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

It is expected that there will be two 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. See Annex 3 for tentative list of participants.

Venue:
The Sukosol, Bangkok - A Sukosol Hotel
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FAO Second International Technical Seminar/Workshop on Acute hepatopancreatic necrosis disease (AHPND): There is a way forward!
23-25 June 2016, Bangkok, Thailand
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 (Pir A 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.

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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**

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 (Pir A 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.

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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.
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. monodon*) and fleshy prawn (*P. chinensis*).

**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:

- movement 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$_{\text{AHPND}}$ 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\textsubscript{AHPND} have shown co-infection with other shrimp pathogens, for example, Monodon baculovirus (MBV), White spot disease (WSD), Hepatopancreatic virus (HPV), \textit{Enterocytozoon hepatopenaei} (EHP) and unidentified gregarine-like entities.

**Disease management**

Several innovations in shrimp management have been targeted at reducing the number of VP\textsubscript{AHPND} in the shrimp and its environment by promoting bacterial diversity and control high numbers of pathogenic VP\textsubscript{AHPND}. This has 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 disinfected 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\textsubscript{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 $V_{P_{\text{AHPND}}}$ (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. A second interregional seminar/workshop under this TCP will be held in Asia in 2016 (Bangkok EMS/AHPND June 2016).

**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 21” 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)
Annex 2

Tentative Programme

22 June 2016 (Wednesday):
- Arrival of participants
- Registration open from 15:00-19:00

23 June 2016 (Thursday)
- Opening Session
  o FAO Regional Office of Asia and the Pacific (Mr Vili A. Fuavao, Deputy Regional Representative for Asia and the Pacific)
  o Thailand Department of Fisheries (Mr Adisorn Promthep, Director-General or representative)
  o Self-introduction of participants
  o Introduction to the Technical Seminar/Workshop and Highlights of the Panama Workshop (June 2015)
- Technical Session 1: Industry Update on AHPND
  o Basic pond management to reduce current disease risks (Dr Pornlerd Chanratchakool)
  o New paradigm for controlling AHPND in intensive culture systems: what works, what doesn’t and why (Mr David Kawahigashi)
  o Living with EMS/AHPND: an Asian experience (Dr Mati Nitibhon)
  o The industry response to AHPND in Mexico: a case study (Mr Dan Fegan)
  o Infection trials with both AHPND and EHP: a hatchery protocol that can exclude AHPND (Dr Loc Tran)
  o AHPND: Interaction, control and pond management (Dr Pikul Jiravanichpaisal)
  o Transforming farm managers into the “family physicians” of their own ponds (Dr Grace Chang)
  o Specific pathogen-free, Specific pathogen-resistant and Specific pathogen-tolerant as part of a biosecurity strategy (Dr Victoria Alday)
- Industry Panel Discussions

24 June 2016 (Friday)
- Technical Session 2: Academic Update on AHPND
  o Update June 2016 on AHPND and EHP research in Thailand (Dr Tim Flegel)
  o Polychaetes as a potential risk for shrimp pathogen transmission (Ms Dresdina)
  o Latest research on AHPND and measures to combat it (Dr Ikuo Hirono)
  o Genomic analysis of the strains of AHPND-Vp (global) and genomic target (Dr. Bruno Gomez-Gil)
  o Ecology, virulence factors and global spread of pathogenic *Vibrio parahaemolyticus* and related *Vibrio* spp. (Dr Iddya Karunasagar)
Research progress and biosecurity control strategies against EHP and AHPND for shrimp farming in the People’s Republic of China (Dr Huang Jie)

Epidemiology of AHPND: experiences in Viet Nam and Thailand (Dr Visanu Boonyawiwat)

Characterization of non-vibrio bacteria as potential associates of AHPND bacteria in *Penaeus vannamei* (Dr Kallaya Dangtip)

Heritability, cross-breeding and inbreeding effects on resistance of *Penaeus vannamei* to AHPND and WSSV in Mexico (Dr Hector Castillo – to be confirmed)

Asian shrimp production and the economic cost of disease (Dr Andy Shinn)

### 25 June 2016 (Saturday)

#### Technical Session 3: National and International Framework Update

- Global production and trade in shrimp and risks of pathogen transfer (Dr Rohana Subasinghe)
- Aquaculture biosecurity challenges in the light of the Ballast Water Management Convention (Dr Guillaume Drillet)
- Dealing with AHPND: Viet Nam experience (Dr Dang Thi Lua)
- Dealing with AHPND: Thailand update from 2015 (Dr Putth Songsangjinda)
- AHPND biosecurity measures: Kingdom of Saudi Arabia experience (Dr Saad)
- Avoiding AHPND: Iran experience (Dr Ghahari and Dr Reza)
- Dealing with shrimp diseases in Brasil (Dr Rodrigo Roubach and Dr Thales Passos de Andrade)
- The World Organisation for Animal Health (OIE): relevant activities regarding AHPND (Prof. Mohammed Shariff)
- Status of AHPND in the NACA/FAO Quarterly Aquatic Animal Disease Reporting System (Dr Eduardo Leano)
- Progress on AHPND actions in OIRSA countries: 2015 onwards (Dr Vielka Morales)
- Updates on AHPND in Southeast Asia: outcomes on a recent regional technical consultation on AHPND (SEAFDEC, Dr Ayson and Dr Rolando Pakingking)
- AHPND Contingency Plan under the framework of the Multinational Andine Community Program (Dr Margy Aliethe Villanueva Soto on behalf of Andean)

#### Closing Session

- Presentation of Seminar Highlights and Discussions on the Way Forward
- Thailand Department of Fisheries Closing Remarks
- FAO Closing Remarks
## Tentative List of Participants

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<th>#</th>
<th>Name</th>
<th>Country/Organization</th>
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<tr>
<td>TCP/INT/3502 (AHPND) government delegates</td>
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<tr>
<td>1</td>
<td>Ms Margy Aliethe Villanueva Soto</td>
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<td>Mr Jorge Erraez Cisneros (to be confirmed)</td>
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<td>Ms Nadia Lucia Moreira Olivet</td>
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**Regional and International intergovernmental organizations**

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There is a way forward!
23-25 June 2016, Bangkok, Thailand
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