Status of Acute Hepatopancreatic Necrosis Disease (AHPND) of Cultured Shrimps in the Philippines

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Abstract

Shrimp is the fourth most important aquaculture commodity in the Philippines in terms of production quantity and second in terms of export value. The two species of shrimp being cultivated in the Philippines are the black tiger shrimp (*Penaeus monodon*) and Pacific white shrimp (*P. vannamei*). Although shrimp production markedly declined in the 1990's due to luminescent vibriosis and white spot disease caused by *Vibrio harveyi* and white spot syndrome virus (WSSV), respectively, the industry was able to recover due to collaborative and participatory efforts of both the public and private sectors. Recovery programs focused on improving culture technologies, prevention and control of disease introduction and outbreaks and environmental enhancement. However, serious outbreaks of an emerging transboundary disease named acute hepatopancreatic necrosis disease (AHPND) caused by unique strains of *V. parahaemolyticus* have recently caused heavy economic losses among shrimps growers in some major shrimp producing provinces in the country, thereby threatening production growth and export expansion of the Philippine shrimp industry. This paper presents the status of AHPND in cultured penaeids and activities of the National Shrimp Health Management Program (NSHMP) of the Bureau of Fisheries and Aquatic Resources (BFAR) on importation policies, disease surveillance, monitoring and reporting, disease diagnosis, and preventive and control measures against AHPND and other transboundary diseases of cultured penaeids in the Philippines.

Introduction

Shrimp mortalities among cultured Pacific white shrimp (*Penaeus vannamei*) and black tiger shrimp (*P. monodon*) due to acute hepatopancreatic necrosis disease (AHPND) caused by unique strains of *Vibrio parahaemolyticus* (VP_{AHPND}) have recently stirred great concerns among growers, hatchery operators, traders, exporters, importers, government agencies, and researchers in the Philippines (Dabu *et al.*, 2015; dela Peña *et al.*, 2015). Amidst the economic losses experienced by the major shrimp-producing farms in the country, the Philippine Bureau of Fisheries and Aquatic Resources (BFAR) through its National Shrimp Health Management Program (NSHMP) has intensified its commitment to the shrimp industry in coming up with practical and effective strategies, i.e. exclusion and containment, aimed at controlling and preventing the further spread of AHPND and other emerging transboundary diseases of penaeid shrimps in the country (BFAR, 2015).

The exclusion strategy of the program includes the issuance of policies against AHPND, strict implementation on importation regulations of specific-pathogen-free (SPF) *P. vannamei* and *P. monodon* broodstocks and postlarvae (PL) to prevent entry of the shrimp pathogens, and accreditation/certification and registration of shrimp hatcheries and grow-out farms in compliance with biosecurity and health status monitoring of farmed shrimps. On the other hand, the containment strategy includes: (a) disease detection and diagnostic; (b) surveillance and reporting; (c) health certification for in-country transboundary movement of live (all stages) shrimps; (d) institutionalization of the sectors involved in the shrimp industry, especially the extensive farms; (e) promotion of biosecurity practices; (f) capacity building; and (g) massive information education campaign (IEC) and participation in training programs and proficiency testing.

The NSHMP has been in place prior to the occurrence of AHPND in the country. It is a

regular and continuing program of BFAR that was officially launched in 1998 and formally introduced to the industry in 2000. It was well supported by the private sector and other associated government agencies. It aims to increase and expand production and exports through intensification of shrimp health management. It adheres to the principles of the two-pronged approaches, i.e. exclusion and containment. Prior to the occurrence of AHPND in the country, shrimp diseases included in the program were white spot disease (WSD), Taura syndrome (TS), yellowhead disease (YHD)/gill-associated disease (GAD), infectious hypodermal haematopoietic necrosis (IHHN), infectious myonecrosis (IMN), white tail disease (MrNv), necrotising hepatopancreatitis (NHP), *P. vannamei* nodavirus (PvNV) infection, monodon baculovirus (MBV), and luminescent vibriosis.

The inclusion of AHPND in the NSHMP started in December 2013 when shrimp productions were rapidly declining in neighboring countries due to serious outbreaks of AHPND. BFAR organized a meeting with the private sector and agreed to suspend the processing and approval of permit to import live shrimps from neighboring countries with confirmed cases of AHPND and infectious myonecrosis (IMN). Following the suspension, BFAR continued to encourage hatcheries to apply for accreditation/certification and registration for proper monitoring and surveillance of AHPND and other shrimp diseases.

History of AHPND occurrence and its prevalence

The first record of AHPND in the Philippines was documented among Pacific white shrimp (*P. vannamei*) and black tiger shrimp (*P. monodon*) obtained from selected sites in Luzon (Bulacan, Pampanga, Bataan, and Batangas provinces), Visayas (Cebu and Bohol provinces), and Mindanao (General Santos and Saranggani provinces) islands. The occurrence of the disease was determined by microbiological methods and polymerase chain reaction (PCR) and was further confirmed by histopathology of the hepatopancreas of infected shrimp (Dabu *et al.*, 2015). AHPND-infected shrimp exhibited an empty midgut and stomach with pale hepatopancreas (Dabu *et al.*, 2015; Tran *et al.*, 2013). Compared to shrimps with normal hepatopancreas, infected shrimps have necrotic symptoms in their hepatopancreas. Some infected shrimp samples manifested acute phase of infection as evidenced by loosening and minimal degradation of the tubule epithelial cells. In addition, some shrimp samples examined demonstrated signs of increased sloughing around the tubule area leading to greater spaces around it whereas, others have enlargement of the nuclei and formation of bacterial colonies in hepatopancreas tubule epithelial cells denoting an early terminal and terminal phases of AHPND, respectively. The prevalence of VP_{AHPND} were 35% (39 positive/113 samples examined) in Luzon, 21% (40/194) in Visayas, and 5% (3/60) in Mindanao (Dabu *et al.*, 2015). The presence of AHPND in the Philippines was also confirmed among pond-cultivated *P. monodon* and *P. vannamei* sampled from a farm in Bohol in early 2015 and late 2014 by another group of researchers (dela Peña *et al.*, 2015). Interestingly, unlike classical AHPND in which typical disease signs and concomitant mortalities occur among PLs within 20 to 30 days after stocking in ponds, these authors noted that disease signs and mortality could still occur during the later stage of the culture cycle, i.e. between 46 and 96 days after pond stocking, suggesting that even older shrimp life stages are apparently susceptible to AHPND (dela Peña *et al.*, 2015).

BFAR laboratories and BFAR-recognized laboratories conducted a total of 2,606 analyses for AHPND in shrimps for the year 2015 (Table 1). AHPND positive samples totaled to 98, constituting 3.8 % of the total samples analyzed. Positive samples originated from 14 provinces namely Pangasinan, Cagayan, Bulacan, Batangas, Negros Occidental, Bohol, Zambales, Pampanga, Cebu, Iloilo, Leyte, Oriental Mindoro, Marinduque and Davao del Sur.

Table 1. AHPND analysis conducted by the Bureau of Fisheries and Aquatic Resources (BFAR) Laboratories and BFAR-recognized Laboratories in 2015.

Laboratory	Method of analyses	No. of positive/Total no. of samples analyzed (%)	Origin of positive samples (province)	Type of samples analyzed
BFAR Central Laboratory	a) IQ2000 AHPND/ EMS Toxin 1 (Detection and Prevention System) b) IQPlus AHPND/EMS2 plasmid c) IQPlus Toxin 1 d) IQPlus Pockit e) *Vibrio parahaemolyticus* compact dry f) Culture on thiosulfate-citrate-bile salts-sucrose (TCBS) agar	48/893 (5.4)	a) Pangasinan b) Cagayan c) Bulacan d) Batangas e) Negros Occidental f) Bohol	*Penaeus vannamei* & *P. monodon* zoea, mysis, postlarvae *P. vannamei* juvenile, water *P. vannamei* & *P. monodon* juveniles, water, broodstock *P. vannamei* postlarvae, juvenile soil, water *P. vannamei* juvenile,water *P. vannamei* postlarvae, juvenile, broodstock, water
BFAR Region I	IQPlus AHPND/EMS Toxin 1	0/17 (0)	-	*P. vannamei* postlarvae, juveniles, polychaetes, broodstocks
BFAR Region III	IQPlus AHPND/EMS Toxin	2/32 (6.3)	a) Zambales b) Pampanga	*P. vannamei* juvenile *P. vannamei* juvenile
BFAR Region IV-a	IQPlus Toxin 1	0/43 (0)	No AHPND positive	*P. monodon* & *P. vannamei* nauplii, mysis, zoea, postlarvae, juveniles
BFAR Region V	IQPlus Toxin 1	0/31 (0)	-	*P. monodon* & *P. merguiensis* postlarvae, juveniles; crab (*Scylla serrata*)
BFAR Region VII	a) IQPlus Toxin I b) IQPlus AHPND/ EMS2 plasmid c) API NE20 d) Culture on TCBS agar	13/384 (3.4)	a) Bohol b) Cebu c) Leyte	*P. vannamei* juveniles *P. vannamei* & *P. monodon* juvenile, broodstocks *P. vannamei* post-larvae
BFAR Region X	IQPlus Toxin 1	0/17 (0)	-	*P. vannamei* & *P. monodon* postlarvae, juveniles; polychaetes
BFAR Region XI	IQPlus Toxin 1	0/4 (0)	-	*P. vannamei* & *P. monodon* postlarvae, juveniles; polychaetes
BFAR Region XIII	IQPlus Toxin 1	0/25 (0)	-	*P. vannamei* & *P. monodon* postlarvae, juveniles; polychaetes

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Laboratory	Method of analyses	No. of positive/Total no. of samples analyzed (%)	Origin of positive samples (province)	Type of samples analyzed
Southeast Asian Fisheries Development Center / Aquaculture Department (SEAFDEC/ AQD)	IQ 2000 Toxin 1	28/188 (14.9)	a) Pampanga b) Iloilo c) Cebu d) Leyte e) Davao del Sur f) Marinduque g) Oriental Mindoro	*P. vannamei* juvenile *P. monodon* postlarvae *P. vannamei* & *P. monodon* juveniles *P. vannamei* juvenile *P. vannmaei* juvenile *P. monodon* juvenile (wild) *P. vannamei* & *P. monodon* juvenile
Negros Prawn Producers Cooperative (NPPC)	a) IQ2000 Toxin 1 b) IQPlus Toxin 1	7/972 (0.7)	a) Bohol b) Cebu c) Negros Occidental	*P. vannamei* adult, postlarve, broodstock, *P. vannmaei* postlarvae, juveniles; polychates (bloodworms), *P. vannamei* & *P. monodon* postlarvae, juvenile, broodstock, nauplii
Total		**98/2,606 (3.8)**		

Diagnostic methods

BFAR Laboratories located in major shrimp-producing regions have the capability to diagnose AHPND by molecular methods including IQ2000 AHPND/EMS Toxin 1 Detection and Prevention System, IQPlus Toxin 1, and IQ Plus Pockit micro PCR. There are 17 shrimp disease diagnostic laboratories operating nationwide (Figure 1). Fifteen of these laboratories are being operated by BFAR while the other 2 BFAR-recognized laboratories are being operated by the Aquaculture Department of the Southeast Asian Fisheries Development Center (SEAFDEC/AQD) and the Negros Prawn Producers Cooperative (NPPC), respectively.

Disease prevention and control

The strategies of the National Shrimp Health Management Program against AHPND include the exclusion and containment.

Exclusion strategy

This strategy involves the prevention of entry and spread of shrimp disease vectors into the country, including AHPND and other transboundary diseases. Because live shrimps (PL or broodstocks), annelids (polychaetes) and clams are potential carriers of pathogens, policies on their importation have been strictly practiced. The existing policies to prevent the entry and spread of shrimp disease vectors have been approved years before the occurrence of AHPND in the country. These policies include (a) Fisheries Administrative Order (FAO) 189 s. 1993 which prohibits the importation of live shrimps of all stages. This was later amended to include prohibition of culture of imported shrimps through FAO 207 s. 2001; (b) Republic Act 8550 of 1998 as amended by RA 10654 in 2015 known as the Philippine Fisheries Code; Section 61.d on importation and exportation of fishery products, i.e. *No person, shall import and/or export fishery products of whatever size, stage or form for any purpose without securing a permit from the Department*; (c) FAO 207 s. of 2001 prohibiting the importation and culture of imported live shrimp of all stages; and (d) FAO 221 s. of 2003 further regulating the importation of live fish and fishery or aquatic products under FAO 135 s. 1981 to include microorganisms and biomolecules.

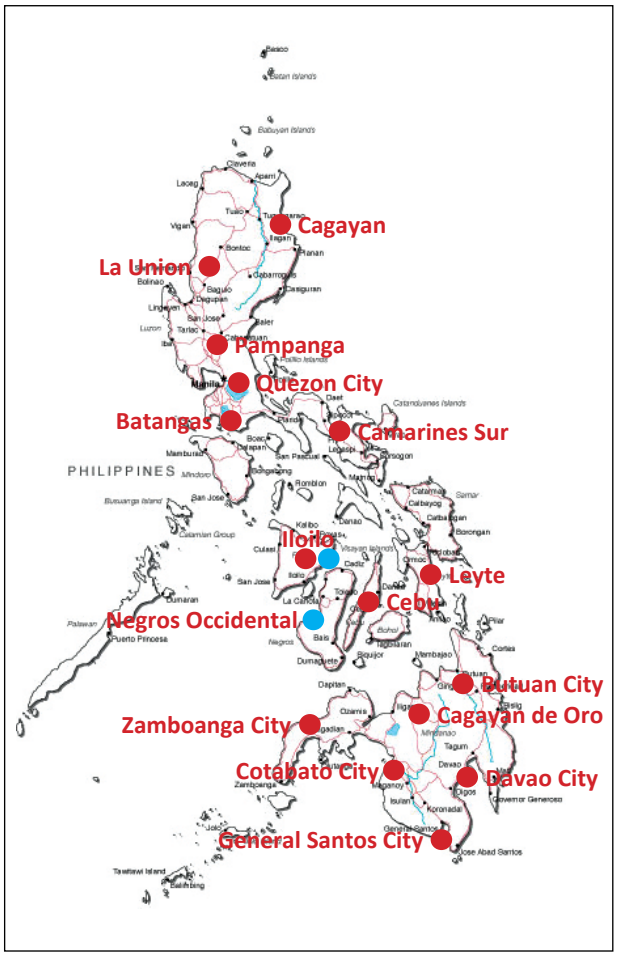

Figure 1. Distribution of BFAR (●) and BFAR-recognized (●) Diagnostic Laboratories for shrimp disease detection in the Philippines.

It was in 2005 when experiments on culture and breeding of *P. vannamei* were conducted at the BFAR facility in Dagupan City, Pangasinan Province (Luzon). After 2 years of successful experimentation, importation and subsequent cultivation of *P. vannamei* in hatcheries and grow-out farms in the Philippines was permitted by the BFAR. In 2009, FAO 230 s. of 2009 was issued to allow the importation of specific-pathogen-free (SPF) *P. monodon* broodstock and PL. It should be noted however, that (a) FAO 225 s. of 2007 (Amending FAO 207), i.e. allowing the importation of *P. vannamei* broodstock and culture of the offspring and (b) FAO 225-1 s. of 2007 stipulating the guidelines for the importation of *P. vannamei*, were among the administrative orders issued by BFAR to regulate the importation, breeding and culture of *P. vannamei*, and prevention of the introduction of transboundary diseases of penaeid shrimps in the country.

In 2013, when the shrimp production in China, Viet Nam, Thailand and Malaysia declined as a consequence of AHPND outbreaks (Tran *et al.*, 2013; Joshi *et al.*, 2014), Fisheries Office Order (FOO) 146 and Fisheries General Memorandum Order (FGMO) 03 were issued. FOO 146 Series of 2013 strictly stipulated the suspension of processing and approval of applications for permit to import all live shrimps and other susceptible crustaceans from Asian countries and other countries affected by AHPND. On the other hand, FGMO 03 s. 2013 enumerated the guidelines on accreditation of *P. vannamei* and *P. monodon* hatcheries for proper monitoring and surveillance of cultivated shrimps for AHPND and other equally important shrimp diseases. The abovementioned rules and regulations were presented through public consultations, i.e. dialogue between the government and private sector representatives.

Importation of specific-pathogen-free (SPF) ***P. vannamei and P. monodon***

In order to prevent the entry of shrimp pathogens into the country, the requirements for broodstock importation of BFAR accredited hatchery for submission to BFAR Fisheries Regulatory and Quarantine Division (FRQD) include the following: (a) letter of intent to import; (b) certificate of compliance issued by BFAR; (c) disease history of the broodstock facility where the broodsotck originated from the time of its commercialization; and (d) two years disease-free status of the broodstock facility as certified by the competent authority of the country of origin, and travel details. Upon submission, the permit to import will be issued by the Director of BFAR and will be only valid for 10 days upon issuance.

P. vannamei importation started in 2007. Only few hatcheries were accredited or certified during that time. As the demand for *P. vannamei* culture increased, more hatcheries applied for accreditation/certification and the number of imported broodstocks consequently increased. Importations of SPF *P. vannamei* broodstocks by BFAR-accredited hatcheries are shown in Table 2. The importation of SPF *P. vannamei* broodstocks significantly increased by more than two folds, i.e. from 6,818 to 14,519 individuals in 2010 and 2015, respectively.

Table 2. Number of specific-pathogen-free (SPF) *P. vannamei* broodstocks imported by BFAR-accredited hatcheries from 2010 to 2015.

Importation Data	Year					
	2010	2011	2012	2013	2014	2015
No. of importation	22	20	21	33	20	25
No. of female *P. vannamei* broodstocks imported (pcs)	3,424	3,538	2,768	6,960	3,967	7,410
No. of male *P. vannamei* broodstocks imported (pcs)	3,394	3,538	2,768	6,915	3,960	7,109
Total no. of *P. vannamei* broodstocks imported (pcs)	6,818	7,076	5,536	13,875	7,927	14,519

Source: BFAR, 2015

Table 3. Number of specific-pathogen-free (SPF) *P. monodon* broodstocks imported by a BFAR-accredited hatchery from 2013 to 2015.

Importation Data	Year		
	2013	2014	2015
No. of importation	1	1	2
No. of female *P. monodon* broodstocks (pcs)	150	250	262
No. of male *P. monodon* broodstocks (pcs)	125	170	266
Total no. SPF *P. monodon* broodstocks (pcs)	275	420	528

Source: BFAR, 2015

There is only one BFAR Accredited SPF *P. monodon* hatchery that imported a total of 528 *P. monodon* broodstocks in 2015 (Table 3). The increase in the importation of *P. vannamei* and *P. monodon* broodstocks from 2013 up to 2015 has been attributed to the rising demand for better quality shrimp PL.

Accreditation and registration of SPF P. vannamei *and* P. monodon *hatcheries*

The minimum biosecurity standards for hatchery facilities of SPF *P. vannamei* and *P. monodon* are stipulated in FOO 225-1 s. 2007, FOO 225-2 s. 2008, 230, 230-1 and 231 s. 2009. It includes: (a) treatment of incoming water through filtration, water disinfection and sedimentation, and water conditioning; (b) disinfection of effluent water; (c) physical isolation for each section in the hatchery; (d) provision of aeration with controlled airflow in each section of the facility; and (e) sanitation and disinfection of workers within the premises. The BFAR-accredited SPF *P. vannamei* and *P. monodon* hatcheries totaled to 23 and 2, respectively in 2015. To ensure compliance to biosecurity, frequent inspections of hatchery facilities have been conducted by BFAR officers.

BFAR-accredited and registered SPF P. vannamei *and* P. monodon *grow-out farms*

To date, the total number of BFAR-accredited/certified and registered shrimp farms is 324. This number corresponds to a total area of 4,820.43 hectares of cultivation ponds. An updated list of accredited/certified and registered hatcheries and grow-out farms is posted monthly at BFAR website (www.da.bfar.gov.ph) depending on whether there are newly accredited/certified hatcheries or grow-out farms. The list includes the name of grower or hatchery, address of the farm and contact number(s) of grower/hatchery operator or representative, and accreditation/certification or registration number.

Containment strategy

The principle of this strategy is to prevent the spread of AHPND in the country, i.e. to contain AHPND in affected areas and to protect the yet uninfected areas free of AHPND.

Table 4. Health certificates issued for in-country transboundary movement of shrimp postlarvae from 2012-2015 for aquaculture purposes.

REGION	YEAR							
	2012		2013		2014		2015	
	No. of HC issued	No. of PL transported	No. of HC issued	No. of PL transported	No. of HC issued	No. of PL transported	No. of HC issued	No. of PL transported
Region 3	23	Nr	56	34,288,000	11	12,504,500	13	20,000,000
Region 6	335	Nr	190	58,250,000	119	38,270,000	67	15,196,000
Region 7	1,261	770,480,455	1,634	1,099,791,400	1,661	1,429,102,345	1,616	762,417,600
Region 11	1	300,000	53	10,385,000	39	10,225,000	22	12,625,000
Region 12a	-	-	-	-	-	-	50	60,500,000
Central	23	19,400,000	31	29,600,000	31	101,055,000	32	121,400,000
Total	**1,643**	**790,180,455**	**1964**	**1,142,318,400**	**1,858**	**1,681,156,845**	**1,800**	**992,138,600**

HC: health certificate; PL: postlarvae

Disease surveillance, monitoring, and reporting

BFAR has been actively involved in the Quarterly Aquatic Animal Disease Reporting System that has been adopted region-wide within Asia-Pacific under the joint program of United Nations-Food and Agriculture Organization (UN-FAO), Network of Aquaculture Centres in Asia-Pacific (NACA), and the World Organization for Animal Health (OIE). Laboratories conducting disease diagnosis are required to submit quarterly reports to the OIE focal point for consolidation and report submission to the OIE. In order to prevent AHPND, it was recommended that the hatcheries and grow-out farms practice strict biosecurity measures such as longer drying up of tanks or ponds prior to stocking, regular water quality monitoring and health status of shrimps, and adoption of the 'green water' technology.

Health Certification for in-country transboundary movement of live shrimps

The issuance of health certificate (HC) for in-country transboundary movement of live shrimps is primarily based on the results of the BFAR-prescribed laboratory tests that are either conducted at BFAR or BFAR-recognized laboratories. This is to ensure that shrimps to be cultured are free from diseases. There is a 3% decrease in the number of HC issued in 2015. One thousand eight hundred fifty-eight and 1,800 HC were issued by BFAR in 2014 and 2015, respectively. A decrease of 59% was observed in the number of PLs transported, i.e. from 1.68 billion in 2014 to 992 million in 2015. This was due to a decrease in the demand for PLs in 2015 (Table 4).

Way forward

AHPND is already present in the Philippines. The Philippines was able to curb massive outbreaks through the application of 'green water' technology, biosecurity, health certification and disease surveillance, monitoring, and diagnosis (Usero *et al.*, 2015). Thus, to prepare the country's shrimp industry for another possible inadvertent emergence of a devastating disease, the NSHMP of BFAR recommends the following: (a) promotion and strict implementation of Shrimp Good Aquaculture Practices among hatcheries and grow-out farms throughout the country which should be harmonized among ASEAN member states as part of the ASEAN Economic Cooperation/ Integration; (b) issuance of health certificates by the competent authority of the country where live shrimps originate; (c) accurate laboratory results with accompanying lot number or batch of the sample being transported to prevent pathogen spread within the region; (d) proper communication among growers particularly with regard to prompt reporting of any possible or suspected disease outbreak in their farms; BFAR's confirmation of the disease outbreak will serve as basis for an advisory to prevent and control further spreading of the disease;

and (e) active participation of the private sector in disease surveillance and reporting of any disease incidence to the competent authority.

Acknowledgements

Special thanks to BFAR Director Atty. Asis G. Perez, BFAR Assistant Director Drusila Esther E. Bayate, Dr. Donald D. V. Lightner and his staff, Aquaculture Pathology Laboratory, University of Arizona, USA, BFAR Regional Director Juan D. Albaladejo, DVM, Dr. Joselito R. Somga, BFAR Fish Health Officer, Ms. Roselyn Usero of NPPC and SEAFDEC AQD.

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