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Report of the

FAO/MARD Technical Workshop on Early Mortality Syndrome (EMS) or Acute Hepatopancreatic Necrosis Syndrome (AHPNS) of Cultured Shrimp (under TCP/VIE/3304)

Hanoi, Viet Nam, 25–27 June 2013

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Report of the

FAO/MARD TECHNICAL WORKSHOP ON EARLY MORTALITY SYNDROME (EMS) OR ACUTE HEPATOPANCREATIC NECROSIS SYNDROME (AHPNS) OF CULTURED SHRIMP (UNDER TCP/VIE/3304)

Hanoi, Viet Nam, 25–27 June 2013

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
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PREPARATION OF THIS DOCUMENT

This document is the final report of the FAO/MARD Technical Workshop on Early Mortality Syndrome (EMS) or Acute Hepatopancreatic Necrosis Syndrome (AHPNS) of Cultured Shrimp, held in Hanoi, Viet Nam, from 25 to 27 June 2013, under the project TCP/VIE/3304 “Emergency assistance to control the spread of an unknown disease affecting shrimps in Viet Nam”.

The preparation of this report was led by Dr Melba B. Reantaso, Aquaculture Branch (FIRA), Food and Agriculture Organization of the United Nations (FAO), Fisheries and Aquaculture Department (FI), based on deliberations and outcomes of the above-mentioned technical workshop. Additional contributions were received from Dr Rohana Subasinghe (FIRA), Prof. Iddya Karunasagar (FAO Products, Trade and Marketing Branch) and Prof. Claude Boyd (Auburn University).

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The experts who participated in the technical workshop (Annex 2 of this report) are very gratefully acknowledged for their excellent presentations and active participation particularly during the finalization of recommendations on specific and generic actions and management measures to reduce the risk of EMS/AHPNS including comments received post-workshop. Special thanks are due to Ms Dao Thi Thanh Hue of Vietnam’s Regional Animal Health Office No. 6 and Prof. Donald V. Lightner and Mr Loc Tran of the University of Arizona for providing photographs as well as Dr Flavio Corsin for additional information.

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ABSTRACT

Under the auspices of the FAO TCP/VIE/3304 (E) Emergency assistance to control the spread of an unknown disease affecting shrimps, being implemented by Vietnam's Ministry of Agriculture and Rural Development (MARD), the FAO/MARD Technical Workshop on "Early Mortality Syndrome (EMS) or Acute Hepatopancreatic Necrosis Syndrome (AHPNS) of Cultured Shrimp" held in Hanoi, Viet Nam from 25 to 27 June 2013 was attended by 63 experts and shrimp aquaculture stakeholders from public and private sectors.

The Workshop participants were informed of: (i) relevant findings and outcomes of the work carried out under TCP/VIE/3304 project and (ii) updates on EMS/AHPNS situation and experiences in affected Asian countries. To assist in further understanding this disease in terms of its aetiology additional technical presentations from other experts were given. Nineteen technical presentations provided the basis for discussions on actions and measures to reduce the risk of EMS/AHPNS.

The Workshop recognized that complacency in the shrimp aquaculture sector resulting in that laxity, during a period of relatively trouble-free shrimp production, led to vulnerability of the sector to any newly emerging pathogen that might arise unexpectedly, as is the case of EMS/AHPNS. Poor management practices, weak compliance with standard, good biosecurity and good aquaculture practices both at farm and hatchery facilities were evident. It is now clear that shrimp aquaculture needs to improve and continue to develop into a sector that implements responsible and science-based farming practices.

With the current understanding that EMS/AHPNS has a bacterial aetiology, a strain of *Vibrio parahaemolyticus*, the Workshop recommended that a proper name be now given to EMS/AHPNS, i.e. acute hepatopancreatic necrosis disease (AHPND).

The Workshop drew a number of recommendations on specific and generic actions and measures for reducing the risk of AHPND, directed to wider shrimp aquaculture stakeholders (public and private sectors) pertinent to important areas such as: AHPND diagnosis; AHPND notification/reporting; international trade of live shrimp, shrimp products (frozen, cooked), and live feed for shrimp; advice to countries affected and not affected by AHPND; measures at farm and hatchery facilities; advice to pharmaceutical and feed companies and shrimp producers; actions on knowledge and capacity development; AHPND outbreak investigation/emergency response; and specific AHPND-targetted research on various themes (i.e. epidemiology, diagnostics, pathogenicity and virulence, public health, mixed infections, non-antimicrobial control measures, environment, polyculture technologies).

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General background on early mortality syndrome/acute hepatopancreatic necrosis syndrome (EMS/AHPN)

1. An unknown disease of cultured shrimp commonly known as early mortality syndrome (EMS) or more technically known as acute hepatopancreatic necrosis syndrome (AHPNS) appeared to have been infecting the shrimp aquaculture sector in Asia. The susceptible species are *Penaeus monodon*, *P. vannamei* and *P. chinensis*. This disease was considered idiopathic, i.e. it is not known whether the cause is infectious or toxic. Some of the earlier hypotheses pointed to a range of agents and other causes such as cypermethrin (an insecticide), other pesticides, pollution, something in the feed, parasites, harmful algae, probiotics, inbreeding.

2. The earliest publication on EMS/AHPNS was an article[1] by Prof. Donald V. Lighter and his team at the Aquaculture Pathology Laboratory, University of Arizona (APL/UAZ), which reported the first occurrence of this disease in southern China and Hainan Island in 2010 and subsequently in Viet Nam and Malaysia in 2011. This article described the gross signs, susceptible species, and pathology of EMS/AHPNS. It also reported on different experiments (e.g. testing for feed and crusticide; use of frozen samples for infectivity) which all failed to induce lesions consistent with those observed in EMS-infected shrimp.

3. Initially, the role of bacteria was suggested to be secondary; bacterial colonization was prominent at the latter stage of the disease. The role of bacteria *Vibrio parahaemolyticus* came up on several occasions. *Vibrio parahaemolyticus* was consistently isolated from EMS/AHPNS-infected shrimp[2.] Based on the work done in China (Zhang *et al.*, 2012)[3], Chinese researchers reported on a virulent strain of *V. parahaemolyticus* isolated from *P. vannamei* suffering from this early mortality disease in 2010 in Guangxi Province.

4. The potential involvement of a phage (a virus infecting bacteria) was highlighted, during the TCP/VIE/3304 First Joint Technical Workshop conducted in Hanoi, Viet Nam from 5–8 November 2012, by Prof. Timothy W. Flegel of Mahidol University (MU). Although the DNA sequencing work done at MU at that time did not reveal a phage-bacterium relationship, a phage evidence from TCP/VIE/3304 project samples was shown by transmission electron microscopy. In this workshop, ongoing studies at MU also reported on the isolation of phage from two cases: (i) most probably from *V. parahaemolyticus* samples from Rayong, Thailand, and (ii) from *V. fluvialis* (anonymous source).

5. The breakthrough finding of Prof. Lightner's team pointing to a strain of *V. parahaemolyticus* as the causative agent of EMS/AHPNS and with potential phage involvement was revealed through two

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[1] Lightner, D.V., Redman, R.M., Pantoja, C.R., Noble, B.L. & Tran, L. 2012. Early mortality syndrome affects shrimp in Asia. Global Aquaculture Advocate (January/February 2012).

[2] The work carried out under TCP/VIE/3304 conducted by Can Tho University had consistently isolated *Vibrio parahaemolyticus* from field samples collected in September–October 2012 (source: TCDC Consultant Mission Report, unpublished); see para. 36 of this report.

[3] Zhang, B., Liu, F., Bian, H., Liu, J., Pan, L. & Huang, J. 2012. Isolation, identification, and pathogenicity analysis of a *Vibrio parahaemolyticus* strain from *Litopenaeus vannamei*. Progress in Fishery Sci. (in Chinese), 33(2): 56–62.)

international press releases (May 2012 by the Global Aquaculture Alliance[4] and FAO[5]) and a publication in the scientific journal, Diseases of Aquatic Organisms in June 2013 (Tran *et al*., 2013)[6].

6. Recognizing the importance of this newly emerging disease, since 2011, many institutions and agencies (national, regional, international; public and private sectors) have exerted efforts in order to understand and find solutions to this shrimp disease that had caused significant impacts to the shrimp aquaculture sector.

Regional initiatives

7. The Network of Aquaculture Centres in Asia and the Pacific (NACA) and the Department of Agriculture, Fisheries and Forestry of Australia (DAFF) organized an "Emergency Regional Consultation on EMS/AHPNS of Shrimp" from 8 to 10 August 2012, in Bangkok, Thailand[7,8.] The consultation brought together over 87 participants consisting of international shrimp health experts, representatives of regional governments and industries and shared information on this emerging disease, its occurrence, pathology and diagnosis.

8. This consultation identified issues and recommendations addressing four themes: (i) current knowledge, knowledge gaps and research priorities, (ii) detection, reporting and surveillance, (iii) biosecurity, emergency response and disease management and (iv) national/regional disease response. These recommendations included:

- **Case definition**: the case definition developed by Prof. D.V. (APL/UAZ) be used in all epidemiological studies; presumptive diagnosis, i.e. gross clinical signs be used at farm/pond level; development of disease card with case definitions for wider dissemination was recommended.
- **Diagnosis**: all potential causative agents should be considered supported by robust challenge tests.
- **Epidemiology and risk factors:** a regionally-coordinated epidemiological study be done.
- **Capacity building**: at the national and regional levels, expertise on shrimp disease outbreak investigation, epidemiology and histopathology should be further developed and strengthened.
- **Local, national and regional response mechanisms and lessons learned**: there should be more effective pooling, particularly of human and financial resources within countries and regionally; national and regional coordination can be improved; make more effective use of regional bodies, such as NACA, for more cost effective outputs and outcome.

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[4] GAA Press Release (May 2013): Cause of EMS shrimp disease identified
www.gaalliance.org/newsroom/news.php?Cause-Of-EMS-Shrimp-Disease-Identified-107

[5] FAO Press Release (3 May 2013): Culprit behind massive shrimp die-offs in Asia unmasked
www.fao.org/news/story/en/item/175416/icode/

[6] Tran, L., Nunan, L., Redman, R.M., Mohney, L.L., Pantoja, C.R., Fitzsimmons, K. & Lightner, D.V. 2013**.** Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp. Diseases of Aquatic Organisms 105:45-55.

[7] NACA, 2012. Report of the Asia Pacific emergency regional consultation on the emerging shrimp disease: early mortality syndrome (EMS)/ acute hepatopancreatic necrosis syndrome (AHPNS), 9–10 Aug 2012. Published by the Network of Aquaculture Centres in Asia and the Pacific, Bangkok, Thailand. August 2012.

[8] The AHPNS news story as well as the audio recordings of 19 technical presentations are available at the following links:
www.enaca.org/modules/news/article.php?article_id=1952
www.enaca.org/modules/podcast/programme.php

- **Knowledge sharing and communication**: recommended that NACA orchestrate a community dialogue hub for AHPNS for all researchers to share experiences (web-based, List-serve, Wiki, Facebook, etc.); seek to host biannual meetings for researchers and other key stakeholders to share experiences and research outputs, as well as provide a platform for simple steps, e.g. promoting the wider exchange of key research materials and exchange of histopathology slides.
- **Regional emergency fund:** NACA and its partners seek to develop a community emergency fund mechanism that can be accessed by all member countries in the face of an aquatic animal disease emergency.

Impacts of early mortality syndrome[9]

9. Some information on the impacts of EMS/AHPNS are available from virtual, government and other sources.

10. In terms of impacts on production, the Department of Animal Health (DAH) of Viet Nam reported on the affected hectarage of shrimp farms in the Mekong Delta (Figure 1): Tra Vinh Province (6 200 ha in 2011); Soc Trang Province (20 000 ha in 2011); Ca Mau Province (15 000 ha in 2010–2011); and Bac Lieu Province (11 000 ha in 2011). The total affected hectarage of shrimp farms in the Mekong Delta is about 39 000 ha. In Malaysia, the Department of Fisheries estimated production losses at USD0.1 billion in 2011. The GAA estimated that losses to the Asia shrimp culture sector could be USD1 billion[10]. The shortage of shrimp supply subsequently had an impact on shrimp prices.

11. In terms of impacts on private sector enterprises, a few examples from Thailand are found from virtual sources, as below:

a. From the Thai Frozen Foods Association (www.thai-frozen.or.th/), it was reckoned that EMS could cut the country's usual annual output to half in 2013 to 300 000 tonnes and this shortage impacts some of the country's largest companies[11].
b. From the Charoen Pokphand Foods (www.cpf.co.th), it was reported that its first-quarter operating profit dropped 70 percent from a year earlier to 3.44 billion baht (USD109.9 million)[12].
c. From the Thai Union Frozen Products PCL (www.thaiuniongroup.com), the country's biggest exporter, indicated that "sales and earnings in the company's shrimp division will decline by 30 percent this year, after first-quarter profit fell to 54 percent to 674 million baht and a difficult time for the local shrimp sector through 2013[13]".

12. In terms of impacts on trade, several countries have suspended or banned the importation of live shrimp and/or all forms of shrimp products (e.g. Dominican Republic[14], Ecuador[15], Mexico[16], Nicaragua[17], the Philippines[18], United States of America[19]) from countries affected by EMS.

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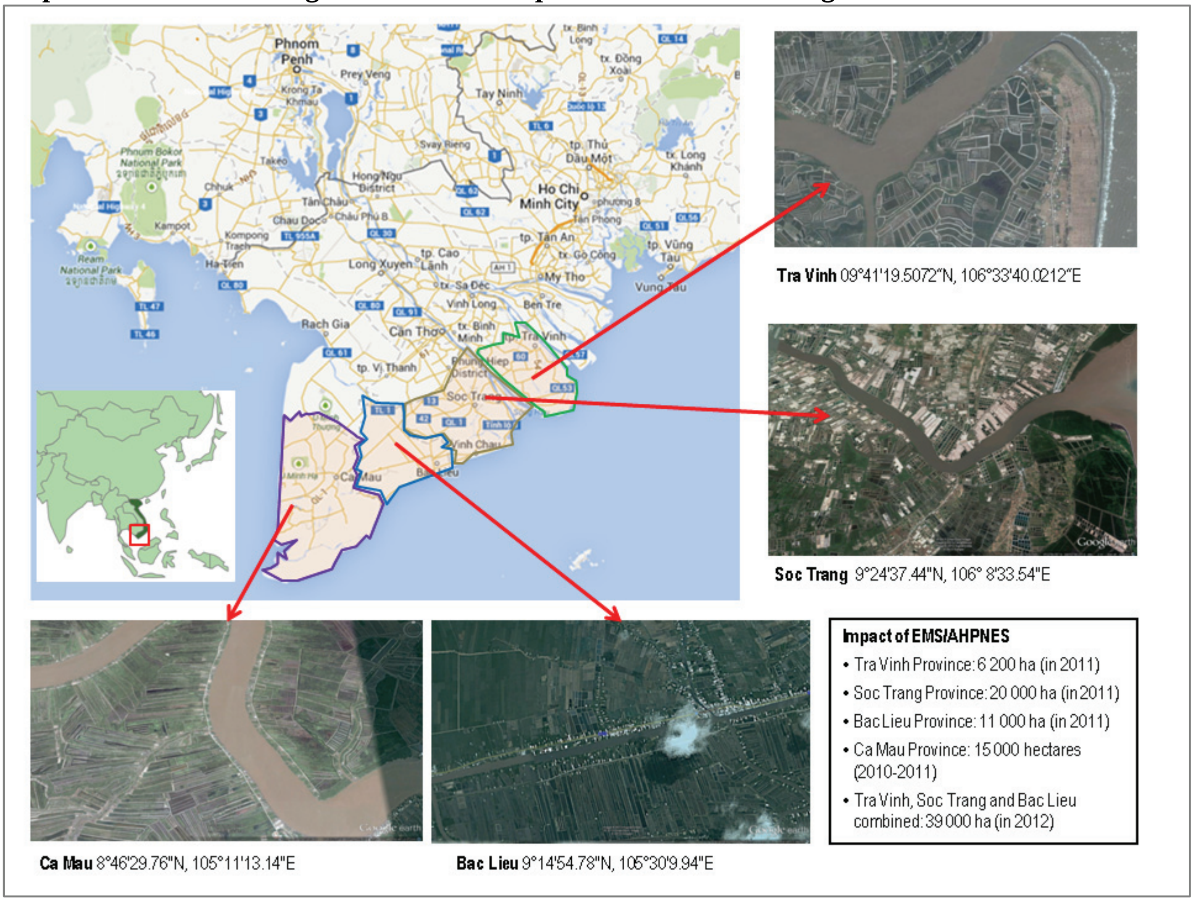
[9] Information on impacts of EMS/AHPNS were gathered from virtual, government and other government sources.
[10] Source: online.wsj.com/article/SB10001424127887323998604578565201120674008.html
[11] Source: online.wsj.com/article/SB10001424127887323998604578565201120674008.html
[12] Source: online.wsj.com/article/SB10001424127887323998604578565201120674008.html
[13] Source: online.wsj.com/article/SB10001424127887323998604578565201120674008.html
[14] Source: Prensa Latina. Dominican Republic Bans Shrimp Imports. 29 July 2013.
[15] Source: www.shrimpnews.com/FreeReportsFolder/NewsReportsFolder/EcuadorBansImportsEMScountries.html
[16] Source: Business Times (Vietnam). Mexico Stops Vietnamese Shrimp Imports. 21 April 2012.
[17] Source: www.thefishsite.com/fishnews/20575/nicaragua-bans-shrimp-imports-over-ems-fears

Figure 1
Map of Viet Nam showing the affected four provinces of the Mekong Delta.



13. In May 2013, two international press releases (GAA[20] and FAO[21]), pointed to a bacterial aetiology based on the findings of Prof. D.V. Lightner and his team at the APL/UAZ. There were also a number of other local (Vietnamese[22] and Chinese) press releases. Because of the significant impacts of EMS/AHPNS, it became the subject of intense debate in web-based discussion and news groups (e.g. Shrimp News International (www.shrimpnews.com), The Shrimp List (finance.groups.yahoo.com/group/shrimp/), etc.) and international meetings (e.g. Global Aquaculture Alliance's GOAL 2012, www.gaalliance.org/GOAL2012NEW; GAA's GOAL 2013, www.gaalliance.org/GOAL2013)

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[18] Source: www.gmanetwork.com/news/story/306163/economy/agricultureandmining/phl-suspends-importation-of-live-shrimps-from-asian-neighbors

[19] Source: The Nation. Rep Warns of US Bid to Ban EMS Shrimp. Petchanet Pratruangkrai. 22 July 2013

[20] GAA Press Release (May 2013): Cause of EMS shrimp disease identified. www.gaalliance.org/newsroom/news.php?Cause-Of-EMS-Shrimp-Disease-Identified-107

[21] FAO Press Release (3 May 2013): Culprit behind massive shrimp die-offs in Asia unmasked www.fao.org/news/story/en/item/175416/icode/

[22] Tìm ra tác nhân gây hội chứng hoại tử gan tụy trên tôm nuôi www.baophuyen.com.vn/Kinh-te-82/3306005905605605154; Cách phòng hội chứng hoại tử gan tụy cấp tính trên tôm Sú và tôm Thẻ nongnghiep.nguontinviet.com/2013/07/cach-phong-hoi-chung-hoai-tu-gan-tuy.html

FAO technical assistance

14. Based on a request from the Government of Viet Nam for technical assistance, a Rapid Deployment Team (RDP)[23], fielded by FAO in July 2011, through the Crisis Management Centre – Animal Health (CMC-AH), made a quick assessment of this unknown disease affecting cultured shrimps in the Mekong Delta provinces of Viet Nam. The findings of the CMC-AH mission based on epidemiological observations and other relevant field data, confirmed that an outbreak occurred (since early 2010 and continued in 2011) with high mortalities among tiger black shrimp (*P. monodon*) and white-leg shrimp (*P. vannamei*). The pattern of disease spread was consistent with an infectious agent, i.e. starting in one pond in one location and subsequently spreading to several ponds within the farm, followed by spread in neighbouring farms. The agent was not known, i.e. the spread pattern and the symptoms were not similar to those of any known major shrimp viral or bacterial disease elsewhere nor in Viet Nam prior to 2010. Table 1 below shows a list of diseases from penaeid shrimps. Shrimps affected by this unknown disease consistently showed the clinical signs of abnormal hepatopancreas (HP), e.g. shrunken, small, swollen and discoloured.

Table 1
List of diseases of *Penaeus monodon*, *P. vannamei* and *P. chinensis*

Disease	***Penaeus monodon***	***Penaeus vannamei***	***Penaeus chinensis****	**Viet Nam****
Infectious hypodermal and haematopoietic necrosis (IHHN) (virus)	√	√	√	√
Infectious myonecrosis (virus)	√	√		
Taura syndrome (virus)		√	√	√
White spot disease (virus)	√	√	√	√
Yellowhead disease (virus)	√	√	√	√
Necrotising hepatopancreatitis (bacteria)		√		
Vibriosis (bacteria)	√	√		√

**P. chinensis* is included in this table because it is one of the EMS/AHPNS susceptible species.
** based on information collected during the mission and other sources (Vietnamese experts).

15. A short epidemiological survey was done during the CMC-AH mission and analysis of farm-level and pond-level questionnaires provided some preliminary insights on a number of risk factors which may play a role in this "unknown disease (syndrome)" (Table 2).

16. The CMC-AH mission recommended the development of an emergency project to be funded by FAO's Technical Cooperation Programme (TCP). Subsequently, an official request to FAO for technical assistance was received in July 2011 from the Department of Animal Health (DAH), Ministry of Agriculture and Rural Development (MARD), the Directorate of Fisheries (D-Fish/MARD) and the Vietnamese Fisheries Society. A TCP project was jointly developed by FAO and MARD in October 2011 and a fully-pledged TCP was submitted to the FAO Representation in Viet Nam in December 2011.

[23] The CMC-AH RDT was composed of Dr Melba Reantaso, Mr Andrew Sobey, Mr Koji Yamamoto and Mr Flavio Corsin; local team from MARD/DAH led by Dr Le Van Khoa assisted the team.

Table 2

Determinants (risk factors) observed during a short epidemiological survey undertaken during the Crisis Management Centre-Animal Health Rapid Deployment Team mission, July 2011, suspected to be playing a role in the "unknown shrimp disease".

	Factors increasing the risk of EMS/AHPNS	Factors reducing the risk of EMS/AHPNS
Farm location	-	• inland location
Farm characteristics and farming system	• intensive system	• rotational shrimp-rice system • use of reservoir • older ponds • farms with fewer ponds
Species	*P. monodon*	-
Pond preparation	• filtering water or washing the pond during pond preparation • use of saponin during pond preparation	-
Post-larvae (seed) source	• seed certified by government authorities • seed from Bac Lieu Province or other provinces outside the Mekong Delta or from unknown sources	• seed from known hatchery or from Soc Trang Province
Water quality	• temperature fluctuations • high salinity	• low salinity
Stocking and stocking season	• stocking more ponds or a larger area • higher stocking density	• stocking *P. monodon* later in the year • stocking older *P. monodon* seed
Feed, supplements and drugs	• adding antibiotics, vitamins, minerals or ferments in feed • use of a particular brand of feed • use of chemicals [insecticides, chlorine]	• having experienced White Spot disease during production
Equipment use	• use of benzalkonium chloride (BKC) for cleaning equipment • common use of equipment	-

17. Officially approved in July 2012, the FAO project TCP/VIE/3304 "Emergency assistance to control the spread of an unknown disease affecting shrimps in Viet Nam" is providing technical assistance to the Government of Viet Nam to support the following outputs: (1) confirm the diagnosis of this unknown disease, currently known as EMS or AHPNS, (2) improve shrimp on-farm biosecurity through farmer training, (3) improve capacity on preparedness to aquatic animal disease emergencies, and (4) develop a national aquatic animal health management (NAAHM) strategy to support long-term improvement of aquatic biosecurity governance capacity of the country. All above four outputs are expected to contribute to a better understanding of this disease, possibly halt its spread, protect the livelihoods of shrimp farmers and sustain shrimp culture in Viet Nam through effective biosecurity. The project is being implemented by MARD through the DAH.

18. Since April 2012, through the joint coordination and facilitation by FAO and DAH and the Department of Fisheries (D-Fish) of MARD, a number of activities were implemented. These include several training/workshops on NAAHM strategy development, review of emergency preparedness guidelines, on-farm shrimp biosecurity training for affected shrimp farmers and design and implementation of a number of studies to better understand EMS/AHPNS in terms of diagnosis

(infectious aetiology, pathology, transmission and infectivity studies, metagenomics, role of environmental toxins) and identification of risk factors through cross-sectional epidemiological studies.

19. In November 2012, a joint technical workshop was held in Hanoi which updated the progress of the work carried out under TCP/VIE/3304 and the MARD National Task Force on EMS and identified additional follow-up studies, followed by a brief progress reporting to the MARD Minister.

20. This FAO/MARD Technical Workshop on EMS/AHPNS of Cultured Shrimp (under TCP/VIE/3304) is part of the implementation of TCP/VIE/3304 and a follow-up from the November 2012 joint technical workshop.

Highlights of the FAO/MARD Technical Workshop on Early Mortality Syndrome (EMS) or Acute Hepatopancreatic Necrosis Syndrome (AHPNS) of Cultured Shrimp (under TCP/VIE/3304), Hanoi, Viet Nam, 25–27 June 2013

Opening session

21. Mr Ken Shimizu, Programme Coordinator of the FAO Representation in Viet Nam, welcomed the participants to this Workshop on EMS/AHPNS. He noted the fairly good representation from Vietnamese authorities and other shrimp aquaculture stakeholder groups as well as foreign experts from both EMS/AHPNS-affected and unaffected countries. He informed the participants of the historical backgound of FAO's assistance to this problem which began during the CMA-AH mission in July 2011 up until the development and implementation of TCP/VIE/3304. An important finding of the CMC-AH mission was the observation of the infectious nature of the agent and a number of determinants or risk factors which may have association with the agent causing the disease. The aspiration of FAO is that the outcomes of this emergency TCP, together with the findings of the work carried out in Viet Nam and other affected countries, can lead to measures that will assist in minimizing the risk associated with this disease and that shrimp farmers are able to bounce back and recover from this unfortunate disease incursion. The good work from colleagues from the APL/UAZ, the outcomes of the TCP, the lessons and approaches from other countries in dealing with EMS/AHPNS can assist in making the governments and the shrimp aquaculture stakeholders better prepared in facing similar disease emergencies in the future. He wished everyone a productive meeting and thanked MARD, DAH, D-Fish and the local and provincial partners for their kind support and collaboration in implementing the TCP/VIE/3304.

22. Dr Tran Dinh Luan, Deputy Director of DAH, welcomed the participants to this workshop. He narrated the history of the request to FAO from the Government of Viet Nam in June 2011 and the objectives of the ongoing TCP/VIE/3304. He was very pleased to see such great speakers present in this Workshop and looked forward to a productive and interesting session. He expressed his appreciation to all experts, representatives of institutions and individuals for their generous support to this endeavour.

23. Dr Melba Reantaso (FAO) presented the purpose, process, participation and expected outcomes of the Workshop. The Workshop was informed of all relevant project findings and the activities carried out under the TCP/VIE/3304 project (as of June 2013), the work carried out by other organizations, particularly APL/UAZ, and updates of EMS/AHPNS-related work done in other affected countries (i.e. China, Malaysia, Thailand). Additional invited presentations provided other relevant technical knowledge which assisted in further understanding EMS/AHPNS as a basis for drawing recommended actions, risk management measures and other follow-up work.

24. The first two days (25–26 June 2013) were devoted to technical presentations; a final session discussed and gathered collective recommendations on specific and generic actions and measures to manage the risk of EMS/AHPNS. The third day (27 June 2013), presentations were made to key MARD

and provincial officials and other shrimp aquaculture stakeholders. The Workshop programme is attached as Annex 1.

25. The Workshop was attended by 63 participants consisting of key personnel involved in TCP/VIE/3304 project, members of the MARD National Task Force on Shrimp Diseases. From Viet Nam, participants included MARD (DAH, DFish, RIAs, DOST, provincial agencies, private sector representatives). From FAO, participants included FAO staff (FIRA, FIPM, FAO Regional Office for Asia and the Pacific, and FAO Representation in Viet Nam) and project consultants. The Workshop also invited key experts involved in the work on EMS/AHPNS in other countries (China, Malaysia, Thailand) and other resource experts (FAO, United States of America). The workshop was facilitated by FAO and MARD. A list of participants can be found in Annex 2.

26. The expected outcomes of the Workshop were achieved, i.e.:

- understanding of the aetiology and epidemiology of EMS/AHPNS was updated and enhanced;
- consensus built on recommendations for actions and measures to manage and reduce the risk of EMS/AHPNS;
- recommendations agreed upon for follow-up work, i.e. publication of a workshop report for wide dissemination as soon as possible.

Session 1: Outcomes of the FAO/MARD Technical Cooperation Programme TCP/VIE/3304 (E) "Emergency assistance to control the spread of an unknown disease affecting shrimps in Viet Nam"

27. During this session various presentations were made pertaining to the work carried out with reference to achieving the following project outputs:

- Output 1: Confirm the diagnosis of the unknown disease (seven presentations);
- Output 2: Farmer training on biosecurity and good aquaculture practices (GAPs);
- Output 4: National Aquatic Animal Health Strategy (NAAHS) for Viet Nam.

28. **"Emergence of a new disease in Southeast Asian Shrimp Farms".** The first presentation from Prof. D.V. Lightner (APL/UAZ) set the scene through a full documentation of the emergence of EMS/AHPNS in Southeast Asian shrimp farms. Prof. Lightner highlighted the main outcomes of the work[24] carried out by his team[25] from the APL/UAZ, Department of Veterinary Science and Microbiology[26], particularly the discovery of the causative agent of EMS/AHPNS.

29. The shrimp farming industry of Southeast Asia constitutes the largest and most productive shrimp farming region in the world. In 2009, an emerging disease called "covert mortality disease" began to cause significant mortalities in China. It is not known whether this ''covert mortality disease'' and EMS/AHPNS are the same or not. By 2010, the range of affected farms in China had expanded, and by 2011 EMS/AHPNS was confirmed in Viet Nam and Malaysia. The disease reached farms in southeastern Thailand in 2012 and caused serious losses in the areas affected by the disease; it has also caused secondary impacts on employment, social welfare and international market presence.

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[24] The work carried out by Prof. D.V. Lightner's team was supported by the following: World Organisation for Animal Health for travel; Department of Animal Health, MARD, Vietnam for local arrangements in Vietnam; Uni-President feed company in Vietnam for funding toxicity and infectivity studies; CP Foods, Thailand for funding recent work on EMS; World Bank and Global Aquaculture Alliance for travel; FAO and Minh Phu Seafood for partial funding; Grobest for partial funding of this work and for molecular biology work.

[25] R.M. Redman, C.R. Pantoja, B.L. Noble, L.M. Nunan and Loc Tran.

[26] OIE Reference Laboratory for Shrimp Diseases.

30. The principal gross signs (see Figure 2) presented by shrimp affected by EMS/AHPNS typically begin within 10–30 days post-stocking of post-larvae (PL) into a newly prepared pond. Affected shrimp typically present a pale to almost white, often atrophied HP. Black spots or streaks are often visible in such HPs, which following excision, may not squash easily between the thumb and forefinger. Affected shrimp may also present soft shells and partially full to empty guts.

31. Histopathology of EMS/AHPNS-affected shrimp shows an acute progressive degeneration of the HP from medial to distal with dysfunction of B-, F-, R- and eventually, E-cells. Also present is a prominent karyomegaly and necrosis and sloughing of these tubule epithelial cells. The sloughing of HP tubule epithelial cells is the principal diagnostic feature of EMS/AHPNS. The terminal stage of EMS/AHPNS shows marked inter- and intra-tubular hemocytic inflammation and development of secondary bacterial infections that occur in association with necrotic and sloughed HP tubule cells (Figure 3).

Box 1

General information about *Vibrio parahaemolyticus*

V. parahaemolyticus is a common inhabitant of coastal and estuarine environments all over the world. Hence they are often found naturally associated with shrimp aquaculture systems. Certain environmental conditions may be more favourable for the establishment, survival and growth of the organism such as temperature, salinity, zooplankton, tidal flushing and dissolved oxygen. *V. parahaemolyticus* is closely related to shrimp pathogenic luminous bacteria such as *V. harveyi*, *V. campbelli* and *V. owensii*. These along with other closely related *Vibrio* spp form a "*V. harveyi* clade" (Cano-Gomez *et al*., 2009)[27]. Bacteria within this clade have a very high degree of similarity at phenotypic and genotypic level. Certain strains of *V. parahaemolyticus* can cause gastroenteritis in humans and clinical strains are characterised by the ability to produce a thermostable direct hemolysin (TDH) or a TDH-related hemolysin (TRH). The genes encoding these hemolysins (*tdh* and *trh* genes) are generally used as markers for human pathogenic strains of *V. parahaemolyticus* (FAO/WHO, 2011)[28]. Human pathogenic strains possessing these markers account for 1–2 percent of environmental strains of *V. parahaemolyticus*. All strains (both clinical and environmental) produce a thermolabile hemolysin (TLH) encoded by *tlh* gene and this is generally used as a marker for *V. parahaemolyticus* in diagnostic tests (Keysner and Depaola, 2004)[29]. The *tdh* and *trh* genes encoding the virulence factors are present in "pathogenicity islands", which are discrete genetic units present only in virulent strains; having a Guanine + Cytosine (G + C) content that is different from the rest of the chromosomal DNA and are generally acquired by horizontal gene transfer.

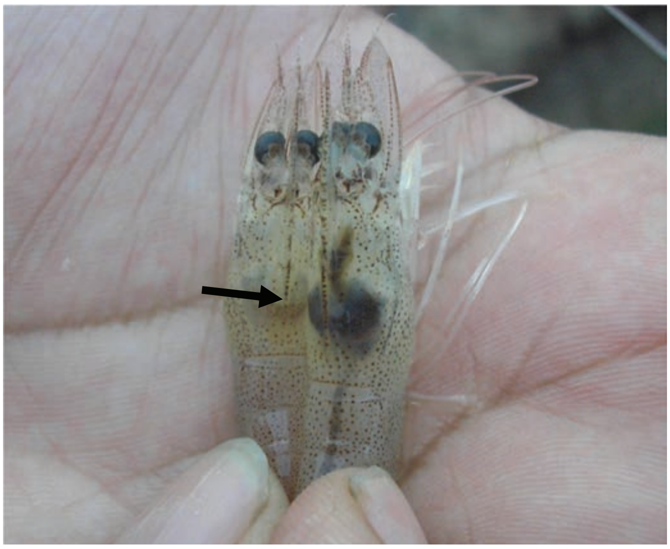
[27] Cano-Gomez, A., Bourne, D.G., Hall, M.R., Owens, L. & Hoj, L. 2009. Molecular identification, typing and tracking of *Vibrio harveyi* in aquaculture systems: Current methods and future prospects. Aquaculture, 287:1-10.

[28] FAO/WHO, 2011. Risk assessment of *Vibrio parahaemolyticus* in seafood: Interpretative Summary and technical Report. Microbiological Risk Assessment Series 16. FAO, Rome, 183p.

[29] Kaysner, C.A. & DePaola, A. 2004. *Vibrio*. In Bacteriological Analytical Manual, US FDA, Chapter 9. www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070830.htm.

Figures 2 a, b, c and d

Typical gross signs of EMS/AHPNS showing normal and abnormal hepatopancreas. (All photos courtesy of Prof. D.V. Lightner [UAZ]).



a. Juvenile *Penaeus vannamei* from Viet Nam showing gross signs of EMS/AHPNS, specifically a pale atrophied hepatopancreas and an empty stomach and midgut.

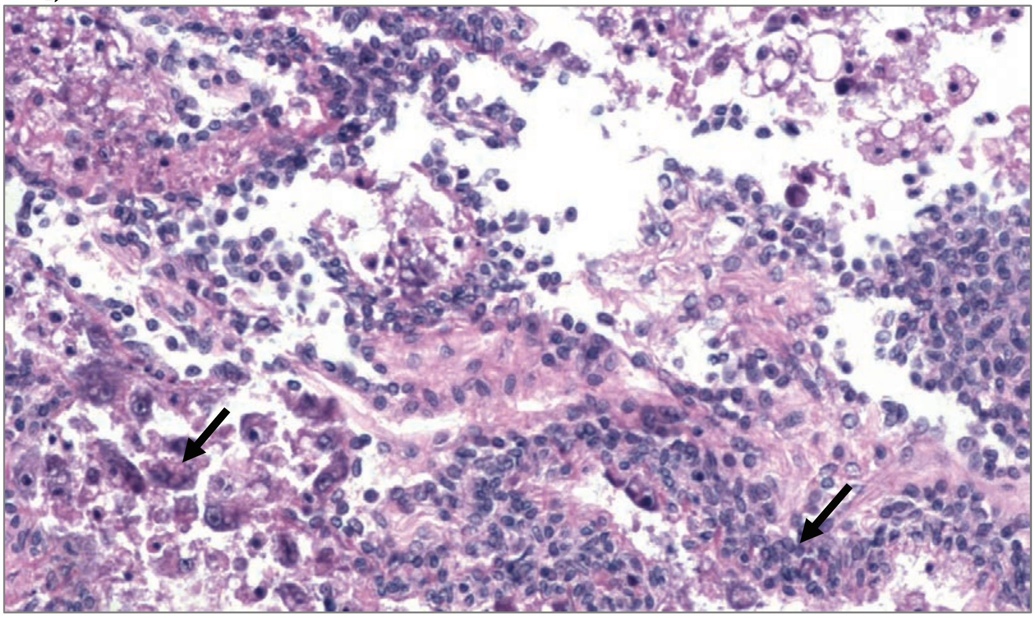
b. *Penaeus monodon* from Viet Nam. Shrimp shows normal hepatopancreas (arrow). Two *P. monodon* show signs of severe EMS/AHPNS as shown by the significantly atrophied hepatopancreas (arrows).

c. Juvenile *Penaeus vannamei* from Viet Nam showing atrophied hepatopancreas indicative of EMS/AHPNS.

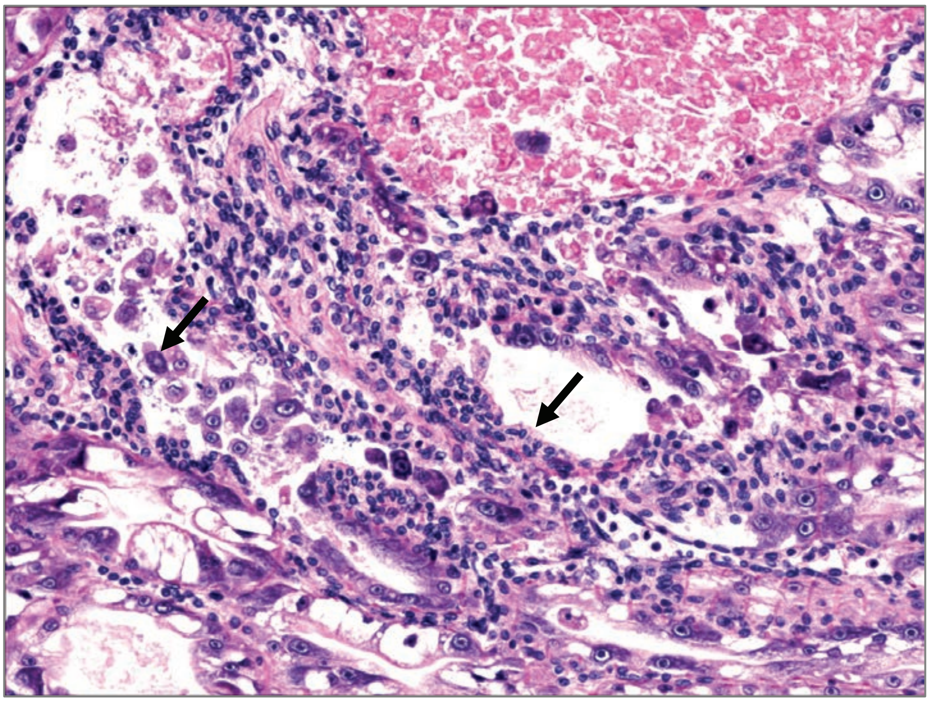
d. Juvenile *Penaeus monodon* from Viet Nam infected with EMS/AHPNS. The hepatopancreas is pale and atrophied; the midgut is empty.

Figures 3 a, b, c and d

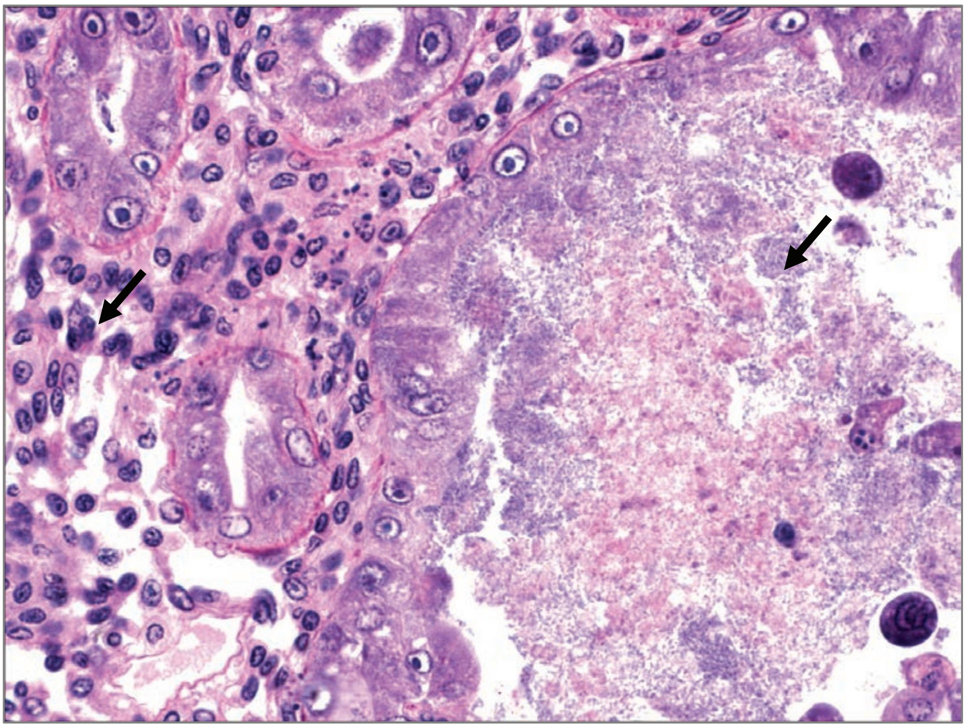
Histological sections showing the main pathological features of both natural infection (a and c) and experimentally-induced EMS/AHPNS shrimps (b and d). (All photos courtesy of Mr Loc Tran [APL/UAZ]).



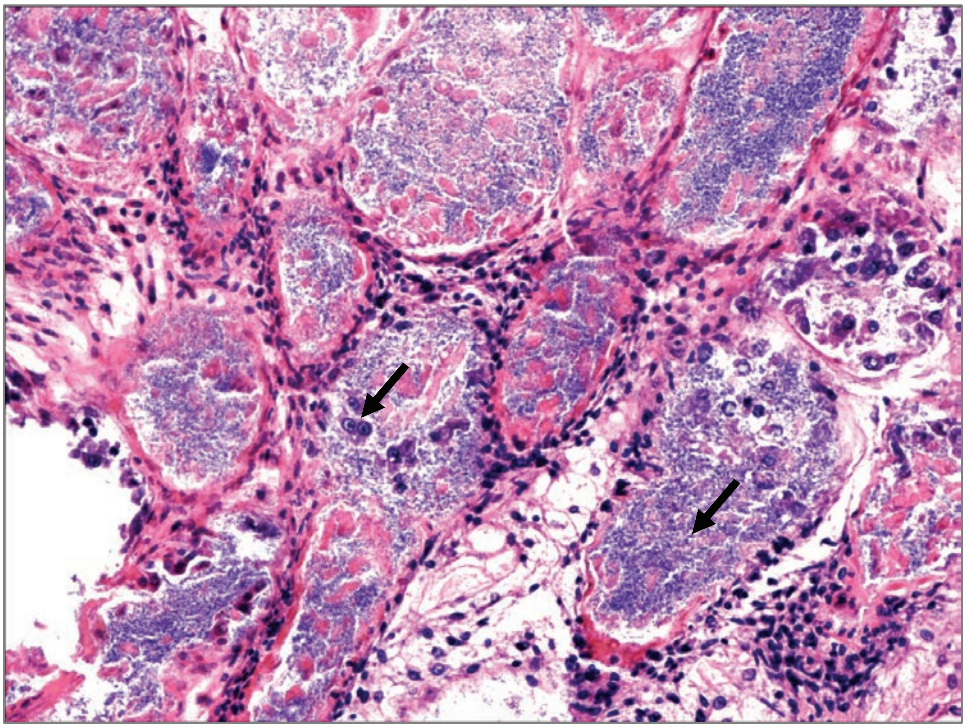
a. EMS natural infection of *P. vannamei*, acute phase. Arrows (L to R): acute sloughing of tubular epithelial cells, haemocyte infiltration. 20x magnification, H&E stain.



b. EMS experimentally induced *P. vannamei*, acute phase. Arrows (L to R): acute sloughing of tubular epithelial cells, haemocyte infiltration. 20x magnification, H&E stain.



c. EMS natural infection of *Penaeus vannamei*, terminal phase. Arrows (L to R): haemocyte infiltration, bacterial colonization. 40x magnification, H&E stain.



d. EMS experimentally induced *P. vannamei*, terminal phase. Arrows (L to R): sloughed tubular epithelial cells, intratubular bacterial colonization, haemocytic infiltration. 40x magnification, H&E stain.

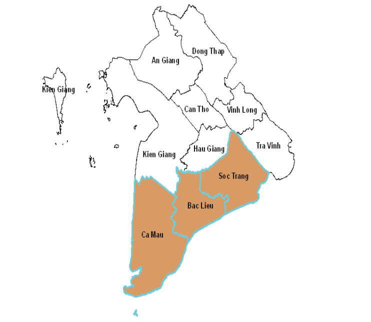
32. The aetiology of EMS/AHPNS has been determined to be caused by the bacterium, *Vibrio parahaemolyticus*. This was confirmed by a series of studies which confirmed *V. parahaemolyticus* as the cause of the disease. Koch's four postulates were completed in establishing the aetiology of the disease. Of interest is that metagenomic sequencing of the agent found to cause EMS/AHPNS, and of a non-pathogenic *V. parahaemolyticus* have indicated the agent of the disease has several extra-chromosal elements that may be the source of the extreme virulence of this bacterium.

33. **"Histology and bacterial metagenomics"**. Prof. T.W. Flegel's presentation on the work carried out by the MU-Centex under TCP/VIE/3304, reported that a total of 224 shrimp specimens from 60 ponds (mean 3.7 ± 1.7 specimens/pond) were received. These samples, in Davidson's fixative and prepared for histological examination by standard procedures for paraffin sections stained with hematolylin and eosin (H&E), were examined for EMS/AHPNS status and for the presence of White Spot syndrome virus (WSSV), Monodon baculovirus (MBV) and Hepatopancreatic virus (HPV). Out of the 60 ponds, 24 (40 percent) exhibited normal HP tissues while the other 28 ponds showed at least some of the specimens exhibiting EMS/AHPNS alone or together with WSSV and/or MBV and/or HPV. Using results from the APL/UAZ as the gold standard, the sensitivity and specificity of the histological diagnosis of pond status for EMS/AHPNS were 79 percent and 90 percent, respectively, with the relatively high level of false negative (low sensitivity) due to the smaller number of specimens (mean=3.7) provided for analysis to the MU-Centex laboratory when compared to the APL/UAZ laboratory (6.7). With respect to metagenomic analysis, six confirmed EMS/AHPNS pond samples and three confirmed normal pond samples were chosen based on the combined histology results from APL/UAZ and MU-Centex. From the mass sequencing result, a total of 21.7 million bases in 42 564 fragments of 511 bp average length were obtained. Using four different algorithms, a total of 19 to 48 organizational taxonomic units (OTU) were obtained for further analysis. This revealed no unique bacterial species associated only with EMS/AHPNS ponds (i.e. not found also in normal ponds). In addition, principal component analysis (PCA) revealed no evidence for the association of the bacterial profiles of EMS/AHPNS. However, two OTUs (201303-350-0011 and 201303-350-0002) showed a significantly higher proportion in EMS/AHPNS ponds than in normal ponds ($p<0.05$) and nucleic acid alignment revealed that these differed in sequence by 4 percent suggesting that they originated from different bacterial species. BLASTn analysis of these sequences gave 99 percent identity to *V. proteolyticus* and *V. parahaemolyticus*, respectively. The results suggested that further research should be focused on these two bacterial species.

34. **"Diagnostic studies: histopathology and PCR".** Ms Dao Thi Thanh Hue, of the Regional Animal Health Office No. 6 (RAHO 6), provided a summary of the work carried out by Can Tho University (CTU) and RAHO 6 under TCP/VIE/3304, consisting of a description of field sampling activities (Table 3) and methodologies, and the results of histopathology examination (Table 4) and PCR tests (Table 5). Field sampling activities were carried out between 11 September until 6 October 2012, with three rounds of sample collection in each of the three Mekong Delta provinces (Soc Trang, Bac Lieu and Ca Mau).

Table 3

Location and dates of sampling of shrimp samples used for histopathology analysis and PCR tests



Sampling No.	Sampling time	Location
1	11/09/12–12/09/2012	Soc Trang: Round 1
2	14/09/12–15/09/2012	Bac Lieu: Round 1
3	17-09/12–18/09/2012	Ca Mau: Round 1
4	20/09/12–21/09/2012	Bac Lieu: Rround 2
5	23/09/12–24/09/2012	Ca Mau: Round 2
6	26/09-12–27/09/2012	Soc Trang: Round 2
7	29/09/12–30/09/2012	Bac Lieu: Round 3
8	02/10/12–03/10/2012	Ca Mau: Round 3
9	05/10/12–06/10/2012	Soc Trang: Round 3

35. At each sampling location, samples were collected from three infected and three non-infected ponds. At the end of the sampling campaign, there were 36 infected and 24 non-infected ponds where samples (Figure 4) were collected. A total of 60 samples (each sample has >20 shrimps) were preserved in Davidson's fixative for histological analysis and 60 samples (pooled gills and pleopods of 10 individuals) were preserved in 95 percent ethanol for PCR analysis. Ten individuals from each pond were subjected to fresh smear examination, fixed and subsequently used for Gram-staining. In addition, HP samples from five individuals were taken for *Vibrio* spp. bacterial and fungal isolations. The remaining live shrimp of each pond were snap-frozen in liquid nitrogen. Samples were transferred to -80°C at the laboratory of CTU. A total of 175 *Vibrio* spp. bacterial isolates were recovered from the HP of shrimp specimens. Isolates were stored at -80°C in Tryptone Soy broth (Oxoid) containing 15 percent glycerol and supplemented with 1 percent (w/v) sodium chloride. Five fungal isolates were recovered from HP of shrimp samples. Isolates were kept on Peptone Yeast Extract Glucose Starch (PYGS) agar plates at 4°C. Tables 4 and 5 below show the results of histopathology and PCR analyses.

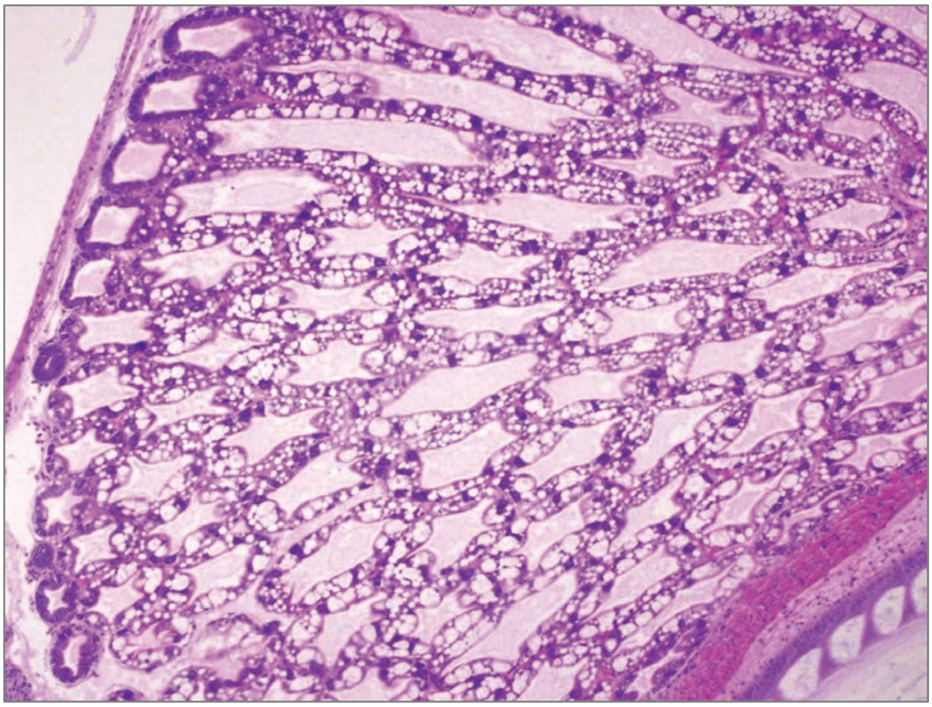
Table 4

Results of histopathology (n=60 ponds)

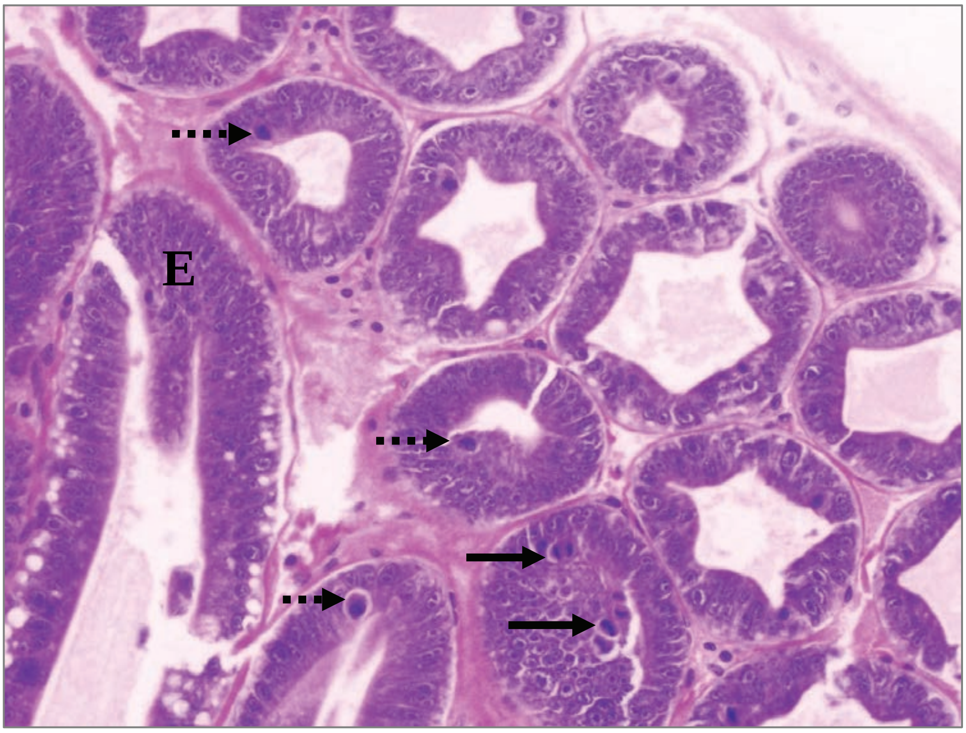
Pathogen	**Laboratory**											
	APL/UAZ				**MU-Centex**				**RAHO 6**			
	Soc Trang	Bac Lieu	Ca Mau	Summary	Soc Trang	Bac Lieu	Ca Mau	Summary	Soc Trang	Bac Lieu	Ca Mau	Summary
EMS/AHPNS	3	16	10	29	6	17	7	30	4	18	9	31
WSSV **(Fig. 5)**	7	4	1	12	7	1	1	9	10	7	5	22
MBV **(Fig. 6)**	0	18	1	19	0	8	2	10	1	7	0	8
HPV **(Fig. 7)**	0	2	0	2	0	1	0	2	0	1	0	1
Gregarine	-	-	-	-	-	-	-	-	1	6	1	8
Gregarine-like	-	-	-	-	12	8	15	33	6	2	2	10

Figure 4 a, b, c and d

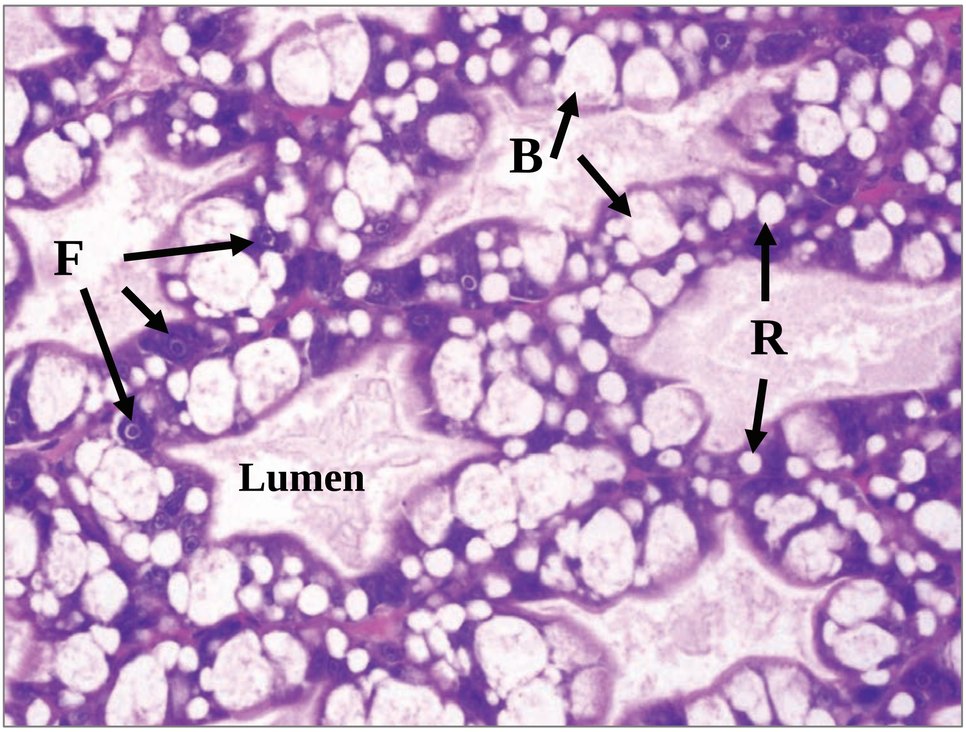
Histological sections of normal hepatopancreas of shrimp (*Penaeus vannamei*) showing E-, R-, B- and F-cells. (All photos courtesy of Ms Dao Thi Thanh Hue [RAHO 6]).



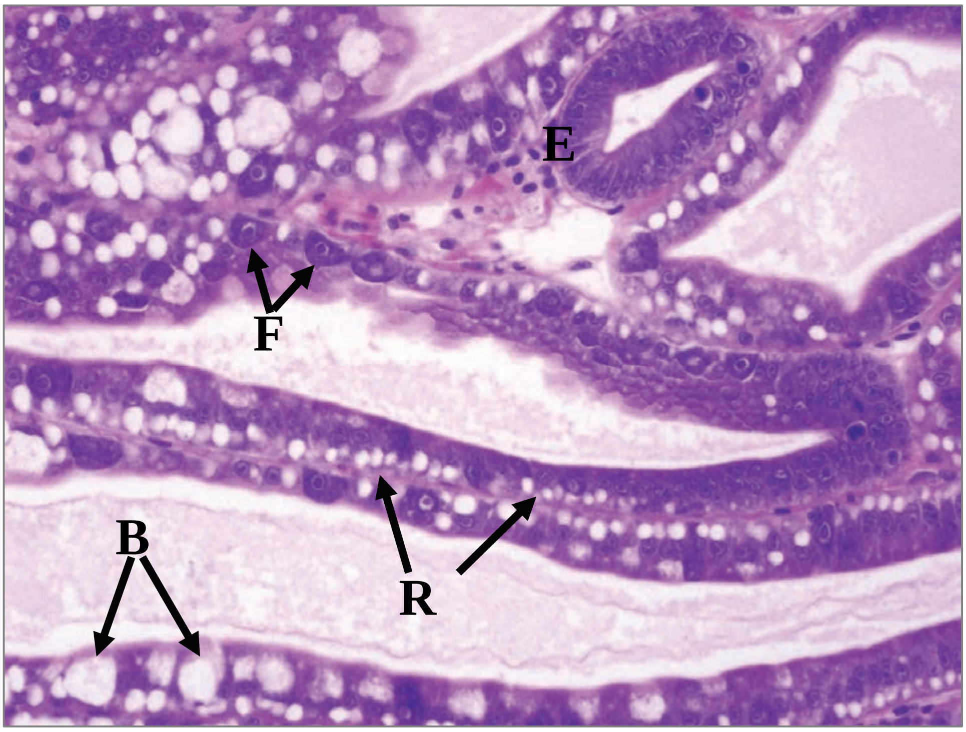
a. Normal hepatopancreas, *P. vannamei*, Ben Tre Province, Viet Nam, 20x magnification, H&E stain



b. Normal hepatopancreas, *P. vannamei* (Ben Tre Province, Viet Nam) showing normal E-cells: E-cell division (arrow); metaphase (broken arrows), 20x magnification, H&E stain



c. Normal hepatopancreas, *P. vannamei* (Ben Tre Province, Viet Nam) showing lumen, normal developing B-, R-, and F-cells, higher magnification at 40x magnification, H&E stain



d. Normal hepatopancreas, *P. vannamei* (Ben Tre Province, Viet Nam) showing normal developing B-, R-, and F-cells, lower magnification at 20x, H&E stain

Figure 5

Histological sections of EMS/AHPNS-infected *Penaeus monodon* from Bac Lieu Province, Viet Nam, showing mixed infection with white spot disease (arrows show inclusion bodies) in the stomach. Upper photo (20x magnification). Lower photo (40x magnification). HP=hepatopancreas; A=stomach and midgut). H&E stain. (Photos courtesy of Ms Dao Thi Thanh Hue [RAHO 6]).

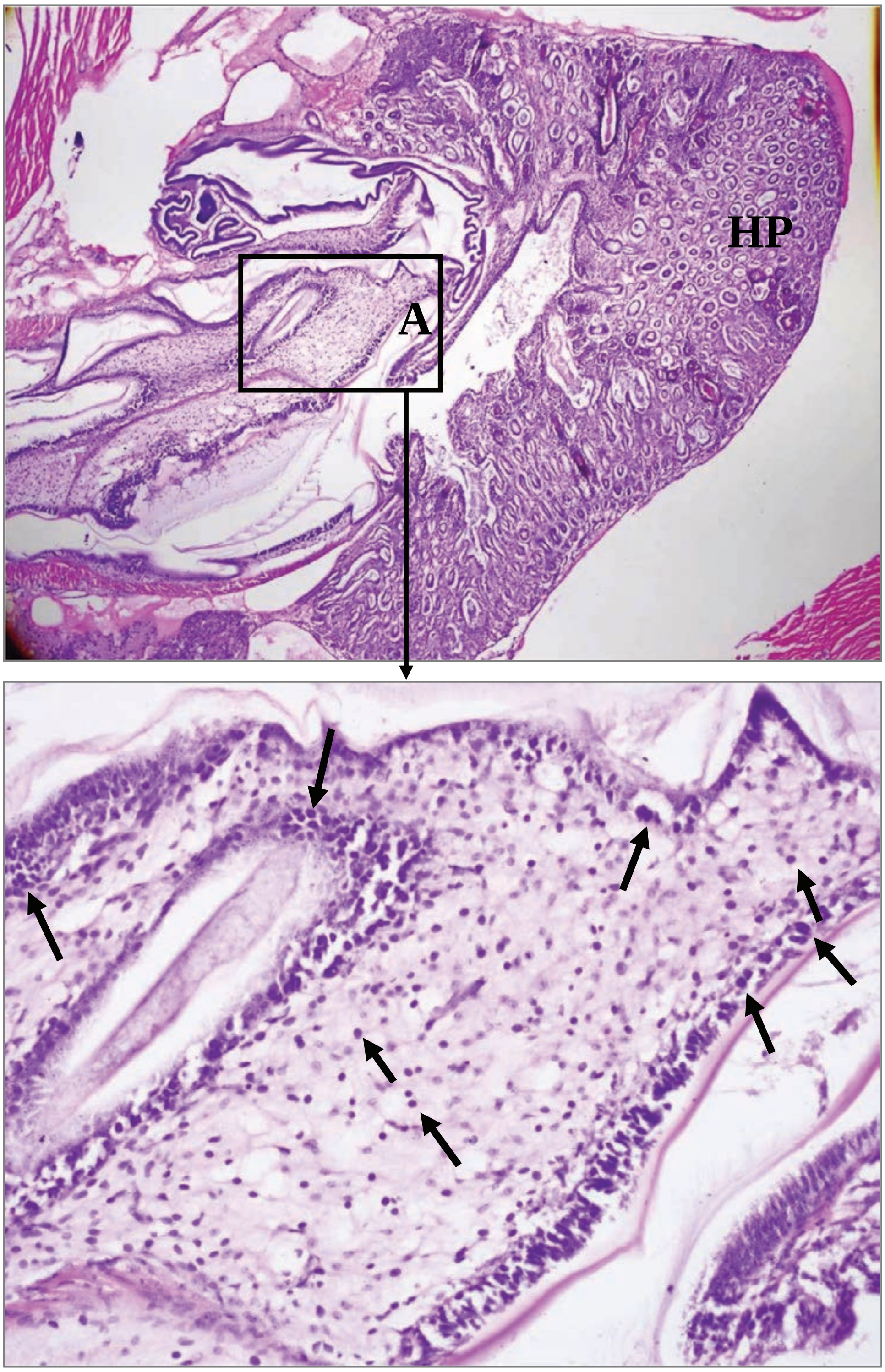
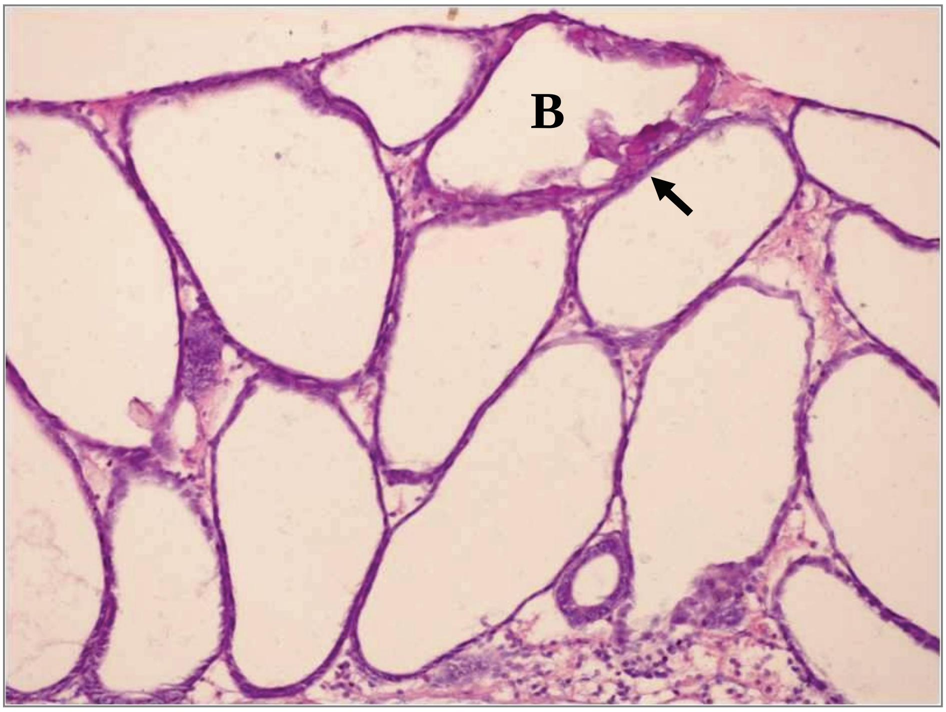


Figure 6

Histological sections of EMS/AHPNS-infected Penaeus monodon from Bac Lieu Province, Viet Nam, showing mixed infection with Monodon baculovirus (MBV) occlusion bodies (arrows) in B area. Upper photo (20x magnification; Lower photo (40x magnification). H&E stain.
(Photos courtesy of Ms Dao Thi Thanh Hue [RAHO 6]).



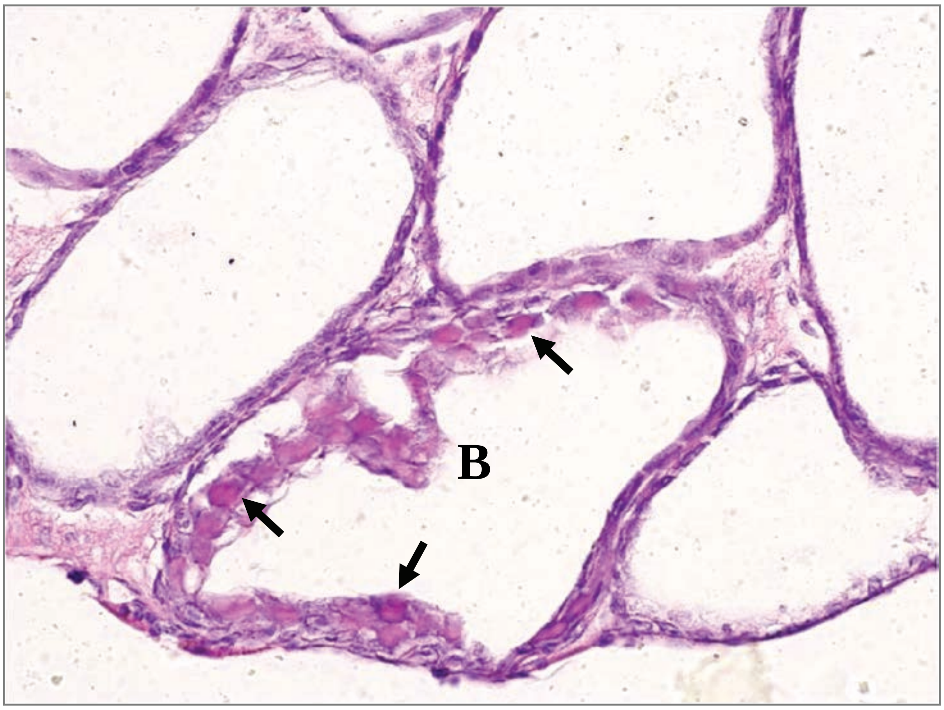


Figure 7
Histological sections of EMS/AHPNS-infected Penaeus monodon from Bac Lieu Province, Viet Nam, showing mixed infection with Hepatopancreatic virus (HPV) intrainclusion bodies (arrows) in the E (upper photo) and F area (lower photo). 40x magnification. H&E stain. (Photos courtesy of Ms Dao Thi Thanh Hue [RAHO 6]).

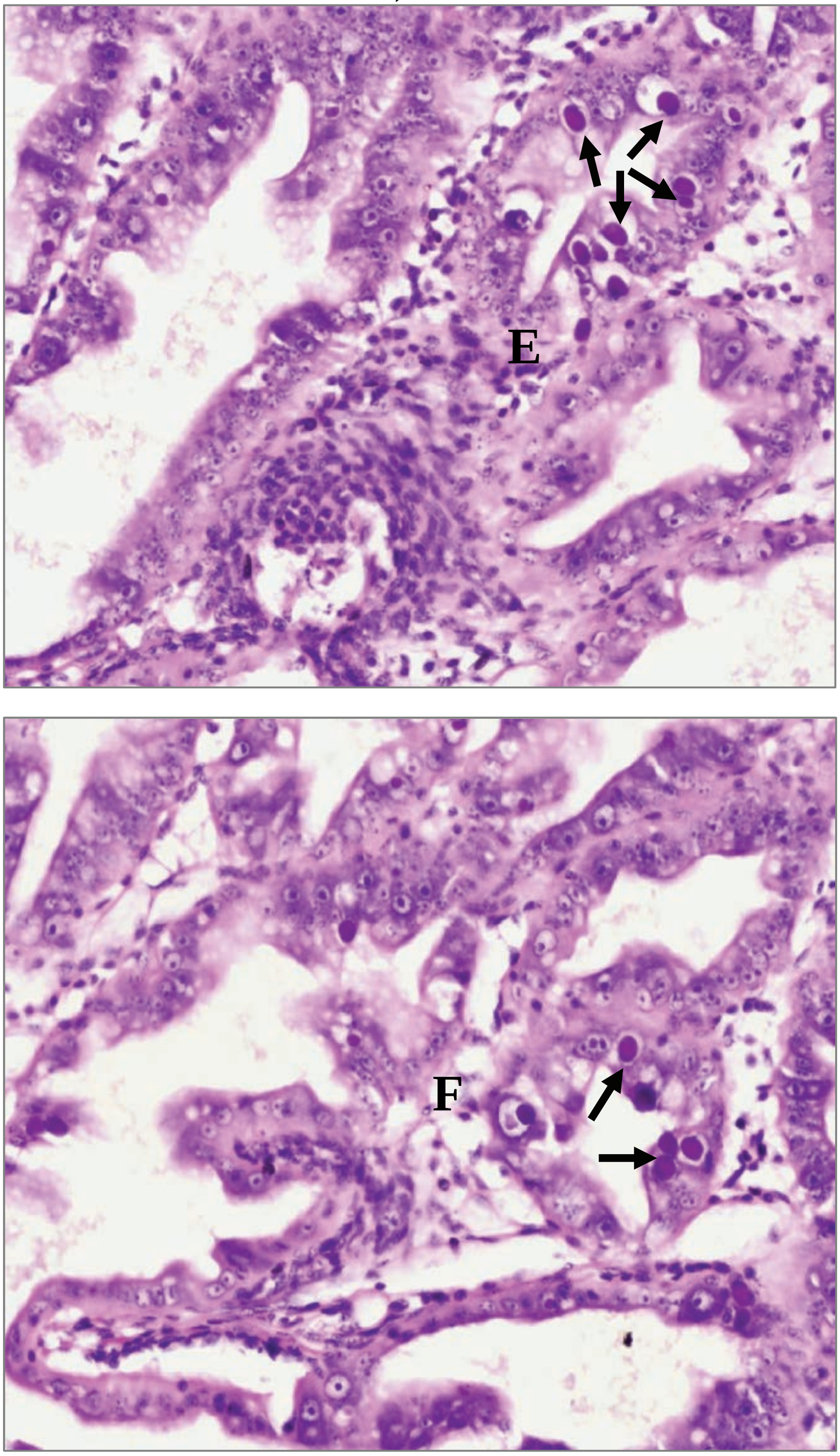


Figure 8

Histological sections of EMS/AHPNS-infected Penaeus monodon from Bac Lieu Province, Viet Nam, showing mixed infection with gregarine entities found in the gut; C=sloughing cells; D=entities infected area. Upper photo (20x magnification). Lower photo (40x magnification). H&E stain. (Photos courtesy of Ms Dao Thi Thanh Hue [RAHO 6]).

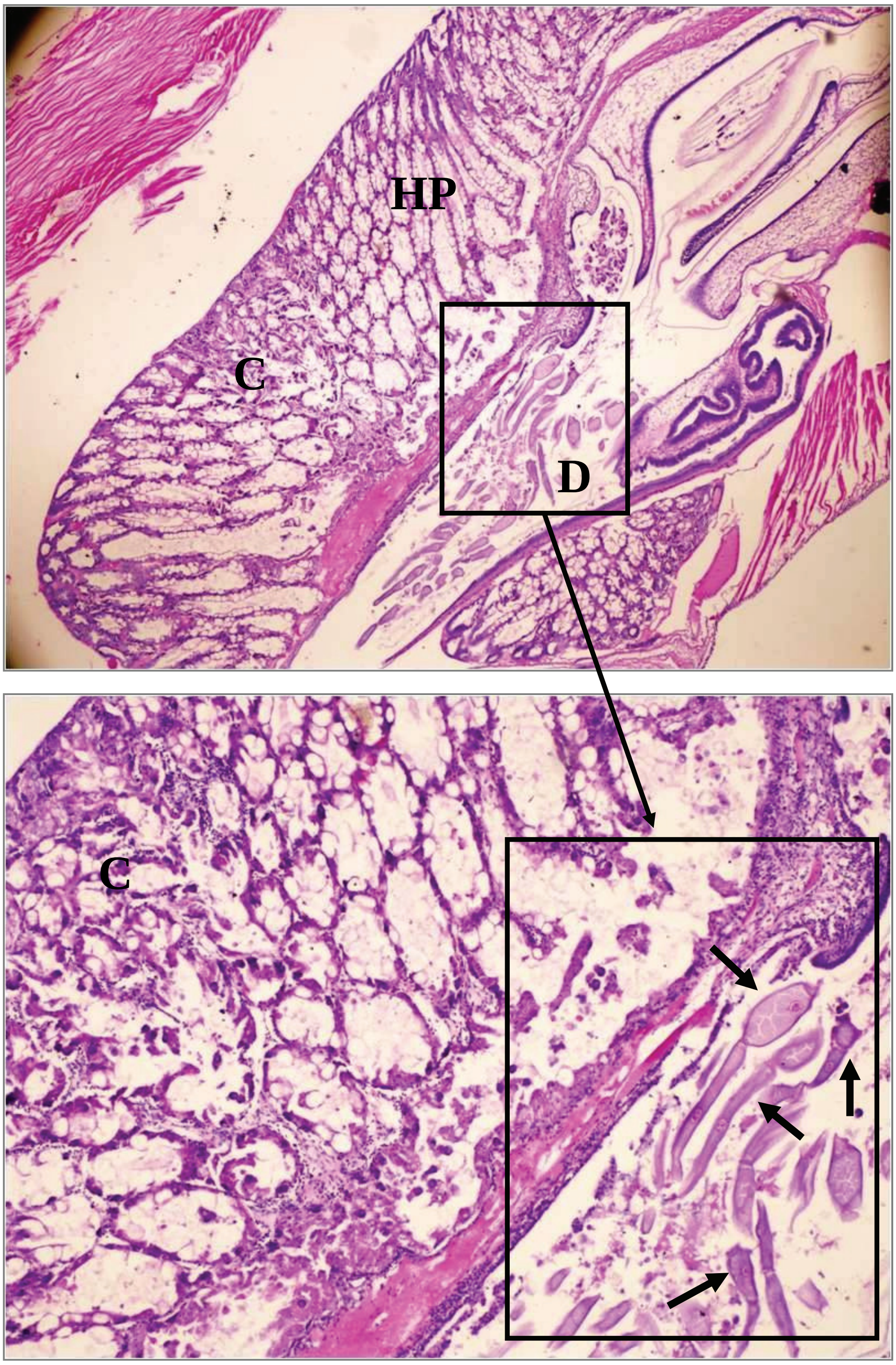


Table 5
Results of PCR test

	PCR test results	
	Positive	**Negative**
WSSV	23	37
YHD	0	60
IMNV	0	60

36. **Diagnostis studies: parasites, fungal and bacterial agents.** Dr Dang Thi Oanh (CTU) presented the outcomes of the work carried out by CTU and RAHO 6 under TCP/VIE/3304 on parasitological, fungal and bacterial agents. Observations on disease signs at the pond level include: pale to white and significant atrophy of HP, soft shells and guts with discontinuous or no contents. The onset of clinical signs and mortality started as early as 10 days post-stocking; moribund shrimps came to the pond side or sink to bottom. Gram-staining of fresh smear of HP from affected shrimp clearly showed the presence of Gram-negative rod-shaped bacteria. In addition, gregarine-like entities (Figure 8) were found in samples from both EMS/AHPNS-infected and non-infected ponds. A total of 175 *Vibrio* spp. bacterial isolates were recovered from shrimp HP. Isolates were identified at species level using API 20E kit. The majority of them belong to *V. parahaemolyticus*. Identification of *V. parahaemolyticus* isolates was confirmed by 16S rRNA sequencing. All *V. parahaemolyticus* isolates revealed haemolysis after two days of incubation on blood agar plates (Blood agar base, Merck with 5 percent calf blood) and three isolates were found to carry phages. Ninety *V. parahaemolyticus* isolates which were selected from three sampling locations (30 isolates each from Soc Trang, Bac Lieu and Ca Mau provinces) and subjected to repetitive element palindromic PCR (rep-PCR) analysis. At the same time, these isolates were used for detection of *tdh* and *trh* genes. Results from rep-PCR analysis resulted in at least four different DNA profiles of tested isolates. Thermolabile hemolysin (TLH) encoded by *tlh* gene was detected from all tested isolates but neither *tdh* nor *trh* genes. In addition, 10 fungal isolates were recovered from shrimp HPs.

37. **"Field study on transmission in EMS/AHPNS-infected pond".** Dr Dang Thi Oanh (CTU) presented the outcomes of the transmission studies carried out under TCP/VIE/3304 to determine if EMS/AHPNS can be transmitted by co-habitation. Experimental hapas containing healthy *P. vannamei* were set up in EMS/AHPNS-infected ponds to study transmission of EMS/AHPNS via water. Hapas containing healthy shrimps and EMS/AHPNS-infected shrimp were set up in AHPNS-infected ponds to study transmission in EMS/AHPNS by co-habitation. Thirty shrimps per treatment were checked for EMS/AHPNS by histology after 10 experimental days. Bacterial isolation was carried out to recover *V. parahaemolyticus* in AHPNS-affected shrimp and these were used for DNA profiling and detection of *tdh* and *trh* genes. Results from both gross signs and histopathology observations revealed typical EMS/AHPNS clinical signs and lesions in experimental shrimps. Transmission of EMS/AHPNS occurred via water (16/60 tested shrimp) and cohabitation (27/60 tested shrimp) of healthy shrimp in EMS/AHPNS-infected ponds. A total of 74 bacterial isolates (~ *Vibrio*) were recovered from HP of shrimp samples. Among these, 31 isolates were identified as *V. parahaemolyticus* by API 20E. Rep-PCR analysis of recovered isolates resulted in at least four different DNA profiles. Thermolabile hemolysin (TLH) encoded by *tlh* gene was detected from all tested isolates but neither *tdh* nor *trh* genes.

38. **"Environmental studies: assessment of toxins in feed, sediment, and water".** Prof. Claude Boyd (Auburn University) presented the outcomes of environmental studies carried out under TCP/VIE/3304. Results of a third and final round of environmental sampling conducted in late September and early October 2012 from shrimp ponds in Soc Trang, Bac Lieu, and Ca Mau provinces of the Mekong Delta, did not differ from those of the first and second rounds of environmental sampling. Average concentrations of major water quality variables, i.e., salinity, pH, dissolved oxygen, un-ionized ammonia, nitrite, hydrogen sulfide, and trace elements did not differ between ponds with EMS/AHPNS-infected shrimp and those with EMS/AHPNS-free shrimp. The concentrations of water quality variables were

within acceptable ranges as frequently in ponds with EMS/AHPNS-infected shrimp as in ponds with healthy shrimp. Moreover, there were no differences in average concentrations of pesticides between waters of ponds related to EMS/AHPNS status of shrimp. Algal toxins and PCBs were not detectable in water samples. Sediment from ponds with healthy shrimp did not differ from ponds with EMS/AHPNS-infected shrimp in average concentrations of trace elements and pesticides. Feed samples from farms affected by EMS/AHPNS had higher average concentrations of two antioxidants, i.e. ethoxyquin and butylated hydroxytoluene, than did feed samples from farms unaffected by EMS/AHPNS. The antioxidant concentrations were below toxic levels, but they possibly could have stressed shrimp.

39. Findings did not show an association between environmental conditions (water quality, sediment condition, pollution, or feed ingredients) and EMS/AHPNS-status of shrimp. It is also important to note that these results do not support the pH-trigger hypothesis for EMS/AHPNS that was recently proposed. Good management practices should be applied to maintain high quality water in ponds and good quality feeds should be used in order to avoid stressing shrimp and making them more susceptible to any disease – including EMS/AHPNS. Moreover, pond effluents often are discharged into the same canals that serve as farm water supplies. This practice favors the spread of disease from one farm to the other. Thus, disinfection of pond water before stocking PL should be a priority issue.

40. **“Environmental studies: pesticide/pathogen combination challenge test”.** Dr Truong Quoc Phu (CTU) presented the results of the environmental studies carried out under TCP/VIE/3304, to determine whether EMS/AHPNS will develop in *P. vannamei* held in water-sediment systems containing *V. parahaemolyticus* and pesticides. The experiment used three kinds of pesticides (i.e. deltamethrin [0.2 μg/L], fenitrothion [0.5μg/L] and hexaconazole [0.5 μg/L]) found in sediments (0.12-27.00μg/kg) and water (0.034-0.05μg/L) in ponds with EMS/AHPNS-infected shrimp in the Mekong Delta. The experiments were conducted using 20L glass tanks with pumping air stones, shrimp density was 1 pc/L, and salinity was 25-27 ppt. Three experiments, each with nine treatments: seven experimental and two control treatments (control (+) and control (-)); sediment (+) and water (+) from EMS/AHPNS-affected shrimp pond; sediment (-) and water (-) from non-EMS/AHPNS-affected shrimp pond. *Vibrio parahaemolyticus* (10^6 CFU/mL) was used on challenge tests of the experiment (shrimp [+])) and shrimp size was 35-40mm (TL). Sampling was conducted twice: first sampling (after 5 days) and second sampling (after 10 days).

41. Results showed that physical parameters were stable among the different treatments during the first and second sampling. Chemical parameters increased during the second sampling, but they are still suitable for shrimp aquaculture with the exception of total nitrogen (TN) and total phosphorus (TP). Results also showed that deltamethrin was not detected after five days; fenithrothion and hexaconazole were detected during the second sampling, but decreased with time. Combination of pesticides and bacteria increased mortality of shrimp. Shrimp exposed to pesticides without bacteria did not show typical EMS/AHPNS pathology.

42. **“Epidemiology of EMS/AHPNS based on cross-sectional epidemiological studies”.** Dr Visanu Boonyawiwat (Kasetsart University) presented the outcomes of cross-sectional epidemiological studies carried out under TCP/VIE/3304 using completed farm and pond-level questionnaires, i.e., 1 195 of 1 254 (95.3 percent) and 1 920 of 2 508 (76.6 percent), respectively. Univariate logistic regression analysis indicated some risk factors, on farms with EMS/AHPNS problems (P-value <0.05), such as: larger farms, water supplied from brackish water compared to seawater, farm using semi-closed system, cleaning of pond bottom by soil removal, pond drying, and farms located in nearby EMS/AHPNS-affected farms. On the other hand, factors such as ratio of water reservoir area compared to culture area larger than 30 percent, using chlorine lime during pond preparation, conditioning of the water before pumping into the culture pond by holding water longer than 35 days and controlling feeding rate by use of feeding tray could reduce the risk of EMS/AHPNS occurring in the farm. Univariate logistic regression analysis

indicated some risk factors, on ponds with EMS/AHPNS problem (P-value <0.05), such as: larger pond size, extreme change in weather conditions, and source of PL. On the other hand, factors such as shrimp ponds deeper than 1.8 metres, use of aerator, reduced feeding rate during first month after stocking, lower density stocking of PL and applying probiotic into the pond during culture period could reduce the risk of EMS/AHPNS occurrence in the pond. The results of this cross-sectional study are similar to the observations gathered during the CMC-AH mission in July 2011 and the ongoing study in Thailand. It can be suggested that the main source of infection (of the *V. parahemolyticus* causing EMS/AHPNS*)* were infected PL and contaminated water. Farm management such as application of good aquaculture practices (GAPs) could be a strategy to reduce the effect of EMS/AHPNS in shrimp culture in Viet Nam.

43. **"Progress on the work related to Output 2: Farmer training on biosecurity and good aquaculture practices (GAPs)".** Dr Lê Văn Khoa (DAH) and Mr Koji Yamamoto (FAO) presented the progress related to Output 2 of TCP/VIE/3404. A joint DAH-FAO farmer training course on "Shrimp on-farm biosecurity" was convened in Soc Trang, Bac Lieu and Ca Mau provinces, from 12 to 17 May 2013. The training courses were attended by 302 trainees, consisted of 234 farmers (including five inputs supplier cum farmers), and 68 representatives from the provincial departments of Agriculture and Rural Development (DARD). The aim was to increase the knowledge of affected shrimp farmers regarding on-farm shrimp biosecurity and shrimp GAPs to assist in optimizing farming practices so that risks and negative consequences of this shrimp disease can be reduced.

44. The training course consisted of the following topics: (1) introduction to biology of shrimp, shrimp culture systems, (2) farmer organization, (3) control of key inputs in shrimp farming; seed, feed, chemicals, drugs and therapeutants, (4) pond environment, water management, and pond preparation, (5) VietGAP, (6) shrimp health management and diseases, (7) health monitoring and record keeping, and (8) EMS/AHPNS updates, disease notification, sample collection, preservation, and transportation. The farmer training participants raised a number of issues during the course such as: (1) field level diagnosis of EMS/AHPNS, (2) use of antibiotics and probiotics, (3) rapid transition from *P. monodon* to *P. vannamei* and associated risks of disease, and (4) adoption of VietGAP. Detailed responses were provided by the trainers on each topic. An evaluation of the conduct of the training course showed a high level of satisfaction and improved the understanding on shrimp on-farm biosecurity (average score of 4.7 out of 5).

45. **"Progress on the work related to Output 4: National Aquatic Animal Health Strategy (NAAHS) for Viet Nam".** Dr Eduardo Leano (NACA), presented the progress regarding the preparation of the NAAHM strategy, Output 4 of TCP/VIE/3304. Preparation of this NAAHM strategy was initiated during a workshop, held in Can Tho City, Viet Nam on 18 August 2012. A Technical Working Group (TWG) was established to take a lead on its development. Prior to the development of the strategy, the FAO Aquatic Animal Health Capacity and Performance Survey questionnaire[30] was undertaken to gather information on the capacity and performance concerning AAH management in the country. Two follow-up workshops of the TWG (10 May 2013 and 20–21 June 2013) were held to complete the survey

[30] This survey questionnaire was first developed by FAO in 2007 during the incursion of epizootic ulcerative syndrome (EUS) in southern Africa as basis for assessing national level performance and capacity on aquatic biosecurity (aquatic animal health). The questionnaires consist of 18 sections pertaining to (1) international trade in live aquatic animals and national border controls, (2) control of domestic movement of live aquatic animals and other domestic activities that may spread pathogens, (3) policy and planning, (4) legislation, (5) disease surveillance/monitoring, (6) disease diagnostics, (7) emergency preparedness and contingency planning, (8) extension services, (9) compliance/enforcement, (10) research, (11) training, (12) expertise, (13) infrastructure, (14) linkages and cooperation, (15) funding support, (16) current challenges, (17) constraints and (18) additional information. Survey results help guide regional and national strategic planning for improving aquatic animal health and assuring adequate and rational support services to achieve sustainable aquaculture development.

and the strategy document. The purpose of the NAAHM strategy is to assist the government in formulating policy and planning towards improving the national aquatic animal health status, achieving international recognition of the high quality aquaculture products and strengthening the country's position in the international markets. The draft strategy contains a Statement of Purpose, Vision, Guiding Principles, Implementation, and Programme Elements such as: (1) Policy, legislation and institutional framework; (2) Epidemiology (surveillance, monitoring), emergency preparedness and reporting; (3) Diagnostics and laboratory systems; (4) Quarantine, inspection and health certification; (5) Veterinary drugs/chemicals and vaccine management; (6) Capacity building; (7) Research and development; (8) Communication and international collaboration; and (9) Resources and funding. The NAAHS will be further developed until approval process by the DAH/MARD afterwhich funding will be sourced for its implementation.

Session 2: Status and experiences dealing with early mortality syndrome (EMS) or acute hepatopancreatic necrosis syndrome (AHPNS) from other countries and other technical presentations

46. This session consisted of eight technical presentations on the current status of distribution of EMS/AHPNS, updates on EMS/AHPNS work carried out in China, Malaysia, Thailand and the USA with additional presentations on vibriosis and bacteriophages (in *Vibrio* and *Aeromonas*).

47. **"Known distribution of EMS/AHPNS: current status".** The current status of the spread of EMS/AHPNS was presented by Dr. Eduardo Leaño of NACA. Outbreaks of EMS/AHPNS were officially reported from China and Viet Nam in 2010, although shrimp mortalities due to what was referred to as "covert mortality disease" have been observed in China as early as 2009. The disease spread to Malaysia in 2011 and to Thailand in 2012. In May 2013, outbreak of EMS/AHPNS was confirmed in Mexico (from Prof. D.V. Lightner's presentation) (Figure 9).

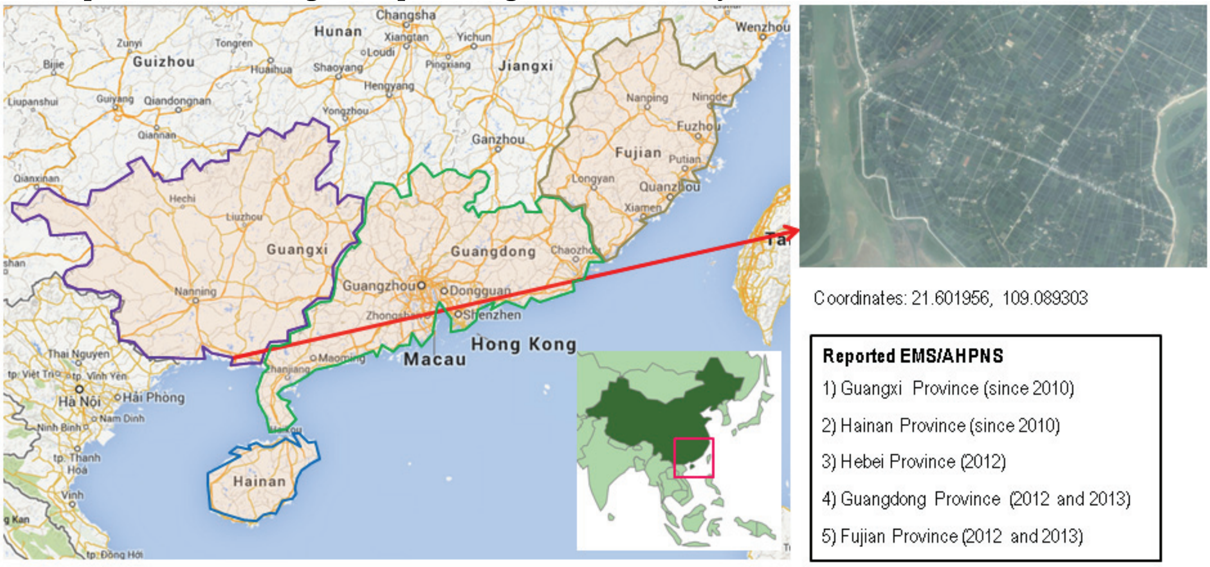
48. When the first outbreak was reported in Viet Nam in 2010, NACA's Asia Regional Advisory Group on AAH (AG) recognized the importance of this emerging disease affecting the shrimp industry of the region. Thus in May 2012, active information dissemination was undertaken by NACA by releasing Official Circular and Disease Advisory to all NACA member countries, key organizations in the region as well as to the private sectors.

49. NACA organized the Asia-Pacific Emergency Regional Consultation of EMS/AHPNS in August 2012 (see also paragraphs seven and eight of this report). A disease card was also developed, published and widely disseminated throughout the region to serve as a guide in the proper diagnosis of the disease. The AG also assessed the listing of this new disease in the NACA/FAO Quarterly Aquatic Animal Disease (QAAD) Reporting system. Starting in January 2013, EMS/AHPNS was included in the list of reportable diseases under the category of "non-OIE listed diseases" for crustaceans. The listing was made for the purpose of gaining more information on EMS/AHPNS within the region. As of the first quarter, EMS/AHPNS reports received in the middle of June 2013 (six reports in total), were only from Viet Nam. Reports from other countries affected by EMS/AHPNS (e.g. China, Malaysia and Thailand) are still pending at the time of this presentation. A follow-up Disease Advisory was also published in March 2013 to address the many circulating false and baseless speculations on the effects and spread of EMS/AHPNS in the region.

Figure 9
Map of Mexico showing main shrimp farming areas in Sonora and Sinaloa

50. **"Progress of the research on EMS/AHPNS in China: *Vibrio parahaemolyticus* and suspected Yellowhead virus isolated from EMS/AHPNS cases".** Dr Jie Huang (Yellow Sea Fisheries Research Institute of China) reported on the isolation of a virulent *V. parahaemolyticus* which showed green colonies on TCBS agar from EMS/AHPNS-infected shrimp samples collected in Guangxi in 2010 (Figure 10). The bacteria do not utilize sucrose and showed high antibiotic resistance. Several Chinese investigators supported *V. parahaemolyticus* as the causative agent of EMS/AHPNS. Some practices were suggested to be used in farms for the control of the disease. These include: regular bottom disinfection with 1,3-Dibromino-5,5-dimethylhydantoin (DBDMH) particles, water disinfection before stocking, lowering salinity, and reducing pH in water by probiotics and in gut using feed additives.

Figure 10
Map of China showing shrimp farming areas affected by EMS/AHPNS



51. Samples from Hebei, Guangdong, and Fujian provinces in 2012 and 2013 (Figure 10) were investigated by histopathological methods, nested RT-PCR following the OIE protocol for Yellowhead virus (YHV), and a highly sensitive and rapid detection kit for YHV. All of the samples had symptoms similar to the Chinese "covert mortality disease", such as pale and partially atrophied HP, empty stomach and gut, and slightly whitish abdominal muscle. Histopathology showed the following observations: lesions characterized by infiltration of hemocytes; loosened HP tubule epithelium; secondary infection of bacteria in the HP; and pyknosis, karyorrhexis, and cytoplasmic inclusions in the hemocytes and lymphoid organ. Eight of the 12 samples were detected as YHV-positive by the nested RT-PCR protocol; sensitivity for the samples seems very low. The sequences of the RT-PCR products showed 76.5 percent to 89 percent similarity with the reported YHV, after sequencing and BLAST on NCBI. The phylogenetic tree of the target sequence showed that the three samples shared the same branch which is different from the known YHV/GAV (Gill-associated virus) strains. Using a newly developed detection kit for YHV, all of the samples were detected as strongly positive. It is suspected that the "covert mortality disease" of shrimp may include infection with other causative agents in addition to the strain of *V. parahaemolyticus* that causes EMS/AHPNS.

52. Research on probiotic-enhanced biofloc technology showed encouraging results to control the disease. It is recommended that early detection and quarantine of bloodstock and PL, disinfection of water and pond bottom, use of probiotics and biofloc technology, and polyculture technology can be used for disease prevention. Dr Huang thanked FAO for the invitation and for supporting his participation to the workshop. This work was supported by the Special Fund for Agro-scientific Research in the Public Interest "Research and demonstration of the rapid diagnosis and biological control technology for the viral diseases in farmed shrimp" (Grant: 201103034) and China Agriculture Research System "the tasks for diseases control scientists in the Farmed Shrimp Research System" (Grant: CARS-47).

53. **"Status of EMS/AHPNS in Malaysia: diagnostic cases and investigations".** Dr Kua Beng Chu, National Fish Health Research Centre (NaFisH), Fisheries Research Institute on behalf of co-workers[31] presented the findings of studies on EMS/AHPNS conducted since 2011 which is currently on Phase IV. The presentation highlighted some results from Phase II and III of EMS/AHPNS investigation in farmed white-leg shrimp, *P. vannamei*. Sentinel surveillance based on reports of mortality cases in farmed white-leg shrimp to NaFisH showed an increasing number of cases since 2011. The prevalence of EMS/AHPNS was 50 percent (6/12) and 26 percent (5/14) in 2011 and 2012, respectively (Figure 11). Confirmation of EMS/AHPNS was based on clinical signs observed at pond level and the characteristic pathology of acute and terminal stages of EMS/AHPNS in the HP. A farm experiment was carried out using five common treatments in two sites in Peninsular Malaysia showed that the treated farmed *P. vannamei* had typical signs of EMS/AHPNS pathology and multiple bacterial infection (*Vibrio fluvialis, V. alginolyticus, V. parahaemolyticus, V. cholerae, Aeromonas hydrophila, Enterobacter cloacae, Pseudomonas* sp. and *Photobacterium damsela).*

54. **"Current status of Early Mortality Syndrome (EMS) in Thailand".** Dr Jiraporn Kasornchandra (Department of Fisheries, Thailand) on behalf of her co-workers[32], started her presentation with a background on shrimp farming in Thailand which had been practiced more than 30 years. Intensive shrimp farming, however, took off in 1988/1989 using hatchery-reared seed and formulated feeds. It required high financial and technical inputs. Survival rates were usually around 60–80 percent. *Penaeus monodon* reared in intensive systems resulted in decreased production during 2000–2003 due to diseases and poor growth rate. In 1997, *P. vannamei* was first introduced but the cultivation was not successful. Disease outbreaks frequently occurred in *P. monodon* culture areas, resulting in significant economic losses. The disease appeared to be the major constraint for the black tiger shrimp industry, therefore, the cultivation of the Pacific white shrimp *P. vannamei* was reconsidered. Development of *P. vannamei* cultivation began in 2002, expanded rapidly and *P. vannamei* is now the main species (approximately 99 percent) of shrimp cultured in Thailand.

55. EMS/AHPNS was first reported in shrimp farm located in the eastern Gulf of Thailand in late 2011 and affected *P. vannamei* during the first 15-35 days of post-stocking in grow-out pond, with high mortalities (approaching 100 percent in some ponds). It was claimed that those farms used the same stock of PLs. In early 2012 (January-April), EMS/AHPNS was reported in the east coast (Gulf of Thailand). At present, approximately five percent of shrimp farms in Thailand have been affected by EMS/AHPNS, mostly occurring in the eastern Gulf of Thailand in Chachoengsao, Rayong, Chantaburi and Trad provinces, and in the southern part of the country including Surattani and Songkhla provinces.

56. Affected shrimps showed clinical signs of soft or loose shell, pale coloration, lethargy and anorexia; with abnormal HP, mostly shrunken or atrophied. Histological findings revealed that pathology is limited to the HP, and included lack of mitotic activity in E-cells and dysfunction of B-, F-, and R-cells, followed by marked inflammatory response (hemolytic infiltration) in the HP. Prominent karyomegaly and massive sloughing of central HP tubule epithelial cells were observed. Finally shrimp die because of secondary bacterial infection mostly by opportunistic *Vibrio* spp. Cross-sectional epidemiological studies revealed that pond-level risk factors involved in EMS/AHPNS are a source of PL and feed quantity; chlorine treatment before stocking helps promote EMS/AHPNS protection.

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[31] Mohd. Iftikhar M.A A & Norazila J (National Fish Health Research Centre), Thavalingam M.P (Sovereign Innovations Sdn Bhd), Choong F.C (Agromerit Sdn. Bhd) and Mohd. Afiq M.R (Arca Biru Sdn Bhd).
[32] J. Kongkumnerd and C. Komvillai.

Figure 11
Map of Malaysia showing site of the outbreak mortality cases from shrimp farming areas

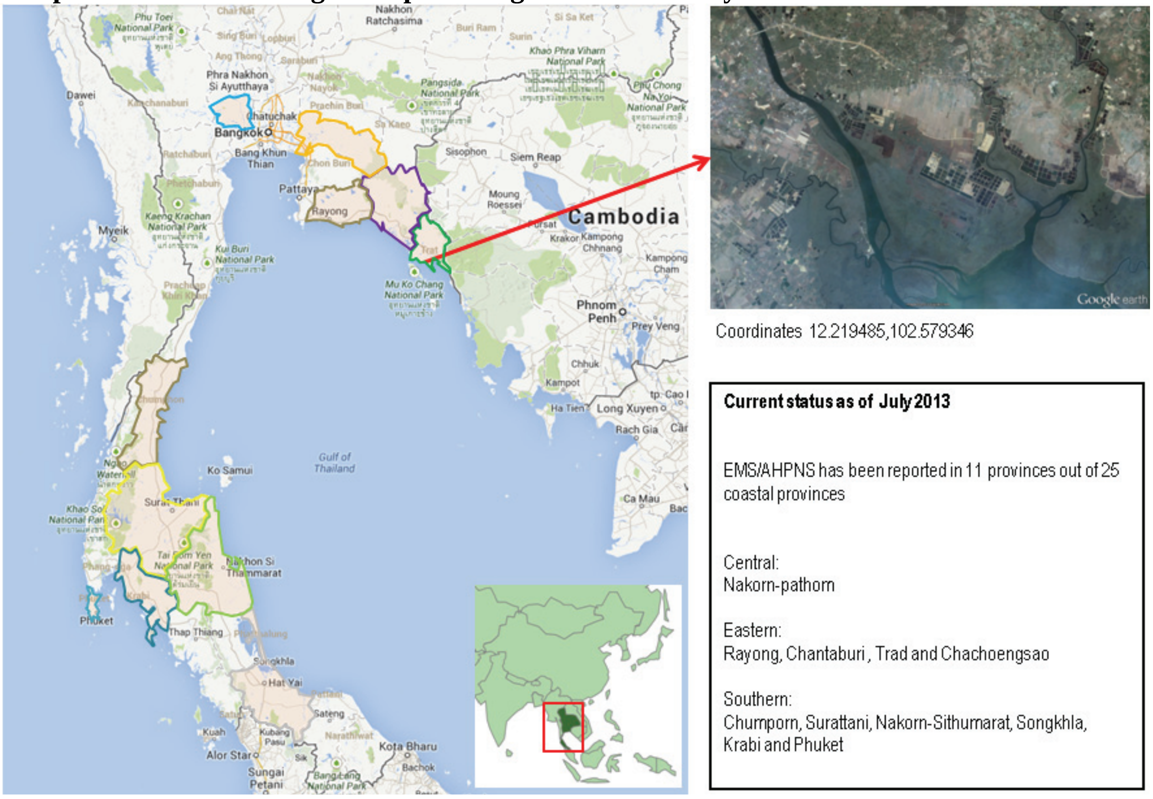
State	Cases received by NaFisH		
	2011	2012	2013
Kedah	2	5	7
Perak	5	4	-
Penang	2	-	-
Pahang	3	-	-
Terengganu	-	-	2
Sabah	-	5	-
Sarawak	-	-	8
Total	12	14	17

Coordinates 4 14'20.29"N, 100 38'0.12"E

57. In January 2013, the Department of Fisheries (DoF) established several programmes to control the disease spread starting at shrimp hatchery and nursing facilities. However, the most effective programme was the “STOP EMS Program”. In case of EMS/AHPNS occurrence within the farm, shrimp farmer must inform DoF local staff within 24 hours according to the Animal Control Act. If disease is confirmed, eradication procedure should be applied to stop the spread. The DoF also implemented disease surveillance and monitoring, established communication regarding the disease situation between officers and shrimp farmers, provided diagnostic services and increased public awareness.

58. Eleven provinces in Thailand (Figure 12) have been affected by EMS/AHPNS. Measures to control disease spread include improving shrimp farm management and shrimp health management practices, and application of probiotics, traditional herbal medicine and molasses.

Figure 12
Map of Thailand showing shrimp farming areas affected by EMS/AHPNS



59. **"Mass mortality of shrimp due to vibriosis in the Philippines: similarities with EMS and lessons learned".** Dr Celia Lavilla-Pitogo (Integrated Aquaculture International LLC, Brunei Darussalam), in her presentation, gave details on the case of vibriosis in the Philippines affecting *P. monodon* hatcheries during the late 1980s, and grow-out ponds in the 1990s. Dr Pitogo shared the approaches taken to prevent and/or control the bacterial disease and lessons learned from the experience. The hatchery system for *P. monodon* evolved from the Japanese community culture system to the modified Galveston method. The shift in culture technique triggered the outbreak of bacterial diseases due to conditions that created a niche for opportunistic bacteria. Although several types of vibrios have been implicated in the epizootics, the dominant species were non-sucrose-fermenting types, mainly luminescent *V. harveyi.*

60. To understand the course of infection, the portals of entry of bacteria in the hatchery were investigated by determining the components and additives that encouraged their growth and dominance. *Vibrio harveyi* entered the culture system through the spawners, untreated seawater and live zooplankton feed. As a result, several approaches to prevent and control bacterial disease were implemented including water treatment, hygienic spawning and egg handling, maintaining ecological balance within the system, and chemotherapy. In the last quarter of 1993, mass mortality was observed in grow-out ponds during the first 60 days of culture and this contributed to the collapse of shrimp grow-out sector. The target organ was HP where intense inflammation was observed. Bacterial enumeration of pond water showed that bacterial diversity began to change two to three weeks after flooding and resulted to dominance of vibrios. Shrimp mortality occurred when the *V. harveyi* population in the water exceeded 10^2 colony-forming units/ml. This observation highlighted the importance of knowing the bacterial composition of the rearing water before stocking of PLs and throughout the first 60 days of culture.

61. Several approaches to prevent or control the problem were attempted. These included the use of reservoirs, water treatment, chemotherapy, hygienic spawning and egg handling, maintaining ecological balance within the system through the application of probiotics, and other system modifications. A comparison of microbial load of hatchery-reared and wild-caught *P. monodon* post-larvae showed that the latter have less *V. harveyi* load. This highlighted the fact that there are hatchery practices that influence larval quality.

62. **"Research on EMS/AHPNS, conducted in Viet Nam and at the University of Arizona since 2011".** The presentation of Mr Loc Tran, APL/UAZ, centered on a breakthrough finding of the causative agent of EMS/AHPNS, a new emerging disease in shrimp, first reported in 2009, affecting both Pacific white shrimp (*P. vannamei*) and black tiger shrimp (*P. monodon*), which had caused significant losses in Southeast Asian shrimp farms. This unknown disease was first classified as idiopathic because no specific causative agent had been identified. However, since early 2013, the APL/UAZ was able to isolate the causative agent of EMS/AHPNS in pure culture. Immersion challenge tests were employed for infectivity studies, which induced 100 percent mortality with typical EMS/AHPNS pathology to experimental shrimp exposed to the pathogenic agent. Subsequent histological analyses showed that EMS/AHPNS lesions were experimentally induced in the laboratory and were identical to those found in EMS/AHPNS infected shrimp samples collected from the endemic areas. Bacterial isolation from the experimentally-infected shrimp enabled recovery of the same bacteria colony type found in field samples. In three separate immersion tests using the recovered isolate from the EMS/AHPNS positive shrimp, the same EMS/AHPNS pathology was reproduced in experimental shrimp with consistent results. Hence, AHPNS has a bacterial etiology and Koch's Postulates have been satisfied in laboratory challenge studies with the isolate of the agent.

63. **"Bacteriophages and pathogenic *Vibrio* spp in the aquatic environment".** The presentation of Prof Iddya Karunasagar (FAO) helped in better understanding bacteriophages and pathogenic vibrios in the aquatic environment. *Vibrio* spp. are autochthonous flora of the aquatic environments and over 80 species are recognized. Some of them have been implicated in diseases of aquatic animals e.g. *V. anguillarum*, *V. harveyi* and *V. penaecida*. A few species such as *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* are recognized as human pathogens. But within these species, pathogenic and non-pathogenic strains are recognized and generally pathogenicity is associated with presence of certain acquired genes. For example, of the over 200 serovars of *V. cholerae* recognized, only serovars O1 and O139 can cause the disease cholera and the cholera toxin genes in these serovars are derived from a filamentous bacteriophage. Human pathogenic strains of *V. parahaemolyticus* carry genes coding for a thermostable direct hemolysin (TDH encoded by *tdh* gene) or a TDH-related hemolysin (encoded by *trh* gene). These genes are present in a "pathogenicity island". FAO/WHO have carried out risk assessments of these pathogens in seafoods and developed a guidance document on methods for detection and enumeration of these pathogens.

64. Bacteriophages are viruses infecting bacteria and these are considered to be the most abundant life forms in the aquatic environment, where they play a role in the ecology of the bacteria. Once they infect bacterial cells, they replicate in these cells and are released to the environment by lysing the host bacteria. Sometimes, bacteriophage genes, on entering the host, get integrated into the host genome and remain dormant there. Such bacteria are referred to as "lysogenic". Lysogenic bacteria may express bacteriophage derived genes and there are several examples of human pathogenic bacteria, in which virulence genes are derived from bacteriophages, e.g. *Corynebacterium diptheriae*, Enterohamorrhagic *Escherichia coli*.

Box 2

Virulence gene acquisition in pathogenic bacteria

Many bacterial species have pathogenic and non-pathogenic strains. For example, there are over 200 serotypes of *V. cholerae*, but strains of only two serotypes, *V. cholerae* O1 and O139 cause the disease cholera (FAO/WHO, 2005)[33]. Non-pathogenic strains may acquire certain virulence genes through bacteriophages (viruses infecting bacteria) or plasmids (extrachromosomal genetic elements that can replicate independently of the chromosome). In the case of *V. cholerae* O1/O139, the gene encoding the cholera toxin gene comes from a filamentous bacteriophage. Bacteriophages derived from highly pathogenic *V. owensii* can confer virulence to certain strains of *V. harveyi* (Busico-Salcedo and Owens, 2013)[34]. In many pathogenic strains, the virulence genes are present in distinct "pathogenicity islands" that are acquired by horizontal gene transfer. Pathogenicity islands are characterised by the presence of flanking inverted repeats, insertion sequences or tRNA genes that act as sites for recombination into the genome. In *V. parahaemolyticus*, the virulence genes *tdh* and *trh* are present in pathogenicity island. In the case of fish pathogenic bacterium, *V. anguillarum*, the virulence genes are found in a plasmid (Di Lorenzo *et al.*, 2003)[35].

65. **"Experience of fish mortalities caused by epidemic clonal strains of *Aeromonas hydrophila* carrying a lysogenic bacteriophage".** Dr Wes Baumgartner, Mississippi State University, on behalf of his co-workers[36] presented their experience in dealing with fish disease outbreaks caused by a bacteria infected by a lysogenic bacteriophage. An epidemic of motile aeromonad septicemia (MAS) due to *Aeromonas hydrophila* in channel catfish (*Ictalurus punctatus*) spread through West Alabama and East Mississippi in 2009 and has been seasonally recurrent, with losses in the millions of pounds. No other primary etiologic agent has been found. Mortalities as high as 60 percent occur in ponds, primarily affecting the larger fish.

66. A multi-state research group (Mississippi, Alabama, Louisiana, Arkansas) examined possible causes and *A. hydrophila* isolates. Isolates from pond epidemics were found to be highly clonal by phylogenetic analysis of seven gene sequences (*atpD*, *dnaJ*, *dnaX*, *gyrA*, *gyrB*, *recA*, *rpoD*). A representative epidemic strain, ML09-119 was found to be more virulent in channel catfish than reference *A. hydrophila* strains by intraperitoneal injection (90 percent survival for reference strain, five percent survival for ML09-119). Bar coded sequencing of seven epidemic isolates and five reference isolates found unique genomic regions in the epidemic isolates that were not present in reference strains. These regions were >99 percent identical within epidemic strains. Three-hundred and seven predicted genes were found, many in genomic islands that suggest lateral gene transfer. A diagnostic PCR targeting a unique epidemic associated genetic locus has been developed to differentiate epidemic strains from reference strains. Some of these genes were associated with *myo*-inositol metabolism, which corresponds with the ability of epidemic strains to utilize *myo*-inositol. Genes from lysogenic bacteriophage, O antigen biosynthesis genes, and transposases were also uniquely present in the epidemic strains.

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[33] FAO/WHO, 2005. Risk Assessment of choleragenic *Vibrio cholera* O1 and O139 in warm water shrimp in international trade: Interpretative Summary and technical Report. Microbiological Risk Assessment Series 9. FAO, Rome, 90p.

[34] Busico-Salcedo, N. & Owens, L. 2013. Virulence changes to *Vibrio harveyi* clade bacteria infected with bacteriophage from *Vibrio owensii*. Indian J. Virol. DOI 10.1007/s13337-013-0136-1 (ahead of print).

[35] Di Lorenzo, M., Stork, M., Tolomasky, M.E., Actis, L.A., Farrel, D., Welch, T.J., Crosa, L.M., Werthiemer, A.M., Chen, Q., Salinas, P., Walbesr, L. & Crosa, J.H. 2003. Complete sequence of virulence plasmid pJM1 from marine fish pathogen *Vibrio anguillarum* strain 775. J. Bacteriol., 185: 5822-5830.

[36] M. Liles (Auburn University Department of Biological Sciences, Fisheries and Allied Aquaculture and M.L. Lawrence (Mississippi State University College of Veterinary Medicine).

67. These data support the conclusion that lateral gene transfer has contributed to the pathogenicity of epidemic *A. hydrophila* strains. Further research will need to be conducted to determine the specific contribution of the unique genetic loci to *A. hydrophila* virulence.

Session 3: Actions and measures for reducing the risk of EMS/AHPNS

68. In this session, Dr Melba Reantaso (FAO) presented a draft document listing a number of specific and generic actions and measures to reduce the risks of EMS/AHPNS. This document was prepared and circulated to participants and other experts to seek opinion and suggestions, prior to the workshop.

69. With the current understanding that Early Mortality Syndrome/Acute Hepatopancreatic Necrosis Syndrome (EMS/AHPNS) has a bacterial aetiology and based on the outcomes of specific work on EMS/AHPNS carried out under the FAO TCP/VIE/3304 as well as other ongoing EMS/AHPNS work, and supported by the expertise (on aquatic animal health, bacterial diseases, shrimp diseases and shrimp aquaculture) of participants, the experts participating in the consultation discussed and agreed on a list of specific and generic actions and measures that may help reduce and manage the risks of EMS/AHPNS, directed to various stakeholder groups (public and private sectors).

Recommendations on specific and generic actions and risk management measures for dealing with early mortality syndrome/acute hepatopancreatic necrosis syndrome (EMS/AHPNS)

70. Complacency in the shrimp aquaculture sector resulting in that laxity, during a period of relatively trouble-free shrimp production, led to vulnerability of the sector to any newly emerging pathogen that might arise unexpectedly, as is the case of EMS/AHPNS. Poor management practices, weak compliance with standard good biosecurity and good aquaculture practices (GAP) both at farm and hatchery facilities have also been evident. It is now clear that shrimp aquaculture needs to improve and continue to implement responsible and science-based farming practices. Twelve recommendations were drawn from the Workshop, namely:

i. **Disease nomenclature.** Since the causative agent, a strain of *Vibrio parahaemolyticus*, has now been identified and Koch's postulates satisfied through challenge tests (Tran *et al*., 2013)[37], the workshop proposed to change EMS/AHPNS to a more proper disease name, acute hepatopancreatic necrosis disease (AHPND)[38].

ii. **Diagnosis of AHPND**. The workshop recommended that the method for the isolation of pure bacterial cultures of the strain of *V. parahaemolyticus* causing AHPND, the challenge test procedure and the AHPND pathology description should be based on Tran *et al*. (2013). There is a need to develop, as soon as possible, a rapid and reliable pond-side method both for diagnostic and surveillance purposes. The workshop also recommended that a reference laboratory for AHPND be designated.

iii. **Disease notification/reporting**. Currently, AHPND is listed in the NACA/FAO Quarterly Aquatic Animal Disease (QAAD) reporting system www.enaca.org/modules/library/publication.php?tag_id=454&label_type=1&title=2012). Because AHPND is not yet in the OIE List of Notifiable Diseases for Aquatic Animals, official reports from other affected countries have not been forthcoming. The workshop recommended that a submission to OIE for listing of AHPND as a notifiable disease be made

[37] Tran, L., Nunan, L., Redman, R.M., Mohney, L.L., Pantoja, C.R., Fitzsimmons, K. & Lightner, D.V. 2013**.** Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp. Diseases of Aquatic Organisms 105:45-55.
[38] From this section onwards, AHPND will be used with reference to EMS/AHPNS.

through: (1) a country who participated in this workshop that is not yet affected by AHPND (e.g. Indonesia) and AHPND-affected countries (i.e. China, Malaysia, Thailand and Viet Nam); and (2) through a member of the OIE Aquatic Animal Health Standards Commission (AAHSC) (i.e. Dr Huang Jie). The workshop report can be used as basis, for consideration, for this submission. The workshop also recommended that the submission be done preferably in August 2013 before the September 2013 meeting of the OIE AAHSC. The consultation noted that if not done this year, submission should be done before the next OIE AAHSC meeting in February 2014. The workshop noted that based on previous experience, since it takes a while and a tedious process is involved for a disease to be listed, the sooner the request is made, the better. Mandatory reporting will enable more reliable information on the current geographical distribution of AHPND. The AHPND disease card prepared by NACA should also be updated.

iv. **International trade of live shrimp, shrimp products (frozen, cooked) and live feed**. Results of preliminary investigations of AHPND did not detect[39] the human pathogenic strains (FAO/WHO, 2011)[40] producing thermostable direct haemolysin (TDH) and TDH-related haemolysin (TRH), in the *V. parahaemolyticus* causing AHPND. There has been no report so far of human-related disease (e.g. gastroenteritis) linked to the consumption of affected shrimp from any of the affected countries since the emergence of AHPND. Because of the concern expressed related to ongoing trade bans of live shrimp by some countries (see paragraph 12 of this report), the following recommendations, targeted to both Competent Authority and producers, are provided concerning the international trade of live shrimp, shrimp products and live feeds:

- Live shrimp: There should be no movement of post-larvae (PL) and broodstock from AHPND-affected countries unless stocks originate from AHPND-free zone or AHPND-free compartment, where disease freedom has been established through risk analysis and surveillance.
- Shrimp products: There should be no measures against movement of frozen and cooked shrimp products prepared for human consumption since there is no reported public health risk of AHPND nor pathogen transfer from these products. Efforts to induce AHPND using frozen materials failed; this suggests that the AHPND agent does not remain infectious in freezing and thawing (Tran *et al*., 2013).
- Live feeds: In general, the use of live feed (as well as fresh and chilled aquatic animals) as feed presents a risk of pathogen transmission (Vijayan *et al*., 2005)[41]. Therefore when AHPND diagnostic method becomes available, measures should be taken (especially by hatchery operators) to ensure that imported live/fresh/chilled aquatic animals to be used as broodstock feeds do not carry APHND-causing strain of *V. parahaemolyticus*.

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[39] Based on preliminary studies conducted under the FAO TCP/VIE/3304 (by Can Tho University) and by the University of Arizona.

[40] FAO/WHO. 2011. Risk assessment of *Vibrio parahaemolyticus* in seafood: Interpretative summary and Technical report. Microbiological Risk Assessment Series No. 16. Rome 193pp.

[41] Vijayan, K.K., Stalin Raj, V., Balasubramanian, C.P., Alavandi, S.V., Sekhar, V.T. and Santiago, T.C. 2005. Polychaete worms - a vector for white spot syndrome virus (WSSV). Diseases of Aquatic Organisms 63:107-111.

v. **Countries affected by AHPND**. The Workshop recommended the following actions/measures directed to the Competent Authority of countries affected by AHPND.

- Proactive reporting of suspicion or occurrence of AHPND to the NACA/FAO QAAD be continued, especially from Asian countries;
- Active/targeted AHPND surveillance and survey of antibiotic resistance be conducted; both actions should be part of national strategies to support compliance with international standards on aquatic animal health and food safety;
- While waiting for the process of AHPND listing to be completed, suspicion or occurrence of AHPND be reported/notified to OIE under the category of diseases of concern to international trading partners according to the OIE Aquatic Animal Health Code; suspicion and occurrence should be confirmed;
- National level communication and awareness raising concerning AHPND be actively conducted for aquaculture stakeholders including consuming public. The Department of Fisheries (DoF) of Thailand's "Stop EMS Programme", for example, can be used as a reference. This programme includes improving hatchery sanitation, broodstock management, and quality of post-larvae; screening for shrimp pathogens including AHPND; improving farm management; controlling the use of antibiotics; testing of antibiotic residues; notifying DoF and eradicating infected stocks upon confirmation of AHPND; active surveillance; and and actively engaging the private sector;
- Audit of farms and hatcheries be undertaken to ensure that standard good biosecurity and GAPs are implemented.

vi. **Countries not affected by AHPND.** The Workshop recommended the following actions/measures, directed to the Competent Authority, of countries not affected by AHPND.

- Notification/reporting of suspicion and occurrence of AHPND to the NACA/FAO QAAD (Asian countries) and OIE and followed by confirmation;
- Passive and active targeted AHPND surveillance be conducted;
- Early detection and early response to contain the disease be part of emergency preparedness/contingency plans in the event of an AHPND outbreak;
- National level communication and awareness raising concerning AHPND be actively conducted for aquaculture stakeholders including the consuming public;
- Audit of farms and hatcheries be undertaken to ensure that standard good biosecurity and GAPs are implemented.

vii. **Farm facilities**. The Workshop recommended that standard GAPs (e.g. maintainance of good water quality, appropriate pond preparation, use of good quality PL, good feed, health monitoring, enhancement of natural pond productivity, etc.) and good biosecurity practices (e.g. focus on prevention and protection, health checks prior to stocking, early detection and quarantine) be strictly followed (see also Boxes 3 and 4). The Workshop emphasized the following measures (particularly during the first two months of culture):

- Stop feeding at the first signs of AHPND; gradual resumption of feeding after signs of recovery;
- Rational use of reservoirs to promote optimum water quality;
- Depending on system, use of deeper ponds (generally 1.5–1.8 m) for more stable water quality (i.e. less fluctuation in water quality parameters);

- Use of adequate mechanical aeration;
- No water intake from canals at times when pesticides are used on adjacent land crops;
- Avoid the use of chemicals with long residual life during disinfection of pond water before stocking.

viii. **Hatchery facilities**. The Workshop recommended that standard good hatchery practices (see also Box 4) be strictly followed with emphasis on the following:

- Ensure that imported live/fresh/chilled aquatic animals to be used as broodstock feeds do not carry APHND-causing strain of *V. parahaemolyticus* (see also section iv);
- Treat *Artemia* cysts with chlorine for decapsulation before hatching.

ix. **Pharmaceutical and feed companies and shrimp producers**. Currently, no known treatment is available. The Workshop recommended that the guidelines contained in the OIE Aquatic Animal Health Code and other relevant references on prudent and responsible use of antimicrobials be followed. These include for example: use of antimicrobial treatment based on correct diagnosis, use of correct withdrawal periods, appropriate treatment made under the supervision of an authorized or recognized aquatic animal health professional.

x. **Knowledge and capacity development**. The Workshop recommended the following actions for development of training/skills and production of relevant materials:

- Training on good biosecurity and GAPs be a continuing activity;
- Information on AHPND be updated on a regular basis and disseminated actively;
- Innovative farm-level and extension-type materials (e.g. comics, videos) be produced to promote GAP and biosecurity practices;
- Particularly for Viet Nam, joint planning and development of model shrimp culture demonstration ponds by the government and private sector;
- Dissemination and use of guidelines on *V. parahaemolyticus* testing procedures and references on risk assessment.

xi. **AHPND outbreak investigation/emergency response**. The Workshop recommended that emergency preparedness and contingency plan be the primary responsibility of the Competent Authority, with advanced financial planning or allocation of emergency fund for aquatic disease outbreaks as a joint endeavour by both public and private sectors.

xii. **Research.** There are many knowledge gaps concerning AHPND. Targetted AHPND-research ideas presented during the consultation and directed towards Competent Authorities, research institutes, universities and other interested private sector entities, included the following:

<u>Epidemiology</u>

- Cohort studies focusing on a number of risk factors identified during the cross-sectional studies conducted under the TCP/VIE/3304:
    - At the farm level:
        1. characteristics of AHPND-affected farms (larger farm, water supply from brackish water, semi-closed system);
        2. factors reducing the risk of AHPND (ratio of water reservoir and culture area more than 30 percent, holding of water longer than 35 days before use in ponds, feed control through use of feeding tray);
    - At the pond level:

i. factors increasing the risk of AHPND (larger pond size, extreme weather conditions, and source of post-larvae, [PL]);
ii. factors reducing the risk of AHPND (culture of shrimp in ponds deeper than 1.8 meters, use of sufficient aeration, decreased feeding rates during first month after stocking, lower density stocking of PL and application of probiotics in the pond during culture period).

- Standardization of data collection for AHPND epidemiological studies
- Comparative studies on bacterial populations from AHPND-affected and non-affected shrimp populations

Diagnostics

- Development of PCR and anti-body based diagnostic techniques
- Techniques to differentiate between shrimp disease causing strains and non-pathogenic strains that are widely prevalent in brackish water environments

Pathogenicity and virulence (of *V. parahaemolyticus* causing AHPND)

- Study on the possibility that mobile genetic elements may be involved in transfer of virulence among *Vibrio* spp. found in the aquaculture environment
- Characterization of the bacterial toxin(s) that cause AHPND
- Study on up and down regulation of proteins in AHPND-affected and non-affected shrimp
- Study on the possible role of cell to cell signaling (quorum sensing) in toxin production
- Full genomic sequencing of AHPND causing bacteria to understand the scope of gene transfer/virulence gene acquisition, to allow discriminatory diagnostics, to predict future mutations, to verify changes that can explain virulence and to understand the types of toxins produced and how they may be regulated

Public health

- Continued studies on the possibility of human pathogenic *tdh* and *trd* genes being carried by AHPND *V. parahaemolyticus* isolates
- Vertebrate health risk studies using AHPND-causing *V. parahaemolyticus* isolates with mice

Mixed infections

- Research on other phenomena (pathogens and others) discovered during the investigation of AHPND
  - infection with other shrimp viruses, e.g. WSSV, MBV, HPV (from TCP/VIE/3304 studies); TSV (from China studies)
  - true gregarines and gregarine-like objects (from TCP/VIE/3304 studies)
  - fungi (from TCP/VIE/3304 studies)
  - multiple bacterial infection: several species of Vibrios such as *V. proteolyticus* (from TCP/VIE/3304 studies); *V. fluvialis*, *V. alginolyticus*, *V. cholerae*; *Aeromonas hydrophila*, *Photobacter damsela* (from Malaysia studies)
  - Covert mortality disease and AHPND (from China studies)

Control measures

- Non-antimicrobial therapy:
  - field trials on efficacy of the following products/technology: prebiotics, probiotics, bacterial biofloc, bioremediators, immunostimulants, vitamins, phage therapy
  - microbial population control studies (e.g. use of mollasses, rice bran, brown sugar added to the pond water)
- Trials on the following water quality management practices and other aquaculture practices:
  - water disinfection before stocking
  - regular bottom disinfection with 1,3-Dibromino-5,5-dimethylhydantoin (DBDMH)
  - use of feed additives to reduce pH in the gut
  - use of low organic fertilizer

Environment (see also Box 5)

- Study on toxicity to shrimp of common pesticides used in the Mekong River Delta
- Study on effect of water quality parameters as AHPND triggers (e.g. pH, salinity, etc.)

Polyculture technologies (see Box 4)

- Trials on the use of polyculture technologies (i.e. tilapia, shrimp, seabass) to reduce AHPND

Box 3

Good aquaculture practices (GAP) and biosecurity maintainance in shrimp farming

Implementation of good aquaculture practices (GAP) and biosecurity maintenance depend greatly on the system, the species, recommended stocking season and the country/region where farming is conducted. However, there are some common elements when dealing with GAP in shrimp pond farming. There are also many examples and references on good management practices. Some of them are provided below:

Pond and pond bottom preparation and water management prior to stocking:

- drying of pond for a period of two or more weeks;
- removing sludge and disposal away from the pond site;
- ploughing wet soil if the sludge has not been removed completely;
- water filtration using twin bag filters of 300 mm mesh size;
- maintaining water depth of at least 80 cm at shallowest part of pond;
- water conditioning for 10–15 days before stocking;
- liming;
- fertilization; and
- controlling undesirable species (e.g., finfish, crustaceans, mollusks, amphibians, reptiles, birds, mammals) through physical, chemical and other means.

Seed selection and stocking:

- general health checks of PLs before stocking using traditional and molecular-based methods;
- use of uniform size and coloured PLs, actively swimming against the water current;
- elimination of weak PL before stocking using formalin (100 ppm) stress for 15–20 minutes in continuously aerated water;
- on-farm nursery rearing of PLs for 15–20 days;
- stocking into green water and avoiding transparent water during stocking;
- stocking using the recommended stocking season by the competent authority; and
- stocking of seed in a single area at the same time to avoid the negative impact of juvenile and adult shrimp being affected by disease and transferring those to the newly stocked batches.

Post-stocking management:

- use of water reservoirs, and 10–15 days aging before use on grow-out ponds;
- regular usage of agricultural lime, especially after water exchange and rain;
- no use of any harmful/banned chemicals;
- using of feed check trays to ensure feeding based on shrimp demand;
- feeding across the pond using boat/floating device to avoid local waste accumulation;
- regular removal of benthic algae;
- water quality monitoring to ensure appropriate pH, alkalinity and DO levels;
- water exchanges only during critical periods;
- weekly checking of pond bottom mud for blackish organic waste accumulation and bad smell; and
- growth monitoring using a cast net.

Health management:

- regular shrimp behavior and health checks;
- correct diagnosis when a health problem occurs;
- if the stock is affected by general vibriosis, feed should be reduced and water and pond bottom quality should be improved as necessary;
- if a disease outbreak occurs due to an infectious disease, the risk of spread of the disease to other farms should be prevented (e.g. no water discharge, no movement of infected stocks) and informing appropriate authorities;
- removal and safe disposal of sick or dead shrimp;
- emergency harvesting after proper decision-making;
- no draining or abandoning of diseased/affected stocks; and
- destruction of infected stocks as appropriate.

Box 4

Some examples of references on biosecurity, shrimp aquaculture, shrimp health management and polyculture technologies in disease prevention

Biosecurity in *P. monodon* and *P. vannamei* hatchery

- **FAO**. 2003. Health management and biosecurity maintenance in white shrimp (*Penaeus vannamei*) hatcheries in Latin America. FAO Fisheries Technical Paper. No. 450. Rome. 62p.
  www.fao.org/docrep/007/y5040e/y5040e00.htm
- **FAO.** 2007. Improving *Penaeus monodon* hatchery practices. manual based on experience in India.
  FAO Fisheries Technical Paper. No. 446. Rome.101p.
  ftp://ftp.fao.org/docrep/fao/010/a1152e/a1152e.pdf

General reference on shrimp

- **Alday-Sanz V., ed.** 2010. The shrimp book. UK, Nottingham University Press.

International standard on aquatic animal health

- **OIE Aquatic Animal Health Code**
  www.oie.int/international-standard-setting/aquatic-code/access-online/

Shrimp culture

- **NACA, SEAFDEC-AQD and FAO**. 1986. NACA Training Manual Series No. 2. Shrimp culture: pond design, operation and management.
  www.fao.org/docrep/field/003/ac210e/AC210E08.htm

Shrimp health management

- **Chanratchakool, P., Turnbull, J. F. Funge-Smith, S.J., MacRae, I. H. & Limsuwan, C.** 1998. Health management in shrimp ponds. Aquatic Animal Health Research Institute, Bangkok, Thailand.
- **Lightner D.V., ed.** 1996. A handbook of shrimp pathology and diagnostic procedures for diseases of cultured Penaeid shrimp. USA, World Aquaculture Society.
- **MPEDA/NACA.** 2003. Shrimp health management extension manual. Prepared by the Network of Aquaculture Centres in Asia-Pacific (NACA) and Marine Products Export Development Authority (MPEDA), India, in cooperation with the Aquatic Animal Health Research Institute, Bangkok, Thailand; Siam Natural Resources Ltd., Bangkok, Thailand; and AusVet Animal Health Services, Australia. Published by the MPEDA, Cochin, India.
  http://library.enaca.org/Shrimp/manual/ShrimpHealthManual.pdf

Tilapia-shrimp polyculture in disease prevention

- **Cruz, P.S., Andalecio, M.N., Blivar, R.B., & Fitzsimmons**. 2008. Tilapia-shrimp polyculture in Negros Island, Philippines: a review. Journal of the World Aquaculture Society. 39 (6): 713-725.
- **Paclibare, J. O., Usero, R.C., Somga, J.R. & Visitacion, R**. 2001. Integration of fishfish in shrimp (*Penaeus monodon*) culture: an effective disease prevention strategy, pp. 151–271. In Inui, Y., Cruz-Lacierda, E. R. (eds.) Proceedings of the SEAFDEC-OIE Seminar-Workshop on Disease Control in Fish and Shrimp Aquaculture in Southeast Asia: diagnosis and husbandry techniques. December 4-6, 2001, Iloilo City, Philippines.

Box 5
Environmental measures

Culture environment. Maintaining a good culture environment is one of the basic principles of disease control; it lessens stress that lowers the resistance of cultured animals to disease. The best way to maintain a good culture environment is to stock post-larvae in accordance with the production potential of the culture system; this will vary primarily with the amount of aeration applied and implementation of good aquaculture practices (GAPs). However, as GAPs may not always assure acceptable water quality, water quality monitoring should be conducted in ponds. The most important variables to monitor are: dissolved oxygen, pH, salinity, ammonia nitrogen, and hydrogen sulfide. Wide daily fluctuations of pH suggest that phytoplankton blooms are excessive or that the water should be limed to increase its buffering capacity. The other variables usually will not be outside acceptable limits unless the amount of aeration is insufficient for the amount of feed input used.

Water quality triggers for EMS/AHPNS. Usually, pH is high in shrimp ponds, so the hypothesis of high pH as trigger for EMS should be supported by further studies. Logically, any water quality variable that is at a stressful level could increase the susceptibility of shrimp to any disease including EMS. Thus, the possibility of stress caused by other water quality issues should be considered along with pH as possible trigger mechanisms.

Probiotics in shrimp farming. Although there is much use for probiotics in shrimp farming, there is no evidence that these amendments improve environmental conditions in ponds. Probiotics should not be harmful to the culture environment or to the shrimp, and there is no reason to discourage farmers who believe in probiotics from using them. However, probiotics should not be recommended as a possible measure for lessening EMS/AHPNS risk unless research is done and evidence demonstrates their effectiveness for this purpose.

Disinfection of water in shrimp ponds. Many compounds are used in shrimp farming to disinfect water before stocking shrimp in ponds and to disinfect make up water used to replace evaporation and seepage in ponds. Chlorination is widely used to disinfect municipal waters supplies, and there have been a few studies that showed that chlorine compounds can be effective disinfectants when used at 20 to 30 mg/l in shrimp farm water supplies. The other compounds used as disinfectants in shrimp farming have not been studied. The effective dose rates of disinfectants commonly used in shrimp farming should be established through microbial bioassays tests.

Pesticides. Some pesticides used on rice in the Mekong Delta – particularly the "new generation" synthetic pyrethroid insecticides – are highly toxic to shrimp. Research on the relationships among pesticide residues in pond sediment and water, shrimp stress, and susceptibility to EMS/AHPNS and other shrimp diseases needs to be done.

Conclusions and way forward

71. The participants concluded that the technical workshop significantly improved the understanding of AHPND and that the process taken by the FAO/MARD TCP/VIE/3304 project could serve as a good model for conducting similar unknown disease investigations in the future.

72. There was good consensus on recommendations for actions and measures to manage and reduce the risk of AHPND.

73. The workshop participants strongly recommended that the report of the workshop be finalized as soon as possible for wider dissemination and that the papers presented during this consultation be compiled and be submitted as a special issue of a refereed journal, e.g. Aquaculture.

74. The organizers, FAO and MARD, closed the workshop with a big thank you to all participants for their efforts and contribution to make this endeavour successful.

ANNEX 1
Workshop agenda

Date and Time	Activities
24 June 2013, Monday	
	Arrival of Participants
DAY 1: 25 June 2013, Tuesday	
	FAO/MARD Technical Consultation on "Early Mortality Syndrome (EMS) or Acute Hepatopancreatic Necrosis Syndrome (APHNS) of Cultured Shrimp"
09:00–09:30	Workshop Opening • Welcome statement (Dr Tran Dinh Luan, MARD) • Opening statement (Mr Ken Shimizu, FAO) Self- introduction of participants Group photo
09:30–09:40	Presentation 1. Background, objectives and expected outcomes of the consultation (Dr Melba B. Reantaso, FAO)
Session 1: Outcomes of the FAO/MARD TCP/VIE/3304 Output 1: Diagnostics	
09:40–10:10	Presentation 2. Emergence of a new disease in Southeast Asian shrimp farms: Prof. Don Lightner (University of Arizona, USA)
10:10–10:40	Presentation 3. Diagnostic studies: Histology and bacterial metagenomics: Prof Tim Flegel (Mahidol University, Thailand)
10:40–11:00	Coffee break
11:00–11:20	Presentation 4. Diagnostic studies: histopathology and PCR: Ms Dao Thi Thanh Hue (Regional Animal Health Office 6, Viet Nam)
11:20–12:00	Presentation 5. Diagnostic studies: parasite, fungal and bacterial agents: Dr Dang Thi Oanh (Can Tho University, Viet Nam)
12:00–12:30	Presentation 6. Field study on transmission of EMS/AHPNS-infected pond: Dr Dang Thi Oanh (Can Tho University, Viet Nam)
12:30–13:00	Discussions
12:30–14:00	Lunch break
14:00–14:30	Presentation 7. Environmental studies: assessment of toxins in feed, sediment, and water: Prof Claude Boyd (Auburn University, USA)
14:30–15:00	Presentation 8. Environmental studies: pesticide /pathogen combination challenge tests: Dr Truong Quoc Phu (Can Tho University, Viet Nam)
15:00–15:30	Discussions
15:30–16:00	Coffee break

16:00–16:45	Presentation 9. Epidemiology of EMS/AHPNS based on cross sectional studies: Dr Visanu Boonyawiwat (Kasetsart University, Thailand)
16:45–17:30	Discussions
17:30–17:45	Wrap up and Tasks for Day 2
DAY 2: 26 June 2013, Wednesday	
08:30–08:45	Tasks for the day
Session 2: EMS/AHPNS status and experiences from other countries and other technical presentations	
09:30–09:45	Presentation 10. Known distribution of EMS/AHPNS: current status: Dr Eduardo Leano (Network of Aquaculture Centres in Asia and the Pacific, Thailand)
09:45–10:00	Presentation 11. Progress of the research on EMS/AHPNS in China: *Vibrio parahaemolyticus* and suspected yellowhead virus isolated from EMS/AHPNS cases: Dr Jie Huang (Yellow Sea Fisheries Research Institute, China)
10:00–10:20	Presentation 12. Status of EMS/AHPNS in Malaysia: diagnostic cases and investigations: Dr Kua Beng Chu (Fisheries Research Institute, Malaysia)
10:20–10:40	Coffee break
10:40–11:00	Presentation 13. Current status of Early Mortality Syndrome (EMS) in Thailand: Dr. Jiraporn Kasornchandra (Department of Fisheries, Thailand)
11:00–11:20	Presentation 14. Mass mortality of shrimp due to vibriosis in the Philippines: similarities with EMS and lessons learned: De Celia Lavilla-Pitogo (Integrated Aquaculture International LLC, Brunei Darussalam)
11:20–11:40	Presentation 15: Research on EMS/AHPNS, conducted in Vietnam and at the University of Arizona since 2011: Mr Loc H. Tran (University of Arizona, USA)
11:40–12:00	Presentation 16. Bacteriophages and pathogenic *Vibrio* spp in the aquatic environment: Dr Iddya Karunasagar (FAO)
12:00-12:20	Presentation 17. Experience of fish mortalities caused by epidemic clonal strains of *Aeromonas hydrophila* carrying a lysogenic bacteriophage: Dr Wes Baumgartner (Mississippi State University, USA)
12:30–13:30	Lunch break
13:30–14:00	Discussions

Session 3: Outcomes of the FAO/MARD TCP/VIE/3304 Outputs 2 and 4	
13:30–13:45	Presentation 18. Progress on the work related to Output 2: Farmer training on biosecurity and best management practices (Dr Le Van Khoa/Mr Koji Yamamoto)
13:45–14:00	Presentation 19. Progress on the work related to Output 4: National strategy on aquatic animal health (Dr Eduardo Leano/Dr Le Van Khoa)
14:15–14:30	Discussions
14:30–14:50	Coffee break
Session 4: Managing EMS/AHPNS and Talking Points for 27 June Consultation	
14:50–17:10	Discussions of recommendations on actions and measures to manage the risk of EMS/AHPNS (Facilitated by Dr Melba B Reantaso)
17:30–17:45	Wrap up and Tasks for Day 3
DAY 3: 27 June 2013, Thursday	
National Consultation on "Early Mortality Syndrome (EMS) or Acute Hepatopancreatic Necrosis Syndrome (APHNS) of Cultured Shrimp"	
09:00–12:30	Presentations:
	Technical Presentations: Overview of EMS/AHPNS Diagnosis of EMS/AHPNS: Epidemiology of EMS Managing and reducing the risks of EMS/AHPNS
12:30–14:00	Lunch break
14:00–16:00	TCP/VIE/3304 Project Team Meeting

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ANNEX 3
Workshop group photo

Sixty three participants consisting of key personnel involved in TCP/VIE/3304 project, members of the MARD National Task Force on Shrimp Diseases, representatives from the Ministry of Agriculture and Rural Development (DAH, DFish, RIAs, DOST, provincial agencies, private sector representatives), FAO staff (FIRA, FIPM, FAO Regional Office for Asia and the Pacific, and FAO Representation in Viet Nam) experts involved in the work on EMS/AHPNS in other countries (China, Malaysia, Thailand) and other experts (Indonesia, United States of America), attended the FAO/MARD Technical Workshop on Early Mortality Syndrome (EMS) or Acute Hepatopancreatic Necrosis Syndrome (AHPNS) of Cultured Shrimp, held in Hanoi, Viet Nam, from 25 to 27 June 2013.

As part of the FAO project TCP/VIE/3304 "Emergency assistance to control the spread of an unknown disease affecting shrimps in Viet Nam", being implemented by Vietnam's Ministry of Agriculture and Rural Development (MARD), the FAO/MARD Technical Workshop on "Early Mortality Syndrome (EMS) or Acute Hepatopancreatic Necrosis Syndrome (AHPNS) of Cultured Shrimp" was held in Hanoi, Viet Nam from 25 to 27 June 2013. The Workshop was attended by 63 participants consisting of key personnel involved in the project, members of the MARD National Task Force on Shrimp Diseases, Vietnamese authorities and aquaculture stakeholders and other resource experts from China, Malaysia, Thailand and the United States of America. With the current understanding that Early Mortality Syndrome/Acute Hepatopancreatic Necrosis Syndrome (EMS/AHPNS) has a bacterial aetiology and based on the outcomes of specific work on EMS/AHPNS carried out under the FAO TCP/VIE/3304 as well as other ongoing EMS/AHPNS work, and supported by the expertise (on aquatic animal health, bacterial diseases, shrimp diseases and shrimp aquaculture) of participants, the experts participating in the consultation discussed and agreed on a list of specific and generic actions and measures that may help reduce and manage the risks of EMS/AHPNS, directed to various stakeholder groups (public and private sectors). The Workshop, thus, updated and enhanced the understanding of the aetiology and epidemiology of EMS/AHPNS and built consensus on above recommendations and the ways forward.

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