

# Current Status of Acute Hepatopancreatic Necrosis Disease (AHPND) of Farmed Shrimp in Malaysia

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## Abstract

A report about a disease problem in cultured whiteleg shrimp (*Penaeus vannamei*) was first received by the National Fish Health Research Center (NaFisH) in 2011 from Perak State showing signs of white feces and slow death leading to serious mortality rate. Later, in September of the same year, the Malaysian Shrimp Farmers Association (MSFA) reported to Department of Fisheries (DOF) severe mortalities in almost all of the whiteleg shrimp farms throughout Peninsular Malaysia. Sampling of shrimps for disease diagnosis was then conducted by NaFisH. The bacteriological and histopathological examinations revealed respectively the isolation of *V. parahemolyticus* and massive sloughing of hepatopancreatic epithelial cells. The disease was subsequently identified as acute hepatopancreatic necrosis disease (AHPND). From our 3-year study, the annual prevalence rates of AHPND were 50%, 26% and 73% in 2011, 2012 and 2013, respectively. At present, AHPND still persists in Malaysia but at a lower prevalence. The risk factors associated with the disease were studied, however, varied environmental and management data analyzed were inconclusive to relate any one parameter directly to the disease. To help ensure the early detection of AHPND, an experimental observation study on 'gut scorecard' was carried out and this was confirmed by PCR and histopathology. Validation of this technique has yet to be carried out to ensure its reliability. We also examined the potential use of some commercial products such as probiotics and disinfectants available in the market but unfortunately results showed that they were not effective in controlling AHPND. Control measures applied by the farmers such as the use of probiotics were also verified but data generated likewise appeared to be inconclusive. On the contrary, our preliminary study on the antibacterial property of the plant extracts, i.e. betel and lemongrass, incorporated in the feed showed some prophylactic and chemotherapeutic potential against AHPND. However, comprehensive *in vitro* and *in vivo* trials are still currently being undertaken to elucidate its efficacy and practical applications. To ensure the shrimp industry's sustainability in Malaysia, results of our ongoing and future studies aimed at preventing and controlling unwarranted outbreaks of AHPND and other emerging transboundary diseases of penaeid shrimps will be continually disseminated to shrimp farmers and pertinent stakeholders.

## Introduction

The whiteleg shrimp (*Penaeus vannamei*) is a popular choice species for shrimp farmers in Malaysia. Whiteleg shrimp culture started in Peninsular Malaysia in 2002. The contributions of whiteleg shrimp and tiger prawn (*P. monodon*) in annual production of farmed shrimps were 70% and 30%, respectively in 2007 (Annual Fisheries Statistics, 2005-2014). They have been successfully cultured in earthen ponds. However, in the latter part of 2010 and early part of 2011, farmers in the southern and middle regions of Peninsular Malaysia encountered several disease outbreaks during the early stage of shrimp culture. At that time, the outbreak was known as early mortality syndrome (EMS) or acute hepatopancreatic necrosis syndrome (AHPNS). Early mortality syndrome was later named as acute hepatopancreatic necrosis disease (AHPND) in 2013 (Tran *et al.*, 2013). AHPND is caused by a unique strain of *Vibrio parahaemolyticus* capable of releasing potent toxins that could consequently lead to tissue destruction and dysfunction of the hepatopancreas (GAA, 2013).

Outbreaks of AHPND among the major whiteleg shrimp-producing farms located in five major states in Peninsular Malaysia had consequently resulted in serious economic losses estimated at USD 0.1 billion in 2011 (NACA 2012). In the course of our investigation, i.e. from 2011 to 2013, we found out that AHPND had caused 40 to 100% mortality of cultured whiteleg shrimps. We also discovered that some surviving whiteleg shrimps had slow growth that subsequently succumbed to morbidity and mortality at the latter stage of culture. Occurrence of AHPND has been reported in China (2009), Viet Nam (2011), Thailand (2012) and the Philippines (2015) (Tran *et al.*, 2013; Joshi *et al.*, 2014; dela Peña *et al.*, 2015). Apart from these Asian countries, AHPND has also been reported in Mexico in 2013 (Lightner *et al.*, 2013; Soto-Rodriguez *et al.*, 2015). AHPND has caused significant reduction in the world shrimp production (NACA, 2013; FAO, 2013; Lightner *et al.*, 2012; Leñaño and Mohan, 2012).

In Malaysia, investigations on AHPND episodes since 2011 have been accordingly categorized into 5 phases. Phase I was conducted in 2011 with the main objective of identifying the etiology of the disease. Phase II on the other hand, delved on factors associated with AHPND outbreaks. Moreover, Phase III focused on control and preventive measures. Finally, Phases IV and V have been concurrently conducted to generate substantial data on the epidemiology of AHPND, establish practical and accurate methods for the early detection of AHPND at the farm level, and develop practical and effective preventive and therapeutic methods through the use of herbs or environment friendly products with potent antibacterial properties.

## History of occurrence

In the mid 2011, the Fisheries Research Institute through the National Fish Health Research Division (NaFisH) received reports of two cases of cultured whiteleg shrimps exhibiting white feces and slow death from shrimp operators in Perak State. Subsequently, the Department of Fisheries (DOF) of Malaysia was informed by the Malaysian Shrimp Farmers Association (MSFA) of high mortalities at alarming rates in most of the shrimp farms located throughout Peninsular Malaysia in September 2011. An immediate action was undertaken to determine the possible cause or etiology of the outbreak. Water and shrimps from affected areas were sampled and diagnosed for diseases. Relevant water quality parameters were also determined. During the course of our investigation, we discovered that the outbreak actually occurred in the east coast of Johor in the latter part of 2010. However, no samples were obtained from Johor during that period because before the sampling was about to be carried out, farmers had already done an emergency harvest. In 2011, the affected States included Perak, Penang, Kedah and Pahang. The etiology of the disease was later confirmed as AHPND based on histopathological changes in the hepatopancreas of affected shrimps and affirmation of the diagnostic finding by the

laboratory of Dr. D. Lightner in Arizona State University. Subsequently, samples from Sabah and Sarawak (2012), Terengganu (2013), Melaka and Johor (2014) States were also found positive for AHPND by histopathology (Figure 1).



Figure 1. Chronological order (year) of AHPND occurrences in Malaysia confirmed by histopathology

### Severity and economic impact

Total production of cultured shrimp was 87,000 metric tons (MT) in 2010 and 90% of the production was contributed by whiteleg shrimps (Figure 2). Several outbreaks of AHPND in whiteleg shrimp farms in 2011, 2012 and 2013 resulted in the reduction of total shrimp production to 67,000 MT, 55,000 MT, and 50,000 MT in 2011, 2012, and 2013,

respectively (Annual Fisheries Statistics, 2005-2014). Based on the estimated total shrimp production losses from 2011 to 2014, the total economic losses from AHPND episodes were estimated to reach USD 0.49 billion.

### Species affected

Currently, two major species of farmed shrimps, i.e. whiteleg shrimp (*P. vannamei*) and tiger prawn (*P. monodon*), have been so far affected by AHPND resulting in serious mortalities. It was first detected in whiteleg shrimp and tiger prawn in 2011 and 2014, respectively. Infection rate was higher in whiteleg shrimp than tiger prawn. AHPND was documented in shrimp post larvae (PL) 7 to 12, juveniles, and broodstocks.

### Disease signs and diagnostic methods

On-farm investigations of shrimps for AHPND diagnosis are usually conducted in farms experiencing mortality within 30 days of stocking shrimps in cultivation ponds or when large-scale die-offs commence. Shrimps infected with AHPND exhibit an array of clinical signs including lethargy, soft shells, whitish muscle, empty stomach and midgut, slow growth, and pale atrophied hepatopancreas that often have black streaks. However, these pond-level observations have to be further diagnostically confirmed as AHPND by taking into account

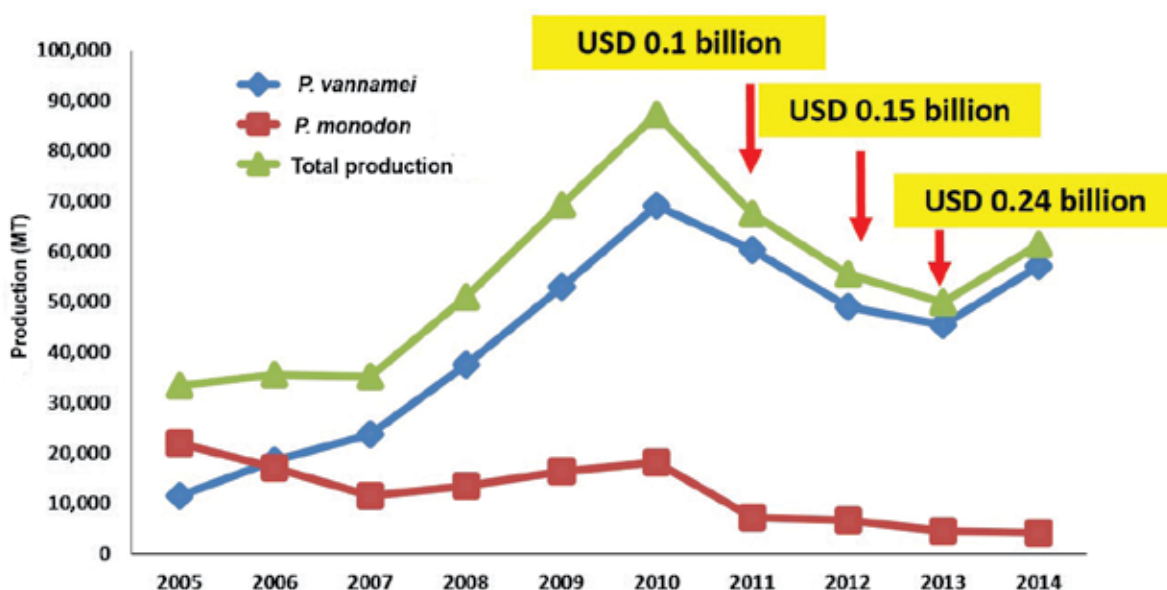


Figure 2. Production of farmed shrimps in Malaysia from 2005 to 2014 and corresponding losses estimated from the reduction of shrimp production.

the pathognomonic histopathological signs of AHPND including the isolation of *V. parahaemolyticus* (biochemically identified using API20E) and massive sloughing of hepatopancreatic tubule epithelial cells of the hepatopancreas of affected shrimps. From 2013 onwards, PCR assay using primers AP2, AP3 and AP4, Real-time PCR, and detection kit (IQ2000) have been employed as confirmatory tests for AHPND.

### Status of AHPND

The current status of AHPND in farmed shrimps in Malaysia is chiefly based on the histopathological findings generated from the number of mortality cases reported to NaFisH, shrimp diagnostic laboratories, and epidemiological work carried out from 2011 to 2015. A total of 4,571 samples of farmed whiteleg shrimps and tiger prawns were obtained from 3 main diagnostic laboratories. The 3 main diagnostic laboratories receiving samples monthly include NaFisH, Fisheries Research Institute, and a private laboratory. The positive cases of AHPND in whiteleg shrimp were 50%, 26%, 34%, 13% and 4% in 2011, 2012, 2013, 2014 and 2015, respectively (Table 1). AHPND was also detected in tiger prawn in 2014 (10%) and 2015 (5%), respectively. At present, AHPND still persists in Malaysia but at a lower prevalence.

### Future directions

Research and development focusing on the early detection of AHPND at the farm level and usage of alternative treatment such as the use herb extracts or environment-friendly products have been carried out since 2013. The risk factors associated with the disease have been also studied, however variations in environmental and management data revealed to be inconclusive to relate any one parameter directly to the occurrence of AHPND. To assist early detection of AHPND in cultivated shrimps, experimental observation study on 'gut scorecard' has been carried out together with PCR and histopathology as confirmatory tests. However, validation of this technique to ensure its validity and reliability is ongoing. Studies on control measures have been also conducted at the farm level through a collaborative effort with the farmers using existing commercial products but the results showed that these commercial products were ineffective in controlling AHPND. Also, control measures formulated by the farmers themselves such as the use of probiotics were analyzed but most of these methods were likewise found to be ineffective. However, our laboratory trial using the betel and lemongrass extracts incorporated in the shrimp's diet conferred protection against an experimental challenge with *V. parahaemolyticus* strains implicated in outbreaks of AHPND in Malaysia. However,

**Table 1. Samples of whiteleg shrimps and tiger prawns examined for AHPND from 2011 to 2015.**

Year	Whiteleg shrimp ( <i>P. vannamei</i> )			Tiger prawn ( <i>P. monodon</i> )		
	Number of samples examined	Number of AHPND positive	Prevalence (%)	Number of samples examined	Number of AHPND positive	Prevalence (%)
2011	394	197	50.0			
2012	584	151	25.9			
2013	661	212	34.4			
2014	1,586	199	12.5	50	5	10.0
2015	1,346	50	3.7	74	4	5.4

more comprehensive studies still need to be conducted to elucidate its efficacy and practical applications. Aside from the imposition of stringent biosecurity measures in hatcheries and grow-out facilities, to realistically ensure the sustainability of the shrimp industry in Malaysia, results of our ongoing and future studies aimed at preventing and controlling unwarranted outbreaks of AHPND and other emerging transboundary diseases of penaeid shrimps will be continually disseminated to shrimp farmers and pertinent stakeholders.

## References

- Annual Fisheries Statistics. 2005-2014. Department of Fisheries Malaysia
- de la Peña, L.D., N.A.R Cabillon, N.A.R, D.D. Catedral, E.C. Amar, R.S. Usero, W.D. Monotilla, A.T. Calpe, D.G. Fernandez, and C.P. Saloma. 2015. Acute hepatopancreatic necrosis disease (AHPND) outbreaks in *Penaeus vannamei* and *P. monodon* cultured in the Philippines. *Dis. Aquat. Org.* 116:251-254.
- FAO Fisheries and Aquaculture Report. 2013. FAO/MARD technical workshop on early mortality syndrome (EMS) or acute hepatopancreatic necrosis syndrome (AHPNS) of cultured shrimp (under TCP/VIE/3304)No. 1053. Hanoi, Vietnam, p.126-130.
- GAA. 2013. Progress in understanding EMS in shrimp. Retrieved May 2, 2013 from the World Wide Web: [www.gaalliance.org/newsroom/weighingInNews.php](http://www.gaalliance.org/newsroom/weighingInNews.php).
- Joshi J., J. Srisala, V.H. Truong, I.T. Chen, B. Nuangsaeng, O. Suthienkul, C.F. Lo, T.W. Flegel, K. Sritunyalucksana, S. Thitamadee. 2014. Variation in *Vibrio parahaemolyticus* isolates from a single thai shrimp farm experiencing an outbreak.
- Lightner D.V, M. Redman, C.R. Pantoja, B.L. Noble and L. Tand. 2012. Early mortality syndrome Affects Shrimp In Asia. *Global Aquaculture Advocate*. 1, 40
- Lightner D.V, C.R. Redman, B.L. Pantoja, L.M. Noble, and Loc Tran Nunan. 2013. Documentation of an Emerging Disease (early mortality syndrome) in SE Asia & Mexico. OIE Reference Laboratory for Shrimp Diseases, Department of Veterinary Science & Microbiology, School of Animal and Comparative Biomedical Sciences.
- Leano E.M and C.V. Mohan. 2012 Emerging threat in the Asian shrimp industry: early mortality syndrome (EMS)/acute hepatopancreatic necrosis syndrome (AHPNS). Network of Aquaculture Centres in Asia-Pacific. Asian fisheries society. Fish health section. Electronic newsletter. No.10.
- 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. Network of Aquaculture Centres in Asia-Pacific, Bangkok, Thailand. August 2012.
- NACA. 2013. Disease advisory: acute hepatopancreatic necrosis syndrome (AHPNS), status update. Retrieved 2013 from the World Wide Web: [www.enaca.org/publications/health/diseasecards/ahpns.disease.advisory-update.march.2013.pdf](http://www.enaca.org/publications/health/diseasecards/ahpns.disease.advisory-update.march.2013.pdf).
- Soto-Rodriguez S.A, B. Gomez-Gil, R. Lozano-Olvera, M. Betancourt-Lozano, and M.S. Morales-Covarrubias. 2015. Field and experimental evidence of *Vibrio parahaemolyticus* as the causative agent of acute hepatopancreatic necrosis disease (AHPND) of cultured shrimp (*Litopenaeus vannamei*) in north- western Mexico. *Appl Environ Microbiol* 81:1689–1699.
- Tran, L., N. Linda, R.M. Redman, L.M. Leone, C.R. Pantajo, K. Fitzsimmons, and D. Lightner. 2013. Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp. *Dis Aquat Organ*. 105, 45-55.