*) are international standards developed by the OIE for aquatic animals (amphibians, crustaceans, fish and molluscs), These standards are recognised under the World Trade Organisation Application of Sanitary and Phytosanitary Measures (SPS Agreement) as the international standards for animal health and zoonoses for safe international trade in aquatic animals and their products. The health measures in the Aquatic Code are used by the veterinary authorities of importing and exporting countries to provide for early detection, reporting and control of agents pathogenic to aquatic animals and, in the case of zoonotic diseases, for humans, and to prevent their transfer via international trade in aquatic animals and aquatic animal products, while avoiding unjustified sanitary barriers to trade.

The Code contains information on the following subjects: glossary; notification of diseases and epidemiological information; criteria for listing aquatic animal diseases; diseases listed by the OIE; import risk analysis; welfare of farmed fish during transport; welfare aspects of stunning and killing of farmed fish for human consumption; and disinfection of salmonid eggs for infectious haematopoietic necrosis, infectious salmon anaemia and viral haemorrhagic septicaemia. This edition includes four new chapters on communication; monitoring of the quantities and usage patterns of antimicrobial agents used in aquatic animals; development and harmonisation of national antimicrobial resistance surveillance and monitoring programmes for aquatic animals; and killing of farmed fish for disease control purposes.

The OIE Member Countries are required to notify to the OIE Headquarters the presence or absence of diseases in their country, noting that the definition for *disease* (as defined in the Glossary of the *Aquatic Code*), means "clinical or non-clinical infection with one or more aetiological agents" In addition Members should also notify the OIE of emerging diseases which have a significant impact on aquatic animal or public health (also a defined term in the Glossary of the *Aquatic Code)*.

Members notify OIE through the World Animal Information System (WAHIS) and this information is then disseminated to Members via the WAHIS platform which is also accessible to the public through the OIE website. The WAHIS provides access to all data submitted by Members on OIE listed diseases and emerging diseases.

In the early warning system, whenever an important epidemiological event occurs in a Member Country, the Member Country must inform the OIE by sending an Immediate Notification which includes the reason for the notification, the name of the disease, the affected species, the geographical area affected, the control measures applied and any laboratory tests carried out or in progress.

Once they have been received, verified and validated by the OIE, the immediate notifications are published in the OIE's three official working languages (English, French and Spanish) under the heading Alerts and sent to everyone on the OIE-Info Distribution List, an electronic distribution list set up to facilitate and widen the dissemination of animal health information. After having informed the OIE of a significant epidemiological event by means of an immediate notification report, the Member must send weekly Follow-up Reports so that the event can be monitored as it evolves. In all cases, the country must submit a final report to notify either that the event has been resolved or that the disease has become endemic.

In both cases, the country will continue to submit information in its six-monthly reports if the disease is on the OIE List.

The monitoring system encompasses a six-monthly Reports that provide information on the presence or absence of diseases on the OIE List and the prevention and control measures applied.

Listed diseases must meet 'Criteria for listing aquatic animal diseases (Ch 1.2.). The first criteria is related to the consequences of the disease. The disease has been shown to affect the health of cultured aquatic animals at the level of a country or a zone resulting in significant consequences, e.g. production losses, morbidity and mortality at a zone or country level OR The disease has been shown to, or scientific evidence indicates that it would effect on the health of morbidity or mortality in wild aquatic animals resulting in significant consequences, e.g. morbidity and mortality at a population level, and ecological impacts OR Natural transmission to humans has been proven, and human infection is associated with severe consequences. The second criteria is related to the spread of the disease. Infectious aetiology of the disease is proven OR An infectious agent is strongly associated with the disease, but the aetiology is not yet known. AND Likelihood of international spread, including via live aquatic animals, their products or fomites. AND Several countries or countries with zones may be declared free of the disease based on the general surveillance principles outlined in Chapter 1.4.. The last criteria is on diagnosis. A repeatable and robust means of detection/diagnosis exists.

There are 9 OIE listed diseases of crustaceans that are notifiable, including acute hepatopancreatic necrosis disease (AHPND) which was listed in 2015. Hence since January 2016, Member Countries have an obligation to notify the OIE, through WAHIS, the presence or absence of AHPND in their country. However, before the listing of AHPND in 2015, countries had an obligation to notify the OIE, through WAHIS, if AHPND was detected because it met the definition for an 'emerging disease'.

All OIE listed diseases have a corresponding disease-specific chapter in the *Aquatic Code* and *Aquatic Manual* providing recommendations to ensure safe international trade. The draft chapter for AHPND for both the Code and Manual have been circulated to OIE Members for comments which will be reviewed by Aquatic Animals Health Standards Commission at their Sept. 2016 meeting and will likely be proposed for adoption in 2017.

OIRSA actions in Central America on Aquatic Animal Health

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OIRSA, as an intergovernmental organization specialized in agro-food safety, has continued supporting the shrimp sector through its Regional Coordination of Animal Health, its Regional Aquatic Animal Health Program and supported by the *ad hoc* Group on Health of Aquatic Organisms, on training in specific issues on shrimp disease control and particularly on Acute Hepatopancreatic Necrosis Disease (AHPND), new disease and best management manuals, shrimp disease pocket booklets, conferences and most importantly, keeping regional warning on the emerging disease AHPND.

In February 2013, OIRSA posted an official notification through the website, to prevent the entry of this emerging disease in the region. This effort was complemented with virtual lectures to the 9 OIRSA member countries by using the GoToMeeting tool. Therefore, OIRSA presented the proposal for a ***Regional Program for AHPND Epidemiological Surveillance (RES),*** in order to have a tool that includes direction for the development and establishment of an AHPND monitoring plan.

Surveillance Plan strategies included training plans, mechanisms for regional harmonization and homologation (sampling, histology samples preparation and criteria for severity index assignment), establishment of a national and regional system for report analysis (suspected cases) and biosecurity enhancement among others.

RES recommendations include the development of a ***Regional Emergency Plan for the prevention and control of AHPND*** where there are described General Principles, structure of the Regional Emergency Plan (notifications to be made if suspicion), legal framework to follow disease notifications, application of Epidemiological Surveillance Systems, disease diagnosis (available laboratories for reliable results), standardized sample reception and analysis, communication & transparency, and monitoring frequency.

The Regional Emergency Plan has a ***Regional Emergency Procedures Manual for the Prevention and Control of AHPND,*** which develops the following subjects:

A) **Biosecurity and best production practices.** Includes restrictions on entering and leaving the farm under surveillance, records management, public prohibited areas advertisements, staff training with operating manuals, parking outside production areas, control of internal displacements, water sources, appropriate stocking densities, water quality, food source, quantity and quality, pests and vector control, staff training and warning awareness, farmed shrimp surveillance, dead organisms removal and diagnosis for death cause, diagnostic farm program, avoiding contact with other species likely to be AHPND carriers, and document organization.

B) **Emergency AHPND.** It has been recommended that each member country has a structure that allows coordination of multidisciplinary activities. Initially a unit responsible for the Emergency Operation with clear functions for coordinating units as Epidemiology, Quarantine, Biosecurity, Assessment, Depopulation & Disinfection, and Communication.

As a complementary process for regional farmed shrimp-health protection, there was a Meeting of the Regional International Council for Agricultural Health, participated by Regional Ministers responsible for Fisheries. It was held on October, 2013 in Dominican Republic and they approved the **"Regulation OSPESCA/OIRSA No. 001-2013 for the Prevention, Control and Eradication of Farmed Shrimp Disease in SICA and OIRSA member countries".**

In summary, OIRSA has made available to veterinary services of the member countries responsible of prevention and control of transboundary diseases such as AHPND, these tools above as a general framework for each nation, on the basis of its legislation and technical knowledge, so they can apply the methodologies recommended in the 3 documents described and the first Regional Regulation implemented by two regional institutions that was approved under the model of governance from SICA/OSPESCA.

It is also crucial that shrimp farmers guilds and organizations as well as technicians, know how does veterinary authority operate in their countries, and join to the effort for AHPND prevention and control.

Surveillance systems should be based on the information contained in the Aquatic Code of the OIE, which also apply to diseases still not listed but considered as emerging and meaning a high risk for penaeid shrimp production such as AHPND.

The documents described above provide a framework for decision making by the competent authorities and may be consulted on the website of OIRSA: www.oirsa.org. These are frequently updated to offer strength tools available to everyone. Nowadays, all these documents are under review by the Directorates of Animal Health of the 9 member countries of OIRSA, in order to have general agreement and to obtain approval at the next meeting of the Technical Committee of OIRSA to be held in October, 2016. It should be noted that national plans presented by OIRSA member countries, have been based mainly in the documents mentioned above.

Updates on AHPND in Southeast Asia: outcomes of a recent regional technical consultation on AHPND

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The most serious problems faced by the aquaculture sector are diseases spread and introduced through movements of hatchery produced stocks, new species for aquaculture, and development and enhancement of the ornamental fish trade. Early Mortality Syndrome (EMS) or currently known as Acute Hepatopancreatic Necrosis Disease (AHPND) is one of the diseases that caused heavy mortalities of cultured shrimp and resulted in significant drop in shrimp production in several Southeast Asian countries, where one million people depend on shrimp aquaculture for their livelihoods. To address this concern, the ASEAN Regional Technical Consultation on EMS/AHPND and other Transboundary Diseases for Aquatic Animal Health Management was organized by the Aquaculture Department of the Southeast Asian Fisheries Development Center (SEAFDEC/AQD) and the Government of the Philippines through the Department of Agriculture-Bureau of Fisheries and Aquatic Resources with financial support from the Government of Japan administered by the ASEAN Secretariat (Japan-ASEAN Integration Fund). The Consultation discussed the EMS/AHPND and other transboundary diseases affecting the region and how these should be managed to improve aquatic animal health and ensure sustainability of aquaculture operations. The outputs of the meeting include the current status of EMS/AHPND and other emerging diseases in farmed shrimps in ASEAN Member States (AMS) and Japan; and policy recommendations and priority areas for R&D collaboration to address the gaps.

The Technical Workshop mainly focused on EMS/AHPND. Outbreaks and incidence of the disease has so far been limited to Malaysia, the Philippines, Thailand and Viet Nam. On the other hand, most countries have in place a system for reporting, monitoring and surveillance for diseases as well as health certification systems for importation and exportation of live aquatic organisms to prevent entry or spread of pathogens. However, considering that AHPND is a relatively new disease, several gaps in our understanding of this health concern still need to be elucidated. Issues identified for R&D include the use of live feeds (specifically polychaetes) as these are potential carriers of pathogens; genetic considerations, e.g. the effect of inbreeding on the shrimps' susceptibility to AHPND and other diseases; vertical transmission of AHPND-causing bacteria; transfer of plasmid carrying the toxin gene to other *Vibrio* species and possibly other bacterial pathogens; environmental risk factors for the spread and outbreaks of AHPND; use of green water technology as well as probiotics in the prevention of AHPND; mixed infection with other shrimp pathogens; development of antibiotic resistance; and development of other strategies for prevention and control of the disease.

Recommendations on policy issues include those that need to be adopted or strengthened including the need for Member Countries to harmonize legislation(s) and regulation(s) related to aquatic animal health management including the legislation for trans-boundary movement of live aquatic animals; compliance with good aquaculture practices to maintain optimal environmental conditions during the culture period; develop and implement the guidelines on health management and good practices to prevent EMS/AHPND and other trans-boundary diseases; adopt capacity building programs that would ensure availability and capacity of public or private laboratory services; strictly implement a monitoring, surveillance and reporting system to relevant authorities and/or Competent Authority at country, regional and international levels; put in place an early warning system and develop emergency preparedness and contingency plans; strengthen cooperation and collaborative arrangements among Member States and other regional and international organization such as OIE, FAO, NACA and SEAFDEC as well as the ASEAN Network of Aquatic Animal Health Centres (ANAAHC); promote and fund public-private partnerships at the national levels as well as cooperation among shrimp industries in the ASEAN Region; promote region-wide capacity building/education and information dissemination programs including technology transfer from AMS to another AMS to enhance awareness of farmers and relevant stakeholders on R&D developments in transboundary diseases especially on management and control.

AHPND Contingency Plan under the framework of the Andean Community of Nations (CAN)

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Given that aquaculture is the fastest growing sector in the production of food of animal origin at global and hemispheric scale, its rapid expansion is threatened by outbreaks of diseases affecting these species. Since 2009 they have been registered outbreaks of the disease called Early Mortality Syndrome/Acute hepatopancreatic Necrosis Disease (EMS/AHPND, of shrimp in China and Southeast Asia and because the globalized trade aquatic animals and their products becomes the primary means of disease transmission, the Andean Community of Nations (CAN) through the General Secretariat conveyed to the Aquatic Animal Health Services of Member Countries to initiate a coordinated effort with the aim of developing a regional plan to evaluate joint actions in order to prevent this disease from reaching Member Countries.

To develop this work it was considered that the trade operations of aquatic animals and their products within the Andean subregion and with third countries, Member Countries shoud implement sanitary measures consistent with the rules of the World Trade Organization (WTO), the World Organization for Animal Health (OIE) and the Codex Alimentarius Commission.

The result of this work included the development of the Andean legal framework for health monitoring of aquatic animals, issued by the Commission of the Andean Community: Decision 808 of 2016 "Measures on the prevention, surveillance, control and eradication of diseases of aquatic animals ", whose purpose is " ... to establish an Andean regional legal framework for the prevention, surveillance, control and eradication of diseases in aquatic animals; approve the Andean Contingency Plan the Early Mortality Syndrome/Acute Hepatopancreatic Necrosis Disease (EMS/AHPND) of farmed shrimp; and harmonize measures and national legislation of member countries on the matter. "

The provisions of the Decision are applicable to all species and products capable of acquiring or transmitting important diseases of aquatic animals and the entire territory of the Member Countries of the Andean Community. Likewise, it has elaborated a draft and adopted the Andean Plan of Contingency against EMS/AHPNDof shrimp farming, in which actions for prevention and monitoring of the disease were established and to strengthen the scientific and technical knowledge of the competent authorities for the benefit of the shrimp farming sector.

For the implementation of the Plan, a Technical Manual was elaborated, approved by resolution 1851 of 2016, as a guide for aquaculture health services in each country, producers and marketers of shrimp products. This manual is a flexible tool that can be adapted by each country according to the importance of the sector in its economy, its environmental and socioeconomic conditions and can be modified according to the evolution of the health situation in the region and new scientific findings on the disease.

Annex 5
Expert Profile

NOTES

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Dr. Reza Mahmoud Alavi was born in 1967 in Western Iran. He graduated from Urmia University with a DVM degree in 1993. Since 2008, he has worked in the Aquatics Health Department as head of the Shrimp health and disease management group in the IVO.

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A Spanish national, she is a veterinarian with an M.Sc and Ph.D. from Stirling University. She has worked for over 25 years on diverse aspects of shrimp health issues covering diseases, diagnostics, sanitary legislation, health management, development of SPF stocks and biosecurity. She has collaborated as an expert for international organizations such as FAO, EU, EFSA, WB and OIE as well as with the private sector such as shrimp producers, aquaculture financial and insurance companies worldwide. She carries out R&D activities for private companies and maintains links to the academic world through teaching at other institutions. She has published over 30 papers in peer review journals, over 50 articles in industry magazines, 5 book chapters, is co-author of the CD-rom Diagnosis of Shrimp Diseases and editor of The Shrimp Book. While working with Pescanova as Director of Aquatic Animal Health, she defined the strategy for development of SPF WSSV tolerant. Presently, she is the Director for Biosecurity for the National Aquaculture Group of Saudi Arabia.

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Expert in international aquatic animal health issues based in British Columbia, Canada specializing in policy development and risk analysis. Career includes periods in Asia with the International Development Research Centre (IDRC) as Fish Health Network Coordinator and as Fisheries Program Officer (Asia and Pacific), and in Canada, as a research scientist and manager in aquatic parasitology with the Institut Marice LaMontagne, Canadian Department of Fisheries and Oceans (DFO). Private consultant since 1997, with experience in more than 50 countries, primarily in Asia, but also with projects in Africa, Latin America, Eastern Europe, the Middle East and the South Pacific. Recent work includes involvement with FAO projects in Oman, Malaysia, southern Africa and Latin America. Frequent editor of scientific and technical publications in aquatic animal health, aquaculture and fisheries for FAO. Recent publications include *Checklist of fish parasites of Malaysia* and *Checklist of fish parasites of Singapore*.

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A DVM graduate from the Veterinary School, Kasetsart University (1994); Ph.D. in Agricultural Biotechnology from the Center of Agricultural Biotechnology, Kasetsart University (2009).

His professional career started at the technical service of a chemical supplier company in the field of shrimp culture where he worked for two years. He joined the Faculty of Veterinary Medicine, Kasetsart University in 1996 with an academic position. At present, he is Head of Department of Farm Resources and Production Medicine, Head of the Aquatic Disease Diagnostic laboratory, Kamphang-saen Diagnostic Center at the Faculty of Veterinary Medicine, Kasetsart University and Adjunct Professor of Atlantic Veterinary College, University of Prince Edward Island, Canada. He has close to 20 years experience working on aquatic animal health in the areas of disease diagnostics, epidemiology and health management.

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Thai nationality, He served as pathologist under Aquatic Animal Health Research Institute, Royal Thai Department of Fisheries during 1986-2004. He led many research works in both laboratory and in the field, focus on balancing animal health and pond environment. He conducted many workshops/training (national and international) on fish and shrimp farm management across the region. He has been invited by inter-government organization as well as private sector as a speaker in many conferences around the world. He has also written several manuals and publications in the field of fish/shrimp health and pond management. He moved to the new position as a Technical Manager under Novozymes since 2005. His responsibility is on conducting research and development trials, for water and soil treatment as well as animal health related products in aquatic animal farms. He has Ph.D. from Institute of Aquaculture, University of Stirling, Scotland in 1992, Bachelor (1985) and Master of Science (1987) in Fisheries from Kasetsart University.

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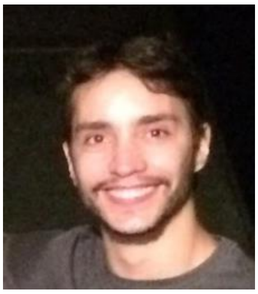
Dr. Kallaya Sritunyalucksana, a Principal Researcher and a head of Shrimp-pathogen interaction (SPI) laboratory of the National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Thailand. She has finished two Ph.D. programs under the supervision of Prof. Kenneth Soderhall at Uppsala University in Sweden and Prof. Timothy W. Flegel at Mahidol University in Thailand. She has been conducting research projects on the control of shrimp immunity, shrimp pathogens and infection mechanism, and shrimp disease control for 24 years with more than 20 scientific projects. Her works has resulted in 42 international publications and an H-index of 18. Her research networks are expanding both at national and international level.

Dr Eduardo DE AZEVEDO PEDROSA CUNHA

edu.cunh@gmail.com



Eduardo de Azevedo Pedrosa Cunha is an official veterinarian of the Ministry of Agriculture, Livestock and Food Supply of Brazil - MAPA with masters in animal health focused on aquatic animal risk analysis. He is currently the Brazilian focal point for aquatic animal of the World Organization of Animal Health – OIE and the head of the National Aquatic Animal Health Coordination of MAPA. His entire career has been devoted to policy making on aquatic animal health.

Dr DESRINA

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Desrina (Dr), Fisheries Department, Faculty of Fisheries and Marine Sciences, Diponegoro University, Semarang, Indonesia.

Dr. Desrina is a fish pathologist with 20 years experience as a lecturer and researcher in fish diseases and fish health management. Her areas of interest are bacterial diseases of fish especially vibriosis, bacterial fish vaccine, fish parasites ecology and control, and white spot syndrome virus (WSSV). She has been the principle investigator of research in above areas funded by Ministry of Research, Technology and Higher Education through various funding schemes. She is currently investigating role of polychaetes in shrimp diseases epidemiology in shrimp ponds focusing on genetic diversity of WSSV in polychaete *Dendronereis* spp and polychaetes as carrier of *Enterocytozoon hepatopenaei* in the hepatopancreatic microsporidiasis (HPM) outbreak in the shrimp pond. She was an invited guest speaker on the 7th Annual Workshop of National Reference Laboratories for Crustacean Diseases held by CEFAS Weymouth, UK, in November 2015 where she presented her research findings on WSSV in polychaete. She is also active in the Commission of Fish Health and Environment, a scientific advisory council in fish diseases and health and a partner of the Directorate General of Aquaculture Fisheries, MMAF, Indonesia. She published papers in peer reviewed scientific journals.

Dr Guillaume DRILLET

gdr@dhigroup.com

Dr Guillaume Drillet has 15 years of experience working within the field of aquatic ecology in the public and private sectors. Dr Drillet holds a Master degree on management of coastal resources in Normandy (France). Expatriated for 15 years, Dr Drillet has lived and participated in many national and international research projects on plankton ecology and physiology in Denmark, France, Scotland, Singapore, Taiwan, Slovakia and in the USA. He took a PhD funded by the European Network of Excellence (Eur-oceans) and the graduate school of stress studies in Denmark. In 2011, Dr Drillet received a Young Elite Scientist Award from the Danish Ministry of Independent research. Dr Drillet is based in Singapore for the last 5 years and work at DHI Water and Environment where he supported the development of the Ballast Water Technology and Innovation Center as well as the DHI Environmental Laboratories. Dr Drillet is section head of a team dealing with ecological processes, ballast water issues and aquaculture; he is lecturing at Temasek Polytechnic and was recently elected as the upcoming President of the World Aquaculture Society for Asia Pacific. Dr Drillet has been coordinating discussions on the effects of temperature as well as the use of Standard Test Organisms (STO) for the MEPC Correspondence Group working on the revision of the G8 guidelines of the BWM convention. Together with his team and other collaborators, Dr

Drillet is currently evaluating the concept of same risk area for carrying out risk assessments under regulation A4 of the ballast water management convention.

Mr Dan FEGAN

danfegan@gmail.com

Dan Fegan has been involved in commercial aquaculture for over 30 years, firstly in Latin America, mainly Ecuador, before moving to Asia in 1986. From 1988 to 1996 he managed commercial and technical support operations of Aquastar in southern Thailand. After 2 years in Malaysia he returned to Thailand where he worked as a consultant and set up the Shrimp Biotechnology Business Unit (SBBU) for the Thai National Center for Genetic Engineering and Biotechnology (BIOTEC) to commercialise their research and provide products and services to the aquaculture industry.

In 2004, he joined the US-based biotechnology company, Alltech Inc. where he was involved in establishing the "Alltech Aqua" brand and building the company's technical capacity and sales to the aquaculture industry. In 2007, he joined Cargill to provide support for their aquaculture businesses, mainly in the Asian region before returning to consulting services.

Dan has been closely involved in aquatic animal health issues since the early outbreaks of shrimp virus diseases in the 1990's and has worked closely with academics, research organisations and the private sector in developing strategies to mitigate and prevent disease outbreaks. He was closely involved in planning some of the responses to the AHPND (EMS) outbreaks with Cargill in Asia and Mexico which included development of a programme combining on-farm risk-assessment, biosecurity recommendations and feed-based solutions to minimize the impact of AHPND.

Dan has been an active member of several professional societies including the Asian Fisheries Society and the World Aquaculture Society. He served as a Director and Board Member of WAS from 1998 to 2007, and as WAS President from 2005-2006. In 2002-03, he was President of the WAS Asian Pacific Chapter and organized the APC's first APC meeting in Bangkok in 2003. He has served in several international working groups and committees including the Technical Committee of the NACA/FAO/OIE Asia-Pacific Aquatic Animal Health Programme and in the Technical Advisory Group of the Aquaculture Stewardship Council.

Dr Tim FLEGEL

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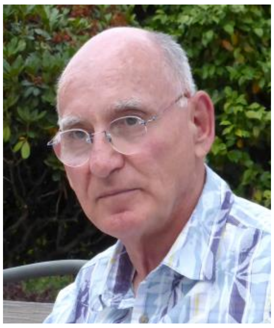

Tim Flegel is currently located at the Center of Excellence for Shrimp Molecular Biology and Biotechnology (Centex Shrimp) that is co-operated by the Faculty of Science Mahidol University and the Thai National Center for Genetic Engineering and Biotechnology (BIOTEC) under the National Science and Technology Development Agency (NSTDA). He was the head of Centex Shrimp from 2001-2012. He has done research on shrimp pathology and shrimp defense mechanisms, particularly for viral pathogens, for the past 24 years. For most of that period, he has been a member of both the Fish Health Section of the Asian Fisheries Society and the World Aquaculture Society and has been active in regional and international activities related to shrimp health management. He has received several national awards for his research on shrimp, the most recent being the 2013 National Research Council of Thailand award as Outstanding Researcher in Agricultural Science and Biology. He became a Thai citizen in 2010. He retired as a Professor in the Department of Biotechnology in 2010 and as head of Centex Shrimp in 2012 and now serves as an advisor to BIOTEC/NSTDA. At Centex Shrimp, he continues to do research on shrimp pathology and on the shrimp response to viral pathogens.

Dr Amrollah GHAHARI

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Dr. Amrollah Ghajari was born in 1973 in the south of Iran. He graduated from Shiraz Uuniversity with a DVM degree in 1999 and started his career as a shrimp expert then after. He has been working on shrimp diseases especially WSSV. As an official veterinarian, hhe works for the Iran Veterinary Organization (IVO) in different levels (provincial to national) and in different places . In 2011, he continued his study for Ph.D. degree on veterinary pathology and now he is a student of veterinary pathology in Tehran uUniversity . His thesis subject is the study of histopathological aspects of VHS in Rainbow Trout in Iran. Currently, he works in the IVO as Director General for the Aquatics Health Department.

Dr Bruno GOMEZ-GIL

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Dr. Gomez-Gil is a lead scientist at the Center for Research in Nutrition and Development (CIAD), Mazatlan Unit for Aquaculture and Environmental Management, Mexico. He received his Ph.D. from the Institute of Aquaculture, Stirling University, Scotland. He established the bacteriology laboratory at CIAD and latter the Microbial Genomics laboratory. For more than 20 years he has studied aquatic microbiology in aquaculture settings and in the environment. His entire career has been devoted to the study of vibrios, their taxonomy, pathogenicity, genomics, and prevalence in marine environments. He has published more than 78 articles in peer-reviewed journals and several book chapters. He has mentored 28 students at all levels and lectures postgraduate courses. Has been responsible for research national and international projects. He is also a curator of the Collection of Aquatic Important Microorganisms (CAIM). He is a member of various scientific organizations.

Dr Ikuo HIRONO

hirono@kaiyodai.ac.jp

Professor, Laboratory of Genome Science, Tokyo University of Marine Science and Technology, Tokyo, Japan.

Prof. Ikuo Hirono obtained his Ph.D. from Kagoshima University in 1993, did a postdoctoral fellowship (JSPS) at Tokyo University of Fisheries in 1993-1994 and was a research scholar at Hopkins Marine Station, Stanford University in 1998. He was Assistant Professor, Tokyo University of Fisheries from 1994-2002, and Associate Professor, Tokyo University of Fisheries, Tokyo University of Marine Science and Technology from 2002-2009. He is a Professor, Tokyo University of Marine Science and Technology from 2009. He is a co-editor in chief of Fish and Shellfish Immunology. He has over 350 publications in several international journals. His main research area is shrimp immune system and development of fish DNA vaccines. He is also conducting research on EMS/AHPND. He has many of students in Thailand, Philippines, Vietnam, Indonesia, Singapore, India, South Korea, China, and Columbia. He has/had many of international research collaborations under governments.

Dr Huang JIE

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Dr. Jie Huang, male, born in 1965, obtained his BSc on virology in Wuhan University (1987), his MSc in the Wuhan Virology Institute, Chinese Academy of Science (CAS) (1990), and his PhD on marine biology in the Ocean Institute, CAS (2004). He is a Senior Researcher of the Maricultural Disease Control and Molecular Pathology Laboratory, Yellow Sea Fisheries Research Institute (YSFRI), Chinese Academy of Fishery Sciences (CAFS); the Chief Scientist for the aquatic animal disease control in CAFS; OIE Designated Expert for White spot disease (WSD) and Infectious and haematopoietic necrosis (IHHN); a doctorial tutor Shanghai Ocean University. He has been conducting research projects on the diagnostics, epidemiology, molecular mechanism of virus infection, and control technology for WSD and other aquatic animal diseases for 26 years. He identified firstly a new virus, called as HHNBV, as the pathogen of white spot disease in China in 1993 and reported the transmission rout of the virus. His group has found several new viruses, new genotypes, or new emerging diseases in marine farming industries of China, including turbot reddish body iridovirus (TRBIV) in turbot, acute viral necrotic virus (AVNV) in scallop, covert mortality nodavirus (CMNV) in shrimp, a new genotype of yellow head virus (YHV-8) in shrimp, an earliest identified virulent strain of *V. parahaemolyticus* in shrimp causing early mortality syndrome (EMS) in 2010. His laboratory established a series of detection techniques, including gene probe, PCR, LAMP, and gene chips, for different aquatic animal pathogens and national standards for shrimp diseases diagnosis. His group has also developed rapid detection kits for more than 20 aquatic animal pathogens, nonspecific immunoenhanceants and probiotic bacteria for shrimp disease prevention, microorganism-enhanced biofloc technology for aquaculture, and marine fish vaccines for *Vibrio anguillarum* and *Edwardsiella tarda*. He proposes the concept of microbiological control technology to prevent aquatic animal disease and actively promotes the concept of biosecurity technological system for aquaculture industry.

He has more than 270 publications of which 57 were published on international journals, obtained 41 patents, published 30 national standards, won 13 national and provincial awards, and trained 94 doctor and master students. He won the Distinguished Expert for TAISHAN scholars of Shandong Province, the Excellence Talent and Innovation Team for Agriculture Research, other national, provincial and ministerial honor titles.

Dr Pikul JIRAVANICHPAISAL

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Dr Pikul Jiravanichpaisal is a Senior Scientist at the FishVet Group (FVG) Asia. Pikul has a BSc in Sanitary Science from Mahidol University, MSc in Microbiology from Kasetsart University, Thailand, Ph.D. in Aquatic Animal Histopathology from Mie University Japan and a Ph.D. in Invertebrate Immunity, Uppsala University, Sweden. Prior to joining FVG Asia Pikul worked as Senior Researcher at BIOTEC, NSTDA. Pikul has more than 20 years' experience working in crustacean microbiology, histopathology and immunology and she is a pioneer in research on intestinalIlmmunity in crustaceans including shrimp and crayfish. She has more than 35 international publications. She is an Academic Editor for PLoS One and reviewer for several international journals. She has been supervisor/asst. supervisor for Master and Ph.D. students and mentor for several young scientists.

Dr Iddya KARUNASAGAR

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Indian national with over 3 decades of experience in the area of aquatic microbiology and food safety. Presently, Senior International Consultant with Products, Trade and Marketing Branch of FAO. Served FAO as Senior Fishery Industries Officer (Quality and Safety) during 2007-2014 and in this position, represented FAO in the meetings of Codex Committee on Fish and Fishery Products. Also served as Leader or Member of FAO/WHO Secretariat for Joint Expert Meeting on Microbiological Risk Assessment (JEMRA). Served as Lead Technical Officer for several FAO projects n Asia, Africa, Eastern Europe, Central Asia, Latin America, Caribbean and South Pacific. Before joining FAO, served as Drafting Member of JEMRA risk assessment of pathogenic Vibrio spp in seafood (2000-2005). Served in academic institutions from 1978-2007 in various capacities starting as Assistant Professor and rising to be the Director of Research of Karnataka Veterinary Animal and Fisheries Sciences University in India and National Professor of Indian Council of Agricultural Research. Authored over 200 papers in refereed international Journals and several book chapters covering food safety and aquaculture. Holds Masters and doctoral degrees in Microbiology from India and experience of postdoctoral research in University of Maryland, USA, University of Wurzburg, Germany.

Mr David KAWAHIGASHI

david@vannamei101.com

David Kawahigashi has over 35 years of technical and management experience in all phases of commercial shrimp aquaculture. He is the founder and Director of Vannamei 101 (2006-present), an aquaculture consulting and training company based out of Hawaii with branches in Thailand and Vietnam.

He has worked in Ecuador during the 1980's and throughout Central and South America up until 2003. Since 2004, he has been instrumental in the rapid development of *Penaeus vannamei* culture in Asia through technology transfer of new culture technologies.

Areas of expertise include broodstock breeding programs, hatchery management, and intensive grow-out systems. Recent technological advances in shrimp aquaculture have expanded business opportunities for Vannamei 101 to regions in the South Pacific, the Americas, the Middle East, and Asia. David attended the University of Hawaii.

His areas of expertise include: genetic improvement and broodstock multiplication programs; design and management of sustainable grow-out systems (polyculture/intensive); biosecurity protocol for farms, hatcheries and breeding facilities (BAP); indoor 2-phase systems using biofloc technology for sustainable shrimp culture; training and manpower recruitment.

Dr Eduardo LEAÑO

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Dr. Leaño is currently the Coordinator of Aquatic Animal Health Programme of NACA. His specialization is on Aquatic Animal Health and Marine and Freshwater Mycology. Before joining to NACA in April 2010, he has worked as Researcher/Scientist at the Fish Health Section of SEAFDEC Aquaculture Department, Philippines (1989-2001), and as Research Fellow at Taiwan Fisheries Research Institute (2002-2003) and National Taiwan Ocean University (2004-2010). He obtained his Ph.D. degree (Applied Microbiology) at the City University of Hong Kong (1999), his M.Sc. (Fisheries) at the University of the Philippines in the Visayas (1993) and his B.Sc. (Inland Fisheries) at the Central Luzon State University (1989). He has co-edited four books in Aquaculture, and has published 59 scientific articles in peer reviewed journals, book chapter and popular aquaculture magazines.

Dr Dang Thi LUA

danglua@ria1.org

Dr. Dang Thi Lua has been an aquatic animal health specialist for more than 16 years and currently responsible as the leader of research group on aquatic animal diseases at Research Institute for Aquaculture No. 1 (RIA1) in Vietnam. She obtained Bachelor degree in Aquaculture from Nha Trang University of Fisheries, Vietnam and MSc, PhD degrees in Applied Marine Biosciences from Tokyo University of Marine Science and Technology, Japan. She was also involved in postdoctoral fellowship program at the Tokyo University of Marine Science and Technology during 2008-2010. Dr. Dang Thi Lua has been an active manager and member of number of research and development projects related to her fields funded by international and national authorities in Vietnam. She is also involved in AHPND research works since this disease has been happened in Vietnam. Her most interests and experiences are aquatic animal disease research, especially fish and shrimp diseases, including disease diagnosis and control. Additional expertise and experiences of Dr. Dang Thi Lua include teaching, training and farming practices.

Dr Vielka MORALES

vielkamorales2003@yahoo.com

Responsible for the Regional Program of Aquatic Animal Health at OIRSA
Panamanian. B.Sc. in Biology with major in Zoology, 14 years of experience in research about shrimp (and other marine species) hatchery and broodstock at Pacific Mariculture Laboratory of the Panamanian Agricultural Ministry. National representative in shrimp affairs in International Organisms as Agricultural Mission of Taiwan, Spanish Agency of International Cooperation and CIID from Canada. Member of the Latin American Aquaculture Association. Vice-president of the Panamanian Association of Aquaculture Professionals (APAPROEA) from 4 years ago. Technical Responsible of the Fisheries and Aquaculture Organization of the Central American Isthmus (OSPESCA) from last 15 years covering subjects related to aquaculture, industrial fishing, artisanal fishing and others. FAO consultant in shrimp nutrition and aquaculture; consultant for PRADEPESCA-EU on issues about shrimp aquaculture. National Project Coordinator for the Regional Plan for Continental Fisheries and Aquaculture (PREPAC) for more than 2 years. She has more than 25 international publications and 17 are related to penaeid shrimp. Participation in several Spanish networks about shrimp and mollusks for the Program of Science and Technology for Countries Development (CYTED). Responsible for the “Vannamei CYTED-Network” for more than 4 years. Main Regional

expert for fisheries management of the Caribbean spiny lobster, from Caribbean Large Marine Ecosystem (CLME). Speaker at courses and conferences on aquaculture issues. Currently she is responsible for the Regional Aquaculture Health Program of the International-Regional Organization of Animal Health (OIRSA).

Mr Mati NITIBHON

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B.Sc. Marine Science Kasetsart University Thailand
M.Sc. Fisheries Biology. U.C.N.W. Bangor, North Wales, U.K.

Started working in the field of shrimp nutrition with Hoffman La Roche (Switzerland) and Pfizer Inc. (USA) as shrimp technical adviser before joining Charoen Pokphand Feedmill (CP groups).

Dr Rolando PAKINGKING

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Rolando Pakingking Jr. is a scientist and the current head of the Fish Health Section, Southeast Asian Fisheries Development Center, Aquaculture Department (SEAFDEC AQD) where he actively engages in researches that delve on immune responses triggered by the interactions of viruses and bacteria in high value marine **fish species. He has conducted research with funding from the Government of** Japan Trust Fund, Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development-Department of Science and Technology (DOST) and National Research Council of the Philippines-DOST among others. He obtained his master's (M.S. Biology) and doctoral (Ph.D. Biological Science) degrees respectively from the University of the Philippines Visayas and Hiroshima University, Japan. He has been a consultant/ fish disease expert of the Food and Agriculture Organization of the United Nations assigned at the Fish Farming Center, Jeddah, Kingdom of Saudi Arabia and visiting professor of University Technology Malaysia. He has authored and co-authored book chapters and several scientific articles published in peer-reviewed journals and serves as referee to a number of scientific journals. He has been a recipient of various awards and has been conferred as Diplomate in Microbiology by the Philippine Academy of Microbiology.

Dr Celia Lavilla-PITOGO

celia.pitogo@fulbrightmail.org

A Filipino scientist with extensive work on vibriosis and other diseases affecting farmed shrimps, Celia was affiliated with SEAFDEC Aquaculture Department (Philippines) for 30 years until July 2010. As Head of the Fish Health Section, she planned, implemented, managed research, and supervised Diagnostic Services for the aquaculture industry. Her work resulted in more than 60 scientific papers, reviews, and manuals as first author and in collaboration with Filipino and international scientists. She co-edited proceedings and books including Diseases in Asian Aquaculture IV, Use of Chemicals in Aquaculture in Asia, and Transboundary Fish Diseases in Southeast Asia. She gave lectures in various seminars, workshops and trainings, and transformed training resources for web-based delivery through AQUAHEALTH Online. She was a member of NACA's Advisory Group on Aquatic Animal Health from 2002-2010. In 2010, she became a member of the *ad hoc* Committee on Antimicrobial Resistance in Aquatic Animals of the World Organization for Animal Health (OIE) that wrote 5 chapters in Section 6 of the OIE Aquatic Code, Recommendations for Antimicrobial Use in Aquatic Animals. An active member of the Fish Health Section, Asian Fisheries Society, she was Chairperson from 1996-1999. Under the 2009 Fulbright Scholarship Program, she conducted research at the University of Arizona, USA with Dr. Don Lightner. In 2010, Celia joined Integrated Aquaculture International LLC, a company based in Nebraska, USA, as Director of Aquatic Animal Health at its Brunei Darussalam operation where she oversees biosecurity and health of SPF *Penaeus monodon* and *P. stylirostris*. She received her PhD in Fish Pathology from Hiroshima University, Japan, MS Marine Biology from the University of the Philippines, and BS Biology from Mindanao State University, Philippines.

Dr Melba B. REANTASO

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A Philippine national, US permanent resident, with 3 decades combined experience on aquaculture, biosecurity governance & aquatic animal health (AAH), Melba led a number of international aquatic disease investigation task forces (e.g. KHV, EUS, AHPND). She had worked for FAO since 1997 in various capacities & as a full-time staff from 2004. Prior to joining FAO, she worked as Molluscan Pathologist (Oxford Laboratory, Maryland Department of Natural Resources, 2002-2004), Regional Aquatic Animal Health Specialist (Network of Aquaculture Centres in the Asia-Pacific, NACA, Bangkok, Thailand, 1999-2002); and retired as Senior Aquaculturist (Philippine Bureau of Fisheries and Aquatic Resources, 1981-1999,). She conducts expert workshops, training courses and develops & implements FAO- and donor-funded projects on various aspects of AAH with a global coverage. Her work also includes development of strategies and frameworks on AAH at national and regional levels. She represents FAO at the OIE Aquatic Animal Health Standards Commission. A published professional, she maintains a network of AAH experts, served as Chief Editor of the FAO Aquaculture Newsletter since 2006-2016, was Secretary (1999-2002) and Chairperson (2002-2005) of the Executive Committee of the Fish Health Section of the Asian Fisheries Society (FHS/AFS) and regularly sought as keynote conference speaker and referee of a number of scientific journals. Melba has Ph.D. (University of Tokyo) and post-doc (Nippon Veterinary and Life Science University) qualifications on AAH, both in Japan, as a Japanese Monbusho government scholar and fellow of the Japanese Society for the. Promotion of Science (JSPS). Her mentors include Dr James Richard Arthur (Canada), Prof Kazuo Ogawa (Japan), Prof Kishio Hatai (Japan), Dr Sharon McGladdery (Canada),Dr Michael Phillips (UK), and Dr Rohana Subasinghe (Sri Lanka).

Dr Rodrigo ROUBACH

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Dr. Roubach was the leading scientist and senior researcher at The National Institute for Research in the Amazon/INPA since 1988, where he has studied several aspects in implementing aquaculture in the Amazon region, including breakthrough papers with native Amazon species. He received his Ph.D. from the College of Fisheries and Allied Aquacultures at Auburn University, United States. He has been granted, responsible and collaborated in several national and international research projects. He is also a member and have collaborated within various scientific organizations acting as chair in scientific meetings. Recently have served as Co-chair during the meeting of the VIII Sub-Committee on Aquaculture of the FAO Fisheries Committee (SCA/COFI 2015) in Brasilia, Brazil. He have authored more than 70 articles in peer-reviewed journals and 9 book chapters in several topics in aquaculture, and in this process, mentored more than 60 students. Currently he is coordinator at the Brazilian Ministry of Agriculture, Livestock and Supply at the Aquaculture Department (since 2005).

Prof. Mohammed SHARIFF

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Mohamed Shariff, Professor, Faculty of Veterinary Medicine, University Putra Malaysia. Prof Shariff obtained his Ph.D. from University of Stirling and has more than 39 years' experience in aquaculture and fish health research. Being one of the pioneers in fish health in Malaysia, his work encompassed various problem solving issues such as identifications of disease causing agents (parasites, bacteria, virus), development of rapid diagnostic tools for the shrimp and fish. He has over 140 papers and filed 10 patents. Shariff is currently working with an industry partner to produce SPF *Penaeus monodon* stocks and enhancing fish immune response with feed supplements. Shariff has collaborated with various EU partners for research project on i) Sustainable Aquaculture, ii) Hazard Analysis of Antimicrobial Resistance Associated with Asian Aquacultural Environments iii) Empowerment of Poor Farmers, Minority Groups and Women in Aquatic Food Production. Shariff served as the President of the Asian Fisheries Society and was a member of the Board of Trustees of the World Fish Centre. He also served on the Aquatic Animal Health for the Asian and the Pacific Region for NACA. He is the Editor-In-Chief of the Asian Fisheries Science Journal. Currently he is the member of the Aquatic Animal Health Commission at OIE

Dr Andy SHINN

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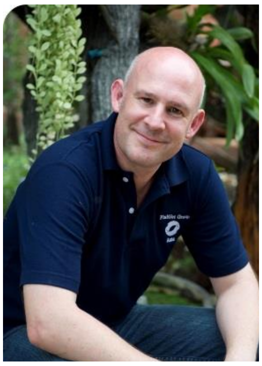

Andy has a Ph.D. in Veterinary Aquaculture from the University of Stirling, Scotland where he conducted aquatic parasitology-focused studies at the Institute of Aquaculture and the Natural History Museum in London. Andy has 27 years' experience in aquatic parasitology and fish disease. He was the former head of Aquatic Parasitology at Stirling before joining Fish Vet Group Asia Limited, part of Benchmark Holdings plc, based in Bangkok, Thailand in 2014 (www.fishvetgroup.com). He now heads up the Fish Vet Group's research aquarium in Chonburi conducting commercial shrimp and fish feed, growth and pathogen challenge trials (e.g. AHPND, EHP and WSSV for shrimp; various bacterial and parasitic pathogens of fish). Andy has authored over 160 published articles, more than 130 of which are in international peer-reviewed scientific **journals (www.researchgate.net/profile/Andrew_Shinn).**

Dr Putth SONGSANGJINDA

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Senior expert on marine shrimp culture, Department of Fisheries, Thailand,
Dr. Putth Songsangjinda has done many researches for more than 30 years in the field of aquaculture system management, particularly on environmental quality, aquaculture eutrophication, effluent treatment, recirculation system, material budget and modeling in marine shrimp production. He had also been invited as a lecturer in many topics related to the experience in development of shrimp farm certification scheme, mangrove friendly shrimp culture and shrimp farm management in many Asian countries by NACA, SEAFDEC and private companies. During 2011- early 2015, He had worked in the position of Director, Marine Shrimp Research and Development Center. He had been involved in the actions of DOF for solving EMS/AHPND problems since the first outbreak occurred in Thailand including the roadmap of restoration of shrimp production that is implementing in Thailand and evaluation of the impact EMS/AHPND on the national shrimp production. As for the new position of Marine Shrimp Culture Expert, He is still involving in this action in particularly the fields of research and technical advisory to DOF, private sectors and shrimp farmers in Thailand.

Dr Rohana SUBASINGHE

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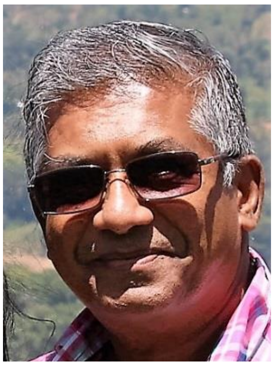

Rohana Subasinghe is a specialist in aquaculture development and aquatic animal health management. He retired from FAO in October 2015, while serving as the Chief of the Aquaculture Brach. Since his graduation in 1980 from the University of Colombo, Sri Lanka, he has worked in all parts of the world, with most experience in Asia. He joined FAO in 1994 and took responsibility in implementing many projects on aquaculture and aquatic animal health at national, regional and international levels worldwide until his retirement. Among others, at FAO, he was also responsible for analyses of trends in aquaculture development globally. A former teacher of the University of Colombo and the Universiti Putra Malaysia, Rohana earned his Ph.D. from Stirling University. Rohana is a strong advocate of sustainable aquaculture and its significant contribution to poverty alleviation and food and nutrition security. His passion is to empower people engaged in small-scale aquaculture value chains. He supports responsible certification of aquaculture and is also responsible for developing the globally approved FAO technical guidelines on aquaculture certification. He served as the Technical Secretary to the Sub-Committee on Aquaculture of the Committee on Fisheries of the FAO, the only global inter-governmental forum on aquaculture. His spearheaded the development of a global partnership for aquaculture development in FAO, particularly addressing the need for a concerted effort to ensure future aquaculture

Mr Shuaib THAIPARAMPIL MUHAMMED

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Shuaib T. Muhammed is Head of Department for Biosecurity at the National Aquaculture Group, NAQUA for the last 8 years. NAQUA is one of the aquaculture giants and leader of desert aquaculture in Saudi Arabia with about 30,000 tons/year. Shuaib develops surveillance and animal health monitoring programs for early detection of diseases. He has got Masters in Marine Biology from Cochin University of Science and Technology, India and trainings at Mahidol University Thailand, University of Arizona USA, on Shrimp Biology and Pathology and also from Autonomous University of Barcelona Spain, Murdoch University Australia on Fish pathology.

Dr Loc TRAN

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Loc Tran, a junior researcher graduated at The University of Arizona with a PhD that focused on shrimp pathology in 2013. In 2014, Loc returned to Vietnam to serve for Nong Lam University at Ho Chi Minh as Assistant Professor teaching courses in aquaculture pathology. At the same time he founded the ShrimpVet Lab, an independent aquaculture pathology research center. The Shrimpvet now has 3 laboratories with more than 30 researchers and crews working on various fields including: disease diagnostics, new technologies in shrimp farming developments, challenge studies on new emerging diseases, technology transfer, and consultancy.

Dr Margy Aliethe VILLANUEVA SOTO

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Professional Biologist, Master in Medical Microbiology at the Javeriana University of Colombia, with experience in Health of live aquatic organisms (fish and shellfish).

Since 2003 she is the Head of Health National Aquatic Species Program of the Technical Department of Animal Health of the Colombian Agricultural Institute (ICA), Colombia Veterinary Service. She also worked in the Technical Department of Quarantine ICA, in formulating protocols and animal health requirements for import and export inspection shipments and issuance of health certificates for live aquatic animals (fish and shellfish) for export and import and the base health regulations for these species. She worked for 7 years in the Aquatic Pathology Laboratory of the National Institute of Fisheries and Aquaculture (INPA), doing diagnostics and microbiological research on fish and shellfish. She has experience in developing programs of prevention and control of aquatic animal diseases, with knowledge of biosecurity measures, quarantine and Best Practices in Aquaculture production as a measure of health management. Since 2003, she is the OIE focal point on Aquatic Animals for Colombia and is part of the Network Group OIRSA and an active contributor to Andean Community of Nations (CAN) aquatic animal health activities.

NOTES

Acute hepatopancreatic necrosis disease (AHPND), currently the most important non-viral disease threat for cultured shrimp, is characterized by mass mortality during the first 35 days of culture where affected shrimp show massive sloughing of hepatopancreatic epithelial cells followed by death. This emerging disease is unlike most diseases affecting farmed penaeid shrimp, in that it is caused by the ingestion of toxins (PirA and PirB) generated by a specific plasmid carried by certain strains of *Vibrio parahaemolyticus*, a bacterium that is ubiquitous in marine and brackishwater environments. The pathogen can thus be present both in cultured shrimp and in the water, sediments and associated organisms of the culture ponds.

TCP/INT/3502 Reducing and managing the risks of Acute Hepatopancreatic Necrosis Disease (AHPND) of cultured shrimp is an inter-regional technical cooperation project being participated by Colombia, Ecuador, Guatemala, Honduras, India, Iran, Mexico, Panama, Peru, the Philippines, and Sri Lanka. This project attempts to raise awareness and disseminate the most up-to-date information about AHPND to better understand the disease and its prevention, control and management. Two major outputs of this inter-regional TCP, include: Output 1: Awareness and technical knowledge on AHPND at national, regional and inter-regional levels enhanced; and Output 2: National and regional actions/strategies and inter-regional framework for cooperation developed to manage and reduce the risks of AHPND.